Microbiology

The Study of Microorganisms

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quizzes: cannot negatively affect your grade,available on fridays from noon until 3

Definition of a Microorganism

- Derived from the Greek: *Mikros*, «small» and *Organismos*, «organism»
  - Microscopic organism which includes either a single cell (unicellular) or a group of identical cells (non differentiated)
  - Includes bacteria, fungi, viruses and protozoans

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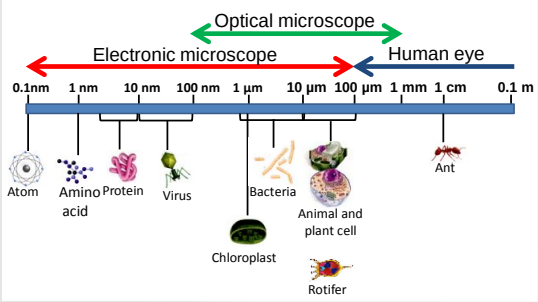
Micro_?



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_Organism?

- That is living
  - Has a metabolism
- That can live independently
  - Is not the unit of a multicellular organization
    - Ex. A liver cell is not an organism
    - Exception: Obligate parasite
      - Cannot survive independently, but is an organism!

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Properties of Life

- Living organisms:
  - Are composed of cells
  - React to their environment
  - Can feed
  - Can obtain and use energy
  - Maintain an internal equilibrium
  - Can grow and reproduce
  - Are subject to evolutionary adaptations

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you can have growth without reproduction, they are not mutually exclusive.

Some organisms cannot generate their own energy, but they can obtain it. this is sufficient to be alive.

~~They also must be able to get rid of waste products.~~

Unit of Life – The Cell

- Structural components :
  - Plasma membrane
  - +/- Cell wall
  - Nucleus or nucleoid
  - Cytoplasm
- Chemical components :
  - Proteins
  - Lipids
  - Polysaccharides
  - Nucleic acids

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Eukaryotes: nucleus

Prokaryotes: nucleoid, general region where you find the genetic material.

Eukaryotic Vs Prokaryotic cells

Eukaryote	Prokaryote
Nuclear membrane Nucleus	No nuclear membrane Nucleoid
More than one DNA molecule	Single DNA molecule
Mitotic division	Non-mitotic division Binary fission
Organelles	No organelles

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Binary fission: does not take measures to make sure each daughter cell receives the appropriate materials. Completely random.

React to its Environment

- Organisms interact with their environment and other organisms
- The responses help ensure the survival of the organism et to continue its biological activities
  - Ex. To move from an area lacking nutrients to an area with more nutrients
  - Chemotropism
  - Phototropism

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Chemotrophism: moving towards a chemical
phototropism: moving towards a light source

Feeding

- Metabolism:
  - Absorption and transformation of chemical compounds
    - Anabolism + Catabolism
      - Anabolism : Macromolecular synthesis
        - » Construction
      - Catabolism : Macromolecular degradation
        - » Destruction
  - Obtain, generate and use energy
  - Elimination of waste products

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Anabolism: synthesis of macromolecules (creating own proteins for survival)
Catabolism: break down of macromolecules (eating)

Maintaining an Internal Equilibrium

- The cell attempts to maintain a constant internal environment
  - Ex. pH, solute concentration, osmotic pressure, temperature, *etc.*
- The organism attempts to maintain a constant internal environment
  - Homeostasis

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They try to maintain an internal equilibirum
Homeostatsis: when you change your environment, your body will take measures to keep a constant internal envt (temperature, water concentration etc.)

Reproduction

- Reproduction:
  - Ability of any organism to generate another organism such as itself
    - Unicellular organisms, such as bacteria, simply divide in two
      - Binary fission
  - Multicellular organisms are often the result of the union of two different cells from different individuals
    - Ex. Sperm + ovum

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Binary fission: there is no exchange of info, they will be identical to the original.

Reproduction – The Genetic Code

- The instructions for the organization and the development of an organisms are encoded in the genes
- Genes are composed of DNA
  - The genome
    - Decoding (Transcription + Translation)
      - Generating the cellular machinery
      - Generating new cells
      - Maintain the cell

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Transcription: production of RNA
translation: reading RNA and making proteins

Evolutionary Adaptation

- Organisms acquire changes which are transmitted to future generations allowing them to better respond to their environment
  - Changes at the level of the genome
    - Maintaining these changes depends on selective pressures
      - Ex. Acquiring resistances to antibiotics

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Selective pressure determines whether or not the mutation in maintained through generations.

Fields of Microbiology

- Bacteriology
  - The study of bacteria
- Environmental microbiology
  - The study of microbial processes in the environment
- Food Microbiology
  - The study of pathogenic microorganism which cause diseases associated with food and the spoilage of food
- Industrial microbiology
  - Use and development of microorganisms in biotechnology
- Medical microbiology
  - The study, diagnosis, and treatment of microbial diseases

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Indistrial microbiology: the production of insulin, creating it from bacteria other than getting it from pigs.

Fields of Microbiology *(Cont'd)*

- Mycology
  - The study of fungi
- Protozoology
  - The study of protists
- Virology
  - The study of viruses
- Epidemiology
  - The study of the role of microorganism in the health and the diseases of populations
- Immunology
  - The study of the defense mechanisms of the body against viruses, bacteria and fungi

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Epidemiology: how micro-organisms cause diseases in a population, not in the individual.

History

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The origins of Life – The Debate?

- **300 B.C.-** Aristotle
  - Belief that living creatures are created spontaneously from non living matter
  - Life is not required to create life
    - Flooding of fields in the spring results in pools of water that generates frogs
    - Meat left outside rots and generates flies
    - Grain crops stored under damp conditions rot and generate mice
    - This belief remains unchallenged for over 2000 years

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(doesnt care about dates)

Theories were based on simple observations.

Hypothesis on the Origins of Life

- No matter can be created or destroyed
- All that exists is the result of transformations
- Life occurs spontaneously by transforming appropriate ingredients
  - Spontaneous generation

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17th Century- Jan Baptista Van Helmont

- Recipe
  - Open container with soiled underwear + wheat
  - After 21 days the smell changes and the ferment from the underwear reacts with the wheat transforming it into adult mice
    - Mice of both sexes are created
    - The adult mice can reproduce themselves

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generated experiments to prove that life could happen spontaneously. The wheat got transformed into mice. He also believed that life could generate life, aristotle did not believe so.

Conclusion

- Wheat + ferment from soiled underwear create mice
- Life can be created from inert materials
- Life created spontaneously can also propagate life

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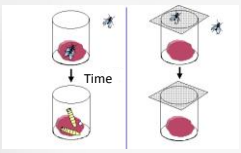
17th Century - Francesco Redi

- The first to challenge the theory of spontaneous generation
  - Redi's question: From where maggots come from?
  - Hypothesis: Maggots come from flies
  - Experiment:



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Original theory: meat + heat=maggots
he did not believe this, he believe they came from flies. When ~~he covered the meat, spontaneous generation did not~~ occur. Spontaneous generation does not occur for complex ~~organisms, but it does for smaller things. He did obse~~rve that the meat began to rot.

17th Century - A. Van Leeuwenhoek

- The use of a microscope allows him to observe life which is invisible to the naked eye
  - The **animalcules**
- First to observe microorganisms with a microscope
  - First to describe bacteria and protists

They occured by spontaneous generation.

18th Century; Controversy: Needham Vs. Spallanzani

- Question: What causes living organisms to appear in a broth?
  - Needham's hypothesis: Spontaneous generation
  - Spallanzani hypothesis : Microbes come from the air. Boiling the broth will kill them

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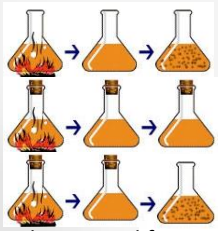
If you leave a broth outside for a while, it generations microorganisms. Where do they come from?

Controversy : Needham Vs. Spallanzani

- Experiment



Compounds essential for spontaneous generation are destroyed by heat!?

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They took a broth, boiled it to kill anything that was there before. they left it for a while and there was growth. (couldnt determine if it as spontaneous or if they were in the air and fell in).
They repeated the experiment but this time they sealed the flask. This time there was no growth. Needham says that there was an essential ingredient in the air to create life.
They repeated the experiment again, sealed. When they opened the flask, they observed growth. At this point they still don't know who's right and who's wrong.

19th Century - Louis Pasteur

- New theory : **Germ theory**
  - Observation
    - Treating milk with heat prevents it from going sour
    - Heat treatments prevents wine fermentation
      - **Pasteurization**
  - Hypothesis
    - Microorganisms in the air fall and grow if they find an appropriate medium such as food

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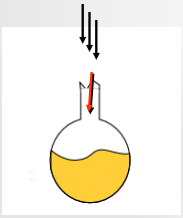
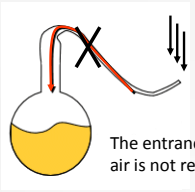
Heat kills microorganisms.

L. Pasteur - Experimental Method

The microorganisms can reach the medium



The microorganisms cannot reach the medium



The entrance of air is not restricted

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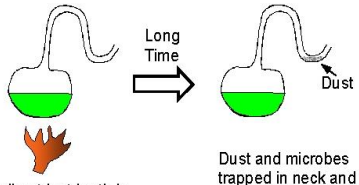
If you leave the flask open, things can simply fall in. With the swam neck flask they microorganisms would not be able to travel to the medium. The entry of air is not restricted.

L. Pasteur - Experimental Method



Boil nutrient broth in swan-necked flask. Open to air.

Dust and microbes trapped in neck and never reach broth. No microorganisms grow in the broth.

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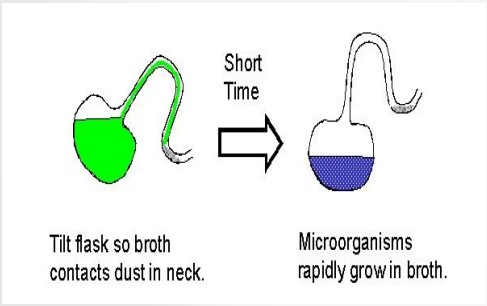
There was no growth even though there was access to air. he did realize there was an accumulation of dust, showing that things did fall in.

L. Pasteur –Experimental Method



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when he tilted it so it came in contact, there was growth.

18 -19th Century - Diseases

- It is generally accepted that there is a link between dirt and disease
- Belief: There are bad seeds in the air called **miasma**
- The miasma that cause disease have a bad smell

Miamas are the cause of bad smell and diseases.

Lister –Father of Antisepsis

- Observation:
  - Notices a high incidence of wound infections following surgeries
    - Proposes that microorganisms in the air are responsible for the infections
- Lister uses carbolic acid, which is used to deodorize sewers, to treat the instruments, wounds, and bandages
  - Observes a large reduction in the incidence of gangrene
    - This eventually leads to sterile surgeries

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lower incidence of infection when using a medium that is supposed to get rid of bad smells.

Pasteur – Germ Theory *(Cont'd)*

- If germs can spoil wine and beer, then the same thing can occur in animals and humans
  - Germs are the cause of diseases
- The French silk industry asks Pasteur to find the cause of the high mortality rate of silk worms
  - Pasteur determines that germs are responsible, but never demonstrates that germs cause diseases in humans

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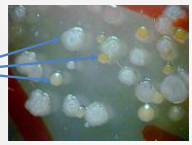
Shows a link between disease and microorganisms, but doesnt do so in humans.

19th Century - Robert Koch

- Observation:
  - Heaps of bacteria (**colonies**) of different sizes, colors, and shapes grow on potato slices exposed to ambient air

Colonies



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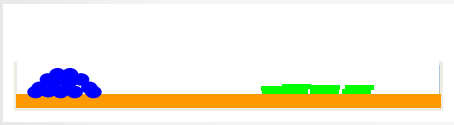
Determined that the little mountains on the potatoes after being left outside were microorganisms. They reproduce so much that we can see them. The potatoe represents a solid medium, where they fall they are stuck. this is opposed to liquid medium where the cells can move around.



- Conclusion:
  - Colonies are pure cultures arising from single cells of different bacteria since a colony spread repeatedly generates identical colonies

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Colonies: a pure culture, only one type of cell.

Koch *(Cont'd)*

- Problem :
  - Several bacteria cannot grow on potatoes!
- Solution :
  - Uses gelatin as a solidifying agent
  - Creates different solid media from liquids such as blood

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Many cells dont like to grow on potatoes, they wanted to find a different medium to grow more types. With gelatin, anything liquid became solid, this allowed him to use pretty much anything as a medium. (blood, tomatoes etc.)

Koch *(Cont'd)*

- Disadvantage of gelatin
  - It is **digested** by several microorganisms
  - It is **liquid** at temperatures above 28°C
- Solution – Agar
  - Polysaccharide derived from an algae
  - Remains solid at temperatures >37°C
  - Melts at 100°C
  - Is not digested by most bacteria

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Gelatin did not stay solid above 28C, this is a problem bc humans are 37C and we were interested in micro-organisms in humans. Also, after the gelatin is disgested it returns to liquid form so it defeats the purpose.
~~Agar stays solid at much higher temperatures, and it~~ is not digested by the majority of organisms.

19th Century- Robert Koch

- Studies anthrax which kills livestock
- Grows the bacteria obtained from diseased animals in pure culture
  - *Bacillus anthracis*
- Observations:
  - The blood from diseased animals transmits the disease
  - The microorganism can only be found in diseased animals
  - The microorganism grown in the lab transmits the disease to healthy animals

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Something in the blood that transmits the disease.

Robert Koch

- Demonstrates a direct link between specific germs and a given disease:
  - 1875 – Identifies the germ responsible for **anthrax**
  - 1882 – Discovers the germ responsible for tuberculosis **(TB)**
  - 1883 – Discovers the germ responsible for **cholera**

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all possible due to his method to make pure cultures.

Robert Koch *(cont'd)*

- Conclusion: Microorganisms are responsible for the diseases
  - **Pathogens**
- These results lead Robert Koch to formulate a set of directives for the association of a microorganism with a given disease
  - **Koch's postulates**

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Koch's Postulates

- The microorganism must be present in each case of disease, but absent from healthy individuals
- The suspected microorganism must be isolate and grown as a pure culture
- The disease must occur when the isolated microorganism is inoculated in a healthy individual
- The same microorganism must be isolated again from the diseased host

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Q.

- Can Koch's postulates be applied to all microorganisms which cause diseases?

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No. Because some hosts are carriers and now show the disease. Pathogens: a microorganism that causes a disease. All microorganisms are opportunists. If given the opportunity, they will harm you.
~~A lot of diseases are caused by viruses, it is not pos~~sible to grow a virus in pure culture.
~~Some people are immune to certain diseases, micro~~organisms. No one wants to be infected with a disease, to be injected with it for study.

Postulates Modified for Viruses

- The virus must be isolated from the diseased host
- The virus must be grown in cells from the host
- The pathogenic power of the virus can be eliminated by filtration
- The virus must cause a disease with similar symptoms when inoculates in a compatible host
- An immune response against the virus must be induced following the infection

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20th Century- Antimicrobial Agents

- Dr. Gerhard Domagk discovers that a stain, Prontosil, is effective against a broad spectrum of bacteria
  - The sulfanilamide portion of Prontosil is responsible for the antimicrobial action

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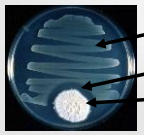
20th Century- Antimicrobial Agents *(cont'd)*

- Alexander Fleming discovers a natural product from a fungi that kills bacteria
  - Penicillin



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noticed that the bacteria would not grow close to the fungi. The 2 cultures were accidentally contaminated.

Virology – Viruses

- 19th Century
  - Charles Chamberland invents a filter whose pores are smaller than bacteria
  - Demitri Ivanowsky shows that an extract from an infected plant is still infectious after filtration with the Chamberland filter
    - Concludes that the agent is a bacterial toxin
  - Martinus Beijerinck (Father of virology) demonstrates that the Chamberland agent can only replicate in cells
    - Names the agent "contagium vivum fluidum" or living contagion

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There is an organism smaller than bacteria that can propagate and cause disease. Initially they thought it was a protein, but they realized that a protein cannot propagate itself.

Immunology

- 1796: A young milk maid informs the physician Edward Jenner that she could not get smallpox because she had already been sick from the cattle vaccine
- 1796: Edward Jenner inoculates a person with the bovine vaccinia virus
  - The person was protected from smallpox
    - Since the virus is called *Vaccinia,* he names the method - vaccination
      - The protection is referred to as immunization

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Milk maids were usually resistant to the disease. It was believed that cows had a disease that was similar, but less harmful, bc they were getting sick from the cow disease, they were immune to the human disease.

Classification of Organisms

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Levels of Classification

- Hierarchical divisions
  - Kingdom (Not used by microbiologists)
    - Microbiologists use the division « **domains** »
  - Phylum
  - Class
  - Order
  - Family
  - Genus
  - Species

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Domains and Kingdoms

- All organisms originate from a common ancestor the **Progenote**
- Organisms derived from the progenote are grouped in three domains or 6 kingdoms

Domains	Kingdoms
Archaea	*Archaeabacteria*
Eubacteria	*Eubacteria*
Eukarya	*Animalia*
	Plantae
	Protista
	Fungi

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domains are based on molecular criteria, kingdoms are based on morphological and physiological criteria.

Definition of a Species

- Basic taxonomic unit which represents a specific type of organism
  - In the case of organisms that reproduce sexually the fundamental definition is reproductive compatibility
- This definition cannot be applied to several microbial species (such as bacteria) since they do not reproduce sexually

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Q. True or False

- All microorganism reproduce asexually?
  - Ex. macroorganisms
- All microorganisms reproduce sexually?
  - Ex.

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Both are false . Microorganisms that can reproduce sexually. Yeast can reproduce sexually and asexually. Bacteria can reproduce through conjugation, exchange of DNA.

Macro-organisms that can reproduce asexually. Fungi, a lot of plants, protozoa

Definition of a Species

- Microbiology:
  - A set of microbial strains that share several characteristics and which are significantly different from other sets of strains
  - Species are identified by comparisons with known standard reference strains

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Definition of a Species

- Microbiology *(cont'd):*
  - Strain:
    - Population of microbes resulting from a unique individual or a pure culture
    - Different strains represent genetic variations within a species
      - **Biotypes**: strains with biochemical or physiological differences
      - **Morphotypes**: Strains with morphological differences
      - **Serotypes**: Strains with antigenic differences

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Within a species you can have differences, under species you have strains. Same species but there are slight differences. Serotypes: they have different antigens, they are recognized by different antibodies.

Nomenclature

- Scientific name – Binomial system
  - Name of genus + name of species
    - The genus name always starts with a capital letter
    - Can be abbreviated
    - The genus name can be used alone
    - The name of the species is never abbreviated
    - The name of the species is never used alone
      - ex: *Bacillus subtilis*
      - *B. subtilis*
      - *Bacillus sp.*
      - *Bacillus*

(use this nomenclature for final exam)

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Properties Used for Classification

- Colony morphology
- Cell shape and grouping
- Structure of the cell wall
  - Gram stain
- Specific cell structures
- Biochemical/metabolic characteristics
  (how to they obtain energy, what carbon do they use)

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Properties Used for Classification

- Serological testing
  - Uses antiserums specific against a group of microorganisms
    - The antiserum contains proteins (antibodies) which react with antigens on the organism
  - Advantages:
    - Very specific
    - Does not require pure cultures
    - Allows the identification of microorganisms that cannot be grown in the lab

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Antiserums (serum with antibodies), the antibody will only react with certain antigens, and certain bacteria, So it does not require a pure culture.

Properties Used for Classification

- Molecular properties
  - G + C content
  - Nucleic acid hybridization
  - Nucleic acid sequencing

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G+C content in the DNA to identify is.

what is the capacity of 2 strands of nucleic acid to anneal to one another.

Identifying the sequence of bases in the genome.

Molecular Properties Used for Classification

- G + C content

$$\text{Mol\%}(G+C) = \frac{G+C}{G+C+A+T} \times 100\%$$ (on one strand)

  - Estimate obtained based on the melting point of DNA
  - A higher G + C content results in a higher melting point

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Melting point is the temperature where is goes from solid to liquid. Bc G-C has 3 bonds, the higher the GC content, the higher the melting point. Above melting point=single stranded. Below-double stranded. If 2 DNA samples have the same melting point, they have similar GC content.

Molecular Properties Used for Classification

- Nucleic acid hybridization
  - Allows to determine what percentage of single stranded DNA from one species can anneal to the single stranded DNA of a different species to generate double stranded hybrids
  - The higher the percentage the more closely related are the species

above Tm (melting point)

below Tm

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2 different species with similar DNA sequences can anneal to one another and create a hybrid. The higher the percentage of annealing, the more closely related they are They higher the percentage of hybrids, the more closely related they are. ~~EXAM QUESTION: In the red, its the DNA from a man~~. In Blue, it is a human, but it can be from a man or a women. The ~~highest percentage of hybridization would be with a man~~? a women? or the same? 2 Males would be higher bc they have the extra Y chromosome.

Molecular Properties Used for Classification

- Nucleic acid sequencing
  - Sequences of genes for specific enzymes
  - Sequences for complete genomes
  - Sequences of 5s and 16s ribosomal RNA genes
    - The comparison of these sequences is commonly used to determine the relationship between different groups of microorganisms

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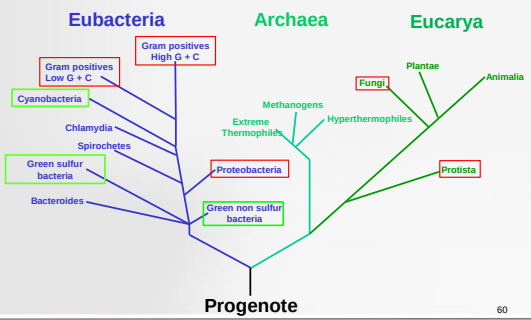
Sequencing is much more precise. Comparing the similarities between 2 genome sequences.

Three Domain Classification



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Boxed in red: find most of human pathogens
Boxed in green: they do photosynthesis.

Domain: *Eubacteria*

- Prokaryote
- The largest group of organisms on Earth
  - Classified according to their…
    - Shape
    - Oxygen requirements (some need O, some dont)
    - Diseases they cause
  - Energy production: photosynthesis or chemosynthesis from organic compounds

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The Proteobacteria

- Largest group of bacteria
  - Gram negative
- Have a cell wall made of peptidoglycan
- Have a second membrane that is external to the cell wall
- Obtain their energy by chemosynthesis from organic compounds
- Largest group of pathogens

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All bacteria except for one have a cell wall. They also have a membrane made of lipids on the inside and the outside of the cell wall. This group has the highest percentage of human pathogens.

The Bacteroids

- Characteristics similar to Proteobacteria
- Do not tolerate oxygen
- Membrane contains **sphingolipids**
- Mostly **mutualistic**
  - Predominant bacteria in the intestines
- Opportunistic pathogens

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Usually associated with a host, they both get benefits. They are opportunistic, they will become harmful if they have the change

Gram Positive Bacteria

- Have a cell wall made of peptidoglycans
- Do not have a membrane external to the cell wall
- Predominant shapes: Spheres or rods
- Obtain their energy by chemosynthesis from organic compounds
- Several species make spores
- Several pathogenic species

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Because they can make spores, they are very effective pathogens. The most lethal. They can survive for long periods of time bc of spores, also permits easy dispersal.

Photosynthetic Bacteria

- Includes Cyanobacteria, green sulfur and green non sulfur bacteria
- Obtain their energy by photosynthesis
- Varied oxygen requirements
- Use inorganic electron source
- Use inorganic carbon source

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Atypical Bacteria

- Chlamydia
  - Gram negative
  - Cell wall is not based on peptidoglycan
  - Obligate intracellular parasite
  - Cannot generate energy
- Mycoplasms
  - No cell wall
  - Undefined shape
  - Obligate intracellular parasite
  - Cannot generate energy

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The host generates the energy

Atypical Bacteria

- Spirochetes
  - Cork screw shaped
  - Too thin to be observed with a traditional microscope
  - Pathogen of syphilis and lime disease
- Mycobacteria
  - Classified amongst high G+C Gram positives
  - Cell wall with mycolic acid which is impermeable to stains
  - Pathogens of Tuberculosis and leprosy

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Mycolic acid: extremely impermeable, similar to wax.

Domain *Archaea*

- Prokaryotes, but more closely related to eukaryotes
- Cell wall does not have peptidoglycan
- Lipid membrane with hydrocarbons
- Extremophiles
  - Methanogens, Halophiles, Thermophiles, Psychrophiles (low temp)
- Oxygen is not required
- Energy production: Chemosynthesis from inorganic sources

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Domain Eukarya: Kingdom Fungi

- Unicellular/multicellular
- Cell wall
- Not organized as tissues
- Energy production: Chemosynthesis
  - Molds, yeast and mushrooms
- Absolute requirement for oxygen

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Domain *Eukarya:* Kingdom *Protista*

- Eukaryotic organisms which cannot be classified in any of the other kingdoms
  - Mostly unicellular, some are multicellular
  - Mostly non photosynthetic
  - Mostly motile
  - Absolute oxygen requirement

- Amoeba, green algae, brown algae diatoms, euglena, myxomycetes, ciliated protozoans

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Domain *Eukarya:* Kingdom *Plantae*

- Multicellular eukaryotic organisms
  - Organized into tissues
  - Perform photosynthesis
  - Cells have cell walls
  - Absolute oxygen requirement
    - Mosses, ferns, conifers, angiosperms, etc.

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Domain *Eukarya:* Kingdom *Animalia*

- Multicellular
- No cell wall
- Organized into tissues
- Absolute requirement for oxygen
- Energy production: Chemosynthesis
- Obtain their nutrients by **ingestion**
  - Sponges, worms, insects, rotifers, vertebrates

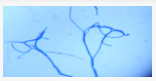
72

Microorganisms

- Prokaryotes :
  - Eubacteria
  - Archeobacteria
- Eukaryotes :
  - Algae
  - Protozoans
  - Fungi
- Viruses ?
  not in a domain or in a kingdom.

73

Microscopy



Staining

74

Stains

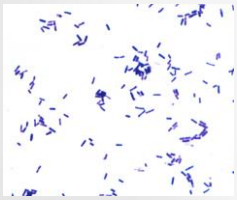
- Basic : Positively charged
  - Interacts with negative groups
    - Ex. Plasma membrane
- Acid : Negatively charged
  - Interacts with positive groups
    - Ex. Glass

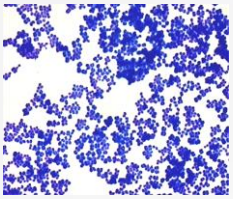
75

positively charged: will react with something that is negatively charged. The plasma membrane is - charged, so it will react with the stain.

Glass is positively charged.

Positive Staining





- Staining of the specimen

76

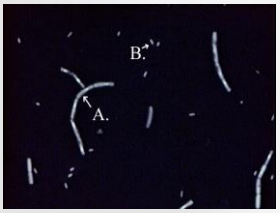
A stain-a chemical

staining-a method, can be done with + or - stain.

Positive staining, reacts with what you want to see and nothing else. Reacts with the bacteria but not the background.

Negative Staining

- Staining of the background



A. Large rod
B. Small rod

77

Negative staining, reacted with the background and not the bacteria.

Method

- Simple Staining
  - Single staining agent
  - Basic or acid stain
  - Positive or negative staining
  - Allows to determine the size, shape, and grouping of cells

78

Simple staining: no descrimination on colour. You can't determine what something is based on the colour.

Cell Shapes

- Coccus:
  - Spheres
  - Division along 1,2 or 3 axes
  - Number of axes along which division occurs gives rise to different groupings
  - Typical groupings according to bacterial genus

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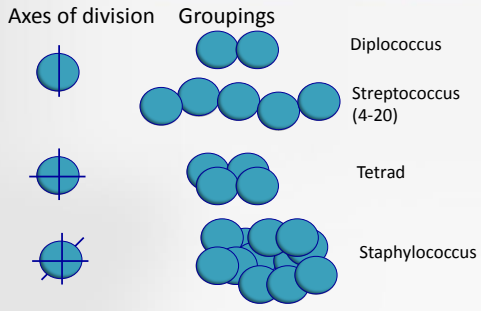
Cocci (Coccus)



80

KNOW THIS*

neisseria:typically diplococcus

streptococcus: typical of the genome streptococcus

Tetrad: typical of micro-coccus

Staphylococcus: more than 2, typical of staphylococcus

Cell Shapes *(cont'd)*

- Rods :
  - Division is only along one axis
  - Typical groupings according to bacterial genus

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Rods

Axes of division

Groupings

Diplobacilli

Streptobacilli

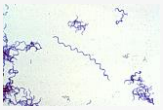
82

Other Cell Shapes

Curved rods

Typical of *Vibrio*

Spirals

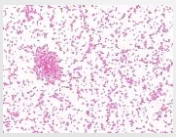


Typical of Spirochetes

83

Differential Staining - Gram Staining

- Divides bacteria into two groups
- Gram Negatives & Gram Positives



84

Gram Positive

- Colored purple
  - Low G + C
    - Rod or bacillus
      - Sporulating: Genera *Bacillus* and *Clostridium*
      - Non sporulating: *Lactobacillus and Listeria*
    - Coccus or sphere
      - Genera *Streptococcus*, *Staphylococcus* and *Micrococcus*
  - High G + C
    - Rod or bacillus
      - Genus *Mycobacterium*

85

Gram Negative

- Colored red
  - Proteobacteria, Bacteroids, Chlamydia, Spirochetes, Cyanobacteria, green sulfur bacteria, etc.
  - Mostly rods
  - Some genera are cocci:
    - Genera: *Neisseria*, *Moraxella*, & *Acinetobacter*

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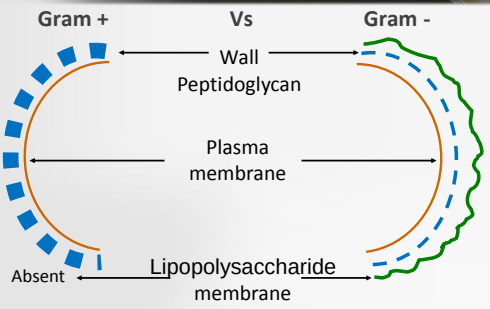
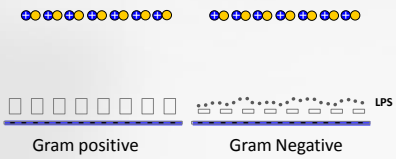
Cell Wall



87

Method – Primary Stain

1. Staining with **crystal violet**
2. Add Gram's **iodine** (Mordant)



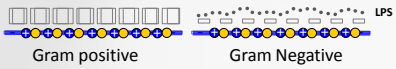
88

Method – Differential Step

3. Alcohol wash

Wall is dehydrated
– Stain + iodine complex is trapped

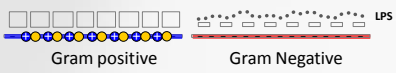
Wall is not dehydrated
– Complex is not trapped



89

Method – Counter Stain

4. Staining with **Safranin**



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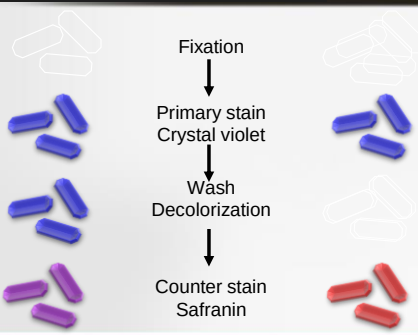
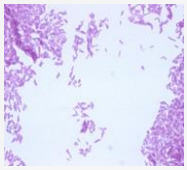
Summary



91

Acid Fast Staining

- Diagnostic staining of Mycobacteria
  - Pathogens of Tuberculosis and leprosy
  - Cell wall with mycolic acid



92

Method

- Principal:
  - High content of compounds similar to waxes, **mycolic acid**, in the cell wall, make these bacteria highly impermeable to stains

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Method *(cont'd)*

- Permeabilization of cell wall with heat
- Staining with basic fuschin
  - Cooling of the cell wall returns it to its impermeable state
    - Stain is trapped
- Acid alcohol wash
  - Differential step
    - **Mycobacteria** retain stain
    - Other bacteria lose stain

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Spore Staining

- Spores:
  - Differentiated bacterial cell
  - Resistant to heat, dehydration, ultraviolet, and different chemical treatments
  - Typical of Gram positive rods
    - Genera ***Bacillus*** and ***Clostridium***
  - Unfavorable conditions induce **sporogenesis**
    - Differentiation of the vegetative cell into an endospore

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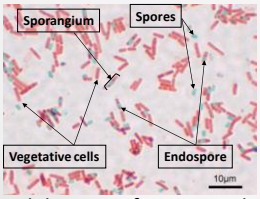
Malachite Green Staining

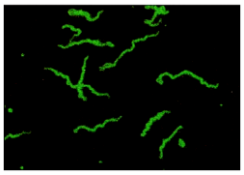


- Permeabilization of spores with heat
- Primary staining with **malachite green**
- Wash
- Counter staining with **safranin**

96

Fluorescent Staining

- Uses UV light
- Fluorescent compound absorbs UV light and emits visible light
  - Fluorescent stains
    - Vital stains
    - Metabolic stains
  - Conjugated antibodies
    - Immunofluorescence

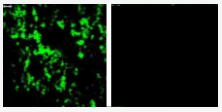


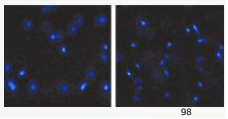
97

Fluorescent Stains– Vital Staining

- Combination of 2 stains
  - SYTOX green
    - Green stain
    - Stains DNA
    - Stains only dead cells
      - Live cells – membrane is impermeable to the stain
      - Dead cells – membranes are damaged and therefore permeable to the stain
  - DAPI
    - Blue stain
    - Stains DNA
    - Stains live and dead cells

Dead Live





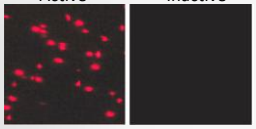
98

Fluorescent Stains – Metabolic Stainning

- CTC
  - Only stains cells which perform respiration
    - The stain is reduced by succinate dehydrogenase to a red fluorescent product

Metabolism

Active Inactive



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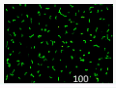
Immunofluorescence

- Makes use of an antibody that recognizes a specific characteristic at the surface of the microorganism
- The antibody is conjugated to a fluorochrome
- The fluorochrome emits visible light when it is excited by UV
- Allows the discrimination of different bacteria



100

Bacterial Anatomy

101

Dimensions

- 1-4µm
  - High ratio of surface area relative to volume
    - Favors diffusion and absorption
      - Nutrient uptake and waste elimination
  - Rapid growth
  - High cell density

102

Plasma Membrane

- Properties and functions
  - Surrounds the cell
    - Approx. 8nm thick
  - Separates exterior from interior
  - Selective barrier
  - Allows the concentration of some compounds
  - Allows the excretion of waste products
  - Site of several metabolic processes
    - ex. Respiration
  - Site of ATP generation

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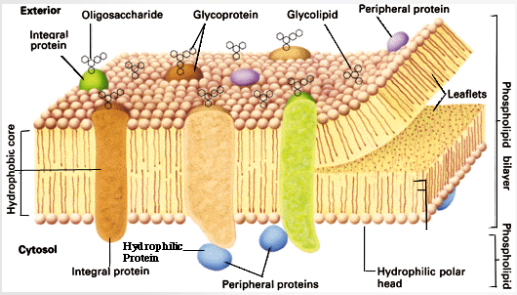
The Lipid Bilayer



104

Plasma Membrane

- Permeability Barrier
  - Maintains the intracellular environment:
    - **Hypertonic**
  - Problem:
    - How does the cell obtain nutrients?

