**19** - (9700-S 2012-Paper 4/2-Q2) - *IMMUNITY*

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.

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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**20** - (9700-S 2012-Paper 4/1-Q2) - *IMMUNITY*

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

**step 1**
A mouse is injected with an antigen, A.

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**step 2**
The mouse is left for a few weeks to allow an immune response to occur.

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**step 3**
Plasma cells (effector B lymphocytes) are extracted from the mouse's spleen.

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**step 4**
Hybridoma cells are formed.

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**step 5**
Each hybridoma cell is isolated and allowed to grow and divide.

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**step 6**
The hybridoma cells producing anti-A antibodies are identified and cultured on a large scale.

**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.

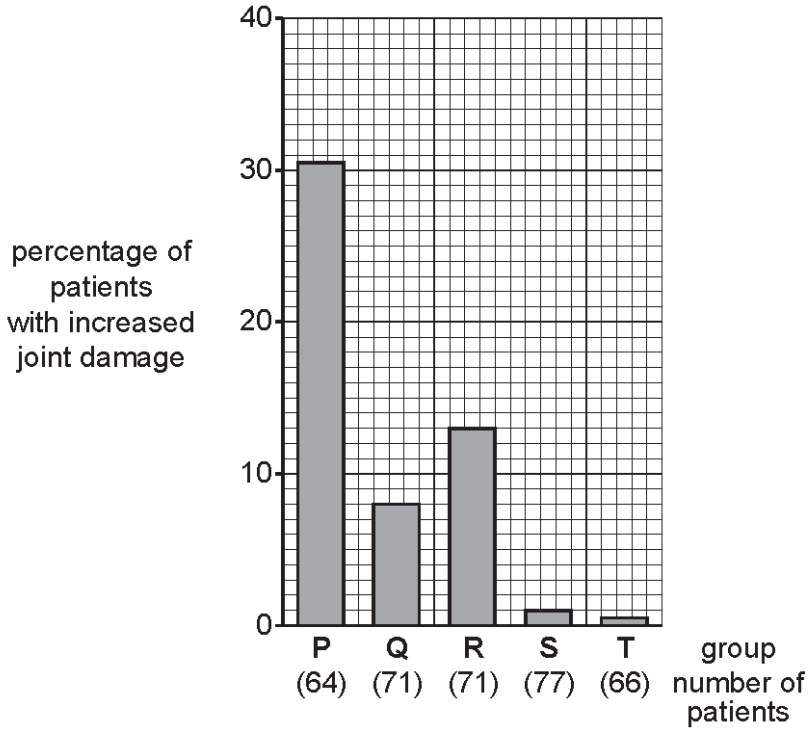


**Fig. 2.2**

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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**21** - (9700-S 2012-Paper 4/1-Q4) - *IMMUNITY, INHERITED CHANGE, SELECTION AND EVOLUTION*

**(a)** Fig. 4.1 shows the structure of a male flower of maize, *Zea mays*.

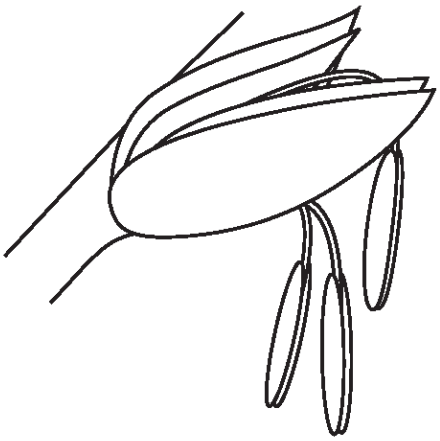

**Fig. 4.1**

With reference to Fig. 4.1, explain how **two** features of this flower adapt it for wind pollination.

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**(b)** The corn borer, *Ostrinia nubilalis*, is an insect pest of maize. The larvae are caterpillars that eat the leaves of the maize plants. The adults can fly. Adult corn borers do not feed on maize plants.

Much of the maize that is grown in the USA has been genetically modified to produce *Bt* toxin, which is lethal to insects that feed on the leaves. However, many populations of the corn borer have now evolved resistance to the *Bt* toxin.

Explain how this resistance could have evolved.

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**(c)** The recessive allele, **r**, of the gene in corn borers confers resistance to *Bt* toxin. Larvae that are homozygous for the normal, dominant allele **R**, or that are heterozygous, are killed when they feed on *Bt* maize.

