CAMBRIDGE INTERNATIONAL AS & A LEVEL BIOLOGY · 9700

BIOLOGY



EXAM QUESTION PACK

19 GENETIC TECHNOLOGY

SCORE	PERCENTAGE
/	%

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Chapter 17

Genetic technology

17.1 Genetically modified organisms in agriculture

1621. 9700_w15_qp_11 Q: 40

Why does the application of nitrate fertilisers cause an increase in crop production?

A Green plants manufacture more protein.

B More nitrogen is fixed in leguminous plants.

C The fertiliser adds energy to the ecosystem.

D The number of denitrifying bacteria decreases.

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Q1.

3 **(a)** Three different strains, **A**, **B** and **C**, of a species of bacterium were grown on nutrient agar in a divided petri dish until they formed 'lawns' covering the agar.

Three discs of filter paper were soaked in a solution of a penicillin antibiotic and one disc placed in contact with each of the bacterial strains for 10 minutes.

After 24 hours, zones of clearance, indicating bacterial cell death, were seen in the 'lawns' of strains **A** and **B**.

The appearance of the petri dish 24 hours after addition of antibiotic is shown in Fig. 3.1.

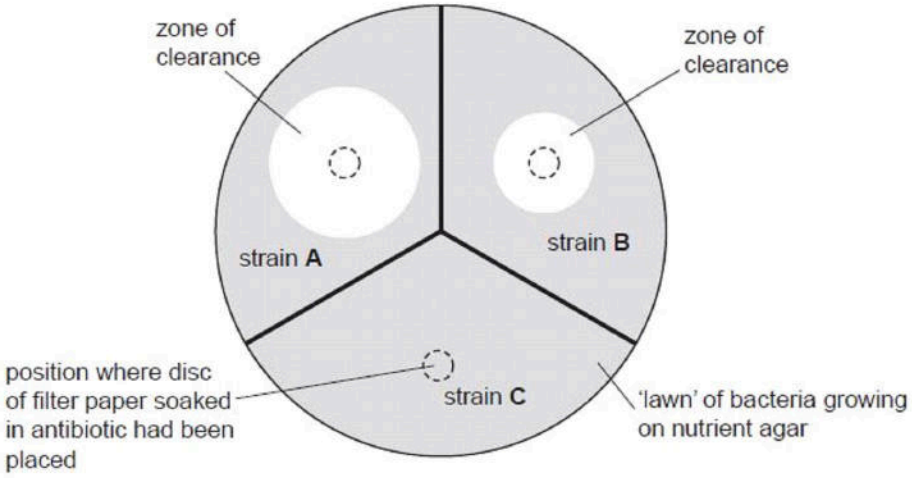


Fig. 3.1

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(i) The effectiveness of the antibiotic is proportional to the area of the zone of clearance.

Measure the diameters (**d**) of each of the zones of clearance of bacterial strains A and B and record them to the nearest mm in Table 3.1.

Using πr^2, calculate the **area** in mm^2 of the zone of clearance for each strain of bacterium and record them in Table 3.1.

Calculate the ratio of the **area** for strain **A** to the **area** for strain **B** and record the ratio in Table 3.1.

Table 3.1

bacterial strain	**A**	**B**
diameter (**d**) of zone of clearance / mm	.....	.....
area of the zone of clearance / mm^2	.....	.....
area for strain **A** : **area** for strain **B**	.....	

[3]

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(ii) Explain the different effects of the antibiotic on bacterial strains **A**, **B** and **C**.

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(iii) Describe the role of natural selection in the spread of bacterial strains, such as **A** and **B**, when an antibiotic is used.

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(b) The β-lactam antibiotics, such as penicillin, are similar shaped molecules to the substrate of a bacterial enzyme, transpeptidase.

Explain the mode of action of β-lactam antibiotics on susceptible bacteria.

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[Total: 15]

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Q2.

5 A significant quantity of the metallic copper produced in some countries is obtained using the chemoautotrophic bacterium, *Thiobacillus ferro-oxidans*, which is present in rocks in many countries.

The process, known as bioleaching, is shown in Fig. 5.1.

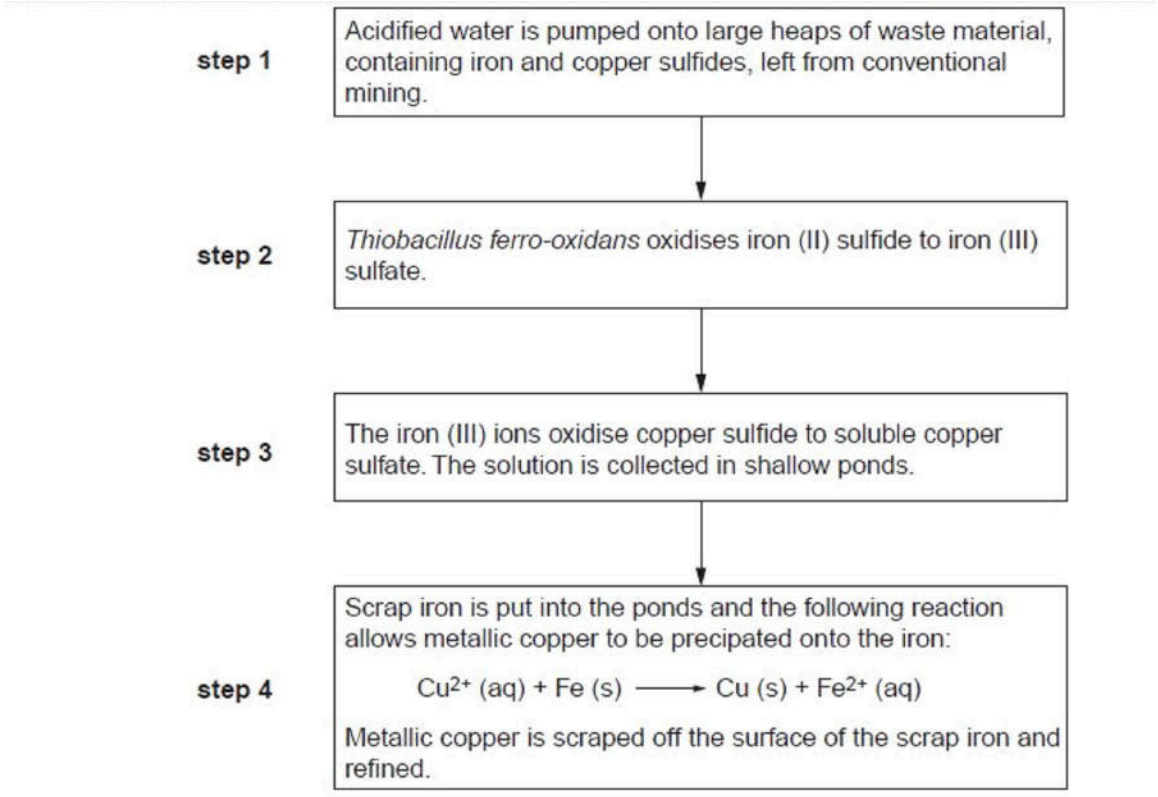


Fig. 5.1

(a) Suggest the benefit to the bacterium of **step 2**.

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(b) State two environmental disadvantages of extracting metals by bioleaching.

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(c) Explain why the production of metallic copper by bioleaching can be cheaper than using conventional mining methods.

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[Total: 8]

Q3.

5 The fungus *Penicillium chrysogenum* is grown in fermenters on an industrial scale to produce penicillin, using a batch culture system.

(a) Explain why batch culture, rather than continuous culture, is used for the production of penicillin.

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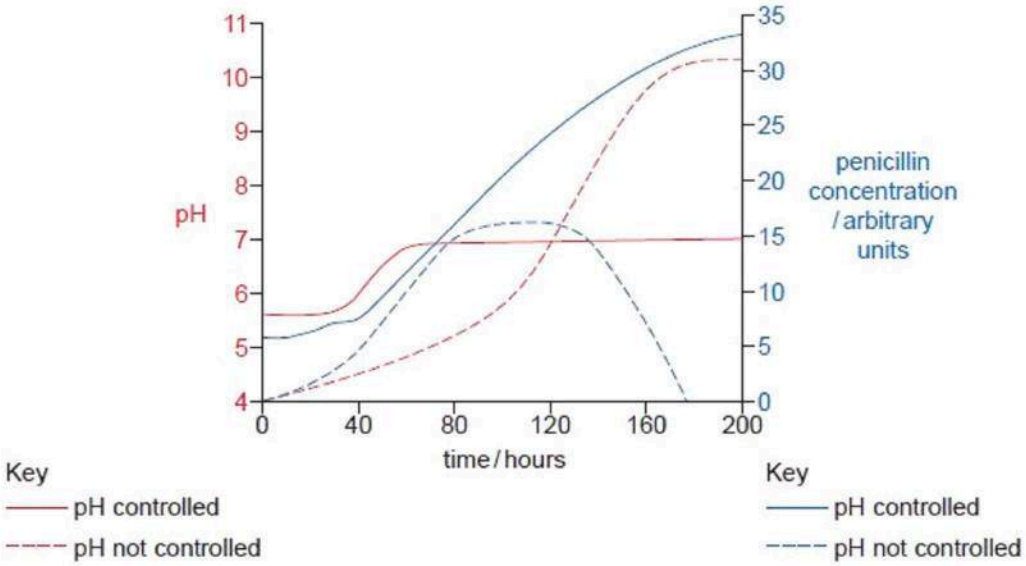
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(b) Temperature and pH are normally controlled in the fermenter. Temperature is kept constant, while pH is held at a value of 5.5 for the first stage of the fermentation and then raised to 6.8 and kept constant for the remainder of the fermentation period.

Fig. 5.1 shows how the pH and the concentration of penicillin in the culture change over time, when the pH is controlled and when the pH is not controlled.



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With reference to Fig. 5.1, describe and explain the differences in the concentration of penicillin in the culture when the pH is controlled and when the pH is not controlled.

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(c) Explain why penicillin affects bacteria but not viruses.

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[Total: 9]

Q4.

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2 **(a)** The glycoproteins CD28 and CD40 are found on the surface of T-lymphocytes (T-cells). They are binding sites for cell-signalling molecules and are essential for triggering the cloning of T-cells in an immune response.

A monoclonal antibody (mAb), which could block the CD40 signalling pathway, was produced from hamsters using the hybridoma method.

Outline the procedure, starting with a hamster, for producing mAbs suitable for use in another mammal, such as a mouse.

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(b) The ability of the mAb produced in **(a)** to prevent rejection of transplanted hearts in mice was compared with that of a protein, **P**, which blocks the CD28 signalling pathway.

Four groups of mice were treated as follows:

- group **A** – no treatment
- group **B** – treated with protein **P** only
- group **C** – treated with mAb only
- group **D** – treated with both mAb and protein **P**.

Fig. 2.1 shows the percentage survival of the transplanted hearts in the four groups of mice over a period of 80 days.

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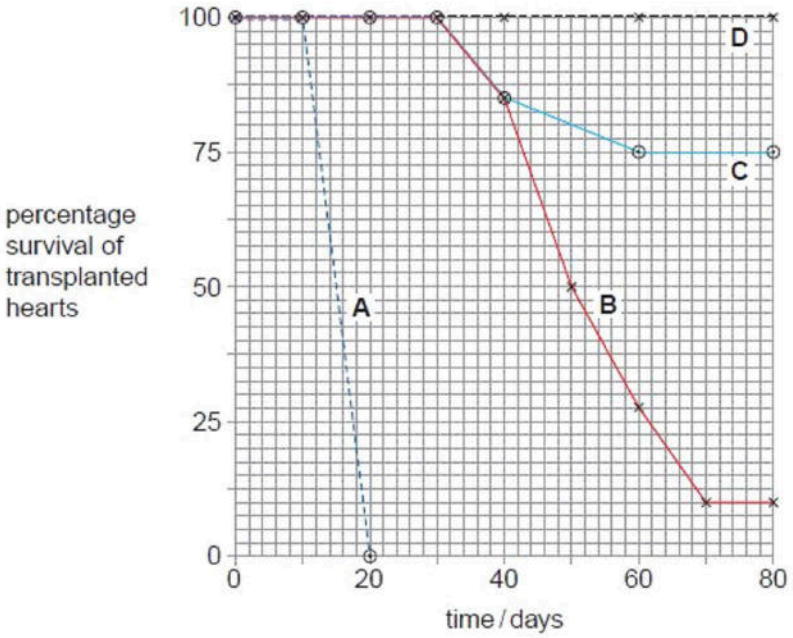


Fig. 2.1

With reference to Fig. 2.1

(i) describe the effectiveness of the four different treatments

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(ii) suggest an explanation for the differences in survival of the transplanted hearts in groups **B** and **D**.

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(c) Examination of the transplanted hearts showed that the hearts in group **A** had significant damage to their coronary arteries, whereas in group **D** these blood vessels appeared normal.

Explain the importance of the coronary arteries of the heart.

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(d) State two uses of mAbs in humans, other than preventing rejection of transplanted tissue.

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[Total: 15]

Q5.

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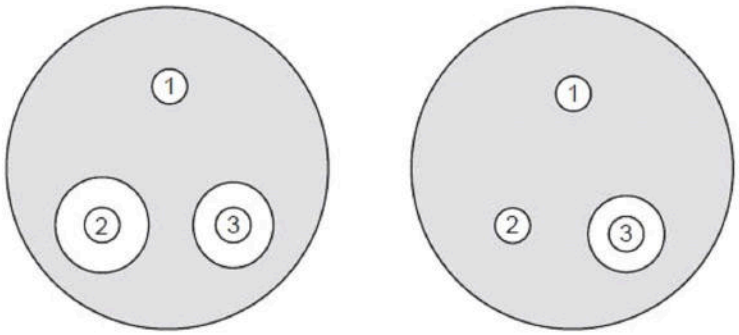
3 The sensitivity of bacteria to antibiotics can be tested using the disc diffusion method. An inoculum of the bacteria is spread onto agar culture plates and then filter paper discs impregnated with antibiotic are pressed onto the surface of the agar. The plates are incubated. Bacteria grow as a 'lawn' across the agar, but a circular zone (the zone of inhibition) appears around any disc where bacterial growth is inhibited.

For Examiner's Use

Two species of bacteria, **A** and **B**, were grown on separate culture plates in the presence of three types of filter paper disc:

1 – no antibiotic (control)
2 – penicillin V, a natural penicillin
3 – carboxypenicillin, a synthetic penicillin.

The appearance of the incubated plates is shown in Fig. 3.1.



bacterium **A** bacterium **B**

Fig. 3.1

(a) With reference to Fig. 3.1, explain the effect of penicillin V on bacterium **A**.

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Bacteria **A** and **B** have different outer layers, as shown in Fig. 3.2.

For Examiner's Use

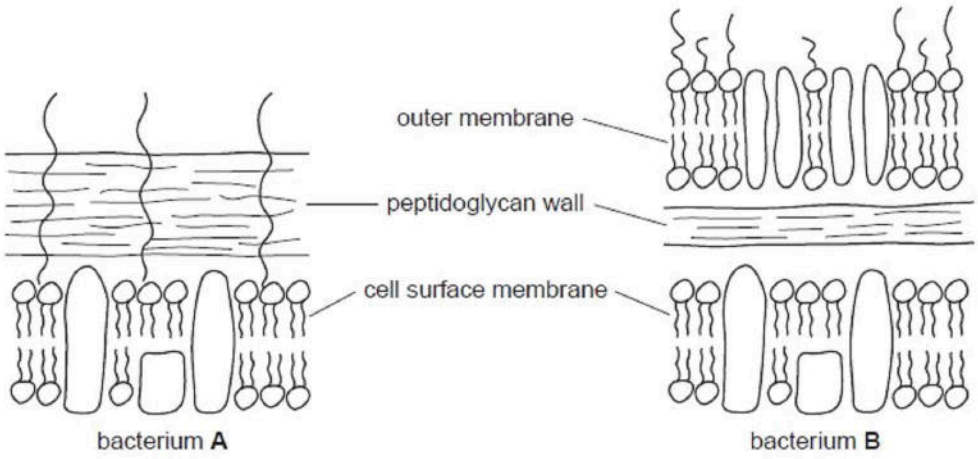


Fig. 3.2

(b) With reference to Fig. 3.1 and Fig. 3.2

(i) describe how the outer layers of bacterium **B** differ from those of bacterium **A**

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(ii) explain the different effects of penicillin V on bacteria **A** and **B**

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(iii) suggest how the synthetic penicillin, carboxypenicillin, is able to affect the growth of bacterium **B**.

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(c) Distinguish between batch culture and continuous culture of microorganisms.

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(d) Explain why batch culture rather than continuous culture is used in the production of penicillin.

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[Total: 15]

Q6.

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2 When gold is associated with mineral ores such as iron sulfide, the sulfides must be oxidised to release the gold particles. Since the mid 1990s, gold has been extracted from such ores by bioleaching.

For Examiner's Use

Suitable bacteria oxidise iron sulfide to soluble iron sulfate, releasing Fe^{3+} and SO_4^{2-} ions. The reaction releases heat energy and temperatures within a heap of ore that is being bioleached (a bioheap) can reach 70 °C or higher.

Examples of bacteria used in this bioleaching are shown in Table 2.1.

Table 2.1

example of bacterium	temperature range for growth / °C	activity	natural habitat
Acidithiobacillus ferrooxidans	35 – 45	oxidise iron and sulfur compounds	acid springs
Sulfobacillus thermosulfidooxidans	45 – 65		
Sulfolobus metallicus	65 – 95		

(a) With reference to Table 2.1, suggest

(i) a natural habitat for organisms such as *S. thermosulfidooxidans* and *S. metallicus*

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(ii) why all three species of bacteria, rather than just one species, are mixed with ore in a bioheap.

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(b) The rate of oxidation of the iron in iron sulfide ore was compared in the presence and absence of *A. ferrooxidans* at pH 2.0.

The results are shown in Fig. 2.1.

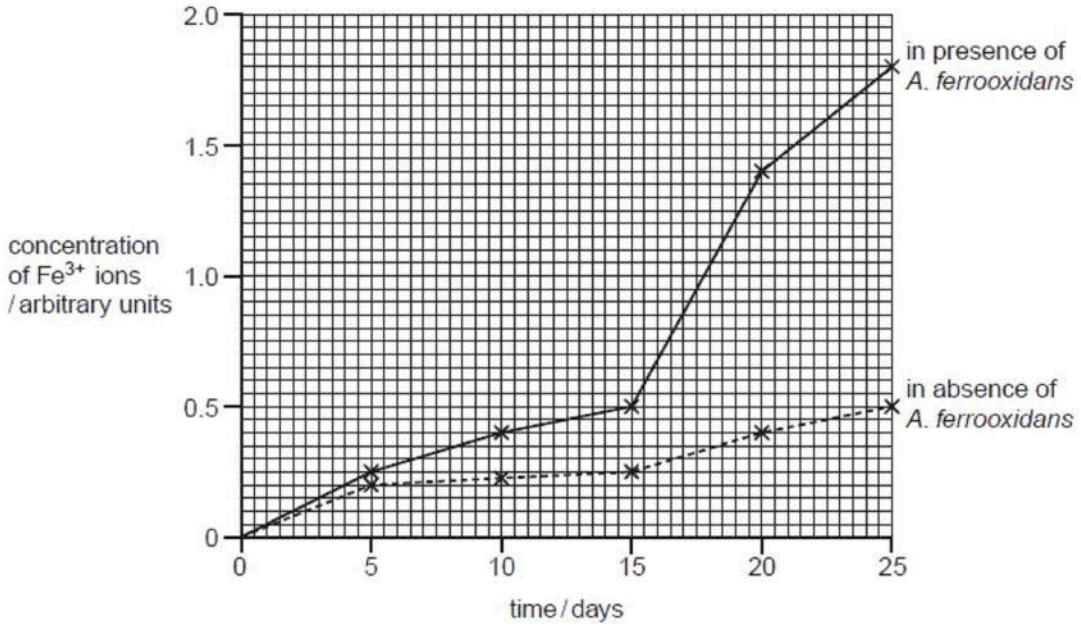


Fig. 2.1

(i) With reference to Fig. 2.1, describe the effect of *A. ferrooxidans* on the oxidation of the ore.

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(ii) Explain why bioleaching is now used on a large scale throughout the world.

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(c) Gold-bearing sulfide ores often contain arsenic, which is potentially toxic to the bacteria used in bioleaching. However, arsenic-resistant strains of *A. ferrooxidans* have been found in some mines.

The activity of two strains of the bacterium, in the presence and absence of arsenic ions, is shown in Table 2.2.

Table 2.2

	oxidation rate of iron in the ore / $mg\,dm^{-3}\,h^{-1}$	
strain of *A. ferrooxidans*	arsenic ions absent	arsenic ions present
1	16	15
2	48	47

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Describe the results shown in Table 2.2 **and** explain the role of natural selection in the evolution of arsenic-resistant bacteria.

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[Total: 14]

Q7.

2 **(a)** Outline how an enzyme can be immobilised in alginate.

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(b) State two advantages, other than stability, of using an immobilised enzyme in an industrial process compared with the same enzyme that has not been immobilised.

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(c) Papain is a protease enzyme. Its activity at different temperatures, when immobilised onto an inert support, was compared with its activity in solution.

The results are shown in Fig. 2.1.

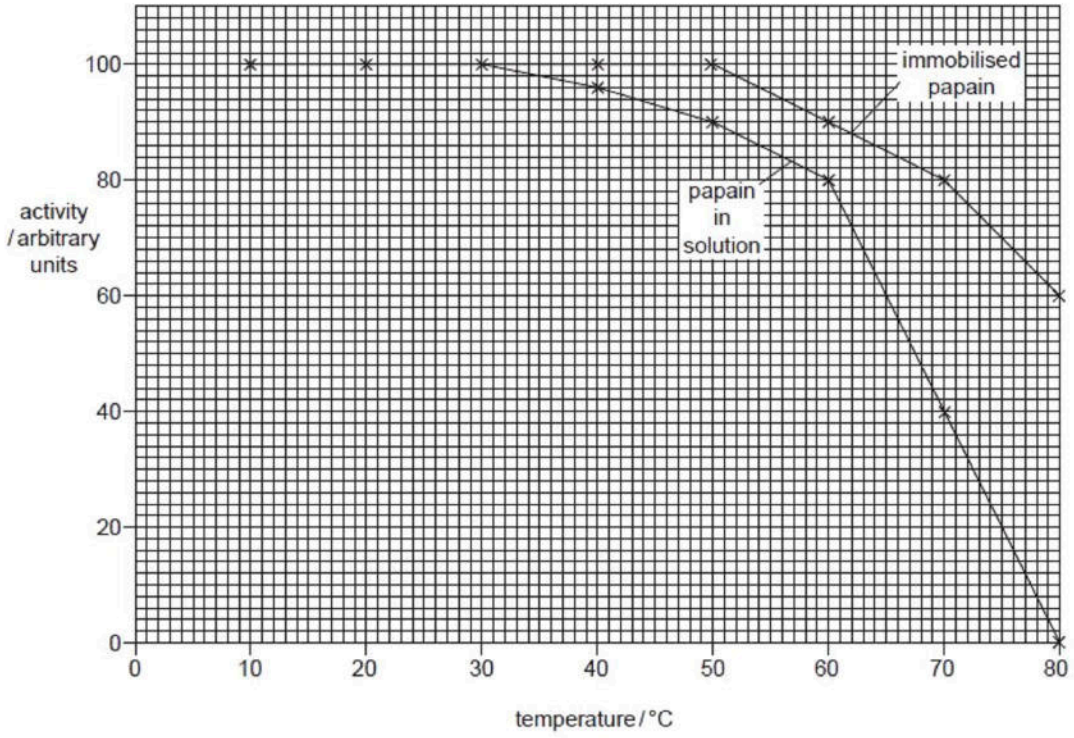


Fig. 2.1

With reference to Fig. 2.1, describe **and** explain the differences in activity of immobilised papain and papain in solution.

For Examiner Use

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[Total: 8]

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Q8.

5 In the USA, about 35% of all maize that is grown has been genetically modified to produce a toxin, called Bt toxin, derived from the bacterium *Bacillus thuringiensis*. The genetically modified plants are known as Bt maize.

(a) Explain the advantages of growing Bt maize.

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(b) An investigation was carried out into the potential effects of dead leaves from Bt maize on organisms living in streams that flow through areas where the maize is grown.

The researchers conducted a laboratory-based experiment in which larvae of one species of aquatic caddis fly, *Lepidostoma liba*, were fed on non-Bt maize leaves, or on leaves from Bt maize. The growth rates of the larvae were measured.

The results are summarised in Fig. 5.1.

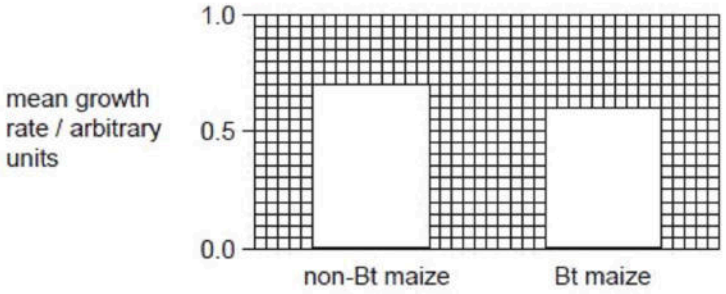


Fig. 5.1

Describe the effect of eating leaves from Bt maize on the growth rate of *L. liba* larvae.

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(c) In a second laboratory-based experiment, three groups of larvae of a different species of caddis fly, *Helicopsyche borealis*, were fed on pollen containing:

A no Bt toxin

B Bt toxin at concentrations found in streams in maize-growing areas

C Bt toxin at concentrations twice as high as found in those streams.

The researchers measured the mortality rates of the caddis fly larvae.

Their results are summarised in Table 5.1.

Table 5.1

groups compared	difference in mortality rate
groups **A** and **B**	no significant difference
groups **A** and **C**	significantly greater mortality in **C** than in **A**

The researchers were careful to state that their results showed the '**potential** ecological effects' on the caddis fly larvae of growing Bt maize.

Suggest **two** reasons why 'potential ecological effects' is a suitable description of any conclusions that could be drawn from the results of this experiment.

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(d) When the results of the experiments described in **(b)** and **(c)** were published, many other scientists criticised the research very strongly.

Suggest why some scientists might wish to suppress results such as these, even if there is no fault with the investigation itself.

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[Total: 7]

Q9.

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2 Some of the steps in the production of monoclonal antibodies are shown in Fig. 2.1.

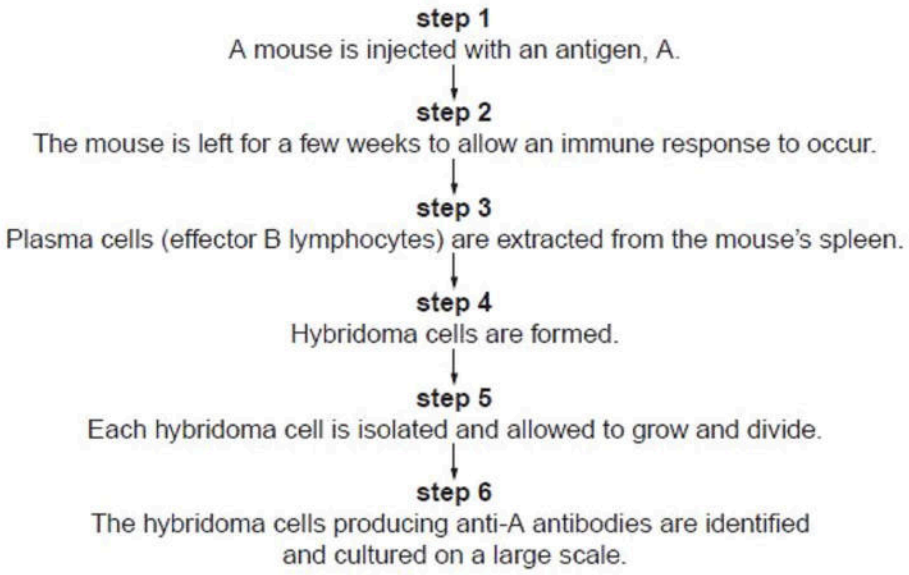


Fig. 2.1

(a) With reference to Fig. 2.1, explain:

(i) what happens during an immune response (**step 2**)

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(ii) what is meant by a hybridoma cell (**step 4**)

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(iii) why hybridoma cells need to be formed (**step 4**)

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(iv) how hybridoma cells producing anti-A antibody can be identified.