105

Permeability of the Membrane

Substance	Rate of uptake
• Water	100
• Glycerol	0.1
• Tryptophan	0.001
• Glucose	0.001
• Chloride ions	0.000001
• Sodium ions	0.0000001

106

Passive Passage

- Passage of molecules through the membrane without an investment of energy
- Rate of passage is a function of the concentration gradient
- Can not operate against a concentration gradient
- Can not create a concentration gradient
- Two types: Passive diffusion
  - Transporter independent
    - Dependent on membrane permeability
  - Facilitated diffusion
    - Transporter dependent
      - Independent of membrane permeability

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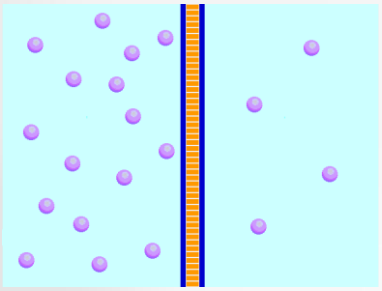
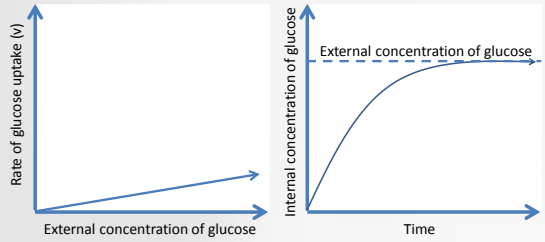
Passive Diffusion



108

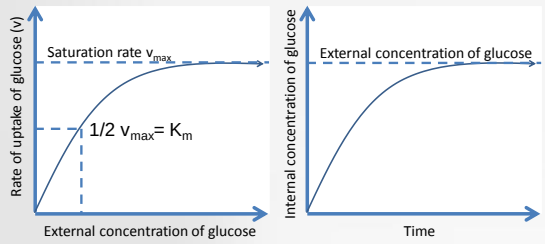
Kinetics of Passive Diffusion



109

It will level out when the concentrations on both sides are equal.

Kinetics of Facilitated Diffusion



K_m: Represents the affinity of transporter for the substrate
* The lower the Km the higher the affinity

110

using a transporter, the rate is much faster. at a certain point you will reach a maximal rate when the transporters are saturated, this is called maximal velocity. Half of the max is Km, Km is the affinity of the transporter. The lower the Km, the higher the affinity, the steeper the slope will be, you will reach Vmax faster. It will stop when the concentration are equal on both sides.

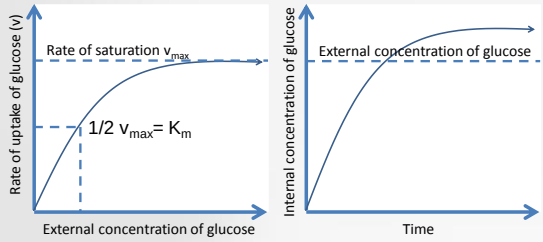
Transport - Active

- Passage of molecules which is dependent on an energy investment
  - ATP or proton gradient
- Passage dependent on a transporter
- Rate of passage is not a function of a concentration gradient
- Can operate against a concentration gradient
- Can create a concentration gradient

111

Transporter requires energy. Can work in both directions, high to low concentrations or low to high concentrations. The concentration does not have to equal on both sides.

Kinetics of Active transport



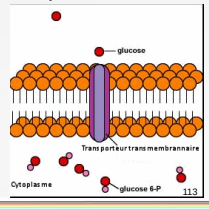
K_m: Represents the affinity of transporter for the substrate
* The lower the Km the higher the affinity

112

Transport will even continue after the concentrations are equal on both sides. So you can create a gradient.

Transport – Group Translocation

- Passage and conversion of molecules as they pass through the membrane with an investment of energy
- Passage is dependent on a transporter
- Independent of a gradient



113

Requires energy, a transporter. When the compound is transported, the compound is modified within the transporter. There is no gradient involved in this transport bc they are being transformed. Different compounds on each side of the membrane.

The Transporters

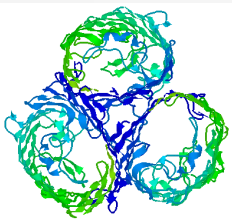
- Amphipathic proteins
- Transmembrane proteins (Integral)
  - Porins
  - Uniporter
  - Symporter
  - Antiporter
- Used for the transport…
  - Facilitated
  - Active
  - Group translocation

114

Amphipathic: hydrophilic and hydrophobic regions
The transporters contain amphipathic proteins, The hydrophobic will associate with the lipid membrane, hydrophilic- internal and external surfaces.

Porins

- Structures in the external membrane of Gram-negative bacteria
- Facilitated diffusion of low molecular weight compounds
- Channels for small hydrophilic molecules
- Protein trimers
- Semi-selective
  - Size
  - Ionic properties

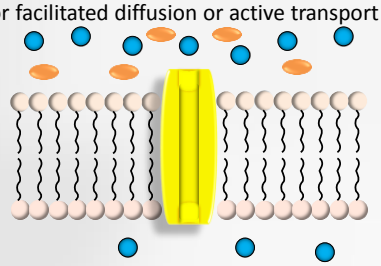


115

allow faciliated diffusion. They create a channel, they are made out of 3 identical proteins. They all small hydrophillic things through the membrane. They are size selective and on ionic properties.

UNIporters

- Selective
- Used for facilitated diffusion or active transport



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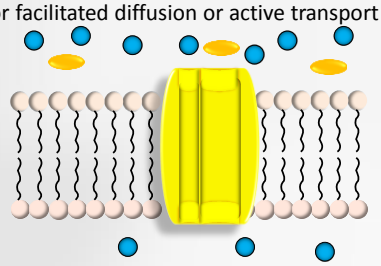
Very selective, transport a very specific compound or compounds that are extremely similar. Some require energy, others dont. They are an integral protein and unidirectional.

SYMporters

- Selective
- Used for facilitated diffusion or active transport

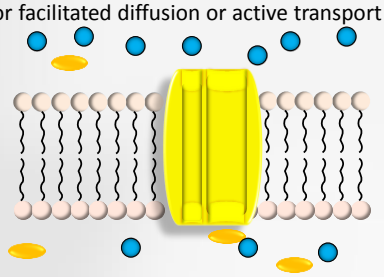


117

Amphipathic, integral, very selective. Transport 2 different things. They cannot transport one without the other. Transported at the same time. They are also unidirectional.

ANTIporters

- Selective
- Used for facilitated diffusion or active transport



118

Amphipathic, selective, unidirectional. Must carry 2 things simultaneously, can't carry one without the other. The transport of the 2 compounds are in different directions. Each compound can only go in one direction. It is an exchange of molecules.

Summary

Properties	P. D.	F. D.	Active T.	Transloc.
Transporter	-	+	+	+
Works against a gradient	No	No	Yes	Not applicable
Specificity	No	Yes	Yes	Yes
Energy expense	No	No	Yes	Yes
As a function of permeability	Yes	No	No	No
Transformation	No	No	No	Yes

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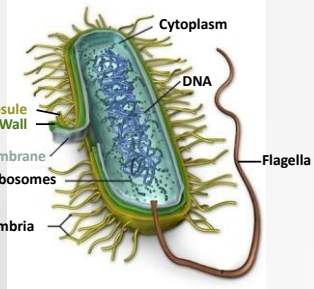
Structures External to the Membrane

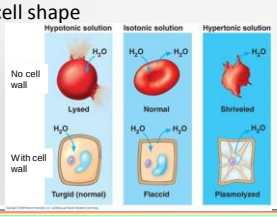


120

Bacteria. Some bacteria have a capsule after the cell wall.

Cell Wall

- Functions:
  - Resist osmotic pressure caused by the entry of water
    - Osmosis (Passive diffusion of water)
  - Defines cell shape



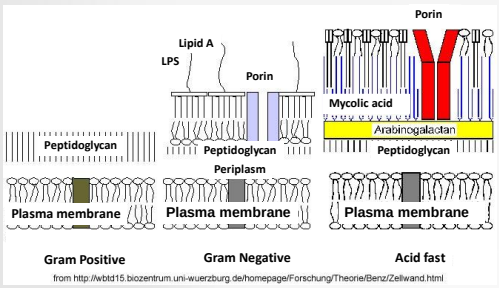
121

In bacteria

With no cell wall there is nothing protected against osmotic pressure and the cell will burst or shrivel up. They have to be in a isotonic solution where the conc. of water and solutes are equal on both sides.

The cell wall can prevent too much water from coming in or going out. Turgid state: when the solute concentration is lower outside the cell.

Summary of Cell Walls

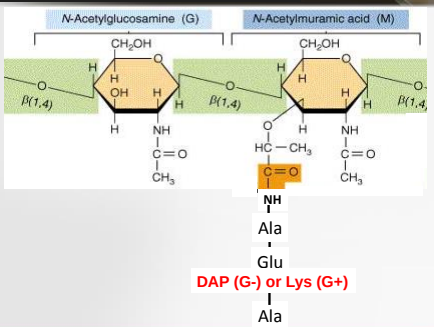


122

IN gram negative, there are 2 layers on plasmamembrane, they use porins and transporters to permeate this.

Acid Fast- very impermeable cell wall, they have transporters to permeate this.

Wall – Units of Peptidoglycan

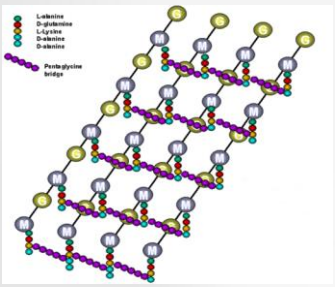


123

Cell wall

Made of units of peptidoglycan, its a disaccharide, has an amino acid peptide. In gram negative cell wall there is DAP, there isnt this in gram positive, there is Lysine instead. This represents one unit.

Wall- Multiple Layers of Peptidoglycan Polymers

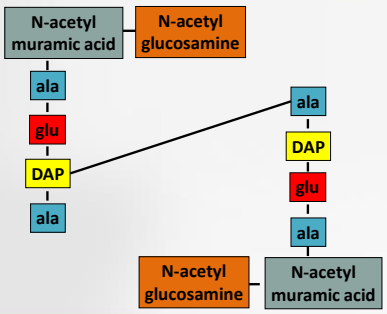


- Gram negative
  - 1-2 layers
- Gram positive
  - 10-30 layers

124

In bacteria, the layers are linked by a bridge (purple balls), linked by amino acid peptide chain.

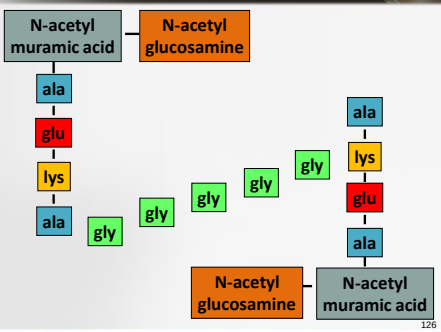
Bridges Between Peptidoglycan Polymers of Gram -



125

Gram negative- it is a direct link, originates from the DAP

Bridges Between Peptidoglycan Polymers of Gram +

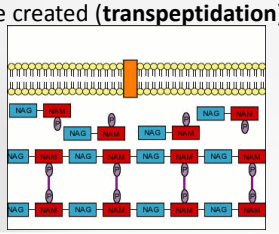


126

Gram positive, it is a glycine bridge, from the alanine to lysine.

Assembly of the Cell Wall

- Cleavage by **autolysin**
- Preformed subunits are added
- Bridges are created (**transpeptidation**)



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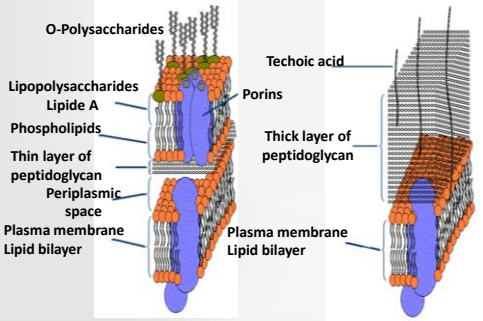
If the bacteria wants to grow, they have to break the cell wall to make it bigger. The cell will use an enzyme (autolysine) will cleave the links between the disaccharides. One they have been cleaved, they will add another unit of peptidoglycan in ~~between them to make it longer. Once the new units~~ have been added, transpeptidation-creates bridges between the ~~polymers. This will only occur if the bacteria are growing~~ bigger.

Walls of Gram – and + Bacteria



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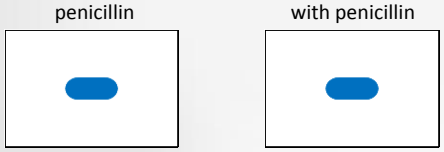
Gram - bacteria, found in the outermost lipid layer, Lipid A is a red flag in the human body, not found in gram + bacteria.

Compounds which Act on the Wall

Beta-Lactams: Inhibit transpeptidation

Growth without penicillin

Growth with penicillin



ß-lactams only act on actively growing bacteria

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Beta-Lactams: ex. penicillin.

They all work the same way, inhibit transpeptidation. They do ~~not cleave anything. If a bridge is already made between~~ polymers, they cannot do anything, they can just prevent new ~~bridges from being made. When it grows and synthesizes~~ the cell wall, the wall gets weaker and weaker bc there are no ~~bridges being made and will eventually die.~~

Compounds which Act on the Wall

- Lysozyme
  - Cleaves ß-1-4 linkages between N-glucosamine and acetyl-muramic acid
  - Mode of action similar to autolysins
  - Acts on growing or non growing bacteria

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Important defense mechanism used by humans, its cleaves the links between disaccharides. Works whether the bacteria is growing or not.

Autolysins do the same things, cleave the linkage.

LPS Layer

- Characteristics :
  - External membrane in only in Gram negative bacteria
  - Lipopolysaccharides implicated in the pathogenic potential
    - Lipid A
  - Impermeable to large proteins, polysaccharides and H+

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Permeability barrier

Gram- are usually not suceptible to betalactams and lysozymes bc they cannot reach the cell wall.

Glycocalyx

- Polysaccharide or polypeptide layer surrounding the cell
  - Also called **Extracellular polysaccharide (EPS)**
- Synthesized inside the cell and then excreted
- Two types:
  1. Mucoid layer
     - Poorly organized and attached
  2. Capsule
     - Highly organized and firmly attached
- Functions:
  - Protects against dehydration
  - Protects against phagocytosis
  - Allows adhesion
  - Resistance to the environment

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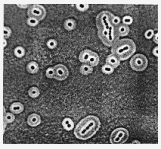
Mucoid layer- biofilm

Prevents phagocytosis bc it makes it hard to grab on to.

Glycocalyx - Capsule

- EPS firmly attached to cell wall
- Allows adhesion to surfaces
- Protection against phagocytosis
  - Virulence factor



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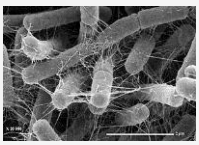
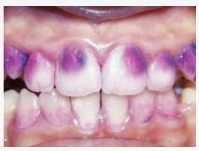
Virulence factor- helps the bacteria hurt you. Allows it to avoid defense mechanisms.

Glycocalyx – Mucoid Layer

- EPS loosely attached to cell wall
- Scaffold of **biofilms**
- Allows adhesion to surfaces
- Protection against phagocytosis
  - Virulence factor
  - Protection against environmental conditions
    - Dehydration
    - Antibiotics





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Creates communities of bacterial cells. They can be composed of the same or different species. Some bacteria may join that ~~they themself cannot produce a mucoid layer. An exa~~mple of a mucoid layer is dental plaque.
~~The mucoid layer makes them harder to attack, defen~~se against dehydration and antibiotics. If they are in a group, the ~~bacteria on the periphery will die and protect the bac~~teria on the inside.

Fimbriae

- Thin hollow fibers
- Composed of protein subunits
- Allows adhesion
- Associated to pathogenic power

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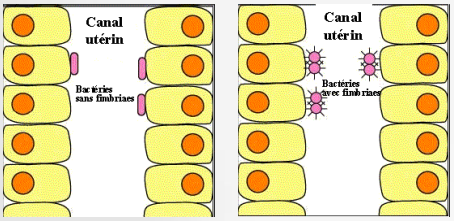
Allows them to cause harm.

Pathogenic Power of Fimbriae



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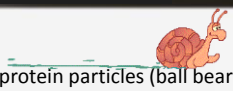
The fimbriae allow attachment to the walls of the urinary tract and cause an infection. If the bacteria didnt have this, they wouldnt be able to.

Bacterial Motility

- Sliding
  - Small rotating protein particles (ball bearings) or secretion of surfactants
- Swimming
  - Flagella
    - Long rigid appendages composed of a single polymer of a single protein; la flagellin



liquid environments

prokaryotes- rigid structures

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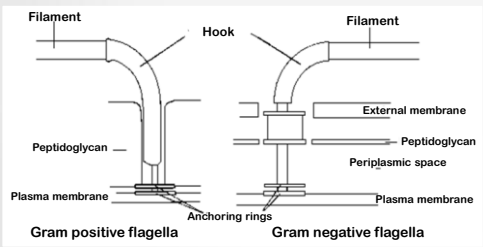
Sliding is affective on solid surface. Surfactants-chemicals that reduce surface friction.

Structure of Flagella



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Does not originate from within the cell, it is anchored to the plasma membrane. Gram positive-only one point of anchorage, gram- there is 2 (due to 2 lipid layers of the membrane)

Bacterial Motility *(cont'd)*

- Gas vesicles – Floatation apparatus
  - Small hollow rigid cylinders composed of two proteins
  - Impermeable to water
  - Permeable to atmospheric gases
  - Found in aquatic bacteria
  - ex. Cyanobacteria

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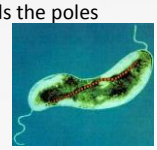
Allow up and down movement.
Beneficial to phytobacteria, to get closer or farther from the light source in a water column.

Bacterial Motility *(cont'd)*

- Magnetosomes - magnetotactic bacteria
  - Chains of magnetite particles
    - Fe_3O_4
  - Each particle represents a miniature magnet
  - Allows to orient themselves towards the poles



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Does not allow movement, They have structures called magnetosomes that contribute to the direction of the bacteria. They have iron particles that work like magnets, based on where they place them in the cell it will direct them by directing themselves with the poles.

Anatomy of Eukaryotic Cells

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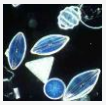
Cell Wall

- Present in several eukaryotic microorganisms

Yeast Fungi Algae Diatoms



- Present in some macroorganisms
- Composed of various polysaccharides, such as cellulose, chitin and glucans

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Cell wall are never permeability barriers unless microbacteria (??)

EUKARYOTES

Plasma Membrane

- Same architecture as that of prokaryotes
  - Lipid bilayer
- Problem - smaller surface area to volume ratio
  - Less efficient passage
  - Solution - Contains **sterols**
    - **Cholesterol**
    - Increases fluidity and permeability to non polar compounds

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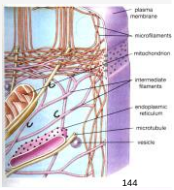
Sterols are not found in prokaryotic membranes, just in eukaryotic plasma membranes.

Cytoskeleton

- Internal protein network of microfilaments, intermediate filaments and microtubules
  - Confers cell shape
  - Allows the creation of compartment
  - Used for internal transport
  - Allows motility



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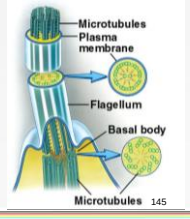
Network of filaments, allows contraction for movement.

Eucaryotic Motility

- Flagella and cilia
  - Flexible cylinders
  - Composed of tubulin
- Extension of the cytoskeleton
- Covered by the plasma membrane



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flexible flagella

Eucaryotic Organelles

- Architecture:
  - Compartments enveloped by lipid bilayers

Mitochondria/Chloroplast	ATP synthesis
Nucleus	Genome
Golgi	Transport-Export
Endoplasmic Reticulum	Protein synthesis
Lysosome	Digestion

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Nutrition

MACRONUTRIENTS: C,H,N,O,P,S

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'Chnops'

Carbon

- Required for the synthesis of all organics
  - Carbohydrates
  - Lipids
  - Proteins
  - Nucleic acids
- Sources
  - Organic
    - Monosaccharides, disaccharides, polysaccharides, proteins, lipids, nucleic acids, phenols, etc.
  - Inorganic
    - CO_2 and CO

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Water and light are not carbon sources, it has to contain carbon to be a carbon source.

Phosphorous

- Required for the synthesis of:
  - Nucleic acids
  - Phospholipids
  - ATP
  - Used as a buffer for the control of the pH
- Sources:
  - Organic and inorganic
    - The inorganic form is the most used

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Organic source of phosphorous: nucleic sources, phospholipids, ATP (contains P and C, organic source of P)

Nitrogen

- Required for the synthesis of:
  - Amino acids
  - Nucleic acids
  - Peptidoglycan
- Sources:
  - Organic: amino acids
  - Inorganic: NH_3, NO_3 & N_2

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Sulfur

- Required for the synthesis of:
  - Amino acids (Cysteine/Methionine)
  - Vitamins (thiamine and biotin)
- Sources:
  - Organic: amino acids
    - Cysteine and methionine
  - Inorganic:
    - S, SO_4

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Exam question: Do I require sulfur to synthesize gas vesicles? Yes because they are made of proteins, that are composed of amino acids- could contain cysteine methionine.

Hydrogen and Oxygen

- Required for the synthesis of all organics!
  - Carbohydrates
  - Lipids
  - Proteins
  - Nucleic acids
- Sources:
  - Organic:
    - Any organic compound
  - Inorganic:
    - H_2 (Methanogens only)

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Oxygen is required for biosynthesis, not oxygen used for breathing. It does not get incorporated into tissues.
You need an organic source H and O to synthesize anything organic within the cell.
~~Methanogens need an inorganic source of H2.~~

Nutritional Classification

- Carbon source
  - Heterotrophs :
    - Preformed organic molecules
  - Autotrophs:
    - Inorganic molecules
      - CO_2 and CO

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Nutritional Classification *(cont'd)*

- Source of energy
  - Phototrophs:
    - Light
  - Chemotrophs:
    - Oxidation of either organic or inorganic compounds
- Source of e-
  - Organotrophs:
    - Reduced organic molecules
  - Lithotrophs:
    - Reduced inorganic molecules

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It has to be a reduced compound for it to be a source of electrons (have electrons that it can give away)

Nutritional Types

- Nomenclature:
  - Source of **C**arbon-**E**nergy-**E**lectrons
    - Ex. Autotroph photolithotroph
    - Heterotroph photoorganotroph
    - Autotroph chemolithotroph
    - Heterotroph chemoorganotroph

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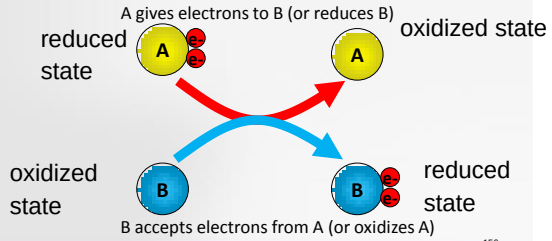
Any combination is possible. If given a medium, you should be able to give an combination possible with that medium.
Exam question: You have (first one), form a media that can sustain this organism. Do you need anything organic? Yes, you need an organic source of oxygen and hydrogen.

Obtaining Energy

- Obtaining energy depends on oxidoreduction reactions



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To be able to transfer electron, there has to be an acceptor and a giver?. A reduces B, B oxidizes A.
You can compare redox potential between different molecules.

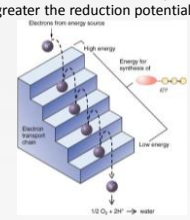
Biologically Important Redox Couples

- E_0 : Relative measure of the reduction potential (to donate electrons!)

Couple Redox	E'_0 (Volts)
$2H^+ + 2e^- \rightarrow H_2$	− 0.42
Ferredoxine (Fe^{3+}) + $e^- \rightarrow$ ferredoxine (Fe^{2+})	− 0.42
$NADP^+ + H^+ + 2e^- \rightarrow$ NADPH	− 0.32
$S + 2H^+ + 2e^- \rightarrow H_2S$	− 0.274
Acétaldéhyde + $2H^+ + 2e^- \rightarrow$ éthanol	− 0.197
Pyruvate$^- + 2H^+ + 2e^- \rightarrow$ lactate^{2-}	− 0.185
$FAD + 2H^+ + 2e^- \rightarrow FADH_2$	− 0.18[b]
Oxaloacétate$^{2-} + 2H^+ + 2e^- \rightarrow$ malate^{2-}	− 0.166
Fumarate$^{2-} + 2H^+ + 2e^- \rightarrow$ succinate $^{2-}$	0.031
Cytochrome *b* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *b* (Fe^{2+})	0.075
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinone H_2	0.10
Cytochrome *c* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *c* (Fe^{2+})	0.254
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.421
$NO_3^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$	0.44
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	0.815

Reduction

The more negative is the E_0 the greater the reduction potential



The more positive is the E_0 the greater the oxidation potential

↑ΔEo = ↑Energy = ↑ d'ATP

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The table illustrates dif. redox table, the higher up on the table you are, the more you want to give away electrons, the lower you are on the table, the more you want to take electrons. The higher you are, the greater your reducing power. The lower you are, the higher your oxidation power. If you want to go up the stairs (giving electrons to a molecule that have a high reducing power) then there has to be energy invested. Going down the stairs doesnt take any energy, going up the stairs takes energy. The greater the distance,the energy Inox delta E0. the more energy is involved.

Obtaining Energy *(cont'd)*

- Oxidative-Respiration
  - Aerobic
    - O_2 used as a final e- acceptor
  - Anaerobic
    - Inorganic final e- acceptor other than O_2 is used
- Fermentation
  - Organic final e- acceptor is used

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Respiration does not necessarily imply that you need oxygen. O2 used in respiration is not involved in biosynthesis.]
The most common inorganic e- acceptor (other than oxygen) is Nitrate.

Microbial Energy Metabolism

- Glycolytic Pathways
- Respiration
- Fermentation
- Chemolithotrophy
- Photosynthesis

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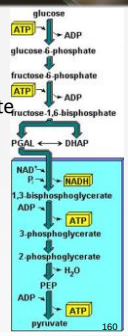
Glycolytic Pathways

- Glycolysis:
  - Most common glycolytic pathway
  - Partial oxidation of glucose to pyruvate
  - Net production of 2 ATP
  - 2 NAD are reduced to NADH

Each of these steps is carried out **twice** for each glucose molecule



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Dont need to know structures or names, just understand what it does. Typically starts with glucose or a 6 carbon compound, It in then converted into 2, 3-carbon compounds. It will cost 2 ATP. No oxido-reduction in the first part of the pathway. The oxidoreduction will occur in the blue box. BLUE BOX Starts and begins at the 3 carbons, the initial 3 carbon compound is going to give electrons, NAD will take the electrons and become NADH. This oxidoreduction pathway will generate 2 ATP per 3 carbon compound. So in total it will generate 4 ATP. Used 2 ATP, generated 4 ATP, reduced 2 NAD+. Net production of ATP- 2. There needs to be a way of regenerating NAD for the pathway to continue.

Glucose, NAD- Which one is higher on the table-Glucose.bc it is more likely to give electrons, higher reducing power. Favorable reaction, energy is being released.

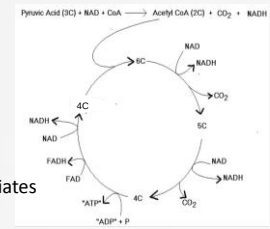
Respiration

- Features
  - Pyruvate is completely oxidized to CO_2
  - NADH is oxidized to NAD
    - Essential for continued operation of glycolytic pathways
  - Uses an inorganic electron acceptor
    - Aerobic respiration: O_2 is the final e- acceptor
    - Anaerobic respiration: An inorganic substance other than O_2 is the final e- acceptor
      - Ex. nitrate, nitrite, sulfate
  - Additional ATP are made

NADH is recycled to NAD.

Stages of Respiration - Krebs Cycle

- Summary/1 pyruvate molecule:
  - Energy equivalents
    - 1 ATP
  - Reducing equivalents
    - 4 NADH
    - 1 FADH
  - One carbon compounds
    - 3 CO_2
  - 4C Biosynthetic intermediates
    - α-ketogluterate
    - Succinate
    - Oxaloacetate



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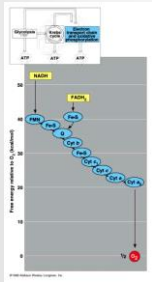
The main function of the pathway is to generate 4 and 5 carbon compounds. In the process, you will use up more NAD, and FAD, making a larger deficit in reducing power, but you will generate some ATP.

Release some CO2.

The 4C biosynthetic intermediates are the point of this pathway, used to make amino acids and other things.

Stages of Respiration – Electron Transport



- Aerobic respiration :
  - Final e- acceptor: O_2
  - 3 ATP/NADH
  - 2 ATP/FADH
- Anaerobic respiration :
  - Final e- acceptor other than O_2:
    - NO_3, NO_2, SO_4, etc.

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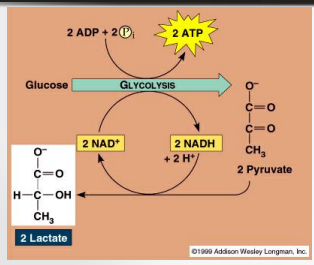
~~This is used to recycle reducing equivalents.~~
The NADH and FADH are passed down the electron transport ~~chain to eventually reduce the O2 to water. HIgher up~~ on the graph-more negative, lower on the graph-more positive. ~~3 ATP are made per NADH bc it enters the electron t~~ransport chain earlier. The Delta E0 between NAD and O2 is greater than the E0 between FAD and O2. The inorganic acceptors will be higher on the graph than O2, so it will generate less energy. Glucose, NAD, Fad, O2, Sulfate, pyruvate put in order from the most positive (e- acceptor) to negative E0. (on quiz and exam) oxygen, sulfate, FADH, NAD, pyruvate, glucose.
Exam question: 2 organisms will use the same e- source, lactose. The 2 organisms derive a different quantity of energy from what they are using. How is that possible? One is using aerobic respiration and one is using anaerobic respiration. The terminal electron acceptor have different E0. One is higher than the other.

Fermentation

- Features
  - Pyruvate is reduced to organic acids or alcohols
  - Final e- acceptor is organic
  - NADH is oxidized to NAD:
    - Essential for continued operation of glycolytic pathways
  - O_2 is not required
  - No additional ATP made
  - Gasses (CO_2 and/or H_2) may be released

Fermentation is used to recycle NADH. No ATP is generated.

Fermentation - Lactic



- Organic electron acceptor - **Pyruvate**
- Regeneration of NAD +

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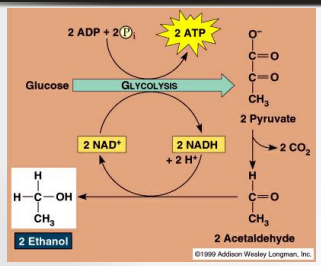
Pyruvate acts as a terminal electron acceptor. No energy generated in the process. Generates lactate.

Fermentation - Ethanolic



- Organic electron acceptor- **Acetaldehyde**
- Regeneration of NAD $^{+}$

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Regenerates NAD.

Chemolithotrophy

- Features
  - Use a reduced inorganic e- donor
    - Ex. Nitrite, sulfur, hydrogen
  - e- go through an electron transport pathway
    - Coupled to the synthesis of ATP and NADH
  - e- are used to reduce a final e- acceptor
    - $O_2 \rightarrow H_2O$ or $CO_2 \rightarrow$ Methane
  - ATP and NADH are used to convert CO_2 to sugars
    - Calvin cycle - autotrophs

Inorganic e- source.

The donor will give to the electron transport chain, you will generate ATP. These organisms dont want to recycle NAD, they want to produce NADH. Important in a lot of biosynthetic pathways.

Lithotrophs are frequently autotrophs, t. They use single C compound as a carbon source and sometimes need to build compounds with more carbon using the calvin cycle. they need a lot of ATP and reducing power to do this.

There is an electron transport chain, a source of e-, the donor will give to something in the transport chain. After this, the lithotrophs do 2 things, 1 the e- will be transferred down to a final acceptor, generate ATP. They also want to produce NADH, to do this they have to do reverse electron transport. This requires energy. (slide on website)

?NAD, iron, oxygen- negative to positive.

Photosynthesis

- Features:
  - An e- donor reduces a photosynthetic pigment
  - Light energy is used to make the redox potential of reduced photosynthetic pigment more negative
  - e- go through an electron transport pathway
    - Coupled to ATP synthesis
  - e- are used to reduce a final e- acceptor
  - ATP is used to convert CO_2 to sugars
    - Calvin cycle - autotrophs

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Photosynthesis – 2 Types

- Anoxygenic - Cyclic
  - Electron donor: Organic or inorganic (H_2S, S or H_2)
  - Final e- acceptor : Oxidized photosynthetic pigment
- Oxygenic- Non-cyclic
  - Electron donor: H_2O
    - $H_2O + Pig_{ox} \rightarrow ½\ O_2 + Pig_{red}$
  - Final e- acceptor: NAD or NADP

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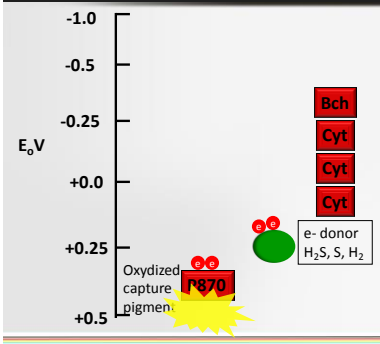
oxygenic-creates oxygen.

Anoxygenic Photosynthesis



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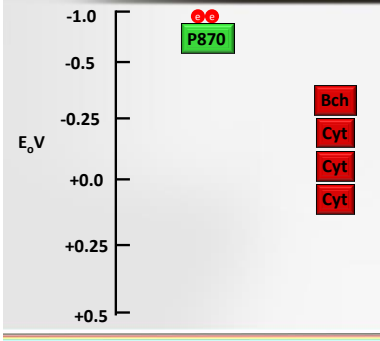
higher on table, higher reducing power. The delta E betwen the P870 and the electron accepter in small, but not big enough to generate ATP. The e-donor will be reduced by the pigment, this will change the redox level of the pigment so that it becomes much more negative.

