Name	CTG

YISHUN JUNIOR COLLEGE
JC 2 PREMINARY EXAMINATION 2017

BIOLOGY **9744/02**

HIGHER 2 **28 August 2017**

Monday 1400 – 1600 hr

Paper 2 Structured Questions

2 hours

Candidates answer on the Question Paper.
No Additional Materials are required.

READ THESE INSTRUCTIONS FIRST

Write your name and CTG in the spaces at the top of this page and on all separate answer paper used.

Write in dark blue or black pen only.

You may use a soft pencil for any diagrams, graphs or rough working.
Do not use paper clips, highlighters, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.
The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
Q1	/11
Q2	/9
Q3	/14
Q4	/10
Q5	/16
Q6	/9
Q7	/12
Q8	/10
Q9	/9
Total	**/100**

This paper consists of **26** printed pages.

Answer **all** questions.

1. Palmitoyl tripeptide-1 is made of three amino acids bonded to a molecule of palmitic acid, a component of one form of a triglyceride. It is used in anti-ageing creams to stimulate collagen repair in skin.

The diagram below shows the structure of palmitoyl tripeptide-1.

X

palmitic acid

The structural formulae of the amino acids present in this tripeptide are shown below.

histidine

lysine

glycine

Fig. 1.1

(a) **(i)** Name the bond labelled **X** on Fig. 1.1. [1]

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(ii) Use the diagrams of the individual amino acids in Fig. 1.1 to identify the primary structure of this tripeptide. [1]

Palmitic acid - __________ - __________ - __________

(iii) The molecule is claimed to be better at penetrating the skin due to it having hydrophilic and hydrophobic properties. [1]

Name the part of the molecule which is hydrophobic.

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Collagen is one of the main structural proteins found in skin and contains over 30% glycine. Each collagen molecule contains about 1000 amino acids.

Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin, which is a linear polypeptide composed of about 700 amino acids that are primarily small and nonpolar (for example, glycine, alanine, and valine).

Elastin is also rich in proline and lysine, but contains only a little hydroxyproline and hydroxylysine.

Tropoelastin is secreted by the cell into the extracellular space. There it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited.

Fig. 1.2 on the next page shows the synthesis of elastin and its eventual use in the formation of elastic fibers.

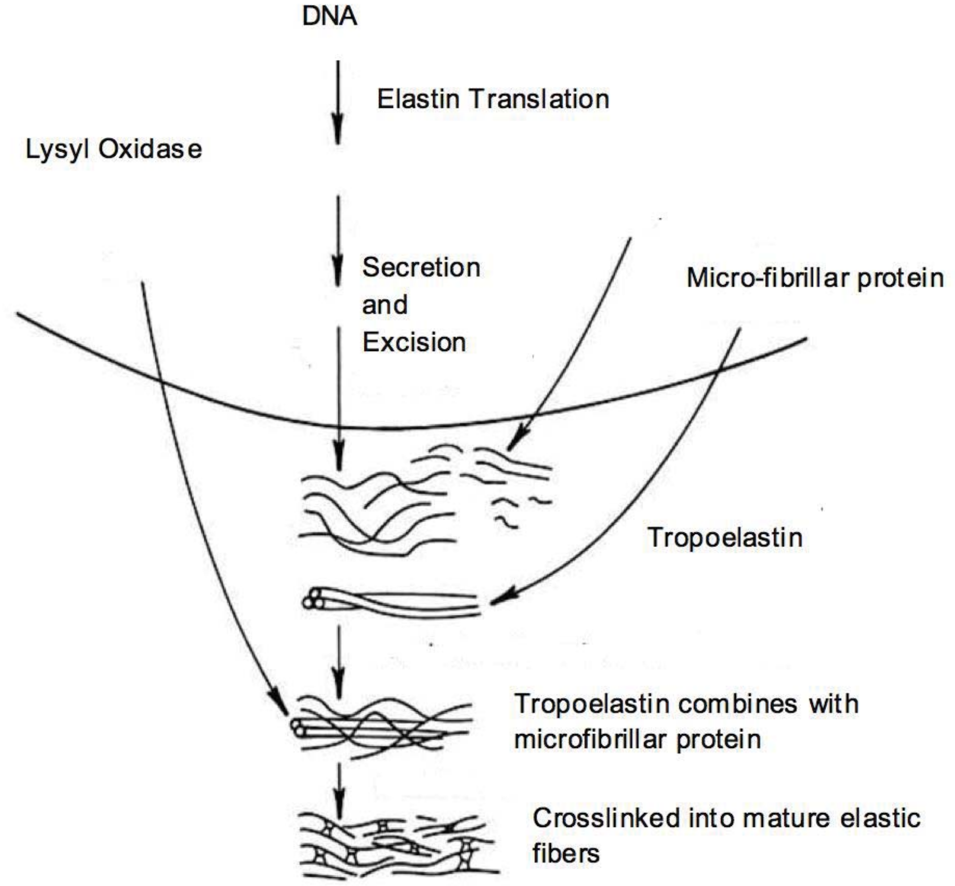

Fig. 1.2

(b) Using the information provided on elastin and collagen, and your own biological knowledge on collagen, state three differences between collagen and elastin. [3]

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(c) State precisely where hydroxylation of amino acids occurs in the biosynthesis of collagen. [1]

(d) Describe how the arrangement of the polymers in collagen differs from cellulose. [2]

(e) The cell surface membrane of a plant cell contains cellulose synthase enzymes. These enzymes make cellulose microfibrils. The synthesised microfibrils pass out through the enzymes as they leave the cell. [2]

CesA gene codes for the catalytic subunit of cellulose synthase. Two alleles have been identified for this gene, allele *A* and *a*.

1. Allele *A* codes for a functional catalytic subunit of cellulose synthase.

2. Allele *a* codes for a non-functional catalytic subunit of cellulose synthase.

Based on observations, plants with genotype *Aa* are usually more susceptible to cell lysis when immersed in hypotonic solution as compared to those with genotype *AA*. Suggest a reason for this observation.

[Total: 11]

2 **(a)** Explain the mode of action of enzymes in terms of enzyme specificity using the induced-fit hypothesis. [3]

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Pepsin is an endopeptidase enzyme found in stomach. It hydroloyses peptide bonds which amino groups are contributed by aromatic amino acids such as tyrosine, tryptophan and phenylalanine.

Fig 2.1 show the graph of how pepsin activity varies with its protein substrate.

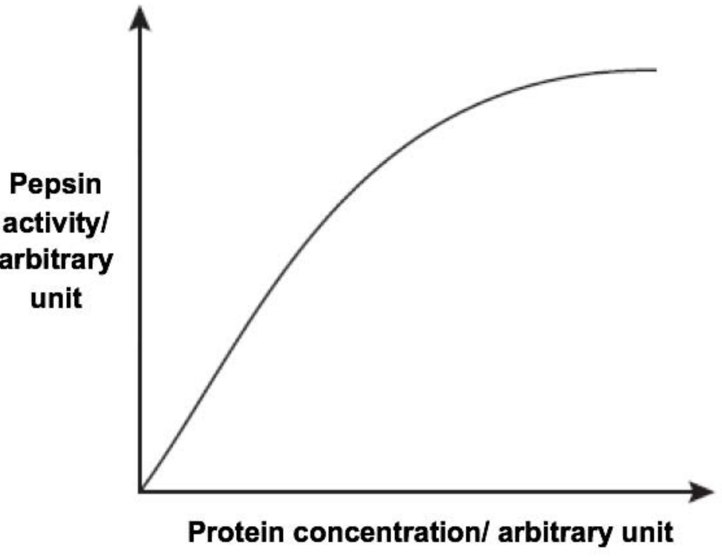

Fig. 2.1

(b) Pepstatin is known to be a non-competitive inhibitor of pepsin.

(i) Explain the effects of pepstatin on pepsin. [4]

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(ii) Draw on Fig 2.1 the graph of how pepsin activity varies with its protein substrate in presence of pepstatin. Annotate the maximal velocity V_{max} and Michaelis constant K_m on the graph you have drawn. [2]

[Total: 9]

3. The discovery in 1953 of the double helix, the twisted-ladder structure of deoxyribonucleic acid (DNA), by James Watson and Francis Crick marked a milestone in the history of science.

Fig. 3.1 shows two deoxyribonucleotides that are commonly found in DNA (a) deoxyadenosine and (b) deoxycytidine.

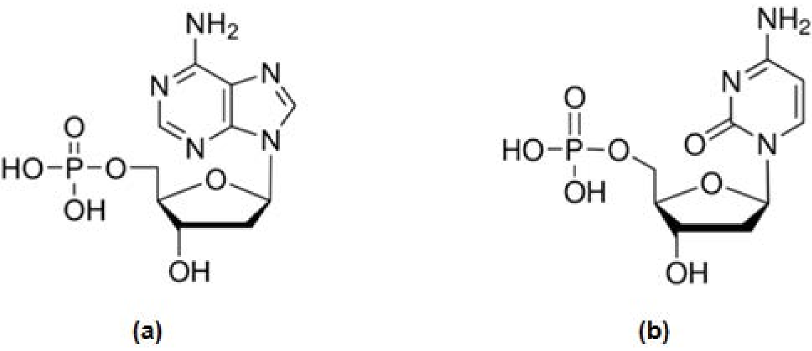

Fig. 3.1

(a) Using Fig. 3.1, draw how deoxyadenosine and deoxycytidine can be used to form a dinucleotide. [2]

(b) Explain the significance of complementary base pairs in DNA. [3]

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Fig. 3.2 is an electron micrograph showing the process of protein synthesis in a prokaryote.

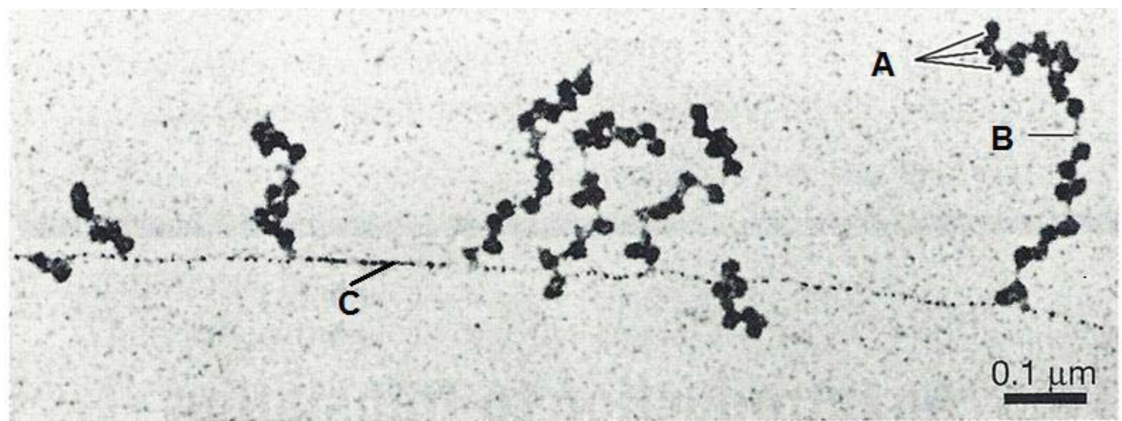

Fig. 3.2

(c) Identify structures **A**, **B** and **C**. [3]

A: ____

B: ____

C: ____

(d) Describe how structure **B** is synthesised in prokaryotes. [3]

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(e) Describe how structure **A** is adapted to its function in protein synthesis. [3]

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[Total: 14]

4 **(a)** In the context of the *lac* operon, describe the organisation of a typical operon. [3]

A student would like to study chemotaxis in bacteria. Chemotaxis is a phenomenon in which a bacterium moves towards a certain chemical (e.g. glucose) or moves away from a certain chemical (e.g. a poison). A bacterium moves by propelling its flagellum and the energy required for this is obtained from bacterial respiration.

The student used a species of bacteria that moves linearly. In one of her experiments, she suspended 2 different strains of the bacteria in strips of semi-solid agar that is soft enough to allow bacterial motility. She is able to observe the tracks made by the moving bacteria. The semi-solid agar contains lactose.

Fig 4.1 shows the results she observed on Day 1 and Day 2.

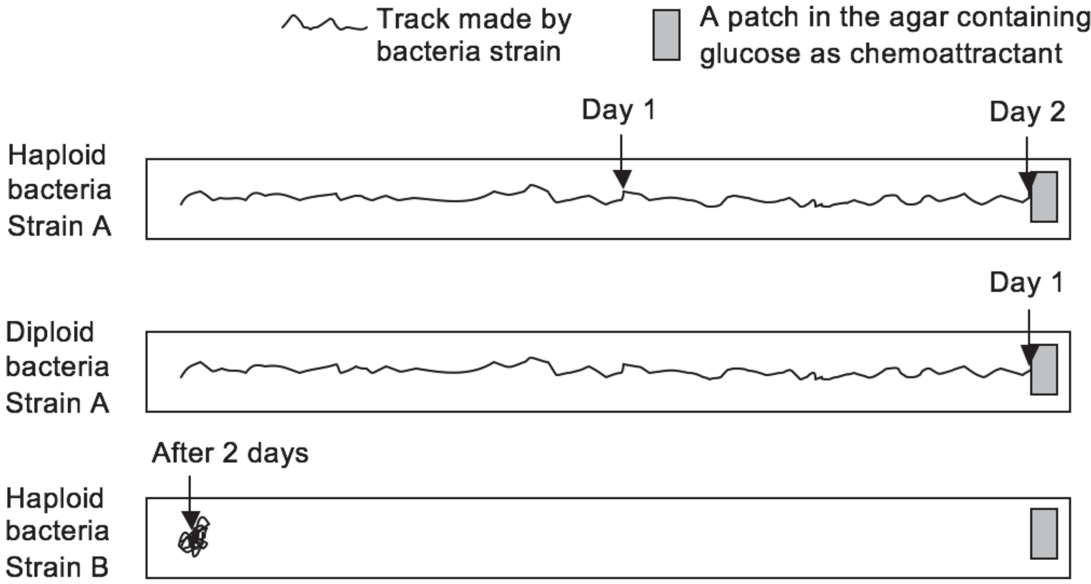

Fig. 4.1

(b) **(i)** Explain why bacteria are able to propel through the semi-solid agar which contains lactose. [3]

(ii) Explain the difference in the results obtained for haploid bacteria strain **A** and diploid bacteria strain **A**. [2]

Haploid bacteria strain B did not show any chemotactic movement after 2 days and the student deduced that strain B has a mutation at the *lac I* gene.

