



**Answer ALL questions.**

**Write your answers in the spaces provided.**

**1** The heart pumps blood around the circulation system.

(a) Explain why many animals need a heart **and** circulation system.

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(b) If the heart of an animal is removed from its body, the heart will continue to beat for a period of time.

The left atrium stops contracting if it is separated from the rest of the heart.

However, the right atrium and the rest of the heart will continue to beat.

Eventually, the right atrium and the rest of the heart will also stop beating.

(i) Give a reason why the heart will continue to beat after being removed from the body.

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(ii) Explain why the left atrium stops beating when it is separated from the right atrium.

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(iii) Explain why the right atrium and the rest of the heart eventually stop beating.

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**(Total for Question 1 = 7 marks)**

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2 White blood cells are involved in the non-specific and specific immune responses.

Neutrophils and monocytes are two types of white blood cell.

The table provides information about the neutrophils and monocytes of one individual.

(a) Complete the table to show the volume of each neutrophil.

$$\text{Volume of a sphere } V = \frac{4}{3} \pi r^3$$

(2)

Information	Neutrophil	Monocyte
Number of cells per mm <sup>3</sup> of blood	3000	400
Diameter of the cell / μm	10	20
Volume of each cell / μm <sup>3</sup>		314



(b) The table shows some properties of neutrophils and monocytes.

Activated monocytes are called macrophages.

Property	Neutrophil	Monocyte
Material taken up by phagocytosis	Bacteria and fungi	Dead cell debris, bacteria and fungi
Antigen presentation	No	Yes
Production of cytokines	No	Yes

(i) State what is meant by the term phagocytosis.

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(ii) Explain why monocytes play a role in the antigen specific immune response to viruses but neutrophils do not.

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**(Total for Question 2 = 6 marks)**



3 The photograph shows a three-spined stickleback (*Gasterosteus aculeatus*).



(Source: © ABS Natural History/Shutterstock)

Magnification  $\times 1.8$

This fish feeds on small invertebrates such as the brine shrimp (*Artemia salina*).

(a) Calculate the length of the stickleback between lines A and B.

Give your answer to **two** significant figures.

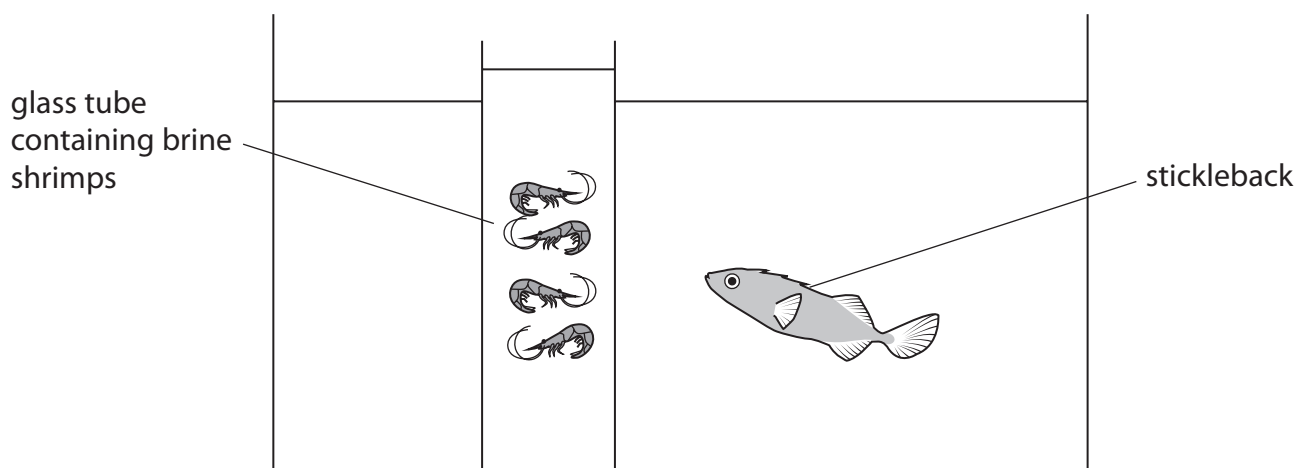
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Answer ..... mm

(b) An investigation studied the food biting response of the three-spined stickleback.

Individual fish were allowed to acclimatise in their own tanks.

