MICROBIOLOGY (BT-102)

Table of Contents

READ THIS BEFORE YOU READ ANYTHING ELSE IN THIS DOCUMENT!

These handouts have been prepared very carefully and provide a concise but conceptual understanding of Fundamentals of Microbiology. Listen to the video lectures first, and then read the corresponding material provided in these handouts. Information covered in the video lectures and the handouts will essentially equip you with enough understanding that studying the material later from the book (Microbiology by Tortora et al) will become an enjoyable experience for you. These lectures and accompanying handouts are in no way enough alone; therefore studying the material from the book is essential to maximize comprehension about Microbiology. I hope you will enjoy learning with me.

The recommended book for this course is **"Microbiology: An Introduction, 10th or 11th Edition, authored by Tortora, Funke, and Case".** Both of these books can be had from Amazon. Some bookshops at Mall Road, Lahore, also carry the original copies of the books. I must tell you that there are many books on Microbiology; however, Tortora's Microbiology is unmatchable. **Please note that the layout of the lectures and the handouts is based on the 10th Edition of the book.**

The diagrams and figures have been taken from the net and the use of these diagrams and figures is purely for educational purpose (intended for a class in a university setting). Again, I highly recommend that the relevant chapters of the book must be read in order to have a good grasp of the subject. Good luck!

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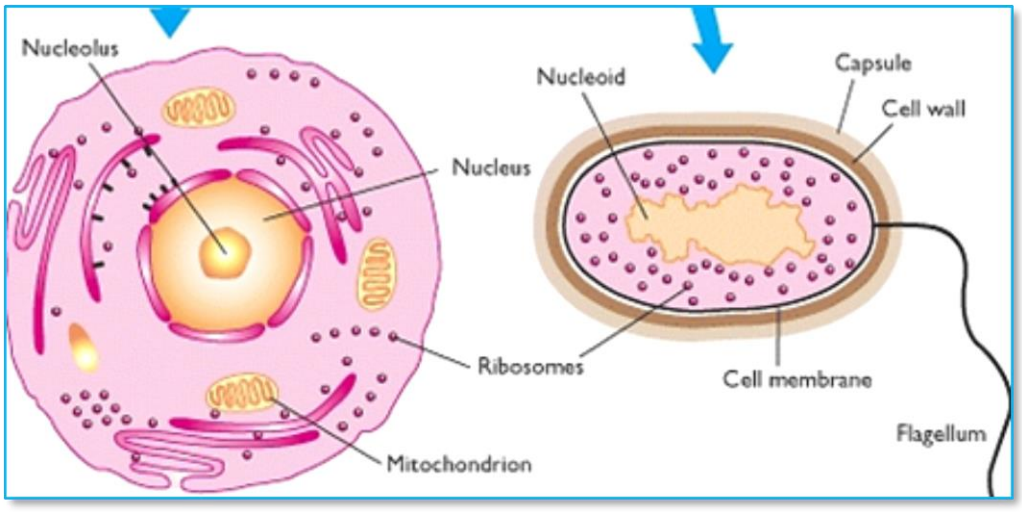
(Ph.D., DVM)

Lesson 1. Read pages 2, 6, and 274 (Chapter 10).

LESSON 1. MICROBES AND THEIR CLASSIFICATION

- **What are Microbes?**

- Also called germs, microorganisms are tiny living beings, usually found as single cells (usually procaryotes), although some are multicellular (usually eucaryotes).
- They are too small to be seen with the unaided or naked eye.
- A microscope is needed to see them.
- Studying microbes is microbiology.
- Microbes are diverse; hence classification has been a real challenge for the microbiologists.
- Most microbes are good for us, however, some cause diseases too.
- Procaryotes have no nuclear membrane while eucaryotes have their DNA enclosed in a nice nuclear membrane.
- The following is an image that illustrates procaryotes versus eucaryotes depicting a few other differences as well: (we will talk in more details about these differences in another lecture)



- **Why do we Classify Organisms?**

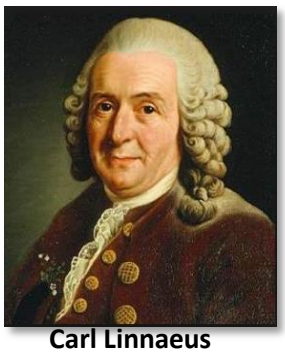
- Studying them becomes convenient if organisms are classified into various groups based on their similarities.
- Easy Identification
- Easy to establish relationship between organisms
- Evolutionary relationships between organisms can be established. Phylogenetic trees can be made or deduced which help biologists to study and understand the organisms better.

Lesson 2. Read pages 3-6, 281, 393-394.

LESSON 2. FIVE KINDOMS AND MICROBIAL CLASSES

- **What are the bases of classification?**
- Similarities and dissimilarities among them are noted.
- Morphology and metabolic enzymes used to be the main features prior to the advent of molecular biology.
- Genetic similarities have become the most important criteria for classifying the organisms now a day.
- Using ribosomal RNA genetics, strong evolutionary relationships can be established.

- **Classification of Organisms**
- From the time of Aristotle, living organisms were placed into just two groups: Plants and Animals
- Carl Linnaeus in 1735 proposed the first classification system with the two kingdoms: Plantae and Animalia.


Carl Linnaeus

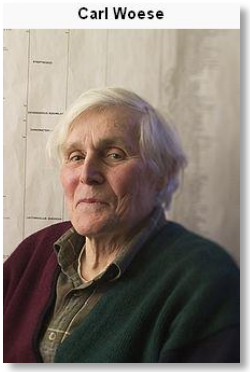
- So, before microbes were known, all organisms were either placed in animal or plant kingdom.
- However, with the invention of the microscope, the scope of organisms changed. Microbes were also discovered.
- In 1857, Carl von Nageli proposed that bacteria and fungi be placed in kingdom Plantae.
- However, in 1866, **Ernst Haeckel** bacteria, protozoa, fungi and algae were placed in another kingdom, Protista.
- Edouard Chatton, in 1937 introduced the term prokaryotes for the first time to distinguish cells with no distinct nucleus.
- In 1959, the fungi were placed in their own kingdom, Fungi.
- In 1968, Robert Murray proposed the kingdom Prokaryotae for bacteria.

Fig

In 1969, *five kingdom classification* was proposed by Robert Whittaker as under:

- **Plantae: plants**
- **Animalia: Animals**
- **Fungi: Yeasts, molds and mushrooms**
- **Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.**

- In 1977, based on the fact that ribosomes are not the same in all cells, Carl Woese proposed three domain classification system as under:



  - Domain Bacteria: contains true bacteria, also called Eubacteria
  - Domain Archea: Archea prokaryotes are included in this domain.
  - Domain Eukarya: This include the following kingdoms:
    - Kingdom Animalia
    - Kingdom Plantae
    - Kingdom Fungi
    - Kingdom Protista
  - Note that prokaryotes (Eubacteria and Archea) have not been yet assigned any kingdoms.

- **Types of Microorganisms: An Overview**

- **BACTERIA:** These are prokaryotes, usually single-celled organisms. Their cell wall is made of **peptidoglycan.** These are true bacteria.

- **ARCHEA:** These are also prokaryotes but their cell wall **lacks peptidoglycan** (may have special lipids).

They also vary in their ribosomal RNA sequence from Eubacteria. They live in extreme conditions. They do not cause diseases.

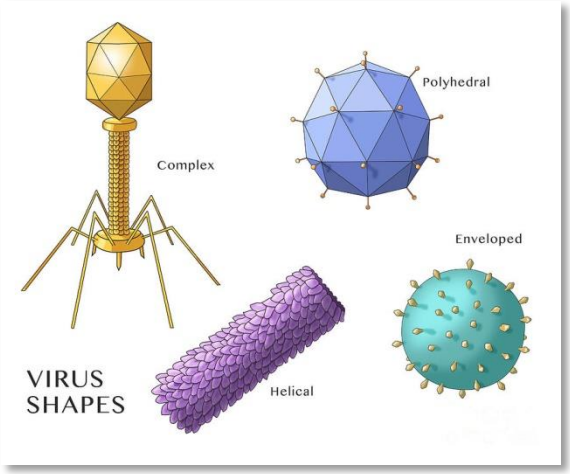
- **PROTISTS:** This group includes algae, protozoa, slime molds and water molds.
  - **Algae:** These are eukaryotes. Their cell wall consists of cellulose. These are photosynthetic and produce oxygen. They are usually unicellular, but multicellular algae are also common. Seaweeds and pond scum are some of the examples. These will be studied in detail in a separate lecture.

  - **Protozoa:** These are also eukaryotes, unicellular and have no cell wall. They move by pseudopods, flagella or cilia. Some are photosynthetic.
  - **Slime Molds:** They are fungus like organisms at one time that may assume a protozoon like nature at another time. Basically, they are single celled but aggregate to form multicellular reproductive structures.

  - **Water Molds:** These are protists that grow on the surface of fresh water and humid soils. Also known as mildews and white rusts. They look like fungus.

- **FUNGI:** Usually unicellular (but also multicellular), their cell wall consists of chitin.

- **VIRUSES:** They are acellular structures that consist of a protein capsid enclosing either DNA or RNA (but never both) and sometimes may contain an extra membrane called envelope.



- **VIROIDS:** They are composed only of circular single stranded (ss) RNA.

- **VIRUSOIDS:** They contain circular ssRNA that need helper viruses for replication and encapsidation. They are also called satellite viruses.

- **PRIONS:** These are basically infectious proteins formed inside the body. They behave like organisms as they can be transmitted from one individual to another. Extremely resistant to heat.

Lesson 3. Read pages 2, 278-9

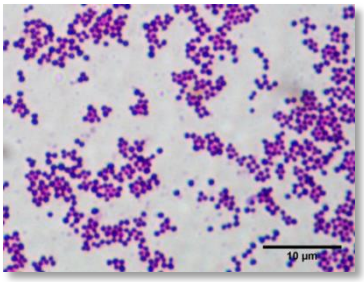
LESSON 3. NAMING MICROBES

- **Binomial Nomenclature**
- **Carl Linnaeus** established the system of scientific nomenclature. According to his system, each organism has two names: The genus and specific epithet (descriptive word that tells some quality of a thing).
    - Example is ***Escherichia coli*** (coli means belonging to the colon).

- The names are italicized or underlined. The genus is capitalized, the specific epithet is lowercased.

- The names are "Latinized" and used worldwide.
- The names may be descriptive or may honor a scientist.

- Another example:
    - ***Staphylococcus aureus***: Describes the clustered (*staphylo-*) spherical (*cocci*) cells
    - Describes the gold-colored (*aureus*) colonies



- After the first use, scientific names may be abbreviated with the first letter of the genus and the specific epithet:

    - ***Escherichia coli*** and ***Staphylococcus aureus*** are found in the human body. ***E. coli*** is found in the large intestine, and ***S. aureus*** is on skin.

- **Difference between bacterium versus bacteria:**
- **Bacterium is a single organism or a single species of organism.**
- **The word "Bacteria" is plural and refers to many cells of the same species or it could mean many different species of organisms too. So be careful in the use of these words.**
- **Similarly the word "medium" and "media" are singular and plural respectively. There is no word "medias".**

Lesson 4. Read pages 6-9.

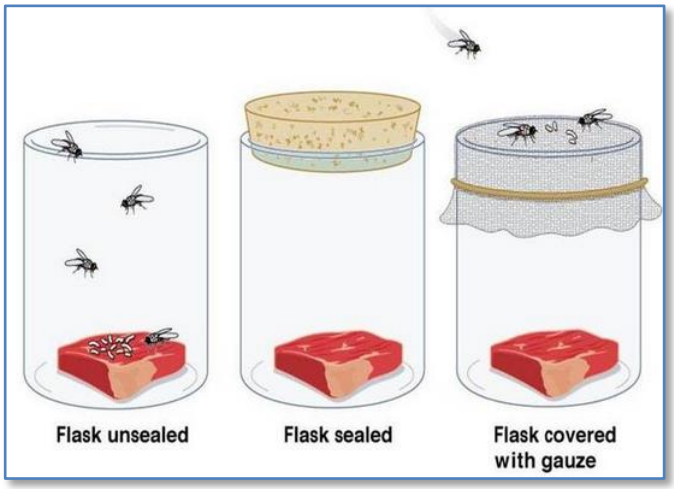
LESSON 4. SPONTANEOUS GENERATION AND ITS BASIS

- **Spontaneous Generation and its Basis**
- From the time of Aristotle, this was a general belief that some forms of life could arise spontaneously from nonliving objects or things. Not long ago, people thought that toads, snakes, and mice could be born from the soil.

- Similarly, flies can emerge from manure and maggot can come out of decaying corpses.

- In 1668, an Italian scientist, Francesco Redi, challenged this spontaneous generation theory. He performed a famous experiment by taking some jars and, by placing meat in them; he sealed some jars and left out others open.

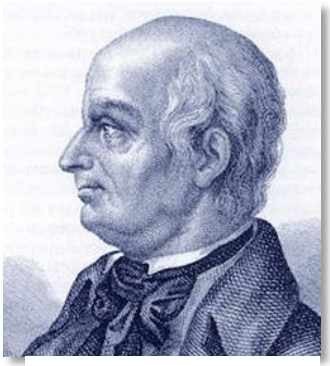

Francesco Redi



- The ones that were left open had maggots in them while the sealed one did not have any life. No doubt, his experiment proved that there is no life without pre-existing life, however, people objected by saying that at least Leeuwenhoek's "Animalcules" (microbes) can arise spontaneously.

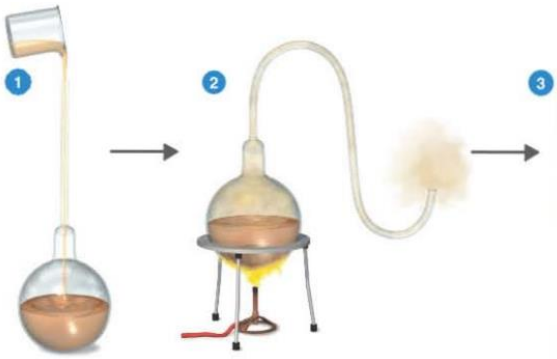
- In 1745, John Needham reported that microbes can arise spontaneously from heated nutrient fluids when cooled and sealed in flasks.

- However, in 1765, another Italian scientist, Lazzaro Spallanzani, proposed that the organisms in the broth prepared by John Needham may have entered from the air after they were boiled and cooled before putting them in flasks. Spallanzani demonstrated this by doing an experiment in which no microbes were seen. John Needham responded that Spallanzani did not allow the vital force to enter in the flasks by sealing the flasks before heating. So vital force from the air did not enter into the flasks, there was no life visible.



Lazzaro Spallanzani

- This vital force was given even more credit when Anton Laurent Lavoisier showed the importance of oxygen to life.
- The issue of spontaneous generation was finally resolved by French scientist, Louis Pasteur in 1861.
- Pasteur made a glass flask with a long narrow S-shaped neck (shown below in the diagram) and filled with the broth and heated the contents to kill microbes present already in the soup. He also took some other flasks (not s-shaped necked flasks) and also heated broth/soup in them but left them opened much like the S-shaped neck flask. No microorganisms were seen in the S-shaped neck flasks, but it did allow the entry of O_2 (vital force).

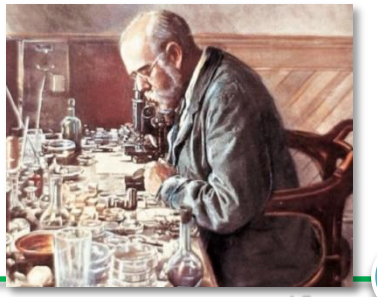


Louis Pasteur

Lesson 5. Read pages 9-12 and 404.

LESSON 5. THE GOLDEN AGE OF MICROBIOLOGY

- **The Golden Age of Microbiology**
- Once the issue of spontaneous generation was resolved, and once the scientists were convinced that microorganisms do exist, they turned their attention and energies for finding out more about microorganisms. Here is a brief overview of what followed in the period from 1857 to 1914, which was rightly called as the **Golden Age of Microbiology**:
    - **Fermentation and Pasteurization:** French wine and beer industry approached Louis Pasteur to fix beer spoilage problem. Many at the time thought that air results in the spoilage of the wine, however, Pasteur demonstrated that wine and beer are fermentation products made by the yeast, and spoilage is done by a bacterium instead. So, he solved the issue of spoilage by low heating of the beer and wine to kill that bacterium. He named this technique for preserving fluids as Pasteurization which we use today for many fluids such as milk, juices etc. In fact, Pasteur demonstrated a link between the causative agent and a disease when he proved that a bacterium is involved in the spoilage.
    - **The Germ Theory of Disease:** Many scientists at the time started thinking (after Pasteur) that microbes may be involved in causing diseases in humans, animals and plants, and they started searching for them. This idea led to the germ theory of the disease.
        - **In 1865, Pasteur** established the causative agent (a protozoan) for silkworm disease.
        - **Joseph Lister,** who was already practicing good hygiene at the time for handling his patients, started using disinfectants for the first time. A disinfectant with the name "Listerine" is still in use today.
        - **In 1876, Robert Koch,** a German contemporary of Pasteur, provided the first proof that a bacterium, *Bacillus anthracis*, actually causes the disease in animals. He was the first to propose what we know today as Koch's Postulates that provided the guidelines



for establishing organisms as causative agents of diseases.

- **Vaccination:** In 1880, Pasteur discovered why vaccination works when he went for demonstrating that *Pasteurella multocida*, a bacterium, causes fowl cholera in chickens. Unfortunately, the culture of the organisms was old and did not cause the disease in the birds. So he took a fresh culture of the organism second time but used the same birds that he had used earlier. To his surprise, the birds did not die even this time. So, he concluded that the first culture (old) was weak and did not cause the disease; however, it stimulated the body to make substances (we now know as antibodies) that provided protection against the fresh culture of bacterium. Pasteur gave this technique the name "vaccination" to honor Edward Jenner who used a similar technique with small pox in 1796.

- **The Birth of A Magic Bullet**
- Some scientists started looking for ways to kill microbes that were found to cause diseases in humans. Paul Ehrlich, a German, was a pioneer in this field. His observation that various stains can bind to the organisms without binding to the background led to testing those stains for antibacterial activities. He thought if stains spare the background (and only binds the organisms), they will not harm the person if injected into the person who is suffering from an infection. He suggested that such a chemical will be called as a **magic bullet** because it would spare the body but destroys the microbe. The use of chemical for treatment is called chemotherapy.
- In 1910, he made the first chemotherapeutic agent, **salvarsan**, for treating humans against syphilis.
- **Sulfonamide** was also synthesized in that time period.
- In 1928, **Alexander Fleming** accidently discovered the first antibiotic, penicillin, from a fungus, *Penicillium chrysogenum*.
- We now have thousands of antibiotics, although, microbes are becoming resistant to most of them.

Lesson 6. Read pages 13-16.

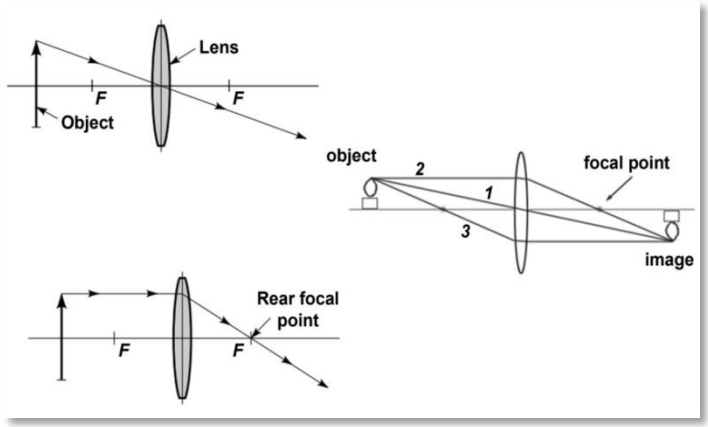
LESSON 6. MODERN DEVELOPMENTS IN MICROBIOLOGY

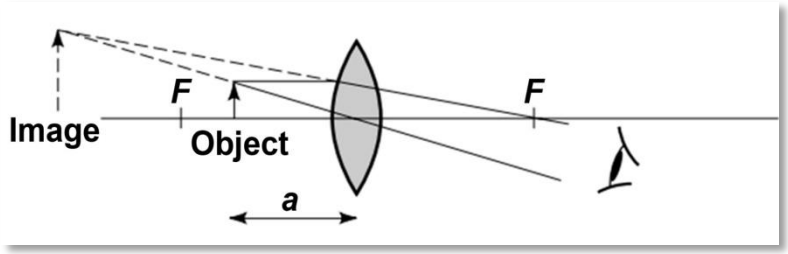
- **Modern Developments in Microbiology**
- The foundation laid during the Golden Era led to burst of knowledge in the field of Microbiology resulting in the creation of many disciplines such as:
    - Virology: The study of viruses
    - Bacteriology: The study of bacteria
    - Mycology: The study of fungi, yeast and mushrooms
    - Immunology: The study of antigens and antibodies
    - Genetic Engineering and Biotechnology: Gene manipulation and biologics production etc.
    - Various other branches have been mentioned in the lecture. Just listen to the lecture. These are just definition of various fields of Microbiology.
    - Epidemiology will be studied in a separate lecture or two in the coming session soon.

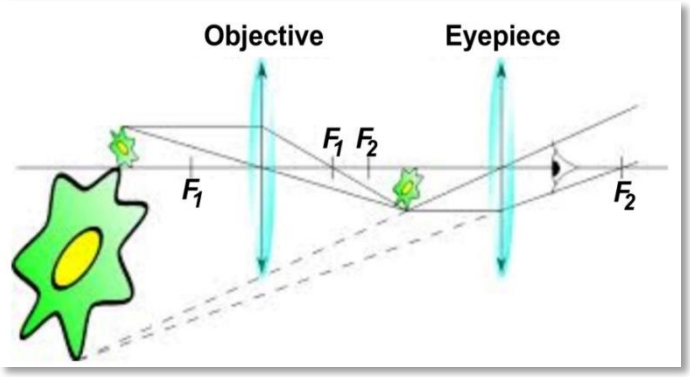
Lesson 7. Read pages 55-59 (Ch 3).

LESSON 7. LIGHT MICROSCOPE AND ITS PRINCIPLE

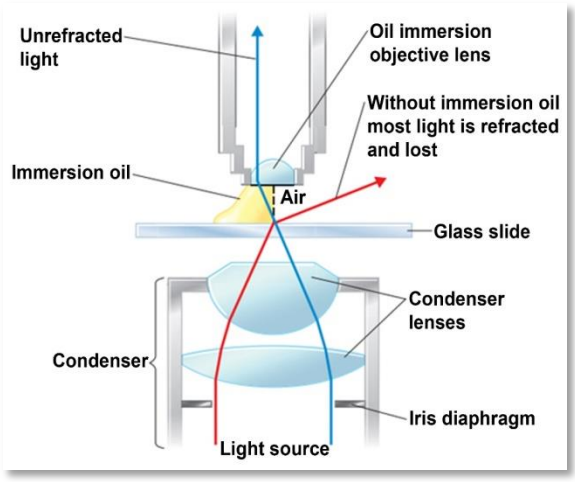
- **Microscopes and Microscopy**
- Illuminator: a light source
- Condenser: Directs the light through the specimen
- Objective Lenses: Close to the specimen
- Ocular Lens (Eyepiece): Close to the eye
- **How a microscope works**
  - Microscope is a combination of convex lenses.
  - If an object lies farther from the focal point of a convex lens, it forms an image on the opposite side of the lens. This image is real and always inverted.
  - Secondly, if the object lies within the focal point of a lens, it forms a virtual image which is on the same side of the lens where the object is located. This is not inverted image.
  - Now, in a microscope, these two lenses are arranged in such a way that the image formed by the first lens is positioned within the focal length of the second lens. Study the diagrams given below for a better concept:







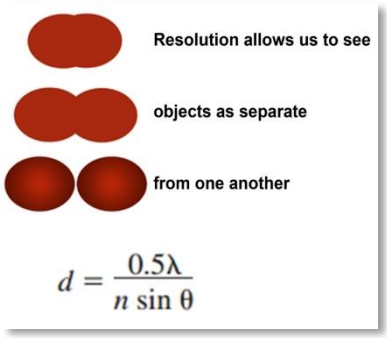
- **What is total magnification?**
  - Total magnification = Magnification of objective lens x magnification of ocular lens
  - Objective lenses come in three sizes mostly:
    - Low: 10 X
    - High: 40 X
    - Oil immersion: 100 X
  - Ocular lens: 10 X
  - Low magnification power: 10 x 10 = 100
  - Oil immersion power: 100 x 10 = 1000
- **Refractive Index and the Image**
  - Refractive Index: Ability of a medium to bend the light
  - Light rays move in a straight line through a single medium
  - Light rays bend when the pass from one medium to another
  - We use cedar wood oil to eliminate this bending of light in microscopy. Cedar wood oil has the same refractive index as that of the glass (1.5), so that the light rays do not bend when they pass through the specimen into the objective lens of the microscope. This provides a brighter and better image.



  - To further increase the image contrast, we stain the specimen with various dyes.

- You can appreciate the path of the light using immersion oil and without the oil in the diagram shown above (last page).

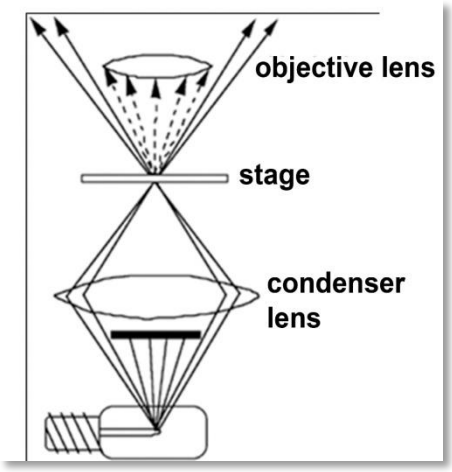
- **What is Resolution of a Lens?**
  - Resolution is the ability of the lenses to distinguish between two closely lying objects as separate. See the accompanying diagram for visual concept of resolution.
  - Light microscope resolving power is 0.2 μm.
  - n = refractive index, 1.5 for immersion oil
  - Resolution (d) in the formula given in the diagram is directly proportional to the wavelength of the light used. As resolution is the smallest distance between two closely lying points, use of light with smaller wavelength gives better resolution. That is why you would find blue filters for blue light as illumination for the microscope, as blue light has the shortest wavelength in the visible spectrum.



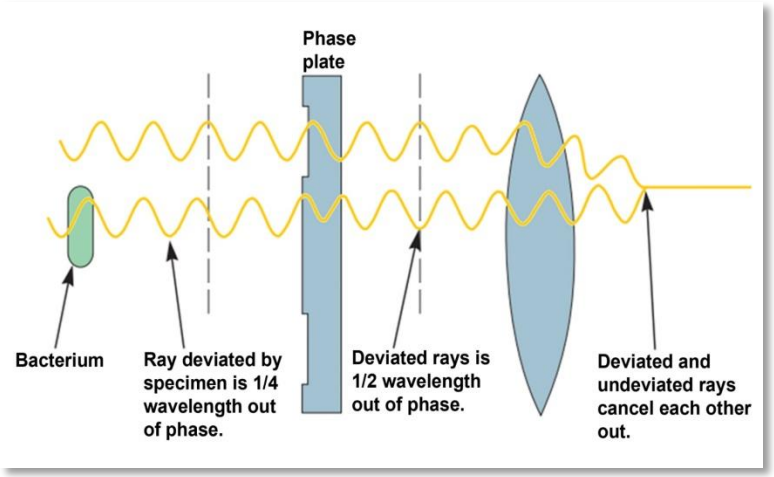
Lesson 8. Read pages 59-66.

LESSON 8. TYPES OF MICROSCOPES

- **Various Types of Microscopes**
- **Darkfield Microscopy**
  - This microscope makes use of a special condenser that provides illumination from the sides only as depicted in the diagram below:
  - This special arrangement of the condenser throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night.



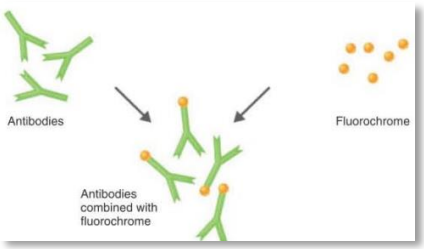
  - The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope.
- **Phase Contrast Microscopy**
- This instrument is built on the concept of phases of light waves. Please follow the accompanying diagram for conceptual understanding of phase microscopy. The light ray that passes through an organism gets retarded about ¼ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the center than the ends. This arrangement causes the

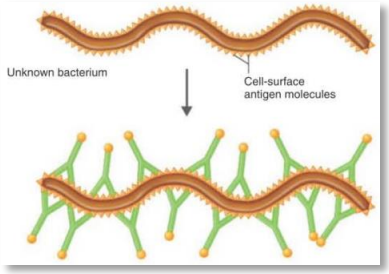


retarded light to further slow down about ¼ of its wavelength, which when recombines with the un-retarded light creates a contrast which makes the cells/organisms visible.

- This method for visualizing organisms also does not involve staining.

- **Fluorescent Microscopy**
  - This microscope is equipped with UV light that provides the source of light for illumination. However, this light source does not pass through the slide, it instead falls over the slide (having specimen) and creates fluorescence.





  - Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum)
  - But how is this fluorescence created? Well, the fluorochromes are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies. See the accompanying diagrams for details. Look for UV lamp (Mercury Lamp).

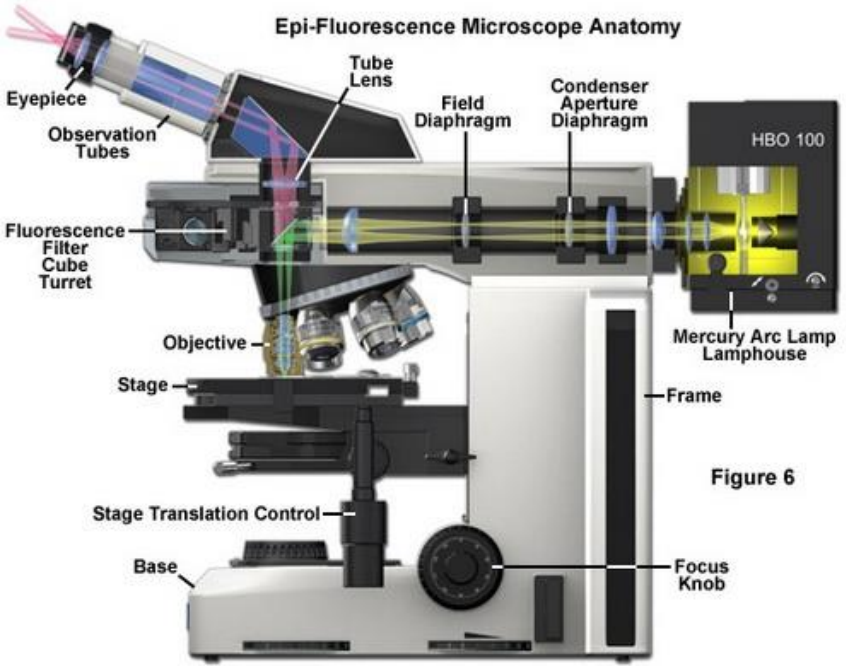
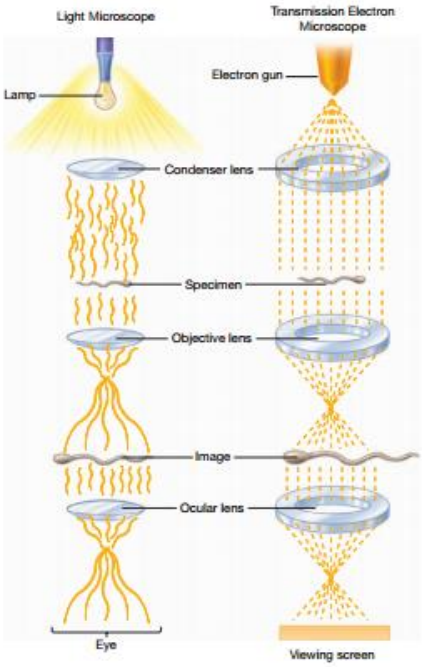
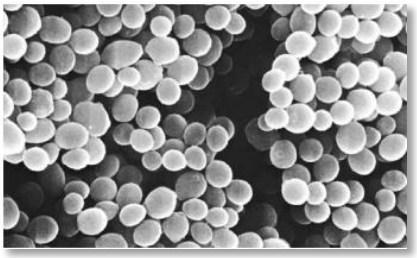


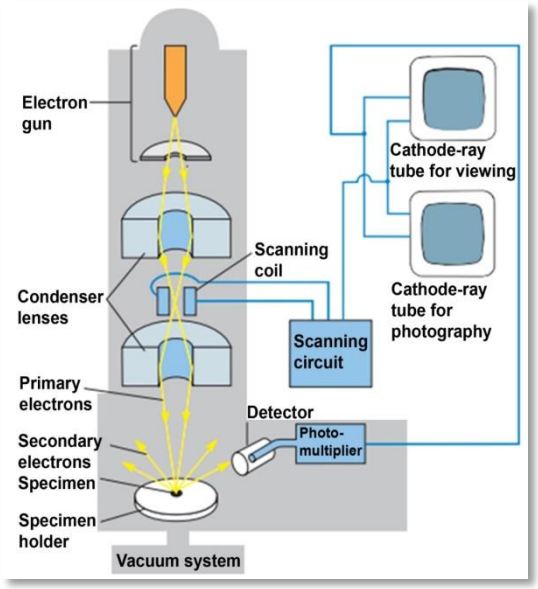
Figure 6

- **Transmission Electron Microscopy**
  - Electron microscope (EM) makes use of electron beam as an alternative to light rays. Since electron beam has much shorter wavelength, resolution of EM is 0.1nm compared with light microscope which is 200nm.
  - Second difference of EM with light microscope is that focusing of electron beam is done not by glass lenses but by electromagnets that acts like lenses.
  - The third difference in EM is that the image of the specimen is formed on a fluorescent screen like a TV screen.



- **Scanning Electron Microscopy**
  - This is a modified version of electron microscope. Electron beam is allowed to fall on to the specimen and the image is formed from the reflected electrons that are collected and assembled into an image by the detector. It gives a three dimensional picture as shown for *Staphylococcus aureus:*

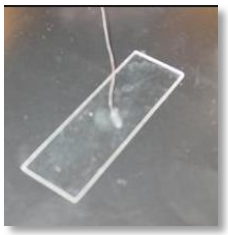




Lesson 9. Read pages 68-69.

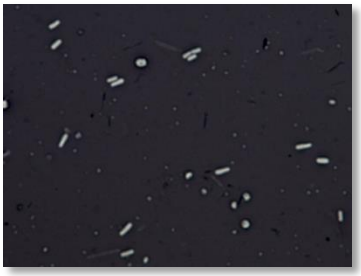
LESSON 9. STAINS AND STAINING (GENERAL)

- **Why do we stain organisms**
  - Most organisms appear colorless when seen under a microscope.
  - Staining emphasizes certain structures of the organisms.
  - Staining is just coloring with a dye.
  - Staining increases visibility of microbes because staining increases contrast.
  - Shape, size and arrangements of the organisms can be readily seen.
  - Purity or contamination of a culture could be determined.
  - Differentiation and classification of microbes is possible. For example, microbes can be categorized into Gram positive or Gram negative groups.
  - Structures such as flagella, capsule and spores etc. of bacteria can be detected with staining.
- **How to make a smear?**
  - Staining starts with making a smear using a glass slide.
  - Using a platinum loop, a colony of a drop of broth culture can be smeared into a thin film on a glass slide for making a smear.



  - The specimen is spread into a thin film (smear).
  - Smear is air-dried.
  - Smear is fixed (attached) to the slide before staining.
  - **Heating** the slide is one way of fixing the smear.
  - **Methyl alcohol** can be used.
- **Why do we fix the smear before staining?**
  - The purpose of fixing the smear is to physically attach the specimen onto the slide. It is important step before the smear is stained.
  - Physical method: Heat
  - Chemical Methods: Ethanol or formaldehyde
  - Stain is applied and then washed off with water.
  - The slide is air dried or blotted.

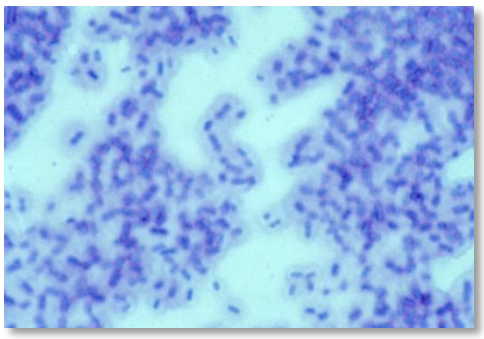
- **What is a stain?**
  - Stains are salts composed of a negative and a positive ion.
  - Chromophore: A colored ion.
  - **Basic dye:** The color is in the positive ion.
  - Acidic dye: The color is in the negative ion.
  - Bacteria are slightly negatively charged at pH 7.
  - Basic dyes are most commonly used for bacteria.
- **Basic Dyes: Examples of positive Stains:**
  - Crystal violet
  - Methylene blue
  - Malachite green
  - Safranin
- **Acidic Dyes: Examples of negative stains:**
  - Eosin
  - Acid fuchsin
  - Nigrosin
- **Negative Staining:**
  - As the cells/organisms are negatively charged, acidic dyes will not bind to them. However, these dyes are used to stain the background. Well, the trick is that the dye is applied to the smear but is never washed off.
  - Organisms with negative stain look colorless in the dark or colored background as seen in the accompanying photograph:



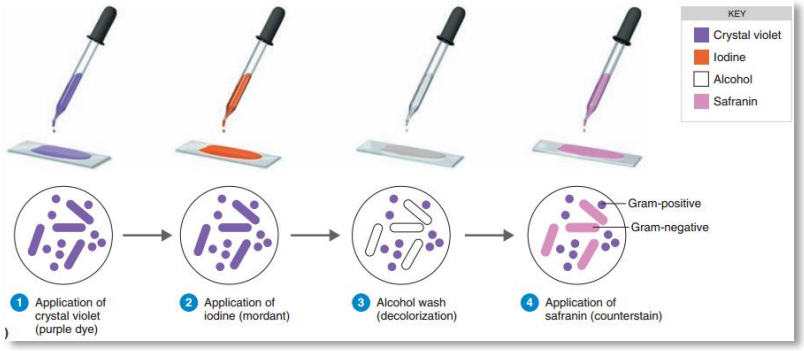
Lesson 10. Read pages 69-70.

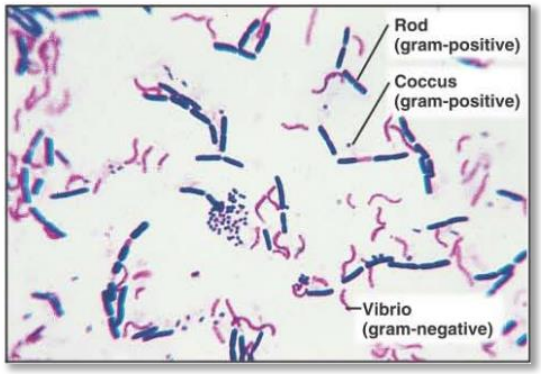
LESSON 10. TYPES OF STAINING

- **Types of Staining**
  - Simple Staining: only one stain is used.
    - Methylene Blue staining
  - Differential Staining: A couple of stains used.
    - Gram Staining
  - Special Staining:
    - Endospore staining



- **Simple Staining (in detail):**
  - A single stain is used.
  - Stain is washed off.
  - A mordant is added, sometimes.
  - Microbes seen under the microscope.
    - Examples of simple stains:
      - Methylene blue
      - Carbolfuchsin
      - Crystal violet, and safranin
- **Differential Staining (Gram's Staining)**
  - Differential stains react differently with different kinds of bacteria.
  - Most common is Gram staining.
  - 1884, by Hans Christian Gram
  - Follow the diagram given below to see the steps involved in Gram's staining:

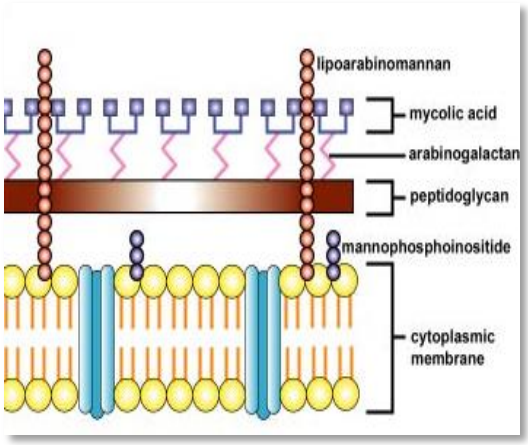


- Crystal violet is added.
  - It is a primary stain
- Crystal violet is washed off.
- Iodine is added to enhance binding.
  - Iodine is a mordant.
- The slide is washed off with alcohol.
  - Decolorizing agent
- Gram positive bacteria retain crystal violet if seen at this stage.
- Gram negative bacteria appear colorless if seen at this stage.
- Alcohol is rinsed off and stained with safranin.
  - Safranin is a counterstain
- The smear is washed again with water.
- Blotted dry and examined microscopically.
- 

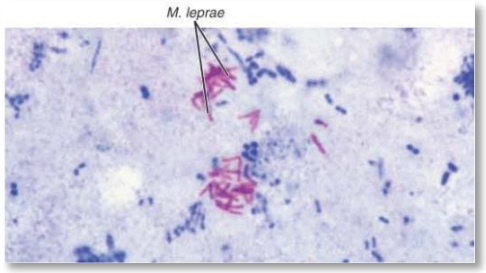
Lesson 11. Read pages 70-72.

LESSON 11. SPECIAL STANING

- **Acid-Fast Staining**
  - Some bacteria such as Mycobacteria have a waxy material in their cell wall.
    - Specific name for this waxy substance is mycolic acid
    - *Mycobacterium tuberculosis*: Causative agent for tuberculosis
    - *M. leprae*: Causative agent for leprosy

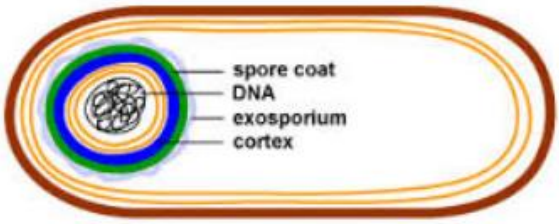


  - **Principle of Acid-fast staining:**
    - Mycobacteria are lipophilic, not easy to stain
    - But once stained, they are resistant to acidic alcohol decolorization process.
  - Overall Procedure for Acid-fast staining:
    - Carbol fuchsin: It is a primary stain
    - Heat is used to enhance penetration of carbol fuchsin.
    - Acid alcohol is a decolorizer.



    - Methylene blue is a counterstain.
    - Please note, Mycobacteria are gram positive, however, they look red in acid-fast staining.
- **Special Staining:**
  - Used for coloring specific parts of an organism. Examples include:
    - Endospore
    - Flagella
    - Capsule

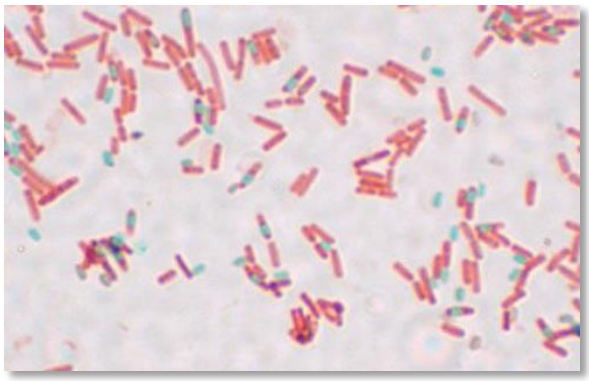
- Endospore: A resistant dormant structure within a cell



- Position of the endospore varies within the cell

- Position of spores can be central, terminal or subterminal. The spores can be bulging or non-bulging.
- Spores can also be seen with Gram's staining. However; they appear as a clear hallo in Gram stained smears. See the accompanying figure.

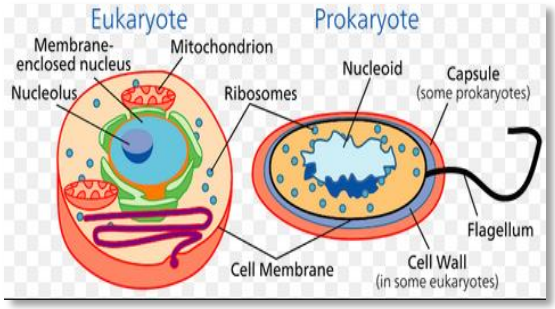
- **Endospore Staining Procedure**
  - Malachite green: Primary stain
  - Heated to steaming for 5 min.
  - Washed with water.
  - Counterstained with safranin.
  - See under the microscope. Spore looks green and vegetative cells as red.



Lesson 12. Read pages 77.

LESSON 12. DIFFERENCES IN PROKARYOTIC AND EUKARYOTIC CELLS

- Prokaryotes and eukaryotes are chemically similar. All made up of nucleic acids, protein, lipids, and carbohydrates. All metabolically similar. So, how we can differentiate them from each other?



