2 MOLECULAR BIOLOGY

Introduction
Water is the medium for life. Living organisms control their composition by a complex web of chemical reactions that occur within this medium. Photosynthesis uses the energy in sunlight to supply the chemical energy needed for life and cell respiration releases this energy when it is needed. Compounds of carbon, hydrogen and oxygen are used to supply and store energy. Many proteins act as enzymes to control the metabolism of the cell and others have a diverse range of biological functions. Genetic information is stored in DNA and can be accurately copied and translated to make the proteins needed by the cell.

2.1 Molecules to metabolism

Understanding

- Molecular biology explains living processes in terms of the chemical substances involved.
- Carbon atoms can form our bonds allowing a diversity of compounds to exist.
- Life is based on carbon compounds including carbohydrates, lipids, proteins and nucleic acids.
- Metabolism is the web of all the enzyme catalysed reactions in a cell or organism.
- Anabolism is the synthesis of complex molecules from simpler molecules including the formation of macromolecules from monomers by condensation reactions.
- Catabolism is the breakdown of complex molecules into simpler molecules including the hydrolysis of macromolecules into monomers.

Applications

- Urea as an example of a compound that is produced by living organisms but can also be artificially synthesized.

Skills

- Drawing molecular diagrams of glucose, ribose, a saturated fatty acid and a generalized amino acid.
- Identification of biochemicals such as carbohydrate, lipid or protein from molecular diagrams.

Nature of science

- Falsification of theories: the artificial synthesis of urea helped to falsify vitalism.
Molecular biology

Molecular biology explains living processes in terms of the chemical substances involved.

The discovery of the structure of DNA in 1953 started a revolution in biology that has transformed our understanding of living organisms. It raised the possibility of explaining biological processes from the structure of molecules and how they interact with each other. The structures are diverse and the interactions are very complex, so although molecular biology is more than 50 years old, it is still a relatively young science.

Many molecules are important in living organisms including one as apparently simple as water, but the most varied and complex molecules are nucleic acids and proteins. Nucleic acids comprise DNA and RNA. They are the chemicals used to make genes. Proteins are astonishingly varied in structure and carry out a huge range of tasks within the cell, including controlling chemical reactions of the cell by acting as enzymes. The relationship between genes and proteins is at the heart of molecular biology.

The approach of the molecular biologist is reductionist as it involves considering the various biochemical processes of a living organism and breaking down into its component parts. This approach has been immensely productive in biology and has given us insights into whole organisms that we would not otherwise have. Some biologists argue that the reductionist approach of the molecular biologist cannot explain everything though, and that when component parts are combined there are emergent properties that cannot be studied without looking at the whole system together.

Synthesis of urea

Urea as an example of a compound that is produced by living organisms but can also be artificially synthesized.

Urea is a nitrogen-containing compound with a relatively simple molecular structure (figure 2). It is a component of urine and this was where it was first discovered. It is produced when there is an excess of amino acids in the body, as a means of excreting the nitrogen from the amino acids. A cycle of reactions, catalysed by enzymes, is used to produce it (figure 3). This happens in the liver. Urea is then transported by the blood stream to the kidneys where it is filtered out and passes out of the body in the urine.

Urea can also be synthesized artificially. The chemical reactions used are different from those in the liver and enzymes are not involved, but the urea that is produced is identical.

\[
\text{ammonia} + \text{carbon dioxide} \rightarrow \text{ammonium carbamate} \\
\rightarrow \text{urea} + \text{water}
\]

About 100 million tonnes are produced annually. Most of this is used as a nitrogen fertilizer on crops.
Urea and the falsification of vitalism

Falsification of theories: the artificial synthesis of urea helped to falsify vitalism.

Urea was discovered in urine in the 1720s and was assumed to be a product of the kidneys. At that time it was widely believed that organic compounds in plants and animals could only be made with the help of a “vital principle”. This was part of vitalism – the theory that the origin and phenomena of life are due to a vital principle, which is different from purely chemical or physical forces. Aristotle used the word psyche for the vital principle – a Greek word meaning breath, life or soul.

In 1828 the German chemist Friedrich Wöhler synthesized urea artificially using silver isocyanate and ammonium chloride. This was the first organic compound to be synthesized artificially. It was a very significant step, because no vital principle had been involved in the synthesis. Wöhler wrote this excitedly to the Swedish chemist Jöns Jacob Berzelius:

In a manner of speaking, I can no longer hold m scattered, chemical water. I must tell you that I can make urea without the kidneys of an animal, be it man or dog.

An obvious deduction was that if urea had been synthesized without a vital principle, other organic compounds could be as well. Wöhler’s achievement was evidence against the theory of vitalism. It helped to falsify the theory, but it did not cause all biologists to abandon vitalism immediately. It usually requires several pieces of evidence against a theory for most biologists to accept that it has been falsified and sometimes controversies over a theory continue for decades.

Although biologists now accept that processes in living organisms are governed by the same chemical and physical forces as in non-living matter, there remain some organic compounds that have not been synthesized artificially. It is still impossible to make complex proteins such as hemoglobin, for example, without using ribosomes and other components of cells. Four years after his synthesis of urea, Wöhler wrote this to Berzelius:

Organic chemistry nowadays almost drives one mad. To me it appears like a primeval tropical forest full of the most remarkable things; a dreadful endless jungle into which one dare not enter, for there seems no way out.
Activity

Carbon compounds

Can you find an example of a biological molecule in which a carbon atom is bonded to atoms of three other elements or even our other elements?

Titin is a giant protein that acts as a molecular spring in muscle. The backbone of the titin molecule is a chain of 100,000 atoms, linked by single covalent bonds.

Can you find an example of a molecule in your body with a chain of over 1,000,000,000 atoms?

Carbon compounds

Carbon atoms can form our bonds allowing a diversity of compounds to exist.

Carbon is only the 15th most abundant element on Earth, but it can be used to make a huge range of different molecules. This has given living organisms almost limitless possibilities for the chemical composition and activities of their cells. The diversity of carbon compounds is explained by the properties of carbon.

Carbon atoms form covalent bonds with other atoms. A covalent bond is formed when two adjacent atoms share a pair of electrons, with one electron contributed by each atom. Covalent bonds are the strongest type of bond between atoms so stable molecules based on carbon can be produced.