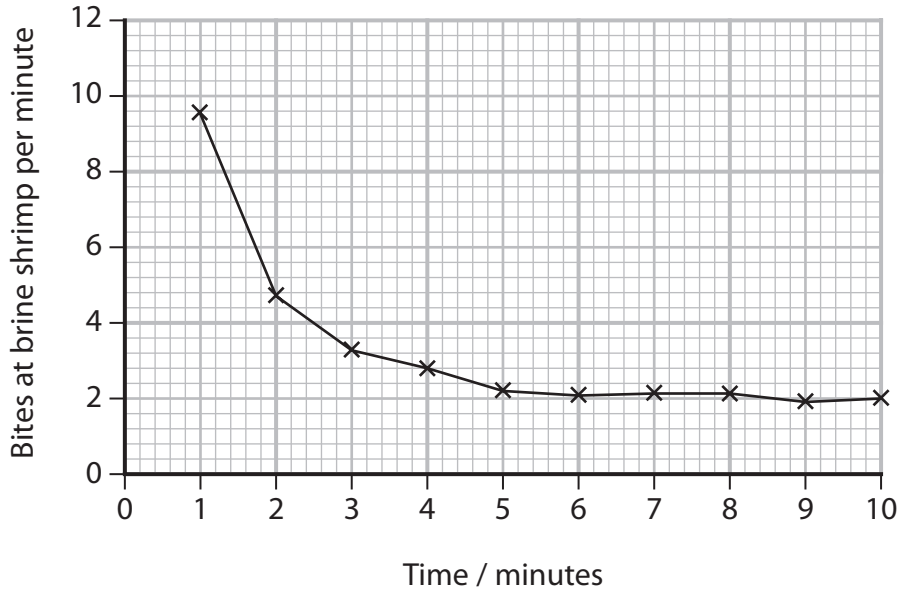
A glass tube containing several live brine shrimps was placed in the middle of the tank, as shown in the diagram.



The stickleback tried to bite the brine shrimps in the tube.

The number of bites made by the stickleback was recorded each minute for 10 minutes.

The graph shows the results of this investigation.



- (i) Calculate the percentage decrease in the number of bite responses from 1 to 6 minutes.

(2)

Answer .....



(ii) Explain the results of this investigation.

(3)

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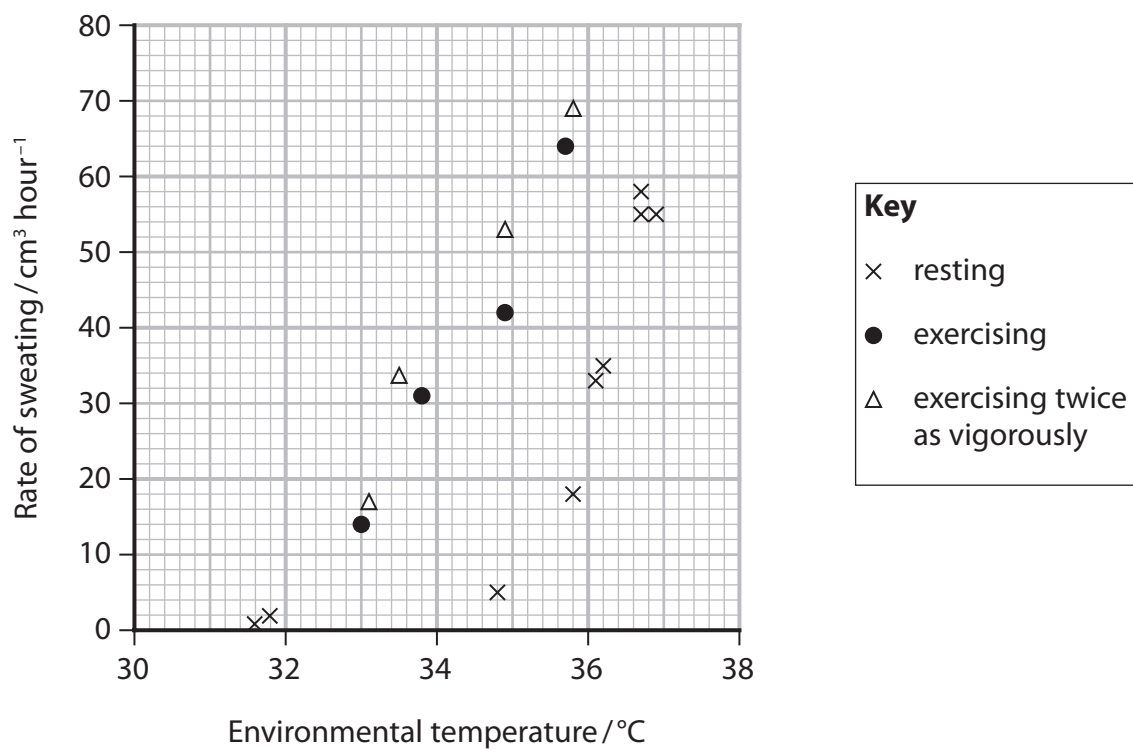






- 4 The effect of environmental temperature and exercise on the rate of sweating was studied.