(c) Explain how the mutation at the *lac I* gene results in no movement of the haploid bacteria strain **B** as shown in Fig 4.1. [2]

[Total: 10]

5. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a transmembrane protein which serves as a channel for the movement of chloride ions in and out of cells. This regulates movement of salt and water balance in epithelial cells. Changes in the CFTR genes result in defective CFTR channel proteins which lead to the disease cystic fibrosis (CF).

Although CF patients exhibit the same symptoms, the genetic cause of CF may differ. There are more than 1500 genetic mutations that can result in CF.

The CFTR gene has 27 exons and encodes for 1480 amino acids. Fig. 5.1 shows the effect of some mutations in the CFTR gene on the CFTR channel protein.

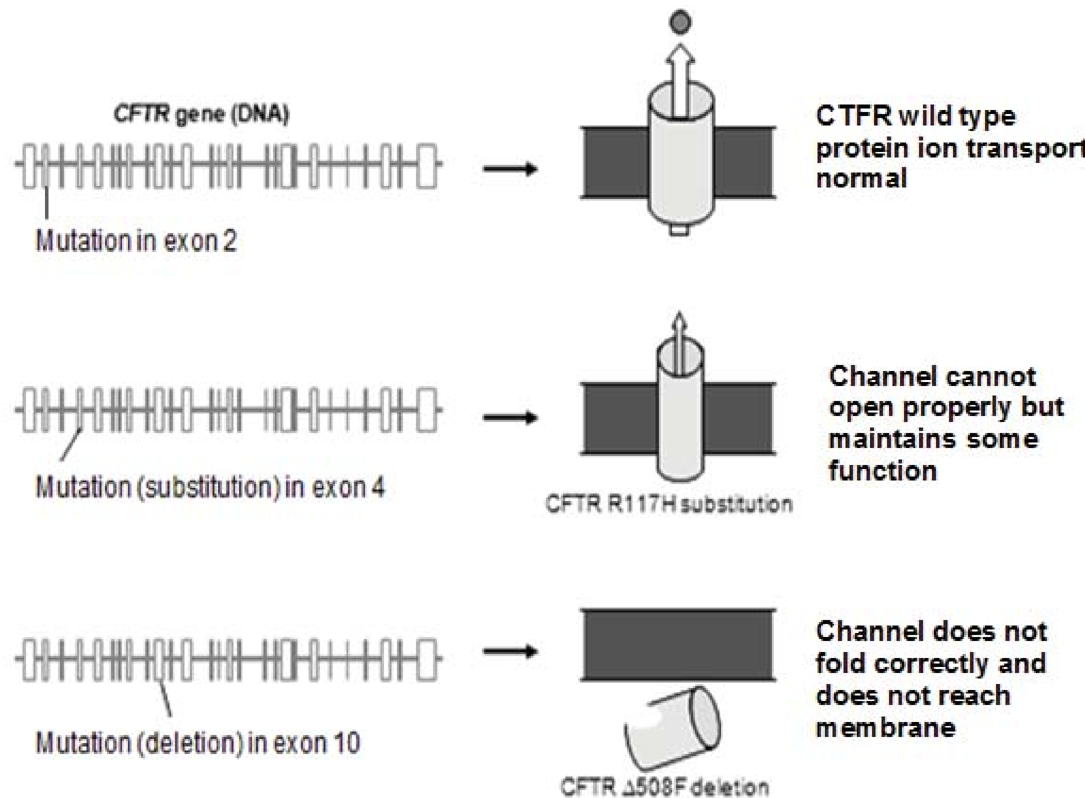

Fig. 5.1

(a) With reference to Fig 5.1,

(i) suggest the type of mutation occurring in exon **2** of the CFTR gene. [1]

(ii) explain why substitution in exon **4** of the CFTR gene results in CFTR channel proteins which cannot open properly but still retain some of the function. [3]

(iii) explain why deletion of one deoxyribonucleotide in exon **10** of the CFTR gene results in misfolded CFTR channel proteins. [3]

Some of the gene mutations resulting in the absence of CFTRs can be detected through the use of an appropriate restriction enzyme. Fig. Fig. 5.2 shows the restriction site of *HpaI*, which coincides with the location of two of these gene mutations.

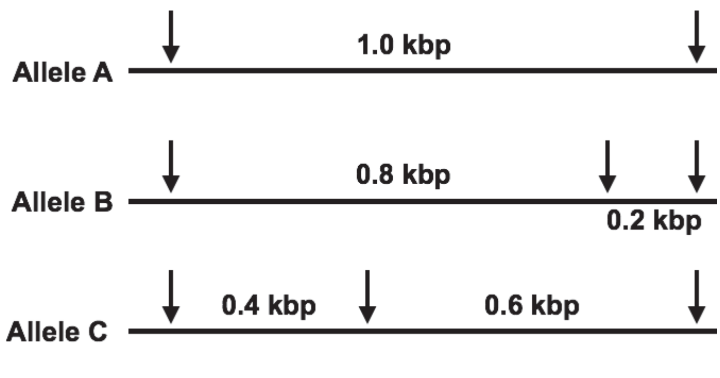

Fig. 5.2

A pedigree of the family where the disease is transmitted through three generations is shown in Fig. 5.3. It corresponded with a Southern blot analysis (shown below the pedigree), where DNA samples from each individual in the family were pre-digested with *HpaI* and probed with an appropriate DNA oligonucleotide sequence.

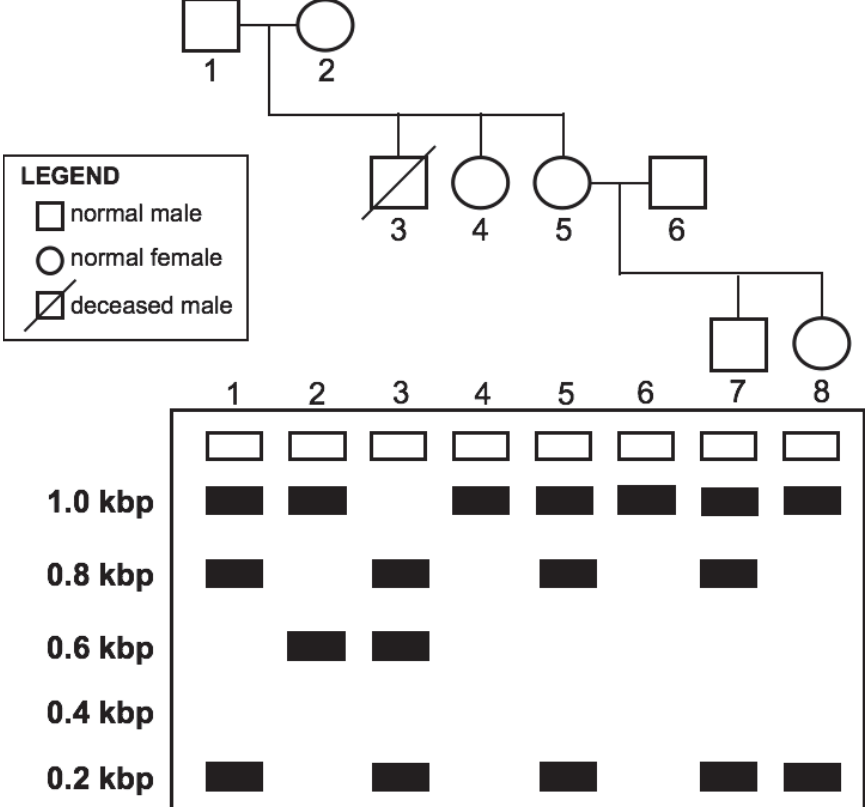

Fig. 5.3

(b) **(i)** Indicate on Fig. 5.2, the position of the probe that gave rise to the above banding patterns. [1]

(ii) Describe the process of Southern blotting to obtain the autoradiograph seen in Fig. 5.3. [4]

(c) With reference to Fig. 5.2 and Fig. 5.3,

(i) state the allele(s) that will result in CF in an individual. [1]

(ii) explain the banding pattern for that individual. [3]

[Total: 16]

6 Fig 6.1 shows how the amount of DNA per nucleus of a coconut plant cell changes over time.

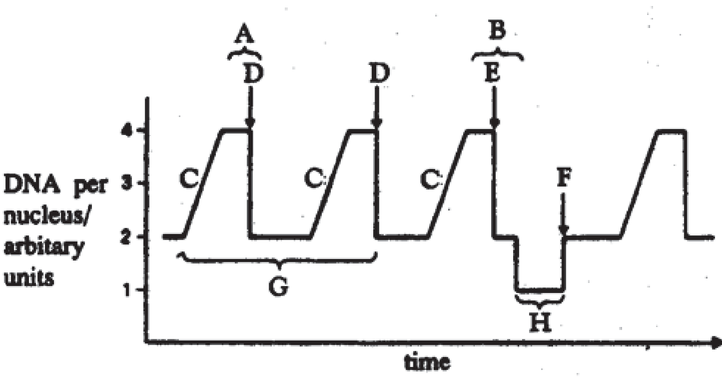

Fig. 6.1

(a) With reference to Fig 6.1,

(i) Contrast the behaviour of chromosomes in stages **A** and **B**. [4]

(ii) Explain the significance of stage **F**. [2]

(iii) Which stage accounts for the double structure of chromosome? Justify your answer. [2]

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(b) Another coconut plant cell went into senescence after stage **F** due to dysregulation cell cycle checkpoints. [1]

Draw on Fig 6.1 the graph of DNA per nucleus the cell against time after stage **F**.

[Total: 9]

7. The common primrose has flowers that vary in the position of their anthers and the length of their styles. These characteristics are controlled by single genes as shown below:

Low anther position	*A*	Long style	*T*
High anther position	*a*	Short style	*t*

Plants, pure breeding for long style and low anther position, were crossed with plants that were homozygous recessive for both characteristics. All the F_1 produced flowers that had low anther positions and long styles.

One of the F_1 offspring was back-crossed with the double homozygous recessive parent. The results of this back-cross are shown below.

Low anther, long style	24
Low anther, short style	10
High anther, long style	13
High anther, short style	25

(a) Use a chi-squared test to determine if the back-cross follows standard Mendelian dihybrid inheritance or otherwise, by completing the table below. [2]

Phenotypes	**Observed (O)**	**Expected (E)**	$\frac{(O-E)^2}{E}$
Low anther, long style	24		
Low anther, short style	10		
High anther, long style	13		
High anther, short style	25		
		χ^2 calculated:	

where χ^2 calculated = $\sum = \frac{(O-E)^2}{E}$

Part of the critical values of the chi-squared distribution is shown below.

Degrees of freedom	**0.90 90%**	**0.80 80%**	**0.70 70%**	**0.50 50%**	**0.30 30%**	**0.20 20%**	**0.10 10%**	**0.05 5%**	**0.02 2%**	**0.01 1%**
1	0.026	0.06	0.15	0.46	1.07	1.64	2.71	3.84	5.41	6.64
2	0.21	0.45	0.71	1.39	2.41	3.22	4.61	5.99	7.82	9.21
3	0.58	1.01	1.42	2.37	3.67	4.64	6.25	7.82	9.84	11.34
4	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	13.39	15.09

(b) Conclude if the observed results follow the expected phenotypic ratio at 5% level of significance. [2]

(c) Draw a genetic diagram using the symbols provided to illustrate the observed results of the back-cross. [5]

(d) Explain the observed results of the back-cross. [3]

[Total: 12]

8. In the MAPK (Mitogen Activated Protein Kinase) pathway, epidermal growth factor (EGF) hormones circulating in the blood are able to trigger transcription within a cell, even though they are unable to enter the cell. This eventually results in the switching on of genes switching and the start of transcription.

Fig. 8.1 shows part of the MAPK pathway.

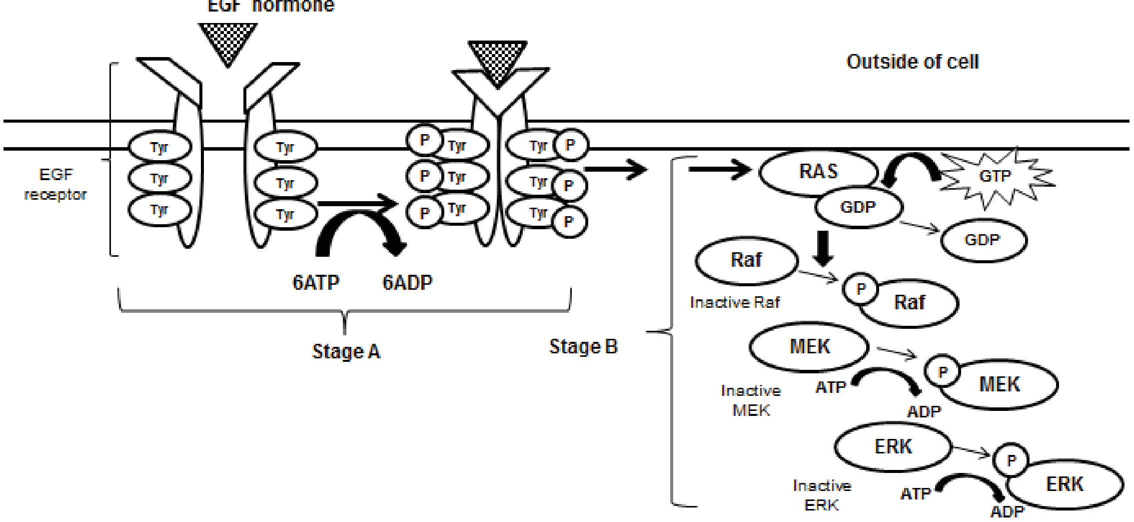

Fig. 8.1

(a) **(i)** Explain why the EGF hormone is unable to enter the cell. [2]

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(ii) With reference to Fig. 8.1, describe stages **A** and **B**. [4]

Stage A: ____

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Stage B: ____

(b) State two advantages of such cell signalling mechanism. [2]

(c) Describe how signalling can be terminated in a GPCR pathway. [2]

[Total: 10]

9. Dengue fever is commonly transmitted by mosquito vector *Aedes aegypti* in Singapore. Factors increasing dengue incidence in Singapore include higher temperature and rapid urbanisation with population growth.

