Testing penicillin

Florey and Chain's experiments to test penicillin on bacterial infections in mice.

Howard Florey and Ernst Chain formed a research team in Oxford in the late 1930s that investigated the use of chemical substances to control bacterial infections. The most promising of these was penicillin, discovered by Alexander Fleming in 1928. Florey and Chain's team developed a method of growing the fungus *Penicillium* in liquid culture in conditions that stimulated it to secrete penicillin. They also developed methods for producing reasonably pure samples of penicillin from the cultures.

The penicillin killed bacteria on agar plates, but they needed to test whether it would control bacterial infections in humans. They first tested it on mice. Eight mice were deliberately infected with *Streptococcus* bacteria that cause death from pneumonia. Four of the infected mice were given injections with penicillin. Within 24 hours all the untreated mice were dead but the four given penicillin were healthy. Florey and Chain decided that they should next do tests on human patients, which required much larger quantities.

When enough penicillin had been produced, a 43-year-old policeman was chosen for the first human test. He had an acute and life-threatening bacterial infection caused by a scratch on the face from a thorn on a rose bush. He was given penicillin for four days and his condition improved considerably, but supplies of penicillin ran out and he suffered a relapse and died from the infection.

Larger quantities of penicillin were produced and five more patients with acute infections were tested. All were cured of their infections, but sadly one of them died. He was a small child who had an infection behind the eye. This had weakened the wall of the artery carrying blood to the brain and although cured of the infection, the child died suddenly of brain hemorrhage when the artery burst.

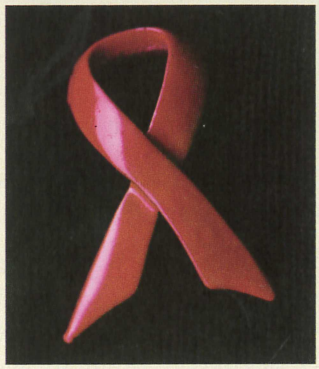
Pharmaceutical companies in the United States then began to produce penicillin in much larger quantities, allowing more extensive testing, which confirmed that it was a highly effective treatment for many previously incurable bacterial infections.

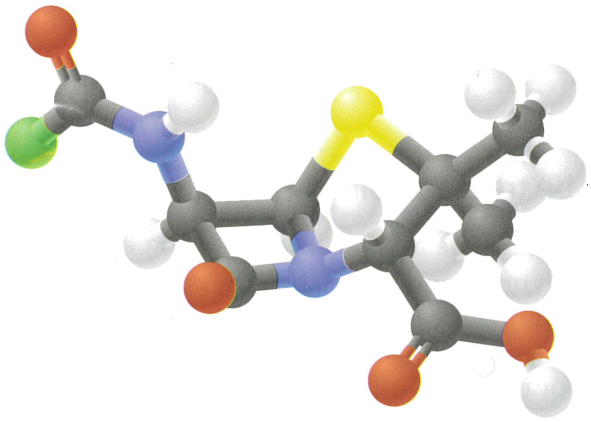
Activity

World AIDS Day

The red AIDS awareness ribbon is an international symbol of awareness and support for those living with HIV. It is worn on World AIDS Day each year – December 1st.

Are you aware how many people in your area are affected and what can be done to support them?





▲ Figure 8 Penicillin – the green ball represents a variable part of the molecule

Penicillin and drug testing

Risks associated with scientific research: Florey and Chain's tests on the safety of penicillin would not be compliant with current protocols on testing.

When any new drug is introduced there are risks that it will prove to be ineffective in some or all patients or that it will cause harmful side effects. These risks are minimized by strict protocols that pharmaceutical companies must follow. Initial tests are performed on animals and then on small numbers of healthy humans. Only if a drug passes these tests is it tested on patients with the disease that the drug is intended to treat. The last tests involve very large numbers of patients to test whether the drug is effective in all patients and to check that there are no severe or common side effects.

There are some famous cases of drugs causing problems during testing or after release.

- Thalidomide was introduced in the 1950s as a treatment for various mild conditions but when it was found to relieve morning sickness in pregnant women it was prescribed for that purpose. The side effects of the drug on the fetus had not been tested and more than 10,000 children were born with birth deformities before the problem was recognized.
- In 2006 six healthy volunteers were given TGN1412, a new protein developed for treatment of autoimmune diseases and leukemia. All six rapidly became very ill and suffered multiple organ failure. Although the volunteers recovered, they may have suffered long-term damage to their immune systems.

It is very unlikely that Florey and Chain would have been allowed to carry out tests on a new drug today with the methods that they used for penicillin. They tested the drug on human patients after only a very brief period of animal testing. Penicillin was a new type of drug and there could easily have been severe side effects. Also the samples that they were using were not pure and there could have been side effects from the impurities.

On the other hand, the patients that they used were all on the point of death and several were cured of their infections as a result of the experimental treatment. Because of expeditious testing with greater risk-taking than would now be allowed, penicillin was introduced far more quickly than would be possible today. During the D-day landings in June 1944 penicillin was used to treat wounded soldiers and the number of deaths from bacterial infection was greatly reduced.

▲ Figure 9 Wounded US troops on Omaha beach 6 June 1944

Viruses and antibiotics

Viral diseases cannot be treated using antibiotics because they lack a metabolism.