Anoxygenic Photosynthesis



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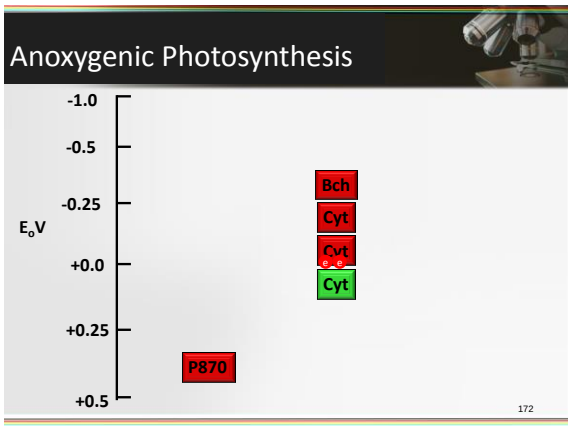
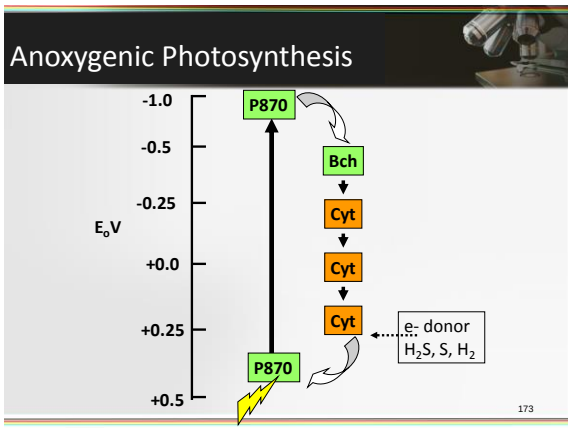
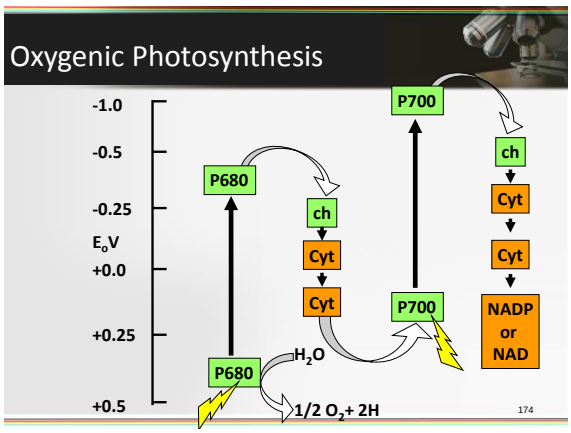
The pigment becomes much more negative.



The electrons can be used to reduce by cytochromes. Once the last cytochrome is reduced, it is used to reduce the photosynthetic pigment, its a cycle. There is a reduce delta E between the inactive and active form of P870, so it can generate ATP.



The amount of delta E generated is based solely off the P870, not the donor.



What can be used to reduce the photosynthetic pigment, this will create oxygen. After it has been reduced, the light will affect its redox potential and make it much more negative. When the last cytochrome is reduced in the first chain, it will reduce the second photosynthetic pigment that is also activated by light to make its redox potential much more negative. It will go through another transport chain. Final e accepter is NAD

Comparison of Photosyntheses

Characteristics	Non cyclic	Cyclic	
	Cyanobacteria	Green/purple sulfur bacteria	Green/purple nonsulfur bacteria
e- donor	H_2O	Sulfur compounds	Organic or H_2
Production of O_2	Yes	No	No
e- acceptor	NAD or NADP	Photosynthetic pigment	Photosynthetic pigment
Environnement	Aerobic	Anaerobic	Anaerobic

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Non-cyclic, usually lives in aerobic env;t, they dont need it they just generate it.
Organotroph-can use an organic electron source, so it would be nonsulfur bacteria.

Culture Media

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Nutritional Complexity

- Nutritional complexity is a function of the biosynthetic capacity
- The greater the biosynthetic capacity, the lower the nutritional requirements

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Nutrional complexity-nutrional requirements, things you need for the organism to grow.
The number of different things you have to provide the organism with is a function of how many things the organism can do on its own. For example, humans have to be provided with vitamin C, humans cannot create it. Therefore they have lower biosynthetic capacity and higher nutrional requirements than things that can produce vitamin C.

Complex Media

- Composed of rich and complex ingredients
  - Ex. Soya protein extracts
  - Milk protein extracts
  - Blood products
  - Tomato juice, etc.
- Exact chemical composition is unknown
- Can be **selective** and/or **differential**

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Not a defined medium bc you dont know the exact ingredients of the medium. Many organisms can grow in these media. Can be made to be selective or differential.

Defined Media

- Known chemical composition
  - Can contain up to 80 different ingredients
  - Can be very simple
  - Allows the growth of a small number of microorganisms
  - Composition is highly variable according to the microorganism
- Can be **selective** and/or **differential**

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Selective Media

- Contain compounds which **inhibit** or **kills** the undesired organisms
  - Ex. Medium containing penicillin only allows the growth for penicillin resistant microorganisms

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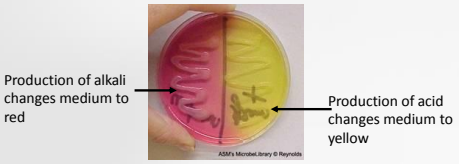
Differential Media

- Allows to discriminate different species
- Often contain pH indicators
  - Allows to discriminate different metabolisms



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Allows visual discrimination. Based on their metabolism, they generate different waste products and they can identify them with pH indicators.

Environmental Parameters

- Oxygen availability
- pH
- Temperature
- Solute concentration

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They trie to maintain solute concentration that is higher than its envt.

Oxygen Requirements

- Aerobic:
  - Absolute requirement of oxygen for survival
  - Oxygen is used as a final electron acceptor
  - Oxygen is used by bacteria which have an oxidative metabolism or perform aerobic respiration
- Microaerophilic:
  - Absolute requirement for low oxygen concentrations
  - High concentrations are deadly

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Oxygen Requirements *(cont'd)*

- Anaerobic/Aerotolerant:
  - Oxygen is tolerated but not required
- Facultative anaerobes:
  - Facultative oxygen requirement
  - Can choose to use oxygen or not
  - Have an oxygen dependent and an oxygen independent metabolism
- Strict or obligate anaerobes:
  - Oxygen is not used or tolerated; cannot survive in the presence of oxygen

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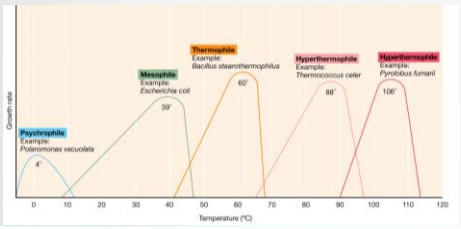
Facultative: it has more than one way to achieve each state. Organisms that can make a choice. They are offered more than one option. If you put them in a env't that does not have oxygen, you are not giving them a choice.
Oxygen dependent aerobic respiration
Oxygen independant- fermentation, photosynthesis

Temperature

- Microorganisms are poikilothermic
  - Do not control their temperature
- Different species have different optimums



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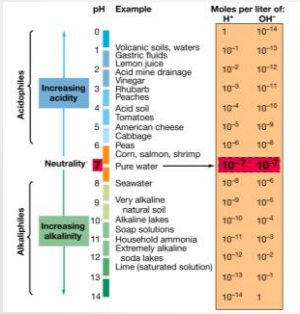
They don't have a way of controlling their temperature.
Thermophile: something that lives in something above 50
PSychrophile- they like cold temperatures, below 10.
Mesophiles- around 40C.
Phile- what they prefer, just because they prefer a certain temperature doesnt mean they can't survive in other temperatures.

pH



186

pH7-neutral, neutraphile
below 7-acid, acidophile
above 7, alkaline, alkalophile
Most human pathogens are neutraphiles, some are acidophiles are affect the stomach.

Controlling pH

- Selective permeability of the membrane
- Antiporters K^+/H^+ or Na^+/H^+
- Protein buffers
- Chaperones
- Excretion of acid or alkaline waste products

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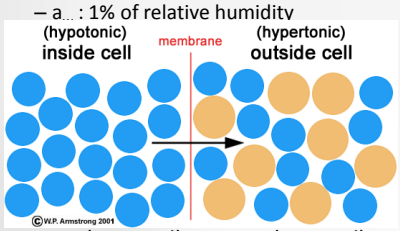
Higher concentration of H+, lower the pH. Protein buffers resist pH changes.
Chaperones: proteins that protect other proteins, keeps them folded properly.
~~Excretion allows them to control the pH of their envt if~~ they live in a tiny envt. For example if they are living in a biofilm.

Water Activity (a_w)

- Measure of water availability
  - a_w : 1% of relative humidity



a_w (External) > a_w (Internal)

Why?

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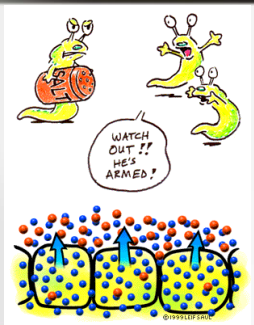
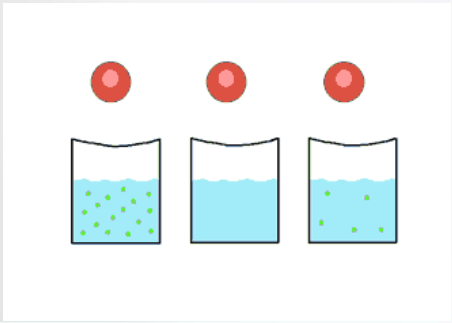
Aw is a measurement of how much water is available. How much water is available is a concentration of solutes. The ~~greater the concentration of solutes, the less water a~~vailable, the lower the Aw. All bacteria try to maintain an Aw inside that ~~is lower than outside, maintain a higher concentration~~ solute inside the cell.
~~Exam question: Highest to lowest Aw= distilled water~~,human blood, sea water, honey, tea spoon of sugar.
Hypo and hypertonic are relative to something, you have to be comparing 2 things



189

Create a high soluted envt, the water leaves the cells and the bacteria dont grow or die.

Osmosis – Diffusion of water



190

Hypertonic- higher solute on the outside, water will leave the cell. When there is more water on the inside of the cell, has a higher solute concentration on the inside then the water will enter the cell. Bacteria like this scenario.
~~Microplasms dont have cell walls, so they want equal~~ solute concentrations on each side.

Control of the a_w

- Control the internal concentration of **compatibles solutes** :
  - Solute that allows metabolism to occurs even when its concentration is high
    - Amino acids, carbohydrates

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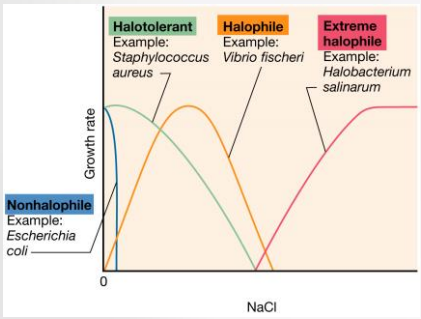

192

Halophile-prefer high solute concentrations in their envt.
Halotolerant-they can tolerate it.
Gram positive are more tolerant to high solute concentrations than gram negative, why? Gram positive can retain their water ~~better, why? They have a cell wall, their peptidoglycan~~ is thicker, Its easier for them to trap something inside, the ~~outermost layer is sugars, the first thing to lose water~~ is the cell wall. Gram postive will keep water better, if the gram negative ~~the cell wall will not become dehydrated, the cell will~~ just lose water.

The Suffix “phil” Vs “tolerant”

- -phil
  - The suffix "phil" describes optimal conditions where the microbe can grow at maximum speed
    - Ex. Thermophile: growth of the microbe occurs at maximum speed at a high temperature rather than a low one
- -tolerant
  - The suffix "tolerant" describes a non-optimal condition where the microbe can survive
    - There is no growth or growth occurs at a highly reduced speed
      - » Ex. Thermotolerant: the microbe survives elevated temperatures, but prefers lower temperatures

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Bacterial Growth

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Bacterial Growth

- Increase in the number of cells
- The bacterium reproduces by binary fission
  - (1→2, 2→4….2n)
- Growth measurements monitor changes in the total number of cells or the mass of cells

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Binary Fission

- Asexual reproduction
  - DNA replication → cellular elongation→ septum formation→ septum completed and cell wall formation→ cellular separation and creation of daughter cells
    - The quantity of all molecules doubles : proteins, DNA, RNA, lipids for membranes, cell wall materials, etc.
    - Everything is distributes almost equally

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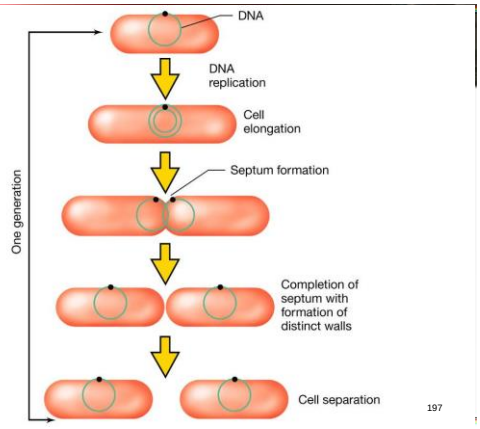

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Growth Parameters

- Generation: 1 cell → 2 cells
  - The population doubles
- Generation time (g):
  - Time required for one cell division
  - g = time/n (n: number of generations)
- Growth rate (μ):
  - Change in cell number or mass/time

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Growth in Batch Cultures

- CLOSED system
  - No addition of new nutrients
  - No elimination of waste products
  - Cells are not withdrawn
    - Ex. Production of yogurt, beer fermentation, blood infection
- Cell density increases until something becomes limiting

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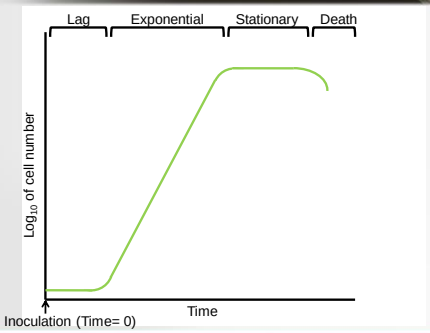
Something may become toxic

Growth Profile of Batch Cultures



200

Lag phase- no change in pop during time, an adaptation phase, they will asses the envt they are in and prepare ~~themselves for growth in that medium. The length of~~ this phase is a function of where they are coming from. If they are coming ~~from a similar media, the adaptation time will be shor~~t. Inversely, if they are switching to a very different media the ~~adaptation phase will be longer. Growth and deaths~~ are both exponential.

Lag or Adaptation Phase

- No increase in the number or the mass of cells
- Active synthesis of components required for growth in the given medium
  - Metabolic adaptation

201

Exponential or Log Phase

- Development and cellular division occurs at maximum speed
- The number and mass of cells doubles at regular intervals
- The population is in physiological and biochemical equilibrium
- Division occurs at an **exponential** rate

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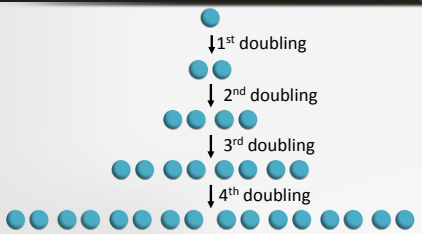
Exponential Division



Final number of cells (N) = Initial number of cells (N_0) X (2^n)

n = number of doublings

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Know how to use this formula, it will be given on the exam.

Exponential Division

Time (h)	Number of generations (n)	Number of cells (N)	Time (h)	Number of generations (n)	Number of cells (N)
0	0	1 (2^0)	4.5	9	512 (2^9)
0.5	1	2 (2^1)	5	10	1024 (2^{10})
1	2	4 (2^2)	5.5	11	2048 (2^{11})
1.5	3	8 (2^3)	6	12	4096 (2^{12})
2	4	16 (2^4)	6.5	13	8192 (2^{13})
2.5	5	32 (2^5)	7	14	16384 (2^{14})
3	6	64 (2^6)	7.5	15	32768 (2^{15})
3.5	7	128 (2^7)	8	16	65536 (2^{16})
4	8	256 (2^8)	8.5	17	131072 (2^{17})

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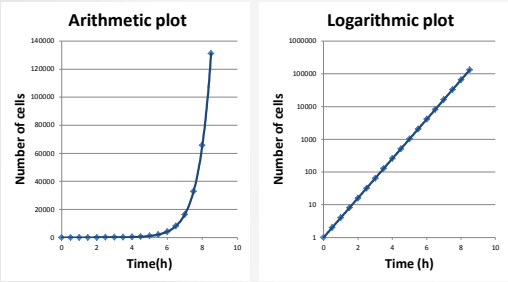
Bacterial Growth Curve



205

make sure you can read a log plot. The scale in between each point on the y axis changes. The scale is log, not the numbers.

Growth Calculations

- If you start with one cell, how many will you have after 4 generations?
  - N_o= Initial number of cells
  - N = Number of cells after n generations
  - n = number of generation
    - Formula : **$N = N_o(2^n)$**
    - N = 1 (2^4) = 16 cell
- How many would you have if you started with 100 cells?
- How many would you after 5 generations if you started with 100 cells?

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Growth Calculations

- *E. coli* has a generation time of 20 minutes. If you start with 1 cell, how many will you have after 2 hours?
  - g=generation time and t=time
  - Formula: **n=t/g**
  - n=(2 hours x 60 minutes/hour)/20 minutes= 6
    - **$N = N_o(2^n)$**
    - N=1(2^6)=64 cells
- After 5 hours?

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Calculating Generation Time

- You start with 2 cells and end up with 2,000 cells after 2 h.
  - How many generations were there?
  - What is the generation time?
    - Formula : **$n=3.3(\log N-\log N_o)$**
    - Thus n=3.3(log (2000)– log (2)) = 9.9 generations
    - Formula : **g=t/n**
    - g=120 minutes/9.9 generations = 12.12 minutes/g

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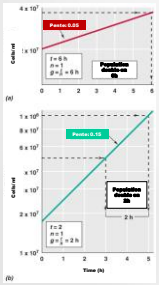
When using these formulas, make sure both values are in the exponenitial phase

Growth Rate - μ



- Growth as a function of time:
  - The shorter is the generation time, the faster is the growth
  - The faster is the growth, the steeper is the slope
  - g=6 hours; slope 0.05
  - g=2 hours; slope 0.15

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Generation time: time it takes to double
Growth rate: it's the speed at which it grows, The shorter the generation time, the steeper the slope will be.

Calculating μ

- After 4 h of growth, an *E.coli* culture goes from 100 cells to 6.6 X 10^6 cells
  - What is the growth rate under these conditions?
    - Formula: **$\mu = ((\log_{10} N - \log_{10} N_0) 2.303) / (t - t_0)$**
    - Thus μ = (log 6.6 X 10^6 – log 100) 2.303/ 4 = 2.8 cells/h
  - What is the generation time?
    - Formula: **$\mu = \ln 2/g$** or **$g = \ln 2/\mu$**
    - Thus g = 0.69/2.8 = 0.25h or 15 minutes

210

All these calculations will be on the exam

Growth rate Constant (K)

- K= Number of generations per unit time during exponential growth
  - Unit of time: h^{-1}
- **K= n/t**
  - n = number of generations
  - t = number of hours

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K- number of generations in one hour

Stationary Phase

- Arrest in cell growth
- The population is no longer in equilibrium
- Arrest due to a lack of nutrients, oxygen, or an excessive accumulation of waste products, etc.
- Represents the maximum yield under the given conditions
  - Yg : Mass of microorganisms formed/mass (g) of consumed substrate
  - Ym: Mass of microorganisms formed/mole of consumed substrate

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Yg is used mostly by industry , they want to know how to get the maximum yield for the minimum cost. How much of on microorganism can you produce for a certain amount of substrate.

Ym is a measurement of efficiency. How efficient the substrate is. You can figure out from substrate it extracts the most energy.

Death Phase

- Exponential loss of viability due to a prolonged lack of nutrients or a prolonged exposure to waste products
- Not necessarily a loss in mass

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If you cause lysis of the cell, your mass will go down.

Measurements of Growth

- Counting microorganisms
  - Relative abundance
    - Turbidity measurements
  - Direct counts
    - Absolute counts
  - Viable counts
    - Absolute number of growing bacteria

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Relative-comparing 2 things.
When measuring relative abundance, you do not get an absolute number. Turbidity measures the amount of light that goes through a sample, measures the cloudiness essentially.
~~Viable counts- number of live organisms, this is quant~~ified by the number of bacteria that can grow or reproduce.
~~We are most interested in viable counts because its~~ mostly alive pathogens that can harm us, not dead pathogens.

Turbidity Measurements

- Measures the quantity of light that can go through a sample
- The less light that passes the more dense is the population
- Measurements of optical density or percent transmission

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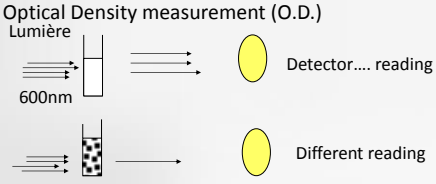
Optical density- how much light does not go through.
Percent transmission-how much light does go through.

Turbidity Measurements

- Spectrophotometer (A600):
  - Optical Density measurement (O.D.)



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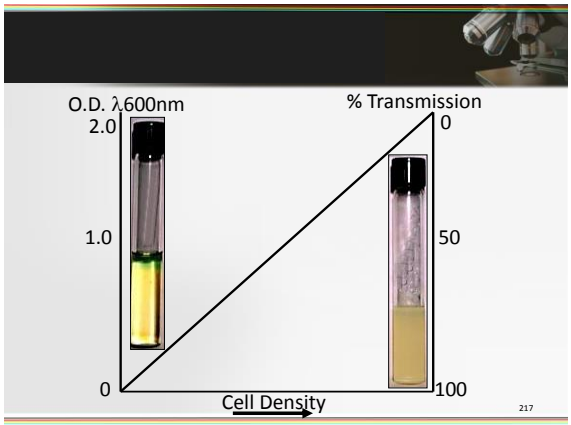

higher cell count, lower the transmission.

•Advantages:
- Quick

• Limits:
- Relative measurement
- Does not discriminate between dead and living
- Does not discriminate between bacteria and detritus
- Does not discriminate between different microbes

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Exam question: 2 bacterial cultures, same number of cells, same media etc.. In the one culture it is a very small rod, the other is a big rod. Compare the optical density of the 2 cultures. The optical density would be much higher in the one with the bigger rods. So when using this method, everything must be the same, including the bacterial species.

Direct Counts

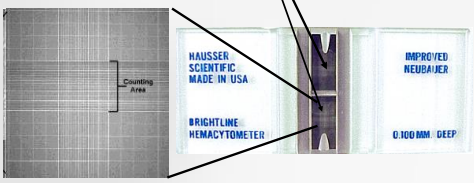
- The sample to be counted is applied to a **hemacytometer** slide which holds a **fixed volume** in a counting chamber
  - The number of cells is counted
  - The number of cells for a given volume is determined

219

How to determine the volume: 2 dimensions, L, W,H. 1cm2 is equal to 1mL. 1mm is equal to 0.1cm.

Hemacytometer Counts

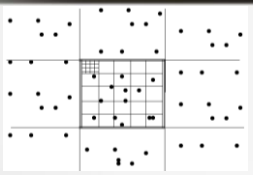
- This slide has **2** independent counting chambers



220

Counting chamber is divided by counting squares.

Determining the Direct Count

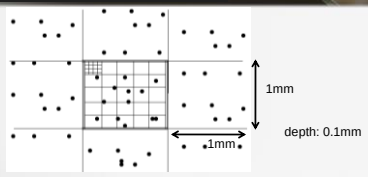


- Count the number of cells in 3 independent squares
  - 8, 8 and 5
- Calculate the average
  - (8 + 8 + 5)/3 =7
  - Therefore 7 cells/square

221

You have to change the square to a volume, mL In your final answer.

Determining the Direct Count *(cont'd)*



- Calculate the volume of a square:
  = 0.1cm X 0.1cm X 0.01cm= 1 X 10^{-4}cm^3 or ml
- Divide the average number of cells/square by the volume of a square
  - Therefore 7/ 1 X 10^{-4} ml = 7 X 10^4 cells/ml

222

***ON THE EXAM

- Advantages:
  - Quick
  - Growth is not required
  - No information about organism required
- Limits:
  - Does not discriminate between live and dead
  - May be difficult to distinguish bacteria from detritus

223

You could use a biostain to allow you to descriminate alive from dead.

Problem

- A sample is applied to a hemacytometer slide whose counting chamber has the following dimensions: 0.1mm X 0.1mm X 0.02mm. Counts of 6, 4 and 2 cells were recorded in three independent squares. What was the number of cells per milliliter in the original sample if the counting chamber has 100 squares?

224

V of counting chamber:0.0002mm x 0.1^3= 2x10^-7
V of one square:2x10-9
Average # of bacteria per square:4
Average # of bacteria per counting chamber: (4x100=400)
Number of bacteria per mL :

Viable Counts

- Serial dilutions of the sample
- Spreading of the dilutions on an appropriate medium
- Each single colony originates from a **colony forming unit (CFU)**
- The number of colonies is equivalent to an approximation of the number of **live** bacteria in the sample

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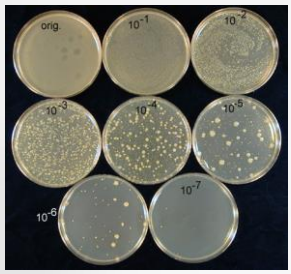
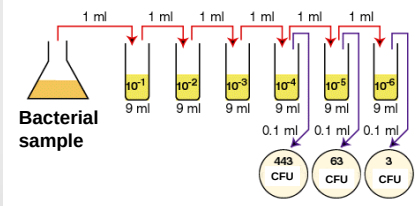
Dilutions for a Viable Count



226

the higher the dilution, the less colonies. Each colonie arose from a single bacteria, so we can estimate the amount of bacteria in the solution.



- Choose a count between 30 – 300 CFU
  - 63 UFC/0.1ml of 10^{-5}
  - 630UFC/1.0ml of 10^{-5}
  - 630UFC/ml X 10^5 = 6.3 x 10^7/ml in the original sample

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UFC=colony forming units.

- Advantages:
  - Determines the number of live organisms
  - Can discriminate between different microorganisms
- Limits:
  - No universal medium
  - Requires the growth of the microorganism
  - CFU $\stackrel{?}{=}$ one bacteria
    - Ex. One CFU of *Streptococcus* ≠ one CFU of *E.coli*

228

You can make media that can discriminate in between different species of bacteria.
There isn't one media that will satisfy all bacteria, and it also requires incubation time.
Bacteria cells depending on the genera has different groupings. So the CFU of one species isnt necessarily equal to the CFU of another species.
Q: The number of cells in both cultures is the same, everything is done the same way. The difference is the first one is streptococcus, the second culture is of the genus micrococcus. How will the CFUs compare to eachother? Micrococcus-always contain 4 individuals, so you will have less in streptococcus. (i think? Check this)
Q: Find an antibody that only recognizes E-coli. (if they want to know the amount of E.Coli, dead or alive).
Problems: questions are on the exam. Due wednesday.

Someone wants to calculate the number of bacteria in the lake, What is the most accurate way? Turbidity measurements is irrelevant because you aren't comparing it to anything. There is no universal medium so that won't work. This would be an underestimate bc your medium is restricting the growth of some bacteria. Direct count would be the most accurate.

Q: Best method to calculate the amount of E.Coli in lake.
Not turbidity-not comparing
not direct count-can't determine if its E.coli or something else
Viable count-knowing is E-coli, you can make a medium that discriminates.
Also he wants a live number (knows if it can harm you)

Control of Microbial Growth

Disinfectants and Antiseptics

229

Method

- Three approaches for the control of microbial growth
  - Chemical
    - Disinfectants and antiseptics
  - Physical
    - Heat
    - Ultraviolet
    - Irradiations
  - Mechanical elimination
    - Cleaning
    - Filtration

230

Terminology

- Cleaning
  - The elimination of visible adherent dirt (blood, proteins and debris), dust or other foreign matter by manual or chemical processes
    - Does not infer the presence or absence of microorganisms
      - Cleanliness ≠ Sterility

231

There are no measurements of cleanliness.
Sterility=absence of microorganisms.

Disinfection

- The use of chemical or physical agents to kill or inhibit the growth of microorganisms
  - Disinfectants
    - Chemical products used on inanimate objects
  - Germicides
    - Chemical products which can be used on either animate (living) or inanimate things
  - Antiseptics
    - Chemical products used on living tissues

232

Other Definitions

- Contamination
  - Contaminant:
    - Non-intentional presence of a microorganism
  - Decontamination:
    - Operation used to reduce or eliminate a contaminant
  - Sanitation: Reducing the level of microbial contamination to prevent transmission in public establishments
    - Restaurants, bathrooms, etc.

233

Factors which Influence the Efficacy

- Microbial load
  - Number of microbes
- Environment
  - Presence of organic matter
  - Concentration of the agent
  - Temperature
  - pH
- Length of exposure

234

Greater the number of organisms- the less efficient it will be

The greater the concentration-more effective MOST of the time

work better at higher temperatures.

Depending on the chemical, theres an optimal pH.

The longer its exposed, the better it will work.

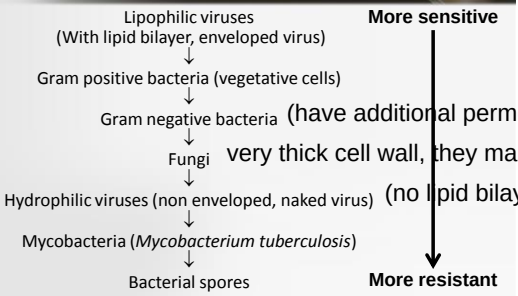
Factors which Influence the Efficacy

- Microbial characteristics
  - Glycocalyx
  - Biofilms
  - Cell wall
  - Resistances
  - Spores

235

Cell walls- very impermeable, makes them very resistant to chemical treatments.
Spores are extremely resistant as well.

Order of Sensitivity



236

(have additional permeability barrier)
very thick cell wall, they make spores(not as resistant as bacterial cells tho)
(no lipid bilayer)

Disinfectants and Antiseptics

- Ideal characteristics
  - Broad action spectrum
  - Powerful
    - Small amount required for a high efficiency
  - Low toxicity in humans
  - Non corrosive
  - Stable
  - Hydrophilic and hydrophobic
  - Low surface tension
  - Odorless or with a pleasant smell

237

The broader the spectrum of the chemical, the better
Stable- long shelf life.
When using these products, you are usually spreading them, so you want them to be easy to spread (low surface tension)

Modes of Action of Chemical Agents

- Denaturation of proteins or DNA
- Mutagenesis of DNA
- Modification of proteins or of DNA
- Interference with the plasma membrane
- Oxidation of functional groups

238

Types of Chemical Agents

- Seven major categories:
  - Phenol and phenolic compounds
  - Alcohols
  - Halogens
  - Oxidative agents
  - Heavy metals
  - Aldehydes
  - Surfactants

239

Phenol and Phenolics

- Phenol (carbolic acid)
  - Used for the first time by Lister
  - Rarely used, since it's an irritant and has a strong odor
- Phenolics: Chemical derivatives of phenol
  - Cresols: Lysol
  - Bisphenols
    - Used in hospital centers
- Denatures proteins and destroys membranes
- Bactericide, fungicide, sporicidal
- Very toxic
- Caustic
- Antiseptic/disinfectant

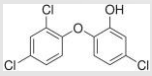
240

Dont say phenols are alcohols on the exam.
Used mostly as a disinfectant, not really an antiseptic.
Dissolves lipid layers, effective on spores

Examples of Phenolic

O-phenylphenol

Thymol



Triclosan

Turpineol

241

Alcohols

- Ethanol (60-95%) and isopropanol
  - Effective against bacteria, fungi, and enveloped viruses
    - Inefficient against spores and naked viruses
  - Denatures proteins and dissolves lipids

242

Halogens

- Four members :
  - Iodine
  - Chloride
  - Bromide
  - Fluoride
- Bactericide, fungicide and viricide
- Iodine inactivates proteins by interacting with disulfur linkages
- Chlorine, bromine and fluoride are strong oxidizing agents

243

Iodine Breaks di-sulfur linkages, so only acts on proteins that have di-sulfur linkages
Oxidizing-they remove the electrons.

Oxidative Agents

- Release hydroxyl free radicals which inhibit bacterial metabolism
  - Very effective against anaerobic organisms
    - Very effective against deep tissue infections

244

Most common are peroxides, very useful against anaerobic organisms. They dont have the ability to remove free radicals, so the free radicals released by the peroxide is very effective against them.

Oxidative Agents

- The three most commonly used are:
  - Hydrogen peroxide
    - Common household antiseptic
  - Ozone
    - Very reactive form of oxygen used for the treatment of water
  - Peracetic acid
    - Peroxide of acetic acid
      - Very effective sporicidal
        Used to sterilize surfaces and medical materials

Can achieve sterilization

245

Has to be able to reduce microrganism by 10^5 fold to be classified as a disinfectant

Heavy Metals - Hg, Ag, Zn, Cu

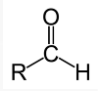
- Interact with proteins causing their denaturation and inactivation
  - Mercury
    - Mercury and silver used to be used in clinical situations
      - Mercury is not used anymore
      - Silver is still used for surgical bandages
  - Zinc
    - Used in mouth washes
    - Used as antifungal in paints
  - Copper
    - Algaecide; used in pools

246

No microorganism has ever developed a tolerance to silver.

Aldehydes

- Compounds with a terminal –CHO group
  - Denatures proteins and inactivates nucleic acids
- Two very reactive aldehydes are used as antimicrobials
  - Glutaraldehyde and fomaldehyde
- Bactericide, sporicidal, fungicide and viricide

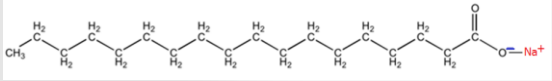


247

Not used on living tissue, broad spectrum, smells very bad.

Surfactants

- Soaps
  - Sodium or potassium salts of fatty acids
  - Effective for the mechanical elimination of microbes from surfaces
  - Ineffective as an antimicrobial

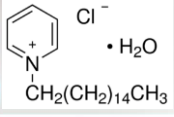


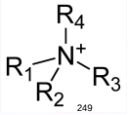
248

Not really an microbial, its good for removing them.

Surfactants

- Detergents
  - Positively charged organic compounds
  - Ex. Quaternary ammonium compounds
  - Dissolves lipid membranes
  - Bactericide, fungicide, and viricide against enveloped viruses
  - Ineffective against spores and naked viruses





249

Much more effective antimicrobials.,

Control of Microbial Growth

Physical Methods: Sterilization

250

Definitions

- Sterilization
  - Killing or removing all forms of microbial life (including endospores)
    - The method most commonly used for sterilization is the use of heat
- Commercial sterilization
  - Thermal treatment which kills *Clostridium botulinum* spores, the causative agent of botulism, in canned foods
    - Does not kill spores of thermophiles which are not pathogenic

251

Clostridium botulism is a known human pathogen, it has spores. It has to make sure to sterilize both of these.

Modes of Heat Sterilization

- Humid heat
  - Kills microbes by denaturing proteins
    - Boiling (100°C)
    - Pasteurization (65 - 140°C)
    - Autoclave (121°C)
- Dry heat
  - Kills by oxidation
    - Pasteur oven (121 - 250°C)
    - Incineration (870 - 1200°C)

252

Dont memorize the temperatures, just know that dry heat has much higher temperatures then humid heat. Humid heat has a shorter duration, dry heat has longer treatment periods. Humid heat has a shorter duration time because water tranducts heat much better than air.

