Lab 3 (10/3/18) - Microbial Growth 1/2

Temp

0-15	**0-30**	**25-40**	**50-60**	**80**
Psychrophile **can grow at 0 but optimum 15** *arctic organisms*	Psychrotroph **can grow at 0 but prefer 20-30** *fridge spoilage*	Mesophile	Thermophile	Hyperthermophiles
		Growth at 25 but better at 37 ***S. aureus*** ***E. coli*** ***B. subtilis***	 ***S. thermophilus***	

pH

Acidophile	Neutrophile	Alkalinophile
	Best growth at pH7 ***S. aureus*** ***E. coli*** ***B. subtilis*** Some growth at pH7 *E. Faecalis*	Best growth at pH9 ***E. faecalis*** Some growth at pH9 *S. aureus* *E. coli* *B. subtilis*

Osmotic Pressure (NaCl)

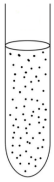
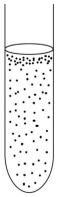
low	**high**
non halophile can't grow in salt ***S. cerevisiae***	obligate halophile aka strict halophile needs salt ***H. salinarium***
facultative halophile (halotolerant of up to 2% NaCl altho S. aureus grew all the way to 15% and E. coli grew up to 5%) ***S. aureus, E. coli***	

0%	5%	10%	15%	25%
S. cerevisiae S. aureus E. coli	S. aureus E. coli	S. aureus	S. aureus	**H. salinarium**

O_2

Superoxide free radicals (O2-) are formed in small amounts during normal aerobic respiration. It's neutralized by a couple of enzymes. Organisms that produce these enzymes can survive in oxygen.

O2- ---(superoxide dismutase)---> H_2O_2 or O_2
H_2O_2 -----(catalase)----> 2 H_2O + O_2

Obligate anaerobes die in oxygen b/c they don't produce the 2 enzymes  *Clostridium*	**Aerotolerant anaerobes dont** use oxygen but they tolerate its presence b/c they **can produce those 2 enzymes** 	**Facultative anaerobes thrive with oxygen but can also survive without oxygen** b/c they can switch to fermentation  *E. coli & E. faecalis*	**Obligate aerobe** need oxygen to grow  *M. luteus*
			M**icroaerophiles** ***are aerobic. They do require oxygen*** but are poisoned by high concentrations. They gather in the upper part of the test tube but not the very top. 

Chemical Requirements for Microbial Growth

Carbon

- ½ dry weight is C
- Chemoautotrophs use inorganic sources of C to make organic compounds
- Chemoheterotrophs must take C in from outside

Nitrogen for the AA backbone.
Sulfur is used to synthesize sulfur-containing AA and vitamins
Phosphorus synth of nucleic acids / phospholipid membranes / ATP
Trace elements Fe, Cu, Zn, Molybdenum

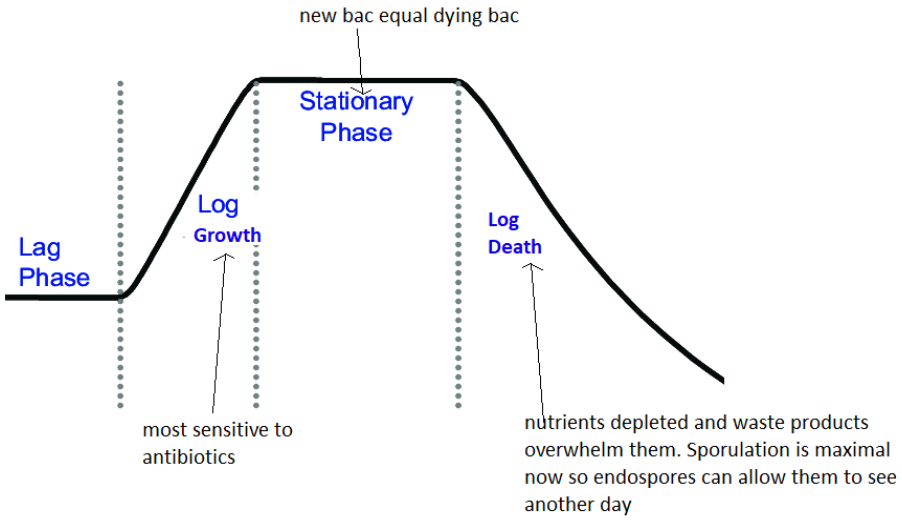
Lab 4 - 10/10/18 - Microbial Growth 2/2

Binary fission generally involves four steps.

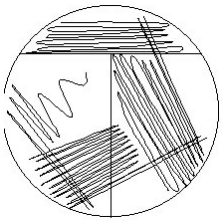
1. Cell replicates chromosomes
2. Cell elongates
3. Cell forms a new cytoplasmic membrane and wall (septum) across the midline.
4. When the septum completed, the daughter cells may remain attached or they may separate completely.

Generation time = time for bacterial population to double

Bacteria grow logarithmically and note that there are TWO log phases here



Streak plate method can be used to isolate different bacteria from a mixed culture



S. marcesens produces red colonies
S. aureus produces yellow colonies
E. coli produces white colonies

Plate Counts:

Purpose: Measure bacterial populations in terms of **colony forming units (CFU).**

Advantage- measures the number of *viable* cells.

Disadvantage - takes 24 hours + for visible colonies to form.

Performance: one of two methods may be used:

Spread Plate Method: 0.1 ml inoculum is added to the surface of a prepoured, solidified

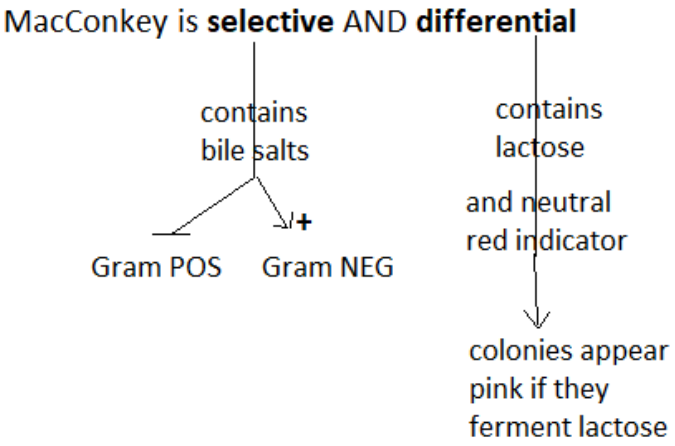
Pour Plate Method: 0.1 or 1.0 mL of a serial dilution of bacteria is poured into a petri dish and then liquefied nutrient medium poured over it. The drawback is that some heat sensitive organism will be damaged by the melted agar and those colonies that form under the surface won't help with surface ID.

Interpretation: If I count 11 colonies on a serial dilution diluted 10^7 times then I had $11x10^7$ bacteria in the original suspension

Selective media - promotes growth of one organism while inhibiting another

Differential media - enables us to characterize closely related organisms based on certain characteristics (eg. blood agar is differential and it differentiates based on bacterial ability to break down RBCs)

Alpha hemolytic streptococcus kills RBC and degrades components partially
Beta hemolytic streptococcus kills RBC and degrades components completely
Gamma hemolytic streptococcus does not kill RBC



Q: what color colonies will you see on a plate inoculated with lactose fermenting staph aureus?
A: no colonies!

We found that e.coli are gram neg and ferment lactose

<u>Preserving Bacterial Cultures</u>:

Two common methods of preserving microbial cultures for long periods are the following:

1. **Deep freezing** is a process in which a pure culture of microbes is placed in a suspending liquid and quick-frozen at temperatures ranging from -50 degrees to -95 degrees Celsius. The culture can usually be thawed and cultured even years later.
2. **lyophilization (freeze-drying)** a suspension of microbes is quickly frozen at temperatures ranging from –54 to -72 degrees Celsius, and the water is removed by a high vacuum (sublimation). The remaining powder like residue that contains the surviving microbes can be stored for years. The organism can be revived at any time by hydration with a suitable liquid nutrient medium.

<u>2nd Quiz will be on:</u>
microbial growth & results
Viable plate count
Generation time
Microbial count
Macconkey agar

Lab 5 - 10/17/18 - Control of Microbial Growth

Semmelweis and Lister developed antimicrobial practices for medical procedures (hand washing, aseptic surgery to prevent contamination)

Physical methods of controlling microbial growth			
Method	**Outcome**	**Example**	**Note**
Sterilization	Destruction of **all life including endospores**	Heat/Autoclave	-Boiling alone won't ensure sterility -Dry heat kills most vegetatives but endospores survive. -Moist heat is the best killer as it causes protein coagulation
Commercial sterilization	**Kill Clostridium botulinum and its endospores**	heating	Preserve taste of food
Antiseptic	Used on **living** organism to kill harmful **vegetative pathogens**	Usually **chemical** but can also be UV, etc	Numbers of microbes, their character, presence of organic matter, environment, pH, etc effect how well it'll work
Disinfectant	Used on **inanimate** object to kill harmful **vegetative pathogens**		
Degermination	Mechanical removal of debris	Alcohol swabbed skin prior to injection	
Sanitization	Decrease microbe counts to safe public levels		
Cidal agent	Kills bacteria		
Static agent	Slows down bacterial growth	Refrigerators	

The **Rate of Microbial Death** is a constant rate (90% in the first min; 90% in the 5th, etc)

Many biocides have **difficulty killing GRAM NEG** b/c they have an LPS layer which protects them *(eg. **Pseudomonas** are very resistant to biocides b/c they have porins in their cell wall which are highly selective of molecules that they permit to enter the cell)*

Thermal death Point - lowest temp at which all organisms in a liquid suspension killed in 10'
Thermal death time - minimal length of time at which all organisms in a liquid suspension killed at given temp
Decimal reduction time - time it takes to kill 90% of em (in minutes)

Physical Methods of Microbial Control:

Moist heat kills primarily by the **coagulation of proteins aka denaturation (destruction of H bonds)** Boiling is not the most reliable form of sterilization as it kills vegetative but can leave endospores. You'd need temps above boiling and that's accomplished by autoclave.

Dry heat kills by oxidation effects (flame)

Pasteurization – heating kills bacteria in a liquid so it takes longer to spoil (also ultra high temp treatment kills so well that liquid can be stored w/o refrigeration)

Dessication – (example lyophilization aka freeze drying)

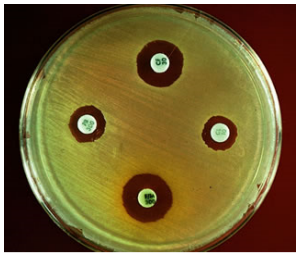
Ionizing radiation (Gamma rays, X rays) are high energy and ionize water to form hydroxyl radicals which react with organic cell components like DNA

Nonionizing radiation (UV) is lower energy. Form Thymine dimers

Results:

Bacillus subtilis	**Staph aureus**	**Pseudomonas Aeruginosa**	**E. coli**
Less sensitive to UV b/c this forms endospores		Yes sensitive to UV	Yes sensitive to UV
		Very resistant to antibiotics and disinfectants	
	Most common cause of cellulitis (yellowish color)	Greenish tint	
Sensitive to Erythromycin		Only sensitive to Nitrofurantoin	Sensitive to Tetracycline & Nitrofurantoin

Kirby-Bauer uses **disk-diffusion method** to determine efficacy of antibiotics (in our case on **Mueller-Hinton Agar**)

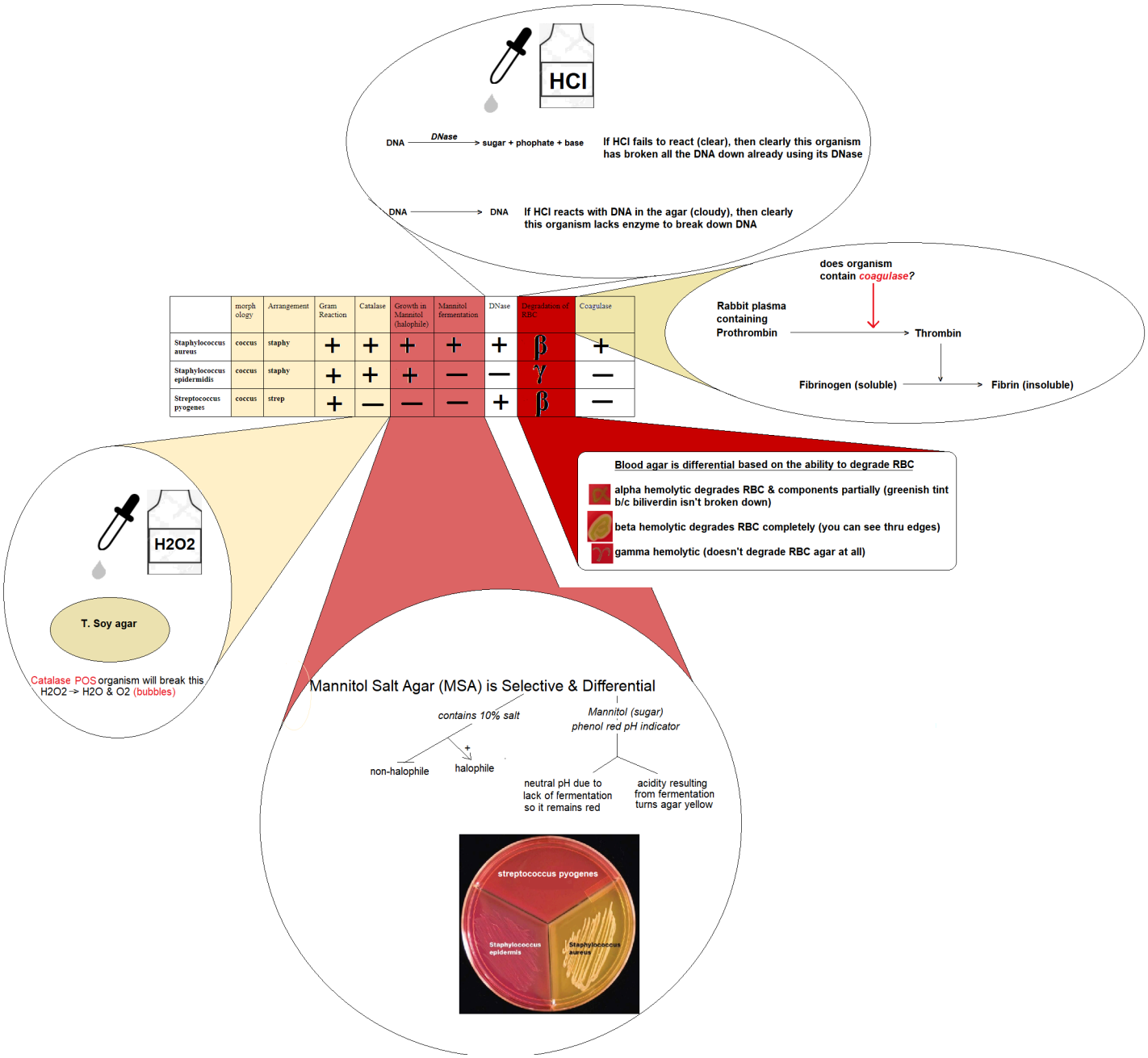


Chemical Methods of Microbial Control - don't achieve sterility but help reduce to safe levels of vegetative microbes	
Phenol	-control surgical infections in OR **-controls sewage odor**
Phenolics	-damage plasma membrane (lysol)
Iodine	oldest and effective antiseptics. It is effective against all kinds of bacteria, many endospores, various fungi, and some viruses. Iodine **tincture:** Iodine in aqueous alcohol. Iodine **iodophor:** combo of iodine and an organic molecule, from which iodine is released slowly (betadine and isodine)
Chlorine	disinfectant
Biguanide	broad spectrum of activity. -frequently used for microbial control on skin and mucous membranes -surgical hand scrubs and preoperative skin prep -biocidal against vegetative organisms but endospores **not affected.**
Alcohols	-optimum concentration of ethanol is 70% (not 100% b/c you need some H2O to work) **-**can't kill endospores. -denatures protein & dissolves lipids -used in degermination of skin prior to injection -unsatisfactory antiseptic when applied to wounds
surfactants	**soap just help mechanically remove stuff (eg. Quaternary ammonium)**

History: **Fleming** saw staph aureus inhibited by penicillin mold on a petri dish.

