Biology 1290B: An introduction to general microbiology.

1. Microbes, an introduction.

The scale of the "invisible world'; There are a thousand millimetres in a metre. There are a thousand microns (micrometres) in a millimetre, an *E. coli* bacterium is about a micron long – so a million of them lined up form a line a metre long, a cell of bakers yeast (a fungus) is about 10-15 microns in diameter. Some microscopic pond life is invisible to the naked eye, some are "just" visible.

Viruses are very tiny, only a fraction of a micron (say 20 - 100 nanometres - billionths of a metre).

Bacteria, fungi, and protozoa can be seen in a light microscope, but except for the larger protozoans, not with much internal detail. Viruses cannot be seen using a light microscope. Viruses can easily be seen using a transmission electron microscope, extensive details of cells can be analysed with an electron microscope.

The "Branches" of microbiology;

Bacteriologists - study bacteria, there are medical, agricultural, biotechnological specializations.

Mycologists - study fungi, there are medical, agricultural, biotechnological specializations.

Protozoologists, study small "animal - like" single celled organisms such as amoeba, and various disease causing parasites.

Phycologists study algae.

The study of **lichens** can also be regarded as a sub discipline of microbiology

Parasitologists- a term generally used to describe those who study small animals as agents of disease (like some microscopic worms for instance) but also used to describe those who study protozoan pathogens.

Immunology is often taught and researched in microbiology faculties.

Key figures in the history of microbiology

Robert Hooke *(*1635 - 1703**)** was a "polymath' he made many scientific discoveries in the 17th century, including making one of the first microscopes and also using a copy of one of Leeuwenhoek's microscopes to see and draw details of the structure of plant cells and some microbes.

Antony van Leeuwenhoek (1632-1723) made the first useful microscopes in the 19th century, they were fiendishly difficult to make and use, they were essentially a lens held in a metal clip, the lens was made from a tiny drop of molten glass, and he used such a microscope to see the first microscopic cells.

Ilya Metchnikoff (1845-1916) was the first to realize that animals such as us had a defence system against infection, what we now call the immune system

Paul Ehrlich (1854-1915), searched for the "magic bullet" against infectious disease, he synthesized the first successful (but very toxic) drug against a disease – syphilis, it was an arsenic derivative he called salvarsan.

Gerhardt Domagk (1895-1964) developed the first useful drug against a variety of bacterial infections, the first sulfa drug –prontosil. Ironically, he died of an infection!

Sir Alexander Fleming (1881-1955) and **Selman Waksman** (1888-1973) discovered the first relatively safe and effective antibiotics (of natural origin) – isolated from microorganisms. Fleming discovered penicillin, Waksman discovered streptomycin and a number of other antibiotics.

Louis Pasteur (1822-1895) was a chemist, he made many great discoveries, and he performed a crucial experiment using a swan necked flask that proved that new life did not just spontaneously arise from substances like rotting meat. For centuries before Pasteur, many people believed in Spontaneous Generation- the belief that life is generated spontaneously from dead organic matter. **Robert Koch** (1843-1910) and his colleagues made many important discoveries in microbiology, Koch initiated the use of the seaweed polysaccharide gel called **agar** as a stable material for the formation of a gel on which separated and pure (single species) colonies of bacteria and fungi could be grown (actually it was the wife of a colleague of his who suggested it), this was a critical advance, and he also stated and used his **Koch's postulates** (discussed later) required to prove that a given organism caused a given disease.

The nutrient medium in the flask shown on the left was first sterilized by prolonged boiling, and it then remained sterile for months even though the tip of the swan neck section was open to the air, because the neck section traps any spores or contaminants that could otherwise get down and into the nutrient broth. If the swan neck piece of the flask is broken off, the nutrient medium gets cloudy and bubbly because contaminant spores and other microbial forms can now get into the nutrient medium, and they grow. As long as nothing gets into the medium nothing grows, this showed once and for all that nutrient medium does not spontaneously form new life – Pasteur thus disproved spontaneous generation.

2. Cells

All living beings are **cellular** (most biologists do not regard viruses as being "alive'). The broadest definition of the structure of a cell is that it is a bag made of lipid enclosing a thick water based soup of lifes chemicals and processes. This is an absurdly inadequate definition though, which fails to impress on you how extremely complex and dynamic a cell is. The cell is bounded by a **plasma membrane** which is made of special phospholipids and is studded with many complex protein pores, channels, gates, receptors, recognition proteins etc. Within is the **cytoplasm** which contains water, and many chemicals and special structures, as well as the **genome**, the sum total of the cells genetic information - in the form of **genes** - linear DNA sequences, or in the case of bacteria – a single circular DNA macromolecule. Cells are exceedingly complex - they possess thousands of different chemicals undertaking thousands of different but interrelated reactions, simultaneously, at enormous speed and under exquisite control.

All cell membranes are based on what is known as the **lipid bilayer** structure, and are "studded' with very large numbers and many types of proteins, some of these span the membrane and are **transport proteins**, some are **receptor** or **recognition** proteins. There is a high capacity for these protein molecules to move around in the cell membrane, as can the actual lipid molecules of the membrane to some extent, so that the modern understanding of the general properties and structure of the cell membrane is referred to as the fluid-mosaic model.

The prokaryotic and eukaryotic cell membranes perform similar functions but differ in the chemical nature of their lipids, carbohydrate, and protein components. Cell membranes function in protection, transport, cell to cell recognition, and especially in bacterial cells they also participate in the biochemical reactions of metabolism.

Surface area to volume ratio (SA/V). The ratio of a cells surface area to its volume places critical limits on its lifestyle - its activities, and its size. As a cell increases in size (let us assume that the cell is a sphere), its volume increases far more than its surface area (in other words, as the radius of the cell increases by an amount X, its surface area increases by an amount proportional to X^2, but its volume increases by an amount proportional to X^3). This places a limit on how large a cell can grow, because the huge increase in volume relative to the increase in surface area means that there is now too little membrane surface area to cope with the increased import and export processes across the cell membrane required by the hugely increased cell volume. Eukaryotic cells have evolved some means to cope with this, principally by means of membrane bound organelles, but particularly by means of an extensive proliferation of internal membrane surface area called the endoplasmic reticulum, but the SA/V ratio does place fundamental limits on how large a cell can become. Bacteria do not 'solve" this SA/V problem, they remain small – this has it's advantages, a high surface area to volume area allows rapid growth for instance, and when this is coupled with the fact that their DNA is much more subject to mutation than is the case for eukaryotic cells– the result is rapidly proliferating growth and many mutation derived variants in the bacterial population that can quickly adapt to changing ecological circumstances.

There are two basic types of cell: **prokaryotic** and **eukaryotic.**

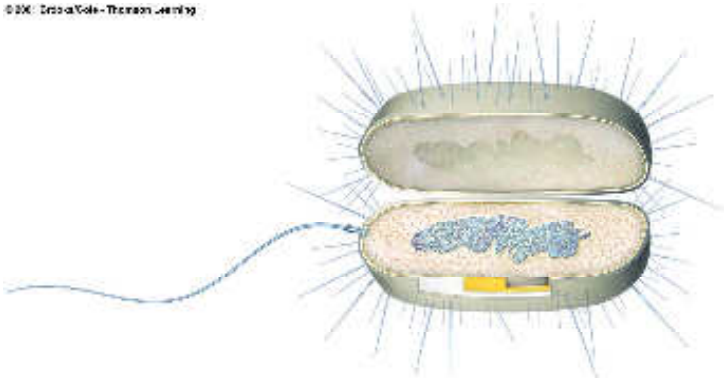
(Viruses are not cellular and most biologists consider them to be biological entities of course, but as not being alive in the generally accepted sense of the word. Do not make the common mistake of thinking of viruses as being cells, they are not!)

Prokaryotic cells do NOT have a discrete membrane bound nucleus that contains chromosomes, this is the defining feature of a prokaryotic cell. Prokaryotic cells have a none membrane bound **single circular** DNA chromosome (properly speaking it should not be referred to as a chromosome, but it in practice is often called a chromosome). ONLY the Archaea, Cyanobacteria and the Eubacteria are prokaryotic, ALL other cellular life forms are eukaryotic. Prokaryotes are absolutely and fundamentally defined on the basis of NOT having a nucleus, the region where their circular DNA macromolecule exists is called the **nucleoid**. Unlike eukaryotic DNA, bacterial DNA is not associated with special cationic proteins called **histones**.

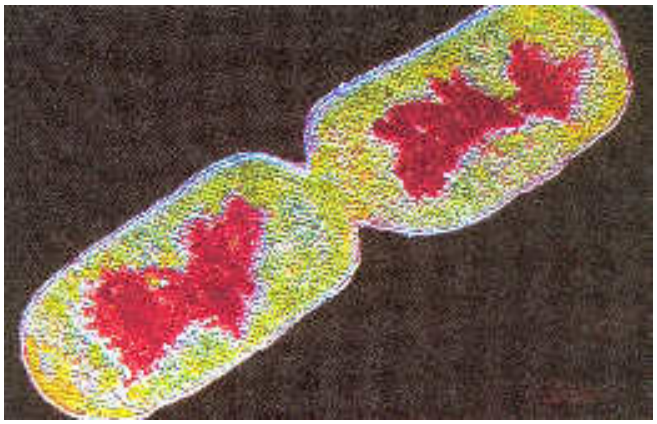
Eukaryotic cells DO have a *membrane bound* genome - consisting of more than one LINEAR chromosome, bounded by a double layered nuclear membrane.
Fungal, protist, plant and animal cells are eukaryotic.

Prokaryotic cells are much smaller than eukaryotic cells and though they can assemble together to form masses or chains, prokaryotic organisms are NOT multicellular in the accepted sense, which is that multicellularity involves association of different cell types in a discrete organism, and that the cells undertake division of labour, they have differing functions, this is seen only in a very simple way in some bacteria, which are fundamentally UNI-cellular. A typical size for a bacterium is that of a cell of *Escherichia coli*, about 1 um (one micron or one micrometer) diameter. A typical yeast cell (a fungus) might have a diameter of 6-8 um or more, and contains much more cytoplasm.