(a) **(i)** Explain how higher temperatures lead to increased dengue incidence. [3]

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(ii) Explain how rapid urbanisation with population growth leads to increased dengue incidence. [2]

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The National Environment Agency promotes the 'Do the Mozzie Wipeout' campaign to urge the community to actively check for, and get rid of stagnant water in their homes by practicing the 5-step Mozzie Wipeout illustrated in Fig 9.1.

Fig. 9.1

(b) Suggest why getting rid of stagnant water in homes using the 5-step Mozzie Wipeout may not necessarily prevent dengue from recurring. [1]

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(c) Outline anthropogenic activities that lead to global warming. [3]

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[Total: 9]

Answer **all** questions.

1. Palmitoyl tripeptide-1 is made of three amino acids bonded to a molecule of palmitic acid, a component of one form of a triglyceride. It is used in anti-ageing creams to stimulate collagen repair in skin.

The diagram below shows the structure of palmitoyl tripeptide-1.

X

palmitic acid

The structural formulae of the amino acids present in this tripeptide are shown below.

histidine

lysine

glycine

Fig. 1.1

(a) **(i)** Name the bond labelled **X** on Fig. 1.1. [1]

- Peptide bond

(ii) Use the diagrams of the individual amino acids in Fig. 1.1 to identify the primary structure of this tripeptide. [1]

- **Palmitic acid - glycine - histidine - lysine**

(iii) The molecule is claimed to be better at penetrating the skin due to it having hydrophilic and hydrophobic properties. [1]

Name the part of the molecule which is hydrophobic.

- **Palmitic acid**

Collagen is one of the main structural proteins found in skin and contains over 30% glycine. Each collagen molecule contains about 1000 amino acids.

Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin, which is a linear polypeptide composed of about 700 amino acids that are primarily small and nonpolar (for example, glycine, alanine, and valine).

Elastin is also rich in proline and lysine, but contains only a little hydroxyproline and hydroxylysine.

Tropoelastin is secreted by the cell into the extracellular space. There it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited.

Fig. 1.2 on the next page shows the synthesis of elastin and its eventual use in the formation of elastic fibers.

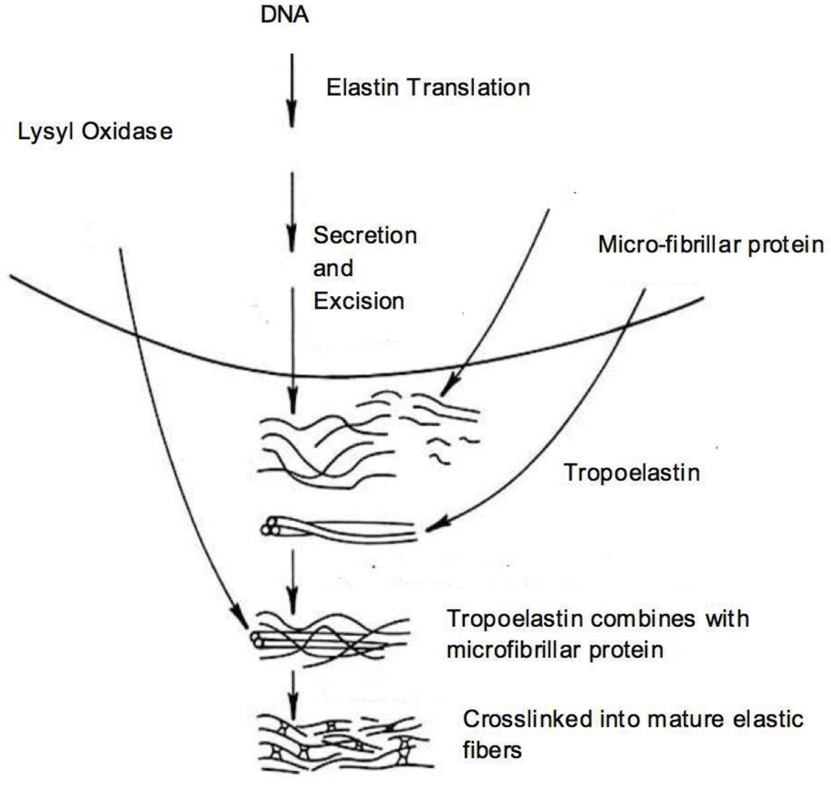


Fig. 1.2

(b) Using the information provided on elastin and collagen, and your own biological knowledge on collagen, state three differences between collagen and elastin. [3]

1. Collagen: repeating units of Glycine-X-Y, where X is Proline and Y is hydroxylysine and hydroxyproline or lysine whereas elastin has no repeating unit.

2. Collagen: hydroxylysine and hydroxyproline are commonly present whereas in elastin - little hydroxylysine and hydroxyproline but has variety of non-polar amino acids such as alanine, glycine and valine.

3. Collagen comprises of a triple helix whereas no triple helix exists in elastin.

4. Tropocollagen units aggregate and self-assemble to form fibrils and fibers, whereas elastin needs microfibrillar protein to desposit the tropoelastin.

5. Each collagen molecule is made of a polypeptide of about 1000 amino acids whereas each elastin molecule is made up of a polypeptide of about 700 amino acids.

(max 3)

(c) State precisely where hydroxylation of the amino acids occurs in the biosynthesis of collagen. [1]

- Lumen of Golgi apparatus / body

(d) Describe how the arrangement of the polymers in collagen differs from cellulose. [2]

- Tropocollagen arranged in **staggered manner** in fibrils where the tropocollagen is held together by **covalent cross links** between C and N terminals of **R groups of lysine and hydroxylysine residues** on adjacent tropocollagen.

- Cellulose chains are arranged parallel to each other, held together by hydrogen bonds formed between the OH groups attached to C3 (of glucose on 1 chain) and C6 of the glucose on adjacent chains.

(e) The cell surface membrane of a plant cell contains cellulose synthase enzymes. These enzymes make cellulose microfibrils. The synthesised microfibrils pass out through the enzymes as they leave the cell. [2]

CesA gene codes for the catalytic subunit of cellulose synthase. Two alleles have been identified for this gene, allele *A* and *a*.

1. Allele *A* codes for a functional catalytic subunit of cellulose synthase.

2. Allele *a* codes for a non-functional catalytic subunit of cellulose synthase.

Based on observations, plants with genotype *Aa* are usually more susceptible to cell lysis when immersed in hypotonic solution as compared to those with genotype *AA*.

Suggest a reason for this observation.

- In plant with with Aa genotype, there is only **1 copy of allele A** for the **expression of functional cellulose synthase.**
- The **concentration of cellulose** in the cell **decreased**, **cell wall** is **weakened**

[Total: 11]

2 **(a)** Explain the mode of action of enzymes in terms of enzyme specificity using the induced-fit hypothesis. [3]

1. The initial conformation of the active site of an enzyme might **not** be **complementary** to the shape of the substrate molecule. When a substrate combines with the enzyme at the active site, it **induces a conformational change** in the **enzyme** structure. This new conformation of the active site is catalytically active and the conformational change causes the amino acids which form the **active site to be moulded into a precise conformation and position.**

2. It **stretches critical bonds in the substrate**, or brings reacting groups on the substrate in **close proximity**, enabling the substrate to fit more snugly into the active site.

3. This **stabilises the transition state** structure and **enables alignment of chemical groups of catalytic amino acid residues in the active site close to the chemical bonds** in the substrate, for the reaction to take place more effectively.

Pepsin is an endopeptidase enzyme found in stomach. It hydroloyses peptide bonds which amino groups are contributed by aromatic amino acids such as tyrosine, tryptophan and phenylalanine.

Fig 2.1 show the graph of how pepsin activity varies with its protein substrate.

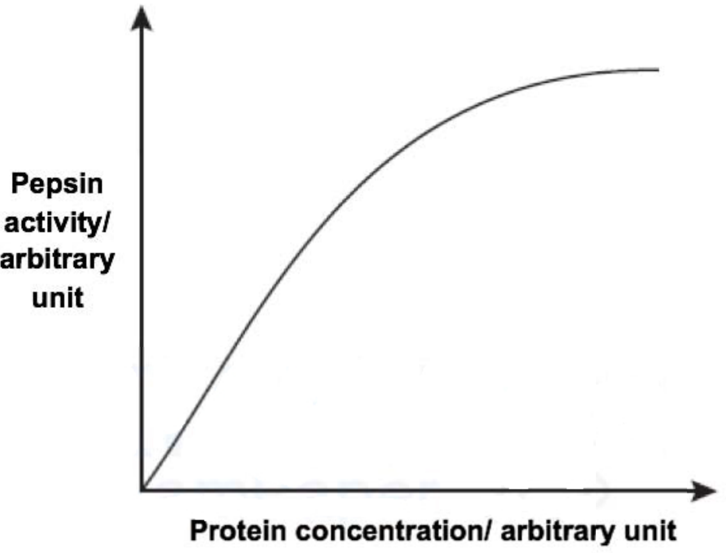


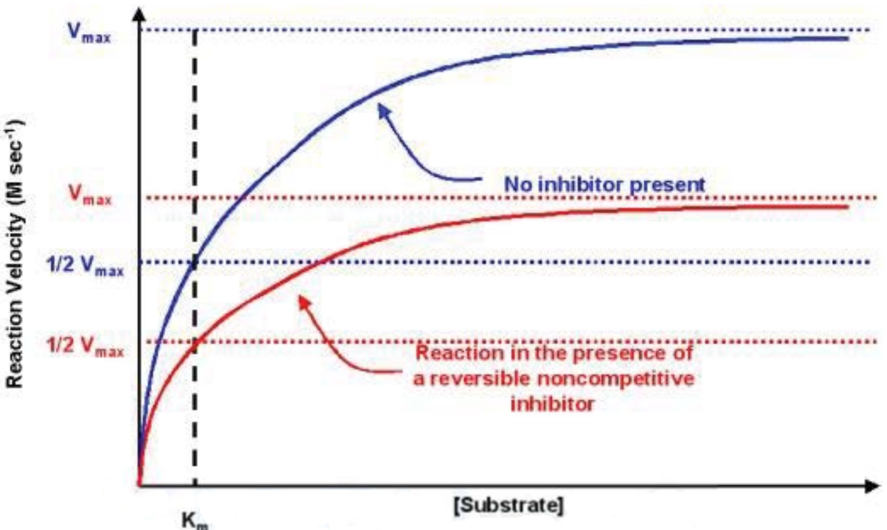
Fig. 2.1

(b) Pepstatin is known to be a non-competitive inhibitor of pepsin.

(i) Explain the effects of pepstatin on pepsin. [4]

1. Pepstatin has **no close structural resemblance to the protein substrate.**
2. Pepstatin **binds to a region other than active site** of pepsin and induces change in its globular **three-dimensional conformation** altering its **active site.**
3. Protein **substrate is still able to bind to the active site** but catalysis cannot take place due to **changes in the nature of the catalytic groups at the active site.**
4. This puts a proportion of pepsin out of action, **lowering rate of pepsin** activity. **Increasing the substrate concentration does not increase rate** of reaction.

(ii) Draw on Fig 2.1 the graph of how pepsin activity varies with its protein substrate in presence of pepstatin. Annotate the maximal velocity V_{max} and Michaelis constant K_m on the graph you have drawn. [2]



[Total: 9]

3. The discovery in 1953 of the double helix, the twisted-ladder structure of deoxyribonucleic acid (DNA), by James Watson and Francis Crick marked a milestone in the history of science.

Fig. 3.1 shows two deoxyribonucleotides that are commonly found in DNA (a) deoxyadenosine and (b) deoxycytidine.

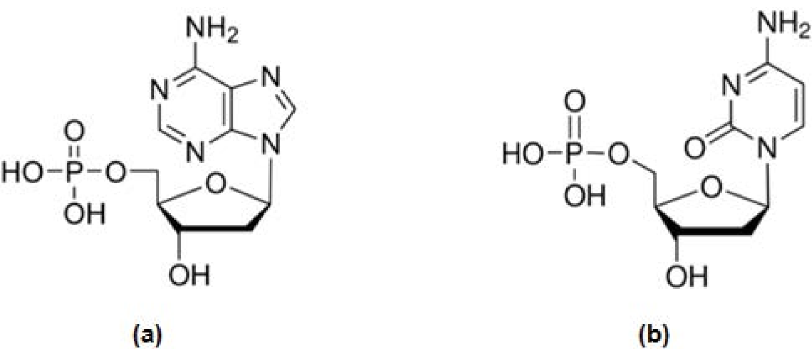


Fig. 3.1

(a) Using Fig. 3.1, draw how deoxyadenosine and deoxycytidine can be used to form a dinucleotide. [2]

(1 mark, indicating removal of 1 H_2O molecule) condensation reaction

+ H_2O

(label) phosphodiester linkage – 1 mark

(b) Explain the significance of complementary base pairs in DNA. [3]

- The **distance between two complementary bases is kept constant** throughout the DNA double helix such that the DNA double helix has a constant diameter.

- The large number of **hydrogen bonds** between complementary bases along the length of the DNA molecule contributes to its **stability**.

- This allows the cell to use **each of the two strands** in the double helix as a **template** for the replication of new DNA strands via complementary base pairing, and for the transmission of genetic information stored in the DNA molecule.