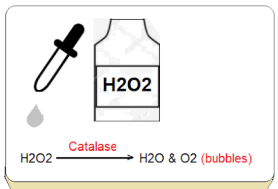
Target	Name	Details
Cell Wall	Penicillin	-beta lactam ring -prevent the cross-linking of peptidoglycan, which interferes with construction of the cell wall.
	Cephalosporin	Like the above but even more effective
	Carbapenems	Like the above
	Bacitracin	-primarily against gram-pos -inhibits cell wall at an even earlier stage than the above **-superficial application only**
	Vancomycin	**g**lycopeptides antibiotics. -toxic -last line of defense against resistant staph aureus
	Isoniazid	stops TB but messing up mycolic acid integration into cell wall
	Ethambutol	-only works against mycobacteria (prevents mycolic acid from forming their cell wall)
Protein Synthesis	Aminoglycosides	group of antibiotics in which amino sugars are linked by glycoside bonds. Examples are streptomycin, neomycin, and gentamycin.
	Tetracyclines	-broad spectrum -especially valuable against intracellular rickettsias and chlamydias. -**discolor baby teeth** or damage pregnant livers
	Chloramphenicol	-comes from Streptomyces but cheaper to synthesize chemically -suppress bone marrow activity
	Macrolides	-erythromycin -not able to penetrate the cell walls of most gram-negative bacilli.
Plasma Memb	Polymyxin B	-topical treatment of superficial skin infections
Nucleic Acid	Rifamycin	-Rifampin -inhibits synthesis of mRNA -treat TB & leprosy -penetrate tissues and reach therapeutic levels in CSF -orange tinted bodily wastes
	Quinolone	Nalidixic acid -inhibits DNA gyrase used for DNA replication
comp. inhib. synthesis of essential metabolites	Sulfonamides	**-Sulfa drugs are the first synthetic antimicrobials** -diminished use now but still used in burn patients

Lab 6 - 10/24/18 - Unknown Lab #1



	morph ology	Arrangement	Gram Reaction	Catalase	Growth in Mannitol (halophile)	Mannitol fermentation	DNase	Degradation of RBC	Coagulase
Staphylococcus aureus	coccus	staphy	+	+	+	+	+	β	+
Staphylococcus epidermidis	coccus	staphy	+	+	+	—	—	γ	—
Streptococcus pyogenes	coccus	strep	+	—	—	—	+	β	—

11/7/18 Lab Unknown # 2 (unknown 15) quiz + chart due next week



T. Soy agar

Phenol Red Test & Durham Tube

ferments sugar?

yes → pH < 6.5 (yellow)

no → pH > 6.5 (red)

Determining Mixed Acid Fermentation using Methyl Red Test

some produce multiple acids as a consequence of fermentation. This is called Mixed Acid Fermentation

Mixed Acid Fermentation occurs?

yes pH < 4.5 → methyl red turns red

no → broth stays yellow

Determining Alcohol Fermentation using Voges-Proskauer Test

Some org. ferment sugar to produce alcohol byproducts like acetoin

Add 8 drops alpha naphthol and KOH (Barritt's Reagent A & B)

acetoin present → Pinkish

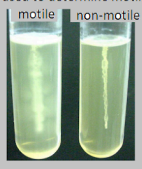
nope → yellow

	Gram Reaction	Shape	Arrangement	Catalase	Ferments Glucose?	Ferments Lactose?	Ferments Mannitol?	Disulfurase	Citrase	Tryptophanase	Mixed Acid Fermentation	Alcohol Fermentation	Motility
					gas	gas	gas						
Escherichia coli	-			+	+ Gas	+ Gas	+ Gas	-	-	+	+	-	+
Staphylococcus aureus	+			+	+ NO	+ NO	+ NO	-	-	-	+	-	-
Enterobacter aerogenes	-			+	+ Gas	+ Gas	+ Gas	-	+	-	-	+	+
Salmonella enteritidis	-			+	+ Gas	- NO	+ Gas	+	+	-	+	-	+
Enterococcus faecalis	+			-	+ NO	+ NO	+ NO	-	-	-	+	-	-

Agar deep

used to determine motility

motile | non-motile



Kligler's Iron Agar (disulfurase)

disulfurase allows org. to extract S from sulfer containing compounds

sodium thiosulfate in agar

disulfurase → Produces hydrogen sulfide which recombines with ferrous sulfate in agar to yield a **BLACK PRECIPITATE**

nope → No black precipitate

Simmons Citrate Agar (contains Bromthymol blue)

citrase allows the organism to obtain C from citrate for use in the Krebs Cycle. One of the byproducts of the Krebs Cycle is produced by decarboxylation (CO_2)

Citrate

citrase → CO_2

Reacts with Na^+ in agar to form $NaHCO_3$ (sodium bicarbonate) which raises pH

Agar turns **BLUE**

No enzyme? → Agar remains **GREEN**

Determining Presence of Tryptophanase using Tryptone Broth

Tryptophan

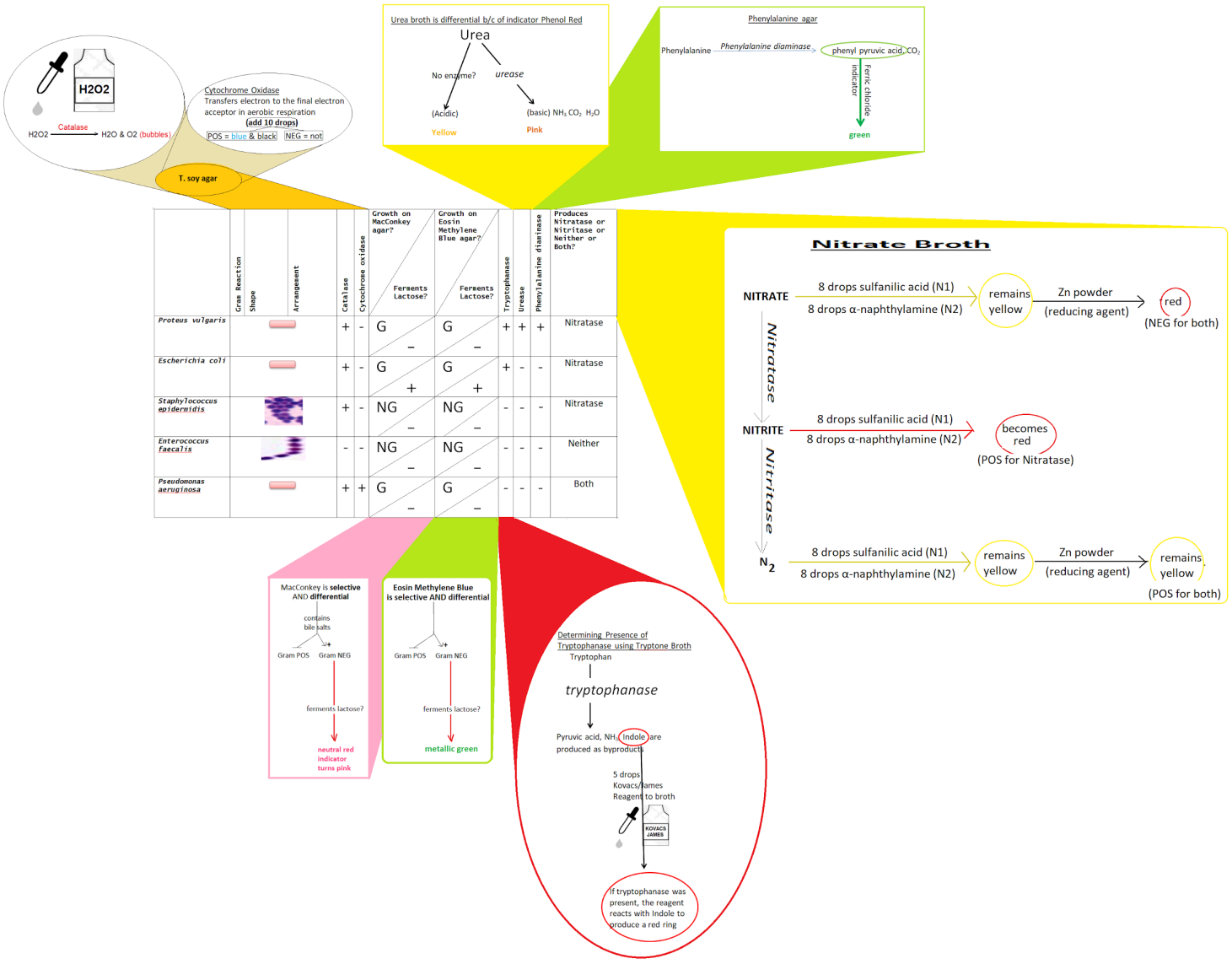
tryptophanase

Pyruvic acid, NH_3, Indole are produced as byproducts

5 drops Kovacs/James Reagent to broth

If tryptophanase was present, the reagent reacts with Indole to produce a red ring

11/21/18 Lab Unknown #3 (unknown 6)



	Gram Reaction	Shape	Arrangement	Catalase	Cytochrome oxidase	Growth on MacConkey agar? Ferments Lactose?	Growth on Eosin Methylene Blue agar? Ferments Lactose?	Tryptophanase	Urease	Phenylalanine diaminase	Produces Nitratase or Nitritase or Neither or Both?
Proteus vulgaris				+	-	G / –	G / –	+	+	+	Nitratase
Escherichia coli				+	-	G / +	G / +	+	-	-	Nitratase
Staphylococcus epidermidis				+	-	NG / –	NG / –	-	-	-	Nitratase
Enterococcus faecalis				-	-	NG / –	NG / –	-	-	-	Neither
Pseudomonas aeruginosa				+	+	G / –	G / –	-	-	-	Both

Bacterial Anatomy

Describe the four major processes of living cells.

Growth (in size)
Reproduction (growth in numbers
Responsiveness (react to conditions - eg. taxis towards attractant or away from repellant)
Metabolism (take in nutrients and use them in controlled chemical reactions)

Compare and contrast Prokaryotic and Eukaryotic cells.

	Eukaryotes	Prokaryotes
DNA	-In cell nucleus -Multiple double stranded chromosomes -DNA with histones	-In cytoplasm -Single round, double stranded chromosome -Not associated with histones -often have extrachromosomal plasmids (antibiotic resistance) which replicate independent of cells DNA
Organelles	Membrane enclosed organelles	No membrane enclosed organelles
Reproduction	Mitosis	Binary Fission
Cell wall	Most lack cells wall but plants do & it's composed of cellulose	Most have cell wall composed of the complex polysaccharide **peptidoglycan**

Describe composition, function, relevance to human health of glycocalyces & capsules vs slime layer.

Glycocalyx is a gelatinous, sticky polymer external to cell wall that can protect against dehydration.

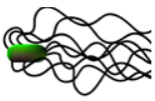
Capsule - if the glycocalyx is well organized and firmly attached to the cell wall
Slime layer - if the glycocalyx is unorganized and loosely attached to the cell wall

It's a **virulence factor** (a characteristic that increases ability of organism to cause harm) b/c it also protects against phagocytosis by cells of the host. Three examples include *Bacillus anthracis, Streptococcus pneumoniae, Klebsiella pneumoniae.*

A glycocalyx made of sugars is an **extracellular polysaccharide** (**EPS**). This helps the bacterium attach to various surfaces which may help it survive.

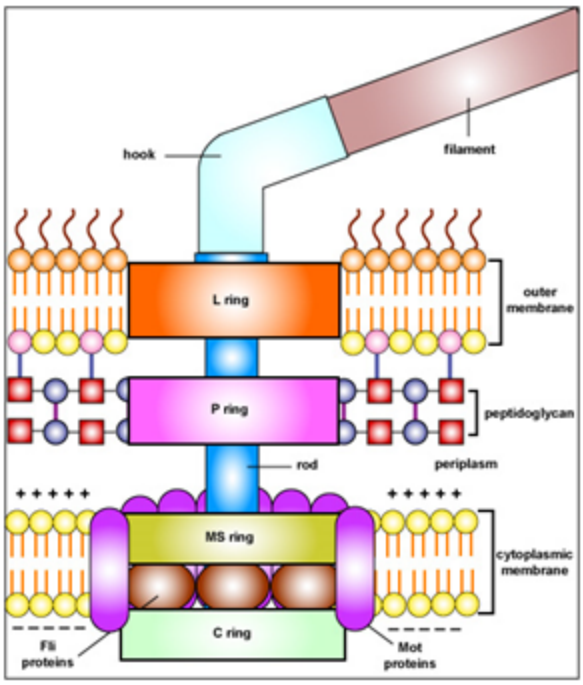
Discuss the structure, functions and arrangements of prokaryotic flagella.

Some prokaryotic cells have **flagella** for propulsion. There are four arrangements as follows:

Monotrichous		single polar flagellum
Amphitrichous		single flagellum at each end
Lophotrichous		two or more flagella at one pole
Peritrichous		flagella distributed all over

A flagellum has three basic parts: 1. **The filament** **2. The Hook** **3. The basal body**
Each prokaryotic flagellum is a semi-rigid, helical structure that moves the cell by rotating from the basal body.

Structure of a Bacterial Flagellum: Gram Negative



Note:
Gram NEG: 4 discs attach flagella to cell
GRAM POS: 2 discs attach flagella to cell

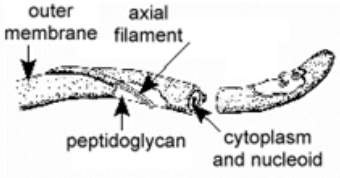
Structure of a Bacterial Flagellum: Gram Negative

*The filament of the bacterial flagellum is connected to a **hook** which, in turn, is attached to a **rod**.*
*The **basal body** of the flagellum consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane. In gram-negative bacteria, the **L ring** anchors the flagellum to the lipopolysaccharide layer of the outer membrane while the **P ring** anchors the flagellum to the peptidoglycan portion of the cell wall. Gram-negative bacteria contain two pairs of rings; the outer pair of rings is anchored to various portions of the cell wall, and the inner pair of rings is anchored to the plasma membrane. In gram-positive bacteria, only the inner pair of rings is present. One ring is embedded in the cell membrane and another in the cell wall.*

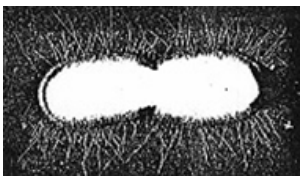
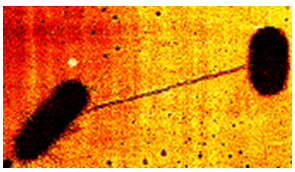
*The **MS ring** is located in the cytoplasmic membrane and the **C ring** in the cytoplasm. The **Mot proteins** surround the MS and C rings of the motor and function to generate torque for rotation of the flagellum. Energy for rotation comes from proton motive force. Protons moving through the Mot proteins drives rotation.*
*The **Fli proteins** act as the motor switch to trigger either clockwise or counterclockwise rotation of the flagellum and to possibly disengage the rod in order to stop motility.*

Axial Filaments aka **endoflagella** are bundles of fibrils that spiral around the inside of the cell. They help the **spirochete** move in a spiral motion *(eg. Treponema pallidum,* the causative agent of syphilis, and *Borrelia burgdorferi,* the causative agent of Lyme disease).

Spirochete Axial Filaments

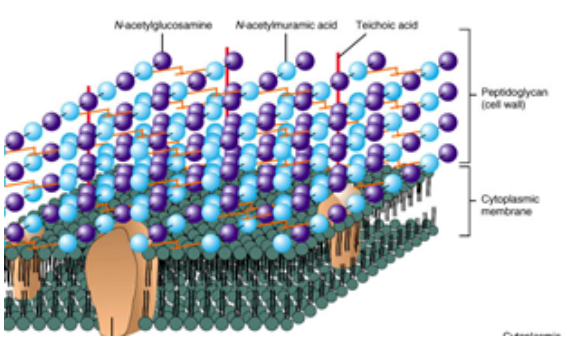
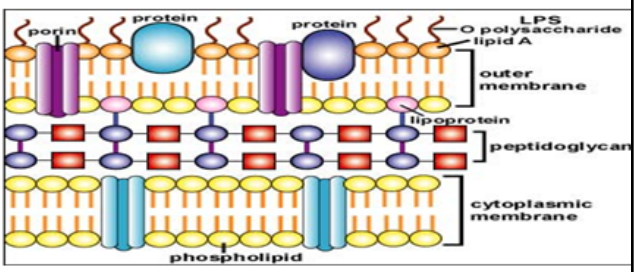


Compare and contrast the structures and functions, of fimbriae, pili, and flagella.

	Flagella	**Fimbriae** 	**Pili** 
structure		Short & numerous	Long & only 1-10 per cell
function	movement	Enable adhesion to surfaces (eg. N. gonorrheae can colonize mucous membranes)	Join together bacterial cells for transfer of genetic material

Compare cell walls of gram pos & gram neg bacteria in terms of structure and clinical indications.

-The major functions of the cell wall include the following: Prevent cell rupture; Maintain shape: Anchor flagella **-Clinically,** the cell wall is important because it contributes to the ability of some species to cause disease and is the site of action of some antibiotics. Plus, the chemical composition of the cell wall is used to differentiate major types of bacteria.

	GRAM POS	**GRAM NEG**
		
Structure	**Thick layer of peptidoglycan**	Outer membrane **and then a thin layer of peptidoglycan** and finally the cell membrane.
Finer points	Cell wall is composed of a macromolecular network called **peptidoglycan** (chains of NAG & NAM are covalently attached to other chains via tetrapeptides). Cell wall also contains **teichoic acids.**	Outer membrane is composed of **lipopolysaccharide.** Peptidoglycan layer doesn't contain teichoic acids.
Of special note	**teichoic acids** are covalently linked to lipids, forming **lipoteichoic acids** that anchor the peptidoglycan to the cell membrane. The teichoic acid is **responsible for cell walls antigenic specificity**	Lipo polysaccharide of outer membrane: 1. Polysaccharide portion provides antigenic specificity for gram neg 2. Lipid portion (**Lipid A**) is referred to as **endotoxin** and is toxic b/c it triggers vasodilation and shock Outer membrane provides a **barrier to certain antibiotics** (eg. penicillin) or **lysozyme** which acts on cell wall. Outer membrane also has a strong negative charge which helps the cell evade complement and phagocytosis
Cell wall of acid fast bacteria?	Special note: ***mycobacterium*** have mycolic acid around them which helps them survive dessication but also makes them difficult to gram stain. The **acid-fast stain** can stain them.	
Lysozyme	great at damaging peptidoglycan of cell wall. So it's very good against GRAM POS bacteria. If lysozyme destroys the cell wall but the cell membrane is still intact, it's called a **protoplast**.	If lysozyme is used against GRAM NEG bacteria it leaves some of the cell wall intact and that's called a **sphereoplast**. For lysozyme to REALLY do damage to GRAM NEGs you need to treat first with **EDTA** to destroy the outer membrane

Describe staining techniques such as simple, differential, and special.

Differential Stain

Gram Stain

Prep smear (drop of water on slide; aseptically place organism on slide; heat fix organism to the slide)
Crystal violet primary stain (1 min) (rinse)
Iodine mordant (1 min) (rinse)
Alcohol (4 squirts) (rinse immediately) ←---------- **disrupts LPS layer of gram neg so the color complex washed out**
Safranin counterstain (1 min) (rinse)
Gram POS = violet Gram NEG = pink

Acid-Fast Stain to differentiate among GRAM POS bacteria

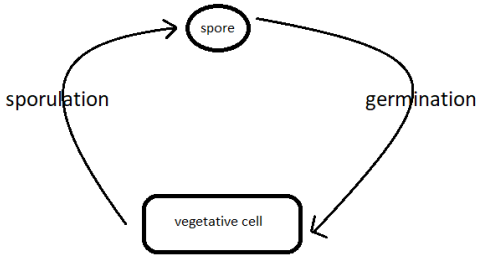
Some GRAM POS organisms *(eg. mycobacterium smegmatus)* have a layer of mycolic acid w/in the peptidoglycan layer which helps keep it hydrated. To differentiate these GRAM POS bacteria from other GRAM POS, you use the acid fast stain (carbolfuschin) which stains acid fast bacteria red.

Prep smear (drop of water on slide; aseptically place organism on slide; heat fix organism to the slide)
Carbolfuschin (5 mins)
Acid Alcohol (4 squirts) (rinse immediately)
Brilliant Green counterstain (1 min) (rinse)
GRAM POS ACID FAST = red GRAM POS NON ACID FAST = green

Endospore Stain

Prep smear (drop of water on slide; aseptically place organism on slide; heat fix organism to the slide)
Malachite Green over blot paper in steam for 3 mins (rinse)
Safranin counterstain (1 min) (rinse)
SPORE = round & green Vegetative bacteria = rodlike and pink

Describe the structure and function of bacterial endospores.



Contrast types of archaeal cell walls with one another and with bacterial cell walls. (not covered)

-Mycoplasma are the smallest bacteria and have no cell walls. They have sterols in cell membrane to protect from lysis.
-Archaea cell walls don't have peptidoglycan in cell wall but do have something similar called pseudomurein.

Describe the functions of the cytoplasmic membrane as they relate to permeability.

-Prokaryotic cell membranes aren't capable of endocytosis and exocytosis
-Proteins in the cell membrane are either **extrinsic** (lie at the inner or outer surface of the membrane) or **intrinsic** (located between the bilayer of the membrane)
-selectively permeable

Compare and contrast the passive and active processes by which materials cross the membrane.

Active Transport (low to high concentration). Another form of active transport is **Group Translocation** which occurs exclusively in prokaryotes. The substance being transported into the cell is **chemically altered** (eg. a phosphate is added to a sugar as it is being brought into the cell and now the sugar can't get out).