- **Prokaryotes have the following features:**
  - DNA is not enclosed in a nuclear membrane.
  - Chromosome: mostly Single, circular
  - DNA not associated with histones
  - No membrane enclosed organelles
  - Cell wall has peptidoglycan, a complex carbohydrate.
  - Divide by binary fission.

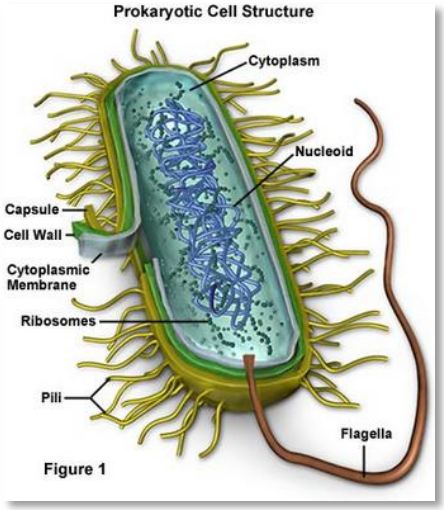
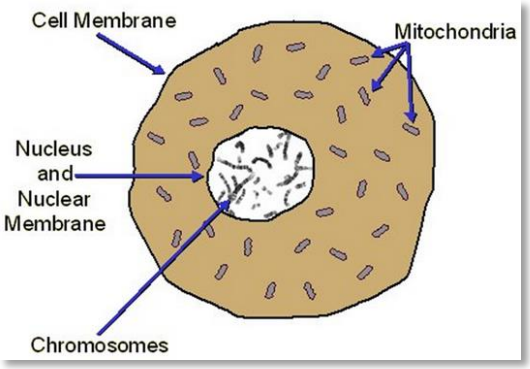
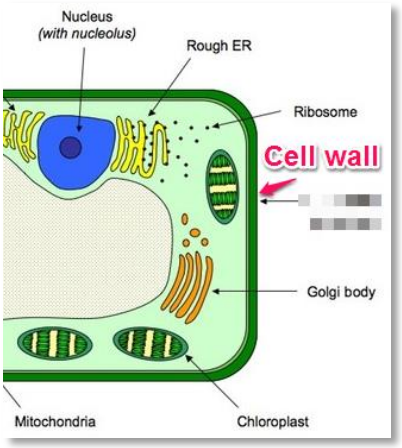


Figure 1

- **Eukaryotes have the following features:**
  - DNA is enclosed in a membrane-bound nucleus.
  - DNA is found in multiple chromosomes.
  - Chromosomes are linear, thread-like structures.
  - DNA is associated with histones.



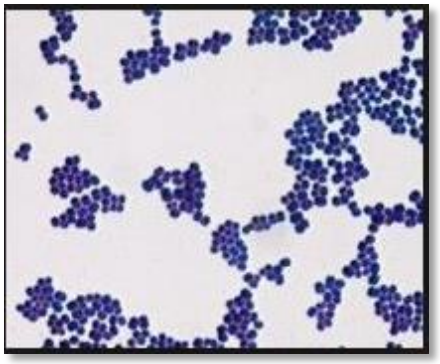
- Cell wall, if present, is chemically simple.
  - Cellulose
  - Chitin
- They have membrane enclosed organelles.
  - Mitochondria or chloroplasts
  - Endoplasmic reticulum
  - Golgi apparatus
  - Lysosomes



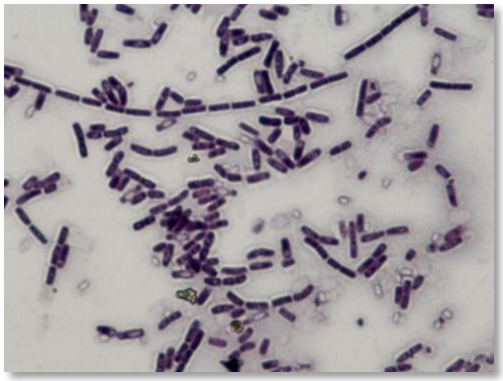
Lesson 13. Read pages 77-79.

LESSON 13. SHAPES AND ARRANGEMENTS OF BACTERIA

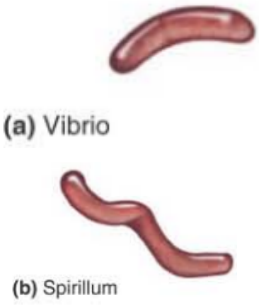
- **Three basic shapes of bacteria:**
  - **Coccus:** These are circular or spherical in shape.



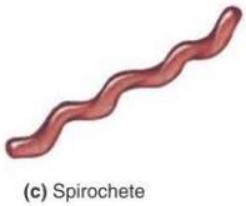
  - **Bacillus:** These rod shaped bacteria.



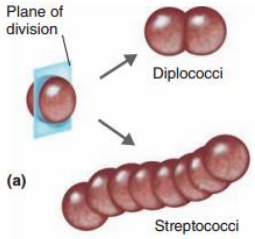
  - **Spiral:** These are curved shaped bacteria. They are further divided into 3 more subgroups.
    - **Vibrio:** curved rods
    - **Spirillum:** Helical but **rigid**



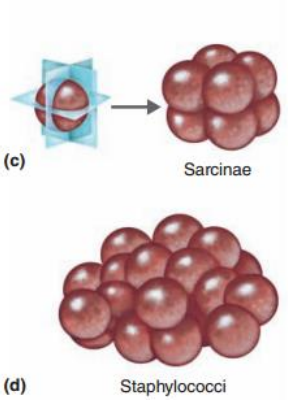
    - **Spirochete:** Helical but **flexible**
      - Spirochetes move by axial filaments which are enclosed by the outer membrane.



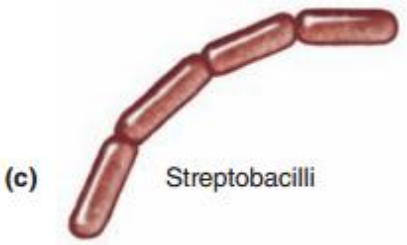
- **Arrangement of Cocci:**
  - If cocci divide in one plane, this division gives rise to two cells. And if these cells remain attached to each other, we call these two cells diplococci.



  - If this division continues and cells remain attached with each other, this arrangement gives rise to a chain of cocci called streptococci.
  - Division in two planes leads to tetrads.
  - Division in 3 planes is called sarcinae.
  - Division in multiple planes is called staphylococci.



- **Arrangement of Bacilli:**
  - Single rod is simply called a **bacillus**.
  - If two bacilli remain attached end to end, this arrangement is called a **diplobacilli**.
  - If bacilli are arranged one after the other, this gives rise to a chain called **streptobacilli.**
  - **Coccobacillus:** It is a special morphology. The cell is neither clearly a bacillus nor a coccus, rather it is an intermediate morphology between the two extremes.

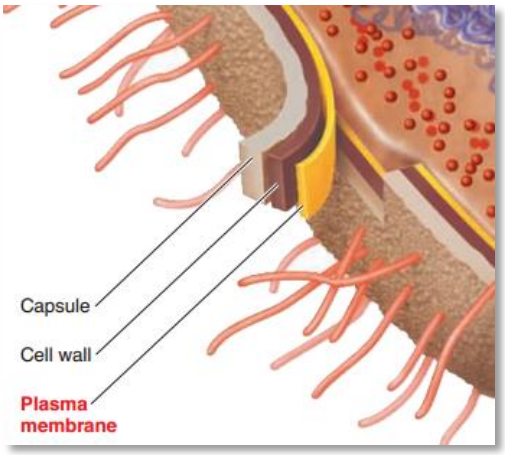


Lesson 14. Read pages 79-81, and 83.

LESSON 14. STRUCTURES EXTERNAL TO THE CELL WALL

- **Structures outside the cell wall (outermost to innermost):**

1. Glycocalyx (Capsule or slime)
2. Flagella
3. Fimbriae
4. Pili



1. **Glycocalyx**

It is the outermost layer, viscous and gelatinous in nature that surrounds the cells. It is composed of polysaccharide and polypeptide or both.

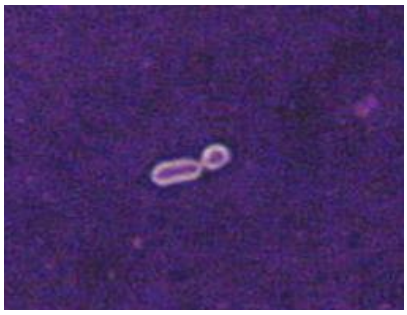
- **Capsule**: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.
  - Plays important role in virulence (degree of pathogenicity)
  - Can also be a part of the vaccine against the bacteria to which it belongs.
  - Can be demonstrated by negative staining technique.
  - Capsulated organism make glistening colonies as seen in the accompanying diagram below:

- *Bacillus anthracic, streptococcus pneumoniae* and Klebsiella are examples of capsulated organisms.
- Thickness of the capsule depends upon the culture conditions. Capsules are mostly are water soluble. Also remember that capsular organisms usually make the broth viscous and stringy.

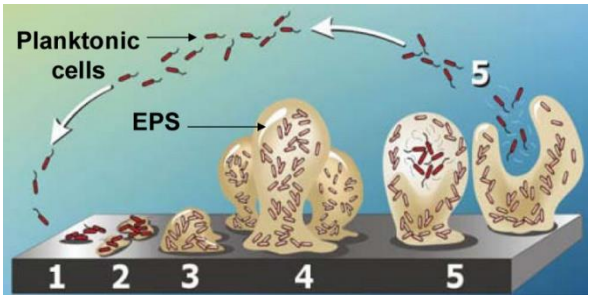
- **Capsular Staining Procedure**

1. Prepare a thick smear in a loopful of congo red (1%) stain

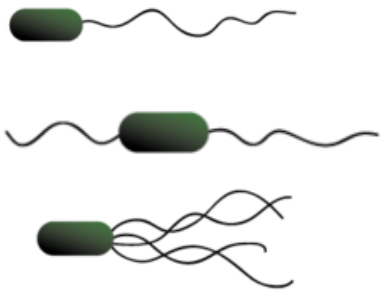
2. Fixation in acid alcohol for 15 sec
3. Wash with dH2O
4. Cover it with acid fuchsin for 1 min.
5. Wash with water
6. A bacterium stains red, capsule remains colorless and is seen in the dark blue background as seen in the accompanying diagram.



- **Glycoclalyx as Biofilm**
  - Glycocalyx is also part of biofilms that bacteria make to attach to surfaces. Bacteria secrete extracellular polymeric substance (EPS)
  - Biofilm protects cells within it.
  - It also facilitates communication amongst cells.



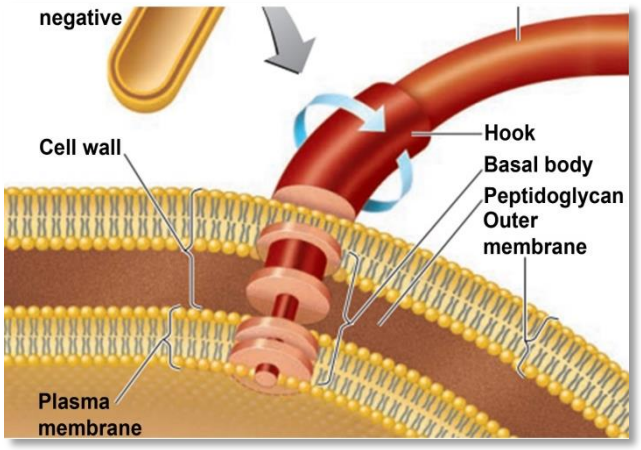
  - **Slime**: If glycocalyx is loosely attached to the cell, it is called slime.
- **FLAGELLA**
  - **Flagellum:** Long filamentous structure that propels bacteria
  - **Classification of bacteria based on flagella**
    - **Atrichous:** No flagellum
    - **Peritrichous**: Distributed over the entire cell
    - **Polar:**
      - Monotrichous
      - Amphitrichous
      - Lophotrichous



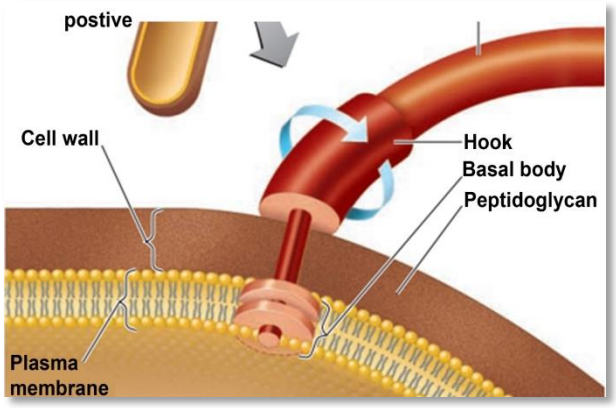
Lesson 15. Read pages 81-83.

LESSON 15. STRUCTURE AND FUNCTION OF FLAGELLA

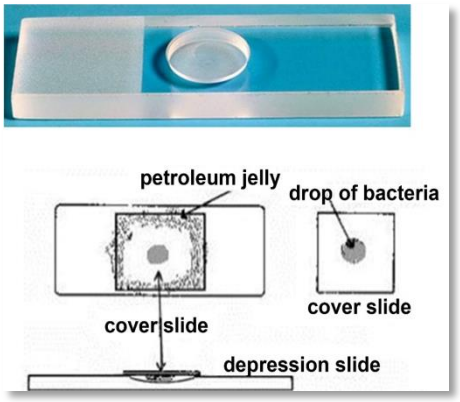
- **Anatomy of Flagella**
- A flagellum consists of the following 3 parts as shown in the accompanying diagram below:
  - Filament: It is made of a protein called flagellin, which makes H antigen that can be used for serovar identification in gram negative bacteria.
  - Hook: A different protein
  - Basal Body: The most complex
- This is the diagram of a gram negative flagellum:
- It has 4 rings in the basal body.
- Gram negative cells have two plasma membranes (outer membrane is in addition to the actual inner plasma membrane).



- Gram's positive organisms have two rings in the basal body and a single plasma membrane with a thick peptidoglycan layer as seen below:

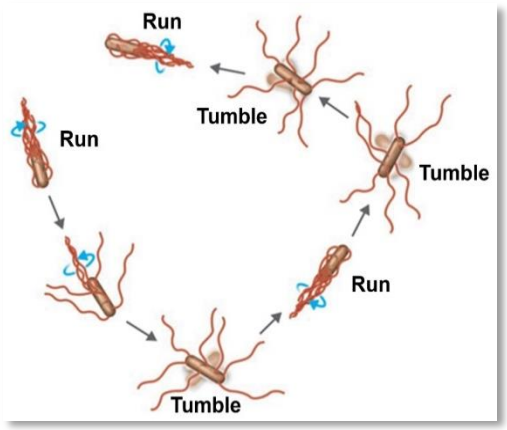


- Flagella are used for motility. Motility can be demonstrated by use of a cavity slide and a coverslip by hanging drop method shown in the diagram below:



- Bacteria exhibit two kinds of motility patterns:

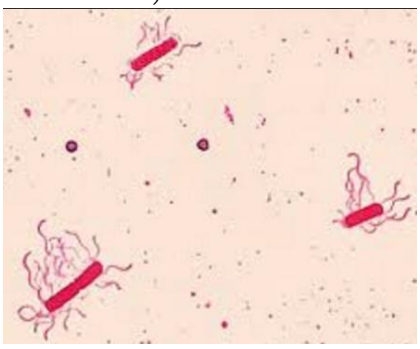
  Run and Tumble as seen below.



- Flagella move counterclockwise for forward thrust (run or swim)
- Clockwise when tumbling

- **Flagellar Staining**

1. Transfer a drop of fresh culture on one end of a slide.
2. Allow the drop to trickle down slowly
3. Air dry the smear. Do not heat fix it.
4. Cover the smear with mordant (tannic acid 10% in 5% NaCl) for 10 to 30 min.
5. Rinse the smear gently with H_2O.
6. Add carbol fuchsin and let it sit for 5-15 min.
7. Rinse off the stain with water
8. Air dry the smear
9. See under the microscope
10. See the figure for how flagella look like.



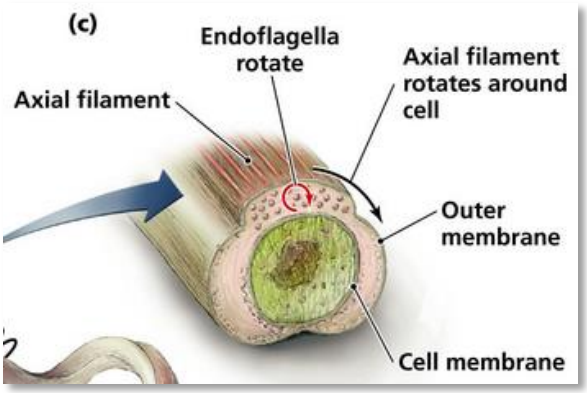
Lesson 16. Read pages 82-84.

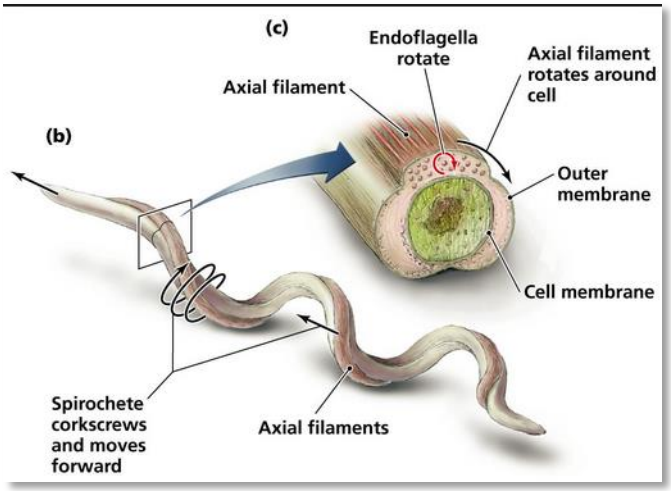
LESSON 16. AXIAL FILAMENTS, FIMBRIAE, AND PILI

- **Structure inside the cell wall**

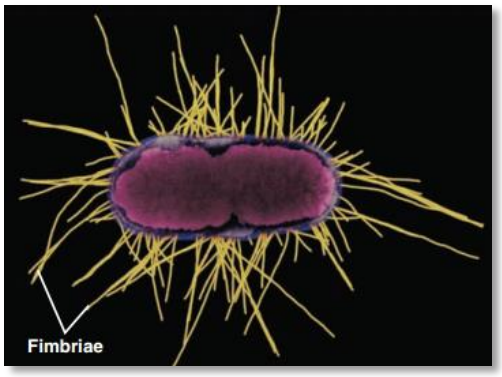
1. Plasma membrane
2. Gas vacuoles
3. Ribosomes
4. Inclusion bodies
5. Nucleoid
6. Periplasmic space

- **AXIAL FILAMENT**
- **Learning Objectives**
- **Structure of Axial Filament**
  - It is not any different from the flagella. The only difference is that these filaments are enclosed by the outer membrane of the cell. That is the reason, it is also called endoflagella. Since it is enclosed by a membrane (not free to move with freedom), this restricted movement creates a corkscrew type motility in spirochetes. It may be noted that axial filaments are present only in the spirochetes.
  - Axial filament has a basal body much like a flagellum.

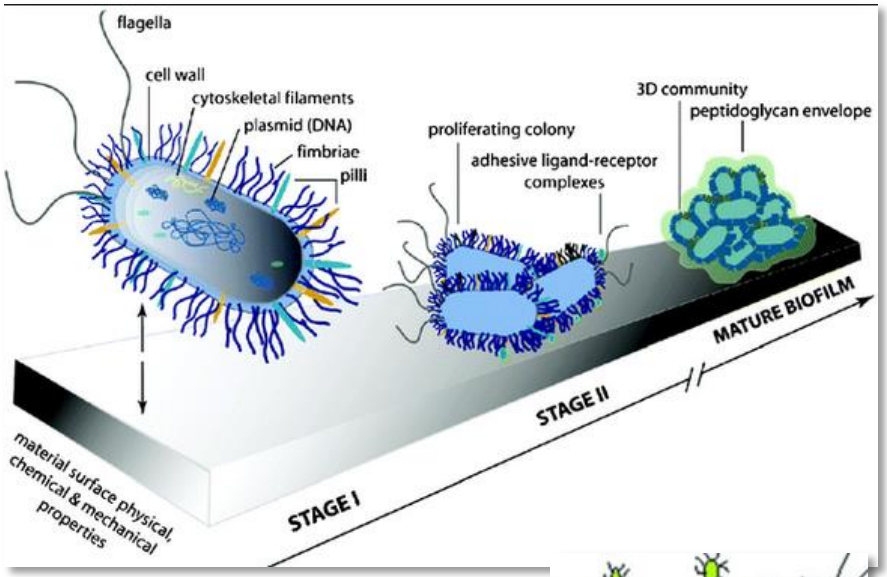




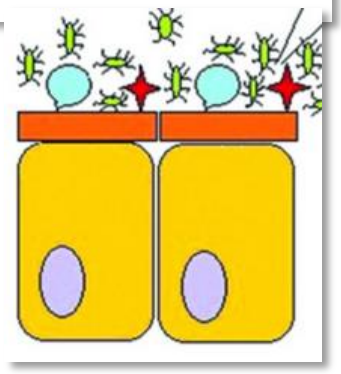
- **Structure and Function of Fimbriae**
  - Hair-like small appendages on G-neg cells
  - Composed of pilin, a protein
  - Can be at one pole or around the entire cell
  - Used for attachment to surfaces or epithelial cells
  - Fimbria: Singular



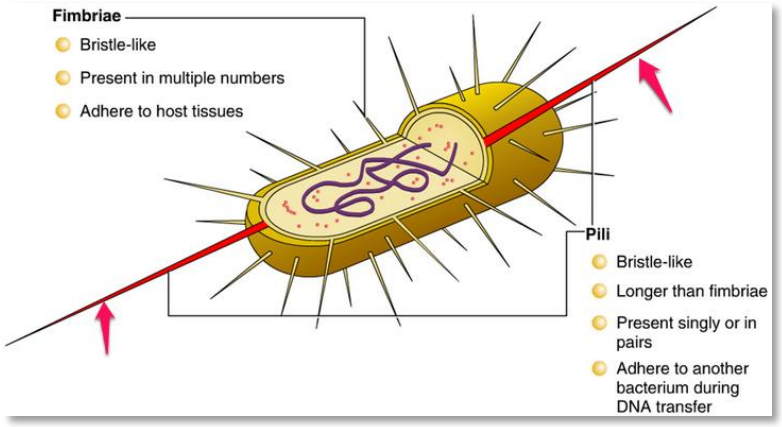
- Fimbriae help bacteria attach to surfaces before they can secrete biofilm as seen below:

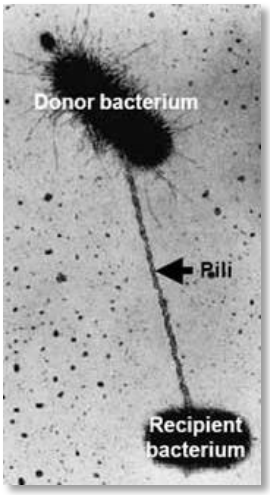


- Fimbriae also help attach to epithelial cells
  - *Neisseria gonorrhoeae*
  - E.coli O157: Diahrrea



- **Structure and Function of Pilus**
- These are hair-like structure composed of pilin, usually one to ten in number.
- Longer than fimbriae
- Used for attachment to:
    - Host cells
    - Bacteria
- Used for DNA transfer from on bacterium to another: Conjugation (Sex pili)
- Also function in twitching Motility
- Gliding Motility is also the function of the pili.

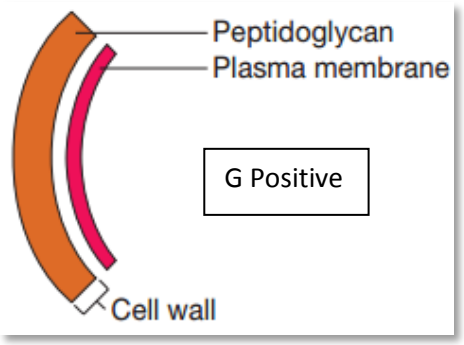




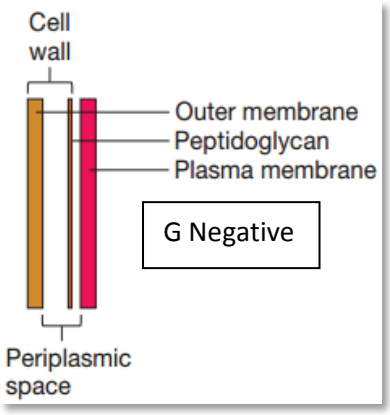
Lesson 17. Read pages 84-87.

LESSON 17. STRUCTURE AND FUNCTION OF CELL WALL

- **Anatomy of Cell Wall**
- Cell wall is the most important layer in bacteria. It is rigid layer just outside the plasma membrane.
- Most important structure in prokaryotes
- It provides shape to the organism.
- It provides protection from osmotic lysis.
- It is involved in pathogenicity (ability of the organism to cause the disease).
- Several antibiotics act on it.
- In 1884, Gram staining was developed by Christian Gram. He did not know at the time the basis of this differential staining procedure now commonly employed in Microbiology. However, when electron microscope became available, scientists learned that it was the cell wall (peptidoglycan) which made Gram staining as a differential staining. Cell wall is much thicker in G-positive bacteria than in G-negative bacteria.

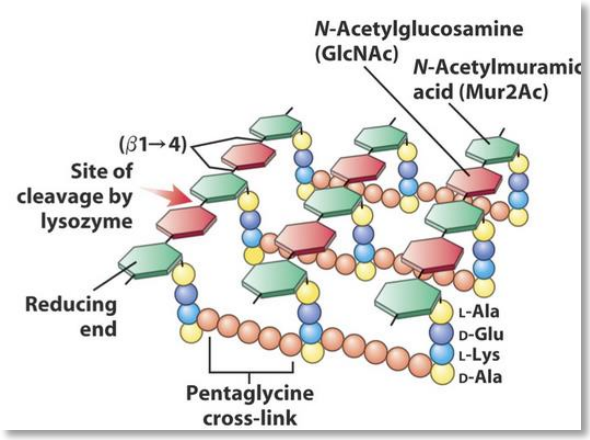


- TEM revealed true differences
- Peptidoglycan thick in G +ive cells
- Thin in G –ive cells

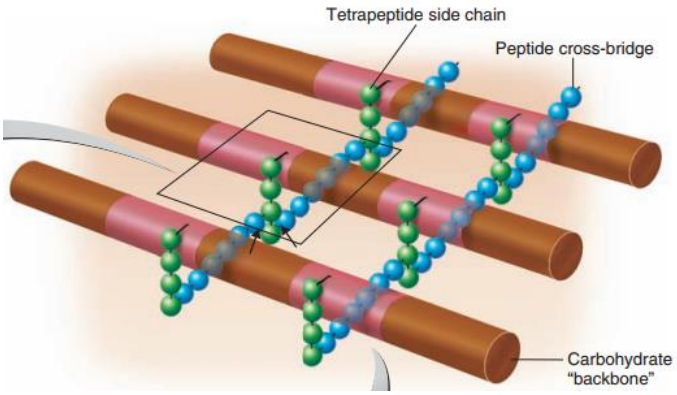


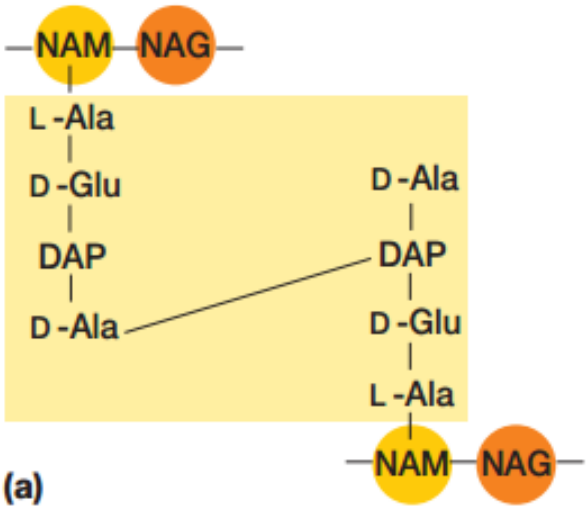
- **Peptidoglycan Structure**
- Peptidoglycan is arranged as a mesh-like polymer
- Peptidoglycan is basically composed N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) which are arranged end to end from 10 – 65 molecules.
- Cell wall also has two chains of amino acids:

- One is called tetrapeptides (composed of 4 or so amino acids).
- The other one is composed of 5 amino acids (pentaglycine). These form cross bridges with tetrapeptides. **Penicillin interferes with cross bridges.**

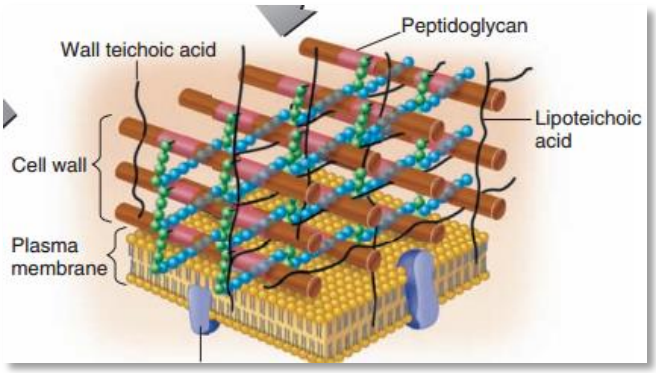


- These two peptides are arranged vertically and horizontally forming part of the mesh-work.





- **Cell Wall of Gram Positive Bacteria**
- Peptidoglycan layer is thicker in gram positive bacteria.
- G-positive cell wall contains teichoic acid which may be divided into wall teichoic (remains within the cell wall) acid and lipoteichoic acid (is inserted into the cell membrane).



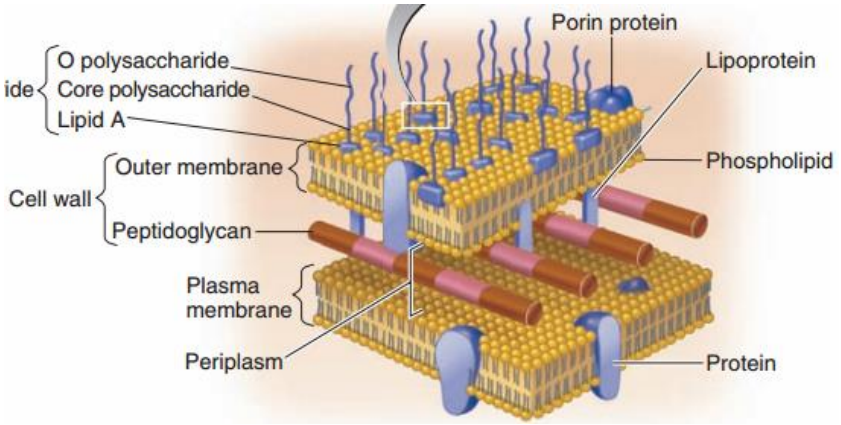
- Teichoic acid is antigenic in nature and helps the cell wall by providing rigidity.

Lesson 18. Read pages 85-87.

LESSON 18. CELL WALL OF GRAM NEGATIVE BACTERIA

- **Cell wall of Gram Negative Bacteria**

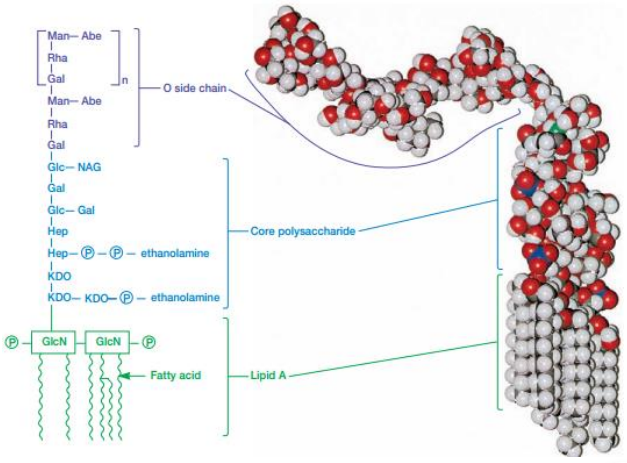
- Gram negative cell wall contains very thin layer of peptidoglycan. However, there are two membranes in gram negative bacteria. The space between two



phospholipid membranes is called periplasmic space. As you can see that the cell wall is very thin and there are two lipid layers (inner and outer), when such an organism is stained with the Gram's procedure, it is not able to retain crystal violet, hence gets washed away with alcohol treatment during the procedure leaving the cell clear of any stain. Counter stain is then applied to make the cell look red.

- Moreover, lipopolysaccharide is also integral part of Gram negative bacteria. This substance is exclusively present in the outer membrane and has pyrogenic properties. This is the reason that infection with Gram negative bacteria leads to relatively high fever. Please remember that there are other cytokines (Tissue necrosis factor, IL-1 and IL-6) also that cause fevers too.

- Lipopolysaccharide molecule consists of 3 parts:



1. Lipid A: endotoxin
   - Fever, vasodilation and shock
2. Core polysaccharide

a. Structural support

2. O side chain

a. Antigenic much like teichoic acid in gram positive bacteria

- **Comparison of G-ive and G+ive Bacteria**

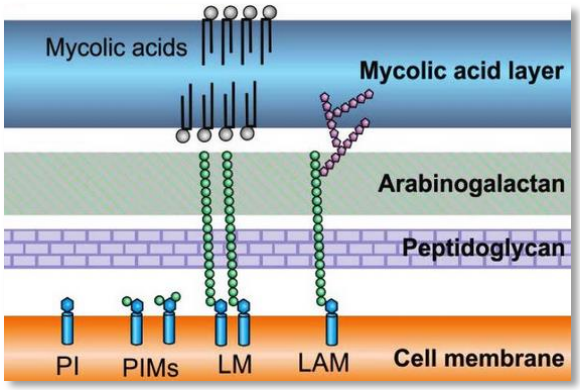
Characteristics	Gram Positive	Gram Negative
Gram reaction	Retain CV dye	Decolorized
Peptidoglycan layer	Thick	Thin
Teichoic Acid	Present	Absent
Periplasmic Space	Absent (generally)	Present
Outer membrane	Absent	Present
LPS	Virtually none	High
Lipid and lipoprotein contents	Low	High
Flagellar structure	2 rings	4 rings
Toxin Produced	Exotoxins	Exo and endotoxins
Susceptibility to penicillin	High	Low
Overall resistance	High	Low

Lesson 19. Read pages 87-89.

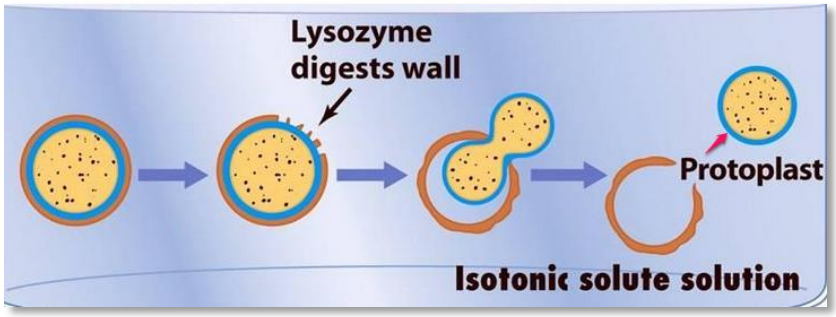
LESSON 19. STRUCTURE AND FUNCITON OF ATYPICAL CELL WALLS

- **Structures of Atypical Cell Walls**
- Some bacteria have no cell wall or have very little, such as **Mycoplasma**.
    - Plasma membrane of **Mycoplasmas** has sterols which provide rigidity to the membrane.
- **Archea: Cell Wall Composition**
- Archea have a cell wall that contains a glycosylated proteinaceous surface layer **(S-layer).** It is certainly not peptidoglycan in nature.
- In some Archea, the cell wall is composed of glycan polymers called **Pseudomurein**.
- No peptidoglycan, so behave like G –ive bacteria when stained with Gram's staining.

- **Acid-Fast Cell Wall**
- Mycobacteria and Nocordia have a special structure called mycolic acid, a waxy lipid.
- Mycolic acid and peptidoglycan layers held by carbohydrate, arabinogalactan.
- Carbolfuchsin stains these bacteria and heat is used to make the stain penetrate the cell.



- **Damage to Cell Wall**
- This is interesting to note that what will happen if cell wall is damaged either physically or chemically (by lysozyme).
- Lysozyme breaks the sugar-derived backbone of cell wall in bacteria.
- Obviously, if cell wall is removed or broken, it leaves the cell with the plasma membrane as its outer membrane, and the cell assumes a



spherical shape. If the cell is Gram positive, and the cell wall is removed, the cell is called a **protoplast**. If the cell is Gram negative, and the cell wall is removed, the remaining cell (with the plasma membrane) is simply called a **spheroplast**. Some cells, called **L-form** organisms, are naturally found without the cell wall. In other words, normally, they have cell wall but under certain conditions, they may lack a cell wall. These cells can be created when grown in the presence of penicillin which inhibits cell wall synthesis.

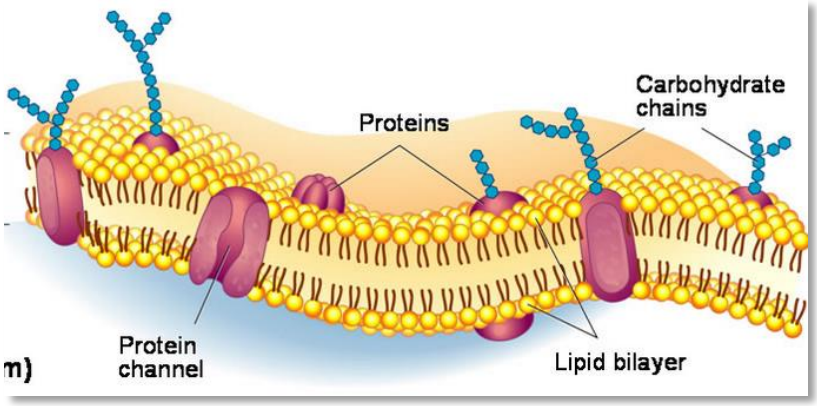
- **Uses of protoplasts and spheroplasts in science**
  - The cells without the cell wall are easy to manipulate with respect to gene transferring because the cells are more receptive to such intakes.
  - After the DNA is transferred, these cells can resynthesize their cell walls when placed in nutrient medium for growth.

Lesson 20. Read pages 89-91.

LESSON 20. STRUCTURE AND FUNCITON OF CELL MEMBRANE

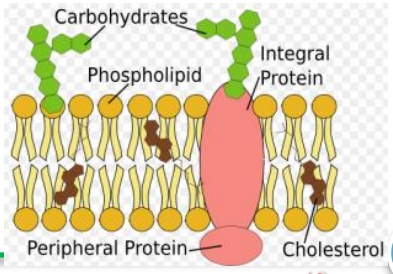
- **Cell Membrane Structure and Function**

- We will not discuss cell membrane in detail as this is covered fully in basic biology course. So briefly, cell membrane is composed of a phospholipid molecules arranged tail to tail with heads facing away from each other. This makes lipid molecules to appear as a bilayer. So all membranes enclosing the organelle in the cell or the cell itself look as bilayer structures. This is the basic design of the plasma membrane. Protein and carbohydrates are also inserted into the plasma membrane as can be seen in the accompanying diagram.



- **Functions of Cell Membrane**
  - Selective permeable barrier
  - Passive and Active Transport
  - Respiration in microbes (bacteria)
  - Photosynthesis in microbes
  - Lipid synthesis
  - Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
  - PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

- **Types of Membrane Proteins**
  - **Peripheral Proteins, are found hanging outside the membrane**
    - Loosely connected to the membrane
    - Soluble in water



- **Integral proteins are inserted in the membrane or embedded in the membrane**
- Insoluble in water
- Amphipathic in nature

- Cell membrane has been referred to as a mosaic fluid model. This means that integral proteins are not static in position in the membrane. They can diffuse laterally and change positions in the membrane from time to time. So, plasma membrane is like water pond and integral proteins are like plastic bags people throw in the pond. These plastic bags swim and move by air currents. Membranes are much like that.
- Cell membrane lipids can be disrupted by alcohols, quaternary ammonium compounds and polymixins. So, these compounds are used as disinfectants or for controlling microbial growth.

Lesson 21. Read pages 91-94.

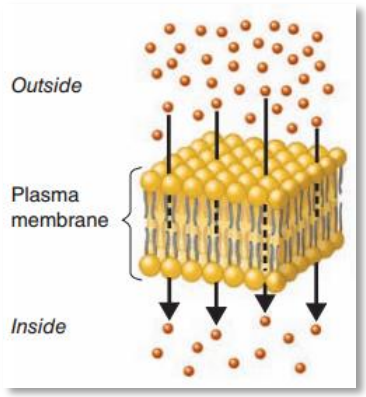
LESSON 21. MOVEMENT OF MATERIALS ACROSS CELL MEMBRANE

- **Transport of substances across cell membrane**
- **Passive Movement**

- Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.
- **Passive Movement is divided into two groups:**

1. Simple Diffusion

- Area of high concentration to low concentration
- This continues until ions or molecules are evenly distributed. When this happens (equal distribution throught), it is called a point of equilibrium.
- O_2 and CO_2 are examples of simple diffusion.

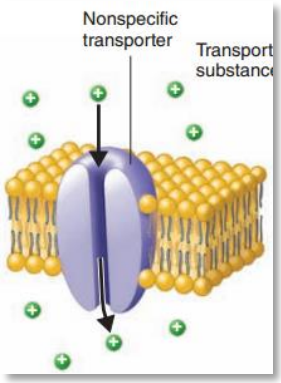


2. Facilitated Diffusion

i. Integral proteins act as channels or carriers in facilitated diffusion
   i. Integral proteins are called transporters or permeases

ii. No energy is required during transportation of substances through these integral proteins.

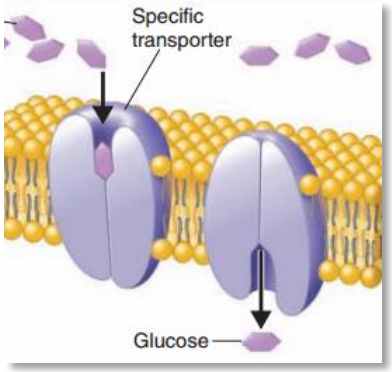
iii. Two kinds of transporters are known:
   i. Nonspecific transporters



1. Ions

ii. specific transporters

1. Change in shape
2. Sugars, vitamins



iv. Examples of facilitated diffusion include:

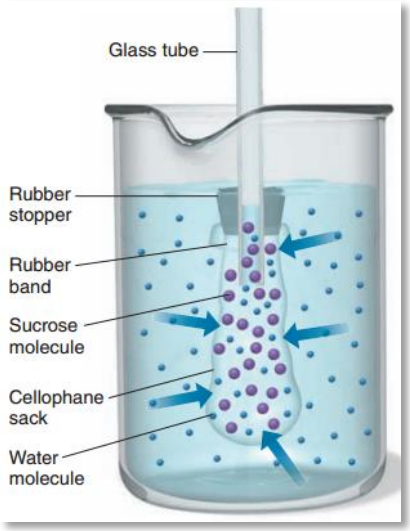
i. Large molecules degraded by extracellular enzymes

ii. Smaller molecules then bind transporters

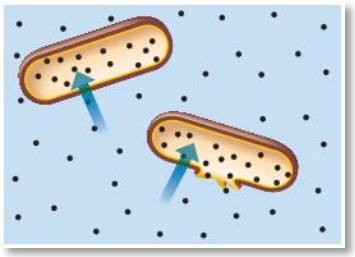
iii. Water molecules can pass through lipid bilayer by *simple diffusion* or though water channels, *aquaporins*.

- **Osmosis:**

- Osmosis is diffusion through a semipermeable membrane that allows some molecules to pass through but others not to pass through. Usually, it is water that passes through the semipermeable membrane.

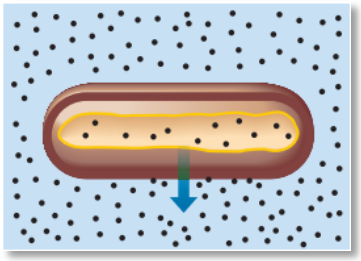


- So, in other words, osmosis is the net movement of solvent from high conc. of solvent to low conc.
- What is osmotic pressure?
  - Pressure required preventing movement of water into a solution containing some solute.
- Consequences of Placing cells in **hypotonic, isotonic or hypertonic** solutions:
  - When cells are placed in hypotonic solution, water moves into the cells as water molecules are more in concentration outside the cells. This causes swelling of the cells and results in their lysis.



  - Keeping the cells in isotonic solutions brings no change in the cells. Tonicity of the solution is important if one wants to store the cells undamaged.

- If cells are placed in hypertonic solution, water will come out of the cells into the hypertonic solution. This will shrink the cells resulting in **plasmolysis** of the cell. Cell will die.