Each carbon atom can form up to four covalent bonds to other atoms, so molecules containing carbon can have complex structures. The bonds can be with other carbon atoms to make rings or chains of any length. Fatty acids contain chains of up to 20 carbon atoms for example. The bonds can also be with other elements such as hydrogen, oxygen, nitrogen or phosphorus.

Carbon atoms can bond with just one other element, such as hydrogen in methane, or they can bond to more than one other element as in ethanol (alcohol found in beer and wine). The four bonds can all be single covalent bonds or there can be two single and one double covalent bond, for example in the carboxyl group of ethanoic acid (the acid in vinegar).

Classifying carbon compounds

Life is based on carbon compounds including carbohydrates, lipids, proteins and nucleic acids.

Living organisms use four main classes of carbon compound. They have different properties and so can be used for different purposes.

Carbohydrates are characterized by their composition. They are composed of carbon, hydrogen and oxygen, with hydrogen and oxygen in the ratio of two hydrogen atoms to one oxygen, hence the name carbohydrate.

Lipids are a broad class of molecules that are insoluble in water, including steroids, waxes, fatty acids and triglycerides. In common language, triglycerides are fats if they are solid at room temperature or oils if they are liquid at room temperature.

Proteins are composed of one or more chains of amino acids. All of the amino acids in these chains contain the elements carbon, hydrogen, oxygen and nitrogen, but two of the twenty amino acids also contain sulphur.

Nucleic acids are chains of subunits called nucleotides, which contain carbon, hydrogen, oxygen, nitrogen and phosphorus. There are two types of nucleic acid: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).
**Drawing molecules**

Drawing molecular diagrams of glucose, ribose, a saturated fatty acid and a generalised amino acid.

There is no need to memorize the structure of many different molecules but a biologist should be able to draw diagrams of a few of the most important molecules.

Each atom in a molecule is represented using the symbol of the element. For example a carbon atom is represented with C and an oxygen atom with O. Single covalent bonds are shown with a line and double bonds with two lines.

Some chemical groups are shown with the atoms together and bonds not indicated. Table 1 gives examples.

<table>
<thead>
<tr>
<th>Name of group</th>
<th>Full structure</th>
<th>Simplified notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxyl</td>
<td>–OH</td>
<td>–OH</td>
</tr>
<tr>
<td>amine</td>
<td>–NH₂</td>
<td>–NH₂</td>
</tr>
<tr>
<td>carboxyl</td>
<td>–COOH</td>
<td>–COOH</td>
</tr>
<tr>
<td>methyl</td>
<td>–CH₃</td>
<td>–CH₃</td>
</tr>
</tbody>
</table>

▲ Table 1

**Ribose**

- The formula for ribose is C₉H₁₀O₅.
- The molecule is a five-membered ring with a side chain.
- Four carbon atoms are in the ring and one forms the side chain.
- The carbon atoms can be numbered starting with number 1 on the right.
- The hydroxyl groups (OH) on carbon atoms 1, 2 and 3 point up, down and down respectively.

**Glucose**

- The formula for glucose is C₆H₁₂O₆.
- The molecule is a six-membered ring with a side chain.
- Five carbon atoms are in the ring and one forms the side chain.
- The carbon atoms can be numbered starting with number 1 on the right.
- The hydroxyl groups (OH) on carbon atoms 1, 2, 3 and 4 point down, down, up and down respectively, although in a form of glucose used by plants to make cellulose the hydroxyl group on carbon atom 1 points upwards.
**Saturated fatty acids**
- The carbon atoms form an unbranched chain.
- In saturated fatty acids they are bonded to each other by single bonds.
- The number of carbon atoms is most commonly between 14 and 20.
- At one end of the chain the carbon atom is part of a carboxyl group.
- At the other end the carbon atom is bonded to three hydrogen atoms.
- All other carbon atoms are bonded to two hydrogen atoms.

**Amino acids**
- A carbon atom in the centre of the molecule is bonded to four different things:
  - an amine group, hence the term amino acid;
  - a carboxyl group which makes the molecule an acid;
  - a hydrogen atom;
  - the R group, which is the variable part of amino acids.

**Identifying molecules**

**Identification of biochemicals as carbohydrate, lipid or protein from molecular diagrams.**

The molecules of carbohydrates, lipids and proteins are so different from each other that it is usually quite easy to recognize them.

- Proteins contain C, H, O and N whereas carbohydrates and lipids contain C, H and O but not N.
- Many proteins contain sulphur (S) but carbohydrates and lipids do not.
- Carbohydrates contain hydrogen and oxygen atoms in a ratio of 2:1, for example glucose is C₆H₁₂O₆ and sucrose (the sugar commonly used in baking) is C₁₂H₂₂O₁₁.
- Lipids contain relatively less oxygen than carbohydrates, for example oleic acid (an unsaturated fatty acid) is C₁₈H₃₀O₂ and the steroid testosterone is C₂₃H₃₆O₂.
Metabolism

Metabolism is the web of all the enzyme catalysed reactions in a cell or organism.

All living organisms carry out large numbers of different chemical reactions. These reactions are catalysed by enzymes. Most of them happen in the cytoplasm of cells but some are extracellular, such as the reactions used to digest food in the small intestine. Metabolism is the sum of all reactions that occur in an organism.

Metabolism consists of pathways by which one type of molecule is transformed into another, in a series of small steps. These pathways are mostly chains of reactions but there are also some cycles. An example is shown in figure 3.

Even in relatively simple prokaryote cells, metabolism consists of over 1,000 different reactions. Global maps showing all reactions are very complex. They are available on the internet, for example in the Kyoto Encyclopedia of Genes and Genomes.

Anabolism

Anabolism is the synthesis of complex molecules from simpler molecules including the formation of macromolecules from monomers by condensation reactions.

Metabolism is often divided into two parts, anabolism and catabolism. Anabolism is reactions that build up larger molecules from smaller ones. An easy way to remember this is by recalling that anabolic steroids are hormones that promote body building. Anabolic reactions require energy, which is usually supplied in the form of ATP.

Anabolism includes these processes:

- Protein synthesis using ribosomes.
- DNA synthesis during replication.
- Photosynthesis, including production of glucose from carbon dioxide and water.
- Synthesis of complex carbohydrates including starch, cellulose and glycogen.

Catabolism

Catabolism is the breakdown of complex molecules into simpler molecules including the hydrolysis of macromolecules into monomers.

