MEGA LECTURE

A2 Biology – Revision Notes
Unit 7 – Microbes And Disease

Bacteria

1. A microbe is any organism that is not visible with the naked eye (requiring a microscope to see). The unaided resolution of the eye is about 0.1mm.
2. Important scientists in microbiology:
    a. Anton Van Leeuwenhoek - used a simple lens and focusing device to see microbes.
    b. Louis Pasteur – disproved the theory of spontaneous generation, using Pasteur flasks. He also demonstrated that heat-treated anthrax bacteria could be injected into sheep to provide them with immunity to anthrax. He developed pasteurisation (for wines).
    c. Edward Jenner – the first person to use vaccination. He studied the role of cowpox in developing immunity to smallpox.
    d. Joseph Lister – the first person to use a disinfectant during surgery. He used a carbolic acid (phenol) spray over the operating area.
    e. Lady Mary Wortley Montague – discovered the variolation technique for vaccination (introducing infected material into a scratch in the skin).
    f. Benjamin Jesty – noticed that people having had cowpox did not get smallpox. He used variolation to infect his family with cowpox, so that they did not contract smallpox.
    g. Robert Koch – developed the conditions for proving that a specific microorganism causes a specific disease, known as Koch's postulates.
    h. Alexander Fleming – discovered penicillin, the first antibiotic.
    i. Christian Gram – developed the Gram staining technique for bacteria.
3. Bacteria are classified according to their shape:
    a. Cocci – spherical bacteria:
        i. Cocci – smallest bacteria, occur as single spheres.
        ii. Diplococci – pairs of spheres, e.g. *Diplococcus pneumoniae* (pneumonia).
        iii. Staphylococci – clusters of spheres, e.g *Staphylococcus aureus* (boils and food poisoning).
        iv. Streptococci – chains of spheres, e.g. *Streptococcus pyogenes* (sore throats).
    b. Bacilli – rod-shaped bacteria:
        i. Individual rods – e.g. *Escherichia coli*, *Salmonella typhi* (typhoid fever).
        ii. Chains of rods – e.g. *Azotobacter*, *Bacillus anthracis* (anthrax).
    c. Spirilla – large, spiral-shaped bacteria (motile with flagella), e.g. *Treponema pallidum* (causes syphilis).
    d. Vibrio – crescent-shaped bacteria (motile), e.g. *Vibrio cholerae* (causes cholera).
4. Bacteria a prokaryotes, i.e. they do not have a nuclear membrane. They do not contain mitochondria (the mesosome is the site of respiration), and have no membrane-bound organelles. Bacterial ribosomes (70S) are smaller than those in eukaryotes (80S). Some bacteria have:
    a. Pili (fimbriae) – projections of cylindrical protein rods (of pilin) on their surface, allowing bacteria to link together.
    b. Flagella – long, fine projections that enable movement. They may be attached to the end of the cell (polar), or around the cell (peritrichous).
    c. Capsule – a layer of polysaccharides or polypeptides outside the cell wall.
5. Gram staining divides bacteria into two distinct groups:
    a. Gram-positive bacteria (take up the stain) have a thick, single-layered, cell wall containing peptidoglycan. These are more susceptible to penicillin and iodine.
    b. Gram-negative bacteria (do not take up the stain) have a thin peptidoglycan layer in the cell wall, surrounded by a second lipoprotein membrane. These are more susceptible to antibodies and complement.
6. Bacteria reproduce at a phenomenal rate. The main form of reproduction is binary fission:
    a. Cell elongation results in the synthesis of additional cytoplasm and nuclear material.
    b. DNA replication takes place (there is no mitotic spindle), and the nuclear material attaches to the plasma membrane or mesosome.
    c. A septum begins to develop, and the nuclear material is distributed to both sides.
    d. The septum is completed, and a cell wall develops to divide the cell into two.
    e. The two daughter cells grow to a critical size, and then repeat this process.
7. New genetic material can be inserted into a bacterium in three main ways:
    a. Conjugation – the bacteria link together by their pili. The donor passes a plasmid called the F-factor (fertility) to the recipient cell (these are often two distinct strains – not