The graph shows the results for one person.



- (a) Explain the importance of the dipole nature of water in sweating.

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(c) Describe how the production of sweat is controlled during exercise in humans.

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**(Total for Question 4 = 10 marks)**

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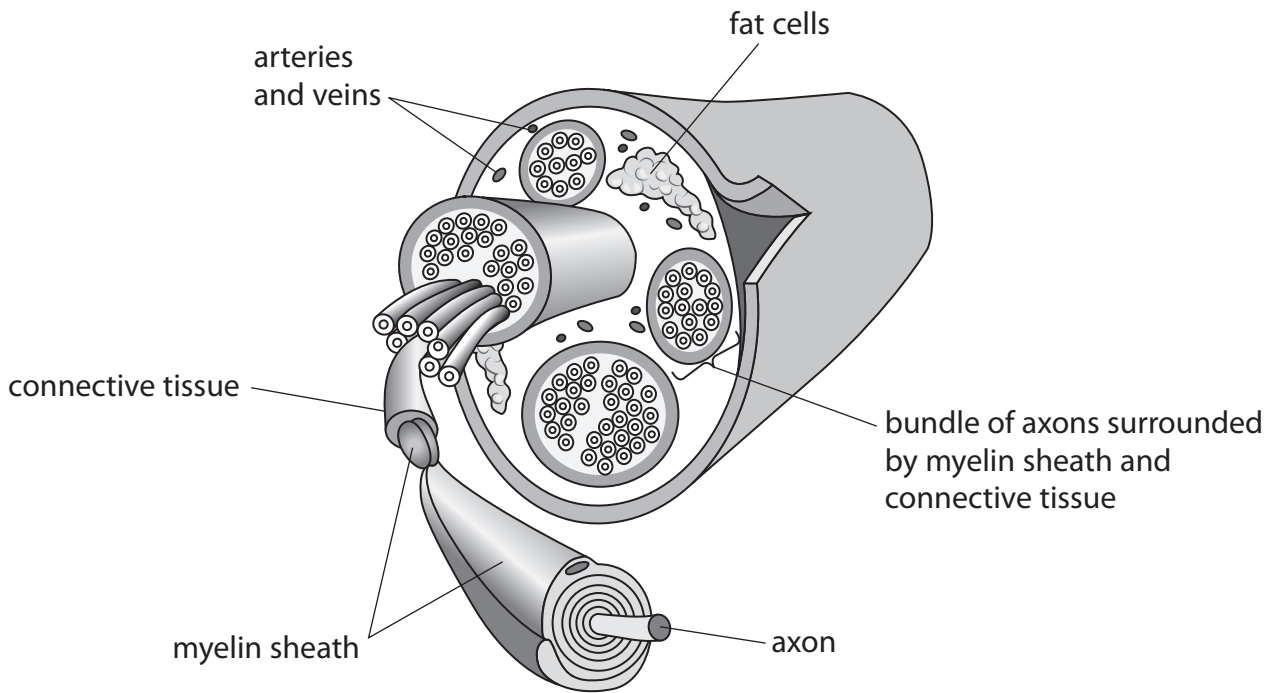


- 5 People with diabetes can suffer from a condition called diabetic peripheral neuropathy (DPN).

In DPN, peripheral nerves become damaged, affecting the transmission of nerve impulses.

Peripheral nerves are nerves that connect the central nervous system to all parts of the body. These nerves are formed from bundles of axons.

The diagram shows the structure of a peripheral nerve.



- (a) Each axon is surrounded by a myelin sheath.  
(i) Name the type of cell that forms the myelin sheath.

(1)





(b) During the early stages of DPN, individuals suffer from nerve pain.

Eventually, the damage results in nerves dying and complete loss of sensation.

Several factors have been linked to an increased risk of DPN in individuals with diabetes.

The table lists some of these risk factors for DPN.

Risk factor	Increase in risk (%)
High triglycerides	100
Smoking	42
High LDL to HDL ratio	67

(i) The information in the table can be used to estimate the increase in risk of developing DPN for an individual with diabetes.

Individual A is a diabetic who smokes and has high triglycerides.

Individual B is a diabetic who smokes and has a high LDL to HDL ratio.

Calculate the ratio of the increase in percentage risk for individuals A and B.

(1)

Ratio .....



(ii) Explain why these risk factors can cause peripheral nerve cells to die.