Catabolism is the part of metabolism in which larger molecules are broken down into smaller ones. Catabolic reactions release energy and in some cases this energy is captured in the form of ATP, which can then be used in the cell. Catabolism includes these processes:

- Digestion of food in the mouth, stomach and small intestine.
- Cell respiration in which glucose or lipids are oxidized to carbon dioxide and water.
- Digestion of complex carbon compounds in dead organic matter by decomposers.
2.2 Water

Understanding

→ Water molecules are polar and hydrogen bonds form between them.
→ Hydrogen bonding and dipolarity explain the adhesive, cohesive, thermal and solvent properties of water.
→ Substances can be hydrophilic or hydrophobic.

Applications

→ Comparison of the thermal properties of water with those of methane.
→ Use of water as a coolant in sweat.
→ Methods of transport of glucose, amino acids, cholesterol, fats, oxygen and sodium chloride in blood in relation to their solubility in water.

Nature of science

→ Use theories to explain natural phenomena: the theory that hydrogen bonds form between water molecules explains water’s properties.

Hydrogen bonding in water

Water molecules are polar and hydrogen bonds form between them.

A water molecule is formed by covalent bonds between an oxygen atom and two hydrogen atoms. The bond between hydrogen and oxygen involves unequal sharing of electrons – it is a polar covalent bond. This is because the nucleus of the oxygen atom is more attractive to electrons than the nuclei of the hydrogen atoms (figure 1).

Because of the unequal sharing of electrons in water molecules, the hydrogen atoms have a partial positive charge and oxygen has a partial negative charge. Because water molecules are bent rather than linear, the two hydrogen atoms are on the same side of the molecule and form one pole and the oxygen forms the opposite pole.

Positively charged particles (positive ions) and negatively charged particles (negative ions) attract each other and form an ionic bond. Water molecules only have partial charges, so the attraction is less but it is still enough to have significant effects. The attraction between water molecules is a “hydrogen bond”. Strictly speaking it is an intermolecular force rather than a bond. A hydrogen bond is the force that forms when a hydrogen atom in one polar molecule is attracted to a slightly negative atom of another polar covalent molecule.

Although a hydrogen bond is a weak intermolecular force, water molecules are small, so there are many of them per unit volume of water and large numbers of hydrogen bonds (figure 2). Collectively they give water its unique properties and these properties are, in turn, of immense importance to living things.
Hydrogen bonds and the properties of water

Use theories to explain natural phenomena: the theory that hydrogen bonds form between water molecules explains water’s properties.

There is strong experimental evidence for hydrogen bonds, but it remains a theory that they form between water molecules. Scientists cannot prove without doubt that they exist as they are not directly visible. However, hydrogen bonds are a very useful way of explaining the properties of water. They explain the cohesive, adhesive, thermal and solvent properties of water. It is these distinctive properties that make water so useful to living organisms.

It might seem unwise to base our understanding of the natural world on something that has not been proven to exist. However this is the way that science works – we can assume that a theory is correct if there is evidence for it, if it helps to predict behaviour, if it has not been falsified and if it helps to explain natural phenomena.

Properties of water

Hydrogen bonding and dipolarity explain the cohesive, adhesive, thermal and solvent properties of water.

Cohesive properties

Cohesion refers to the binding together of two molecules of the same type, for instance two water molecules.

Water molecules are cohesive – they cohere, which means they stick to each other, due to hydrogen bonding, described in the previous section. This property is useful for water transport in plants. Water is sucked through xylem vessels at low pressure. The method can only work if the water molecules are not separated by the suction forces. Due to hydrogen bonding this rarely happens and water can be pulled up to the top of the tallest trees – over a hundred metres.

Adhesive properties

Hydrogen bonds can form between water and other polar molecules, causing water to stick to them. This is called adhesion. This property is useful in leaves, where water adheres to cellulose molecules in cell walls. If water evaporates from the cell walls and is lost from the leaf via the network of air spaces, adhesive forces cause water to be drawn out of the nearest xylem vessel. This keeps the walls moist so they can absorb carbon dioxide needed for photosynthesis.

Thermal properties

Water has several thermal properties that are useful to living organisms:

- **High specific heat capacity.** Hydrogen bonds restrict the motion of water molecules and increases in the temperature of water require hydrogen bonds to be broken. Energy is needed to do this. As a result, the amount of energy needed to raise the temperature of water is relatively large. To cool down, water must lose relatively large amounts of energy. Water’s temperature remains relatively stable in comparison to air or land, so it is a thermally stable habitat for aquatic organisms.

- **High latent heat of vaporization.** When a molecule evaporates it separates from other molecules in a liquid and becomes a vapour molecule. The heat needed to do this is called the latent heat of
vaporization. Evaporation therefore has a cooling effect. Considerable amounts of heat are needed to vaporize water, because hydrogen bonds have to be broken. This makes it a good evaporative coolant. Sweating is an example of the use of water as a coolant.

- **High boiling point.** The boiling point of a substance is the highest temperature that it can reach in a liquid state. For the same reasons that water has a high latent heat of vaporization, its boiling point is high. Water is therefore liquid over a broad range of temperatures from 0 °C to 100 °C. This is the temperature range found in most habitats on Earth.

**Solvent properties**

Water has important solvent properties. The polar nature of the water molecule means that it forms shells around charged and polar molecules, preventing them from clumping together and keeping them in solution. Water forms hydrogen bonds with polar molecules. Its partially negative oxygen pole is attracted to positively charged ions and its partially positive hydrogen pole is attracted to negatively charged ions, so both dissolve. Cytoplasm is a complex mixture of dissolved substances in which the chemical reactions of metabolism occurs.

**Hydrophilic and hydrophobic**

Substances can be hydrophilic or hydrophobic.

The literal meaning of the word hydrophilic is water-loving. It is used to describe substances that are chemically attracted to water. All substances that dissolve in water are hydrophilic, including polar molecules such as glucose, and particles with positive or negative charges such as sodium and chloride ions. Substances that water adheres to, cellulose for example, are also hydrophilic.

Some substances are insoluble in water although they dissolve in other solvents such as propanone (acetone). The term hydrophobic is used to describe them, though they are not actually water-fearing. Molecules are hydrophobic if they do not have negative or positive charges and are nonpolar. All lipids are hydrophobic, including fats and oils.