In terms of "internal architecture" prokaryotic cells are far simpler than eukaryotic cells, as we discuss below, they lack all of the complex internal membrane based structure of eukaryotic cells. Actually, there are some recent reports that do indicate the presence of some simple membrane bound vesicles in some bacteria, so it is no longer an absolute axiom to state that bacteria do not have membrane bound organelles (MBO's), but nonetheless, bacteria are still much less structurally complex, internally, than eukaryotic cells. The lack of complex internal structure in bacteria should not lead you into believing that they are also biochemically simple, bacteria have complex biochemical systems - metabolism in other words, they just do not operate their biochemical events within specialized "walled off" membrane bound organelle structures.



A typical prokaryotic cell, no nucleus, but a region where the DNA exists called a nucleoid, a cell wall, and a cytoplasm with no membrane bound organelles



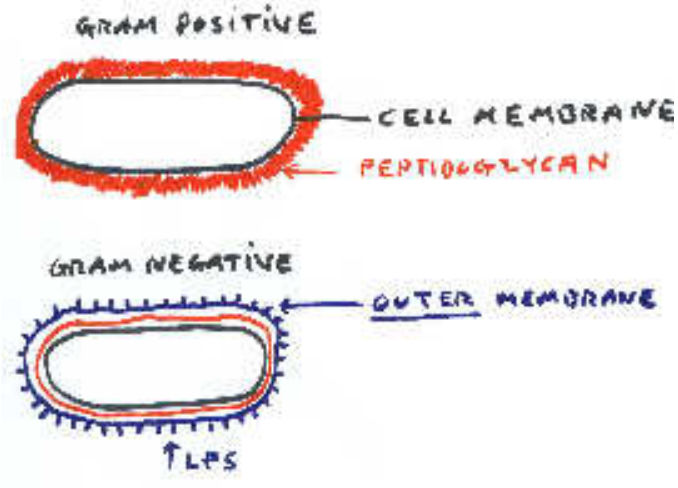
Thin section of a typical dividing rod shaped bacterium, transmission electron microscopy, false colored. The red region is the **nucleoid** where a non membrane bound single circular DNA genome is located.

Nearly all prokaryotes have a rigid **cell wall**, which determines the shape of the cell, and in most cases the wall contains **peptidoglycan**, a unique and complex acidic polysaccharide that lends rigidity to the wall. The bacterial cell wall serves to resist the very high internal osmotic pressure that would burst (lyse) the cell unless the wall was present. The cell wall is metabolically inert, it does not participate actively in transport processes in and out of cells, it is there as a structural strengthening agent – to provide rigidity and shape. Penicillins kill bacteria by interfering with the synthesis of peptidoglycan in cell walls as it is being formed during bacterial reproduction, this weakens the cell wall structure and the bacterial cell bursts. There are a few bacterial genera that do not have cell walls, *Mycoplasma* is a classic example, they are parasites of animals such as cattle and are able to exist at the same osmotic pressure as the animal cells that they live in and among. Archaebacteria DO have cell walls, but peptidoglycan is not a part of their cell wall structure.

Most Gram negative bacteria have a tiny but definite space between their cell walls and the cell membrane, this is called the **periplasmic space**. This space is of significance in pathological behaviour, it is metabolically active in many bacteria, it contains enzymes and transport proteins and other factors that mediate the ability of the bacterium either to cause disease in hosts or to evade destruction, enzymes in this space can, for instance, destroy antibiotics before they can enter the bacterium. A periplasmic space is present in some Gram positive bacteria, but it is much rarer.

From now on, unless I say differently, ALL of my discussion about prokaryotic structure and function should be taken as referring specifically to **eu**bacteria – these are the types of bacteria that cause human disease problems although the vast majority of the eubacteria are not pathogenic to man and in fact, many eubacteria are beneficial to man, either as symbiotic components of the digestive tract, as bacteria involved in food manufacture, or as components of our ecosystem that synthesize new biomass by photosynthesis or that fix nitrogen into soils for use by plants.

There are two major categories of bacteria on the basis of cell wall structure:
Gram positive bacteria have thick walls with a dense homogeneous layer of **peptidoglycan** (a complex polysaccharide polymer composed of two types of sugar subunit, - we look at this later)
Gram negative bacteria have thin cell walls with much less peptidoglycan and an EXTRA outer lipid membrane (not a cell membrane) that contains a toxic compound called lipopolysaccharide (LPS).



A Gram positive bacterium with a thick exposed layer of peptidoglycan. Gram positive walls also contain a unique substance called **teichoic acid**

A Gram negative bacterium with an extra outer lipid membrane covering a thin layer of peptidoglycan. The outer membrane contains lipopolysaccharide (**LPS**) also known as **endotoxin**

The term "Gram stain" refers to a dye staining *procedure* involving sequential treatment of bacteria on a slide with crystal violet dye, iodine, alcohol and safranin dye. We will look at this in detail in a later tutorial. The completed Gram stain has one of two results, the dyed cell is red when viewed under the microscope and is termed as Gram negative, or it is purple when viewed under the microscope and is termed as Gram positive. The particular Gram result is correlated with a particular cell wall structure, and it is the iodine treatment step that distinguishes between Gram positive and Gram negative, but the alcohol is the point where Gram positive and Gram negative are differentiated. The nature of the cell wall, Gram negative or Gram positive, has a high relevance to the general type of human disease the bacterium might cause and what kind of antibiotic might be used to treat it, and thus the Gram stain is the FIRST bit of knowledge one needs when analyzing a bacterial infection. For instance, many skin and wound infections are caused by gram positive bacteria, whereas many gut dwelling bacteria are gram negative.

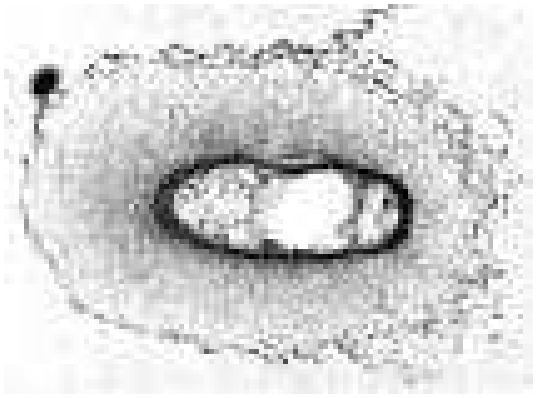
There are also a few bacteria which do not stain well by the Gram stain, such as the **acid-fast** bacteria, they have a lot of waxy lipid in their cell walls and capsules that interferes with the Gram stain. The tuberculosis and leprosy causing genus *Mycobacterium* which we will look at later, is an example of an acid fast bacterium which is initially identified in sputum or other body fluids by means of an acid-fast dye staining procedure. As mentioned, very few bacterial species lack a cell wall, *Mycoplasma* species are the best example, these commonly infect meat and are nuisance contaminants of cell cultures in research laboratories, *Mycoplasma* cannot be identified

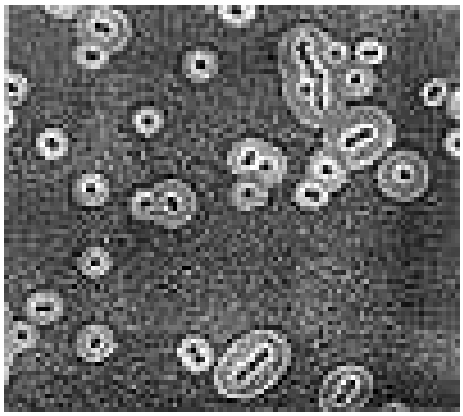
with a gram stain, they will accept the dyes involved, but the result is meaningless in the absence of a cell wall.

All cells, prokaryotic and eukaryotic, contain **ribosomes** (non-membrane bound organelles) which synthesize proteins, but those of the prokaryotes are smaller, this is an important consideration in treating bacterial infections, some antibiotics such as tetracycline attack and inactivate the smaller bacterial ribosome but not our larger eukaryotic ribosome. Ribosomes are complex macro (very large) molecules composed of a smaller and a larger subunit, they consist of a special nucleic acid (ribosomal nucleic acid) and protein and their function is to synthesize proteins by reading the mRNA copy of sections of DNA (more detail later on this topic). Ribosomes <u>are</u> organelles but they are NOT membrane bound. There are two sizes of ribosomes, 80S in eukaryotic cells and 70S in bacteria and mitochondria (don't worry about what is meant by 80S and 70S, just understand 80S is heavier than 70S). It is no accident that mitochondria have bacterial sized ribosomes - mitochondria evolved from bacteria.

Bacteria may have **flagella**, which are whip like structures that extend through the cell wall from the cell membrane, they allow movement, and some bacteria have much smaller flexible rod like extensions which are called **pili** (not found in eukaryotic cells) and function in reproduction and adherence to surfaces. When bacteria can move, either by means of flagella or a process called gliding which does not involve flagella, they are referred to as being **motile**.

Many bacteria have a thick layer called a **capsule**, which can help the bacteria to evade immune detection and make them more **virulent** (able to cause disease more aggressively).





The photo on the left shows a single bacterium surrounded by a thick capsule, the photo on the right shows a nigrosin negative stain which outlines unstained bacteria and their capsules which appear like surrounding halo's. The capsule may be composed of modified amino acids, small peptides, waxy lipids, or carbohydrate substances.

Membrane bound organelles are found in eukaryotic cells.

Membrane bound organelles (MBO's) serve to compartmentalize certain reactions into separated spaces in eukaryotic cells, this is important because there are many essential cellular biochemical reactions which are incompatible with other essential reactions, and organelles allow such reactions to be physically isolated so that they can occur at the same time, and the small specialized organelles also allow reactants to be more concentrated, this can produce faster more efficient reactions which can be better controlled. Remember - prokaryotes (bacteria) do not have membrane bound organelles (with the rare exception noted earlier), and this means that bacteria often have to separate essential reactions in time by performing one reaction after the other - this is less efficient and bacterial metabolic components cannot be localized in concentrated form in the cell in vesicles (they don't have any), which may slow down metabolic reactions.