- It allows any **anomalies in the base pairing** to be **easily detected** where there is a **sudden increase/decrease in diameter of the molecule**, thus allows for proofreading in DNA replication.

Fig. 3.2 is an electron micrograph showing the process of protein synthesis in a prokaryote.

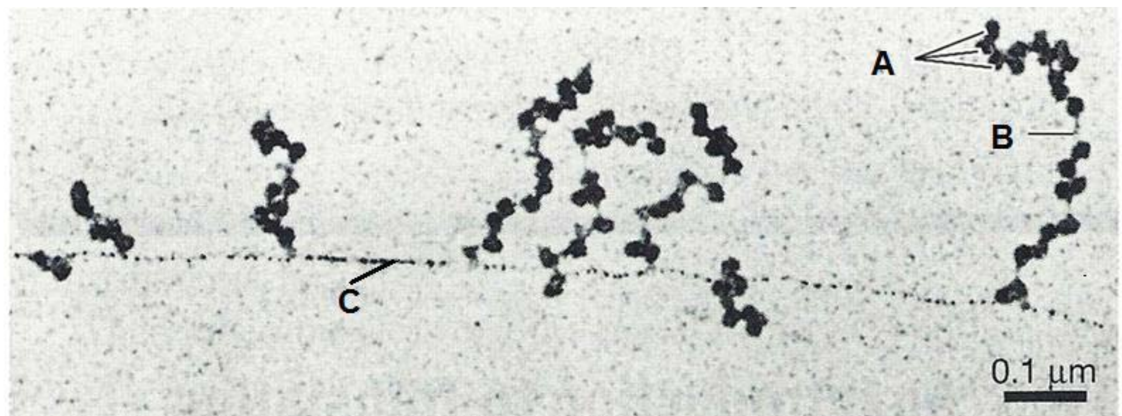


Fig. 3.2

(c) Identify structures **A**, **B** and **C**. [3]

A – 70S ribosomes
B – messenger RNA
C – Deoxyribonucleic acid

(d) Describe how structure **B** is synthesised in prokaryotes. [3]

- **RNA polymerase** recognises and binds to the **promoter** of the gene, causing the DNA double helix to **unwind and separate**;
- 1 of the two DNA strands / the non-coding strand / strand that is read 3' → 5' direction serve as **template strand**;

- Ribonucleotides are added by **complementary base pairing** with the template DNA strand;
- RNA polymerase catalyses **formation of phosphodiester bonds** between adjacent ribonucleotides;

- Transcription proceeds until after the RNA polymerase transcribes a **termination sequence**.

(max 3)

(e) Describe how structure **A** is adapted to its function in protein synthesis. [3]

- **small ribosomal subunit** with recognition / binding site for **initiatior amino acyl tRNA;**
- binding site for **5' UTR of mRNA** to initiate translation;

- **Large ribosomal subunit** has 2 binding sites, P (name) and A (name) sites, to facilitate **complementary base-pairing between anti-codon and codon;**
- **large subunit with P site** to bind **amino-acyl tRNA with growing polypeptide chain;**
- with **A site** to bind **incoming amino-acyl tRNA to be added** to the growing polypeptide chain;
- with **E (exit) site** to allow free **tRNA exit**;

- **large ribosomal subunit** contains **peptidyl transferase** to **catalyse formation of peptide bond;**
- binding site for **translation factors / GTP / release factor** etc.

(max 3)

[Total: 14]

4 **(a)** In the context of the *lac* operon, describe the organisation of a typical operon. [3]

Operon is a sequence of DNA with the following components:

- **Promoter** is located **upstream** of structural genes;
- **Operator** is located downstream of the promoter and upstream of structural genes;

Structural genes

- 3 structural genes (LacZ, LacY and LacA) that **codes for 3 enzymes involved in lactose metabolism;**

A student would like to study chemotaxis in bacteria. Chemotaxis is a phenomenon in which a bacterium moves towards a certain chemical (e.g. glucose) or moves away from a certain chemical (e.g. a poison). A bacterium moves by propelling its flagellum and the energy required for this is obtained from bacterial respiration.

The student used a species of bacteria that moves linearly. In one of her experiments, she suspended 2 different strains of the bacteria in strips of semi-solid agar that is soft enough to allow bacterial motility. She is able to observe the tracks made by the moving bacteria. The semi-solid agar contains lactose.

Fig 4.1 shows the results she observed on Day 1 and Day 2.

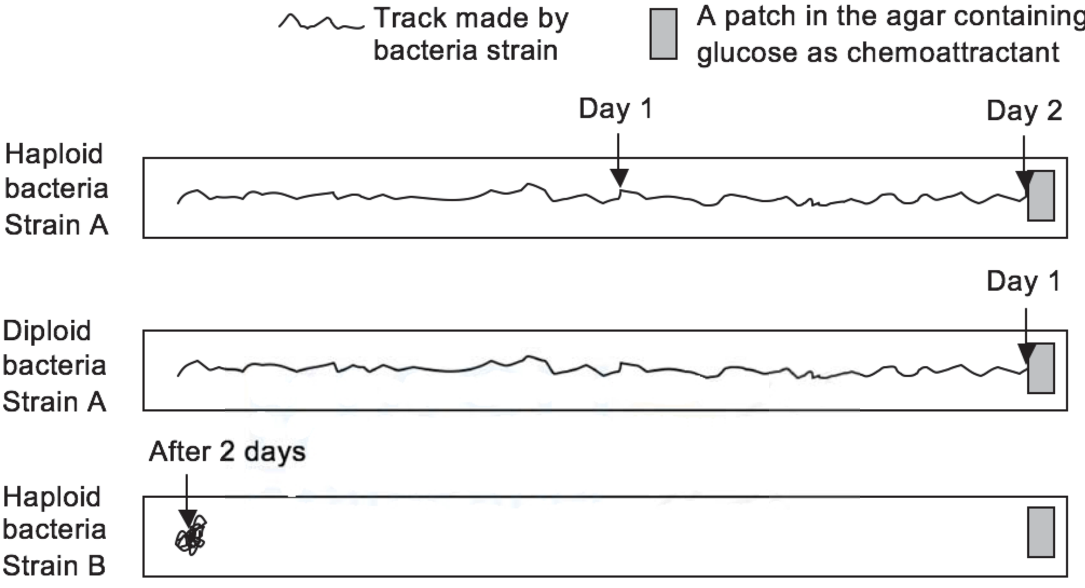


Fig. 4.1

(b) **(i)** Explain why bacteria are able to propel through the semi-solid agar which contains lactose. [3]

- When lactose is present, some lactose is transported into the bacteria cell and converted into inducer, allolactose;
- Allolactose binds to the active repressor protein so that it's three-dimensional conformation is altered and ∴ cannot bind to the operator site;
- RNA polymerase can now transcribe the structural genes, forming a polycistronic mRNA;
- β-galactosidase is synthesized to hydrolyze lactose to release energy to propel flagella;

(max 3)

(ii) Explain the difference in the results obtained for monoploid bacteria strain **A** and diploid bacteria strain **A**. [2]

- Diploid bacteria strain A has two copies of *lac* operon;
- As a result, diploid bacteria can synthesize twice as much β-galactosidase as compared to monoploid bacteria;

Haploid bacteria strain B did not show any chemotactic movement after 2 days and the student deduced that strain B has a mutation at the *lac I* gene.

(c) Explain how the mutation at the *lac I* gene results in no movement of the haploid bacteria strain **B** as shown in Fig 4.1. [2]

- Binding site of repressor protein is no longer complementary to allolactose;
- Presence of lactose did not inactivate repressor;
- Transcription of structural genes cannot take place;
- No enzymes formed, unable to metabolise lactose for energy;

(max 2)

[Total: 10]

5. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a transmembrane protein which serves as a channel for the movement of chloride ions in and out of cells. This regulates movement of salt and water balance in epithelial cells. Changes in the CFTR genes result in defective CFTR channel proteins which lead to the disease cystic fibrosis (CF).

Although CF patients exhibit the same symptoms, the genetic cause of CF may differ. There are more than 1500 genetic mutations that can result in CF.

The CFTR gene has 27 exons and encodes for 1480 amino acids. Fig. 5.1 shows the effect of some mutations in the CFTR gene on the CFTR channel protein.

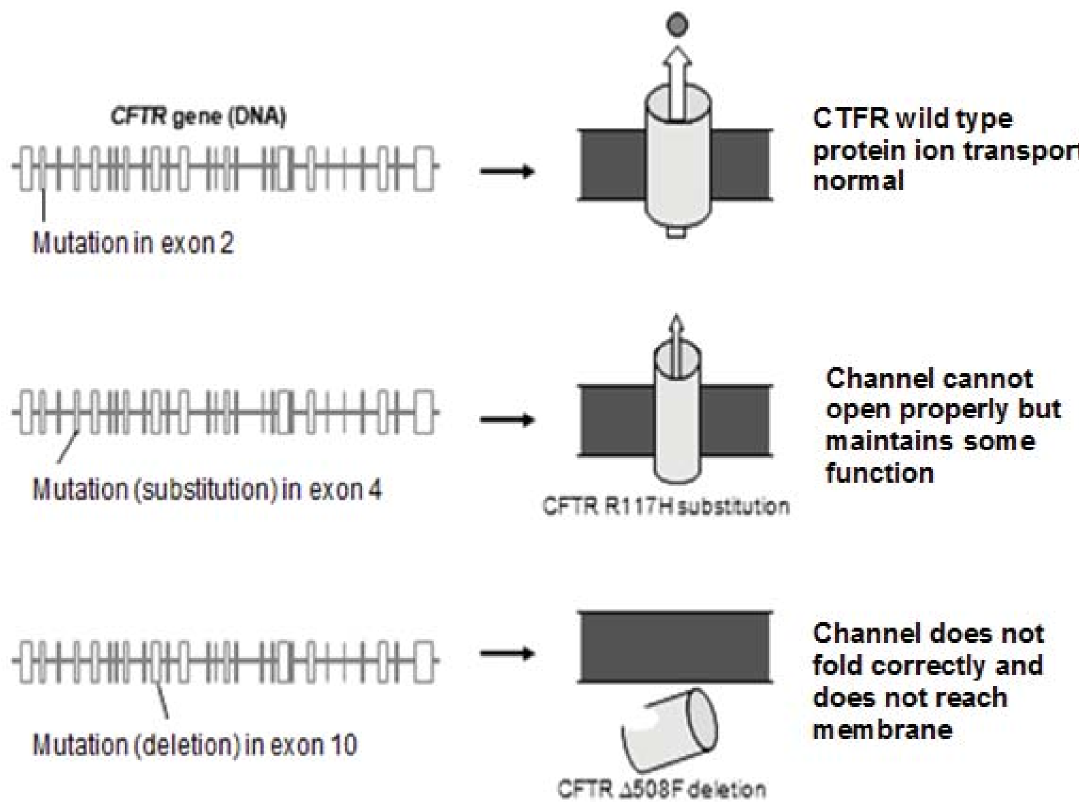


Fig. 5.1

(a) With reference to Fig 5.1,

(i) **s**uggest the type of mutation occurring in exon 2 of the CFTR gene. [1]

- Silent mutation

(ii) explain why substitution in exon 4 of the CFTR gene results in CFTR channel proteins which cannot open properly but still retain some of the function. [3]

- A substitution mutation in the first or second base of the triplet DNA code could result in a new codon;
- which could also result in the formation of a new amino acid;
- with properties similar to the original amino acid so that some function is maintained;

(iii) explain why deletion of one deoxyribonucleotide in exon 10 of the CFTR gene results in misfolded CFTR channel proteins. [3]

- Deletion of one deoxyribonucleotide leads to frameshift mutation;
- Causing a change in subsequent codons on mRNA, resulting in different amino acids *(Reject proteins)* with different R groups;
- Affect the types of bonds formed between amino acids in polypeptide chain;
- Changes three-dimensional conformation of CFTR protein leading to misfolding/ leads to truncated protein due to nonsense mutation;

Some of the gene mutations resulting in the absence of CFTRs can be detected through the use of an appropriate restriction enzyme. Fig. Fig. 5.2 shows the restriction site of *HpaI*, which coincides with the location of two of these gene mutations.

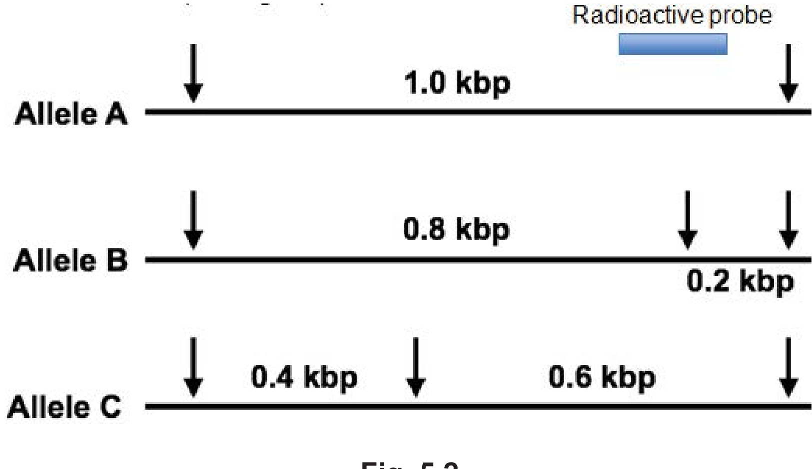


Fig. 5.2

A pedigree of the family where the disease is transmitted through three generations is shown in Fig. 5.3. It corresponded with a Southern blot analysis (shown below the pedigree), where DNA samples from each individual in the family were pre-digested with *HpaI* and probed with an appropriate DNA oligonucleotide sequence.