![Figure 3: When two nonpolar molecules in water come into contact, weak interactions form between them and more hydrogen bonds form between water molecules](image)
If a nonpolar molecule is surrounded by water molecules, hydrogen bonds form between the water molecules, but not between the nonpolar molecule and the water molecules. If two nonpolar molecules are surrounded by water molecules and random movements bring them together, they behave as though they are attracted to each other. There is a slight attraction between nonpolar molecules, but more significantly, if they are in contact with each other, more hydrogen bonds can form between water molecules. This is not because they are water-fearing: it is simply because water molecules are more attracted to each other than to the nonpolar molecules. As a result, nonpolar molecules tend to join together in water to form larger and larger groups. The forces that cause nonpolar molecules to join together into groups in water are known as hydrophobic interactions.

**Comparing water and methane**

**Comparison of the thermal properties of water with those of methane.**

The properties of water have already been described. Methane is a waste product of anaerobic respiration in certain prokaryotes that live in habitats where oxygen is lacking. Methanogenic prokaryotes live in swamps and other wetlands and in the guts of animals, including termites, cattle and sheep. They also live in waste dumps and are deliberately encouraged to produce methane in anaerobic digesters. Methane can be used as a fuel but if allowed to escape into the atmosphere it contributes to the greenhouse effect.

Water and methane are both small molecules with atoms linked by single covalent bonds. However water molecules are polar and can form hydrogen bonds, whereas methane molecules are nonpolar and do not form hydrogen bonds. As a result their physical properties are very different.

The data in table 1 shows some of the physical properties of methane and water. The density and specific heat capacity are given for methane and water in a liquid state. The data shows that water has a higher specific heat capacity, higher latent heat of vaporization, higher melting point and higher boiling point. Whereas methane is liquid over a range of only 22 °C, water is liquid over 100 °C.

<table>
<thead>
<tr>
<th>Property</th>
<th>Methane</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>CH₄</td>
<td>H₂O</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Density (g per cm³)</td>
<td>0.46</td>
<td>1</td>
</tr>
<tr>
<td>Specific heat capacity (J per g per °C)</td>
<td>2.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Latent heat of vaporization (J/g)</td>
<td>760</td>
<td>2257</td>
</tr>
<tr>
<td>Melting point</td>
<td>−182 °C</td>
<td>0 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>−160 °C</td>
<td>100 °C</td>
</tr>
</tbody>
</table>

▲ Table 1 Comparing methane and water

▲ Figure 4 Bubbles of methane gas, produced by prokaryotes decomposing organic matter at the bottom of a pond have been trapped in ice when the pond froze.
Cooling the bod with sweat

Use of water as a coolant in sweat.

Sweat is secreted by glands in the skin. The sweat is carried along narrow ducts to the surface of the skin where it spreads out. The heat needed for the evaporation of water in sweat is taken from the tissues of the skin, reducing their temperature. Blood flowing through the skin is therefore cooled. This is an effective method of cooling the body because water has a high latent heat of vaporization. Solutes in the sweat, especially ions such as sodium, are left on the skin surface and can sometimes be detected by their salty taste.

Sweat secretion is controlled by the hypothalamus of the brain. It has receptors that monitor blood temperature and also receives sensory inputs from temperature receptors in the skin. If the body is overheated the hypothalamus stimulates the sweat glands to secrete up to two litres of sweat per hour. Usually no sweat is secreted if the body is below the target temperature, though when adrenalin is secreted we sweat even if we are already cold. This is because adrenalin is secreted when our brain anticipates a period of intense activity that will tend to cause the body to overheat.

Transport in blood plasma

Methods of transport of glucose, amino acids, cholesterol, fats, oxygen and sodium chloride in blood in relation to their solubility in water.

Blood transports a wide variety of substances, using several methods to avoid possible problems and ensure that each substance is carried in large enough quantities for the body’s needs.

**Sodium chloride** is an ionic compound that is freely soluble in water, dissolving to form sodium ions (Na+) and chloride ions (Cl−), which are carried in blood plasma.

**Amino acids** have both negative and positive charges. Because of this they are soluble in water but their solubility varies depending on the R group, some of which are hydrophilic while others are hydrophobic. All amino acids are soluble enough to be carried dissolved in blood plasma.

**Glucose** is a polar molecule. It is freely soluble in water and is carried dissolved in blood plasma.

**Oxygen** is a nonpolar molecule. Because of the small size of the molecule it dissolves in water but only sparingly and water becomes saturated with oxygen at relatively low concentrations. Also, as the temperature of water rises, the solubility of oxygen decreases, so blood plasma at 37 °C can hold much less dissolved oxygen than water at 20 °C or lower. The amount of oxygen that blood plasma can transport around the body is far too little to provide for aerobic cell respiration. This problem is overcome by the use of hemoglobin in red blood cells. Hemoglobin has binding sites for oxygen and greatly increases the capacity of the blood for oxygen transport.
**Fats molecules** are entirely nonpolar, are larger than oxygen and are insoluble in water. They are carried in blood inside lipoprotein complexes. These are groups of molecules with a single layer of phospholipid on the outside and fats inside. The hydrophilic phosphate heads of the phospholipids face outwards and are in contact with water in the blood plasma. The hydrophobic hydrocarbon tails face inwards and are in contact with the fats. There are also proteins in the phospholipid monolayer, hence the name lipoprotein.

**Cholesterol** molecules are hydrophobic, apart from a small hydrophilic region at one end. This is not enough to make cholesterol dissolve in water and instead it is transported with fats in lipoprotein complexes. The cholesterol molecules are positioned in the phospholipid monolayers, with the hydrophilic region facing outwards in the region with the phosphate heads of the phospholipids.

**Figure 5** Arrangement of molecules in a lipoprotein complex

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### 2.3 Carbohydrates and lipids

**Understanding**

- Monosaccharide monomers are linked together by condensation reactions to form disaccharides and polysaccharide polymers.
- Fatty acids can be saturated, monounsaturated or polyunsaturated.
- Unsaturated fatty acids can be cis or trans isomers.
- Triglycerides are formed by condensation from three fatty acids and one glycerol.

**Applications**

- Structure and function of cellulose and starch in plants and glycogen in humans.
- Scientific evidence or health risks of trans- and saturated fats.
- Lipids are more suitable or long-term energy storage in humans than carbohydrates.
- Evaluation of evidence and the methods used to obtain evidence or health claims made about lipids.