Boiling

- 10 minutes at 100°C at sea level
  - Kills vegetative form of bacterial pathogens
  - Kills most viruses and fungal spores
  - Endospores and some viruses are not destroyed
    - Hepatitis virus: Can survive up to 30 minutes
    - Endospores: Can survive for periods of 20 hours or more

253

Not useful for the food industry, boiling will not be affective.

Pasteurization

- Used for drinks
- Kills pathogenic agents without affecting the taste of the food
  - Does not sterilize
  - Classical pasteurization
    - 63°C for 30 seconds
  - HTST Pasteurization
    - 72°C for 15 seconds
  - UHT Pasteurization
    - 140°C for 3 sec. under vacuum

254

Reduces the amount of microbes to an acceptable level.

Autoclave

- Makes use of humid heat under pressure
- Temperature of 121°C at twice the atmospheric pressure
- All organisms and endospores are killed in 15 minutes

255

Dry Heat Sterilization

- Pasteur oven
  - Temperature between 121 and 250°C
  - Long exposure times (2-12 hours)
  - Not recommended for chemical products
  - Can be used for some metals and glass
- Incineration
  - 870 to 1200°C
  - Burns and physically destroys
  - Used for needles, glass, corpses, etc.

256

Parameters of Death by Heat

- Thermal death point (TDP)
  - Lowest temperature at which all bacteria are killed within 10 minutes
- Thermal death time (TDT)
  - Length of time required to kill all the bacteria at a given temperature
- Decimal reduction time (DRT– D value)
  - Time required to kill 90% of a microbial population

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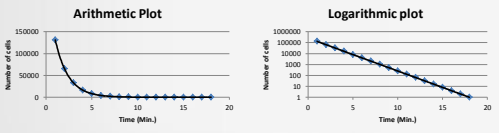
Profile of Thermal Mortality



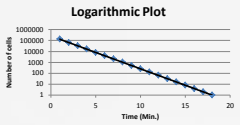
- Death is exponential
  - It's therefore impossible to reach zero
    - Established standards:
      - TDP and TDT: < 1 cell
      - Sterility in the lab: 10^{-6} cells or spores
      - Sterility for food: 10^{-12} cells or spores

know these numbers

258

Calculations of Mortality

- Mortality constant (k)
  - Rate of mortality
    - Negative slope
  - Formula: **$-kt = \ln N_o/N$**
    - T: Length of time
    - N: Number of surviving cells
    - N_o: Initial number of cells
      - N_o/N: Inactivation factor



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Example

- A treatment at 100°C for 1h reduced a bacterial population from 10^8 to 10^2 cells
  - What is the mortality rate?
    - Inactivation factor = $N_0/N = 10^8/10^2 = 10^6$
    - $-kt = \ln N_o/N$; thus $-k = (\ln N_o/N)/t$
    - $-k = \ln 10^6/60$ minutes = 0.23 cells/min
    - k= -0.23 cells/min

260

k has to be a negative value.

Calculating TDT

- If you start with 10^8 cells and k at 100°C = -0.23 cells/min, what would be the TDT?
  - You wish to calculate the shortest amount of time to reduce the population to <1 cell
  - Inactivation factor you want
    - $10^8/0.99 = 10^8/0.99 = 10^8/1 = 10^8$
  - Since $-kt = \ln N_o/N$; $t = (\ln N_o/N)/-k$
    - Thus $t = \ln 10^8 / -(-0.23)$ = 80 minutes

261

All these calculations are on the exam.

Decimal Reduction Time – D value

- Time required to kill **90%** of microorganisms at a given temperature
- Time required to reduce the population by a **factor of 10** at a given temperature
- Time required to achieve an inactivation factor (N_o/N) of 10

262

(move the decimal place by one spot)

Calculating the Decimal Reduction Time

- If you start with 10^8 cells and k at 100°C = -0.23 cells/min., what is the D_{100} value?
  - D = Time required for an inactivation factor of 10
  - $-kt = \ln (N_o/N)$ or $t = \ln (N_o/N)/-k$
    - Thus t= ln 10/ -(- 0.23) = 10 minutes

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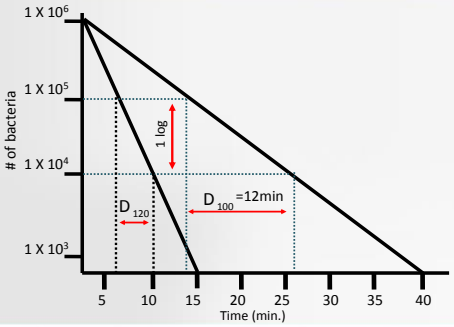
Calculating the Decimal Reduction Time

- If you start with 10^8 cells and k at 100°C = -0.23 cells/min., what is the D_{100} value?
  - How much time would be required to reduce the population to 10^2?
    - Inactivation factor = $10^8/10^2 = 10^6$
    - $-t = (\ln N_o/N)/-k$ Thus $-t = \ln 10^6 /-0.23$ = 60 min.
    - Or the time require for an inactivation factor of 10^6 = 6X the time requires for an inactivation factor of 10
      - Thus 6D = 6(10 min) = 60 minutes

264

The amount of time it takes to achieve sterility. You will divide 10^8 by 10^-6. 10^14 is equal to the amount of D values. You multiply 14 (10minutes)= 140 minutes.

Determining D from a Graph



265

On the exam** be able to get data from the graph.
D-value is higher under dry heat.

Problem

- 18 minutes are needed at 75°C to reduce a population from 10^9 to 10^6
  - What is D_{75} ?
    - Inactivation factor = $10^9/10^6 = 10^3$
    - Thus three times the time required for one inactivation factor = 18 min.
    - Thus $3D_{75}$ = 18 min.
    - Thus D_{75} = 18 min./3 = 6 min.

266

10^3, 3- 3xD
D=3D=18min.
18/3= 6 minutes.

Calculating D *(cont'd)*

- The D value can be calculated without knowing the mortality constant (k)
  - Formula : **$D = t/(\log N_o - \log N)$**
    - 18 minutes are needed at 75°C to reduce a population from 10^9 to 10^6
    - What is D_{75}
      - $D = t/(\log N_o - \log N)$
      - Thus $D_{75} = 18/(\log 10^9 - \log 10^6) = 18/3 = 6$ minutes

267

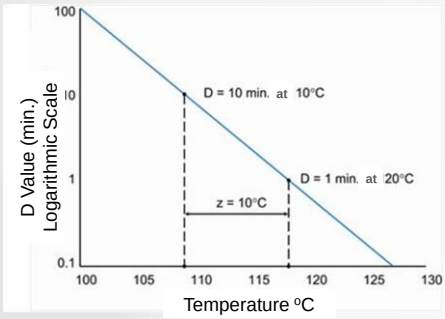
Relative Resistance of Microorganisms

- Z value
  - Temperature change required to reduce the D value by 90%
  - Temperature change required to reduce the D value by a factor 10
    - The smaller the Z value, the more sensitive to heat is the microorganism

268

D value is ALWAYS a unit of time
Z value is a unit of temperature.
~~Z changes D.~~
If at 100 C it took 10 minutes to reduce. At 90C, it would take ~~longer, you would move the decimal place to the right~~. at 150C , its would be shorter, so you would move it to the left.

Heat Sensitivity – Z Value



269

For every 10C, the value of D will change.

Relationship Between D and Z

- If Z =10°C
  - Each 10°C increment change of temperature would change the D value by one log
  - Thus if D at 110°C = 10 minutes
    - At 120°C it would be = to 1 minutes
    - At 130°C it would be = to 0.1 minutes
    - At 140°C it would be = to 0.01 minutes
  - What would be the D value at 80°C?

270

At 80C it would be 10 000minutes.

Problem

- The Z value of an organism is 2°C. If 18 min are required at 75°C to reduce the population from 10^9 to 10^6; to what temperature should this organism be subjected to achieve the same result in10.8 seconds?
- 18 minutes = 1080 seconds
  - Thus to go from 1080 to 10.8 sec. = 2 log
    - 2 log = 2Z = 4°C
    - Since we want to **reduce** the amount of time, we must **increase** the temperature by 4°C
      - Thus 75 + 4 =79°C

271

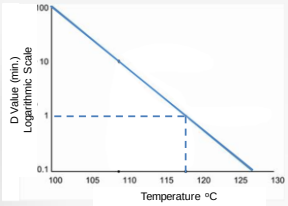
Thermal Death Point (TDP)

- Minimum temperature required to reduce a population to less than one cell in 10 minutes
  - Ex. What is the TDP of a culture which has 10^8 cells?
    - Calculate the inactivation factor wanted : $10^8/0.99 = 10^8$
    - Calculate the number of decimal reductions wanted : 8D
      - » Thus 8D = 10 minutes or D = 1.25 minutes
    - Determine form the graph illustrating the level of temperature sensitivity the temperature which corresponds to the D value you want

272

Determining the TDP

- Want a D value equal to 1.25 minutes
  - According to the graph this corresponds to a minimum temperature of…
    - ~ 117°C



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Radiation Sterilization

- 3 types of radiations kill microbes
  1. Ionizing radiations
     - Ionizing radiation : Gamma rays and X rays
     - Cause mutations in DNA
       - Used for the sterilization of pharmaceutical products and disposable medical supplies
  2. Ultra violet light
     - Damages DNA and causes mutations
     - Used to sterilize surfaces
       - Ex. Operating room

274

UV penetration is extremely low, only about a mm.

Radiation Sterilization

3. Microwave radiations
   - Causes water molecules to be heated
     - Can kill vegetative cells in humid food products
     - Endospores, which do not contain water, are not damaged
     - Ineffective on solid foods
       - » Unequal penetration

275

Only works with things that have a high water content.

Filtration

- Exclusion principle using filters
  - Elimination of microbes by the passage of a liquid or gas through a filter whose pore size prevents the passage of…
    - Bacteria: pores of 0.22 and 0.45µm
      - Does not retain mycoplasms and viruses
    - Virus : pores of 0.01µm

276

Have to use much smaller pore sizes to remove viruses.

Filtration

- Used for the sterilization of thermosensitive materials
  - Vaccines, enzymes, antibiotic, and some culture media
  - High efficiency filters for particles in the air (HEPA; High Efficiency Particulate Air)
    - Used in operating rooms to eliminate bacteria in the air

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EXAM MATERIAL ENDS HERE

Control of Microbial Growth

In Vivo: Antibiotherapy

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Antimicrobial Drugs

- Antibiotic or Antibacterial
  - Against bacteria
- Antifungal
  - Against fungi
- Antiviral
  - Against viruses

279

The Drugs: Antibiotics

- Definitions:
  - Literal: Anti (against) biotic (life)
  - Old def.: Any compound made by a microorganism which inhibits or kills bacteria
  - New def.: Any compound which inhibits or kills bacteria

280

Desired Characteristics

- **High** selective toxicity
  - Must kill or inhibit the targeted organism with minimal deleterious effects on the host
    - **Penicillin**: (High selective toxicity)
      - Targets the cell wall
    - **Cyanide**: (Low selective toxicity)
      - Targets electron transport of eukaryotes/prokaryotes

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Desired Characteristics *(cont'd)*

- **High** toxic or lethal dose (LD50)
  - Concentration of the agent qui that is toxic for the **host**
    - Penicillin: (3 000 mg/Kg)
    - Arsenic: (15 mg/Kg)
- **Low** therapeutic dose
  - Concentration of the agent required for the clinical treatment of an infection
    - Penicillin : 12.5 mg/Kg
    - Garlic: 300 mg/Kg

282

Low therapeutic dose: amount to kill to microbial infection, you want this to be low as possible.
High lethal dose: You want this to be as high as possible, the amount of the agent that hurts the host taking the medication.

Therapeutic index

- Toxic dose/Therapeutic Dose
  - Want a therapeutic dose which is?
    - ❖**High**

283

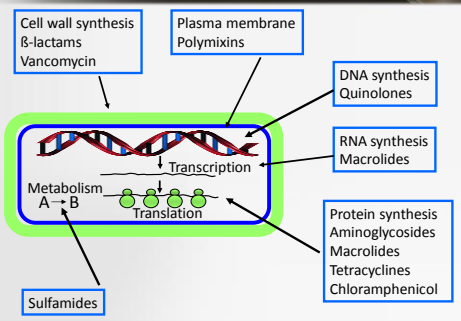
Action Spectrums

- Narrow:
  - Restricted efficacy against some types of microorganisms
    - Ex. Only acts against Gram -
- Broad:
  - Effective against a wide diversity of microorganisms
    - Ex. Acts on Gram + and -

284

In general, the broader the spectrum, the lower the therapeutic index.

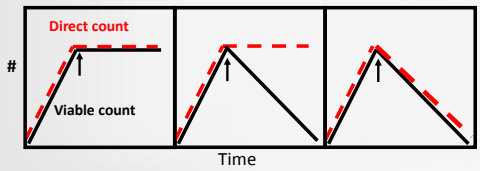
Antibacterial Targets



285

Know this i think** the types of antibacterials and the cell locations they will target.
Antimetabolites; also know as analogues, they are agents that resemble the substrate, they will compete the substrate for the enzyme and prevent the metabolic reaction from taking place.
Ex. Sulfamides that inhibit folic acid synthesis.

Modes of Action



- Bacteriostatic
  - Inhibits growth
  - Non lethal
  - Reversible
- Bactericide
  - Kills
  - Irreversible
- Bacteriolytic
  - Kills
  - Cell lysis
  - Irreversible

286

These are growth curves, time vs cell number. Arrow represents the moment in time where the antibiotic is added. ~~Blac line is the viable count, red is the direct count.~~ In the first one, the numbers stop increasing, if you were to ~~remove the antibiotic growth would resume and start~~ over again.

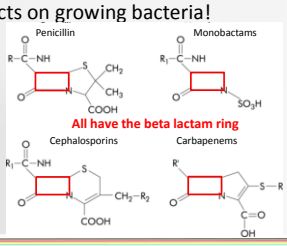
~~2nd graph-cell number decrease, same direct count~~ as the first one, the cell number stops increasing the cells are still there, ~~they are just dead. If you removed the antibiotic at the~~ end of the curve, the pattern would stay the same.

3rd graph-Cause death by cell lysis, irreversible, decrease in both counts, conditions have to appropriate for them to be effective.

How would these compare in optical density. bacteriostatic: same pattern as the red line. Bacterioside:same as the red line Bacteriolytic: same as the red line

Beta-Lactams

- Bacteriolytic
- Inhibit synthesis of the cell wall
  - Only acts on growing bacteria!



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Midterm: indentify the antibiotic by its structures and know their characteristics.

~~Beta-lactam ring: box (4 sides, does not necessarily~~ have to be a rectangle) with a nitrogen group on one corner=beta lactam.

~~Inhibit cell wall synthesis, so they only work on active~~ly growing cells.

~~Be able to recognize the different types of beta-lactam~~s as well. (on exam)

Penicillins & Cephalosporins

- Natural penicillin – penicillin G
  - Narrow spectrum; only acts on Gram positives
- Aminopenicillin – ampicillin and amoxicillin
  - Broad spectrum; acts on Gram positives and negatives
- Cephalosporins – Ex. Cefepime & Ceftazidime
  - Developed to have a broader action spectrum as compared to penicillins

288

penicillins:means a class of antibiotics

Penicillin: talking specifically about penicillin G, discovered by flemming, rarely used now bc it has a narrow spectrum, only works on gram +, has lps something, permeability barrier.

~~Aminopenicillin: have been modified in the lab to have~~ a broader spectrum

~~Cephalosporins: synthetic, have the broadest spectru~~m among penicillins.

Monobactams & Carbapenems

- Monobactams
  - Very narrow action spectrum; useless against Gram positives and anaerobes
- Carbapenems – Last generation of beta lactams
  - Very broad action spectrum
  - Acts against Gram positives, negatives, anaerobes and aerobes

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Monobactams: limited use

All penicillins cause problem in the gastral intestinal track. Can

Adverse Effects of Beta Lactams

- Severe allergies amongst 10% of the population
- Gastro-intestinal problems
  - Vomiting, nausea, diarrhea
- Immune effects
  - Immunodepression
- Neurological problems (carbapenem)
  - Irritability, confusion, seizures

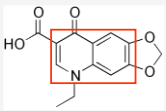
290

The most tosix ones have the lowest therapeutic index, they are ordered from least to most toxic in the lecture.

more susceptible to opportunistic infections.

Quinolones

- Bactericides
  - Inhibit DNA synthesis
  - Broad spectrum
  - Side effects:
    - Severe gastrointestinal problems
  - Ex. Ciprofloxacin

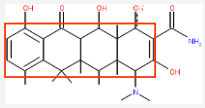


291

Much more toxic than beta lactams

Tetracyclines

- Bacteriostatic
  - Inhibits protein synthesis
  - Broad spectrum
  - Side effects:
    - Hepatic toxicity
    - Renal toxicity
    - Vitamin deficiency

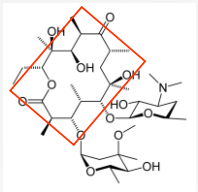


292

Specifically act on ribosomes.
Much more toxic, we have the same ribosomes as bacteria, much lower selective toxicity.

Macrolides

- Bacteriostatic
  - Inhibits protein synthesis
  - Narrow spectrum
  - Side effects
    - Diarrhea
    - Hepatic damage
  - Ex. Erythromycin & Clarithromycin



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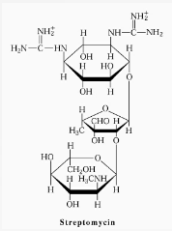
Big irregular ring, more than 6 sides.

Aminoglycosides

- Bactericides
  - Narrow spectrum
  - Inhibit protein synthesis
  - High level of toxicity
  - Side effects:
    - Allergies
    - Renal damages
    - Deafness
  - Ex. Gentamycin, streptomycin



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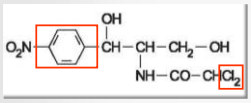
sugar based structure, targets protein synthesis, even more toxic.

Chloramphenicol

- Bactericides
  - Narrow spectrum
  - Inhibit protein synthesis
  - Side effects:
    - Only used in extreme cases
    - Hematological toxicity

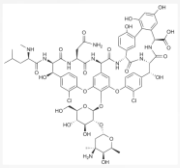


295

Has a chlorine ring, so toxic that it is a last resort. Very narrow spectrum, but it acts on something that is common to us, which is why it is so toxic.

Glycopeptide Antibiotics

- Composed of polycyclic amino acids
- Inhibits cell wall synthesis
- Acts mostly against Gram Positives
- Used as a last recourse
  - Ex. Vancomycin



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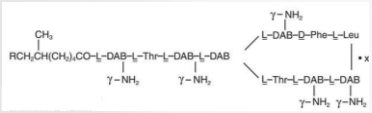
Cyclic amino acids, work on cell wall, very toxic, last resort. A lot of microbes have built a resistance to the other antibiotics, now they are starting to build a resistance to this one.

Peptide Antibiotic –Polymixin B

- Disrupts the plasma membrane
  - Binds lipid A and phospholipids
  - Only acts against gram negatives
- Topical use only
  - Can cause lysis of eukaryotic cells

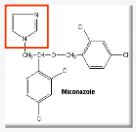


297

Very toxic, polypeptide. It is so toxic, acts on gram negative and phospholipids. Doesnt work on gram positive. Only used topically, cannot be ingested.

Topical Antifungals

- Imidalzole derivatives:
  - Miconazole, Clotrimazole, Cetoconazole
- Targets and modes of action:
  - Extraction of sterols from plasma membrane
  - Inhibition of plasma membrane synthesis
  - Low therapeutic index

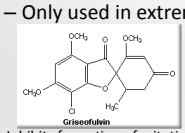


298

Much more difficult to target things that we dont have, used externally, 5 member ring with nitrogen. Causes extraction of sterols in the plasma membrane. Sterols in the membrane is a characteristic unique to eukaryotic cells. This is as toxic for us as it is to the fungi, which is why it is only used topically.

Systemic Antifungals

- Targets and modes of action:
  - DNA replication
  - Cell division
  - Very low therapeutic index
  - Only used in extreme cases



- Inhibits formation of mitotic microtubule fibers

- Nucleoside analog: Inhibits DNA synthesis

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Antifungals that you ingest, you dont usually need to use this bc fungi are easily dealt with by the immune system.
Will inhibit DNA synthesis by competing for the polymerase. It still works bc the only cells that are doing DNA synthesis are those that are dividing, so the effects on us are minimal bc there is not alot. A pathogen is usually actively dividing so thats why it works on them.
Works just as well on fungal cells as our cells.

Antimicrobial Therapies

- Empirical
  - The infectious organism is unknown
  - Broad spectrum antibiotic recommended
- Definitive
  - The infectious agent was identified
  - A specific therapy is chosen
  - Narrow spectrum antibiotic recommended
- Prophylactic or preventive
  - Prevent an initial infection or a reinfection

more toxic

300

Preventative therapies are present in hospital workers. Using broad spectrum antibiotics.

Choice of Appropriate Antibiotherapy

- Determine the site of the infection
- Sample and isolate the pathogen
- Determine sensitivity
- Choose the administration route
  - Oral
  - Intravenous
  - Topical

301

The Infection Site

- Most important criteria for choosing the appropriate antimicrobial!
  - Allows to determine the presumptive identity of the infectious organism
    - Particularly useful for empirical therapies
  - Allows the determination of the dose and administration route
    - The effectiveness of the therapy depends on the concentration of the antibiotic at the site of the infection and its relationship to the MIC
      - **The concentration at the site must be higher than the MIC**

302

For any microbial to be effective, the concentration of it at the infection must be higher than the MIC (minimal inhibitory concentration)

Sensitivity: Kirby Bauer Assay

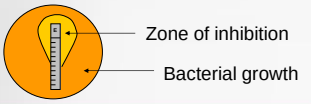
- Medium is inoculated with bacteria to be tested
- Discs containing antibiotics are deposited on the medium
- A concentration gradient is established due to the diffusion of the antibiotic in the medium
- Following the incubation, the inhibition zones are measured
- The sizes of the zones are compared to those established to determine whether the organism is sensitive or resistant

303

Closer to disk-higher concentration of antibiotic. INhibition zone=no growth, the bigger the inhibition zone, the less tolerant the microorganism is to the antibiotic. You cannot use this to conclude whether or not a antimicrobial is effective in the human body. All three types of antimicroobials show the same result, so you cant differenciate between them with this.

E-Test

- Same principal as the Kirby Bauer assay
- Makes use of a plastic strip with a predefined gradient of antibiotic concentrations
- The results are read directly on the strip
  - The intersection point of the zone of inhibition and the strip



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Antibiotic has different known concentrations. Gives you the concentration that is the boundary between tolerant and non-tolerant.

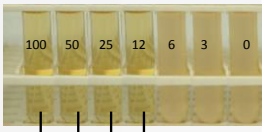
E-Test

305

Determining Efficacy

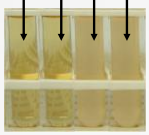
- Minimal Inhibitory Concentration

Cultures with different concentrations of antibiotique



MIC=12µg/ml

Subculture without antibiotics



MBC=50µg/ml

- Minimal Bactericide Concentration

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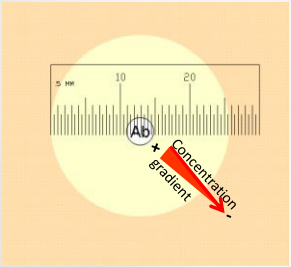
The MIC is 12 and higher. There was no growth at 12 and higher, this would be the therapeutic dose.
This could be because you either killed it or inhibited it. You cant tell with this method. You remove the antibiotic to see if they resume growth.
MBC= minimum bacterialcide concentration: the minimum at which it is not reversible, not reversible.
**ALL antibiotics have a MIC, not all antibiotics have a MBC, only bacterialcidal and bacteriolytic.
In this example they do not grow back so its one of those ^

Diameters of Inhibition Vs Concentration



27mm = to MIC
< 27mm = Conc. > MIC
> 27mm = Conc. < MIC

307

The further away from the disc, the lower the concentration, the periphery represents the threshold that the microb. can tolerate. The minimal inhibitory concentration. Past 27mm, concentration below the MIC.

Pharmacodynamics of Antibiotics

- Behavior of antibiotics *in vivo*
  - Interaction of antibiotics with the bacteria
    - The antibiotic must reach the site where the microbe resides
    - The concentration of the antibiotic at the infection site must be above the MIC
    - The antibiotic must occupy a sufficient number of sites on the target
    - The antibiotic must remain in contact with the target for a sufficient amount of time

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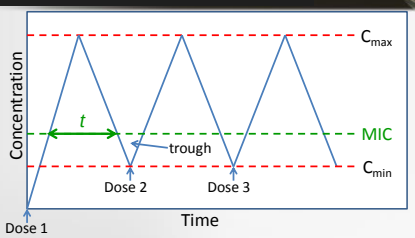
Concentration of Antibiotics *In Vivo*



- C_{max}: Maximum concentration attained for a given dose
- C_{min}: Minimal concentration attained between doses
- *t*: Time during which the concentration is maintained above the MIC

309

In Vivo Sensitivity

- **Sensitive** pathogen
  - MIC is lower than C_{min}
- **Resistant** pathogen
  - MIC is higher than C_{max}
- **Intermediate** sensitivity pathogen
  - MIC is between C_{min} and C_{max}
    - A combination of antibiotics is recommended

310

Example

- *In vivo conc.* of antibiotic "A"
  - C_{min}: 5 µg/ml
  - C_{max}: 40 µg/ml
    - Therefore:
      - CMI < 5 µg/ml = **Sensitive** microorganism
      - CMI > 40µg/ml = **Resistant** microorganism
      - CMI entre 5 -40 µg/ml = **Intermediate** sensitivity microorganism

311

In Vivo Modes of Action of Antibiotics

- Concentration independent activity
  - Time dependent activity
  - The effect is a function of the length of time that the target is in contact with the antibiotic at a concentration above the MIC
    - Efficacy is evaluated by t > CMI
  - Efficacy remains the same at all concentrations above the MIC
    - Typical of ß-lactams, vancomycin, macrolides, and tetracyclines

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In Vivo Modes of Action of Antibiotics

- Concentration dependent activity
  - Time independent activity
  - The effect is a function of the quantity of antibiotic that is in contact with the target
    - Efficacy is evaluated by the ratio of : C_{max}/MIC
  - The efficiency is not affected by the amount of time of the exposure
    - Typical of quinolones and aminoglycosides

313

Drawbacks of Antibiotherapy

- Kills the natural flora
- Does not act on bacterial toxins
  - Ex. cholera, diphtheria, botulism, tetanus
- Creates a selective pressure for antibiotic resistant strains
- Favors opportunistic infections
  - Ex. *Clostridium difficile* Associated Diarrhea (DACD)

314

Antibiotic Resistance

315

Midterm short answer questions: 10 antibiotic structures, 10 descriptions, match them together
KNow therupeutic index, medicinal applications of the antibiotics
Viral growth curve

Consequences of an Antibiotherapy

- Treatment of an invading organism
  - A life is saved
- Establishment of a selective pressure
  - If the invading microorganism does not develop a resistance it will die
  - 1945- A. Fleming warns that the inappropriate use of penicillin will lead to the selection of resistant bacteria
    - A few years later the first resistant strains appear

316

The Number of Resistant Bacteria is increasing at an Alarming Rate

- To what is this increase attributable?
  - 1990: 300 metric tons of antibiotics are used in humans
  - Approx. 30X more in agriculture
  - 70% are not used appropriately
    - Doses which are too low
    - Prescribed for too short durations
    - Prescribed for viral infections
    - Prescribed for bacterial infections which would have resolved themselves

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Reasons for Such a High Consumption

- The patient
  - Wants a rapid treatment
  - Publicity
- The physician
  - Wants to satisfy the patient
  - Avoid legal proceedings
  - Cost
    - Antibiotherapy is less expensive than other tests and treatments
- Industry
  - Publicity and pressure from pharmaceutical companies

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Natural or Innate Resistances

- Resistance is not acquired; **Natural trait**
  - Streptomycetes are resistant to the antibiotics they produce
  - Gram negative bacteria: LPS layer acts as a permeability barrier
  - Some bacteria do not possess the antibiotic's target
    - Mycoplasms – No cell wall; therefore resistant to penicillins and cephalosporins
    - Mycobacteria – No peptidoglycan; therefore resistant to penicillins and cephalosporins

319

Acquired resistance

- The microorganism **acquires** a resistance to one or more antibiotics
  - Following the administration of a given antibiotic or of an antibiotic with similar properties
    - Occurs mostly in the **trough** below the MIC
  - After an encounter of a microorganism that acquired a resistance
    - Transfer of resistance

320

How are Resistance Acquired?

- Microorganisms normally and randomly acquire spontaneous mutations
- The presence of antibiotics exerts a selective pressure
  - The presence of the antibiotic creates a selective environment for microorganisms that acquired by chance a favorable mutation

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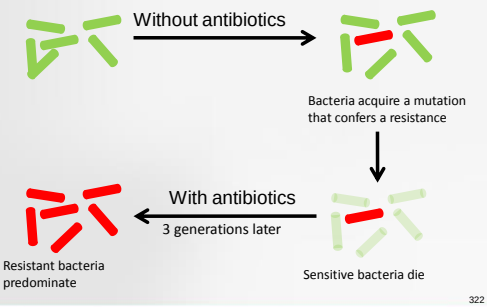
Spontaneously Acquired Resistance



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Mechanisms of Resistance

- Impermeability:
  - Reduced number of porins
  - Biofilms
- Inactivation:
  - Proteins that bind and inactivate the antibiotic
- Transport:
  - Pumps the antibiotic outside the cell
    - The internal concentration is below the MIC
- Degradation of the antibiotic
- Modification of the target
  - The antibiotic cannot bind its target

323

Multiple Resistance Organisms

- The MROs
  - Microorganisms resistant to one or several classes of antimicrobial agents
    - Most common MROs:
      1. Methicillin resistant *S. aureus* (MRSA)
      2. Vancomycin resistant Enterococci (VRE)
      3. Enterobacteria which produce broad spectrum beta lactamases (ESBLs)
      4. Multiple drug resistant tuberculosis (MDR-TB)
      5. Nouveau Delhi Metallo-Beta-Lactamase (NDM-1)

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Transfer of Resistances

- Exchange of genes/mutations between different strains or species
  - No selective pressure required

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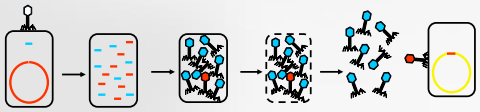
Transfer Mechanisms - Transduction

- Viral infection of donor bacteria
- Viral DNA replication and fragmentation of bacterial DNA
- Assembly and packaging of DNA
- Release of phage
- Infection of recipient bacteria
- Integration into the genome

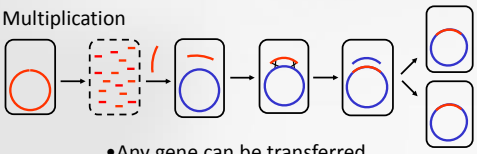


•Any gene can be transferred

326

Transfer Mechanisms - Transformation

- Death and lysis of donor bacteria
- DNA fragmentation
- Uptake of DNA by recipient bacteria
- Recombination
- DNA exchange
- Multiplication

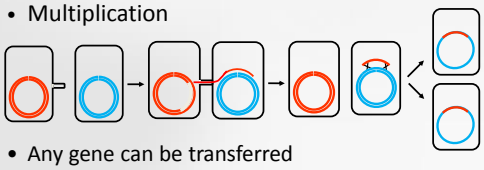


•Any gene can be transferred

327

Transfer Mechanisms - Conjugation

- Donor bacteria with pilus
- Mating with recipient bacteria
- DNA transfer
- Recombination
- Multiplication



- Any gene can be transferred

328

Preventing Acquired Resistances

- Education
- More effectively controlled usage
- Eliminate the prophylactic usage of antibiotics in livestock
- Reduce usage in household products
- Reduce prophylactic usage
- Discovery and synthesis of new antibiotics
- Use a combination of antibiotics

329

Combination Antibiotherapy

- Simultaneous administration of two antibiotics
  - Reduce the probability of acquiring a resistance
  - Use antibiotics for which the microorganism has an intermediate sensitivity
  - Treatment of an infection involving multiple microorganisms

330

Results of Combination Antibiotherapy

- Additive effect (indifferent) :
  - The activity of a combination of two antibiotics is equal to the sum of the individual activities
- Synergistic effect:
  - The activity of a combination of two antibiotics is higher than the sum of the individual activities
- Antagonistic effect:
  - The activity of a combination of two antibiotics is less than the sum of the individual activities

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Determining Effect of the Combination

- Determine fractional inhibitory concentration (FIC)
  - [(MIC of drug A in combination)/(MIC of drug A alone)] + [(MIC of drug B in combination)/(MIC of drug B alone)].
- Synergism, FIC ≤0.5
- Indifference, FIC >0.5 and ≤4
- Antagonism, FIC >4

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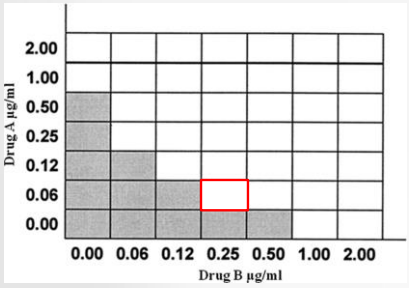
Example of Combination Therapy



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Probiotics

An alternative or complement to antibiotherapy

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Definition

- For (Pro) life (biotic)
- Live microbial feed supplements that have beneficial effects on the host by improving its intestinal microbial balance

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Newborn Microbiota

- Mother's microbiota
  - Maternal vaginal and intestinal flora constitutes the source of bacteria, which colonizes the intestine of new born
- Mode of delivery
- Birth environment

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Factors Affecting Intestinal Microbiota

- Antibiotics and other drug intake
- Microbial infections
- Diet (highly processed, low fiber foods)
- Chronic diarrhea
- Stress
- Radiation and chemotherapy

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Characteristics of Effective Probiotics

- Able to survive passage through digestive tract
- Able to attach to the intestinal epithelia and colonize
- Able to utilize the nutrients in a normal diet
- Non pathogenic and non toxic
- Capable of exerting a beneficial effect on host
- Anti-inflammatory, antimutagenic, immunostimulatory

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Probiotics: proposed mechanisms

- Adherence and stimulation of immune system
  - Up-regulation of mucin gene
  - Enhance secretory antibodies
- Competition for adhesion sites and essential nutrients
- Production of antimicrobial factors
  - Acidophilin, bacteriocin, etc.
- Provide favorable environment for growth of other beneficial bacteria

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Most Common Probiotic Strains

- *Lactobacillus* species
  - *L. acidophilus*
  - *L. plantarum*
  - *L. casei*
  - *L. brevis*
  - *L. delbreuckii*
- *Bifidobacterium* species
  - *B. adolescentis*
  - *B. bifidum*
  - *B. longum*
  - *B. infantis*
  - *B. breve*

340

Probiotics: Proposed uses

- Infectious diarrhea
- Antibiotic-associated diarrhea
- Irritable bowel Syndrome (IBS)
- Bacterial vaginosis
- Recurrent UTI's
- *H. pylori* infections
- Radiation induced diarrhea
- Constipation

341

Probiotics: Prescribing

- Which organism to use?
  - *Lactobacillus sp.* best studied
- What dose?
  - 10 billion organisms/day
- For How long?
  - Probiotics do not permanently colonize the intestine, thus, daily consumption required

342

Probiotics: Prescribing (*cont'd*)

- Any side effects?
  - 2% risk bloating/gas
  - May cause health problems in immunocompromised individuals
    - Skin rash, fever, bloody stools etc.
  - May interact with immunosuppressive drugs

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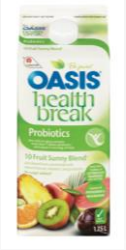
Probiotic Foods



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Virology

345

Characteristics of Viruses

- Obligate parasite
- Incapable of multiplying independently
- DNA **or** RNA genome
- Absence of DNA and RNA together in the same virion
- Genomes packaged in a protein capsid

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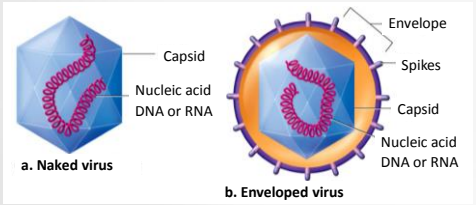
Virion Anatomy



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Envelope viruses have an extra layer

Capsid

- Protein shell that encloses the genome
- Protects the genome from physical, chemical and enzymatic conditions
- Has receptor proteins (spikes) that enable the recognition and attachment to the host cell
- For some viruses, the capsid is surrounded by a lipid bilayer
  - Has enzymes or receptors (spikes) required for attachment and penetration

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Spikes allow them to recognize and attach to a given cell. Envelope viruses have spikes on the envelope instead.