Passive Transport (high to low concentration without spending any ATP)

Simple Diffusion (may not involve membrane) - Simple diffusion is the net movement of molecules or ions from an area of high concentration to an area of low concentration until a state of equilibrium has been established. Cells rely on simple diffusion to transport certain small, molecules, such as oxygen and carbon dioxide

Facilitated Diffusion (involves membrane) - the substance (glucose for example) to be transported combines with a plasma membrane protein called a **transporter.**

Osmosis (refers only to movement of water) - note that the side with higher [ ] of solutes is the one with the greater osmotic pressure.

Define osmosis and distinguish among isotonic, hypertonic, and hypotonic solutions.

Isotonic - medium has same concentration of solutes as the other side
Hypotonic - medium has lower concentration of solutes than the other side
Hypertonic - medium has higher concentration of solutes than the other side

Define inclusions and give examples.

Inclusions are reserve deposits within the cytoplasm of prokaryotic cells. May be used in identification of bacteria.

Metachromatic Granules are a reserve of inorganic phosphate that can be used to make ATP or plasma membranes. They stain red with blue dyes.

Polysaccharide Granules consist of glycogen and starch

Lipid Inclusions represent a reserve of energy and can be stained with fat soluble dyes like **Sudan**

Sulfur Granules can be used to form S-containing AA's like MET & CYS

Carboxysomes are inclusions that contain **enzyme ribulose 1,5-diphosphate carboxylase** which allows utilization of **carbon from inorganic sources** like CO_2. Note that chemoautotrophs get C from inorganic sources while chemoheterotrophs get C from organic sources.

Gas Vacuoles maintain buoyancy so cell can remain at water depth it likes

Magnetosomes are inclusions of iron oxide that decompose hydrogen peroxide which may protect cell from H_2O_2 accumulation

And some random notes about Ribosomes

Ribosomes are composed of two subunits. Prokaryotic ribosomes are called 70S (composed of a 50S+30S) while eukaryotic ribosomes are 80S. Several antibiotics work by targeting the ribosomes to interfere with protein synthesis.

Streptomycin/Gentamicin attack the 30S
Erythromycin attacks 50S

Bacterial Genetics

As discussed in an earlier lecture, The DNA in bacteria has several chief distinguishing characteristics:

1. *Their DNA is not enclosed within a membrane and is one circular chromosome.*
2. *The DNA is not associated with histones.*
3. *There is only one copy of DNA per cell. Thus bacterial DNA exists in the haploid state.*

Instead of discussing certain details of bacterial genetics, such as replication, transcription, and translation, our lecture will primarily focus on the mechanisms of bacterial exchange of genetic information.
It is important to note that bacterial do not actually engage in sexual union with other bacteria. They undergo gene replication, forming an exact copy of their genome, and then split in two, taking a copy with each half (binary fission).

In eukaryotes, on the other hand, a set of gametes is contributed by each parent and thus ensure genetic diversity.

Vertical gene transfer occurs when genes are passed from an organism to its offspring. Bacteria are unique in that not only are they able to pass their genes to its offspring, but also laterally to other microbes of the same generation! This is known as **horizontal gene transfer**.

If bacteria do not engage in sex, how are they able to undergo genetic change that is necessary for survival?
There are five mechanisms that ensure genetic variability among bacteria.

1. **Simple mutations**
2. **Transformation**
3. **Transduction**
4. **Conjugation**
5. **Transposon insertion**

It is important to note that the exchange of genetic material allows for the sharing of genes that code for proteins, such as those that provide antibiotic resistance, exotoxins, enzymes, and other virulent factors (pili, flagella, and capsules).

Let us discuss each.

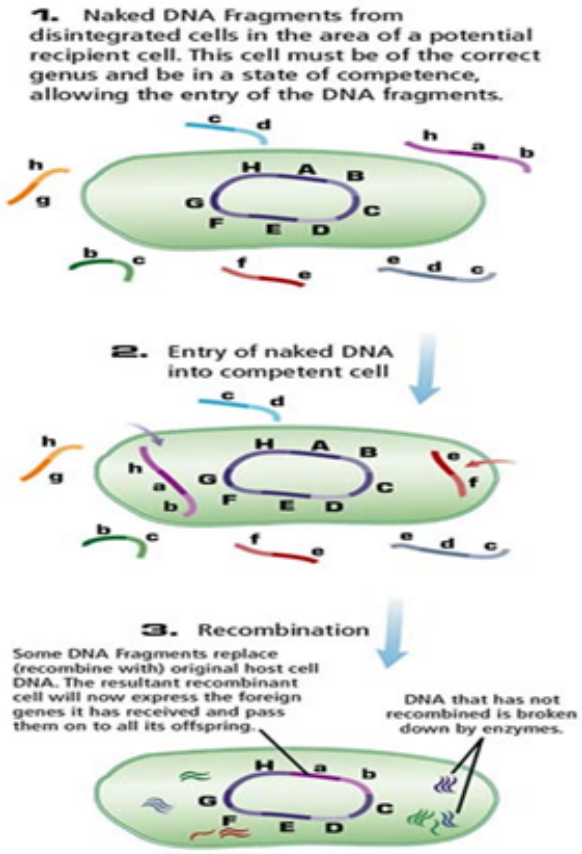
Simple Mutations:

Although simple mutations may account for some variability, it is rare for a single point mutation to change an organism in a helpful manner. Point mutations usually result in nonsense or missense. Missense mutations occur as a result of an insertion of an incorrect amino acid in a protein, as a result of an incorrect base substitution. The effects may be dramatic and usually has negative effects.

Missense mutation: A genetic change involving the substitution of one base in the DNA foranother which results in the substitution of one amino acid in a polypeptide for another. A missense mutation is a "readable" genetic message although its "sense" (its meaning) is changed. This is in contrast to a nonsense mutation which has no meaning except to halt the reading of the genetic message.
The first missense mutation discovered in humans was found to be responsible for sickle hemoglobin, the molecular basis of sickle cell trait and sickle cell anemia. The mutation causes an amino acid change from glutamic acid to valine, converting normal adult hemoglobin (hemoglobin A) to sickle hemoglobin (hemoglobin S).

<u>Transformation</u>:
During the process of **transformation,** genes are transferred from one bacterium to another as "naked" DNA in solution. The naked DNA fragments are released during cell lysis. However, the recipient bacteria **must be competent**. This means that the structures on the recipient's cell wall can bind to the DNA and take it up intracellularly. Thus, recipient bacteria are usually of
the same species as the donor.

The DNA that has been brought in can then incorporate itself into the recipient's genome if there is enough homology between the strands. This is another reason why this transfer can only occur between closely related bacteria.



The initial experiment on transformation was performed by **Frederick Griffith** in England in 1928 while working with two strains of *Streptococcus pneumoniae.*

Griffith used **smooth encapsulated pneumococci**, which cause violent infection and death in mice, and **rough nonencapsulated pneumococci**, which do not kill mice.

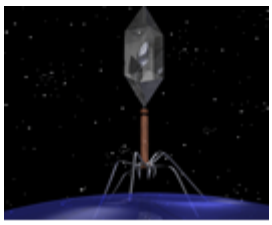
Griffith heat-killed the smooth encapsulated pneumococci and injected them, along with the live rough nonencapsulated pneumococci into mice. As a result, the mice died!

Griffith then cultured out bacteria from the blood of the dead mice, however, all he could find was **live smooth encapsulated** pneumococci.
What happened?

The gene encoding the capsule had been released from the heat-killed bacteria and became incorporated into the living rough nonencapsulated bacteria. Thus, the rough bacteria were **transformed** into virulent encapsulated smooth bacteria.

Transduction:

During the process of transduction, a virus that infects bacteria, called a bacteriophage, carries a piece of bacterial DNA from one bacterium to another.



In order for us to understand the process of transduction, let us discuss the role of **bacteriophages**.

Bacteriophages resemble most viruses in having a protein called a **capsid** that surrounds a molecule of DNA or RNA. The bacteriophage looks like a spider with a long skinny neck.

The phage will bind by its tail fibers to specific receptors on the bacterial cell surface. This process is called **adsorption.**

After adsorption, the phage then undergoes **penetration.** During penetration, the phage pushes a long hollow tube under its neck through the bacterial cell wall and cytoplasmic membrane. DNA in the head is injected through the tube into the bacterium.

Following adsorption and penetration, the injected DNA takes over the host bacteria's RNA polymerase for the transcription of the phage DNA to messenger RNA. As a result, new capsids, DNA, and enzymes are formed, and the bacterial cell fills with new phages. At some point the cell can hold no more viral particles and undergoes lysis, thus releasing the phages.

There are two types of Bacteriophages:

Virulent Phages infect the bacteria, the reproduce inside the bacteria leading to lysis resulting to the death of the bacteria.

Temperate Phages on the other hand, do not immediately lyse the bacteria they infect. The temperate phage undergoes adsorption and penetration like the virulent phage but the, rather than undergoing transcription, its DNA becomes incorporated into the bacterial chromosome awaiting activation.

The integrated temperate phage genome is called a prophage. Bacteria that have a prophage integrated into their chromosome are called **lysogenic** because at some time the repressed prophage can become activated.

Once activated, the prophage initiates the production of new phages, beginning a cycle that ends with bacterial cell lysis. You may think of temperate phages like little genetic time bombs.

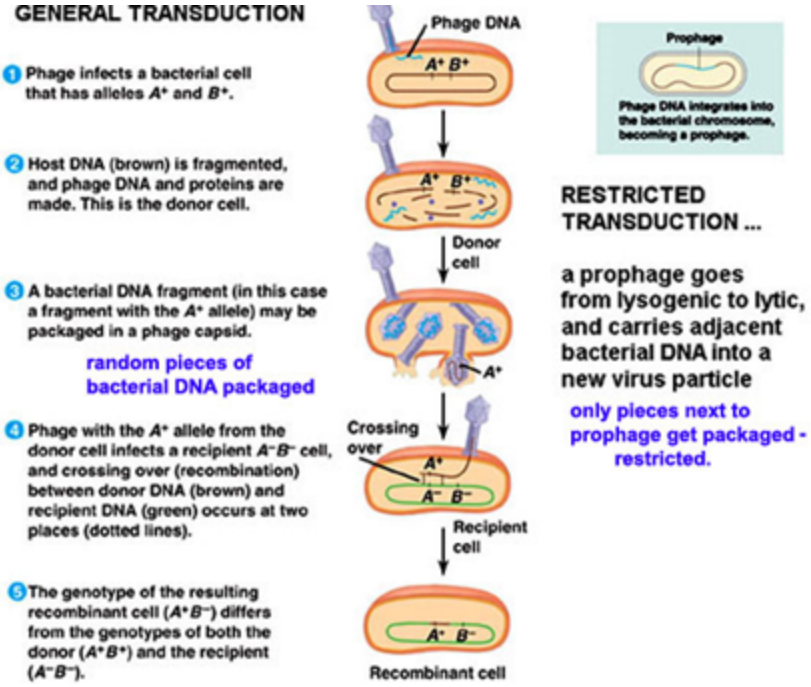
During the period when the prophage is still not activated, is it possible for another similar phage to infect the bacteria again?

No. Why?

The first temperate phage to infect a bacterium produces a repressor protein which blocks a subsequent infection by a *similar* phage. This is often referred to as **lysogenic immunity.** Lysogenic immunity ensures that the first temperate phage is the bacteria's sole occupant.

Ok, now that we have some basic understanding of what bacteriophages are, let's discuss how these phages can carry bacterial DNA from one bacterium to another. This process is called **transduction.**

Since there are two types of phages, there are also two types of transduction. **Virulent phages** are involved in **generalized transduction. Temperate phages** are involved in **specialized transduction**.



<u>Generalized Transduction</u>:
The phage penetrates into a host bacterium.

The phage DNA is transcribed, replicated, and translated into capsids and enzymes. At the same time the bacterial DNA is repressed and eventually destroyed.

However, sometimes pieces are the same size as the phage DNA and may accidently be packed into the phage capsid head. Following lysis of the bacterial cell and the release of the phages, the one phage with the piece of bacterial DNA can then infect another bacterium. Thus, it will inject the piece of bacterial DNA that it is "accidently" carrying.

If there is some homology between the newly injected strand and the recipient bacterial genome, the piece may become incorporated. The gene on that piece may encode a protein that the recipient did not originally have, such as a protein that inactivates an antibiotic.

It is important to note that in generalized transduction, the bacteriophage is only carrying bacterial DNA, so the recipient cell will survive. Thus, one may argue that generalized transduction is more effective than transformation because the transferred DNA piece is protected from destruction during the transfer by the phage capsid that holds it.

Specialized Transduction:
As mentioned, specialized transduction occurs with temperate phages. Recall that the temperate phage penetrates, and then its DNA becomes incorporated into the bacterial chromosome. It is then called a prophage, and the bacterium is now lysogenic.

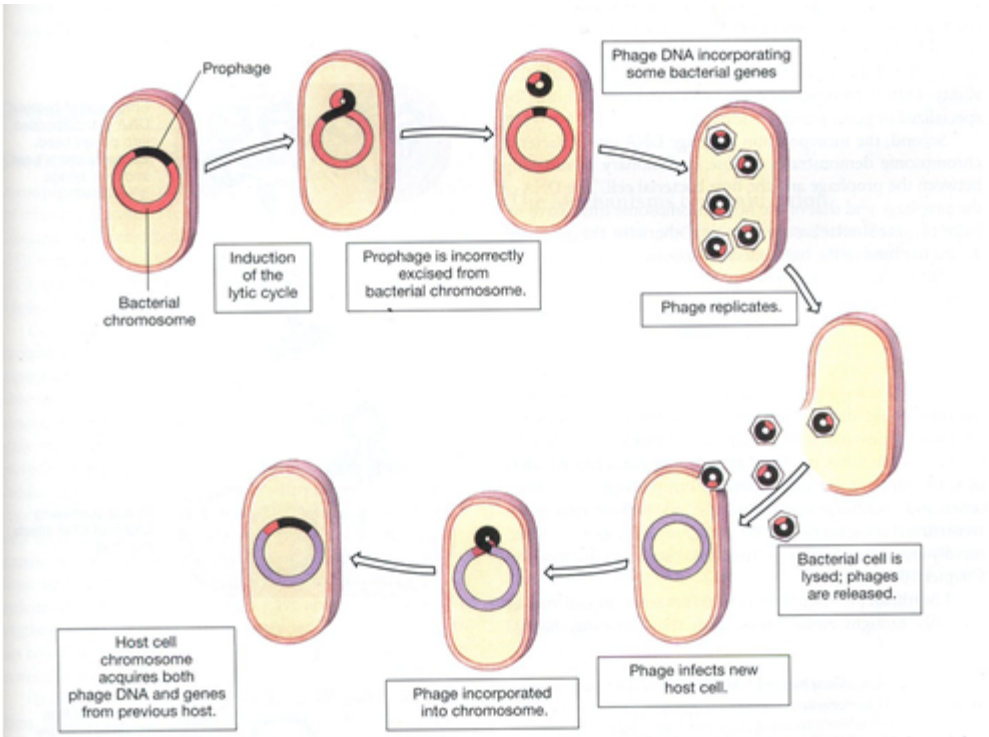


FIGURE 8.4 **Specialized transduction by lambda phage in *E. coli*.** In this process, phage DNA always inserts itself into the bacterial host chromosome at a particular site. When the phage DNA replicates, it takes bacterial genes from either side of the site and packages them with its own DNA into new phages. Only genes adjacent to the insertion site, not genes from other parts of the host chromosome, are transduced. These genes can then be introduced into the phage's next host cell, where they will confer new genetic traits.

Normally the prophage is inactive, however if it becomes active, the prophage DNA is spliced out of the bacterial chromosome and is then replicated, translated, and packaged into a capsid.

Sometimes there is an error in splicing, and a piece of bacterial DNA will be cut, replicated, and packaged with the phage DNA. This may result in a transfer of that piece of bacterial DNA to another bacterium.

The Significance of Transduction:

1. It transfers genetic material from one bacterial cell to another and alters the genetic characteristics of the recipient cell.

1. The incorporation of the phage DNA into a bacterial chromosome demonstrates a close evolutionary relationship between the prophage and the host bacterial cell. The DNA of the prophage and that of the host chromosome must have regions of quite similar base sequences.

1. The discovery that a prophage can exist in a cell for a long period of time suggests a possible mechanism for the viral origin of cancer. If a prophage can exist in a bacterial cell and at some point alter the expression of the cell's DNA, this could explain how animal viruses cause malignant changes.

1. An interesting thought is that some animal viruses probably brought along genes from their previous host(s) when they infected new human hosts. These previous hosts were not necessarily humans. In that sense, you may not be entirely human now!

1. For molecular genetics, transduction provides a way to study gene linkage. By studying different phage transduction, scientists can determine where they were inserted on the chromosome and which adjacent genes they are capable of transferring. The findings of such studies, may allow identification of the sequence of genes in a chromosome. This procedure is known as **chromosome mapping.**

<u>Conjugation</u>:
In conjugation DNA is transferred directly by cell-cell contact; an efficient exchange of genetic information.

It is important to note that unlike transformation and transduction, conjugation (aka exchange of genetic info) may occur between unrelated bacteria and is a **major** mechanism for the transfer of antibiotic resistance.

For conjugation to occur, one bacterium must have a **self-transmissible plasmid,** also called an **F (fertility) plasmid.**

Plasmids are circular double-stranded DNA molecules that lie outside the chromosome and can Carry many genes, including those for drug resistance.

Bacteria that carry F plasmids are called F(+) cells. In conjugation, an F (+) donor cell will pass its F plasmid to an F (-) recipient cell, thus making the recipient F(+).

Plasmids during conjugation are transmitted via a sex pilus. As discussed during an earlier lecture this is a long protein structure that protrudes from the cell surface in some cells.

As one DNA strand is passed through the "conjugal" bridge, the remaining strand is paired with new nucleotide bases. The same thing happens to the strand that passes to the other cell. At the end of the union, the bridge breaks down and both bacteria have double-stranded F plasmids.

Transposons and Transposition:

Transposons are segments of DNA that transpose (move) themselves from one location in a DNA molecule to another location in the same or different molecule. The result of the action of a transposon is called **transposition.**

The American geneticist Barbara McClintock (1902-1992) discovered these so called “jumping genes through an analysis of the colors of the kernels of corn, however they occur in all organisms and have been studied most thoroughly in microorganisms.

Transposons may move from one site to another site on the same chromosome, or to another chromosome or plasmid.

As you may imagine, the frequent movement of transposons could wreak havoc inside a cell. For example, as transposons move about on chromosomes, they may insert themselves within genes, inactivating them.

Fortunately, transposition occurs relatively rarely. The frequency of transposition is comparable to the spontaneous mutation rate that occurs in bacteria.

In addition to being transferred on resistance plasmids by conjugation, resistance genes can move from one plasmid to another in a cell or even become inserted in the chromosome.