The **endoplasmic reticulum (ER**) MBO found in eukaryotic cells is a complex folding of flattened membrane bound spaces, which often "crams' the cytoplasm of highly active cells and provides a large surface area for reactions that occur on or across membranes. Reactions also occur in the spaces in the ER, **rough** ER is "rough" in appearance because it is extensively "studded" on the exterior of the membrane with ribosomes which synthesize and release proteins into the volume enclosed by the ER membranes, **smooth** ER does not have associated ribosomes and is generally involved in producing steroids and other lipids or in reactions not involving protein synthesis. **Golgi bodies** are MBO's composed of a nested set of flattened vacuoles (a "stack of pancakes") which receive proteins and other molecules from the ER and prepare, modify (by addition of sugars or phosphates etc) and transport them in MBO's called **vesicles** (small membrane bound spheres), either to other places in the cell, or often, transport molecules out of the cell, Golgi bodies are also involved in forming lysosomes (below).

Other vesicles (a general term used for any small membrane bound sphere in a cell): L**ysosomes** contain destructive digestive ozidizing enzymes, **peroxisomes** neutralize hydrogen peroxide, and there are many small vesicles which pinch off to enclose and import materials (**endocytosis**) or which export substances from the cell (**exocytosis**).

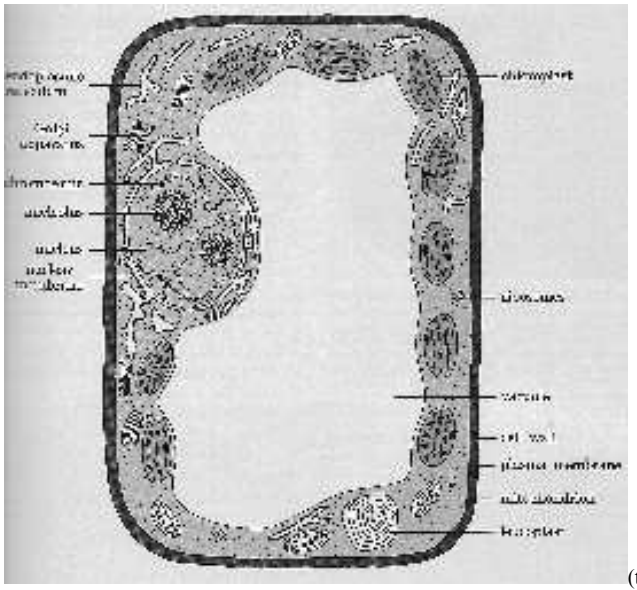
The **nucleus** is an MBO, because it is a spherical lipid double membrane enclosing the chromosomes The eukaryotic cell nucleus is a spherical double membrane structure which contains many pores, within it are the linear chromosomes (a pair each of the 23 different chromosomes in human body cells), anchored to the inner membrane by specialized proteins and stabilized by special cationic (positively charged) proteins called **histones**. The **nucleolus** found in the nucleus is where mRNA molecules are formed, which are formed into ribosomes which pass through the nuclear pores and function in the cytoplasm to synthesise proteins.

Eukaryotic cells have a **cytoskeleton**, a network of scaffolding formed from interacting protein filaments, **microtubules** and **microfilaments**, it gives the cell some internal structure and participates in transport of materials around the cell. The cytoskeleton is

constantly assembling and disassembling in various areas of the cell, a process that in some creatures such as amoeba plays a significant role in movement, prokaryotic cells do not have a cytoskeleton.

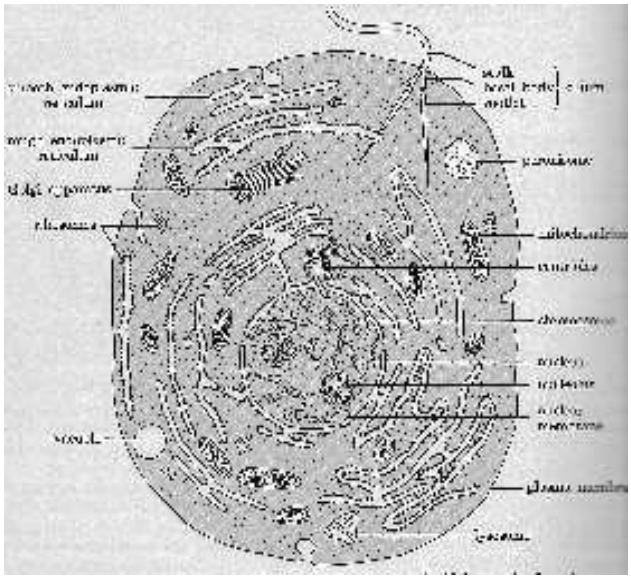
Eukaryotic cells may also have flagella, but they are of different structure and evolutionary origin than those of prokaryotic cells and they show a characteristic 9+2 arrangement of microtubules in cross section which is never found in prokaryotic flagella. Eukaryotic cells may also possess **cilia**, much shorter extensions that beat in a co-ordinated way. Protozoa commonly have flagella and cilia. Bacteria *do not* have cilia Eukaryotic cells (fungi, algae, plants) may have cell walls, but do they NOT contain peptidoglycan. Any eukaryotic cell that can photosynthesize must have **chloroplasts** (algae, some protists, plants) since these bodies perform photosynthesis in eukaryotes, prokaryotic cells never have chloroplasts (why?).

Mitochondria are the "energy factories" of the cell. It is the mitochondria in cells which require the oxygen we breathe, and they use it in the efficient metabolism of glucose and other molecules to produce energy in the form of ATP. It is these bodies which require the oxygen we breathe. Most of the ATP in a eukaryotic cell is produced by the mitochondria. Mitochondria have a double membrane, and the inner membrane is extensively infolded to form cristae which act to increase the surface area available for enzymatic metabolic reactions which are oriented on membranes. **Aerobic respiration** (which we discuss below) is the most efficient and the major energy yielding process, and it takes place in mitochondria.



This is a cross section drawing of a typical eukaryotic plant cell, containing chloroplasts as well as the other MBO's, and note the large central vacuole which is typical of many plant cells

(taken from Biological Science, W.T. Keeton 1972.)



This is a cross section drawing of a typical generalized animal cell, showing the usual features; many vacuoles, extensive ER, mitochondria, Golgi bodies and the large double membrane bound nucleus

(taken from Biological Science, W.T. Keeton 1972.)

Convincing evidence suggests that eukaryotic cells are the evolutionary consequence of a symbiotic relationship between an ancient large cell (a so-called ur-karyote) and bacteria that were engulfed by that larger cell but not destroyed. This is the **endosymbiont** theory for the origin of mitochondria and chloroplasts and other MBO's. Mitochondria and chloroplasts (and some other MBO's) are believed to be evolutionary modifications of ancient engulfed bacteria - this is the endosymbiont theory. Like bacteria, mitochondria and chloroplasts have their own genome, though they are no longer able to live free of the parent cell, and the genome is bacterial in type - a circular chromosome. Mitochondria and chloroplasts also produce their own ribosomes, but these are of bacterial size and structure. The inner membrane of chloroplasts and mitochondria shares chemical structures and features of bacterial membranes

Transport

Transport is a critical process in and out of all cells. Nutrients need to be taken in and wastes removed, and this includes gases such as oxygen and carbon dioxide. Transport involves an understanding of the basic features of diffusion and osmosis, which I will mention briefly here but illustrate more deeply in class.

Diffusion involves the movement of atoms and molecules across the membrane in response to their concentration gradient, only from an area of high to an area of low concentration.

Osmosis is a special case of diffusion where solutes move (diffuse) as described, BUT there is a **semi permeable membrane** in place - a membrane which has holes (pores) in it which allow some solutes to pass but not others. Think about what this interposition of a semi permeable membrane means in terms of the effect it has on the movement of water as well as the movement of solutes across the membrane. Imagine a long glass tube which we bend into a "U" shape, having glued a semi permeable membrane in place, inside, at the bottom of the "U". Now we fill the left side of the "U"

tube with pure water (no solutes in it), and the right side with the same volume of a 10% solution of sugar. For purposes of illustration here we will assume that water molecules are very tiny and easily cross through the holes in the membrane, but the sugar molecules are too big to cross through the holes in the membrane.

Now imagine you could easily count the number of water molecules in a given volume in either solution, what would you find? In the solution that contains sugar, say in one milliliter, you would find that there are less water molecules to count than in the one milliliter of the pure water solution in the other half of the tube. Why? because the space occupied by water molecules in the 10% sugar solution is now partly occupied with sugar molecules which have displaced some of the water - got it? Now you have a situation in which in effect there is more water per volume on one side of the membrane than the other, so, the water will move (as in the case of simple diffusion) from where it is in highest concentration - the pure water solution - to where it is in lowest concentration - which is the sugar solution. This will mean that as water moves across the semi-permeable membrane into the sugar solution the level of water in the sugar solution side of the tube will rise, and it will continue to rise until the osmotic force which is causing the water to cross the membrane from the pure water side to the sugar solution side is exactly opposed by the pressure exerted by the weight of the rising column of water in the tube. Alternatively, you could prevent the passage of the pure water across the membrane into the sugar solution by exerting a pressure on the surface of the sugar solution, and the amount of pressure you have to exert to prevent the movement is the **osmotic pressure**, and that pressure is dependent on the number of solute molecules present.