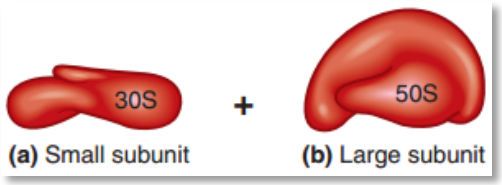


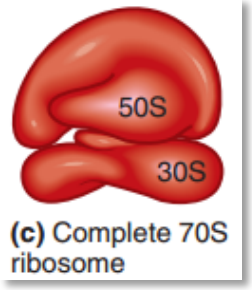
- **Active Movement**
- Movement against the concentration gradient
- From low concentration to high concentration of a substance
- Requires energy (ATP)
- Substances that are actively transported into the cells undergo either no change or they are chemically modified to keep them there in the cell. Remember, active transport brings substances inside the cell against the gradient; hence molecules brought in can go out. That is why these molecules may be modified to keep them inside the cells. In other words, this modification avoids passive diffusion of those molecules from the cells outwards.
- **Group Translocation** is also part of active transport. Such substances are more prone to chemical alterations during transport. *Glucose is an example that undergoes alterations to keep this molecule inside the cell.*

Lesson 22. Read pages 94-96.

LESSON 22. STRUCTURES INSIDE THE PROKARYOTIC CELLS

- **What Lies inside the Cell?**
- We will talk about a few organelles that are more important with respect to the organisms. They include the nucleoid, ribosomes and inclusion bodies.
- **The Nucleoid**
- Bacteria have circular dsDNA (bacterial chromosome), although there are a few that have linear dsDNA too.
- However, dsDNA in bacteria is not enclosed by a nuclear membrane as we see in eukaryotic cells.
- There are no histones in bacterial cells. However, there are histone-like proteins present.
- dsDNA in bacteria is attached to inside of plasma membrane.
    - Bacteria have extrachromosomal DNA called plasmid.
    - Plasmids are not needed for bacteria but are advantageous when present.
- **Ribosomes**
- Composed of proteins + ribosomal RNA
- 2 subunits
    - 70S ribosomes (when two units are combined into one unit)
        - 50S: one rRNA
        - 30S: two rRNA





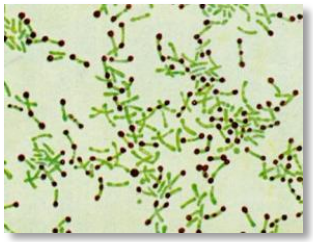
- Several antibiotics act on ribosomes and inhibit protein synthesis
    - Streptomycin attach to 30S

- **Inclusion Bodies**
- Inclusions are reserved deposits of nutrients. Bacteria accumulate nutrients when nutrients are plentiful.
- Concentrating nutrients in the form of inclusions avoids the increase in osmotic pressure due to accumulation of nutrients in one place. Not all organisms accumulate them. So,

inclusions vary from bacteria to bacteria. They can also serve as markers for bacterial identification as some are limited to specific organisms. The followings are some of the important inclusions:

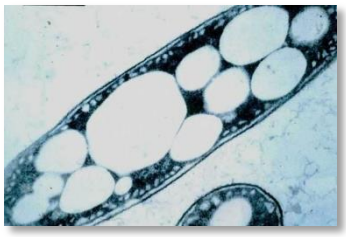
- Metachromatic granules
- Polysaccharide granules
- Lipid inclusions
- Sulfur granules etc.

- **Metachromatic Granules**
  - Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red).



  - Large inclusions
  - These inclusions contain inorganic phosphates. Inorganic phosphates are used up in ATP synthesis.
  - Characteristics of *Corynebacterium diphtheria: This bacterium can be identified by the presence of these granules in it.*

- **Gas Vacuoles**
  - Aquatic bacteria needs gas vacuoles to float to a certain level in water.
  - Such microbes maintain buoyancy for obtaining:
    - Nutrients
    - O_2
    - Light



- **Magnetosomes**
  - Inclusions of iron oxide
  - Surrounded by invaginations of plasma membrane
  - Present in G negative bacteria

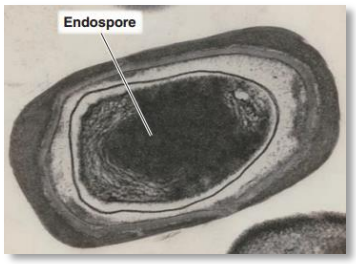
- Act like a magnet. Bacteria can stick to iron containing rocks for nutrition.
- Decompose H_2O_2 which is toxic for cells.

- **Polysaccharide Granules**
  - Typically consists of glycogen or starch
  - Can be demonstrated by iodine which makes glycogen appear reddish brown while starch appears blue.

Lesson 23. Read pages 96-98.

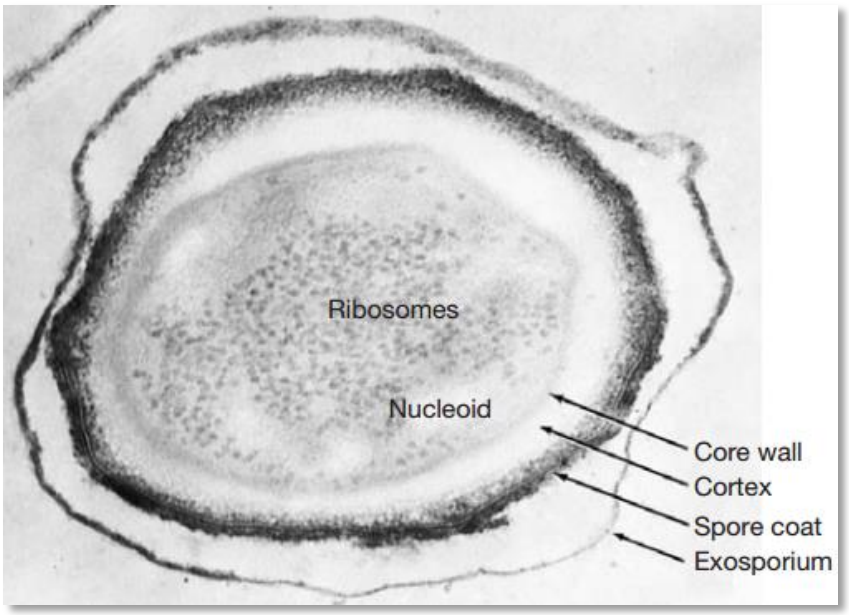
LESSON 23. ANATOMY OF ENDOSPORE

- **Anatomy of Endospores**
  - What are endospores and how they form?
  - What are the advantages of having an endospore?
- **Characteristics of Endospores**
  - Specialized resting cells
  - Highly durable, dehydrated forms of bacteria
  - Formed inside a bacterium
  - Survive extremes of temperatures; resistant to heat; needs to be autoclaved in order to be killed.
  - Survive lack of water; can survive in high salt concentrations. Clostridia spores can survive in honey and that is one reason, honey is not recommended for infants, because spore can cause tetanus in babies.

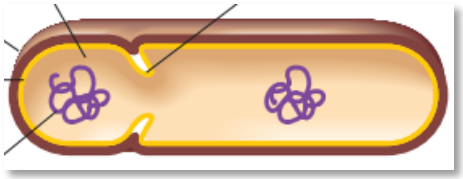


  - Survive radiation danger
  - Survive in the toxic environment as well.
  - Mostly formed by Gram positive bacteria
    - Genus Clostridium
    - Genus Bacillus
  - One gram negative: *Coxiella burnetii* also has it.
  - Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.
- **Endospore and its various parts/structures**
  - **Exosporium:** A thin delicate outermost covering of the spore
  - **Spore coat:** 2nd layer underneath the exosporium. It is thick and composed of several protein layers.
    - Resistant to chemicals
    - It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favorable for their growth.
  - **Cortex:** It is the 3rd layer from outside in. It has peptidoglycan in it.

- **Spore cell wall or core wall:** Surrounds the protoplast or spore core
- **Spore core:** Contains nucleoid and ribosomes
- Please see the diagram given below for details.



- **The Process of Sporogenesis**
  - In-growth of plasma membrane (spore septum) is the first step when an organism chooses to go for sporulation.



  - Spore septum becomes double layered.

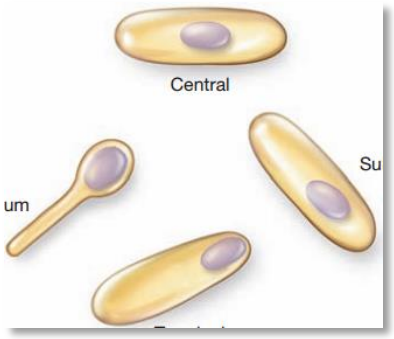
  - Peptidoglycan is laid down between the two membranes.

  - A thick spore coat is formed and the spore is released from the cell.

- It keeps lying there in the environment until it finds suitable environment for its germination and growth.

- What makes the spore so resistant?
  - The core has dipicolinic acid complexed with calcium.
    - Thought to stabilize DNA
  - The spore also has small acid-soluble acid binding proteins.
    - These protect DNA from heat, desiccation and chemicals

- **Location of the Spore can be used for Classifying the organisms**
  - Spore can be **bulging or non-bulging**. Bulging means that the size of the spore is bigger than the vegetative cell in which the spore is developing.
  - Position of the spore can be:
    - Central
    - Terminal
    - Subterminal
  - Used for identification
  - *Clostridium tetani*
    - *The spore is terminal in position and looks like a drum-stick.*



- **Germination of Spore**
  - Spore germinates when it finds a conducive environment for its growth. Germination has 3 phases or stages:
    - **Activation:** It prepares the spore for germination.
      - Heat can activate the spore when appropriate moisture and nutrients are present in the environment.
    - **Germination:** Spore starts swelling and loosing its coats etc.
      - It becomes metabolically active.
    - **Outgrowth**: New components are made.

- **Spore and Sterilization**
  - It is not easy to kill the spore. They are quite resistant to heat and desiccation. However, there are two methods that can destroy these spores completely.

1.**Autoclaving:** 121 ^{0}C at 15 psi for 20 min

But this much temperature can destroy sugars get damaged at this temp.

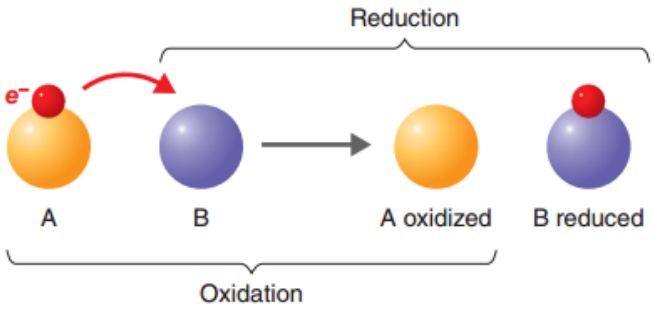
2. **Tyndallization:** This is a special method used for killing spores in solutions that can get degraded at high temperature such as autoclaving. Let's say, we want to sterilize 5% glucose solution. Autoclaving is not the option as it will destroy the sugar. Although filtration could be used to sterilize it, however, heat can be also be used to achieve sterilization. Here is how it is done:

    a. On day 1, boil the solution. Cool it to 37^0C and incubate it overnight. Incubation will cause the spores to germinate and they will convert them into vegetative forms.
    b. On day 2, boil the sugar solution again. Since spores will have germinated, it will be easy to kill them just by boiling the solution. Just to ensure that no spores have been left in the solution, one can incubate them for one more night.
    c. On day 3, boil the solution again and all the vegetative form will be killed leaving no organisms in the sugar solution.

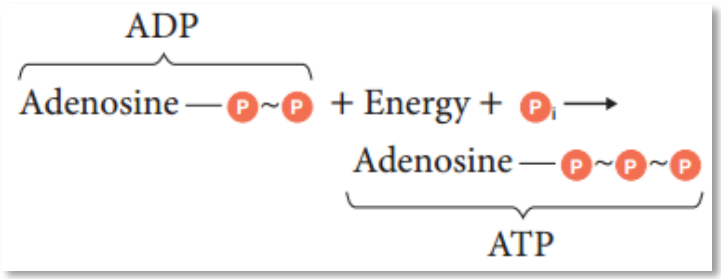
Lesson 24. Read pages 114, 117, 121-123.

LESSON 24. HOW ENERGY IS CAPTURED FROM FOOD

- **Energy in Nutrients**
- Energy is associated with electrons that form bonds between their atoms. It is interesting to note that nutrients are rich in hydrogen. And it is the hydrogen atoms that move from various substrates during metabolism providing electron force for reactions in the body. This energy is ultimately concentrated into bonds of ATP. It is the property of ATP molecules that they can easily release energy to coupled reactions during metabolism.
- **What is metabolism?**
  - Metabolism = sum of all chemical reactions within an organism
  - Metabolism = reactions that release energy (aka catabolism) + reactions that require energy (anabolism)
- **Oxidation-Reduction Reactions**
- **Oxidation** = loss of electrons, or gain of oxygen, or loss of hydrogen
- **Reduction** = gain of electrons, or loss of oxygen, or gain of hydrogen



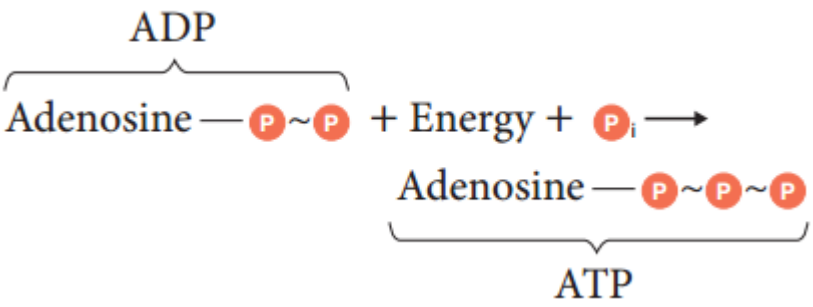
- In metabolism, hydrogen and electrons are harvested from organic molecules such as carbohydrates, fats and proteins which are used to concentrate this energy into ATP.



- Coenzymes such as NAD, NADP, FAD, FMN etc. are used as carriers of these electrons in oxidation reduction reactions.

- In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time.
- This is equivalent to removal of hydrogen atoms. (Hydrogen has one electron and one proton).
- Most biological oxidations involve the loss of hydrogen atoms, they are also called dehydrogenation reactions.

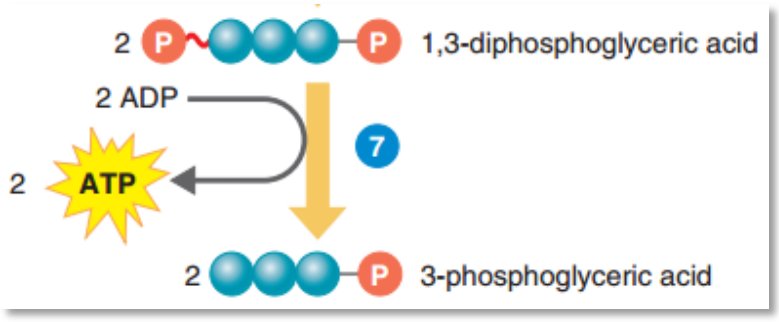
- **Phosphorylation and ATP Production**
- The addition of an inorganic phosphate group to a chemical compound is called phosphorylation. So, a phosphate is added to ADP to form ATP molecules in cells. In other words, energy derived from hydrogen containing organic molecules such as carbohydrates and fats is captured and concentrated in the form of ATP molecules. This reaction can be summarized as under:



- **There are three ways to make ATP in various organisms:**

1. **Substrate level phosphorylation**

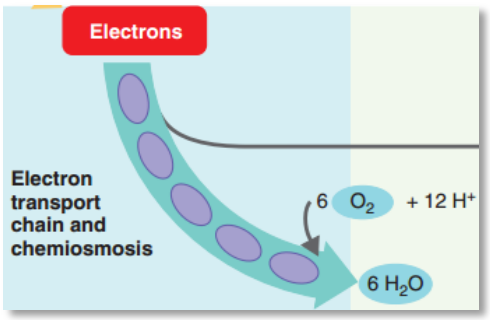
Substrate level phosphorylation occurs when the high energy phosphate is transferred directly from an already phosphorylated compound to ADP. We see this as an example during glycolysis when 2 ATPs are generated during conversion of 1,3 diphosphoglyceric acid to 3-phosphoglyceric acid as seen below in the diagram:



2. Oxidative Phosphorylation

a. Electrons captured from foods are transferred to co-enzymes such as NAD+ or FAD etc.

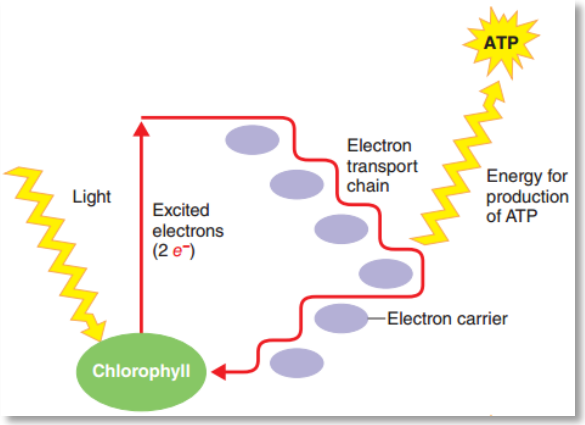
b. Then these electrons pass through a series of electron carriers and then ultimately to the last electron acceptor molecules such as O_2 or other inorganic compounds (nitrate, sulphate, carbonate etc) through a series of these electron carriers in system called electron transport chains.



c. During these events, ATP is generated by chemiosmosis.

3. Photophosphorylation

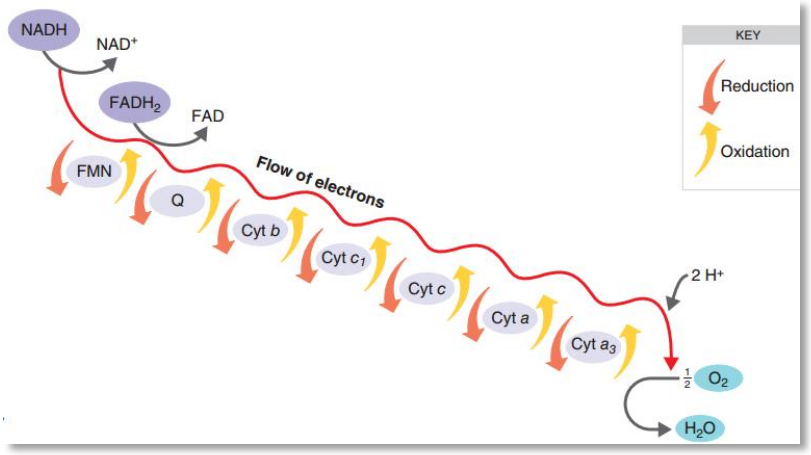
a. This occurs in plants and chlorophyll containing microbes or photosynthetic cells. In these cells, light energy is finally converted into ATP. An electron transport chain is also involved here. This is summarized in the accompanying diagram above.



Lesson 25. Read pages 124-125, 127-136.

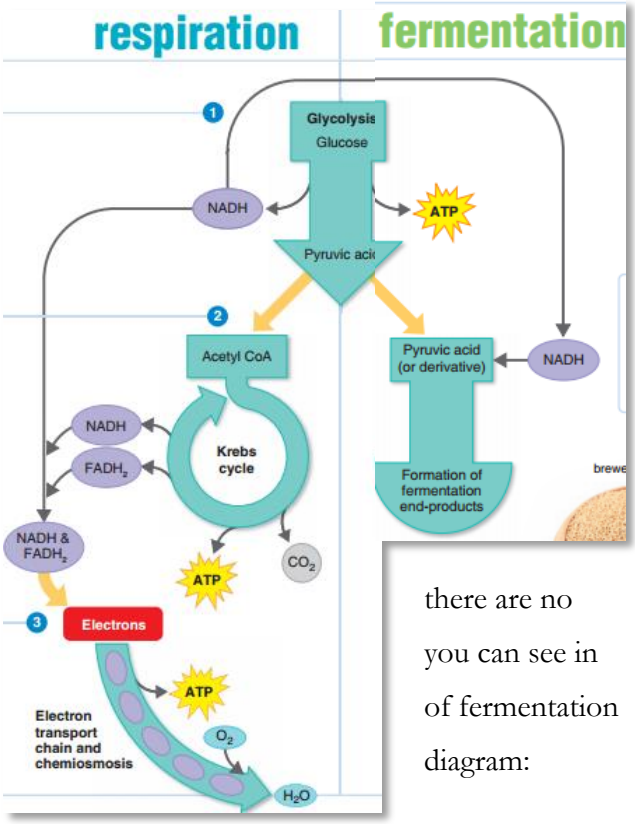
LESSON 25. CELLULAR RESPIRATION AND FERMENTATION

- **Carbohydrate Catabolism, Respiration and Fermentation**
- We will briefly discuss carbohydrate catabolism as a model for deriving energy in cells. Please note that organism can use fats and proteins too for energy production.
- Glucose is the most commonly used carb for all organisms. Microbes can derive energy from glucose in two ways:
    1. Respiration (just remember, it is not breathing!)
    2. Fermentation
- Both these processes start with glycolysis but follow different subsequent pathways for deriving energy from glucose.
- Respiration of glucose takes place in three general steps:
    - Glycolysis: Oxidation of glucose to pyruvic acid
    - Krebs cycle: Oxidation of acetyl CoA to CO2
    - Electron Transport Chain: Coenzymes that carry electrons from Krebs cycle or glycolysis are oxidized to create ATP.
    - Respiration is further divided into two types:
        - Aerobic Respiration: If in the electron transport chain, the final electron acceptor is oxygen, it is called an aerobic respiration.

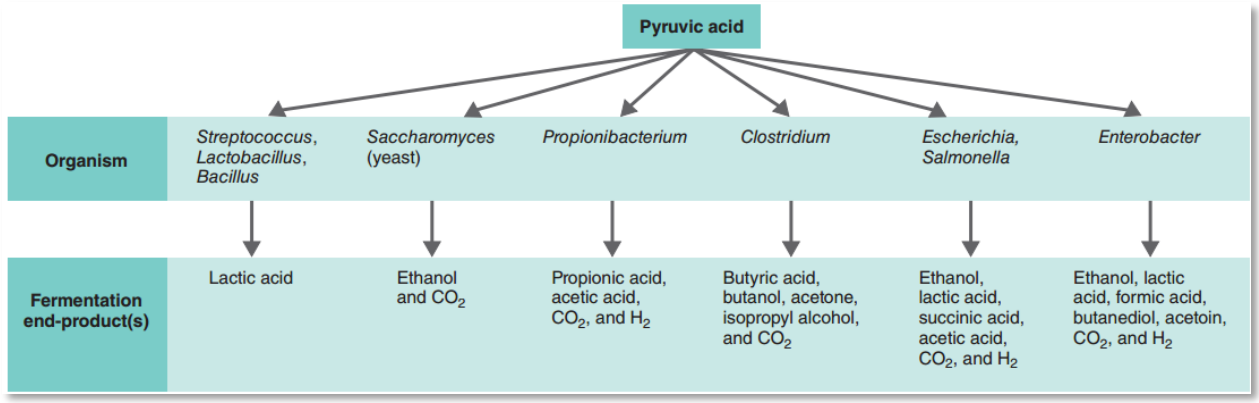


        - Anaerobic Respiration: If in the electron transport chain, the final electron acceptor is any inorganic compound other than oxygen, it is called an anaerobic respiration. This final electron acceptor may be a nitrate ion, a sulfate ion, or a carbonate ion.

- Fermentation: Initial steps for glucose oxidation by fermentation are the same as they occur in glycolysis for respiration. However, when pyruvate is generated through glycolysis, electrons are also captured by NADH and this NADH needs to be regenerated into NAD^+ for recycling. If the final electron acceptor is one of the end products such as ethanol, lactic acid, acetic acid etc, the process is called fermentation. Both anaerobic respiration and fermentation do not use oxygen during these processes, and during fermentation, electron transport chain involved as the accompanying diagram. Examples are given in the accompanying



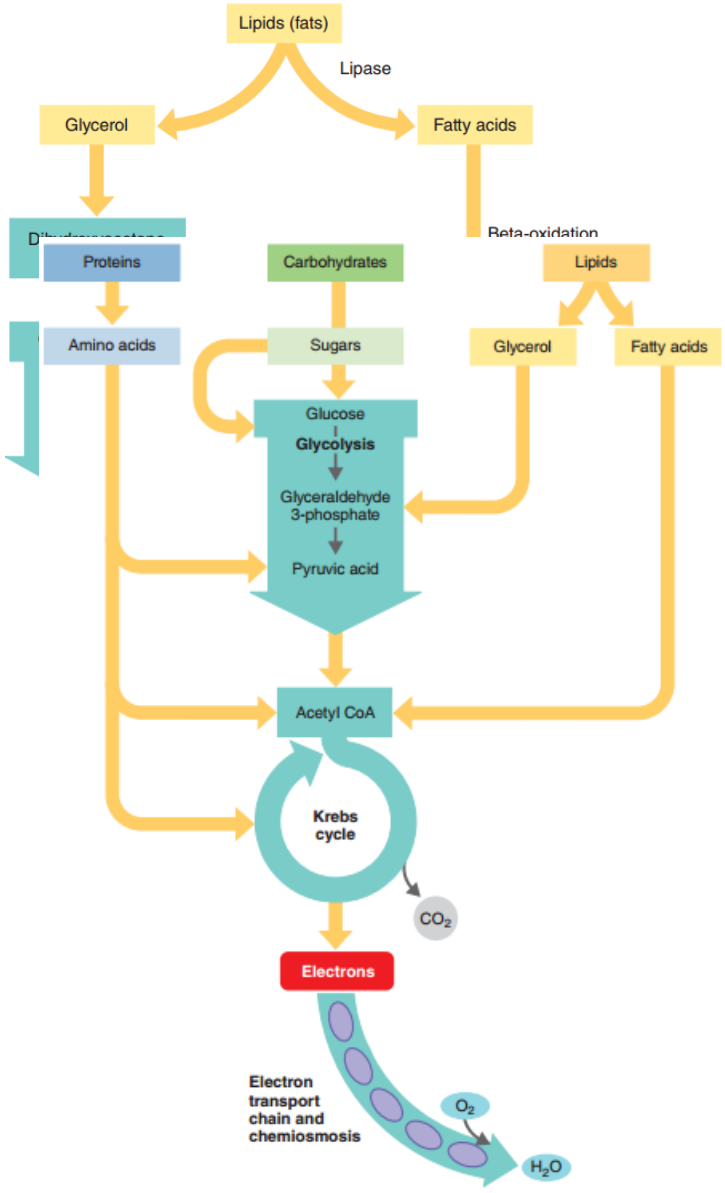
there are no you can see in of fermentation diagram:



Lesson 26. Read pages 136-139, 142-145.

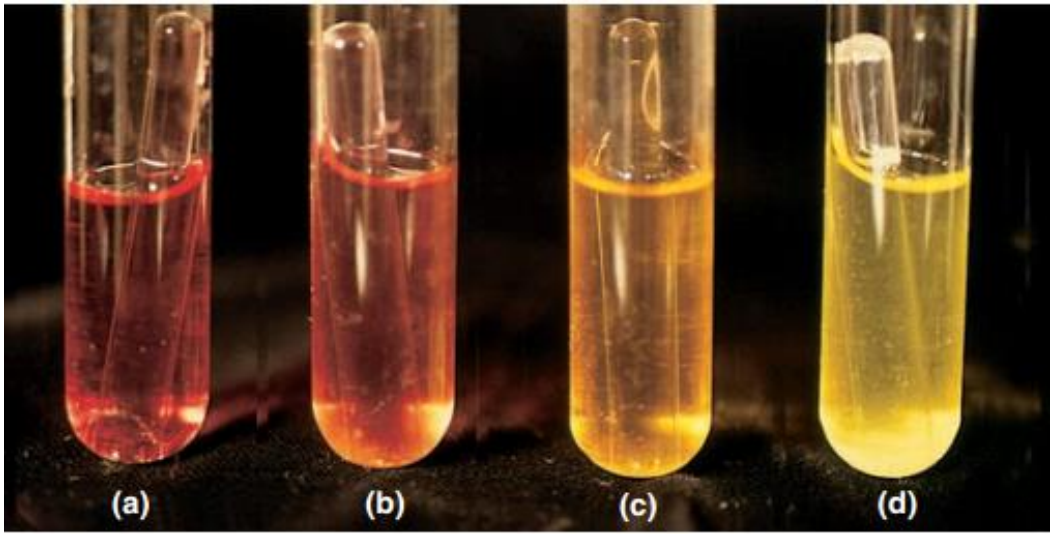
LESSON 26. ANEROBIC RESPIRATION, LIPID AND PROTEIN CATABOLISM

- **Lipid and Protein Catabolism**
- Glucose is not the only food that organisms can use to capture electrons and drive production of ATP. Fats and proteins can also be used by organisms for ATP production. These organic compounds enter at various steps in the glycolytic and Krebs cycle and become part of these cycles in driving ATP production. The diagram given below illustrates these points. Read the book.



- **How is fermentation test carried out?**

- One can test the ability of an organism to ferment a particular carbohydrate by providing the organism with a protein source, a single carbohydrate and a pH indicator in the medium. It is important that the organism must a purified culture (it should consist of only one type of organism), otherwise results of the test will not be valid. The reason that only one sugar should be present in the medium is that if there are two or more sugars, you would not know which one was fermented. An inverted Durham tube is also used to see if the organism produces gas. As the gas accumulates in the tube, it displaces the fluid medium



leaving an empty space at the top which is an indication of gas production. Change of color of the medium is an indication of fermentation of the sugar in the tube by the organism. Change in the color and presence of gas can be seen in the photo above.

- **Classification of Organisms based on electron and carbon sources**

- Organisms can be classified on the basis of electron source into two categories:
  - **Phototrophs:** If the electrons are derived from the light, the organisms are called phototrophs.
  - **Chemotrophs:** If the electrons are extracted from the chemicals such as the food the organisms eat or use, the organisms are called chemotrophs.
- A second pattern of classification comes from the use of carbon. Again, organisms can be placed into two groups:
  - **Autotrophs:** If organisms use CO2 as their carbon source.

- **Heterotrophs:** If the organisms use various chemicals as a source of carbon.

- And when you combine all these groups together the following four patterns of organisms emerge as classes:
  - Photoautotrophs
  - Photoheterotrophs
  - Chemotautotrophs
  - Chemotheterotrophs

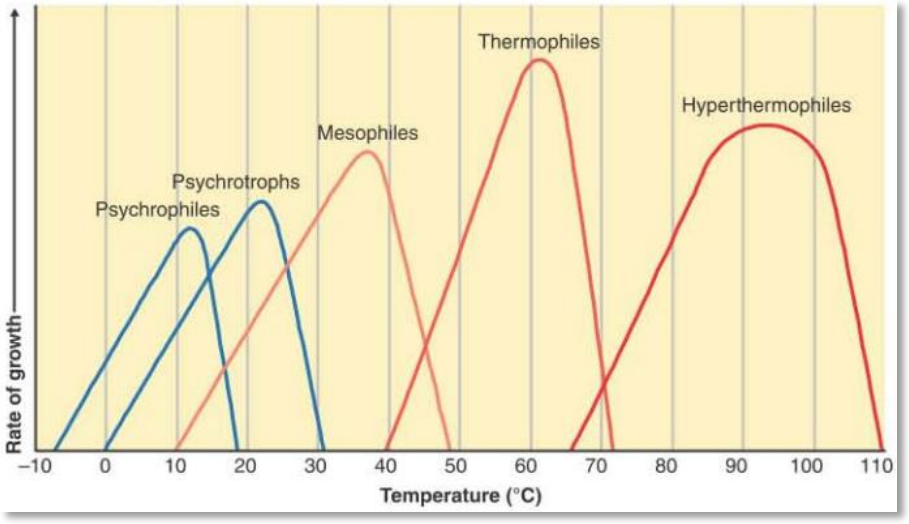
Lesson 27. Read pages 157-158.

LESSON 27. GROWTH REQUIREMENTS FOR MICROBES AND THEIR CLASSIFICATION

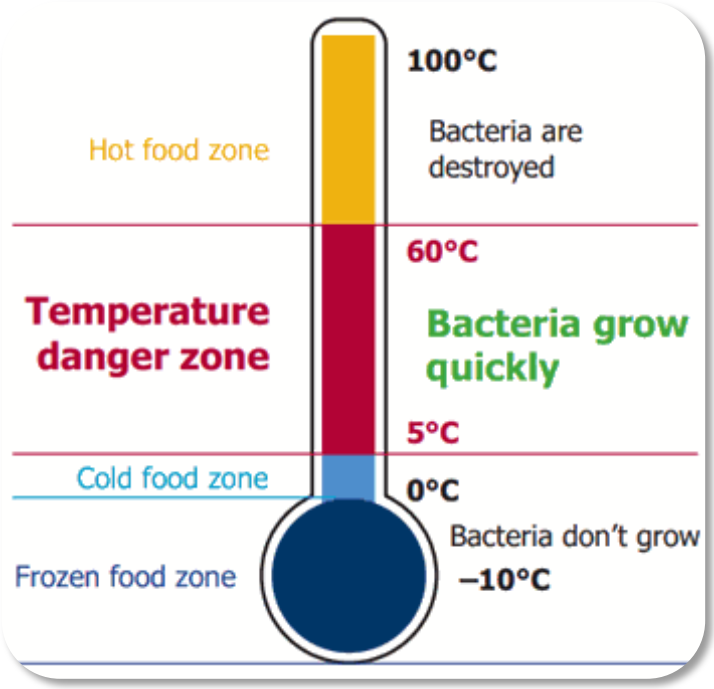
- **Growth Requirements for Microorganisms:**
- **Physical:**
  - Temperature
  - pH
  - Osmotic Pressure
- **Chemical:**
  - Source of C
  - Source of electrons
  - N, S, P, O
  - Trace elements
  - Growth factors
  - Water
- **Classification of Microbes based on Temperature Requirement**
- Organisms are basically classified into three groups based on the temp requirements:

1. **Psychrophiles:** These are further divided into strict psychrophiles and psychrotrophs:
   - **Psychrotrophs:** Cold loving: 15 ^{0}C
   - **Psychrotrophs:** Optimum temp is 20-30 ^{0}C. Food spoilage bacteria that can spoil food during refrigeration.
2. **Mesophiles:** 25 – 40 ^{0}C: These are the ones that cause diseases in animals and humans.
   - Moderate temp loving organisms
   - Optimum: 37 ^{0}C as this is the body temperature of humans and animals.
3. **Thermophiles:** 50 – 60 ^{0}C
   - Heat loving
   - Important in organic compost piles
   - These are further divided into hyperthermophiles that grow optimally at 80 ^{0}C. They live in hot springs.

- The following diagram depicts various groups of organisms classified on the basis of temperature.



- Every group of these organisms has a:
  - Minimum growth temp
  - Optimum growth temp.
  - Maximum growth temp.
- Min and max temperatures are mostly 30^0C apart.
- Now that we know that microbes have a range of temperature for growth that varies from minus 10^0C to plus 110^0C, it also brings something important to our mind. How can we keep our food safe from getting spoiled? Well, if the food after being prepared is stored below 5^0C or above 60^0C, it will not be spoiled (see the attached diagram below for the visual concept of temperature and the food safety). However, you should also know that you cannot keep the food at these temperatures for long. The food must be consumed within a few hours of preparation; otherwise, it will start to get spoiled. For long term storage, the food must be stored below zero degree in the freezer.


100°C
Bacteria are destroyed
Hot food zone
60°C
Temperature danger zone
Bacteria grow quickly
5°C
Cold food zone
0°C
Bacteria don't grow
Frozen food zone
–10°C

Lesson 28. Read pages 158-160.

LESSON 28. PH AND OSMOTIC PRESSURE REQUIREMENTS OF MICROBES

- **The second requirement for microbial growth is pH (-log of pH).**
  - Acidity:
    - If the pH is < 7
  - Alkalinity:
    - > 7
  - Enzymes need certain pH for optimal function.
  - Most organisms grow at pH between 6.5 and 7.5
  - Very few grow below pH 4
    - Pickles and Many Cheeses not spoiled at low pH because low pH discourages microbial growth. Fungus can grow though.
  - Acid is produced by bacteria during fermentation
  - **Acidophiles**
  - Molds and yeast grow At pH of 5 to 6
  - Alkalinity also inhibits microbial growth; however, it is rarely used for preservation of food. Mostly, acidity is used to suppress microbial growth.
- **Buffers in the culture**
  - Bacteria grown in lab produce acids
  - Growth inhibited by acids
  - Buffers are used to maintain desired pH
  - Peptones and amino acids are used as buffers
  - Phosphates are also used for buffers
- **Osmotic Pressure is the third requirement for microbial growth**
  - Isotonicity is required to keep the cells in good shape and health. Osmotic pressure is maintained mostly by adding sodium chloride in the medium for growth.
  - If cells are placed in hypertonic solutions, water leaves the cells shrinking the cells and damaging them. This process is called plasmolysis.
  - High salt concentrations are used for preserving foods.
  - If placed in hypotonic solutions, microbes will burst because water will enter into the cells.

- **Extreme halophiles**
- There are microbes (Archea) that require high salt concentrations for growth:
  - Obligate halophiles require very high salt concentration to grow.
    - 30 % salt
  - Facultative halophiles: These do not require very high concentration of salt for growth, however, they do not mind the presence of high salt concentrations. They grow happily.
    - 2% Salt (optimum growth)

Lesson 29. Read pages 160.

LESSON 29. ELEMENTS NEEDED FOR MICROBIAL GROWTH

- **Elements Needed for Microbial Growth**
- **Carbon**
  - Besides water, C is the most important requirement.
  - It provides structural backbone for living matter.
  - All organic compounds have it.
  - Half the dry wt of microbes is C.
  - **Chemoheterotrophs**
    - Use C from energy sources such as fats, carbs, and proteins
  - **Chemoautotrophs and photoautotrophs**
    - Use CO_2 as carbon source
- **Nitrogen, Sulfur and Phosphorus**
  - Nitrogen and Sulfur are needed for protein synthesis.
  - Nitrogen and phosphorus needed for DNA and RNA synthesis and ATP.
  - **How bacteria get Nitrogen?**
    - By decomposing proteins
    - From $NH4^+$ ions
    - From Nitrates
    - Use Gaseous N_2
      - These are nitrogen fixing bacteria.
  - **How bacteria get sulfur?**
    - From Sulfate ions
    - From Hydrogen sulfide
    - From Sulfur containing amino acids
  - **From where is phosphorus obtained by microbes?**
    - From phosphate ions mostly
  - Trace elements are not usually added in the growth medium, they are assumed to be present in complex media when you add meat extracts etc.

Lesson 30. Read pages 161-162.

LESSON 30. OXYGEN AS A REQUIREMENT FOR MICROBIAL GROWTH

- **Microbial Classification based on Oxygen Requirement**
- Oxygen is needed for aerobic respiration.
- Use of O_2 means more ATP
- NADH and $FADH_2$ during Krebs cycle oxidized via Electron Transport Chain which generates 36 or 38 ATP molecules in the organisms that use O_2 as the final electron acceptor.
- Based on the use and concentration of Oxygen, microbes can be classified into five groups:
    - **Obligate Aerobes**: Oxygen must be present for their growth. These organisms do not grow if O_2 is not present in the medium. If you test these organisms in a tube containing nutrient agar, the growth will remain confined to the surface only (see the diagram for the concept).
        - Mycobacterium tuberculosis is an example of such an organism.

    - **Facultative Anaerobes:** These organisms prefer aerobic respiration; however, if oxygen is not available, they can use anaerobic respiration or fermentative modes for generating ATPs. These organisms will be present throughout the tube, however, more growth will be seen on the top (close to the surface) because these organisms prefer using oxygen if it is available.

    - **Obligate Anaerobes:** Although, these organisms do not use oxygen, they cannot grow in the presence of oxygen. The reason is that oxygen creates toxic compounds which can kill these cells. Normally, oxygen toxic compounds are eliminated by the cells, however, strict anaerobe do not have a system to get rid of these toxic compounds when they are made in the cells. So using or not using oxygen is one thing and growing in the presence of oxygen (and not utilizing it) is another. The growth will remain confined to the bottom which has little oxygen. See the attached diagram.

- **Aerotolerant Aerobes:** These organisms do not use oxygen and also are not bothered by the presence of oxygen. In other words, they have a better system to dispose of toxic oxygen compounds. They will grow evenly throughout the tube of medium as seen in the accompanying diagram.

- **Toxic Compounds Generated by Oxygen**
- Oxygen generates highly reactive substances which can react with any molecule that they come in contact harming the organism or the body. The following four compounds are important to know (see the attached diagram for the concept):
  - **Singlet Oxygen:** It is molecular oxygen that has acquired an extra electron and is now in a high energy state and can react aggressively with any other molecule.
  - **Superoxide Radicals or Superoxide Anions:** These are created whenever oxygen is present in the cells. However, cells have superoxide dismutase that converts them into oxygen and H_2O_2.

$$O_2 + e^- \rightarrow O_2^{\bullet -} \text{ (superoxide radical)}$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2 \text{ (hydrogen peroxide)}$$

$$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH\bullet \text{ (hydroxyl radical)}$$

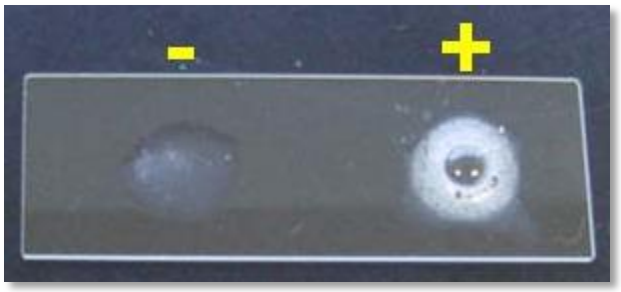
  - **Peroxide Anions:** When H_2O_2 is produced, it gives rise to peroxide anions which are toxic to the cells. Cells have evolved to deal with this toxic H_2O_2 by an enzyme called catalase as under:

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

  - Hydroxyl Radical: It is another intermediate product of oxygen radicals.

- **How to test organisms for the presence of catalase activity?**
  - Mix a drop of fresh culture with a drop of H2O2. If the organism is catalase positive, oxygen bubbles will be generated which can be seen easily as are visible in the accompanying figure.

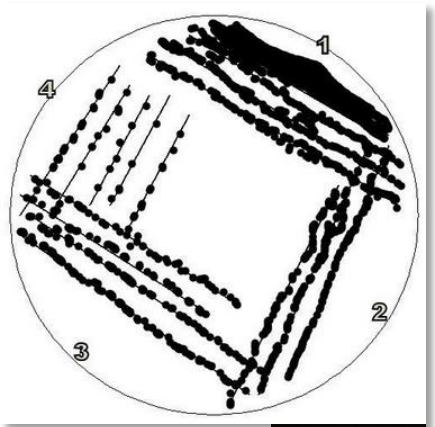


Lesson 31. Read pages 164, 170.

LESSON 31. CULTURE AND CULTURE MEDIA

- **What is a culture?**
  - This term is used to define growth of microbes. In other words, microbial growth in the lab is called a culture.
- What is a culture medium?
  - A nutrient material that supports the growth of microbes in the lab is called a culture medium.
- **What is an inoculum?**
  - Microbes introduced into a culture medium that initiate growth of organisms.
- **Types of media**
  - **Solid medium:** Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other. A well isolated colony is assumed to be the progeny of a single cell.

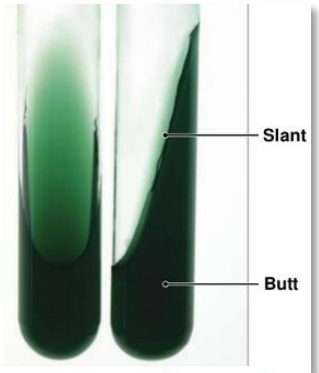
Purification of organisms is done by streaking the organisms as can be seen in the diagram on the right. Secondly, the solid media are used to study the colony characteristics.

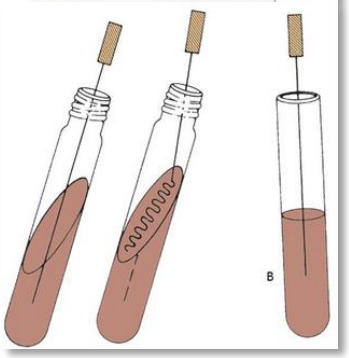


  - **Liquid medium:** It is fluid in nature. It is used for growing an organism as a stock (at large scale).

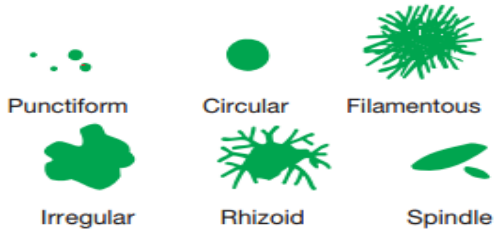
- **Characteristics of Agar (Solidifier of a medium):**
  - Agar is derived from a sea weed.
  - It is a complex carbohydrate which is not used by bacteria during the growth.
  - It liquefies at 85^0C and solidifies at 43 ^{0}C
  - Agar could be used to solidify medium in Petri plates or slants.