The ability of a genetic sequence to move from one location to another is called **transposition.** Such a mobile genetic sequence is called a **transposable element.**

These transposable elements can carry genes for antibiotic resistance and virulence factors.

It is important to note that like conjugation, these elements can insert into a donor chromosome **without having DNA homology.**

Transposons insert into the DNA of phages, plasmids, and bacterial chromosomes. They **do not** replicate independently but are copied during their host’s DNA transcription.

When Transposons leave the DNA they are incorporated in, there is frequently aberrant excision and the transposon can carry new DNA away to another site.

Clinical significance of Transposons:

The importance of transposons clinically is that transposon gene that confers a particular drug resistance can move to the plasmids of different bacterial genera, resulting in the rapid spread of resistant strains.

Bacterial Metabolism

Distinguish among metabolism, anabolism, and catabolism.

metabolism - sum of all chemical reactions within a living organism and consists of:

catabolism releases energy (**exergonic**) from the breakdown of complex organic compounds (eg. cells break sugars down via hydrolysis to CO_2 and H_2O and release energy)

anabolism uses energy (**endergonic**) to build complex organic molecules from simpler ones (eg. synthesis of proteins from AA and nucleic acids from nucleotides and synthesis of polysaccharides from sugars)

The body takes the energy from catabolic reactions to drive anabolic reactions using the currency of ATP

Define activation energy, enzyme, apoenzyme, cofactor, coenzyme, active site, substrate, and describe their role in enzyme activity.

Enzymes:- biological catalysts which are specific for a certain substrate. The specificity of enzymes is made possible by their tertiary structures.

- **Primary Structure** – determined by sequence of AA
- **Secondary Structure-** localized, repetitious twisting or folding of the polypeptide chain due to H-bonds joining atoms of polypeptide bonds at different locations along the polypeptide chain. The two types of secondary protein structures are clockwise spirals called helices and pleated sheets.
- **Tertiary Structure-** overall 3D structure which gives the enzyme its specificity. **Messing with this is called denaturation.** This folding is not repetitive or predictable and involves several interactions between various amino acid side groups in the polypeptide chain.

Enzyme Components:

Active enzyme **Haloenzyme** = protein portion called **Apoenzyme** + nonprotein portion called **Cofactor** (or **coenzyme** if it is organic)

Examples of cofactors may include Zn, Mg, Ca, etc.

Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions (eg. NAD+ NADP+, FAD, FMN, CoA)

Describe how various factors affect enzyme activity.

Temperature: higher temp means faster reaction up to a point (often 35-40C for pathogenic bacteria). Drastic increase in temp will **denature enzymes (mess with their tertiary structure)**

pH:ditto

Substrate Concentration: There is a maximum rate at which a certain amount of enzyme can catalyze a specific reaction. This max rate is achieved only when **substrate concentration is very high (substrate level saturation) b/c** the active site of the enzyme is always occupied by substrate. **Thus, the rate of reaction is influenced by the substrate concentration.**

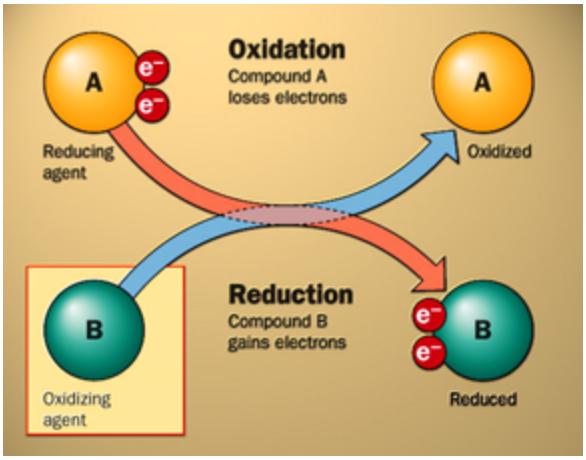
Inhibitors:

Competitive inhibitors fill the active site and compete with the normal substrate for the active site.
Noncompetitive inhibitors do not compete with the substrate for the enzyme's active site; instead, they interact with another part of the enzyme via **allosteric inhibition.**

Contrast reduction and oxidation reactions.

We will be considering two general aspects of energy production: oxidation-reduction reactions and mechanism of ATP production.

Oxidation-Reduction Reactions: are ***always paired***; in other words, each time one substance is oxidized, another is simultaneously reduced.



In many cellular oxidations, electrons and protons (hydrogen ions, H+) are removed at the same time. This is equivalent to the removal of hydrogen atoms, because as you already know, a hydrogen atom is made up of one proton and one electron. Thus, because most biological oxidations involve the loss of hydrogen atoms, they are also called **dehydrogenation reactions.**

The Generation of ATP:

Adding the P to ADP is called **phosphorylation.** Organisms use the following mechanisms of phosphorylation to generate ATP from ADP.

1. **Substrate-level phosphorylation -** P transferred directly to an ADP, creating ATP
2. **Oxidative Phosphorylation -** ATP is formed as a consequence of a bunch of oxidation/reduction rxns in the electron transport chain. This process is called chemiosmosis.

	Substrates	**Gross Products**	**Net Products**	**Net Energy Production including oxidative Phos. in ETC**
Glycolysis	2 ATP used	4 ATP 2 NADH	2 ATP (substrate level Phosph.) 2 NADH	2 ATP (sub. Lvl. Phos.) 6 ATP (oxidative Phos.)
Prep		2 NADH	2 NADH	6 ATP (oxidative Phos.)
Krebs cycle		2 ATP 6 NADH 2 FADH2	2 ATP (substrate level Phosph.) 6 NADH 2 FADH2	2 ATP (sub. Lvl. Phos.) 18 ATP (oxidative Phos.) 4 ATP (oxidative Phos.)
Summary	In aerobic respiration among prokaryotes, a total of 38 ATP molecules can be generated from one molecule of glucose. **34 + 4 = 38 ATP**			

FADH2 goes to ETC → each yielding 2 ATP by oxidative phosphorylation
NADH goes to ETC → each yielding 3 ATP by oxidative phosphorylation

Metabolic Pathways of Energy Production:
Microorganisms first conduct **glycolysis**. After this, they can employ either **cellular respiration** or **fermentation**.

Overview: Cellular respiration of Glucose:
The respiration of glucose occurs in three principal stages:

1. **Glycolysis -** oxidation of glucose to two molecules of pyruvic acid with the production of some ATP and NADH.
2. **The Krebs Cycle -** oxidation of acetyl CoA (a derivative of pyruvic acid) to carbon dioxide, with the production of some ATP, NADH, and $FADH_2$.
3. **The Electron Transport Chain (System)** - NADH and $FADH_2$ are oxidized; contributing electrons in a "cascade" of oxidation-reduction reactions involving a series of electron carrier molecules. Energy from these reactions is used to generate most of the ATP of Cell Respiration in a process called **chemiosmosis.**

Overview: Fermentation
Once glycolysis has taken place, the pyruvic acid is converted into one or more different organic products (**alcohol or lactic acid**) depending on the type of cell. Unlike cellular respiration, there is **no Krebs cycle or ETC in fermentation.** Thus, the **ATP yield comes only from glycolysis and is low yield**. Fermentation is defined as:

1. releases energy from sugars or other organics.
2. Does not require oxygen
3. Does not require use of the Krebs cycle or an electron transport chain
4. Produces only a small amount of ATP because much of the original energy in glucose remains in the chemical bonds of organic end-products, such as lactic acid or ethanol.

A few key points should be noted about glycolysis:

1. **During glycolysis NAD+ is reduced to NADH**
2. **There is a net production of 2 ATP by substrate-level phosphorylation**
3. **Glycolysis does not require oxygen**

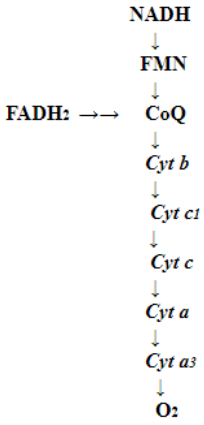
In studying glycolysis, particular focus should be placed on the following:

1. **Two ATP- consuming steps.**
2. **Two ATP-generating steps.**
3. **Two oxidation-reduction reactions that involve NAD + - NADH.**
4. **Three irreversible (rate limiting) steps, involving the enzymes hexokinase, phosphofructokinase, and pyruvic kinase**

A few key points should be noted about Krebs cycle. For every **two** molecules of acetyl CoA that enter the cycle:

1. **Four molecules of CO_2 are liberated by decarboxylation.**
2. **Six molecules of NADH are produced by oxidation-reduction reactions.**
3. **Two $FADH_2$ molecules are produced by oxidation-reduction reactions.**
4. **Two ATP molecules are produced by substrate-level phosphorylation**

<u>Electron Transport Chain</u>: A series of carrier molecules (Flavoproteins, Cytochromes, Ubiquinones) capable of oxidation-reduction rxns. As electrons pass through, ATP is generated.



<u>Identify three useful products of fermentation, and explain how fermentation reactions are used in the identification of bacteria.</u>

<u>Biochemical Tests and Bacterial Identification</u>:

test can help ID bacteria b/c different ones produce different enzymes.
*An example of such a test is a **fermentation test.** We will be conducting this test in our laboratory. The test medium contains protein, a single carbohydrate, a pH indicator, and a fermentation tube (Durham tube) to capture gas.*

Bacteria inoculated into the tube can use the protein or carbohydrate as a carbon and energy source. If they catabolize the carbohydrate and produce acid, the pH indicator changes color.

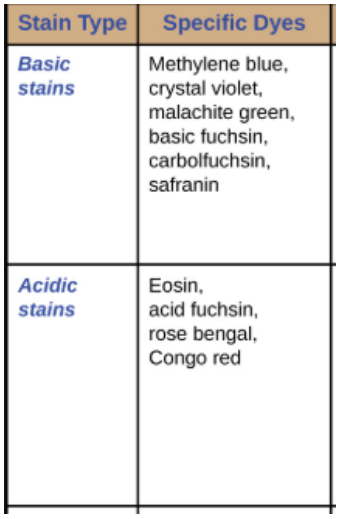
Some organisms produce gas as well as acid from carbohydrate metabolism. The presence of a bubble in the Durham tube indicates gas formation.

There will be a host of other biochemical test we will be performing in our laboratory this semester.

Post Exam 1 Notes

Know which bacteria are gram pos vs neg and shapes b/c many questions

	organization	shape	Gram stain
Staph aureus	staph 	coccus	POS
Bacillus subtilis	strept 	bacillus	POS
E. coli	individual 	bacillus	NEG

Which dye is acidic vs basic?  	*How do you stain endospore?*  *Schaffer Fulton stain (malachite green)*	*What's especially different about archaea vs prokaryotes?* Archaea cell wall doesn't have peptidoglycan	*Who forms endospores?* Bacillus, clostridium
	How do you stain capsule? Negative staining	*Polymyxins target what part of cell?* LPS of Gram NEG bacteria	*Cephalosporins/Penicillin target what part of cell?* Prevent them from forming a new cell wall so the bacteria get bigger and bigger and POP

Innate Immunity

Differentiate innate and adaptive immunity.

	Innate	**Adaptive**
Present at birth?	Yes	No – requires an initial breach of innate defense
Speed of response?	Rapid	Slow
Responds to specific microbe?	No	**Yes**
Memory?	No	**Yes**
		VERSATILITY (will make different Ab's against a host of different antigens)
		TOLERANCE (won't attack your own cells)
	First line defense **a) Skin** **b) Mucous membranes** **Second line defense** **a) Natural killer cells** **b) Phagocytes** **c) Inflammation** **d) Fever** **e) Antimicrobial substances**	**1. T Lymphocytes** **2. B Lymphocytes**

Describe the role of the skin and mucous membranes in innate immunity.

first line of D based on **physical (barriers)** and **chemical (substances that inhibit microbes)**

Differentiate physical from chemical factors and discuss several examples.

Physical Factors:

Skin layers containing keratin slough off shedding microbes. Dryness also prevents microbial growth.

Mucous membranes – epithelial layer and connective tissue under it. Lines GI, Resp and genitourinary tracts.

Mucus, a thick glycoprotein produced by goblet cells. Traps many microorganisms. Respiratory tract mucosa also has mucus coated hairs that stop inhaled particles, and cilia sweep dust and bacteria laden mucus toward the mouth, preventing it from entering the lower resp passages. Urine flow also sweeps away microbes.
Lacrimal apparatus (innate mechanical immunity) – makes/drains tears which clean eyes
Saliva – washes away microorganisms
Peristalsis, defecation, and vomiting also expel microbes.

Chemical Factors:
-Sebaceous glands of the skin produce **sebum** which has unsaturated fatty acids that inhibit microorganisms. The low pH of the skin (3-5) is due to these **fatty acids** and **lactic acid**.
-**Perspiration** flush microorganisms from the surface of the skin and also has **lysozyme which breaks the cell walls of gram-positive bacteria** and a little bit of gram negs.
-Saliva not only contains lysozyme but also urea and uric acid which contribute to the control of microbial growth. The pH of saliva (6.55-6.85) also inhibits some microbes. **Immunoglobulin A, an antibody** is also a constituent of saliva.
-Gastric juice in stomach is a mix of **HCl, enzymes,** and mucus. Acidity destroys most bacteria and their toxins except *Clostridium botulinum* and *Staphylococcus aureus.*
-Vaginal secretions of g**lycogen** are catabolized by *Lactobacillus acidophilus* into lactic acid so low pH inhibits microbes.

Classify leukocytes, and discuss the roles of granulocytes and monocytes.
Our body uses a variety of nonspecific cellular devices to protect itself (the Second Line of Defense) and this includes:

Phagocytes like **macrophages/APC** (which are derived from monocytes). They can either be **Free Macrophages (**wandering tissues looking for debris or foreigners) or **Fixed Macrophages (histiocytes)** which are permanent residents of certain tissues (eg. **Microglia in CNS & Kupffer in liver and Langerhans in skin). T**hey work how?

-Engulf foreigner and destroy with lysosomal enzymes or
-Repiratory Burst of toxins to kill em

Neutrophils (most abundant WBC) is the **first responder** and becomes **phagocytic** upon encountering infectious material in the tissues.
Eosinophils defends against **parasites** by discharging destructive granules.

Discuss the process of phagocytosis.

An hour after inflammation initiated, phagocytes arrive at the site of injury. As the flow of blood gradually decreases, phagocytes (neutrophils and monocytes) stick to the inner surface of the endothelium of blood vessels (**margination)** and then squeeze between the endothelial cells of the blood vessels to reach damaged area (**diapedesis).**

To ingest the foreign antigen, it must first be recognized and adhered to. This is more probable if **complement proteins** or **antibodies** coat the foreign particles (**opsonization)** b/c that coating entices phagocyte receptors to bind. Then, cytoplasmic extensions bring the organism inside, enclosing it in a vacuole (**phagosome)** which then fuses with a lysosome (**phagolysosome).** Additionally, the **RESPIRATORY BURST (NO, superoxide, H2O2) will F that pathogen up. But the phagocyte/neutrophil will take this victory to the grave as it also dies.** *The respiratory burst may also enable potassium to enter the phagolysosome; the phagolysosome becomes hyperosmotic resulting in the activation of protein-digesting enzymes that digest the invader. Neutrophils also produce antimicrobial chemicals called* ***defensins*** *that pierce the pathogen's membrane.*

Note: things like pneumococcus have capsules – remember? Those capsules prevent phagocyte binding.

Immunological Surveillance:

Unlike other lymphocytes (B&T) Natural Killer cells (NK) are capable of murdering any abnormal cells in our bodies in a nonselective manner (bacteria, virally infected cells, cancerous cells displaying **tumor specific antigens**) via a constant monitoring process called **immunological surveillance. How?**

1. Recognition of unusual stuff in the membrane
2. Adherence
3. Golgi points at the abnormal cell and secretory vesicles containing **perforins** shoot out and hit the abnormal cell and perforate it.

The process of avoiding detection or neutralizing the NK cells is called **immunological escape.** Once immunological escape has occurred, cancer cells can multiply and spread without interference by NK cells.

Complement:

Blood plasma contains 11 special **complement proteins** that work with antibodies in a cascading fashion. There are two routes:

1. **The Classical Pathway (arguably part of the specific immune response)**
   a. **Rapid** way of activating the complement system.
   b. **Triggered when C1 binds to an Ab already attached to an Ag.** The C1 acts an enzyme, catalyzing a series of reactions involving other complement proteins.
   c. **Finally, inactive C3 is converted to active C3b**
2. **The Alternative Pathway (Properdin) (totally nonspecific)**
   a. **Slower** way of activating complement.
   b. **Triggered by exposure to any type of foreign material** like capsule of a bacterium or virally infected cell.
   c. **Finally, inactive C3 is converted to active C3b**

***Effects of Complement Activation:**

1. **Stimulation of inflammation:** Activated complement proteins enhance the release of **histamine** by mast cells and basophils, which accelerates blood flow to the region.
2. **Attraction of Phagocytes:** Activated complement proteins attract neutrophils and macrophages to the area.
3. **Enhancement of Phagocytosis: A coating of complement proteins and antibodies (opsonins)** attract phagocytes and makes the target easier to bind to and engulf.
4. **Destruction of Target Plasma Membranes:** In the presence of C3b, five complements proteins (C5-C9) bind to the membrane, form a **membrane attack complex (MAC)** which punches holes in the membrane.