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**(Total for Question 5 = 12 marks)**

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6 The zebrafish (*Danio rerio*) has been studied as a model for vertebrate development. Zebrafish have a variety of types of stripes and fin shape phenotypes. Wild type zebrafish are homozygous for the black stripe and short fin phenotypes. The photograph shows a wild type zebrafish.



(Source: © Mirko\_Rosenau/Shutterstock)

(a) The black stripes of zebrafish are produced by cells called melanophores. Melanophores produce a black pigment called melanin.

Explain how a specialised cell such as a melanophore is produced from a stem cell.

(3)

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- (b) Zebrafish with different phenotypes have been produced by introducing gene mutations into the zebrafish genome.

Two of these phenotypes, spots and long fin, are described in the table.

Phenotype name	Photograph	Description
spots	 <p>(Source: © Grigorev Mikhail/Shutterstock)</p>	melanin produced in spots instead of stripes
long fin	 <p>(Source: © Grigorev Mikhail/Shutterstock)</p>	long fins instead of short fins

In an investigation, the inheritance of these two phenotypes was studied.

In cross 1, one parent was homozygous for stripes and the other parent was homozygous for spots.

In cross 2, one parent was homozygous for short fins and the other parent was homozygous for long fins.

The table shows the results of this investigation.

Cross	Genotype of parent 1	Genotype of parent 2	Phenotype of the offspring
1	homozygous for stripes and short fins	homozygous for spots and short fins	all had stripes and short fins
2	homozygous for stripes and short fins	homozygous for stripes and long fins	all had stripes and long fins

- (i) State what is meant by the term gene mutation.

(1)



(ii) Deduce the pattern of inheritance of the spots and long fin phenotypes.

(2)

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(c) Melanin pattern (stripes or spots) and fin length (long or short) are controlled by different genes.

Parents heterozygous for both these genes (DdNn) were crossed.

The table shows the expected genotypes of the parental gametes and the genotypes and phenotypes of the offspring.

The expected genotypes and phenotypes assume that genes for spots and long fins are inherited independently of each other.

(i) Complete the table by filling in the missing genotypes and phenotypes.

(2)

		Parent 1 gametes			
		DN	Dn	dN	dn
Parent 2 gametes	DN	DDNN	DDNn	DdNN	DdNn
		stripes and long fins	stripes and long fins	stripes and long fins	stripes and long fins
	Dn	DDNn	DDnn	DdNn	Ddnn
		stripes and long fins	stripes and short fins	stripes and long fins	stripes and short fins
	dN	DdNN	DdNn	ddNN	ddNn
		stripes and long fins	stripes and long fins	spots and long fins	spots and long fins
	dn				

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(ii) The table contains the observed and expected results of this cross.

Phenotype	Observed number	Expected number
stripes and long fins	270	288
stripes and short fins	87	96
spots and long fins	115	96
spots and short fins	40	32
Total	512	512

Calculate a value for chi squared ( $\chi^2$ ).

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

(3)

Answer .....

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(iii) Some critical values for the chi squared test ( $\chi^2$ ) are given in the table.

degrees of freedom	Critical value	
	p = 0.05	p = 0.01
1	3.84	6.64
2	5.99	9.21
3	7.82	11.35
4	9.49	13.28
5	11.07	15.09

State a conclusion that can be drawn from the results of this cross.

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**(Total for Question 6 = 13 marks)**

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7 Tuberculosis is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*.

(a) Describe how *M. tuberculosis* bacteria evade the immune system.

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(b) The transcription factor STAT3 is involved in regulating the activity of macrophages and T cells.

(i) State what is meant by the term transcription factor.

(1)

