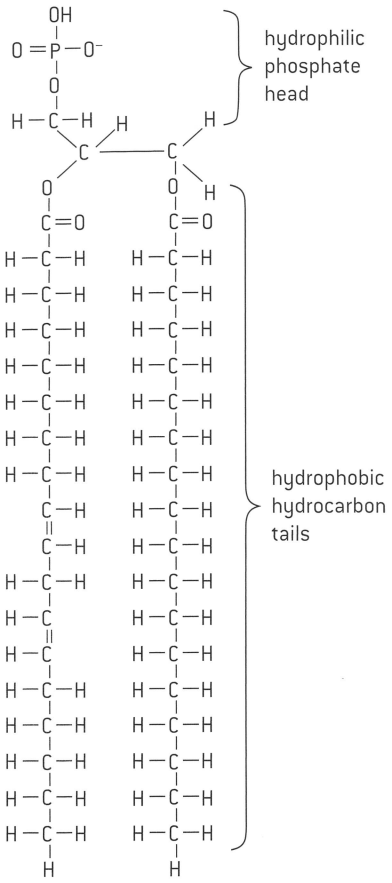


▲ Figure 1 The molecular structure of a phospholipid. The phosphate often has other hydrophilic groups attached to it, but these are not shown in this diagram

Phospholipid bilayers

Phospholipids form bilayers in water due to the amphipathic properties of phospholipid molecules.

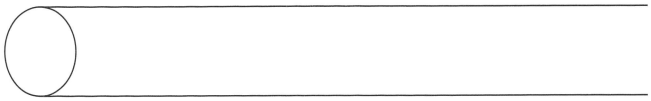
Some substances are attracted to water – they are **hydrophilic**.

Other substances are not attracted to water – they are **hydrophobic**.

Phospholipids are unusual because part of a phospholipid molecule is hydrophilic and part is hydrophobic. Substances with this property are described as **amphipathic**.

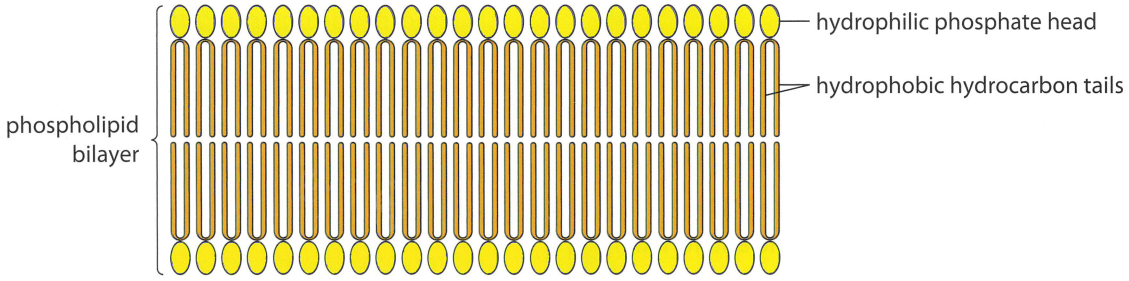
The hydrophilic part of a phospholipid is the phosphate group. The hydrophobic part consists of two hydrocarbon chains. The chemical structure of phospholipids is shown in figure 1.

The structure can be represented simply using a circle for the phosphate group and two lines for the hydrocarbon chains.



▲ Figure 2 Simplified diagram of a phospholipid molecule

The two parts of the molecule are often called phosphate heads and hydrocarbon tails. When phospholipids are mixed with water the phosphate heads are attracted to the water but the hydrocarbon tails are attracted to each other, but not to water. Because of this the phospholipids become arranged into double layers, with the hydrophobic hydrocarbon tails facing inwards towards each other and the hydrophilic heads facing the water on either side. These double layers are called phospholipid bilayers. They are stable structures and they form the basis of all cell membranes.



▲ Figure 3 Simplified diagram of a phospholipid bilayer

Models of membrane structure

Using models as representations of the real world: there are alternative models of membrane structure.

In the 1920s, Gorter and Grendel extracted phospholipids from the plasma membrane of red blood cells and calculated that the area that the phospholipids occupied when arranged in a monolayer was twice as large as the area of plasma membrane. They deduced that the membrane contained a **bilayer** of phospholipids. There were several errors in

their methods but luckily these cancelled each other out and there is now very strong evidence for cell membranes being based on phospholipid bilayers.

Membranes also contain protein and Gorter and Grendel's model did not explain where this is located. In the 1930s Davson and Danielli proposed layers of protein adjacent to the phospholipid bilayer, on both sides of the membrane. They proposed this sandwich model because they thought it would explain how membranes, despite being very thin, are a very effective barrier to the movement of some substances. High magnification electron micrographs of membranes were made in the 1950s, which showed a railroad track appearance – two dark lines with a lighter band between.Proteins appear dark in electron micrographs and phospholipids appear light, so this appearance fitted the Davson-Danielli model.

Another model of membrane structure was proposed in 1966 by Singer and Nicolson. In this model the proteins occupy a variety of positions in the membrane. Peripheral proteins are attached to the inner or outer surface. Integral proteins are embedded in the phospholipid bilayer, in some cases with parts protruding out from the bilayer on one or both sides. The proteins are likened to the tiles in a mosaic. Because the phospholipid molecules are free to move in each of the two layers of the bilayer, the proteins are also able to move. This gives the model its name – the fluid mosaic model.

Problems with the Davson–Danielli model

Falsification of theories with one theory being superseded by another: evidence falsified the Davson–Danielli model.

The Davson–Danielli model of membrane structure was accepted by most cell biologists for about 30 years. Results of many experiments fitted the model including X-ray diffraction studies and electron microscopy.