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(b) Rheumatoid arthritis (RA) is an autoimmune disease in which T lymphocytes attack the cartilage of joints by secreting a protein, TNFα. When RA is untreated, joint damage increases considerably.

The monoclonal antibody, infliximab, is used to treat RA. Infliximab specifically binds to TNFα.

A trial was set up to compare the effectiveness of infliximab and a standard treatment for RA, the anti-inflammatory drug, MTX.

Five groups of people with RA received the following treatments for one year:

- group **P** – MTX only
- group **Q** – MTX plus low dosage of infliximab at intervals of eight weeks
- group **R** – MTX plus low dosage of infliximab at intervals of four weeks
- group **S** – MTX plus high dosage of infliximab at intervals of eight weeks
- group **T** – MTX plus high dosage of infliximab at intervals of four weeks.

At the end of the year's treatment, the proportion of people in each group with increased joint damage was determined.

The results are shown in Fig. 2.2.

The number of people in each group is shown in brackets.

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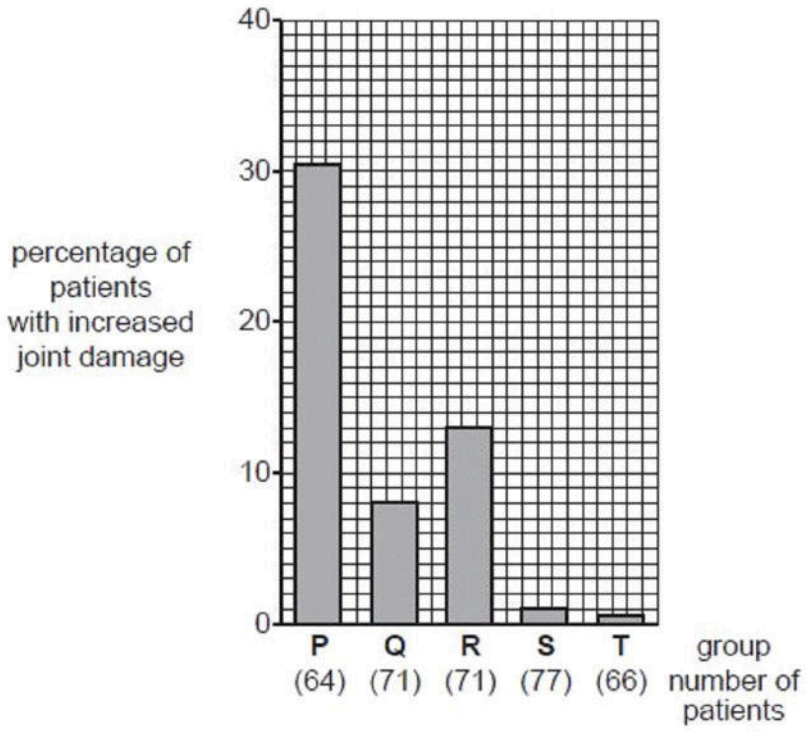


Fig. 2.2

With reference to Fig. 2.2:

(i) describe the effect of infliximab treatment on these people

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(ii) suggest why the results in groups **Q** and **R** do not follow the general trend.

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(c) Explain the advantages of the use of monoclonal antibodies, compared with conventional methods, in the **diagnosis** of disease.

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[Total: 15]

Q10.

2 The bacterium, *Treponema pallidum*, causes the sexually-transmitted infectious disease, syphilis. If left untreated, the disease can be fatal, but early diagnosis can lead to successful treatment. One of the difficulties of diagnosing this disease in its early stages is the problem of recognising *T. pallidum* among the other species belonging to the genus *Treponema* that live in humans. These other treponemes are harmless. E

A mouse was injected with some cells of *T. pallidum*.

(a) Outline the steps that would then be necessary to produce a clone of hybridoma cells secreting an antibody against this bacterium.

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(b) A monoclonal antibody, H9-1, has been developed that is specific to a surface protein on *T. pallidum*, but which is not present on four other species of treponemes found in humans.

Each molecule of H9-1 carries a fluorescent yellow marker.

One of the first visible signs of syphilis is a painless sore.

Suggest how H9-1 is used in the diagnosis of syphilis, using a sample taken from a sore and placed on a microscope slide.

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(c) Before the development of H9-1, two tests for the presence of *T. pallidum* were commonly used:

- dark-field microscopy (in which treponemes could be seen moving against a dark background)
- testing for the presence of anti-treponemal antibodies in the blood plasma.

Suggest why, in the **early** stages of an infection, the presence of *T. pallidum* might not be detected by either of these tests.

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(d) The accuracy of the diagnosis of infection by *T. pallidum* using H9-1 was compared with that using dark-field microscopy and with blood testing. The results are shown in Table 2.1.

A positive test result indicated that *T. pallidum* is present and a negative test result that it is absent.

Table 2.1

test	test results of 30 people later confirmed to have the infection	test results of 31 people later confirmed not to have the infection
H9-1	all positive	all negative
dark-field microscopy	one negative	two positive
blood test	three negative	two positive

With reference to Table 2.1:

(i) compare the accuracy of diagnosis of the presence of *T. pallidum* using the different tests

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(ii) suggest why blood testing for anti-treponemal antibodies gave two positive results in patients later found not to have the infection.

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(e) Describe briefly **one** use of a monoclonal antibody in the **treatment** of disease.

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[Total: 15]

Q11.

3 The filamentous fungus, *Fusarium venenatum*, can be grown in a fermenter and harvested as mycoprotein. It is sold as a food in a number of different countries.

The fungus is grown in continuous culture in 150 000 dm^3 airlift fermenters, in which the introduction of bubbles of compressed air both oxygenates and stirs the contents. The fungus grows as narrow, branched filaments, giving the harvested mycoprotein a naturally chewy, fibrous texture. Approximately 300 kg of fungus can be harvested per hour.

(a) Explain what is meant by the term *continuous culture*.

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(b) After about six weeks, mutants may appear in the fungal population, for example, a more highly-branched form of the fungus.

The fermenter is emptied, cleaned and repopulated with the original strain of *F. venenatum* every six weeks.

Explain why the fermentation process should be stopped before mutants appear.

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(c) Approximately 12% of the harvested fungus is protein.

Calculate the approximate mass of protein harvested in one day during continuous culture.

Show your working.

answer ..... [1]

[Total:7]

Q12.

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3 The filamentous fungus, *Fusarium venenatum*, is grown in continuous culture in large fermenters to provide mycoprotein for human consumption.

For Examiner Use

(a) Explain what is meant by the term *continuous culture*.

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(b) In an investigation into the growth of the fungus in culture, several factors were varied including:

- temperature
- concentration of the carbon source
- concentration of the nitrogen source.

Some of the results are shown in Table 3.1.

Table 3.1

temperature /°C	concentration of carbon source / g dm^{-3}	concentration of nitrogen source / g dm^{-3}	dry mass of fungus / g dm^{-3}
25	7.0	2.9	3.1
25	14.0	3.5	4.3
30	7.0	3.5	4.8
30	14.0	2.9	4.2

(i) Describe the effect of temperature on the growth of the fungus at the different concentrations of the carbon source.

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(ii) Explain why the fungus needs sources of carbon and nitrogen.

carbon .....

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nitrogen .....

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[Total: 8]

Q13.

4 **(a)** Outline the hybridoma method for the production of a monoclonal antibody.

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(b) Herceptin is a monoclonal antibody used in the treatment of some breast cancers. It binds strongly to molecules of a receptor protein, HER2, that is produced in abnormally large quantities in the plasma (cell surface) membranes of about 30% of human breast cancers.

Investigations have been made into the most effective way to use Herceptin to treat breast cancer.

One experiment investigated the ability of different treatments to induce cell death in breast cancer cells.

Herceptin and X-ray treatment were used both separately and together. The results are shown in Fig. 4.1.

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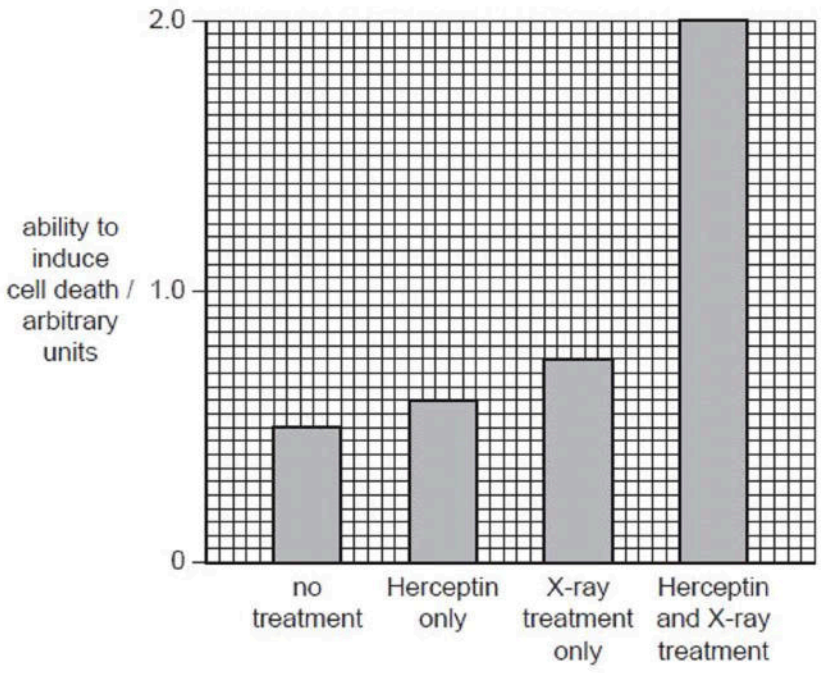


Fig. 4.1

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With reference to Fig. 4.1,

(i) compare the effects on breast cancer cells of the different treatments

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(ii) calculate the percentage increase in the ability to induce cell death of using Herceptin **and** X-ray treatment compared with using Herceptin only.

Show your working.

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(c) A second experiment investigated the effect of increasing doses of X-rays on the survival of breast cancer cells in the presence and absence of Herceptin. The results are shown in Fig. 4.2.

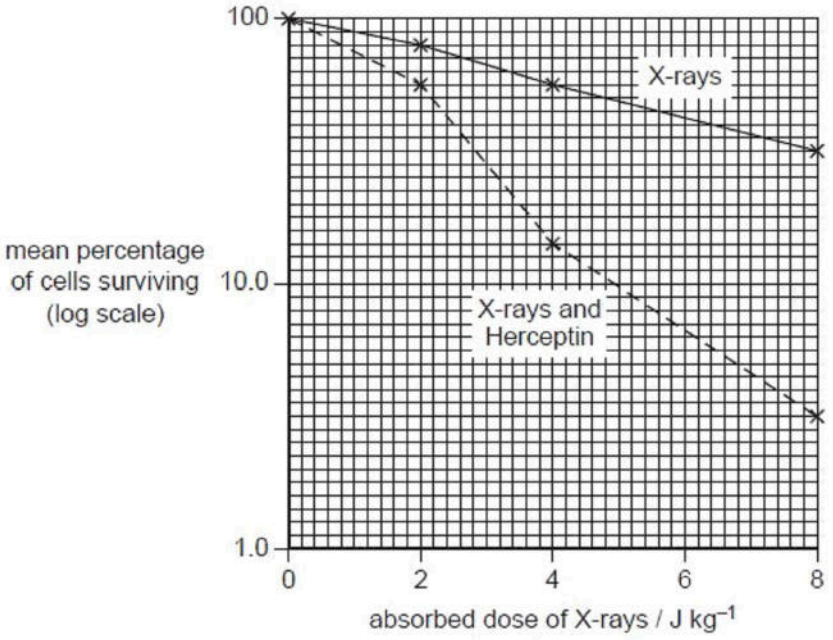


Fig. 4.2

With reference to Fig. 4.2,

(i) compare the effects of increasing doses of X-rays on cells in the presence and absence of Herceptin

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.....[3]

(ii) suggest an explanation for the effect of Herceptin.

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.....[2]

[Total: 14]

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Q14.

3 Proteases that work in alkaline conditions are made in large quantities for use in the detergent industry. The microorganism that is generally used for this is the bacterium *Bacillus subtilis*.

An investigation was carried out to compare three potential production methods:

- using free cells of *B. subtilis*
- using *B. subtilis* cells immobilised in cubes of agar
- using *B. subtilis* cells immobilised in beads of sodium alginate.

To immobilise the cells in agar, the agar was dissolved and cooled. A suspension of *B. subtilis* was then added. The agar-bacterium mixture was poured into sterile dishes and allowed to solidify. It was then cut into cubes with sides of 2 mm.

(a) (i) Explain why the agar was cooled before the suspension of *B. subtilis* was added.

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.....[1]

(ii) Describe how cells of *B. subtilis* could be immobilised in beads of alginate.

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.....[3]

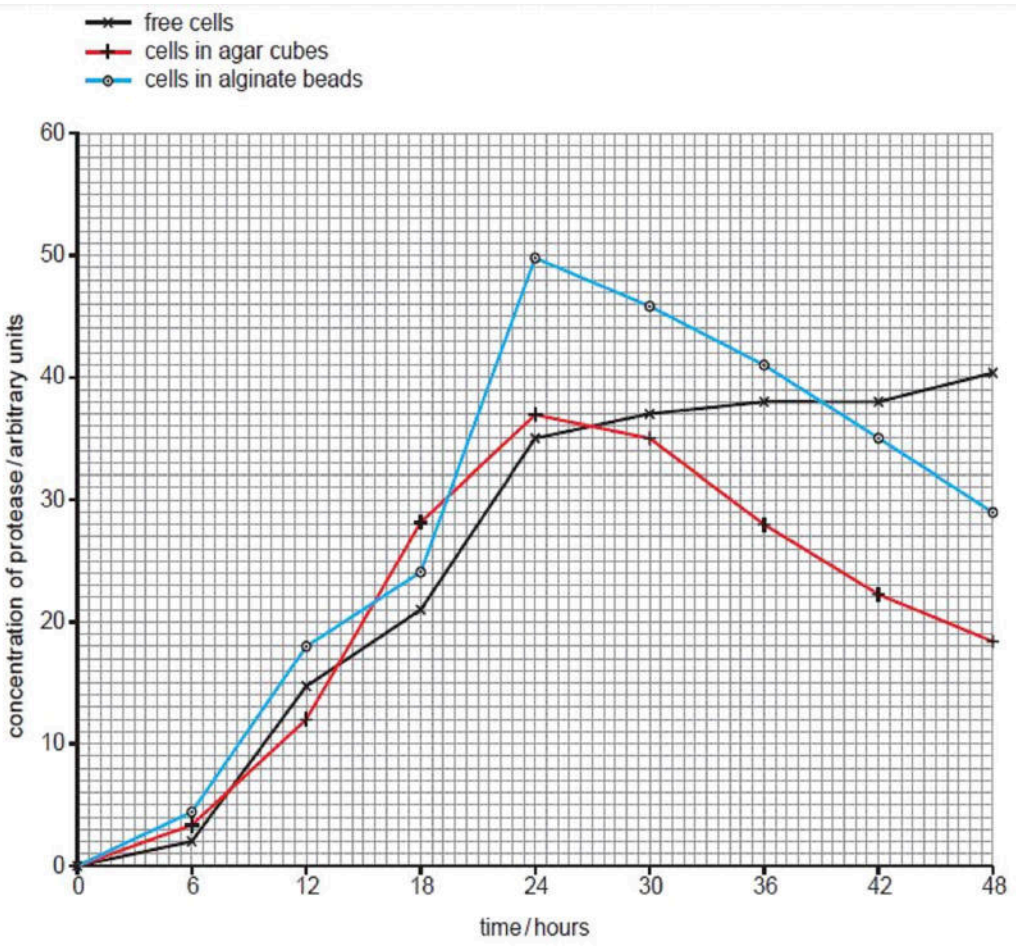
(b) A liquid medium containing glucose, a nitrogen source and various mineral ions was made up, and 50 cm^3 placed into each of three flasks.

Samples of a culture of free cells of *B. subtilis*, agar cubes containing immobilised *B. subtilis* and alginate beads containing *B. subtilis* were placed in the three flasks. Each flask contained the same number of bacteria. All the flasks were incubated at 37 °C for 48 hours.

Samples of the liquid medium in each flask were taken at six hourly intervals and the concentration of protease measured.

The results are shown in Fig. 3.1.

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(i) With reference to Fig. 3.1, compare the results for the free cells of *B. subtilis* and cells immobilised in alginate beads.

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.....[4]

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(ii) Suggest why lower concentrations of protease were produced by *B. subtilis* immobilised in agar cubes than *B. subtilis* immobilised in alginate beads.

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.....[2]

(c) Two new cultures of immobilised *B. subtilis* were set up as described in **(b)**.
However, this time a repeat batch fermentation method was used, in which the liquid medium was replaced every 24 hours. This was continued until the cubes or beads had begun to disintegrate.

The results are shown in Table 3.1.

Table 3.1

	number of batches before cubes or beads disintegrated	total fermentation time / hours	total protease produced / arbitrary units	mean productivity of protease / arbitrary units per hour
agar cubes	6	144	1792	12.44
alginate beads	9	216	3264	15.11

With reference to Table 3.1

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(i) calculate the percentage increase in the total protease produced when the bacteria were immobilised in alginate rather than agar.

Show your working.

.....[2]

(ii) explain why using bacteria immobilised in alginate rather than agar would be a more cost-effective production of protease.

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.....[3]

[Total: 15]

Q15.

3 (a) Cell walls of bacteria contain peptidoglycans. Peptidoglycans are long chains of the sugars N-acetylmuranic acid (NAM) and N-acetylglucosamine (NAG) which alternate along the chain. A short peptide chain of three to five amino acids is attached to each NAM and these form cross-links with similar peptide chains from adjacent strands.

Fig. 3.1 shows a diagram representing part of a peptidoglycan structure.

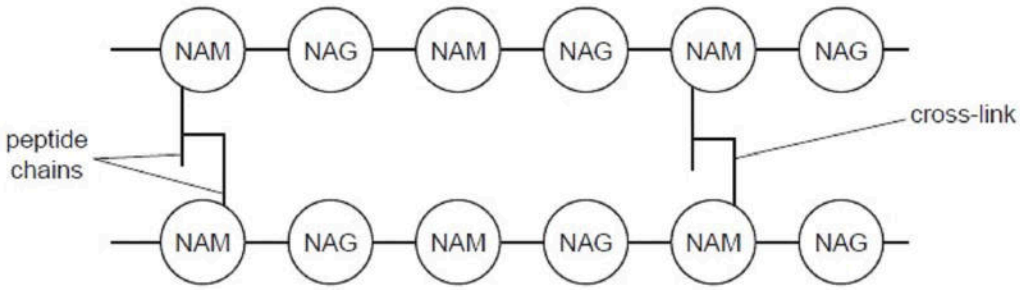


Fig. 3.1

(i) Name the type of reaction that takes place to assemble the peptide chains that form the cross-links.

.....[1]

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(ii) Describe the mode of action of antibiotics, such as penicillin, on bacteria.

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.....[4]

(iii) Suggest the name of the type of enzyme that assembles the peptide chains that form the cross-links in peptidoglycans.

.....[1]

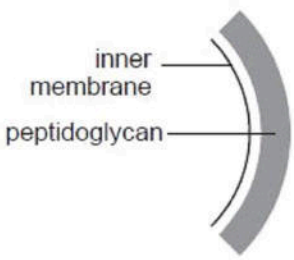
(b) State why antibiotics, such as penicillin, have no effect on viruses.

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.....[1]

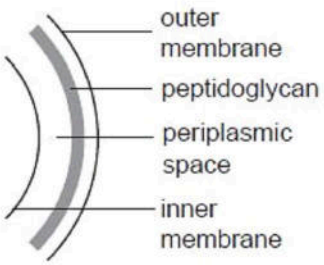
(c) Bacteria may be Gram-positive or Gram-negative.

Fig. 3.2 shows a diagram of part of the cell walls of both Gram-positive and Gram-negative bacteria.



Gram-positive

Gram-positive bacteria cell walls have a peptidoglycan content of 50%



Gram-negative

Gram-negative bacteria cell walls have a peptidoglycan content of 10 – 20%

Fig. 3.2

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Suggest why Gram-positive bacteria are more susceptible to the action of penicillin than Gram-negative bacteria.

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.....[2]

(d) There is evidence that some bacteria have developed resistance to antibiotics.

One form of pneumonia, a serious lung disease, is caused by the bacterium *Streptococcus pneumoniae*. The Canadian Health Service has carried out a survey to show how the resistance of *S. pneumoniae* to penicillin has changed over the last 20 years.

Fig. 3.3 shows the results of this survey.

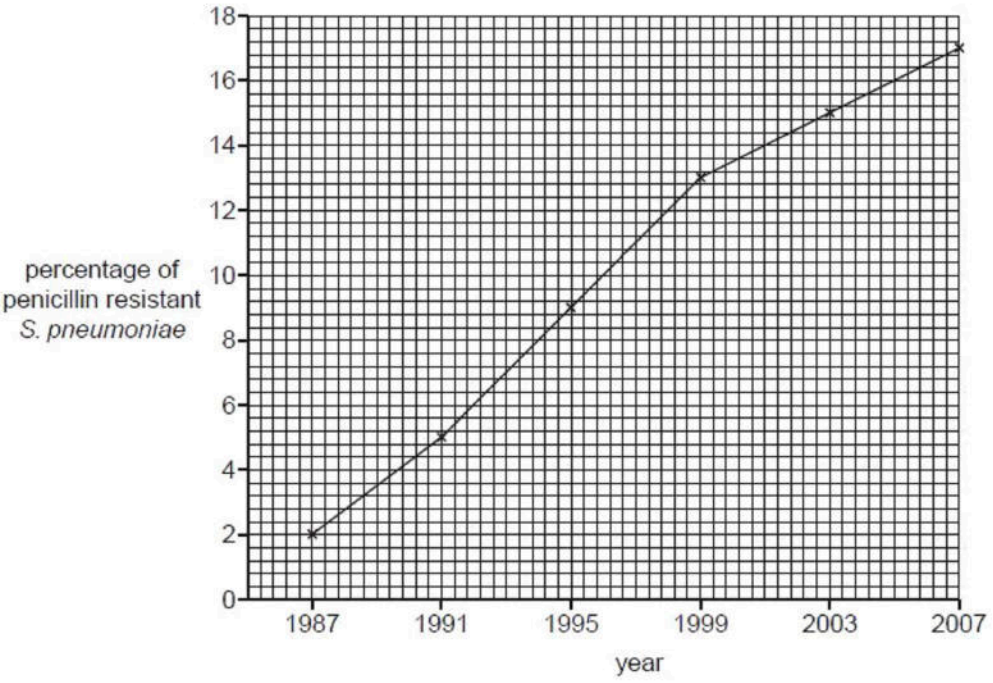


Fig. 3.3

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Describe the results shown in Fig. 3.3 **and** explain how some strains of *S. pneumoniae* may have become resistant to penicillin.

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.....[5]

[Total: 14]

Q16.

2 The disease-causing bacterium, *Pseudomonas aeruginosa*, may occur in the form of a 'biofilm'. A biofilm consists of a layer of bacteria, growing on a surface and attached to one another. Such biofilms are difficult to control by antibiotics.

A mutant strain of *P. aeruginosa* has been found which produces biofilms that are indistinguishable from those of the wild-type bacteria. However, the mutant strain differs from the wild-type in its resistance to an antibiotic, **A**.

(a) Antibiotic **A** belongs to a group of antibiotics known as anti-pseudomonal penicillins.

(i) Describe the mode of action of penicillin on bacteria.

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..... [3]

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(ii) Explain why penicillin does not affect viruses.

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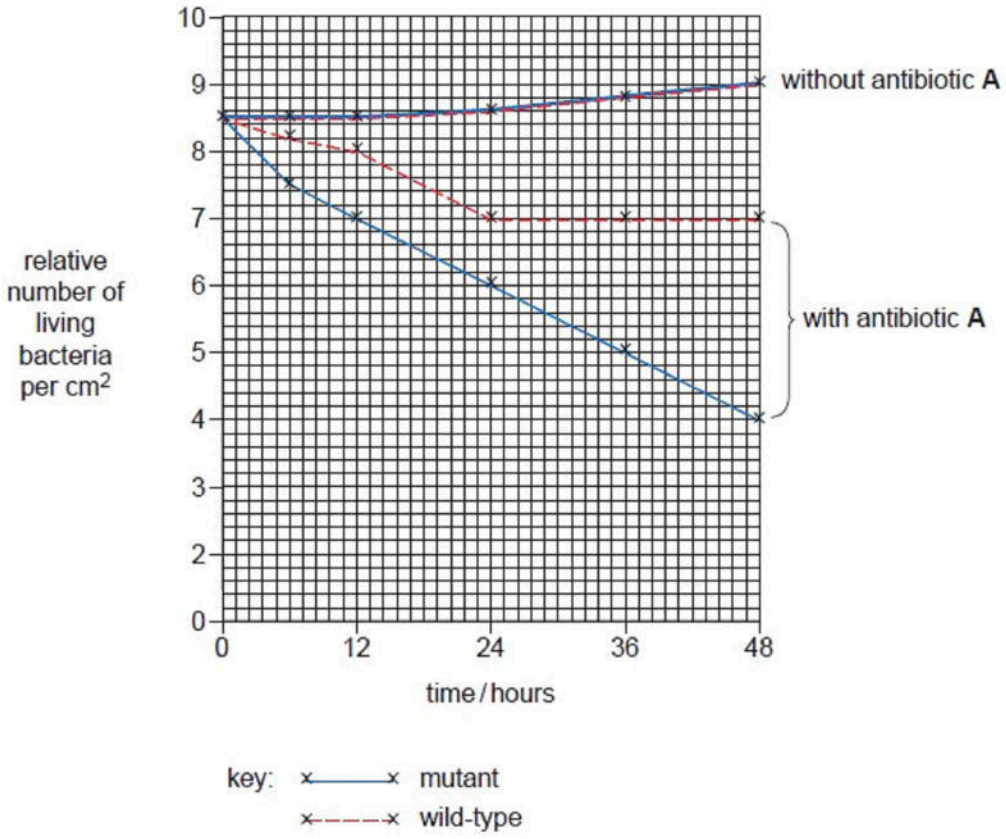
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..... [2]

(b) Wild-type and mutant bacteria were grown on solid culture media both with antibiotic **A** and without antibiotic **A**.

The subsequent change in numbers of living bacteria is shown in Fig. 2.1.



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With reference to Fig. 2.1, describe the changes in numbers of the wild-type and mutant bacteria on culture media with antibiotic **A and** without antibiotic **A**.