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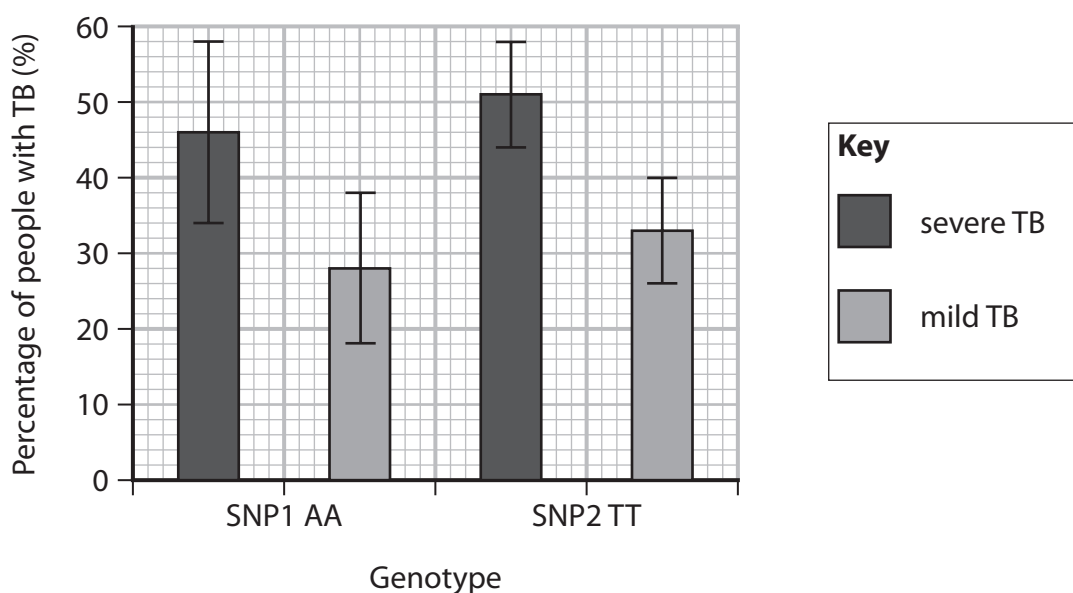
\*(ii) The gene for STAT3 can have alternative bases at two sites, SNP1 and SNP2.

- The base at SNP1 is either A or G and the base at SNP2 is either T or C.
- The SNP1 and SNP2 sites are in the introns of the STAT3 gene.

In one investigation, the association of different bases at these two SNP sites with tuberculosis (TB) infection was studied.

The table and graph show some of the results of this investigation.

SNP	Bases present in each allele	Chance of this SNP in a person with TB compared with a healthy person
SNP1	AA	increased chance
	AG or GG	same chance
SNP2	TT	increased chance
	TC or CC	same chance



In a second investigation, white blood cells with different SNP1 genotypes were grown in culture with cells infected with *Mycobacteria*.

After three days the number of surviving bacterial cells was determined.



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(Total for Question 7 = 12 marks)



8 The scientific article you have studied is adapted from a chapter in the book *Oxygen*.

Use the information from the scientific article and your own knowledge to answer the following questions.

(a) The mass of 'carbon turned into sugars by photosynthesis' is a measure of the gross productivity of photosynthetic organisms (paragraph 2).

Write an equation that describes the relationship between total carbon turned into sugar and the carbon turned into sugar that becomes available to primary consumers.

(1)

(b) Name one product, other than ATP and oxygen, produced by the light dependent reactions of photosynthesis (paragraph 4).

(1)

(c) Explain why cells use 'chemical energy in the form of ATP' (paragraphs 4 and 5).

(3)







(f) Explain how chloroplasts are adapted for their role in photosynthesis (paragraphs 9 and 10).

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(g) Explain how energy of the electrons passed along an electron transport chain is used to power the synthesis of ATP (paragraph 10).

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(h) Explain how 'tiny changes' in chlorophyll molecules could result in a change in the wavelength of light absorbed (paragraphs 12 and 13).

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(i) The structure of the oxygen-evolving complex is similar to that of catalase, 'it looks as if it evolved from two catalase enzymes lashed together' (paragraph 17).

Explain how proteomics and genomics could be used to support the suggestion that the oxygen-evolving complex evolved from catalase.

(4)

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(j) Sketch a graph to compare the effect of substrate concentration on the rate of reaction of catalase and peroxidase (paragraphs 19 and 20).

Include suitable units in the labels for each axis.

(3)



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**(Total for Question 8 = 30 marks)**

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**TOTAL FOR PAPER = 100 MARKS**

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**Pearson Edexcel Level 3 GCE**

**Wednesday 21 June 2023**

Morning (Time: 2 hours)