male/female, but plus/minus). The F-factor may be in a plasmid (replicating independently), or incorporated into the main bacterial chromosome.
   b. Transformation – one bacterium releases DNA which is absorbed by a second bacterium, allowing it to acquire new characteristics.
   c. Transduction – new genes can be inserted into the bacterial chromosome by a bacteria phage (a virus acting as a vector).
8. Plasmids that carry genes allowing the bacteria to survive adverse conditions are called R-factors. Antibiotic resistance can therefore be transferred from one species to another, resulting in multiple drug resistance. Bacteria phages can be used to treat bacteria, but these are not effective internally.
9. The energy for bacterial growth comes from two main sources:
   a. Sunlight – photosynthesis (using bacteriochlorophyll) by photoautotrophic bacteria. The plasma membrane of phototrophic bacteria is extended into thylakoids within the cell.
   b. Oxidation of chemical compounds:
      i. Heterotrophs – feed on organic matter (usually decomposers) by respiration (aerobic or anaerobic).
      ii. Chemoautotrophs – oxidation of simple inorganic compounds for energy source.
10. When culturing bacteria it is important to use aseptic techniques:
   a. Equipment can be sterilised using gamma irradiation, or by placing in an Autoclave (this is also used to sterilise nutrient agar) for about 15 minutes.
   b. A transfer chamber is used to avoid contamination.
11. When counting bacteria, the total cell count includes all the living and dead cells, whereas the viable cell count is only a measure of the living cells:
   a. A haemocytometer is a slide with two grooves, and an island between the grooves. A cover slip is placed over the island, and a sample is introduced by capillary action. There is an etched grid on the slide, so the bacteria can be counted using a powerful microscope. The volume is known, so the concentration and hence total cell count can be estimated.
   b. A colorimeter can be used (after calibration with a haemocytometer) to estimate total cell count, by measuring the turbidity of the solution.
   c. A serial dilution of a broth can be taken, and each dilution plated onto agar, until individual colonies can be observed and counted. Assuming that each colony arises from a single bacterium, the number of colonies can be scaled up by the dilution factor to give an estimate of the viable cell count.
12. The bacterial population growth curve occurs in four main phases:
   a. Lag phase – the cells are active, but there is little increase in number. The cells accommodate to the new conditions, take in water and synthesise ribosomes and enzymes.
   b. Log (exponential) phase – Nutrients and space are in plentiful supply, so there is little competition, and the bacteria multiply at their maximum rate.
   c. Stationary phase – the carrying capacity (maximum number of bacteria that the environment can support) is reached, so intraspecific competition takes place between bacteria. Hence the death rate balances the population growth rate, and the number of bacteria remains roughly constant.
   d. Death phase – the nutrient supply is running out, and the waste products accumulate, resulting in increased toxicity of the environment. The organisms are killed, and the population size eventually falls to zero. Spores may be produced during the stationary phase, that are resistant to the adverse conditions.
13. Factors affecting the growth of bacteria include nutrients, space, temperature, pH (drops due to the production of CO_2, converted into carbonic acid), oxygen, waste products, and the presence of antibiotics/poisons. These must be controlled when growing bacteria commercially.
14. Bacterial growth can be controlled using physical methods (gamma irradiation or in an Autoclave using high temperatures) or by chemical means:
   a. Disinfectants – reduces the number of bacteria on inanimate surfaces, but may not remove all spores and microorganisms (e.g. phenolic compounds).
   b. Antiseptics – can be used on living tissues to kill or inhibit the growth of microorganisms:
      i. Bactericidal (or fungicidal) chemicals kill bacteria (or fungi).
      ii. Bacteriostatic (or fungistatic) chemicals inhibit the growth of bacteria (or fungi).
15. Bioassays (using bacterial growth to assess something) can be used to determine the effectiveness of bactericidal chemicals:
   a. Phenol coefficient test – take a series of dilutions of phenol, and a series of dilutions of the disinfectant. Inoculate each of the dilutions and incubate in a water bath at 20°C. Take a subculture every 5 minutes on an agar plate, and incubate for 2 days. The phenol

coefficient is the concentration of disinfectant is the concentration needed to kill all the bacteria after 10 minutes (but not after 5 minutes), divided for that needed by the phenol.

b. Use-dilution test – take a series of concentrations of a bacterial broth, and contaminate stainless steel cylinders with each concentration. Place these in various concentrations of the test agent for 10 minutes, and then into a culture medium. The growth pattern indicates the 95% effective concentration of the test disinfectant.
c. Dilution plate technique – pour and inoculate a number of agar plates, and add increasing concentrations of disinfectant. Incubate for 48 hours, to find the minimum concentration needed to inhibit growth.
d. Disc diffusion test – pour and inoculate a number of agar plates, and place wells around the plate. Fill the wells with varying concentrations of disinfectant, and inoculate. The diameter of the clear zone around each well is an indication of the effectiveness of the disinfectant.

16. Antibiotics can be either narrow-spectrum (affecting a few types of bacterium) or broad-spectrum (affecting a wide range of bacteria):
    a. Therapeutic dose – the dose of antibiotic needed to kill or inhibit the bacteria.
    b. Toxic dose – the level that causes damage to the host.
    c. Therapeutic index – the ratio of the therapeutic and toxic doses.
    d. Minimum inhibitory concentration (MIC) – the lowest concentration that prevents growth of a specific bacterium.
    e. Minimum lethal concentration (MLC) – the lowest concentration that kills a specific bacterium.