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..... [4]

(c) The wild-type and mutant strains of this bacterium have different DNA sequences in part of a gene coding for an enzyme which is needed to produce polymers of glucose, called glucans. Glucans are secreted by bacteria and can bind to various molecules, including those of antibiotic **A**. *E*

Explain

(i) how a mutation of a gene coding for an enzyme may result in an enzyme with reduced activity,

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..... [2]

(ii) the different effects of antibiotic **A**, shown in Fig. 2.1, on the wild-type and mutant strains of bacteria.

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..... [2]

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(d) Explain the role of natural selection in the evolution of antibiotic resistance in bacteria.

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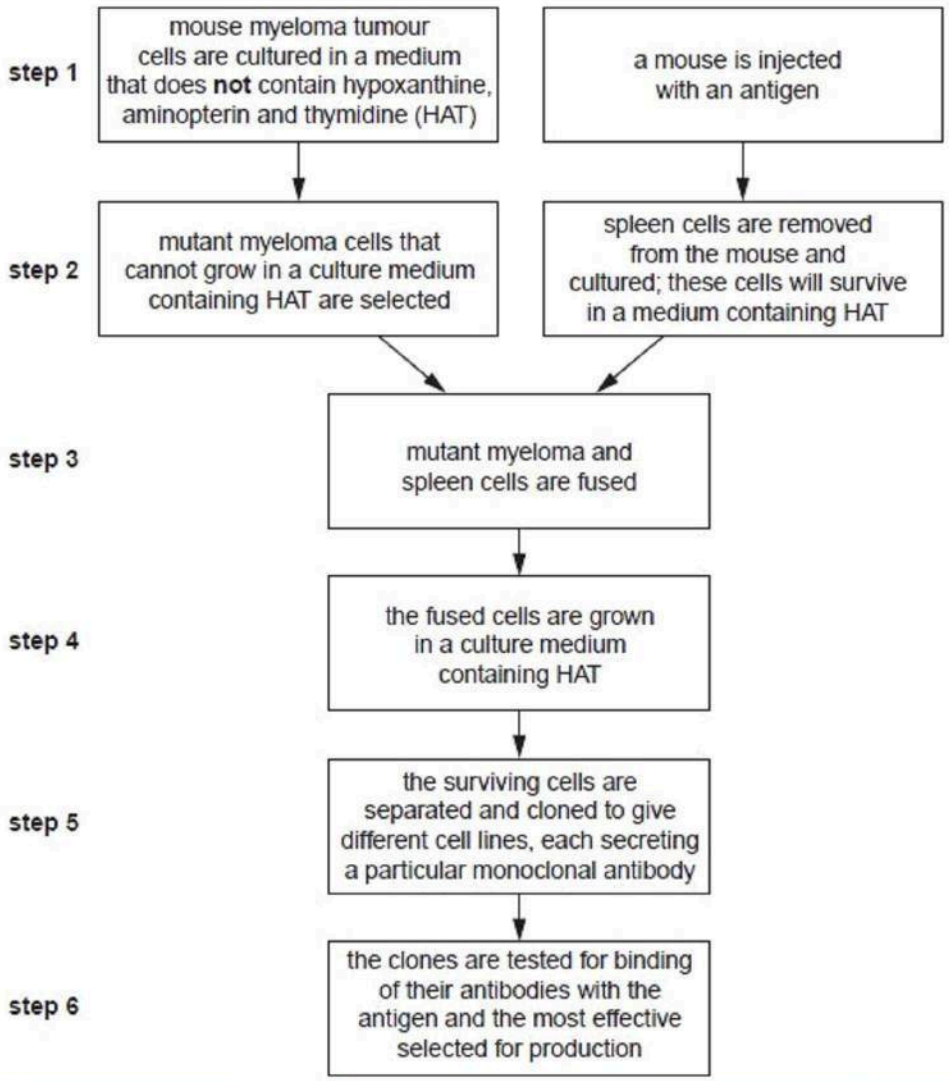
..... [3]

[Total: 16]

Q17.

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3 Fig. 3.1 shows a method of producing monoclonal antibodies.



(a) **(i)** Name this method of producing monoclonal antibodies.

.....[1]

(ii) Describe what is meant by the term *monoclonal antibody*.

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.....[2]

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(iii) Describe, in detail, the molecular structure of one of the antibodies produced in **step 6**. You may wish to use an annotated diagram to answer the question.

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..... [3]

(b) The mutant myeloma cells used in **step 2** are myeloma cells with a gene mutation that prevents them from growing in a culture medium containing hypoxanthine, aminopterin and thymidine (HAT).

(i) Suggest why cells with this gene mutation **cannot** grow in a culture medium containing HAT.

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..... [2]

(ii) Explain why the mutant myeloma cells **can** grow in a culture medium containing HAT after they have been fused with mouse spleen cells (**steps 3** and **4**).

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..... [2]

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(iii) Suggest why growing the fused cells in a culture medium containing HAT (**step 4**) is an important part of the procedure shown in Fig. 3.1.

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..... [2]

(c) Suggest advantages of using monoclonal antibodies for pregnancy testing.

Exa

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..... [4]

[Total: 16]

Q18.

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2 **(a)** In the small intestine, the enzyme lactase hydrolyses the disaccharide lactose into the monosaccharides glucose and galactose. A deficiency of lactase can lead to a condition known as lactose intolerance. The lactose passes undigested into the large intestine resulting in diarrhoea. Some babies are born with congenital lactase deficiency, which is an inherited condition, and require lactose-free milk from birth.

Suggest how two parents, who can digest lactose, can have a child with congenital lactase deficiency.

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.....[2]

(b) The enzyme lactase can be produced by biotechnology and then used to produce lactose-free dairy products. Lactase is frequently used immobilised in alginate beads.

Fig. 2.1 shows a comparison between the activity of lactase free in solution and lactase immobilised in alginate beads, over a range of temperatures. Equal concentrations of free lactase and immobilised lactase were used.

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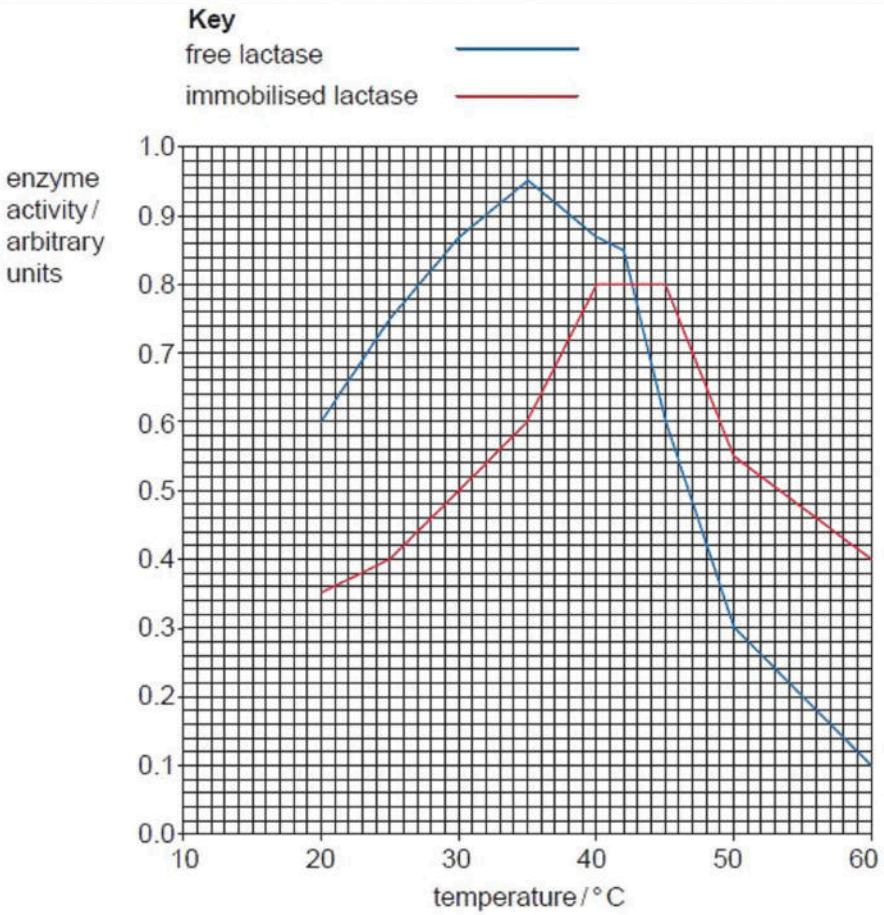


Fig. 2.1

With reference to Fig. 2.1:

(i) describe the effect of immobilisation on the activity of lactase

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.....[3]

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(ii) suggest explanations for the differences between the activity of immobilised lactase and free lactase up to 40 °C.

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.....[2]

(c) State the advantages of using immobilised enzymes instead of free enzymes.

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.....[3]

[Total: 10]

Q19.

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2 **(a)** Penicillin belongs to a group of antibiotics known as β lactams, which all act in the same way on bacteria.

Describe how penicillin kills non-resistant bacteria.

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.....[4]

(b) One of the ways in which a bacterium may be resistant to an antibiotic, such as a β lactam, is by having protein pumps in its cell surface membrane which expel the antibiotic from the bacterium.

The gene coding for such an efflux pump is carried on a plasmid.

Outline how the bacterium produces an efflux pump from a gene on a plasmid.

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.....[3]

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(c) A strain of the bacterium *Pseudomonas aeruginosa*, strain **R**, has a gene coding for an efflux pump and is resistant to a β lactam antibiotic.

The minimum inhibitory concentration (MIC) of the β lactam for strain **R** was determined. The MIC is the lowest concentration of antibiotic that prevents a colony of the bacterium from growing.

The MICs were also determined for two mutant strains derived from strain **R**, mutant strain **1** and mutant strain **2**. Each of these strains differs from strain **R** in the expression of the gene coding for the efflux pump.

The MICs for the three strains of *P. aeruginosa* are shown in Table 2.1.

Table 2.1

strain of *P. aeruginosa*	**MIC of β lactam / μg cm^{-3}**
resistant strain **R**	64
mutant strain **1**	0.5
mutant strain **2**	256

With reference to Table 2.1, suggest:

(i) why the MICs for mutant strains **1** and **2** differ from that for strain **R**

*mutant strain **1*** .....

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*mutant strain **2*** .....

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.....[4]

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(ii) how a population of strain **R** of *P. aeruginosa* could be replaced by mutant strain **2**.

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.....[4]

[Total: 15]

Q20.

3 Penicillin-binding proteins (PBPs) are proteins found in the cell surface membranes of bacteria. PBPs catalyse the final steps in the production of a peptidoglycan cell wall.

(a) From the information given above, describe the likely molecular structure of a PBP.

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.....[2]

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(b) Penicillin-resistant mutants of the bacterium, *Staphylococcus aureus*, produce a PBP, PBP2a, that does not bind well with penicillin.

Suggest how the presence of PBP2a in the cell surface membrane provides *S. aureus* with resistance to the effects of penicillin.

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.....[3]

(c) Explain why penicillin does not affect viruses.

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.....[2]

[Total: 7]

Q21.

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2 Many tumours release a protein growth factor called VEGF. This is a chemical signal that causes nearby blood vessels to grow new branches into the tumour.

The monoclonal antibody, bevacizumab (Avastin®), specifically binds to VEGF.

(a) Suggest how Avastin® can prevent the growth and spread of a tumour.

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.....[2]

(b) Avastin® is made by the hybridoma method.

State:

(i) the antigen that is injected into a mouse to produce this monoclonal antibody

.....[1]

(ii) what is meant by a *hybridoma*.

.....

.....

.....[1]

(c) The monoclonal antibody made by the hybridoma method is modified to obtain humanised mouse antibody. This type of antibody molecule resembles those produced by humans.

Suggest advantages of using humanised mouse antibody rather than mouse antibody.

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.....[3]

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(d) A second monoclonal antibody, ranibizumab (Lucentis®) is used to treat eye diseases. Lucentis® is a fragment of Avastin® and is shown in Fig. 2.1.

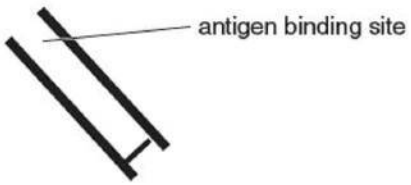


Fig. 2.1

Complete Fig. 2.1 to show a molecule of Avastin®.

Labels are **not** required. [2]

[Total: 9]

Q22.

5 (a) The pollutants in waste water from the textile industry may include azo-dyes, which give colour to textiles. Azo-dyes are organic pollutants that can be carcinogenic.

White-rot fungi are useful for the treatment of environmental pollution as they produce extracellular enzymes that are able to break down a number of organic pollutants, such as azo-dyes.

The extracellular enzymes produced by white-rot fungi are primary metabolites that are mass produced by continuous culture.

(i) Outline the main operating conditions of continuous culture.

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.....[2]

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(ii) State **three** advantages of using continuous culture and not batch culture, for the mass production of these enzymes.

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.....[3]

(b) (i) The ability of the white-rot fungi to break down azo-dyes was investigated. A suspension of the intact fungal cells was added to water contaminated with various concentrations of an azo-dye.

The results are shown in Table 5.1.

Table 5.1

	percentage breakdown of azo-dye	
azo-dye concentration / mg dm^{-3}	**after 7 days**	**after 10 days**
50	21.9	100.0
100	53.1	93.7
150	61.7	89.6
200	51.0	87.3

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Describe the results shown in Table 5.1.

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.....[4]

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(ii) When a similar experiment was performed using the free enzymes from the white-rot fungal cells, all concentrations of azo-dye shown in Table 5.1 were broken down within 12 hours.

Suggest why free enzymes break down the azo-dye more quickly than intact white-rot fungal cells.

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.....[2]

(c) These extracellular enzymes may be immobilised on an inert support.

Outline the advantages of using immobilised enzymes in the treatment of textile waste water.

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.....[3]

[Total: 14]

Q23.

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1 (a) Define the term *biotechnology*.

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..... [2]

(b) The extent to which the potential of biotechnology will be realised depends on public attitudes towards the technology involved.

Describe the factors that influence public opinion in determining the acceptability of the technology.

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..... [4]

(c) Fig. 1.1 shows the ethene production of a normal tomato and a 'Flavr Savr' genetically modified tomato left on a plant for three weeks prior to picking. Naturally produced ethene in fruits speeds up the ripening process.

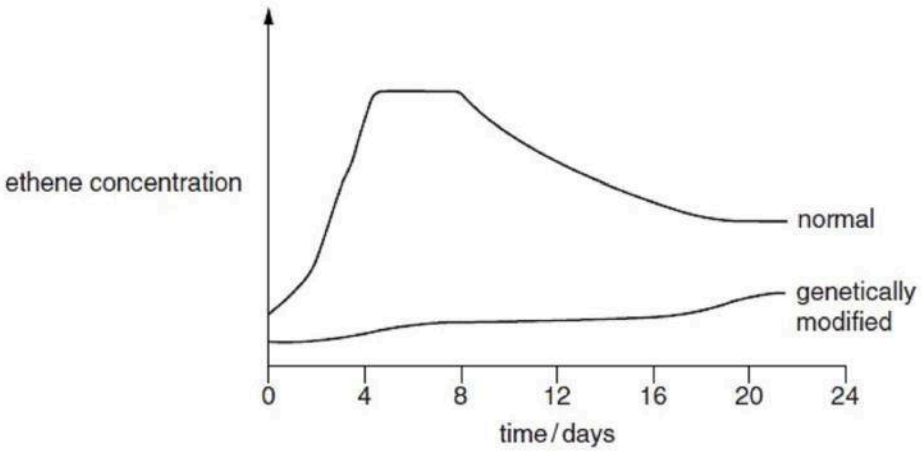


Fig. 1.1

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(i) Compare the production of ethene in normal tomatoes with that in genetically modified tomatoes.

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..... [3]

(ii) Suggest what effect this genetic modification will have on the ripening of the tomatoes.

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..... [2]

(iii) Explain the benefits that these genetically modified tomatoes might have for the grower and for the shopkeeper.

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..... [2]

(iv) Outline the ethical implications of consuming transgenic food products.

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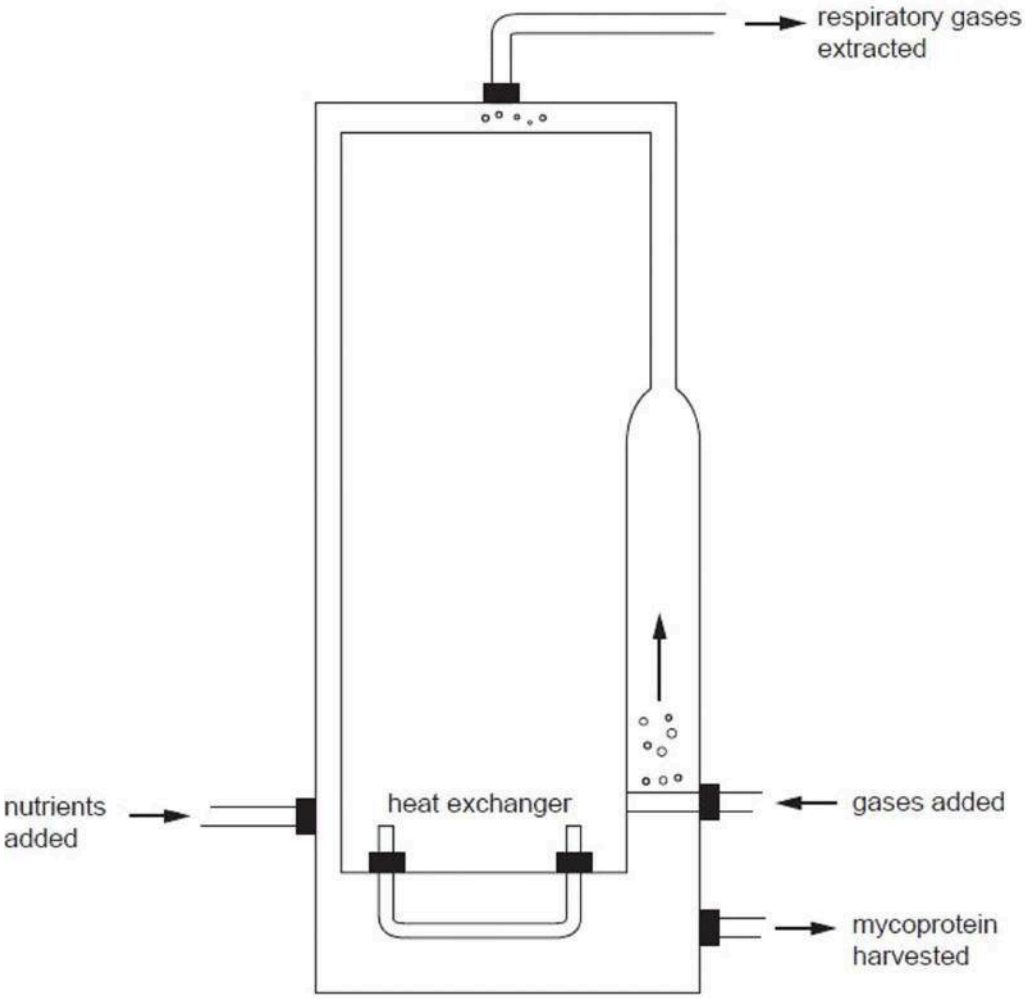
..... [2]

[Total: 15]

Q24.

2 Mycoprotein, an alternative to meat, is produced by growing the fungus, *Fusarium graminearum*, in an air-lift fermenter, similar to the one shown in Fig. 2.1.

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(a) (i) With reference to Fig. 2.1, explain how an air-lift fermenter works.

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.....[3]

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(ii) List three nutrient growth requirements of *Fusarium*.

1. .....

2. .....

3. .....[3]

(b) Describe two ways in which *Fusarium* must be processed **after** fermentation to make it acceptable as a human food product.

1. .....

.....

2. .....

.....[2]

[Total : 8]

Section-B

1.

9 **(a)** Bacteria are members of the kingdom *Prokaryota*. Describe the main features of a bacterial cell. [8]

(b) Outline the use of bacteria in the extraction of metals from ores. [7]

[Total:15]

2.

10 **(a)** Explain how the *lac* operon is involved in the metabolism of lactose in *Escherichia coli*. [9]

(b) Describe the role of gibberellin in the germination of barley. [6]

[Total: 15]

3.

10 **(a)** Describe the action of penicillin on bacteria. [8]

(b) Outline the use of microorganisms in the extraction of heavy metals from their ores. [7]

[Total: 15]

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4.

10 (a) Describe the production of penicillin using the batch culture method. [8]

(b) Mycoprotein is produced using a continuous culture method.

Describe the advantages of the batch culture method **and** the continuous culture method. [7]

[Total: 15]

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Q1.

1 Factor VIII is a glycoprotein synthesised in liver cells. Many haemophiliacs, who are deficient in Factor VIII, are now treated by regular injections of genetically engineered Factor VIII. Fig. 1.1 shows the molecular structure of Factor VIII.

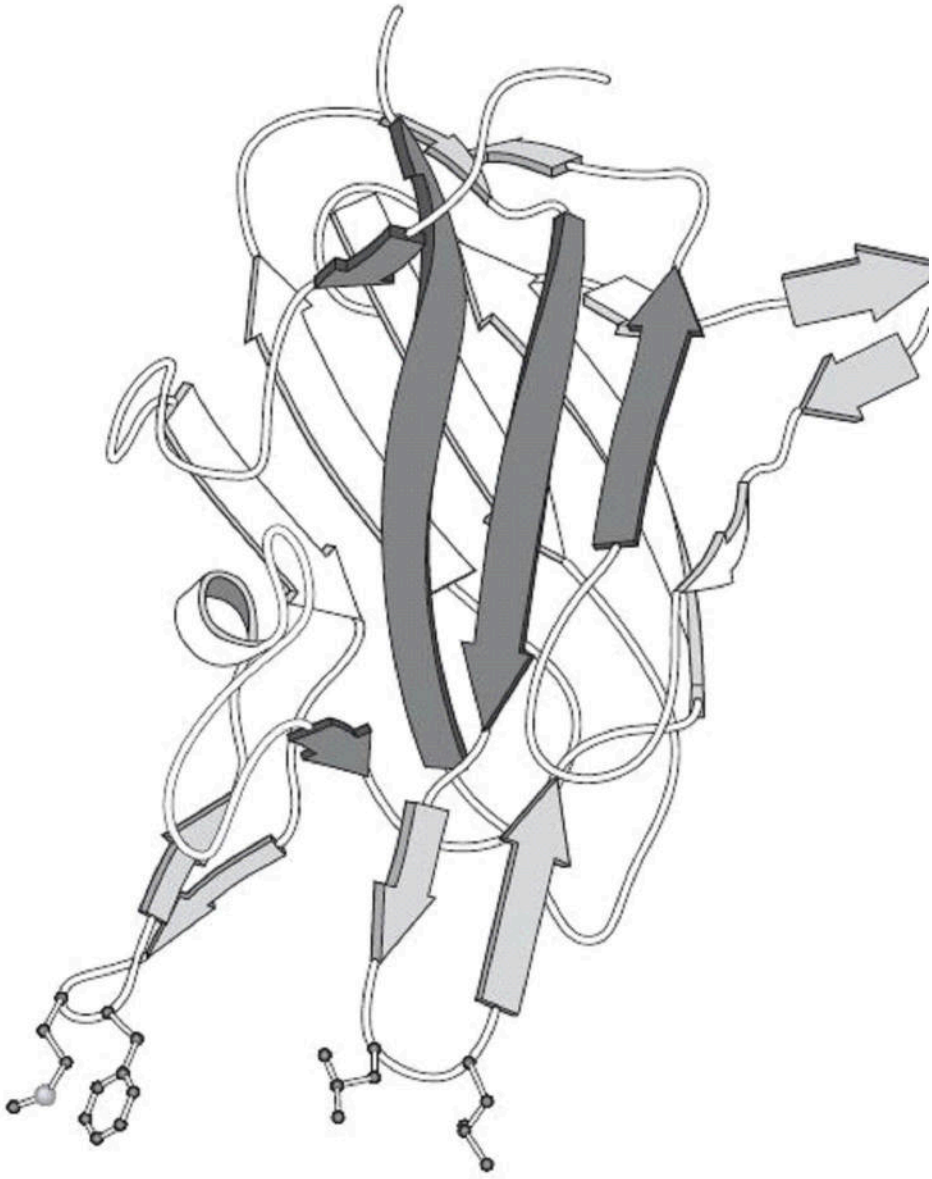


Fig. 1.1

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Use

(a) Explain how the shape of the Factor VIII protein molecule shown in Fig. 1.1 is maintained.

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.....[3]

(b) Outline how the isolated gene for human Factor VIII is obtained and inserted into a host cell.

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.....[4]

(c) State **one** advantage of using recombinant Factor VIII instead of blood derived Factor VIII.

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.....[1]

(d) Suggest why the host cell used to produce genetically engineered Factor VIII must be a mammalian cell and not a bacterial cell.

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.....[1]

[Total: 9]

Q2.

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(b) Fig. 4.1 shows some of the steps involved in the production of bacteria capable of synthesising human insulin.

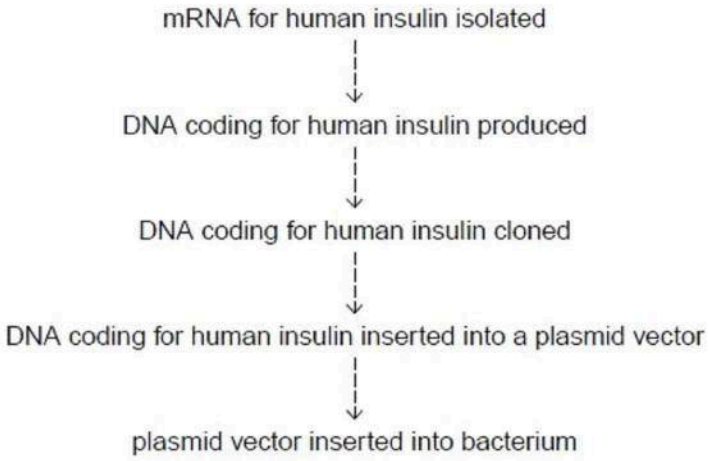


Fig. 4.1

State the role of each of the following enzymes in the production of bacteria capable of synthesising human insulin,

reverse transcriptase .....

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DNA polymerase .....

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restriction enzymes (restriction endonucleases) .....

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DNA ligase. .....

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.....[6]

Q3.

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(b) Cultivated maize is related to a wild Mexican grass, teosinte. Teosinte looks very different from maize. In particular, teosinte has a very hard layer surrounding the fruit, making it impossible to use as an edible grain.

Archaeological excavations have found that maize in its edible form dates back at least 4000 years. It has been argued that the structural differences between teosinte and maize grains are so great that it is unlikely that maize could have been bred from teosinte so long ago.

Investigations have been carried out into the genetic differences between teosinte and maize. A gene on chromosome 4, *tga 1*, containing 1042 base pairs, was found to be responsible for all of the structural differences between teosinte and maize.

Fig. 3.2 shows the DNA base sequence of the only part of *tga 1* that always differs between teosinte and maize.

teosinte	GAT	TGG	GAT	CTC	AAG	GCG	GCG	GGC	GCG	TGG
maize	GAT	TGG	GAT	CTC	AAC	GCG	GCG	GGC	GCG	TGG

Fig. 3.2

(i) Outline the principles of electrophoresis as used in sequencing this DNA.

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.....[4]

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(ii) Suggest how the difference in the base sequence of the *tga 1* gene shown in Fig. 3.2 could cause large differences in phenotype between teosinte and maize.