In the 1950s and 60s some experimental evidence accumulated that did not fit with the Davson–Danielli model:

- **Freeze-etched electron micrographs.** This technique involves rapid freezing of cells and then fracturing them. The fracture occurs along lines of weakness, including the centre of membranes. Globular structures scattered through freeze-etched images of the centre of membranes were interpreted as transmembrane proteins.
- **Structure of membrane proteins.** Improvements in biochemical techniques allowed proteins to be extracted from membranes. They were found to be very varied in size and globular in shape so were unlike the type of structural protein that would form continuous layers on the

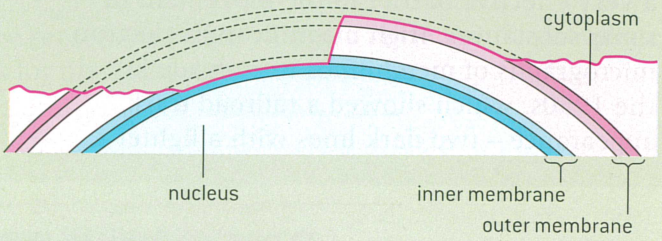
▲ Figure 4 Freeze-etched electron micrograph of nuclear membranes, with nuclear pores visible and vesicles in the surrounding cytoplasm. The diagram on page 28 shows the line of fracture through the centre of the inner and outer nuclear membranes. Transmembrane proteins are visible in both of the membranes

periphery of the membrane. Also the proteins were hydrophobic on at least part of their surface so they would be attracted to the hydrocarbon tails of the phospholipids in the centre of the membrane.

- **Fluorescent antibody tagging.** Red or green fluorescent markers were attached to antibodies that bind to membrane proteins. The membrane proteins of some cells were tagged with red markers and other cells with green markers. The cells were fused together. Within 40 minutes the red and green markers were mixed throughout the membrane of the fused cell. This showed that membrane proteins are free to move within the membrane rather than being fixed in a peripheral layer.

Taken together, this experimental evidence falsified the Davson–Danielli model. A replacement was needed that fitted the evidence and the model that became widely accepted was the Singer–Nicolson fluid mosaic model. It has been the leading model for over fifty years but it would be unwise to assume that it will never be superseded. There are already some suggested modifications of the model.

An important maxim for scientists is "Think it possible that you might be mistaken." Advances in science happen because scientists reject dogma and instead search continually for better understanding.



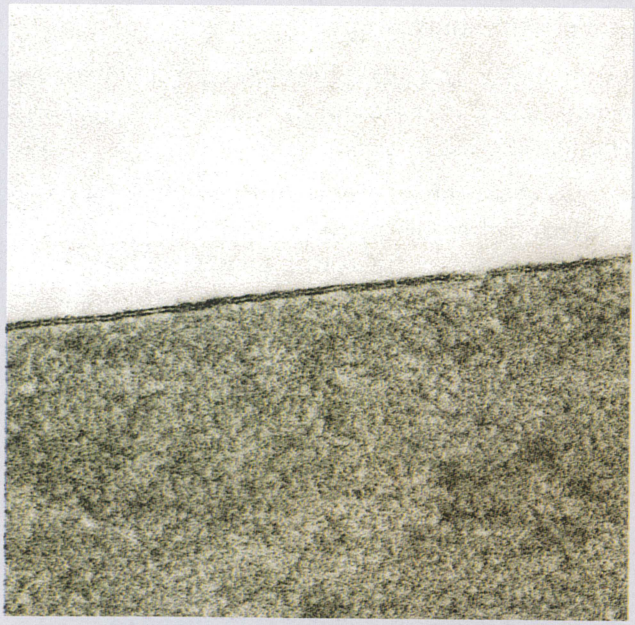
Evidence for and against the Davson–Danielli model of membrane structure

Analysis of evidence from electron microscopy that led to the proposal of the Davson–Danielli model.

Figure 5 shows the plasma membrane of a red blood cell and some of the cytoplasm near the edge of the cell.

1. Describe the appearance of the plasma membrane. [2]
2. Explain how this appearance suggested that the membrane had a central region of phospholipid with layers of protein on either side. [2]
3. Suggest reasons for the dark grainy appearance of the cytoplasm of the red blood cell. [2]
4. Calculate the magnification of the electron micrograph assuming that the thickness of the membrane is 10 nanometres. [3]

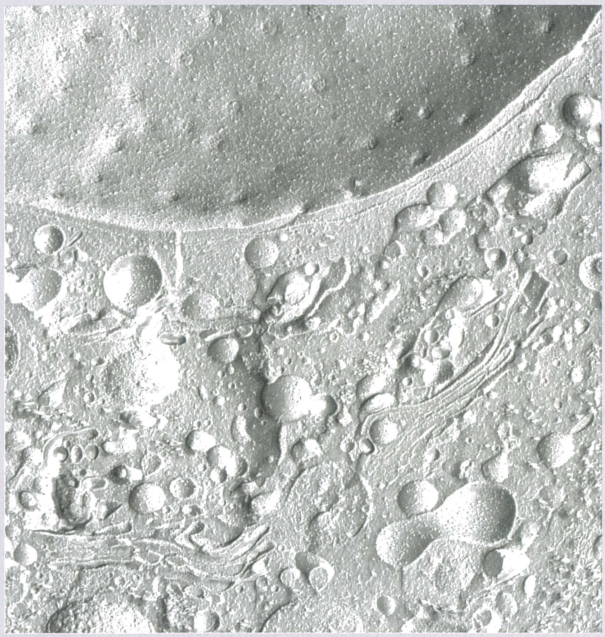
The two sets of data-based questions that follow are based on the types of data that were used to falsify the Davson–Danielli model of membrane structure.



▲ Figure 5 TEM of plasma membrane of a red blood cell

Data-based questions: Membranes in freeze-etched electron micrographs

Figure 6 shows a freeze-etched electron micrograph image of part of a cell. It was prepared by Professor Horst Robenek of Münster University.



▲ Figure 6

1 In all of the fractured membranes in the micrograph small granules are visible.

a) State what these granules are. [2]

b) Explain the significance of these granules in the investigation of membrane structure. [3]

2 One of the membranes that surround the nucleus is visible at the top of the micrograph. Deduce whether it is the inner or outer nuclear membrane. (Always give your reasons when asked to deduce something.) [2]

3 Identify three mitochondria visible in the micrograph, either using labels or by describing their positions. [2]

4 Explain the evidence from the micrograph that this cell was processing proteins in its cytoplasm. [2]

Extension questions on this topic can be found at www.oxfordsecondary.co.uk/ib-biology

Diffusion of proteins in membranes

Frye and Edidin used an elegant technique to obtain evidence for the fluid nature of membranes. They attached fluorescent markers to membrane proteins – green markers to mouse cells and red markers to human cells. In both cases, spherical cells growing in tissue culture were used. The marked mouse and human cells were then fused together. At first, the fused cells had one green hemisphere and one red one, but over the minutes following fusion, the red and green markers gradually merged, until they were completely mixed throughout the whole of the cell membrane. Blocking of ATP production did not prevent this mixing (ATP supplies energy for active processes in the cell).