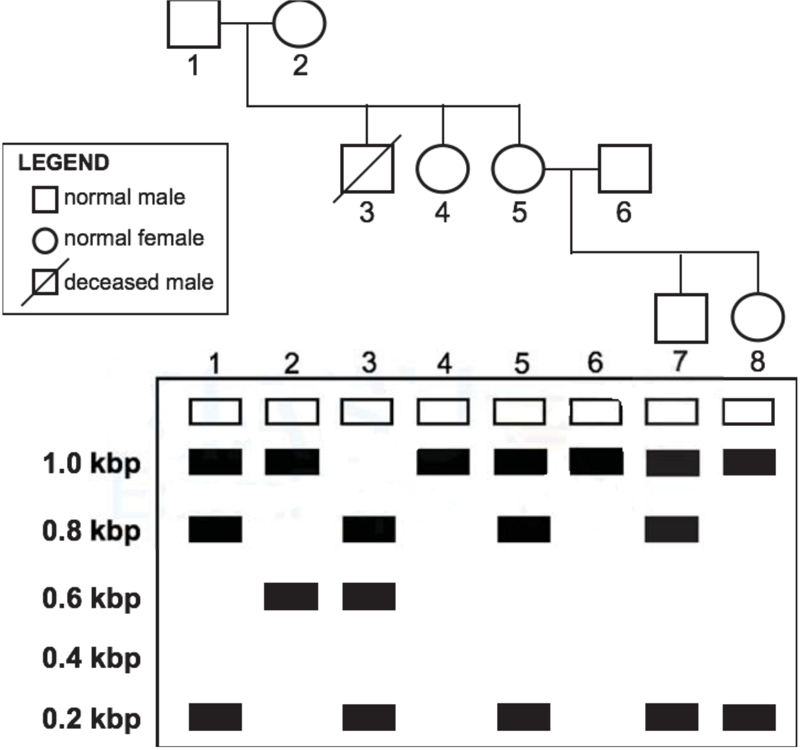


Fig. 5.3

(b) **(i)** Indicate on Fig. 5.2, the position of the probe that gave rise to the above banding patterns. **Ref. to Fig. 5.2** [1]

(ii) Describe the process of Southern blotting to obtain the autoradiograph seen in Fig. 5.3. [4]

Restriction digestion and gel electrophoresis:
- Isolate the DNA segment required from the genomic DNA using *HpaI*;
- Restriction fragments are separated using gel electrophoresis, **according to molecular size** with larger fragments moving a shorter distance.

Denaturation of double-stranded DNA in agarose gel to single-stranded DNA:
- A replica of the DNA bands is made by transferring (or blotting) the DNA on the gel onto a membrane made of nitrocellulose.

- The double-stranded DNA molecules must be denatured by exposing the gel to **alkaline denaturing conditions.**

Hybridisation of DNA with labelled gene probe:
- **Single-stranded radioactively / fluorescent labelled probes** specific for the disease causing gene are allowed to **complementary base-pair** to the target DNA on the nitrocellulose membrane via **nucleic acid hybridization**;

Visualisation of DNA bands:
Either
- **If radioactively labelled gene probes are used,** the DNA bands with probes bound to them are **visualised by autoradiography;**

OR

If **fluorescent labelled gene probes** are used, the DNA bands with probes bound to them are **visualized under ultraviolet light.**

(max 4)

(c) With reference to Fig. 5.2 and Fig. 5.3,

(i) state the allele(s) that will result in CF in an individual. [1]

- Alleles B and C

(ii) explain the banding pattern for that individual. [3]

- 3 fragments observed in the banding pattern of **individual 3**;
- Individual 3 inherited Allele B from father and Allele C from mother;
- Allele B from Individual 1 / father has a restriction site separating the fragments which is detected by the probe into 0.8 and 0.2 kbp;
- Allele C from Individual 2 / mother has a restriction site at another location, resulting in a 0.6kbp fragment detected by the probe;

[Total: 16]

6 Fig 6.1 shows how the amount of DNA per nucleus of a coconut plant cell changes over time.

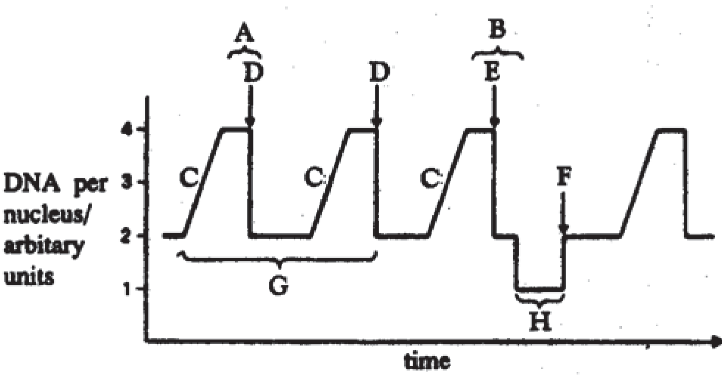


Fig. 6.1

(a) With reference to Fig 6.1,

(i) Contrast the behaviour of chromosomes in stages **A** and **B**. [4]

Stage A (Mitosis)	**Stage B (Meiosis)**
Homologous chromosomes **do not pair up** in prophase	Homologous chromosome **pair up** to form a **bivalent** in **prophase I**
There is **no chiasma formation and crossing** over occurring between homologous chromosomes	**Chiasma formation and crossing over** occur between **non-sister chromatids** of homologous chromosomes
Chromosomes are arranged in a **single row** at the equator of the spindle during metaphase	Chromosomes are arranged in a **double row** / two rows at the equator of the spindle during metaphase I
1. It involves the separation of **sister chromatids** at anaphase 2. amount of DNA per nucleus decreased from 4 to 2 arbitrary units	1. It involves the separation of **homologous chromosomes** at anaphase I and separation of **sister chromatids** at anaphase II. 2. Amount of DNA in the nucleus decreased from 4 to 2 arbitrary units and subsequently to 1 arbitrary unit.

(ii) Explain the significance of stage **F**. [2]

- During stage F (fertilisation), the nuclei of the haploid male and female gametes fuse to produce a zygote with diploid number of chromosomes for each species, **restoring the diploid condition** of the plant cell + data quoting: 1 AU to 2 AU
- **Random** fertilisation of gametes further **increases genetic variation** within a population.

(iii) Which stage accounts for the double structure of chromosome? Justify your answer. [2]

- Stage C
- DNA replication during S phase of interphase results in **two genetically identical sister chromatids** joined at the centromere of each chromosome + data quoting: 2 AU to 4 AU

(b) Another coconut plant cell went into senescence after stage **F** due to dysregulation cell cycle checkpoints. [1]

Draw on Fig 6.1 the graph of DNA per nucleus the cell against time after stage **F**.

- **Horizontal straight line graph after time point indicated by stage F**

[Total: 9]

7. The common primrose has flowers that vary in the position of their anthers and the length of their styles. These characteristics are controlled by single genes as shown below:

Low anther position	*A*	Long style	*T*
High anther position	*a*	Short style	*t*

Plants, pure breeding for long style and low anther position, were crossed with plants that were homozygous recessive for both characteristics. All the F_1 produced flowers that had low anther positions and long styles.

One of the F_1 offspring was back-crossed with the double homozygous recessive parent. The results of this back-cross are shown below.

Low anther, long style	24
Low anther, short style	10
High anther, long style	13
High anther, short style	25

(a) Use a chi-squared test to determine if the back-cross follows standard Mendelian dihybrid inheritance or otherwise, by completing the table below. [2]

Phenotypes	Observed (O)	Expected (E)	$\frac{(O-E)^2}{E}$
Low anther, long style	24	**18**	**2.00**
Low anther, short style	10	**18**	**3.56**
High anther, long style	13	**18**	**1.39**
High anther, short style	25	**18**	**2.72**
		χ^2 calculated:	**9.67 (3 s.f.)**

1. **1 mark for correct calculation of values in both columns**
2. **1 mark for correct χ^2 value to 3 s.f.**

where χ^2 calculated = $\sum \; = \frac{(O-E)^2}{E}$

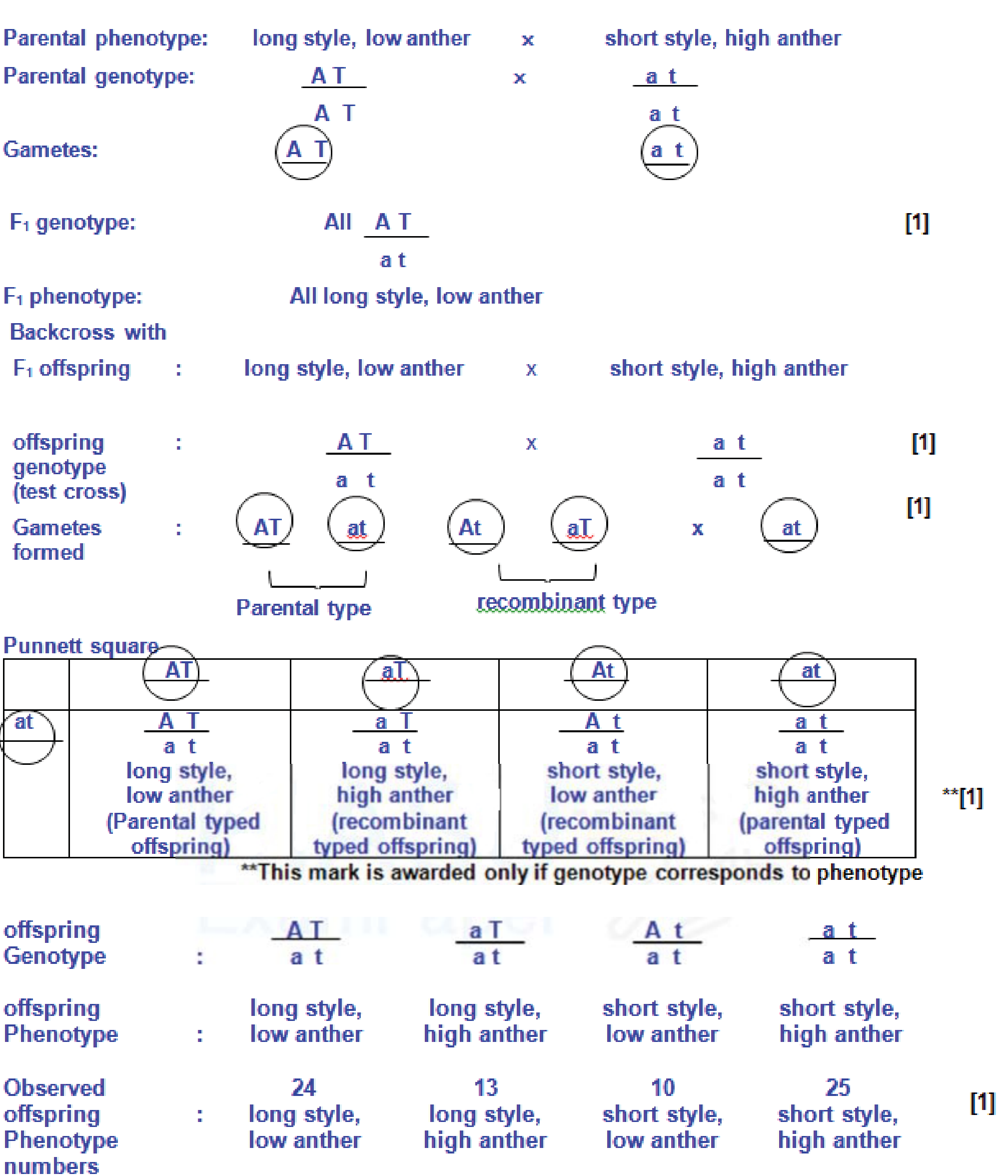
Part of the critical values of the chi-squared distribution is shown below.

Degrees of freedom	0.90 90%	0.80 80%	0.70 70%	0.50 50%	0.30 30%	0.20 20%	0.10 10%	0.05 5%	0.02 2%	0.01 1%
1	0.026	0.06	0.15	0.46	1.07	1.64	2.71	3.84	5.41	6.64
2	0.21	0.45	0.71	1.39	2.41	3.22	4.61	5.99	7.82	9.21
3	0.58	1.01	1.42	2.37	3.67	4.64	6.25	7.82	9.84	11.34
4	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	13.39	15.09

(b) Conclude if the observed results follow the expected phenotypic ratio at 5% level of significance. [2]

- Given that X^2 calculated is greater than X^2 critical, 9.67 > 7.82 there is **significant difference** between observed and expected, and is not due to chance.
- The null hypothesis is therefore rejected and the observed phenotypic ratio does not follow the expected phenotypic ratio of 1:1:1:1, and the difference is due to **incomplete autosomal linkage** between the 2 genes.

(c) Draw a genetic diagram using the symbols provided to illustrate the observed results of the back-cross. [5]



(d) Explain the observed results of the back-cross. [3]

1. The **majority of the flowers** produced from the back-cross have parental phenotypes of long style, low anther position and short style, high anther position.

   This means that the two genes for length of style and position of anther are **incompletely linked** and found on the **same chromosomes**.

AND any 2 below:

2. Since the 2 gene loci are linked, there is a higher probability that parental phenotypes will predominate. This is because linked genes **do not assort independently** but tend to stay together in the **same combinations** as they were in the parents.

3. The offspring phenotypes: **long style, high anther position and short style, low anther position**, are **recombinant phenotypes** as a result of **crossing over**.
4. **Since crossing over is a chance event**, which may or may not occur during prophase I of meiosis, **probability of recombinant phenotypes is lower.**

OR
Point #1 AND any 2 below:

2. **Crossing over in prophase I** occurs as chiasma is formed between the loci of the two genes coding for length of style and position of anther, parental gametic genotype combinations are **AT** and **at**.

3. This allows for the exchange of alleles between non-sister chromatids of homologous chromosomes so that a new combination of alleles is formed in the resultant gametes: **At** and **aT**.

4. giving rise to recombinant offspring with long style, high anther position and short style, low anther position, when the gamete with the genotype **At**, or with the genotype **aT** fuse with the gamete with the genotype **at** from a with plant that is homozygous recessive for both characteristics used in the test cross, forming an offspring with the genotype **At / at** and **aT/ at**, respectively.