Localized Inflammation:

is a localized tissue response to injury. (Contrast to fever which is systemic) 5 signs:

Redness (Rubor)	After damage, **blood vessels dilate** and this increases blood flow to the damaged area and you'll also see redness (erythema) and **heat** associated with inflammation.
Heat (calor)	
Pain (dolor)	**Increased permeability** permits defensive substances typically found in the blood vessels to leak into the injured tissue (along with fluid so you get swelling). The pain is due to **damaged tissue releasing bradykinins which increase nerve sensitivity**. You take it easy and this helps it heal.
Swelling (Tumor)	
Loss of function	

Vasodilation *and increased blood vessel permeability are caused by chemicals released by damaged cells:*

1. Histamine *is a chemical present in many cells of the body, especially in* ***mast cells*** *in connective tissue, circulating* ***basophils,*** *and blood* ***platelets.*** *As mentioned earlier, it is also released in response to stimulation by components of the complement system.*

2. Kinins *also cause vasodilation and increased permeability. They are present in blood plasma, and once activated, play a role in chemotaxis by attracting phagocytic granulocytes, chiefly neutrophils, to the injured area.*

3. Prostaglandins *are released by damaged cells and intensifies the effects of Histamine and Kinins and assist phagocytes to move through capillary walls.*

4. Leukotrienes *are produced by mast cells and basophils. Leukotrienes cause increased permeability of blood vessels and assist in attaching phagocytes to pathogens.*

5. Cytokines *secreted by activated fixed macrophages contribute to vasodilation and increased permeability. This in turn helps deliver clotting elements of blood to the injured area. The blood clots that form around the site of activity prevents the microbe (or its toxins) from spreading to other parts of the body.*

As a result of all of these chemical mediators of inflammation, there may be a localized collection of ***pus,*** *a mixture of dead cells and body fluids, in a cavity formed by the breakdown of body tissues. This focus of infection is called an* ***abscess.*** *Common abscesses include pustules and boils.*

Fever:

Systemic response to bacterial infection. Normally body temp set by **hypothalamus (thermostat).** But if, for example, phagocytes ingest a gram neg bacteria and LPS endotoxin released, then cytokines **interleukin-I,** along with **tissue necrosis factor alpha** might be released. These cytokines cause the hypothalamus to reset at a higher temperature. *Eg. If the body thermostat increases to 102 F, to adjust to the new thermostat setting, the body responds by constricting blood vessels, increasing the rate of metabolism, and shivering, all of which raise the body temperature. Even though body temperature is climbing higher than normal, the skin remains cold, and shivering occurs. This condition is called a* ***chill,*** *and is a definite sign that body temperature is rising. When the body temperature reaches the new setting of the thermostat, the chill disappears. The body will continue to maintain its temperature of 102 F until the cytokines are eliminated. At this point, the thermostat is then reset to 98.6 degrees F. As the infection subsides, heat-losing mechanisms such as vasodilation and sweating begin. This phase of the fever is called the* ***crisis*** *and is an indication that body temperature is falling.*

Up to a certain point, fever is a considered to be a defense against disease. Interleukin-I helps step up the production of T cells. High body temperature also intensifies the effect of antiviral interferons. The increase in body temperature speeds up the body's reaction and may contribute in a more rapid tissue repair.

***The absence of fever doesn't rule out infection. The person could just be immunocompromised.**

Discuss the role of interferons.

Compare and contrast the actions of interferon alpha, beta, and gamma.

Interferons are released by activated lymphocytes and macrophages and cells infected with viruses. They interfere with **viral replication inside the cell.** In addition to their role in slowing the spread of viral infections, interferons stimulate macrophages and NK cells. At least three types of interferons exist:

Alpha interferons *are produced by several types of leukocytes, attract and stimulate NK cells.*

Beta interferons*, secreted by fibrocytes slow inflammation in a damaged area.*

Gamma interferons*, secreted by T cells and NK cells, stimulate macrophage activity.*

THERAPEUTIC USES OF INTERFERONS

>I-interferons-alpha and -beta have been used to treat various viral infections. One currently approved use for various types of interferon-a is in the treatment of certain cases of acute and chronic hepatitis C and chronic hepatitis B.

>Interferon-gamma has been used to treat a variety of disease in which macrophage activation might play an important role in recovery, eg. lepromatous leprosy, leishmaniasis, toxoplasmosis.

>Since interferons have anti-proliferative effects, they have also been used to treat certain tumors such as melanoma and Kaposi's sarcoma.

Adaptive Immunity

The Adaptive Immune System (Specific Defenses):

Specific defense is provided by the coordinated activities of **T cells** and **B cells,** which respond to the presence of specific antigens.

-T cells responsible for **cell-mediated immunity (cellular immunity),** which defends against abnormal cells and pathogens inside cells.

-B cells provide **antibody-mediated immunity (humoral immunity),** which defends against antigens and pathogens in body fluids.

4 general properties exhibited by specific immunity:

Specificity – specific antigen activates a specific defense and the immune system hits that target and no others. Each T cell or B cell has receptors that will bind to one specific antigen, ignoring all others.

Memory – During an initial response to an antigen, lymphocytes that are sensitive to it produce 2 groups of cells:

1. **One group of cells attacks the invader immediately.**
2. **Another group (memory cells) remains inactive unless it is exposed to the same antigen at a later date.** At that point it can launch a faster, stronger counterattack.

Versatility – There are millions of antigens and you'll encounter only a few. You must be ready to confront any antigen at any time. That's why you have a huge diversity of lymphocytes, each specific for one antigen.

Tolerance – The immune response targets foreigners but leaves normal tissues alone.

Cell Mediated Immunity (T Cells):

1. **Cytotoxic Killer T cells:** These cells enter peripheral tissues and directly attack antigens physically and chemically (using perforins, lymphotoxins and by activating genes in the target cell nucleus that prompt apoptosis). Contrast this cell to NK cells which are part of the innate response.

2. **Helper T cells (CD4): B cells must be activated by helper T cells** before B cells can produce antibodies. Helper T cells are the **primary target of HIV.**

3. **Suppressor T cells:** These cells inhibit T cell and B cell activities to moderate the immune response.

MHC/HLA is a glycoprotein inside the infect cell. It binds to antigen and the entire complex goes to the cell surface (antigen presentation).

MHC I is present on the membranes of all cells

MHC II is present only in the membranes of antigen presenting cells

Brief summary of what follows

Before an immune response can begin, T cells must first be activated by exposure to an antigen in a process called Antigen Presentation. Basically, an **MHC-Antigen complex** must be recognized by the **CD8 or CD4 marker bound to CD3**. In addition, in order for a T cell to be activated, the T cell also needs to see other markers on the cells (**costimulation**) in order to confirm the initial activation signal.

Discussing which type of T cell?	Recognizes which MHC-Antigen complex?	What does T cell do?	And Gives Rise to?	And then?
CD8 T cell	MHC I	Binds to MHCI-Ag complex	**Active Cytotoxic Killer Tc**	Kill the cell carrying the antigen
			Memory Tc	Lies in wait for reappearance of the Ag
			Suppressor Tc	Controls and moderates the immune response by B's & T's
CD4 T cell	MHC II	Binds to MHCII-Ag complex	**Active Helper T**	Gets rid of the antigen only
			Memory T_H	Lies in wait for reappearance of the Ag

<u>Activation of CD8 T Cells</u>:
Two different classes of CD8 T cells are activated by exposure to antigens bound to Class I MHC proteins.

- One type of CD8 T cell responds quickly, giving rise to large numbers of:
  - **Cytotoxic Killer T cells** which take 2 days to ramp up and start killing
  - **Cytotoxic memory T cells** which will quickly differentiate into cytotoxic T cells the ***next*** time you're infected, producing a fast/effective response b4 the organism gets too comfy in your body.

- The other type of CD8 T cells responds slowly and produces small numbers of:
  - **Suppressor T cells** which suppress the responses of other T and B cells

<u>Activation of CD4 T Cells</u>:
Upon activation, CD4 T cells undergo a series of divisions that produce

- **Active helper T cells** which do the following:
  1. **Stimulate T cell divisions that produce memory helper T cells and accelerate the maturation of cytotoxic T cells.**
  2. **Attract & stimulate macrophages to the area (enhancing nonspecific defense).**
  3. **Attract and stimulate the activity of NK cells, providing another mechanism for the destruction of abnormal cells and pathogens.**
  4. **Promote the activation of B cells, leading to B cell division, plasma cell maturation, and antibody production.**

- **Memory T helper cells** which remain in reserve.

<u>Antibody Mediated Immunity (Humoral Immunity)</u>:
The body has lots of different B cells, each carrying its own particular antibody molecule. If the corresponding antigen appears, they bind (**sensitization**) and get ready to activate.

B cell plasma membranes contain Class II MHC proteins. During the process of sensitization, antigens are brought into the cell by endocytosis and then displayed on the surface, bound to Class II MHC proteins. At this point, the sensitized B cell is on "standby" but **will not** undergo activation unless it receives the "OK" from a helper T cell.

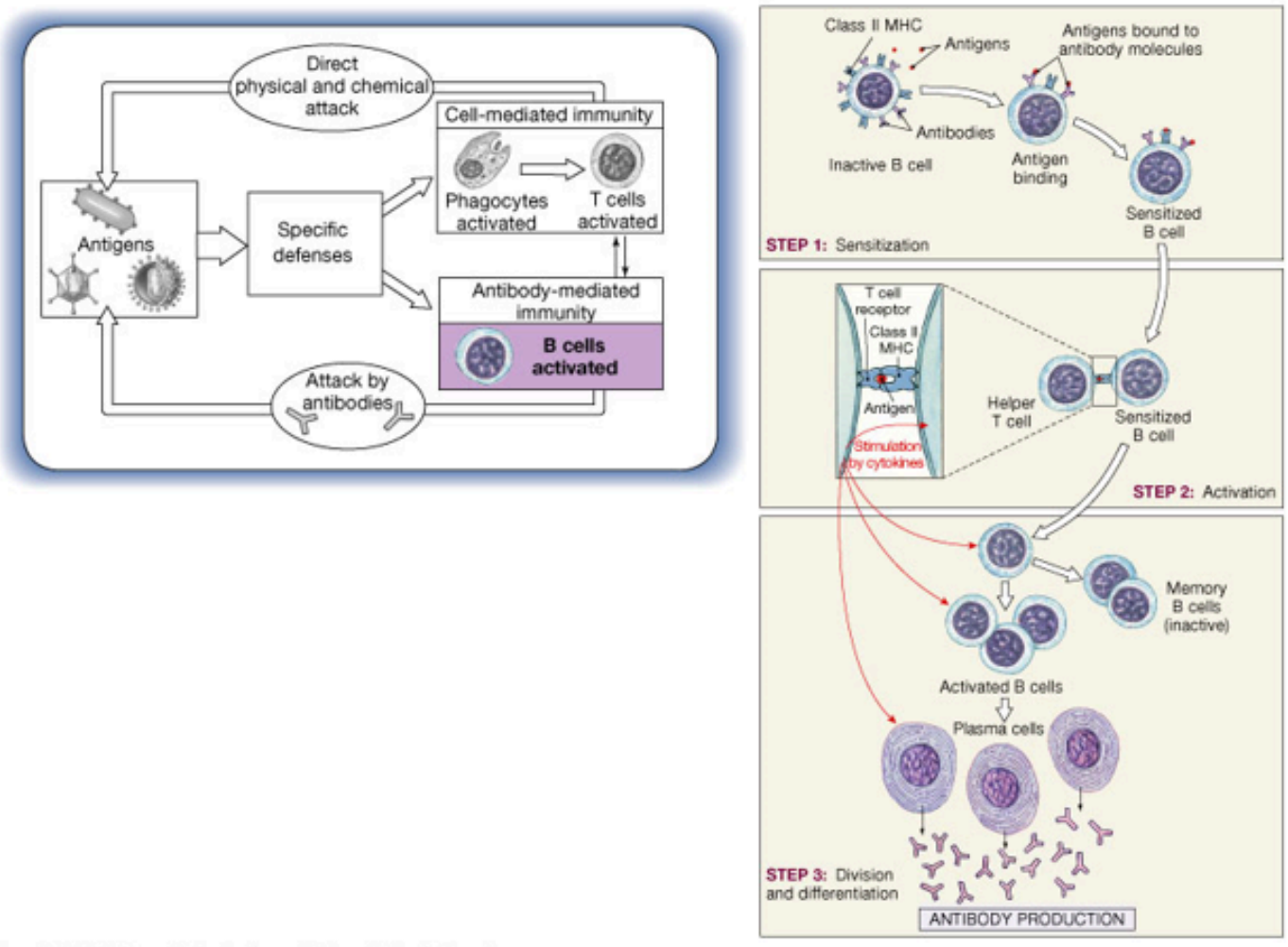
When a sensitized B cell encounters a helper T cell already activated by antigen presentation, the helper T cell binds to the MHC complex, recognizes the presence of the antigen, and begins secreting cytokines that promote B cell activation.

B lymphocyte differentiates into:

Plasma cells which secrete antibodies

Memory B cells which remain in reserve to deal with future infections with the same Ag. On subsequent exposure, the memory B cells respond by differentiating into plasma cells that secrete antibodies in massive quantities.

Below you find an illustration depicting the event of B Cell Sensitization & Activation:



Antibody Structure:
-An antibody molecule consists of one pair heavy chains & one pair light chains.
-The **base** is a **constant segment** and used to **structurally classify** the antibody (IgG, IgE, IgD, IgM, IgA).
-The **variable region/antigenic determinant** is responsible for the antibody's **versatility**. There should be at least two antigenic sites.

The structure of the constant segments of the heavy chains determines the way the antibody is secreted and how it is distributed within the body. *For example, antibodies in one class circulate in body fluids, whereas those of another class bind to the membranes of basophils and mast cells.*

The Antigen-Antibody Complex:
When Ab binds to Ag, it's an **antigen-antibody complex**. A **complete antigen** is an antigen with at least **two** antigenic determinant sites. Exposure to a complete antigen can lead to B cell sensitization and a subsequent immune response.

The formation of an antigen-antibody complex may cause the elimination of the antigen in several ways:	
Neutralization	Antibody binds to the part of the virus or toxin that is actually used to attach to body cells. In this way, the invader is rendered incapable of attachment.
Precipitation and Agglutination	Antibody can bind to multiple antigens, forming extensive **immune complexes (agglutination)** which might even precipitate out of solution.
Activation of Complement	Antibody binds to the antigen and portions of the **heavy chain segment** binds to complement proteins, activating the complement system and destroying the antigen.
Attraction of Phagocytes	Antibodies covering the antigen attract Eosinophils, neutrophils, and macrophages which phagocytize and destroy.
Opsonization	A coating of antibodies and complement proteins increases the effectiveness of phagocytosis. How? Some bacteria have slick coverings so phagocytes must be able to hang onto their prey before they can engulf it.
Stimulation of Inflammation	Antibodies may promote inflammation through the stimulation of basophils and mast cells.
Prevention of Bacterial and Viral Adhesion	Antibodies dissolved in saliva, mucus, and perspiration coat epithelia, providing an additional layer of defense. A covering of antibodies makes it difficult for pathogens to attach to and penetrate body surfaces.

Classes of Antibodies:

Body fluids have five classes of antibodies, or **immunoglobulins.**

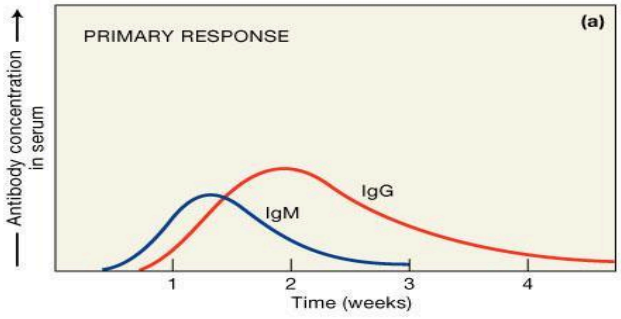
Class	Description	Clinical Relevance
IgG	-Most abundant and diverse -Cross placenta to provide immunity to baby	Responsible for hemolytic disease of newborn (erythroblastosis fetalis). Ab production starts late in the 3rd trimester so it won't affect the first baby.
IgE	Attached to surface of basophils and mast cells. When suitable Ag is then bound, the cell is stimulated to degranulate.	Allergic Rxn, Parasitic infections
IgD	On the surface of B cells where it can bind to antigens in ECF. This binding prompts activation of B cells and helper T cells	
IgM	-Pentamer -Appears in acute stage -First to be made by baby	ABO transfusion reactions
IgA	-secretory antibody found in glandular secretions	attack pathogen b4 it can get inside

Primary and Secondary Responses to Antigen Exposure:

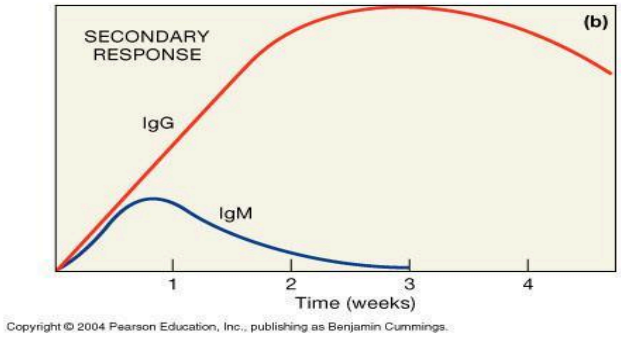
The initial response to exposure to an antigen is called the **primary response.** When the antigen appears again, it triggers a more extensive and prolonged **secondary response.** Primary and secondary responses are characteristics of both cell-mediated and antibody-mediated immunities.

Primary Response

Takes a bit of time to develop **(titer doesn't peak for 1-2 weeks!)** b/c the appropriate B cells need to be "touched" and then differentiate into plasma cells. **IgM are the first to appear in the bloodstream while IgG appears more slowly.** That's b/c the plasma cells responsible for IgG production begin producing IgG only after large numbers of memory B's are produced.

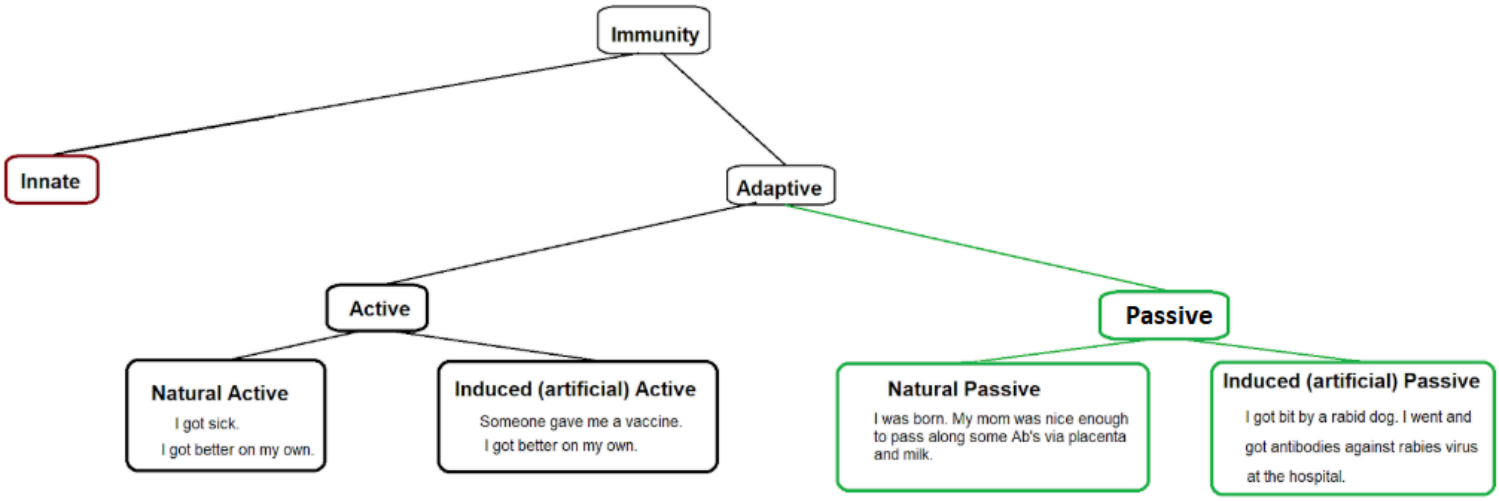


Then, the titer goes down (b/c **plasma cells have a high metabolic rate, they die quickly. Further production of plasma cells is inhibited by suppression factors released by suppressor T cell.)**



The Secondary Response:

Upon second exposure, memory B cells respond real quickly to even low antigen levels, differentiating into plasma cells and mass producing very destructive antibodies. Titers ramp up quicker and higher than upon first exposure. It's so effective that if you managed to survive the first infection, you are GOOD and will hardly feel the second exposure (reasoning for vaccines).