When two solutions are separated by a semi-permeable membrane the solution which has a higher concentration of solute is referred to as being **hypertonic** (or hyperosmotic), the solution of lower solute concentration is referred to as being **hypotonic** (or hypo-osmotic). If the two solutions either side of the semi-permeable membrane are of equal solute concentration then both solutions are said to be **isotonic** (or iso-osmotic). Water always moves from the hypotonic to the hypertonic solution if there is no opposing pressure. If the solutions are isotonic there is NO net movement of water across the membrane. How much water moves when osmosis occurs is of course determined by the magnitude of the solute concentration difference across the semi permeable membrane.
Osmosis is a critically important factor for living cells. In some cases it is essential, kidneys rely on osmosis for proper function, plants rely on osmosis for proper movement of water and nutrients. In other cases osmotic forces can be lethal, bacterial cells cannot actively counteract the osmotic force of water entering the cell and oppose that tendency by having a tough cell wall that resists the huge pressures generated by osmosis, so that bacterial cells are under very high pressures. Animals cells avoid osmotic problems by living in fluids which are of the same osmotic strength as the fluids inside the cells.

Facilitated diffusion across a membrane involves special protein molecules in the membrane that allow movement of specific atoms or molecules across the membrane in response to their concentration gradient, atoms or molecules cannot be accumulated

from an area of low concentration to an area of high concentration by this process– facilitated diffusion does not accumulate materials into the cell from where they are in scarcer amounts outside the cell, this is why the cell does NOT *expend* energy to perform facilitated diffusion.

Active transport is the transport of a specific molecule across a cell membrane *against* its concentration gradient (from an area of low to an area of higher concentration –you can think of this as an accumulation process in the cell, taking scarce nutrients into the cell and accumulating them) and this DOES require the expenditure of energy in the form of ATP. Generally, a given active transport protein in a cell membrane only transports a given molecule.

Endocytosis occurs when a small fragment of cell membrane captures food material from the exterior, pinches off to form a sphere and enters the cell. **Exocytosis** is the reverse process where small vacuoles from the cytoplasm travel to and then merge with the cell membrane and release material to the cell exterior, such as waste molecules. ONLY eukaryotic cells undertake these processes, and not all eukaryotes do it.

3. Taxonomy

Taxonomy is the science of the classification of organisms.

Taxonomy is a system of orderly classification of organisms into categories called **taxons**.

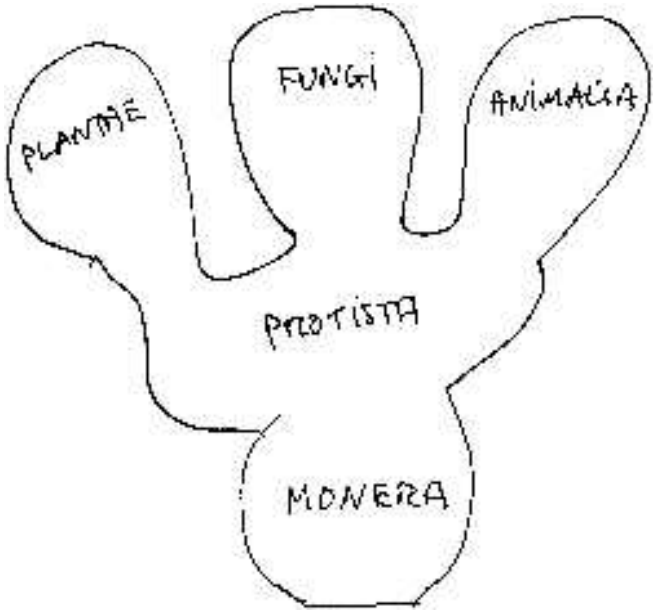
Taxonomy is based on the **Linnaean binomial system.** The original rationale behind this system is not used now, Carolus Linnaeus (he was Swedish, but he latinized his name) lived in a time centuries ago when it was not appreciated that evolution occurred. He classified organisms mostly according to similar appearance, but this can be misleading, in the absence of evolutionary theory fish and whales are grouped together for instance, because they look alike. The formal binomial naming method created by Linnaeus is still used, but the modern classification rationale is based on evolutionary relatedness.

All living cellular things (biological entities other than viruses and prions) have a **species** and a **genus** designation, and organisms are placed into groupings that reflect their **evolutionary relationships**.

For us, *Homo sapiens*, our genus is Homo and our species is sapiens. Note the correct form used, genus name is underlined and capitalized, species name is not capitalized and is underlined. Alternatively the two words can be separately underlined or can both be entirely capitalized (though this is rarely done). These look like really fussy, picky rules, but this is essential, serious misunderstandings can occur if this convention is not followed.

Escherichia coli is incorrect (not in bold or underlined or italicized). *Escherichia Coli* is incorrect (species epithet "coli" first letter must not be capitalized). *escherichia coli* is incorrect, the term is italicized but the genus must begin with a capital letter. It is acceptable and a usual practice to just use the first letter of the genus of a species PROVIDING that in any section of text the entire taxonomic name has FIRST been given in full, thus once the name *Escherichia coli* has been given in a written work it can then be referred to as as *E. coli*.

The Five Kingdom Classification System; Animalia, Fungi, Plantae, Protista, Monera.



This is *one* of the possible classification "trees", there are others, and as usual in taxonomy, this is a contentious issue, but the five Kingdom system is consistent with numerous sources of evidence.

Within each Kingdom each organism is nested into a **hierarchical classification** of taxons in the order - Kingdom, Phylum-Division, Class, Order, Family, Genus, Species. The order of this list is important (which accounts for use of the term hierarchical), each taxon holds progressively more numbers of taxonomically different organisms as one moves up the list from species level, thus a genus contains a number of species, a family contains a number of genera and thus contains more species than a single genus in that classification since each family contains a number of genera each with their own species.

Taxonomists - those who study the classification of organisms can be a HIGHLY argumentative bunch, put two of them together and you may get three opinions at to how an organism should be classified.

The **Prokaryotes** (Kingdom Monera) are:

1) The **eubacteria** (so called "true" bacteria - in future lectures, when I use the word bacteria - it refers to the eubacteria unless I state otherwise). Some of the eubacteria cause human disease, and this is why the eubacteria are those bacteria that are of main interest to medical microbiologists.

2) The **cyanobacteria** (so called and improperly referred to as blue-green algae) - common photosynthesising bacteria often noted as the green scum on ponds in summer months.

3) The **purple photosynthetic bacteria**, these perform photosynthesis but they do not use chlorophyll, they use special purple pigments instead, they are found in brine ponds for instance.

4) the **Archaebacteria**, a group of evolutionarily ancient bacteria which are adapted to living in extreme environments such as high salt, intense cold, high temperature, high acidity etc

ALL the other Kingdoms contain **eu**karyotic organisms:

The Kingdom **Protista**.

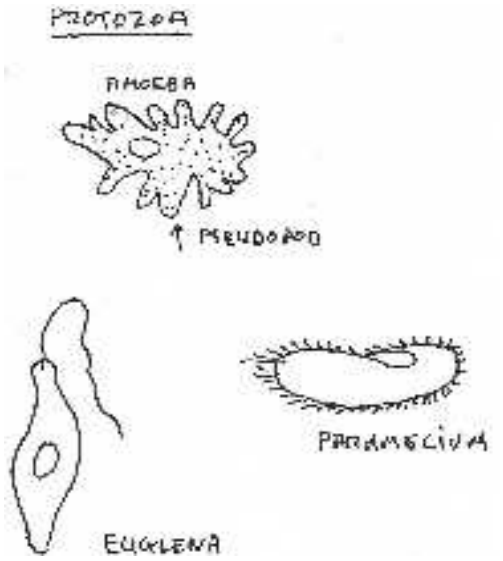
These are a diverse set of organisms

Algae - fresh and saltwater, single cells to simple multicellular photosynthesising organisms. Autotrophs, most have cell walls.

Slime molds- they are NOT fungi, look like fungi but can also be animal or even plant-like in morphology. Heterotrophs

Protozoa, a diverse group of single celled creatures that look and act like animals often, but are not, includes amoeba, paramecia etc, they are heterotrophs (some have autotrophic algae as symbiotic partners).

Some protozoa cause human disease. Some protists have plant and animal-like characteristics and are hard to classify - the Euglenoid protista are Heterotrophic and Autotrophic.



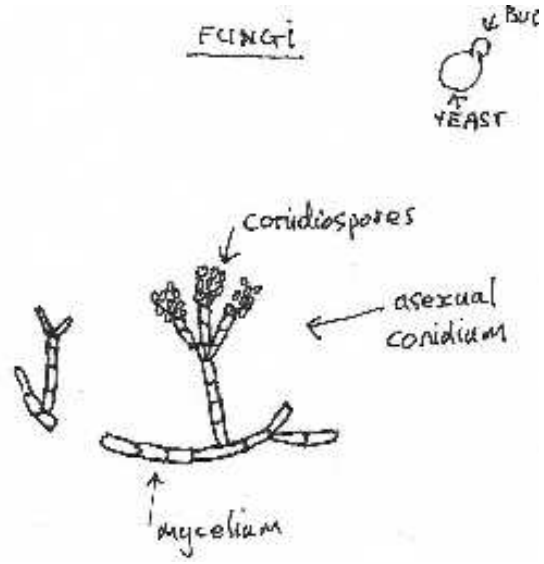
To the left are typical types of protozoa, many other forms exist. Amoeba flow around slowly by forming "false feet" called pseudopodia and drawing their contents up into them, *Euglena* is highly motile, and has chlorophyll. *Paramecium* is, like amoeba, predatory, it has a semi-rigid exterior covered in cilia and is highly motile.

The Kingdom **Fungi**.

Non photosynthesising single celled to multicellular organisms most of which have cell walls. Heterotrophs.

Includes **yeasts** (which are unicellular) and **mycelial** (filamentous) organisms such as bread mold and the mushroom forming fungi.

Some fungi cause human disease, some are important in food preparation.



To the left is a sketch of typical fungal morphology, a **mycelial** fungus is shown that is forming a non sexual spore generating body called a conidium, as found in *Penicillium* for instance. In the upper right is a **yeast** which is a single celled fungus, and this example is producing a bud, this is typical of the Brewers yeast *Saccharomyces cerevisiae*

The Kingdom **Plantae** – autotrophs.

Multicellular photosynthesising organisms with cell walls.

Trees, shrubs, bushes, grasses, moss, ferns etc.

The Kingdom **Animalia**. – Heterotrophs.