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.....[2]

(iii) With reference to Fig. 3.2, explain how these results support the suggestion that it would have been relatively easy for early farmers in Mexico to have bred maize from teosinte.

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.....[3]

Q4.

6 **(a)** Describe the role of insulin in the regulation of blood glucose concentration.

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..... [3]

(b) State two advantages of treating diabetes with insulin produced by gene technology.

1 .....

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2 .....

..... [2]

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(c) One of the steps in the production of bacteria capable of producing human insulin is the insertion of the gene coding for human insulin into a plasmid vector.

Fig. 6.1 shows one of the artificial plasmids constructed to act as a vector.

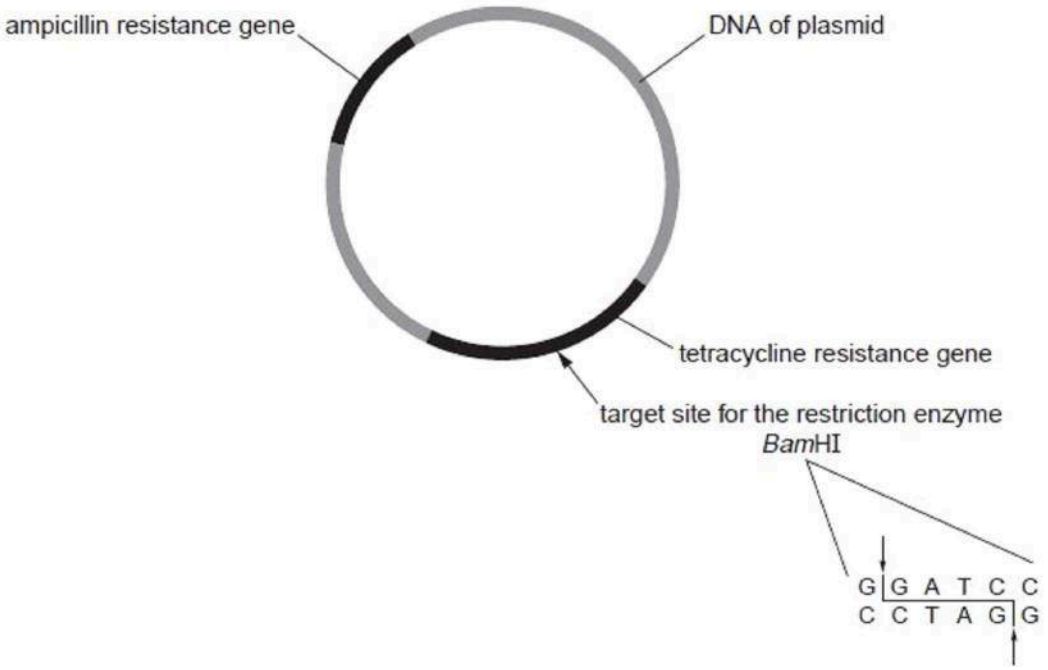


Fig. 6.1

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(i) With reference to Fig. 6.1, explain the importance of the plasmid having a single target site for a particular restriction enzyme, such as *Bam*HI.

.....

.....

.....

..... [2]

(ii) The genes for ampicillin resistance and tetracycline resistance on the plasmid allow the genetic engineer to distinguish between bacteria that have taken up different circles of DNA.

Complete the table to show whether bacteria which have taken up each different circle of DNA are, or are not resistant to ampicillin, to tetracycline or to both. Show presence of resistance with a tick (✓) and absence of resistance with a cross (✗).

circle of DNA taken up by bacteria	bacteria resistant to ampicillin	bacteria resistant to tetracycline
unaltered plasmids		
recombinant plasmids that have taken up the wanted gene		
circles of the wanted gene		

[3]

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(d) (i) Explain why genes for antibiotic resistance are now rarely used as markers in gene technology.

.....

.....

.....

.....

.....

..... [3]

(ii) Describe the use of **one** alternative marker gene that can be used instead of an antibiotic gene.

.....

.....

.....

..... [2]

[Total: 15]

Q5.

8 (a) Cystic fibrosis (CF) is an inherited disease.

For Examiner Use

(i) Explain briefly how two parents who do not have CF may have a child with CF.

.....

.....

.....

..... [2]

(ii) Describe **two** ways in which CF affects the lungs.

.....

.....

.....

..... [2]

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(b) One potential way of treating CF is by using gene therapy.

(i) Outline, with reference to CF, what is meant by *gene therapy*.

.....

.....

.....

..... [2]

(ii) Describe one possible advantage and one possible disadvantage of using gene therapy to treat CF.

advantage .....

.....

disadvantage .....

..... [2]

[Total: 8]

Q6.

8 Gene technology has many uses including the production of substances such as insulin.

(a) (i) Outline what is meant by *gene technology*.

.....

.....

.....

..... [2]

(ii) Explain why genes for enzymes that produce fluorescent substances are used as makers in gene technology.

.....

.....

.....

..... [2]

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(b) There is much controversy throughout the world regarding the use of genetically modified (GM) crops.

(i) Suggest **two** advantages of growing GM rice with an enhanced vitamin A content.

.....

.....

.....

..... [2]

(ii) Suggest **two** disadvantages of growing GM crops.

.....

.....

.....

..... [2]

[Total: 8]

Q7.

3 Variable number tandem repeats (VNTRs) are repetitive, non-coding sections of DNA. A particular VNTR is located at the same locus in different individuals, but the number of repeats in that VNTR varies between individuals.

(a) Explain how, in the process of genetic fingerprinting, gel electrophoresis is able to distinguish between the VNTRs that occur at the same loci of different individuals.

.....

.....

.....

.....

.....

.....[3]

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(b) Gel electrophoresis is also used in genetic screening.

The mutation of the β-globin gene which gives rise to sickle cell anaemia removes a recognition site of a restriction enzyme, **R**, as shown in Fig. 3.1. **R** cuts DNA at the sites indicated by arrows (↓). The lengths of the resulting fragments are shown in kilobases (kb).

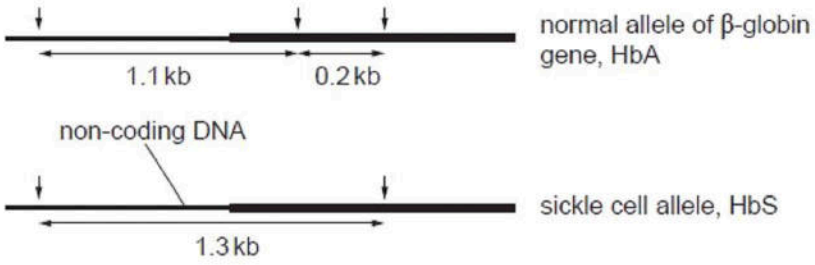


Fig. 3.1

Fig. 3.2 shows an electrophoresis gel with a stained band of DNA from an individual who was homozygous for the normal allele for β-globin, HbA HbA. This band is the 1.1 kb fragment shown in Fig. 3.1. The 0.2 kb fragment is **not** shown.

Complete Fig. 3.2 by drawing the stained DNA that would result from an individual who is heterozygous for the sickle cell allele, HbA HbS.

Put your answer on to Fig. 3.2. [2]

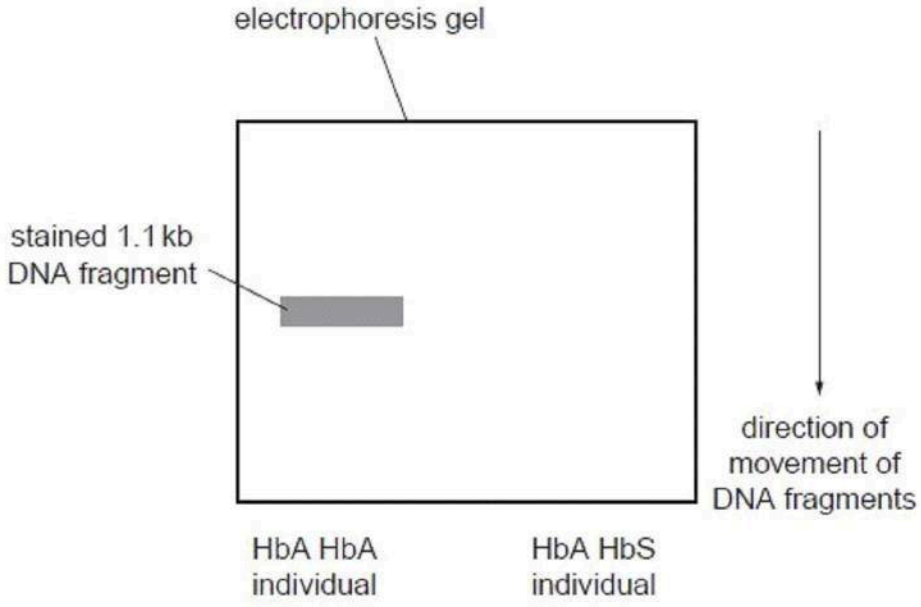


Fig. 3.2

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(c) Describe the different circumstances in which this genetic screening for the sickle cell allele, HbS, might be used.

.....

.....

.....

.....

.....

.....[3]

[Total: 8]

Q8.

3 In order to sequence the DNA of a gene, it is first denatured to separate its two strands.

Then, in the presence of a large supply of each of the four nucleotides, the single-stranded DNA is replicated by DNA polymerase.

(a) Explain what determines the sequence of nucleotides in the newly replicated strand of DNA.

.....

.....

.....

.....[2]

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(b) A low concentration of specially prepared nucleotides is also present. Once added to the chain, these nucleotides do **not** allow the chain to continue growing.

Each special nucleotide is labelled with a fluorescent dye, using a different colour for each of the four bases.

Fig. 3.1 shows a replicated DNA chain ending with one of the special nucleotides.

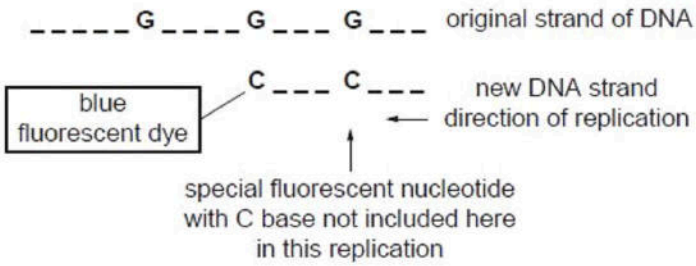


Fig. 3.1

With reference to Fig. 3.1 and to the information given, suggest why a special nucleotide with a C base was **not** included by DNA polymerase at the first site requiring a C nucleotide.

.....

.....

.....

.....[2]

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(c) This method of sequencing a gene produces as many DNA fragments as there are nucleotides in the gene, each fragment differing in length by one nucleotide.

Fo
Exami
Us

Fig. 3.2 shows part of a set of such fragments.

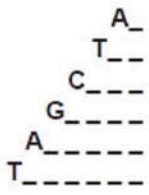


Fig. 3.2

These fragments are loaded onto a sequencing gel, shown in Fig. 3.3, and separated by electrophoresis.

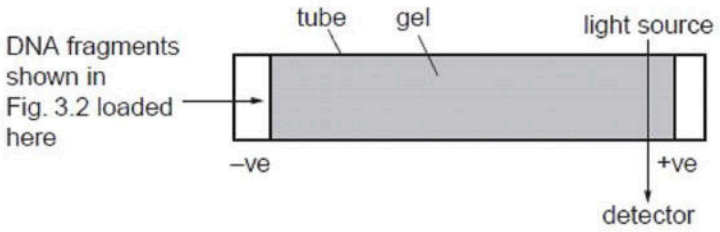


Fig. 3.3

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(i) In what order will the fragments shown in Fig. 3.2 reach the light source and detector shown in Fig. 3.3?

.....

.....[1]

(ii) Explain how gel electrophoresis separates these fragments of DNA.

.....

.....

.....

.....

.....

.....

.....

.....[3]

[Total: 8]

Q9.

4 Golden Rice™ is a genetically modified form of rice that produces relatively large amounts of β carotene in the endosperm. β carotene is metabolised in the human body to produce vitamin A.

(a) Explain why rice has been genetically modified to produce extra β carotene.

.....

.....

.....

.....[2]

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(b) The first types of Golden Rice™ produced only a very low mass of β carotene per gram of rice. Research continued to try to increase this.

Fig. 4.1 shows the metabolic pathway by which β carotene is synthesised in plants, and the enzymes that catalyse each step of the pathway.

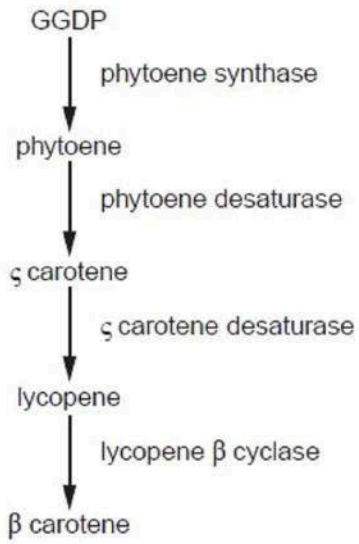


Fig. 4.1

The first types of Golden Rice™ contained a phytoene synthase gene, *psy*, from daffodils and a gene *crtI*, which produced the two desaturase enzymes, from the bacterium *Erwinia uredovora*.

Measurements of the quantities of intermediates in this metabolic pathway in rice endosperm showed that there was always a large amount of GGDP present, and that no phytoene accumulated in the tissues.

Explain how this suggests it was **not** the enzymes produced by the *crtI* gene that were limiting the production of β carotene.

.....

.....

.....

.....[2]

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(c) Investigations were carried out to see if *psy* genes taken from species other than daffodils would enable rice endosperm to produce greater quantities of β carotene than the first types of Golden Rice™.

- *Psy* genes were isolated from the DNA of maize, tomatoes, peppers and daffodils. The genes were inserted into different plasmids.
- The promoter *Ubi*1, and *crtI* genes from *E. uredovora*, were also inserted into all of the plasmids.
- The four types of genetically modified plasmids were then inserted into different cultures of rice cells.
- The quantity of β carotene produced by these rice cells was measured.

The results are shown in Table 4.1.

Table 4.1

source of *psy* gene	total β carotene content of rice cells / arbitrary units
maize	14
pepper	4
tomato	6
daffodil	1

(i) Name the type of enzyme that would have been used to cut the *psy* gene out of the DNA of the plant cells.

.....[1]

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(ii) Explain why a promoter was inserted into the plasmids.

.....

.....

.....

.....[2]

(iii) Explain whether or not these results support the hypothesis that the *psy* gene, not the *crtI* gene, was limiting the production of β carotene in genetically modified rice.

.....

.....

.....

.....[2]

(d) The original choice of a *psy* gene from daffodils was made because daffodils produce large amounts of β carotene in their yellow petals, and because they are monocotyledonous plants, like rice.

Suggest explanations for the much lower production of β carotene in rice containing the *psy* gene from daffodils than in rice containing the *psy* gene from maize.

.....

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.....[2]

(e) Describe the possible disadvantages of growing Golden Rice™.

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.....

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.....

.....[3]

[Total: 14]

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Q10.

2 A number of diseases, such as dengue fever, are spread by mosquitoes. The incidence of this disease has increased dramatically in recent years and this has been linked with the spread of the mosquito, *Aedes aegypti*.

In an attempt to reduce the numbers of *A. aegypti*, genetically modified (GM) male mosquitoes were produced. One of the genes added to these mosquitoes, **when switched on**, results in the production of a protein which is toxic to mosquitoes.

In 2010, in the Cayman Islands and in Malaysia, GM male mosquitoes were released into the wild to mate with females. All the resulting offspring died in the larval stage.

(a) About 3 million GM male mosquitoes were released in the Cayman Islands.

Suggest why releasing such large numbers of male mosquitoes did not immediately increase the risk of transmission of dengue fever.

.....

..... [1]

(b) In Malaysia, both GM male and non-GM male mosquitoes were released in order to compare their dispersal and life span in the wild. The GM mosquitoes could be identified because they also carried a gene for green fluorescent protein (GFP).

Explain why, in many examples of gene technology, fluorescent markers are used in preference to antibiotic resistance genes.

.....

.....

.....

..... [2]

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(c) In addition to the gene for GFP, the DNA that has been added to the GM mosquitoes consists of

- a promoter
- a gene coding for a toxic protein, tTA
- a binding site for tTA.

When a GM mosquito larva hatches from an egg, the promoter induces the production of only a small amount of tTA, so that the larva does not die immediately. In a process of positive feedback, the tTA produced binds to the DNA as shown in Fig. 2.1. This increases the expression of the gene until the increased concentration of tTA kills the larva.

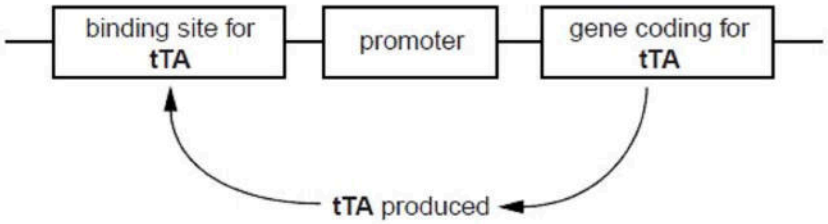


Fig. 2.1

(i) Suggest why this process is called *positive feedback*.

.....

..... [1]

(ii) Explain why, in gene technology, a promoter needs to be transferred along with the desired gene.

.....

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.....

.....

..... [3]

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(iii) Switching on the gene coding for tTA in the mosquito larvae, rather than in the eggs, increases the effectiveness of this method of controlling mosquito numbers.

Suggest why this is so.

.....

.....

.....

..... [2]

(d) GM mosquitoes carrying the tTA gene can live and reproduce normally when fed on a diet containing an added chemical, **A**.

With reference to Fig. 2.1:

(i) suggest how **A** could prevent death of the GM mosquitoes

.....

.....

.....

..... [2]

(ii) suggest how large numbers of adult GM male mosquitoes can be produced for release into the wild, from an original stock of GM males

.....

.....

.....

..... [2]

(iii) suggest why there is little danger of the gene carried by these GM mosquitoes being passed to other organisms from GM mosquitoes which escape or are released into the wild.

.....

.....

.....

..... [2]

[Total: 15]

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Q11.

2 The pink bollworm moth, *Pectinophora gossypiella*, is a pest of cotton crops. The size of its population can be reduced by releasing large numbers of sterile male moths into cotton fields. The sterile male moths mate with wild females from the cotton fields, but no offspring are produced.

Over a period of three years, 20 million genetically modified (GM) sterile male moths were released in the USA. Each insect contained a gene coding for a red fluorescent protein (DsRed) taken from a species of reef coral. The added DNA also included a promoter.

(a) Explain why, in gene technology:

(i) genes for fluorescent proteins such as DsRed are now more commonly used as markers than are genes for antibiotic resistance

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.....

.....

..... [2]

(ii) a promoter needs to be included when transferring a gene from a coral into an insect.

.....

.....

.....

.....

.....

.....

.....

..... [3]

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(b) DsRed is visible at all stages of the life cycle of the moth, but the presence of the gene in a particular individual can be confirmed by genetic fingerprinting, using gel electrophoresis.

For Examiner Use

(i) Outline the principles of gel electrophoresis.

.....

.....

.....

.....

.....

.....

.....

..... [4]

(ii) Explain how the presence of the gene for DsRed in a moth can be confirmed once electrophoresis is complete.

.....

.....

.....

..... [2]

(c) DsRed allows sterile male moths to be distinguished from wild moths when caught in an insect trap in a field of cotton plants.

Suggest why it is important to be sure whether a moth caught in such a trap is a released sterile male or a wild insect.

.....

.....

.....

..... [2]

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(d) The United States Department of Agriculture has ruled that the release of sterile males to control insect pest numbers is environmentally preferable to all other alternatives.

Suggest what information would be needed to determine whether the release of the sterile male moths, carrying the gene for DsRed, has a damaging effect on the environment.

.....

.....

.....

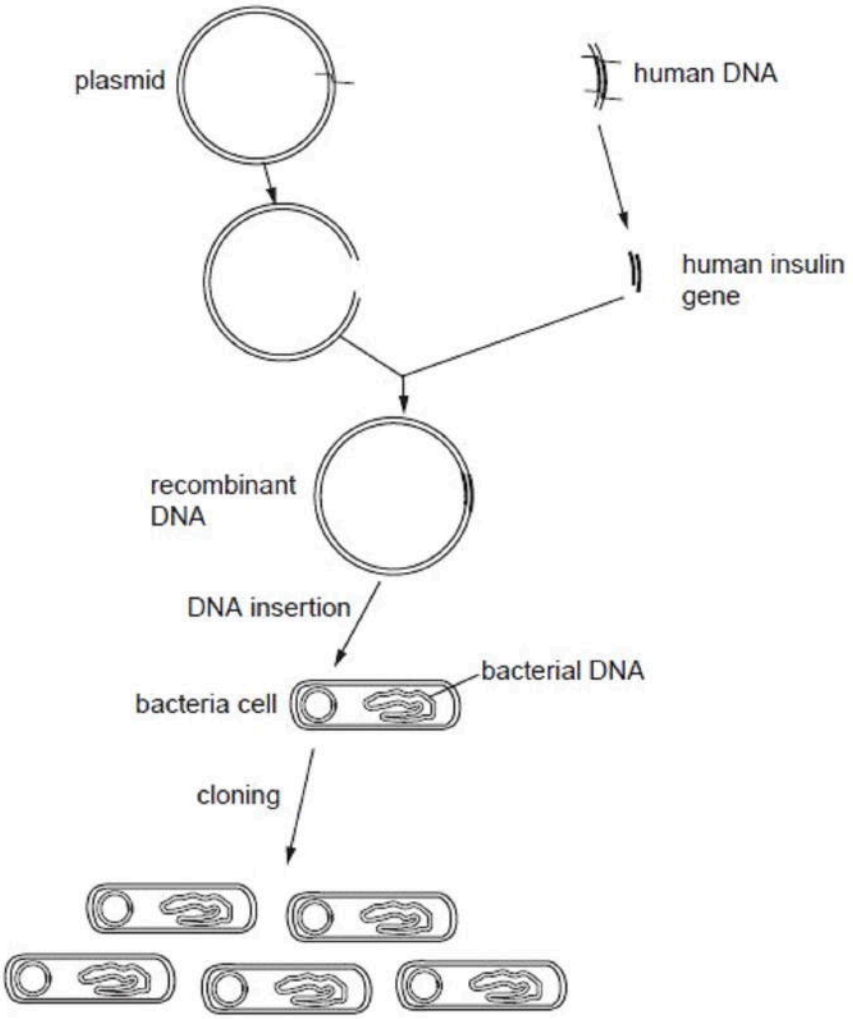
..... [2]

[Total: 15]

Q12.

5 Fig. 5.1 outlines the way in which the gene for human insulin is incorporated into plasmid DNA and inserted into a bacterium.

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plasmid
human DNA
human insulin gene
recombinant DNA
DNA insertion
bacterial DNA
bacteria cell
cloning

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(a) Describe how the plasmid DNA is cut.

.....

.....

.....

.....[3]

(b) Explain how the human insulin gene is joined to the plasmid DNA.

.....

.....

.....

.....[3]

(c) List **two** advantages of treating diabetics with human insulin produced by genetic engineering.

1. .....

.....

2. .....

.....[2]

[Total : 8]

Q13.

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2 Fig. 2.1 shows the CFTR (cystic fibrosis transmembrane conductance regulator) protein in a plasma (cell surface) membrane.

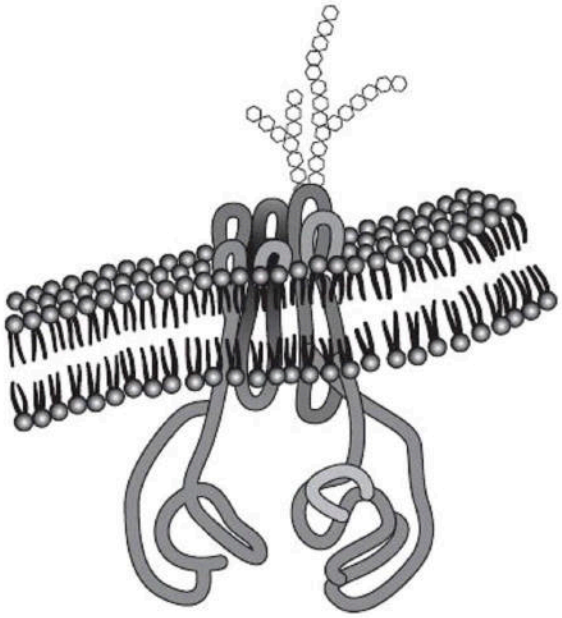


Fig. 2.1

(a) (i) Describe the normal function of the CFTR protein.

.....

.....

.....[2]

(ii) On Fig. 2.1, use the letter **E** to indicate the external face of the membrane.
State how you identified this face.

.....

.....[1]

(b) Cystic fibrosis is caused by a recessive allele of the *CFTR* gene.

(i) Explain the meaning of the term *recessive allele*.

.....

.....

.....[2]

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(ii) Explain how cystic fibrosis affects the function of the lungs.

.....

.....

.....

.....

.....[3]

(c) As cystic fibrosis is caused by a recessive allele of a single gene, it is a good candidate for gene therapy. Trials were undertaken in the 1990s, attempting to deliver the normal allele of the *CFTR* gene into cells of the respiratory tract, using viruses or liposomes as vectors.

Explain how viruses deliver the allele into cells.

.....

.....

.....[2]

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(d) In some people with cystic fibrosis, the allele has a single-base mutation which produces a 'nonsense' (stop) codon within the gene.

(i) Explain how this mutation would prevent normal CFTR protein being produced.

.....

.....

.....[2]

(ii) A new type of drug, PTC124, enables translation to continue through the nonsense codon. Trials in mice homozygous for a *CFTR* allele containing the nonsense codon have found that animals treated with PTC124 produce normal CFTR protein in their cells. The drug is taken orally, and is readily taken up into cells all over the body.

Using your knowledge of the progress towards successful gene therapy for cystic fibrosis, suggest why PTC124 could be a simpler and more reliable treatment for this disease.

.....

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.....

.....

.....[3]

[Total: 15]

Q14.

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6 **(a)** A husband and wife who already have a child with cystic fibrosis (CF) elected to have their second child tested for the condition while still a fetus in very early pregnancy. The results of the test, a DNA banding pattern, were discussed with a genetic counsellor.

The relevant DNA banding pattern produced by electrophoresis is shown in Fig. 6.1.

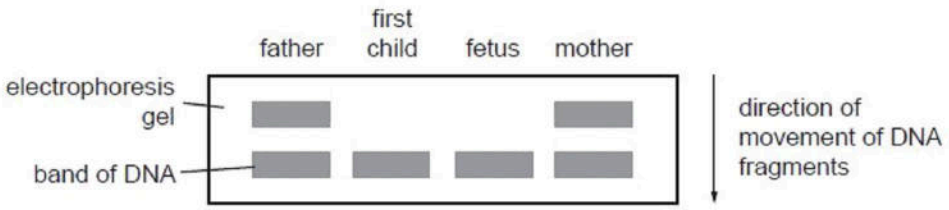


Fig. 6.1

With reference to Fig. 6.1, explain why,

(i) the fetus will develop CF,

.....

.....[1]

(ii) the positions of the bands of DNA of the first child and of the fetus indicate that the mutant allele for CF has a deletion in comparison with the normal allele.

.....

.....

.....

.....[2]

(b) Explain briefly the need to discuss the result of the test with a genetic counsellor.

.....

.....

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.....

.....

.....

.....