Time after fusion / minutes	Cells with markers fully mixed/%				
	Result 1	Result 2	Result 3	Result 4	Mean
5	0	0	–	–	
10	3	0	–	–	
25	40	54	–	–	
40	87	88	93	100	
120	100	–	–	–	

1 Calculate the mean percentage of cells with markers fully mixed for each time after fusion. [4]

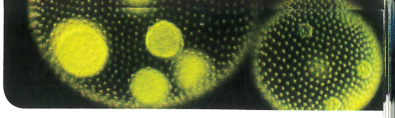
2 Plot a graph of the results, including range bars for times where there was variation in the results. To do this you plot the highest and lowest results with a small bar and join these bars with a ruled line. You should also plot the mean result with a cross. This will lie on the range bar. [4]

3 Describe the trend shown by the graph. [1]

4 Explain whether the results fit the Davson–Danielli model or the Singer–Nicolson model more closely. [2]

5 Explain the benefit of plotting range bars on graphs. [2]

6 During this experiment the cells were incubated at 37 °C. Suggest a reason for the researchers choosing this temperature. [1]



Spontaneous generation and the origin of cells

Verifying the general principles that underlie the natural world: the principle that cells only come from pre-existing cells needs to be verified.

Spontaneous generation is the formation of living organisms from non-living matter. The Greek philosopher and botanist Theophrastus reported that a plant called Silphium had sprung up from soil where it was not previously present and described this as an example of spontaneous generation. Aristotle wrote about insects being formed from the dew falling on leaves or from the hair, flesh or faeces of animals. In the 16th century the German-Swiss botanist and astrologer Paracelsus quoted observations of spontaneous generation of mice, frogs and eels from water, air or decaying matter.

It is easy to see how ideas of spontaneous generation could have persisted when cells and microorganisms had not been discovered and the nature of sexual reproduction was not understood. From the 17th century onwards biologists carried out experiments to test the theory that life could arise from non-living matter. Francesco Redi showed that maggots only developed in rotting meat if flies were allowed to come into contact with it. Lazzaro Spallanzani boiled soup in eight containers, then sealed four of them and left the others open to the air. Organisms grew in the containers left open but not in the others.

Some biologists remained convinced that spontaneous generation could occur if there was access to the air. Louis Pasteur responded by carrying out carefully designed experiments with swan-necked flasks, which established beyond reasonable doubt that spontaneous generation of life does not now occur. Pasteur's experiments are described in the next section of this sub-topic.

Apart from the evidence from the experiments of Pasteur and others, there are other reasons for biologists universally accepting that cells only come from pre-existing cells:

- A cell is a highly complex structure and no natural mechanism has been suggested for producing cells from simpler subunits.
- No example is known of increases in the number of cells in a population, organism or tissue without cell division occurring.
- Viruses are produced from simpler subunits but they do not consist of cells, and they can only be produced inside the host cells that they have infected.

Spontaneous generation and Pasteur's experiments

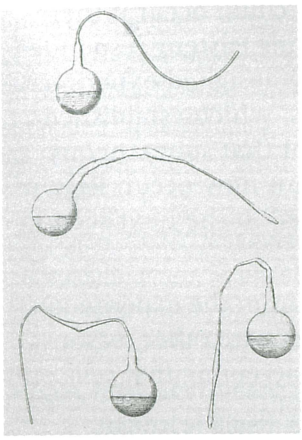
Evidence from Pasteur's experiments that spontaneous generation of cells and organisms does not now occur on Earth.

Louis Pasteur made a nutrient broth by boiling water containing yeast and sugar. He showed that if this broth was kept in a sealed flask, it remained unchanged, and no fungi or other organisms appeared. He then passed air though a pad of cotton wool in a tube, to filter out microscopic particles from the air, including bacteria and the spores of fungi. If the pad of cotton wool was placed in broth in a sealed flask, within 36 hours, there were large number of microorganisms in the broth and mould grew over its surface.

The most famous of Pasteur's experiments involved the use of swan-necked flasks. He placed samples of broth in flasks with long necks and then melted the glass of the necks and bent it into a variety of shapes, shown in figure 3.

Pasteur then boiled the broth in some of the flasks to kill any organisms present but left others unboiled as controls. Fungi and other organisms soon appeared in the unboiled flasks but not in the boiled ones, even after long periods of time. The broth in the flasks was in contact with air, which it had been suggested was needed for spontaneous generation, yet no spontaneous generation occurred. Pasteur snapped the necks of some of the flasks to leave a shorter vertical neck. Organisms were soon apparent in these flasks and decomposed the broth.

Pasteur published his results in 1860 and subsequently repeated them with other liquids including urine and milk, with the same results. He concluded that the swan necks prevented organisms from the air getting into the broth or other liquids and that no organisms appeared spontaneously. His experiments convinced most biologists, both at the time of publication and since then.



▲ Figure 3 Drawings of Pasteur's swan-necked flasks

Origin of the first cells

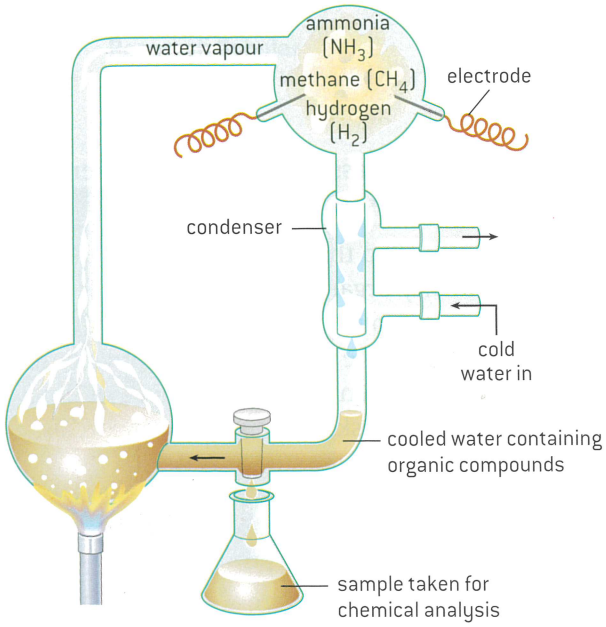
The first cells must have arisen from non-living material.