Paper  
reference

**9BN0/03**

**Biology A (Salters Nuffield)**

**Advanced**

**PAPER 3: General and Practical Applications in Biology**

**Scientific article for use with Question 8**

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**Pearson**

## Scientific article for use with Question 8

### 'Green Planet': Radiation and the Evolution of Photosynthesis

1. The world is so dominated by the green machinery of photosynthesis that it is easy to miss the wood for the trees – to overlook the conundrum at its heart. Photosynthesis uses light to split water, a trick that we have seen is neither easy nor safe: it amounts to the same thing as irradiation. A catalyst such as chlorophyll gives ordinary sunlight the destructive potency of x-rays. The waste product is oxygen, a toxic gas in its own right. So why split a molecule as robust as water to produce toxic waste if you can split something else much more easily, such as hydrogen sulphide or dissolved iron salts, to generate a less toxic product?
2. The immediate answer is easy. For living organisms, the pickings from water-splitting photosynthesis are much richer than those from hydrothermal activity, the major source of hydrogen sulphide and iron salts. Today, the total organic carbon production deriving from hydro-thermal sources is estimated to be about 200 million tonnes (metric tons) each year. In contrast, the amount of carbon turned into sugars by photosynthesis by plants, algae and cyanobacteria is thought to be a million million tonnes a year – a 5000-fold difference. From a biological point of view, if you can split water, you can split anything. Such a powerful weapon must be caged in some way lest it run amok and attack other molecules in the cell. If, when the water-splitting device first evolved, it was not yet properly caged, as we might postulate for a blindly groping first step, then it is hard to see what advantage it could offer.
3. Why, and how, then, did oxygenic photosynthesis evolve? There is good circumstantial evidence that oxidative stress, produced by solar radiation as on Mars, lies behind the evolution of photosynthesis on the Earth. The details are fascinating but also reveal just how deeply rooted is our resistance to oxygen toxicity: part and parcel, it seems, of the earliest known life on Earth. The earliest known bacteria did not produce oxygen by photosynthesis, but they could breathe oxygen – in other words they could apparently generate energy from oxygen-requiring respiration before there was any free oxygen in the air. To understand how this could be, and why it is relevant to our health today, we need to look first at how photosynthesis works, and how it came to evolve.
4. Of the different types of photosynthesis carried out by living organisms, only the familiar oxygenic form practised by plants, algae and cyanobacteria generates oxygen. All other forms (collectively known as anoxygenic photosynthesis) do not produce oxygen and pre-date the more sophisticated oxygenic form. Despite our anthropocentric interest in oxygen, plants are not much concerned with the gas – what they need from photosynthesis is energy and hydrogen atoms. The different forms of photosynthesis are united only in that they all use light energy to make chemical energy (in the form of ATP) needed to cobble hydrogen onto carbon dioxide to form sugars. They differ in the source of the hydrogen, which might come from water, hydrogen sulphide or iron salts, or indeed any other chemical with hydrogen attached.
5. Overall, plant photosynthesis converts carbon dioxide (CO<sub>2</sub>) from the air into simple organic molecules such as sugars (general formula CH<sub>2</sub>O). These are subsequently burnt by the plant in its mitochondria (see Chapter 3) to produce more ATP, and also converted into the wealth of carbohydrates, lipids, proteins and nucleic acids that make up life. We met the enzyme that cobbles hydrogen onto carbon dioxide in Chapter 5 – RUBISCO, the most abundant enzyme on the planet. But RUBISCO needs to be spoon-fed with its raw materials – hydrogen and carbon dioxide. Carbon dioxide comes from the air, or is dissolved in the oceans, so that is easy. Hydrogen, on the other hand, is not readily available – it reacts quickly (especially with oxygen to form water) and is so light that it can evaporate away into outer space. Hydrogen therefore needs a dedicated supply system of its own. This is, in fact, the key to photosynthesis, but for many years the lock resisted picking. Ironically, the mechanism only became clear when researchers finally understood where the oxygen waste came from.

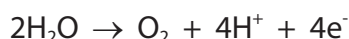


6. At first, scientists guessed that the oxygen came from carbon dioxide – a perfectly reasonable and intuitive assumption, but quite wrong as it turned out. The fallacy was first exposed in 1931, when Cornelis van Niel showed that a strain of photosynthetic bacteria used carbon dioxide and hydrogen sulphide (H<sub>2</sub>S) to produce carbohydrate and sulphur in the presence of light – but did not give off oxygen:



7. The chemical similarity between H<sub>2</sub>S and H<sub>2</sub>O led him to propose that in plants the oxygen might come not from carbon dioxide at all, but from water, and that the central trick of photosynthesis might be the same in both cases. The validity of this hypothesis was confirmed in 1937 by Robert Hill, who found that, if provided with iron ferricyanide (which does not contain oxygen) as an alternative to carbon dioxide, plants could continue to produce oxygen even if they could not actually grow.
8. In oxygenic photosynthesis, then, hydrogen atoms (or rather, the protons (H<sup>+</sup>) and electrons (e<sup>-</sup>) that constitute hydrogen atoms) are extracted from water, leaving the 'husk' – the oxygen – to be jettisoned into the air. The only advantage of water is its great abundance, for it is not easy to split in this way. The energy required to extract protons and electrons from water is much higher (nearly half as much again) than that needed to split hydrogen sulphide. Controlling this additional energy requires special 'high-voltage' molecular machinery, which had to evolve from the 'low-voltage' photosynthetic machinery previously used to split hydrogen sulphide. To understand how and why this voltage jump was made, we need to look at the structure and function of the machinery in a little more detail.
9. The interaction of light with any molecule always takes place at the level of the photon. In photosynthesis, chlorophyll is the molecule that absorbs photons. It cannot absorb any photon – it is constrained by the structure of its bonds to absorb photons with very particular quantities of energy. Plant chlorophyll absorbs photons of red light, with a wavelength of 680 nanometres. In contrast, the anoxygenic purple photosynthetic bacterium *Rhodobacter sphaeroides* has a different type of chlorophyll, which absorbs less-energetic infrared rays with a wavelength of 870 nanometres.
10. When chlorophyll absorbs a photon, its internal bonds are energized. The energetic vibrations force an electron from the molecule, leaving the chlorophyll short of one electron. Loss of an electron creates an unstable, reactive form of chlorophyll. However, the newly reactive molecule cannot simply take back its missing electron. That is snatched by a neighbouring protein and is whipped off down a chain of linked proteins, putting it beyond reach, like a rugby ball being passed across the field by a line of players. On the way, its energy is used to power the synthesis of ATP in a manner exactly analogous to that in mitochondrial respiration. The theft of an electron is half way to stealing an entire hydrogen atom, as hydrogen consists of a single proton and a single electron. Little extra work is needed to steal the proton. Electrostatic rearrangements draw a positively charged proton (from water in the case of oxygenic photosynthesis) after the negatively charged electron. The proton and then electron are eventually reunited by RUBISCO as a hydrogen atom in a sugar molecule.
11. What happens to the chlorophyll? Having lost an electron, it becomes far more reactive, and will snatch an electron from the nearest suitable source. The source of suitable electrons includes any plentiful sacrificial chemical, such as water, hydrogen sulphide or iron. Devouring an electron settles the chlorophyll back into its normal equable state, at least until another photon sets the whole cycle in motion again.

12. Which electron donor is used in photosynthesis – hydrogen sulphide, iron or water – ultimately depends on the energy of the photons that are absorbed by the chlorophyll. In the case of purple bacteria, their chlorophyll can only absorb low-energy infrared rays. This provides enough energy to extract electrons from hydrogen sulphide and iron, but not from water. To extract electrons from water requires extra energy, which must be acquired from higher-energy photons. To do this requires a change in the structure of chlorophyll, so it can absorb red-light photons instead of infrared light.
13. According to Robert Blankenship of Arizona State University and Hyman Hartman of the Institute for Advanced Studies in Biology at Berkeley, California, tiny changes in the structure of bacterial chlorophylls can lead to large shifts in their absorption properties. Two small changes to the structure of bacteriochlorophyll *a* (which absorbs at 870 nm) are all that it takes to generate chlorophyll *d*, which absorbs at 716 nanometres. In 1996, an article in *Nature* by Hideaki Miyashita and colleagues of the Marine Biotechnology Institute in Kamaishi, Japan, reported that chlorophyll *d* is the main photosynthetic pigment in a bacterium called *Acaryochloris marina*, which splits water to generate oxygen. Thus, an intermediate between bacteriochlorophyll and plant chlorophyll is not only plausible: it actually exists. From chlorophyll *d* another trifling change is all that is required to produce chlorophyll *a*, the principal pigment in plants, algae and cyanobacteria, which absorbs light at 680 nanometres.
14. Technically, then, the evolutionary steps required to get from bacteriochlorophyll to plant chlorophyll are simply achieved. The question remains, why? A chlorophyll that absorbs light at 680 nanometres is less good at absorbing light at 870 nanometres. It is therefore less efficient at splitting hydrogen sulphide, and so bacteria carrying it are at a competitive disadvantage compared with the bacteria that kept their original chlorophyll. Even worse, switching chlorophylls to split water poses the problem of what to do with the toxic oxygen waste, as well as any leaking free-radical intermediates – the same as those produced by radiation. Without foresight, how did life manage to cope with its dangerous new invention?
15. Chlorophyll extracts electrons from water one at a time. To generate oxygen from water, it must absorb four photons and lose four electrons in succession, each time drawing an electron from one of two water molecules. The overall water-splitting reaction is:



Only in the final stage is oxygen released. The rate at which chlorophyll extracts electrons depends on how quickly the photons are absorbed. As the successive steps cannot take place instantly, a series of potentially reactive free-radical intermediates must be produced, if only transiently.

16. If photosynthesis is to work at all, the reactive intermediates from water must be sealed inside a structure that immobilizes them, preventing them from escaping before oxygen is released. Needless to say, they are sealed in such a cage, this is how photosynthesis works. The cage is made of proteins and is called the oxygen-evolving complex (or sometimes the water-splitting enzyme). Water is bound tightly inside the protein cage while the electrons are extracted one at a time. But this is no ordinary cage. Its structure conceals a secret that is much older than the hills, which transports us back to the time before oxygenic photosynthesis evolved, to a time more than 2.7 billion years ago, before there was any oxygen in the atmosphere. This structure is the key to life on Earth, for without it the Earth would have remained as sterile as Mars.