.....[4]

[Total: 7]

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Q15.

4 The secretion of insulin by the islets of Langerhans in the pancreas stimulates the liver to reduce the blood glucose concentration.

(a) Describe how the **liver** reduces blood glucose concentration, when insulin is secreted.

.....

.....

.....

.....

.....

..... [3]

(b) Almost all insulin used to treat type I diabetes is produced by genetically engineered bacteria or yeast. A summary of this procedure is shown in Fig. 4.1.

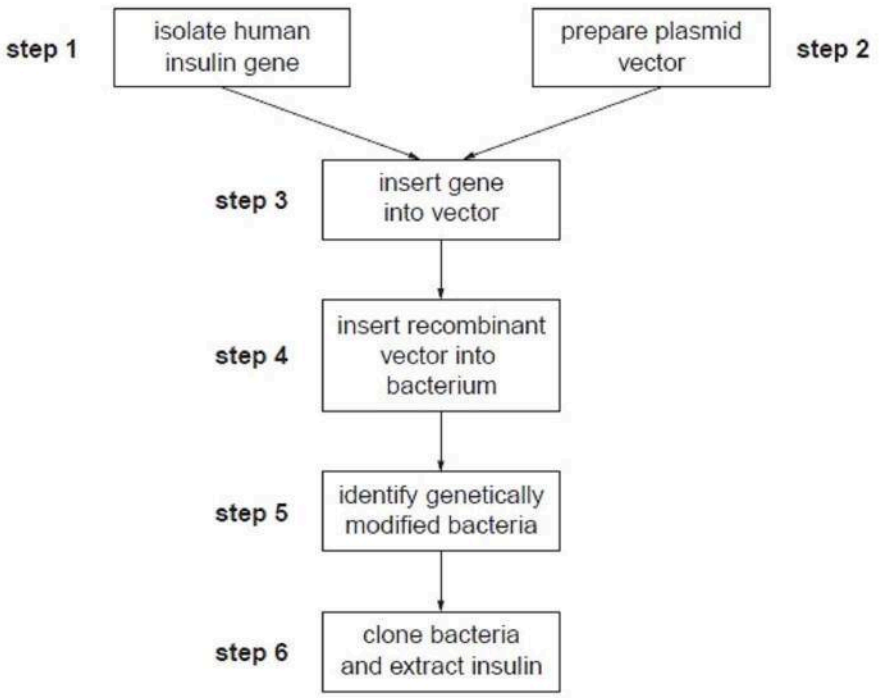


Fig. 4.1

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(i) One way of carrying out **step 1** is to collect mRNA from β cells from the pancreas. The relevant mRNA is then isolated and used to make DNA.

Suggest why isolating the mRNA coding for insulin in a β cell is easier than isolating the DNA for insulin in a β cell.

.....

.....

.....

..... [2]

(ii) Outline the use of restriction enzymes in **step 2**.

.....

.....

.....

..... [2]

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(c) Most people with type I diabetes inject insulin. A recent product contains insulin that can be administered using a nasal spray. The spray is inhaled and the insulin is taken up through the lungs.

Fig. 4.2 shows the concentration of insulin in the blood plasma in the 480 minutes after injecting or inhaling insulin. In both cases, the insulin was of the same type, obtained from genetically engineered *Escherichia coli*.

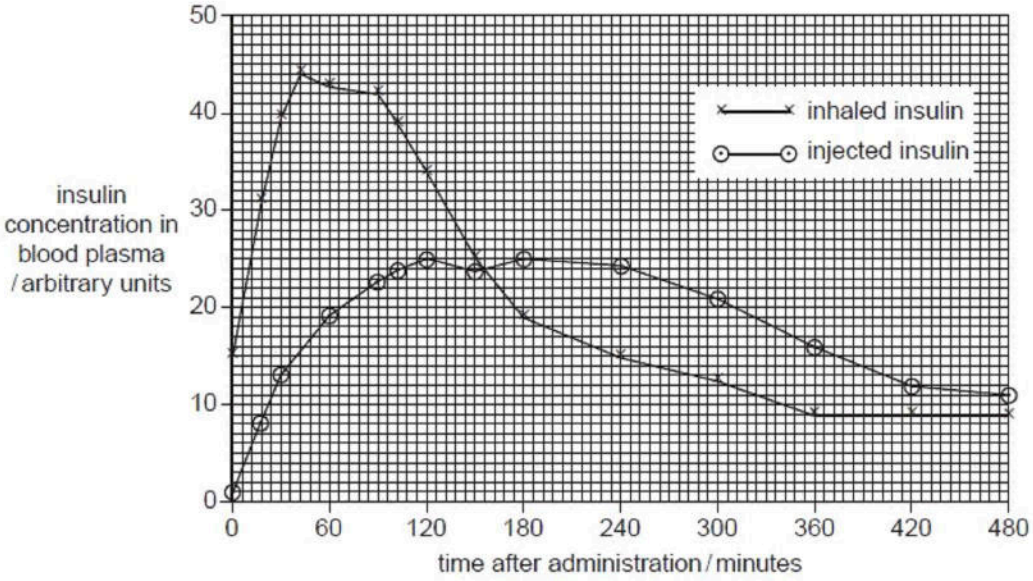


Fig. 4.2

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Fig. 4.3 shows the concentration of glucose in the blood plasma in the 480 minutes after injecting or inhaling insulin.

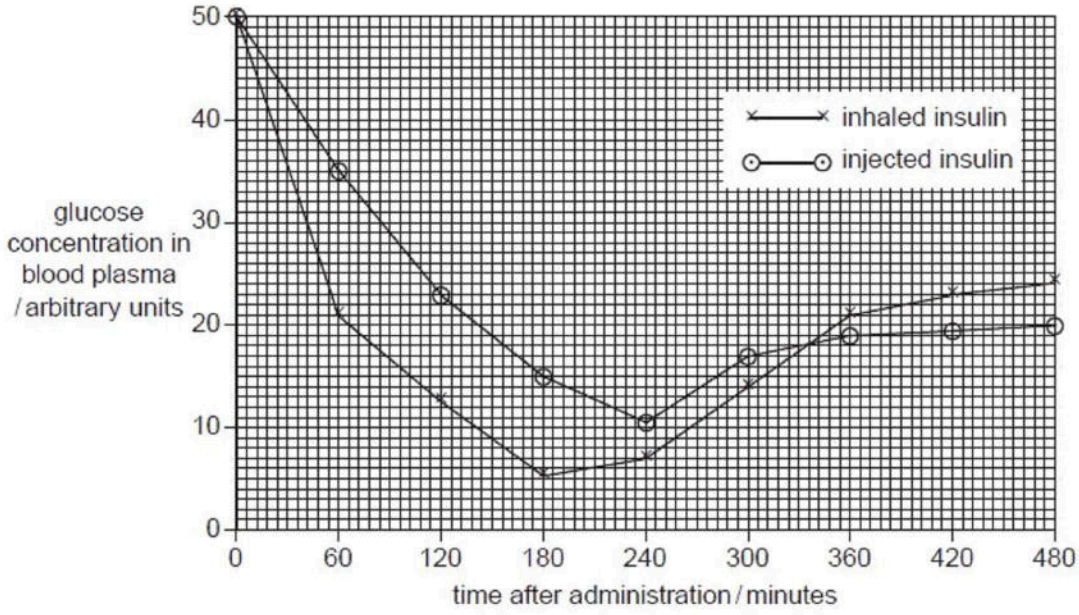


Fig. 4.3

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(i) Compare the results for injected insulin and inhaled insulin shown in Fig. 4.2.

Ex

.....

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..... [3]

(ii) With reference to Fig. 4.2, explain the differences in the blood glucose levels after injecting or inhaling insulin shown in Fig. 4.3.

.....

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..... [3]

(iii) With reference to Figs. 4.2 and 4.3, suggest one advantage and one disadvantage of inhaling insulin rather than injecting it.

advantage .....

.....

disadvantage .....

..... [2]

[Total: 15]

Q16.

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5 Many attempts have been made to find methods of using gene therapy to treat cystic fibrosis. One approach uses viruses to deliver normal alleles of the CFTR gene into epithelial cells of the airways. Viral delivery systems have two main problems:

- The virus may trigger an immune response which destroys the infected cells.
- Most non-pathogenic viruses are not very good at getting into cells, so very few cells receive the allele.

A team of researchers in the USA developed a new strain (AAV2.5T) of AAV, a non-pathogenic virus. AAV2.5T has an improved ability to bind with epithelial cells of the airways. Genes for the CFTR protein and for an enzyme, luciferase, were added to the DNA of the viruses. Luciferase produces a fluorescent green protein when luciferin is added.

The normal AAV strain and the AAV2.5T strain were added to cultures of epithelial cells from the airways. After adding luciferin, the numbers of cells that had taken up the viral genes was estimated using the intensity of the green fluorescence which developed.

The results are shown in Fig. 5.1.

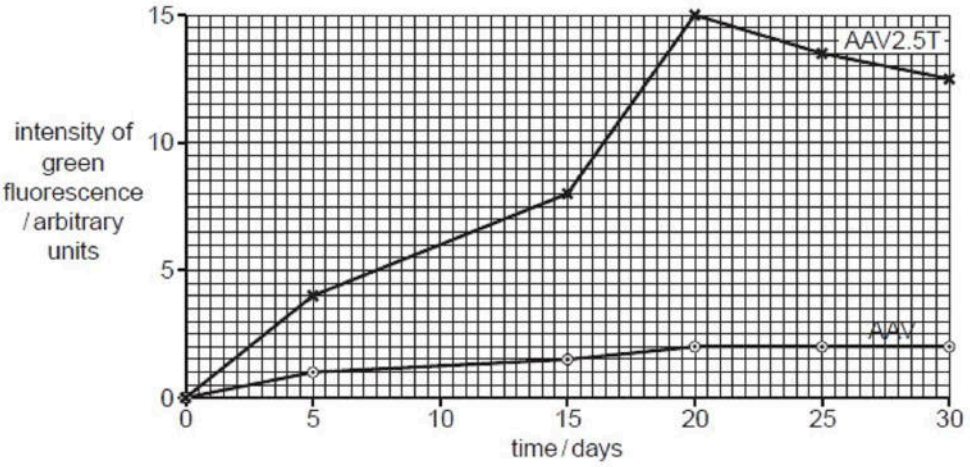


Fig. 5.1

(a) With reference to Fig. 5.1, compare the ability of the two viral strains, AAV and AAV2.5T, to infect epithelial cells from the airways.

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.....

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..... [2]

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(b) Explain why the researchers added a gene for luciferase to the viral DNA.

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.....

..... [2]

(c) Suggest how delivering normal alleles of the CFTR gene into epithelial cells in the airways could relieve the symptoms of cystic fibrosis.

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..... [4]

[Total: 8]

Q17.

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5 In humans, the gene *RPE65* encodes a protein responsible for regenerating visual pigment in rod and cone cells after they have been exposed to light. A recessive allele of this gene causes impaired vision from birth, progressing to complete blindness in early adulthood. This condition is called LCA.

In 2008, trials were carried out into the possibility and safety of treating LCA using gene therapy.

(a) Suggest and explain why LCA is suitable for treatment using gene therapy.

.....

.....

.....

.....

.....

.....[3]

(b) Six adults with this condition were used in the study. Genetically modified adenoviruses (a type of virus that can cause respiratory infections) were used as vectors. The vectors were injected beneath the retina of one eye of each of the participants.

Suggest two ways in which the genome of the adenoviruses used as vectors would differ from that of normal adenoviruses.

1. .....

.....

2. .....

.....[2]

(c) Improvements were found in the vision of all the participants, but the small number in the trials made most of these improvements not statistically significant.

Suggest why these trials were designed to include such a small number of participants.

.....

.....

.....

.....[2]

[Total: 7]

Q18.

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7 Insulin can be produced on a large scale using gene technology and prokaryotes such as *Escherichia coli*.

Table 7.1 summarises the sequence of steps in one method of production of insulin by *E. coli*.

Complete Table 7.1 by adding one statement in each of the empty boxes.

Table 7.1

step	reason for step
obtain copies of gene with sticky ends	the gene codes for the synthesis of insulin
	acts as a vector for the transfer of the gene into the host
use restriction endonuclease enzyme	
mix vector and gene	
	to seal the sugar-phosphate backbone
	to obtain transformed host *E. coli* cells
screen for, and obtain, successfully transformed cells	
	to obtain large amounts of insulin for extraction and purification

[7]

Q19.

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5 **(a)** The steps involved in a method of production of human insulin by gene technology are listed in Table 5.1.
The steps are **not** listed in the correct order.

Exa

Table 5.1

step	description
A	DNA coding for human insulin inserted into cut plasmid vector
B	genetically modified bacteria identified
C	mRNA for human insulin isolated in β cells
D	plasmid vector inserted into bacterium
E	genetically modified bacteria cloned
F	DNA for human insulin cloned
G	human insulin harvested
H	cDNA coding for human insulin synthesised

(i) Complete Table 5.2 to show the steps in the correct order.

Two of the steps have been done for you.

Table 5.2

correct order	letter of step
1	C
2	
3	
4	
5	D
6	
7	
8	

[4]

(ii) Name the enzymes responsible for the following steps:

step A .....

step H ..... [2]

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(b) Explain **two** advantages of treating diabetes with human insulin produced by gene technology rather than using insulin from animals.

E

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.....[2]

[Total: 8]

Q20.

3 Green fluorescent protein (GFP) is a small protein that emits bright green fluorescence in blue light. It was first isolated from the jellyfish, *Aequorea victoria*.

E

The gene coding for GFP can be expressed in bacteria, such as *Escherichia coli*, and so it is often used as a marker to show successful uptake of a gene by the bacterium.

(a) (i) Outline how a gene from another species can be inserted into *E. coli*.

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(ii) Explain how a marker gene, such as the gene for GFP, is used to show successful uptake of a gene for a wanted protein.

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.....[3]

(b) Genes for enzymes that produce fluorescent substances are often used as markers in gene technology.

GFP is **not** an enzyme.

Suggest **one** disadvantage of using the gene for GFP to produce easily detectable fluorescence, rather than using a gene for an enzyme that produces a fluorescent substance.

Explain your answer.

disadvantage .....

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explanation .....

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[Total: 8]

Q21.

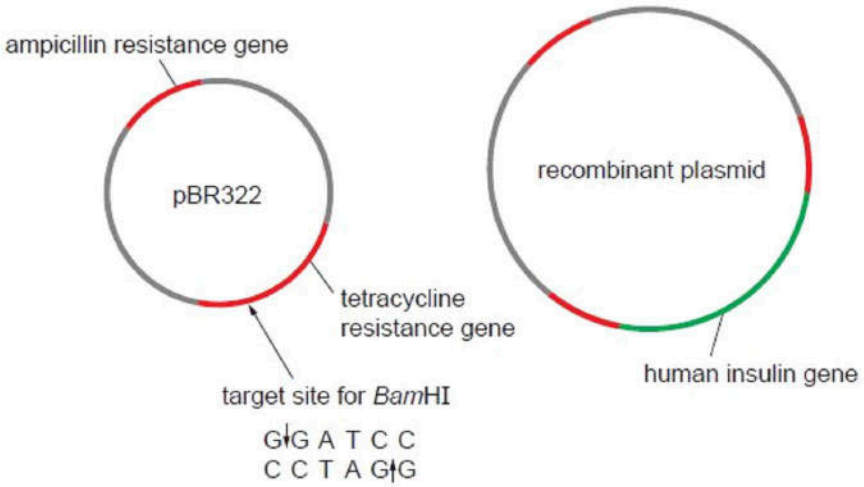
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2 The artificial plasmid, pBR322, was constructed to act as a vector. It has often been used to insert human genes, such as the human insulin gene, into the bacterium, *Escherichia coli*.

The plasmid was constructed to include two genes, each giving resistance to a different antibiotic: an ampicillin resistance gene and a tetracycline resistance gene. The plasmid also has a target site for the restriction enzyme, *Bam*HI, in the middle of the tetracycline resistance gene.

A pBR322 plasmid was cut using *Bam*HI and the cDNA gene for human insulin inserted into it.

Fig. 2.1 shows pBR322 and the recombinant plasmid.



(a) With reference to Fig. 2.1, describe how a cDNA human insulin gene can be inserted into pBR322 that has been cut by *Bam*HI.

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..... [4]

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(b) Bacteria were then mixed with the recombinant plasmids. Those bacteria which had successfully taken up recombinant plasmids were identified using the following steps:

step 1 – the bacteria were spread onto culture plates containing nutrient agar and ampicillin and incubated to allow colonies to form

step 2 – some bacteria from each of the colonies growing on these plates were transferred to plates containing nutrient agar and tetracycline, as shown in Fig. 2.2.

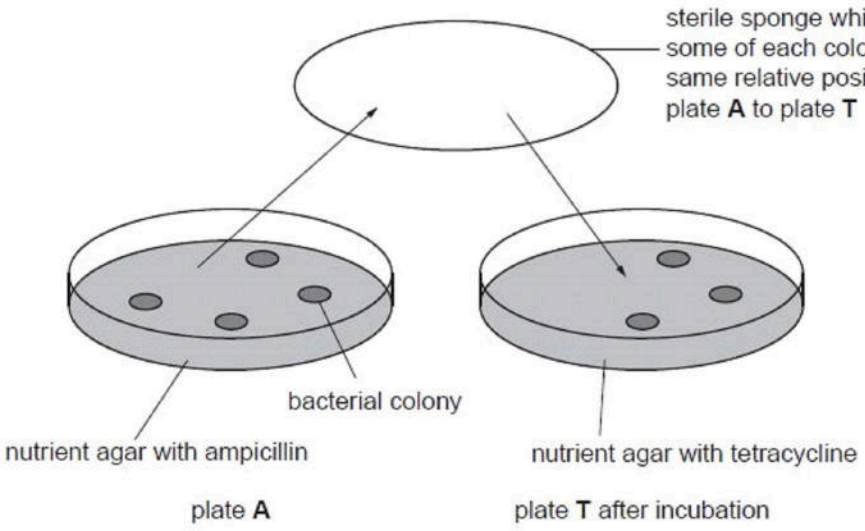


Fig. 2.2

(i) Explain why the bacteria were first spread onto plates containing ampicillin.

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(ii) Explain why it is important, for identifying bacteria that have successfully taken up the recombinant plasmid, that on pBR322 the target site for *Bam*HI is in the middle of the tetracycline resistance gene.

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(iii) Use a label line and the letter **C** to identify, on Fig. 2.2, a colony of bacteria that contain the recombinant plasmid.

Put your answer onto Fig. 2.2 on page 5. [1]

(c) Plasmid vectors carrying antibiotic resistance genes are now rarely used in gene technology.

(i) Explain why antibiotic resistance genes are now rarely used.

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.....[2]

(ii) State one type of gene that has replaced antibiotic resistance genes in plasmid vectors and indicate how its presence can be detected.

type of gene .....

.....

detection.....

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.....[2]

[Total: 15]

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Q22.

2 Russian scientists have discovered the fruits of a flowering plant, *Silene stenophylla*, in the food store in a burrow of a ground squirrel in frozen sediments in Siberia.

Dating techniques suggest that the fruits were stored by the ground squirrel about 32 000 years ago, shortly before the ground became permanently frozen.

Tissue samples were taken from the fruits and grown in a nutrient culture medium. After treatment with plant hormones to stimulate the growth of roots and shoots, 36 complete plants were produced.

These 'regenerated' plants, which looked identical to one another, flowered and after cross-pollination, produced seeds that were able to germinate.

(a) Explain why cross-pollination produces more genetic variation among the offspring than self-pollination.

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.....[3]

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(b) The flowers of modern-day *S. stenophylla* look similar, but not identical, to the flowers of the 'regenerated' plants.

Outline how DNA sequencing could be used to compare the DNA of modern-day and 'regenerated' *S. stenophylla*.

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(c) Suggest a simple experiment, using plants of modern-day and 'regenerated' *S. stenophylla* to find out whether, after 32 000 years, they are still the same species.

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.....[2]

[Total: 9]

Q23.

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3 A group of membrane proteins which transport sugars out of cells have been identified and called SWEETs. They are found in the cell surface membranes of both animal and plant cells, including mammalian liver cells and rice mesophyll cells.

Each SWEET is a protein with seven coiled regions which together make a pore through a membrane bilayer as shown in Fig. 3.1.

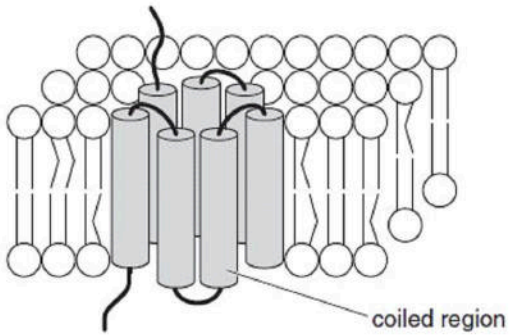


Fig. 3.1

(a) (i) Explain why, to enter or leave a cell, sugars need molecules such as SWEETS.

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.....[2]

(ii) Suggest how a SWEET is held within the membrane bilayer.

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(b) The bacterium, *Xanthomonas oryzae* (Xoo), causes the disease known as bacterial leaf blight in rice plants. It infects the intercellular spaces of the leaves of the host plant. Then, by switching on *SWEET* genes in the mesophyll cells, it stimulates the secretion of glucose into the intercellular spaces.

Several different **recessive** alleles have been found, in rice plants from different countries, which give resistance to bacterial leaf blight. All these alleles have a mutation in the promoter of the *SWEET* gene.

The effect of Xoo on wild type and resistant rice plants is compared in Fig. 3.2.

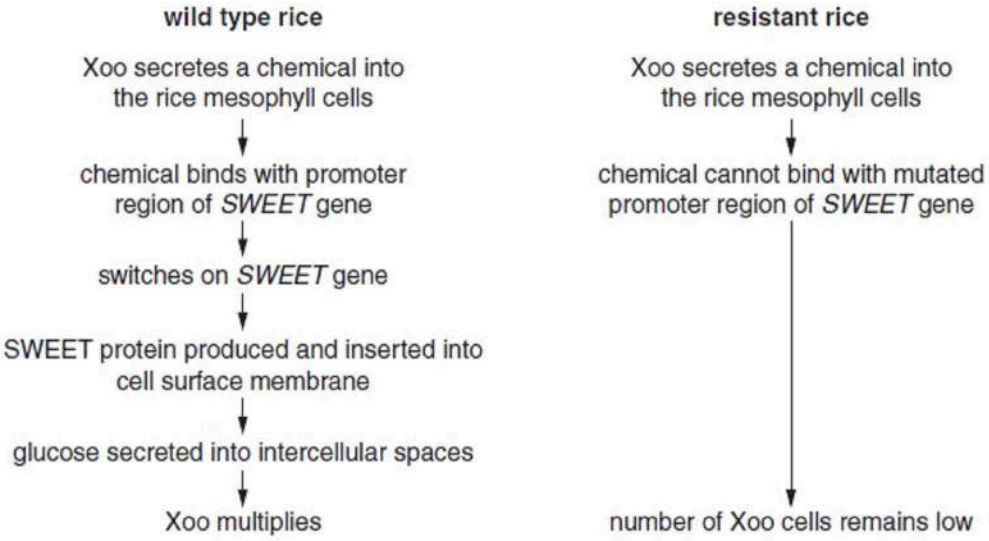


Fig. 3.2

(i) Using the information in Fig. 3.2, explain this resistance of rice plants to Xoo.

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(ii) Explain why it would be difficult to transfer this resistance into susceptible rice plants by genetic engineering.

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.....[2]

(iii) Explain why the presence of large numbers of Xoo in the intercellular air spaces of rice plants affects the ability of the plants to grow with their roots submerged in water.

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(ii) Explain why it would be difficult to transfer this resistance into susceptible rice plants by genetic engineering.

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(iii) Explain why the presence of large numbers of Xoo in the intercellular air spaces of rice plants affects the ability of the plants to grow with their roots submerged in water.

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.....[4]

[Total: 14]

Q24.

3 Human insulin can be synthesised in a laboratory strain of *Escherichia coli* using recombinant DNA (rDNA) technology.

The starting point for the process is mRNA coding for insulin, isolated from human pancreas cells.

Four enzymes are needed:

- reverse transcriptase
- DNA polymerase
- restriction enzyme
- DNA ligase.

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(a) (i) State the role of each of these enzymes in producing rDNA carrying the gene for human insulin.

reverse transcriptase

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DNA polymerase

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restriction enzyme

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DNA ligase

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.....[4]

(ii) Outline the role of insulin in a healthy human.

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.....[3]

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(iii) Describe and explain **one** advantage of treating diabetics with human insulin produced by rDNA technology.

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.....[2]

(b) It is possible to use rDNA technology to produce insulin with a slightly different structure from that of human insulin. The effect of the changed structure can then be investigated.

The activities of equal quantities of two insulins, both produced by *E. coli*, were compared in healthy, non-diabetic subjects:

- human insulin
- insulin **X**, in which the positions of two amino acids, lysine and proline, were exchanged. Lysine has a hydrophilic R group and proline has a hydrophobic R group.

The results of the investigation are shown in Fig. 3.1.

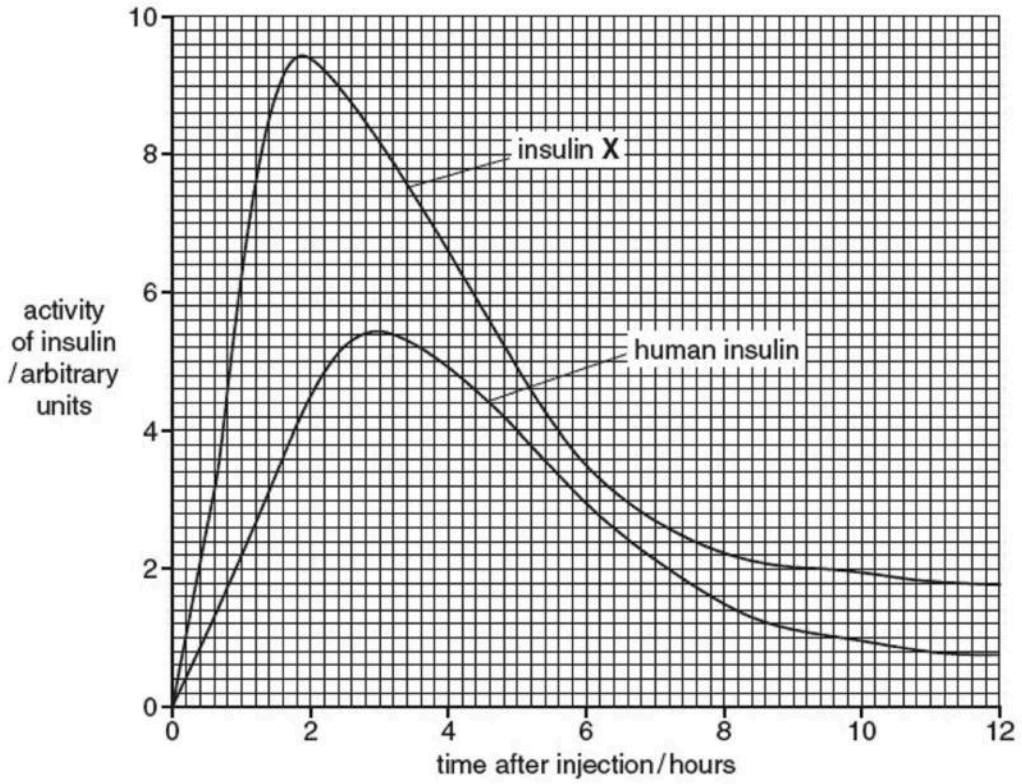


Fig. 3.1

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(i) With reference to Fig. 3.1 describe the differences in activity between human insulin and insulin **X**.