- **What are a slant culture and a butt?**
  - A culture made on an angled surface in a tube as shown in the accompanying diagram is called a slant.
  - Butt is the bottom portion of the slant. Butt is used for stab cultures. Stab culture is made by a straight inoculating line. Motility of organisms can be demonstrated by stab culture in a semisolid nutrient agar (you will see this in your practical classes).

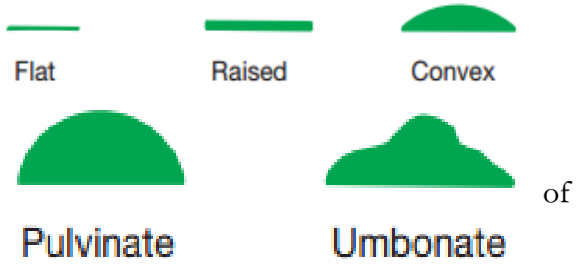




- **Characteristics of Bacterial Colony**
  - **Forms of Bacterial Colonies**
    - **Punctiform:** These are pinpoint colonies.
    - **Circular:** These colonies look like round or circular in form.
    - **Filamentous:** Threads running in all directions.
    - **Irregular:** The form of the colony is irregular. There is no regular shape.
    - **Rhizoid:** The colony has branched filaments.
    - **Spindle shaped:** these colonies are thicker in the middle and thinner at the edges.

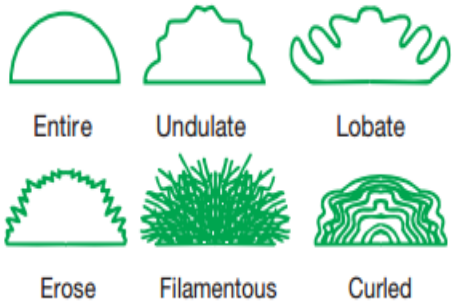


- **Elevation of Bacterial Colonies**
  - **Flat:** Slightly raised from the surface (when you see them from the side).
  - **Raised:** Elevated from the surface more than the flat colonies.
  - **Convex:** thicker from the middle or raised in the middle than at the edges.
  - **Pulvunate:** bigger version convex colonies.



of

  - **Umbonate:** overall convex but further raised in the middle.
- **Margins of Bacterial Colonies**
  - **Entire:** Look circular
  - **Undulate:** slightly wavy margins
  - **Lobate:** Look like as if they are divided into lobes
  - **Erose:** Serrated edges
  - **Filamentous:** threadlike
  - **Curled:** Series of wave patterns



Lesson 32. Read pages 165-170.

LESSON 32. CLASSIFICATION OF CULTURE MEDIA

- **What is a chemically defined medium?**
  - If you know the exact composition of a growth medium, such a medium is called a chemically defined medium. Chemically defined media are used for research work mostly.
- **What is a complex medium?**
  - When the ingredients of the growth medium are not exactly known, the medium is called a complex medium. Nutrient agar and nutrient broth are examples of complex media. Such media have yeast extract, meat extract and peptone etc as important ingredients. What is present in the yeast or meat extract is not exactly known, and this is the reason these media are called complex media.
- Defined or Complex media can further be divided into **General purpose media, Selective media, Differential media, and Enrichment media.**
- **What is a General Purpose Medium?**
  - A medium that supports the growth of most of bacteria is called a general purpose medium. Nutrient agar is such a medium.
- **What is a Selective Medium?**
  - These media suppress the growth of unwanted bacteria and supports the growth the desired microbes. Inclusion of bismuth sulfite in a medium suppresses the growth of gram-positive bacteria and most gram-negative intestinal bacteria. However, it spares the growth of *Salmonella typhi*. Another example is MacConkey's agar which contains bile which inhibits bacteria that do not belong to the gut.
- **What is a Differential Medium?**
  - A medium that enables differentiation of microbes on a single medium is called a differential medium. So, colonies on the medium will look different enabling visual differentiation of microbes. An example of such a medium is blood agar. Hemolytic microbes can be differentiated from non-hemolytic microbes by the look of the colonies and areas around them as is seen in the

accompanying figure.

- β-hemolysis: clear zone
- *Strep pyogenese*
- α-hemolysis: greenish halo

- **What is an Enrichment Medium?**
  - Bacteria that are present in small numbers can be missed easily, so such bacteria are encouraged to grow while others are not supported. Such a medium that achieves this purpose is called enrichment medium. Selenite broth is enrichment medium for Salmonella.
- **What is a Reducing Medium?**
  - This is also a special medium for growing obligate or strict anaerobes. The medium contain a reducing agent such as sodium thioglycolate which combines with the dissolved oxygen depleting the medium of oxygen.

- **How to Preserve a Culture?**
  - **Refrigeration:** It can be used for short term storage of organisms.
  - **Deep Freezing:** for long term storage, samples can be stored at minus 40 or minus 80°C.
  - **Lyophilization:** Also called freeze drying. This method removes water (by sublimation under vacuum) from a frozen culture (at minus 54 to minus 72 °C) of microbes that can then be stored for a long time.

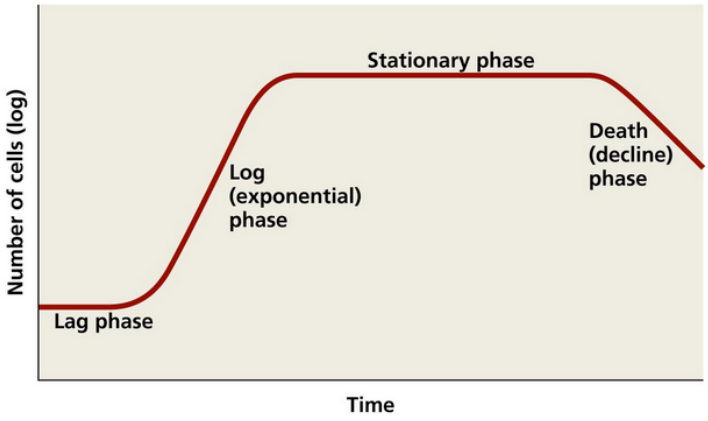
- **How to obtain a Pure Culture?**
  - The most commonly used method for obtaining a pure culture is streaking on the solid medium which is also called as **streak plate method**. However, this is not the only method to purify a culture. Dilution method can also be used to purify colonies from each other. Serial 10-fold dilutions can be made from a mixed culture and the last few dilutions in the series can be spread onto the plate with an L-shaped sterile glass rod. Although, this technique is mostly used for counting the viable number of bacteria in a culture, however, this could be used for picking up isolated colonies which can further be cultured for downstream testing of the organism to establish the identity of the organism under question.

Lesson 33. Read pages 171-173.

LESSON 33. BACTERIAL GROWTH CURVE

- **Generation Time and Growth Curve**

- Let us first discuss what a batch culture is. Well, when you place a liquid medium in a closed vessel such as a flask, and inoculate this medium with a bacterium, the bacterium will keep on multiplying until nutrients are depleted and toxic wastes are accumulated. If the medium is not refreshed, the growth of microbes will stop ultimately. This is called a batch culture.
- In a batch culture, organisms go through various phases of growth. If we follow this growth with respect to time, and make a graph of this growth pattern, it will give us what we call as a growth curve. See the given figure for a conceptual understanding of the growth curve:



- There are four distinct phases of this curve.
  1. **The Lag Phase:** cells prepares for growth in this phase. No growth is observed during this period or phase, however. Cells are metabolically very active during this phase.
  2. **The Log Phase:** During this phase, organisms multiply exponentially or logarithmically. Generation time becomes constant during this phase and that is the reason, the log graph will show a straight line. Cells are in the most active stage during this phase of growth curve. For commercial applications such as vaccine production, cells have to remain in this phase in order to reproduce most efficiently resulting in increased cell mass or number. Another application of this phase of growth is to determine the generation time.
  3. **The Stationary Phase:** This is also called a period of equilibrium as microbial deaths equal production of new cells. In other words, organisms start dying during this phase, however, the number of dead organisms is replaced by new organisms because there is still replication of cells going on. So, overall number of organisms does not change. This is the reason, it is called a stationary phase.

4. **The Death Phase:** The number of deaths exceeds the number of new cells formed during this phase. In other words, overall number decreases. It is also called logarithmic decline phase. Why there is a decline phase or death phase. The reason is simple: Nutrients are depleted and waste products which are toxic to the cells accumulate suppressing the growth and killing the cells.

Lesson 34. Read pages 174-179.

LESSON 34. METHODS FOR ESTIMATION OF MICROBIAL GROWTH

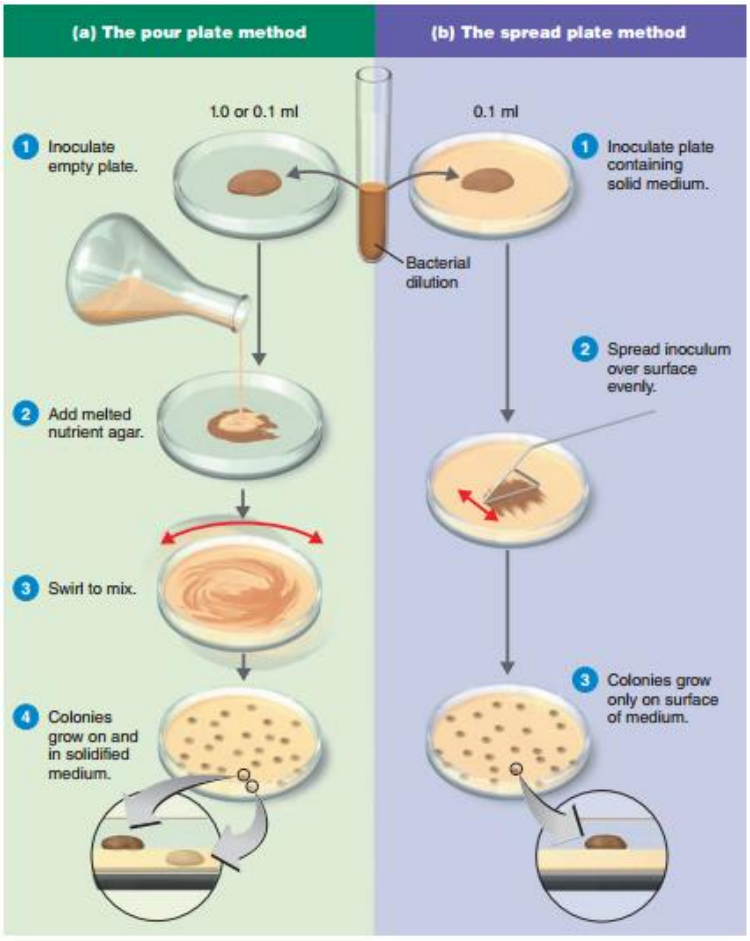
- **Direct Measurement of Microbial Growth**

- Microbial populations can be measured by two ways: 1. By the **number** or 2. By the **mass** (which is directly proportional to a pre-calculated or pre-determined number). Because the organisms are high in number, counting methods work by diluting the samples and counting a small portion of the diluted sample. The number of organisms in that small volume of diluted sample is then multiplied by the dilution factor to calculate the original number of organisms in the sample. This number is usually reported as the number of organisms in one ml of volume of the sample or one gram of weight of the sample. Now let's discuss various methods for microbial counts. At first, we will focus on **direct methods**.

1. **Plate Counts:** It is the most frequently used method for counting microbes. Please note that this method can only count the viable number of microbes that will grow and form colonies on the plate. It does not give you a total count. Total count of microbes in a sample includes viable and dead microbes. Total count can be determined by other methods discussed below. The sample is serially diluted and small volumes from the last three dilutions are used to determine the number. Small volumes from the last 3 dilutions are spread on to the surface of nutrient agar plates and the plates are incubated overnight. Each viable microbe will form a colony which can be counted visually. Plate count can further be divided into two methods based on the way the inoculum is applied onto the plates:

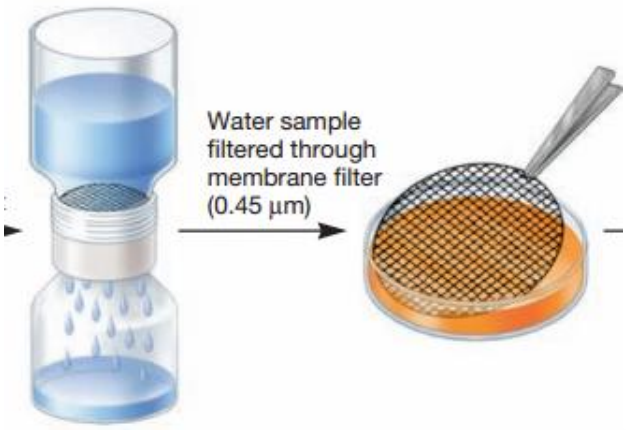
    a. **Pour Plate Method:** Small volumes (0.1 ml) from the last 3 dilutions are poured into the nutrient agar which is held at 50°C (molten agar) in a water bath. After thorough mixing of the inoculum, agar is poured into petri dishes and dishes are incubated overnight. The numbers of colonies are counted and the number is multiplied by the dilution factor to determine the number in one ml of original sample. Optimum number of colonies should be in the range of 30 to 300 colonies per plate for the procedure to be valid.

b. **Spread Plate Method:** The difference between this method and the method



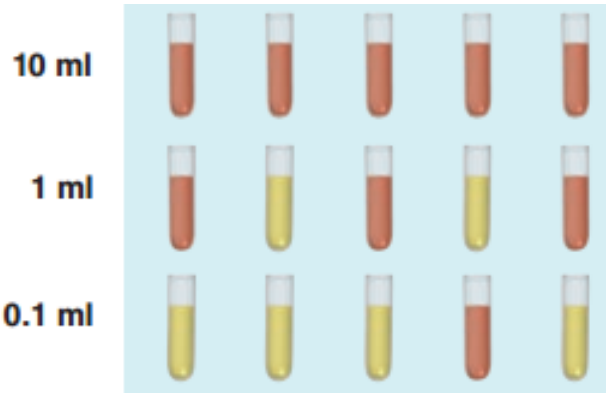
mentioned above is that the inoculum is spread with a sterile L-shaped rod as shown in the accompanying diagram. Colonies are counted and the number determined by multiplying with the dilution factor. This is the most commonly used methods of all.

2. **Filtration:** This method is used to count the microbes when they are in very low number (For example, if it is water sample that has only a few microbes). So, about 100 ml of water samples is filtered through a porous membrane that does not allow microbes to pass through it. The membrane is then inserted onto a nutrient agar plate and the plate is incubated overnight for colonies to



appear. Organisms can absorb nutrients through the membrane and grow on top of the membrane filter.

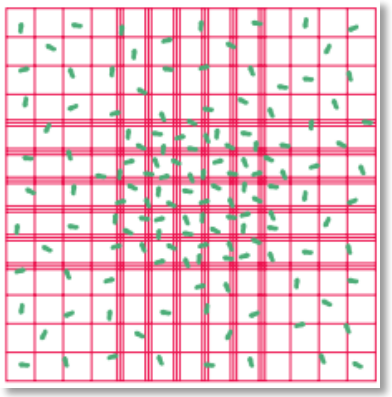
3. **The most Probable Number Method:** This method makes use of a statistical estimating technique. A chart has been worked out to determine the most probable number in the sample with 95% chances that the number falls within a range given in the chart. Mostly, our sample is drinking water. In this method, three sets of tubes with 5 tubes in each set are used (see the diagram for details). Each tube in the first set receives 10 ml of water sample as an inoculum. Each tube in the 2nd set receives 1 ml of water, while each tube in the 3rd set receives 0.1ml of water as inoculum. All tubes are incubated and growth in the tubes of each set is recorded which generates a code. This code is read from a chart to determine the most probable number as shown in the diagram: Brown tubes are showing growth. So this generates a code that is read as 5-3-1. This code is then followed in another chart which provides the count. See the chart below: This gives a



count of 110 microbes in 100 ml of water samples.

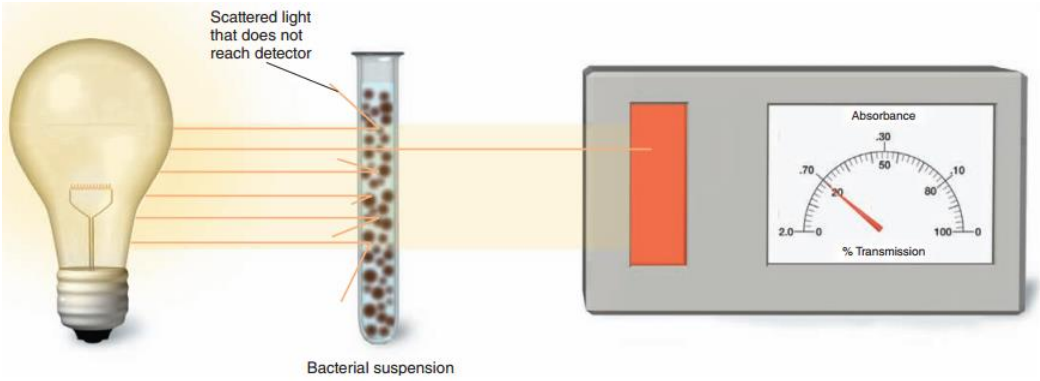
Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

4. **Direct Microscopic Count:** This method uses a hemocytometer which takes a specific volume of sample and the number of microbes can directly be counted under a light microscope. The number of microbes in one ml of original sample may be determined by multiplying with appropriate number (you will learn in your labs). No incubation is required, so this method is quicker and it also gives a total count (viable as well as dead microbes). There is an auto version of this method where the cells are counted electronically. The name of the instrument is Coulter counter.



- **Indirect Methods for Counting Microbes**
  - These methods are based on estimates (not direct counting). Turbidity, metabolic activity and dry weight are considered estimating procedures for bacterial counts. With all these methods, the number of bacteria is determined once and then that number is used in future to estimate the population by matching the values with previously created charts.

1. **Turbidity:** When bacteria multiply, they create turbidity in the liquid medium. This turbidity can be determined (optical density) by spectrophotometry using a specific wavelength. Optical density is then matched with a chart that has already been created by counting the number of bacteria versus optical density.



2. **Metabolic Activity:** This method assumes that a product of metabolic activity is directly proportional to the number of bacteria in the sample. Acid or CO_2 can be measured and compared with a preformed chart to know the number of microbes.
3. **Dry Weight**: Organisms are grown and filtered to get rid of water contents. Filter is air-dried and weighed to determine the dry mass of microbes. The dry mass is then compared with a predefined chart that gives the number of bacteria or fungi etc. in the sample.

Lesson 35. Read pages 185-186.

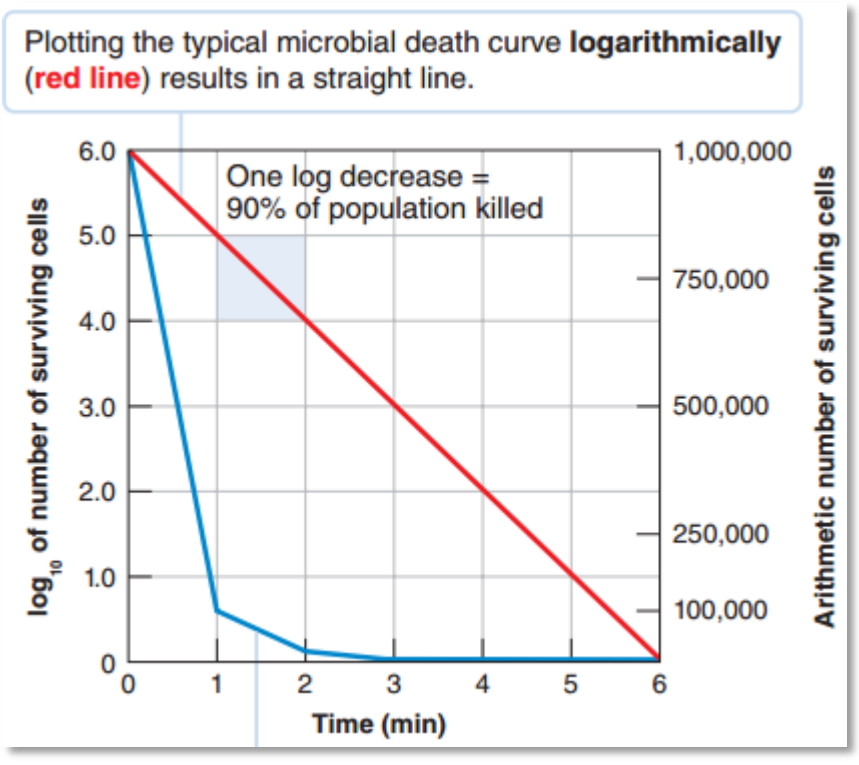
LESSON 35. TERMS USED FOR MICROBIAL CONTROL

- **The Terminology of Microbial Control**
  - **Sepsis:** Microbial contamination
  - **Asepsis:** Absence of significant contamination
    - Aseptic surgery techniques prevent microbial contamination of wounds
  - **Sterilization**: Removing all microbial life including spores
  - **Disinfection**: Removing pathogens significantly
  - **Antisepsis**: Removing pathogens from living tissue
  - **Degerming**: Removing microbes from a limited area (injection site)
  - **Sanitization**: Lowering microbial counts on eating utensils
  - **Biocide/germicide**: Kills microbes
  - **Bacteriostasis**: Inhibiting, not killing, microbes
  - **Chemotherapy:** killing microbes within the host tissue

Lesson 36. Read pages 186-188.

LESSON 36. FACTORS AFFECTING MICROBIAL CONTROL

- **The Rate of Microbial Death**
  - When microbes are treated with antibiotics or disinfectants, they usually die at a constant rate. In other words, if you heat a culture of bacteria and determine the cells that die in 1 min, you will find a specific number of organisms get dead. And now, if you extend the heat for another min, leftover microbes will die at the same rate again. This is what we call a constant rate of death. This will be clearer with a numerical example. Suppose a population of 1 million microbes has been treated for 1 minute, and 90% of the population has died. We are now left with 100,000 microbes. If the population is treated for another minute, 90% of *those* microbes die, and we are left with 10,000 survivors. In other words, for each minute the treatment is applied, 90% of the remaining population is killed. If the death curve is plotted logarithmically, the death rate is constant, as shown by the straight line in the figure below:



- **Decimal Reduction Time:** Time required to kill 90% of microbes at a given temperature

- Also called D value (time to drop the value by 1 log or 10-fold)
- Decimal reduction time will vary from temperature to temperature for a given species of microorganism.

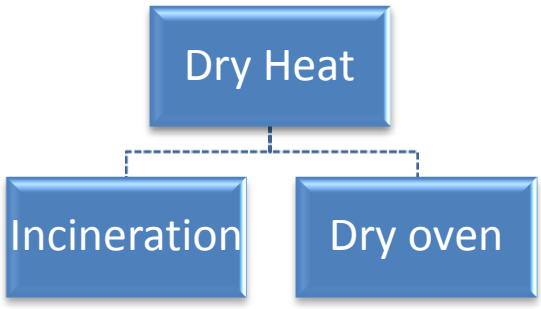
- **Factors that influencing the effectiveness of Antimicrobials**
  - **The number of microbes:** It means if there are more numbers of germs in an environment, they will take more time to kill.
  - Environmental Influences: Presence of organic matter such as pus, blood etc. makes antimicrobial less effective. Fats and proteins are especially protective.
  - **Time of Exposure:** Endospores take more time to get killed.
  - **Microbial Characteristics**: Generally, Gram positive bacteria are more sensitive to disinfectants than gram negative microbes. It is because of LPS present in G-negative bacteria. Mycobacteria are more resistant than other gram positive microbes.
  - **Concentration of Antimicrobial**: Concentrated antimicrobial will be more effective than the diluted one.

Lesson 37. Read pages 187-192.

LESSON 37. METHODS OF MICROBIAL CONTROL

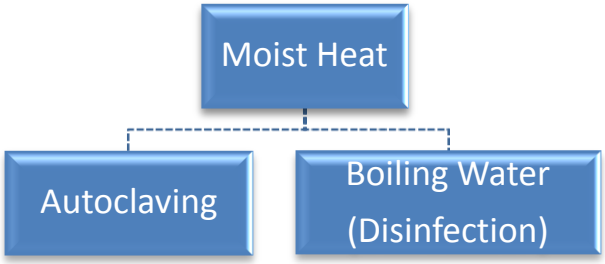
- **Actions of Microbial Control Agents**
  - **Alteration of membrane permeability:** Damage to the lipids and proteins of plasma membrane causes the leakage of cell contents killing the organism.
  - **Damage to Proteins and Nucleic Acids:** Enzymes in the microbes are the targets of microbial control agents. Similarly DNA or RNA damage by heat, radiation or chemicals is also seen as one mechanism of action.

- **Various Methods for Controlling Microbial Growth:** These methods can be broadly classified into four groups: Physical, Mechanical, Chemical and Biological
- **Physical Methods of Microbial Control**
  - **Physical methods include heat and radiation**
- **Heat:** It is the cheapest sources of all and easily available to control microbial growth. There are a few heat related concepts that we must appreciate. Heat denatures enzymes.
  - **Thermal Death Point:** It is the lowest temperature at which all cells in a culture are killed in 10 min. It will be a specific temperature for a specific species of organism.
  - **Thermal Death Time:** Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures.
- Methods that use heat can further be classified into **dry and moist** heat methods.



- Heat kills by oxidation

- Form of dry heat include: 1. Dry heat (hot-air oven), 2. Flaming (Platinum loop sterilization in the flame of Bunsen burner), 3. Incineration (burning to ashes).
- Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at 100°C, and Tyndallization (100°C for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than 100°C).



- **Pasteurization:** This technique typically employs low heat for killing pathogenic and food spoiling bacteria in milk. However, it does not kill thermoduric organisms. Thermoduric organisms are not pathogenic though. Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. Dairy industry tests phosphatase to determine if pasteurization of dairy product has occurred effectively because phosphatase present in raw milk gets inactivated with effective pasteurization temperatures. The following three equivalent heat treatments can be given to achieve pasteurization of milk:
  - 63°C for 30 min
  - High-temperature short-time: 72°C for 15 sec
  - Ultra-high-temperature: 140°C for 4 sec
- **Other Physical Methods**
  - Low temperature inhibits microbial growth by slowing down chemical reactions.
    - **Refrigeration:** Materials stored at 2-8°C can last for a day or two.
    - **Deep-freezing:** Long term storage of materials is possible at minus temperatures in the deep freezers.
    - **Lyophilization**: Freeze drying is another method for long term storage of food and other materials.
  - **High pressure** deshapes and denatures proteins: This technique is used for preserving juices and wines.

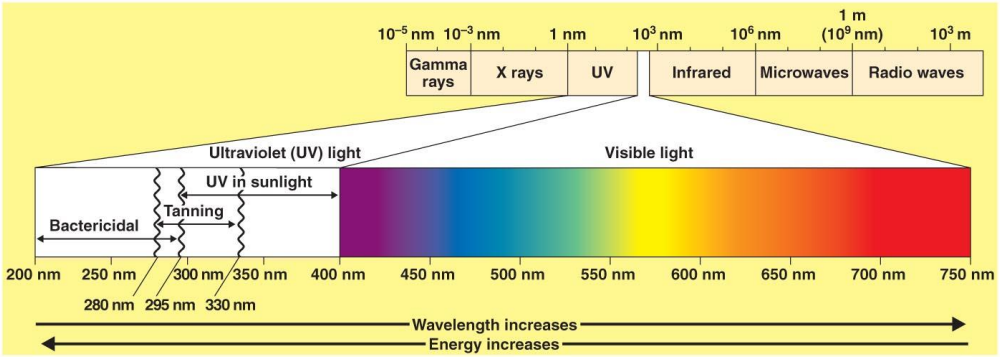
- **Desiccation** prevents metabolism: This method is very old and still in use today. Food stored in high concentrations of salt or sugar solution can keep for long.
- **Osmotic pressure** causes plasmolysis. High salt concentrations for example can take the water content of the cells out leaving them starved of water.

Lesson 38. Read pages 192-194.

LESSON 38. PHYSICAL METHODS OF MICROBIAL CONTROL

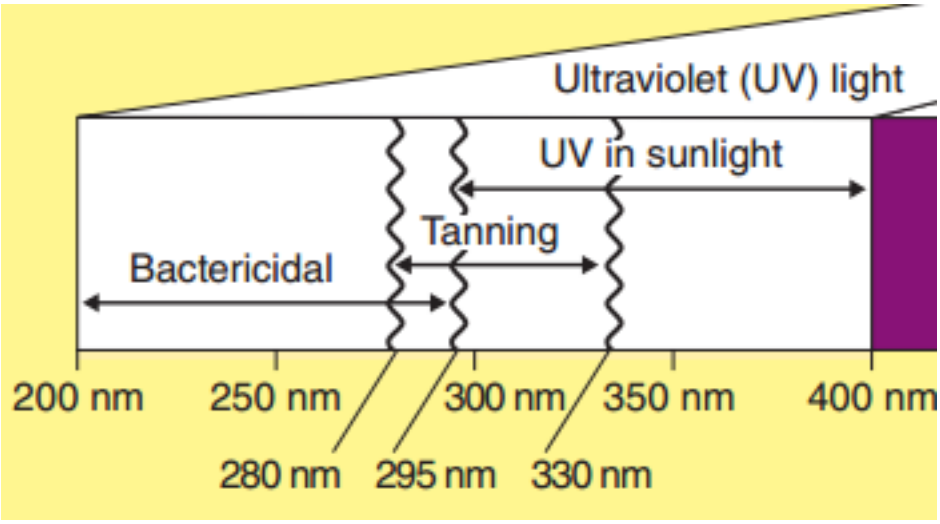
- **Filtration:** It is also a physical method. It can be used to filter air (using high efficiency particulate air filter) or liquid medium using membrane filters.

- **Radiation:**
  - Ionizing and non-ionizing radiation
  - Ionizing Radiation: High energy waves that destroys microbes.



- Ionization radiations include gamma rays, X rays, or high-energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.
- Non-ionizing Radiation: wavelengths longer than 1nm fall into non-ionizing radiation. The best example is UV light. UV light causes mutations in the DNA by dimerization of thymidine bases. These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The most useful wavelength is 260 nm. This wavelength is specifically absorbed by DNA. UV radiation is used to control organisms in the air mostly. However, surfaces are also sterilized by UV radiation. UV light cannot penetrate deep into

the fluids, so only surface bacteria are killed. See the range of UV light that is useful for microbial killing.



Lesson 39. Read pages 200.

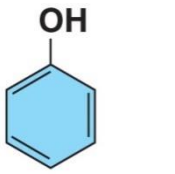
LESSON 39. ETHYLENE OXIDE FOR MICROBIAL CONTROL

- **Gases with Antimicrobial Activity:**
  - **Ethylene Oxide:** Many heat-sensitive items such as disposable plastic, petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized by ethylene oxide. Ethylene oxide is a strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity. It rapidly penetrates packing materials, even plastic wraps. EtO is explosive, supplied in a 10% to 20% concentration mixed with either CO_2 or dichlorodifluoromethane. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L.

Lesson 40. Read pages 195-197.

LESSON 40. CHEMICALS AS ANTIMICROBIAL AGENTS I

- **Chemical Methods of Microbial Control**
  - Chemicals are used to control microbial growth. Factors that influence the efficacy of a chemical disinfectant include the concentration of the disinfectant, presence of organic matter in the environment (matrix) where the disinfectant is being used, pH of the environment (disinfectants are more effective at acidic pH), and time of exposure to the disinfectant.
- **Properties of an Ideal Disinfectant**
  - It should be effective at low concentration.
  - It should be nontoxic to body tissues.
  - It should be effective in organic matter.
  - It should have a broad spectrum of activity.
- There is no ideal disinfectant available that provides all of the above features.
- **Types of Disinfectants**
  - **Phenol and Phenolics**
    - Lister was the first to use phenol (carbolic acid) as a disinfectant to control microbes in surgery. Derivatives of phenols are called phenolics. Phenols disrupt plasma membranes and are effective in the presence of organic matter. They are mostly used microbes in Pus, saliva, feces. The structure of phenol is shown to the right of this page. Phenol is irritating to the skin. Derivatives of phenols are less irritant.



(a) Phenol

    - **Cresol** is an example of such a derivative and o-phenylphenol is an example of a cresol.
    - **Bisphenols:** These are also derivative of phenol. Hexachlorophene is an example of a bisphenol. This disinfectant is very commonly used in surgeries in hospitals. Trichlosan is another example of bisphenol. Trichlosan inhibits synthesis of lipid that are needed in the plasma membrane of microbes.
  - **Biguanide:** Made by condensation of two guanidine molecules. These compounds disrupt plasma membrane. Examples include chlorhexidine and alexidine.

- **Halogens:** Iodine and chlorine are very good antimicrobial agents. Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

    - **Tincture of Iodine:** A solution of iodine in alcohol is called a tincture.
    - **Iodophor:** A combination of iodine with an organic molecule from which iodine is released slowly. An example is Betadine.
    - **Choline:** when chlorine gas is mixed with water, it forms **hypochlorous acid** which has germicidal activity. It is a very strong oxidizing agent which inactivates cellular enzymes.
        - **Calcium Hypochlorite:** Commonly used as disinfectant for dairy equipment.
        - **Sodium Hypochlorite:** Bleach consists of this compound. It is also is very good disinfectant for inanimate objects.
        - **Chloramine:** It is a combination of chlorine and ammonia. Used for dairy equipment mostly.

Lesson 41 . Read pages 197-200.

LESSON 41. CHEMICALS AS ANTIMICROBIAL AGENTS II

- **Alcohols:** Ethanol, isopropanol are examples. Alcohols denature proteins, and dissolve lipids. Alcohols require water for being more effective. This is the reason that seventy percent alcohol is more effective than 100%. Please note that alcohols can effectively kill vegetative form of bacteria, and fungi but not spores. Alcohols are not very effective on wounds. Commonly used in hand sanitizers.
- **Heavy Metals and Their Compounds:** Silver, zinc, mercury and copper have biocidal and antiseptic activity. Heavy metals can be effective at very low concentration. This property is called oligodynamic action. Heavy metals denature proteins.
    - **Silver nitrate (1%):** Commonly used as a disinfectant.
    - **Silver and sulfadiazine combination:** used on burns.
    - **Mercuric chloride:** It is bacteriostatic in nature. This compound is toxic though and its use is not favored very much.
    - **Copper sulfate:** A commonly used disinfectant for controlling green algae in water ponds.
    - **Zinc chloride:** Commonly used in mouthwashes.
- **Surface Active Agents (Surfactants):** They decrease surface tension among molecules of a liquid. Soap and detergents fall into this category.
    - **Soaps and Detergents:** These have no antiseptic activities. They cause mechanical removal of germs by emulsification. This mechanical removal is also called degerming activity.
    - **Quaternary Ammonium Compounds (Quats**): The most common ones are cationic detergents. They do not kill endospores and mycobacteria though. They are more active against gram positive than gram negative bacteria. The affect plasma membrane, inhibit enzymes and denature proteins. Benzalkonium chloride is an example of a Quat.
- **Chemical Food Preservatives:** These chemicals are used in the food to retard food spoiling bacteria. These chemicals are either simple organic acids or their salts (that are easily metabolized in the body if eaten, therefore are safe) and include sodium benzoate,

sorbic acid, and calcium propionate. Sodium nitrate and sodium nitrite are also used to preserve meat and meat products.

- **Antibiotics:** Although antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese.

- **Aldehydes:** These are the most effective antimicrobials. Two examples are formaldehyde and glutaraldehyde. They inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—NH_2, —OH, —COOH, and —SH).

Lesson 42 and 43. Read pages 186-187.

LESSON 42 AND 43. MECHANISMS OF ACTION OF ANTIMICROBIALS

- **Mechanisms of Action of Physical and Chemical Methods**
- Mechanisms are discussed at various places in the chapter, so you have to study almost all the pages that discuss the use of chemical and physical methods. I have summarized them all in the two video lectures.
- Most of these methods target the plasma membrane and proteins. The phospholipids of the plasma membrane are disrupted and proteins are denatured while nucleic acids are mutated.

Lesson 44 . Read pages 233-236.

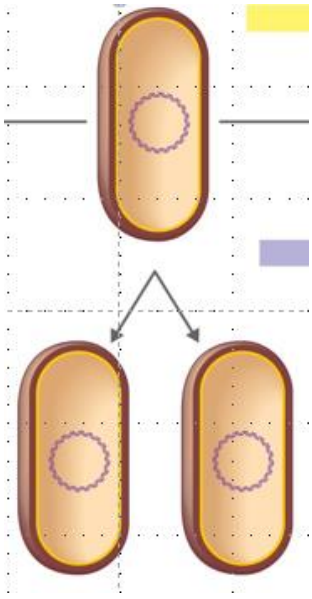
LESSON 44. GENE TRANSFERRING AMONG MICROBES

- We all know that everything in cells is controlled by genes. All metabolic activities, movement of the cells from one place to the other, interaction with the environment or with other cells etc. are all carried by the genes. One of the most important aspects that concern us as humans is the development of antibiotic resistance among microbes especially bacteria that cause diseases in humans, animals, and birds etc. This chapter covers the basics of DNA replication and gene manipulation in an overview style which is good for a general understanding; however, our focus is on the gene transfer that transmits antibiotic resistance from one organism to the other.

- **Gene Transfer and Recombination**

  - **Vertical Gene Transfer**

    - This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.



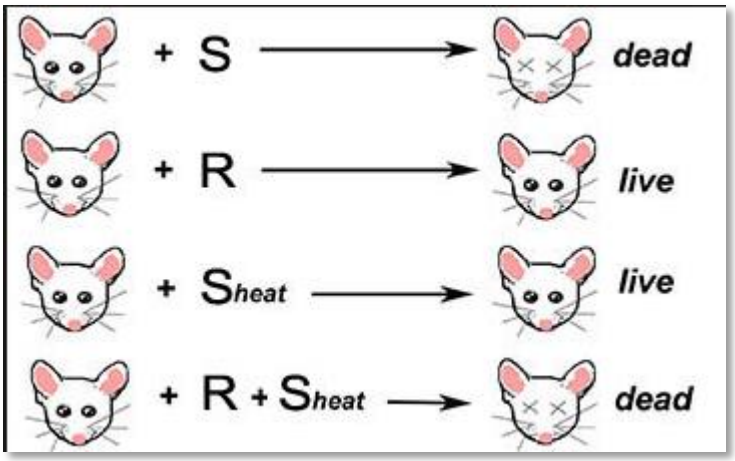
  - **Horizontal Gene Transfer**

    - When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

      1. Transformation

2. Conjugation
3. Transduction

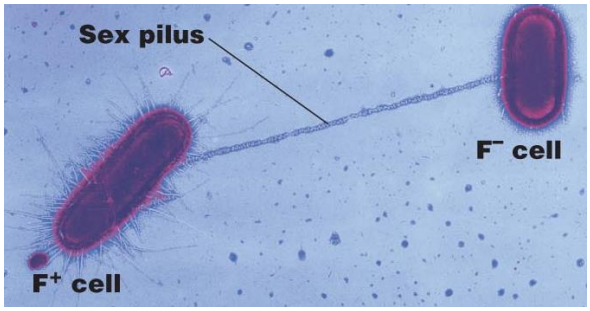
- **Transformation in Bacteria**
  - Naked DNA in solution is transferred from one bacterium to another during this process. Frederick Griffith observed this process in 1928 when he was working with *Strep pneumonia* in his lab. This bacterium has two strains: one is capsulated and is called a Smooth strain (which is pathogenic) and the other is Rough strain (non-pathogenic).
  - When live encapsulated bacteria are injected into a mouse, the mouse gets killed by the microbe. When rough strain is injected, the mouse is not killed. Moreover, when heat-killed encapsulated bacteria are injected, it also does not kill the mouse. However, when heat-killed strain is mixed with rough live strain, and a mouse is injected with this mixture, the mouse dies. If you isolate the bacterium from these dead mice, the bacteria resemble with encapsulated strain of *Strep pneumonia*. The interpretation of this experiment led to the mechanism that rough live bacteria take up DNA from the heat-killed encapsulated strain and becomes pathogenic by acquiring the ability to form a capsule. Very few bacteria have this property of accepting foreign DNA and most of it is degraded (only small fragments of DNA get incorporated in the genome of the recipient. The cells that can take up DNA are called competent cells. We can create such microbes in the lab that can accept a given DNA during gene transfers that we undertake in our DNA work. The cells that take up the DNA and incorporate this in their genome successfully are called transformed cells.



Lesson 45. Read pages 236-237.

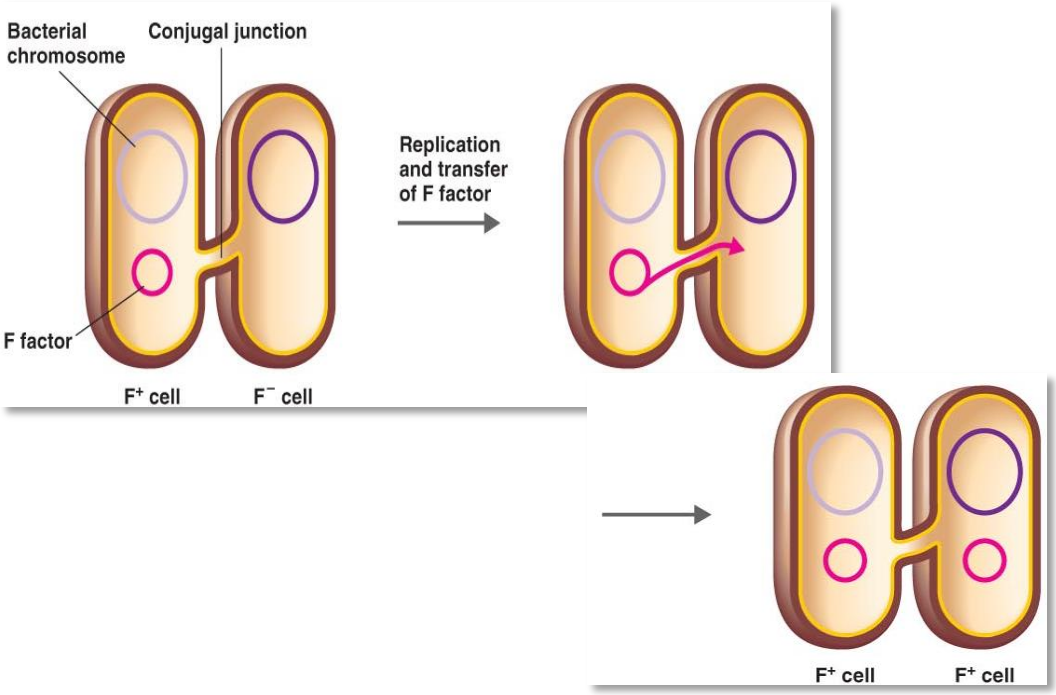
LESSON 45. CONJUGATION OF BACTERIA

- **Conjugation of Bacteria**
  - Genes transfer process can be mediated by plasmids. Plasmids are extra-chromosomal circular DNA fragments that replicate independently of bacterial chromosome. In other words, a given bacterial cell can have multiple copies of plasmid with the same cell. Plasmids often have genes that are not strictly needed by the microbe for its growth. However, the genes on the plasmids provide some advantages to the bacterium. For example, antibiotic resistant genes offer a mechanism for the bacterium to resist the presence of that antibiotic. In other words, bacterium that has a plasmid with an antibiotic gene on it can still multiply in the presence of that antibiotic.
  - Conjugation requires direct cell to cell contact and cells have to be opposite mating types. Plasmids in G –ive cells have genes that code for sex pili because pili are needed to make a bridge between the two mating type cells. Since there is no pilus present in G +ve bacteria, they secrete sticky surface molecules instead which create contact between the two cells. The pilus along with two mating cells is shown in the diagram below:
  - These plasmids are transmissible from one bacterium to another during conjugation.



  - Plasmid is replicated and a single copy of the plasmid is transferred to the recipient bacterium through the pilus.
  - A complementary copy is then made in both the cells to make the DNA of the plasmid double stranded molecule.

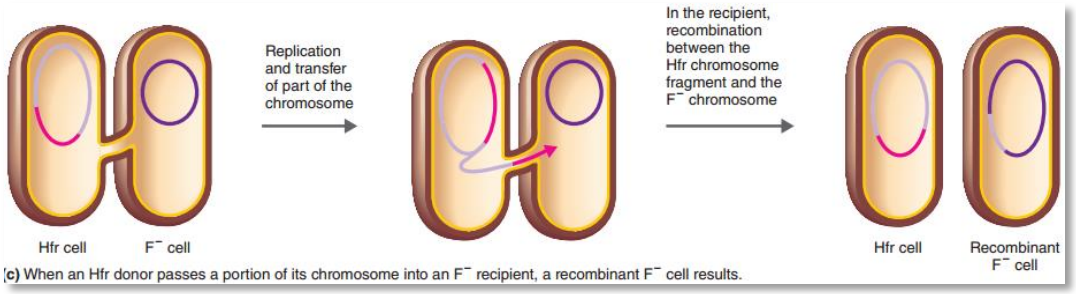
- In E. coli, fertility factor (F factor) plasmid was the first plasmid observed to be transferred from one organism to the other, hence those bacteria that have this plasmid are called F^+ cells to differentiate from those that do not have one (F^-). However, once F^- cells acquire F^+ plasmid, they also become F^+.