Classification

- 3 primary means of classification:
  - Morphology of protein coat (capsid)
  - Presence or absence of envelope
  - Genome: Nucleic acid

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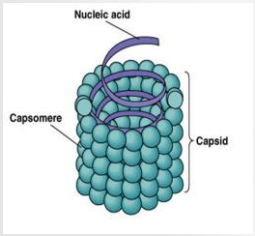
Capsid Shapes

- Helical
  - Ex. Ebola virus
- Isometric or Polyhedral
  - Ex. Influenza
- Pleomorphic
  - Ex. Bacteriophages

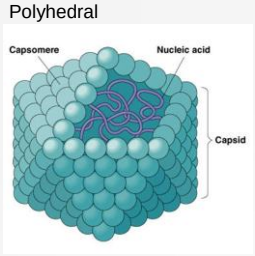
350

Capsid Shapes *(cont'd)*

Helical



Polyhedral



351

Genomes

- Single stranded DNA
- Double stranded DNA
- Single stranded RNA
- Double stranded RNA
- One or more molecules (segmented)
- Circular or linear

352

Molecules=chromosomes, some viruses have one copy of their genome, some have multiple copies of their genome.

Classification of Viruses

- Groups
  - I. (ds DNA)
  - II. (ss DNA)
  - III. (ds RNA)
  - IV. (+ ss RNA)
  - V. (- ss RNA)
  - VI. (RNA rev. trans.)

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+ss RNA, positive is synonymous to coding
-ss RNA=non-coding
Reverse transcriptase=retro viruses

Viral Taxonomy

- Family names end in -viridae
- Genus names end in -virus
- Viral species: A group of viruses sharing similar genetic information and ecological niche (host)
  - Common names are used
- Subspecies are designated by a number

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Viral Taxonomy Example: Herpesvirus

- **Classification:**
  - Herpesviridae (Family)
  - Herpesvirus (Genus)
  - Herpes simplex type 1 / type 2 (Species)
- **Structure:**
  - non-seg., lin., dsDNA, helical, env.

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Groupings Based on Transmission Route

- Not a taxonomic grouping
  - More than one family may be included in one transmission grouping

Virus Group	Transmission Route
Enteric	Fecal-oral
Respiratory	Respiratory
Zoonotic	Animal to human
Sexually transmitted	Sexual contact

(cannot be transmitted from human to humans)

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Ultimate Goals of Viruses

- Generate copies of the original genome
  - Replication
    - Double stranded DNA/RNA = 2 strands; 1+ and 1-
      - Each strand acts as a template for the synthesis of opposing strand
    - Single stranded DNA/RNA = 1 strand; + or –
      - The synthesis of an identical copy of the original strand requires the synthesis of the strand of opposite polarity!!
        - » + → - → + or - → + → -
        - » + ~~→~~ + or - ~~→~~ -
- Produce capsid proteins and assembly
  - Translation
    - Always performed by the cellular machinery

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Makes the copy, not the complementary strands. There is no known enzyme that can make a copy, just the complementary strand.

+=sense

-=antisense

The positive strand is a template for the -, and vice versa

Single stranded-they can't make a copy, so they have to make an intermediate form of the positive into a negative, and then make a copy of the positive from that.

They will package their DNA in capsids.

They all rely on cellular machinery to do translation, this is why they are obligate parasites.

Overview

- Replication
  - Generate copies of the genome (DNA or RNA)
  - Done by nucleic acid polymerases
    - Nucleic acid → nucleic acid
      - DNA dependent DNA Pol.: DNA→ DNA
      - RNA dependent DNA Pol.: RNA→ DNA
      - RNA dependent RNA Pol. RNA → RNA
- Transcription
  - Generate a complementary strand of RNA

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What follows dependant is what it synthesizes.
DNA dependant DNA=uses DNA to synthesize DNA.

Overview

- Translation – Cytoplasm
  - Protein synthesis from mRNA (+RNA)
  - Done by ribosomes
    - Nucleic acid→ Amino acids

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mRNA=coding RNA
This is all done by the cell, the virus does not contribute at all.

Replication & Transcription of DNA Genomes

- Small DNA viruses (ex. parvovirus):
  - Host's enzymes perform replication, transcription and translation
  - The viral DNA must be replicated in the nucleus
  - Viral replication requires actively growing cells
  - Host functions are not inhibited

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inhibition would prevent the virus from replicating

Replication & Transcription of DNA Genomes *(cont'd)*

- Large DNA viruses (ex. Herpesvirus)
  - Using host enzymes to transcribe the genes required for replication of viral DNA
  - Viral replication occurs in the nucleus
  - Require actively growing cells
  - Inhibit the host DNA synthesis

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They provide their own DNA polymerase, so they inhibit DNA synthesis by the host so they can monopolize the resources of the cell.

Replication & Transcription of DNA Genomes *(cont'd)*

- Very large DNA viruses (ex. Poxvirus)
  - Nucleocapsid contains DNA and RNA polymerases
  - Viral genome is replicated in the cytoplasm
  - Do not necessarily require actively growing cells

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They can replicate in the cytoplasm, not the nucleus because they can provide their own DNA and RNA polymerases.

Single Stranded RNA Viruses

Positive or Negative strand

- Positive strand:
  - Sequence is that of mRNA
  - Can be translated into proteins
- Negative Strand:
  - Sequence is complementary to that of mRNA
  - Cannot be translated

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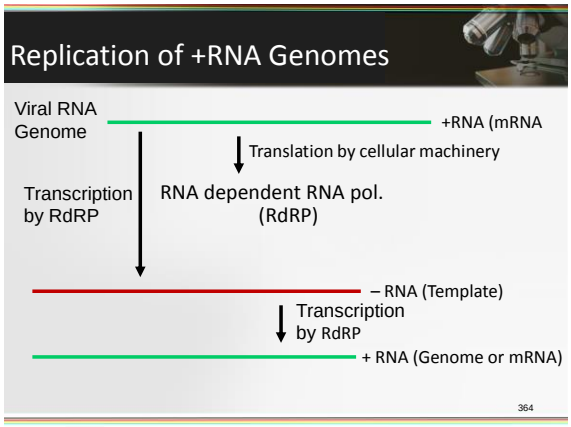
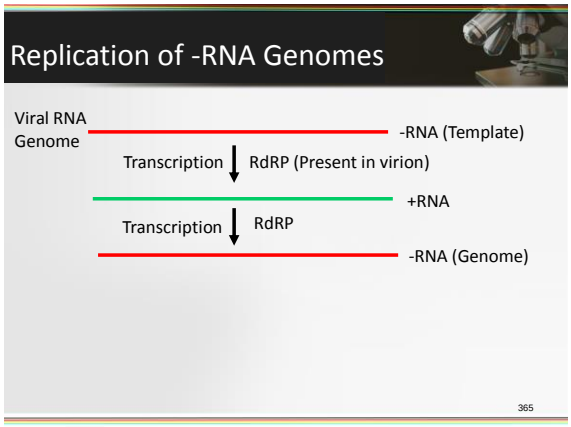
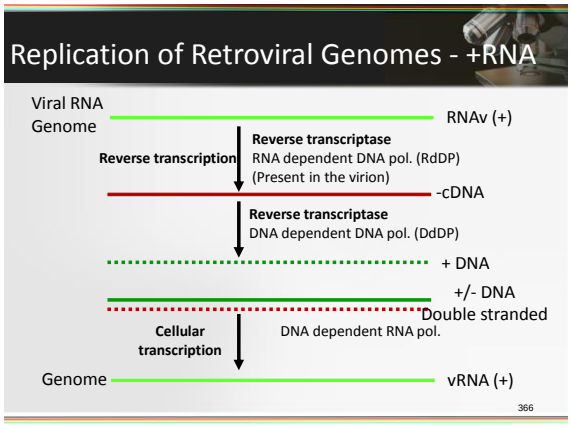
Positive RNA, coding RNA, same as mRNA, can be recognized and translated by the cell.
- cell, does not correspond to mRNA, the cell will not recognize the RNA and not translate it.



- RNA will act as a template, the polymerase will read the RNA and replicate it into the + RNA.



Cannot be read by the cell. The virus has to bring its own polymerase with it in the nuclear capsid in order to replicate the - RNA. It will synthesize a + RNA, and then use that to create a - RNA. The + RNA will be translated by the cell.



It will allow it to read the RNA and create a cDNA (complementary DNA). From the -cDNA, it will make a complementary strand of DNA that is +.

Protein Synthesis

- Generate proteins for assembly
  - Translation
    - DNA: Require the synthesis of an mRNA that can be translated
    - Single stranded RNA = 1 strand; + or –
      - The (+) strand represents an RNA that can be translated
      - The (-) strand represents the antisense of the mRNA; it cannot be translated
        - » Requires the synthesis of the complementary strand
        - » - → +
        - » + → +

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Viral Growth – Infectious Cycle

- Attachment
- Penetration
- Replication of genome & Synthesis of viral proteins
- Assembly and Release

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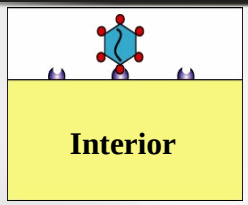
Attachment to the cell, it has to obtain entry (penetration). It will then carry out processes to replicate the genome and synthesize proteins. They then have to use strategies to be released from the cell, and then restart the process on a new cell.

Attachment and Penetration



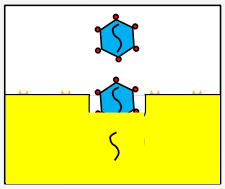
- Bacterial viruses – Naked
  - Attachment to receptor
    - Receptor determines **tropism**
  - Injection of genome

369

The spikes (viral receptor) are analogous to a key that only allows entry to certain rooms. The viral receptor has to be recognized by a corresponding cellular receptor. It then injects its genome into the cell.

Attachment and Penetration

- Naked viruses
  - Attachment to receptor
  - Endocytosis of naked virus
  - Release of genome

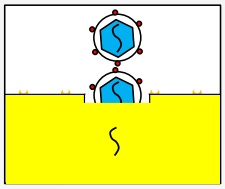


370

They evolved a strategy to attack to a receptor associated with endocytosis. This causes it to engulf the entire virus. The naked virus acquires an envelope after being engulfed. Theres no indication on the outside of the cell that it has been infected by a virus. The virus has to go through steps to release its genome. Uncoating-to get rid of the envelope, decapsidation=to get rid of the capsid

Attachment and Penetration

- Enveloped viruses
  - Attachment to receptor
  - Endocytosis of env. virus
  - Uncoating
  - Release of genome

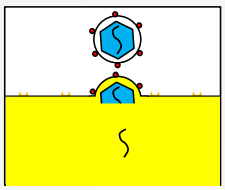


371

Associated with endocytosis, this causes an enveloped virus to acquire a 2nd lipid bilayer. Uncoating to remove both bilayers, decapsidation to remove the capsid and release the genome.

Attachment and Penetration

- Enveloped viruses
  - Attachment to receptor
  - Fusion of envelope
  - Penetration of nucleocapsid
  - Release of genome

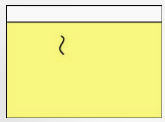


372

The envelope is identical to that of the cell membrane, the envelope is derived from the cell membrane, they are one in the same. Given that the bilayers are identical, they fuse and what ~~enters the cell is only the nucleocapsid. Uncoating hap~~pens at the same time as penetration. whatever proteins the cell had on the membrane stay on the membrane on the outside of the cell. It then has to go through decapsidation to release its genome.

Assembly and Release

- Capsid is assembled around the genome
- Disintegration of plasma membrane
- Release

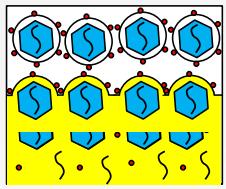


373

They will replicate the genome and it will act as a scaffold around which they grow their capsid. Once they have accumulated enough viruses in the cell, they will cause lysis of the cell. In bacterial cells, They disentegrate the membrane and break down the cell wall. If its a eukaryotic virus, lysis will be accomplished by disintegrating the plasma membrane.

Assembly and Release

- Synthesis of proteins of viral spikes
- Migration of proteins of spikes to the membrane
- Assembly of capsid
- Insertion of genome
- Migration and exocytosis of virions
  - Acquire envelope



374

They will export the spike proteins to the surface of the membrane, there is a lot of evidence that this cell was infected. Capsid is assembled independent of the genome. Release will done by exocytosis. Release is not associated with death of the cell, they can continuously create new viruses. The envelope will the be the cell membrane with the spikes. The cell will eventually die, but not due to release. These processes are not possible if they have a cell wall.

Viroids

- Naked single circular strand of RNA that codes for a single protein
- No protein coat
- Replicated by RNA dep. RNA pol.
- Most infect plants
- Only one known to infect humans
  - Hepatitis D
    - Requires coinfection by Hepatitis B

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Positive RNA, no capsid, RNA only codes for one protein. Most infect plant, there is one known to humans.

Prions

- "Infectious proteins"
- Normal proteins (PrPc) that get converted into an alternate configuration by contact with other prion proteins (PrPsc)
- No DNA or RNA
- Inherited and transmissible diseases
  - Spongiform encephalopathies
    - Sheep scrapie, Creutzfeldt-Jakob disease, Kuru, mad cow disease

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They are not organisms, they can cause disease and can be transmitted. Every mammal has this within their genome to make this protein. They usually make the normal version PrPc if they are not sick. If it comes into contact with an abnormal version (folded differently) it will get converted into the abnormal folding.
You can acquire the disease if there is a mutation in your genome, and transmit this to their offspring. It is also transmitted by ingestion. All your normal proteins will be converted to the abnormal when they come into contact.
The disease if named based on what organism the disease is found in.
Kuru: you got it by eating another human with the disease.

Viruses Within Everyone's Reach

Human papilloma, Influenza and the Human Immunodeficiency Virus

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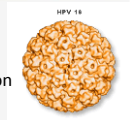
Human Papillomavirus

- Small naked icosahedral
  - 51 types known
    - Types 6, 11, 16 and 18 are the most common
- Tropism:
  - Differentiating cells of the epidermis
- Receptor:
  - unknown
- Double stranded DNA genome
  - Only 6 genes



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Naked virus, small DNA virus, capsid shape:icosahedral
Tropism, what cells does it infect,
It is very dependant on this host so it requires actively growing cells.
It affects much deeper layers of the skin, they are the actively growing cells.

HPV Infectious Cycle

1. Attachment to cellular receptor (infection has been initiated)
2. Endocytosis
3. Penetration of enveloped virus in the nucleus
4. Fusion, uncoating, release of genome
5. Transcription and synthesis of early proteins
   - Non structural regulatory proteins
6. Recruitment and modification of host's DNA polymerase
7. Replication of viral genome
8. Transcription and synthesis of late proteins
9. Assembly of capsid
10. Packaging of genome
11. Cell lysis and viral release

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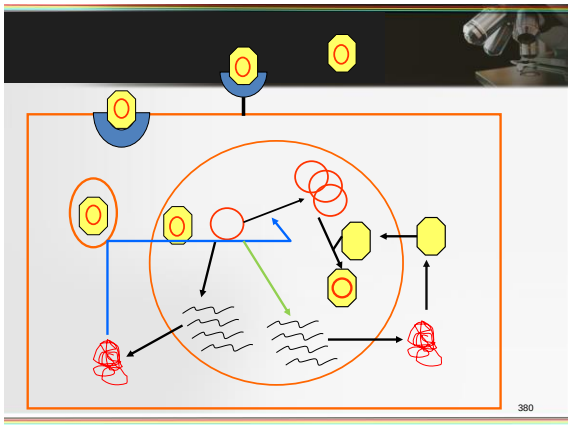
The virus penetrates the cell by endocytosis, the whole viral ~~particle enteres the cell and acquires an envelope. It~~ enters the nucleus by fusing with the membrane of the nucleus. The ~~envelope it acquired allows it to do this. Once in the~~ nucleus, you get decapsidazion, the virus is released in the nucleus. It ~~then carries out transcription with the cells machinery~~, it will create early proteins (do not contribute to the structure of the ~~virus) the goal of these proteins is to monopolize the~~ replication machinery of the cell. Some of these proteins with ~~be used to modify the host's DNA polymerase, The h~~osts polymerase will preferentially replicate viral DNA. The genome ~~of the virus is being replicated, synthesis of the prote~~ins, they will make capsid proteins to allow the virus to makes its ~~capsid, it will introduce its DNA its the capsid. There~~ will be cell lysis, the whole process will repeat itself in a new cell.



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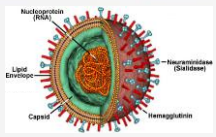
Once the virus is inside the nucleus, it cannot detect it anymore.

Influenza

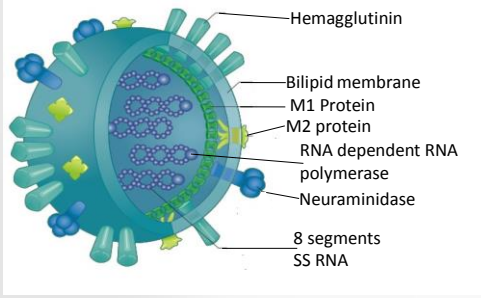
- Enveloped virus
- Three types
  - A, B and C
- Tropism:
  - Epithelial cells of the respiratory passage
- Receptor:
  - Sialic acid
- Segmented (-) RNA
  - 8 segments



381

It will bind to the sialic acid of the membrane of the cells. The virus has an antisense genome, so it cannot be translated by the cell. Influenza has 8 different RNA chromosomes that code for different proteins.

Influenza Virus - Anatomy



382

Acquires envelope from the cell, hemagglutinin recognizes the sialic acid on the cell. Neuraminidinade controls the release of the virus when it attached to the cell. In the capsid, all - non-coding RNA. Once it infects the cell, the cell cannot read negative RNA. The virus must bring with it an RNA dependant RNA polymerase, this will be able to read RNA and synthesize RNA. It has one RNA associated with each of its chromosomes.

Influenza Virus – Virion Proteins

- Hemagglutinin
  - Viral receptor
- M1 Protein
  - Scaffold protein - capsid
- M2 protein
  - Involved in uncoating
- Neuraminidase
  - Involved in release
- RNA dependent RNA polymerase
  - Allows viral transcription

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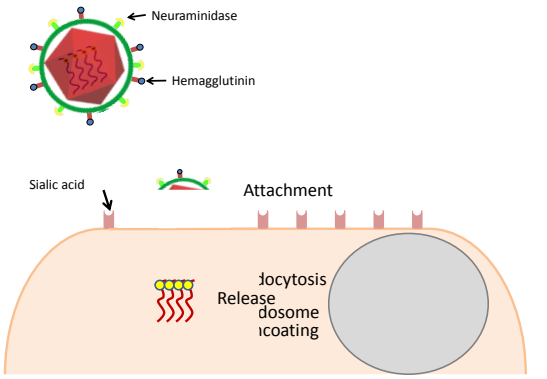
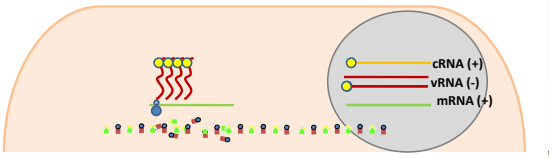
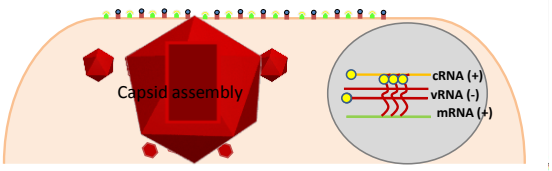


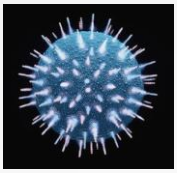
Influenza enters the cell by endocytosis, it will acquire a second envelope. It will be covered by 2 lipid bilayers=endosome. There will be uncoating of the virus, and decapsidation. With each RNA molecule there will be as associated RNA polymerase. The genome with the polymerase goes to the nucleus, the associated polymerase will read the RNA and synthesize a + RNA, mRNA. The cell can translate this and generate proteins. IT will also synthesize another + RNA, complementary RNA, cRNA, and this will be used to make copies of the -RNA, copies of the viruses genome. The spikes, hemaglutinin will be synthesized and exported to the membrane of the cell. This cell can then be recognized as infected because there are new proteins on the cell. Proteins needed to synthesize the capsid will be made, 8 of the RNA chromosomes will be packaged in the capsid. It will then be released by exocytosis, and acquire the membrane with the spikes. It can generate more than one virus in one cell.


cRNA (+)
vRNA (-)
mRNA (+)


Capsid assembly
cRNA (+)
vRNA (-)
mRNA (+)

HIV

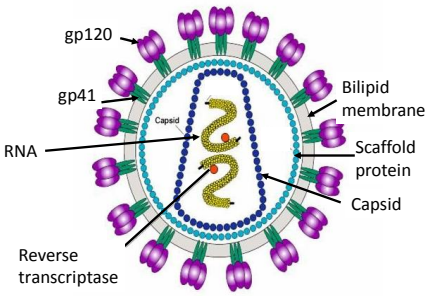
- Enveloped icosahedral virus
- Tropism:
  - Cells of the immune system
    - T, monocytes and macrophage
- Receptor:
  - CD4
- +RNA Genome
  - 2 copies



388

HIV can infect any cell that expresses CD4. HIV is a diploid virus, 2 copies of the chromosome.

HIV- Anatomy



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Envelope virus, lipid blayer derived from the cell. gp120 adn gp41 are the viral receptors. It is known as a retrovirus, it has a reverse transcriptase. HIV brings its own reverse transcriptase within the nuclear capsid.

HIV–Virion Proteins

- Capsid proteins – P6, P7 and P24
- Scaffold protein – P17
- GP120 and GP141 gp41
  - Make up viral receptor
- Reverse transcriptase
  - RNA dependent DNA pol. and DNA dependent DNA pol.
- Integrase
  - Allows integration of viral genome into cellular genome
- Viral protease
  - Allows maturation of viral polyprotein

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~~Whent he genome is transcribed and translated it cre~~ates a polyprotein (a big protein) that it chopped up into smaller proteins, this requires a viral protease.

Infectious cycle of HIV

1. Attachment of gp41/120 to CD4
2. Fusion of the envelope to cell membrane
3. Uncoating and release from capsid
4. Replication and integration of viral DNA genome
5. Transcription of mRNA by cell
6. Translation of polyprotein by the cell
   - gag, pol and env
7. Maturation of polyproteins by protease
   - gag → p6, p7, p17 et p24
   - pol →reverse transcriptase and integrase
   - env → gp120 and gp41
8. Capsid assembly
9. Packaging of genome
10. Exocytosis and release

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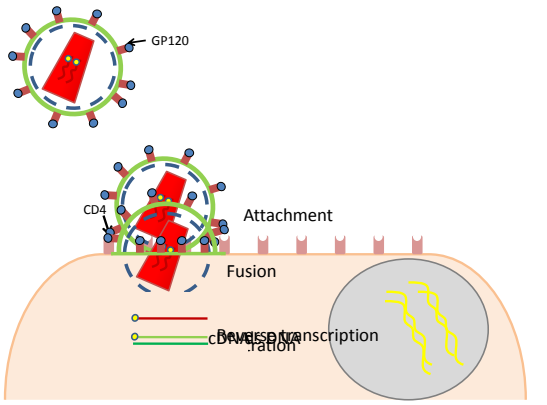
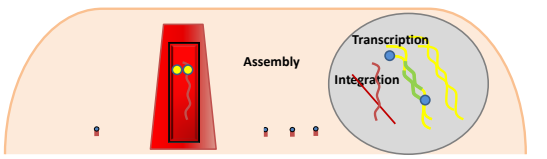
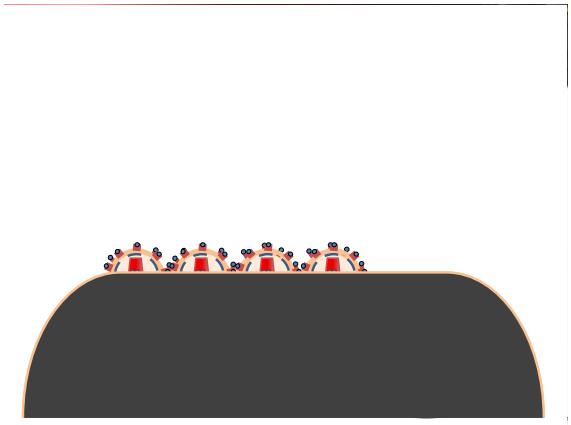
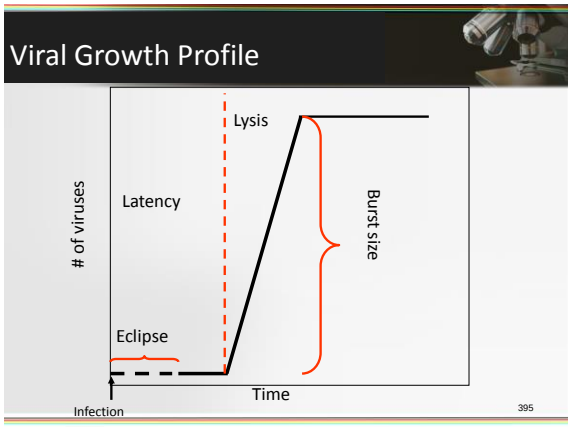
Uncoating is simultaneous to penetration. There is decapsidation to realease the genome. The genome is replicated and integrated into the host genome. The cell will then use its own polymerases to trancribe the virus genome and make viral mRNA. It will then be translated by the cell to make polyproteins, the viral protease will chop this up to make all the viral proteins. Once the proteins are synthesized, they can be enveloped in the capsid and released by exocytosis.



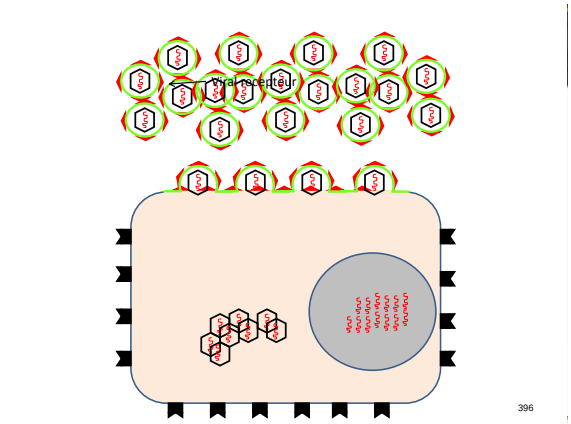
When the cell enters by endocytosis, the spike proteins remain on the membrane. It will create cDNA, the reverse transcriptase will create a complementary strand of the cDNA, it will become double stranded, integrase will allow it to be integrated into the genome of the host. The cell will transcribe the DNA to make mRNA, it will be translated by the cell, spike proteins will be exported to the membrane, it can now be recognized by the immune system as infected. IT will synthesize capsid proteins and assemble the capsid. The cell polymerase will transcribe DNA into RNA, two copies will be packaged in the capsid with a reverse transcriptase. The cell wil accumulate a lot of viruses, when they are exported by exocytosis they will acquire the spikes.







**EXAM arrow-when the virus meets the cell. If it meets the appropriate cell with the right receptor, infection is initiated. Following attachment, the virus is disassembled, the eclipse period is when you cannot detect the virus anymore. Eclipse period, dissassembly of the virus, RNA replication, proteins are synthesized. Once they accumulate viruses, the cell will start releasing them. Burst size: how many viruses were produced by one infected cell.



Eclipse period:virus does not exist anymore. Replication and translated, synthesize the proteins, stock assembly. When the virus reappears, you are out of the eclipse period. Once enough are accumulated, they are released.

Burst Size

- Definition:
  - Number of viruses generated by **one** infected cell following **one** complete infectious cycle
- Parameters:
  - Number of infected cells is determined according multiplicity of infection (M.O.I.)
    - M.O.I.: Number of infectious viruses available per **one** cell

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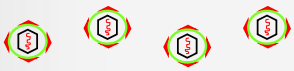
2 cells infected by 2 viruses, and they generate together 2 viruses, you would say your burst size is 1.
1 virus is the presence of 2 cells, the MOI is 0.5.
1 virus in the presence of 1 cell, the MOI is 1.

Multiplicity of Infection

Number of viruses = 4



M.O.I. = 4/6 = 0.67
or
4 of 6 cells will be infected

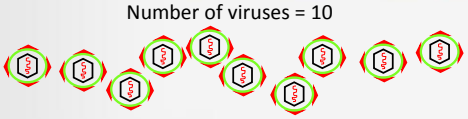
Number of cells = 6

•398
398

6 cells with 4 viruses, 4 cells will get infected. MOI: 4/6. If you get a number that is less than 1 when calculating MOI, you can conclude 2 things: not all cells will get infected. 2. the number of cells that will be infected is equal to the number of viruses.

Multiplicity of Infection

Number of viruses = 10



M.O.I. = 10/6 = 1.6
or
6 of 6 cells will be infected
Number of cells = 6

399

The number is greater than or equal to one, you can conclude 3 things. 1. all cells will get infected. 2. no cell will remain uninfected. 3. the number of infected cells will be equal to the number of available cells, no the number of viruses.

Calculating the M.O.I.

- 10^8 cells are exposed to 10^4 viruses
  - What is the multiplicity of infection?
- How many cells will be infected?

400

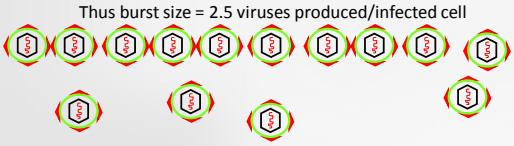
Calculating Burst Size

Number of viruses produced =10

or

10 viruses/4 infected cells

Thus burst size = 2.5 viruses produced/infected cell



Number of infected cells = 4

401

Calculating Burst Size

- M.O.I.=0.0001
- Number of cells = 10^8
- After 10 hours there are 10^6 viruses
  - What is the burst size?

402

Burst size:100, every cell produced 100 viruses, you have 100X more viruses than you did at the beginning. At the beginning of the second cycle, the MOI will be 0.01 (multiplied by 100) MOI, less than 1, not all cells will get infected, given that there are cells that remain uninfected a second cycle will start. 10^4 cells are infected.

Counting and Typing Viruses

- Direct count
  - Determines the total number of viruses (infectious and non infectious)
- Plaque assay
  - Determines the number of infectious viruses (analogous to viable count)
- Hemagglutination assay
  - Determines the total number of viruses
  - Only possible with hemagglutinating viruses
    - Which have hemagglutinin – Ex. influenza (binds to red blood cells)
    - Infectious and non infectious
- Hemagglutination inhibition assay
  - Antigenic typing of hemagglutinating viruses (want to know what is the virus type)

403

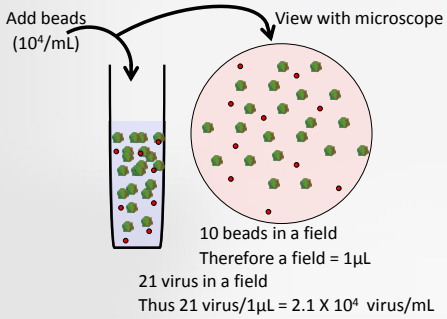
Direct Counts

- Negative Staining
- Electronic microscope
- Does not discriminate between infectious and non-infectious particles
- Useful for viruses that cannot be grown in the lab
- Use beads at a known concentration to obtain an estimate of the volume of a field of vision

404

Most viral pathogens cannot be grown in the lab, this is the only strategy we have to count them.
Use something of a known concentration to calculate the concentration of an unknown.

Viral Direct Counts



405

You add to the sample some counting beads to have a known concentration. You look at the sample under the microscope. You first count the number of beads you observe. ex. you see 10 beads, 10^4 beads represents 1 ml, you cross multiply. You then know 10 beads represents 0.001ml. You count the number of viruses, you find out the number per 1 ul, you then multiply this to get the concentration in mL.

Plaque Assay

- Infect monolayer of cells or bacterial lawn with different dilutions of virus
- Determine the number of plaques for each dilution
  - Each plaque is the result of a single viral particle infecting one cell initially
    - 1 **PFU**

406

The assay must represent what the virus usually infects, you will add different dilutions of the virus. When the virus infects the cells, you will generate a plaque. You determine that 1 plaque is equal to 1 virus infecting 1 cell.

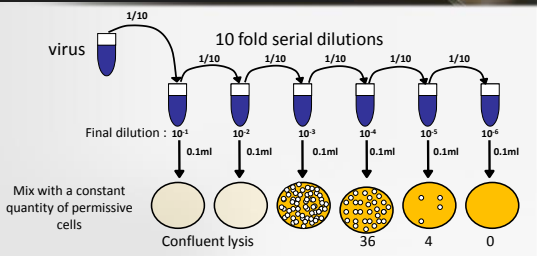
Plaques

- Localized cytopathic effect
  - Cell lysis
- Each plaque represents an infection site
  - Each infection site is initiated by a single infected cell

407

The virus completed many cycles until it generated a whole in the lawn. The MOI must have been less than 1 because not all cells are infected. If it was more than 1, all the cells would have been infected and just 1 big whole. Each plaque represents an infection site.