**Nature of science**

- Evaluating claims: health claims made about lipids need to be assessed.

**Skills**

- Use molecular visualization software to compare cellulose, starch and glycogen.
- Determination of body mass index by calculation or use of a nomogram.
Carbohydrates

Monosaccharide monomers are linked together by condensation reactions to form disaccharides and polysaccharide polymers.

Glucose, fructose and ribose are all examples of monosaccharides. The structure of glucose and ribose molecules was shown in sub-topic 2.1. Monosaccharides can be linked together to make larger molecules.

- Monosaccharides are single sugar units.
- Disaccharides consist of two monosaccharides linked together. For example, maltose is made by linking two glucose molecules together. Sucrose is made by linking a glucose and a fructose.
- Polysaccharides consist of many monosaccharides linked together. Starch, glycogen and cellulose are polysaccharides. They are all made by linking together glucose molecules. The differences between them are described later in this sub-topic.

When monosaccharides combine, they do so by a process called condensation (figure 1). This involves the loss of an –OH from one molecule and an –H from another molecule, which together form H₂O. Thus, condensation involves the combination of subunits and yields water.

Linking together monosaccharides to form disaccharides and polysaccharides is an anabolic process and energy has to be used to do it. ATP supplies energy to the monosaccharides and this energy is then used when the condensation reaction occurs.

![Figure 1 Condensation and hydrolysis reactions between monosaccharides and disaccharides](image-url)


**Imaging carbohydrate molecules**

Use of molecular visualization software to compare cellulose, starch and glycogen.

The most widely used molecular visualization software is Jmol, which can be downloaded free of charge. There are also many websites that use Jmol, which are easier to use. Suggestions of suitable websites are available with the electronic resources that accompany this book.

When Jmol software is being used, you should be able to make these changes to the image of a molecule that you see on the screen:

- Use the scroll function on the mouse to make the image larger or smaller.
- Left click and move the mouse to rotate the image.
- Right click to display a menu that allows you to change the style of molecular model, label the atoms, make the molecule rotate continuously or change the background colour.

Spend some time developing your skill in molecular visualization and then try these questions to test your skill level and learn more about the structure of polysaccharides.

**Questions**

1. Select glucose with the ball and stick style with a black background.
   - What colours are used to show carbon, hydrogen and oxygen atoms? [2]

2. Select sucrose with sticks style and a blue background.
   - What is the difference between the glucose ring and the fructose ring in the sucrose molecule? [1]

3. Select amylose, which is the unbranched form of starch, with the wireframe style and a white background. If possible select a short amylose chain and then a longer one.
   - What is the overall shape of an amylose molecule? [1]
   - How many glucose molecules in the chain are linked to only one other glucose? [1]

4. Select amylopectin, with the styles and colours that you prefer. Amylopectin is the branched form of starch. Zoom in to look closely at a position where there is a branch. A glucose molecule must be linked to an extra third glucose to make the branch.
   - What is different about this linkage, compared to the linkages between glucose molecules in unbranched parts of the molecule? [1]
   - How many glucose molecules are linked to only one other glucose in the amylopectin molecule? [1]

▲ Figure 2 Images of sugars using molecular visualization software – (a) fructose, (b) maltose, (c) lactose
Pol saccharides

Structure and function of cellulose and starch in plants and glycogen in humans.

Starch, glycogen and cellulose are all made by linking together glucose molecules, yet their structure and functions are very different. This is due to differences in the type of glucose used to make them and in the type of linkage between glucose molecules.

Glucose has five –OH groups, any of which could be used in condensation reactions, but only three of them are actually used to link to make polysaccharides. The most common link is between the –OH on carbon atom 1 (on the right hand side in molecular diagrams of glucose) and the –OH on carbon atom 4 (shown on the left hand side). The –OH on carbon atom 6 (shown at the top of molecular diagrams) is used to form side branches in some polysaccharides.

Glucose can have the –OH group on carbon atom 1 pointing either upwards or downwards. In alpha glucose (α-glucose) the –OH group points downwards but in beta glucose (β-glucose) it points upwards. This small difference has major consequences for polysaccharides made from glucose.

Cellulose is made by linking together β-glucose molecules. Condensation reactions link carbon atom 1 to carbon atom 4 on the next β-glucose. The –OH groups on carbon atom 1 and 4 point in opposite directions: up on carbon 1 and down on carbon 4. To bring these –OH groups together and allow a condensation reaction to occur, each β-glucose added to the chain has to be positioned at 180° to the previous one. The glucose subunits in the chain are oriented alternately upwards and downwards. The consequence of this is that the cellulose molecule is a straight chain, rather than curved.

Cellulose molecules are unbranched chains of β-glucose, allowing them to form bundles with hydrogen bonds linking the cellulose molecules. These bundles are called cellulose microfibrils. They have very high tensile strength and are used as the basis of plant cell walls. The tensile strength of cellulose prevents plant cells from bursting, even when very high pressures have developed inside the cell due to entry of water by osmosis.

5 Select glycogen. It is similar but not identical to the amyllopectin form of starch.
   • What is the difference between glycogen and amyllopectin? [1]

6 Select cellulose.
   • How is it different in shape from the other polysaccharides? [1]

7 Look at the oxygen atom that forms part of the ring in each glucose molecule in the chain.
   • What pattern do you notice in the position of these oxygen atoms along the chain?
Starch is made by linking together α-glucose molecules. As in cellulose, the links are made by condensation reactions between the –OH groups on carbon atom 1 of one glucose and carbon atom 4 of the adjacent glucose. These –OH groups both point downwards, so all the glucose molecules in starch can be orientated in the same way. The consequence of this is that the starch molecule is curved, rather than straight. There are two forms of starch. In amylose the chain of α-glucose molecules is unbranched and forms a helix. In amylopectin the chain is branched, so has a more globular shape.

Starch is only made by plant cells. Molecules of both types of starch are hydrophilic but they are too large to be soluble in water. They are therefore useful in cells where large amounts of glucose need to be stored, but a concentrated glucose solution would cause too much water to enter a cell by osmosis. Starch is used as a store of glucose and therefore of energy in seeds and storage organs such as potato cells. Starch is made as a temporary store in leaf cells when glucose is being made faster by photosynthesis than it can be exported to other parts of the plant.

