Lesson Four

Respiration and Enzymes

Aims

By the end of this lesson you should be able to:

- understand how the process of respiration produces ATP in living organisms, and know that ATP provides energy for cells
- describe the differences between aerobic and anaerobic respiration, and know these equations:
  - the word equation and the balanced chemical symbol equation for aerobic respiration in living organisms.
  - the word equations for anaerobic respiration in plants and animals
- understand how to investigate the evolution of carbon dioxide and heat from respiring seeds or other suitable living organisms
- understand the role of enzymes as biological catalysts in metabolic reactions.
- understand how enzyme function can be affected by changes in temperature, including changes to the shape of the active site, and how this can be investigated experimentally
- understand how enzyme function can be affected by changes in pH altering the active site, **and how this can be investigated experimentally**.

Context

This lesson covers parts (f) and 2.10 – 2.14B of Section 2: 'Structure and functions in living organisms' of the Edexcel specification.

Edexcel International GCSE (9-1) Biology Student Book, pages 6-15.

Introduction

In Lesson One we learned that the function of mitochondria is to release energy for use by the cell in a process called **respiration**. In the first part of this lesson we find out what respiration involves.

Respiration is only one of a large number of chemical reactions going on in cells. Each of these reactions is controlled and speeded up by special protein molecules called **enzymes**. In the second part of the lesson we discover how enzymes work, and about two of the factors which affect their performance.

Respiration and Energy

Every cell, in every single organism on the planet, needs a continual supply of energy if it is to remain alive. All the activities of life – growing, moving, thinking and all the rest – require energy. Without energy cells and organisms stop and die.

The energy needed is released in the process called **respiration**. Respiration is absolutely crucial to our survival: if respiration stops, life stops.

So – what is this mysterious yet crucial process?

Definition: Respiration is a set of chemical reactions, going on inside cells, which releases energy for use by the cell during the breakdown of food.

Several points in this definition require some explanation:

- Respiration is a set of *chemical reactions*: in everyday speech "respiration" is another name for "breathing". This is <u>not</u> how the term is used in biology.

- Respiration goes on *inside cells*: every single cell in an organism needs energy to live, and every single cell releases its own energy by respiration. It is sometimes called **cell respiration** to emphasise this point.

- Respiration involves *the breakdown of food*: it always involves the breakdown of larger molecules into smaller ones, with the release of the chemical energy stored in these larger molecules. The most important of these larger molecules is **glucose**.

To hammer home the point: every single cell, in every single organism on the planet, releases its own energy from food using a set of chemical reactions called "respiration".

Get it right! Respiration *releases* the energy stored in food, it does not *make* energy. Energy cannot be created (made) or destroyed, only changed from one form to another.

Activity 1	Explore the labels on foods in your kitchen Can you discover the **units** used to measure energy in biology?

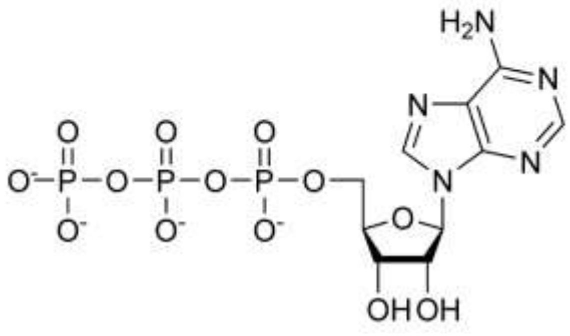
Energy and ATP

Respiration releases energy in two different forms:

- as **heat**. It is this heat which keeps your body warm, and which makes you overheat if you do vigorous exercise (which needs extra energy and therefore extra respiration to release it)

- as chemical energy trapped in a special molecule called **ATP**. It is this energy which cells use for all the other processes of life.

ATP stands for **adenosine triphosphate**. One of its molecules looks like this:



An ATP molecule
(You do not need to remember the details of this structure)

It has three **phosphate groups**: the three repeat structures on the left of the molecule with phosphorus (P) and oxygen (O) atoms.