Plaque Assay



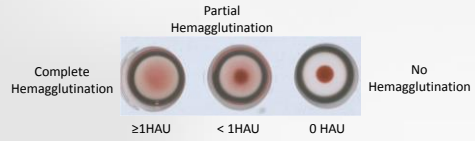
36 PFU for 0.1ml of a 10^{-4} dilution
Original conc. = 36PFU/0.1 X 10^4 = 3.6 X 10^6 PFU/ml

408

You can samples with each dilutions, you count your plaques. 36= 10^-4 dilution, you cross multiply to get your original concentration.

Hemagglutination Assay

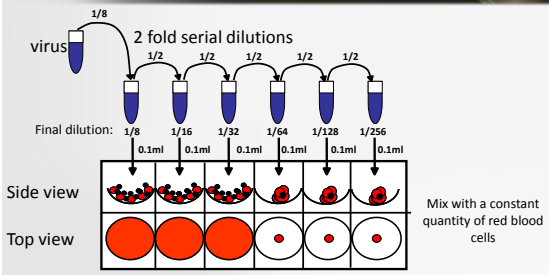
- Measures the minimum quantity of virus required to agglutinate all the red blood cells
  - 1 hemagglutination unit (HAU)



409

If you have complete hemagglutination, it is more than 1HAU. A button, a compact group of red blood cells. In this situation you will have 0HAU. If there is a button and diffuse precipitate, you have less than 1 HAU.

Hemagglutination Assay

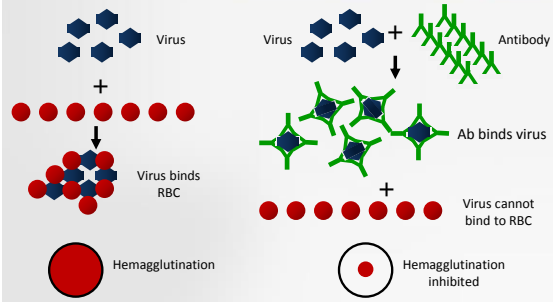


The most dilute sample with complete hemagglutination = 0.1ml of 1/32 = 1HAU
Original conc. = 1 HAU/0.1 X 32 = 320 HAU/ml

410

You add a different dilution to a FIXED number of red blood cells, observe the results. When you look at your assay, you look at the most dilute sample that gets complete hemagglutination. 0.1ml equals 1 HAU.

Hemagglutination Inhibition Assay



411

If you expose teh virus to an antibody before the reb blood cells, either the antibody will not recognize the virus and you will get the same result. If the antibody does recognize it, it will bind to the spikes on the virus, the virus will not be able to bind so there is no longer any hemagglutination. This is called a neutralizing antibody.

Antiviral Drugs

- Several antivirals are prodrugs
  - They must be phosphorylated by viral enzymes to be activated
- Must act against an essential viral function
- Must have an acceptable toxicity for the host

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Given that viruses are dependant on the host, it is very hard to target the virus and not the host. Most antivirals these days are prodrugs. They are an inactive form of the drug. In order to work they have to be activated. They usually require viral specific enzymes to be activated. If its an uninfected a host, it will have little to no effect. They have to kill the infected cell in order to kill the virus, so they always have some level of toxicity.

Antiviral Drugs

- Created to inhibit functions which are essential to the viral infectious cycle
  - Entry
    - Attachment
    - Penetration
  - Uncoating
  - Replication
  - Protein maturation
  - Assembly
  - Release

413

Antiviral Drugs

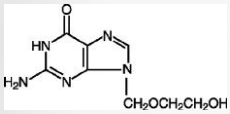
- Current antivirals have no effect on viruses which are not reproducing!
- A host immune response is essential for recovery from a viral infection

414

They only act on the viral cycle, not on the virus itself. All antiviral drugs are bacteriostatic, as soon as you remove the antiviral drug the virus will start again. The drug will stop the viral cycle but not the virus, the immune system must get rid of the virus. If the immune system does not get rid of the virus after being treated, the cycle will start again.

Current Antivirals

- Purine and pyrimidine nucleoside analogs
  - Ex. Valacyclovir
    - Prodrug of acyclovir
    - Guanine analog
  - Inhibits polymerase and reverse transcriptase



415

A prodrug, when activated that will prevent certain polymerases, reverse transcriptase. They will prevent our polymerases, but since they are only activated by viral enzymes, theoretically it should only affect infected cells.

Mode of Action of Acyclovir

Acyclovir + ATP —(Viral Kinase)→ Acyclovir phosphate

Acyclovir phosphate —(Cellular Kinase)→ Acyclovir triphosphate

Acyclovir triphosphate → Incorporated in DNA
Inhibits replication

Similar mechanisms for all nucleoside analogs

416

Inhibits polymerases, kills the cell. NOT the virus.

Current Antivirals

- Uncoating inhibitors
  - Ex. Amantidine
    - Inhibits M2 protein of influenza
- Release inhibitors
  - Ex. Oseltamivir (Tamiflu)
    - Inhibits neuraminidase of influenza
- Protein maturation inhibitors
  - Viral protease inhibitors (**PI**)
    - Ex. Atazanavir

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None of these drugs are absolutely specific to the virus.

Antiviral Drugs

- Non desirable characteristics associated with antivirals:
  - Low selective toxicity
  - High therapeutic dose
  - Low toxic dose
  - Low therapeutic index

418

High theurapeutic dose, when you ingest the drug, the drug has to target the cell, it cannot discriminate between a normal cell and an infected cell. Toxic dose is usually very low. We very rarely use antiviral drugs for these reasons.
10^8cells infected tihe 10^2 virus. After 1 hour, 5x10^3 viruses produced. What is initial MOI: 10^-6.
How many cells get infected: 10^2
What is burst size: 50
How many cells remain uninfected: 10^8.
What is MOI for second cycle: viruses= 10^2x50=5000
The MOI is 50x 10^-6.
Viruses at the end of second cycle:2.5x10^5.
(EXAM)How many cycles can be initiated: 5
MOI is less than 1, so theres more than 1 cycle.
10^2= cycle #1
you will generate 50X more viruses
10^-6 x50. This is not the last cycle, <1 cycle #2
50X whatever it was before=2.5x10^-4. still less than 1, cycle3 repeat until you get an MOI greater than 1. That will be the last cycle. When everything is done, how many viruses will you have? 50x10^8.

Immunology

The Immune System

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Immunity

- All the mechanisms used by the human body to protect itself against foreign invaders – The **antigens**
  - 3 lines of defense :
    - 1st – The barriers
      - **Innate system**– no education
      - Active at all times
      - Goal: prevent entry
    - 2nd –Phagocytic system
      - **Innate system** – no education
      - Prevent propagation
      - Destroy foreign entity
      - Recruit and activate the 3rd line of defense
    - 3rd - Acquired system
      - **Must be educated**
      - Destruction/Neutralization specific to the entity
      - Prevent future invasions

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Foreign invader=antigen=something foreign to the human body.
1st barrier-innate-you have immediately form birth, always active, prevent entry into the body (not cell).
2nd -part of the innate system (avaiable since you've been conceived) comes into action if the first line doesnt work, something gained entrance, wants to destroy the antigen and prevent it from spreading. Activates the 3rd line, sort of like an alarm.
3rd. adaptive system, has to be educated, anything other than who it is is considered an antigen, not active immediately. Education happens during 7 months into gestation, to 2 months after birth. It learns to inactive anything that is foreign. Capable of remembering, when it meets the same invasion it will act faster and better, it is able to improve.

Characteristics of the Two Systems

Innate	Acquired
• Antigen independent	• Antigen dependent (it will only activate when it has assessed that there is an antigen.
• Immediate	• Delayed (can take upto a month before it actually operates after detecting the antigen)
• Not specific to the antigen	• Specific to the antigen (evaluates the best way to deal with the antigen)
• No immunological memory	• Immunological memory (the next time it will act faster and better)

always active
act the same way
idependant of what
the antigen is

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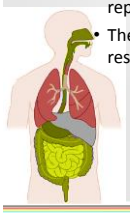
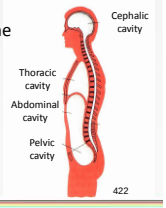
1st Line of Defense - Barriers

- Prevent entry inside the body
  - The interior is sterile unless there is an infection
    - In the man, all cavities represent the interior
    - In the woman, all the cavities, except for the pelvic cavity, represent the interior
    - The gastrointestinal tract and part of the respiratory tract are external



green part of respiratory tract is exterior



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Barriers – The Skin

Physical	Chemical
• Thick keratinized cells	• High salt concentration
• Sloughing of cells	• Fatty acids inhibit bacterial growth
	• **Defensins**-peptide antibiotics
	• Low pH
	• Antimicrobial produces by microflora

423

Surface of the skin is sort of a wall the prevents entrance. Constantly shedding skin, so if something falls on your skin it will eventually fall off. Very difficult for a microorganism to establish itself. Your skin also has a very high salt concentration. Antibacterial fatty acids, defensins. It has a low Ph, some pathgogens prefer a neutral pH. Microflora inhibit microbial growth.

Barriers – The Eye

Physical	Chemical
• Tears – Wash/elimination	• Tears-Lysozyme • Lactoferrin – Protein that binds iron • Low a_w

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Lysozyme:

Lactoferrin:makes sure there is no free iron available, lactoferrin binds to iron and makes it unavailable, iron is necessary for many type of microbial growth,

Low water concentration. (Aw)

Barriers – Respiratory and Gastrointestinal Tracts

Physical	Chemical
• Nasal hairs and cilia – Filtration • Mucus secretions – Traps particles • Mucociliary escalator • Sneezing and coughing • Secretion of surfactants by A cells – Prevents attachment	• Lysozymes • Lactoferrin • Antimicrobial peptides • Thiocyanate secreted by salivary glands • Gastric acids • Bile salts • Digestive enzymes • Microflora

425

Filtration prevents particles from propagating farther down the respiratory tract. Mucociliary escalator constantly moves the mucus back up. Sneezing or coughing is the alternative if the escalotor isnt working. This is used to expel things that have built up, not re-enter the body.

Surfactants make it very difficult for microbes to establish themselves.

Thiocyanate prevents respiration

Gastric acids make the pH very low, greatly dissolves lipids and bilipid membranes. Bile salts are even more effective as dissolving lipids. Digestive enzymes, digest invaders. The microflora will monopolize all the resources, food, space, creates a lot of antimicrobial compounds to prevent other microbes from establishing themselves.

Barriers – Natural Flora

- Microorganisms located within specific sites of healthy individuals
  - All external surfaces are colonized
  - Competition against pathogens
  - Source of pathogenic organisms (**Opportunists**)
  - Stimulates the immune system
    - Stimulates the production of antibodies against **shared epitopes** common to other pathogens

426

Microflora will be different depending on the area of the body it is found on.

Pathogen:a microbe that ALWAYS causes harm, you will never have a pathogen that it part of the microflora. The flora represents a source of opportunistic pathogens. There are 3 stipulations between the microflora and the immune system. If the microorganism goes to a different site (wrong area of body), the immune system will automatically attack. The immune system will tolerate the presence of the flora as long as it does not go beyond a certain level. If this happens, this immune system will attack. Under no circumstance is the microflora allowed to access the interior. The immune system will attack immediately if this happens. The microflora is recognized as foreign to the immune system, to its serves to educate the immune system. They have characteristics that are similar to pathogens. These shared characteristics are called epitopes.

Innate system – 2nd Line of Defense

- Cells and serum substances predisposed for an immediate attack of exogenous and endogenous antigens
  - Alternative complement cascade
  - Blood cells– Leucocytes
    - Polymorphonuclear cells – Granulocytes
    - Monocytes/macrophages
    - Natural killer cells
  - Inflammatory response

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endogenous-antigen inside of the cell, exogenous-outside of the cell. LEucocytes-white blood cell, monocytes and macrophages and in the same cell, macrophages are in your tissues, monocytes are in your blood.

Alternative Complement Cascade

- Involves a collection of serum proteins
  - Complement proteins:
    - C2, C3, C4, C5, C6, C7, C8 and C9
  - Accessory proteins: B and P
- Initiated by binding to components common to a great variety of bacterial cells:
  - Alternative: Binding of C3b to lipid A or techoic acid

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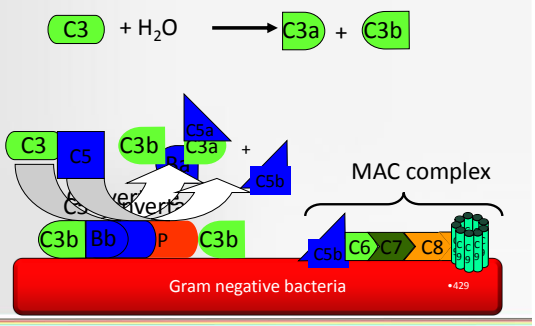
proezymes: c2-9, they are inactive
To initiate the cascade is by binding to something that is common to a lot of different bacteria, (ex. lipid A found on all gram negative bacteria).

Alternative Complement Cascade



A gram negative bacteria enters your cells, it has lipid A. C3 proteins gets spontaneously hydrolized with water to C3a abd C3 b, this happens all the time independent of whether or not there is an invader present. This hydrolysis is very inefficient, produces very little of the active form of C3. The C3b will bind to the bacteria, the cascade is initiated. B will then bind C3b. This will only happen if C3b bound to the bacteria. If B binds, then B is cleaved into Bb and Ba. Bb stays bounds, Ba leaves. P then binds to the complex (Bb +C3b, not bacteria). Only a very little portion of bacteria get bound by C3b bc the hydrolysis is very inefficient. The cascade has amplification steps to deal witht he increasing number of bacteria. C3 convertase (the whole complex), will do the exact same thing as the beginning, the convertase will activate the C3, much more efficient than hydrolysis. you will create very high levels of C3b. C3b will bind to a greater number of bacteria, initiate the cascade. Or the C3b will add itself to the complex, the second complex is another amplification step that we call C5 convertase. This will be used to activate C5. It will be converted to C5a and C5b. C5b can bind ANY LIPID BILAYER. It will either bind to the

same bacteria that initiated the cascade, or other bacteria. Or it can bind to a parasite, a enveloped virus. Binding of C5b is independent of the complex. If C5 is bound to a lipid bilayer, there will be C6, C7, C8 and multiple C9. This is called the MAC (membrane attack complex), it attacks membranes. This cannot occur on gram positive bacteria (outer layer is sugars, not lipid bilayer, not naked viruses, MAC complex has to occur on a membrane. This is used to create a channel/pore through the lipid bilayer. Gives access to cell wall. Peptide antibiotics and such will be able to act on these cells, this gives us access to the cell wall.

Consequences

- Opsonization
  - C3b and C4b
    - Bacteria, viruses and parasites
- Anaphylatoxins
  - C3a and C5a
    - Activate granulocytes and monocytes
      - Induce the inflammatory response
      - Degranulation
- Membrane attack complex (MAC)
  - Osmotic lysis
    - Gram negative bacteria – Allows lysozyme to reach cell wall
    - Parasites – Loss of solutes

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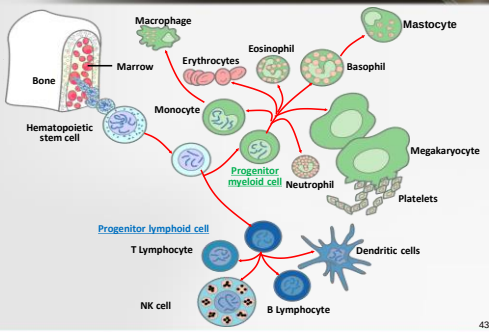
Opsonization is synonymous to labelling. Tells the body that this antigen is foreign. Anaphylatoxins, C3a and C5a, they induce an inflammatory response.released in the serum, bind to white blood cells, causes the imflammatory response, and causes the cells to empty their contents into your body. They are used to tell the body that something is wrong.
MAC complex, causes osmostic lysis, causes cell to lose its

Blood Cells



431

All these cells come from a hematopoetic cell, from your bone marrow. Blue ones-lymphocytes. Those is green predominantly work as the 2nd line of defense. They act immediately. They are incapable of descriminating who they are as opposed to anything else. The lymphocytes work predominantly in the 3rd line of defense, they are capable or learning, discriminating, identifying foreign cells.

Polymorphonuclear Cells - Granulocytes

- Neutrophil
  - Phagocytic cell
  - High concentrations are indicative of an infection
  - Normally absent in healthy tissues
- Eosinophil
  - Fights parasitic infections
  - Involved in allergic reactions
- Basophile/Mastocyte
  - Involved in allergic reactions

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Phagocytic cell can ingest and digest, following infection they start proliferating, these cells and inclusively found in the blood stream, found also in the tissues if their is an infection.
Eosinophil, they are not phagocytic, they take care of parastic infections bc they are too big to be ingested. They release to the exterior digestive enzymes, causes an inflammatory response.
Basophile: in blood stream, Mastocyte: in the tissues releasing granules and initiating the inflammatory response.

Roles of Granulocytes

- Destruction of invading entity
  - Phagocytosis
    - Enzymatic digestion
    - Chemical destruction
      - Antiseptics: peroxide and hypochlorite
  - Release of granules
    - Inflammatory mediators
    - Digestive enzymes
    - Antiseptics: peroxide and hypochlorite
- Recruit non granular leukocytes
- Recruit lymphocytes – 3rd line of defense

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Release granules, there will be mediators of the inflammory response, release antiseptics and digestive enzymes. These chemicals cannots tell the difference between bacteria and your tissues, this response is very damaging to the host and the antigen. This also acts as an alarm to call non granular leukocytes, they use chemotatic compounds, these compounds attract other cells. You will also recruit lymphocytes that are mainly the 3rd line of defense.

Non Granular Leukocytes

blood tissues

- Monocytes/Macrophages/Dendritic cells
  - Phagocytes
  - **Antigen presenting cells (APC)**
    - Ingestion/Digestion/Presentation
      - Presentation of epitopes from exogenous antigens
      - Presentation on **MHCII**
      - Presentation **T helper** lymphocytes
    - Activate the 3rd line– Acquired response
- Natural killer cells (NK)
  - Lymphocyte which kills infected and tumor cells

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They are APC, their job is to ingest whatever, could be an antigen or a dead cell, they ingest anything. Anything they digest, they will present. To be able to present, they use MHCII. If the ingested something, it has to exogenous. MHCII is used to present anything that is exogenous, it is only found on these cells. When they present to the TH cell, it will evaluate, it is this me? Either it doesnt recognize what it being presented, then it is not a threat, no action needed. If it does recognize that its an antigen, it must mount and attack. 3rd line of defense is initiated. Chose the most appropriate way to deal with a foreign invader. The APC does not discriminate, the TH cell does it. NKs constantly patrol the body, deals with endogenous antigens. Kills a cell that has been infected, or kills abnormal cells (ex. tumors).

NK Cells

- Kill cells which lack or have low levels of **MHCI**
  - Virus infected cells
  - Cancer cells
- Two cell surface receptors
  - Inhibitory receptor
    - Interacts with MHCI of nucleated cells
  - Activating receptor
    - Interacts with stress induced glycoproteins

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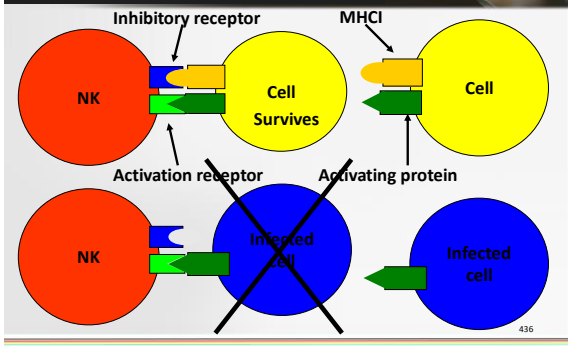
Viruses inhibit the hosts cells protein synthesis, causing low MHC1, Cancer cells go to a more primitive state, causing them to have low MHC1.
MHc1 is typically found on all cells that have a nucleus.

NK Cell Activity



If the cell doesnt have MHC1, theres nothing to interact with the inhibitory receptor, only the activation receptor, the NK cell will attack the infected cell.

Inflammatory Response

- Goals:
  - Neutralize or destroy the invader
  - Alert the third line of defense
- Initiators
  - Tissue damage
  - Infections
  - Toxins
  - Anaphylatoxins
- Symptoms
  - Redness, heat, pain, edema, loss of function

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The inflammatory response is also alerting you that theres a problem so you can help, sleep, go to the doctor etc..
You don't need an antigen to initiate it, could be due to physical harm to the body. (cut, bruises, etc...)
Increased blood flow=makes the area red.
2 types of heat, localized heat due to blood flow increase, inflammatory response will act on your thermoregulatory response, increase temperature, to make conditions unfavourable for growth (most bacteria are mesophiles).
Pain: advising the individual there is a problem.
Adema: chemicals make blood vessels much mroe permeable, allowing the fighting cells to leave the blood and into your tissues. Liquid in your tissues=edema.
Try to prevent movement, minimize propagation of the invader.

Physiological Inflammatory Response

- Release of mediators by leukocytes:
  - Histamines
    - Cause vasodilatation and vasopermeability
      - Allows passage of leukocytes from the blood into the tissues
  - Prostaglandins
    - Act on the thermoregulatory center (hypothalamus)
      - Fever
  - Cytokines
    - Chemotaxis and activation of leukocytes

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Chemotaxis: attract other leukocytes, informing other cells where the problem is, activates the leukocytes to make them more efficient.

3rd Line of Defense – Acquired System

- Characteristics:
  - Specific
  - Acquires memory after a first encounter
  - Improvement of response for subsequent encounters (learns)
  - Discrimination between "Self" and Non-Self"

439

non-self= antigen, an invader, something it has to fight off, not synonymous to harmful.

Non-Self

- What is external and which gains access to my interior is probably non-self
- Most of my cells are labelled to identify myself
  - **MHCI** and **MHCII**
- I have cellular receptors which recognize epitopes which I do not possess
  - BCR and TCR
- I label what is non-self with opsonins
  - Complement proteins and antibodies

440

Different levels of MCH1 and MHC11 depending on the individual.
B-cell receptor and T-cell receptor, receptor of lymphocytes and that do discrimination, determine if its self or non-self. The repertoire of receptors are big enough to recognize EVERYTHING, anything that has, does, or could exist. The problem is that everything includes me... . So they can recognize everything except self cells. So if they recognize something, they automatically assume it is an antigen.
Opsonins-They can also label things as non self, tell the body to destroy them.

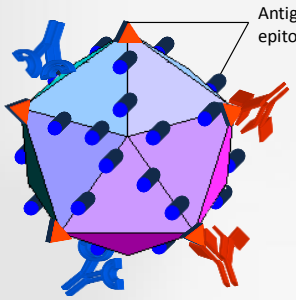
Non-Self

- Antigens
  - Entities recognized by receptors B (BCR) or T (TCR) lymphocytes
    - Exogenous antigens
      - Extracellular entities
    - Endogenous entities
      - Intracellular entities **synthesized** within the cell
  - Antigens have **epitopes** (or antigenic determinants) that interact with **paratopes** of BCR and TCR

441

These receptors recognize is the epitopes on the antigens. Paratopes is what recognizes the epitope.

Non-Self – Antigen



442

Red + blue=epitopes. The epitopes must be recognized by the cell receptors because they are non self.
The receptor is specific to the epitope, not the antigen. If it were to be antigen specific, it would have to recognize an epitope that is unique to the antigen.

Presentation

- Epitopes from antigens must be presented in order to be recognized as non-self

- **MHCI**
  - Present on most nucleated cells
    - Exceptions: red blood cells, neurons and spermatozoa
  - Presents epitopes from endogenous antigens to TCR of T_c lymphocytes
- **MHCII**
  - Present only on APC
    - Monocytes, macrophages, dendritic cells, B lymphocytes
  - Presents epitopes from exogenous antigens to TCR of T_H lymphocytes

443

If sperm had MHC1, the women would attack the sperm bc it would have differnt MCH1 then her. If something is synthesized in MHC1, presents on endogenous epitopes to Tc lymphocytes. Tc lymphocytes will evaluate whatever is being presented, they will discriminate. If they recognize it, they will determine its non-self.
MHCII. exclusively present on antigen presenting cells. Presents only exogenous epitopes. Has to be something that you ingest. MHCii will present any exogenous cell to Th lymphocytes, they will determine whether its self or non self.
Exam Q: B lymphocytes gets infected by a virus, on what MHC will it present? B lymphocytes have Both MCH1&2. MHC1 is present on all cells.

3rd line of defense – Two Fronts

Humoral response		Cellular response	
Target	Participants	Target	Participants
Exogenous Ag	T_H lymphocytes – TH2	Endogenous Ag	NK cells
	B Lymphocytes		T_H Lymphocytes – TH1
	Antibodies		T_c Lymphocytes
	Classical complement cascade		

444

Humoral response acts on extracellular antigens. B lymphocyte will produce antibodies that will deal with the antigen.
Cellular response: deal with things within the cell, synthesized by the cell. Th

Humoral Response

- The humoral response requires **two** signals to be activated
  1. A T_H (TH2) lymphocyte must **determine that an epitope** presented by **MHCII** of an APC from the innate system represents non-self
     - Consequence : T_H is activated
  2. A T_H (TH2) lymphocyte, which was activated, must **confirm** that an epitope presented by **MHCII** of a B lymphocyte represents non-self
     - Consequence : B lymphocyte is activated

445

MHCII presents only to TH2 cells. It will evaluate, the TCR will do this, if it concludes it is foreign, it will secrete compounds that will activate the TH cells.
A B lymph. must recognize something as being nonself, it will ingest it and present it on MHCII. It will present to Th cells, a second presentation to the cell that has already recognized it as non self. If it recognizes it as non self a second time, the TH2 will secrete compounds that activate the B lymph.

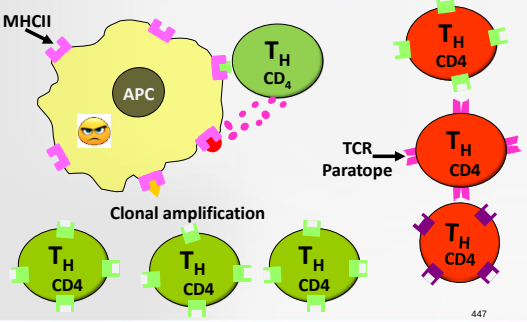
T_H Lymphocytes

- **One** T_H Lymphocyte has TCRs with **one** paratope which can recognize **one** epitope presented by the **MHCII** of APCs
  - Macrophages, monocytes, dendritic cells and B lymphocytes
- Responsible for the discrimination of non-self

446

They only recognize antigens that are presented to them on MHCII of antigen presenting cells (APC).
All t h cells are CD4

1. Presentation to T_H Cells

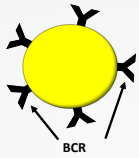


447

if recognition occurs, it will excrete cytokines that will activate that particular Th cell. It will undergo clonal amplification.

B Lymphocytes

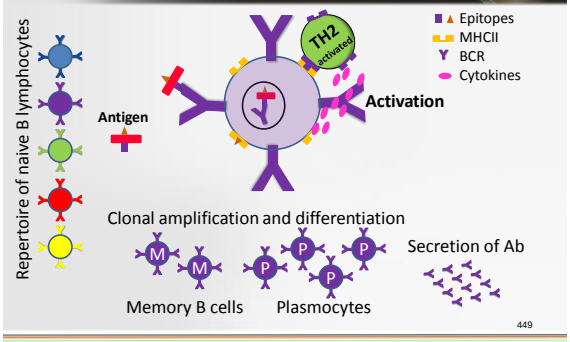
- Have **one** BCR which can recognize **one** epitope from exogenous antigens
- Do endocytosis of Ag-BCR complexes
- APC: Present epitopes on **MHCII**



448

BCR- cell surface receptor. Each BCR can recognize one epitope. recognizes and binds free antigens, doesnt have to be presented.
B lymphocyte, It will ingest the whole complex, following ingestion it will present it on MHCII. This will present only to TH cells.

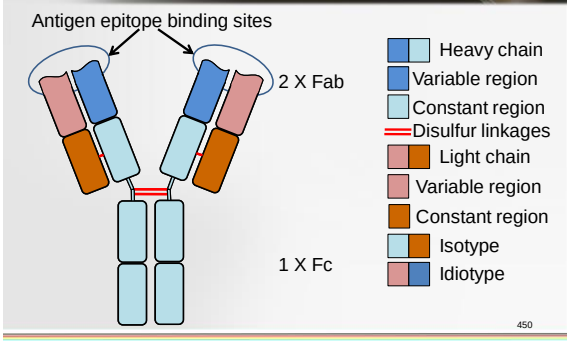
2. Activation of the Humoral Response



449

What its presenting on MHCII is not necessarily the epitope that it recognized, it could be that epitope or other epitopes present on the antigen. After the lB lymphocyte ingests, it will have to present to the TH cell through the MHCII, it has to be the TH2 that has already evaluated the antigen before. The Th2 will secrete cytokines that will activate B lymphocytes. The b cells will do clonal amplification.
Memory cells: on the second encounter of appropriate B lymphocytes and BCR, when it happens again it will happen much faster. The plasmocytes will secrete the Antibodies, that have that recognize the same epitopes, try to deal with the antigen.

Antibodies (Immunoglobulins)



450

Composed of polypeptide chain, composed of 2 chaines. Variable and constant region, 2 identical heavy chains that are linked by disulfide bridges. There is another polypeptide chain (light chain) is linked to heavy chain by disulfide linkage, there are 2 identical light chains, they also have a variable and a constant region. The constant region is called the isotype of the antibody, this confers the function of the antibody. There are 5 possible isotypes, and a given b cell can make any of the 5. All b lymphocytes make all the same 5 isotypes. The idiotype (the top part) contains the paratope that confers the specificity of the antibody, what epitope it can bind. All 5 isotypes will have the same idiotype. If you cleave an antibody, you will create an Fc (constant fragement) and 2 Fab (antibody binding fragment) Fab can still bind epitopes, but no function will be associated.

Antibodies *(cont'd)*

- Variable regions
  - Unique to each B lymphocyte
  - Ab Idiotype
  - Paratope region
  - Confers specificity towards epitope
- Constant regions
  - Ab Isotype
    - IgM, IgA, IgD, IgG, IgE
  - Confers mode of action of Ab

451

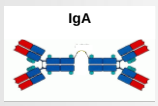
Isotypes

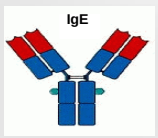
- Membrane bound: BCR
- Monomer; membrane bound: BCR
- Pentamer: Serum
  - First antibody secreted following a first encounter
- Serum antibody:
  - 80% of serum Ab
  - Crosses placenta

452

IgM: pentamer, secreted form, has a valency of 10, can bind 10 copies of an epitope, the monomer IgM and the igD have a valency of 2, can only bind 2 copies of the epitope.
igD, crosses placenta, gives protection to the fetus. also has a valency of 2.

Isotypes





- Dimer:
  - Associated with mucus linings
    - Urogenital tract
    - Respiratory tract
    - Gastrointestinal tract
  - Secreted in colostrum
- Monomer:
  - Membrane receptor of basophils and mastocytes

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IgA: valency of 4, antibody associated with all surfaces that have a mucus lining, all points of entry. If prevents entrance, adds to the barriers. Also secreted in breast milk, protection for the baby after the 2 months after birth.

Modes of Action of Antibodies

- Agglutination
- Neutralization
- Opsonization
- ADCC
- Degranulation of granulocytes
- Complement fixation
  - Classical complement pathway

IgM and IgA (valency >2)

IgM, IgA and IgG inactivation

IgG and IgM label things

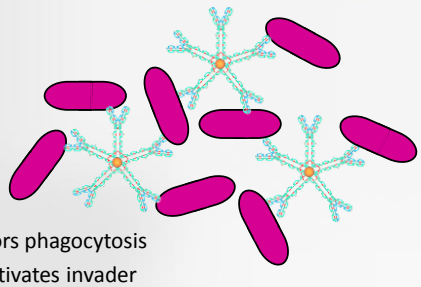
IgG and IgM antibody dependant cell mediated cytotoxicity. involves NK cells

IgE associate with granular leukocytes, causes degranulation

IgM and IgG initiates the classical cascade.

454

Agglutination

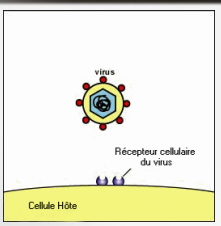


- Favors phagocytosis
- Inactivates invader

Cross link different antigens, favour phagocytosis, can lead to inactivation of the invader.

455

Neutralization



- Binding to essential components resulting in inactivation

Prevents virus from binding by binding to all of its receptors.

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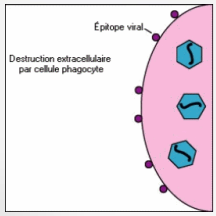
Opsonization

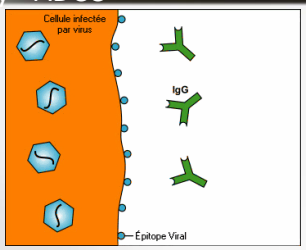


- Fc regions are opsonins recognized by phagocytic cells

457

anitbodies that recognize spike proteins on virus infected cells. Once the antibody binds, it acts as a flag to phagocytic cells, binds to the Fc receptor, it will empty the contents of the cell, it willl kill the cell and anything in proximity.

Antibody Dependent Cell Mediated Cytotoxicity - ADCC

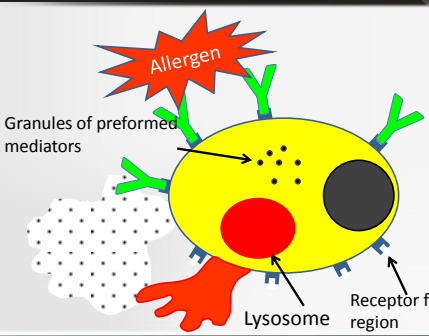


- Fc regions are recognized by receptors on NK cells

458

NK cell, has Fc receptors that can recognize bound antibodies, targeted killing, only kills the cell it has identified.

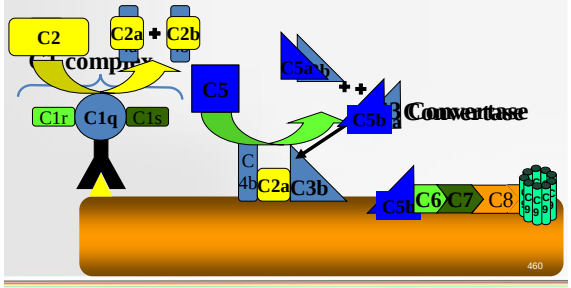
Degranulation



459

The IGe will bind to the FC cell region, if an allergen is recognized, it will release the inflammatory response.

Classical Complement Cascade



The antibody has to bind ot the epitope, it will alloy the binding of C1q, and then C1r and C1s, which is called the C1 complex. It allows to do 2 conversions, C2 to C2a +c2b, and C4 into C4b and C4a, C2a and C4b will bind. They will convert C3 into c3a and c3b, c3b will bind to the complex, you will then convert C5, C6, c7, C8, C9 etcc.. the same as the others, except this can only occur on the lipid bilayer.