Commercial Biotechnology

1. Microorganisms may be cultured commercially in order to obtain a substance that they produce. They must be able to:
    a. Produce the substance in large quantities in a small amount of time.
    b. Be available in pure culture, and be genetically stable.
    c. Grow rapidly in large-scale culture.
    d. Not be harmful to humans.
    e. Be capable of easy removal from culture.
    f. Grow on readily available and cheap raw materials.
    g. Have growth conditions that do not require extremes of temperature.
2. Yeasts are widely cultured commercially, and are used in the production of:
    a. Food substances in their own right, and in bread production.
    b. Beers, ales, lagers and wines (fermentation to produce alcohol), and alcohol as a biofuel.
3. Substances produced by microorganisms are classified into two groups:
    a. Primary metabolites – produced during the growth of the organism (normal growth products). These include amino acids, nucleotides, acids, ethanol and enzymes:
        i. *Acetobacter sp.* – production of ethanoic acid.
        ii. *Aspergillus niger* – production of pectinases.
        iii. *Bacillus subtilis* – production of proteases (tenderising meat).
    b. Secondary metabolites – chemicals that are not directly involved in normal growth, e.g. antibiotics. These are produced after the main growth period is completed.
4. There are two main types of process for the production:
    a. Batch processes – raw materials and microbes are placed together in a container vessel. The microbes are then allowed to grow to their maximum population size, then the fermenter is emptied and the products are extracted and purified.
    b. Continuous process – the nutrients are continually inputted into the fermentation vessel, and the material is continually removed and processed. This may be more economical, as it doesn't have to be shut down on a regular basis.
5. In order to develop a large industrial fermentation process, four main stages are required:
    a. Isolation of the microorganism – the organism must be able to be purified easily, and give the optimum product yield. The fungus used to produce Quorn was selected from 20-30 different strains.
    b. Culture preparation – the master culture is stored by freeze-drying (lyophilising) in a small space. Small samples are taken from the master culture in order to produce stock cultures, from which working cultures can subsequently be produced.
    c. Laboratory scale ($200cm^3$ fermenter) – the optimum conditions (pH, temperature, aeration, mineral balance etc.) are determined using a small-scale fermenter.

   d. Pilot plant (200 to 500 dm^3 fermenter) – the laboratory fermenter is scaled up to make sure that it works on a larger scale. There may be problems with heating/aeration etc, which may require electric mixers, cooling mechanisms etc.

6. There are two types of fermenter:
   a. Surface culture – the microorganism is contained on a flat surface, and the substrate runs over the surface. In the production of citric acid, shallow trays are stacked to give a cascade system – a mixture of the medium and citric acid is extracted from the base of the column. The medium is acidified to pH 2.5 in order to stop the production of oxalic acid, and to prevent contamination.
   b. Submerged culture – the organism is kept submerged in a nutrient medium. Sterile air is blown through the medium to provide oxygen, in a process called sparging. The mixture must be continually stirred to keep the organism in contact with the medium. This can be done in two ways:
      i. Air-lift fermenter – the air enters at the bottom of the fermenter, and is used to mix the medium and the organism, by causing them to follow a circular pathway (like a convection current).
      ii. Stirred fermenter – a series of large paddles continually rotate, to mix and to break up any lumps of fungus forming. Sterile air is still introduced at the base, but it is not used to do the mixing.
   c. Surface culture uses simple technology, and is cheap to run, but has high labour costs and a low overall yield. Submerged culture uses complex technology, and is expensive to run, but has low labour costs and a high overall yield.
7. In the production of penicillin, the fungus *Penicillium chrysogenum* is used in a batch process:
   a. The fungus is cultured in cornsteep liquor, with an additional nitrogen source, a carbon source, and buffers to maintain a pH of 6.5.
   b. New nutrients are added after the main growth phase, as penicillin is a secondary metabolite. The penicillin is secreted into the medium, so the medium is extracted, and the fermenter is batch filled again. Downstream processing then takes place.
8. Downstream processing is the extraction and purification of the product, and accounts for up to 50% of the total cost. For penicillin production:
   a. The slurry is run off from the tank and filtered, to extract the microorganisms.
   b. The filtrate is processed to extract the penicillin, using a countercurrent flow system. The filtrate is run in the opposite direction to butylacetate, separated by a membrane. The penicillin is absorbed by the butylacetate, and is dissolved in it. Potassium salts are added to precipitate out the penicillin, which is then washed, dried and filtered.
9. In the commercial production of enzymes, screening must take place to select the best strain. Extracellular enzymes (ones that are secreted by the microorganism) have three main advantages over those that are intracellular:
   a. The enzyme is already outside the cell, so the cells do not need to be broken open.
   b. Only a limited number of enzymes are secreted, so isolation is much easier.
   c. Extracellular enzymes are less likely to be broken down by heat/chemicals (more robust).
10. Screening can be used to detect the presence of extracellular enzymes:
   a. Amylases are detected by plating on agar containing starch. After incubating, iodine is added, so that the microbes that break down the starch have clear zones around them.
   b. Proteases are detected by plating on agar containing casein (makes the plates look cloudy). The microbes that break down the casein have clear zones surrounding them.
   c. Antibiotic production can be detected by plating the microbes with a test organism such as *Staphylococcus aureus*. The presence of areas in which the test organism doesn't grow indicates the presence of antibiotics.
11. Enzymes are produced using submerged culture techniques. They are produced on a commercial scale when the organism is in the post-exponential growth phase. In downstream processing:
   a. The mixture of nutrient broth, cells and extracellular enzymes is filtered and centrifuged to remove cellular material.
   b. The liquid enzyme mixture is concentrated by evaporation, to give the bulk enzyme.
   c. The bulk enzyme may be:
      i. Concentrated by chromatography to give the pure enzyme.
      ii. Added to stabilisers to give the bulk liquid enzyme.
      iii. Precipitated and filtered, then sprayed and grinded to give the powdered enzyme.
12. Food production uses *Aspergillus niger* to produce pectinases. Pectin is found in cell walls to provide strength, and forms a gel with fruit juice (it also causes jams to set). Pectinase prevents the

gel from forming, so much more juice can be obtained from the fruit when crushing. This also clears the fruit juice. It is particularly important in wine making, otherwise the gel persists through the whole fermentation process.