If we trace back the ancestry of cells over billions of years, we must eventually reach the earliest cells to have existed. These were the first living things on Earth. Unless cells arrived on Earth from somewhere else in the universe, they must have arisen from non-living material. This is a logical conclusion, but it gives perhaps the hardest question of all for biologists to answer: how could a structure as complex as the cell have arisen by natural means from non-living material?

It has sometimes been argued that complex structures cannot arise by evolution, but there is evidence that this can happen in a series of stages over long periods of time. Living cells may have evolved over hundreds of millions of years. There are hypotheses for how some of the main stages could have occurred.

1. Production of carbon compounds such as sugars and amino acids

Stanley Miller and Harold Urey passed steam through a mixture of methane, hydrogen and ammonia. The mixture was thought to be representative of the atmosphere of the early Earth. Electrical discharges were used to simulate lightning. They found that amino acids and other carbon compounds needed for life were produced.



▲ Figure 4 Miller and Urey's apparatus

2. Assembly of carbon compounds into polymers

A possible site for the origin of the first carbon compounds is around deep-sea vents. These are cracks in the Earth's surface, characterized by gushing hot water carrying reduced inorganic chemicals such as iron sulphide. These chemicals represent readily accessible supplies of energy, a source of energy for the assembly of these carbon compounds into polymers.

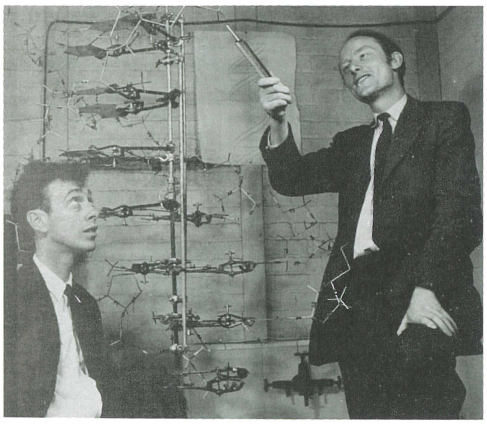
▲ Figure 5 Deep sea vents

TOK

What is the relative role of competition and cooperation in scientific research?

Three prominent research groups openly competed to elucidate the structure of DNA: Watson and Crick were working at Cambridge; Maurice Wilkins and Rosalind Franklin were working at Kings College of the University of London; and Linus Pauling's research group was operating out of Caltech in the United States.

A stereotype of scientists is that they take a dispassionate approach to investigation. The truth is that science is a social endeavour involving a number of emotion-influenced interactions between science. In addition to the joy of discovery, scientists seek the esteem of their community. Within research groups, collaboration is important, but outside of their research group competition often restricts open communication that might accelerate the pace of scientific discovery. On the other hand, competition may motivate ambitious scientists to work tirelessly.



▲ Figure 8 Crick and Watson and their DNA model

Crick and Watson's models of DNA structure

Crick and Watson's discovery of the structure of DNA using model-making.

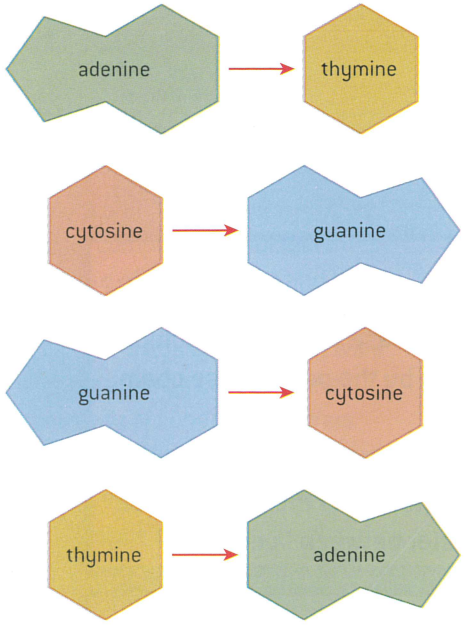
Crick and Watson's success in discovering the structure of DNA was based on using the evidence to develop possible structures for DNA and testing them by model-building. Their first model consisted of a triple helix, with bases on the outside of the molecule and magnesium holding the two strands together with ionic bonds to the phosphate groups on each strand. The helical structure and the spacing between subunits in the helix fitted the X-ray diffraction pattern obtained by Rosalind Franklin.

It was difficult to get all parts of this model to fit together satisfactorily and it was rejected when Franklin pointed out that there would not be enough magnesium available to form the cross links between the strands. Another deficiency of this first model was that is that it did not take account of Chargaff's finding that the amount of adenine equals the thymine and the amount of cytosine equals the amount of guanine.

To investigate the relationship between the bases in DNA pieces of cardboard were cut out to represent their shapes. These showed that A-T and C-G base pairs could be formed, with hydrogen bonds linking the bases. The base pairs were equal in length so would fit between two outer sugar-phosphate backbones.

Another flash of insight was needed to make the parts of the molecule fit together: the two strands in the helix had to run in opposite directions – they must be antiparallel. Crick and Watson were then able to build their second model of the structure of DNA. They used metal rods and sheeting cut to shape and held together with small clamps. Bond lengths were all to scale and bond angles correct. Figure 8 shows Crick and Watson with the newly constructed model.