During respiration, some of the energy released is used to join the third of the three phosphate groups onto the molecule:

ADP + P + energy → ATP + water

In this equation, **ADP** stands for **adenosine diphosphate** (the same molecule as ATP, but with just two rather than three phosphate groups attached) and P stands for **phosphate**.

This energy is later released when the third phosphate group drops off again:

ATP + water → ADP + P + energy

This second reaction is linked to other processes in the cell which need energy to drive them, and the energy released from the ATP makes these processes run.

There are three main types of process that need energy provided by ATP:

- **biosynthesis**: the making of larger molecules from smaller ones, which occurs during growth
- **active transport**: the pumping of particles across membranes against a concentration gradient (see Lesson Two)
- muscle contraction in animals, and other forms of cell **movement**.

Almost all other life processes depend upon one of these three, and therefore on energy released during the breakdown of ATP.

Aerobic and Anaerobic

Respiration can occur in two different ways, both starting with glucose:

- In **aerobic respiration** glucose is broken down *using oxygen*. In this case, it is broken down completely to carbon dioxide and water, and most of the chemical energy it contains is released.

- In **anaerobic respiration** glucose is only partly broken down, without the help of oxygen, and only a fraction (roughly 1/40th) of its chemical energy is released.

Aerobic respiration is clearly the more efficient of the two, and it is always done by cells provided they have a good supply of oxygen. Anaerobic respiration only occurs when cells run short of oxygen. We shall now look at the two forms of respiration in turn.

Activity 2	If a marathon runner sets off too fast, they always take longer to cover the 26.2 miles than if they run at a slower steady pace. Use the information above to suggest why.

Aerobic Respiration

Aerobic respiration can be described by the following **word equation**:

glucose + oxygen ----> carbon dioxide + water (+ energy)

This means that glucose and oxygen are used up, while carbon dioxide and water are made. The **chemical energy** stored in the glucose molecule is released, and some of it is captured as ATP and used by the cell. (The energy is written in brackets because it is not a chemical substance like the other four names.)

In actual fact this equation is only a summary of a long and complex process. The large glucose molecule is really dismantled in a series of small steps, the first few of which occur in the cytoplasm and the later ones (the ones which make use of the oxygen) in the mitochondria. But the equation correctly gives the starting point (the glucose and oxygen) and the end point (the carbon dioxide and water) of the whole process.

As well as the word equation, you also need to know the **chemical symbol equation** for aerobic respiration. This requires some knowledge of chemistry, so if you get lost refer to Appendix 1 at the end of the lesson for some extra help.

The symbol equation for aerobic respiration is:

$C_6H_{12}O_6$ + $6O_2$ ------> $6CO_2$ + $6H_2O$ (+ energy)

This means that each glucose molecule is broken down with the help of six oxygen molecules to produce six carbon dioxide molecules plus six water molecules.

Anaerobic Respiration

Whereas aerobic respiration is performed in essentially the same way by all organisms, anaerobic respiration can take a variety of forms. In each case:

- oxygen is *not* used
- glucose is *not* fully broken down to water and carbon dioxide
- only a fraction of the chemical energy stored in the glucose is released and trapped as ATP.

There are three important forms of anaerobic respiration you should know about. In each case the cells involved can also do aerobic respiration, and only resort to anaerobic respiration when they run short of oxygen:

Yeast

Yeast and plants break down glucose to **ethanol** (alcohol) and carbon dioxide. This is useful in breadmaking and brewing, something we will explore in a later chapter. The chemical formula for ethanol is C_2H_5OH, and the word equation for this reaction is:

glucose ----> ethanol + carbon dioxide (+ <u>some</u> energy)

Other Microorganisms

Bacteria, protozoa and some plants break down glucose to **methane**. This happens in the digestive systems of cows, in rubbish tips, in marshes and in rice paddy fields. The release of methane from these contributes to global warming – again something we will explore later. The chemical formula for methane is CH_4.