Complex multicellular organisms of diverse types, no cell walls, showing characteristic irritability and movement.

Some cause human disease (parasitic infections, worms etc or act as vectors for other disease causing organisms).

Viruses

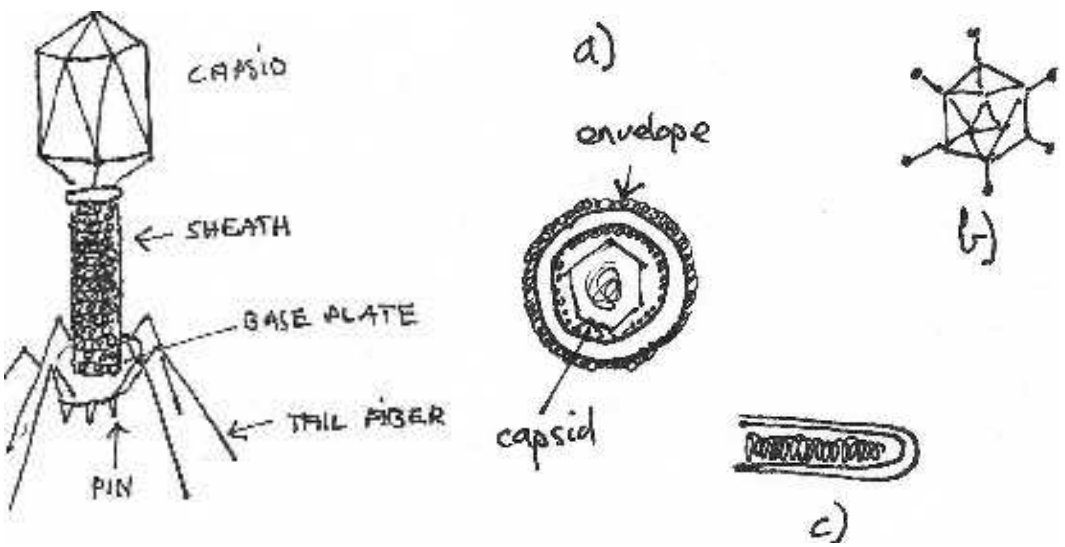
Viruses are not classified by using the Binomial naming system and do not belong to any of the five kingdoms.

Viruses are not cellular and are dependent on host cells for their replication.

Viruses are classified in two different ways:

1) according to their structure - genetic (DNA or RNA?) and physical (shape etc) – this scheme is favoured by scientists doing fundamental work.

2) according to the type of disease they cause, this scheme is favored by medical workers who need to correlate given viruses with given diseases.



To the left is a **T4 bacteriophage**, a virus that infects bacteria, it is unusually complex in structure. There are other types of this virus – or phage as they are often called, that lack "tails". Phage do not enter bacterial cells, the cell wall is too rigid, they "inject" just their nucleic acid instead and the nucleic acid initiates "hijacking" of the bacterium and causes many copies of itself to be synthesized.

To the right are more examples of virus shapes, a) is a retrovirus (the AIDS virus (HIV) is a **retrovirus)**, it has an outer lipid envelope derived from the host cell membrane as it leaves the host cell.
b) is an **adenovirus**, this virus is being used in experiments designed to use viruses to carry human DNA to target cells, as part of genetic engineering therapy for inherited diseases.
c) is a **rhabdovirus** (rhabdo- more or less means bullet shaped), rabies virus is the best example.

4. Growth and culture of bacteria.

First, a reminder from general high school microbiology:

Organisms can be categorized based on how they obtain nutrition:

autotrophs form their own organic molecules from simple inorganic compounds, there are two types of autotrophs (this word literally means self-feeder):

> **photoautotrophs** - use light energy to form organic compounds from simple inorganic materials such as carbon dioxide, water and salts (plants, algae, cyanobacteria).
>
> **chemoautotroph** - use energy obtained by breaking down simple inorganic molecules (some bacteria).

Heterotrophs must use pre-formed organic compounds (such as glucose) that were formed by the autotrophs. Fungi, animals, many bacteria, protozoa are heterotrophs.

Bacterial growth

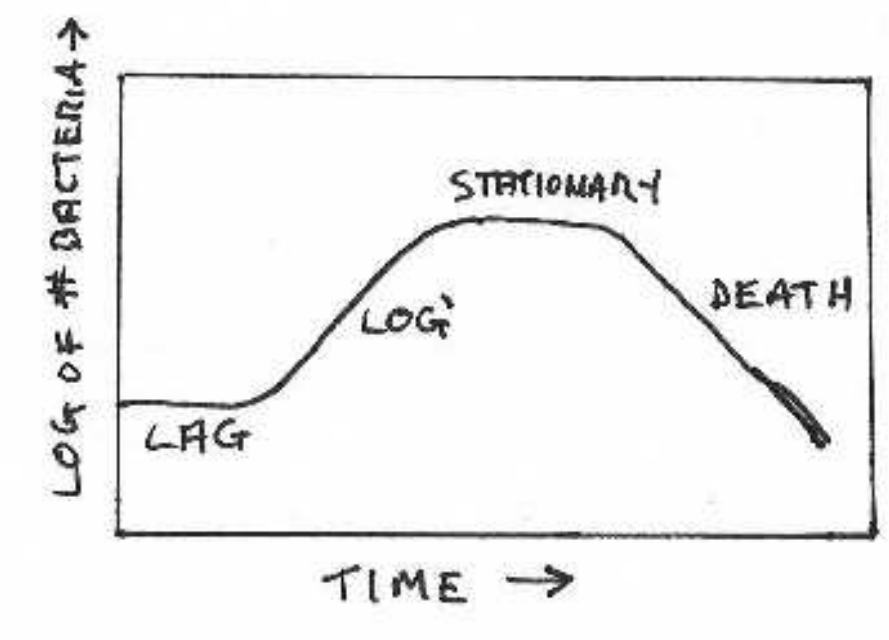
A typical cell of the bacterium *E. coli* can reproduce in as little as twenty minutes under good conditions (lots of nutrient and a warm temperature - 37 C). This is much faster than the average eukaryotic cell (Bakers yeast for instance, might manage to reproduce asexually by a process called budding once an hour, at best). If you do the math, this means that ONE bacterial cell becomes millions within a day, this has great significance, potentially, for your health and safety. Bacterial cells attain a very limited cell size and they reproduce frequently, so that bacterial growth is defined as the orderly increase in numbers of bacteria over time. Bacterial cells do not have multiple linear chromosomes as our eukaryotic cells do, so they do not undergo mitosis or meiosis.

If a single bacterium the size of *E. coli* has a generation time of 20 minutes, and nothing impedes the division of successive progeny, within 48 hours a mass of bacteria will be formed that is 4000 times as great as the mass of the earth! Obviously, there are factors which limit this growth! – principally availability of resources, presence of competitors, buildup of toxic metabolites etc.

Bacterial cells undergo a replication of their **single circular chromosome** and then the cell "splits in two" in a process called **binary fission**. Many bacteria also contain much smaller circular DNA molecules, these are classified as extrachromosomal DNA structures, and they are called **plasmids,** they commonly hold genes for antibiotic resistance or toxin production, and these also replicate as the bacterium undergoes reproduction, the plasmids are a key concern in the pathogenesis and virulence of many bacteria that cause human disease. Extrachromosomal DNA is also introduced into bacteria by some infecting viruses, this is called **transduction**, these viruses can errantly include bacterial genes in their capsids as new viruses form, and then they introduce these pieces of DNA into the bacterium they infect. So, bacteriophage can transfer bacterial genes from one bacterial species to another, In some cases genes transferred by transduction can increase the virulence of a disease caused by the receiving bacterium.

A rod shaped bacterium (technically, a rod shape is described as a bacillus) will grow in length while binary fission is underway, and then produce a "cross wall" called a **septum** which results in two individual bacteria. The name "binary fission" was given by researchers because this is what was observed (a literal translation of binary fission into simple English is "splitting in two), binary fission is a complex event mechanically and chemically but it looks like a simple splitting in two under a microscope. Imagine that you have inoculated a flask of culture fluid (containing nutrients and agitated to mix oxygen into the fluid) with a small loop full of bacteria (it will hold millions of bacteria even though there does not appear to be anything on the loop). One can take samples regularly and count the number of bacteria present. The typical results of bacterial growth when plotted in a graph with numbers of bacteria expressed in logarithmic form will be what you see below.

Note that the vertical axis is the number of bacteria counted, and it is on a logarithmic scale, the horizontal axis is time.



The time it takes for a bacterium to undergo one cell division is called the **generation time**. There will often be a short **lag phase** in the culture before one sees an increase in bacterial numbers. This lag phase is generally seen while the inoculated bacteria adjust to the new culture fluid, but if one were to inoculate bacteria from one active culture into a culture fluid of the same composition at the same temperature then one often does not see a lag phase.

The growth will then be **exponential** – a straight line on a semi log graph indicates this, this phase is also referred to as **logarithmic** or **log** phase, and it is in this phase that microbes are growing rapidly in numbers and are most metabolically active, and it is in this phase that they are most susceptible to an antimicrobial agent.

Later, the graph will begin to curve, this is a transition phase as the culture fails to provide needed nutrients to allow bacterial cell division, then the graph will show a horizontal line for some time, this is **stationary phase** – as many bacteria are dying as are being generated, or maybe in some cases bacteria are neither reproducing nor dying.

Finally the curve descends, this indicates **death phase** in the culture. It is possible for some bacteria to be reproducing in the death phase, but remember that the growth curve represents the behaviour of a huge population of bacteria, in the death phase many more bacteria are dying than are reproducing.

In some bacterial species (notably the Gram positive genera *Bacillus* and *Clostridium*), **endospores** may be formed as the culture nears death phase, these are tough dry spherical bodies that can endure long periods (often many years) in harsh chemical and climatic

conditions, and will germinate to produce a new bacterial cell when conditions are suitable.