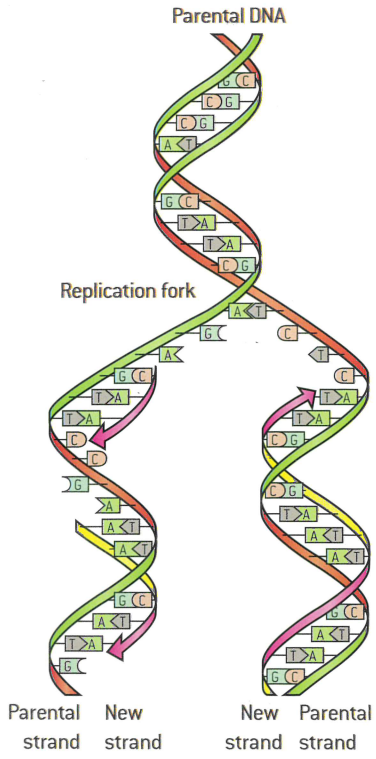
The model convinced all those who saw it. A typical comment was "It just looked right". The structure immediately suggested a mechanism for copying DNA. It also led quickly to the realization that the genetic code must consist of triplets of bases. In many ways the discovery of DNA structure started the great molecular biology revolution, with effects that are still reverberating in science and in society.



▲ Figure 1

The base sequence on the template strand determines the base sequence on the new strand. Only a nucleotide carrying a base that is complementary to the next base on the template strand can successfully be added to the new strand (figure 1).

This is because complementary bases form hydrogen bonds with each other, stabilizing the structure. If a nucleotide with the wrong base started to be inserted, hydrogen bonding between bases would not occur and the nucleotide would not be added to the chain. The rule that one base always pairs with another is called **complementary base pairing**. It ensures that the two DNA molecules that result from DNA replication are identical in their base sequences to the parent molecule that was replicated.



▲ Figure 2 Semi-conservative replication

Obtaining evidence for the theory of semi-conservative replication

Obtaining evidence for scientific theories: Meselson and Stahl obtained evidence for the semi-conservative replication of DNA.

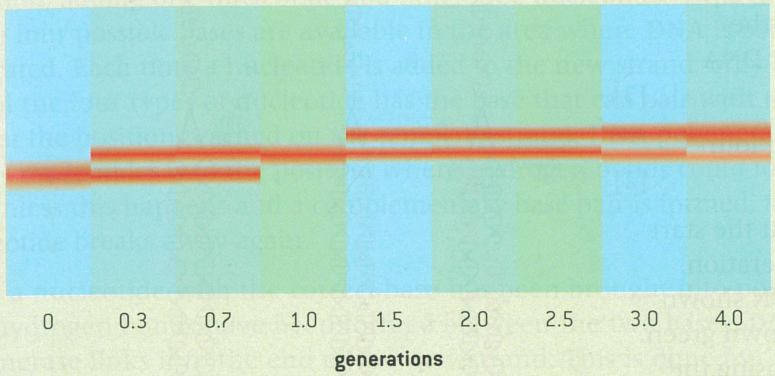
Semi-conservative replication is an example of a scientific theory that seemed intuitively right, but nonetheless needed to be backed up with evidence. Laboratories around the world attempted to confirm experimentally that replication of DNA is semi-conservative and soon convincing evidence had been obtained.

In 1958 Matthew Meselson and Franklin Stahl published the results of exceedingly elegant experiments that provided very strong evidence for semi-conservative replication. They used ^{15}N, a rare isotope of nitrogen that has one more neutron than the normal ^{14}N isotope, so is denser. In the 1930s Harold Urey had developed methods of purifying stable isotopes that could be used as tracers in biochemical pathways. ^{15}N was one of these.

Meselson and Stahl devised a new method of separating DNA containing ^{15}N in its bases from DNA with ^{14}N. The technique is called caesium chloride density gradient centrifugation. A solution of caesium chloride is spun in an ultracentrifuge at nearly 45,000 revolutions per minute for 20 hours. The dense caesium ions tend to move towards the bottom of the tube but do not sediment fully because of diffusion. A gradient is established, with the greatest caesium concentration, and therefore density, at the bottom and the lowest at the top of the tube. Any substance centrifuged with the caesium chloride solution becomes concentrated at a level corresponding with its density.

Meselson and Stahl cultured the bacterium *E. coli* for fourteen generations in a medium where the only nitrogen source was ^{15}N. Almost all nitrogen atoms in the bases of the DNA in the bacteria were therefore ^{15}N. They then transferred the bacteria abruptly to a medium in which all the nitrogen was ^{14}N. At the temperature used to culture them, the generation time was 50 minutes – the bacteria divided and therefore replicated their DNA once every 50 minutes.

Meselson and Stahl collected samples of DNA from the bacterial culture for several hours from the time when it was transferred to the ^{14}N medium. They extracted the DNA and measured its density by caesium chloride density gradient centrifugation. The DNA could be detected because it absorbs ultraviolet light, and so created a dark band when the tubes were illuminated with ultraviolet. Figure 3 shows the results. In the next part of this sub-topic there is guidance in how to analyse the changes in position of the dark bands.



▲ Figure 3

Activity

New experimental techniques

Meselson and Stahl used three techniques in their experiments that that were relatively new. Identify a technique used by them that was developed:

a) by Urey in the 1930s

b) by Pickels in the 1940s

c) by Meselson and Stahl themselves in the 1950s.

Activity

Modelling helicase activity

To model helicase activity you could use some two-stranded rope or string and a split key ring. The strands in the rope are helical and represent the two strands in DNA. Open the key ring and put one strand of the rope inside it. Close the ring so that the other strand is outside. Slide the ring along the string to separate the strands. What problems are revealed by this model of the activity of helicase? Use the internet to find the solution used by living organisms.

Meselson and Stahl's DNA replication experiments

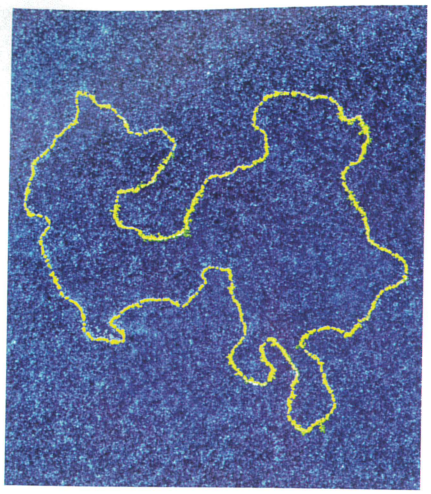
Analysis of Meselson and Stahl's results to obtain support for the theory of semi-conservative replication of DNA.