State the genotype of the corn borers that successfully turn from larvae into adults in the fields where *Bt* maize is grown.

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**(d)** In order to reduce the number of corn borers resistant to *Bt* toxin, farmers in the USA are required to grow up to 50% of their maize as non-*Bt* varieties. The non-*Bt* maize is grown in separate areas, called 'refuges', close to the fields of *Bt* maize. This is called the HDR strategy.

Almost all corn borer larvae feeding on this non-*Bt* maize have the genotypes **RR** or **Rr**. The HDR strategy assumes that, when these become adults, they will interbreed with the adults developing in the *Bt* maize fields.

Explain how the HDR strategy could reduce the proportion of corn borers that are resistant to the *Bt* toxin.

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**(e)** The HDR strategy works only if a high proportion of the adult corn borers developing in the *Bt* fields mate with adult corn borers from the non-*Bt* refuges. An investigation was carried out to determine the extent to which female corn borers mate with males from their own field, or from outside that field.

- Several hundred male and female adult corn borers were marked and then released into a maize field that contained no corn borers.
- After 36 hours, as many corn borers as possible were recaptured from the field and the number of marked and non-marked male and female corn borers was recorded.
- The percentage of the marked females that had mated with marked males was also recorded.
- This was repeated on four more occasions.

The results are shown in Table 4.1.

**Table 4.1**

| trial | percentage of recaptured males that were marked | percentage of recaptured females that were marked | percentage of marked females that had mated | percentage of marked females that had mated with marked males |
|---|---|---|---|---|
| **1** | 30 | 19 | 96 | 10 |
| **2** | 43 | 96 | 100 | 38 |
| **3** | 67 | 83 | 90 | 67 |
| **4** | 25 | 9 | 67 | 50 |
| **5** | 18 | 21 | 100 | 35 |

**(i)** With reference to the two shaded columns in Table 4.1, explain what the results indicate about the degree of mixing between corn borers from different fields.

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**(ii)** With reference to Table 4.1, suggest and explain the implications of the results of this investigation for the effectiveness of the HDR strategy.

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**22** - (9700-W 2012-Paper 4/1-Q2) - *IMMUNITY, INHERITED CHANGE, GENETIC TECHNOLOGY*

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

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**23** - (9700-W 2012-Paper 4/3-Q3) - *BIOLOGICAL MOLECULES, IMMUNITY*

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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**(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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**(c)** Explain why penicillin does not affect viruses.

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**24** - (9700-W 2014-Paper 4/1-Q2) - *IMMUNITY*

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

State:

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

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

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**(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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**(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.

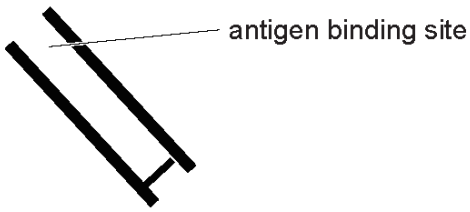


**Fig. 2.1**

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

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

**25** - (9700-S 2015-Paper 4/1-Q10) - *IMMUNITY*

**(a)** Describe the **advantages** of using batch culture for penicillin production and continuous culture for mycoprotein production. [8]

**(b)** Outline the hybridoma method for the production of a monoclonal antibody. [7]

[Total: 15]

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**26** - (9700-W 2015-Paper 4/3-Q2) - *IMMUNITY*

A vaccine, NicVAX, is being developed to help people stop smoking tobacco. Injection of NicVAX into the body causes production of antibody molecules that bind to nicotine.

**(a)** Outline the immune response that leads to the production of these anti-nicotine antibodies.

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**(b)** Mice injected with NicVAX produce B-lymphocytes that mature into cells responsible for the production of antibody (plasma cells).

Outline how these B-lymphocytes can be used to produce monoclonal antibody.

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**(c)** Tobacco smoking during pregnancy has adverse side-effects on the developing fetus.

An investigation was carried out to find out whether vaccinating pregnant women with NicVAX might offer some protection for the developing fetus.

Two different monoclonal antibodies, produced in response to NicVAX, were used in this investigation:

- Nic-IgG
- Nic311.

Nicotine, or nicotine plus one of the monoclonal antibodies, was injected into the maternal circulation. The concentrations of nicotine in the fetal circulation were measured at intervals.