13. Commercial enzymes can be used in detergents – these are mostly proteases, as most stains are derived from proteins. They have to be thermostable, and tolerant of a wide pH range, as well as not attacking keratin (which wool consists of) or cotton/silk.
14. Immobilised enzymes are widely used commercially – they can be immobilised in three ways:
    a. Cross linkage – the enzymes are linked together by gluteraldehyde, to form a mesh. This may damage some enzymes, but those that are not damaged remain very active.
    b. Entrapment – the enzymes are trapped in gel microcapsules or in a fibrous polymer mesh. This does not damage the enzymes, but may slow their action due to the substrate having to diffuse in.
    c. Adsorption – the enzymes are held by weak bonds on the surface of an adsorbing agent (e.g. glass bead, carbon particle, collagen). The enzymes easily come into contact with the substrate, but it is expensive, and the enzymes may become detached.
15. Biosensors use immobilised enzymes to test for substances:
    a. Clinistix uses glucose oxidase enzymes attached to the end of a plastic strip. Glucose is oxidised to gluconic acid and hydrogen peroxides, which changes the colour of the chromagen dye from blue to brown.
    b. Some biosensors work electronically, by having a selectively permeable membrane in front of a biological recognition membrane. This may contain enzymes, antibodies, a membrane component, organelles, or living cells on it. A transducer measures a physical or chemical change on the biological recognition membrane. It may be potentiometric (e.g. a pH meter) or amperometric (e.g. an O_2 electrode).
16. Therapeutic enzymes are used to treat diseases, for example Asparaginase is used in the treatment of acute lymphatic leukaemia.
17. Whole microbial cells may be immobilised in a similar way to enzymes, and are used in industrial processes such as the bioconversion of steroids, the production of amino acids, and the recovery of precious metals from dilute solutions. The production of cortisone (a steroid hormone given to arthritis sufferers to reduce the swelling in the joints) uses immobilised cells of ***Rhizopus arrhizus.***

Bacterial Disease

1. Most bacteria are either beneficial or harmless to humans – those that cause disease are pathogens:
    a. The symptoms of the disease are usually caused by waste products of the pathogens.
    b. An infection is when the effects are noticeable on the body.
    c. Transmission is when an infection is passed on to somebody else.
2. Koch's postulates – conditions to prove that a specific microorganism causes a specific disease:
    a. The organism thought to be causing the disease should always be present in animals suffering from the disease, and absent in healthy ones.
    b. The organism must be cultivated in pure culture outside the body of the infected animal.
    c. The culture, when inoculated into healthy animals, should cause the disease symptoms.
    d. The organism should be re-isolated from the experimental animals, and be cultured again in the laboratory. This new culture should be the same as the original one.
3. Diseases such as typhoid and cholera are transmitted through water, and can cause diarrhoea. The major cause of diarrhoea, however, is *Escherichia coli* in water contaminated with human faeces:
    a. Pathogenic strains of *E. coli* produce toxins in the small intestine, causing gastroenteritis and the secretion of large amounts of fluid into the digestive tract resulting in diarrhoea.
    b. Oral rehydration therapy (ORT) can be used to replace the water lost through diarrhoea, by providing an isotonic drink to replace water, salts and sugars.
4. To avoid water contamination, water-treatment processes take place. Sewage is treated by first removing solids, then spraying the sewage onto a deep bed of stones, providing a surface for bacteria to culture, and break down organic materials in the sewage. Treatment of water from reservoirs also takes place before being suitable for drinking:
    a. Screens remove large fragments, e.g. dead leaves and litter.
    b. A settlement tank allows sand and mud to settle out.
    c. Alum (potassium aluminium sulphate) is added to remove clay by causing particles to flocculate (stick together) so that they sediment out more quickly.
    d. Lime is added to neutralise acid water.
    e. Chlorine is added to kill all remaining bacteria, and make the water safe to drink.
5. Food-borne infections include *Salmonella* food poisoning, botulism, enteritis, cholera and typhoid:

   a. *Salmonella* food poisoning is spread mainly in two ways:
      i. By not cooking food thoroughly (e.g. raw eggs – newly laid eggs may be contaminated with poultry faeces).
      ii. By contaminating cooked meat from handling raw meat first (e.g. chicken).
   b. *Salmonella* infects the colon and the lower part of the ileum. The body reacts to this by increasing fluid production, and reducing the reabsorption of water, resulting in diarrhoea. Other symptoms are stomach pain, nausea, vomiting, chills, headaches, fatigue and fever.
6. Air-borne infections are spread when an infected person coughs, sneezes, talks or breathes, as the pathogens are passed into the air in small droplets saliva, mucus and water. These can remain suspended in the air for long periods. Larger droplets fall to the ground and dry out fairly quickly, exposing the pathogens to air currents. If fresh air entering a building contains pathogens, the pipes in ventilation systems can become reservoirs of pathogens, leading to sick building syndrome.
7. Infections that can be transmitted by direct contact are said to be contagious. Sexually transmitted diseases are transmitted by sexual contact (condoms reduce the risk of infection). A person with a cold blowing their nose will cause some viruses to end up on their hands rather than the tissue, so they can be spread by shaking hands (washing hands reduces the risk of infection).
8. Insect bites can transmit pathogens through the saliva of the insect (e.g. malaria is caused by a protozoan parasite, and is spread by mosquitoes; sleeping sickness is spread by the tsetse fly). Insect vectors are insect organisms that carry disease.
9. Pathogenicity is the ability of a bacterium to cause disease. The main factors are:
   a. The way in which the bacterium attaches and gains entry to host cells.
   b. The types of toxin produced by the bacterium.
   c. The infectivity of the bacterium (the number needed to cause an infection).
   d. The invasiveness of the bacterium (its ability to spread within the host).
10. After infection, a pathogen must do three things in order to produce a disease:
   a. Attachment – ligands on the cell wall of the pathogen (often polysaccharides) bind to receptors on the membranes of specific human tissues (usually epithelial). This matching is called host specificity, and the process of attachment is called specific adherence. Some bacteria have a glycolalyx (sticky layer) on their cell wall, which helps attachment. Because ligands and receptors are genetically controlled, different strains of pathogen have different disease-causing characteristics, and different people have different susceptibilities to diseases.
   b. Entry (penetration) – some pathogens produce enzymes that damage the membrane of the host cell, allowing it to enter. In other cases, the host cell may engulf the pathogen by endocytosis. Phagocytes engulf bacteria in an attempt to destroy them, however some bacteria may survive, due to having a protective capsule, and then reproduce inside.
   c. Colonisation – the pathogen multiplies and colonises the tissue. The host's immune system will attempt to fight the pathogen, but if the defence is overcome, then the disease becomes established.
11. There are two types of toxin, produced by bacterial pathogens:
   a. Exotoxins – proteins that are secreted by, or leak from, bacteria. These are responsible for the symptoms of many diseases. Exotoxins are detected as foreign antigens by the body, which triggers an immune reaction. Heat treatment of exotoxins can produce toxoids, which are similar in structure to the exotoxin but without harmful effects (can be used in vaccinations):
      i. Diarrhoea (*Escherichia coli*) – exotoxins affect the lining of the intestines.
      ii. Tetanus (*Clostridium tetani*) – exotoxins affect the function of nerve cells that normally prevent muscle contraction, causing all muscles to contract at once (spastic paralysis). The bacterium is picked up easily from anything dirty.
      iii. Botulism (*Clostridium botulinum*) – exotoxins cause all muscles to relax. The bacterium is anaerobic, and the toxin is thermostable (difficult to destroy).
   b. Endotoxins – complex compounds (lipopolysaccharides), usually found in bacterial cell walls. They are released when the bacterium dies, and are picked up by macrophages. The macrophages produce proteins to increase the core body temperature and cause weakness and aching. Endotoxins are not affected by heat, so cannot be converted into toxoids.
12. Infectivity is a measure of the number of organisms needed to cause an infection. In the normal body flora, no one organism is present in very large numbers. Antibiotics can destroy these cells, allowing pathogens to multiply without competition. *Salmonella enteritidis* (food poisoning) does not have a high infectivity, and needs to be present in large numbers for the disease to occur.

Salmonella typhi (typhoid fever) has a high infectivity, and only a few cells are needed to start the infection, but the symptoms take about a week to develop.

13. Invasive bacteria can penetrate cells and break into the blood and lymph vessels, to carry the pathogens and toxins around the body. *Corynebacterium diphtheriae* (diphtheria) has a low invasiveness and only affects those cells close to the site of infection (the toxin prevents protein synthesis in host cells). *Clostridium tetani* (tetanus) has a high invasiveness, and the toxin travels throughout the whole body, to prevent muscle relaxation.

