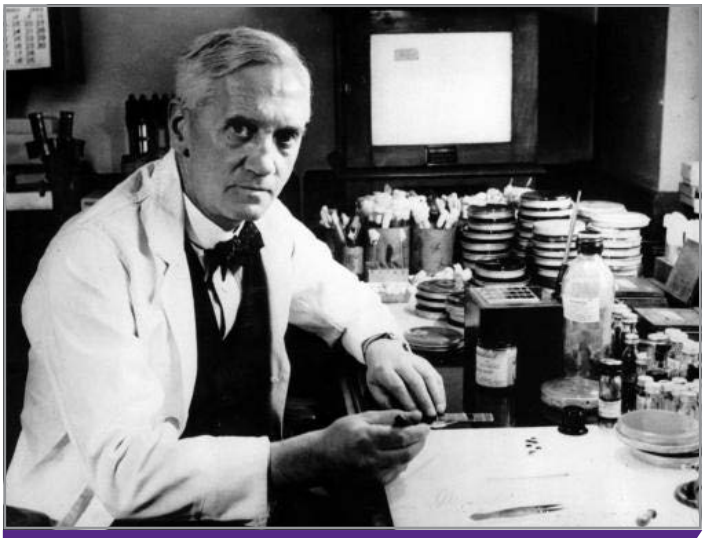
Penicillin – a life-saving antibiotic

Penicillin

History happened at a London university in 1928 when Alexander Fleming noticed that a culture of *Staphylococcus* bacteria had been contaminated by a *Penicillium* mould. Wherever there were *mould* particles, the bacterial colonies had died. Fleming speculated that the fungus was producing an antibacterial substance. The idea of using biological rather than chemical agents against bacteria was not new. Pasteur had noted that *anthrax* bacteria multiplied in urine but stopped developing when other bacteria were added. In Italy, Arnaldo Cantani had some success in displacing *tubercle bacteria* when he painted the throats of infected people with other bacterial strains.



Alexander Fleming (Wikipedia)

A wonder drug

Fleming's experience included treating war wounds, often ineffectively, with *antiseptics*. Previously, he had discovered that *lysozyme enzyme* (from nasal mucus and tears) killed some types of bacteria. His subsequent work on penicillium showed penicillin extracts to be non-toxic, effective against *gram-positive* bacteria but very hard to produce in quantity. It was another 10 years before Howard Florey and his team isolated an active substance, penicillin G. Working with different penicillium strains, they used beer-brewing technology to improve output. UV / X-ray irradiation was used to develop a *mutant* penicillium strain that was a relatively prolific producer of the drug. Early experiments on mice and humans showed it to be very effective in treating infections. An initial problem arose from the fact that penicillin is eliminated rapidly from the body - it was necessary to collect patient urine in order to recycle the drug. However, sufficient quantities were produced to save the lives of thousands of Allied soldiers in World War II; in previous wars, most wound-related deaths were from infection. The structure of penicillin was elucidated by Dorothy Hodgkin in 1945 and, in 1957, the first penicillin was produced by wholly chemical means.

Prontosil, discovered in 1933, was the first drug successfully used to treat infections. It was not always effective and side-effects could be serious. However, penicillin transformed everything. Before its general availability, deaths from *scarlet fever*, *pneumonia*, *syphilis*, *blood poisoning* and *gangrene* were far more common. To this day, penicillin is used to control and prevent disease. Childhood deaths from bone, stomach, heart and throat infections have been greatly reduced. It must be stated, however, that the reduced impact of infectious disease is primarily due to improved diet, hygiene and living conditions. Today, penicillins are just one of many types of *antibiotic*.

Penicillium mould on an orange (Wikipedia)

How it works

Chemically, all penicillins contain *beta-lactam* rings, with different side-chains attached. Natural penicillins are generated by moulds in a *bioreactor*. Semi-synthetic penicillins with non-naturally occurring side-chains are produced by adding specialised chemicals to the reaction mixture. Synthetic penicillins are produced wholly by chemical means.