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.....[4]

(ii) Suggest how exchanging the position of two amino acids in the insulin molecule can result in differences in activity.

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.....[2]

[Total: 15]

Q25.

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3 (a) The diagram outlines how a gene coding for human insulin is produced by genetic engineering techniques.

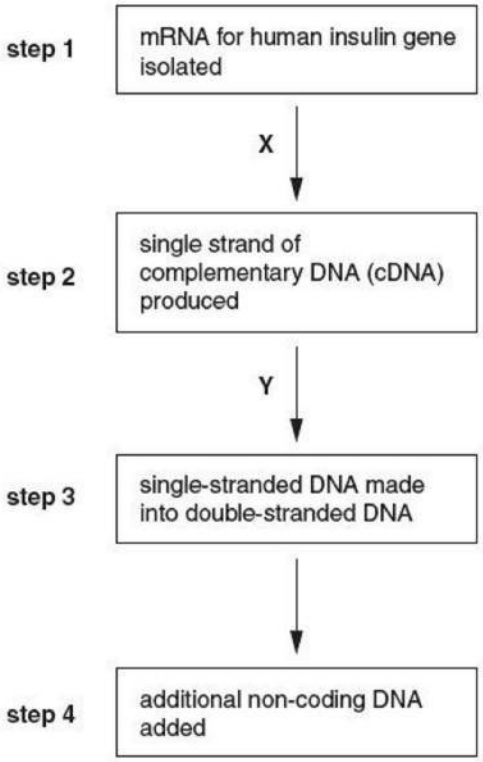


Fig. 3.1

(i) Name the enzymes **X** and **Y**.

X .....

Y ..... [2]

(ii) Explain why the starting point in this procedure is mRNA.

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.....[2]

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(b) Suggest why some methods of manufacturing genetically engineered insulin use eukaryotic yeast cells rather than prokaryotic bacterial cells.

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(c) State **three** advantages of using human insulin produced by genetic engineering.

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.....[3]

[Total: 9]

Q26.

1 (a) Repeated self-pollination in some species of flowering plants leads to inbreeding.

Describe briefly the effect of inbreeding on genetic diversity.

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..... [3]

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Some species of tropical ginger plants have two distinct phenotypes, **A** and **B**, that coexist in all populations. Phenotypes **A** and **B** differ in the behaviour of the anthers and stigmas of their flowers. In both phenotypes, the flowers open for only one day and the behaviour of the flowers of each phenotype is synchronised in all plants in the population. The differences between the two phenotypes are shown in Table 1.1.

Table 1.1

time	phenotype	behaviour of flower
0600 – 1145	**A**	stigma above anther, avoiding contact with insects anther split to release pollen
	B	receptive stigma below anther anther **not** split to release pollen
1145 – 1330	**A**	stigma moves downwards below anther
	B	stigma elongates and moves above anther
1430 – 1500	**A**	receptive stigma below anther anther no longer releasing pollen
	B	stigma above anther, avoiding contact with insects anther split to release pollen

(b) With reference to Table 1.1,

(i) state the time of day at which phenotype **A** may be pollinated by phenotype **B**;

..... [1]

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Use

(ii) explain how the behaviour of the two phenotypes of ginger plant helps to avoid inbreeding.

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..... [4]

(c) The number of phenotypes **A** and **B** in a natural population of one species of ginger plant was found to be 86 and 78 respectively. The ratio expected in the population was 1 : 1. The χ^2 (chi-squared) test was performed on these data, giving a calculated χ^2 of 0.39.

(i) State the number of degrees of freedom applicable to these data.

..... [1]

(ii) Use the calculated value of χ^2 and the table of probabilities provided in Table 1.2 to find the probability of the observed ratio departing significantly from the expected ratio.

probability ..... [1]

Table 1.2

degrees of freedom	probability, p				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

(iii) State what conclusion may be drawn from the probability found in **(ii)**.

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.....

..... [2]

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(iv) Suggest, using suitable symbols, genotypes for phenotypes **A** and **B** that would enable both phenotypes to be maintained in the ginger plant population for many generations. Explain the symbols you select.

explanation of symbols .....

.....

*genotype **A*** .....

*genotype **B*** .....[3]

[Total: 15]

Q27.

2 (a) Cystic fibrosis (CF) is caused by mutations of a gene coding for a transmembrane protein (CFTR) which acts as an ion pore. A large number of different mutations of the gene have been found.

(i) Describe briefly the symptoms of CF in humans.

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..... [3]

(ii) Explain how CF is inherited.

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..... [3]

(iii) Explain why a gene test for CF may not reveal the presence of the disease.

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..... [2]

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(b) Ion transport by cells expressing the normal allele for CFTR was compared with that by cells expressing mutant alleles. The mutant cells chosen for study were all capable of producing a CFTR protein and correctly inserting it into their cell surface membrane. The abilities of the cells to transport HCO_3^- and Cl^- were measured and expressed as a HCO_3^- : Cl^- transport ratio. The results are shown in Table 2.1.

Table 2.1

CF allele expressed by cells	HCO_3^- : Cl^- transport ratio of cells
normal alleles	1.0 : 1.0
mutant alleles associated with CF with inadequate functioning of the pancreas	all < 0.1 : 1.0
mutant alleles associated with CF with adequate functioning of the pancreas	0.3 – 0.46 : 1.0

(i) Explain why it was important that the CFTR protein was produced and correctly inserted into the cell surface membrane of all mutant cells chosen for study.

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..... [2]

(ii) With reference to Table 2.1, explain the cause of inadequate functioning of the pancreas in CF.

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..... [3]

(iii) Suggest **two** consequences of inadequate functioning of the pancreas in CF.

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..... [2]

[Total: 15]

Section_B

1.

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7 **(a)** Describe the use of recombinant DNA technology in the synthesis of human insulin by bacteria. [9]

(b) Explain the advantages of treating diabetics with human insulin produced by genetic engineering. [6]

2.

10 **(a)** Explain what is meant by a **gene** mutation and outline the possible consequences of a gene mutation for an organism. [9]

(b) Explain how faulty CFTR proteins in cell surface membranes can lead to the symptoms of cystic fibrosis. [6]

[Total: 15]

3.

10 **(a)** Cystic fibrosis (CF) is a genetic disease caused by an autosomal recessive allele. Gene therapy has been attempted to treat CF since 1993. Outline the basic principles of gene therapy for the treatment of CF. [8]

(b) Describe the role of a genetic counsellor in dealing with genetic diseases in humans and discuss the circumstances in which a couple might be referred to a genetic counsellor. [7]

[Total: 15]

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1. Nov/2018/Paper_41/No.5

(a) Leber's congenital amaurosis (LCA) is an autosomal recessive eye disease. LCA results in eye disorders, including severe loss of vision, at birth. LCA has been successfully treated by gene therapy, using a virus instead of a plasmid as the vector.

Adeno-associated virus (AAV) vectors containing the therapeutic allele were injected directly into the retina, the layer at the back of the eye containing the photoreceptor cells. People who had been blind from a young age were able to see again.

There is a risk associated with the injection method used to deliver the vectors, as it might cause the retina to detach, damaging vision. This method of delivery was first used for LCA before being trialled on other retinal diseases that gradually reduce the vision of people as they get older.

(i) Suggest the main steps involved in creating recombinant DNA for this example of gene therapy.

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(ii) Explain why the fact that LCA is an autosomal recessive genetic disease makes it suitable for treatment with gene therapy.

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..... [2]

(iii) Suggest why the retinal injection method of gene therapy was used for LCA before it was trialled on other retinal diseases that gradually reduce the vision of people as they get older.

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.....[2]

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(b) Scientists tried to create an improved virus vector for gene therapy.

step 1 – The scientists used a special form of the polymerase chain reaction (PCR). This form of PCR causes mutations in the DNA sequence of AAV by base substitution.

step 2 – The viruses containing different base substitutions were tested. This was done by using the different viruses to deliver a new gene, the gene for green fluorescent protein (GFP), into the photoreceptor cells of mice, using the retinal injection method.

step 3 – The best virus, known as 7m8, caused the photoreceptor cells in the retina of the mouse to fluoresce brightly, even when the recombinant virus was injected into the fluid inside the eye instead of into the retina itself.

step 4 – The 7m8 virus was used to cure a mouse with LCA by injecting this virus containing the therapeutic allele into the fluid inside the eye of the mouse.

(i) Suggest how errors occurring during PCR can cause base substitution mutations in the DNA sequence of AAV.

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..... [3]

(ii) Explain why the photoreceptor cells of the mouse fluoresced in step 3.

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..... [2]

(iii) Predict the impact of the 7m8 AAV on treatment for age-related retinal diseases.

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.....[1]

[Total: 14]

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1. Nov/2019/Paper_41/No.3

Therapeutic proteins are used to treat disease. The first purified therapeutic protein used was insulin, in 1922. The insulin was extracted from animal pancreases. Since 1982 most insulin has been made by recombinant DNA technology.

(a) Explain the advantages of producing human therapeutic proteins, such as insulin, by recombinant DNA technology.

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..... [4]

Calcitonin is a small protein hormone consisting of 32 amino acids. One of its functions is to inhibit the activity of cells, called osteoclasts, that break down bone tissue.

Calcitonin has been used as a therapeutic protein to treat osteoporosis. In osteoporosis too much breakdown of bone tissue in older people leads to reduced bone density and a risk of breaking bones.

(b) Fig. 3.1 shows the amino acid sequences of human calcitonin and calcitonin from the salmon fish, *Salmo salar*.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
human	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe
salmon	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu

position	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
human	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro
salmon	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro

Fig. 3.1

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(i) The two amino acid sequences shown in Fig. 3.1 can be compared. The number of amino acids that occur at the same position in both sequences can be counted and expressed as a percentage of the total number of amino acids present in one sequence. This is called the percentage sequence similarity.

Use Fig. 3.1 to calculate the percentage sequence similarity of human and salmon calcitonin.

Show your working.

..... % [2]

(ii) Compared to human calcitonin, salmon calcitonin is more biologically active. It remains active in the human body for longer and binds to calcitonin receptors more readily.

Bioinformatics was used to identify this more biologically active form of calcitonin to treat osteoporosis.

Explain how bioinformatics helped identify salmon calcitonin as a suitable form of calcitonin to treat human osteoporosis.

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..... [2]

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(c) Salmon calcitonin to treat osteoporosis is made by genetically engineered *Escherichia coli* bacteria. To produce these bacteria, a plasmid was cut and joined to the new gene to form a recombinant plasmid. The recombinant plasmid was then introduced into the bacterial cells.

(i) Name an enzyme that can:

- cut plasmid DNA

.....

- join the salmon calcitonin gene with plasmid DNA.

..... [2]

(ii) Identify **and** explain two properties of plasmids that allow them to be used as vectors in gene cloning.

1 .....

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.....

.....

2 .....

.....

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..... [2]

(d) The gene coding for salmon calcitonin is introduced into bacteria in a specially designed plasmid called an expression vector. An expression vector must contain a prokaryotic promoter, such as the *lac* promoter.

Explain why differences in the control of gene expression in prokaryotes and eukaryotes mean that expression vector plasmids must contain a prokaryotic promoter.

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..... [2]

[Total: 14]

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2. Nov/2019/Paper_42/No.3

Therapeutic proteins are used to treat disease. The first purified therapeutic protein used was insulin, in 1922. The insulin was extracted from animal pancreases. Since 1982 most insulin has been made by recombinant DNA technology.

(a) Explain the advantages of producing human therapeutic proteins, such as insulin, by recombinant DNA technology.

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..... [4]

Calcitonin is a small protein hormone consisting of 32 amino acids. One of its functions is to inhibit the activity of cells, called osteoclasts, that break down bone tissue.

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(b) Fig. 3.1 shows the amino acid sequences of human calcitonin and calcitonin from the salmon fish, *Salmo salar*.

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
human	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe
salmon	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu

position	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
human	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro
salmon	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro

Fig. 3.1

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(i) The two amino acid sequences shown in Fig. 3.1 can be compared. The number of amino acids that occur at the same position in both sequences can be counted and expressed as a percentage of the total number of amino acids present in one sequence. This is called the percentage sequence similarity.

Use Fig. 3.1 to calculate the percentage sequence similarity of human and salmon calcitonin.

Show your working.

..... % [2]

(ii) Compared to human calcitonin, salmon calcitonin is more biologically active. It remains active in the human body for longer and binds to calcitonin receptors more readily.

Bioinformatics was used to identify this more biologically active form of calcitonin to treat osteoporosis.

Explain how bioinformatics helped identify salmon calcitonin as a suitable form of calcitonin to treat human osteoporosis.

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..... [2]

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(c) Salmon calcitonin to treat osteoporosis is made by genetically engineered *Escherichia coli* bacteria. To produce these bacteria, a plasmid was cut and joined to the new gene to form a recombinant plasmid. The recombinant plasmid was then introduced into the bacterial cells.

(i) Name an enzyme that can:

- cut plasmid DNA

.....

- join the salmon calcitonin gene with plasmid DNA.

..... [2]

(ii) Identify **and** explain two properties of plasmids that allow them to be used as vectors in gene cloning.

1 .....

.....

.....

.....

2 .....

.....

.....

..... [2]

(d) The gene coding for salmon calcitonin is introduced into bacteria in a specially designed plasmid called an expression vector. An expression vector must contain a prokaryotic promoter, such as the *lac* promoter.

Explain why differences in the control of gene expression in prokaryotes and eukaryotes mean that expression vector plasmids must contain a prokaryotic promoter.

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..... [2]

[Total: 14]

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3. Nov/2019/Paper_43/No.3

Therapeutic proteins are used to treat disease. One example of a therapeutic protein is human growth hormone (hGH).

hGH has important roles in growth during childhood and in regulation of metabolism in adulthood.

Children described as hGH-deficient do not produce enough hGH and grow more slowly than other children. People who were hGH-deficient when they were children have a mean adult height that is 32 cm shorter than the population mean.

Daily injections of hGH are a treatment for hGH-deficient children that can increase growth rate, resulting in an increased adult height.

(a) The growth rate of three children was measured.

- The growth rate of the hGH-deficient child who did **not** receive daily hGH injections was 2.5 cm $year^{-1}$.
- The growth rate of the hGH-deficient child who received daily hGH injections was 10.0 cm $year^{-1}$ in the first year after starting treatment.
- The growth rate of the child who is **not** hGH-deficient was 5.0 cm $year^{-1}$.

Calculate the percentage increase in growth rate of the hGH-deficient child treated with hGH injections compared to the child who is **not** hGH-deficient.

Show your working.

..... % [2]

When this form of treatment started in 1958, hGH could only be obtained from the pituitary glands of people who had died.

In 1981, a plasmid containing hGH cDNA was constructed and inserted into *Escherichia coli* bacteria. This allowed recombinant hGH protein to be produced by the bacteria.

(b) Name the type of enzyme that:

- cuts plasmid DNA

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- makes cDNA from hGH mRNA.

..... [2]

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(c) Identify **and** explain **two** properties of plasmids that allow them to be used as vectors of hGH cDNA into cells of *Escherichia coli*.

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(d) In 1985, several cases of a rare brain disease were discovered in people who had been treated many years previously with hGH obtained from pituitary glands. It was decided, from 1985 onwards, that only recombinant hGH should be used to treat patients.

Explain the advantages of producing human therapeutic proteins, such as hGH, by recombinant DNA technology.

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(e) hGH is one of many proteins in the body whose secretion or production is controlled by a person's sleep-wake pattern.

The sleep-wake pattern describes when, during a 24 hour day, a person is asleep and when they are awake. For example:

- pattern 1 – asleep during the night and awake during the day (normal)
- pattern 2 – asleep during the day and awake during the night.

Researchers used microarray analysis to identify which genes have their expression changed by a person's sleep-wake pattern. They collected mRNA from:

- a group of people with sleep-wake pattern 1
- the same group of people whose sleep-wake pattern was changed to pattern 2.

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A summary of the results is shown in Table 3.1.

Table 3.1

sleep-wake pattern	number of genes with increased expression		
	during the day	during the night	all the time
pattern 1	661	733	108
pattern 2	134	95	8

(i) Describe how changing the sleep-wake pattern from pattern 1 to pattern 2 affects the number of genes expressed.

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(ii) Explain how eukaryotic genes can be switched on and off, for example, at certain times of day.

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(iii) Explain how bioinformatics can help to identify whether the genes whose expression is changed by moving from pattern 1 to pattern 2 are important to health.

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[Total: 15]

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4. June/2019/Paper_41/No.2

The interpupillary distance (IPD) is the distance in millimetres between the centres of the pupils of the eyes. Fig. 2.1 shows how IPD is measured.

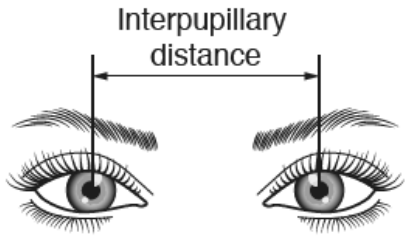


Fig. 2.1

IPD is one example of a characteristic of human facial structure that shows variation.

Fig. 2.2 shows the pattern of variation in IPD in a large sample of adults.

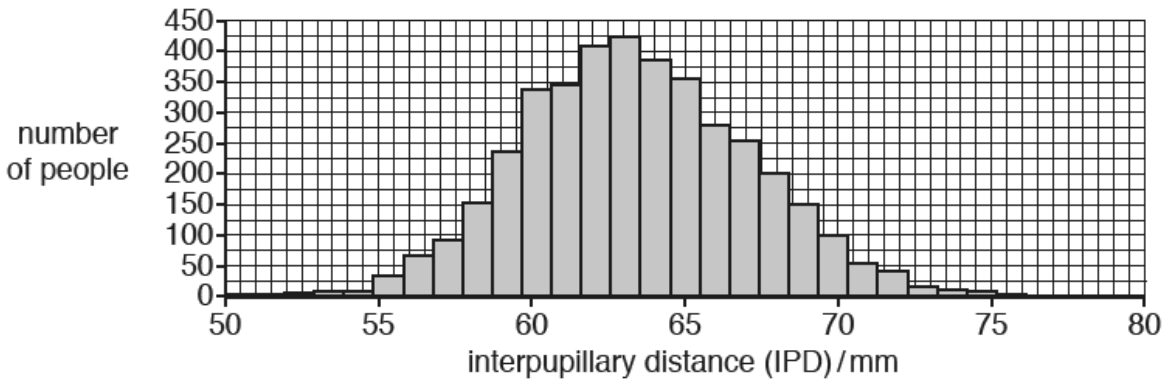


Fig. 2.2

(a) (i) Name the type of variation shown in Fig. 2.2.

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(ii) Suggest **and** explain how genes and the environment contribute to variation in IPD in humans.

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(b) Individuals with an IPD of 70 mm or more have a mutation in the *PAX3* gene that results in less PAX3 protein being made.

The normal role of the PAX3 protein is to increase the expression of many other genes involved in embryonic development. These genes affect a range of phenotypic features such as facial structure, hearing and eye colour.

(i) State the term that is used to describe a gene, such as *PAX3*, that controls the expression of other genes **and** suggest how the PAX3 protein controls the expression of other genes.

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(ii) Describe how microarray analysis could be used to identify the genes switched on by PAX3 in embryonic cells.

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(iii) The chimpanzee, *Pan troglodytes*, has DNA that is 98.5% similar to humans, including possession of the *PAX3* gene. Investigations show that chimpanzees express higher levels of the PAX3 protein during embryonic development than humans.

Fig. 2.3 shows a chimpanzee, *Pan troglodytes*.



Fig. 2.3

Suggest how knowledge of the *PAX3* gene helps scientists explain how humans and chimpanzees are very different in facial structure, even though they have very similar DNA.

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[Total: 15]

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5. June/2019/Paper_41/No.6

Maize, *Zea mays*, is an important food crop for human consumption and for feeding to animals.

Two varieties of maize are MON810 and Justina. Justina has been developed in the traditional way by selective breeding (artificial selection) and MON810 is an example of a genetically modified (GM) organism.

(a) MON810 produces a chemical that is toxic to insect pests. It is described as insect-resistant.

Outline how genetic engineering gave MON810 the trait of insect resistance.

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(b) Countries vary in the extent to which they grow GM varieties such as MON810, instead of traditional varieties such as Justina.

- In the USA, 88% of the total maize that is grown is GM.
- In most European countries, 0% of the maize that is grown is GM.

Scientists used computer models to predict the effect of two different changes in agricultural practice on maize crop yields:

- a global ban that reduces the cultivation of GM maize to 0% everywhere
- all countries increasing the cultivation of GM maize to the 88% level of the USA.

Table 6.1 shows the results of this modelling for four countries.

Table 6.1

country	percentage change in yield of maize	
	decrease GM maize cultivation to 0% of total	increase GM maize cultivation to 88% of total
Argentina	–8.86	+2.90
Honduras	–1.26	+16.75
Spain	–3.82	+5.39
USA	–7.63	0.00

Explain what the data in Table 6.1 suggests about the social and ethical implications of growing GM maize.

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[Total:8]

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6. June/2019/Paper_42/No.2(c)

(c) **(i)** Describe how microarray analysis can detect differences in the expression of many genes when comparing two samples, such as the offspring of wild and captive-bred fish.

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(ii) Explain how gene expression is controlled in eukaryotes such as fish.

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7. June/2019/Paper_42/No.3(a, b)

Soybean, *Glycine max*, is an important food crop for human consumption and for feeding to animals.

Two varieties of soybean are Vinton 81 and GTS 40-3-2.

Vinton 81 has been developed in the traditional way by selective breeding (artificial selection) and GTS 40-3-2 is an example of a genetically modified (GM) organism.

(a) GTS 40-3-2 is described as herbicide-resistant, as it can withstand the application of glyphosate herbicide. Glyphosate is sprayed on the crop to kill weeds.

Outline how genetic engineering gave GTS 40-3-2 the trait of herbicide resistance.

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(b) Countries vary in the extent to which they grow GM varieties, such as GTS 40-3-2, instead of traditional varieties such as Vinton 81.

- In the USA, 94% of soybeans grown are GM.
- In Europe, 0% of soybeans grown are GM.

Scientists used computer models to predict the effect of two different changes in agricultural practice on soybean crop yields:

- a global ban that reduces the cultivation of GM soybeans to 0% everywhere
- all countries increasing the cultivation of GM soybeans to the 94% level of the USA.

Table 3.1 shows the results of this modelling for four countries.

Table 3.1

country	percentage change in yield of soybeans	
	decrease GM soybean cultivation to 0% of total	increase GM soybean cultivation to 94% of total
Argentina	0.00	+6.23
Bolivia	–10.82	0.00
Brazil	0.00	+6.23
USA	–5.87	0.00

Explain what the data in Table 3.1 suggests about the social and ethical implications of growing GM soybeans.

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8. June/2019/Paper_43/No.4

Rice, *Oryza sativa*, is an important food crop for human consumption.

Two varieties of rice are T2A-1 and Shanyou 2.

Shanyou 2 has been developed in the traditional way by selective breeding (artificial selection) and T2A-1 is an example of a genetically modified (GM) organism.

(a) T2A-1 rice is described as insect-resistant as it produces a chemical that is toxic to insect pests.

Outline how genetic engineering gave T2A-1 rice the trait of insect resistance.

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(b) Countries vary in the extent to which they grow GM varieties instead of traditional crop varieties.

- The USA, Canada and countries in South America plant GM varieties on a large proportion of their crop-growing land, reaching 94% for soybeans in the USA.
- In most European countries, 0% of the crop-growing land is planted with GM varieties.

Scientists used computer models to predict the effects of a global ban that reduced the cultivation of GM crops to 0% everywhere.

Table 4.1 shows the results of this modelling on world crop yields and the purchase price of each crop.

Table 4.1

crop	percentage change in crop yield	percentage change in purchase price
rapeseed	–0.14	+1.96
rice	–0.12	+1.58
soybeans	–1.40	+4.05

(i) Explain what the data in Table 4.1 show about the **social** implications of growing GM crops.

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(ii) A worldwide ban on growing GM crop varieties would mean that more land would be needed to grow traditional crops, such as Shanyou 2. This would involve converting forest and grassland to crop-growing land.

Table 4.2 shows the predicted changes in carbon dioxide emissions associated with this change in use of land.

Table 4.2

change in use of land	change in carbon dioxide emissions /million kg CO_2
forest to crop-growing land	+ 608 726
grassland to crop-growing land	+ 276 042

Discuss what the data in Table 4.2 indicate about the environmental implications of growing GM crops.

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[Total: 8]

9. Feb/2019/Paper_42/No.3(a, d)

During an immune response, only B-lymphocytes with receptors that are specific to the antigens present are activated. Activation occurs when an antigen binds to a receptor of a B-lymphocyte.

Activated B-lymphocytes grow in size and then divide by mitosis. Many further mitotic cell divisions occur, increasing the number of B-lymphocytes with receptors specific to the antigen. Eventually, cells produced in this process will develop into either plasma cells that secrete antibodies or memory B-cells.

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Fig. 3.1 is a summary of B-lymphocyte activation and the events that follow.

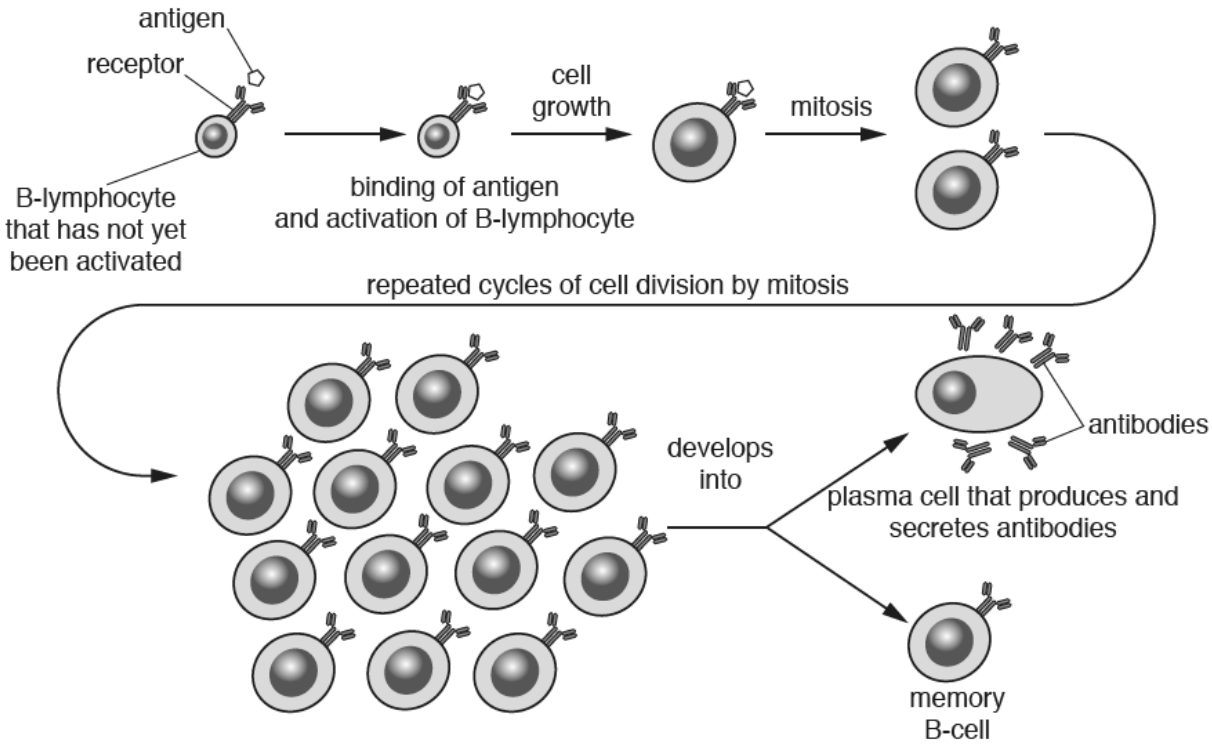


Fig. 3.1

The development of plasma cells and memory B-cells in this process depends on transcription factors.