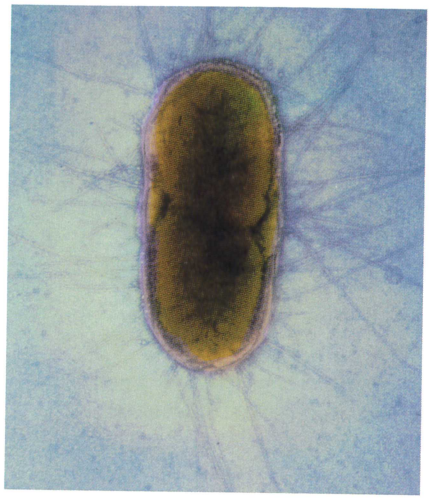
The data-based question below will guide you through the analysis of Meselson and Stahl's results and help to build your skills in this aspect of science.

Data-based questions: The Meselson and Stahl experiment

In order for cell division to occur, DNA must be duplicated to ensure that progeny cells have the same genetic information as the parent cells. The

to a ^{14}N medium. Samples of the bacteria were taken over a period of time and separated by density gradient centrifugation, a method in





▲ Figure 1 (a) Circular DNA molecule from a bacterium (b) Bacterium preparing to divide

Because only one chromosome is present in a prokaryotic cell, there is usually only a single copy of each gene. Two identical copies are present briefly after the chromosome has been replicated, but this is a preparation for cell division. The two genetically identical chromosomes are moved to opposite poles and the cell then splits in two.

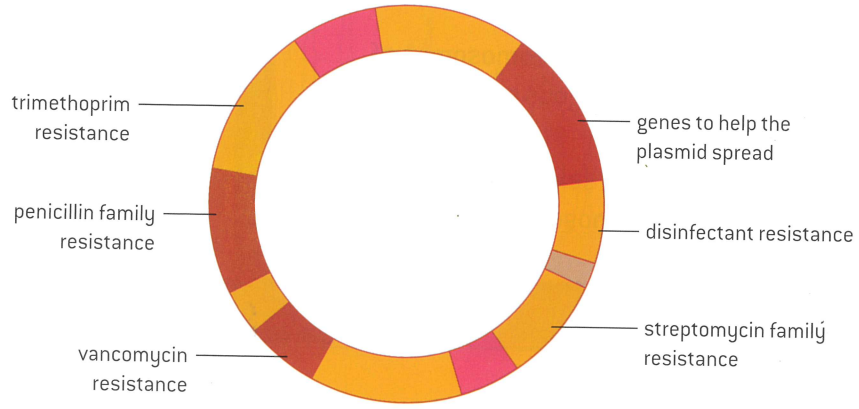
Plasmids

Some prokaryotes also have plasmids but eukaryotes do not.

Plasmids are small extra DNA molecules that are commonly found in prokaryotes but are very unusual in eukaryotes. They are usually small, circular and naked, containing a few genes that may be useful to the cell but not those needed for its basic life processes. For example, genes for antibiotic resistance are often located in plasmids. These genes are beneficial when an antibiotic is present in the environment but are not at other times.

Plasmids are not always replicated at the same time as the chromosome of a prokaryotic cell or at the same rate. Hence there may be multiple copies of plasmids in a cell and a plasmid may not be passed to both cells formed by cell division.

Copies of plasmids can be transferred from one cell to another, allowing spread through a population. It is even possible for plasmids to cross the species barrier. This happens if a plasmid that is released when a prokaryotic cell dies is absorbed by a cell of a different species. It is a natural method of gene transfer between species. Plasmids are also used by biologists to transfer genes between species artificially.



▲ Figure 2 The pLW1043 plasmid

Using autoradiography to measure DNA molecules

Developments in scientific research follow improvements in techniques: autoradiography was used to establish the length of DNA molecules in chromosomes.

Quantitative data is usually considered to be the strongest type of evidence for or against a hypothesis, but in biology it is sometimes images that provide the most convincing evidence.

Developments in microscopy have allowed images to be produced of structures that were previously invisible. These sometimes confirm existing ideas but sometimes also change our understanding.

Autoradiography was used by biologists from the 1940s onwards to discover where specific substances were located in cells or tissues. John Cairns used the technique in a different way in the 1960s. He obtained images of whole DNA molecules from *E. coli* bacteria. At the time it was not clear whether the bacterial chromosome was a single DNA molecule or more than one, but the images produced by Cairns answered this question. They also revealed replication forks in DNA for the first time. Cairns's technique was used by others to investigate the structure of eukaryote chromosomes.

Measuring the length of DNA molecules

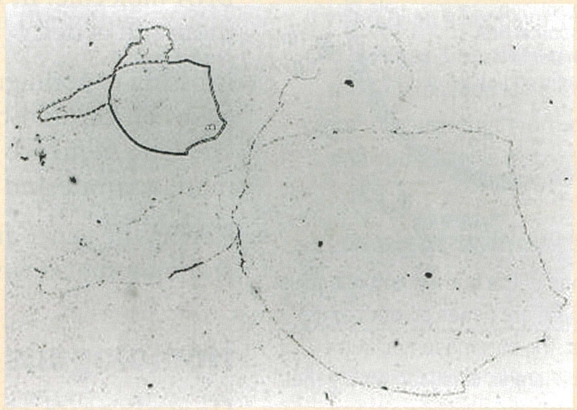
Cairns's technique for measuring the length of DNA molecules by autoradiography.

John Cairns produced images of DNA molecules from *E.coli* using this technique:

- Cells were grown for two generations in a culture medium containing tritiated thymidine. Thymidine consists of the base thymine linked to deoxyribose and is used by *E. coli* to make nucleotides that it uses in DNA replication. Tritiated thymidine contains tritium, a radioactive isotope of hydrogen, so radioactively labelled DNA was produced by replication in the *E. coli* cells.
- The cells were then placed onto a dialysis membrane and their cell walls were digested using the enzyme lysozyme. The cells were gently burst to release their DNA onto the surface of the dialysis membrane.
- A thin film of photographic emulsion was applied to the surface of the membrane and left in darkness for two months. During that time some of the atoms of tritium in the DNA decayed and emitted high energy electrons, which react with the film.
- At the end of the two-month period the film was developed and examined with a microscope. At each point where a tritium atom decayed there is a dark grain. These indicate the position of the DNA.