Viral Disease

1. Viruses cannot survive without a living host (they have no organelles and no metabolism of their own), and are very small compared to bacteria. They can be crystallised like a chemical, and stored. Viruses consist of a strand of nucleic acid (DNA or RNA) surrounded by a protein sheath called a capsid, built of many identical capsomeres (they have very few genes, so they cannot encode many proteins). As such, the capsid forms a symmetrical structure as either a helical or an icosahedral capsid. The capsid protects the genetic material and is vital in attaching to the host. Some viruses have an outer membranous envelope derived from the host as it leaves the cell – this helps to penetrate the host cell membrane.
2. Poliomyelitis is a viral disease damaging the nervous system, causing paralysis of voluntary muscles, which can affect breathing. Once nerves are destroyed, they cannot be replaced, so it can result in permanent damage to the nervous system. The polio virus is very small and has a small amount of nuclear material in comparison to other viruses.
3. Foot and mouth disease affects cloven-hoofed animals (not horses or poultry), severely damaging the feet and forming lesions in the mouth. It is not fatal, but there is no cure, it reduces the animals' productivity, and it is very easily transmitted – the virus can survive for up to two days in a carrier.
4. Influenza affects the upper respiratory tract (it can sometimes reach the lungs):
    a. As the body is in a weakened state, it is prone to secondary infections.
    b. The virus is usually transmitted through droplets in human breath, and through mucus. It can also be carried by some animals.
    c. Ligands in the outer coat of the virus combine with receptors in the host membrane, allowing the virus to attach to epithelial cells. The viral RNA and RNA polymerase separate from the viral coat, and the RNA enters the nucleus of the host cell. The viral RNA polymerase is activated, and viral mRNA is formed – these move into the cytoplasm where they form new viral coats from proteins and lipids. The new virus particles are self-assembled. Finally, a late-produced enzyme causes cell lysis, and releases the viruses that can proceed to infect other cells.
    d. There are three major types of influenza – Type A is the most common, and Type C is the most rare, but Type B causes pandemics (world-wide epidemics) every five years.
    e. Liposomes can be used in the vaccination against influenza, as a purified viral protein is encased in a hollow lipid sphere. These are inhaled, and fuse with cell membranes to release the viral protein into the epithelial cells. The viral protein passes into the blood capillaries, stimulating the production of antibodies against the influenza virus.
5. Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV), and results in the immune system breaking down so that it can no longer defend the body against disease. HIV consists of two copies of the RNA genome (and reverse transcriptase enzymes) surrounded by an inner core protein shell, a protein nucleocapsid, and a lipid envelope, containing glycoprotein spikes:
    a. People who are infected with HIV produce antibody to combat the virus – if this is detected in the blood, the individual is said to be HIV-positive.
    b. The HIV virus attaches to the cell membrane of a helper T cell, releasing the viral RNA and reverse transcriptase enzymes into the lymphocyte. This produces viral DNA, which is incorporated into the DNA of the host cell.
    c. A person showing the symptoms of tiredness, fever, weight loss and diarrhoea is said to have AIDS-related complex (ARC). The person is only said to have AIDS when the symptoms of one of the diseases known to be related to AIDS appears.
    d. In an HIV-infected person, a pathogen entering the bloodstream activated infected helper T cells, which activates the viral DNA. Instead of the normal immune response, the helper T cell produces more HIV particles, and then is lysed, allowing the HIV particles to infect other T cells. Thus the immune system is weakened further, and there is no destruction of the pathogen.

e. Diseases that infect people with a weakened immune system (AIDS-related diseases) are opportunistic diseases. The most common of these are Kaposi's sarcoma (a rare form of cancer causing black tumours) and a form of pneumonia. People suffering from AIDS die as a result of the opportunistic diseases, not as a result of AIDS itself.
f. HIV is transmitted only by the introduction of blood, semen or vaginal secretions from an infected person, as these contain high numbers of T lymphocytes, which may be infected (in urine and saliva, the HIV particles are isolated, and do not cause infection). HIV may be transmitted in three main ways:
    i. Transfusion of infected blood – since 1985 all blood donations have been tested for HIV – before, many people became infected (particularly haemophiliacs).
    ii. Sharing needles – blood cells from a previous use remain in the needle, so that if the person is HIV-positive, it can be passed on to the next user. This is common between drug users, and with tattooists reusing needles.
    iii. Sexual activity – HIV is spread through infected semen and vaginal secretions. Oral, vaginal and anal intercourse are all high-risk activities. People with genital herpes are at a greater risk, as the skin in the genital area is broken.
g. The transmission of HIV can be reduced by providing needle exchange facilities for drug users, and ensuring tattooists are reputable. The use of condoms reduces the incidence of transmission during sexual intercourse.

6. Viral diseases can be very difficult to treat because the viruses have no metabolism of their own; hence antibiotics have no effect on them (they target the metabolism of bacteria). The viruses are inside the host cell during the infection, and thus they cannot be damaged without damaging the host. There are a few antiviral drugs, however, which can work by:
    a. Inhibiting the production of viral DNA/RNA by altering the host cell's DNA.
    b. Preventing the enzymes essential for the production of new virus particles from working.
    c. Preventing the viral particles from entering the cells in the first place (these tend to have little effect, as many cells are already colonised by the time the symptoms appear).
7. Bacteriophages are viruses that infect bacteria, and can be used as an alternative to antibiotics (it is a lot more difficult for the bacteria to become resistant). There are problems, however, in that the antigens on viruses are able to change rapidly as the virus mutates, thus it may be dangerous to introduce genetic material into dividing cells. There is also the danger of crossing the species barrier, which could cause damage to humans.