(a) Explain the role of transcription factors in gene expression in eukaryotic cells.

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(d) Microarrays can be used to analyse the effect of transcription factors, such as BLIMP-1, on gene expression.

(i) Describe how a microarray is used in the study of gene expression.

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(ii) BCL6 is another transcription factor found in B-lymphocytes. The effect of BCL6 on gene expression was compared in two samples of B-lymphocytes.

- Sample 1 consisted of B-lymphocytes that were producing BCL6.
- Sample 2 consisted of B-lymphocytes that were **not** producing BCL6.

Suggest why a microarray is suitable for identifying the function of the transcription factor BCL6 in these two samples.

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10. Feb/2019/Paper_42/No.5

Fig. 5.1 shows the area of land that was used to grow genetically modified (GM) crops in the USA, Brazil, India and China from 2004 to 2015.

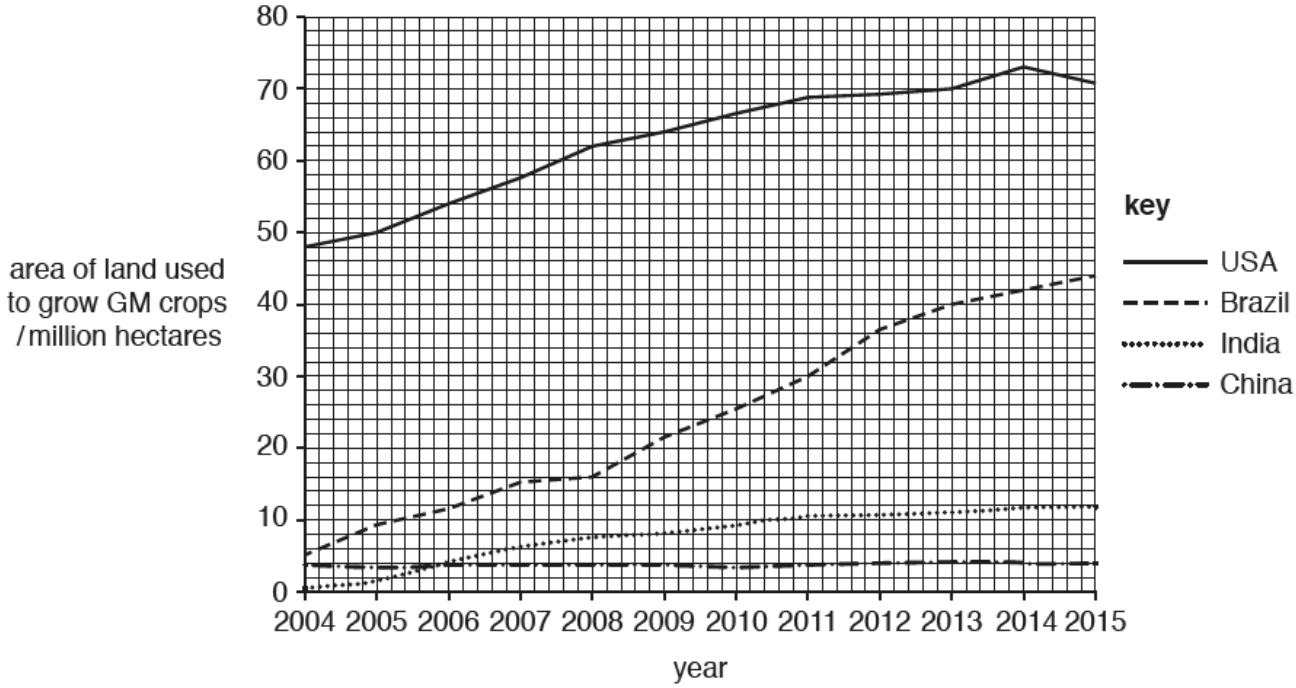


Fig. 5.1

(a) (i) Suggest reasons why the area of land used to grow GM crops in the USA is greater than the area of land used to grow GM crops in Brazil.

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(ii) Describe the differences in the area of land that was used to grow GM crops in China and the area of land that was used to grow GM crops in India, over the time shown in Fig. 5.1.

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(b) **(i)** Genetic modifications in crops can provide resistance to insect pests. Bt maize is one type of GM crop that has been produced to be resistant to insect pests.

State **two** benefits to farmers of insect resistance in crops.

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..... [2]

(ii) Describe the difference between Bt maize and non-GM maize that explains why Bt maize is resistant to insects.

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..... [2]

(c) **(i)** State **two** reasons why people may have objections to the growth of insect-resistant GM crops.

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(ii) In 2015, the percentage of the USA population that objected to the use of GM crops was lower than in 2005.

Suggest why a smaller percentage of the USA population objected to the use of GM crops in 2015.

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[Total: 12]

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1. Nov/2021/Paper_41/No.4

(a) In 1984, the geneticist Alec Jeffreys invented a DNA testing technique, known as DNA profiling, that produces a DNA banding pattern on a gel. The DNA banding pattern (profile) is unique to each individual.

DNA profiling can be used in police forensic work to catch criminals.

Since 1987, police in many countries have collected and stored DNA from crime scenes to create DNA profiles, which they try to match with the DNA profiles of criminal suspects.

(i) DNA at a crime scene may be obtained from hairs and traces of blood, semen and saliva.

Explain why PCR may be needed before DNA from a crime scene can be profiled.

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(ii) Explain why electrophoresis produces a DNA banding pattern on a gel.

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(b) GEDmatch is described as 'an open data personal genomics website'. It can be used by people who want to upload their DNA data to trace their ancestors and other relatives.

In 2018, police in the USA solved a large number of serious crimes. Some of these crimes had been unsolved for over thirty years. The police used GEDmatch to profile DNA taken from crime scenes and to look for matching DNA profiles. In many cases the police found partial matches to the relatives of criminals. This allowed the criminals to be identified and then charged on the basis of a complete DNA profile match.

(i) Suggest why the police strategy of comparing crime scene DNA with the GEDmatch database was so successful.

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(ii) Explain why GEDmatch is an example of bioinformatics.

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(iii) The first successful conviction resulting from the use of GEDmatch by the police was widely reported.

Some journalists and broadcasters thought that the GEDmatch website should not have been used by the police in this way.

In the days following the news, the number of citizens choosing to upload their DNA data to GEDmatch increased from 1500 to 5000 a day.

Comment on the social **and** ethical issues raised by this first successful conviction.

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[Total: 10]

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2. Nov/2021/Paper_42/No.4

(a) Genome-wide association studies find links between single nucleotide polymorphisms (SNPs) and phenotypic features such as human diseases. SNPs are points on the DNA that vary in the population because of DNA base substitutions.

A genome-wide association study investigates the effect of genetic variation on a disease. A large number of people with the disease and a large number of healthy control individuals provide DNA. Microarray chips are used to identify the genotype of each individual at many SNPs.

The Wellcome Trust Case Control Consortium (WTCCC) study was an important genome-wide association study.

- The study used a microarray chip that identified each person's genotype at 500 000 different SNPs.
- The study looked for links between SNPs and 7 different diseases.
- For each disease, 2000 people with the disease were tested.
- Their results were compared with the results of 3000 healthy control individuals.

(i) Outline how microarrays are used in the analysis of genomes.

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(ii) Explain why bioinformatics was important to the WTCCC study.

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(b) Fig. 4.1 summarises results for three diseases in the WTCCC study. The 22 human autosomes and the X chromosome (chromosome 23) are shown.

Chromosome locations with SNPs that are associated with a disease at a statistically significant level (greater than 5 arbitrary units) are shown in black.

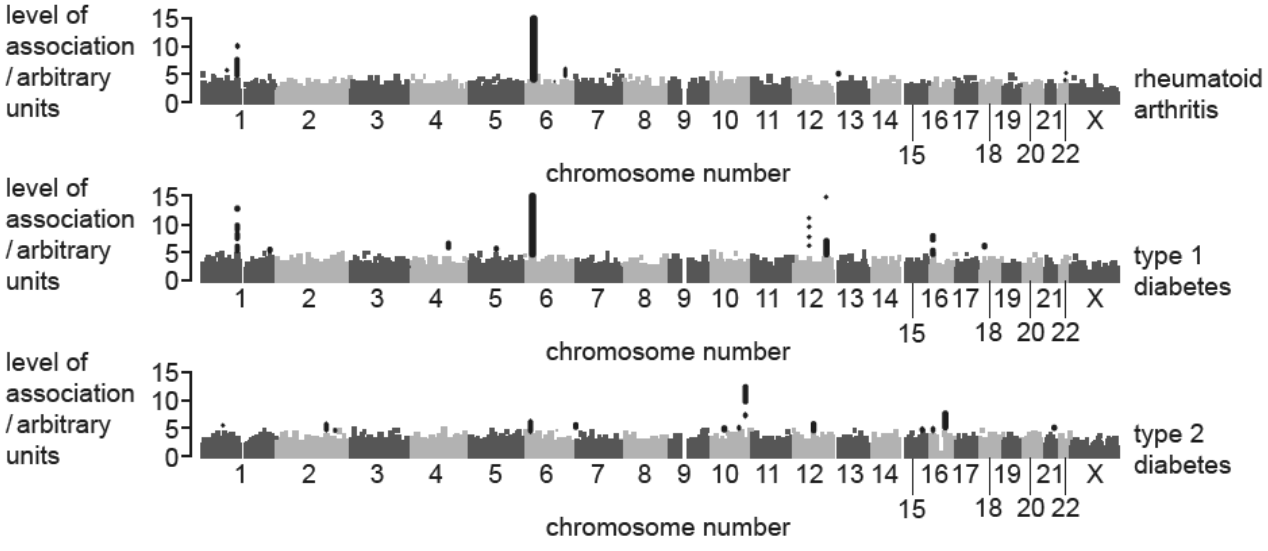


Fig. 4.1

(i) Identify the chromosomes that contain SNPs that have a high level of association with **both** rheumatoid arthritis and Type 1 diabetes.

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(ii) With reference to Fig. 4.1, compare the genetic basis of the three diseases.

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..... [2]

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(c) Individuals can choose to have their DNA analysed on a microarray chip to predict their risk of developing different diseases.

Outline the social **and** ethical considerations of this type of DNA analysis.

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[Total: 12]

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3. Nov/2021/Paper_42/No.7

(a) The Labrador is a variety of domestic dog. Labradors have fur that can be brown, black or yellow.

In Labradors, *TYRP1* is one gene that codes for fur colour. This gene has two alleles, **B** and **b**.

- The dominant allele, **B**, codes for the enzyme tyrosinase that functions in the pathway to produce melanin, leading to black fur.
- The production of melanin in Labradors is very similar to the production of melanin in humans.
- The recessive allele, **b**, codes for an enzyme that results in the production of a brown form of melanin, leading to brown fur.

Outline how melanin may be produced in Labradors to produce black fur.

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..... [2]

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(b) Another gene, *MC1R*, interacts with *TYRP1*.

MC1R has two alleles, **E** and **e**.

- The dominant allele, **E**, allows the alleles of *TYRP1* to be expressed.
- The recessive allele, **e**, prevents the alleles of *TYRP1* from being expressed.
- When no form of melanin is produced the Labrador will have yellow fur.

(i) Construct a genetic diagram to show the ratio of possible offspring from a cross between a black male Labrador, heterozygous for both genes, and a yellow female Labrador, heterozygous for *TYRP1*.

parental phenotype: black × yellow

parental genotype

gametes

offspring genotypes

offspring phenotypes

ratio .....

[6]

(ii) State the term used to describe a protein that is involved in the control of gene expression in eukaryotes.

..... [1]

[Total: 9]

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1. June/2022/Paper_41/No.4

In 1973 a technique for genetic engineering was used for the first time. Recombinant DNA was made using a plasmid and this was successfully transferred into an organism.

In 2012 a new technique for genetic engineering, called gene editing, was developed.

(a) Table 4.1 lists some statements about the two genetic engineering techniques.

Complete Table 4.1 to compare the original genetic engineering technique using a plasmid vector with the newer technique of gene editing. For each row, place a tick (✓) in the correct column if the statement applies and leave a blank if the statement does not apply.

Table 4.1

statement	genetic engineering using a plasmid	gene editing
It can produce a transgenic organism.		
It can modify the characteristics of an organism.		
It can delete unwanted DNA.		
It uses an enzyme that cuts DNA.		
It can use RNA to precisely locate the target gene.		

[5]

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(b) Orange trees, *Citrus sinensis*, produce fruits that are an important food crop. The functional leaf area of orange trees may be reduced by the growth of citrus canker bacteria. These bacteria cause citrus canker disease.

Scientists used gene editing to develop two types of orange tree with different mutations (changes to the DNA). The mutant orange tree leaves showed resistance to citrus canker disease.

Fig. 4.1 shows the area of leaf with citrus canker disease in wild type (not gene edited) and gene edited orange tree leaves after they have been exposed to citrus canker bacteria.

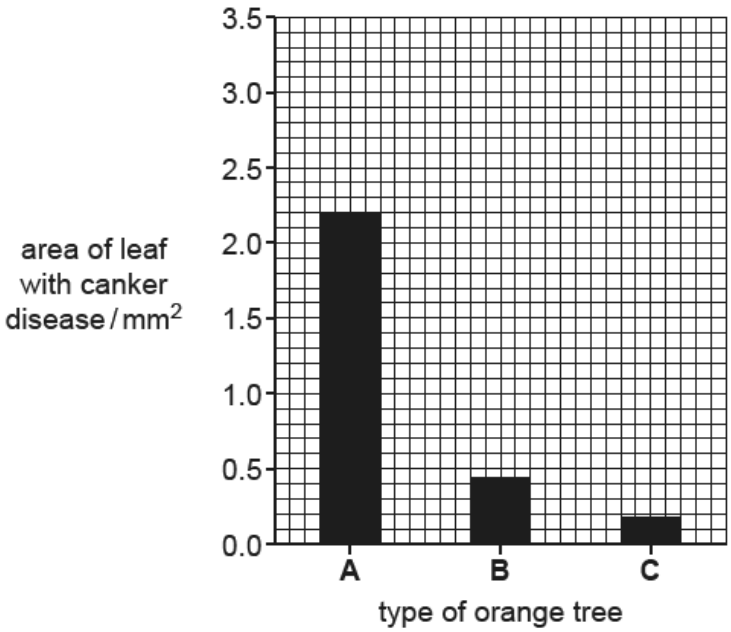


Fig. 4.1

(i) Identify the letter that represents the wild type orange trees on Fig. 4.1.

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(ii) Explain the social benefits of this example of gene editing.

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[Total: 9]

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2. June/2022/Paper_42/No.4

In 1973, a technique for genetic engineering was used for the first time. Recombinant DNA was made using a plasmid and this was successfully transferred into an organism.

In 2012, a new technique for genetic engineering, called gene editing, was developed.

(a) Table 4.1 lists some statements about the two genetic engineering techniques.

Complete Table 4.1 to compare the original genetic engineering technique using a plasmid vector with the newer technique of gene editing. For each row, place a tick (✓) in the correct column if the statement applies and leave a blank if the statement does not apply.

Table 4.1

statement	genetic engineering using a plasmid	gene editing
It may cause the organism to produce a different protein.		
It may cause a single base pair in a gene to be changed.		
The success of the technique can be evaluated using marker genes.		
It may use the CRISPR system.		
It uses DNA ligase.		

[5]

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(b) Cassava plants, *Manihot esculenta*, produce roots that have a high starch content. These roots are an important food source in tropical regions. The growth of cassava plants is reduced by competition from weeds.

Scientists used gene editing to develop two types of cassava plant with different mutations (changes to the DNA). The gene edited cassava plants showed resistance to the herbicide glyphosate. In susceptible plants, glyphosate prevents synthesis of three amino acids from a precursor molecule called shikimate.

Fig. 4.1 shows the concentration of shikimate in the wild type (not gene edited) and the two types of gene edited cassava plant after they were exposed to three different concentrations of glyphosate.

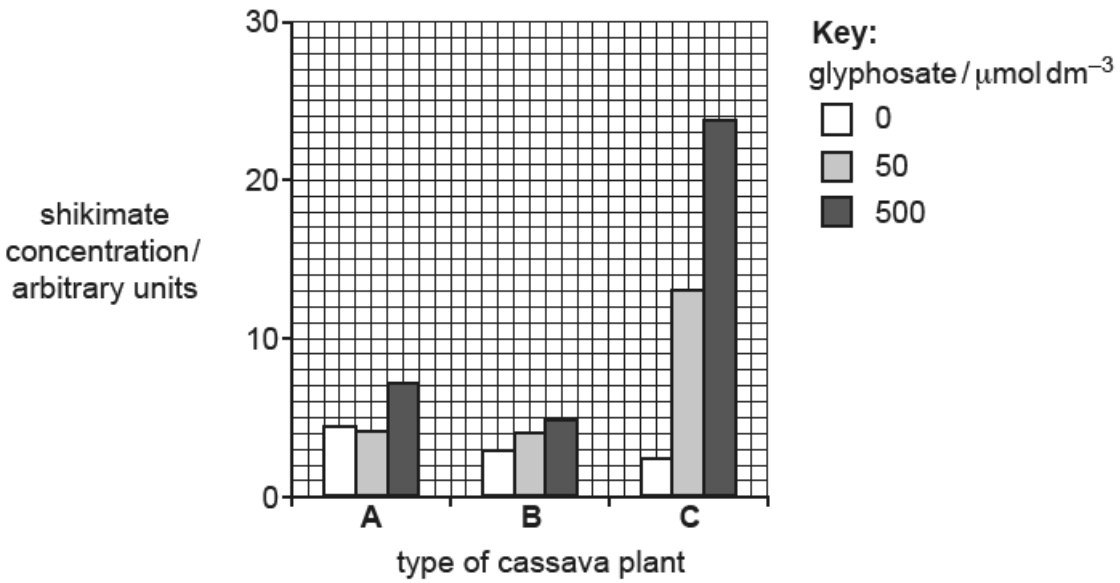


Fig. 4.1

(i) Identify the letter in Fig. 4.1 that represents the wild type cassava plant.

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(ii) Explain the social benefit of this example of gene editing.

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..... [3]

[Total: 9]

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3. June/2022/Paper_43/No.4

In 1973, a technique for genetic engineering was used for the first time. Recombinant DNA was made using a plasmid and this was successfully transferred into an organism.

In 2012, a new technique for genetic engineering, called gene editing, was developed.

(a) Table 4.1 lists some statements about the two genetic engineering techniques.

Complete Table 4.1 to compare the original genetic engineering technique using a plasmid vector with the newer technique of gene editing.
For each row, place a tick (✓) in the correct column if the statement applies and leave a blank if the statement does not apply.

Table 4.1

statement	genetic engineering using a plasmid	gene editing
It can add a new phenotypic characteristic to an organism.		
It can change an A–T base pair to C–G.		
It can inactivate a desired selected gene in an organism.		
It may change DNA in a way that cannot be distinguished from a natural mutation.		
It requires a DNA donor and a recipient.		

[5]

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(b) *Camelina sativa* is a fast-growing plant with oil-rich seeds.

C. sativa grows in dry and poor soils and so it may be important as a food crop in the future. The oil from its seeds has a high content of polyunsaturated fatty acids. This shortens the time that the oil can be stored for, which is a disadvantage.

Scientists used gene editing to develop two types of *C. sativa* with different genetic changes. The gene edited *C. sativa* seeds produced oil with longer storage times.

Fig. 4.1 shows the percentage composition of fatty acids in the oil extracted from seeds of gene edited and wild type (not gene edited) *C. sativa*.

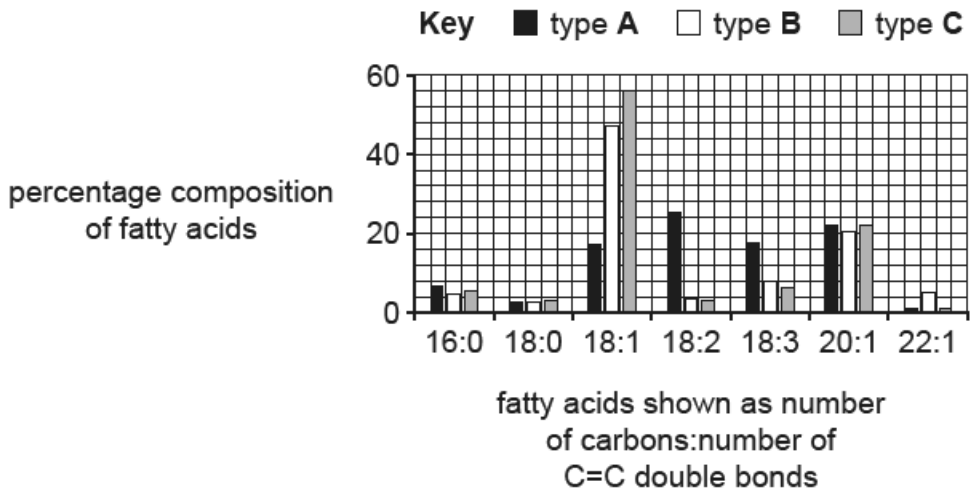


Fig. 4.1

(i) Identify the letter that represents the oil of the wild type *C. sativa* on Fig. 4.1.

..... [1]

(ii) With reference to Fig. 4.1, discuss the social benefits of this example of gene editing.

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..... [3]

[Total: 9]

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1. Nov/2022/Paper_41/No.6

Selective breeding has been used to improve the characteristics of crop plants, including maize and rice.

(a) Outline how vigorous, uniform varieties of maize were developed by selective breeding.

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..... [4]

(b) IR8 is a variety of rice plant that was developed in the 1960s.

IR8 was developed by breeding together two different varieties of rice:

- PETA, which produces a high yield
- DGWG, which is a dwarf variety.

Fig. 6.1 is a diagram showing the three varieties of rice.

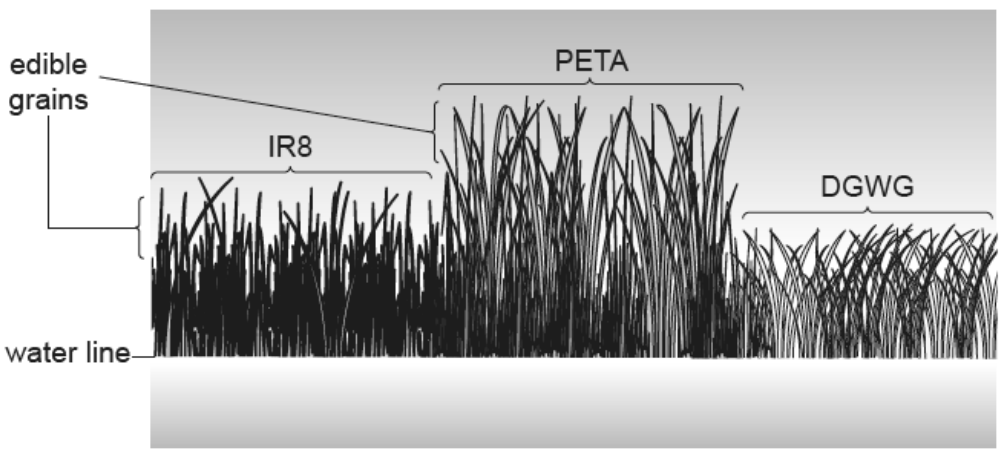


Fig. 6.1

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(i) Suggest why IR8 is an improvement on the PETA variety of rice.

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..... [1]

(ii) In 2009, scientists produced a new variety of rice, IR64-Sub1, by breeding together:

- FR13A, a variety which has a low yield but has an allele for flood tolerance
- IR64, a variety which produces a high yield.

The scientists sequenced the DNA of these three rice varieties.

Suggest the benefit of sequencing the DNA of IR64-Sub1.

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..... [1]

(iii) After a few generations of breeding, the scientists crossed IR64-Sub1 with IR64.

Explain why the scientists crossed IR64-Sub1 with IR64.

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..... [1]

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(c) Thale cress, *Arabidopsis thaliana*, is another plant that has dwarf varieties.

Scientists treated three varieties of *A. thaliana* with gibberellin:

- the wild type (normal, non-dwarf variety)
- dwarf variety **A**
- dwarf variety **B**.

Fig. 6.2 shows the responses of the three varieties to treatment with gibberellin.

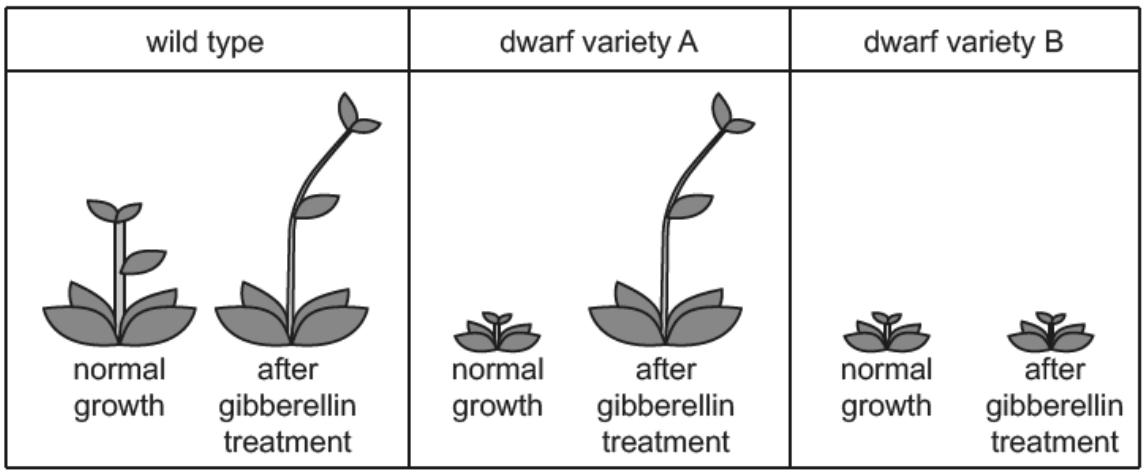


Fig. 6.2

(i) With reference to Fig. 6.2, suggest explanations for the different responses to gibberellin shown by dwarf variety **A** and dwarf variety **B**.

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(ii) BZR1 is a transcription factor that helps to regulate growth and development in *A. thaliana*.

Outline the features of a transcription factor such as BZR1.

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..... [2]

[Total: 13]

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2. Nov/2022/Paper_42/No.4

Haemophilia is a blood clotting disorder in humans caused by a mutant allele on the X chromosome.

Table 4.1 compares two forms of haemophilia: haemophilia A and haemophilia B.

Table 4.1

	haemophilia A	**haemophilia B**
gene	*F8*	*F9*
clotting factor protein	factor VIII	factor IX
proportion of males born with haemophilia	1 in 5000	1 in 30 000
length of functional gene (exons only)/kilobase pairs	7.0	1.6

(a) Genetic engineering is used to make recombinant human proteins to treat people with haemophilia A and haemophilia B.

Outline the principles of genetic engineering.

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(b) Scientists are working towards a goal of treating haemophilia by gene therapy. They plan to use a common, harmless virus to introduce the functional gene. The virus has a genome that is 4.7 kilobase pairs long.