Lesson 46. Read pages 236-238.

LESSON 46. HIGH FREQUENCY OF RECOMBINATION

- **High Frequency of Recombination**
- In some cells that carry F^+, F^+ (Fertility factor) gets incorporated in the chromosomal DNA which converts F^+ cells to high frequency of recombination cells (Hfr cells). When conjugation occurs between an Hfr cell and an F^- cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the



(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

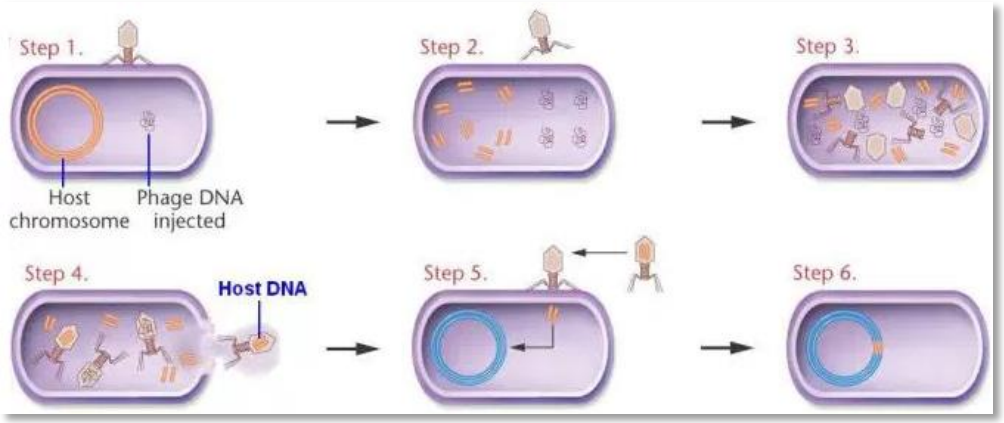
recipient cell. Since this transfer starts from the middle of F factor gene, and most of the time, this transfer is not complete; the whole chromosome of Hfr cell is not transferred. However, some genes can be transferred during this process. When these genes become integrated into the genomic DNA of the recipient cells, the recipient cells acquires new versions of genes that were not part of its genome previously. It may be noted that F^- cells remains F^- negative cell because F factor is not transferred completely. This whole process is illustrated in the accompanying diagram. Also remember that these newly acquired genes can be mapped easily with respect to the time they get transferred to F^- cells.

  - So, conjugation can be used to map the genes on the bacterial chromosome. Video clip in the lecture illustrates this mapping very well.

Lesson 47. Read pages 237, 239.

LESSON 47. TRANSDUCTION IN BACTERIA

- **Transduction in Bacteria by a Bacteriophage**
  - Transferring of a gene from a bacterium to another bacterium via a virus is called transduction.
  - When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released. What happens is that when phages cause lysis of infected cells, bacterial chromosomal DNA also gets fragmented and such fragments can sometimes be packed into the viral capsids much like the viral DNA. Now, the capsid carrying the bacterial gene or genes in that fragment can infect another E. coli and can inject its DNA (bacterial DNA fragment) into it resulting in the transfer of genes from one bacterium to another. The mechanism is illustrated in the figure below:



Lesson 48. Read pages 274-282.

LESSON 48. THREE DOMAIN SYSTEM

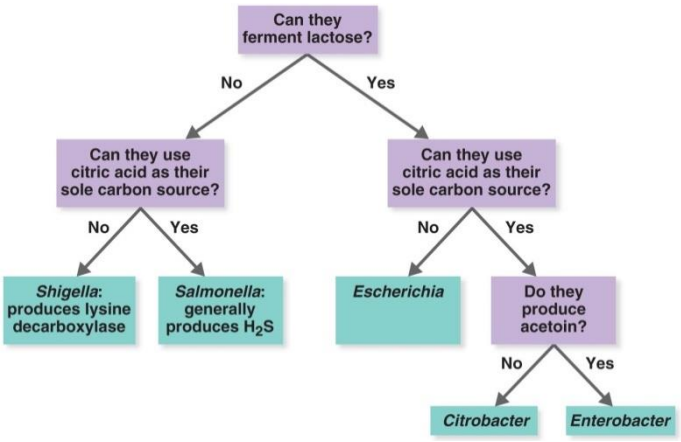
- **Some Definitions we must know.**
- **What is taxonomy:** The science of classifying organisms or orderly arrangements of organisms is called taxonomy.
- **Systematics, or Phylogeny:** The study of the evolutionary history of organisms is called systematics or phylogeny.
- Clone: Population of cells derived from a single cell that are genetically identical.
- **Strain:** A genetic variant of a clone is called a strain.

- **Classification of Microbes**
  - Please see pages 4-6 of these handouts for information on the classification of microbes.
- **Taxonomic Hierarchy**
  - Microbes are placed in groups based on similarities that they share with each other. All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. A bacterial **species** represents "a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminative phenotypic property" (definition taken from the internet). A **genus** consists of various specie; however, these species differ from each other in certain ways, although these are related by descent with each other. Related genera make up a **family**. A group of similar families constitutes an **order,** and a group of similar orders makes up a **class.** Related classes, in turn, make up a **phylum.** All phyla that are related to each other make up a **kingdom**, and related kingdoms are grouped into a **domain**.

Lesson 49. Read pages 282-286.

LESSON 49. CLASSICAL METHODS OF BACTERIAL IDENTIFICATION

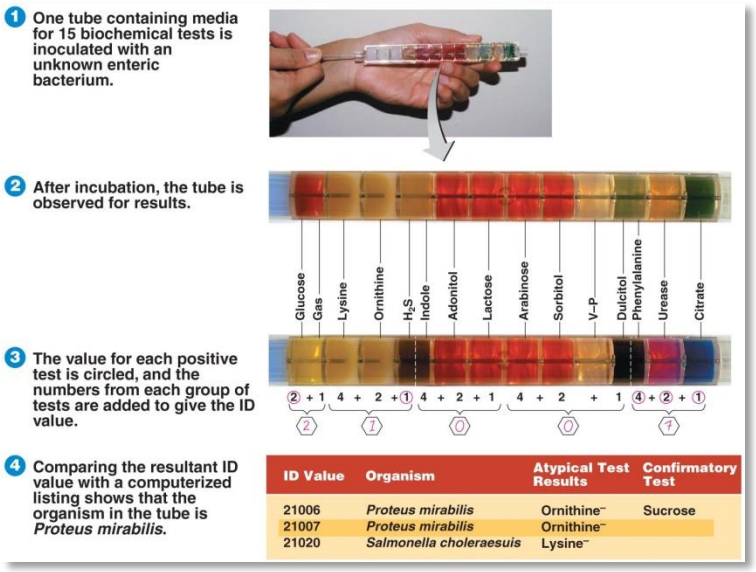
- **Methods of Classifying and Identifying Microorganisms**
  - A classification system provides a basis for characterization and comparison that ultimately helps us in their identification as an individual. Although, the methods for classifying microbes may be different from the methods that are used for identification of microbes for treatment purposes, however, both share the same basic theme: grouping organisms based on their similarities with each other.
  - It may also be noted that large organisms such as plants and animals can be grouped on the basis of morphology alone; such a characteristic does not strictly apply to the bacteria which more or less have the same shape. In other words, morphology alone cannot help microbes in their classification or identification. That is the reason, classification and identification of microbes involves detection of enzymes of various metabolic pathways in these cells. So, biochemical testing is integral part of microbial (procaryotes) classification and identification.
  - Most of these identification schemes have a dichotomy. In other words, if an enzyme or a character is present, we call this as positive for that enzyme, and if the organism is negative with respect to the character under question, we place the organism into another group (negative group). This type of yes and no situations leads to a classification or identification scheme as is depicted in the accompanying figure for identifying selected genera of enteric bacteria. See the next page:



- Microbes especially bacteria (disease causing) can be identified by three methods:

- Classical or Conventional Method
- Serological Methods
- Nucleic Acid based Methods

- Classical methods of microbial identification involve differential staining of the sample before it its culturing, culturing the sample onto nutrient agar, blood agar and MacConkey's agar, purifying the culture (colonies expected to be involved in the disease) and detection of various enzymes that belong to various metabolic pathways. Classically, such methods used

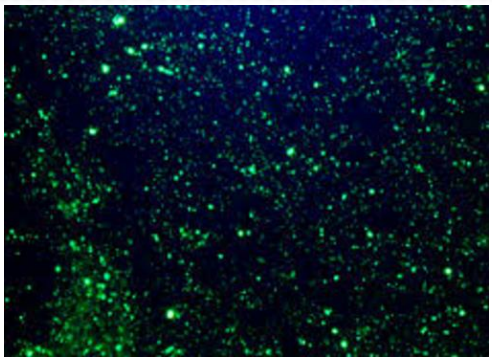


to take a long time to perform (3 days at least); however, rapid identification methods have become available now which use preformed media that can be used for testing the presence of metabolic enzymes. Such a kit is shown in the figure above (previous page).

Lesson 50. Read pages 287.

LESSON 50. SEROLOGICAL METHODS FOR BACTERIAL IDENTIFICATION

- **Serological Methods for Identification of Bacteria**
  - Serological methods involve antibodies and antigen interactions. Antibodies are produced by B lymphocytes against any foreign antigens such as bacteria and their toxins that enter the body of animals or humans. Antibodies are very specific in their interaction. In other words, antibodies made against E. coli do not bind or interact with Staphylococci or vice versa. Although, there are many serological techniques that can be successfully used for identification of microbial infections, we will focus on only a few of them. The main advantage of using serological method is the speed and economy. In other words, serological methods are quick to do (take only about an hour) and can work directly on the sample (sample does not have to be cultured and purified as is needed in conventional methods of identification). Serological methods can be performed on cultured microbes as well which again speed up the diagnosis. Also remember that in all these serological tests, either the serum or the antigen should be known to us.
  - **Fluorescent Antibody Test (FAT):** This test identifies an antigen in the sample. So, antibodies should be known to you. For examples, a brain sample of rabid dog has been submitted to you in the lab and you need to know if the dog was suffering from rabies (caused by a virus). As we know the virus is an antigen in this test, we will supply antibodies from the lab and these antibodies, as I mentioned earlier, will be known to us to be specific for rabies virus. So, in order to see if rabies virus is present in the brain, we will rub the brain on a glass slide (it will make a smear), air dry it, and then the smear is stained with the rabies specific antibodies that have already been tagged with some fluorescent dye. We then wash these antibodies after a few minutes to remove unbound antibodies and observe the smear under the UV equipped microscope. If rabies virus is present in the brain, we will see fluorescent spots in the smear as depicted in the figure below. Sample tested by this method does not have to be a purified sample. In other words, it could be mixed culture. Because antibodies are specific to the antigen, therefore, purification of the antigen is not necessary.



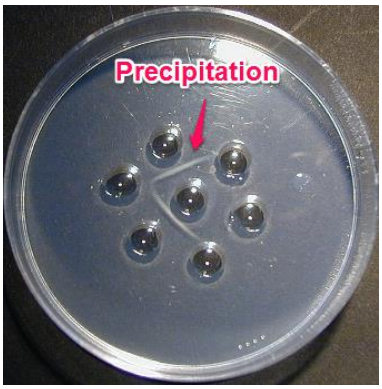
- **Slide Agglutination Test:** Let's first talk about a few definitions that will make our life easier. There are two words we must know about them: They are agglutination and precipitation. **Agglutination** is a process in which soluble antibodies interact with a particulate antigen. **Precipitation** is a process in which soluble antibodies interact with a soluble antigen. Now let's talk about a few examples of agglutination tests:
    - **Blood Grouping:** This is a typical agglutination test which involves antibodies (that are always soluble in nature) and RBCs (that are particulate in nature).
    - **Rose Bengal Plate Agglutination Test:** This test is used to diagnose Brucellosis in animals in which serum of animals suspected for Brucellosis is mixed with Brucella antigen (bacterim). The resulting positive interaction is seen as flocculation as are shown in the accompanying figure:

    - **Widal Test:** This test involves Salmonella cells (Antigen) and Salmonella antibodies.
    - **Coombs Test:** This involves antibodies coated RBCs (antigen) and antiantibodies.

Lesson 51. Read pages 287.

LESSON 51. PRECIPITATION TEST

- **Precipitation Tests:** These involve soluble antigens and soluble antibodies. Examples include:
    - **Agar Gel Diffusion Test:** This test is performed in agarose gel which is poured in a petri dish and wells are created which are used to place antigens and antibodies for interactions with each other. Antigens and antibodies diffuse from their respective wells and, where these molecules meet each other, a precipitation line is formed as shown in the accompanying figure.



    - **Precipitation of proteins:** This method can also be used to isolate a specific protein from cells by using protein specific antibodies which precipitate out that protein from a mixture of cellular proteins.
- It may also be noted that all antibody and antigen interactions can be grouped into two categories: Directly visible and indirectly visible tests.
- Directly visible tests are those that we can see with our naked eyes directly. For example, slide agglutination, rose Bengal plate test, Widal test, all these interactions are visible to the eye directly.

Lesson 52. Read pages 287-288.

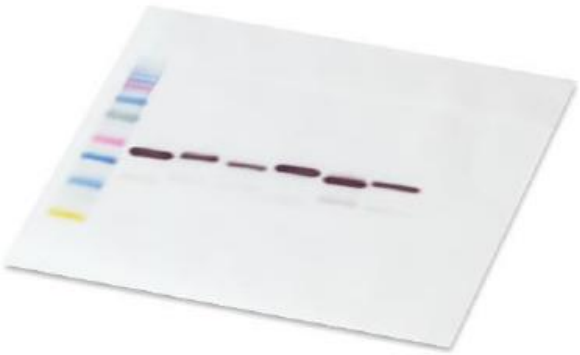
LESSON 52. ELISA

- **Enzyme-linked Immunosorbent Assay (ELISA)**
  - There are some interactions (Antigens and Antibodies) that are not directly evident. Interactions may have taken place, but there is no visible direct clue if the interaction has taken place or not. For such interactions, we use indirect methods. Say, there is big body of smoke visible to you from a kilometer ahead of you. What is your guess? Yes, you guessed it right. There is a big fire there. Did you see the fire? Probably not. You guessed on the basis of smoke that there is a fire there. This is called indirect way of interpretations. A beautiful example of such a test is ELISA where we do not know if the interaction has taken place or not. However, we can see it indirectly. We will see that in the following paragraph which discusses ELISA.
  - Enzyme-Linked Immunosorbent Assay: This test is done by coating the ELISA plastic plates either with the antigen or the antibodies. Suppose we have Brucella antigen coated ELISA plates that can detect antibodies from the serum of a Brucella infected animal. You take the serum and put into the wells of ELISA plate. You allow time for antigen and antibody to interact and then wash these wells with some buffer to remove unbound antibodies. These bound antibodies are not visible at this step. So, how do we see this interaction? Well, we use another antibody that has been made against the antibody bound to the antigen. This special antibody has been tagged with an enzyme. After allowing time for interaction of this 2^{nd} antibody with the 1^{st} antibody, 2^{nd} antibody is also given a washing to remove unbound antibody molecules. Again, we cannot say if interaction has taken place or not. Here comes an indirect way of detecting this interaction. Remember that the 2^{nd} antibody has a molecule of an enzyme attached to it. Now, if we provide substrate to the enzyme in the wells, enzyme will convert the substrate into a color compound visible to the eyes. The color change can be measured by a spectrophotometer as well which gives a quantitative data. See a photo below for ELISA plate.

Lesson 53. Read pages 287, and 289.

LESSON 53. WESTERN BLOTTING

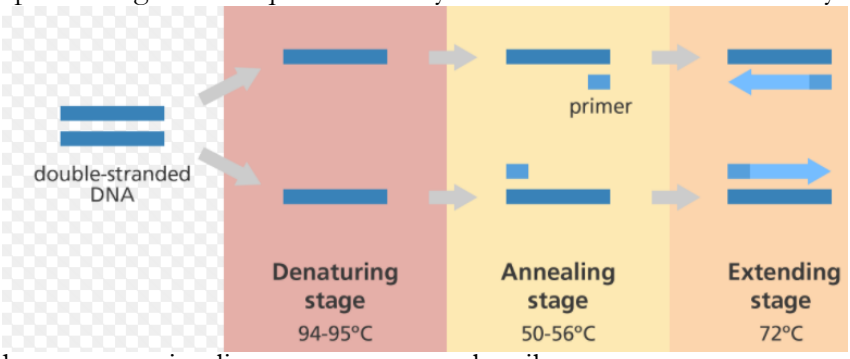
- **Western Blotting:** We can use this technique for the detection of antigen in the serum. Microbial proteins can be separated on SDS-PAGE by electrophoresis and the presence of these proteins can be detected by enzyme-tagged antibodies specific to those proteins. A color band will be seen where the specific protein (antigen) is present on the gel. Please remember that proteins in the gel are first transferred to a paper strip before they could be detected by specific antibodies as seen in the accompanying figure.



Lesson 54, 55. Read pages 290-293.

LESSON 54, 55. NUCLEIC ACID BASED TESTS

- **Nucleic Acid Based Tests**
- There are many tests that involve nucleic acid sequence detection for the presence of microbes in a clinical sample. Please remember, as antibodies are specific to the organisms, DNA or RNA sequences must be uniquely specific for the microbe that we are going to detect. Such sequences that are specific to an organism are called **signature sequences**. In other words, it is these sequences that provide the specificity for the organisms.
- There are many such tests, however, we will only focus on polymerase chain reaction (PCR).
    - PCR goes through 3 step cycles for about 30 to 35 times. Each cycle consists of a denaturing stage (about 95°C), primer annealing (50 to 56°C) and extending stage (72°C). A primer is a fragment of DNA which is specific to the signature sequence. Two primers span the signature sequence actually. At the end of 30 or so such cycles, enough DNA

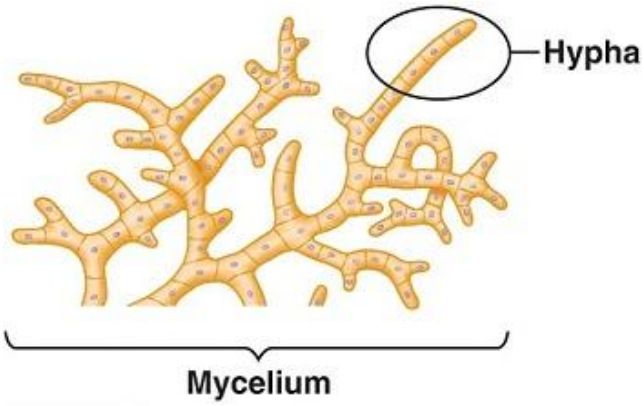


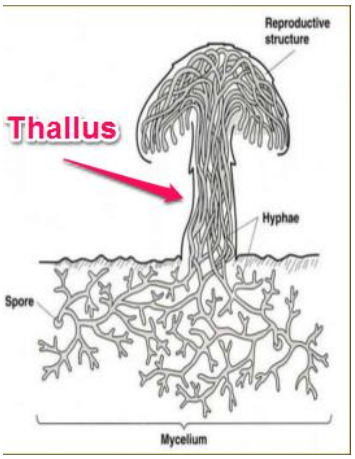
accumulates that one can visualize on an agarose gel easily.

Lesson 56. Read pages 330-331.

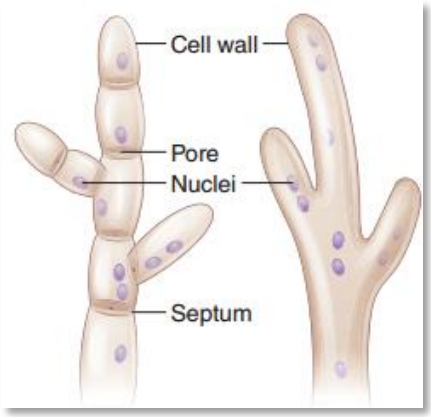
LESSON 56. WHAT ARE FUNGI

- Fungi are eukaryotic multicellular organisms. Some cause diseases in humans and animals; others are beneficial for us (we eat mushrooms and we use yeasts for bread making) and for the environment as they decay dead plants and organic matter. Yeasts are unicellular eukaryotes. Some pathogenic fungi are dimorphic. It means that they could be found as molds or filaments at room temperatures; however, they can assume single cell morphology (yeast) when incubated at 37°C.
- **Characteristics of Fungi**
- Please note that yeasts could be diagnosed by biochemical testing much like bacteria; however, filamentous fungi are identified by morphology under the microscope, colony characteristics and the color of the spores.



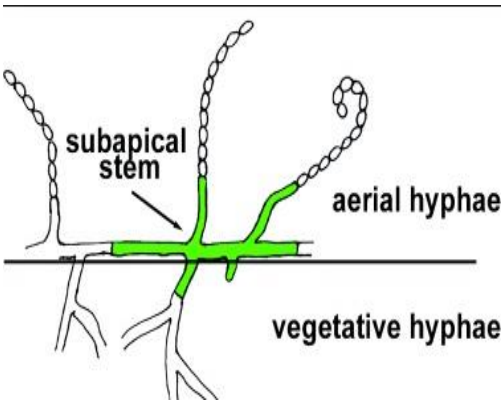


- **Thallus:** The mass of hyphae constituting the thallus of a fungus is called the mycelium. Also, the entire vegetative structure or body of a fungus, lichen, or alga is called a thallus.
- Fungi do not have chlorophyll, and their cell wall contains chitin.
- Thread-like filaments of fungi are called hyphae (singular is hypha). A collection of hyphae constitutes mycelium (plural is mycilia). Please see the above figure for these structures.



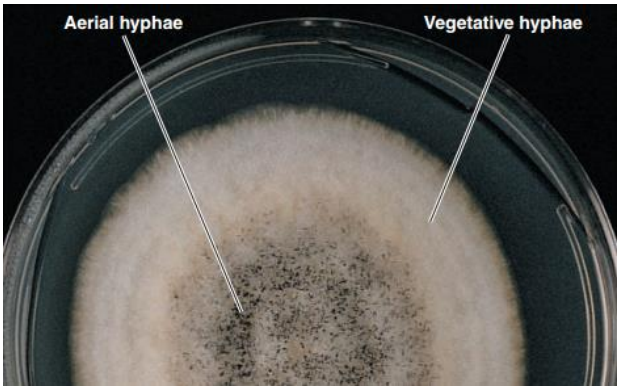
- Hyphae come in two types: Septate and non-septate (coenocytic). Septate hypha has partitioning which separates cells from each other while coenocytic looks like a long tube with no partitioning anywhere. A spore germinate into a hyphal tip which elongate to a hypha. Interestingly, a hypha can grow from a fragment of another hypha as well. In the figure below, septate hypa is on the left.

- Vegetative Hyphae: Hyphae that are embedded in the medium and are used to obtain food are called vegetative hyphae.



- Aerial or Reproductive Hyphae: The portion of hyphae that is concerned with reproduction. This portion is projected into the air. They bear reproductive spores.

- Hyphae look like a cottony growth on the medium while yeast growth looks like a powder as shown in the figures to the right:

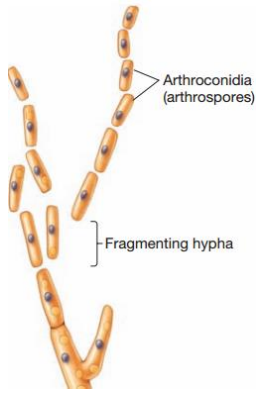


Lesson 57. Read pages 332-333.

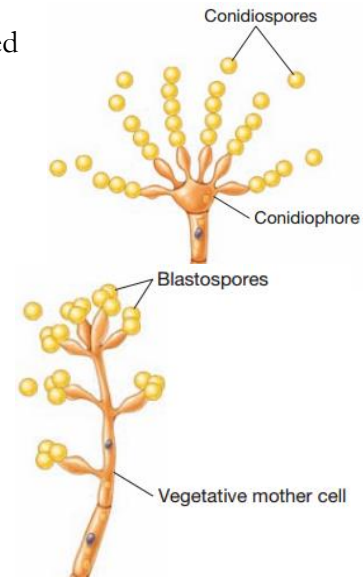
LESSON 57. ASEXUAL REPRODUCTION OF FUNGI

- **Life Cycle of Filamentous Fungi**
  - Filamentous fungi can reproduce asexually by fragmentation of their hyphae. This fragmentation takes various names according to the species of the fungus (we will see them in the bullet below). Spores can also be produced sexually by these fungi; however, this happens only when food is scarce and environmental conditions are not favorable for further growth of filamentous fungi.
  - **Types of Asexual Spores**

1. **Arthroconidia (Arthrospores):** Formed when hyphae fragment through splitting of the cell wall or the septum as shown in the figure:

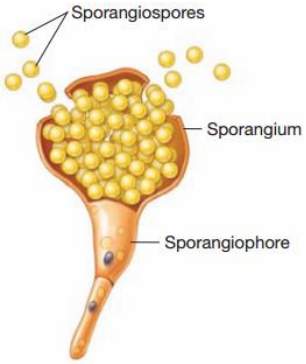


2. **Conidiospores:** This type of spore is not enclosed in a sac. It is produced in a chain at the end of a conidiophore (hypha that bears conidiospores). Aspergillus produces such spores.



3. **Blastoconidia or Blastospores:** Produced at the tip of hypha by budding. Candida albicans produces such spores asexually.

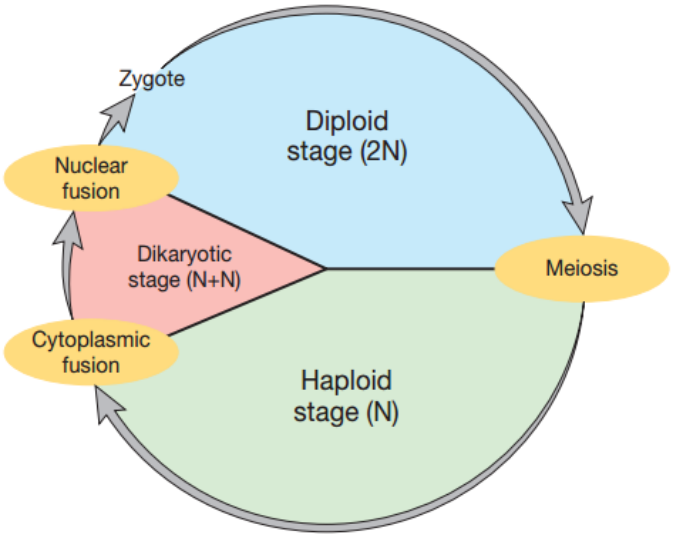
4. **Sporangiospores:** Develop in a sac at the tip of the hypha. See the figure for the sac and the spores. Rhizopus makes spores in a sac.



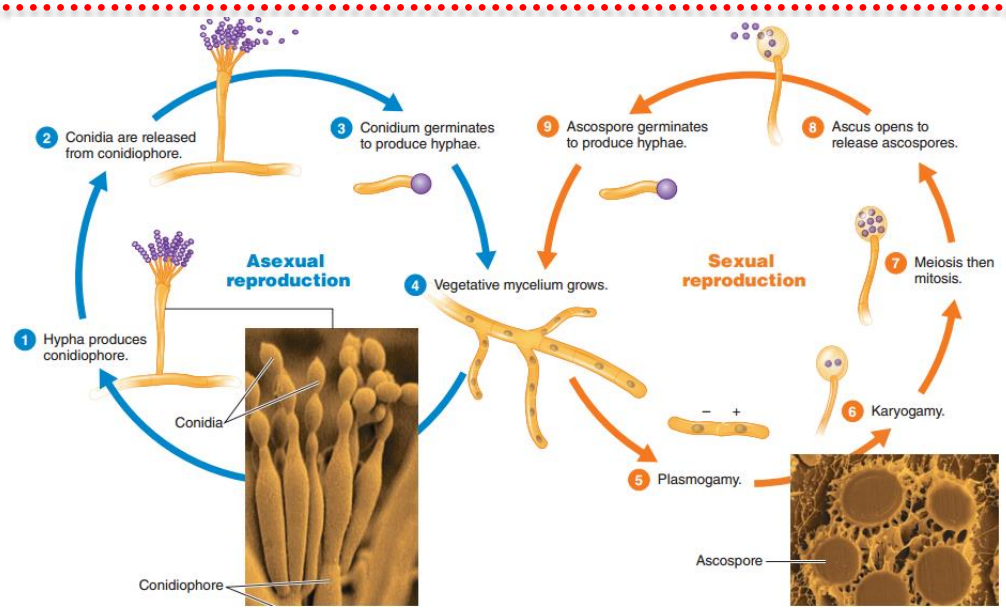
Lesson 58. Read pages 333-335.

LESSON 58. SEXUAL REPRODUCTION IN FUNGI

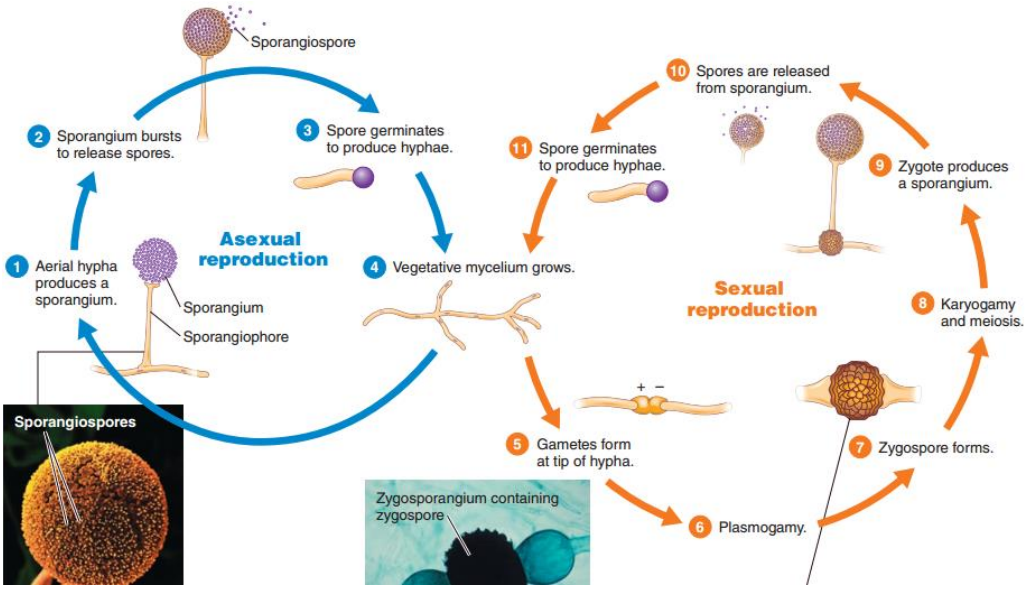
- **Sexual Reproduction of Spores in Filamentous Fungi**
- Sexual spores are produced in three phases:
    1. **Plasmogamy:** A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-). Both cells are haploid (one set of chromosomes)
    2. **Karyogamy:** The (+) and (-) nuclei fuse to form a diploid zygote nucleus.
    3. **Meiosis:** The diploid nucleus gives rise to haploid nuclei (sexual spores).



- Fungi could be classified into four phyla based on how they produce their sexual spores. Please note, in the lab, nutrition is abundant and most fungi only make asexual spores. So, clinical identification of fungi is based on microscopic examination of asexual spores (their size, shape, arrangements, if they are enclosed in a sac or not etc.).
    1. **Phylum Ascomycota:** These fungi have septate hyphae and both sexual and asexual spores are formed. Asexual spores are mainly conidia; however, arthrospores and chlamydospores can also be formed. Sexual spores are formed in a sac called ascus, hence are called ascospores. *Aspergillus flavus, Saccharomyces cerevisiae,* and *Candida albicans* are examples.

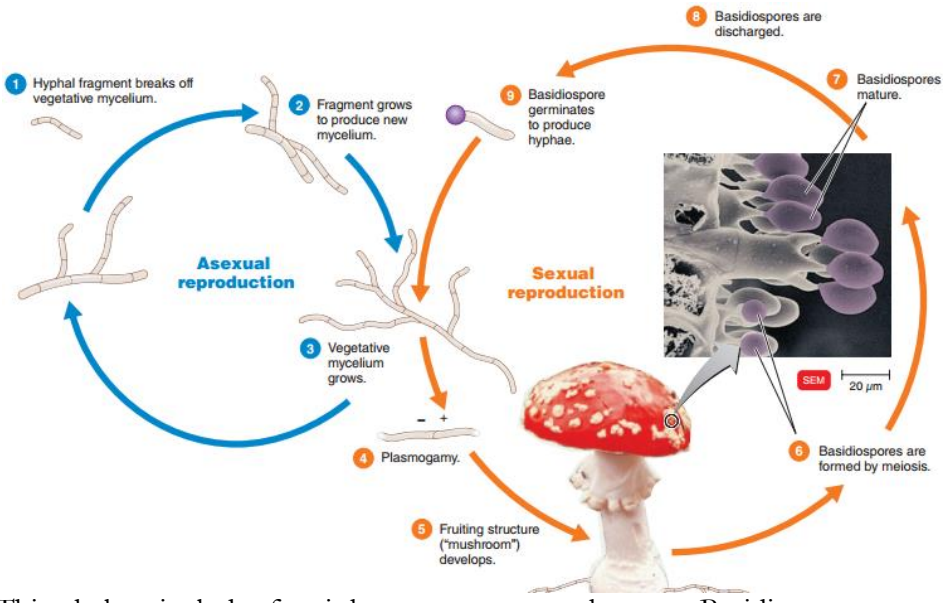


2. **Phylum Zygomycota:** These are non-septate fungi. Asexual spores are called



sporangiospores and they are formed in a sac called sporangium. Sexual spores are called zygospores. Common black bread mold, *Rhizopus stolonifer* is an example.

3. **Phylum Basidiomycota:** These fungi have septate hypyae. Sexual spores are called basidiospores while asexual spores are known as conidia or arthrospores.



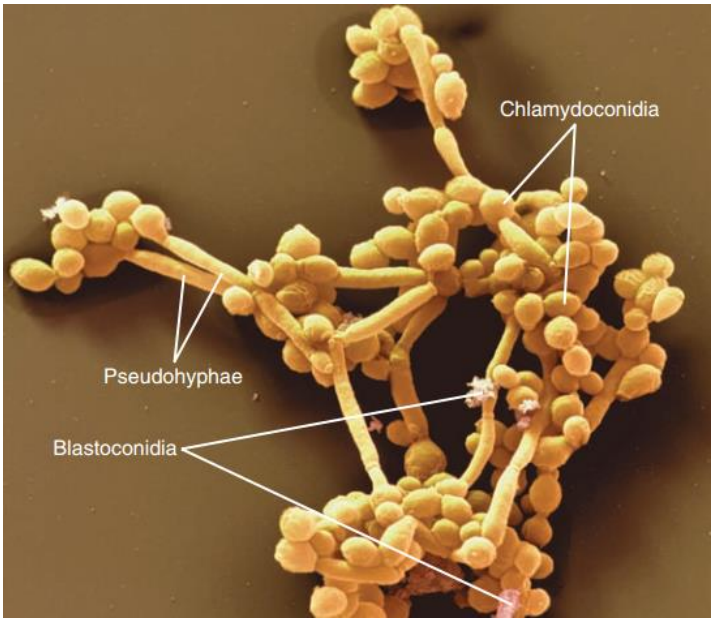
This phylum includes fungi that are seen as mushrooms. Basidiospores are formed on a club shaped base called basidium. Each basidium bears usually fours basidiospores. Cryptococcus and Malassezia are examples of pathogenic basidiomycetes.

4. **Phylum Deuteromycota (Fungi Imperfecti):** These are septate hyphae but a clear sexual spore stage is not present. Asexual spores include conidia, arthrospores, blastospores and chlamydospores. It may also be noted please that fungi that produce both sexual and asexual spores are called teleomophs; while those that only produce asexually are called anamorphs. Penicillium is an anamorph.

Lesson 59. Read pages 332.

LESSON 59. WHAT ARE YEASTS

- Yeasts divide or multiply by budding which leaves uneven cells. If cells remain attached with each other after budding, it may form a short chain of cells called pseudohypa. Candida albicans needs these pseudohyphae to penetrate deeply into tissue in human infection. See the figure given below for pseudohyphae:



- Some yeasts divide by fission which is even division compared with budding yeast which is a kind of uneven division.

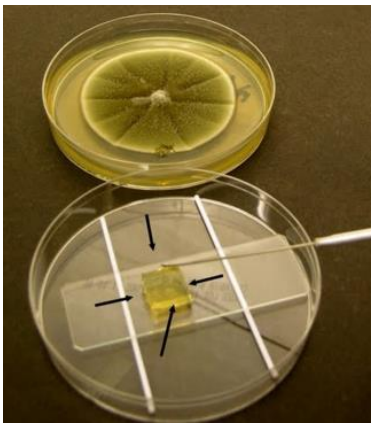
- Yeast if grown on solid medium grows like bacteria. In other words, colonies of yeast look like bacterial colonies but are bigger in size.

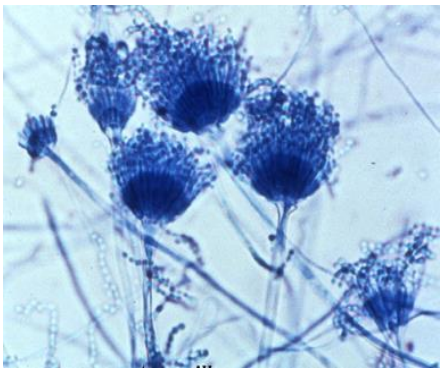
- Yeasts can grow as facultative anaerobes. Saccharomyces if given oxygen to grow, it converts carbohydrates aerobically and produces CO_2 and water; however, if oxygen is denied, carbohydrates get converted fermentatively into ethanol and CO_2.

Lesson 60. Read pages 332-333.

LESSON 60. IDENTIFICATION OF SELECTED FUNGI

- **Growth and Identification of Selected Filamentous Fungi**
- Filamentous fungi are mainly identified by morphology, and not by biochemical testing. Fungi are cultured using a slide (slide culture) on top of Sabouraud's agar. Spores are placed on top of the medium or embedded in the medium. A block of the medium can be used to grow fungi or a glass coverslip can be used to grow spores into hyphae and mycelium and spore arrangements can be studied under the microscope by staining with lactophenol cotton blue. Slides stained with lactophenol cotton blue can also be seen under the microscope. See the figure to understand how fungus is grown in a petri dish on top of a slide. Stained slides can show specific arrangements of spores, their sizes and other morphological features as seen in the figure for Aspergillus.





Lesson 61. Read pages 335-339.

LESSON 61. BENEFITS AND DISEASES OF FUNGI

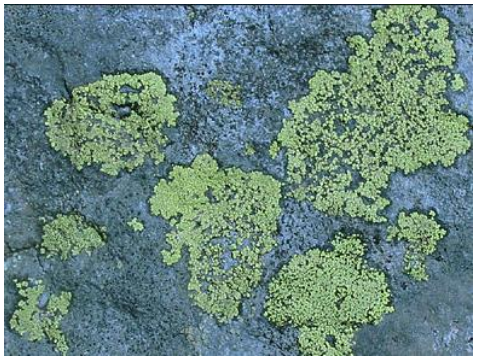
- **Economic Benefits of Fungi**
  - Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentatively). However, if Saccharomyces are incubated aerobically (for bread making), they metabolize glucose to produce CO_2 (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise. This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from *Aspergillus niger*; cellulase from Trichoderma, antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products. Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.
- **Examples of Pathogenic Fungi**
  - Fungal diseases are called mycoses. Mycotoxicosis on the other hand is ingestion of fungal toxins made in the food or feed that humans or animals consume. Fungal diseases mostly are superficial (cutaneous); however, some fungi can cause systemic sickness as well, especially in immunocompromised individuals. Histoplasmosis and coccidioidomycosis are examples of deep systemic fungal infections that can occur both in humans and dogs and cats. Sporotrichosis in gardeners and farmers**,** and ringworm infection (cuased by Trichophytons and Microsporum species that use keratinase to degrade keratin in nails, hair and skin) are examples of cutaneous or superficial fungal infections. Ringworm is shown in the figure to the right:

Lesson 62. Read pages 339-340.

LESSON 62. LICHENS AND THEIR USES

- **What are Lichens and how useful they are to us?**
- Lichens are a combination of green alga (or a cyanobacterium) and a fungus. Green alga or cyanobacterium is also called a phycobiont and the fungus is called a mycobiont in this relationship. Both partners have a symbiotic relationship with each other. If separated apart, none can exist for long suggesting that both are benefited from each other. Lichens are the only microorganism that can grow where no other microbes can grow. This is the reason that you will see lichens growing on bare rocks, tree trunks and house roofs and bare soils. They typically grow extremely slowly (only 1 mm to 3 cm per year). Lichens are classified on the basis of the fungus. There are three types of lichens in general that are based on the physical appearance:

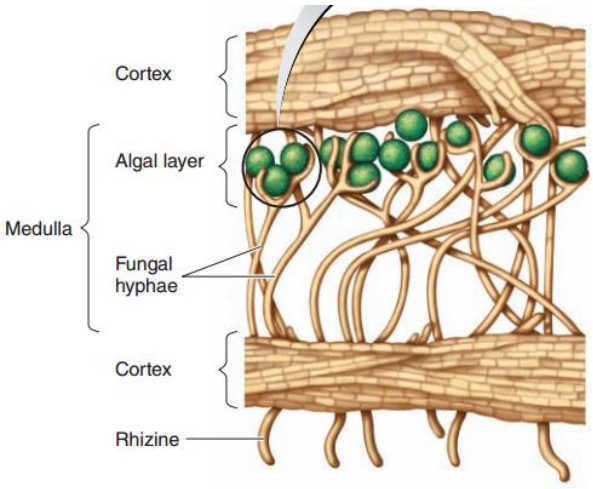
1. **Crusotse:** These lichens form a thin crust on the surfaces of objects on which they grow (see the figure).



2. **Foliose:** These look like a leaf or they are leaf-like growths.

3. **Fruticose:** These are coral-like shrubby or bushy growths.

- **Lichen Thallus or Body**
- Lichen thallus consists of 3 parts:
  - **Medulla:** It is mostly the middle portion. Microscopically, it consists of fungal hyphae and algal cells or cyanobacteria. In other words, fungal hyphae grow around algal cells in a tangled mass.
  - **Cortex:** This may divide into two layers: upper and lower layer (see the accompanying figure for details).
  - **Rhizine:** These are roots or holdfasts through with fungal hyphae obtain nutrients and moisture.



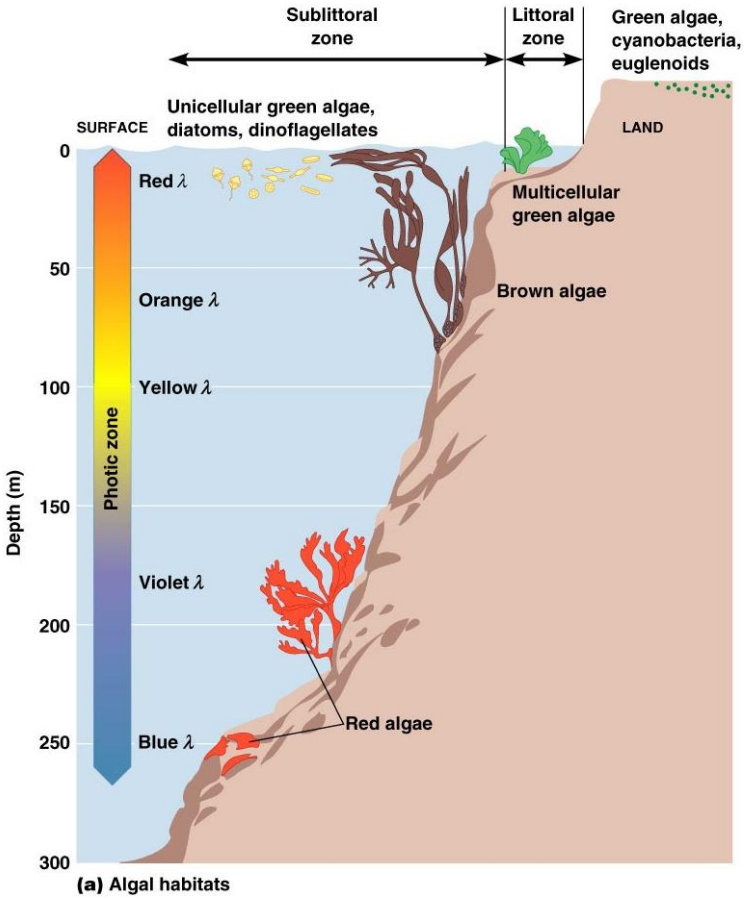
- **Economic Importance of Lichens**
  - Various dyes for clothes are obtained from lichens.
  - Usnic acid is obtained from a lichen and used as antiseptic in China.