Consequences of the Classical Pathway

- Opsonization
  - C3b & C4b
- Anaphylatoxins
  - C3a & C5a
- MAC
  - Osmotic lysis

461

Membrance attack complex (MAC)

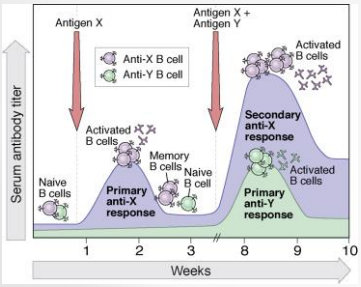
Immune Responses: 1st and 2nd Encounters

Element	Primary response	Secondary response
Maximal response	Weaker	Stronger
Antigen dose required	Relatively high	Low
Delayed response	Between 10-21 days	Between 1-3 days

462

1st and 2nd encounters of an antigen. You need more of an antigen to activate the response the first time it will take a long time the appropriate Ta cell to find the right Tr cell. Same to find the right b cell to find the right bcr. After this, you get clonal replication, so you have way more of these cells. So the second time, you have more probability to find the appropriate cells.

Immune Responses: 1st and 2nd Encounters



463

Exam question: Expose and ind. to a second antigen for the first time, but instead you see the response shown in purple. How is this possible? If the 2nd antigen has shared antigens with the 1st antigen, you'll get a response as if it were a second exposure. (crossimmunity)

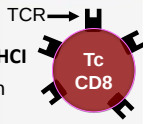
Cell Mediated Response

- The cell mediated response requires **two** signals to be activated
  1. A T_H (TH1) lymphocyte must **determine** that an epitope presented by **MHCII** of an APC of the innate system represents non-self
     - Consequence : T_H is activated
  2. A Tc (naive) lymphocyte must also **determine** that the epitope presented by **MHCI** of the same APC represents non-self
     - Consequence : Tc lymphocyte is armed

464

Endogenous means it found a way to get in the cell and found and replicate in the cell. There is a way to identify cells that have been compromized. MHCII only presents exogenous cells.
TH1 is doing the discrimination, it concludes it is antigen. The second cell called Tc lymphocytes. They are unable to kill (naive), to be able to kill, you have to arm them (remove the safety). If ANY cell, which gets infected by a pathogen and synthesizes proteins from that pathogen, anything synthesized by that cell will be presented by MHC1, (only presents endogenous), only presents to Tc cells, if i does recognize it will determine it is non-self. The Tc cell will be armed to kill.

Tc Lymphocytes

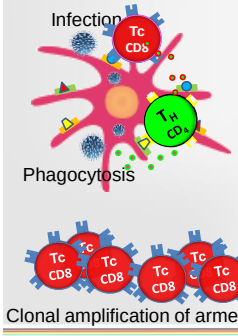


- Cellular receptor – TCR
  - Recognition of non-self presented by **MHCI**
  - **One** Tc lymphocyte has **one** TCR that can recognize **one** epitope complexed with **MHCI**
- Naïve Tc must be armed
  - **Two** signals are required :
    - Recognition by a TVR of a specific epitope complexed with **MHCI**
    - Activating cytokines produced by TH1

465

Does not recognize free antigen. In order to carry out their function, Tc must be armed- 2 signals.

Cell Mediated Response



Presentation of endogenous epitopes on MHCI

Presentation of exogenous epitopes on MHCII

Recognition by TCR of TH1 - activation

Recognition by TCR of Tc - arming

466

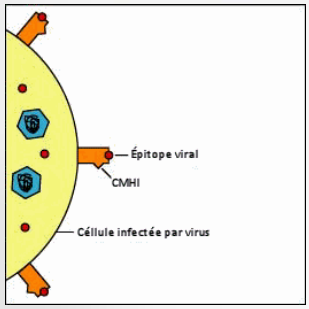
Dendridic cells, nucleated, they Have MHCI. Given that it is an antigen presenting cell, it also has MHCII. It can present both endogenous and exogenous antigens. This can present simultaneously to Th and Tc cells. They have receptors that allow them to be infected by most viruses. The virus will go through its infectious cycle, synthesizing viral proteins (endogenous antigens), The cell will present epitopes on MHC1. This cell can also do phagocytosis, it can ingest a cell infected by a virus (exogenous cell), this will then be presented on MHCII. Whatever is presented on MHCII, it will be presented by Th cell, if it recognizes it as nonself, it will be activated by the APC.

MHC1 is evaluated by Tc cells, if it determines it is non self, the APC will secrete cytokines to activate the Tc cell, the Tc will be amplified. They are ready to kill. They will asses with their TCR anything presented on MHCI, if they recognize it, they will kill the cell that presents them.

Q: The MHC1 and MHCII dont represent necessarily the same epitope.

There are viruses that can be presented on MHCII but never on MHC1 (vice versa).

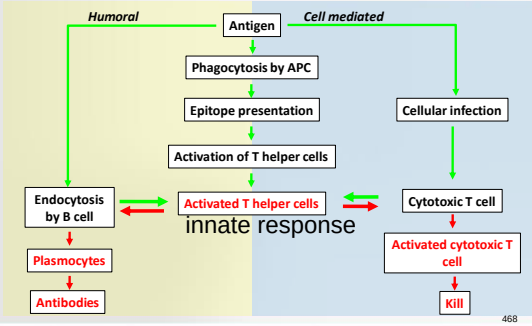
Attack by Armed T Cell



467

The T cell will secrete enzymes and proteins to kill the infected cell. After it has killed the cell it will look for another cell to kill.

Overview of Acquired Immune Responses



468

Innate response will activate the third line of response.

Attack of the Immune System - HIV

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HIV

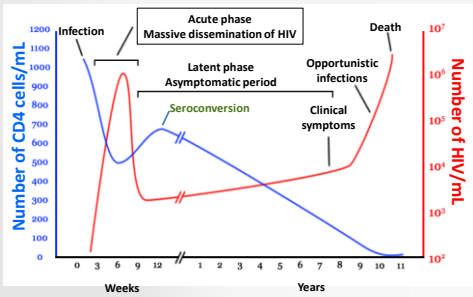
- Viral receptor
  - Gp120
- Cellular receptor
  - CD4
    - T cells, monocytes and macrophages
- Preferential replication in activated T cells
- Lifespan of a T cell which is actively replicating HIV: 2.2 days

470

Cd4 is found on t cells, monocytes, and macrophages. IT handicaps phagocytosis by APC and activating the T helper cells. Has a huge impact on humoral and cell mediated response.

HIV integrates its genome into the genome of the host cell. Anytime a t cell gets activated, that will simultaneously activate HIV. A tell cell that actively replicates HIV has a very short life span of about 2 days,

HIV Infection Time Course



471

Blue- cells targeted by HIV, CD4.

Red-number of HIV virus particles

WHen you get infected, the number of HIV viruses will increase, its killing off a lot of CD4 cells (a huge drop). This is about 3 weeks, it takes 3 weeks for the immune system to react. The cell mediated and humoral response will start acting, the HIV numbers will greatly drop. Consequently, the number of CD4 cells go back up. Partial recovery. The symptoms disappear and you enter a asympotomatic period, you are dealing with the infection. There will be a state of seroconversion, you will be sero-positive. This means you go to state that you have detectable antibodies against an antigen. Seropositive for HIV, not synonymous to saying someone is HIV positive or -. Seropositive-they have seen the antigen in the past, or it is there right now.

The body slows down the number of increase of HIV viruses, so there is a progressive increase of HIV viruses, decline in CD4. Your immune system is slowly getting weaker and weaker. When the number of Cd4 cells get to about 500, this makes the body very susceptible to opportunisitc infections. If it goes below the threshold of 200, they have AIDS. Opportunistic infections are much more severe, you can die from these infections. There are other things that can cause a CD4 level below 200. Retroviral drugs allow the CD4 levels to stay above 200, can reduce the HIV levels to below detectable levels.

HIV Infection Time Course

- Primary syndrome (6-12 weeks following the infection)
  - Symptoms similar to mononucleosis
  - CD4 count: Rapid drop 1000 → 500
- Clinical latent period (Up to 10 years)
  - Asymptomatic period
  - CD4 count: Progressive drop 650 → 0
    - From 500 – 200 : at risk of opportunistic infections
- AIDS
  - CD4 count below 200
    - From 200 – 50: High risk of severe opportunistic infections
    - Less than 50 : immuno-incompetence → death

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Retroviral drugs keep them in the primary syndrome phase.

Acquired Immunity

Vaccines

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Immunizations

Type of Immunity	Mode of acquisition
• **Active (Prophylactic)**	
➢ Natural	➢ Infection
➢ Non intentional	
➢ Artificial	➢ Vaccination
➢ Deliberate	
• **Passive (Therapeutic)**	
➢ Natural	➢ Ab transfer – mother to child
	➢ Transplacental or colostrum
➢ Artificial	➢ Administration of Ab

474

Active- to prevent the infection, to expose you to some form of the antigen so you can memorize the antigen, so the second time you get it is will be a secondary response.
Natural- get exposed to the antigen in a natural fashion, ex. getting the flu
artificial- getting a needle, deliberate introduction of an antigen
Passive- not to teach, analogous to cheating on the exam, 2 people, the first person meets the antigen, they learn and remember, the first person then transfers the info to a second person that never saw the antigen.
Natural- a mother can transfer her antibodies to a fetus, does not have to learn the defense frist. Transplacental IGG, Can also be transferred through breast milk. (IGA) Both these processes are temporary bc there is not memory.
The goal of passive is to treat, not to prevent.

Artificial- take the antibodies from somewhere else and injecting them into someone else. They are not learning, but taking advantage of the tool the other person developed to fight off the infection. Antivenom are produced by injecting a horse with the venom, you then bleed the horse after a few weeks. You take the antibodies from the serum of the horse, this is the antivenom. You use this to treat the person. The horse acquired active artificial immunity.

Antitoxins and antivenoms are typically neutralizing antibodies.

Definitions

- Vaccine:
  - Suspension of attenuated or killed microorganisms or a fraction of these administered to induce an immune response and thus prevent the infectious disease
- Anatoxin:
  - Modified non toxic version of a toxin which retains its immunogenicity
- Adjuvant :
  - Compound added to vaccine preparations which increases the immune response

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Vaccine: intentionally expose someone to an antigen to acquire memory and prevent future infections.
Anatoxins: exposing to the non harmful version of the toxin and acquire a memory, so when you are exposed you can fight it.
Adjuvant: makes the antigen much more noticeable, to activate the third line of response. Induce the 2nd line of defense, the inflammatory response.

Content of Vaccine Preparations

- Proteins, polysaccharides, nucleic acids
- Preservative
  - Thiomersal (an organomercurial)
    - Antibacterial and antifungal
- Adjuvant
  - Aluminum salts
- Organism or some component of it

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Preservatives to increase shelf life.
Aluminum salts: similar to little knifes, very damaging to the cells. Activates inflammatory response.

Types of Vaccines

- Attenuated (live)
  - Less virulent, but live, version of a pathogen
- Inactivated (dead)
  - Bacteria or viruses killed with heat or formaldehyde
  - Anatoxin vaccines
  - Subunit vaccines
    - Protein or other purified component from the pathogen

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Attenuated: less virulent (mean), they are alive, they can carry out the infection and can be transmitted to a vaccinated person to a non vaccinated person.
Inactivated: Anatoxins-inactivated versions of toxins
subunit vaccines- you use only one part of the organism, not the entire thing.
These forms of vaccines cannot be transmitted from a immunized person to a not immunized person.

Goals of Vaccination

- Protecting the individual – Protection efficacy
- Protecting the population – Herd Immunity

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Herd immunity: if you immunize a high fraction of the population, that will allow you to protect non immunized individuals.

Attenuated Vaccines (*live*)

- Attenuated or eliminated virulence
  - Attenuation is a consequence of mutations
    - Ex. Measles, mumps, rubella, influenza, tuberculosis
- Advantages:
  - High efficacy
  - Mimics infection
  - Structures remain unchanged
- Disadvantages:
  - May induce symptoms
  - May cause the disease in immunodepressed individuals
  - Can revert to wild type

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Advantages: very high level of protection, it exactly mimicks the real infection so the body will learn. The epitopes are the same as the real thing.
These vaccines can mutate back to the wild type.

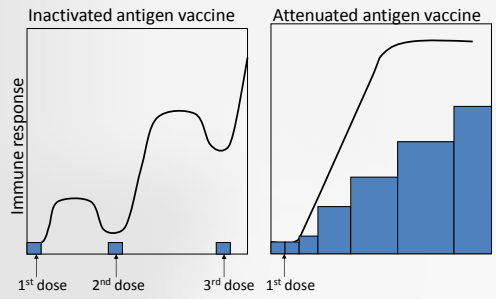
Inactivated Vaccines

- Inactivation by physical methods
  - Heat treatment
  - Formaldehyde treatment
    - Anthrax, Cholera, Pertussis, Influenza
- Advantage:
  - Very safe
- Disadvantages:
  - Antigenic structures may have changed
  - Short term and weak immunity
  - Does not mimic infection
  - Allergic reactions
  - Booster shots usually required
  - Adjuvants required

480

Disadavantages: you may change the structures of the epitopes when you kill them.
Allergic reactions due to additives.
You need several doses bc they are not alive.
You require adjuvants to activate the inflammatory response.

Immune Response – Inactivated Vs Attenuated



481

Need 3 doses to get a big enough response.

Smallpox Vaccine

- Infectious agent: Smallpox Virus
  - Single host: Humans
- Vaccine: Vaccinia virus (cowpox)
  - Live virus (infectious, attenuated)
  - The vaccine strain could transmit itself from immunized to non immunized individuals!!
  - Allowed the eradication of the virus and its disease
    - Last case 1977

482

cowpox: the disease is much less severe in humans, shares a lot of epitopes with the smallpox virus. Its an infectious virus, so it could be transmitted from immunized to non immunized individuals. Allows us to eliminate the virus very effectively.

Influenza Vaccines

- 2 versions
  - Inactivated
    - 2 type A strains and 1 type B strain
    - Virus is grown in chicken embryos and then killed
    - Combined treatment of heat and formaldehyde
    - Only induces a humoral response
  - Attenuated (LAIV)
    - Attenuated by cold adaptation at 25°C
    - Induces a humoral as well as a cell mediated immunity

483

Exclusively an exogenous antigen bc its dead, generates only a humoral response. This vaccine will protect you against the infection, not against the disease. This means that following the humoral response you will create the antibodies, this will neutralize the virus from the infecting the cells. But if the cell does get infected, they cant do anything about it.
LAIV (you grow it in chicken embryos, has a a lot of mutations to allow it to grow at 25%, humans have a T of 37, so the virus is slowed down. It is infectious, it will activate the humoral and cell reponse, the ind. will acquire memory, much more effective.

DPT Vaccine

- D - Diphteria
  - *Corynebacterium diphtheriae*
    - Diphteric anatoxin
- P – Pertussis (whooping cough)
  - *Bordetella pertussis*
    - Pertussis antigen
- T - Tetanus
  - *Clostridium tetani*
    - Tetanic anatoxin

484

DPT: protects against more than 1 antigen.
Diphteric anatoxin, a different version of the anatoxin.
P- you do not use the organism, you use a component of the bacteria. sub unit vaccine
T- doesnt cause any harm, the vaccine does not contain the bacteria, contains just the anatoxin.

Gardasil - HPV

- Preparation of the L1 capsid protein
  - Protein L1 auto-assembles generating non-infectious virus like particles - **VLP**
  - The VLPs induce a strong humoral response
    - Protects against the infection not the disease
    - Non-therapeutic

485

Does not contain the virus, contains a single capsid protein. This is produced by genetic engineering. Once you purify it, it assembles to create a capsid with no genome. Its similar to a virus except its just a capsid. It will induce a humoral response, very good at preventing. PRevent the infection, not the disease.

MMR Vaccine

- Multivalent live attenuated vaccine against measles, mumps and rubella
  - Measles RNA virus propagated in human cells
  - Mumps RNA virus propagated in chick embryos
  - Rubella RNA virus propagated in human cells
- Efficacy 95%
- Lifelong Immunity

486

3 pathogens that are RNA viruses, in all 3 cases it is attenuations, they are live. They are very efficient, one immunization=immune for life. There are several side effect associated.

MMR Adverse Reactions

• Fever	5%-15%
• Rash	5%
• Joint symptoms	25%
• Thrombocytopenia	<1/30,000 doses
• Deafness	Rare
• Encephalopathy	<1/1,000,000 doses

487

exam material ends here

problem set due tuesday after the midterm

Medical Microbiology

Host-Pathogen Relationship

488

What is a Disease?

- Any change to a healthy state in which the whole or part of the body of the host is not in perfect balance
  - **Infectious** - A diseased state due to the presence of a pathogen or its products
  - **Non Infectious -** A diseased state due to non-living causes
    - Genetics, poisoning, environmental, etc.

489

Host is not in perfect balance. Infectious: caused by a pathogen, or a product of the microbe
non-infectious: envtal factors, genetics, poison (ex. diabetes)

The Pathogen and the Infection

- Pathogen :
  - Any organism which has the ability to cause an infectious disease
- Infection :
  - State when a pathogen grows and multiplies in the host
    - An infection can cause or not cause a disease
    - **Infection is not synonymous with disease**

490

Infection means that you have a pathogen that grows and multiplies in the host, does not necessariliy mean it will lead to the disease. You have to demonstrate symptoms.

Types de Pathogens

- Primary pathogens
  - Cause disease after infection
  - Are not normally associated with the host
    - Ex. TB (Mycobacterium tuberculosis), Influenza
- Opportunistic pathogens
  - Cause illness in certain circumstances
  - Can be part of the natural flora
    - Ex. *Enterococcus faecium, Candida albicans* (natural flora)
    - Ex. *Pseudomonas aeruginosa, Serratia marcescens* (Environmental microorganisms)

491

Primary=true pathogens, always cause an infectious disease. Not microbes associated with the flora.
Opp: not always associated with the disease, usually when the immune system is weakened, may be part of the natural flora.
Environmental organisms: dont usually cause the disease, but igiven the opportunity they will

Classes of Microbial Pathogens

- Bacteria (Primary and opportunistic)
  - Ex. Primary: TB; Opportunistic : *E.coli*
- Fungi (Mostly opportunistic)
  - Ex. Yeast (candidosis)
- Protozoa (Primary)
  - Ex. *Plasmodium spp*. (malaria), *Trypanosoma spp*. (sleeping sickness)
- Virus (Primary)

492

Protozoa=parasites

The Infectious Disease

- How does it establish itself?
- 3 Requirements :
  - A susceptible host
  - A pathogenic agent
  - A favorable environment for the pathogen

493

Susceptible:provide the appropriate envt for the pathogen, not have a resistence to the pathogen (immune memory).
Pathogenic agent: a microorganism that can grow and survive in the host, archea would not be a pathogenic agent.

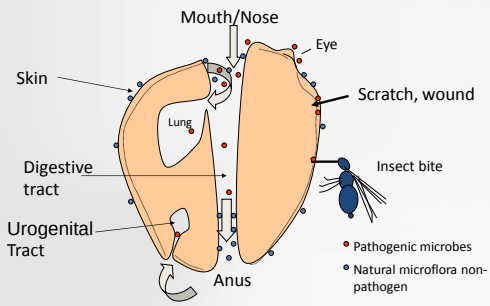
To Become a Disease Causing Agent

- 7 Commandments
  - Find an appropriate host
  - Obtain access to the interior
    - Penetration
  - Find a site of establishment
    - Adherence and Propagation
  - Multiplication
  - Ability to cause harm
  - Exit
  - Transmission to a new host

494

The pathogen has to meet the host, an encounter
2. some means to get in
3. be able to propagate and find a place it wants to establosh itself, have ways to adhere to that site.
4.grow
(these steps, called establishing the infection, the harm is not always caused by the microbe, caused by the host fighting the microbe)
The microbe has to exit the host and be transmitted to a new source.

Penetration & Propagation

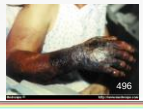


495

respiratory, gastro-intestinal tract, through the skin (cut and scratches), entrance through an insect, microbes that enter the skin are more likely to cause harm because they bipass the first barrier and enter immediately the interior. The other entrances have the first line of defense so the microbe has to get through that. Safer to breath through nose bc you have way more barriers, mucus, filtration, chemicals. If you breath through your mouth you are bypassing a lot of these barriers.

Enzymes which Aid in the Invasion

- Collagenase - Digests connective tissue proteins that hold the cells together(*Ex. C. perfringens*)
- Hyaluronidase - breaks down hyaluronic acid, polysaccharide that retains cells together(*Ex. S. pyogenes*)
  - Cause necrosis and blackening of tissues (progression of a few inches in a few hours



496

Collagenase: allows them to dig through the tissues, move around, a lot of damage to the host, favours exit.

Adherence

- Pathogens have structures that allow adhesion
  - Ex. Pili, fimbriae and glycocalyx
- Pathogens compete with the natural flora for attachments sites

497

Harming the Host

- Production of poisons such as toxins and enzymes which damage or kill cells and tissues
- Direct invasion and destruction of host cells
- Initiate an immune response of the host, which leads to symptoms

498

inflammatory response is extremely damaging to the host.

Toxins

- Endotoxin:
  - Structural component of the membranes of Gram-negative bacteria (LPS)
    - Only toxic if it is released
      - Cell lysis
- Exotoxins:
  - Proteins synthesized and secreted by bacteria
    - Not a structural component

499

All gram - bacteria have endotoxin bc its part of the LPS layer, its not secreted, it a strucutal component. Completely harmless unless its released (cell lysis). Bacteriolytic antibiotic will cause cell lysis and be extremely damaging to the host. What causes the harm is that the inflammatory response recognizes lipid A. Exotoxins: not specifically associated with bacteria, synonymous to a poison. The use of antibiotics is completely useless, typically you would use passive immunity (antibodies) to inactivate the toxin.

Properties of Toxins

Endotoxins	Exotoxins
– Thermostable	– Thermolabile (60-80°C)
– Low toxicity (mg/Kg)	– High toxicity (µg/Kg)
– Inflammatory	– Effects associated to specific symptoms
• Non specific	
– Weakly immunogenic	– Highly immunogenic
– Pyrogenic (fever)	– Non pyrogenic

500

~~Endotoxins: cant identify the toxin by the symptoms b~~c that are all the same, in exotoxins have very specific symptoms. Second line recognizes endotoxins well, not the third line. vice versa for the exotoxin. Much easier to acquire memory an immunity for an exotoxin.

(very little to be harmful)

Endotoxins

- Lipopolysaccharide:
  - Structural component of gram negative bacteria
    - Lipid A
  - Only active when released as a consequence of cell lysis
  - Causes **endotoxemia** :
    - Free endotoxin in the blood stream
  - Cause **Septicemia** (septic shock)
    - Systemic inflammatory response

501

Endotoxemia: which leads to septic shock, an extreme inflammatory response that will lead to death in a couple hours. The immune system decides its better to kill the individual to save the species.

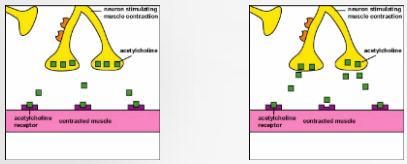
Classes of Exotoxins

- Neurotoxins
  - Interfere with synaptic transmission of neurons
- Enterotoxins
  - Interfere with the reabsorption of water by mucosa
    - Respiratory and intestinal tracts
- Cytotoxins
  - Inhibit specific cellular functions (kill cells)
    - Ex. Protein synthesis

502

Neurotoxins (*cont'd*)

- Tetanic toxin (*Clostridium tetani*)
  - Inhibits neurotransmitter secretion by inhibitory neurons
  - Causes spastic paralysis

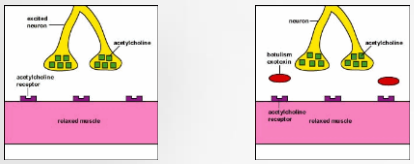


503

gram positive, rod, forms spore, strict anarobe, exotoxin produced by the bacteria causes the harm, the toxin inhibits the release of inhibitors that causes the muscles to be in a constant flexed state: Spastic paralysis. know for the exam.

Neurotoxins (*cont'd*)

- Botulinum toxin *(Clostridium botulinum)*
  - Inhibits discharge of acetylcholine
  - Cause flaccid paralysis



504

Gram +, rod, spore former, strict anarobe. Prevents the release acetylcholine, the muscles are always in a relaxed state, Flaccid paralysis. KNow for the exam

Enterotoxins - Cholera Toxin

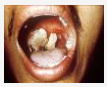
- The toxin stimulates the production of cAMP
- An increase in cAMP levels causes loss of electrolytes and water from cells lining the intestine
- Causes very abundant watery diarrhea (1L / h)
  - Severe dehydration
  - Death (18 hours to days)

505

Vibrio choloeras, gram negative. Stimulate the production of cAMP, causes the lose of electrolyes and ways from intestinal tract, or respiratory tract,

Cytotoxins – Diphtheria Toxin

- Produced by *Corynebacterium diphteriae*
  - Inhibits protein synthesis causing cellular death:
    - Localized (mucous membrane of the throat)
      - Degeneration of the epithelial cells of the throat
      - Inflammation and edema
      - Pseudomembrane formation
    - Systemic
      - Heart failure
      - Inhibition of the nervous system - paralysis



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If its localized, swelling of the respiratory passage, a lot of scar tissue =pseudomembrane, this can eventually block the respiratory passage.

Transmission of the Infectious Agent

- Essential steps:
  1. Exit of the infectious agent from the host
  2. Find a new host
  3. Enter a new host

507

Exit Routes

- Upper respiratory tract - the nose and mouth
  - Aerosols - speech, cough, sneeze
- Gastrointestinal tract - intestine
  - Feces
- Skin
  - Dead skin layers, exudates or crusts
- Urogenital tract
  - Urine, semen, vaginal secretions
- blood

508

Least common=blood, involves articifial transmission, sharing needles, biting etc.

Transmission of the Infectious Agent

- By droplets
  - Talking, coughing and sneezing
- By formites: inanimate objects
- Direct contact from person to person
- Fecal - Oral
- By arthropod vector
  - Flies, mosquitoes

509

Formites: you cough, ends up on a table, then someones touches the table and contracts it.
Fecal-oral: anus to mouth

Transmission of the Infectious Agent

- Airborne particles (dust)
- Parenteral
  - Direct transmission through blood
- Horizontal - from mother to child
  - Prenatal
    - Passage through the placenta
  - Perinatal
    - At birth

510

Consequences of the Infectious Disease

- The result of the infectious disease depends
  - Properties of the host
    - The immune response
  - Properties of pathogen
    - virulence

The host is sick He dies	The host is sick He recovers	The host is not sick Infection without disease
Defenses failed	Defenses eventually worked	Defenses worked
No immunological memory	No or poor immunological memory	Immunological memory from a previous encounter
Highly virulent agent	Virulent agent	Non-virulent agent

511

Whether the immune response works or not depends if you have a memory of it, and how quickly it reacts. You want to have memory which is why we use vaccines.
Virulence:any characteristics of the pathogen that allows it to carry out the 7 commandments, the meanness of the pathogen.

Virulence

- Infectious dose (ID_{50})
  - Minimum number of organisms to cause the disease in 50% of hosts
    - Apparition of symptoms
- Lethal dose (LD_{50})
  - Minimum number of organisms required to kill 50% of hosts

Bacillus anthracis

Dose / Route of entry	ID_{50}	LD_{50}
Skin	10-50	50 - 250
Inhalation	1 000 - 8 000	8 000 – 10 000
Ingestion	25 000-100 000	150 000-500 000

512

ID: relates to the apparition of symptoms, minmum amount to cause symptoms
depending on the route of entry, the ID or LD will be very different. Skin as mode of entry, ID and LD is much lower than if they use ingestion. By using the skin, you get past all the first lines of defense. Can take up to 3 weeks for the second line to act.

Host:Pathogen Relationship

The Infectious Disease

513

Classification of Infectious Diseases

- As a function of duration
  - Acute - characterized by sudden onset, rapid progression, often with severe symptoms
  - Chronic - characterized by late onset and slow progression. Less severe symptoms
  - Latent - characterized by periods without symptoms between flare-ups

514

Classification of Infectious Diseases

- As a function of the site of infection
  - Local
    - Confined to a specific area of the body
  - Systemic
    - Infection with a generalized distribution in the tissues
- As a function of the order of declaration
  - Primary
    - Initial infection in a healthy individual
  - Secondary
    - Occurs in a person weakened by a primary infection

515

The primary establishes conditions that makes the host susceptible to the secondary infection. ex HIV.

Progression of the Acute Disease

- Incubation period:
  - Includes meeting, the penetration, the spread and growth
  - Average duration of 2-3 days, several weeks or months
- Prodromal period:
  - Precedes the expression of specific symptoms
    - Ex. Headache, dizziness, gastrointestinal pain
  - Represents the beginning of the pathogenic activity

516

incubation period: most dangerous period, you cannot identify the person so you wont takes measures to avoid the person, the person themselves doesnt know that they are sick, no symptoms.
Prodromal: non specific symptoms, caused by immune system, inflammatory response. They know that they are sick. 2nd line of defense telling the 3rd line that theres a problem. Body knows theres a problem.

Progression of the Acute Disease

- Acute period
  - Interactions between pathogen and host are maximal
    - Active contribution of the inflammatory response
    - Specific and non-specific symptoms
      - 1st encounter
        - » The acquired immune response is initiated, there are no antibodies present
      - 2nd encounter
        - » High level of activity of the acquired system
        - » High levels of antibodies

517

1st encounter, 3rd line takes a while to response
2nd encounter, respond very quickly.
At this point, the doctor can guess what the pathogen is.

Progression of the Acute Disease

- Convalescence:
  - Recovery from the acute period
  - Decrease of specific and non-specific symptoms
  - Antibody level is at its peak

518

Host recovers, defenses worked. This stage implies that the person survived.

Food Microbiology

519

The Good, the bad, and the Ugly

- The good
  - Important in the production of many foods
    - Improving texture and taste
- The bad
  - Foodborne diseases
    - Infections et intoxications
- The ugly
  - Cause food spoilage

520

Factors which Affect Microbial Growth in Food

- Intrinsic:
  - Conditions inherent to the food product
    - Water availability (a_w)
      - Most foods> 0.98; Most microbes require> 0.90
    - pH: pathogens can not grow at a pH <4.5
    - Nutrients
    - Antimicrobial compounds garlic, spices
    - Biological structures
      - Peel, bark, shells first line of defense

521

Bacteria need a lot of water to grow, so foods low in aw (water availilbility) will not be as good for bacteria to grow.

Factors which Affect Microbial Growth in Food

- Extrinsic:
  - Environmental conditions
    - Temperature
    - Water availability (aw)
    - Oxygen availability
      - Vacuum packaging
    - Antimicrobial compounds
    - Preservatives
    - Treatments
      - Pasteurization
      - Radiation
      - Sterilization

522

prevent or slowth bacterial growth, they do not kill microorganisms.
pasteurization: lowers the number of bacteria
radiation:form of sterilization

Microorganisms in the Production of Foods and Beverages

- Lactic fermentation
  - Glucose → pyruvate → Lactic acid
    - Products of fermented milk (cheeses, yogurt)
    - Pickled vegetables (sauerkraut, pickles)
- Alcoholic fermentation by yeasts:
  - Glucose → pyruvate → alcohol + CO_2
    - Beers, wines, distilled spirits
    - Bread
- Fungal growth
  - Soya sauce, blue cheese

523

intrinsic factor, property of the food

Bread: production of CO2 makes the bread rise.

Food Spoilage

- Undesirable changes
  - Taste, odor, appearance
    - Usually harmless, but may be accompanied by the presence of pathogens
  - Bacteria most often associated with spoilage
    - *Pseudomonas*, *Erwinia*, *Acetobacter*, *Lactobacillus*

524

shelf life associated with different microorganisms that can grow on food.

Food Conservation

- Killing microorganisms:
  - Canning, pasteurization, cooking, irradiation
- Inhibit growth:
  - Temperature
    - Refrigeration, freezing
- Reduce aw
  - Dehydration, salt, sugar
- Preservatives
  - Nitrates

525

Foodborne Diseases

- Foodborne diseases are acute diseases associated with the recent consumption of food
  - The food in question contains a pathogen (food infection) or a toxin (food poisoning)
    - Estimates of the CDC
      - 76 million cases annually
      - 325,000 hospitalizations
      - 5,000 deaths

526

Food infections: they involve a pathogen that has to respect the 7 commandments we saw earlier. An infectious disease.
Food intoxication (food poisoning): does does involve an infection, you will get the disease is produced by the toxin that is produced by the micro-organism. The toxin is not produced in the host.

Food Infections

- Food infections are the result of the ingestion of foods that contain the pathogen and the body's reaction to its presence
  - Pathogens: bacterial, fungal and viral
    - Requires growth of the pathogen in the host
    - Relatively long incubation period (24-72h)
    - Often accompanied by a fever

527

Have to ingest the pathogen, the symptoms associated are due to your immune response to the pathogen (inflammatory response). Has to respect 7 commandments, incubation period 1-3 days before you start seeing symptoms.

E.coli Serotypes that Cause Diarrhea

- Classified according to their virulence
  - Entertoxigenic *E. coli (ETEC)*
  - Enterpathogenic *E. coli (EPEC)*
  - Enterohemorrhagic *E. coli (EHEC)*
  - Enteroinvasive *E. coli (EIEC)*
- Antigenic discrimination
  - O antigen : component of the LPS layer
  - K antigen : Capsule
  - H antigen : flagellin

528

E.coli is a usual part of the flora of the human intestine. These serotypes (same species) are harmful. You can discriminate them bc they have different antigens on them.
The capsule makes them much more resistant to phagocytosis. They have different epitopes on their flagellin.
These are all produce exotoxins, the e.coli is your flora dont produce exotoxins.
Endotoxin in synonymous to lipid A (all gram - bacteria), these E.coli also have endotoxins.

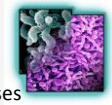
E.coli Serotypes that Cause Diarrhea

- Entertoxigenic *E. coli (ETEC)*
  - Also known as traveler's diarrhea
- Enterpathogenic *E. coli (EPEC)*
  - Similar to ETEC but enters cells and causes the destruction of the microvilli
- Enterohemorrhagic *E.coli (EHEC)*
  - Necrosis, inflammation and ulceration of the colon
- Enteroinvasive *E. coli (EIEC)*
  - The most virulent serotype (O157: H7)
  - Bloody diarrhea; leads to a hemolytic uremic syndrome

529

ETEC; affects your intestinal lining
EPEC-penetrates the cells, the cells will be presented on MHC1 and be killed the T somethings, microvilli killed by your immune system.
EHEC: induces inflammatory response, kills your tissues, lycocytes will empty their contents and create holes in your colon (ulcers)
EIEC: causes lysis of red blood cells,

Bacterial Gastroenteritis - *Campylobacter*



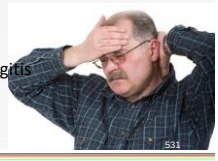
- Gram negative curved rod
  - Found within the natural flora of poultry
  - Responsible for the largest number of cases annually of foodborne diseases
  - Disease characterized by abdominal pain, bloody diarrhea and fever
  - Can lead to Guillain-Barre syndrome
  - Pathology caused by enterotoxin and cytotoxin

530

Found on all surfaces of poultry, gram -, has endotoxin A, enterotoxin-causes diarrhea, cytotoxin-causes cell death. This causes an inflammatory response, Guillan-barre syndrome - inflammation of the nervous system that can lead to paralysis.