Muscle

Human muscles break down glucose to **lactic acid**. This happens when the blood cannot bring oxygen to the muscle fast enough for it to get all of its energy from aerobic respiration, for example during a 100 metres sprint. Afterwards the lactic acid is broken down to carbon dioxide and water using oxygen (but without, unfortunately, releasing any useful energy) – a process called "paying back the oxygen debt". The chemical formula for lactic acid is $C_3H_6O_3$, and the word equation for this reaction is:

glucose ----> lactic acid (+ <u>some</u> energy)

Activity 3	If you go on to a running track and sprint as hard as you can, your legs will hurt and you will be forced to slow up before completing a single lap. After stopping, you breathe hard for quite a long time before recovering. (If you have not experienced this, go and try it!) Use the above information to suggest the reasons for both of these facts.

Investigating the production of carbon dioxide by small living organisms

You need to understand experiments which investigate the production of carbon dioxide by small organisms as they respire. Woodlice, maggots and germinating seeds are often used for these investigations. We will adapt an experiment described in your textbook, so before continuing please read Activity 3 on page 14 of the textbook (If you are not familiar with the concept of pH, refer to Appendix 2 at the end of the lesson now for some extra help).

Three comments on this experiment:

- An **indicator** is a chemical which changes colour to show a solution's **pH**, i.e. how acidic or alkaline it is. **Hydrogencarbonate indicator** is often used in Biology because it is very **sensitive** – which means it changes colour with a very small change in pH.

- Carbon dioxide is a weak acid. So as the organisms in the experiment produce it, the solution becomes slightly more acidic. This turns the indicator from orange to yellow.

- The gauze platform has small holes in it. This allows the carbon dioxide made by the organisms to reach the indicator solution, but without the organisms coming into contact with the solution.

For experiments like this to be valid, they need to include **a control.** In this case, a good control would be an identical set of apparatus set up without any living organisms.

Activity 4

(a) Explain why the control is necessary to make the experiment valid.

(b) If the control indicator stays orange, but the other indicator turns yellow, what would you conclude?

(c) If both turn yellow, what would you conclude?

Activity 5

The experiment could also be used, as in the textbook, to find out which of two organisms was respiring faster.

Supposing you were comparing germinating seeds and maggots in this way to find out which respired faster:

(a) What would you measure to find out the answer?
(b) What important factors would have to be controlled in the two tubes to make it a fair test?

Investigating the production of heat by respiring seeds

This is shown in Activity 4, on page 15 of the textbook. Note the following points:

- Vacuum flasks are used because the amount of heat produced is very small. Without them this heat would just escape, and the temperature would not rise significantly.
- A control (the dead peas) is used so that you can be sure that any temperature rise is due to the living peas respiring.
- The microorganisms on the peas are killed using disinfectant, because they too respire making heat.

Activity 6

Explain how you could adapt this experiment to investigate the relative rates of respiration in germinating pea and wheat seeds.

Be sure to make your design valid and reliable (refer back to Lesson Three if necessary to see how to do this).

Enzymes

Every cell is kept working by a large number of different chemical reactions – **metabolic reactions** – which go on in its cytoplasm and nucleus. These must all go at the right speed, or the cell will malfunction and die.

Each of these reactions is controlled by a special protein molecule called an **enzyme** – a different type for each reaction. Each enzyme has two slightly different roles:

- to *speed up* its reaction. Most of the reactions would go too slowly to sustain life at normal temperatures, and the enzymes make them go fast enough. This means that enzymes are biological **catalysts**: a catalyst is something that speeds up a chemical reaction without being either used up or made during it;

- to *control the rate* of its reaction. The enzyme also ensures it does not go too fast or too slow for the cell's needs.

Twig

Log on to Twig and look at the fact-pack titled: **Enzymes**

www.ool.co.uk/1048yp

How enzymes power the everyday chemical reactions our bodies rely on.