Special microscope slide counting chambers are used to count cells in fluid cultures, and there are a number of automated instruments that will do the same thing. Or, a process called serial dilution and plating onto agar is used and I will describe this in class or tutorial.

How quickly a bacterial culture increases in numbers depends on a variety of factors, such as temperature, pH, oxygen content, amount and type of nutrient. There is an optimum value for all of these factors for the best growth of any given bacterium. Many bacteria are inhibited from growing at acid pH's, but there is an important group of bacteria – the **acidophiles**, of numerous different species, that *prefer* to grow at acid pH (generally down around pH 2-4), indeed many of them actually produce acids, and some acidophiles are important in food production, such as those involved in yogurt production.

Temperature: *Psychrophiles* grow best at lower but not excessively colder temperatures (15-20 C but sometimes as low as 0 C, a few pathogens are psychrophiles), *mesophiles* at 25-40 C (thus most bacteria that are human pathogens are mesophiles), *thermophiles* at >40 C. Some bacteria (like some that associate with deep sea volcanic vents) have to have high temperature in order to grow, sometimes up around the boiling point of water, these are obligate thermophiles, some of these are economically important (one of them provides an essential enzyme used in DNA amplification for criminal DNA fingerprinting). It is obvious that most human pathogenic bacteria are mesophiles, since this is within the temperature range of the human body (approximately 37 C).

Oxygen. Some bacteria will not grow unless oxygen is present, these are **obligate aerobes**. Some bacteria need oxygen to grow, but at very low levels, these are **microaerophiles**, some bacteria must have NO oxygen present in order to grow, these are **obligate anaerobes**, in fact some obligate anaerobes, such as the *Clostridia*, are actually killed by exposure to even small amounts of oxygen. Some bacteria grow with or without the presence of oxygen, these are **facultative**.

Bacteria that can be grown on solid medium (agar, a non nutrient polysaccharide gelling agent extracted from seaweed) will form colonies, the shape, colour, texture etc of these colonies will vary with temperature and the type of medium used, but within controlled conditions the nature of a colony on visual inspection, accompanied with simple colour reactions for oxidase and other enzymes, can give good preliminary clues as to what type of bacterium is present when combined with the performance of a Gram stain. Proper formation of colonies requires that few enough bacteria are applied to the medium so that one can be statistically certain that the colony grew from a single bacterium, if you don't do this properly it is possible to get more than one type of bacterium forming a mixed colony in the same spot and this can lead to confused interpretation.

Bacteria have specific nutritional requirements, and there is wide variation as to what any given bacterial species needs in its "diet". I will discuss this more in class. The differences in the ability of bacteria to utilize given chemicals as nutrient forms the basis of diagnostic tests used to identify their species (taxonomic analysis). Many bacterial (and fungal) organisms **cannot** be grown on general laboratory culture media, some will not grow on any kind of medium. Some microbes are **fastidious**, they have special nutritional needs that are not met by standard growth media, some bacteria need high salt conditions to grow, these are called **halophiles**. Many bacteria will not grow on standard culture plates because they are strict anaerobes and have to be grown in an oxygen free atmosphere. Some bacteria are described as fastidious because they need a specialized growth medium, as with the bacteria that cause gonorrhea, they grow best on a blood based medium. The point here is that one must NOT assume that use of standard media will allow growth of the bacteria that may be responsible for a particular problem or infection, careful analysis of the situation has to take place so that other tests and growth conditions can be applied, if there is a suspicion that a causative organism may not show up using the faster and more convenient aerobic nutrient agar and other media.

Bacteria can be grown in liquid medium, or more conveniently on the surface of a growth medium gelled with a seaweed polymer called **agar**. Agar is a polysaccharide purified from certain marine algae, and it has an unusual property that makes it ideal for use as a solid growth base. When agar powder is placed in water it is insoluble until the water is brought to boiling point, when it will melt and dissolve into solution (agar is very expensive, but you only need 1.5 to 2% in the medium). This molten agar based solution will NOT set solid until the temperature drops to below about 44 C. The great advantage of agar is that once it has set, it will NOT melt until it is heated all the way up to boiling point again. What this means is that there is a wide temperature range at which the agar based medium will remain solid and stable. This was not the case with other media used early on in microbiology, such as gelatin, which would form a gel base, but this would melt if the temperature was raised much more than a few degrees. Agar itself is NOT a nutrient in microbiological growth media, if it was, it would be no use because it would break down as colonies grow on it. It is the substances that are dissolved in solution along with the agar that are the nutrients, agar just provides a stable nutrient containing gel platform on which colonies can grow.

Complex growth media contain rich organic materials such as beef extract, yeast extract, peptone (hydrolysed beef), malt extract etc, these are rich in the nutrients many bacteria need. They are called complex media because, while we know from experience that they are good substances for growth media, they contain many different compounds and we are not always sure of the exact composition of these media.

Defined media consist of mixtures of known compounds (salts, glucose, minerals, vitamins etc) in known amounts. Defined media are used to test for the ability of an unknown bacterium to use known compounds, such as nitrogen or carbon compounds as sole sources of that element, and as such, defined media can be used to identify bacteria according to what sources they can and cannot use.

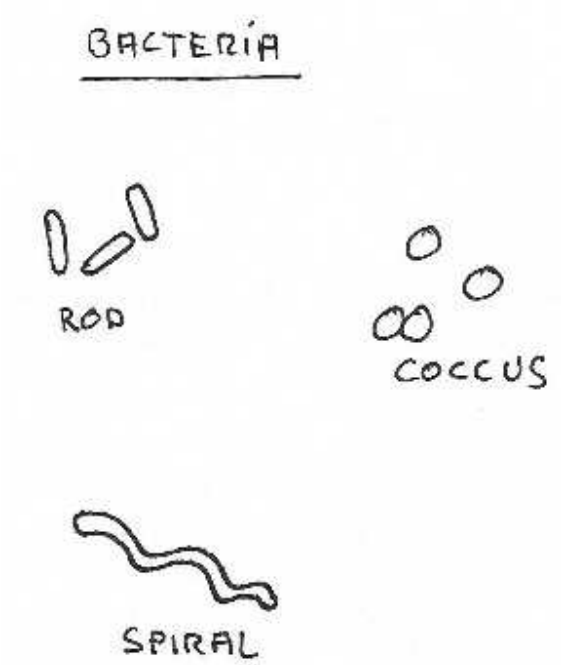
Irrespective of whether a growth medium is complex or defined, it is also sometimes possible to compose a medium so that it excludes growth of a given bacterial type (selective medium), or so that it allows different bacteria to grow but differentiates between them in some way (differential medium).

Selective media (which may be complex or defined) allow a particular type of bacterium to grow in preference to another, an example is certain media that contain dyes that will selectively allow one kind of bacterium to grow and not another - based on what their Gram type is (Rose Bengal agar for instance, which selects for the growth of yeasts and molds)). **Differential** media (which may be complex or defined) allow more than one type of bacterium to grow but identify one of the bacterial colonies - usually by means of a colour change caused by a chemical reaction that a particular bacterium can undertake but the others on the plate cannot, an example of this is EMB agar (Eosin-Methylene Blue) which specifically gives colonies of *E. coli* a metallic dark sheen, while other colonies of bacteria are darkish but do not have the metallic sheen.

Strictly anaerobic organisms have to be grown in the absence of oxygen, I will discuss this further in class or tutorial

5. Identifying bacterial species.

The first thing to do (if it is possible) is to grow them on agar to form pure colonies and then take a sample of these and look at them under the microscope, this gives you their morphology – their shape which gives a first clue used in identification. Growing them in culture may not always be possible, they must then be observed directly from samples, this is more difficult and you cannot be certain that the bacterium or other microbe you are looking at, amidst the others in the field of view, is the one of interest, it takes experience in such a case to know that you are viewing the correct organism.



The sketch to the left shows typical bacterial shapes. In some cases bacteria can become associated to form filaments, clumps etc. Rods (this shape is technically referred to as a **bacillus**) can associate to from chains, **cocci** (spherical bacteria) can associate to form chains (as with *Streptococcus*), or grape like clusters as with *Staphylococcus*), or packages of two or four contained within a membrane. Some bacteria are spiral in form, others are slightly curved – this is called a vibrio shape. In some cases a single bacterial species may show a wide variety of shapes, in this case the bacterium is said to be **pleomorphic**, and this is an important point to remember when trying to identify a bacterial pathogen from its shape.

1) **Identification of bacteria by their physiological profile ("diet")**. Bacteria are grown on agar plates which contain all that is necessary for bacteria to grow except for a particular sole target nutrient. Microorganisms need a source of nitrogen, a source of carbon etc, and species differ as to what form of nitrogen or carbon etc they can use. The tested nutrient that is added to allow growth, to make the medium complete in effect, might be a given amino acid since some bacteria might use the nitrogen from it and not from another amino acid, or a given sugar might be used as a carbon source while another sugar may not be used by a given bacterium, or a compound might be included in test media which identifies a given enzyme the bacterium may or may not possess. Testing of physiological profiles is often accompanied by microscopy to verify the shape of a bacterium since whether or not it is a coccus or a bacillus or even pleomorphic (which can add confusion!) can give initial identifying features along with the result of the Gram stain

Performing such a physiological profile can involve dozens of different types of plates. Each plate usually holds a **defined synthetic medium**, which is a medium where one knows the chemical identity of all of the constituents and can tailor the composition of the medium so that one ingredient is used to test to see if the bacterium growing on the medium can use that ingredient. Perhaps you design the medium so that the only carbon source is lactose, some bacteria will use the lactose as sole carbon source and will thus grow and form colonies, others cannot grow to form colonies because they cannot use the lactose as sole carbon source. In this way a profile can be developed for a given bacterium, of carbon and nitrogen and other sources that it can use. The pattern of use of individual components will differ for each bacterium and this can be used to identify the bacterium.

So, the plates are checked to see if the bacterium grew using a given nitrogen or carbon source, or in the presence of other compounds, or if it gave a positive colour reaction for presence of a given compound or enzyme. The enzymes that can be tested for include aminases, phosphatases, polysaccharidases such as cellulase, catalase, proteases, lipases, various oxidases etc.