The images produced by Cairns showed that the chromosome in *E. coli* is a single circular DNA molecule with a length of 1,100 µm. This is remarkably long given that the length of the *E coli* cells is only 2 µm.

Autoradiography was then used by other researchers to produce images of eukaryotic chromosomes. An image of a chromosome from the fruit fly *Drosophila melanogaster* was produced that was 12,000 µm long. This corresponded with the total amount of DNA known to be in a *D. melanogaster* chromosome, so for this species at least a chromosome contains one very long DNA molecule. In contrast to prokaryotes, the molecule was linear rather than circular.



▲ Figure 3

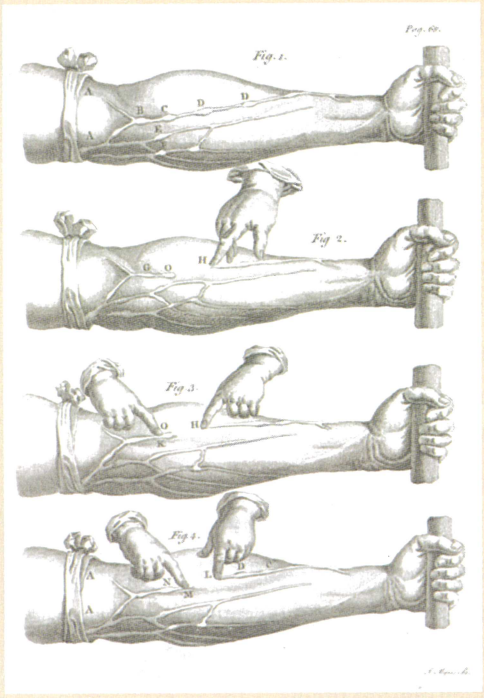
William Harvey and the circulation of blood

William Harvey's discovery of the circulation of the blood with the heart acting as the pump.

William Harvey is usually credited with the discovery of the circulation of the blood as he combined earlier discoveries with his own research findings to produce a convincing overall theory for blood flow in the body. He overcame widespread opposition by publishing his results and also by touring Europe to demonstrate experiments that falsified previous theories and provided evidence for his theory. As a result his theory became generally accepted.

Harvey demonstrated that blood flow through the larger vessels is unidirectional, with valves to prevent backflow. He also showed that the rate of flow through major vessels was far too high for blood to be consumed in the body after being pumped out by the heart, as earlier theories proposed. It must therefore return to the heart and be recycled. Harvey showed that the heart pumps blood out in the arteries and it returns in veins. He predicted the presence of numerous fine vessels too small to be seen with contemporary equipment that linked arteries to veins in the tissues of the body.

Blood capillaries are too narrow to be seen with the naked eye or with a hand lens. Microscopes had not been invented by the time that Harvey published his theory about the circulation of blood in 1628. It was not until 1660, after his death, that blood was seen flowing from arteries to veins though capillaries as he had predicted.



▲ Figure 1 Harvey's experiment to demonstrate that blood flow in the veins of the arm is unidirectional

Overturning ancient theories in science

Theories are regarded as uncertain: William Harvey overturned theories developed by the ancient Greek philosopher Galen on movement of blood in the body.

During the Renaissance, interest was reawakened in the classical writings of Greece and Rome. This stimulated literature and the arts, but in some ways it hampered progress in science. It became almost impossible to question the doctrines of such writers as Aristotle, Hippocrates, Ptolemy and Galen.

According to Galen, blood is formed in the liver and is pumped to and fro between the liver and the right ventricle of the heart. A little blood passes into the left ventricle, where it meets air from the lungs and becomes "vital spirits". The vital spirits are distributed to the body by the arteries. Some of the vital spirits flow to the brain, to be converted into "animal spirits", which are then distributed by the nerves to the body.

William Harvey was unwilling to accept these doctrines without evidence. He made careful observations and did experiments, from which he deduced that blood circulates through the pulmonary and systemic circulations. He predicted the existence of capillaries, linking arteries and veins, even though the lenses of the time were not powerful enough for him to see them.

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The following extract is from Harvey's book *On the Generation of Animals*, published in 1651 when he was 73.

> *And hence it is that without the due admonition of the senses, without frequent observation and reiterated experiment, our mind goes astray after phantoms and appearances. Diligent observation is therefore requisite in every science, and the senses are frequently to be appealed to. We are, I say, to strive after personal experience, not to rely of the experience of others: without which no one can properly become a student of any branch of natural science. I would not have you therefore, gentle reader, to take anything on trust from me concerning the Generation of Animals: I appeal to your own eyes as my witness and judge. The method of pursuing truth commonly pursued at this time therefore is to be held erroneous and almost foolish, in which so many enquire what things others have said, and omit to ask whether the things themselves be actually so or not.*

Arteries

Arteries convey blood at high pressure from the ventricles to the tissues of the body.

Arteries are vessels that convey blood from the heart to the tissues of the body. The main pumping chambers of the heart are the ventricles. They have thick strong muscle in their walls that pumps blood into the arteries, reaching a high pressure at the peak of each pumping cycle. The artery walls work with the heart to facilitate and control blood flow. Elastic and muscle tissue in the walls are used to do this.

Elastic tissue contains elastin fibres, which store the energy that stretches them at the peak of each pumping cycle. Their recoil helps propel the blood on down the artery. Contraction of smooth muscle in the artery wall determines the diameter of the lumen and to some extent the rigidity of the arteries, thus controlling the overall flow through them.

Both the elastic and muscular tissues contribute to the toughness of the walls, which have to be strong to withstand the constantly changing and intermittently high blood pressure without bulging outwards (aneurysm) or bursting. The blood's progress along major arteries is thus pulsatile, not continuous. The pulse reflects each heartbeat and can easily be felt in arteries that pass near the body surface, including those in the wrist and the neck.

Each organ of the body is supplied with blood by one or more arteries. For example, each kidney is supplied by a renal artery and the liver by the hepatic artery. The powerful, continuously active muscles of the heart itself are supplied with blood by coronary arteries.