Glycogen is very similar to the branched form of starch, but there is more branching, making the molecule more compact. Glycogen is made by animals and also some fungi. It is stored in the liver and some muscles in humans. Glycogen has the same function as starch in plants: it acts as a store of energy in the form of glucose, in cells where large stores of dissolved glucose would cause osmotic problems. With both starch and glycogen it is easy to add extra glucose molecules or remove them. This can be done at both ends of an unbranched molecule or at any of the ends in a branched molecule. Starch and glycogen molecules do not have a fixed size and the number of glucose molecules that they contain can be increased or decreased.

**Lipids**

**Triglycerides are formed by condensation from three fatty acids and one glycerol.**

Lipids are a diverse group of carbon compounds that share the property of being insoluble in water. Triglycerides are one of the principal groups of lipid. Examples of triglycerides are the fat in adipose tissue in humans.
and the oil in sunflower seeds. Fats are liquid at body temperature (37 °C) but solid at room temperature (20 °C) whereas oils are liquid at both body temperature and room temperature.

A triglyceride is made by combining three fatty acids with one glycerol (see figure 7). Each of the fatty acids is linked to the glycerol by a condensation reaction, so three water molecules are produced. The linkage formed between each fatty acid and the glycerol is an ester bond. This type of bond is formed when an acid reacts with the –OH group in an alcohol. In this case the reaction is between the –COOH group on a fatty acid and an –OH on the glycerol.

Triglycerides are used as energy stores. The energy from them can be released by aerobic cell respiration. Because they do not conduct heat well, they are used as heat insulators, for example in the blubber of Arctic marine mammals.

![Figure 7 Formation of a triglyceride from glycerol and three fatty acids](image)

**Energy storage**

Lipids and carbohydrates are both used for energy storage in humans, but lipids are normally used for long-term energy storage. The lipids that are used are fats. They are stored in specialized groups of cells called adipose tissue. Adipose tissue is located immediately beneath the skin and also around some organs including the kidneys.

There are several reasons for using lipids rather than carbohydrates for long-term energy storage:

- The amount of energy released in cell respiration per gram of lipids is double the amount released from a gram of carbohydrates. The same amount of energy stored as lipid rather than carbohydrate therefore adds half as much to body mass. In fact the mass advantage of lipids is even greater because fats form pure droplets in cells with no water associated, whereas each gram of glycogen is associated with about two grams of water, so lipids are actually six times more efficient in the amount of energy that can be stored per gram of body mass. This is important, because we have to carry our energy stores around with us wherever we go. It is even more important for animals such as birds and bats that fly.

- Stored lipids have some secondary roles that could not be performed as well by carbohydrates. Because lipids are poor conductors of heat, they can be used as heat insulators. This is the reason for much of our stored fat being in sub-cutaneous adipose tissue next to the skin. Because fat
is liquid at body temperature, it can also act as a shock absorber. This is the reason for adipose tissue around the kidneys and some other organs.

Glycogen is the carbohydrate that is used for energy storage, in the liver and in some muscles. Although lipids are ideal for long-term storage of energy, glycogen is used for short-term storage. This is because glycogen can be broken down to glucose rapids, then transported easily by the blood to where it is needed. Fats in adipose tissue cannot be mobilized as rapidly. Glucose can be used either in anaerobic or aerobic cell respiration whereas fats and fatty acids can only be used in aerobic respiration. The liver stores up to 150 grams of glycogen and some muscles store up to 2% glycogen by mass.

**Da a-based ques ions: Emperor peng ins**

During the Antarctic winter female Emperor penguins live and feed at sea, but males have to stay on the ice to incubate the single egg the female has laid. Throughout this time the males eat no food. After 16 weeks the eggs hatch and the females return. While the males are incubating the eggs they stand in tightly packed groups of about 3,000 birds. To investigate the reasons for standing in groups, 10 male birds were taken from a colony at Pointe Geologie in Antarctica. They had already survived 4 weeks without food. They were kept for 14 more weeks without food in fenced enclosures where they could not form groups. All other conditions were kept the same as in the wild colony. The mean air temperature was −16.4 °C. The composition of the captive and the wild birds’ bodies was measured before and after the 14-week period of the experiment. The results in kilograms are shown in figure 8.

a) Calculate the total mass loss for each group of birds. [2]

i) wild

ii) captive

b) Compare the changes in lipid content of the captive birds with those of the birds living free in the colony. [2]

i) Besides being used as an energy source, state another function of lipid which might be important for penguin survival. [1]
**Bod mass index**

**Determination of body mass index by calculation or use of a nomogram.**

The body mass index, usually abbreviated to BMI, was developed by a Belgian statistician, Adolphe Quetelet. Two measurements are needed to calculate it: the mass of the person in kilograms and their height in metres.

BMI is calculated using this formula:

\[
BMI = \frac{\text{mass in kilograms}}{\text{(height in metres)}^2}
\]

Units or BMI are kg m\(^{-2}\).

BMI can also be found using a type of chart called a nomogram. A straight line between the height on the left hand scale and the mass on the right hand scale intersects the BMI on the central scale. The data based questions on page 81 include a BMI nomogram.

BMI is used to assess whether a person’s body mass is at a healthy level, or is too high or too low. Table 1 shows how this is done:

<table>
<thead>
<tr>
<th>BMI</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>below 18.5</td>
<td>underweight</td>
</tr>
<tr>
<td>18.5 – 24.9</td>
<td>normal weight</td>
</tr>
<tr>
<td>25.0 – 29.9</td>
<td>overweight</td>
</tr>
<tr>
<td>30.0 or more</td>
<td>obese</td>
</tr>
</tbody>
</table>

▲ Table 1

In some parts of the world, food supplies are insufficient or are unevenly distributed and many people as a result are underweight. In other parts of the world a likelier cause of being underweight is anorexia nervosa. This is a psychological condition that involves voluntary starvation and loss of body mass.

Obesity is an increasing problem in some countries. Excessive food intake and insufficient exercise cause an accumulation of fat in adipose tissue. The amount of body fat can be estimated using skin old calipers (figure 9). Obesity increases the risk of conditions such as coronary heart disease and type 2 diabetes. It reduces life expectancy significantly and is increasing the overall costs of health care in countries where rates of obesity are rising.

▲ Measuring body mass. What was this person’s body mass index if their height was 1.80 metres?