- Erythrolitmin: for Litmus paper
- Lichens can cause allergic contact dermatitis.
- Lichens are the major food for tundra herbivores.
- They can be used as indicator of environmental pollution.

Lesson 63. Read pages 339-342.

LESSON 63. ALGAE AND THEIR BENEFITS

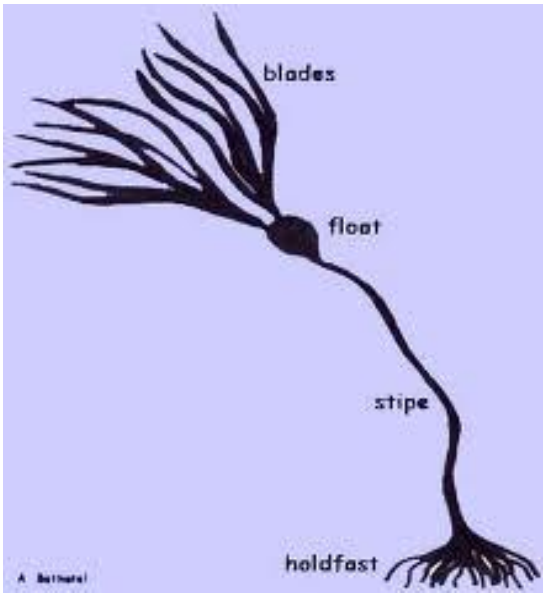
- **Algae**
  - These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O_2 on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such algae include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located



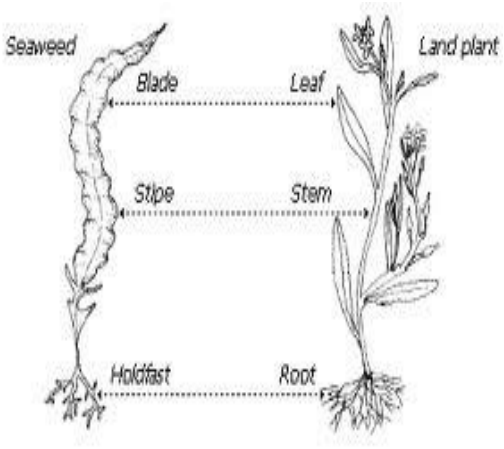
far from the surface and can use blue light from the sun as blue light is of shorter

wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic.

- Body of multicellular alga such as seaweed is called a thallus which consists of branched holdfasts (anchor alga to rock) stemlike hollow stipes and leaflike blades. There is no vascular tissue in these algae. Also, the stipe is not lignified or woody, so it does not provide support to the weed. Surrounding water provides the support for the thallus. Some algae have a gas filled body inside them which keeps them floating in the water. This gas filled structure is called pneumatocyst or float.



- Here is a little comparison between a true plant and a multicellular seaweed (see in the figure to the right).



- **Reproduction in Algae**
- Although multicellular algae look very much like plants, they do not produce seeds as plants do. Instead, there are three ways that these and other algae can reproduce themselves:

1. **Vegetative Reproduction:** Any part of the thallus or the body of algae can develop into a new individual. In fact, it is the most common method of reproduction in algae. Vegetative reproduction can further be divided into:
    a. **Cell division or fission:** The unicellular forms commonly reproduce by binary fission. Diatom is an example.
    b. **Fragmentation:** This commonly happens in multicellular algae in which a thallus breaks into many fragments and each fragment can develop into another thallus or individual.
    c. **Budding:** Bud-like structures are formed that develop into another individual.
2. **Asexual Reproduction:** In this method, spores are formed without any sexual mating.
    a. **Zoospores:** These are motile naked spores provided with flagella.
    b. **Aplanospores:** These are non-motile spores.
    c. **Exospores and endospores:** There are many other but we will not cover them all in this course.
3. **Sexual Reproduction:** gametes to form zygote in sexual reproduction. There are five ways, these gametes can be produced in, but we will not cover them in this course.

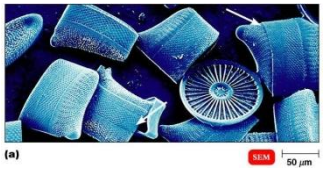
Lesson 64. Read pages 342-345.

LESSON 64. TYPES OF ALGAE AND THEIR USES

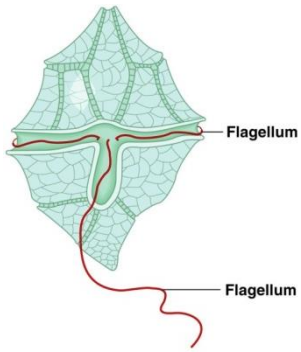
- **Types of Seaweeds**
  - **Phaeophyta (Kelp):** Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll *a* and *c*, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.
  - **Rhodophyta:** Red Alage, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).

  - **Chlorophyta:** Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.
  - **Bacillariophyta:** Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels.
    - Diatoms: Come in beautiful colors and shapes.

  - **Dinoflagellata:** These are unicellular algae collectively called as plankton (free floating



organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll *a* and *c*, and carotene. These algae store starch in them. Dinoflagellates in the genus Alexandrium produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of Alexndrium gives ocean a deep red color commonly known as red tide. See the diagram of a dinoflegellate to the right.



- **Oomycota (Water molds):** They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores. See the cottony growth on the fish in the accompanying figure to the right for a water mold.

- Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s.
- *P. ramorum* causes sudden oak death.

- **Role of Algae in Nature**
  - Algae are important for aquatic life. They are at the bottom of the food chain because they fix CO_2 into organic molecules (Carbs) which could be used by other organisms.
  - They produce 80% of molecular oxygen on earth.
  - Algal blooms which result from fluctuation of temperature sometimes may cause problems for nature but that happens occasionally.
  - Algal blooms tell us about the status of pollution in the environment.

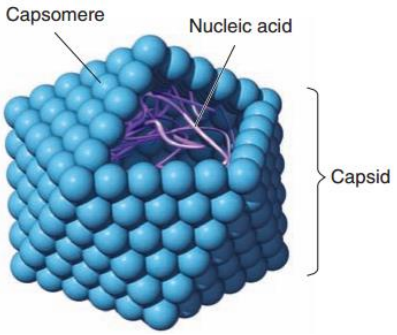
- Planktonic organisms (dead and decaying) are responsible for oil production. Petroleum and natural gas are the residues (hydrocarbons) of diatoms and other planktonic organisms.

Lesson 65. Read pages 368-374.

LESSON 65. VIRUSES AND THEIR STRUCTURES

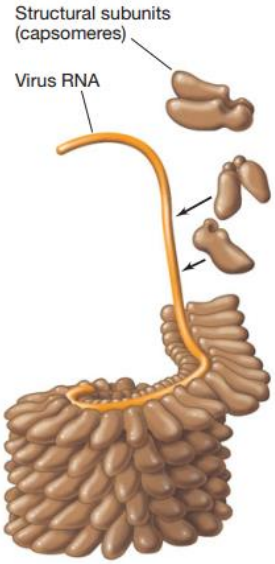
- **How were viruses discovered?**
- Viruses are too small to be seen with the light microscope and secondly, they do not grow without a cell. In 1886, Adolf Mayer, a Dutch chemist, showed that tobacco mosaic disease (TMD) can be transmitted from diseased tobacco plants to healthy tobacco plants. In 1892, Dimitri Iwanoski filtered the sap of a diseased plant to retain the causative agent in the filter but did not succeed to do that because the filtrate caused the disease in healthy tobacco plants. The virus was named as a filterable commodity, also called as a contagious fluid.
- **General Characteristics of Viruses**
- Viruses are composed of a protein capsid which encloses either DNA or RNA inside it. Capsids of some viruses possess an extra layer called an envelope. They can only multiply inside a cell. Since viruses lack any metabolic enzymes and use these enzymes belonging to the cell they infect, making of antiviral drugs is a real challenge because anything that is toxic for the virus can also kill the cell. Viruses can infect humans, animals, plants, insects, and bacteria. Bacteriophages are the viruses that infect bacteria.

- **Viral Structure**
  - A **virion** is an infectious particle composed of nucleic acid that is surrounded by a protein coat (capsid). Viruses are **classified based** on their nucleic acid and protein coat. Viruses have an extracellular and an intracellular state. Outside of a cell, in the extracellular state, a virus is called a **virion**.

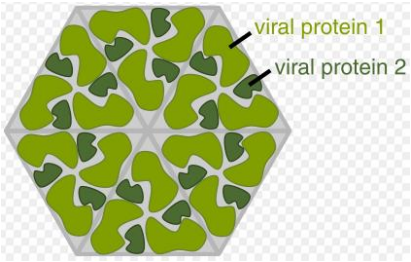


  - A virus could be a DNA virus or a RNA virus. DNA could be double stranded or single stranded. Nucleic acid molecules may be linear or circular. Single stranded DNA may be plus or sense strand. Similarly, single stranded RNA viruses may be plus strand or minus strand. RNA viruses may have their RNA as a single segment or many segments. The viruses may be enveloped or non-enveloped. Symmetry of the virus may be helical, icosahedral or complex based on the arrangement of the nucleic acid and the capsomeres that make up the capsid.

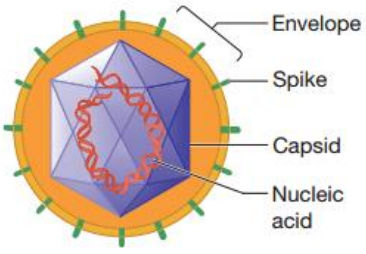
- **Nucleic Acid:** Either DNA or RNA (never both)
  - Single or double stranded
  - Circular or linear
  - One segment or multiple segments
- **Capsid:** Capsid is made up of protein subunits called capsomeres that are arranged in 3 possible ways to give a helical, or icosahedral or complex symmetry to the viruses.
  - Capsomeres: Subunit called protomers aggregate to form capsomeres.
    - See the figure for a single type of capsomeres. Capsomeres are being associated with the nucleic acid (RNA) of the virus.



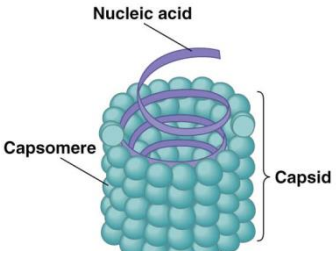
    - Multiple types of subunit proteins that make up the capsid (see the figure for the concept).



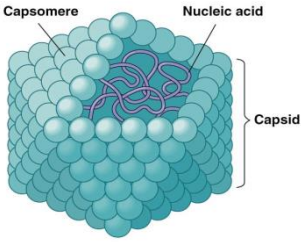
- **Envelope:** In some viruses, the capsid is covered by an envelope which is partially derived from the cell the virus buds off. The envelope is made up of lipid, protein and carbohydrates. The envelope may or may not have **spikes** (carbohydrate-protein complexes) projecting from the surface of the envelope. These spikes help the virus to attach to the host cells. These spikes can also be used for identification of the viruses.



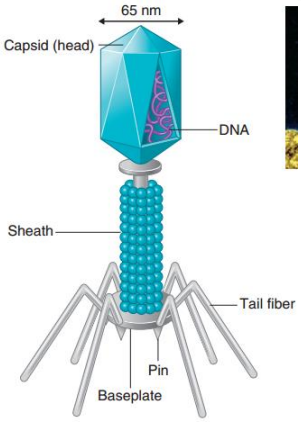
- **Capsid Architecture**
  - **Helical:** The capsid assumes a long rod shape structure which may be rigid or flexible in nature. Rabies virus is an example.



  - **Polyhedral (Icosahedron):** The capsid assumes a regular polyhedron with 20 triangular faces and 12 corners. Poliovirus is an example.



  - **Complex:** This capsid arrangement is seen in bacteriophages. A polyhedral head (that contains the nucleic acid) along with a sheath (long helical tube) that bears tail fibers make this complex capsid structure.



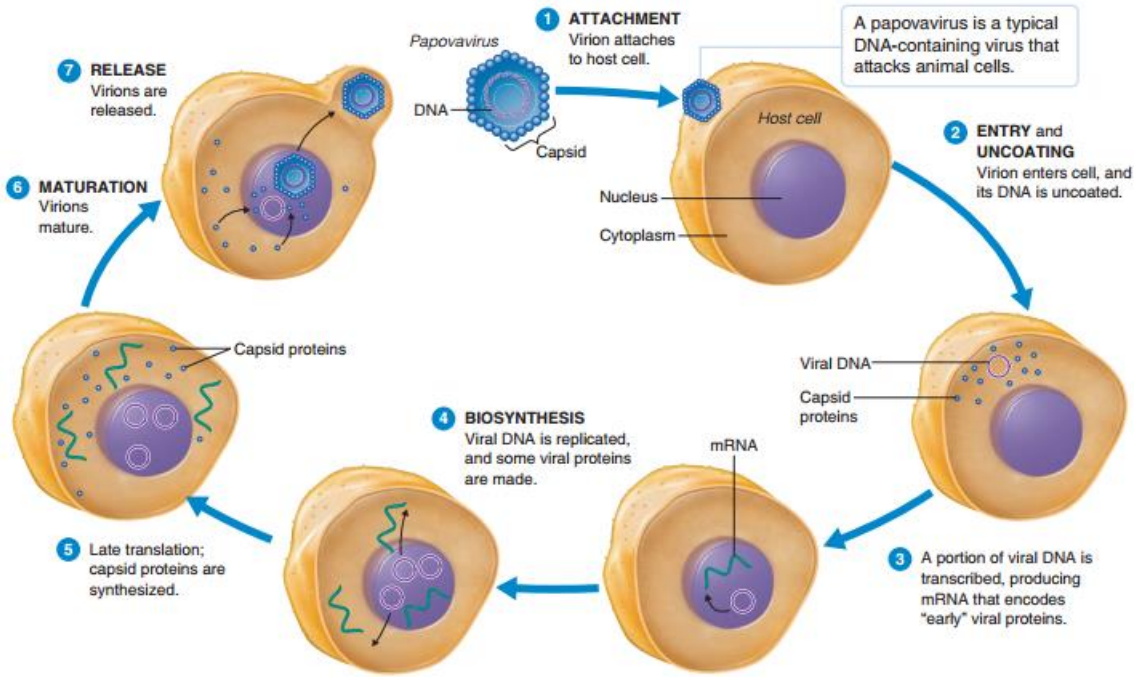
- **Viral Taxonomy**
  - Family names of viruses end in *-viridae.*
  - Genus names end in *-virus.*
    - Herpesviridae (Family)
    - *Herpesvirus (Virus)*

Lesson 66, 67. Read pages 382-389.

LESSON 66, 67. MULTIPLICATION OF ANIMAL VIRUSES

- **Multiplication of Animal Viruses**
  - Viral multiplication goes through various stages in the cell. These include viral attachment, entry into the cell by pinocytosis or fusion with the plasma membrane, uncoating of the virus, biosynthesis of capsids and nucleic acids, maturation (assembly of the virus) and final release of the virus from the cell.
  - **Attachment of the Virus:** Receptors are located on the plasma membrane of animal cells with which the virus makes an initial contact. These complementary sites are located on the envelope in enveloped viruses and on the capsids on naked viruses.
  - **Entry into the Cells:** Many viruses enter into the cells by receptor-mediated endocytosis. Fusion is another process of entry seen in enveloped viruses in which the viral envelope fuses with the plasma membrane of the cells ultimately releasing the encapsidated virus into the cells.
  - **Uncoating of the Virus:** Uncoating is basically removal of the viral capsid and releasing the nucleic acid into the cell. More or less, host cellular enzymes break the capsid freeing the viral nucleic acids.

- **Biosynthesis of DNA Viruses:** Generally, DNA-containing viruses replicate their DNA in the nucleus of the host cell by using viral enzymes, and they synthesize their capsid and other proteins in the cytoplasm by using host cell enzymes. Then the proteins migrate into the nucleus and are joined with the newly synthesized DNA to

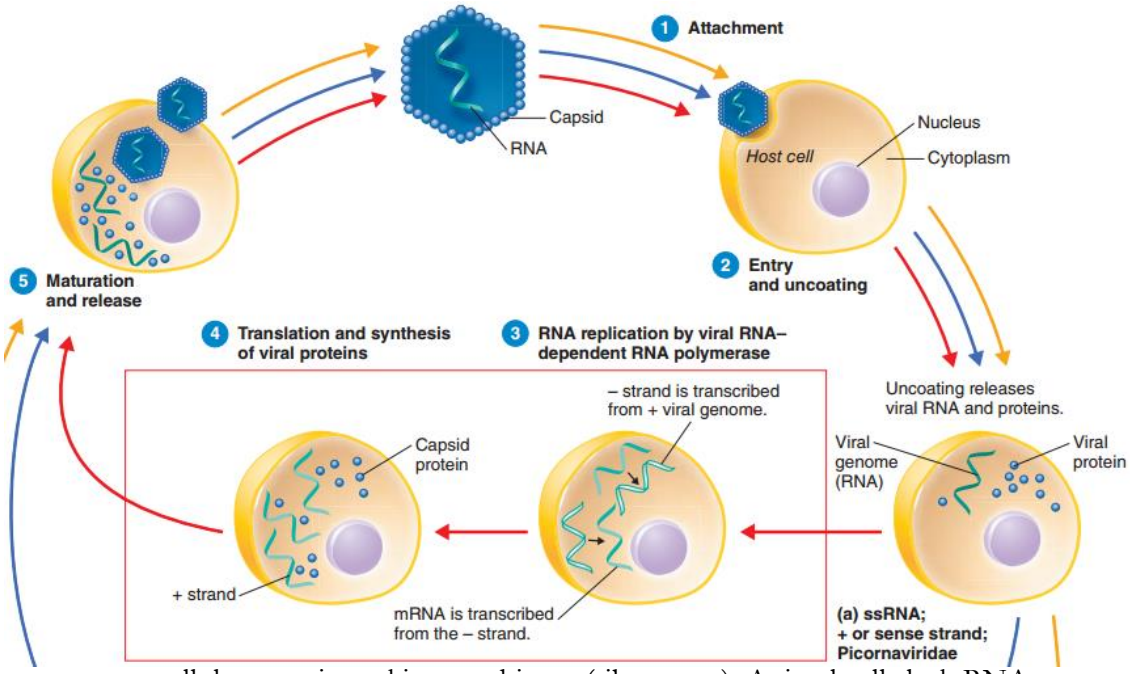


form virions. These virions are transported along the endoplasmic reticulum to the host cell's membrane for release. Herpesviruses, papovaviruses, adenoviruses, and hepadnaviruses all follow this pattern of biosynthesis. Poxviruses are an exception because all of their components are synthesized in the cytoplasm. Replication of a DNA-containing animal virus is shown in the figure above.

- **Biosynthesis of RNA Viruses:** RNA viruses multiply in the host cell's cytoplasm. The major differences among the multiplication processes of these viruses lie in how mRNA and viral RNA are produced. Single stranded RNA viruses are classified as positive or negative depending on the sense or polarity of the RNA. The negative viral RNA is complementary to the mRNA and must be converted to a positive RNA by RNA polymerase before translation. Therefore, the purified RNA of a negative sense virus is not infectious by itself, as it needs to be converted to a positive sense RNA for replication. Also, it is important to understand that minus

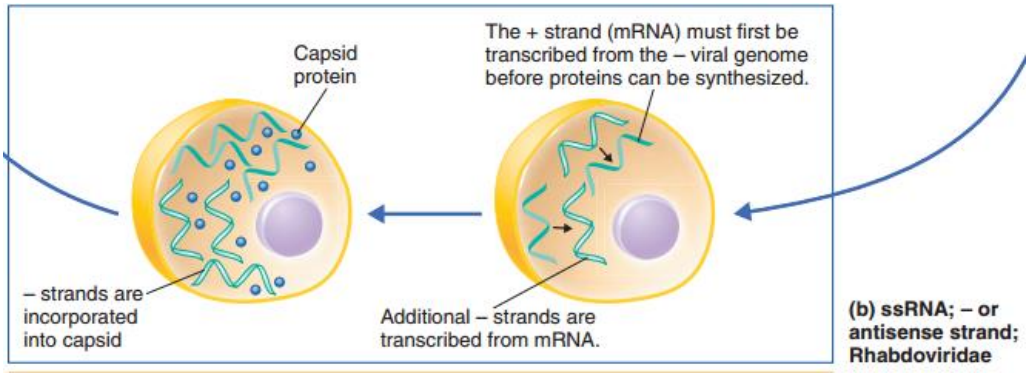
sense ssRNA viruses will bring RNA dependent RNA polymerase with them in their capsids as the host cell does not have such a RNA polymerase to convert antisense RNA to plus strand which can be translated into proteins for making more RNA polymerase to make more copies of plus strands. The plus strand RNA also acts as a template to make minus strands. Let's briefly talk about each RNA type viruses with respect to their biosynthesis.

- **Biosynthesis of a plus strand RNA Virus:** A plus or sense RNA is actually an mRNA because it can be translated directly into a protein molecule by the



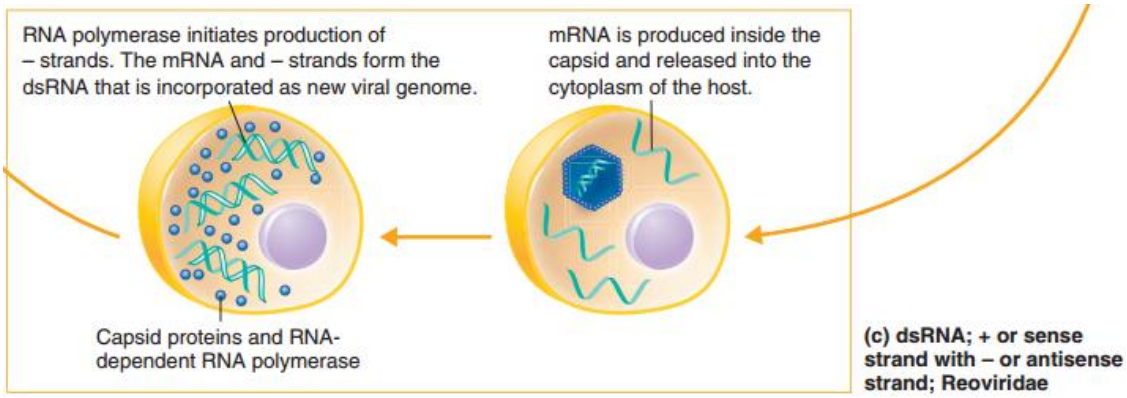
cellular protein making machinery (ribosomes). Animal cells lack RNA dependent RNA polymerase, so RNA viruses bring these genes (on their sense strand) with them and dictate the cellular machinery to make RNA dependent RNA polymerase which will use the plus RNA strands as templates and make many complementary copies (minus strand RNA), which are then used as a template to make plus strand RNA copies. These plus strand RNAs are then packed into the capsid making the virion before they are released from the cells. Please read the text with the figure above to see how it relates to the viral replication in plus strand RNA viruses.

- **Biosynthesis of Minus (antisense) Strand RNA Viruses:** Such viruses are single stranded RNA viruses. As the cells do not have RNA dependent RNA polymerase to convert this RNA into a plus



strand (mRNA), these types of viruses bring this enzyme with them enclosed in their capsids. However, once mRNA (plus strand) is made, this can act as mRNA for more RNA polymerase and also as a template for making more minus stands that will be packed in the capsids for making infectious virions. See the figure for the concept.

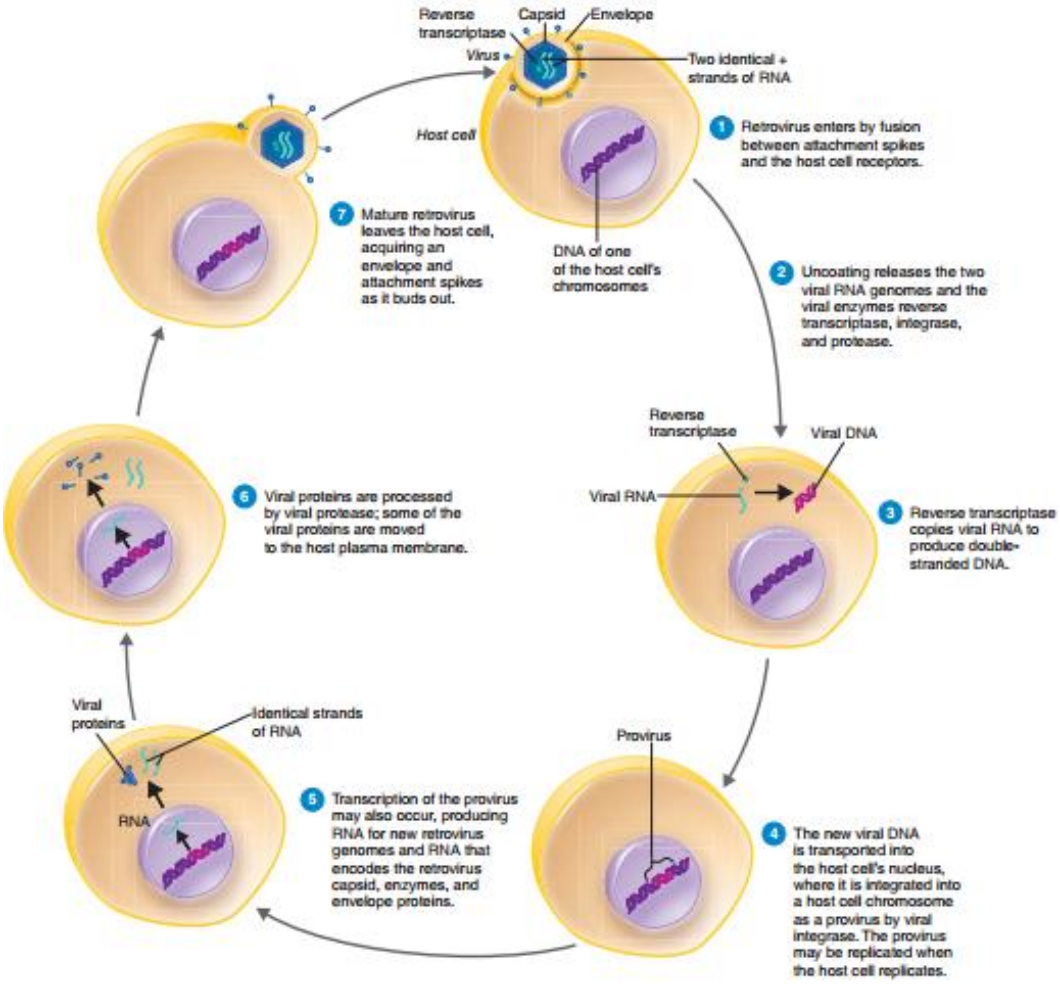
- **Biosynthesis of dsRNA Viruses:** These viruses are very special when it comes to their replication in the cells. Since there is no



double stranded RNA normally present in the cells, the environment of the host is not very conducive for such viruses. Therefore in order to protect its dsRNA from being degraded, these viruses replicate their mRNA while they are still inside the capsid and release those mRNA (plus strands only) into the cytoplasm of infected cells. This plus stand acts as an mRNA as well as a template for minus strands;

both of these strands are then encapsidated as the genome of new virions.

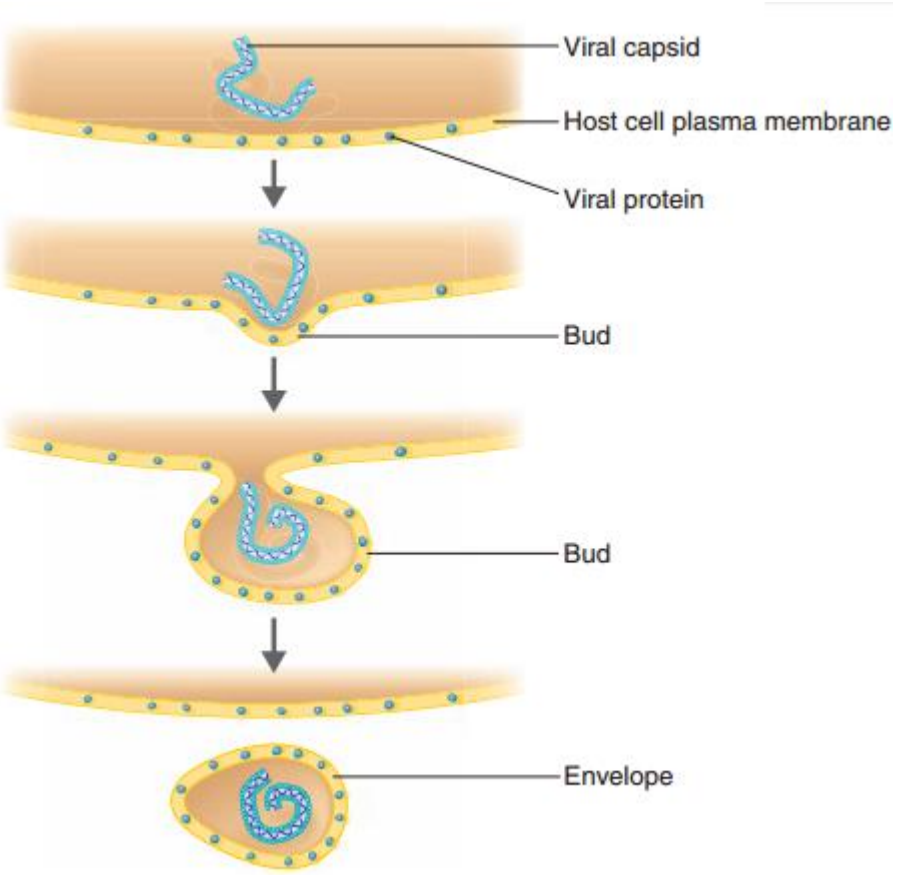
- **Biosynthesis of Retroviruses:** These viruses are basically ssRNA viruses with two identical copies of plus strand only. However, they also have reverse transcriptase in their capsids which converts plus



strands into double stranded DNA molecules. These dsDNA are then are integrated into host genome as proviruses. While still remaining part of host chromosome, mRNAs are generated from the provirus which can make capsids and other proteases along with reverse transcriptase. New virions are assembled with two new copies of plus strands along with reverse transcriptase molecules in the capsid. The newly assembled virions are then released as enveloped

viruses because they take part of the plasma membrane with them. See the accompanying figure for visual impression of retroviruses.

- Maturation and Release: Capsomeres assemble into capsids packed with the nucleic acids ready to be released out of the cell. The viruses that are naked are released by

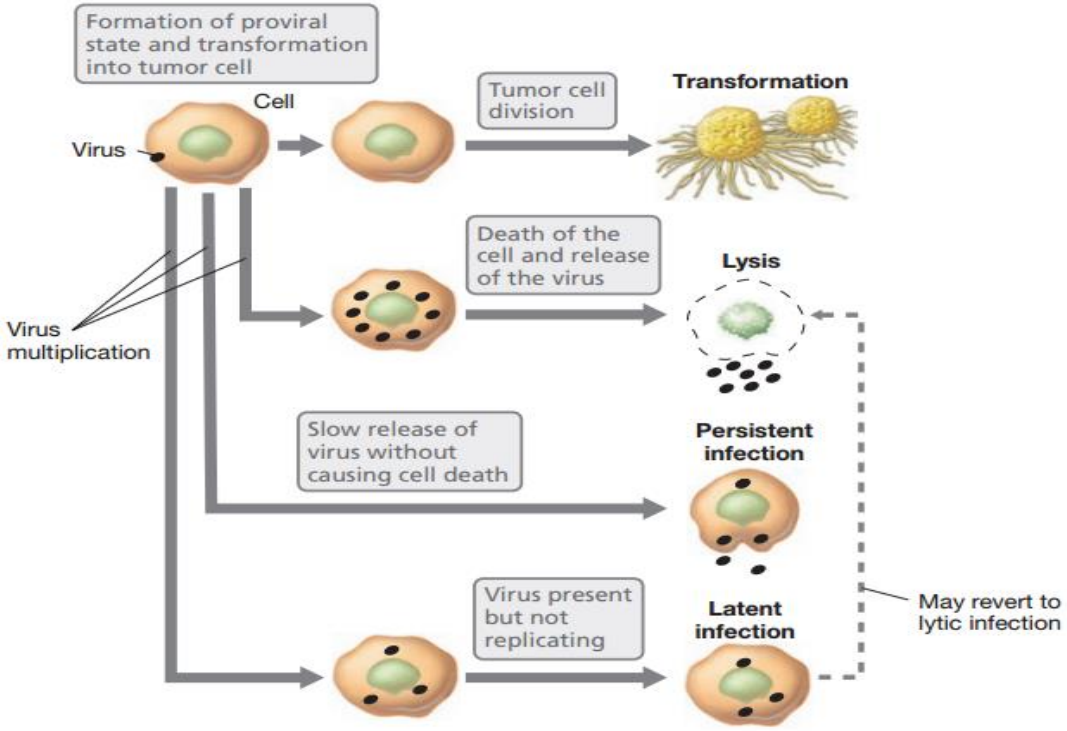


bursting the cells; however, those viruses that are enveloped bud off by sticking to the underside of plasma membrane which encloses the capsid before it is released off the cell. In this type of release, the cell does not get killed or lysed.

Lesson 68. Read pages 389-342.

LESSON 68. CONSEQUENCES OF VIRAL INFECTIONS

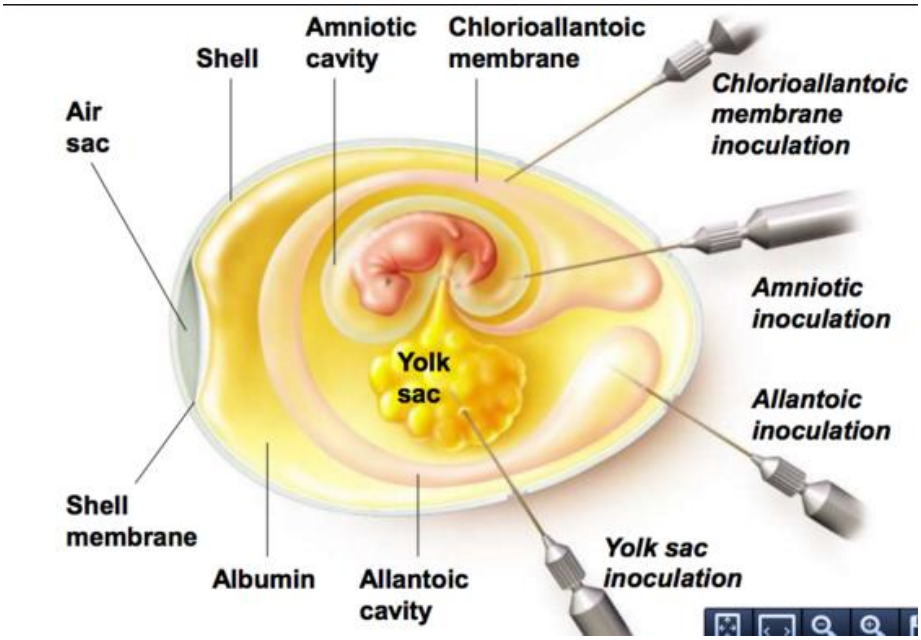
- **Consequences of Viral Infections**
- When virus invades body cells, it brings about microscopic and macroscopic changes called as cytopathic effects (CPE). Some viral infections result in lysis of cells (especially with the naked viruses). Such infections are called virulent infections. Other viruses do not replicate inside the cells and remain latent forever or for sometimes. These are called latent infections. Sometimes, latent viruses keep replicating inside the cells albeit at a slower rate that does not kill the cell. However, such individuals are carriers of the virus and may spread to other people. Some viruses are such that these transform host cells to cancerous cells.



Lesson 69. Read pages 372-378.

LESSON 69. CULTIVATION OF VIRUSES AND THEIR ENUMERATION

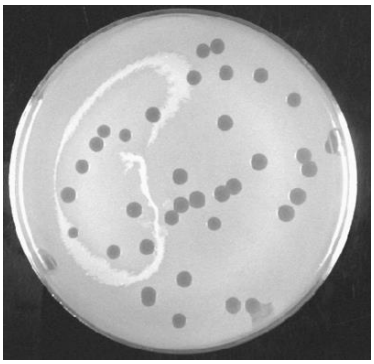
- **Cultivation of Viruses**
- Viruses are obligate intracellular parasites. In other words, virus will only replicate inside a



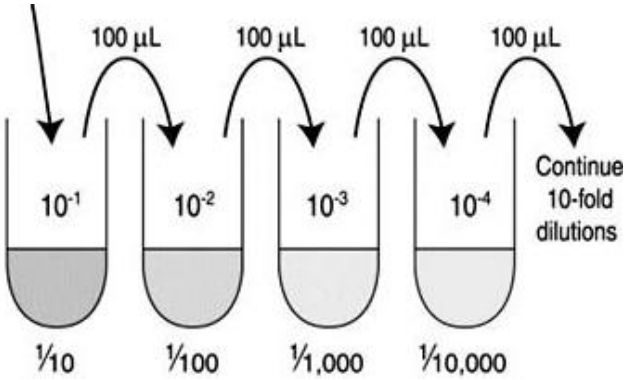
living cell. Hence, viruses can be grown in animals (mice, rabbits etc), embryonated eggs (hen, duck), and cell cultures of animal and plant origin. Various cell lines are available commercially for this purpose.

- **Virus Enumeration Methods**
  - Although the viruses do not grow in a nutrient plate as bacteria do, however, they can be counted directly or indirectly. Direct methods include electron microscopy (although not very commonly used). Indirect methods are the ones that are more common and they include plaque counting, focal CPE counting in cell cultures that are overlaid with semisolid agar, calculation of infectious dose 50% and or lethal dose 50%. In most of these methods, cells are lysed making visible lesions that could be counted individually and each such lesion represents one virus.
  - **Plaque Formation and Counting:** Plaques are equivalent to bacterial colonies. Basically a plaque is an area where the virus infection has lysed the cells. These

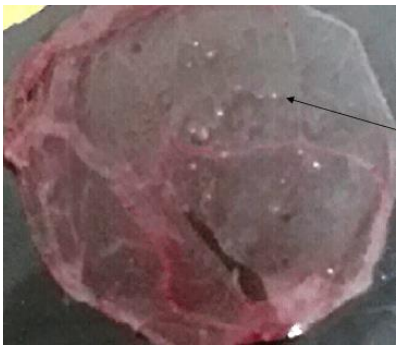
plaques are formed on cultured cells in a culture dish. A monolayer of the cells is infected with the virus to be enumerated and then the culture is overlaid with the semisolid agar so that infected and lysed cells do not move away from the site in the dish. Plaques can be counted easily much like bacterial colonies.



- **Serial Dilution Method:** In this method, a virus suspension is diluted 10-fold serially and a portion from each dilution is inoculated onto a cell culture that has been added into a plate that contains a series of small wells. CPE is noted in microtiter plate wells and the highest dilution giving CPE is recorded. This provides a numerical count of the virus in terms of dilution or concentration in the original sample that produce CPE. Please remember that these counts are based on CPEs and CPE is assumed to be caused by a single virion which replicated in a cell and then spread to adjacent cells. A number of virions will be needed to cause a visible CPE lesion in the cell culture.



- **Plaque Assay for Enumeration of Virus Particles:** In this assay, chorioallantoic membrane is inoculated with a dilution of the virus and lesions that are produced focally (called pock lesions) are counted which represent virus particles. Each white spot in the figure is a pock lesion and represents one virus.



- **Haemagglutination (HA) Units**: Some viruses have the property of agglutinating RBCs. Virus dilutions are made and incubated with RBCs. The highest dilution giving a complete agglutination is called one HA unit. This is also a way to know the quantity of the virus.

- **Infectious Dose 50% Endpoint:** This is another way of determining the number of viral particles needed for causing infection in a population. Serial dilutions of a virus are injected into a susceptible host and 50% end point is calculated statistically.
- **Lethal Dose 50% Endpoint.** Here is another method that determines the number of viruses needed to kill 50% of susceptible population.

Lesson 70. Read pages 392-395.

LESSON 70. VIROIDS, VIRUSOIDS AND PRIONS

- **Viroids**
  - These are infectious agents that consist only of RNA and found in plants only. No capsids, no envelopes, just RNA. They are covalently closed circular ssRNAs about 250 nucleotides long. The circular RNA normally exists as a rodlike shape due to intrastrand base pairing, which forms double-stranded regions with single-stranded loops. They do not encode any gene products, so they are replicated by cellular DNA dependent RNA polymerase (amazingly!). A plant infected with such viroids may not show any signs of illness. However, in other plants, they may cause severe disease. They cause the disease by RNA silencing process.

- **Virusoids**
  - They are similar to viroids; however, they encode one or two gene products and need a helper virus to infect the host cells. The helper virus supplies gene products and other materials needed by the virusoid for completion of its replication cycle. Hepatitis D virusoid is a typical example which uses Hepatitis B virus as a helper.
- **Prions**
  - These are proteinaceous infectious particles that cause neurodegenerative diseases such as scrapie in sheep, bovine spongiform encephlopthy, and Kuru in humans. These are abnormal form of cellular proteins. How these proteins accumulate in the cell is not clear, although, some genetic components are known to be involved.

Lesson 71. Read pages 418-420.

LESSON 71. EPIDEMIOLOGY AND ITS METHODS

- **What is Epidemiology?**
  - The study of where and when diseases occur and how they are transmitted in a population. There are three basic types of investigations relating to epidemiology:
    - **Descriptive Epidemiology:** (recording the data about disease). It includes location and time of cases of disease, gender, health, age of patients etc. (Basically, frequency and distribution of risk factors in population are recorded). Prevalence and incidence of disease are typical examples. Such studies can be retrospective (after they occur) or prospective (before they occur). So, in summary, in descriptive epidemiology, information about an already existing disease is recorded.

    - **Analytical Epidemiology:** It relates to determining the cause of the disease. Analysis of data, mode of transmission of diseases and means to prevent diseases also come in this discipline. Such studies can be done with **case control methods** in which factors that have preceded the disease are determined. A group of people who have the disease is compared with another group of people in the same location who are free of the disease. A **Cohort method** tracks two groups forward from exposure to outcome. Cohort study compares the experience of a group exposed to the factor with another group not exposed to the factor. An association between exposure and its outcome is determined which makes it easy to see the risk factor involved. In other words, cohort studies begin with a group of people (a cohort) free of disease. The people in the cohort are grouped by whether or not they are exposed to a potential cause of disease. The whole cohort is followed over time to see if the development of new cases of the disease (or other outcome) differs between the groups with and without exposure.

    - **Experimental Epidemiology**: A hypothesis relating to a disease is tested in this approach. For example, smoking cause cancer can be tested by designing

an experiment in mice in which they are exposed to certain levels of smoke over time and then the outcome is observed. Another example will be testing of a drug for prevention of a disease.

- **Case Reporting**: Case reporting is essential part of epidemiology. It provides incidence and prevalence of a disease to epidemiologist. Health care workers report specified disease to local, state, and national offices for such purposes. Using these reports, epidemiologists begin various studies of the patients. If an epidemiological study shows that a large enough segment of the population is affected by a disease, an attempt is then made to isolate and identify its causative agent. Identification is accomplished by a number of different microbiological methods. Identifying the causative agent often provides valuable information regarding the reservoir for the disease. Once the chain of transmission is discovered, it is possible to apply control measures to stop the disease from spreading.

- **Definitions Relevant to Epidemiology that We Must Know**
  - **Morbidity:** Incidence of a disease
  - **Morbidity rate:** Number of people affected in relation to the total population in a given time period
  - **Mortality:** Deaths from a disease
  - **Mortality rate:** Number of deaths from a disease in relation to the population in a given time
  - **Pathogenicity:** The ability to cause disease
  - **Virulence:** The extent of pathogenicity

Lesson 72. Read pages 400-404.

LESSON 72. VARIOUS DEFINITIONS RELATING TO DISEASES

- **Pathology**: Study of diseases is called Pathology. It includes cause of the disease, its pathogenesis (how disease establishes itself) and structural and functional changes it brings about in the body.
- **Infection:** invasion by the microbes. May not develop the disease.
- **Disease:** dysfunction in the body
- **Microbiome:** Microbial communities that live in or on humans or animals.
- **Normal microbiota or flora:** These are microbes that are permanent residence but do not cause diseases under normal conditions. Howerver, if for any reason, the host is stressed and the immune system shows some suppression, this normal flora can cause infection.
- **Transient microbiota:** present for several days, weeks or months and then disappears ultimately. One gets them from a different location and as soon as one returns to the normal environment, transient flora disappears.

- **What determines the distribution and composition of it?**
  - Nutrients: Availability of various nutrients determines the type of organisms present in a system. Similarly, physical and chemical factors also influence the type of microbiota. Other factors include pH, O2, and CO2. Host defenses, level of stress and age of an individual also matters for allowing microbiota of an individual.