Activity 7

Extension: If you are interested, you can find out more about catalysts in general from the Wikipedia page on Catalysis at www.ool.co.uk/0411bi.

How Enzymes Work

Each enzyme is a large protein molecule with a particular shape. One part of its surface is called the **active site**. During the chemical reaction, the molecules which are going to be changed, called the **substrate** molecules, bind onto the active site.

Binding onto the active site helps the substrate molecules change into their **products** more easily. These then drop off the active site, and the next set of substrate molecules bind. There is a good diagram of this in your textbook: study Figure 1.5 on page 7 of the textbook now.

The active site is exactly the right shape to fit its substrate molecules, rather like a lock is exactly the right shape to fit its key. This means that each enzyme can control only one chemical reaction, just like each lock will only accept only one shape of key. We say an enzyme is **specific** to its reaction – it does not make reactions *in general* go faster, but only *its particular* reaction.

Enzymes and Temperature

Chemical reactions controlled by enzymes go faster if you warm them up. There are two reasons for this:

- a reaction can only occur when the substrate molecules have reached the enzyme's active site. The higher the temperature the faster the particles move and the less time an enzyme molecule has to wait for the next set of substrate molecules to arrive;

- the higher the temperature the more energy, on average, each substrate particle has. Having more energy makes the substrate molecule more likely to react once it is bound onto the active site.

However if you carry on increasing the temperature above about 40°C the reaction slows down and eventually stops. This is because, at higher temperatures, the enzyme molecule vibrates more and more. The shape of its active site changes, and although the substrate molecules get there faster they cannot bind so well once they arrive. Eventually, at a high enough temperature, the shape of the active site is permanently and completely lost, and the reaction stops. The enzyme is then said to be **denatured**.

The result is the graph shown in Figure 1.6 on page 8 of the textbook, which you should examine now. The temperature at which the reaction goes fastest is called the **optimum temperature**. For many enzymes this is close to, or just above, human body temperature (37°C).

Activity 8

Use the above information to suggest reasons for the following facts:

(a) A human being dies quite rapidly if his/her body temperature gets as high as 45°C.
(b) Lizards can often be seen basking on rocks in the morning sunshine.
(c) The only animals living in the Arctic and Antarctic are mammals (polar bears) and birds (penguins).

Investigating how enzyme action is affected by changes in temperature

Activity 1 on pages 9-10 of the textbook shows one way in which the effect of temperature on enzyme action can be measured. Read this account carefully and note the following:

- starch gives a blue-black colour when mixed with iodine solution (see Lesson Six). As the starch in the starch-amylase mixture is broken down, it gives a less and less blue-black colour with iodine solution (as in the results table on page 10)
- samples must be withdrawn from the reaction mixture to test – the iodine solution must not be added to the reaction mixture in the test tube. This is because the iodine solution would affect the activity of the enzyme, probably stopping it from working
- the reaction mixture is kept in a **water bath** so that it remains at a constant temperature – the temperature being investigated. The individual solutions are left in the water bath for several minutes before mixing to **equilibrate** (reach the temperature of the water bath) – otherwise the reaction will occur at a lower temperature than expected.
- it would be a good idea to repeat the experiment at each temperature: see Lessons Five and Seven.

Enzymes and pH

Changing the pH of a solution changes the electrical charge (positive [+] or negative [-]) on some of the atoms present. This changes the shape of an enzyme molecule and therefore the shape of its active site. This is because the enzyme molecule is largely held together by forces of attraction between electrical charges on it component atoms.

It follows that there must be one particular pH – the **optimum pH** – where the active site is exactly the right shape to fit the substrate molecules and the reaction will go fastest. If the pH becomes higher or lower than this, the active site will be the wrong shape and the reaction will slow up or stop.

Examine a graph of this at Figure 1.7 on page 8 of the textbook.