Protection Against Disease

1. The human body has a number of barriers that prevent pathogens from entering the body:
    a. The skin – outer cells filled with indigestible keratin to inhibit microorganism growth.
    b. Sebum – oily secretion from sebaceous glands, toxic to microorganisms.
    c. Tears, saliva and urine – contain lysosyme enzymes which hydrolyse molecules in the cell walls of microbes (Gram-negative bacteria).
    d. Mucus in respiratory tracts – traps organisms, which are swept up by cilia to the throat and swallowed.
    e. Commensals – harmless bacteria compete more successfully than pathogens for nutrients:
        i. Skin commensals and gut flora competitively exclude pathogens by occupying all ecological niches.
        ii. Vaginal bacteria feed on carbohydrates and produce waste lactate (acidic).
    f. Stomach acid and enzymes – microbes are killed in the stomach, and digested.
2. If the body is injured, a general inflammatory response will take place:
    a. The blood clots at the site of a cut, by fibrinogen converting to a mesh a fibrin, which traps platelets. This seals the cut, to prevent pathogens entering the blood.
    b. Inflammation around the site of the injury takes place.
    c. Macrophages are attracted to the site of injury, where they destroy pathogens by phagocytosis.
3. Histamine is released by basophils and mast cells (beneath the skin and around blood vessels). It has no effect within these cells, but has several effects outside:
    a. Vasodilation – relaxes smooth muscle of arterioles, increasing blood flow to injured area.
    b. Inflammation – increases the permeability of capillary cell walls, resulting in the wound swelling and becoming warm.
    c. Increased sensitivity of sensory neurones:
        i. Prostaglandins trigger pain sensation (inhibited by aspirin).
        ii. Bradykinin cause pain and swelling (due to damaged tissue or blood clots).

4. Allergic responses are immune reactions to substances (allergins):
    a. The allergin stimulates certain plasma cells to produce reagin antibodies.
    b. The reagins bind to the surface of mast cells, to make them hypersensitive.
    c. Hypersensitive cells release histamine when the allergin meets them (allergic response).
5. There are a number of different types of leucocyte (white blood cell):
    a. Granulocytes (have granules in their cytoplasm, and develop from myeloid tissue):
        i. Neutrophils – phagocytic leucocytes.
        ii. Basophils – allergic response and response to parasitic infections.
        iii. Eosinophils – combat effects of histamine, and release toxic proteins against larger pathogens (parasites) – major basic protein (MBP).
    b. Agranulocytes (lack granules in their cytoplasm, and develop from lymphoid tissue):
        i. Monocytes (macrophages) – phagocytic leucocytes.
        ii. T lymphocytes – cell-mediated immune response; migrate to thymus.
        iii. B lymphocytes – antibody-mediated immune response; migrate to bursa in birds.
6. An immune response is initiated due to the body detecting foreign material. A substance causing an immune response is called an antigen. These may be on the cell wall of a bacterium, on the surface of a virus, or individual molecules of toxin. Self antigens do not trigger an immune response, and are encoded by genes called major histocompatability complex (MHC) – the human leucocyte antigen (HLA) group is an MHC protein, and is encoded by five different genes. In order for a transplanted organ to be accepted, the HLA groups must match – otherwise it is rejected.
7. The human immune system includes organs that filter blood and lymph. Lymph nodes contain phagocytes that ingest pathogens in the lymph, and T and B lymphocytes. The spleen has phagocytes to filter the blood in a similar way, also removing worn red blood cells and platelets.
8. Phagocytosis is the general immune response to pathogens – phagocytes are attracted by chemicals released from damaged cells. They squeeze through capillary walls by diapedesis, and engulf bacteria and debris by endocytosis – these are digested by enzymes from lysosomes in vacuoles in the cytoplasm. This process is aided by complement proteins:
    a. Induce cell lysis by forming a giant complex in foreign cell membranes.
    b. Inflammation is enhanced by causing histamine to be released.
    c. Phagocytes are attracted to the site of infection.
    d. Splits into polypeptide fragments with two reactive surfaces – one reacts with the pathogen, and the other matches phagocyte receptors. The phagocyte attaches to the pathogen by the complement, in a process called opsonization.
9. The specific immune response is mediated by lymphocytes. These form from pleuripotent stem cells in the bone marrow. T cells must be activated by the thymus in order to make the cells competent. B cells mature in the bone.
10. Non-self antigens are recognised by antibodies. These are Y-shaped immunoglobulins, with a constant region (the heavy chain) and two variable regions as the antigen binding sites. An antibody is specific to an antigen. Different constant regions of the antibodies result in five different isotypes (the most common is IgG, gamma globulin).
11. Cell-mediated immunity is caused by T lymphocytes. These can detect bacteria, but not isolated viruses (it only detects viruses once they have invaded a host cell, due to viral protein getting into the cell membrane of the host cell):
    a. A competent T lymphocyte is activated by a specific antigen on the pathogen. It then multiplies by mitosis to form a clone of identical cells.
    b. T lymphocytes differentiate into four types:
        i. Cytotoxic T cells – attach to infected or pathogenic cells, and release perforin to kill them
        ii. Helper T cells – attract/stimulate macrophages, B cells, and cytotoxic T cells.
        iii. Memory T cells – remain in the lymph nodes, to respond rapidly to another infection by the same pathogen.
        iv. Suppressor T cells – slow down and stop the immune reaction after about a week.
12. Antibody-mediated (humoral) immunity is caused by B lymphocytes. A competent T helper cell interacts with the appropriate competent B cell, causing it to multiply by mitosis to form a clone of identical cells. B lymphocytes differentiate into two types:
    a. Plasma B cells – secrete the specific antibody against the pathogen, which is transported via the lymph and the blood to the site of infection. The antibody has four main effects:
        i. Neutralisation – the antibody combines with the active part of the toxin and/or virus to prevent attachment to body cells.