Viruses are non-living and can only reproduce when they are inside living cells. They use the chemical processes of a living host cell, instead of having a metabolism of their own. They do not have their own means of transcription or protein synthesis and they rely on the

b) selegeline, which is an inhibitor of monoamine oxidase-B (MAO-B) [1]

c) tolcapone, which is an inhibitor of catechol-O-methyl transferase (COMT) [1]

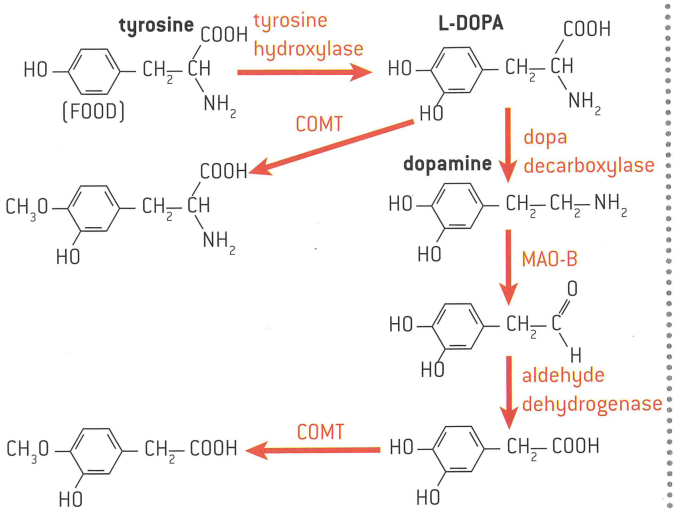
d) ropinirole, which is an agonist of dopamine [1]

e) safinamide, which inhibits reuptake of dopamine by pre-synaptic neurons. [1]

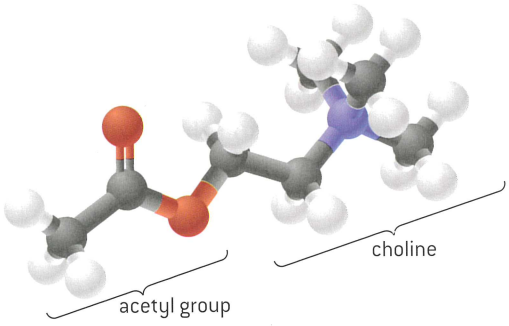
2 Discuss how a cure for Parkinson's disease might in the future be developed by:

a) stem cell therapy [3]

b) gene therapy. [2]



▲ Figure 15 The formation and breakdown of L-DOPA and dopamine. The enzymes catalysing each step are shown in red



▲ Figure 16 Acetylcholine

Acetylcholine

Secretion and reabsorption of acetylcholine by neurons at synapses.

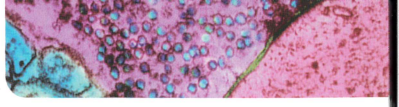
Acetylcholine is used as the neurotransmitter in many synapses, including synapses between neurons and muscle fibres. It is produced in the pre-synaptic neuron by combining choline, absorbed from the diet, with an acetyl group produced during aerobic respiration. The acetylcholine is loaded into vesicles and then released into the synaptic cleft during synaptic transmission.

The receptors for acetylcholine in the post-synaptic membrane have a binding site to which acetylcholine will bind. The acetylcholine only remains bound to the receptor for a short time, during which only one action potential is initiated in the post-synaptic neuron. This is because the enzyme acetylcholinesterase is present in the synaptic cleft and rapidly breaks acetylcholine down into choline and acetate. The choline is reabsorbed into the pre-synaptic neuron, where it is converted back into active neurotransmitter by recombining it with an acetyl group.

Neonicotinoids

Blocking of synaptic transmission at cholinergic synapses in insects by binding of neonicotinoid pesticides to acetylcholine receptors.

Neonicotinoids are synthetic compounds similar to nicotine. They bind to the acetylcholine receptor in cholinergic synapses in the central nervous system of insects. Acetylcholinesterase does not



break down neonicotinoids, so the binding is irreversible. The receptors are blocked, so acetylcholine is unable to bind and synaptic transmission is prevented. The consequence in insects is paralysis and death. Neonicotinoids are therefore very effective insecticides.

One of the advantages of neonicotinoids as pesticides is that they are not highly toxic to humans and other mammals. This is because a much greater proportion of synapses in the central nervous system are cholinergic in insects than in mammals and also because neonicotinoids bind much less strongly to acetylcholine receptors in mammals than insects.

Neonicotinoid pesticides are now used on huge areas of crops. In particular one neonicotinoid, imidacloprid, is the most widely used insecticide in the world. However, concerns have been raised about the effects of these insecticides on honeybees and other beneficial insects. There has been considerable controversy over this and the evidence of harm is disputed by the manufacturers and some government agencies.

Activity

Research updates on neonicotinoids

There are currently intense research efforts to try to discover whether neonicotinoids are to blame for collapses in honeybee colonies. What are the most recent research findings and do they suggest that these insecticides should be banned?

▲ Figure 17 Research has shown that the neonicotinoid pesticide imidacloprid reduces growth of bumblebee colonies

Threshold potentials

A nerve impulse is only initiated if the threshold potential is reached.

Nerve impulses follow an all-or-nothing principle. An action potential is only initiated if the threshold potential is reached, because only at this potential do voltage-gated sodium channels start to open, causing depolarization. The opening of some sodium channels and the inward diffusion of sodium ions increases the membrane potential causing more sodium channels to open – there is a positive feedback effect. If the threshold potential is reached there will therefore always be a full depolarization.

At a synapse, the amount of neurotransmitter secreted following depolarization of the pre-synaptic membrane may not be enough to cause the threshold potential to be reached in the post-synaptic membrane. The post-synaptic membrane does not then depolarize. The sodium ions that have entered the post-synaptic neuron are pumped out by sodium–potassium pumps and the post-synaptic membrane returns to the resting potential.

A typical post-synaptic neuron in the brain or spinal cord has synapses not just with one but with many pre-synaptic neurons. It may be necessary for several of these to release neurotransmitter at the same time for the threshold potential to be reached and a nerve impulse to be initiated in the post-synaptic neuron. This type of mechanism can be used to process information from different sources in the body to help in decision-making.