17. The structure of the oxygen-evolving complex is very similar to that of an antioxidant enzyme called catalase. In fact, the oxygen-evolving complex looks as if it evolved from two catalase enzymes lashed together. If so, then catalase must have evolved *before* the oxygen-evolving complex. If so, the chronology must be as follows. Catalase evolved on the early Earth, in an atmosphere devoid of oxygen. One day, two catalase molecules became bound together to form a cage that enabled the safe splitting of water: the oxygen-evolving complex. This cage allowed the evolution of oxygenic photosynthesis. As a result, the atmosphere filled with oxygen. Life was put under serious oxidative stress. Luckily it could cope: it already had at least one antioxidant enzyme that could protect it – catalase. How convenient! But wait a moment. If catalase came before photosynthesis, then even if there was no atmospheric oxygen, there must have been oxidative stress. Is this plausible? To answer this question, we must take a look at how catalase works.
18. There are several different types of catalase. Most animal cells have a form that has four haem molecules embedded in its core. In contrast, some microbes have a different sort of catalase, which contains manganese instead of haem at its core. Despite their different structures, both enzymes are equally fast, and are correctly called catalase, in the sense that they work in the same way – they both catalyse the reaction of two molecules of hydrogen peroxide with each other to form oxygen and water:



This simple reaction mechanism reveals a great deal about conditions on the Earth 3.5 billion years ago. It is the exact equivalent of the natural reaction between two molecules of hydrogen peroxide, but is speeded up 100 million times by the enzyme. The need for two molecules of hydrogen peroxide means that catalase is extremely effective at removing hydrogen peroxide when concentrations are high, when it is easy to bring two molecules together. It works less well at low concentrations of hydrogen peroxide, when it is harder to find two molecules close together. Catalase is thus swift to remove high concentrations of hydrogen peroxide, but is poor at mopping up trace amounts or at maintaining a stable low-level equilibrium.

19. Today, most aerobic organisms have a second group of enzymes, known as the peroxidases, which can dispose of trace amounts of hydrogen peroxide. These enzymes work better at low levels of hydrogen peroxide because they act in a fundamentally different way. Rather than bringing two molecules of hydrogen peroxide together, they use antioxidants such as vitamin C to convert a single molecule of hydrogen peroxide into two molecules of water, without generating any oxygen. Most aerobic cells have both sets of enzymes, and break down hydrogen peroxide using both mechanisms. Catalase is used for bulk removal, peroxidase for subtle adjustments.
20. Catalase would presumably have been present in the photosynthetic bacteria that generated energy by splitting hydrogen sulphide or iron salts in the era before oxygenic photosynthesis. In fact, hydrogen peroxide has some parallels with these early photosynthetic fuels. To remove electrons from hydrogen peroxide requires a similar input of energy to that required to remove electrons from hydrogen sulphide, and so could have been achieved using the same bacteriochlorophyll. Hydrogen peroxide would therefore have been a good source of hydrogen for photosynthesis. And, while far less plentiful than hydrogen sulphide and iron salts, it was nonetheless formed most readily in the surface waters, closest to the full power of the Sun. If this scenario is true, then catalase could have doubled as a photosynthetic enzyme. Because splitting hydrogen peroxide generates oxygen, this recruitment of catalase to photosynthesis also bridges the evolutionary gap between anoxygenic and oxygenic photosynthesis.



21. If catalase was acting as a photosynthetic enzyme, then it would be natural for a number of catalase molecules to cluster around the photosynthetic apparatus. In these circumstances, it would be simple for two catalase molecules to become associated as a complex: the prototype oxygen-evolving complex. At first it would have continued to use hydrogen peroxide as an electron donor, but given the right energy input, this complex could split water. We know that three small changes in the structure of bacteriochlorophyll can transform its properties, enabling it to absorb high-energy light at a wavelength of 680 nm. We now have a prototype oxygen-evolving complex (the nutcracker that can physically split water) and a chlorophyll that can provide enough energy for it to do so (or the hand that presses the nutcracker). Thus, with no foresight and no disadvantageous steps, we have taken a path leading from anoxygenic photosynthesis to oxygenic photosynthesis.

Adapted from: Nick Lane. 'Green Planet'. In *Oxygen*. Oxford, Oxford University Press, 2002. pp131–146.



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