A **hypersensitivity** or **allergic reaction is** an exaggerated response by our immune system. There are 4 types:

	Mechanism	**Clinical Relevance**
Type I (anaphylactic)	**Most common** Occurs w/in a few minutes after a person previously sensitized to an allergen is re-exposed to it. **IgE mediated**	**First time around, person produces IgE that remains bound to mast cells and basophils.** Upon subsequent exposure, the IgE binds to the antigen and the cells release histamine, prostaglandins, leukotrienes → results in widespread vasodilation, increased capillary permeability, smooth muscle contraction in lungs, and mucus. **Anaphylactic shock** = airway constriction + widespread vasodilation and **low BP (shock)**
Type II (Cytotoxic)	IgG or IgM directed **against antigens on a person's OWN blood or tissue cells which results in complement activation**.	**Hemolytic disease of newborn** or **ABO transfusion rxn.**
Type III (Immune-complex)	You get **strep throat/pharyngitis** and don't treat it. So your body attacks it with Ab and lots of immune complexes form and subsequently get stuck in BM of endothelium. Inflammation and release of toxic chemicals. Hematuria and proteinuria result.	Causes **post-streptococcal Glomerulonephritis and Rheumatoid arthritis**
Type IV (Cell-mediated or delayed hypersensitivity rxn)	Allergens are taken up by APC (eg. langerhans cells in the skin) and taken to lymph nodes where they are presented to T cells. T cells return to the site of origin and stimulate an inflammatory response. **Things are delayed 12-72 hours b/c it's purely cell mediated.**	TB, poison ivy **haptens***, nickel

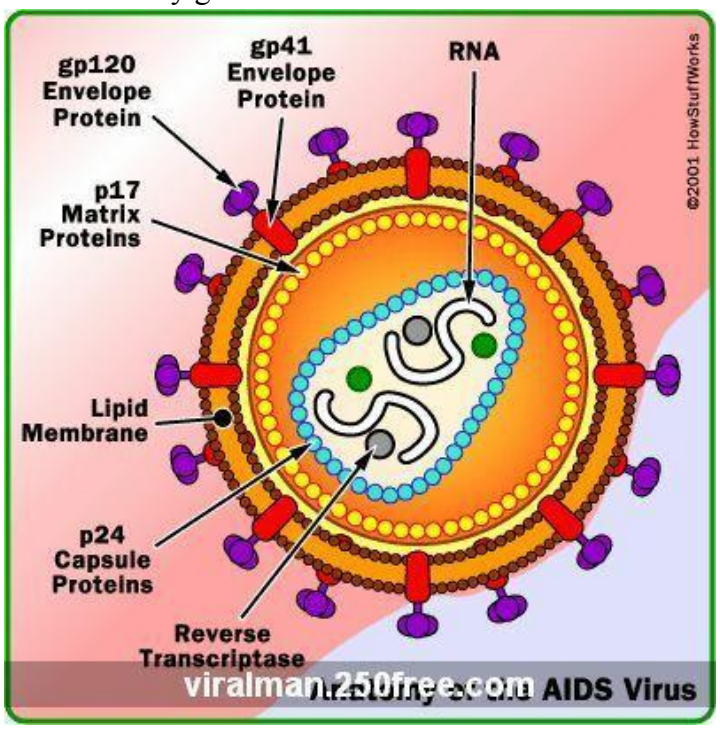
*Haptens are small molecules that by themselves make poor antigens because they evade detection. They can however become antigenic when bound to larger, carrier molecules (often proteins).

Effects of Stress and Age on Immune System
More susceptible to infections and malignancies and tend to produce more auto-Ab's.

For example, T cells become less responsive to antigens, and fewer T cells respond to infections. This may result from age-related atrophy of the thymus or decreased production of thymic hormones. Because the T cell population decreases with age, B cells are also less responsive. Consequently, antibody levels do not increase as rapidly in response to a challenge by an antigen, resulting in increased susceptibility to various infections.

AIDS: Acquired Immunodeficiency Syndrome: (An overview)
-Caused by the **animal virus** HIV (which **kills helper T cells**). **AIDS is the end stage** result.
-HIV is **fragile**. It can't survive for long outside the human body.

HIV Structure and Infection
-It's a **retrovirus** so it contains an inner core of **RNA** covered by a **protein capsid** which protects the nucleic acid from nucleases.
-Protease enzymes chop up other proteins to assemble the protein capsid.
-Once inside the host, the HIV takes off its coat and a viral enzyme **reverse transcriptase reads the RNA to make a DNA copy which integrates into the host chromosome.** Now you've got HIV DNA in your cell! Holy shit! The cell starts producing millions of copies of viral RNA and assembles new protein coats for all of them. Off they go to infect other T cells.



Signs, Symptoms, and Diagnosis of HIV Infection:
Soon after being infected with HIV, you have a brief flu-like illness with fever fatigue, rash, headache, joint pain, sore throat, and swollen lymph nodes and night sweats. Within 3-4 weeks, plasma cells have been secreting enough antibodies that they become detectable in blood (**HIV screening test**).

Progression to AIDS:

After 2-10 years of your body's defenses attacking infected cells and the HIV destroying T cells, the body loses its ability to replace T cells. That's when people start experiencing symptoms of immunodeficiency. They become susceptible to a bunch of opportunistic infections. AIDS is diagnosed when T cell count < 200/microliter

<u>Treatment of HIV Infection</u>:

Vaccines designed to block new HIV infections and to reduce the viral load (the number of copies of HIV RNA in a microlitter of blood plasma) in those who are already infected are in clinical trials. Meanwhile, two categories of drugs have proved successful in extending the life of many HIV-infected individuals:

1. **Reverse transcriptase inhibitors** do what they sound like they do.
    a. **Zidovudine (ZDV, previously called AZT).**
    b. **Didanosine (ddl)**
    c. **Stavudine (d4T)**
    d. **Trizivir**
2. **Protease inhibitors** do what they sound like they do.
    a. **Nelfinavir**
    b. **Saquinavir**
    c. **Ritonavir**
    d. **Indinavir**

HIGHLY ACTIVE ANTIVIRAL THERAPY (HAART) is a cocktail of

2 REVERSE TRANSCRIPTASE INHIBITORS + ONE PROTEASE INHIBITOR

This is highly effective with Pts experiencing huge reduction in viral load and an increase in # of helper T cells.

Disease and Epidemiology

Define pathology, etiology, infection, and disease.

Infection & disease are not the same thing. For example, the body may be infected with a virus that causes AIDS, but there may be no symptoms of the disease.

Define normal and transient microbiota

Animals are free of microbes in utero. But at birth, they begin to establish themselves. Newborn's first contact with microorganisms is with vaginal **lactobacilli,** which take up residence in the newborn's intestine.

After birth, *E. coli* and other food borne bacteria take up shop in the large intestine

We have normal flora/microbiota and other microorganisms called **transient microbiota (**present for several weeks and then gone).

Relationships Between the Normal Microbiota and the Host

Normal microbiota can benefit the host by preventing growth of harmful microorganisms **(microbial antagonism)** by c**ompeting for nutrients, producing substances harmful to the invading microbes, and affecting conditions such as pH.** However, when the balance between normal microbiota and pathogenic microbes is upset, disease can result. For example, normal vaginal microbiota maintains pH 3.5. But if the population is eliminated by antibiotics or excess cleaning, pH reverts to neutral, allowing *Candida albicans* yeast to flourish (**vaginitis).**

Similarly, treatment with antibiotics can kill off normal microbiota of large intestine, leading to ***Clostridium difficile*** **GI infections.**

Examples of symbiosis

Commensalism is when one organism benefits and the other is unaffected (many microorganisms that make up our normal microbiota are commensals).
Mutualism is when both benefit (our intestinal E. coli synthesize vit K and B for us, so we give them a place with some food to hang out in)
Parasitism is when benefits at the expense of the other (disease causers)

List Koch's postulates.

Koch was a German who played a major role in establishing that microorganisms cause specific diseases. For example, he showed that anthrax is caused by B. anthracis and M. tuberculosis causes tuberculosis. Basically, we can study the etiology of any infectious disease thanks to him. Here are his postulates:

1. **The same pathogen must be present in every case of the disease.**

2. **The pathogen must be isolated from the diseased host and grown in pure culture.**

3. **The pathogen from the pure culture must cause the disease when it is inoculated into a healthy, susceptible laboratory animal.**

4. **The pathogen must be isolated from the inoculated animal and must be shown to be the original organism.**

Classifying Infectious Diseases and differentiating a communicable from a noncommunicable disease.

The specific **signs** (objective changes measured by physician) and **symptoms** (subjective changes - like pain - not apparent to an observer) that accompany a disease are called a **syndrome.** The diagnosis of a disease is made on the bases of a clinical history, evaluation of the signs and symptoms, together with the results of laboratory tests.

Communicable disease spreads from one host to another, either directly or indirectly**. Contagious disease** is easily spread from one person to another.
Noncommunicable disease is not spread from one host to another b/c they are normal microbiota or reside outside the body and only produce disease when introduced inside.

Incidence vs Prevalance

Incidence is the frequency of new cases. It may be used as an indicator of the spread of the disease.
Prevalence refers to the frequency of existing cases. Prevalence takes into account both old and new cases so it serves as an indicator of how seriously and how long a disease affects a population.
For example, the incidence of AIDS in the United States in 1999 was 45,000, while the prevalence in that same year was estimated to be 700,000.

Categorize diseases according to frequency of occurrence.

Frequency of occurrence is another criterion for classifying disease:

Sporadic disease happens only occasionally (you would occasionally hear of people overdosing on chemical weed)
Endemic disease is constantly present (but there are enough dumb people in NYC that it will always be happening in one part or another)
Epidemic disease is when many people in a given area acquire a disease in a relatively short period (like that weekend in Harlem when 50 people overdosed in one park)
Pandemic is when the disease goes worldwide (if overdosing on chemical weed was happening all over the world)

The severity or Duration of a Disease

Acute comes on fast and lasts a short time (**influenza**)
Chronic develops slowly and lasts a while (**mono, TB, Hep B**)
Subacute is intermediate between the two (subacute endocarditis)
Latent is caused by an agent that remains inactive and suddenly active (**eg. Shingles)**.

Vaccination provides **herd immunity.**

Identify four predisposing factors for disease.

Gender, Climate, Nutrition, Age, Occupation, Preexisting illness, Habits

The Development of Disease

Once an organism overcomes the defense of the host, development of the disease follows a certain sequence that tends to be similar whether or not the disease is acute or chronic. The sequence occurs as follows:

Incubation period - time btwn initial infection and first appearance of signs/symptoms. Depends on:

- **The specific organism involved.**
- **The virulence of the microorganism.**
- **The number of infecting microorganisms.**
- **The resistance of the host.**

Prodromal period - is the short period that follows incubation. This period is characterized by early, mild symptoms such as aches and malaise

Period of illness - is when disease is most acute. The person exhibits overt signs and symptoms of disease, such as fever, chills, muscle pain (myalgias), sensitivity to light (photophobia), sore throat (pharyngitis), lymph node enlargement (lymphadenopathy, and gastrointestinal disturbances. During this period of illness, the number of white blood cells may increase or decrease. Generally, the patient's immune responses overcome the pathogen, and the period of illness ends.

Period of decline - signs and symptoms subside but pt is vulnerable to secondary infeciton

Period of Convalescence - the person regains strength and body returns to form. Recovery has occurred.

The Spread of Infection

For a disease to perpetuate there must be a reservoir (a continual source of the disease organism) such that the pathogen can survive and continue to infect others.

Human Reservoirs the principal living reservoir for human disease. Those with signs and symptoms can transit the disease, but even those without signs (**carriers**) are important living reservoirs of infection.

Animal Reservoirs. Well the idea is kind of obvious. Diseases that occur primarily in animals and can transmit to humans (rabies, lyme disease) are called **zoonosis.**

Nonliving Reservoirs like soil or water harbor tons of pathogens. *Clostridium botulinum* and *Clostridium tetani* are part of the normal intestinal microbiota of horses and cattle so their fertilizer contaminates soil.

The transmission of Disease

The causative agents of disease can be transmitted from the reservoir of infection to a susceptible host by several principle routes:

Contact Transmission: spread of an agent of disease by **direct contact**, **indirect contact**, or **droplet transmission**.

Direct contact transmission, also known as *person-to-person transmission,* is the direct transmission of an agent by physical contact between its source and a susceptible host; **no intermediate object is involved.** The most common forms of direct contact transmission are

touching, *kissing*, and *sexual intercourse*.

Indirect contact transmission occurs when the agent of disease is transmitted from its reservoir to a susceptible host by means of a nonliving object (**fomite**). Examples include tissues, bedding, drinking cups, utensils, toys.

Droplet Transmission occurs when microbes are spread in mucus droplets (coughing, sneezing, laughing, or talking) (*influenza, pneumonia*, and *pertussis* (whooping cough))

Vehicle Transmission: transmission of disease agents by a medium such as water, food, or air.

Waterborne transmission spread by water contaminated with poorly treated sewage (eg. cholera, waterborne shigellosis, leptospirosis).

Foodborne transmission transmitted in foods poorly cooked, poorly refrigerated, or prepped under unsanitary conditions (eg. food poisoning and tapeworm).

Vectors

Vectors are animals that carry pathogens from one host to another. Arthropods are the most common vectors.

Nosocomial (Hospital-Acquired) Infections

No evidence of being present or even being in incubation at the time pt enters hospital; it's acquired as a result of a stay at a health care facility.

Compromised Host

Is someone one whose resistance to infection is impaired by disease, therapy, or burns.

Epidemiology

Science that studies when and where diseases occur and how they are transmitted in populations**.** An epidemiologist not only determines the etiology of a disease but also identifies other factors and patterns concerning the folks affected by assembling and analyzing data such as age, sex, occupation, personal habits, socioeconomic status, and history of immunization.

Morbidity vs. Mortality

Morbidity refers to the incidence of specific notifiable diseases.
Morbidity rate is the number of people affected by a disease in a given period of time in relation to the total population.
Mortality refers to the number of deaths from specific notifiable diseases.
Mortality rate is the number of deaths resulting from a disease in a population in a given period of time in relation to the total population.

Staphylococcus

Some basics

-warm, dark, moist places (they're on every person but 15% carry the "pathogenic" coagulase Positive *S. aureus* in their **nose and other places like axilla, groin**)

-this can be a serious problem b/c it's aggressive and invades tissues, bones, bloodstream to produce septic shock and DIC (**cellulitis**)

-nosocomial infections

-post-traumatic infections

Family life

There's a family called **Micrococcaceae** and it has two important members: **micrococci** and **staphylococci**. These two genera are both **catalase positive.**

staphylococci have three clinically important species	**Coagulase**	**Ferments Mannitol?**	**Resistant to Novobiocin?**	**DNase?**	**Character**
S. *aureus*	**+**	**Yes**		**+**	**Golden color**
S. *epidermidis*	**-**				
S. *saprophyticus*	**-**	**Yes**	**Yes**		**UTI**

Epidemiology

-Patients who regularly use needles likely to get *S. aureus*.

-Stopping its spread is as simple as washing hands in the hospital

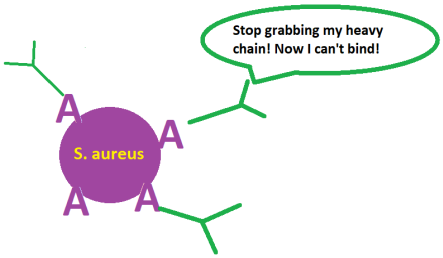
Pathogenesis

-highly virulent organisms can infect regular people

-nonpathogenic (saprophytic) organisms only cause infection in the immunocompromised

Host Defense Aspects

The healthy host defends against Staphylococcus using nonspecific defenses (skin, mucous membranes and leukocytes). But **the presence of a foreign body can really cause trouble**. For example, ***S. epidermidis* infects you if you've got prosthetic heart valves or joints**. Meanwhile, ***S. aureus* infects you if you've got sutures on your skin.**

Microbial Virulence: Healthy immune systems ward off the normal skin flora *S. aureus*, but it can cause disease using a host of virulence factors. We already know it's got **peptidoglycan** in the cell wall for stability and we know that some can produce an outer **"polysaccharide slime layer"** to protect against phagocytosis. But it's got some other nasty virulence factors like the ability to:		
Disable host's immune defenses	Tunnel through the host tissue	Exotoxins
Protein A is a surface protein that grabs the antibodies heavy chain and turns the antibody around so it can't even bind 	**Hyaluronidase** is a protein that breaks down proteoglycan in connective tissue **(joint pain**)	**Exfoliatin** causes skin to slough off (**scalded skin syndrome**) tested using the **Nikolsky sign**
Coagulase causes fibrin "shell" to form around the bacteria, shielding it from phagocytosis.	**lipase** is capable of degrading fats and oils which facilitates colonization of the organism in sebaceous glands. **Can cause something like necrotizing fasciitis (flesh eating disease)**	**Enterotoxins** - food poisoning and vomiting/diarrhea associated with **potato salad or macaroni salad** left out at room temp
Hemolysins are 4 types (alpha, beta, gamma, and delta) that destroy RBCs, neutrophils, macrophages, and platelets.		**TSST-1** Toxic shock syndrome associated with **tampons**
Leukocidins kill WBCs		
Penicillinase destroys the beta-lactam portion of penicillin		

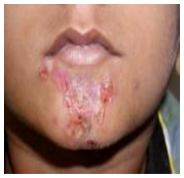
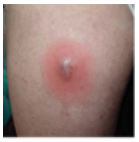
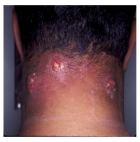
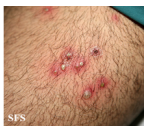
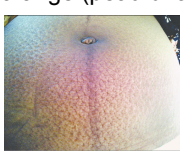
Clinical Manifestations:

1)Diseases caused by **toxins produced by S. aureus**

	Staphylococcal Gastroenteritis	**Toxic Shock Syndrome (TSS)**	**Staphylococcal Scalded Skin Syndrome (SSSS)**
Etiology	*S. aureus* **preforms exotoxin** in custards and cream filled pastries and milk → you eat it	**Tampon** use results in *S. aureus* infection and resulting **exotoxins**	Abrasion might cause a *S. aureus* infection which releases toxin which takes your skin right off
Symptoms	-arrives abruptly w/in 2-8 hours with nausea/vomiting, cramps and diarrhea -It's all over in less than 24 hrs	**-High fever** -Vomiting -Diarrhea **-Confusion** -**Skin Rash** (sunburn-like erythroderma which is red, inflamed skin) -**Hypotension and shock** associated with **ARDS** (acute respiratory distress syndrome) **-desquamation** in the palms **-multi-organ involvement**	-tender skin -Skin Rash (sunburn-like) **-desquamation (Nikolsky's sign)**
Diagnosis	Recognize clinical symptoms with several persons affected in one outbreak	Recognize clinical symptoms	**Infants commonly involved**
Prophylaxis & Treatment	Fluid and electrolyte replacement	Remove foreign object Fluid and electrolyte replacement to prevent hypovolemia, hypotension, shock Antibiotic therapies with Beta-lactamase-resistant anti-staphylococcal agents	Heals quickly