[Total: 12]

8. In the MAPK (Mitogen Activated Protein Kinase) pathway, epidermal growth factor (EGF) hormones circulating in the blood are able to trigger transcription within a cell, even though they are unable to enter the cell. This eventually results in the switching on of genes switching and the start of transcription.

Fig. 8.1 shows part of the MAPK pathway.

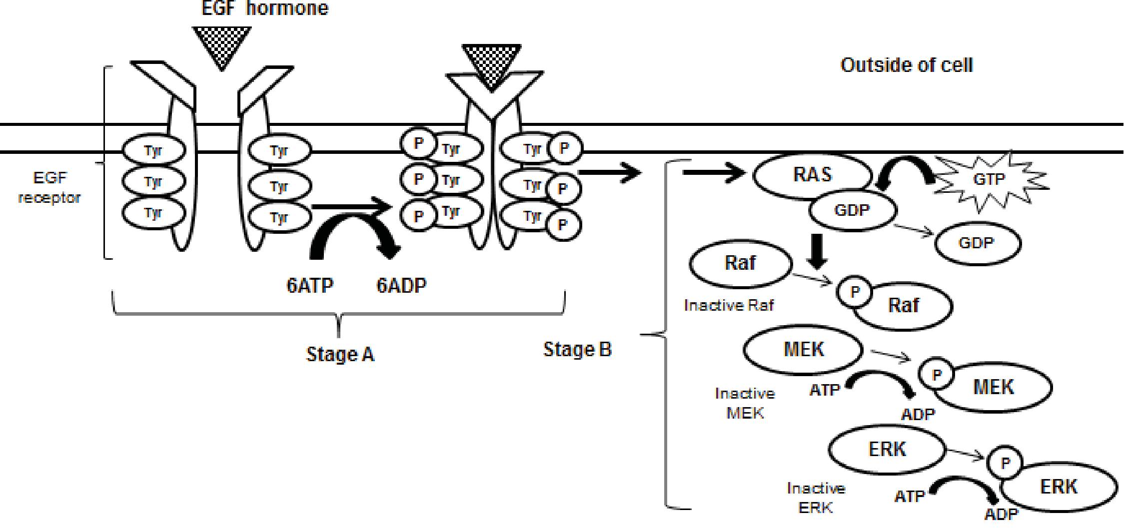


Fig. 8.1

(a) **(i)** Explain why the EGF hormone is unable to enter the cell. [2]

- EGF is a protein hormone
- that are too large to be transported by channel/carrier proteins
- hydrophilic / polar, hence, unable to pass through hydrophobic core of phospholipid bilayer / hydrophobic fatty acid tails

(ii) With reference to Fig. 8.1, describe stages **A** and **B**. [4]

Stage A (2 marks):

- **EGF** binds to the **complementary receptor** to form **dimer**
- This **activates** the catalytic **tyrosine kinase** tail of the receptor protein **by phosphorylating tyrosine residues using ATP**

Stage B (max 2):

- **EGF receptor dimer** causes **Ras protein to GDP with GTP** ; **activate Ras protein**,
- which then activates a **phosphorylation cascade**

Max 1 from the points below:

- **activated Ras in turn activates / phosphorylates Raf**
- **activated Raf activates/phosphorylates MEK**
- which in turn **activate/phosphorylates ERK**

(b) State two advantages of such cell signalling mechanism. [2]

- Signal Amplification (with brief description);
- Coordinated responses, same ligand different cells different gene expressio

(c) Describe how signalling can be terminated in a GPCR pathway. [2]

- GTPases, hydrolysis of GTP to GDP
- Protein phosphatases, removal of phosphate group from the protein kinases.
- Degradation of ligand

[Total: 10]

9. Dengue fever is commonly transmitted by mosquito vector *Aedes aegypti* in Singapore. Factors increasing dengue incidence in Singapore include higher temperature and rapid urbanisation with population growth.

(a) **(i)** Explain how higher temperature leads to increased dengue incidence. [3]

1. Mosquito vectors *Aedes aegypti* are **sensitive to temperature changes** as immature stages in the aquatic environment and as adults. Larvae take a **shorter time to mature** in warmer waters so there is a greater capacity to produce more offspring during the transmission period.
2. In warmer climates, adult female mosquitoes **digest blood faster and feed more frequently,** thus increasing transmission intensity.
3. Dengue viruses complete **extrinsic incubation within the female mosquito in a shorter time** as temperature rises, thereby increasing the proportion of infective vectors.

(ii) Explain how rapid urbanisation with population growth leads to increased dengue incidence. [2]

1. Rapid urbanisation with population growth creates **more habitats** for the mosquito vectors *Aedes aegypti* because it is **well-adapted to living in the urbanised environment**

2. Difficult to monitor multiple hidden corners which could be **water-logged** for female *Aedes aegypti* to **lay eggs** and **hatch larvae**

The National Environment Agency promotes the 'Do the Mozzie Wipeout' campaign to urge the community to actively check for, and get rid of stagnant water in their homes by practicing the 5-step Mozzie Wipeout illustrated in Fig 9.1.

Fig. 9.1

(b) Suggest why getting rid of stagnant water in homes using the 5-step Mozzie Wipeout may not necessarily prevent dengue from recurring. [1]

- *Aedes aegypti* mosquitoes have adapted such that their eggs can **survive dry conditions/ dessication** for several months. If eggs are laid in a dried-up container, new mosquitoes only develop when the container is filled with water e.g. during rainy period + ref step 2, 4, 5 not always useful.

(c) Outline anthropogenic activities that lead to global warming. [3]

1. Burning of fossil fuels linked to increasing energy usage;
2. Clearing of forests representing a loss of carbon sink;
3. Increasing consumption of meat that is more resource-intensive

(any 2)

4. lead to increased emission of greenhouse gases such as carbon dioxide and methane. Enhanced greenhouse effect of Earth leads to global warming.

[Total: 9]

Name	CTG

YISHUN JUNIOR COLLEGE
JC 2 PRELIMINARY EXAMINATION 2017

BIOLOGY **9744/04**

HIGHER 2 **21 AUGUST 2017**
Paper 4

2 hours 30 minutes

Candidates answer on the Question Paper

Additional material: As listed in the Confidential Instructions

YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE

READ THESE INSTRUCTIONS FIRST

Write your name and CTG in the spaces at the top of this page and on all the work you hand in.
Give details of the practical shift and the laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen only.

You may use a HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.

The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	/25
2	/16
3	/14
Total	**/55**

This paper consists of **16** printed pages.

Question 1

The beetroot is a starchy edible root from the *Beta vulgaris* plant. It has long been used for medicinal purposes, primarily for disorders of the liver as it helps to stimulate the liver's detoxification processes. The water-soluble plant pigment that the gives beetroot its rich, purple-crimson colour is betanin and is typically contained in the vacuoles of beetroot cells. Betanin is a glucoside, and hydrolyses into the sugar glucose and its constituent nitrogenous compounds, which are colourless. Fig. 1.0 shows the molecular structure of betanin.

Fig 1.0

You are required to investigate the relationship between temperature and the rate of diffusion of betanin. The use of hydrochloric acid would yield information on the quantity of betanin generated from the experiment.

Safety information:

	You should wear eye protection throughout this practical. The coloured beetroot pigment will stain skin or clothes.
	0.6M hydrochloric acid (HCl) is a potential irritant. Avoid contact with eyes and skin.

You are provided with:

- a piece of fresh beetroot tissue
- a bottle of 0.6M hydrochloric acid

Proceed as follows:

1. Place the fresh beetroot tissue provided on a white tile. Using a cork borer, cut out cylinders of beetroot. Cut 15 pieces of beetroot discs of approximately 5 mm thickness from the cylinder using a scalpel.

2. Place all the cut beetroot discs in a small beaker of distilled water for three minutes.

3. Label five boiling tubes – 30°C, 40°C, 50°C, 60°C and 70°C.

4. Label five test tubes - 30°C, 40°C, 50°C, 60°C and 70°C.

5. Add 10 cm^3 distilled water to each boiling tube.

6. Set up a water bath in the 500 cm^3 beaker with 40°C water (taken from the thermostatically - controlled water bath).

7. Place the boiling tube labelled 70°C into the water bath you prepared. Heat the water gently until the temperature in the boiling tube reaches that of the water bath at 70°C. Use a thermometer to monitor the temperature.

8. When the temperature reaches 70°C, remove the boiling tube from the heat source and place in the rack.

9. Take three of the beetroot discs and impale on a satay stick with space between each disc.

10. Immediately immerse the impaled beetroot discs in the boiling tube labelled 70°C for exactly one minute.

11. Leave the impaled beetroot discs to stand in the boiling tube for a further 5 minutes.

12. When five minutes is up, remove the impaled beetroot discs from the boiling tube.

13. Repeat steps 7 to 12 for the remaining temperatures - 30°C, 40°C, 50°C and 60°C. Add tap water or shaved ice, where necessary, to attain and maintain the temperature. Use the thermometer to monitor the temperature.

14. Stir the solution in the boiling tube labelled 70°C with a clean glass rod and transfer 2 cm^3 of the solution into the test tube labelled 70°C using a clean Pasteur pipette.

15. Using a syringe, draw up 10 cm^3 of 0.6M hydrochloric acid (HCl).

16. Add 1 cm^3 of the 0.6M HCl into the test tube labelled 70°C and shake gently for 20 seconds. Continue adding 0.6M HCl and shaking the reaction mixture following each addition of HCl, until the solution decolourises completely.

17. Note the volume of 0.6M HCl required for complete decolourisation of the solution.

18. Perform another replicate by repeating steps 14 to 17.

19. Repeat steps 14 to 18 for the remaining temperatures - 30°C, 40°C, 50°C and 60°C.

20. Record your observations, **including the volume of 0.6M HCl used,** in the space provided on the next page.

(a) (i) Prepare the space below to record your observations.

[5]

(ii) A similar experiment was conducted by another student and he obtained the following results shown in Table 1.1. Calculate the rate of decolourisation by filling in the table below.

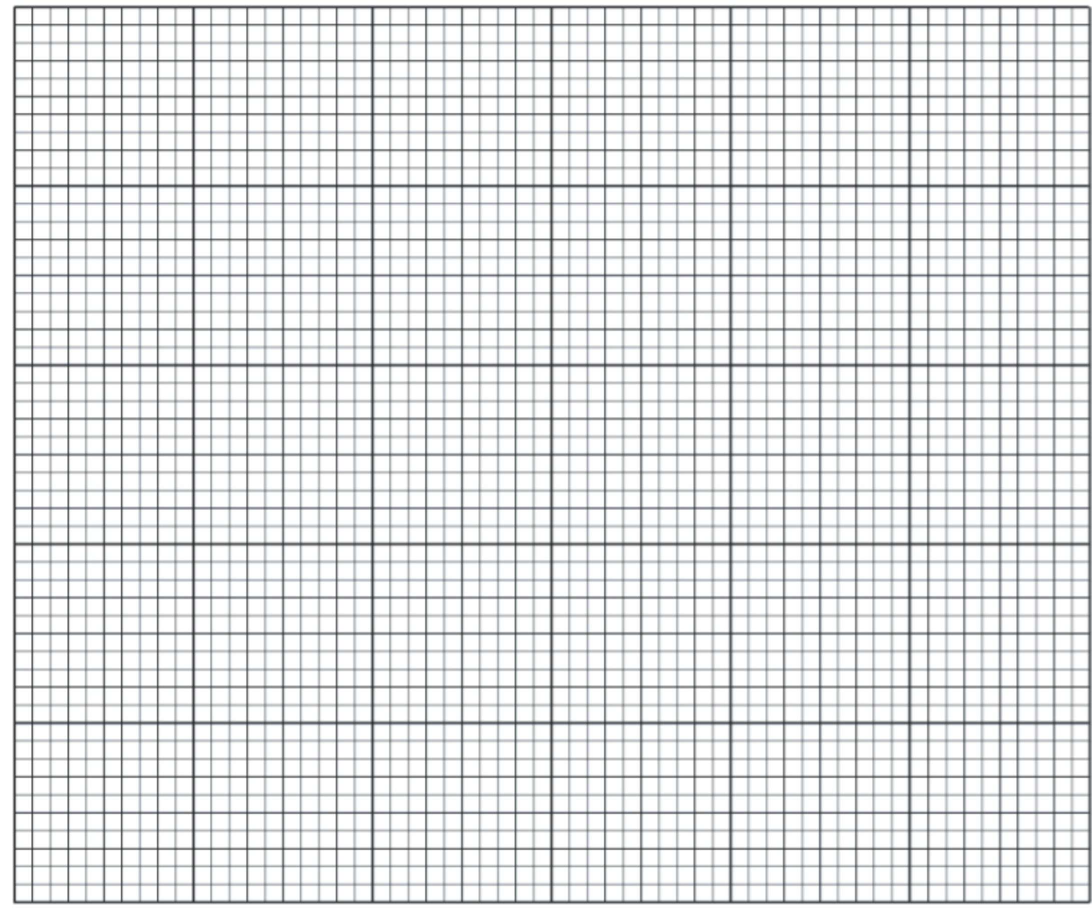
Table 1.1

Temperature/°C	**Time taken for decolourisation / s**	**Rate of decolourisation / s^{-1}**
80	100	
70	80	
60	65	
50	49	
40	40	

[1]

You are required to use a sharp pencil for graphs.

(iii) Using the grid provided below, plot a graph of the rate of decolourisation against temperature.



[4]

(iv) Explain the impact of increasing temperature on the structure and fluidity of beetroot membranes.

____

____

____

____

____

____ [3]

(v) Explain how increasing temperature affects the rate of diffusion of betanin into the water in the boiling tube.

____

____

____

____ [2]

(b) Explain why the beetroot discs must be immersed in distilled water for three minutes in step **2.**

____

____ [1]

(c) Suggest why dilute hydrochloric acid is able to decolourise the solution in step **16** and relate this to the trend observed in the graph plotted in **(a)(iii)**.