Listeria monocytogenese - Listeriosis

- Small Gram positive rod
  - intracellular parasite
  - Responsible for the majority of deaths associated with foodborne diseases
  - Makes a cytotoxin - O listeriolysin
  - Pathogenesis:
    - Bacteremia → sepsis
    - Neurological disorders → meningitis
    - Abortion



531

Opportunistic pathogen, doesn't really like 37, likes colder temperatures. When you store food in the fridge, inhibit growth of other microorganisms and this one can take advantage.
cytotoxin-causes cell death, sepsis-septic shock
inflammation of the nervous system.
Babies in the womb are very susceptible to this virus bc they have no immune system. They body will try to eject the baby to get rid of the infection.

Norwalk Virus – Viral Gastroenteritis

- Infects cells lining the small intestine
  - Damages cells and villi
  - Cause a very watery diarrhea with nausea, vomiting, abdominal cramps and headaches
  - Extremely contagious
    - ID_{50}: 1
- Cannot be grown in the lab

532

One viral particle is sufficient to establish the infection. It will kill the cells it infects, get a strong inflammatory response, which causes the symptom of diarrhea.

Food Intoxications

- Growth of bacteria and production of exotoxin in foods.
- Accumulation of the toxin leads to intoxication
  - Declaration of symptoms between 1-3 hours
- Examples:
  - *Staphylococcus aureus*
  - *Clostridium botulinum*

533

Produced exotoxin within the food, not within the host. When you ingest the food, you ingest the toxin, symptoms can appear in as little as an hour.

gram + cocci, within the natural flora of your skin, not harmful on the skin.

true pathogen, gram + rod,

Staphylococcus aureus

- Gram positive cocci
  - Resident of the natural flora of the skin
    - Produces several enterotoxins that target the gut
    - Exceptionally resistant enterotoxins
    - Poisoning associated with meals prepared in advance
      - Contamination of food by the one who prepares it
      - Food that remains between 25 -28ºC for 2 hours or more allows the growth and accumulation of toxin
  - Reheating does not inactivate the toxin
  - Ingestion → intoxication (1-6h)
    - » Vomiting, diarrhea, severe abdominal cramps

534

As someone prepares food, the bacteria on their skin gets introduced to the food. The is extremely heat resistant. When the food reaches an acceptable temperature, it will start growing, accumulation of the bacteria. Heat does not inactivate the toxin.

Clostridium botulinum - Botulism

- Gram positive rod, sporulating, strict anaerobe
  - Produces a neurotoxin that inhibits the release of acetylcholine
  - Associated with canned food
    - Symptoms: 12-72h after ingestion
      - Vomiting, diarrhea, blurred vision and muscle weakness

535

Not a very good pathogen considering we have oxygen in our body. Canned food is a good place for it to grow (no oxygen), if its not sterilized properly, you will get growth of the bacteria, produce the exotoxin. When consumed you will get the symptoms.

Clinical Microbiology

Diagnostic

536

Diagnostic

- Establish and confirm the etiology of the disease
- Track the progression of the infection in the patient

537

Etiology: whats causing the disease.
Monitor the progression and the treatment of the disease.

Methods

- Microscopy and biochemical tests
- Immunological
  - Precipitation test
  - Agglutination test
  - Complement fixation test
  - ELISA
  - Immunochromatography
- Molecular
  - Hybridization
  - PCR and RT-PCR

538

Taking a sample and looking at it in the microscope.
Biochemical tests: you have to grow the microorganism.
Immunological: monitor the immune response to the microorganism, does not require growth, rapid
molecular: does not require growth, rapid.

Microscopy and Biochemical

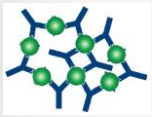
- Microscopy
  - Gram Stain
  - Acid fast stain
- Biochemical tests
  - Identification based on metabolic characteristics
    - Ex. Oxygen Requirements
    - Carbon sources used
    - Oxidative or fermentative metabolism
    - Metabolic byproducts

539

You can identigy the microorganism based on the metabolism it has.

Immunology – Precipitation Tests

- Principal
  - When antibodies react with multiple epitopes on soluble antigens, there is formation of networks which generate an insoluble precipitate
  - Precipitation reactions can take place in solution or in gels such as agar

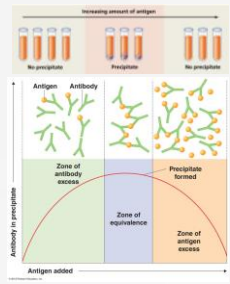


540

If you can form huge agglutinations until it becomes large enough to view with the eye as a precipitate.

Phenomena of Zones

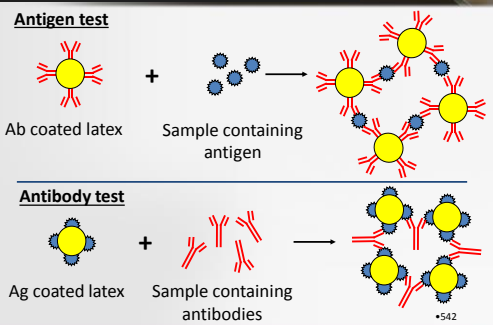
- The precipitate formation is influenced by the concentration of Ag - Ab
- Used for the detection of Ab
  - Ex. Khans Test for Syphilis



541

Make different dilutions of the sample, if you use it to identify the antigen, you add a constant amount of antibodies. If the antigen is limiting, more antibodies then antigen, then it is not sufficent to cause corsslinking, no precipitation.
IF there is an excess of antigen, the antigen will occupy all the sites, no precipitation.
To have precipitation, you need an optimal ratio of antigen and antigen, the zone of equivalence.
If you get precipation you can conclude that you have antigens or antibodies.

Latex Agglutination



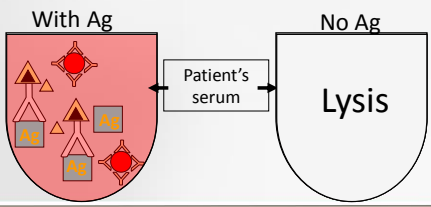
542

Latex beads coated with antibodies, use this to know if an antigen is present or not. With caoted beads, you cn get a valency of several thousand. You mix beads with the sample, if antigen is present you will get cross-linking, precipitate.
You can also coat the beads with antigens, the purpose will be to know if theres antibodies against the antigen. Same principle, if theres precipitate then there are antibodies present. If you are seropositive (detecting presence of antibodies)

ON THE FINAL EXAM

Complement Fixation

- Method
  - Add Ab against Ag to detect
  - Add a limiting concentration of complement
  - Add IgG sensitized RBC



543

Based on the principle of the classic complement cascade. Detecting presence of antigen or antibody. You will add an antibodie that will react with the antigen, they will bind if its present. Formation of antigen-antibody complex. The antibody has to be IGG or IGM. After adding the antibody, you will add complement proteins C1-C9 in limited concentration, not in excess. If you have formation of a complex, the complementation cascade will take place. (no complex-no cascade). Complement fixation occurred or didnt occur. The complement proteins will get used up if the cascade occurs, (no complex-remain unused), Add red blood cells that have been sensitized (have IGG or IGM bound to them). Since there is no cascade proteins remaining, then they cannot complete the cascade on the red blood cells. No lysis of red blood cells. Positive result, negative for lysis. If the complement cascade are available, they will start the cascade on the red blood cells.

Complement Fixation *(cont'd)*

- Test that determines whether complement was used as a result of specific binding of Ab to Ag
  - Complement used – No lysis of RBC
    - Complement fixation
    - Antigen present
  - Unused complement – lysis of RBC
    - No complement fixation
    - Antigen absent
- Test can be performed quantitatively
- Moderate sensitivity

544

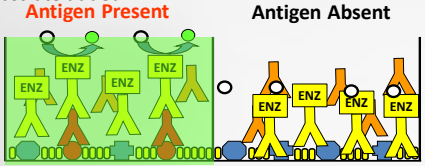
Dosage by ELISA

- Used to detect the presence of antibodies or antigens
  - Very sensitive
  - Quantitative
  - Quick

545

ELISA –Antigen Detection

Serum (source of Ag) Added to wells
Blocking agent added
Ab against Ag added
Wash
Detecting Ab added
Wash
Substrate added

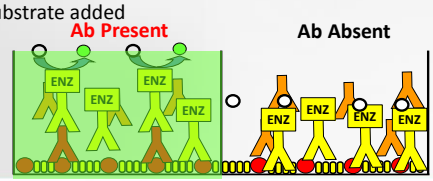


546

A plate that has well made out of plastic, this plastic will bind any protein. Add sample to wells, if they have proteins they will bind to the plastic. The proteins were immobilized. The sample is unknown. Add blocking agent to saturate the plastic, make sure all binding sites are occupied. Add known antibody that can react to the proteins, the antibody becomes immobilized. If you went to do a wash, any antibody that was not immobilized gets removed. You then add a secondary antibody that recognizes the first antibody (binds to the first antibody that is bound to the antigen). The second antibody is immobilized. Any secondary antibodies get removed. You add an enzyme that gets immobilized by attaching to the second antibody. Quantity of enzyme that is immobilized is an indicator of the amount of everything else that was bound. The substrate is free and colourless, the enzyme will convert it into a colour. The amount of colour is a function of how much antigen is present. More colour=more enzyme=more antigen.

ELISA –Antibody Detection

Target Ag for Ab to be detected added to wells
Blocking agent added
Test serum added
Wash
Detecting Ab added
Wash
Substrate added

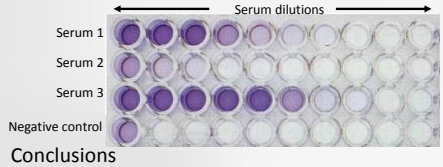


547

Determining the antibody instead of antigen. You coat the plastic with an antigen. You add blocking agent. You then add the sample with the unknown antibodies (serum of a patient). If antibody is present, it will bind to the antigen. You add 2nd antibody that recognizes the second antibody, same process. Exam question: do you have antibody against the antigen, what kind of antibody do you have?Seondary atnibodies that are specific to epitopes. The epitopes are found on the Fc region.

Interpretation of Results

- Serums of patients tested for HIV
  - 1º Ab anti-HIV from mouse
  - 2º Ab anti-Ab of mouse



- Conclusions
  - Patients 1 & 3 are positive for HIV
  - Patient 3 has the highest titer
  - Patient 2 is negative for HIV

548

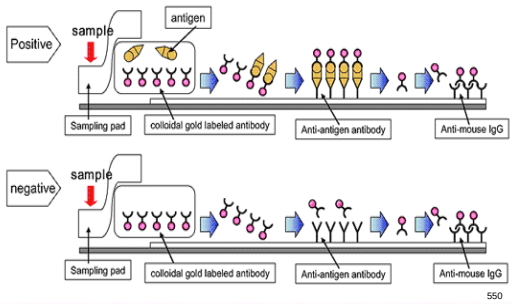
Coated wells with different diluations of serum that you collected from patients. Added an antibody that recognizes HIV, add a second antibody that recognizes the first one. Serum 3 gave you a higher concentration as serum 1. Serum 2 was similar to the control so you know the antigen is not present. This is testing for antigens, not antibodies, so you cant tell which one is seropositive.

Immunochromatography

- Same principal as the ELISA
  - Qualitative rather than quantitative
  - Detects interaction of a specific antibody against an antigen
    - Colorimetric Method
  - Principal of several rapid tests
    - ≤ 15 minutes
    - Used for bacterial and viral pathogen detection
    - Can also be used for antibody detection

549

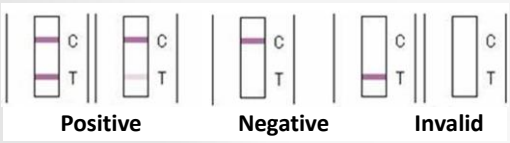
Principal of Immunochromatography



550

A strip, 2 positions on the strip where you have an antibody that has been immobilized. At the 1st position, the antibody has an epitope on the antigen that you want to detect. At the 2nd position, you have a second antibody that is immobilized, but it does not recognize the antigen you want to detect. It detects the antibody used for the test. You apply the sample to the begging of the strip, the sample will diffuse along the strip, it will reach a point where it has a free antibody that will bind to the antigen you want to detect. Usually labelled with something that gives colour. If you are positive for the antigen, you will create antibody-antigen complexes. Diffusion will continue, reach the immobilized antibody that recognizes a different epitope on the same antigen. The complex will be bound to the first immobilized antibody if you have the antigen, you will get a colour reaction. If it is not bound, it will reach the 2nd position. You will get a colour reaction at the second position.
IF you dont have the antibody, you will only get colour reaction at the second position.

Interpretation



551

Hybridization Test

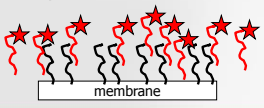
- Used for diagnosis of bacterial and viral infections
- DNA of the sample to be tested is isolated, denatured and then combined with a probe specific to the microbe
  - Several commercial kits are available
    - Very useful for diagnosis of viral infections and organisms which can not or are difficult to grow in laboratory

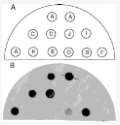
552

use a DNA sequence that is specific to what you want to identify, so you can conclude if the organism has this DNA sequence of not. You will extract DNA, denature it (make it single stranded), immobilize it to the membrane. You then add your single-stranded probe (DNA or RNA), it needs to be labelled so you can detect it. Expose membrane to probe, if it has the same sequences it will anneal to the membrane. If you perform a wash, only annealed probes will remain.

Hybridization

1. Immobilize denatured DNA (target) to a support (membrane)
2. Add DNA or RNA probe
3. Allow renaturation, and then wash to remove free probe
4. Detection of hybrid sequences between probe and target





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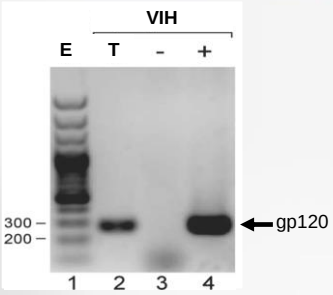
PCR and RT-PCR

- PCR
  - Allows exponential amplification of a specific sequence from genome DNA
    - Based on the replication of DNA
    - Very useful for the diagnosis of viral infections
- RT-PCR
  - Allows exponential amplification of a specific sequence from RNA genome
    - Same as PCR, but requires an initial step to convert RNA into DNA
      - Reverse transcriptase reaction

554

Both techniques are based on the principle of amplifying a sequence. If you can't amplify, the sequence is not present. PCR starts with DNA as a template. RT-PCR starts with RNA as a template, lets you know if the sequence is presence in RNA form. Reverse transcriptase to convert RNA into DNA, then you can amplify it.

PCR



555

RT-PCR, T=control
trying to identify gp120, you got amplification.

Epidemiology

556

Definitions

- **Epidemiology**: Branch of medicine that describes the occurrence, distribution and types of diseases in populations for distinct time periods
- Epidemiology is the study of who, what, when, where and how as they relate to outbreaks of infectious diseases

557

studying at the level of a population, how a micro-organism/ disease is transmitted throughout a population.

Definitions *(cont'd)*

- Infectious disease
  - Disease caused by an infectious agent
    - Ex. Common cold
- Subclinical disease
  - State in which the infection caused tissue damage, but with no clinical signs or symptoms
- Contagious disease
  - Direct or indirect transmission from an infected person
    - Ex. Influenza – The flu
- Transmissible disease
  - Transmission by non-natural means from an infected person
    - Ex. Food intoxication – *S. aureus*

558

Subclinical disease: someones whos infected, but does not show symptoms. ex. in the incubation period.
Transmissble: not contagious, you won't catch it from being around them.

Measures of the Occurrence of a Disease

- Cumulative incidence or attack rate
  - Type of incidence applied to a fraction of the population that is observed over a defined period
  - Measure of the risk of developing the disease
  - Measure of transmissibility

$$CI = \frac{\text{N}^{\text{o}} \text{ of } \textbf{new} \text{ cases of the disease during the specified time}}{\text{Population at risk } \textbf{during} \text{ the period of time}} \times 1\,000$$

559

The risk of developing the disease, how infectious it is. The higher the value, the more contagious it is.
only include cases that happen after the beginning of the study.
Can only look at the people at risk (children, elderly etc..)

Attack Rate: Example

- During the first week of April 2002, the unit of Public Health was called to investigate more than 20 reports of people suffering from gastroenteritis after eating at the Parramatta restaurant. An investigation was conducted and all customers who ate at the restaurant during the week were interviewed. They found 2,000 customers who ate at the restaurant, including 400 who fell ill
  - What was the attack rate?

Attack rate = 400/2000
= 0.2 X 1 000 = 200/1 000 customers

560

2000- population at risk
400/2000 x 1000=

Measures of the Occurrence of a Disease

- Prevalence :
  - Fraction of population at risk that is affected (new cases and preexisting cases) by the disease at a specific point in time

$$P = \frac{\textbf{Total N}^{\textbf{o}} \textbf{ (new + preexisting)} \text{ of cases of the disease during the specified time period}}{\text{Population at risk } \textbf{during} \text{ the time period}} \times 1\,000$$

561

What fraction of the population is expressing the disease.

Prevalence : Example

- A survey among 300 children under 5 years of age revealed that 70 of them had chickenpox
  - What is the prevalence expressed per 1 000 people?
  - How many cases of chickenpox would be expected at a given time if the total population of children under 5 is 25 000?

562

Prevalence: 70/300 x1000= 233

Number of cases predicted amongst 25 000
233/1000 x 25000 =5825 cases of chickenpox

Measures of the Occurrence of a Disease

- Incidence rate
  - Number of new cases in a population divided by the total units of time of each individual observed in the population at risk

$$IR = \frac{\text{Nº of new cases of the disease/event for the specified period of time}}{\text{Sum of the amount of time during which each person was at risk (Person-years)}}$$

563

Similar to a rate of speed.

Person year- follow 1 person for 1 year-1 person years
1 person over 2 years- 2 person years.

If someone dies, leaves the country, gets sick, during a study, after that point you don't count them anymore, no longer part of the population at risk.

Calculating the Number of Person-Years

- Example 1 :
  - At the beginning of a study, with a duration of 5 years, it was determined that among a population of 100 people, 30 were at risk of a particular disease

Year of study	Number of cases
1	2
2	0
3	1
4	0
5	3

Number of person-years =
(27 pers. X 5 years) + (2 pers. X 1 year) + (1 pers. X 3 years)
= 140 **person-years**

564

30x5= 150, number of person years if no one got sick.
3 people have been eliminated (they got sick)
you have 27 left, 27x5 person years
you add them together. You dont have in account the 3 in the last 5 years because they made it the whole time period without getting sick.
IF you were to stop it at 3 years : (1x2 pers) + (28 x 3years) = 86 person years.

Calculating the Number of Person-Years

- Example 2:
  - The incidence of duodenal ulcer was examined in 14 subjects who used a particular drug
    - 4 subjects began the study in January 1990
    - In December 1994, two subjects left the study
    - 10 subjects joined the study in December 1995 and completed it in November 1996
    - The study was completed in December 1996

565

4 subjects started in 1990
2 ended in 1994
2x5 years= 10 person-years
2 continued study until 1996
2x7 years= 14 person years
10 subjects started in dec 1995 and ended in dec 1996
10x1=10 person years
Total: 10+14+10=34 years
This type of study can be done on weeks, months ex.. 12 months, 365 days,

Calculating the Number of Person-Years

- Example 3:
  - The incidence of influenza was examined in a population of 1000 individuals during the period of Jan. - April 2013

Month of study	Number of cases
J	12
F	25
M	100
A	200

(12 pers. X 1/12) + (25 pers. X 2/12) + (100 pers. X 3/12) + (863 pers. X 1/12) = 102.1 person-years

566

montly basis, multiple by 1/12 for each month.
should be 863 x 4/12.

Prediction

- Over a period of 3 years, among 150 people who consumed seafood, 10 contracted a food infection. If on average 25% of a population of 10 000 inhabitants consume seafood, how many people / year will contract a food infection following the consumption of seafood?

567

Solution

- Incidence rate:
  - Nº of person-years = 150 pers. X 3 years= 450 P-Y
  - Nº of new cases = 10
  - I.R.= 10/450= 0.02 cases/person-years
- Prediction:
  - Nº of persons at risk = 0.25 X 10 000 = 2 500
  - Nº of persons predicted to contract a food infection:
    - 0.02 X 2 500 = 50 persons/year

568

Relative Risk

- Often we need to know the association between a result and factors (eg, age, sex, race, smoking status, etc.)
  - Relationship between the probability of contracting a disease when exposed to a factor, and the likelihood of contracting the disease when not exposed to this factor

569

ex. does smoking make you more likely to develop lung cancer.

2 x 2 Table: Calculating the Association

if you got the disease or whatever

behaviour

	Result	
Exposure	Yes	No
Yes	*a*	*b*
No	*c*	*d*

570

2 x 2 Table

a = Number of exposed persons with the result
b = Number of exposed persons without the result
c = Number of persons not exposed with the result
d = Number of persons not exposed without the result

****

a + *b* = Total number of exposed persons
c + *d* = Total number of persons not exposed
a + *c* = Total number of persons with the result
b + *d* = Total number of persons without the result
a + *b* + *c* + *d* = Total population at risk

571

Calculating the Relative Risk

- The relative risk is the risk of the disease in the exposed group divided by the risk of disease in the unexposed group

$$RR = \frac{a/(a+b)}{c/(c+d)}$$

572

RR= (exposed+ disease/total exposed)/ (not exposed+disease/ total not exposed)
Value greater than 1: positive correlation, increased risk of developed the disease when exposed to the factor
value 0f 1: no correlation, probability of the result is the same regardless if you are exposed to the factor or not
less than 1: negative correlation, protective effect. The factor decreases your risk of getting the result

Relative Risk : Example

	Diarrhea?	
Pink hamburger	Yes	No
Yes	23	10
No	7	60

$$RR = \frac{a\ /\ (a+b)}{c\ /\ (c+d)} = \frac{23\ /\ 33}{7\ /\ 67} = 7.0$$

573

The risk of developing diarrhea is you eat pink hamburgers. you're 7 times more likely to get it if you eat pink hamburgers.

Interpretation of the RR

- = 1 – Indicates that is no association
- > 1 – Indicates a positive association
- < 1 – Indicates a negative association
  - Ex.
    - If RR = 5
      - The people exposed are 5 times more likely to have the result as compared to people who have not been exposed
    - If RR = 0,5
      - The people exposed are 2 times less likely to have the result as compared to people who have not been exposed
        - » Protective effect
    - If RR = 1
      - The people exposed are not more or less likely to have the result as compared to people who have not been exposed

574

Odds Ratio

- Only in retrospect
  - Seeks to determine whether a person with the disease was more likely to be exposed to the risk factor than someone without the disease

$$RC = \frac{a/c}{b/d} = \frac{ad}{bc}$$

575

RC= (exposed+disease/notexposed + disease)/(exposed no disease/ no exposed no disease)
Odds ratio is not a predictive value, if calculating how likely you were to have been exposed to the factor if you were sick.

Odds Ratio: Example

	Diarrhea?	
Pink hamburger	Yes	No
Yes	23	10
No	7	60

$$OR = \frac{23/7}{10/60} = \frac{3.3}{0.17} = 19.4$$

576

Interpretation of the OR

- = 1 – No difference in the probability of exposure between the ill person and healthy person
- > 1 – Indicates that the probability that the patient has been exposed is higher
- < 1 – Indicates that the probability that the patient has been exposed is lower
  - Ex.
    - If OR = 5
      - Sick people are 5 times more likely to have been exposed as compared to people who are not sick
    - If OR = 0,5
      - Sick people are 2 times less likely to have been exposed as compared to people who are not sick

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Attributable Risk (AR)

- Measure of the number of cases that could have been prevented if the exposure was eliminated

$$AR = \frac{R_1 - R_0}{R_1} \quad \text{or} \quad AR = \frac{RR - 1}{RR}$$

R_0 : Risk in the absence of exposure
R_1 : Risk with exposure
RR : Relative risk

578

Allows to determine how many cases could have been prevented if a risk factor was eliminated.

Example of AR

	Diarrhea?	
Pink hamburger	Yes	No
Yes	23	10
No	7	60

- R_1 = 23/33 = 0.70
- R_0 = 7/67 = 0.10
- RR = 0.70/0.10 = 7.0
- AR = (7.0 -1)/7.0
- or (0.7 – 0.1)/0.7
- =0.86

Therefore 86% of cases could have been avoided

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86% of cases could have been avoided if they didnt eat pink hamburgers.

Types of Outbreaks

- Sporadic
  - Occasional incidence
  - No defined pattern
- Endemic
  - Regular incidence maintained at a low rate
- Epidemic
  - Sudden increase above the predicted rate
- Pandemic
  - Epidemic disease of which the incidence is world wide

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Sporadic: random,
endemic: disease that you always find some people in the population that have it. Theres always someone that has a cold. Basal rate.
Epidemic:
Pandemic: same as epidemic, but at the international level.

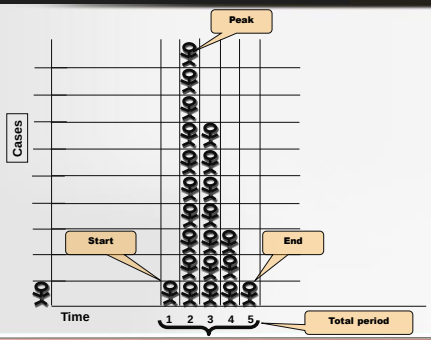
Epidemiological Profiles

- Epidemics linked to a common source
  - Sudden increase in the number of individuals afflicted followed by a progressive decline
- Epidemic Propagation
  - Slow increase in the number of people afflicted
    - Typical of contagious diseases
  - Index-Case: First person that can be found to have contracted the disease

581

Common source: very rapid, someone falls sick at almost the same time. You will then see a progressive decline as everyone gets better. This is characteristic of a transmissble disease, not contagious.

Characteristics of the Curve

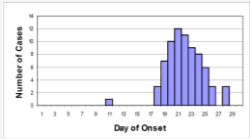


582

cases=total cases, the number of cases that include the cases from the period before. To get new cases, you have to subtract one form the other.
1=one case
In other graphs, it will be indicated new cases. To get total number of new cases, you would have to add them together from different time points.

Epidemics Linked to a Common Source

- People are exposed to the same source for a short defined period of time
- The shape of the curve shows a rapid increase with a defined peak, followed by a gradual decline

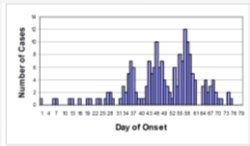


583

Each bar includes the number from the previous bar. To figure out the total number of cases over the study, you would choose the highest bar and figure out its value.

Epidemic Propagation

- One case of disease is the source of the infection
  - Subsequent cases then act as sources for subsequent infections
- The shape of the curve contains a series of successively larger peaks
- This trend may continue until
  - The number of susceptible people is exhausted
  - Herd immunity is acquired
  - Control measures are implemented



584

Progressively higher peaks until you reach the highest peak.
theres a hole, that person doesnt exist anymore, they died or something.
When they are stuck together, its the same people that you counted in the bar prior. Difference between 1 and 7=2. This is because there was a hole, this means that the person doesnt count anymore.
After the highest peak they are recovering. The either acquired immunity, or you are taking measures to slow down the disease.

Basic Reproduction Number - R_0

- Definition: Expected number of secondary cases produced by a single infection in a completely susceptible population
  - If $R_0 < 1$: the infection will die out in the long run
  - If $R_0 > 1$: the infection will spread in a population
- Factors that affect R_0
  - Probability of infection when a susceptible and infected individual meet
  - Rate of contact between susceptible and infected
  - Duration of infectiousness

585

Similar to generation. If you have 1 person that is sick, that person is responsible for transmitting the disease to how many people.
If R0=3, then 1 person is responsible for making 3 people sick.
If R0 is greater than 1, then the disease will continue spreading unitl all people at risk will be infected.
You want the R0 to be less than 1, if you use a vaccine you want it to reduce R0 to less than 1.
Rate of contact: 20 year old has a much higher rate of contact bc they are going everywhere, lots of places, than an old 90 years old that cant walk.
R0 will be very high if someone can remain infected for 20 years.
R0 will be low if someone dies within 24 hours of contracting the disease.

Herd Immunity

- Resistance of a community or group of people to a particular disease
- Herd immunity implies group protection beyond that afforded by the protection of immunized individuals
  - Immunized individuals protect non immunized individuals
    - $R_0 < 1$

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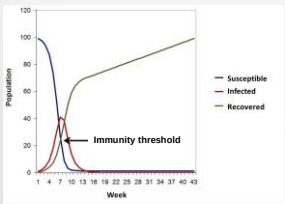
People that do have protection are protecting non-immunized individuals.

Herd Immunity Threshold

- Proportion of immune individuals in a population above which a disease may not persist



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As the disease spreads, the number of susceptible ind. decreases. The number of recovered people increases as the number of susceptible people decreases.
Immunity threshold: herd immunity is reached.

Determining Herd Immunity Threshold

- How many people must be immunized to acquire herd immunity?
  - $V_c = (1-1/R_0)/E$
    - V_c *:* Critical minimum proportion to be vaccinated
    - *E :* Percentage of immunized individuals which are protected (Measure of vaccine efficiency)
  - Need to reach an $R_0 < 1$

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Vc= minimum number of poeple to acquire herd immunity.

Determining Herd Immunity Threshold

- Example
  - A given disease has R_0 of 3 and vaccination provides 100% protection. How many people must be immunized to acquire herd immunity?
  - $V_c = (1-1/R_0)/E$
  - $V_c = (1-1/3)/1$
  - $V_c = (1-1/R_0)/E$
  - $V_c = 0.66$; therefore 66% need to be immunized

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Source of the Infectious Agent

- Inanimate reservoirs
  - Some pathogens are found mainly in non-living habitats
  - ex. *Clostridium tetani*, found in soil
- Animate reservoirs
  - The pathogen is not usually found in nonliving habitats
  - ex. Virus; obligate parasite

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Mostly pathogens that are transmissible but not contagious. Doesnt require a host, survives well in the envt.
Animate: contagious, require a host to survive, Can have more than 1 host, does not necessarily cause the disease in all hosts.

Human Reservoirs

- Active carrier
  - Individual who suffers from the disease and which expresses the symptoms associated it
- Incubating carrier
  - Healthy individuals who harbor the pathogen
  - The individual will be sick at a later date
- Convalescent carrier
  - Individual who has recovered from the illness
  - Expresses no symptoms
  - Harbors a large number of live pathogens
- Healthy carrier
  - Individual was never sick
  - Harbors the pathogen

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Active carrier: they are infected and expressing the disease, they harbour and express symptoms of the pathogen. Considered the safest reservoir because you can identify them. You can isolate, stay away, take necessary measures to treat them.
Incubating: they have the infection, does not have the disease, much more detrimental to the pop. bc you can't identify him. No measures can be taken.
Convalescent: has no symptoms, thinks they are better even though he still has pathogens, still contagious, can't identify him either.
Healthy:Has the infection, does not develop the disease. The pathogen stopped at the commandment that says it has to cause harm. They are still contagious, still has the pathogen. No way to identify them. Most harmful. Responsible for transmitting the disease to the most people. Harbours it for the longest period of time. Immune system in tolerating the pathogen.

Animal Reservoirs

- Healthy carriers
  - Does not cause the disease in animals
  - Can be a resident of the natural flora
    - Ex. *E. coli, Salmonella*
- Diseased carrier
  - Causes the disease in the animal
  - The disease can be transmitted to humans
    - **Zoonosis**
      - Disease that can be transmitted from animals to humans naturally

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The pathogen doesnt cause the disease in the animal because there is a compromise between the pathogen and the immune system of the animal. Can cause the disease in humans.
Diseased carrier: cause disease in animals and in humans.
Zoonosis: pathogen can be transmitted from animal to man, not from man to man.
There are some diseases that can be passed from animal to man, and man to man.

Bacterial Zoonosis

Bacteria	Animal host	Disease
Mycobacterium bovis	Livestock	Tuberculosis
Yersinia pestis	Rodents	Bubonic plague
Bacillus anthracis	Livestock	Anthrax
Borrelia burgdorferi	Cervidae	Lime disease
Chlamydia psittacosis	Birds	Psittacosis

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Control of the Reservoir

- Destruction of the reservoir
  - Domestic animals
    - Ex. Bovine tuberculosis, mad cow disease
  - Wild animals (Very difficult)
    - Rabies, Nile virus
  - Humans (impossible)
  - Inanimate reservoirs
    - Elimination or treatment is possible
      - Ex. Treatment of water

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Best way to get rid of a disease is to destroy the reservoir. This is permitted for domestic animals, not for humans.

Control of Transmission

- Airborne transmission
  - Isolate sick patients
  - Wearing of a mask (frequent in Japan)
  - Filtration system
- Transmission by contact
  - Frequent hand washing
  - Minimize contacts
  - Use of condoms
  - Use of disinfectants
- Transmission by ingestion
  - Chlorine treatment of water
  - Treatment of waste water
  - Cooking food
  - Use of food preservatives

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Wearing a mask for healthy and sick ind. Filtration is effective.

Quarantine

- Goal:
  - Eliminate/restrict propagation
  - The goal is not to save the diseased!
- Diseases for which there is an international agreement which allows quarantine :
  - Smallpox
  - Cholera
  - Plague
  - Yellow fever
  - Typhoid fever
  - SARS

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The purpose of quarantine is to save the people that are not sick. the purpose is to isolate the sick until they die or recover. No intention to save the sick.

Nosocomial Diseases

- Infection acquired in a hospital that was not present or incubating upon admission
- Persons at risk of nosocomial infections:
  - Patients
  - Personnel
  - Visitors

Infection you acquire in a health care establishment. You get it in the establishment, you develop the disease. Anyone who goes to a health care establishment is at risk.

Nosocomial Vs Community Acquired

- Greater risk of acquiring a nosocomial infection compared to a community acquired infection; why?
  - Host defenses depressed by underlying disease or treatment, malnutrition, age
  - Anatomic barriers breached (IV's, catheters, surgery, etc.)
  - Exposure to more virulent pathogens
    - Ex. Many multiple resistance organisms

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More virulent because there is extremely strong selective pressure in hospitals etc..

Source of Pathogens

- Reactivation of latent infection
  - TB, herpes viruses
- Endogenous:
  - Normal flora of the skin, respiratory Tract, GI tract
- Exogenous
  - Inanimate environment
  - Animate environment
    - Hospital staff, visitors, other patients

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Contracts between immune system and natural flora is weakened bc they are sick,
Ex, Ties and beards are very good reservoirs to carry pathogens from one patient to the other.

Preventive Measures

- Hand washing :
  - Before and after contact with a patient
  - Before and after preparing/handling/serving food or medicine
  - After contact with contaminated items
  - After satisfying one's personal needs
  - Before leaving the work area
- Wearing gloves
- The mask
- Isolation of patients

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Gloves act as a transmission vehicle if they are not changed after every patient.