Activity

Discussion questions on William Harvey's methods

1. William Harvey refused to accept doctrines without evidence. Are there academic contexts where it is reasonable to accept doctrines on the basis of authority rather than evidence gathered from primary sources?
2. Harvey welcomed questions and criticisms of his theories when teaching anatomy classes. Suggest why he might have done this.
3. Can you think of examples of the "phantoms and appearances" that Harvey refers to?
4. Why does Harvey recommend "reiteration"

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.

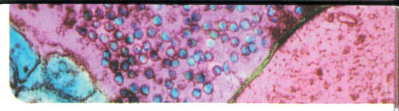
On the other hand, the patients that they used were all on the point of death and several were cured of their infections as a result of the experimental treatment. Because of expeditious testing with greater risk-taking than would now be allowed, penicillin was introduced far more quickly than would 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



In vitro fertilization

The use in IVF of drugs to suspend the normal secretion of hormones, followed by the use of artificial doses of hormones to induce superovulation and establish a pregnancy.

The natural method of fertilization in humans is *in vivo*, meaning that it occurs inside the living tissues of the body. Fertilization can also happen outside the body in carefully controlled laboratory conditions. This is called *in vitro* fertilization, almost always abbreviated to IVF. This procedure has been used extensively to overcome fertility problems in either the male or female parent.

There are several different protocols for IVF, but the first stage is usually down-regulation. The woman takes a drug each day, usually as a nasal spray, to stop her pituitary gland secreting FSH or LH. Secretion of estrogen and progesterone therefore also stops. This suspends the normal menstrual cycle and allows doctors to control the timing and amount of egg production in the woman's ovaries.

Intramuscular injections of FSH and LH are then given daily for about ten days, to stimulate follicles to develop. The FSH injections give a much higher concentration of this hormone than during a normal menstrual cycle and as a consequence far more follicles develop than usual. Twelve is not unusual and there can be as many twenty follicles. This stage of IVF is therefore called superovulation.

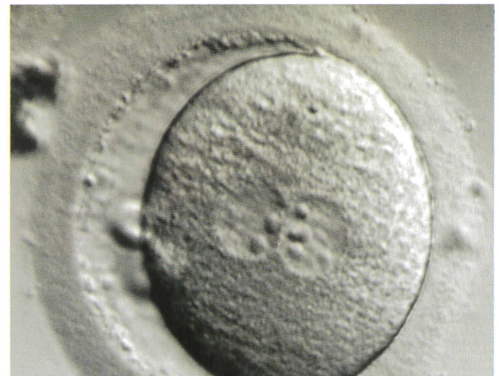
When the follicles are 18 mm in diameter they are stimulated to mature by an injection of HCG, another hormone that is normally secreted by the embryo. A micropipette mounted on an ultrasound scanner is passed through the uterus wall to wash eggs out of the follicles. Each egg is mixed with 50,000 to 100,000 sperm cells in sterile conditions in a shallow dish, which is then incubated at 37 °C until the next day.

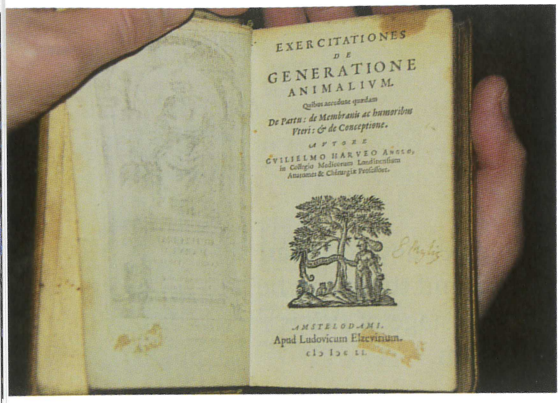
If fertilization is successful then one or more embryos are placed in the uterus when they are about 48 hours old. Because the woman has not gone through a normal menstrual cycle extra progesterone is usually given as a tablet placed in the vagina, to ensure that the uterus lining is maintained. If the embryos implant and continue to grow then the pregnancy that follows is no different from a pregnancy that began by natural conception.

William Harvey and sexual reproduction

William Harvey's investigation of sexual reproduction in deer.

William Harvey is chiefly remembered for his discovery of the circulation of the blood, but he also had a lifelong obsession with how life is transmitted from generation to generation and pioneered research into sexual reproduction. He was taught the "seed and soil" theory of Aristotle, according to which the male produces a seed, which forms an





▲ Figure 13 William Harvey's book on the reproduction of animals *Exercitationes de Generatione Animalium* published in 1651

He regarded his experiments with deer as proof that Aristotle's theory of reproduction was false and concluded "the fetus doth neither proceed from the seed of male or female in coition, nor yet from any commixture of that seed". Although Aristotle's "seed and soil" theory was false, Harvey's conclusion that the fetus did not result from events during coitus (sexual intercourse) was also false.

Harvey was well aware that he had not discovered the basis of sexual reproduction: "neither the philosophers nor the physicians of yesterday or today have satisfactorily explained, or solved the problem of Aristotle."

Improvements in apparatus and research breakthroughs

Developments in scientific research follow improvements in apparatus: William Harvey was hampered in his observational research into reproduction by lack of equipment. The microscope was invented seventeen years after his death.

Harvey was understandably reluctant to publish his research into sexual reproduction, but he did eventually do so in 1651 when he was 73 years old in his work *Exercitationes de Generatione Animalium*. He knew that he had not solved the mystery of sexual reproduction:

> *When I plainly see nothing at all doth remain in the uterus after coition, ... no more than remains in the braine after sensation, ... I have invented this Fable. Let the learned and ingenious flock of men consider of it; let the supercilious reject it: and for the scoffing ticklish generation, let them laugh their swinge. Because I say, there is no sensible thing in the uterus after coition; and yet there is a necessity, that something should be there, which may render the animal fruitful.*

William Harvey failed to solve the mystery because effective microscopes were not available when he was working, so fusion of gametes and subsequent embryo development remained undiscovered. He was unlucky with his choice of experimental animal because embryos in the deer that he used remain microscopically small for an unusually long period. Microscopes were invented seventeen years after Harvey's death, allowing the discovery of sperm, eggs and early stage embryos.

Scientific research has often been hampered for a time by deficiencies in apparatus, with discoveries only being made following improvements. This will continue into the future and we can look forward to further transformations in our understanding of the natural world as new techniques and technology are invented.