____

____

____

____

____

____ [3]

(d) *Other* than repeats, suggest a limitation of the procedure.

____

____ [1]

(e) Suggest two improvements to the procedure that will improve the reliability of your results.

____

____

____

____ [2]

(f) Describe how you would modify this investigation to determine the effect of alcohol concentration on the permeability of the cell membrane of the beetroot tissue.

____

____

____

____

____

____ [3]

[Total: 25]

Question 2

During this question, you will require access to a microscope.

<u>Section I</u>

You are provided with a slide of a stained transverse section through the leaves of three different plants. You are not expected to be familiar with these plant specimens.

These plants are adapted to living in different habitats:

- xerophyte: a plant that lives in an environment where water is scarce;
- mesophyte: a plant that lives in an environment with moderate amount of moisture and
- hydrophyte: a plant adapted to grow in water.

(a) Make a labelled plan drawing of the transverse section of the three leaves in the space below.

Your diagram should include:

1. labels of the cuticle layer and intercellular air spaces, where appropriate, and

2. suitable annotations that suggest the type of plant each leaf is taken from.

[6]

(b) Suggest how the different types of leaves support Darwin's theory of evolution.

[4]

Section II

You will investigate the effect of boiling on a slice of potato tissue.

Proceed as follows:

1. Use a scalpel to slice 2 thin slices from the raw potato sample provided.

2. Take a slice of the raw potato and place it on a microscope slide. Add a drop of iodine to stain it.

3. Observed the sample on the microscope under the low-power objective lens of your microscope.

4. Place the other potato slice into a boiling tube of distilled water and boil it for 1 minute.

5. Retrieve the boiled slice of potato from the boiling tube using a pair of forceps and place it on a white tile to cool for 1 minute.

6. Repeat steps 2 – 3 for the boiled potato slice.

(a) Make a labelled drawing to compare the effect of the two types of treatment on the potato cells, indicating the positions of the blue-black stains, in the space below.

[2]

(b) Using the eyepiece graticule fitted in the eyepiece lens of your microscope, and the stage micrometer, determine the average size of the blue stains observed for the two samples drawn in **(a)**.

Show the measurements that you made and your working.

[2]

(c) Account for the difference in the two types of treatment observed in **(a)**.

___

___

___

___ [2]

[Total: 16]

Question 3

The enzyme β-galactosidase hydrolyses the sugar lactose into glucose and galactose, which can be absorbed into the blood stream. However, it is not easy to measure β-galactosidase activity using the appearance of glucose and galactose.

Iodine binds to a site on β-galactosidase other than the active site and changes the conformation of the active site.

In order to measure the enzyme activity of β-galactosidase, an artificial substrate called o-nitrophenyl-beta-galactopyraniside (ONPG) is used. The enzyme degrades ONPG to produce β-galactose and o-nitrophenol, a yellow coloured compound. This reaction occurs optimally at pH 5.0. The reaction is stopped by adding sodium carbonate solution to increase the pH to 11.

Using this information and your own knowledge, design an experiment to investigate the effect of increasing the concentration of iodine on the rate of hydrolysis of ONPG by β-galactosidase.

You must use:

- 1.0% β-galactosidase solution,
- 0.3% iodine solution,
- distilled water,
- 5mM ONPG
- pH 5.0 buffer,
- 0.5 M sodium carbonate solution.

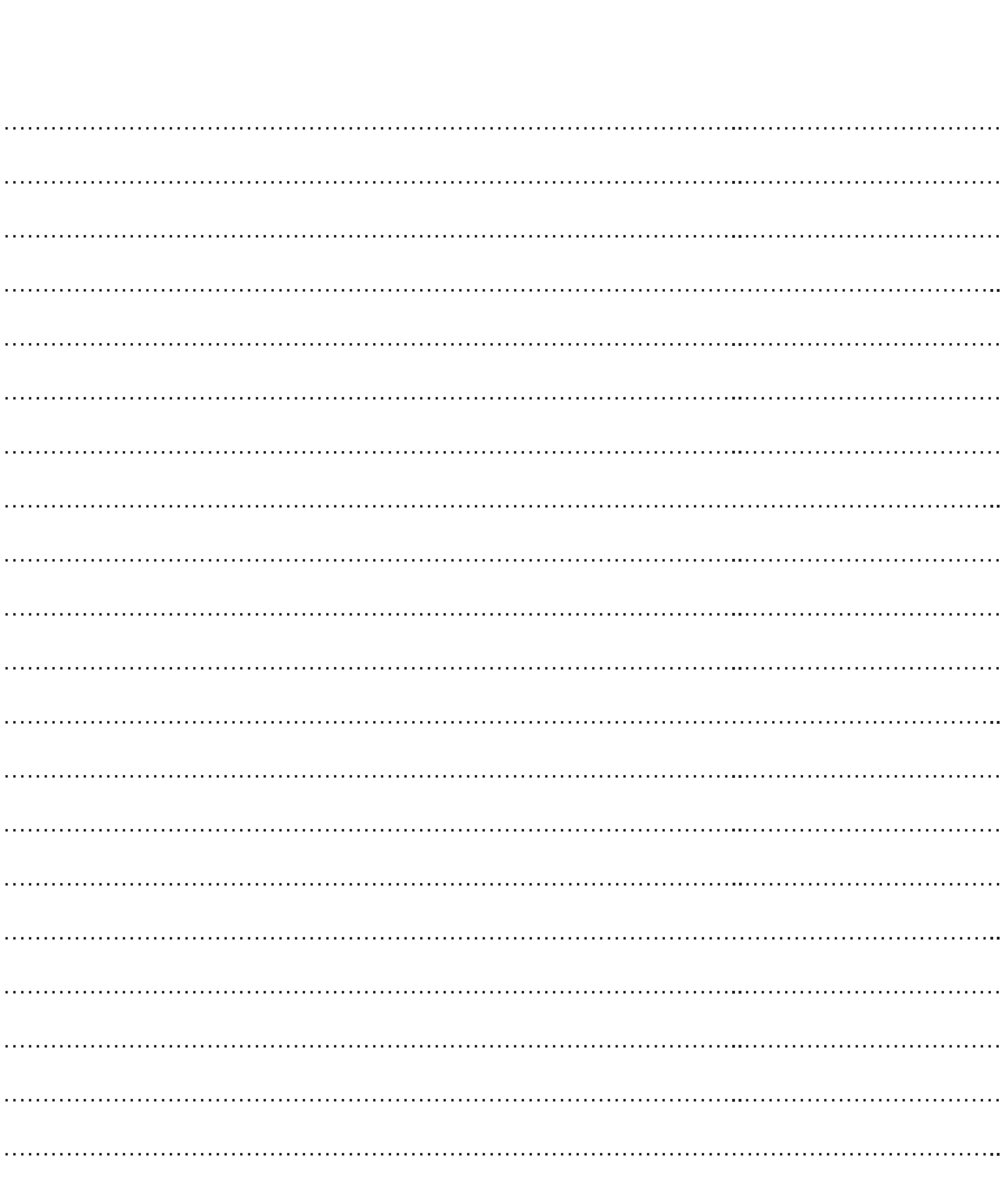
You should select from the following apparatus and use appropriate additional apparatus:

- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, glass rods etc.,
- timer e.g. stopwatch or stop clock,
- thermometer,
- colorimeter,
- thermostatically-controlled water bath.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[Total: 14]



List of Materials and Apparatus for JC2 H2 BIOLOGY Preliminary Examination Paper 4

Question 1

Item	Apparatus / reagents / chemicals	Quantity per student
1	A piece of fresh beetroot tissue, approximately 5 cm in length.	1
2	0.6M hydrochloric acid solution	100 cm^3
3	10 cm^3 syringe	2
4	Graduated Pasteur pipette able to drawn up at least 2 cm^3 of solution	5
5	100 cm^3 beaker	1
6	500 cm^3 beaker	1
7	Satay sticks	5
8	Cork borer	1
9	Scalpel	1
10	White tile	1
11	Glass rod	1
12	Boiling tube	5
13	Test tube	5
14	Wooden rack to hold boiling tubes and test tubes	1
15	Thermometer	1
16	15 cm ruler	1
17	Stopwatch	1
18	Safety goggles	1 pair
19	Paper towels	5
20	Tripod with wire gauze	1
21	Heatproof mat	1
22	Plastic sieve to collect beetroot pieces	1

Item	Apparatus / reagents / chemicals	Quantity per student
22	**Access to water from thermostatically-controlled water bath set at 40 °C**	-
23	**Access to shaved ice – ice buckets to be placed on teacher's bench**	-

Question 2

- Each candidate must have **sole, uninterrupted** use of the prepared slide for **1 hour 15 minute only.**

Item	Apparatus / reagents / chemicals	Quantity per student
1	Microscope with an eyepiece graticule	1
2	Stage micrometer	1
3	Prepared slide, labelled **'mesophytic, hydrophytic and xerophytic' leaf, TS** (placed in a Petri dish)	At least 1 between 2
4	Glass microscope slides	2
5	Cover slips	2
6	Blunt forceps	1 pair
7	Iodine solution	1 bottle
8	Slice of raw potato at least 1 cm in thickness	1

For both questions

Item	Apparatus / reagents / chemicals	Quantity per student
1	Distilled water, labelled **W**	At least 100 cm^3
2	Bunsen burner	1
3	Lighter	1
4	Wooden test tube / boiling tube holder	1 pair
5	Marker for labelling	1

Name	CTG

YISHUN JUNIOR COLLEGE
JC 2 PRELIMINARY EXAMINATION 2017

BIOLOGY **9744/04**

HIGHER 2 **21 AUGUST 2017**
Paper 4

2 hours 30 minutes

Candidates answer on the Question Paper

Additional material: As listed in the Confidential Instructions

YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE YISHUN JUNIOR COLLEGE

READ THESE INSTRUCTIONS FIRST

Write your name and CTG in the spaces at the top of this page and on all the work you hand in.
Give details of the practical shift and the laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen only.

You may use a HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.

The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	/25
2	/16
3	/14
Total	**/55**

This paper consists of **16** printed pages.

Question 1

The beetroot is a starchy edible root from the *Beta vulgaris* plant. It has long been used for medicinal purposes, primarily for disorders of the liver as it helps to stimulate the liver's detoxification processes. The water-soluble plant pigment that the gives beetroot its rich, purple-crimson colour is betanin and is typically contained in the vacuoles of beetroot cells. Betanin is a glucoside, and hydrolyses into the sugar glucose and its constituent nitrogenous compounds, which are colourless. Fig. 1.0 shows the molecular structure of betanin.

Fig 1.0

You are required to investigate the relationship between temperature and the rate of diffusion of betanin. The use of hydrochloric acid would yield information on the quantity of betanin generated from the experiment.

Safety information:

	You should wear eye protection throughout this practical. The coloured beetroot pigment will stain skin or clothes.
	0.6M hydrochloric acid (HCl) is a potential irritant. Avoid contact with eyes and skin.

You are provided with:

- a piece of fresh beetroot tissue
- a bottle of 0.6M hydrochloric acid

Proceed as follows:

1. Place the fresh beetroot tissue provided on a white tile. Using a cork borer, cut out cylinders of beetroot. Cut 15 pieces of beetroot discs of approximately 5 mm thickness from the cylinder using a scalpel.

2. Place all the cut beetroot discs in a small beaker of distilled water for three minutes.

3. Label five boiling tubes – 30°C, 40°C, 50°C, 60°C and 70°C.

4. Label five test tubes - 30°C, 40°C, 50°C, 60°C and 70°C.

5. Add 10 cm^3 distilled water to each boiling tube.

6. Set up a water bath in the 500 cm^3 beaker with 40°C water (taken from the thermostatically - controlled water bath).

7. Place the boiling tube labelled 70°C into the water bath you prepared. Heat the water gently until the temperature in the boiling tube reaches that of the water bath at 70°C. Use a thermometer to monitor the temperature.

8. When the temperature reaches 70°C, remove the boiling tube from the heat source and place in the rack.

9. Take three of the beetroot discs and impale on a satay stick with space between each disc.

10. Immediately immerse the impaled beetroot discs in the boiling tube labelled 70°C for exactly one minute.

11. Leave the impaled beetroot discs to stand in the boiling tube for a further 5 minutes.

12. When five minutes is up, remove the impaled beetroot discs from the boiling tube.

13. Repeat steps 7 to 12 for the remaining temperatures - 30°C, 40°C, 50°C and 60°C. Add tap water or shaved ice, where necessary, to attain and maintain the temperature. Use the thermometer to monitor the temperature.

14. Stir the solution in the boiling tube labelled 70°C with a clean glass rod and transfer 2 cm^3 of the solution into the test tube labelled 70°C using a clean Pasteur pipette.

15. Using a syringe, draw up 10 cm^3 of 0.6M hydrochloric acid (HCl).

16. Add 1 cm^3 of the 0.6M HCl into the test tube labelled 70°C and shake gently for 20 seconds. Continue adding 0.6M HCl and shaking the reaction mixture following each addition of HCl, until the solution decolourises completely.

17. Note the volume of 0.6M HCl required for complete decolourisation of the solution.

18. Perform another replicate by repeating steps 14 to 17.

19. Repeat steps 14 to 18 for the remaining temperatures - 30°C, 40°C, 50°C and 60°C.

20. Record your observations, **including the volume of 0.6M HCl used,** in the space provided on the next page.

(a) (i) Prepare the space below to record your observations.

Teacher's results:

Temperature/ °C	**Volume of HCl required for complete decolourisation/ cm^3**		
	Reading 1	**Reading 2**	**Average**
70.0	**6.0**	**7.0**	**6.5**
60.0	**5.0**	**5.0**	**5.0**
50.0	**3.0**	**3.0**	**3.0**
40.0	**2.0**	**3.0**	**2.5**
30.0	**1.0**	**1.0**	**1.0**

[5]

(ii) A similar experiment was conducted by another student and he obtained the following results shown in Table 1.1. Calculate the rate of decolourisation by filling in the table below.