The results of the investigation are shown in Fig 2.1.

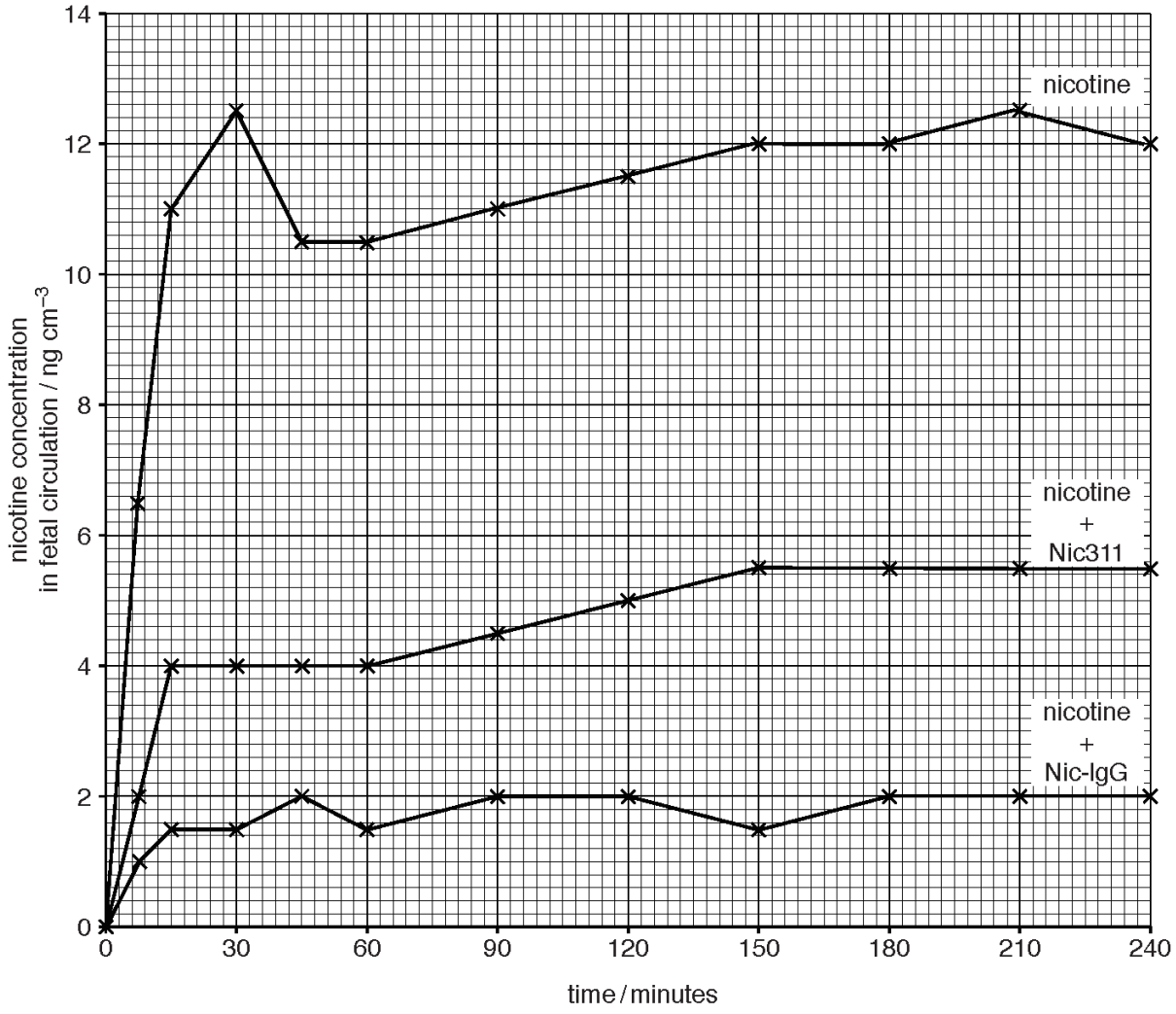


**Fig. 2.1**

**(i)** With reference to Fig. 2.1, describe the results obtained for nicotine only.

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**(ii)** Discuss the extent to which these results support the idea that vaccination with NicVAX could protect the developing fetus of a woman who smokes tobacco.

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**(d)** State **one** medical use of monoclonal antibodies, other than their use in producing vaccines.

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