2)Diseases caused by **direct Staphylococcal Invasion and Spread**

Most minor skin infections are caused by either *S. aureus* or *Group A Beta-hemolytic streptococci*. Direct invasion thru minor breaks in skin/mucous membranes is the hallmark of disease produced by *S. aureus*. A wide variety of dermal and soft tissue infections may result, including any of the following:

	Impetigo is a superficial vesiculopustular infection. **Ecthyma** is an ulcerative form of impetigo. 	**Furuncles** are infected hair follicles that go deep (perifollicular) 	**Carbuncle= cluster of furuncles** with subcutaneous spread of infection 	**Folliculitis** is inflammation of the hair follicles 	**Cellulitis** Is an acute inflammation within solid tissues characterized by hyperemia, WBC infiltration, and edema **without** cellular necrosis and suppuration (formation of pus)	**Osteomyelitis** is an inflammation and destruction of bone
Etiology & Pathogen.	*Staph aureus* (primarily) but also Streptococcus May occur after superficial trauma with **break in skin** The arms, legs and face are more susceptible than unexposed areas Ecthyma penetrates more deeply than impetigo, resulting in ulceration with subsequent scarring.	Occurs in healthy young persons living in crowded quarters.	Occurs commonly in healthy males but also in those with diabetes and debilitating disease and old age	Staph *aureus* **(**primarily**)** but also ***P. aeruginosa* (hot-tub folliculitis)**	*Strep pyogenes (group A Beta-hemolytic streptococcus)* is the **most** common cause **Diffuse infection occurs because streptokinase, DNASE, and hyaluronidase break down barriers that would normally keep the infection localized.** *S. aureus* can also cause it as a result of open wound infection *E. coli* and *P. aeruginosa* also implicated amongst diabetics	Infection of bone is produced by blood-borne organisms spreading from infected tissue, (prosthetic joint, contaminated fractures, and bone surgery) Risk factors: *-debilitating comorbid disease* *-cancer* *-radiotherapy* *-diabetes* *-Hemodialysis* *-IV drug use* Infection of the bone (eg. in the feet of diabetics)and **bone necrosis**
Signs Symptom Dx	**Honey colored, crusted lesions** Itching is common and scratching can spread it	Occur on neck, breasts, face, buttocks. Painful when on the skin attached to underlying structures (nose, ear, fingers). Nodule becomes a pustule that discharges pus	suppuration, slow healing and a scar		Infection is most common in the lower extremities. Local erythema and tenderness.Hot, Red, Edematous skin like an orange (peau d' orange). 	**Febrile**, weight loss, localized warmth, swelling,, erythema, and tenderness Bone pain (back pain) Malaise

Treatment	**Mupirocin**	Incision and drainage H**ot compress** so the lesion can point and drain on its own multiple furuncles should be treated with penicillinase resistant antibiotics Reoccurs	Infected hairs can be removed, but new papules tend to develop. Similar to impetigo (topical antibiotics)	*Penicillin* *Erythromycin* or *Clindamycin* Got MRSA? (methicillin resistant staph aureus) → then you get the antibiotic *Vancomycin*	Antibiotics against both Gram Pos & Gram Neg should be given until results are available Penicillinase resistant Penicillin (nafcillin or oxacillin) Antibiotics parenterally Surgical debridement of necrotic tissue

suppuration=pus forming *erythema=skin redness* *myalgia=muscle pain* *pruritic=itchy*

Staphylococcal Endocarditis		
	Subacute Bacterial Endocarditis	**Acute Bacterial Endocarditis**
Etiology	Streptococcus (95%) **Staphylococcus epidermidis (5%) is the one most associated with prosthetic heart valve infection**	Staphylococcus aureus
Infects?	Bicuspid (mitral) valve	Tricuspid valve
Cause?	Pre-existing valvular heart disease	IV drug use
Signs	Chronic low fever, myalgia, night sweats, weight loss	Rapid onset high fever, myalgia, chills

Exanthemas - are rashes that arise as a result of infectious diseases. They include the following:

	Measles **"Rubeola"**	**"German measles"** **"Rubella"** **"3 day Measles"**	**Roseola (exanthema subitum)**	**Varicella (chickenpox) & Herpes Zoster (shingles)**	**Erythema infectiosum (fifth disease)**
Etiology	**RNA paramyxovirus** that's remained one stable antigenic serotype for many years	**RNA togavirus**	**Herpesvirus 6**	**Varicella Zoster Virus** (dsDNA virus)	**Parvovirus B-19**
Clinical features	Common in kids in developing countries who live in dense populations **a)incubation period** of **8-12 days** after exposure w/o symptoms **b)prodrome** period of malaise, **fever** up to 105F, **cough**, coryza, **conjunctivitis**, photophobia and **Koplik spots** on the buccal mucosa. **c)erythematous maculopapular rash** begins on head and spreads downward; lasts 5 days; resolves from head and downwards also.	2-3 week incubation **Classic finding: posterior auricular, cervical, and suboccipital lymphadenopathy; maculopapular rash** that begins on the face and then becomes generalized Rash lasts 3 days	**"Happy sick baby"** **-Abrupt high fever** that resolves in a few days as a maculopapular rash appears on the trunk -Child appears well	Highly infectious so you get it as a kid. **14 day incubation period.** Theen a 5 day **infectious period that begins just before the blisters show up - until everything crusts over** "**Teardrop**" vesicles appear, become cloudy, break down, and crust over before healing. **Multiple "crops" growing simultaneously** so some areas of the body have the rash popping up while in other parts it's resolving. **Compare to those other three... ←** HZ occurs due to reactivation of the virus in adulthood	**3 stage rash** 1)"**slapped cheek**" appearance of cheeks 2)erythematous maculopapular rash that begins on arms and spreads to trunk 3)rash varies with exposure to heat and sunlight
Dx	Based on characteristic clinical findings: >4X rise in hemagglutin. inhibition antibodies **>Warthin Finkeldy giant cells** containing lots of nuclei	Virus isolation is difficult Dx confirmed by 4X rise in hemagglutin. Inhibition or complement fixing Ab's. Congential rubella diagnosed in neonatal period by the presence of IgM to rubella in the newborn serum (increased IgM in newborn indicates recent rubella infection of the fetus		Based on clinical findings. But if dx is unclear, perform a **Tzanck test** (ulcer scraping) and look for multinucleated giant cells with intranuclear lesions	
Tx	Mainly supportive Ribavirin to immunocompromised Vitamin A if very young or immunodeficient or impaired intestinal absorption or malnutrition or recent immigrant from measles area	**Postnatal rubella** is mild so no treatment. Treatment of congenital rubella is supportive.		Anti-pruritic meds and bathing to prevent secondary bacterial infections	self-limiting
Prevention	Part of the MMR vaccine (live but attenuated). 1st dose at 15	Part of the MMR vaccine (live but attenuated virus) given starting at 15 months			

	months although even sooner in endemic areas (that's why it's uncommon in developed countries) All healthcare workers need to be immune Contraindications to the live vaccine include immunocompromised states, pregnancy, or active TB				
Complications	Rare but occurs in malnourished or immunocompromised kids: 1)Secondary bacterial **otitis media** is very common 2)**Pneumoonia is responsible for most of the deaths** 3)demyelinating encephalopathy is rare but does kill	**Postnatal rubella** often no clinical manifestation **Congenital rubella** results in deafness, cataracts, glaucoma, congenital heart disease, and mental retardation. **Higher risk of congenital defects when the disease occurs earlier in pregnancy:** **1-3 months? High chance of multiple defects** **4 months?** 10% chance of single defect **5-9 months?** Occasional defect		In immunocompro-mised, the disease is called "progressive varicella" and it's serious. The lung, liver and brain are involved, with lung complications leading to death. If such kids get exposed to it, they should receive **prophylaxis with varicella-zoster immune globulin**. If they catch it, they should get **IV acyclovir** Varicella in pregnancy is really bad b/c 10% of these babies have **"varicella embryopathy"** (cerebral damage, scarred, atrophic limbs).	

These 2 diseases placed together at the end of the lecture. Not related to each other...

	Scarlet Fever	**Rocky Mountain Spotted Fever**
Etiology	group *A* streptococcal strains that produces erythrogenic toxin	Caused by ***Rickettsia rickettsii*** (east, southeast & Ohio River valley) Vector that carries it is ***Dermacentor (wood or dog tick)*** that is active in spring & summer. It infects kids (boyscouts and girlscouts)
Clinical features	associated with pharyngeal infections  Erythematous rash which blanches with pressure. It appears initially on trunk and then becomes generalized. Skin rough like **sandpaper;** **"strawberry tongue"**	**1 week (incubation period) after you get bit by a tick**, an abrupt 2 week fever with chills, headache, irritability, confusion, myalgia, conjunctivitis with photophobia Rash on the hands, wrists, feet, and ankles, which spread to involve the entire body. **Can lead to DIC so you end up bleeding all over**
Dx	Clinical presentation and presence of group A streptococci on throat culture	Clinical appearance; **history of tick bite**
Tx	10 days of penicillin	Chloramphenicol or **tetracycline** (to children over 8) *Note: must be treated b/c it's serious*

Acute & Chronic Hepatitis

Hepatitis refers to **liver inflammation** due to a viral infection by the hepatitis virus or alcohol use.

1. *IV drug use(Hepatitis B and C)*
2. *Alcohol use (alcoholic hepatitis)*
3. *Travel to developing countries (hepatitis A and E)*

Hepatitis A	**Hepatitis B**	**Hepatitis C**	**Hepatitis D**	**Hepatitis E**
acute	acute or chronic	acute or chronic	Acute or chronic	acute
ssRNA **picornavirus**	dsDNA hepadnavirus	RNA virus	RNA virus (**delta agent**)	RNA virus
Found in serum, stool, and liver *only* during acute infection	It's a major cause of **hepatocellular carcinoma** worldwide.	major cause of cirrhosis and **post-transfusion hepatitis.**	infectious only in the presence of hep B b/c it can't replicate on its own so if person has hep D, they also have hep B	Mexico, Asia, and Africa self limited but can be fatal in pregnant women
spread via the **fecal-oral route** and can lead to epidemics	spread by blood and other body fluids, including saliva (so it's an STD)	IV drug users (**blood borne transmission**)	Super-infection with a chronic, **severe clinical course**	spread via the fecal-oral route
IgM indicates acute infection; IgG (which stays for life) indicates previous exposure and immunity				

Now some bits about HBV

-intact HBV is called the **Dane particle**

-envelope and capsid make up the **hepatitis B surface antigen (HBsAg).** Antibodies form against this component (**anti- HBsAg**). These antibodies are protective. **Thus having anti-HBsAg means that the patient is immune against HBV.**

-core of the virus is called the **Hepatitis B core antigen (HBcAg).** The core is also antigenic and antibodies form against the core, although **antiHBcAg are not protective.**

-During an active infection, a part of the core (**HBeAg)** is found in the serum

-presence of HBsAg and HBeAg is indicates a highly infectious state

Common Serologic patterns in Hepatitis B virus Infection.

	HBsAg	anti-HBs	anti-HBc	HBeAg	anti-HBe
Acute Hep B (highly infectious)	+	-	IgM	+	-
Chronic Hep B with viral replication (highly infectious)	+	-	IgG	+	-
Chronic Hep B with low viral replication (but can still infect)	+	-	IgG	-	+
Recovery from Hep B (immunity)	-	+	IgG	-	+/-
Vaccinated against Hep B (immunity) (b/c only Ab against surface antigen is protective)	-	+	-	-	-

	Acute Hepatitis	**Chronic Hepatitis**
Sx	-starts with a viral **prodrome of nonspecific symptoms** (malaise, joint pain, fatigue, nausea, vomiting, changes in bowel habits followed by jaundice). -**jaundice, scleral icterus,** hepatosplenomegaly.	Symptoms indicative of chronic liver disease (jaundice, cirrhosis).
Dx	**Labs demonstrate elevated liver enzymes & bilirubin although diagnosis is made based on hepatitis serology**	Findings include elevated liver enzymes lasting more than 6 months and a rise in bilirubin/alkaline phosphatase. Diagnosis is made on the basis of hepatitis serologies and liver biopsy, which may show inflammation, fibrosis, and necrosis involving hepatocytes.
Tx	Rest Administer alpha-interferon for HBV and HCV to decrease the likelihood of chronic hepatitis.	Alpha-interferon and lamivudine have proven efficacy for chronic HBV infection. For HCV you may treat with alpha-interferon and ribavirin. For end stage liver failure transplantation is the treatment of choice.

	Typical Exudate in alveoli Predominantly pulmonary features such as purulent cough and chest pains				Atypical No exudate as alveoli are air filled Predominantly non-pulmonary features such as fever, dry cough			
	Streptococcus pneumoniae	***Staphylococcus aureus* pneumonia**	***Klebsiella pneumonia* (encapsulated gram NEG bacilli)**	***H Influenzae* pneunomonia (gram NEG coccobacilli)**	***Legionella pneumophilia***	***Mycoplasma pneumonia* (*aka 1° atypical pnuenomia)**	***Chlamydia Psittacosis***	***Pneumocystis carinii***
Epidemi	Most common cause of bacterial pneumonia 67% of community acquired Winter especially	Hospitalized pts (especially intubated) and infants/elderly 35% of pts die b/c they're already sick	Causes Friedlanders **pneumonia** **Rare in healthy folks but it happens in infants, elderly, alcoholics, immune compromised** 25-50% mortality	Affects children Strains with **Hib polysaccharide capsule are the most virulent** and likely to cause serious disease	**Pontiac fever** (flu-like w/o pneumonia) **Legionnaire's** (with pneumonia) **Late summer/early fall when AC unit is dirtiest** Middle aged men who smoke and drink alot	Everyone recovers with or w/o treatment	From birds	**Fungus** causes disease **only when immunity compromised** (eg. chemo, **AIDS is big**) when CD4 count<200
Pathog	Inhale pathogen→ inflammatory response in alveoli→ protein rich exudate acts as a culture medium		Colonization of oropharynx→ microaspiration of secretions→			**"Walking pneumonia"**	Inhale dust from infected bird	
Sx	**See description of lobar pneumonia below***		sputum looks like **currant jelly**, tissue necrosis, fulminant course		High fever Bradycardia	Gradual malaise, sore throat, dry cough		
	Upper resp. Infection often precedes fever/chills & pleurisy (painful breathing), cough and **rusty colored sputum**							
Dx	Leukocytosis POS blood culture confirms **Gram POS lancet shaped diplococci**						History of bird exposure and recovery of the agent from body	
Xray	Pulmonary infiltrate shows white **Dense consolidation to a single lobe** is specific for this organism					**Xray changes are dramatic while pt presents as pretty normal**		Diffuse, bilateral perihilar infiltrates
Px Tx	Vaccine containing 23 specific polysaccharide antigens is available **Penicillin**	Penicillinase resistant penicillin Alternatively		Vaccine Trimethoprim-Sulfamethoxazole (TMP-SMX)	Erythromycin	Tetracycline and erythromycin Won't respond to antibiotics that mess	Tetracycline and doxycycline	Trimethoprim-Sulfamethoxazole (TMP-SMX)
		cephalosporin Vancomycin for MRSA				with cells wall (penicillin) b/c it's got no cell wall!		

Lobar Pneumonia occurs in 4 stages

Congestion - extensive serous exudate; vascular engorgement; bacterial proliferation

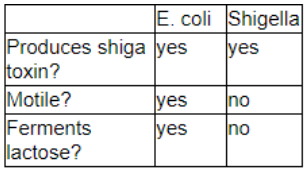
Red hepatization - extravasation of RBCs into alveoli along with neutrophils and fibrin; reddish and solid looking like the liver

Gray hepatization - RBCs disintegrate while neutrophils and fibrin remain; gray and solid looking

Resolution - recovery; exudate gets reabsorbed by the body

Gastroenteritis (GE)

All of the diseases in this lecture cause **inflammation of the lining of the stomach/intestines** with symptoms of **stomach pain, anorexia, nausea, vomiting/diarrhea**. The history is really important b/c alot of them sound pretty similar. For example, think (b. Cereus = reheated rice; staph food poisoning = custard rich food; vibrio parahaemolyticus = seafood poisoning and watery diarrhea)

	Traveler's diarrhea	**E.coli 0157:H7**	**Staphylococcal food poisoning**
Epidem.	Caused by bacteria endemic to local water (3rd world countries)	Found in undercooked beef (especially ground beef) or unpasteurized milk.	**Custard like foods** contaminated by skin handlers with skin infections → staphylococcal **enterotoxin** causes you trouble
Etiology Pathophysio.	Enterotoxigenic E.coli (usually)	Ingest **E. coli 0157: H7** → shiga toxins produced in large intestine → toxins damage endothelial cell walls and mucosa and can also damage vessels in kidney	staphylococcal **enterotoxin** (not the organism) causes you trouble
Sx	**Nausea, vomiting, borborygmi ("blurp blurp"), abdominal cramps,** and **diarrhea** begin 12 to 72 hours after ingesting contaminated food or water. Severity is variable although most cases are mild self-limited. Dehydration is the only real problem you'll have.	Begins acutely with severe abdominal cramps and watery diarrhea that may become very bloody **(*"all blood and no stool"***). Lasts 1-8 dayas. **Complications:** Normally no fever but 5% of cases are complicated by the **hemolytic-uremic syndrome (HUS),** which is characterized by fever and the following: 1. hemolytic anemia 2. thrombocytopenia 3. acute renal failure You might mistake the above syndrome for TTP but note that TTP isn't preceded by diarrhea!	Abrupt onset of nausea/vomiting/diarrhea 2-8 hours after eating the contaminated food. . Acid base imbalance and shock may ensue in severe cases. The attack is brief, often lasting less than 12 hours, and recovery is usually complete.
Dx		***E. coli 0157: H7*** **and shigella both produce shiga toxin. You need to distinguish between them by isolating the organism from stool culture** 	Based on symptoms and seeing multiple people in a single outbreak
Prevention	Avoid street food and ice cubes. Eat only fresh hot food. Eat fruits that can be peeled.	1. Proper disposal of feces, good hygiene, and careful hand washing 2. Pasteurization of milk and thorough cooking of beef prevent foodborne transmission.	Careful food preparation
Tx	Fluid replacement. Shouldn't use antimotility agent b/c the agent will stay inside you longer! Antibiotics not recommended for mild diarrhea. If you must, then use **Ciprofloxacin but this is contraindicated in children less than 16 years of age.**	Supportive care. Antibiotics won't help b/c you're dealing with a friggin toxin! Patients who develop HUS are going to need dialysis and special care	Treatment is usually supportive & involve replacement of fluids and electrolytes which often brings dramatic relief.