The cytoplasm of cells is maintained at a pH of about 7, which is **neutral**, so enzymes which work *inside* cells all have an optimum pH of about 7. However the enzymes which break down foods in our digestive systems are different. They work *outside* cells. The one that digests protein in the stomach – **pepsin** – is adapted to work best in the highly acidic conditions of the stomach at about pH 2. Others, for example **trypsin** which digests proteins in the small intestine, are adapted to work best in alkaline conditions.

Activity 9	Use the above information to suggest why "pickling" can be used to preserve foods.

Investigating how enzyme action is affected by changes in pH

The effect of pH on enzyme action can be measured by slightly adapting Activity 1 on pages 9-10 of the textbook (see above):

- the reaction mixture is still kept in a water bath to keep the temperature constant, and the starch and amylase solutions are still equilibrated before mixing.

- the pH of both solutions is changed before mixing by adding a **buffer**. A buffer is a chemical which produces a particular pH when dissolved in water. It also prevents the pH from changing as a result of the reaction that goes on.
- the pH of the solutions can be measured, before and after mixing, in one of two ways:
  - using an electronic pH probe inserted directly into the solution;
  - withdrawing a sample of solution, and testing it with an **indicator** like universal indicator (see Appendix 2 at the end of this lesson). Indicator must not be added directly to the reaction mixture, as it is likely to slow down the action of the enzyme.

Another investigation into the effect of pH on enzyme activity is given at Activity 2 on pages 11-12 of the textbook. You should study this investigation carefully.

Now is the time to read through *Edexcel International GCSE (9-1) Biology Student Book,* pages 6-15. This covers the same topics as this lesson, so will add to your understanding of the material.

Keywords

respiration
anaerobic
hydrogencarbonate indicator
metabolic reactions
enzyme
substrate
optimum temperature
water bath
buffer
aerobic
lactic acid
control
catalyst
active site
denatured
ATP
ADP
phosphate
equilibrate
indicator

Summary

Lesson Four: Respiration and Enzymes

Respiration ------- energy and ATP

-------- aerobic

-------- anaerobic

Enzymes ---------- catalysts

---------- active site

---------- effect of ------ temperature

------ pH

What you need to know

- the meanings of the technical terms in **bold** print in this lesson
- that respiration releases energy in the form of ATP
- the differences between aerobic and anaerobic respiration
- the word and symbol equations for aerobic respiration, and the word equations for anaerobic respiration
- the explanation of enzyme action in terms of active sites
- the effect of temperature on enzyme activity
- the effect of pH on enzyme activity

What you might be asked to do

- write out the word and symbol equations for aerobic respiration, and the word equations for anaerobic respiration
- explain the importance of ATP in cells
- explain the products of respiration produced in different circumstances

- design or interpret investigations involving respiration and enzymes
- describe and explain the effect of temperature on enzyme action, including the effect of temperature on the shape of the active site
- describe and explain the effect of pH on enzyme action, including the effect of pH on the shape of the active site

Appendix 1: Chemical Formulae

In chemical formulae, each **element** is given a **symbol** of one or two letters. You need to be familiar with these symbols and elements:

Carbon	C	Sodium	Na
Hydrogen	H	Potassium	K
Oxygen	O	Aluminium	Al
Nitrogen	N	Iron	Fe
Sulphur	S	Magnesium	Mg
Phosphorus	P	Calcium	Ca
Chlorine	Cl		
Iodine	I		

Molecules contain two or more atoms joined together. In the formula for a molecule, each atom is represented by its symbol. So, for example:

- A carbon dioxide molecule has the formula CO_2. This means it contains one carbon atom joined to two oxygen atoms.
- A water molecule has the formula H_2O. This means it contains two hydrogen atoms joined to one oxygen atom.
- A glucose molecule has the formula $C_6H_{12}O_6$. This means it contains six carbon atoms joined to twelve hydrogen atoms and six oxygen atoms.

- An oxygen molecule has the formula O_2. This means it contains two oxygen atoms joined together.

A **compound** is a substance whose molecules contain more than one sort of atom. So carbon dioxide, water and glucose are compounds, but oxygen is not.