---

**Activity**

**Estimating body fat percentage**

To estimate body fat percentage, measure the thickness of a skinfold in millimetres using calipers in these four places:

- Front of upper arm
- Back of upper arm
- Below scapula
- Side of waist

The measurements are added and then analysis tools available on the internet can be used to calculate the estimate.

▲ Figure 9 Measuring body fat with skinfold callipers
Da a based qu ę ions: Nomograms and BMI

Use figure 11 to answer these questions.

1. a) State the body mass index of a man who has a mass of 75 kg and a height of 1.45 metres. [1]

   b) Deduce the body mass status of this man. [1]

2. a) State the body mass of the person standing on the scales on the previous page. [1]

   b) The person has a height of 1.8 metres. Deduce their body mass status. [1]

3. a) A woman has a height of 1.50 cm and a BMI of 40. Calculate the minimum amount of body mass she must lose to reach normal body mass status. Show all of your working. [3]

   b) Suggest two ways in which the woman could reduce her body mass. [2]

4. Outline the relationship between height and BMI for a fixed body mass. [1]

Fatt ę acids

Fatty acids can be saturated, monounsaturated or polyunsaturated.

The basic structure of fatty acids was described in sub-topic 2.1. There is a chain of carbon atoms, with hydrogen atoms linked to them by single covalent bonds. It is therefore a hydrocarbon chain. At one end of the chain is the acid part of the molecule. This is a carboxyl group, which can be represented as –COOH.

The length of the hydrocarbon chain is variable but most of the fatty acids used by living organisms have between 14 and 20 carbon atoms. Another variable feature is the bonding between the carbon atoms. In some fatty
acids all of the carbon atoms are linked by single covalent bonds, but in other fatty acids there are one or more positions in the chain where carbon atoms are linked by double covalent bonds.

If a carbon atom is linked to adjacent carbons in the chain by single bonds, it can also bond to two hydrogen atoms. If a carbon atom is linked by a double bond to an adjacent carbon in the chain, it can only bond to one hydrogen atom. A fatty acid with single bonds between all of its carbon atoms therefore contains as much hydrogen as it possibly could and is called a saturated fatty acid. Fatty acids that have one or more double bonds are unsaturated because they contain less hydrogen than they could. If there is one double bond, the fatty acid is monounsaturated and if it has more than one double bond it is polyunsaturated.

Figure 12 shows one saturated fatty acid, one monounsaturated and one polyunsaturated fatty acid. It is not necessary to remember names of specific fatty acids in IB Biology.

### Unsaturated fatty acids

Unsaturated fatty acids can be cis or trans isomers.

In unsaturated fatty acids in living organisms, the hydrogen atoms are nearly always on the same side of the two carbon atoms that are double bonded – these are called cis-fatty acids. The alternative is for the hydrogens to be on opposite sides – called trans-fatty acids. These two conformations are shown in figure 14.

In cis-fatty acids, there is a bend in the hydrocarbon chain at the double bond. This makes triglycerides containing cis-unsaturated fatty acids less good at packing together in regular arrays than saturated fatty acids, so it lowers the melting point. Triglycerides with cis-unsaturated fatty acids are therefore usually liquid at room temperature – they are oils.

Trans-fatty acids do not have a bend in the hydrocarbon chain at the double bond, so they have a higher melting point and are solid at room temperature. Trans-fatty acids are produced artificially by partial hydrogenation of vegetable or fish oils. This is done to produce solid fats for use in margarine and some other processed foods.
Health risks of fats
Scientific evidence or health risks of trans-ats and saturated ats.

There have been many claims about the effects of different types of fat on human health. The main concern is coronary heart disease (CHD). In this disease the coronary arteries become partially blocked by fatty deposits, leading to blood clot formation and heart attacks.

A positive correlation has been found between saturated fatty acid intake and rates of CHD in many research programs. However, finding a correlation does not prove that saturated fats cause the disease. It could be another factor correlated with saturated fat intake, such as low amounts of dietary fibre, that actually causes CHD.

There are populations that do not fit the correlation. The Maasai of Kenya for example have a diet that is rich in meat, fat, blood and milk. They therefore have a high consumption of saturated fats, yet CHD is almost unknown among the Maasai. Figure 17 shows members of another Kenyan tribe that show this trend.

Diets rich in olive oil, which contains cis-monounsaturated fatty acids, are traditionally eaten in countries around the Mediterranean. The populations of these countries typically have low rates of CHD and it has been claimed that this is due to the intake of cis-monounsaturated fatty acids. However, genetic factors in these populations, or other aspects of the diet such as the use of tomatoes in many dishes could explain the CHD rates.

There is also a positive correlation between amounts of trans-fat consumed and rates of CHD. Other risk factors have been tested, to see if they can account for the correlation, but none did. Trans-fats therefore probably do cause CHD. In patients who had died from CHD, fatty deposits in the diseased arteries have been found to contain high concentrations of trans-fats, which gives more evidence of a causal link.
Evaluating the health risks of foods

Evaluating claims: health claims made about lipids need to be assessed.

Many health claims about foods are made. In some cases the claim is that the food has a health benefit and in other cases it is that the food is harmful. Many claims have been found to be false when they are tested scientifically.

It is relatively easy to test claims about the effects of diet on health using laboratory animals. Large numbers of genetically uniform animals can be bred and groups of them with the same age, sex and state of health can be selected for use in experiments. Variables other than diet, such as temperature and amount of exercise, can be controlled so that they do not influence the results of the experiment. Diets can be designed so that only one dietary factor varies and strong evidence can thus be obtained about the effect of this factor on the animal.

Results of animal experiments are often interesting, but they do not tell us with certainty what the health effects are on humans of a factor in the diet. It would be very difficult to carry out similar controlled experiments with humans. It might be possible to select matched groups of experimental subjects in terms of age, sex and health, but unless identical twins were used they would be genetically different. It would also be almost impossible to control other variables such as exercise and few humans would be willing to eat a very strictly controlled diet for a long enough period.

Researchers into the health risks of food must therefore use a different approach. Evidence is obtained by epidemiological studies. These involve finding a large cohort of people, measuring their food intake and following their health over a period of years. Statistical procedures can then be used to find out whether factors in the diet are associated with an increased frequency of a particular disease. The analysis has to eliminate the effects of other factors that could be causing the disease.

Nature of science question: using volunteers in experiments.