- **Relationships between the Normal Microbiota and the Host**
  - Generally speaking, the relationship of microbiota with the host is beneficial for the host. For example, microbiota will antagonize the growth of a new microbe at the same place where microbiota is already there. This phenomenon is called microbial antagonism or competitive exclusion. Normal microbiota competes with the potentially pathogenic organism for the same nutrient. Also, microbiota secretes various chemical that inhibit the growth of pathogenic organisms. The importance of microbiota can be realized from the fact that when this microbiota is disrupted with antibiotics, fungal and yeast infection is very common in patients receiving antibiotics. A very good example is of E. coli secreting bacteriocins which inhibit other organisms (such as Salmonella) in the intestinal tract.
  - Interaction of microbiota with the host leads to a relationship called symbiosis. There are a few outcomes of this symbiosis which we must know. The two participants of this relationship are microbiota and the host. At least one of these participants is benefited and sometimes both can be benefited from this mutual relationship. Different names or terms have been given to these mutual relationships as under:
    - **Mutualism:** both are benefited.
    - **Parasitism:** one is harmed.
    - **Commensalism:** One is benefited, other is unharmed.

Lesson 73. Read pages 406-409.

LESSON 73. DEFINITIONS RELATING TO EPIDEMIOLOGY AND DISEASES

- **Some Definitions Relating to Occurrence of a Disease**
  - **Endemic:** When a disease is consistently present, but with low incidence, we call it an endemic disease. Those that remain infected with the disease organism act as reservoir of infection. Common cold is a typical example.
  - **Epidemic:** Within an area, unusually high incidence (many people get sick in a short period of time) of a disease is called an epidemic. Influenza is a good example.
  - **Pandemic:** When a disease assumes a global occurrence or becomes worldwide, it is called an epidemic. AIDS is a good example of such as epidemic.
  - **Incidence:** # of new cases in a given time period in a population
  - **Prevalence:** New + existing cases

- **Some Definitions Relating to the Severity or Duration of a Disease**
  - **Acute Disease:** One that develops rapidly but lasts only a short time. Influenza is a good example.
  - **Chronic Disease:** The disease develops more slowly but lasts for a long time. Tuberculosis is an example.
  - **Subacute Disease:** Intermediate between acute and chronic disease.
  - **Latent Disease:** Causative agent remains inactive for a time; however, it can get activated under stress and cause the disease. Shingles is an example.
  - **Herd Immunity:** When many individual in a community are immune, the disease does not spread so easily. This is called herd immunity.
  - **Local Infection:** If the invading organism remains confined to a limited small area in the body. An abscess or a boil is a good example.
  - **Systemic (Generlized) Infection:** When a microbe spreads throughout the body by blood or lymph is called a systemic infection. Measles is a good example.
  - **Focal Infection:** Sometimes an organisms gets into the blood or lymph, but then becomes confined to an organ or a system (instead of becoming systemic), this is called a focal infection.

- **Sepsis:** A toxic inflammatory condition arising from the spread of an organism from a focus of infection.
- **Septicemia:** A systemic infection arising from the multiplication of pathogen in the blood. Septicemia is a common example of sepsis.
- **Bacteremia:** The presence of bacteria in the blood.
- **Toxemia:** Presence of toxin in the blood. Tetanus is an example.
- **Primary infection:** caused by the initial infectious organism.
- **Secondary infection:** Caused by an opportunistic organism that invades the body which has been weakened by a primary pathogen.
- **Subclinical (Inapparent) Infection:** An infection which is not visible clinically. Poliovirus and hepatitis A viruses may be carried by individuals without showing any signs.
- **Infection:** When an organism colonizes and grows in the body, it establishes an infection.
- **Incubation period**: time between infection and diseases
- **Acute period:** height of disease when signs or symptoms are at their peaks.
- **Decline period:** Disease symptoms subside
- **Convalescent period:** patient returns to normalcy
- **Reservoir:** Sites where organisms of infections remain viable. It may be animate or inanimate objects. These reservoirs act as source of infection. They are also called as careers of the disease. Soil and water makes two nonliving reservoirs.
- **Zoonosis:** Primarily an animal infection, but can be transmitted to humans. It can assume epidemic proportions in humans and is difficult to control or eradicate.
- **Carrier:** A living carrier is a pathogen infected individual who does not show any signs or symptoms of the disease, but is a potential source of infection for others. Typical example in Salmonella is Typhoid Mary story.

- **Predisposing Factors for a Disease**
  - **Gender**: Males and females have different diseases because of their gender differences.
  - Genetics: Some diseases have a genetic link.
  - Climate: Cold winter and hot summers have different diseases.

- Nutrition: Good nutrition protects a person from getting a disease.
- Fatigue: Any stress increases the chances of infection.
- Age: old age versus young is a factor for various infections.
- Environment: A dirty environment leads to infections.
- Occupation: Many occupations have specific diseases.

- **Reservoirs of Infections**
  - **Humans:** Carriers
  - **Animals: Infected animals are carriers of infection.**
  - **Inanimate Objects:** Soil is also a source of microbes for infection.
  - **Water:** Contaminated by feces may bear Vibrio cholera and Salmonella typhi for infection.

Lesson 74. Read pages 409-414.

LESSON 74. VEHICLES OF INFECTIONS

- **How Diseases are Transmitted?**
  - **Direct Transmission:** person to person physical contact is a direct contact. Influenza spread is a typical example.
  - **Indirect Transmission:** A nonliving object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.
  - **Droplet Transmission:** Mucus droplets are created when you sneeze for example. These droplets carry

organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.
  - **Vehicle Transmission:** Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.
- **What are Vectors?**
  - A **vector** is a vehicle (animate or inanimate) that carries a pathogen from one host to another.
  - Arthropods are vectors for many diseases. There are two ways these arthropods can transmit organisms to susceptible hosts:

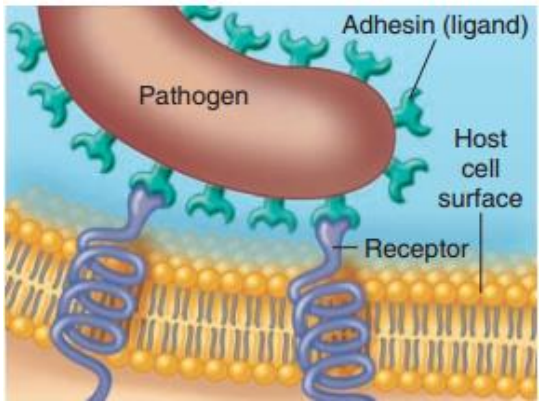
- **Mechanical Transmission**: In this type of transmission, organisms stick to the body parts of insects and get transmitted passively from one location to another.
- **Biological Transmission**: Microbes multiply in the insect and is transmitted actively by insect bites from one individual to another.

- **What are Nosocomial Infections?**
  - Hospital acquired infections are called nosocomial infections.

Lesson 75. Read pages 429-430.

LESSON 75. PORTALS OF ENTRY OF MICROBES

- **How Microorganisms Enter a Host**
  - Most pathogen needs to enter the body before they can cause disease. After entry into the host, the pathogen must be able to evade the host defense mechanisms, adhere to the cell they like to invade, and damage the cells in order to produce the disease. There are several routes by which microbes enter the body. These avenues are called portals of entry.



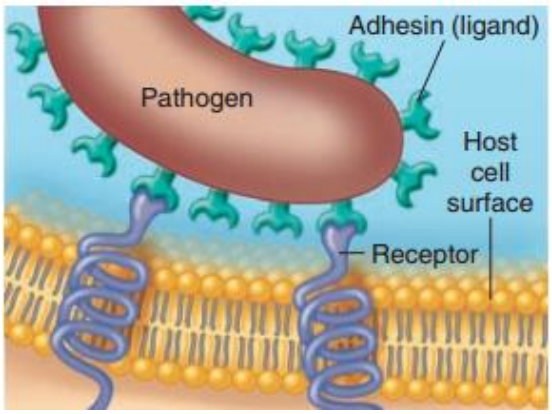
  - **Portals of Entry:**
    - Mucus membranes: These include linings of digestive tract, respiratory tract, urogenital system, and conjunctiva. Most microbes enter through the digestive and respiratory tracts. Diseases that are commonly contracted via the respiratory tract include the common cold, pneumonia, tuberculosis, influenza, and measles. Microbes in the gastrointestinal tract can cause poliomyelitis, hepatitis A, typhoid fever, amebic dysentery, giardiasis, shigellosis (bacillary dysentery), and cholera. HIV infection, genital warts, chlamydia, herpes, syphilis, and gonorrhea are urogenital infections.
    - Skin: Surface area wise, skin is the largest organ of the body. Unbroken skin is impermeable to organisms. However, hair follicles and sweat gland ducts are some of the portals for microbial invasion.
    - Parenteral Route: Injury to the skin or mucous membrane leads to easy access for the organisms in the body. Any cut received on the skin for example becomes a portal of entry. Punctures, injections, bites, cuts, wounds, surgery, and splitting of the skin or mucous membrane due to swelling or drying can all establish parenteral routes. HIV, the hepatitis viruses, and bacteria that cause tetanus and gangrene can be transmitted parenterally. Also note please that most

organisms prefer one route over the other. For example, Salmonella if swallowed will cause the disease; however, if rubbed on the skin will not cause typhoid other than a local inflammatory reaction.

- **Numbers of Invading Microbes**
  - A specific number of microbes is needed for them to cause an infection or disease. A few microbes can be eliminated by the defense mechanisms of the body easily; however, when the number increases, the chances of disease increase proportionally. **Virulence** of microbes is expressed as infectious dose 50% which also depends upon the route of entry. Different microbes have different ID50s. For example, ID_{50} for *Bacillus anthracis* is 10-50 spores if exposed through the skin; this number has to be increased to 10-20 thousands spores through inhalation; and ID_{50} is 250 to 1000 spores if *B. anthracis* has to cause the disease via GI tract.

Lesson 76. Read pages 431-433.

LESSON 76. ENTRY AND EVASION OF MICROBES IN THE BODY



- **How Microbes Adhere to the Cells**
  - **Adhesions or Ligands:** Cells have receptors with which adhesins or ligands of microbes attach. Adhesins may be located on a microbe's glycocalyx or on other microbial surface structures, such as pili, fmbriae, and flagella. These are glycoproteins or lipoproteins composition wise.
  - **Biofilms:** Biofilms are made by some microbes which help them stick to surfaces. These are extracellular substances (basically glycocalyx) released by microbes. Examples include dental plaques on teeth, algae on walls of swimming pools, and scum on shower doors.

- **How Bacterial Pathogens Penetrate Host Defenses**
- **Capsule:** The capsule impairs phagocytosis and help the organisms evade the immune system; however, antibodies can help cells of the immune system in phagocytosis of such encapsulated organisms. Only a few bacteria produce capsule which include *Strep pneumonia, Klbesiella pneumonia.*
- **Cell wall components:**
  - **M protein:** Found on cell surface and fimbriae; it also resists phagocytosis by white blood cells; example includes *Strep pyogenes.* Mycolic acid in Mycobacterium is another waxy substance which also resists phagocytosis by phagocytes.
- **Enzymes (exoenzymes):**
  - **Coagulase:** It clots blood and bacteria are trapped or hide themselves in the clot. Example includes Staphs that produces coagulase.
  - **Hyaluronidase:** It dissolves connective tissues and help organism to spread through the tissues.

- **Collagenase:** This enzyme breaks down collagen which also helps the organisms spread through the tissues.
- **IgA protease:** It digests IgA antibodies making them dysfunctional. These destroyed antibodies are of no use then against the organisms.

- **Antigenic variation:** Some organisms can change their surface antigen, so antibodies made against previous antigens are no more effective. Influenza virus is a typical example which changes its antigenic structures, hence previously made antibodies are ineffective against antigenic variant of influenza. An example of such as bacterium that changes its antigen is *Neisseria gonorrheae.*

Lesson 77. Read pages 434-439.

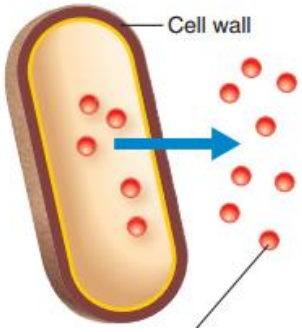
LESSON 77. HOW MICROBES DAMAGE THE BODY AND SETS UP INFECTION

- **How Bacterial Pathogens Damage Host Cells**

- Microbes damage the host cells by four mechanisms:
  - **By using the host nutrients:** Bacteria can deplete nutrients needed by the host cells. For example, bacteria secrete proteins called siderophores which bind and concentrate iron. Iron is needed for bacterial growth. There are 3 proteins belonging to the host which normally carry iron. They include lactoferrin, transferrin, and ferritin. In other words, siderophores may bind all these proteins thus depleting the host cells of iron.

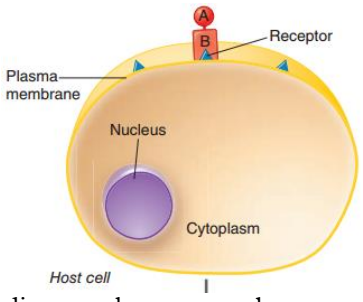
  - **Direct Damage:** Bacteria that grow in host cells rupture the cells eventually to release themselves and spread further. Rupturing obviously cause the damage to the tissues.

  - **Production of Toxins:** Toxins are poisonous substances released by microbes.



  - **Exotoxins:** These are released by bacteria when they are still alive. Antibodies made against them are called antitoxins which can bind with the toxins and can neutralize them. Toxins can be treated chemically to alter them so that they do not exhibit their toxicity, hence such toxins (called toxoids) could be used as vaccines. Exotoxins are disease specific. For example, tetanus toxin is released by Clostridium tetani. Exotoxins are also cell or tissue specific and they are sometimes named according to the cells or tissues they bind to. For example, neurotoxin binds to nerves, cardiotoxins attach heart muscles, hepatotoxins damage hepatocytes and leukotoxins attach leukocytes.

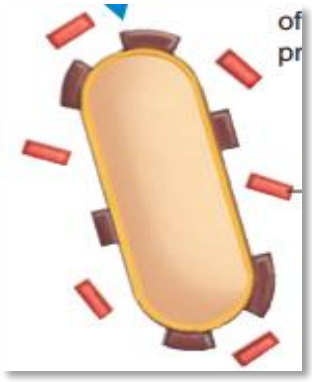
  - **Types of Toxins:** Based on the structure and function, exotoxins are divided into three types:
    - A-B Toxins: Such toxins consist of two parts designated as A and B, both of which are polypeptides. The A part is the active (enzyme) component, and the B part is the binding component. A model example of A-B toxin is diphtheria toxin. B part binds to the receptor on the cell and then A-B toxin is then internalized by the cell thus releasing the toxin in the cell which then shows its action.



    - **Membrane Disrupting Toxins:** These toxins disrupt plasma membrane and cause lysis of the cells. Some make channels in the plasma membrane

while others disrupt phospholipid molecules. Membrane disrupting cells kills phagocytes thus contributing to their virulence. Membrane disrupting toxins that kill leukocytes are called leukocidins. They make membrane channels. Those that lyse RBCs are called hemolysins.

- **Superantigens:** These antigens provoke very intense immune response. These are bacterial proteins that stimulate T cells nonspecifically resulting in widespread release of cytokines. The excessively high levels of cytokines released by T cells enter the bloodstream and give rise to a number of symptoms, including fever, nausea, vomiting, diarrhea, and sometimes shock and even death.
- **Endotoxins:** They are part of the outer portion of cell wall of gram negative bacteria. LPS is an example. LPS is released when gram negative cells die and their cell wall disrupts. Endotoxins cause release of cytokines (IL-1 and TNF) from macrophages. LPS and cytokine result in high fever. Endotoxins cause chills, fever, weakness, generalized aches, and, in some cases, shock (endotoxins cause vasodilation and blood pooling) and even death. Presence of LPS in fluids can be tested by limulus amebocyte lysate assay which contains a clotting protein that clots when reacts with LPS.



Lesson 78. Read pages 450.

LESSON 78. IMMUNITY AND ITS TYPES

- **What is Immunity?**
- **Immunity: Ability of the body to protect itself from harmful effects of diseases.**
- Susceptibility: **lack of immunity which leads to easy establishment of disease.**
- **Types of Immunity**
- **Innate (Natural):**
    - Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific.
    - Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body.
    - Nonspecific: It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there).
    - Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.
    - **First line of defense:** Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside.
    - **Second line of defense:** Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.
- **Adaptive Immunity:** This will be studied in detail in a separate lecture soon.

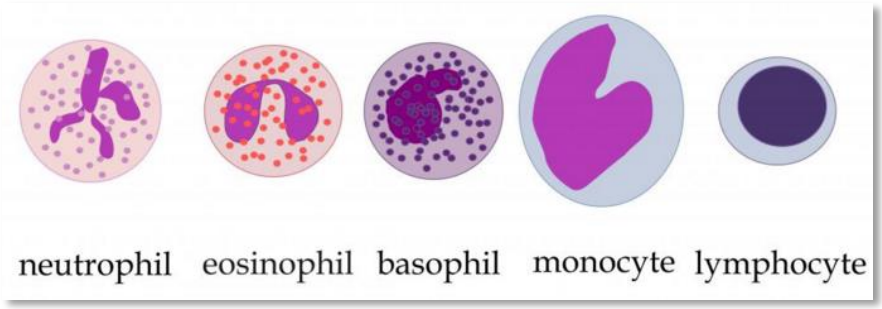
- **Adaptive (Acquired):** It is the third line of defense. Acquired by experience, specific to an organism.

- Specific: It means that antibodies made against E. coli will destroy E. coli only, and will not do any harm to a Staph for example.
- Have memory: It means if antibodies have once been made against E. coli, this exposure will be remembered by the body, and if a second encounter is made with the same type of E. coli again, the body would mount a heightened immune response against E. coli because it has memory cells developed in the body which upon second stimulation proliferate quickly and mount a greater defensive response.
- Because of memory, body's immune response increase with every repeated exposure. It has a limit also!
- Slower to respond: For the first time exposure, the body's response is slower and takes a long time compared with the innate immunity which is immediate.
- Adaptive Immune Response can further be divided into:
  - **Humoral immune response** (antibody response by B cells or lymphocytes)
  - **Cellular immune response** (T cells are made which kills microbial harboring cells).

Lesson 79. Read pages 450-453.

LESSON 79. CELLS AND SECRETIONS OF INNATE IMMUNITY

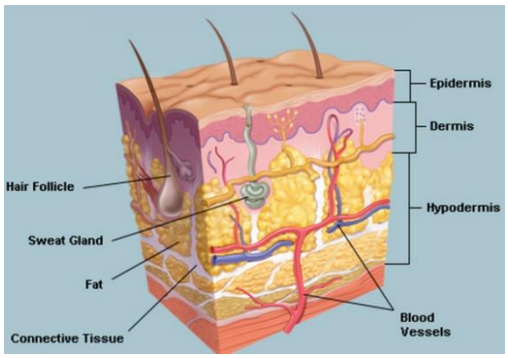
- **Components of Innate Immunity**
  - Cells and Secretions of Innate Immunity
    - **Cells:** Neutrophils, eosinophils, basophils, monocytes, macrophages, natural killer cell (see figure below)



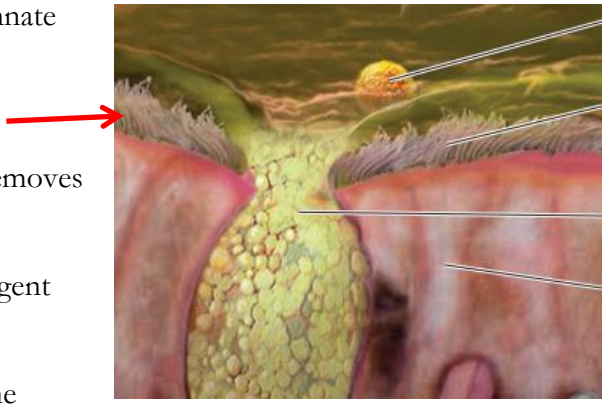
    - **Secretions:** complement proteins, bradykinins, histamine, interleukins (cytokines), acute phase proteins etc
      - More than 30 difference proteins make complement system, and these complement the cells of the immune system in destroying microbes.

  - **Toll like receptors (TLRs):** Protein molecules on defensive cells which act like a receptor binding to pathogen associated molecular patterns (PAMPs) mentioned below.
  - **Pathogen associated molecular patterns (PAMPs):** Formed by LPS, flagellin, peptidoglycan, DNA, RNA, these are repetitive patterns which the cells of the innate immune system recognize and get activated to release cytokines and reactive radicals that kill the organisms.
  - **Cytokines** are released as a result of defense cell stimulation by PAMPs and TLRs interactions.
    - Cytokines recruits more immune cells and also stimulate adaptive immune response.

- **Components of the First Line of Defense**
  - Skin: It is a physical barrier. There are five layers of epithelial cells that microbes have to cross before infecting the body. Acidic pH of the skin also discourages the growth of microbes.



  - Mucus membrane: Epithelial cells that line tubular organs and body cavities such as GI tract, respiratory tract, peritoneal cavity etc. act as a barrier for the entry of microbes. Some mucosal surfaces have cilia on them which beat unidirectionally clearing the organism from the body.
  - Mucus: Mucus traps organisms which are cleared by cilia beating unidirectionally to propel the organism out of the body (as in coughing from respiratory system).
  - Lacrimal apparatus: Washing action of tears and also the presence of lysozymes in tears and urine are examples of innate immunity.
  - Cilia: Beat unidirectionally
  - Urine: Flushing action of urine removes microbes from urinary tract.
  - Earwax: It acts as antimicrobial agent and also traps microbes.



  - Peristalsis: Provides motility to the intestine propelling the digesta caudally and out of the rectum removing the organisms.
  - Vomiting: Also removes microbes.
  - Diarrhea: Also removes microbes with the feces.
  - Defecation
  - Cebum: Fatty acids are antimicrobial.
  - pH of the skin (3-5) which discourages microbial growth.
  - Lysozyme in sweat, tears, saliva, body fluids and urine
  - Acidic pH of stomach also discourages microbial growth.

Lesson 80. Read pages 460-470.

LESSON 80. MORE SECRETIONS OF INNATE IMMUNE SYSTEM

- **Inflammation is also part of the innate immunity.** Damage to body tissues triggers a local defensive response which is called inflammation. It causes redness, pain, swelling, heat, and loss of function of that organ.
    - During inflammation, various mediators of inflammation are released, some of which include:
        - Histamine
        - Kinins: Almost all cells.
        - Prostaglandins by damaged cells
        - Leukotrienes by mast cells
    - Cytokines are released (TNF-alpha), and they cause release of acute phase proteins from the liver.

- **Fever is also part of Innate Immunity:**
    - Fever is a systemic response to pyrogens and some cytokines such as IL-1 and TNF alpha. These affect hypothalamus which sets the thermostat a bit higher resulting in overproduction of heat by body muscles and increased metabolic activity. Growth of microbes is arrested at high temperature, so fever does help reducing the microbial load.

- **Interferons are also part of Innate Immunity**
    - These are antiviral proteins, produced by macrophages and lymphocytes in response to viral infections. Fibroblasts can also secrete it. These proteins interfere with viral replication.
    - Species specific: This means that interferon produced by humans cannot be used in dogs or cats or vice versa.
    - Not viral specific: This means that interferons produced in response to virus A can also be effective against virus B.
    - **Types of Interferons:**

- **Alpha:** It is released by all infected cells.
- **Beta:** Also produced by infected cells.
- **Gamma:** This one is released by lymphocytes only. Lymphocytes belong to adaptive immune system (just remember that).

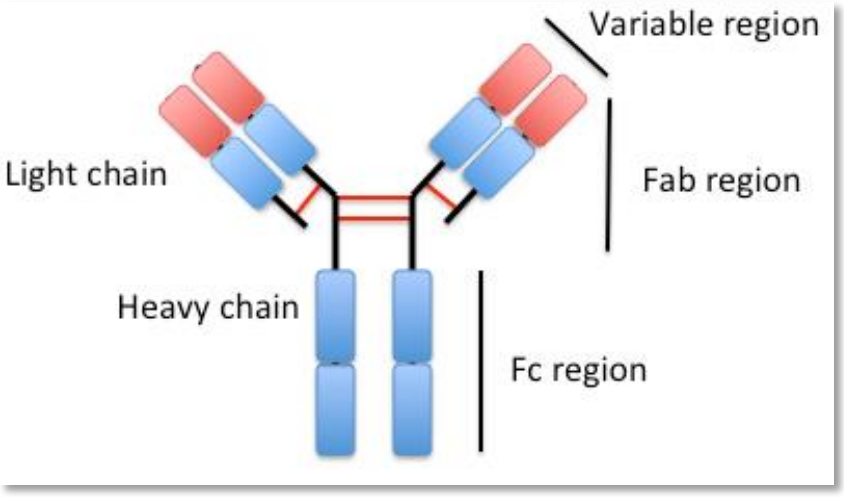
- **Antimicrobial Peptides also belong to Innate Immunity**
  - These are 12 - 50 amino acid long peptides. Many cells secrete them:
    - Defensins by neutrophils
    - Dermicidin by sweat glands
    - Thrombocidins by platelets

Lesson 81. Read pages 477-478.

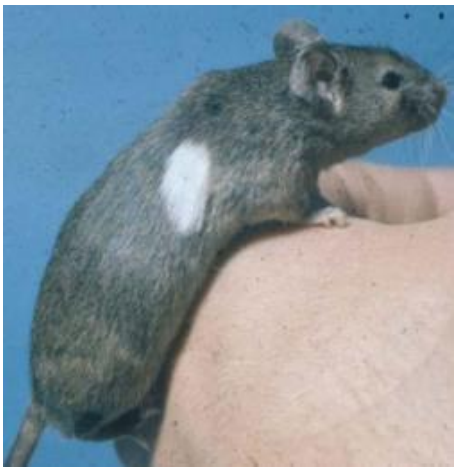
LESSON 81. ADAPTIVE IMMUNE RESPONSE

- Adaptive Immunity provides 3rd line of defense. Adaptive immune consists of B and T cells. B cells upon stimulation by specific antigens transform themselves to plasma cells and start secreting antibodies. T cells provide helper activity to B cells and macrophages, and also act as a fourth line of defense against those intracellular microbes that are not accessible to antibodies and must be eliminated. A subset of T cells (CD8 positive T cells) bind to cells that are harboring intracellular microbes and kill the cells by apoptosis (programmed cell death).

- **How was adaptive immune response discovered?**
  - **Observations:** Individuals once recovered from smallpox, measles, or chickenpox become immune to subsequent exposures with the same microbe.
  - Experiments using vaccines or exposure to pathogenic organism (after recovery from the disease in animals) led to antibody discovery in the serum.
  - It was also observed that antibodies transferred from one animal to another can protect the recipient from getting sick.
  - Later it was discovered that antibodies are made up of four polypeptide chains held together by disulfide bonds and it consists of Fc part and Fab part. Fc part is what determines the type of antibody while Fab portion binds the antigen. See the accompanying figure for details.



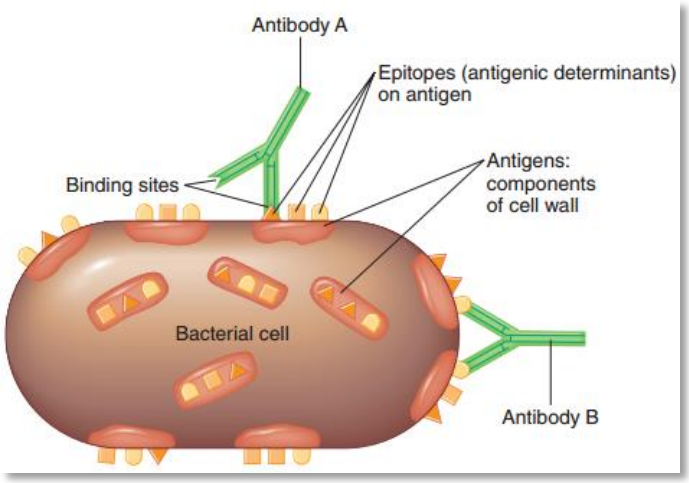
- **Involvement of T cells** was also discovered with transplantation experiments in mice. When the thymus gland was removed from the mice and skin grafts were inserted in those mice, grafts were accepted suggesting the involvement of thymus in this role. Later experiments recognized T cells to be involved in tissue rejection.
- **T cells** were discovered to be responsible for **cellular immunity** which is a part of the adaptive immunity.



Lesson 82. Read pages 478-481.

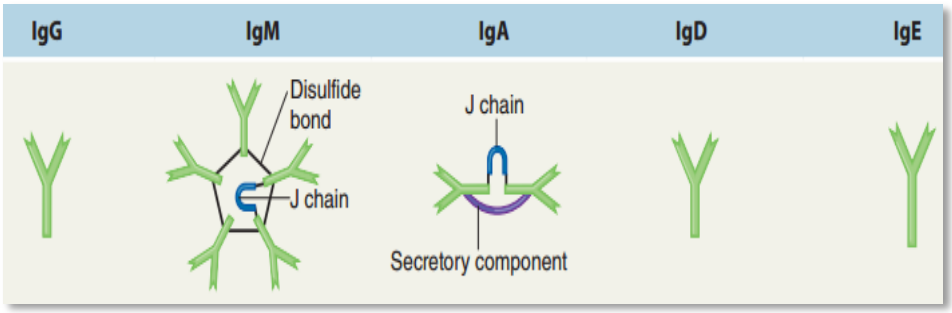
LESSON 82. ANTIGENS AND ANTIBODIES

- **Characteristics of Antigens**
  - **Antigen:** A foreign substance that provokes immunity is called an antigen.
    - More specifically called as an **immunogen**. So immunogen and antigen although slightly different but used interchangeably.
  - Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens.
  - Antigens are foreign to the body.
  - Degradability: Inert molecules are not antigenic.
  - **Epitopes:** Specific regions on an antigen. In other words, any antigen has multiple regions each one of those act as a small antigen. These are called **epitopes**. See the figure that has projections. Each projection is an epitope.



  - These epitopes are also called as antigenic determinants.
  - Most antigens have MW >10,000 D. Molecules that are less than 10,000 D are generally poor antigens.
  - **Haptens:** It is a molecule that by itself does not induce antibody production; however, can bind preformed antibodies. So, it needs a carrier molecule to induce antibody production.
    - Penicillin is an example that acts like a hapten.
- **What are Antibodies?**
  - Antibodies are glycoproteins in nature.
  - Antibodies are present in gamma globulins portion of the serum when serum is electrophoresed. Antibodies are classified according to their Fc part into 5 isotypes and these include:

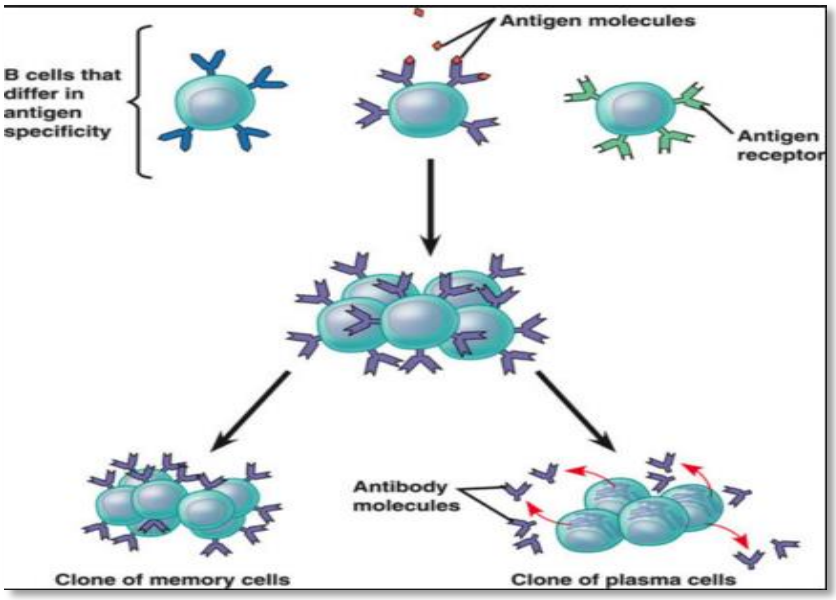
- IgM: Mostly remain in the blood because these are big molecules (pentamers) and do not cross capillaries.
- IgG: This isotype is found in all body secretions as monomer molecules in the serum, peritoneal and pleural fluids.
- IgA (mucus membrane): These are found in mucosal secretions and milk. These are dimers mostly.
- IgE (allergy): IgE is found in the serum and is formed against allergens. These are involved in allergic reactions.
- IgD: Normally remain in the B cells. They are not secreted.



Lesson 83. Read pages 482-483.

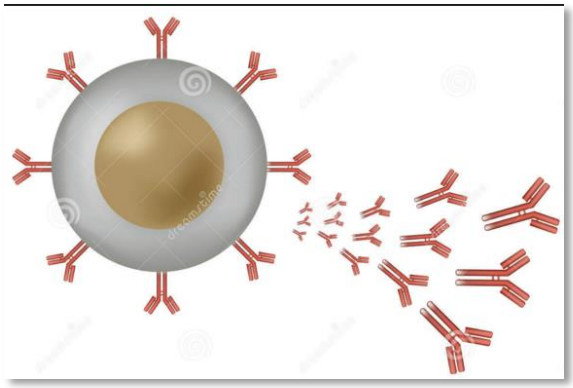
LESSON 83. CLONAL SELECTION THEORY OF ANTIBODIES

- **What is Clonal Selection Theory of Antibodies?**
- There are two theories about antibody production by B cells. One theory called **instructional theory** suggests that an antigen binds to any B cell and instruct to make antibodies that should fit the antigen that previously has interacted with that B cell. However, this model failed because no proof was found to corroborate this idea. The second theory was forwarded with the name **clonal selection theory**. According to this theory, B cells are generated in the bone marrow randomly without seeing the antigen. When such a B cell encounters its specific antigen in the body, it starts proliferating into a clone which ultimately becomes plasma cells and start secreting antibodies. A beautiful example to understand clonal selection theory is like going to a shoe store and select a shoe size that fits you very well. Note, that the shoe that you selected was made randomly without any regard to your foot size. Clonal selection theory is exactly like that. Antigen selects the clone which fits the antigen.



    - B cell never changes its specificity, only isotype of the antibody changes. In other words, a B cell that is secreting IgM can switch over to secreting IgG. This is called class switching.

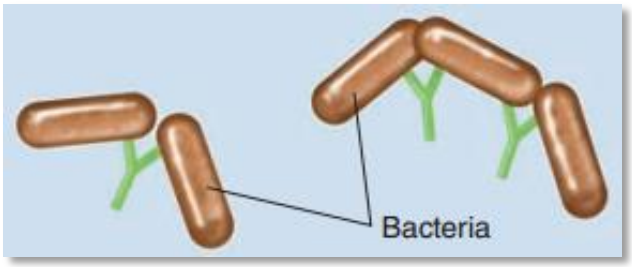
- Each B cell has unique antibody molecules against one epitope only. In other words, all antibody molecules that you see in the accompanying figure have the same specificity. They bind only one epitope of an antigen.



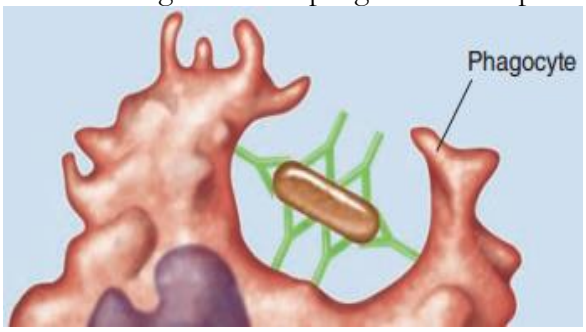
Lesson 84. Read pages 484-485.

LESSON 84. USE OF ANTIBODIES IN BODY DEFENSES

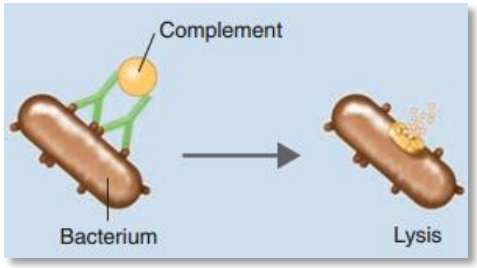
- **How Antibodies Eliminate Antigens from the Body?**
  - **Agglutination:** Antibodies can agglutinate antigens which are then cleared as a group by macrophages.



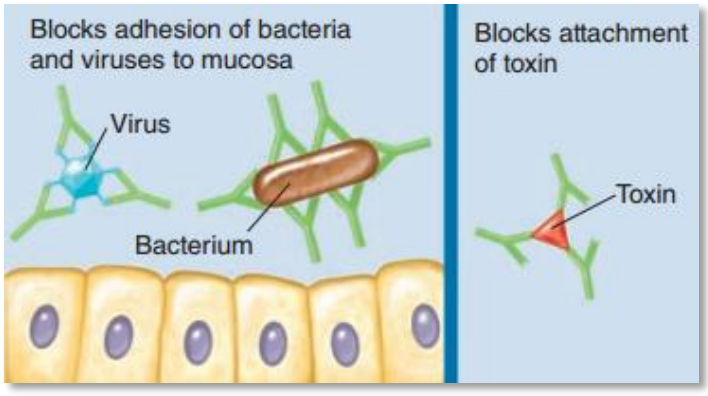
  - **Opsonization:** Antibodies can coat or cover antigens. Macrophages have receptors that can bind Fc portion of antibodies that have bound to antigens, thus engulfing the antigen and clearing it from the body.



  - **Complement Activation:** Antibodies such as IgG and IgM can activate complement which punches holes in the organisms resulting in leakage of the internal contents of microbes killing them.



  - **Antibody-dependent cell-mediated cytotoxicity**: Cells are destroyed by first coating with antibodies.
  - **Neutralization:** Toxins bind with antibodies and they become incapable of binding to their target receptors thus the toxins become harmless.



Lesson 85. Read pages 486-490.

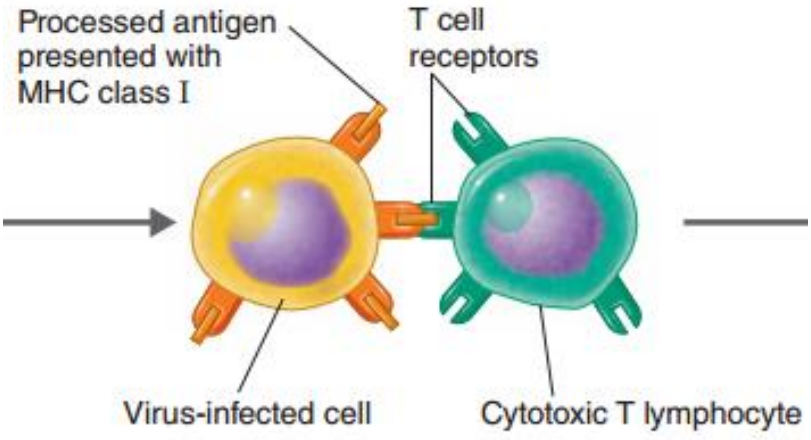
LESSON 85. CELLULAR IMMUNITY AND ANTIGEN PRESENTING CELLS

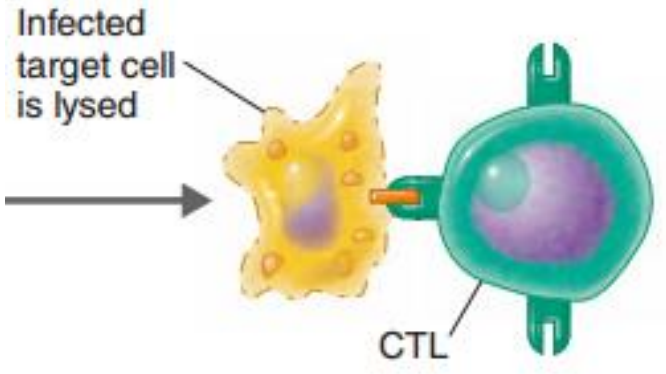
- **What Constitutes Cellular Immune Response**
- If you look into the origin of T and B cells, both B and T cells originate from the stem cells in the bone marrow, but then T cells go to the thymus for maturation. Once they are released, these T cells distribute throughout the body and make part of the cellular immunity. B cells remain in the bone marrow and mature there. After maturation in the marrow, they also leave these places and go to secondary lymphoid organs such as lymph nodes and spleen etc. They also keep circulating between blood and lymph and when these B cells encounter an antigen, they are stimulated to make antibodies.

- **Characteristics of T cells**
  - T cells are MHC restricted. This means that these cells recognize antigens only when these are associated with self MHC molecule. This also means that T cells do not bind or attach to free antigens. As B cells have B cell receptors, T cells have T cell Receptor. B cell receptor binds antigenic epitope while T cells bind T cell specific antigenic epitopes.
- **Distribution of Major Histocompatibility Complex (MHC) Molecules**
  - MHC class I molecules are present on all nucleated cells.
  - MHC class II molecules are resent only on Antigen Presenting Cells.
  - **Antigen Presenting Cells:** These cells are able to present antigens to B cells for making antibodies by B cells.
    - There are three cells that fall into this category: Dendritic cells, macrophages, and B cells.

- **T Cells and their Types**
  - T helper cells
    - TH1 ($CD4^+$)
    - TH2 ($CD4^+$)
  - T cytotoxic cells ($CD8^+$)

- **How Cytotoxic T cells Kill Virus Infected Cells**
  - Antibodies are larger molecules. If a virus succeeds entering into a cell and starts multiplying inside that cell, antibodies cannot penetrate the cells or cannot chase the virus inside the cell. However, cytotoxic T cells bind to virus infected cells through MHC class I molecules that are displaying viral antigens on its surface. T cells bind to virus infected cells and signal them to die.



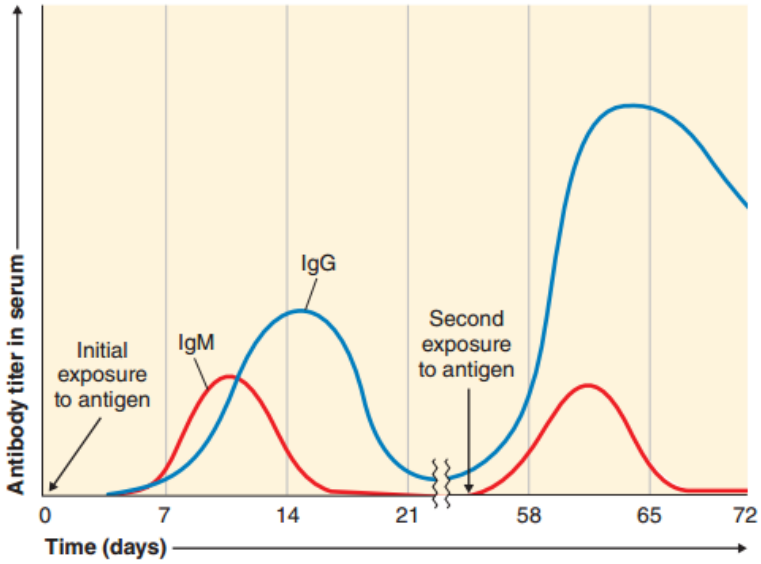


So, when such a cell dies, virus is also released from the dying cell. These viral particles then can be handled by antibodies which bind these viruses and remove them from the body.

Lesson 86. Read pages 493-495.

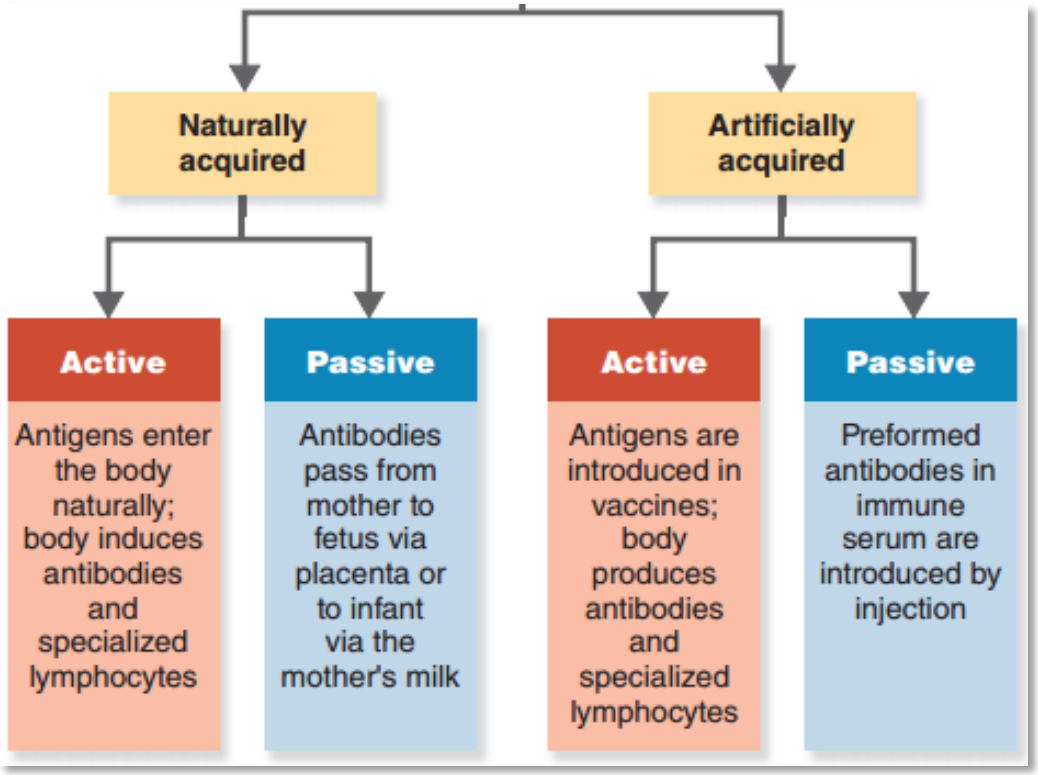
LESSON 86. PRIMARY AND SECONDARY IMMUNE RESPONSES, ACTIVE AND PASSIVE IMMUNITY

- **What is a Primary and a Secondary Immune Response and Role of Memory?**
- When a person is exposed to an antigen (a microbe for example), the antigen is presented to B cells which start making antibodies. Initially, there are no detectable antibodies in the serum. However, after 3-5 days, IgM antibodies are secreted and then 7-10 days after, the same B cell starts making IgG. The level of IgG rises to a level and then it starts declining. This is a typical response of an antigen exposure and is called a primary immune response. It is interesting to note that a second exposure with the same antigen results in a heightened response in terms of very high levels of antibodies that are achieved in a very short time (within 2-7 days). This is called a secondary immune response. It is also called a memory or anamnestic response because memory cells are involved that ensure a quick response to the second exposure by the same antigen (to which the immune system has already been exposed). The accompanying figure illustrates this concept (next page). Blue line in the figure depicts a primary and a secondary response.