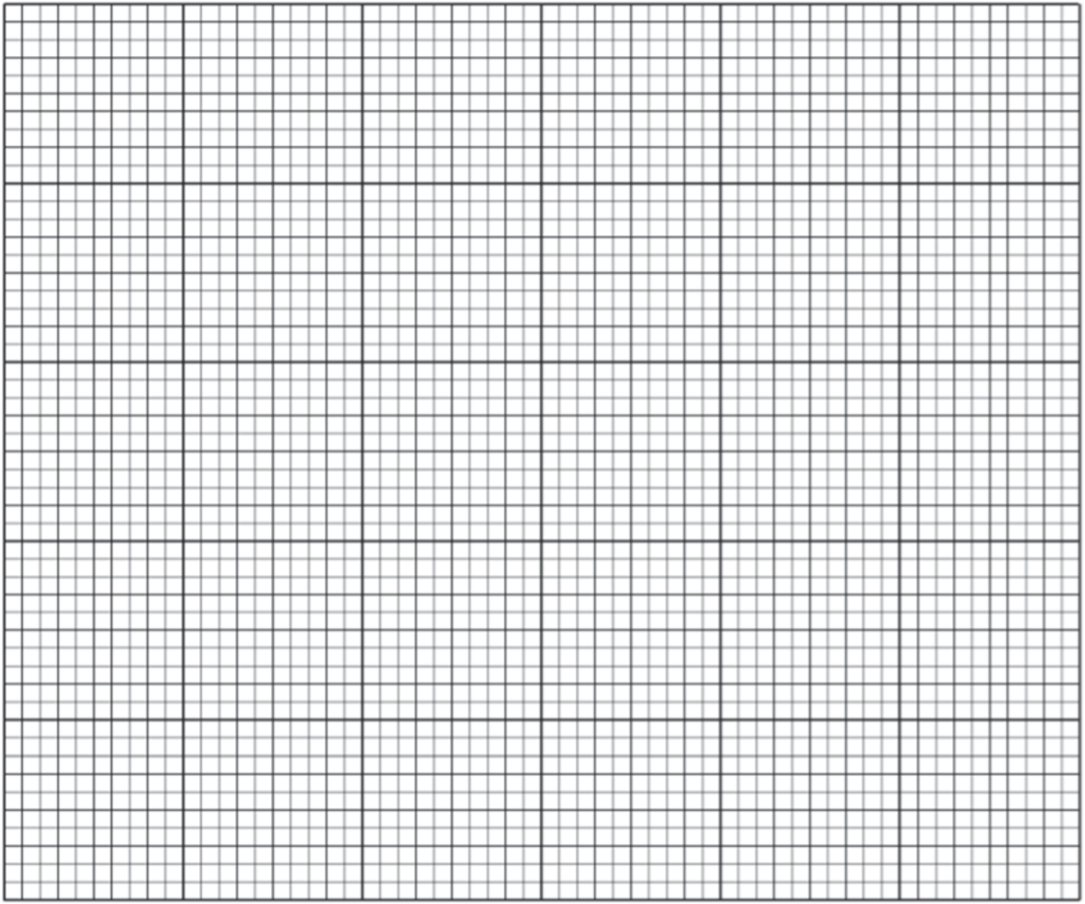
Table 1.1

Temperature/°C	**Time taken for decolourisation / s**	**Rate of decolourisation / s^{-1}**
80	100	0.0100
70	80	0.0125
60	65	0.0154
50	49	0.0204
40	40	0.0250

[1]

You are required to use a sharp pencil for graphs.

(iii) Using the grid provided below, plot a graph of the rate of decolourisation against temperature.



Marking points:

1. Line of best fit
2. Correct labels of the independent and dependent variables with units
3. <u>Sensible</u> equidistant scale using at least 2/3 of the graph paper (reject: half of graph paper)
4. Accurate plotting of the points with no extrapolation of graph in both axes

[4]

(iv) Explain the impact of increasing temperature on the structure and fluidity of beetroot membranes.

1. Increasing temperature increases the kinetic energy of the phospholipids and beetroot membranes becomes increasingly fluid; *(Reject: membrane is flaccid/turgid; expand/contract)*
2. Increasing temperature increases the lateral movement of the phospholipids and membrane proteins along the membrane;
3. Increased intramolecular vibrations leads to disruption of hydrophobic interactions or hydrogen bonds and denaturation of membrane proteins;
4. Further increase in the temperature could lead to complete disintegration/ rupturing of the beetroot membranes / makes the membranes leaky.

[3]

(v) Explain how increasing temperature affects the rate of diffusion of betanin into the water in the boiling tube.

1. Higher the temperature, the greater the increase in kinetic energy of the pigments;;
2. Movement of the betanin / pigments down the concentration gradient at a faster rate

OR

increases the rate of diffusion of betanin / pigments into the water in the boiling tube.

[2]

(b) Explain why the beetroot discs must be immersed in distilled water for three minutes in step **2.**

- To remove any residual pigments that leaked out of the beetroot discs as a result of mechanical damage from cutting with the scapel /use of the cork borer.

[1]

(c) Suggest why dilute hydrochloric acid is able to decolourise the solution in step **16** and relate this to the trend observed in the graph plotted in **(a)(iii)**.

1. High proton concentration could result in the acid hydrolysis of betanin / pigment;
2. disrupting the ionic OR hydrogen bonds in the structure of the pigment.

Reference to trend in the graph:

3. With increasing temperature, a higher concentration of betanin will be present in the water (as a result of increased membrane permeability), leading to more time required for acid hydrolysis / decolorisation of the pigments in the water. This leads to a lower rate of decolourisation with increasing temperature.

[3]

(d) *Other* than repeats, suggest a limitation of the procedure.

Any 1 below:

1. Subjectivity of the naked eye to observe the colour change from red to colourless;;
2. It is difficult to maintain a constant temperature in the water bath by adding hot water or ice, as heat is lost to the surroundings.

[1]

(e) Suggest two improvements to the procedure that will improve the reliability of your results.

1. Use of the colorimeter to measure % light absorbance OR % light transmitted to quantify the degree of decolorisation by HCl.
2. Place the samples into thermostatically controlled water baths set up at the respective temperatures.

[2]

(f) Describe how you would modify this investigation to determine the effect of alcohol concentration on the permeability of the cell membrane of the beetroot tissue.

1. At least 5 concentration of alcohol using simple dilution: negative control 0%, 5%, 10%, 15%, 20%, 25%.
2. Incubation of alcohol solution with beetroot tissue for 5 mins in a 30°C thermostatically-controlled water bath.
3. Measure the volume of HCl required to decolourise the pigments present for each of the concentration.
4. Plot the graph of volume of HCl (cm^3) used against concentration of alcohol (%).

[3]

[Total: 25]

Question 2

During this question, you will require access to a microscope.

Section I

You are provided with a slide of a stained transverse section through the leaves of three different plants. You are not expected to be familiar with these plant specimens.

These plants are adapted to living in different habitats:

- xerophyte: a plant that lives in an environment where water is scarce;
- mesophyte: a plant that lives in an environment with moderate amount of moisture and
- hydrophyte: a plant adapted to grow in water.

(a) Make a labelled plan drawing of the transverse section of the three leaves in the space below.

Your diagram should include:

1. labels of the cuticle layer and intercellular air spaces, where appropriate, and
2. suitable annotations that suggest the type of plant each leaf is taken from.

Marking points:

XEROPHYTE

Plants that live in conditions where water is scare (for example in the desert)

1. Absence of intercellular airspaces to prevent loss of water vapour;;
2. Thick cuticle to prevent loss of water;;

MESOPHYTE

Land plants living in environment with moderate amount of moisture.

1. Intermediate size airspaces allow for gaseous exchange;;
2. Moderate size of the cuticle, thicker than hydrophyte ;;

HYDROPHYTE

A plant adapted to grow in water.

1. Presence of large intercellular airspaces for buoyance / storage of oxygen;;
2. Thin cuticle / absence of cuticle to prevent water loss as plant is found in environment with abundance of water;;

[6]

(b) Suggest how the different types of leaves support Darwin's theory of evolution.

1. Variation occurs in the population, due to mutation and meiosis;
2. Different habitats would have different selection pressure that selects for plants with different traits;
3. For example, varying thickness of the cuticle to reduce / prevent water loss → e.g. leaf from hydrophytic plant has a very thin cuticle / no cuticle to prevent water loss as the plant lives in an environment where water is in abundance;
4. Individuals with the advantageous traits in the particular habitat will have greater reproductive success and pass down the favourable alleles to the offspring;;

[4]

Section II

You will investigate the effect of boiling on a slice of potato tissue.

Proceed as follows:

1. Use a scalpel to slice 2 thin slices from the raw potato sample provided.

2. Take a slice of the raw potato and place it on a microscope slide. Add a drop of iodine to stain it.

3. Observed the sample on the microscope under the low-power objective lens of your microscope.

4. Place the other potato slice into a boiling tube of distilled water and boil it for 1 minute.

5. Retrieve the boiled slice of potato from the boiling tube using a pair of forceps and place it on a white tile to cool for 1 minute.

6. Repeat steps 2 – 3 for the boiled potato slice.

(a) Make a labelled drawing to compare the effect of the two types of treatment on the potato cells, indicating the positions of the blue-black stains, in the space below.

1. Large plan drawing of the cells at least 3 cm in length;;
2. Annotation of starch grains (blue stains) outside the cell for boiled sample.

[2]

(b) Using the eyepiece graticule fitted in the eyepiece lens of your microscope, and the stage micrometer, determine the average size of the blue stains observed for the two samples drawn in **(a)**.

Show the measurements that you made and your working.

- Average size of the starch granules as calculated would be around **3-10 microns;**
- Marks given for accuracy and working (must show the actual length of one eyepiece graticule and the number of eyepiece graticule per sample).

[2]

(c) Account for the difference in the two types of treatment observed in **(a).**

1. Blue-black stains due to the presence of the amylose in starch;;
2. Boiling of the cell ruptures *(reject: more permeable)* the cell wall and cell membranes, releasing starch into the extracellular environment;;

[2]

[Total: 16]

Question 3

The enzyme β-galactosidase hydrolyses the sugar lactose into glucose and galactose, which can be absorbed into the blood stream. However, it is not easy to measure β-galactosidase activity using the appearance of glucose and galactose.

Iodine binds to a site on β-galactosidase other than the active site and changes the conformation of the active site.

In order to measure the enzyme activity of β-galactosidase, an artificial substrate called o-nitrophenyl-beta-galactopyraniside (ONPG) is used. The enzyme degrades ONPG to produce β-galactose and o-nitrophenol, a yellow coloured compound. This reaction occurs optimally at pH 5.0. The reaction is stopped by adding sodium carbonate solution to increase the pH to 11.

Using this information and your own knowledge, design an experiment to investigate the effect of increasing the concentration of iodine on the rate of hydrolysis of ONPG by β-galactosidase.

You must use:

- 1.0% β-galactosidase solution,
- 0.3% iodine solution,
- distilled water,
- 5mM ONPG
- pH 5.0 buffer,
- 0.5 M sodium carbonate solution.

You should select from the following apparatus and use appropriate additional apparatus:

- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, glass rods etc.,
- timer e.g. stopwatch or stop clock,
- thermometer,
- colorimeter,
- thermostatically-controlled water bath.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[Total: 14]

Scientific reasoning / Explanation of theory [3 marks]

- Ref to basic function of enzyme inhibitors, e.g. enzyme inhibitors affect enzyme activity resulting in a decrease in the rate of enzyme catalyzed reactions. i.e. the idea enzyme substrate complex.
- Ref to non-competitive inhibition:
    - does not bind to active site of the enzyme
    - Binds to inhibitor site, e.g. allosteric site
    - Inhibition cannot be overcome by increasing substrate concentration
- Ref to ONPG color / absorbance value; i.e. increasing iodine concentration will decrease enzyme activity, lower rate of reaction and hence resulting in a lowered absorbance. Alternatively, light transmission will be appropriate.

Variables [2 marks]
(1 mark)
- Controlled variables: Temperature, pH, Volume of inhibitor used, with explanation

(Both below present and correct for 1 mark)
- Dependent: absorbance value / colour intensity / product formed (ONP)
- Independent: Iodine volume / concentration used

Procedure [4 marks max, 1 mark each]
- Using serial dilution / simple dilution to obtain at least 5 known concentration of ONPG, diluting with either buffer solution or distilled water, illustrating with dilution table etc.
- Equilibration procedure with appropriate timing (~ 2 to 5 mins)
- Stating that the tube be kept in ice to reduce enzyme activity / maintaining constant temperature during enzymatic reaction
- Stating equal volume of ONPG solution used
- Specific time set for reaction (1 – 2 minutes after addition of enzyme)
- Means of measuring 2 – 3 absorbance value for each tube using colorimeter / spectrophotometer, blanking of the cuvette at the start with distilled water before loading in the colour samples.
- Stating replicates (reliability) and repeats to be performed (Reproducibility)

Positive Control [1 mark]
- Absence of inhibitor (zero iodine, substitute with distilled water) which would result in high abs value due to increase enzymatic activity.

Data recording [Table 2 marks, Graph 1 mark, max. 3 marks]

- Tabulation of data with headings and units;
- Including average value for absorbance value
- Plot graph of absorbance value (abs) against ONPG concentration / volume
- Correct labelling of x and y – axis

Table of results					
Cuvette no.	Concentration of Iodine / %	Absorbance value 1 / abs	Absorbance value 2 / abs	Absorbance value 3 / abs	Average Absorbance / abs
1					
2					
3					
4					
5					
6					
Positive control					

At least 2 Safety Precautions / Risk Hazards [1 mark]

- take care when handling glassware such as beakers and test tubes
- do not allow reagent such as ONPG to have contact with skin or eyes, wash when in contact
- wear goggles throughout the experiment

[14]