Pathogenic bacteria have a cell wall composed of layers of *peptidoglycan*, made secure by *peptide* cross-linkages. When bacteria replicate, or do routine maintenance on the cell wall, penicillin inhibits an enzyme vital for the formation of cross-linkages. With the cell wall damaged, the bacteria can die of *osmotic lysis*. Sometimes, they may grow slowly and abnormally. This reduces their capacity to cause harm. *Gram-positive* bacteria have a much thicker cell wall than *gram-negative* bacteria and depend far more on it for protection. Consequently, natural penicillins have very limited effectiveness against gram-negative bacteria.

Most pathogenic bacteria are gram-positive but gram-negative bacteria can cause an array of illnesses including *cholera*, *plague*, gastrointestinal, urinary and some hospital-acquired infections. Semi-synthetic penicillins have proven effective against gram-negative bacteria. Penicillin's mode of action is destructive to a bacterial rather than a mammalian process. This makes it a safe drug with a high *therapeutic index*. When broken down enzymatically, it can yield *amino acids* which contribute minimally to nutrition.

The fast elimination of penicillin was dealt with by adding chemicals to the dosage to slow down its elimination through the kidneys. Penicillin was often given by injection because of water-solubility problems and its susceptibility to stomach acid attack. Injections have the advantage of requiring less drug amounts and fast delivery but oral administration is convenient. Problems associated with oral administration have been solved by adding protective *buffer* compounds to tablets or by using semi-synthetic drugs. Other drugs such as *contraceptives* have reduced effect if taken with penicillins. Some chemicals, for instance *methotrexate*, are not so easily eliminated from the body if penicillin is present. As with other drugs, allergic reactions are possible; up to 19% of people are *allergic* to penicillin G.

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Penicillin attacks a range of bacteria including some natural bodily flora; occasionally this leads to *superinfection* in which pathogens take advantage of the disruption of bacterial populations in the body. Penicillin therefore is a medicine best taken under medical direction.

Drug resistance

Penicillin-resistant bacteria were identified even before the drug was widely used. Resistant organisms typically produce penicillinases — enzymes that catalyse the *hydrolysis* of the beta-lactam ring, leaving a product with no anti-bacterial activity. Susceptibility to these enzymes varies among the different penicillins. Methicillin, for instance, is a synthetic penicillin that has proven effective against bacteria that are resistant to penicillin G. However, bacteria resistant to methicillin have emerged, the best known example being 'methicillin resistant staphylococcus aureus' (*MRSA*).

Scientists have tried adding penicillinase inhibitors to penicillin medications. Clavulanic acid is a natural product that has been successfully used. Another inhibitor, sulfone, is produced by the oxidation of penicillin itself. Because sulfone molecules resemble penicillin, they are attacked by penicillinases. A stable penicillinase-sulfone complex forms, with no enzymatic activity. Resistant bacteria may also develop other strategies, one of them being to alter their surfaces to prevent binding of penicillin.

The white patches contain antibiotics. The clear areas around some of them show how the growth of the mould was inhibited. (Image: Wikipedia)

A general approach to dealing with antibiotic resistance involves the administration of drug combinations in the hope that bacteria will not be resistant to all of them. Short life-cycles and selection pressure mean that drug resistance will inevitably emerge. Gram-negative bacteria pose a particular difficulty. Not only can they exchange *DNA,* as all bacteria do, but they are also proficient at transferring it between unrelated gram-negative species. Antibiotics like *vancomycin*, whose use was largely discontinued because of harmful side-effects, have been used in last resort cases. New drugs will only solve the problem temporarily.

Hospitals have been forced to adopt measures designed to minimise the transfer of bacteria, and antibiotic resistance, between people. These include screening on admission, isolation, minimising the length of patient stays, vigorous disinfection and personal hygiene procedures. Antibiotic resistance is a natural phenomenon but it can be moderated. Penicillin and other antibiotics serve humankind well and will probably continue to do so for some time yet.