Appendix 2: pH

The pH scale is used in science as a measure of acidity and alkalinity. A liquid which is neither acid nor alkaline is said to be **neutral** and is given a pH of 7. Numbers lower than 7 are acidic, and the lower the number the more acidic it is. Numbers higher than 7 are alkaline, and the higher the number the more alkaline it is:

```
1   2   3   4   5   6   7   8   9   10   11   12   13   14

        more acidic         n      more alkaline
<-------------------------- e   ----------------------------->
                            u
                            t
                            r
                            a
                            l
```

The most-used **indicator** is called **universal indicator**. Universal indicator is green when neutral, blue when alkaline, and turns yellow then orange then red as it becomes more acidic.

Suggested Answers to Activities

Activity 1

Kilojoules, usually abbreviated kJ.

"Calories" is an old measure of energy, no longer used in Biology. 1Calorie = 4.2 kilojoules.

Activity 2

The marathon runner has a limited amount of readily usable energy stored in his/her body (It is, in fact, in the glycogen stored in the liver and muscles). If they set off too fast, they will use some of this in *an*aerobic respiration, which uses their store up 40 times as fast as aerobic respiration. They will run out of usable energy well before the end of the race!

Activity 3

The build-up of lactic acid in your leg muscles (a) makes them hurt and (b) stops them working properly. After stopping, you need to get extra oxygen into your blood to break the lactic acid down. This is achieved by breathing hard for some time after you stop running.

Activity 4

(a) It is possible that the indicator would turn yellow on its own after a few minutes. Or perhaps light makes it turn yellow, or contact with glass. You must eliminate these possibilities before you can be sure that it is extra carbon dioxide which is causing the change.

(b) That the organisms are producing carbon dioxide, or at least producing an acid of some sort.

(c) You cannot be sure why this is happening – no useful conclusion.

Activity 5

(a) The time taken for the indicator to turn yellow in the two tubes.

(b) At least (1) the temperature (increased temperature increases the rate of respiration) (2) the mass of the organisms (3) the volume of indicator solution.

Activity 6

Two sets of apparatus, one with pea seeds and one with wheat seeds. Take the temperatures of both flasks at intervals. the one with the higher temperature rise has the seeds which are respiring faster.

Do several replicates of each flask to increase reliability.

Control the following variables to make the investigation valid: mass of seeds in each flask; time that seeds are soaked for before setting up flasks; age of seeds (older seeds may respire less well); size and shape of vaccuum flask. N.B. It is better to have the same mass of seeds than the same number of seeds.

Activity 8

(a) The person's enzymes work less efficiently, or are denatured, at this temperature, so their vital metabolic reactions slow down or stop.

(b) Lizards are cold blooded. They cool down overnight, so their metabolic reactions slow up, making them sluggish. They bask to warm up, so their metabolic reactions speed up, making them more active.

(c) Mammals and birds are warm blooded. This means their cells are maintained at close to the optimum temperature for the operation of their enzymes. Cold blooded animals would get so cold that their metabolic reactions would be too slow to maintain life.

Activity 9

The pickling fluid is acidic. This means that the enzymes in the microbes which would otherwise make the food go off cannot function, so they don't grow.