	E. coli	Shigella
Produces shiga toxin?	yes	yes
Motile?	yes	no
Ferments lactose?	yes	no

Botulism

-spores are very heat-resistant and may survive boiling for several hours. Exposure to moist heat for 30 minutes will kill the spores.

-Toxins on the other hand, are easily destroyed by heat.

-Toxin can be produced even at low temps and anaerobic conditions (canned foods commonly implicated)

	Foodborne Botulism	**Wound Botulism**	**Infant botulism**
Etiology	C. botulinum produces several types of **neurotoxins** that affect humans (types A, B, E, F) although **type A and B** are very poisonous and resistant to gastrointestinal enzymes so they are the ones which often cause the trouble.		
Pathophysiology	The toxin is a neuromuscular poison which **inhibits Ach**, causing **descending paralysis** and **death from diaphragmatic paralysis**		
	Preformed toxin and organism already present in the canned food → person ingests it	Toxin produced **after organism infects your wound**	Spores are ingested from honey → **toxin is produced afterwards in the baby's intestine**
Sx	Abrupt onset of symptoms including: *a) Nausea* *b) Vomiting* *c) Abdominal cramps* *d) Diarrhea* Next, neurological symptoms characterized by descending weakness and paralysis of respiration, trunk and extremities. Also: *a) Diplopia* *b) Ptosis* *c) Loss of accommodation* *d) Loss of papillary light reflex* **<u>Complications</u>** **respiratory failure caused by diaphragmatic paralysis**	**Neurological symptoms just like the other one, but no GI symptoms** Hx of recent traumatic puncture wound	Constipation and then neuromuscular paralysis, beginning with the cranial nerves and proceeding to peripheral and respiratory musculature. Cranial nerve deficits typically include the following: *a) Ptosis* *b) Weak cry* *c) Poor suck* *d) Expressionless face* The severity varies from mild lethargy to severe hypotonia (low muscle tone) and respiratory insufficiency.
Dx	Botulism may be confused with a number of disorders including *Guillain-Barre, Poliomyelitis, Stroke, Myasthenia gravis, Tick paralysis, Poisoning by belladonna*		
	You're going to dx based on a hx of ingestion of a likely food source and the pattern of neuromuscular disturbances. If multiple pts involved, it's pretty clear. **Dx can be confirmed by demonstrating *C. botulinum toxin* in the serum or feces or by isolating the organism from the feces**.	Dx confirmed by **finding toxin in serum** or isolating the organism on anaerobic culture.	Dx confirmed by **finding toxin in the feces**
Prevention	-Proper canning and adequate heating of home-canned food before serving are essential. -Infants under 12 months shouldn't get honey.		
Tx	-Gastric lavage (pump stomach) with administration of activated charcoal may be helpful. **-Give large doses of antitoxin (A, B, E) before the toxin reaches the synapses and binds to them.** Toxin already bound at the neuromuscular junction won't be unbound so it doesn't reverse rapidly. Ultimate recovery depends on regeneration of nerve endings, which may take weeks or months.		

	-The major threat to life is going to be due to a progressive respiratory paralysis that slowly lowers their vital capacity. In other words, they won't show signs of rapid respiratory distress. That's why their vital capacity should be continually monitored.

***Clostridium Perfringens* Food Poisoning**

-eating food contaminated by this organism causes an acute gastroenteritis
-the enterotoxin produced is sensitive to heat

Etiology

Widely distributed in feces, soil, air, and water, it's often **contaminated meat**  that causes outbreaks. Basically, someone leaves out meat contaminated with *C. Perfringens* at room temp → organism multiplies→ you eat the meat→ *C. Perfringens* produces enterotoxin inside you → the enterotoxin acts on small intestine

Sx

→ less than 24 hrs later, you experience mild gastroenteritis (water diarrhea and abdominal cramps) → all is resolved within 24 hrs

Dx

evidence of eating contaminated food and the isolation of organism from suspected food/stool

Prevention and Treatment:

-To prevent disease, you should obviously quickly refrigerate leftover meat
-reheat leftovers thoroughly before serving *(recall that the toxin is sensitive to heat?)*

Viral Gastroenteritis (Intestinal Flu)

Super contagious cause of infectious diarrhea. You can really feel this in your stomach. Symptoms include *a)Vomiting b)Watery diarrhea c)Abdominal cramps*				
	Rotavirus	**Calicivirus (e.g., Norwalk virus)**	**Enteric adenovirus**	**Astrovirus**
Etiology and Pathophysiology	The viruses cause illness by infecting enterocytes within the villous epithelium of the small intestine. Destruction of the cells in this layer causes the movement of fluids and salts into the intestinal lumen. **Watery diarrhea** results**.**			
Epidemiology	**most common cause of severe dehydrating diarrhea in young children** **Fecal-oral route** **Nov-March**	Older children and adults Principal cause of epidemic viral gastroenteritis **Cruise ships, water and food-borne** Year-round	**Serotypes 40 & 41** second most common cause of childhood viral gastroenteritis **Fecal-oral route** **More in summer**	Usually young children **Fecal-oral route** **More in winter**
Symptoms/Signs	Severe watery diarrhea	Children vomit Adults diarrhea	2 weeks of diarrhea	
Prevention	This is tough b/c of how often it's asymptomatic and how easily it's transmitted from one person to another - particularly babies in diapers. Best thing to be done here is for the caregiver to wash hands thoroughly after changing diapers			
Tx	Mainstay of therapy is oral fluid resuscitation. IV only needed if severely dehydrated			

STDs

Syphilis

Etiology and basics

-***Treponema pallidum*** (spirochete) which can't survive for long outside the human body. It's transmitted sexually or non sexually, or via placenta

-infection does not lead to immunity

-if untreated, itll go thru 1^0, 2^0, 3^0 stages

Pathogenesis

Primary Syphilis

-after infection, up to 90 days of incubation and then a **painless chancre** (as opposed to herpes which hurts) which produces a clear fluid full of spirochetes.

-the areas of infection are wherever it was introduced. If untreated...

Secondary Syphilis

-affects any organ (eyes, bones, kidneys, liver, spleen) although half of people get lymphadenopathy.

-spreads thru bloodstream and causes widespread mucocutaneous lesions

eg. **syphilitic dermatitis** which are symmetric lesions on palms and soles

eg. **condyloma lata** which are flat, grey or pink papules at mucocutaneous junctions and moist areas of skin). **Extremely infectious** although they eventually heal on their own. (note: condyloma acuminata are venereal warts and they are small, multiple and sharply raised)

Latent Syphilis

-**no clinical signs** of syphilis and it may last a lifetime

-can only detect via a positive specific treponemal antibody test on more than one occasion

-Early latency is defined as the first year after infection while late latency is noninfectious except in the case of pregnant women

Tertiary Syphilis

A few folks develop this problem decades later. Several types of lesions can develop:

Benign tertiary gummatous or bone syphilis-inflammatory, destructive masses which can be destructive and painful

Cardiovascular syphilis-causes **aortic insufficiency** or **aortic aneurysm** possibly leading to congestive heart failure

Neurosyphilis (4 types)

1. Asymptomatic neurosyphilis causes mild meningitis
2. Meningovascular neurosyphilis causes meningitis and palsies
3. Parenchymatous neurosyphilis destroys cortical parenchyma so you suffer dementia, mental illness, tremors
4. **Tabes dorsalis** is a progressive degeneration of posterior columns and roots of spinal cord; destruction of large joints (Charcot's joints); incontinence; impotence; optic atrophy (small pupils which fail to constrict in response to light but constrict normally to accomodation aka **Argyll pupils**)

Diagnosis

Darkfield Examination	**Serologic Tests (nonspecific screening test)**	**Treponemal Tests (specific treponemal Ab test)**
Seeing spirochetes on Darkfield is a definitive way of diagnosis syphilis during the primary and secondary stages	**This is a nonspecific screening test** looking for **anticardiolipin Ab's** (it can either be the **VDRL, RPR or USR tests)** It can be false POS in a lot of patients b/c it's detecting a normal human Ag. Meanwhile, it can also be false NEG b/c this test doesn't turn POS until 2 weeks after the onset of a chancre! It basically is neither sensitive nor specific. So then you do the next→	Most commonly used test is the fluorescent treponemal Ab **(FTA-ABS)** test **This is a confirmatory test.** It's both sensitive and specific.

Treatment

-give penicillin or erythromycin (pregnant women MUST get penicillin b/c erythromycin won't work)

Herpes Simplex Virus 2 (HSV2)

-painful vesicular lesions that can appear anywhere
-shedding for 12 days
-recurrent
-acyclovir doesn't cure it. It just decreases length of symptoms
-recent outbreak or visible lesion while pregnant? Needs C section

Human Papilloma Virus (HPV)

-dsDNA virus
-associated with cancer
-STD
-clinical presentation as condyloma acuminatum
-Pap smear can demonstrate the koilocyte (halo cell) which has a wrinkled appearance. Its nucleus is surrounded by a perinuclear clear zone or halo. Pap smears with this change are designated as low-grade squamous intraepithelial lesions.

	Neisseria Gonorrhoeae	Chlamydia Trachomatis
	These two are always competing for most common STD. They are a lot like each other and act like each other and often even hang out in the same place together causing similar havoc (while in men, they'll cause urethritis, in women they'll really get to go places)	
Etiology	-caused by a **GRAM NEG diplococcus** that **loves humans so much it can't live w/o em**	
Pathogenesis	-virulence factors include surface pili (to better attach to your mucosa) and an ability to stave off ingestion/killing by neutrophils. -they also produce IgA protease which chops up secretory IgA antibody	
Clinical Presentation	-looks a lot like chlamydia so treat for both simultaneously -greenish yellow discharge	**-vertical transmission to baby will mess up its eyes (use erythromycin for these pregnant pts)** -**L type causes lymphogranuloma venereum,** a rare systemic disease in 3 stages: **1)painless genital ulcers** **2)painful lymphadenopathy** **3)anal and rectal fistulas and elephantiasis**
Dx	-swab the relevant area and grow on **Thayer-Martin agar** -confirmation is if you see GRAM NEG intracellular diplococci	clinical presentation
Tx	**-condoms are effective prophylaxis** <u>Treat with both:</u> **ceftriaxone (in case its gonorrhea)** **doxycline/azithromycin/macrolide (in case it's clahmydia)**	

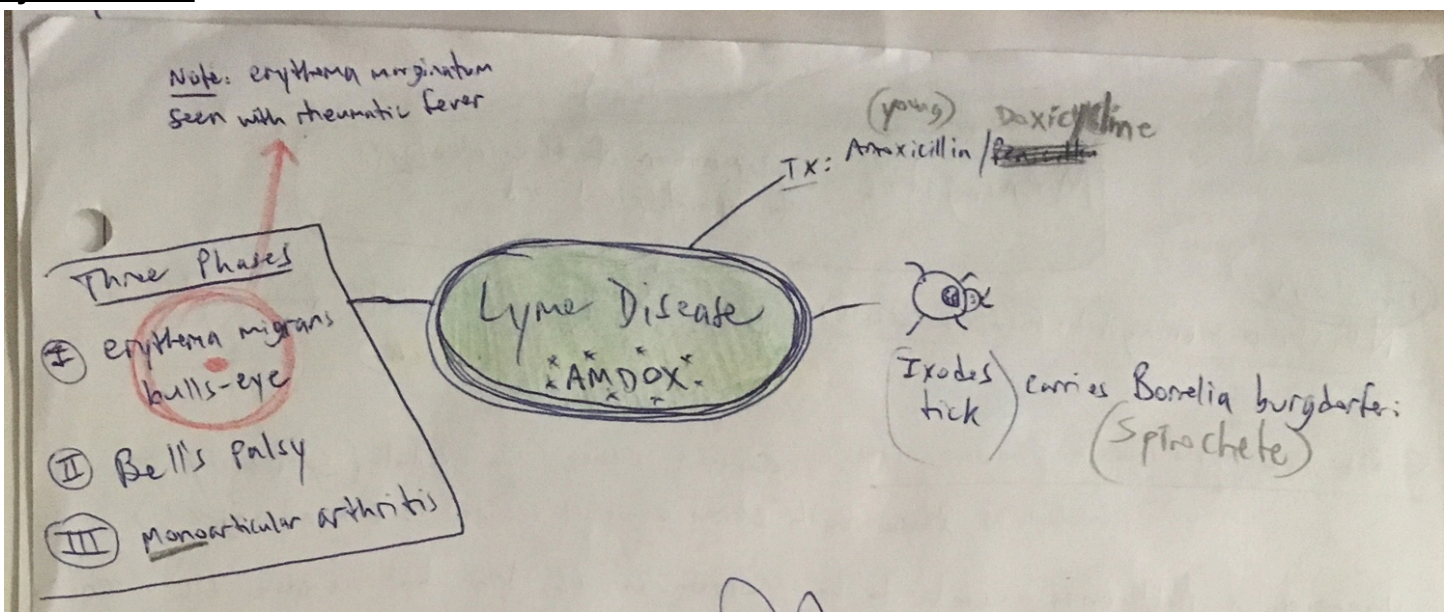
Vaginal physiology

-Estrogen maintains a healthy epithelium/glycogen which lactobacillus uses. This keeps pH acidic down here.
-Excessive cleaning/sex or foreign bodies or low estrogen will mess up the vaginal physiology.

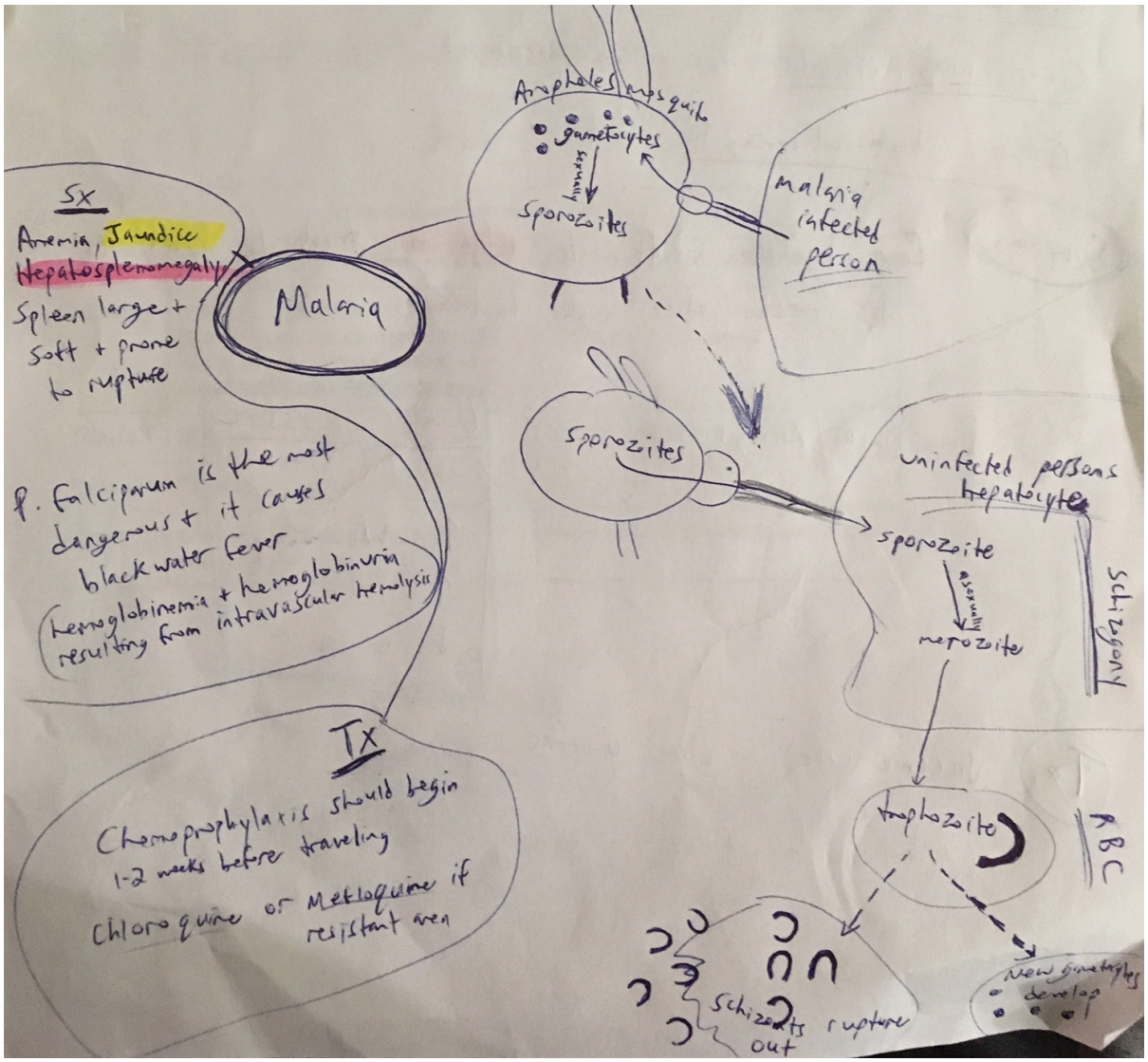
Vaginitis

	Bacterial	Fungal	Protozoan (STD)
Etiology	*Gardnerella vaginalis*	*Candida* (contraceptives, tight clothing)	*Trichomonas*
Clinical Presentation	Most common	-Cheesy discharge -recurrent	Green frothy discharge
Dx	-stinky grey discharge -positive KOH whiff test -pH>4.5 -few leukocytes, lots of bacteria and clue cells which are squamous cells with coccobacilli attached to surface	Pseudohyphae in microscopy	Highly mobile flagellated organism
Tx	Clindamycin, metronidazole	Anything ending with "zole"	Metronidazole (aka flagyl) and NO ALCOHOL

Lyme Disease



Malaria



Meningitis

Meningitis - inflammation of the meninges of brain/spinal cord

Etiology

Neisseria meningitidis (meningococcus) - transmitted by resp droplets from people in close contact (eg. barracks)

Streptococcus pneumoniae (pneumococcus) - common cause in adults (specifically in alcoholics, those with chronic otitis + sinusitis + head injuries)

Haemophilus influenza used to be common in children but no more b/c vaccine

Gram Neg meningitis in immunocompromised

Group B streptococcal meningitis - baby

Symptoms

fever, headache, stiff neck, Waterhouse Friderichsen Syndrome (DIC shock hypotension)

cat's meow (babies), tight fontanelles

DX

Brudzinski/Kernigs signs

Lumbar puncture		
Bacterial		viral
↑	WBC	~
↑	Protein	~
↓	Glucose	~

Tx

Vaccine used in close quarters

Cephalosporin (IV antibiotics)