   ii. Precipitation – the two binding sites on the antibody link to two antigens, to form a lattice complex. Soluble antigens are precipitated and ingested by phagocytes.
   iii. Agglutination – the two binding sites on the antibody link to antigens on two different pathogens, forming an immune complex to aid ingestion by phagocytes.
   iv. Complement reactions – the antigen-antibody complex on the surface of the pathogen triggers the complement reactions and opsonization.
   b. Memory B cells – continue to secrete the antibody for many years, and are able to reproduce rapidly to produce an instant supply of plasma B cells.
13. Monoclonal antibodies are used in medical diagnosis, as they respond to a specific antigen:
   a. Tumour cells and antibody-producing cells are fused to form myeloma cells.
   b. Myeloma cells and competent B lymphocytes are fused to form hybridoma cells.
   c. Hybridoma cells divide to produce a clone of antibody-producing cells.
14. Interferons are produced by lymphocytes as a response to viral infections. They protect cells from viral attack, increase T cell and macrophage activity, stimulate B cell division and antibody secretion by plasma cells, and stop cells from growing. Interferons are useful in the treatment of some illnesses (e.g. cancer treatment), but are cannot be used as general-purpose anti-viral drugs.
15. There are two levels of response with respect to antibody production:
   a. Primary response – the first time an individual comes into contact with a specific antigen, it takes 3-14 days after infection to produce the antibody. This is the latent period, after which the amount of antibody in the blood rises quickly then begins to fall. Memory cells are produced that remain in the blood after the infection.
   b. Secondary response – if a second infection occurs (by the same pathogen), the response is much more rapid (shorter latent period) and much more antibody is produced, due to memory cells. The pathogen is destroyed before the symptoms are displayed.
16. There are four main types of immunity:
   a. Active natural immunity – memory cells develop after natural exposure to antigens.
   b. Active artificially induced immunity – memory cells develop after vaccination.
   c. Passive natural immunity – antibody transfer (e.g. through placenta or breast feeding) results in short-term immunity (a few months), as no memory cells develop.
   d. Passive artificially induced immunity – antibodies are injected (short-term immunity).
17. A vaccine contains antigen derived from pathogenic organisms. This stimulates a primary response, resulting in the creation of memory cells, but without the symptoms of the disease. Children must be vaccinated as they grow up in order to prevent large-scale disease outbreaks – the proportion of individuals who must be immune to a disease in order to prevent an epidemic is called the percentage cover. Booster vaccinations are sometimes needed to re-establish the level of immunity to the disease. The five main types of vaccine are:
   a. Killed virulent organisms, e.g. whooping cough.
   b. Live, non-virulent organisms, e.g. rubella.
   c. Toxoids (heat-treated toxins), e.g. diphtheria.
   d. Isolated antigens (from a pathogen), e.g. influenza.
   e. Genetically engineered antigens, e.g. hepatitis B.
18. It is difficult to become immune to a virus, as they undergo many mutations, resulting in many different forms producing similar symptoms, but with different antigens. The body may become immune to one strain, but a new strain may arise to cause its own primary response. Influenza vaccines have to be prepared from the most likely strain to cause an epidemic.
19. Antibiotics are only effective against bacterial infections (not viruses) – there are roughly 50 different antibiotics available, and these fall into two groups – broad spectrum antibiotics affect a wide range of bacteria, whereas narrow spectrum antibiotics only affect a few types of bacterium.
20. Antibiotics that have been chemically altered to make them more effective or to have fewer side effects are termed semi-synthetic. They can work in a number of different ways:
   a. Inhibiting cell wall synthesis (e.g. penicillin).
   b. Binding to ribosomes and inhibiting protein synthesis (e.g. streptomycin).
   c. Interfering with prokaryotic DNA replication and transcription (e.g. ciprofloxacin).
   d. Binding to the cell membrane to make it more permeable (e.g. polymyxin B).
   e. Inhibiting cell metabolism – antimetabolites (e.g. sulphanomides).
21. Bacteria may become resistant to antibiotics by altering the structure of the antibiotic (e.g. opening the β-lactam ring) or by modifying the bacterial cells. Resistance develops as a spontaneous mutation, and can spread through the population asexually and sexually (conjugation, transformation, and transduction).