(i) With reference to Table 4.1 and the introduction to **(a)**, assess:

- which form of haemophilia, A or B, scientists should try to treat first
- whether they should attempt to treat haemophilia with gene therapy at all.

Explain your reasoning.

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..... [3]

(ii) In gene therapy trials to treat haemophilia, the gene coding for the clotting factor needs to be introduced together with a promoter.

Explain why a promoter has to be introduced as well as the desired gene.

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(iii) Some individuals taking part in gene therapy trials have been naturally exposed to the virus carrying the functional gene, so that their blood already contains antibodies to the virus.

Predict how this will affect the success of the gene therapy treatment.

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(c) Gene editing is a newer technique for modifying DNA. Some scientists are researching the use of gene editing, instead of introducing a functional gene, to treat haemophilia.

State **two** possible advantages of using gene editing as a method of treating haemophilia.

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..... [2]

[Total: 13]

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(a) The *lac* operon of prokaryotes contains a group of structural genes that are under the same control and are transcribed together.

Another operon found in prokaryotes is the *trp* operon.

Fig. 6.1 summarises the structure and control of the *trp* operon.

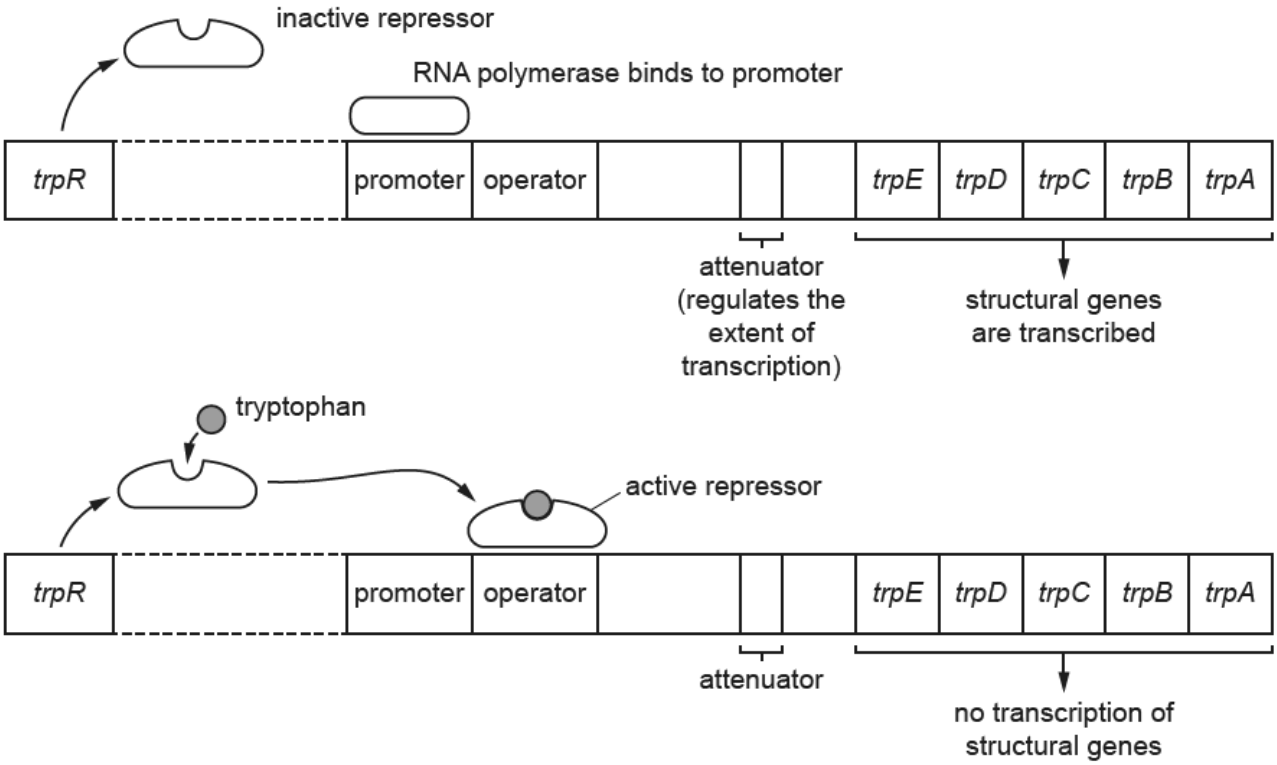


Fig. 6.1

(i) Describe the differences in structure **and** control between the *lac* operon and the *trp* operon.

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(ii) Suggest why structural genes in operons are transcribed together.

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..... [1]

(iii) *trpA* is an example of a structural gene and *trpR* is an example of a regulatory gene.

Describe the differences between the functions of structural genes and regulatory genes.

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..... [2]

(iv) *trpA* codes for the enzyme tryptophan synthase.

Tryptophan synthase catalyses the formation of the amino acid tryptophan.

Explain why tryptophan synthase is an example of a repressible enzyme.

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..... [2]

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(b) Control of gene expression in eukaryotes is more complex than in prokaryotes.

In plants, the control of gene expression can involve plant hormones, such as gibberellin, and proteins known as JAZ and MYC.

(i) Describe how gibberellin activates genes in plant cells.

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..... [4]

(ii) Transcription of some plant genes is prevented when JAZ proteins bind to other proteins known as MYC.

When JAZ proteins are broken down, MYC proteins are free to bind to DNA. This allows transcription to begin.

State the term that is used to describe proteins such as MYC proteins.

..... [1]

[Total: 13]

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1. March/2023/Paper_ 9700/42/No.2

Interferon-alpha (IFN-α) can be produced as a recombinant human protein to treat some types of cancer. The gene *IFNA2* codes for IFN-α.

One method of producing recombinant IFN-α uses genetically engineered *Escherichia coli* bacteria that contain recombinant plasmids. Each recombinant plasmid contains:

- the gene *IFNA2*
- three regulatory sequences of the *lac* operon (promoter, operator and *lacI*)
- a gene for antibiotic resistance, AMP^R.

Each of the sequences for the *lacI* gene and AMP^R gene contains its own promoter. As a result, these genes are always expressed in *E. coli* bacteria that contain this recombinant plasmid.

Fig. 2.1 is a diagram of the recombinant plasmid. The promoter regions of the *lacI* gene and AMP^R gene are **not** shown.

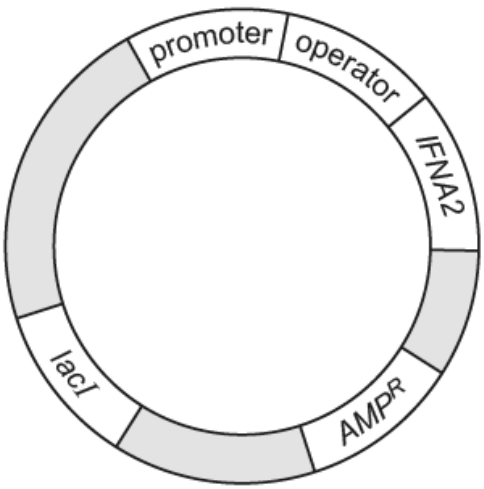


Fig. 2.1

(a) The start of transcription of the gene *IFNA2* by *E. coli* with the recombinant plasmid shown in Fig. 2.1 needs to be controlled to obtain an optimum yield of IFN-α.

Scientists investigated the effect of two inducers of transcription on the production of recombinant IFN-α:

- lactose, which is converted to allolactose in *E. coli*
- IPTG, which is a synthetic molecule with a very similar structure to allolactose. IPTG **cannot** be broken down by *E. coli*.

The scientists grew three cultures of *E. coli* containing the recombinant plasmid in the same growth medium. The growth medium contained glucose, amino acids, essential vitamins and minerals. The growth medium did **not** contain lactose.

After four hours, either lactose or IPTG at the same concentration was added to two of the cultures of *E. coli*. As a control, the third culture of *E. coli* was grown without adding lactose or IPTG.

The concentration of recombinant IFN-α in the cultures was measured at different times over a period of 28 hours. The results are shown in Fig. 2.2.

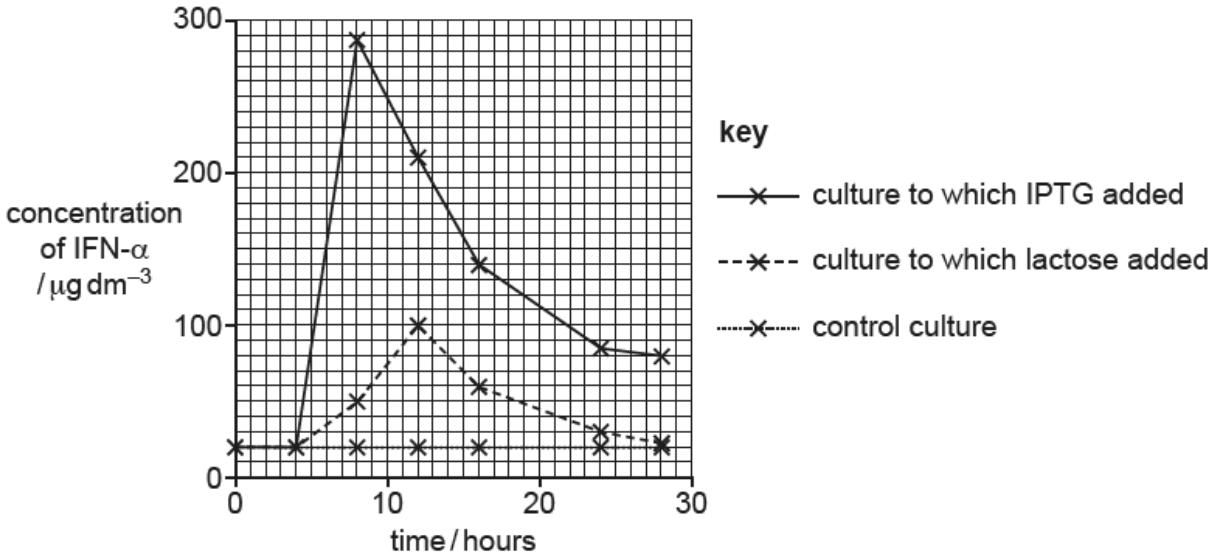


Fig. 2.2

(i) The regulatory sequences of the *lac* operon contained in the recombinant plasmid are involved in the control of transcription of the gene *IFNA2*.

Explain the role of the gene *lacI* in the control of transcription of the *IFNA2* gene between **0 hours** and **4 hours**.

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..... [2]

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(ii) With reference to Fig. 2.2, describe the changes in the concentration of recombinant IFN-α in the culture containing IPTG from when IPTG was added at **4 hours** to the end of the experiment at **28 hours**.

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..... [3]

(iii) Suggest **one** reason for the difference between the concentration of recombinant IFN-α in the culture at **8 hours** in the presence of lactose and the concentration of recombinant IFN-α in the culture at **8 hours** in the presence of IPTG.

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..... [1]

(iv) Suggest **one** reason for the change in the concentration of recombinant IFN-α in the culture containing IPTG from **12 hours** to **16 hours**.

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..... [1]

(b) The gene AMP^R in the plasmid shown in Fig. 2.1 codes for a protein that provides resistance to the antibiotic ampicillin.

Suggest how AMP^R allows genetically engineered *E. coli* containing the recombinant plasmid to be identified.

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..... [1]

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(c) Bacteria can evolve antibiotic resistance through natural processes.

Outline how bacteria can evolve to become resistant to antibiotics.

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[Total: 13]

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2. March/2023/Paper_ 9700/42/No.3

Salmon can be genetically modified (GM) to produce increased quantities of growth hormone, which is a protein. GM salmon modified in this way have a faster growth rate and reach their maximum body mass at a younger age than non-GM salmon.

(a) Within any population of salmon there is variation in body mass. This is an example of continuous variation.

Explain what is meant by continuous variation **and** how it can be caused.

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..... [3]

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(b) Scientists investigated whether injection of very young non-GM salmon with recom growth hormone could cause an increase in the growth rate of the salmon.

The scientists used two groups of non-GM salmon:

- a control group of salmon that were not injected with recombinant growth horr
- an experimental group of salmon that were injected with 1.0 μg of recom growth hormone at the start of the experiment and once a week for the ne weeks.

The mean body mass of the salmon in the two groups at the start of the experiment w same (5.3 g).

After six weeks, the body mass of every salmon was measured again. The resul summarised in Table 3.1.

Table 3.1

		no injection with recombinant growth hormone	**injected with recombinant growth hormone**
number of non-GM salmon (n)		28	27
body mass /g	range	6.5–8.6	7.2–12.7
	mean ($\bar{x}$)	7.7	9.4
	standard deviation (s)	0.4	1.1

A student decided that a *t*-test should be performed on the results shown in Table 3.1.

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(i) Calculate the value of t for the results shown in Table 3.1 using the formula for the t-test:

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Give your answer to **two** decimal places.

Show your working.

t = ...

[3]

(ii) The critical value at $p = 0.05$ for these data is 2.01.

The student used the results in Table 3.1 and the t-test to conclude that the injections of recombinant growth hormone cause an increase in the growth rate of the non-GM salmon.

Comment on the extent to which the conclusion made by the student can be supported.

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(iii) Suggest **one** advantage, **other** than cost, of farming GM salmon that produce increased quantities of growth hormone instead of farming non-GM salmon that are injected with recombinant growth hormone each week.

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..... [1]

[Total: 10]

3. March/2023/Paper_ 9700/42/No.4

Array comparative genome hybridisation (aCGH) is a technique involving the use of a microarray to analyse a genome or sections of a genome.

(a) Outline the steps required to prepare the genome of an individual so that the genome is ready for analysis using a microarray chip.

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..... [3]

(b) DiGeorge syndrome is a dominant inherited disease in humans. DiGeorge syndrome is caused by deletion of a large number of nucleotides from chromosome 22.

The number of nucleotides deleted varies between individuals in a range from 800 000 to 3 100 000. The largest deletions can cause the removal of up to 46 protein-coding genes from the chromosome.

Fig. 4.1 shows the results of aCGH using a microarray specific for the section of chromosome 22 within which the DiGeorge syndrome deletion occurs. The microarray analysed DNA from two individuals:

- one with DiGeorge syndrome
- one who did not have DiGeorge syndrome (control DNA for comparison).

In the aCGH results shown in Fig. 4.1:

- Each small circle represents the results from a single probe on the microarray.
- The *x*-axis shows the position of each probe on chromosome 22. The position is shown as distance along the chromosome in millions of nucleotides.
- A result close to 100% fluorescence on the *y*-axis means that the DNA from the individual with DiGeorge syndrome fluoresces at the same intensity as the control DNA for that probe.
- A result close to 50% fluorescence on the *y*-axis means that the DNA from the individual with DiGeorge syndrome fluoresces half as much as the control DNA for that probe.

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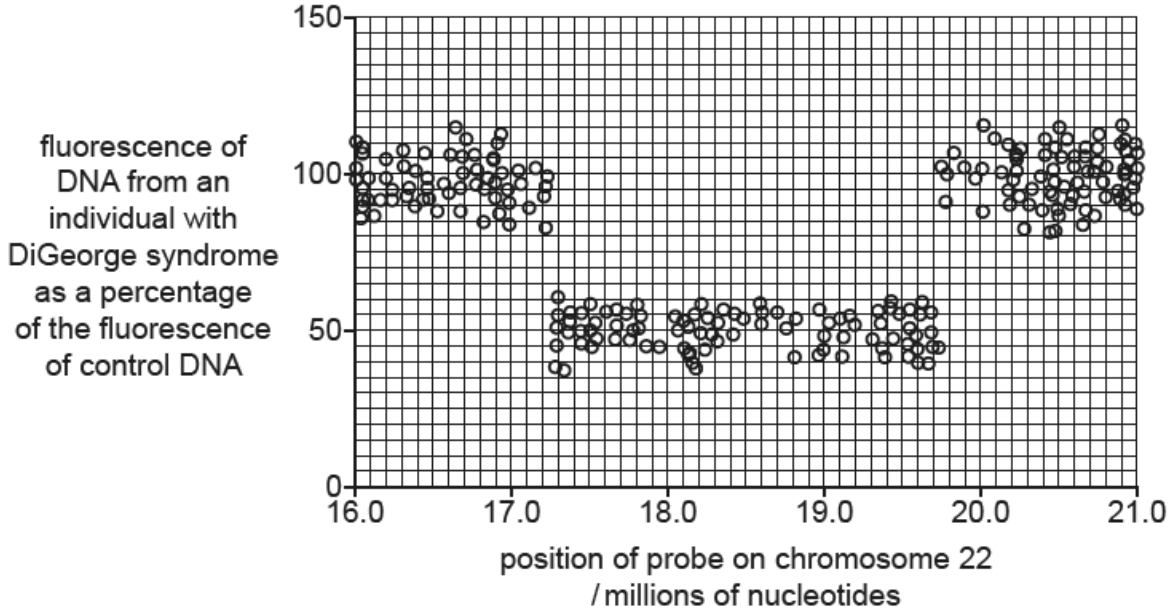


Fig. 4.1

(i) With reference to Fig. 4.1, estimate the number of nucleotides deleted from the affected chromosome 22 in the individual with DiGeorge syndrome.

Give your answer to the nearest 100 000 nucleotides.

..... [1]

(ii) Explain how the microarray technique works to give the results shown in Fig. 4.1.

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(iii) Suggest why the phenotypes of two individuals with DiGeorge syndrome can be different.

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[Total: 10]

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4. June/2023/Paper_ 9700/41/No.4

(a) Recombinant human proteins can be used to treat disease.

(i) Define the term recombinant DNA.

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..... [1]

(ii) From the 1920s until the 1970s, insulin obtained from the bodies of animals was used to treat diabetes. From the 1970s, recombinant human insulin was used instead.

Explain the advantages of using recombinant human insulin to treat people with diabetes.

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..... [3]

(b) Insulin is composed of two polypeptide chains, the A chain and the B chain, that are linked by disulfide bonds.

Variations in amino acid sequence occur:

- in the insulin molecules of different animals
- in new versions of human insulin that have been engineered to control blood glucose concentration more effectively than normal recombinant human insulin. These new versions of human insulin are called analogues.

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Table 4.1 shows the amino acid positions where variation occurs in different animal and human analogue insulin molecules. The dashes indicate a missing amino acid.

Table 4.1

amino acid position	type of insulin					
	human	**cow**	**pig**	**cat**	**short-acting analogue**	**long-acting analogue**
A8	threonine	alanine	threonine	alanine	threonine	threonine
A10	isoleucine	valine	isoleucine	valine	isoleucine	isoleucine
A18	asparagine	–	asparagine	histidine	asparagine	asparagine
A21	asparagine	asparagine	asparagine	asparagine	asparagine	glycine
B3	asparagine	asparagine	asparagine	asparagine	asparagine	–
B28	proline	proline	proline	proline	lysine	proline
B29	lysine	lysine	lysine	lysine	proline	lysine
B30	threonine	alanine	alanine	alanine	threonine	threonine
B31	–	–	–	–	–	arginine
B32	–	–	–	–	–	arginine

(i) Cats with diabetes can be successfully treated with insulin injections. Cat insulin is **not** available, but vets can choose from the other types of insulin shown in Table 4.1.

Identify the type of insulin that is most suitable for treating cats.

..... [1]

(ii) Suggest ways in which analogue insulin molecules can be produced by genetic engineering techniques.

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(c) Information about amino acid and nucleotide sequences is stored in computer databases.

Outline the advantages of using databases of nucleotide sequences to investigate evolutionary relationships between species.

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[Total: 11]

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5. June/2023/Paper_ 9700/42/No.4

Genetic technology uses many different enzymes and techniques.

(a) Restriction endonucleases are used in genetic modification. These enzymes occur naturally in prokaryotic cells. More than 3500 different restriction endonucleases have been identified and it is thought there are many more to discover.

Name **two** domains that are a source of restriction endonucleases.

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..... [1]

(b) Originally, the method used to obtain a restriction endonuclease was to:

- grow large numbers of the specific prokaryotic cells that are the source of the enzyme
- break open the cells and extract and purify the restriction endonuclease.

This original method produced only a small quantity of restriction endonuclease and was **not** economical.

The newer method for large-scale production is to:

- obtain the gene coding for a specific restriction endonuclease
- introduce the gene into *Escherichia coli*, with a promoter that allows the gene to be expressed continuously.

The newer method increases the quantity of specific restriction endonuclease produced.

Suggest **and** explain the steps needed to carry out the newer method for large-scale production of a specific restriction endonuclease.

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(c) Describe the advantages of databases for the study and use of restriction endonucleases.

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(d) Electrophoresis is a technique used in genetic technology.

Paper chromatography is a technique used to investigate the photosynthetic pigments found in chloroplasts.

Compare the similarities and differences between electrophoresis and chromatography.

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[Total: 12]

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6. June/2023/Paper_ 9700/43/No.3

Gene expression in a cell is controlled. When a gene is expressed (switched on), the gene is transcribed. When a gene is **not** expressed (switched off), the gene is **not** transcribed.

Environmental changes can cause some genes to be switched on or switched off.

(a) An example of control of gene expression in prokaryotes is regulation in the *lac* operon.

The *lac* operon is a length of DNA that is made up of different parts.

Fig. 3.1 shows a simple diagram representing the *lacI* (regulatory) gene and the *lac* operon.



Fig. 3.1

(i) Outline the main features of the *lac* operon.

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(ii) Explain the role of the *lacI* gene in the regulation of the *lac* operon.

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(b) The *lac* operon codes for inducible enzymes. Repressible operons code for repressible enzymes.

Suggest **and** explain why it is an advantage to a prokaryote to have a repressible operon.

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[Total: 10]

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7. June/2023/Paper_ 9700/43/No.4

One cause of the genetic disease severe combined immunodeficiency (SCID) is a mutation in the *ADA* gene. This mutation results in a deficiency of the enzyme adenosine deaminase (ADA).

Although ADA is found throughout the body, it is especially active in lymphocytes. The absence of functional ADA causes the build-up of toxic metabolites that kill lymphocytes and damage organs.

Babies are often diagnosed with SCID by six months old. Treatment can greatly improve the life expectancy of children with SCID.

Some treatment options are available.

- Enzyme replacement therapy with recombinant human ADA made by genetically modified (GM) *Escherichia coli*. Weekly intra-muscular injections are given.
- Bone marrow transplant if a well-matched donor, such as a close relative, can be found.
- Gene therapy.

(a) Suggest **and** explain why it may be more appropriate to use enzyme replacement therapy to treat SCID instead of a bone marrow transplant.

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(b) Outline the procedure used for gene therapy treatment of a person with SCID.

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(c) Suggest the social and ethical implications of gene therapy for SCID that need to be considered before treatment is carried out.

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[Total: 10]

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1. Nov/2023/Paper_9700/41/No.4

The potato plant, *Solanum tuberosum*, is an important food crop. Crop yield is reduced if the leaves of the plant are eaten by the larvae (immature stages) of the Colorado beetle, *Leptinotarsa decemlineata*.

Crop scientists used recombinant DNA technology to create two genetically modified (GM) varieties of potato plant. These plants produce proteins that are poisonous to insects.

- GM potato variety **A** contains two new genes, *SN* and *Bt*.
- GM potato variety **B** contains two new genes, *SN* and *OCII*.

The new varieties were tested by having a constant number of Colorado beetle larvae introduced to the plants at time 0 hours. The number of larvae that were alive after 24, 48 and 72 hours was recorded. The percentage of the larvae that had died in each time interval was calculated. This was repeated for potato plants that had not been genetically modified (non-GM).

Table 4.1 shows the percentage of Colorado beetle larvae that had died on the GM potato plant varieties and on non-GM potato plants.

Table 4.1

type of potato plant	percentage of Colorado beetle larvae that had died		
	24 h	48 h	72 h
GM potato variety **A**	50	93	100
GM potato variety **B**	37	70	93
non-GM potato	0	0	0

(a) (i) Suggest what is meant by recombinant DNA technology.

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..... [2]

(ii) Suggest why the scientists created two different types of GM potato plant.

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..... [1]

(iii) State why the scientists also performed the test on non-GM potato plants.

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..... [1]

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(b) Discuss how the results in Table 4.1 provide information that could help to solve the global demand for food.

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[Total: 7]

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2. Nov/2023/Paper_9700/41/No.8

Environmental conditions such as light intensity affect plant physiology.

(a) Use the letter **X** to identify a point on the sketch graph in Fig. 8.1 where light intensity is acting as a limiting factor on the rate of photosynthesis. [1]

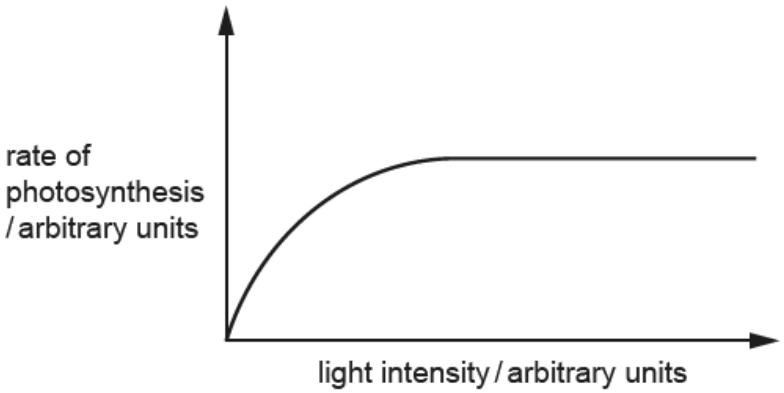


Fig. 8.1

(b) An experiment investigated how light intensity affected gene expression in kale, *Brassica oleracea sabellica*.

- Two groups of kale plants were grown, with one group in high light intensity and one group in low light intensity. All other conditions were standardised.
- After the same period of time, messenger RNA (mRNA) was extracted from each group of kale plants.
- The mRNA was used to produce cDNA.
- The cDNA was hybridised with probes for 89 621 kale genes on a microarray.
- The microarrays showed which genes were switched on (expressed) in each set of conditions.

The results showed that expression of 18% of the genes was affected by light intensity.

- 14% of the genes were switched on only in high light intensity.
- 4% were switched on only in low light intensity.

(i) State the name of the enzyme that produces cDNA from an mRNA template.

..... [1]

(ii) State the name of the type of proteins that control gene expression in plants.

..... [1]

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(iii) Suggest why **more** genes were switched on only in plants growing in **high** light intensity compared to fewer genes that were switched on only in plants growing in low light intensity.

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..... [3]

[Total: 6]

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3. Nov/2023/Paper_9700/41/No.10

Medicine is defined as the diagnosis, treatment and prevention of disease.

Outline the different ways in which genetic technology can be applied to medicine, with reference to named diseases.

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4. Nov/2023/Paper_9700/42/No.4

(a) A marker gene can be used when a gene of interest is introduced into a plant by genetic engineering.

Describe **and** explain how the use of a marker gene coding for a fluorescent product can show that the introduced gene of interest is being expressed in plants.

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(b) Give **two** advantages of genetically engineering herbicide resistance in crop plants such as soybean.

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..... [2]

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(c) DNA microarray analysis is a technique used in genetic technology that involves fluorescence. A DNA microarray has single-stranded probes attached to its surface. These probes hybridise to the fluorescently tagged single-stranded DNA that is added.

(i) Explain why DNA hybridisation occurs between the probe DNA and the added DNA.

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..... [1]

(ii) A DNA microarray analysis can be used to identify the level of expression of some genes.

Describe **and** explain how the level of expression of some genes can be identified using the DNA microarray analysis technique.

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..... [3]

[Total: 10]

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