- Adaptive Immune Response can be acquired **naturally or artificially**. Each of these categories can further be divided either into active or passive type.

- **Naturally Acquired Active Immunity:** This type of adaptive immunity develops in a person who gets infected with a microbe but then recovers from that illness making antibodies.
- **Naturally Acquired Passive Immunity:** A typical example of this type of immunity is transfer of antibodies from colostrum or milk of a mother to the offspring.
- **Artificially Acquired Active Immunity:** When a person receives a vaccine against a microbe and the body makes antibodies in response to that vaccine, it is called artificial acquired active immunity because body makes these antibodies actively by itself.
- **Artificially Acquired Passive Immunity:** When premade antibodies are given to a person in order to protect a person from an illness or a snake bite, this constitutes an artificially acquired passive immunity type. The following figure illustrates these four types.

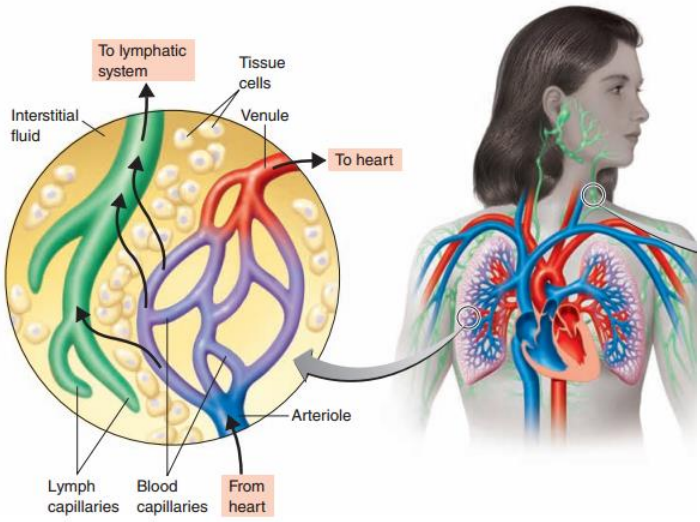


Lesson 87. Read pages 456-457.

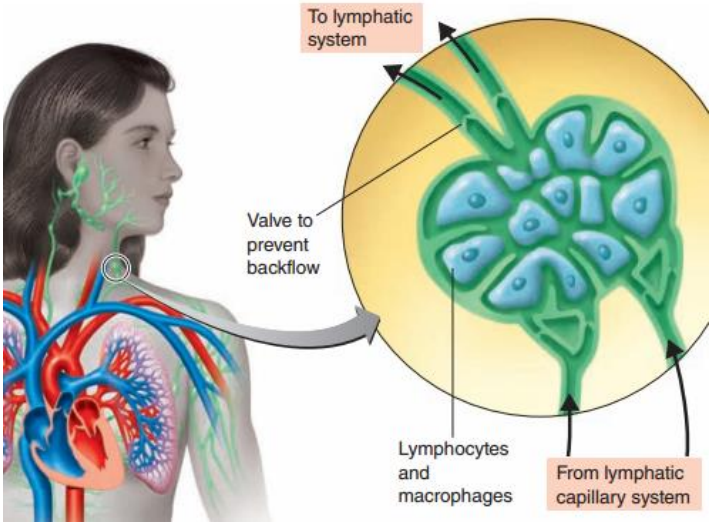
LESSON 87. BODY TRANSPORT SYSTEMS

- **Role of Lymphatics and Cardiovascular System in Infection and Immunity**

Cardiovascular and lymphatics are the two transport systems of the body by which cells of the immune system patrol the whole body. Cardiovascular system consists of the heart and associated blood vessels (arteries and veins) in which blood circulates while lymphatics are channels that start from tissue spaces and collect tissue fluid (called lymph) from tissue spaces and bring them back to the heart.



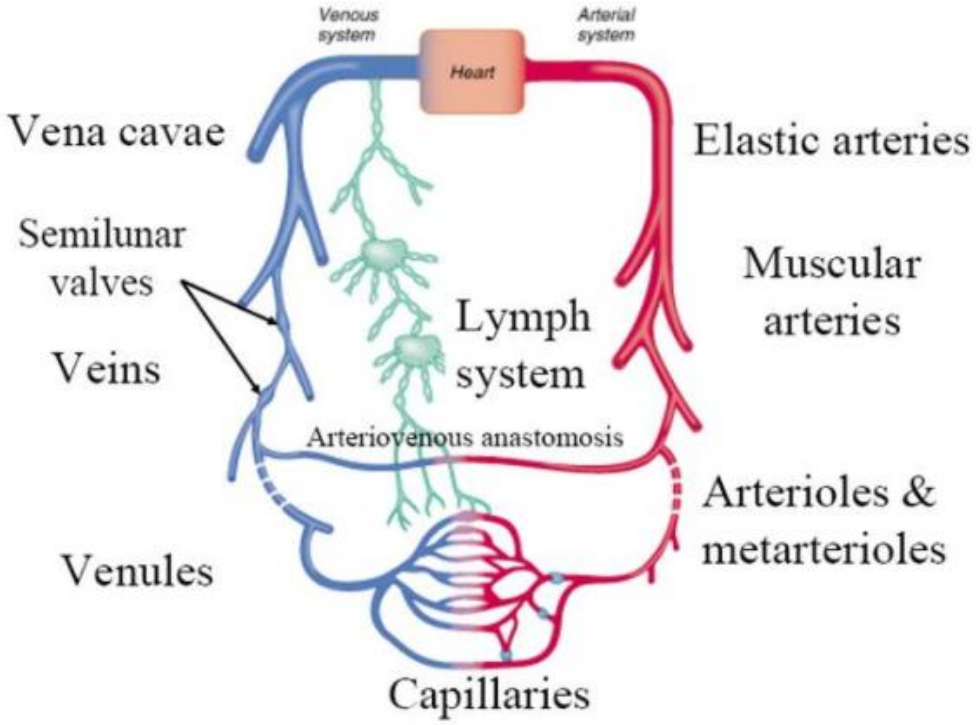
Along these lymphatic channels, there are strategically placed lymph nodes which filter lymph for microbes much like blood is filtered in the spleen. Lymph nodes contain aggregations of B and T cells along with macrophages and dendritic cells which interact with each other inducing adaptive immune responses.



- **Why Cardiovascular and Lymphatics are needed?**
- Well, in unicellular organisms, nutrients can be absorbed directly from the environment in which a unicellular organism is living. However, a multicellular organism does not have this easy access to the nutrients. So, nutrients must surround the cells in a multicellular organism. These nutrients are provided by cardiovascular system which pumps blood to all organs. Remember that only fluid part of blood leaves blood vessels. RBCs never leave blood vessels. Sometimes proteins also leak from these blood vessels; however, once they leak from the blood vessels, they never can enter back into blood vessels because of their bigger size. If proteins remain in the tissue spaces, they exert osmotic pressure which attracts water into these space resulting in edema. However, nature has provided another mechanism in the form of lymphatic channels which start from tissue spaces and can drain tissue fluid and proteins back to the heart. This is the role of blood vessels and lympahtics that they ensure

circulation of nutrients throughout the body without causing any edema or abnormality. B cells and T cells also keep circulating between these two channels (cardiovascular and lymphatics). Unfortunately, microbes can also use these two systems to spread themselves in the body. That is the reason, spleen and lymph nodes are placed in these channels by the nature to deal with these microbes that find their ways into blood and lymph.

- **Lymphatic System consists of the following:**
  - Bone marrow
  - Lymph nodes
  - Spleen
  - Tonsils
  - Thymus
  - Mucosa associated
  - Bursa of Fabricius in birds



Lesson 88. Read pages 501-504.

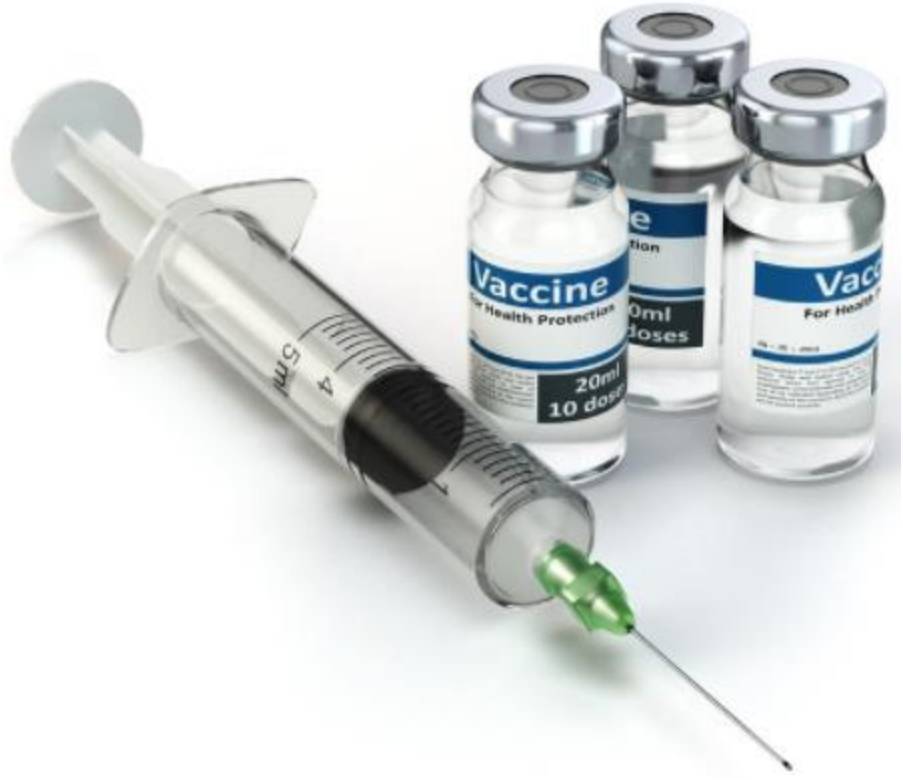
LESSON 88. VACCINES AND TYPES OF VACCINES

- **What is a vaccine?**
    - A product that creates immunity against an infection is called a vaccine. It could be the whole organism or a part of the organism.
- The observation that people or animals once recover from an infection do not get sick easily to that organism again led to the discovery of vaccines and vaccination.
- **Principle of Vaccination**
    - A vaccine mimics the infection by the organism but without causing the disease, so immune system gets stimulated in exactly the same way as is done by an infection. However, since vaccines do not harm the body, they provide a safe way for inducing immunity against real microbes that may be encountered in one's life.
- **Types of Vaccines**
    - **Live Attenuated Vaccines:** These are weakened organisms so that they do not harm the body, but divide or replicate in the same way as pathogenic version of the organism does in the body. So, when a live virus or bacteria multiplies in the body, it stimulates the immune system to create an adaptive immune response which is protective against the disease for which vaccine was given.
        - The benefits of live vaccines are a better immunity because live microbes mimic actual infection (although they do not cause harm to the body). Live vaccines induce both humoral and cellular immune responses together, so live vaccines are a better choice if available. Because live microbes multiply in the body, this replication results in the increase of antigenic amount needed for stimulation of the immune system. Also, this antigen remains for a long time in the body, continuous presence of antigen continuously keeps the immune system active for longer time resulting in long term immunity.
    - **Inactivated Killed Vaccines:** Sometimes a non-pathogenic version of the microbe is not available, and to make a vaccine in this situation, the organism is killed with formalin or phenol and then used as a killed inactivated vaccine. Because organisms in such a vaccine do not replicate in the body, frequent boosters are required to

achieve a good level of immunity (antibodies). Also, remember that this is a killed vaccine, so classical cellular immunity is not created with killed vaccines.

- **Toxoids:** Some diseases are caused by bacterial toxins, and not by bacteria. In such cases, toxin is modified (inactivated) and used as a vaccine. Such a vaccine is known as a toxoid.
  - Tetanus toxoid is a good example.
- **Conjugated Vaccines:** Sometimes special parts of organisms are required for generating protective immunity. However, those substances are poor antigens if used alone. In order to enhance immunity, such poor antigens are attached to proteins, and such vaccines are called conjugated vaccines.
  - *Haemophilus influenzae* type b is a good example.
- **DNA vaccines:** We now know that sometimes only certain proteins are required for generating a good immune response. In such cases, the genes responsible for those proteins can act as vaccines. DNA coding for such a protein is injected into cells, and that DNA gets transcribed by the cells of the body into the required protein and that protein induces the antibody production (because that protein is foreign to the body).
  - West Nile Virus is a good example of DNA vaccine.
- **Subunit vaccines:** Also called recombinant vaccines, such vaccines are composed of a portion of viral or bacterial proteins that can induce immunity. In other words, this is another version of DNA vaccines. In subunit vaccines, antigenic epitopes are included in the genome which is inserted into an expression vector that makes those subunit proteins. These subunit proteins are collected and used as vaccines to stimulate the immune system. Again, these act like a killed vaccine. There is another mechanism being explored in these days for recombinant vaccines. A subunit vaccine for Foot and Mouth disease virus is available.
- Such genes are being incorporated in plants now that are acting like an expression vector. Animals can eat those plants and subunit vaccines can induce immune

response through M cells of the intestine. Success is limited though so far.

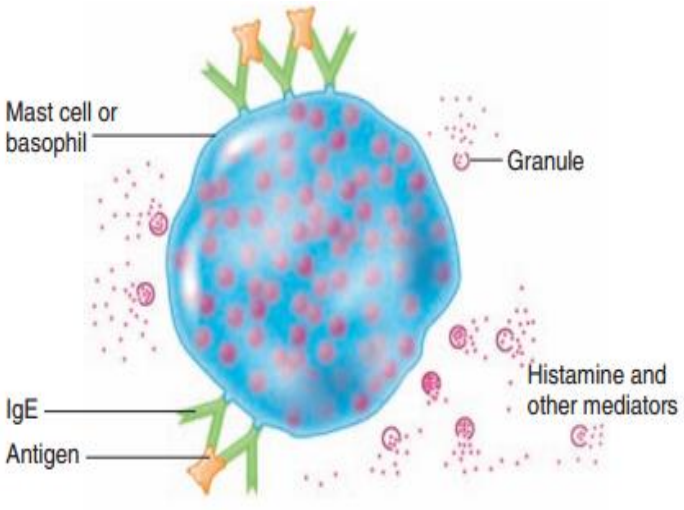


Lesson 89. Read pages 523-526.

LESSON 89. HYPERSENSITIVITY TYPE I

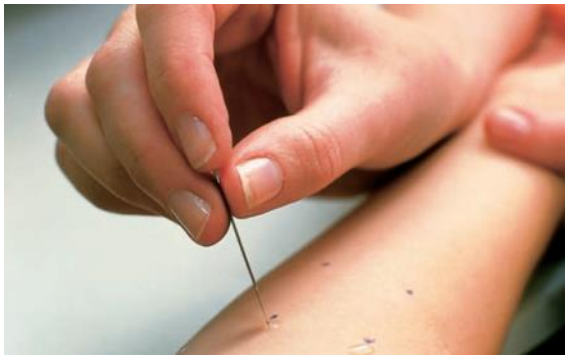
- **What is Hypersensitivity?**
    - It is a normal Immune response but in a damaging way. In other words, immune system is acting in a way for which it has made to. So when an antigen gets into the body, immune system recognizes it as foreign and start making antibodies against the antigen. However, the consequences are bad and damaging to the body. It may be called an abnormal antigenic response. Allergy is another name for hypersensitivity. Those antigens that act in a bad way are called allergens.
- **Types of Hypersensitivities**
- Hypersensitivities can be grouped into 4 types based on the types of antibodies or cells involved.
    - **Type I (Anaphylactic):** Also called anaphylaxis or immediate hypersensitivity. This is IgE mediated.
    - **Type II (Cytotoxic):** IgG and IgM mediated as these antibodies attach to cells and destroy them by activating complement.
    - **Type III (Immune complex mediated):** Immune complexes are formed by IgM or IgG antibodies and these complexes lodge in the capillary beds and cause damage by complement activation.
    - **Type IV (delayed type cell-mediated):** $CD4^+$ helper cells along with macrophages are involved.

- **Type I or Anaphylaxis:** It involves IgE and it occurs within 30 minutes of exposure to the allergen. Antigens called allergens preferentially induce IgE production instead of IgG against the antigens. These IgEs get bound to mast cells in the tissues and a second exposure to the same allergen activates mast cells to secrete histamine and other mediators of inflammation. When allergens bind to two adjacent antibody molecules on the surface of the mast cells, this cross linking activates degranulation of mast cells that secrete histamines and other mediators of inflammation. These chemicals result in vasodilation and shock.



  The patient also experiences difficulty in breathing. It is an emergency as it may kill the person. Anaphylaxis may be local or systemic.

- Shock from drug reactions, venoms, and common allergens causing asthma are some examples of type I hypersensitivity.
- Hypersensitivity can be diagnosed by demonstration of IgE by intradermal injections of allergens one by one. If specific IgE are present in the serum, a reaction will be visible on the skin locally in the form of a wheel of inflammation. Moreover, IgE levels can be measured by ELISA. Measurement of IgE is more practical as nature of allergens is not known most of the time.

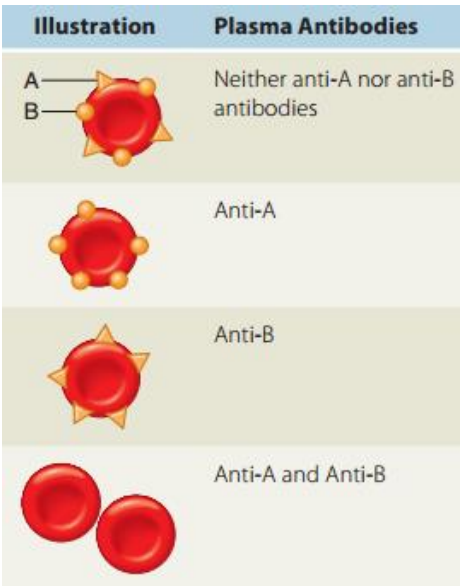


- Rx: Epinephrine & antihistaminic drugs provide a means of treatment.

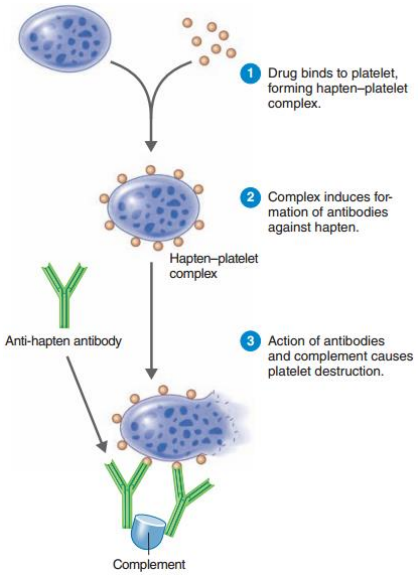
Lesson 90. Read pages 526-528.

LESSON 90. HYPERSENSITIVITY TYPE II

- **Type II Hypersensitivity (cytotoxic)**
  - This hypersensitivity involves antigenic cells and IgG or IgM antibodies that become reactive with these body cells. Whenever, IgM or IgG antibodies bind to their antigens, they always activate complement which then can punch holes in the cells lysing them altogether. Blood transfusion reaction is a good example of type II hypersensitivity.



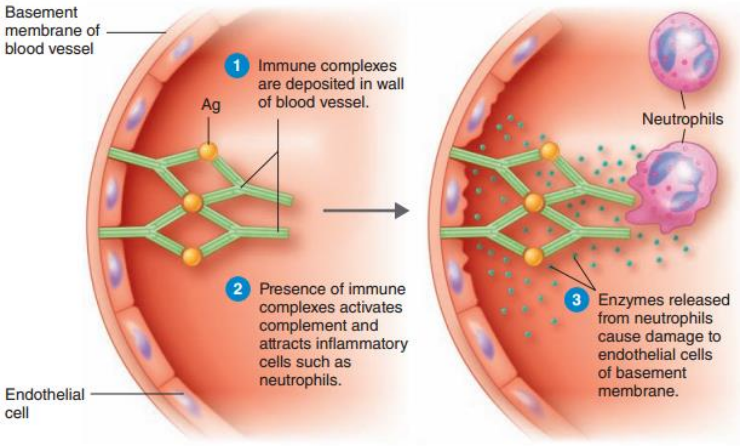
  - Sometimes, drugs bind to body cells and change them in such a way that cells of the immune system start reacting with body's own cells damaging them. See the figure for drug induced cytotoxicity.
  - Since this type of hypersensitivity targets body's own cells, these hypersensitivities can be diagnosed by looking into the number of cells such as RBCs, platelets etc.
  - This hypersensitivity can be treated by avoid those drugs that cause it. Blood transfusion should be done with correct blood types.



Lesson 91. Read pages 528-529.

LESSON 91. HYPERSENSITIVITY TYPES III

- **Hypersensitivity Type III (Immune Complex Mediated)**
  - This hypersensitivity involves soluble antigen circulating in the serum and antibodies (IgG or sometimes IgM). Complement gets activated when these complexes get deposited in capillary beds as we see in the kidneys for example. A typical example will be the use of antivenom (antibodies) that was prepared in horses but given to a human to save the life. What happens is that horse antibodies will be recognized by human body as a foreign protein and human immune system will generate antibodies against this. These antibodies will bind with antivenom antibodies and make complexes. These complexes get deposited when kidney glomeruli filter plasma. Complement binds to these trapped complexes and cause damage to the kidneys.

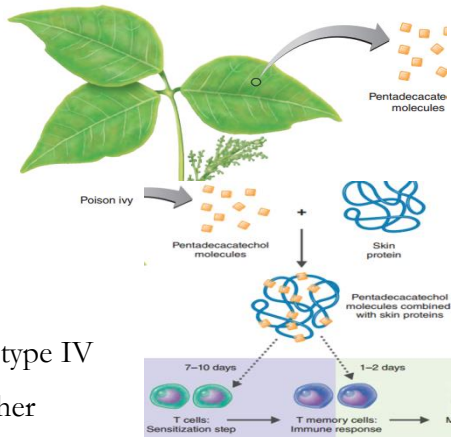


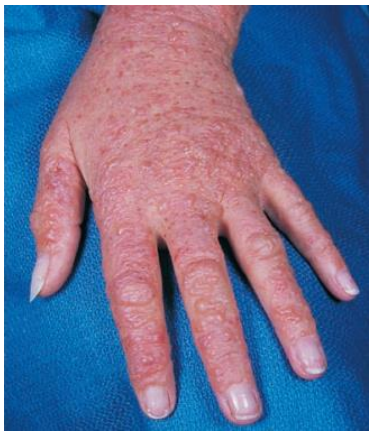
  - This type of hypersensitivity can be diagnosed by history and symptoms. Glomerulonephritis and cellulitis is very common. Deposition of immune complexes in the kidneys can be diagnosed by presence of IgG in kidney cells by using anti-IgG antibodies.

Lesson 92. Read pages 529-531.

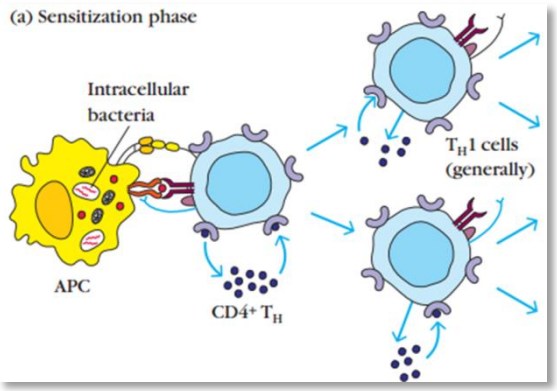
LESSON 92. HYPERSENSITIVITY TYPE IV

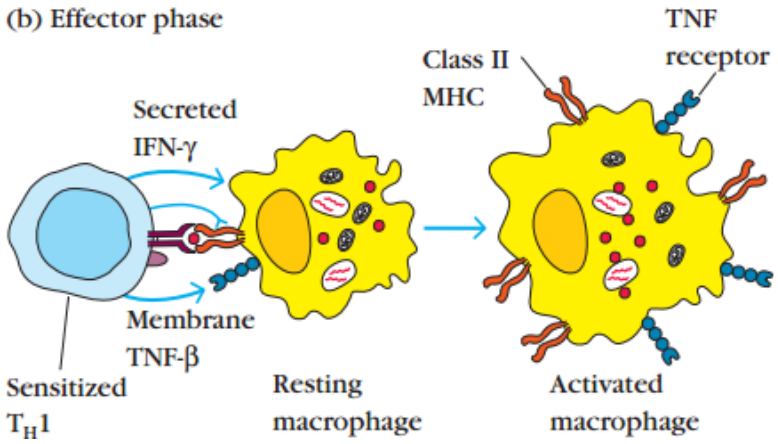
- **Type IV (Delayed Type) Hypersensitivity**
  - It is called as delayed type because its bad effect becomes visible 2-3 days after exposure to the antigen. This is the only hypersensitivity that involves cells rather than antibodies. Robert Koch discovered this when he injected tuberculin in patients. Contact dermatitis is the most common example of this kind of hypersensitivity. This is basically an interaction between an antigen, T cells and macrophages. Transplant rejection is another good example of delayed type IV hypersensitivity. Reaction to hair dyes is another example. Poison Ivy is another example in which contact with this plant initiate type IV hypersensitivity response. Chemicals from poison ivy interact with skin proteins and change them into foreign-looking protein molecules. Body's immune system mounts an immune response against these proteins resulting in a damaging type of intense inflammatory response. This is what happens with contact dermatitis (see the accompanying figure).





- **How Type IV delayed hypersensitivity work**
  - **Sensitization Phase:** On the first exposure, macrophages take up the antigen, and present this antigen to CD^{4+} Th1 cells which become sensitized to the antigen.
  - **Effector Phase:** Upon second exposure, these sensitized $CD4^{+}$ cells again interact with the macrophages that present the same antigen to T cells, which then secrete cytokines that in turn activate macrophages. Activated macrophages become aggressive and start killing the cells and also releasing more chemical mediators of inflammation that attract more macrophages to the area. See the figure below for effector phase.





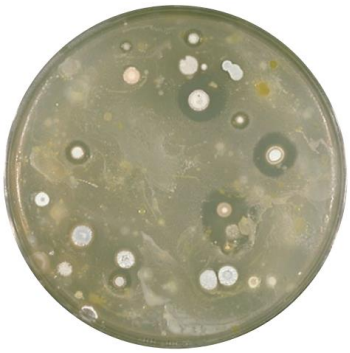
  - This hypersensitivity can be diagnosed by skin testing by injecting the intending antigen intradermally. Treatment is symptomatic and one should avoid contact with the allergen.

Lesson 93. Read pages 554-555.

LESSON 93. HISTORY OF ANTIMICROBIALS

- **A Little History of Antimicrobials**
  - **Paul Ehrlich** coined the word "chemotherapy" for treating diseases with chemicals. His observation that organisms get stained without staining the background led to the notion that chemicals can be used selectively to kill organisms while sparing other body cells.

  - Antimicrobials are used for chemotherapy because of their selective toxicity to microbes (while sparing body cells).
  - 1928: Alexander Fleming working with *Staph aureus* observed colony of a mold with inhibitory effect. Later, this organism was identified to be *Penicillium notatum*. Penicillin was found to be the agent secreted by this organism. Later, *P chrysongenum* was used to produce Penicillin commercially. Penicillin was named as an antibiotic because it was made by an organism and it had inhibitory role against another organism. Antibiotics must not be toxic to the body. Although, many antibiotics are produced by microbes, but only a few are selectively toxic to microbes. See the Table above for a brief list of such microbes and antibiotics they secrete.

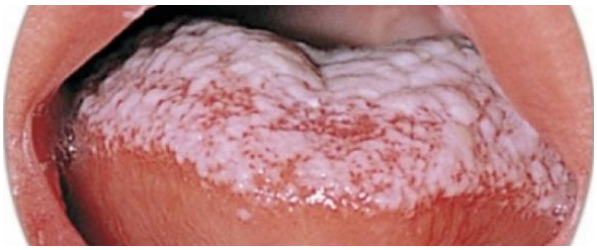


Microbe	Antibiotics
Bacillus subtilis	Bacitracin
Paenibacillus polymyxa	Polymyxin
Streptomyces nodosus	Amphotericin B
Streptomycems venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Saccharopolyspora erythraea	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpurea	Gentamicin

Lesson 94. Read pages 555-556.

LESSON 94. SCOPE AND SPECTRUM OF ANTIMICROBIALS

- **Difficulty in Developing Effective Antimicrobials**
  - Developing drugs against prokaryotes is relatively easy because prokaryotic cell differ from eukaryotic cell with respect to many structures that may be used as targets for antibiotics. However, developing drugs against viruses and fungi is difficult because viruses use eukaryotic cellular machinery for viral replication and fungi are eukaryotic cells. Any attempt to target any structure in the eukaryote basically amounts to killing the cell itself.
- **Spectrum of Antibiotics and their Scope**
  - **Narrow:** Narrow spectrum antibiotics target only a group of organisms. For example, Penicillin kills G+ve bacteria only because Penicillin act on the cell wall which is more pronounced in Gram positive organisms. The advantage of using narrow spectrum is that these spare microflora of the body.
  - **Wide:** These antibiotics target many groups of microbes. For example, tetracycline is used against Gram negative and Gram positive organisms and many others. The main disadvantage of such antibiotics is that they also destroy the normal microflora because they target every single organism. You may have observed that when broad spectrum antibiotics are given especially to kids, they often develop candidiasis (caused by *Candida albicnas*) commonly called thrush in their mouth cavity. It leaves white spots on the tongue and oral cavity.



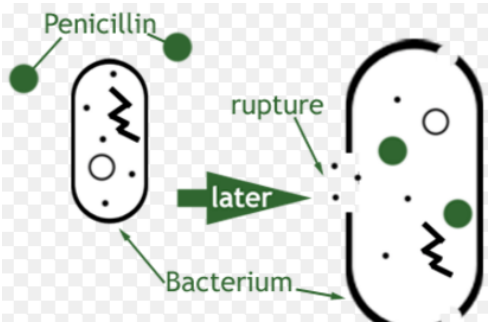
  - **Bactericidal:** Those antibiotics that kill and lyse the cells are called bactericidal.
  - **Bacteriostatic:** Such antibiotics stop the growth of the organisms, they do not kill or lyse the cells. Cells of the immune system then clear them from the body.

Lesson 95. Read pages 556-559.

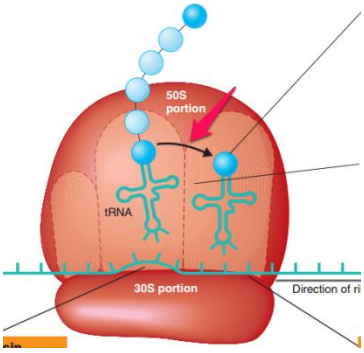
LESSON 95. ANTIBACRIAL DRUG TARGETS

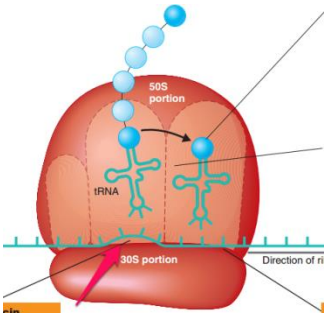
- **Drug Targets for Prokaryotic Cells**

1. **Inhibition of cell wall synthesis:** Many antibiotics inhibit synthesis of peptidoglycan cell wall. Examples include Penicillins, cephalosporins, bacitracin, vancomycin. As the target is the peptidoglycan layer of the cell wall, the cell wall does not get synthesized, and cells swell up and are lysed as a result (cidal). So, Penicillin is a bactericidal drug or antibiotics. Target of such drugs is the growing cells that are actively involved in synthesizing of cell wall.



2. **Inhibition of Protein Synthesis**: Many antibiotic target ribosomes and bind them so that the ribosomes are not able to make proteins. Such drugs include chloramphenicol, erythromycin, tetracycline and streptomycin. Selective cytotoxicity is ensured by the difference in the ribosomes of prokaryotes versus eukaryotes. Eukaryotes have 80S ribosome while prokaryotes have 70S ribosomes. There are many targets in the ribosomes to inhibit protein synthesis. For example, **chloramphenicol** binds to 50S ribosomes and inhibits peptide bonding, hence protein synthesis is stopped which arrests the growth of the organisms (see the figure for where this is happening). Another drug, **streptomycin**, binds to 30S part of ribosome add changes the shape of this part of the ribosome which in turn results in misreading of RNA codes by the ribosomes stopping the synthesis of proteins. **Tetracyclines** interfere with attachment of tRNA to mRNA ribosome complex thus inhibiting protein synthesis.





3. **Inhibition of Nucleic Acid Replication and Transcription**: Drugs such as quinolones and rifampin target DNA synthesis process. So, if DNA does not duplicate in an organism, the organism will not grow further. These drugs interfere with DNA replication and transcription, and are very limited because such drugs can interfere with body cells as well.

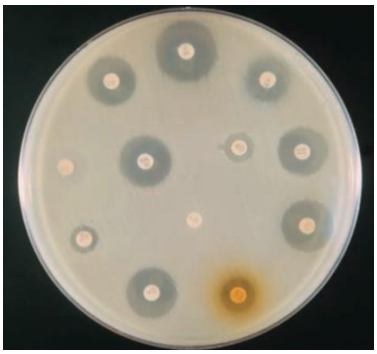
4. **Injury to Plasma Membrane:** Polymixin B is such a drug that targets plasma membrane. Polypeptide antibiotics bring changes in the permeability of plasma membrane and result in the loss of imp metabolite from the cell. Antifungal (Amphotericin B, Ketoconazole) drugs are other examples. These drugs combine with sterols in the plasma membrane. Sterol is not present in bacteria, so these drugs are not effective against bacteria.
5. **Inhibition of Essential Metabolic Synthesis**: Sulfanilamide and trimethoprim act as a substrate needed for making GABA. Sulfanilamide and para-aminobenzoic acid (PABA) look very much like each other. However, PABA is a substrate for an enzyme that is involved in folic acid synthesis. Folic acid is needed for purine and pyrimidine synthesis, hence when sulfa drugs compete with PABA, DNA and RNA are not made, stopping the growth of the organisms.

Lesson 96. Read pages 572-573.

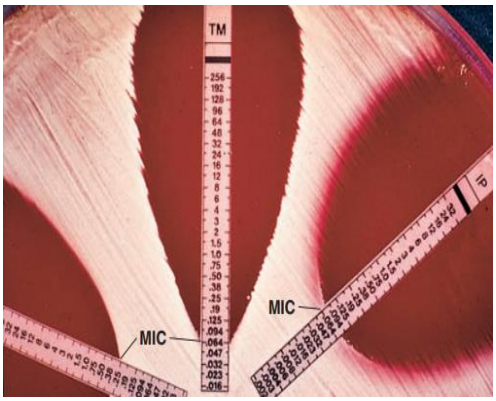
LESSON 96. ANTIMICROBIAL SENSITIVITY TESTING

- **Drug Sensitivity: Tests to Guide Chemotherapy**
- Physicians usually prescribe drugs that they know will work. If the organism has been identified, selection of drug is easy. There are three methods used for determining which drug will work in a patient who needs chemotherapy.

1. **Disk Diffusion Method:** A standardized inoculum of the organisms is spread on agar plate. Filter paper disks soaked with antibacterials are then placed on the lawn of the organism. Plates are incubated at 37°C and results are recorded. Drugs diffuse from the paper disks radially and if the organisms are sensitive to the drug, a clear area with no growth around the disks develops. The size of the zone directly relates to the degree of sensitivity of the drug. This zone is compared to a standard table for recording the results of sensitivity test. Results are reported as sensitive or resistant or intermediate (between the two extremes).



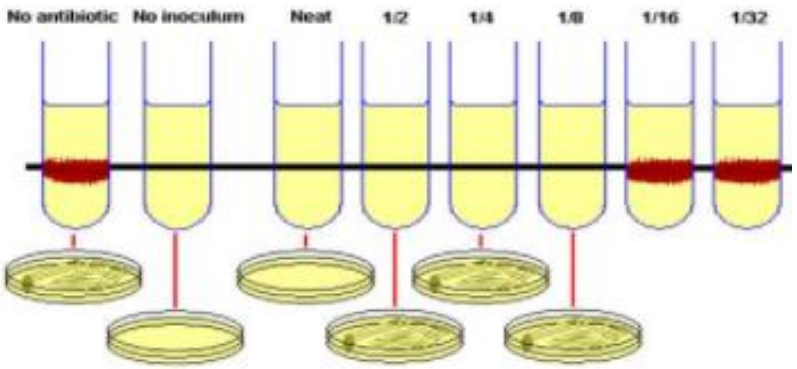
2. **Minimum Inhibitory Concentration (MIC):** This test is also called E test (Epsilometer). MIC is defined as the lowest concentration that inhibits visible growth. A strip has a gradient of concentrations of the drug. It gives the results in ug/mL.



3. **Broth Dilution Method:** Disk diffusion method has limitations; it does not tell you if the drug is bacteriostatic or bactericidal. Broth dilution method has this capacity to tell if an antibacterial is bactericidal or bacteriostatic. Various dilutions of the drug are made and tested with the test organism. This test tells both MIC and minimal bactericidal concentration (MBC). The same number of organisms in placed in all the wells, however, each well has a different concentration of the drug to be

tested. Growth pattern is then noted after overnight incubation with the drug. Minimum concentration of the drug that inhibits the growth of the organism is called the minimum inhibitory concentration.

a. **How to test if the drug is bactericidal or bacteriostatic:** To determine if the drug is bactericidal or bacteriostatic, the wells that do not show growth are important. Take the inoculum from the well which is next (higher concentration of the drug) to the well showing MIC and place this into nutrient broth tube. If growth of the organism is observed from the inoculum, the drug is bacteriostatic, otherwise it si bacteriocidal. The reason is that a bacteriostatic drug simply arrests the growth of the organism and as soon as the organism is inoculated onto a drug free medium, it starts growing. A cidal drug will kill the organism completely, and the organisms do not grow in the new plate or broth when cultured.



- **What is an Antibiogram?**
- Susceptibility of organisms is recorded over a period of time to see if there is a change in response. Drug sensitivity record that belongs to bacteria in a region is called an antibiogram. Such records are important for clinicians because they have to prescribe various antibacterials to patients for immediate treatment. Since sensitivity results become available only after 2-3 days, it is important for physicians to prescribe drugs that are known to work in an area.

Lesson 97. Read pages 559-567.

LESSON 97. EXAMPLES OF ANTIMICRIBIAL DRUGS

- Examples of antibmicrobials that act on the cell wall or inhibit protein synthesis, or disrupt plasma membrane, or interfere with nucleic acid synthesis, or compete with metabolites discussed in this video. Please read the book for details on them.

Lesson 98. Read pages 567-570.

LESSON 98. EXAMPLES OF ANTIFUNGAL AND ANTIVIRAL DRUGS

- Examples of antifungal and antiviral drugs are discussed in these videos. These are just few names that the student must know. Please read the book for details on them.

Lesson 99. Read pages 571-572.

LESSON 99. EXAMPLES OF ANTIPROTOZOAL AND ANTHELMINTHICS

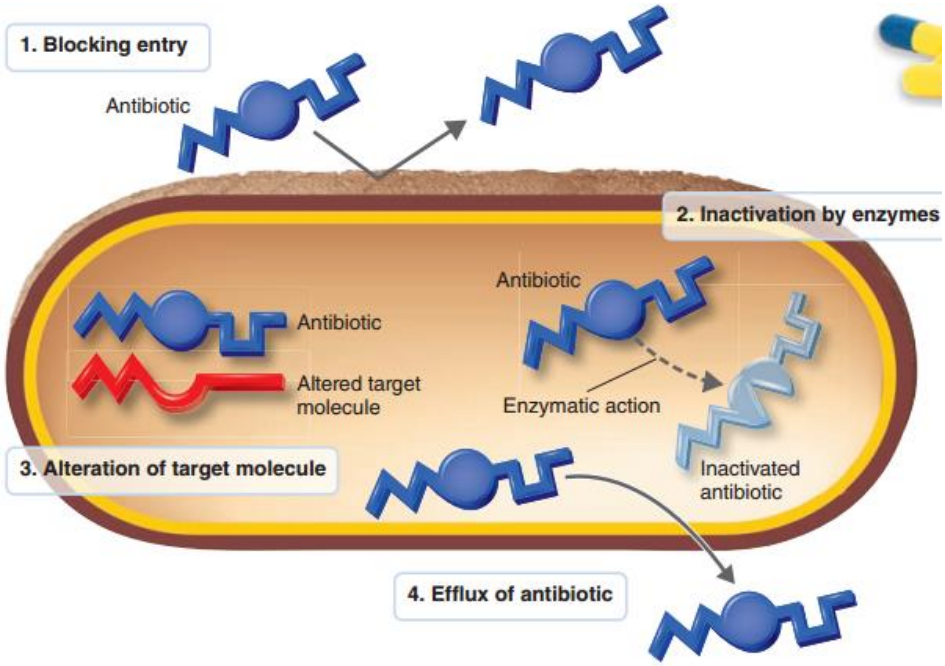
- Examples of antiprotozoal and anthelminthic are discussed in this video lecture. Please read the book for more details.

Lesson 100. Read pages 573-575.

LESSON 100. DRUG RESISTANCE AND HOW THIS DEVELOPS

- **Drug Resistance and its Causes**
- There are four mechanisms by which microbes can carry out resistance against antibacterials.
    1. **Blocking Entry:** Some organisms do not allow the entry of antimicrobials into them. In other words, organisms develop mechanisms to stop the antibacterials from entering into the cells. Some bacteria change their porin channels so drugs cannot penetrate the plasma membrane. It happens more frequently in gram negative cells than in gram positive microbes.
    2. **Inactivation by Enzymes:** Microbes develop specific enzymes that can degrade antibacterials and inactivate them or make them ineffective. A good example is beta-lactamase which degrades penicillins and other derivatives of penicillin.
    3. **Alteration of Target Molecules:** Many antibiotics targets ribosomes and stop protein synthesis in bacteria. However, a little change in the ribosome here and there can knock all those antibiotics that bind to these ribosomes and block them from protein synthesis. In other words, slight modifications in the ribosomes do not affect their functionality in terms of protein making; however, these changes are enough to make antibiotics ineffective because antibiotics cannot bind to the ribosomes anymore.
    4. **Efflux of Antibiotics:** Certain proteins in the plasma membrane especially in gram negative organisms act as pumps which can pump various antibiotics out of

5. the organisms keeping the final concentration of the antibiotic lower than what is needed to kill or stop the growth of the organism. These four mechanisms are depicted in the figure below:



- Please note

  that all these changes create mutants in the organism that outnumber wild type original microbes eventually and resistance becomes evident. This resistance is then transferred or transmitted by conjugation and transduction that we studied in genetic chapter earlier.

- **What is a Superbug?**
- When an organism or bacteria becomes resistant to many antibiotics, it is called a superbug because killing such an organism becomes a daunting task for the physicians. Here is the list of many such organisms that fall into the category of superbugs:
  - MRSA (most commonly): Because of continuous use, resistance to original penicillin emerged after a few years of use. Scientists introduced synthetic penicillin called methicillin which was more effective against penicillin resistant *Staph aureus*. However, soon after the use of methicillin, *Staph aureus* also became resistant to the drug. So these Staphs were named as Methicillin Resistant *Staphylococcus aureus* (MRSA). ***Now the same term MRSA is applied to many organisms that have become resistant to a wide range of Penicillins and Cephalosporins.*** Originally,

MRSA was confined to Staph aureus only which still is the most common MRSA pathogen, however, some other organisms have also been given MRSA status:

- *Enterococcus faecium*
- *Klebsiella pneumoniae*

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