The pattern of negative and positive results obtained by all these tests is correlated with the pattern of results verified for known bacterial species to find out which corresponds with the unknown bacterium. The result is confirmed with other findings, such as Gram stain, morphology, motility etc, and where the bacterium was isolated.

These tests can be run on commercial devices which package the tests into one small strip of test media.

The **Gram stain** result is the primary bit of information for identifying bacteria. In a Gram stain procedure the test bacteria are smeared and heat fixed onto a slide, then in sequence Crystal violet (a purple dye), iodine, alcohol and safranin (a red dye) are applied, the slide is dried and viewed by light microscope at high power (details of this procedure will be given in a tutorial session). Gram positive cells retain the first purple dye and are thus purple, Gram negative cells do not retain the first dye and take up the last dye, which is red, so they are red or pink. The iodine in the second step is critical, it acts as a **mordant** – a fixative- with Gram positive bacteria, it causes the crystal violet not to wash out when the alcohol (the **differentiating** step) is applied. The iodine does not act as a mordant in Gram negative bacteria, in these the crystal violet is washed out by the alcohol so that the red dye is taken up. The cell wall of bacteria is where the iodine exerts its mordant action.

Complex media were mentioned previously, these consist of nutrients such as yeast extract or beef extract etc, which are of complex and generally unanalyzed precise composition and as such are usually not as useful for generation of a physiological profile, but are used for general cultivation of bacteria and fungi.

2) **Identification by DNA and protein analysis**. It is possible to obtain patterns of presence-absence of certain microbial proteins when they are separated on a gel in an electrical current - this is called electrophoresis. This pattern can be identified as belonging to one or a small group of specific bacteria.
The same sort of analysis can be done with microbial DNA. Patterns of bands of DNA on a gel, which have been obtained from certain areas of a bacterial genome and "cut" in a specific way by special enzymes, can be correlated with particular bacteria or groups of bacteria.

Both these methods can be used to perform taxonomic analysis, and in medical and forensic diagnosis. Reaction of microbes with specific antibodies is also used to identify particular strains of a species of bacterium, at one time this was commonly done with *Streptococcus*, using antibodies to identify bacterial strains is called serological testing.

Bergey's Manual is THE "bible" of bacteriology, it is the "place to go" to find the information that allows identification of bacteria. For many years Dr R.G.E. Murray (still going strong in his mid 80's) was the chief editor of this manual, as was his father before him, Dr Murray is a Professor emeritus of the Microbiology department here at Western.

The same types of biochemical tests and DNA and protein analyses can be used to identify fungi. Because fungi are larger than bacteria and can produce filaments, cells, and spores of a wide variety of shapes and sizes, microscopic examination of fungi is a more useful means to identify fungi than it is for bacteria.

6. Viruses

A French-Canadian was one of the early pioneers of the basic biology of viruses - Felix D'Herelle.

"Viruses are bad news wrapped in protein" - Sir Peter Medawar

Viruses are **obligate intracellular parasites**, they have to infect a cell, "hijack" control of the cell and cause it to make new copies of the virus. Viruses are not cells, for that reason most biologists do not regard them as being "alive". Viruses are very small, much smaller than eukaryotic cells, much smaller than bacteria.

Viruses do not have cytoplasm, do not have a metabolism. Some viruses do house one or a few enzymes, but this is not an indication that they have a metabolism, they do not. The AIDS virus(HIV) for instance, is a retrovirus, this means that it copies its RNA genome into DNA that is then integrated with the host cell DNA. In order to achieve this copying from RNA to DNA an HIV virus carries a unique enzyme called reverse transcriptase.

Viruses are essentially nucleic acid within a capsule made of protein.

The viral genome contains far fewer genes than cell genomes, from less than a dozen (as in the influenza virus) to a few hundred at most, as opposed to thousands or tens of thousands in cell genomes.

The viral genome can be DNA or RNA, but not BOTH.

The protein shell or capsule of viruses is called a **capsid**, and is composed of many smaller protein subunits called **capsomeres** (sometimes spelled as capsomers).

Some viruses have many glycoprotein "spikes" which dot their capsid surfaces and act to attach the virus to specific receptor sites on the host cell membrane.

The capsid has a distinctive shape, it can be **Polyhedral**, or **filamentous**, or a **complex** capsid composed of a number of capsid shapes.

Some viruses that infect animals have an outer lipid membrane derived and modified from the cell that was forced to replicate and release the virus - it functions in making the virus more infectious and is called the **envelope**. Viruses that infect cells that also have a rigid cell wall (such as bacteria, fungi, plant cell etc) do not form an envelope. All types of cells have viruses that infect them. Viruses are generally specific as to what type of cell they will infect. Viruses that infect potato do not infect us, viruses that infect one genus of animal generally do not infect another (there are well known exceptions to this rule though – like flu viruses!) So, viruses that infect animal cells have a **host range** and within that range they have a **viral specificity** - which refers to what cell types they can infect.

Note that bacteria are infected by viruses, and bacterial viruses are known as **bacteriophage** or "phage" in common usage. Bacteriophage are of great significance, they can be one means by which new and dangerous bacterial variants arise because bacteriophage can "accidently" pass virulence related genes from one bacterial species to another. And some bacteriophage contain genes for toxins that can be produced in bacteria. Bacteriophage are also critical tools in modern biotechnology research and industry. Many bacteriophage actually carry an enzyme that is found also in human tears and other body fluids, it is called **lysozyme**, it is used by the bacteriophage to break down peptidoglycan in the bacterial cell wall so that the phage can enter the bacterial cell. Much of the modern understanding of how ALL viruses replicate and infect was taken from studies with the **T even phage** that infect bacteria.

When a virus infects a cell and directs that cell to make new copies of itself, it is a very different process than cell reproduction and is therefore NOT called reproduction - it is called **virus replication**.

There are five stages in virus replication: (This was mostly worked out using bacteriophage infections of bacteria, and the terminology applied to the bacteriophage infection cycle is used here, and of course, this is a continuous process, the "steps" are imposed by humans so that we can understand this continuous process);

1. **Attachment**- specific attachment of the virus to the host cell, it attaches because it binds with specific molecules on the cell wall surface of the bacterium, the same principle of specific recognition of a binding or attachment site is found with viruses that infect animal and other cells that do not have a cell wall, and in that case it is molecules projecting from the target cell membrane that act as recognition sites so that the virus will attach to the cell.
2. **Penetration** - the virus (or in some cases - just its genome) enters the cell. When a cell has a cell wall (bacteria, fungi, plant cells, algal cells) the usual strategy is for insertion of just the genome of the virus into the cell cytoplasm, cell walls are rigid and difficult for entire virus particles to penetrate. In animal cells, the entire virus enters.
3. **Synthesis** - viral genes are transcribed and then direct the host cell membrane to make components of the virus. In many cases the first step is the destruction of the host cell

genome, followed by transcription and translation of viral nucleic acid that results in generation of virus components.

4. **Maturation** - the viral components assemble into complete virus copies, this is a thermodynamic process, it is not directly (as far as I can gather) controlled, it is just that there is a thermodynamically favourable tendency for the different virus particle components to associate in the correct way to form a functioning virus particle.

5. **Release** - the new viruses are released, which may or may not kill (**lyse –** this word means to burst in this context) the host cell. In some cases, as with cells that have cell walls, the virus has to direct lytic processes that lyse or burst the cell by first damaging the cell wall. In other cases, as with animal cells that lack a cell wall, there may be an immediate release of virus particles that bursts and kills the host cell, or the virus can escape from the host cell by forming an **envelope** derived from the host cell membrane, and this can be done in a way (essentially exocytosis) that does not cause host cell destruction, at least not immediately, so that many viruses leave the cell in this fashion.

The immediate viral infection, replication and exiting of newly replicated viruses from cells, with the destruction of the host cell is called the **lytic cycle.**

In some cases viruses penetrate and infect a cell but do not immediately direct the synthesis of new viruses, instead, they **integrate** into the host cell genome without killing the host cell, they insert their genes into the DNA in the host cell chromosomes, and the viral genes are "quietly" copied along with the host cell genome as it reproduces. When a bacterial virus genome is in this integrated form it is called a **prophage**. If a bacterium is growing and reproducing while it contains a prophage it is said to be in a **lysogenic cycle** of growth. At some later stage, a matter of perhaps days, and sometimes years, a stimulus (uv light is an example) triggers the virus genome to activate to begin replication of new viruses, when this occurs the bacterium is now in a lytic cycle.

As mentioned, when this delayed integrated mode of virus infection is found in bacteria the practice is to call it a **lysogenic** cycle, but when a similar thing occurs in animal cells such as ours, it is often called a **latent** infection. This terminology can be a bit confusing, since it is possible to have a virus infection described as latent when there are actual free virus particles present but not causing disease, and in other cases the term latent infection is used when no actual virus particles are evident and the viral genome remains integrated and inactive in the host cell genome. Often, in animal virus infections, where the viral genome has integrated with the host genome, the viral genome so inserted is referred to as a **provirus.** The AIDS virus - HIV - undergoes a prolonged provirus phase. As mentioned, the term latent is also applied to whole, formed viruses in animal cells that are not active, they are dormant and can be triggered, often years later, to become active and promote disease, the classic example is shingles, this is caused by viruses that had previously caused chicken pox, and when the patient recovered, viruses remained in some of the nerve cells in the body and activate much later on to cause the painful blistering condition called shingles.

Viruses that cause infections in animals often cause a characteristic and diagnostic **cytopathic effect** (CPE) when they are grown in artificial animal cell tissue culture. The cultured animal cells form a sheet, one cell thick, that adheres at the bottom of the container of nutrient fluid. Viruses added to the culture cause "holes" to appear in the sheet when they infect cells, by causing dead infected cells in the sheet to detach from the bottom of the container, as well as causing the cells to change in shape. Animal viruses in cell culture are also often observed to cause the formation of large multinucleate cells that are a result of the fusion together of numerous cells, these are called **syncytia**, which are commonly observed in the cytopathic effect. The CPE often involves the production of diagnostic **inclusion bodies** inside infected cells, a classic example is the dark tiny Negri bodies, these are granules that are found in nerve cells infected with rabies. Inclusion bodies are often masses of viral nucleic acids or proteins.