Find this and other lessons on **www.sta.ie**

Syllabus References

The appropriate syllabus references are:

Junior Certificate Science

1C8. Microbiology and biotechnology

Leaving Certificate Biology

3.1.2. Microorganisms
3.1.3. Monera
3.1.4. Fungi
3.1.5. Laboratory procedures
H.2.1.4. Cell structure
H.2.2.7. Enzymes
H.3.1.9. Nature of bacteria and fungi
H.3.1.10. Growth curves of microorganisms

Leaving Certificate Chemistry

1A1. General principles of industrial chemistry

Science and Technology in Action is widely used in science modules and project work in **Transition Year**.

Learning Outcomes

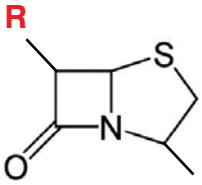
On completion of this lesson, students should be able to:

- Gain an understanding of the nature and mode of action of penicillin drugs
- Recognise that penicillin revolutionised the treatment of infection and is used to this day
- Recognise that penicillin has its limitations and has problems associated with its use.
- Understand how these problems have been and may be dealt with.

General Learning Points

The following points can be used to enhance the lesson content and to inform discussion.

1. Antibiotics are compounds that kill or prevent the growth of microorganisms.
2. Beta-lactam rings are cycloamides. They form the core structure of a number of antibiotic families. These antibiotics generally interfere with bacterial cell wall synthesis. Processes targeted by other antibiotics include cell membrane function and protein synthesis.



3. Penicillin has a high therapeutic index (ratio of lethal dose to therapeutic dose). It is regarded as a bactericidal agent.
4. Broad spectrum antibiotics are effective against a range of gram-positive and gram-negative bacteria. Narrow spectrum antibiotics are effective against a range of one type only.

Student Activities

1. Make a table of diseases caused by gram-positive and gram-negative bacteria. If possible, include a column showing some of the antibiotics that could be used to treat them.
2. Draw a classroom poster showing the molecular structures of the different types of penicillin.
3. Make a list of the different penicillins. Classify them as natural, semi-synthetic or synthetic.
4. Obtain antibiotic multi-discs and place them on different bacterial cultures in petri dishes prior to incubation. After allowing time for colonies to grow, assess the effectiveness of the different antibiotics by the lack, or otherwise, of colony growth.
5. Construct a poster showing the different families of antibiotics, their uses and their modes of action.
6. Picture yourself in Alexander Fleming's lab at the moment of his famous discovery — you are one of his lab assistants. Write a short play or story describing what happened.
7. Explain why people suffering from flu, a viral disease, are sometimes given antibiotics. Discuss why you should not be given too much or too little antibiotic and why you must complete the course, even if you are feeling better.
8. If you are in London, make sure you visit Fleming's old lab, now a museum.
9. For a good read, and an insight into a world without antibiotics, read Ivan Turgenev's classic novel "Fathers and Sons".

True/False Questions

a) Penicillins are the only antibiotics containing a beta-lactam ring. **T F**

b) Penicillins have been effectively used against fungi and other parasites as well as bacteria. **T F**

c) Antibiotics have been administered preventatively to people with compromised immune systems. **T F**

d) Penicillium generally produces penicillin under stress and not during normal active cell growth. **T F**

e) Bactericidal antibiotics do not actually kill the bacteria. **T F**

f) Bacteria have evolved that are resistant to methicillin, a synthetic penicillin. **T F**

g) Oxacillin, cloxacillin, amoxicillin and ampicillin are different types of penicillin. **T F**

h) Penicillins are stored in warm conditions in order to minimise hydrolysis of the beta-lactam ring. **T F**

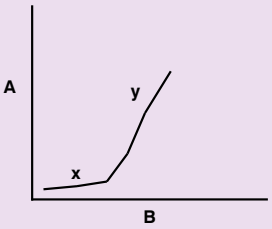
i) Penicillin can induce a severe reaction if taken with alcohol. **T F**

Check your answers to these questions on www.sta.ie.

Examination Questions

Leaving Certificate Biology (HL) 2008, Q.15(c)

The diagram shows a bacterial growth curve.



(i) A and B represent the labels on the axes. What does each of them stand for?

(ii) What term is applied to the part of the curve labelled x? What is happening during x?