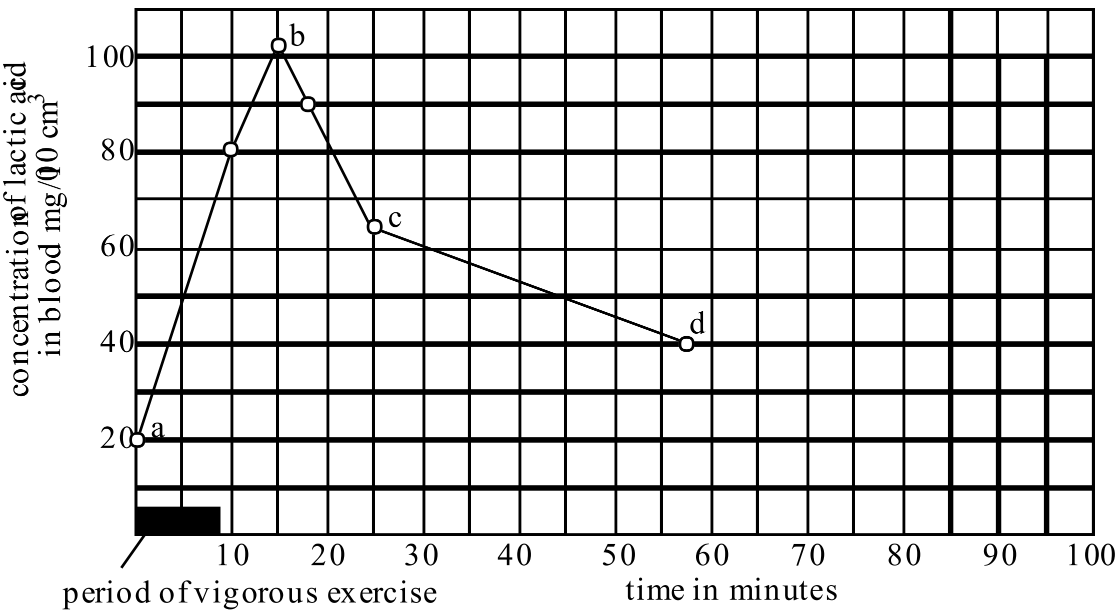
Tutor-Marked Assignment B

Question 1

An experiment was carried out on an athlete who ran fast on the spot for nine minutes (shown by the bar at the base of the graph).

Samples of his blood were taken at regular intervals and analysed. One particular compound in the blood, lactic acid, was found to vary greatly in its concentration, as you can see from the graph below.

Graph to show the effects of vigorous exercise on the concentration of lactic acid in the blood



(a) What is the name for the process which releases energy from glucose in cells? (1 mark)

(b) What process produces lactic acid in human muscle? (1 mark)

(c) Explain why the athlete's leg muscles were producing lactic acid during the vigorous exercise. (3 marks)

(d) The lactic acid levels peaked in the athlete's bloodstream some minutes after the exercise stopped. Suggest a reason for this. (2 marks)

(e) Explain why the lactic acid levels eventually started to fall. (2 marks)

(f) Measurements of the blood of a marathon runner during a marathon race do not show a similar rise in lactic acid levels. Explain fully why this is the case. (3 marks)

Question 2

An investigation was carried out to find the effect of temperature on the activity of the enzyme trypsin, which digests proteins in the small intestine of humans.

(a) Give a suitable hypothesis for this investigation. (3 marks)

(b) Use your hypothesis to generate a prediction which could be tested. (3 marks)

A student added trypsin to seven test tubes containing protein kept at different temperatures. The time taken for digestion of the protein by trypsin to finish was recorded and is shown in the table below:

Temperature ^{0}C	Time taken for digestion of protein in minutes
5	48
15	24
25	12
35	6
45	3
55	6
65	No effect

(c) What was the student's

(i) dependent variable? (1 mark)

(ii) independent variable? (1 mark)

(d) Did the student obtain any anomalous results? Explain your answer. (2 marks)

(e) Using the table

(i) state the optimum temperature for the action of trypsin. (1 mark)

(ii) explain how you arrived at your answer. (2 marks)

(f) Explain the reason for the result at 65°C. (3 marks)

(g) Suggest three important factors which would need to be controlled to make this investigation valid, and in each case explain why. (6 marks)

(h) What could the student have done to make her answer for the optimum temperature

(i) more reliable? (2 marks)

(ii) more precise? (2 marks)

(i) Both skin and the membranes covering the eyes contain proteins.

(i) Suggest two important safety precautions the student should take. (2 marks)

(ii) Explain your answer. (2 marks)

Question 3

Describe an experiment to investigate the effect of training on subjects' ability to run faster without respiring anaerobically.

(8 marks)

Total marks = 50