During the Second World War, experiments were conducted both in England and in the US using conscientious objectors to military service as volunteers. The volunteers were willing to sacrifice their health to help extend medical knowledge. A vitamin C trial in England involved 20 volunteers. For six weeks they were all given a diet containing 70 mg of vitamin C. Then, for the next eight months, three volunteers were kept on the diet with 70 mg, seven had their dose reduced to 10 mg and ten were given no vitamin C. All of these ten volunteers developed scurvy. Three-centimetre cuts were made in their thighs, with the wounds closed up with five stitches. These wounds failed to heal. There was also bleeding from hair follicles and from the gums. Some of the volunteers developed more serious heart problems. The groups given 10 mg or 70 mg of vitamin C fared equally well and did not develop scurvy.

Experiments on requirements for vitamin C have also been done using real guinea-pigs, which ironically are suitable because guinea-pigs, like humans, cannot synthesize ascorbic acid. During trial periods with various intakes of vitamin C, concentrations in blood plasma and urine were monitored. The guinea-pigs were then killed and collagen in bone and skin was tested. The collagen in guinea-pigs with restricted vitamin C had less cross-linking between the protein fibres and therefore lower strength.

1. Is it ethically acceptable for doctors or scientists to perform experiments on volunteers, where there is a risk that the health of the volunteers will be harmed?

2. Sometimes people are paid to participate in medical experiments, such as drug trials. Is this more or less acceptable than using unpaid volunteers?

3. Is it better to use animals for experiments or are the ethical objections the same as with humans?

4. Is it acceptable to kill animals, so that an experiment can be done?
### Analysis of data on health risks of lipids

**Evaluation of evidence and the methods used to obtain the evidence for health claims made about lipids.**

An evaluation is defined in IB as an assessment of implications and limitations. Evidence for health claims comes from scientific research. There are two questions to ask about this research:

1. **Implications** – do the results of the research support the health claim strongly, moderately or not at all?

2. **Limitations** – were the research methods used rigorous, or are there uncertainties about the conclusions because of weaknesses in methodology?

The first question is answered by analysing the results of the research – either experimental results or results of a survey. Analysis is usually easiest if the results are presented as a graph or other type of visual display.

- Is there a correlation between intake of the lipid being investigated and rate of the disease or the health benefit? This might be either a positive or negative correlation.

- How large is the difference between mean (average) rates of the disease with different levels of lipid intake? Small differences may not be significant.

- How widely spread is the data? This is shown by the spread of data points on a scattergraph or the size of error bars on a bar chart. The more widely spread the data, the less likely it is that mean differences are significant.

- If statistical tests have been done on the data, do they show significant differences?

The second question is answered by assessing the methods used. The points below refer to surveys and slightly different questions should be asked to assess controlled experiments.

- How large was the sample size? In surveys it is usually necessary to have thousands of people in a survey to get reliable results.

- How even was the sample in sex, age, state of health and lifestyle? The more even the sample, the less other factors can affect the results.

- If the sample was uneven, were the results adjusted to eliminate the effects of other factors?

- Were the measurements of lipid intake and disease rates reliable? Sometimes people in a survey do not report their intake accurately and diseases are sometimes misdiagnosed.

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### Data-based questions: Evaluating evidence from a health survey

The Nurses’ Health Survey is a highly respected survey into the health consequences of many factors. It began in 1976 with 121,700 female nurses in the USA and Canada, who completed a lengthy questionnaire about their lifestyle factors and medical history. Follow-up questionnaires have been completed every two years since then.

Details of the methods used to assess diet and diagnose coronary heart disease can be found by reading a research paper in the American Journal of Epidemiology, which is freely available on the internet: Oh, K, Hu, FB, Manson, JE, Stampfer, MJ and Willett, WC. (2005) Dietary Fat Intake and Risk of Coronary Heart Disease in Women: 20 Years of Follow-up of the Nurses’ Health Study. *American Journal of Epidemiology*, 161:672–679. doi:10.1093/aje/kwi085

To assess the effects of trans-fats on rates of CHD, the participants in the survey were divided into five groups according to their trans-fat intake. Quintile 1 was the 20% of participants with the lowest intake and quintile 5 was the 20% with the highest intake. The average intake of trans-fats for each quintile was calculated, as a percentage of dietary energy intake. The relative risk of CHD was found for each quintile, with Quintile 1 assigned a risk of 1. The risk was adjusted for differences between the quintiles in age, body mass index, smoking, alcohol intake, parental
history of CHD, intake of other foods that affect CHD rates and various other factors. 

Figure 18 is a graph showing the percentage of energy from trans-fats for each of the five quintiles and the adjusted relative risk of CHD. The effect of trans-fat intake on relative risk of CHD is statistically significant with a confidence level of 99%.

1 Suggest reasons for using only female nurses in this survey. [3]

2 State the trend shown in the graph. [1]

3 The mean age of nurses in the five quintiles was not the same. Explain the reasons for adjusting the results to compensate for the effects of age differences. [2]

4 Calculate the chance, based on the statistical tests, of the differences in CHD risk being due to factors other than trans-fat intake. [2]

5 Discuss evidence from the graph that other factors were having some effect on rates of CHD. [2]

## Data-based questions: Saturated fats and coronary heart disease

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<thead>
<tr>
<th>Populations ranked by % calories as saturated fat</th>
<th>E. Finland</th>
<th>W. Finland</th>
<th>Zephen</th>
<th>USA</th>
<th>Slovenia</th>
<th>Belgium</th>
<th>Crevalc</th>
<th>Zrenjanin</th>
<th>Dalmatia</th>
<th>Crete</th>
<th>Montegiorgio</th>
<th>Velika</th>
<th>Rome</th>
<th>Corfu</th>
<th>Ushibuka</th>
<th>Tanushmanu</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Calories as saturated fat</td>
<td>22</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>9</td>
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<td>8</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Death rate/100,000 yr⁻¹ All causes</td>
<td>992</td>
<td>351</td>
<td>420</td>
<td>574</td>
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<td>1027</td>
<td>764</td>
<td>1248</td>
<td>1006</td>
</tr>
</tbody>
</table>

▲ Table 2

1 a) Plot a scattergraph of the data in table 2. [5]

    b) Outline the trend shown by the scattergraph. [2]

2 Compare the results for:

    a) East and West Finland; [2]

    b) Crete and Montegiorgio. [2]

3 Evaluate the evidence from this survey for saturated fats as a cause of coronary heart disease. [4]