(iii) What term is applied to the part of the curve labelled y? What is happening during y?

(iv) Copy the diagram into your answer book and continue the curve to show the next phase.

Explain why you have continued the curve in this way.

(v) Distinguish between batch and continuous flow food processing using micro-organisms in the food industry.

Leaving Certificate Biology (HL) 2007, Q8 (b)

Answer the following questions in relation to your investigation of the growth of leaf yeast.

(i) It was necessary to use a nutrient medium. What is a nutrient medium?

(ii) Name the nutrient medium that you used.

(iii) The nutrient medium should be sterile. Explain the underlined term.

(iv) Describe, in words and/or labelled diagram(s), how you conducted the investigation.

(v) What was the result of your investigation?

Leaving Certificate Biology (HL) 2012, .Q8

(a) Are fungi prokaryotic or eukaryotic?

Name one structure in plant cells not found in fungi.

(b) What is the purpose of using agar when growing fungi or bacteria in the laboratory?

Suggest one reason why leaf yeasts are more plentiful in July than in March.

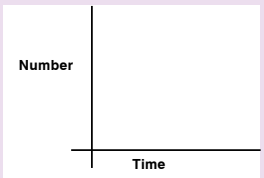
Describe how you introduced the leaf yeasts into agar plates.

What was the precise purpose of a control in this investigation?

How did you recognise the leaf yeasts when they appeared on the agar?

How did you safely dispose of the plates at the end of the investigation?

Using the axes shown, draw a graph to show how the number of leaf yeasts varied following their introduction into the plate.



Did You Know?

- Moulds have been part of folk remedies for centuries in the treatment of cuts and wounds. People knew the use of antibiotics long before they were discovered.
- There is evidence that Joseph Lister, pioneer of antiseptic surgery, had recognised the antibacterial properties of penicillium mould in the 19th century. Contemporary beliefs and technology were not yet ready to exploit that observation.
- Penicillin can sometimes produce harmful side-effects in patients. One mechanism involves the penicillin molecules adsorbing to the surface of red blood cells, forming a complex which is attacked by the immune system. The destruction of red blood cells leads to anaemia. Symptoms disappear once the drug is discontinued.
- With antibiotic resistance a problem, scientists are researching the possibility of using positively charged nanoparticles. These particles would attach to negatively charged bacterial membranes, damaging them irreparably. Depending on the infection, the nanoparticles could be applied in a skin lotion or injected into the bloodstream. Enzymes would subsequently be used to destroy the nanoparticles.

Biographical Notes

Alexander Fleming (1881 – 1955)

He was the seventh in a family of eight born to an Ayrshire farm family. In his early years, he was a member of the Territorial Army. Moving to London, he studied at a polytechnic, worked with a shipping company and finally moved to St Mary's College where he became a professor in 1928. During World War I, he served with distinction in the Army Medical Corps. In 1915 he married Sarah McElroy — from Killala, County Mayo — and their son became a GP. Sarah died in 1949 and in 1953 Fleming married Dr. Amalia Koutsouri-Voureka.

There are anomalous accounts of what happened on the day he initially noticed the action of penicillin. One account had him on the verge of destroying the contaminated culture. There is disagreement on what he said on seeing the bacterial lysis. Was it "that's funny", "that's interesting" or in his own west Scotland dialect "here's a rum go!"

Revise The Terms

Can you recall the meaning of the following terms? Revising terminology is a powerful aid to recall and retention.

allergic, amino acid, anthrax, antibiotic, antiseptic, bactericidal, bacteriostatic, beta-lactam, bioreactor, blood poisoning, broad spectrum antibiotic, buffer, cholera, contraceptive, disinfectant, DNA, gangrene, gram-negative, gram-positive, hydrolysis, lysis, lysozyme, methotrexate, mutant, narrow spectrum antibiotic, osmotic lysis, pathogenic, penicillin, penicillium, peptide, peptidoglycan, plague, pneumonia, Prontosil, scarlet fever, staphylococcus, superinfection, syphilis, therapeutic index, vancomycin.

Check the Glossary of terms for this lesson on www.sta.ie