I mentioned before that some animal viruses possess an outer lipid membrane - the **envelope** (sometimes also called a coat). When such a virus is replicated in an animal cell it is released from that cell in a process of exocytosis, which does not kill the cell. In that case the virus retains that small piece of the host cell membrane that encloses it while it is exiting the cell, inserts some viral proteins into it, and it becomes the viral envelope. Possession of that envelope allows fusion of the virus into the cell membrane of a new host cell - the envelope assists in infection. "Naked" animal viruses do not have an envelope, they generally have many spikes of **glycoprotein** which recognise and bind the virus to host cell receptors.

Some viruses can cause birth defects - eg., Rubella. Some viruses can cause cancer - eg., human papillomavirus, some strains of which cause genital warts, and some types of which cause cervical cancer - there is a new vaccine that is touted as giving excellent protection against cervical cancer caused by the papillomavirus, and there are plans to give mass vaccinations to pre-adolescent females.

Except for extreme cases involving the very old or the immune compromised, until recently there has been little option for treatment of virus infection with drugs. Some powerful anti-nucleic acid (DNA or RNA) replication drugs (these are usually nucleotide analogues) have been used for decades to treat established flu in people who are at very high risk of fatal infection – such as the very old in whom the flu can be life threatening, but they can have serious side effects and there are now many reports of resistance. The last 5 years or so have seen the development and use of some effective drugs against an established flu infection that work in a different way, these tend to be agents that block receptor interactions, and will be mentioned when we discuss the flu – Canada has established large stocks of doses of one of these agents.

Penicillin, tetracycline, and the other antibiotics used for bacterial infections are USELESS for treating viral infections.

The MAIN defence against viral infections is the **immune system**, and also by activation of the immune system to recognise the virus PRIOR to infection, by vaccination (**immunization**).

7. Eukaryotic organisms that cause human disease – parasitic disease.

Generally speaking, although one can fairly say that ALL pathogens are parasites, a term that is applied to an organism that lives at the expense of another, this term has been reserved for protozoans and small animals which infect humans to cause disease.

Since we are soon going to concern ourselves heavily with infectious disease, let's all agree on what we mean when we say pathogen, a pathogen is any biological agent (a microorganism usually) that can actively cause disease in a target organism.

Fungi. Fungi are of two basic forms, one is the **yeast** form – yeasts are defined on the basis of being unicellular spherical or ovoid cells that exist independently and don't normally form filaments, they reproduce asexually by **budding**. The other form is as a **mycelium** (mycelia plural) - a mass of branching filaments each known as a **hypha** (hyphae plural). There are some fairly common infections caused by fungi which are fortunately, superficial, and they tend to be regarded as not being serious (ie, by anybody except those who suffer from them) this includes; vaginal yeast infections, skin infections such as athletes foot, jock itch etc, Thrush (a yeast infection of the throat). Many of these can be treated successfully by application of special creams- topically, though some may require systemic treatment (pills).
Thrush is caused by the yeast *Candida albicans*, a normal constituent of skin and vaginal flora. Contrary to popular belief, there are many species of yeasts, not just *Saccharomyces cerevisiae*, the brewing and baking yeast, and *Candida* is NOT that yeast! *Candida* is one of the yeast species that causes vaginal yeast infections, it is an **opportunistic pathogen** – especially when a woman is on the contraceptive pill, and the increased hormone levels alter the nutrient status of the vaginal secretions to encourage *Candida* to grow, inappropriate vaginal douching can also predispose to yeast infections and prolonged antibacterial antibiotic treatment can cause *Candida* infections because the bacteria killed by the antibiotics normally compete with the yeasts for nutrients and so govern and limit their numbers.

The original version of 1290B was developed by Dr Andre Lachance of UWO's Biology department, he is a justifiably world renowned yeast biologist, I did my PhD with him and I admire his passion about the subject of yeasts. There are MANY more yeast species than *Saccharomyces cerevisiae* – the one that features so prominently in human food and beverage production. There are hundreds of yeast species, many of which are useful to humans, they make bread, beer, wine, vitamins, medicines, industrial chemicals, pigments used to give farm raised salmon a pretty pink colour, and so on. Yeasts are a primary tool in fundamental scientific research, many basic discoveries in human physiology and genetics were made using yeast model systems, and Dr Lachance has made major contributions to the understanding of speciation and microbial ecology using yeasts. Yeasts are important as living creatures "in their own right' just as we are, they participate in many kinds of complex ecologies.

There is one species of yeast that is regarded as a true pathogen – *Cryptococcus*, it is not usually a causal agent of vaginitis, but can be found in some people who are susceptible,

where it can cause serious skin and even lung and internal infections. For reasons that are not clear, a virulent strain of *Cryptococcus* has been found in recent years on Vancouver island and has caused some serious infections. The earliest reported case of infections on the island caused by this species – *Cryptococcus gattii* was in 2000, and since 2004 a hundred cases have been reported in immuno-compromised patients, many of the infections were of the lungs.

In some cases otherwise superficial fungal infections may signify a larger problem, with the immune system, because the person has AIDS for instance (Thrush is a common first symptom).
So-called surface infections CAN be a persistent and debilitating skin problem in very hot and moist climates where they are hard to treat and many of the sufferers are poor and cannot afford treatment.
Some serious and potentially fatal internal (**systemic)** infections can be caused by fungi, they are difficult and risky to treat with drugs, and it can take a long time to cure these diseases. Systemic fungal infections are frequent killers of terminal stage AIDS patients and of people who have compromised (damaged) immune systems because they are aged or are suffering from cancer – anticancer drugs frequently impair the immune system and this allows internal fungal growth.

Histoplasmosis and **Aspergillosis** (pigeons are a key vector) of the lung are classic fungal infections. Immune compromised cancer patients and those in end stage AIDS can suffer systemic (body-wide) and often fatal fungal infections by fungi which are not a danger to people with normal immune systems, many end stage AIDS sufferers are killed by black yeast or black mycelial fungal infections of the brain, these black fungi are similar to the organisms that are the basis of my research.

Fungi also cause problems as producers of **toxins** in foods. The **ergot** fungus *Claviceps purpurea* releases lots of toxins into **sclerotial** bodies it forms on infected wheat, these can cause many problems when ingested in contaminated bread, hallucinations, loss of blood circulation, fever, gangrene and can sometimes be fatal. *Aspergillus flavus* releases **aflatoxins** that can cause liver cancer, a common problem area for this is in peanuts, strict inspections and control apply in Canada, Of course, one must also NOT eat wild mushrooms unless one is very sure which are edible and which are poisonous.

Keep in mind that antibiotics that attack eukaryotic organisms are potentially dangerous - YOU are a eukaryote and in some cases drugs taken internally to treat a eukaryotic infection (fungal, protozoan, animal) may attack and harm YOU.

Protists.
Algae: Believe it or not, algae can be a health hazard, *Pfisteria* is an alga, and in summer months this alga blooms (forms extensive areas of growth, often as dense mats floating on the surface) and can release potent neurotoxins into lake and marine waters (though this is also caused by cyanobacteria), or into seas. Red Tide is another toxic bloom forming alga (genus *Gonyaulax*) found in warm marine waters, it can cause serious and sometimes fatal poisoning if fish or clams etc are eaten that have consumed large

numbers of the alga. Algae do provide food products, Irish Moss is harvested extensively (as is done off the coast of the Maritimes region) because it provides a gum like polysaccharide used in foods and cosmetics, agar is an algal product as well as edible sea weeds such as dulse and nori.

Protozoa. ALL protozoa are unicellular. Many are normal and harmless components of water and soil, but there are a number of protozoa which can cause serious human disease, some of them, such as *Plasmodium* (which causes malaria) are carried to their human hosts by vectors such as mosquitoes. People who have single copies of the genetic sickle cell anemia trait (nearly all are of African origin) are resistant to malaria because their red blood cells do not support survival of the protozoan agent responsible for malaria. Many experts state that malaria is the cause of the greatest number of deaths from infectious disease. Other examples are *Giardia* (causes Giardiasis or Beaver fever) and *Cryptosporidium* which cause serious intestinal problems and are contaminants of surface waters. One protozoan - *Trichomonas*, causes a vaginitis, a sexually transmitted disease (STD).

Some experts maintain that the most prevalent fatal infection in the world today is **malaria**. One new book I have read (The Fever Trail, author M. Honigsbaum) states that there are currently as many as 500 million cases of malaria and up to 1.7 million deaths a year, half of whom are children in sub Saharan Africa (this is 3000 deaths of children a day). Over the last 15 years AIDS has killed about 18 million people, malaria has killed at least 23 million. Some experts maintain that of all the human beings that have ever existed half were killed by malaria.

Small animals. “Worms”, this word is an imprecise term taxonomically, creatures from many genera are described as worms, some of them are serious parasites of humans. Flatworms such as **flukes** and **tapeworms** cause serious liver and intestinal infections. Some of the roundworms (nematodes) cause serious parasitic infections such as hookworms and pinworms, elephantiasis, trichinosis. Many of the “higher” animals are of consequence in human disease (from insects to birds and more) because they act as reservoirs and vectors of disease causing organisms.

Elephantiasis; this is a very serious problem in undeveloped areas of the world, Known as Lymphatic filariasis, this disease currently affects around 120 million people in 80 countries, and 40 million of these people have been seriously infected with the disease. This disease is extremely rare in Western countries and is found almost exclusively in the tropics. The disease is caused by a number of tiny nematode “worms”. A concerted worldwide effort is now underway to try to eradicate this disease, the manufacturers of an effective drug against the disease are giving the medication away free, but political problems are preventing its use in some countries. The grossly swollen tissue damage that is seen with this disease is very often irreversible.