VICTORIA JUNIOR COLLEGE
JC 2 PRELIMINARY EXAMINATION
2025
HIGHER 2

NAME: ……

CT CLASS: ……..……

BIOLOGY

9744 / 03

Paper 3 Long Structured and Free-response Questions

18/09/2025

2 hour

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

READ THESE INSTRUCTIONS FIRST

Write your name and CT class in the spaces at the top of this page.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

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

Section B
Answer any **one** question in the space provided on the Question Paper.

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.

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

Question	Marks
Section A	
1	
2	
3	
Section B	
Total	

This document consists of **20** printed pages.

[Turn over]

1 (a) Outline the roles of oxygen in aerobic respiration. [3]

- Serves as the **final electron acceptor** at the end of the **electron transport chain** in **oxidative phosphorylation**;
- Allows **continuous flow of electrons** along the electron transport chain for **continuous production of ATP** in oxidative phosphorylation;
- Allows the **regeneration of NAD and FAD** for **link reaction and Krebs cycle to continue** in the mitochondria;

Air contains a mixture of gases, which include approximately 78% nitrogen, 21% oxygen and 0.03% carbon dioxide. Fluctuations in these levels have occurred multiple times throughout the evolution of earth's atmosphere. It is known that Earth was once without oxygen, but the gas began to accumulate in the atmosphere around 2.3 billion years ago. The increased presence of oxygen produces a more efficient energy source in the form of aerobic metabolism, producing 16-18 times more adenosine triphosphate (ATP) per hexose sugar than anaerobic metabolism.

Multicellular organisms require efficient oxygen transport systems because the simple process of diffusion is too slow and inefficient to reach all cells within their large bodies, which have a small surface-area-to-volume ratio. Invertebrates like molluscs and arthropods make use of a protein called haemocyanin to transport oxygen throughout their bodies. It is an extracellular protein not contained within cells, but is instead dissolved freely in the haemolymph, which is the invertebrate equivalent of blood.

Haemocyanin is made up of a dimer or hexamer of subunit proteins depending on the species, with each subunit containing two copper ions to bind to one oxygen molecule. Fig. 1.1 shows the structure of a haemocyanin subunit, as well as the arrangement of the oxygen binding site in haemocyanin made up of six histidine amino acid residues (His) and the two copper ions.

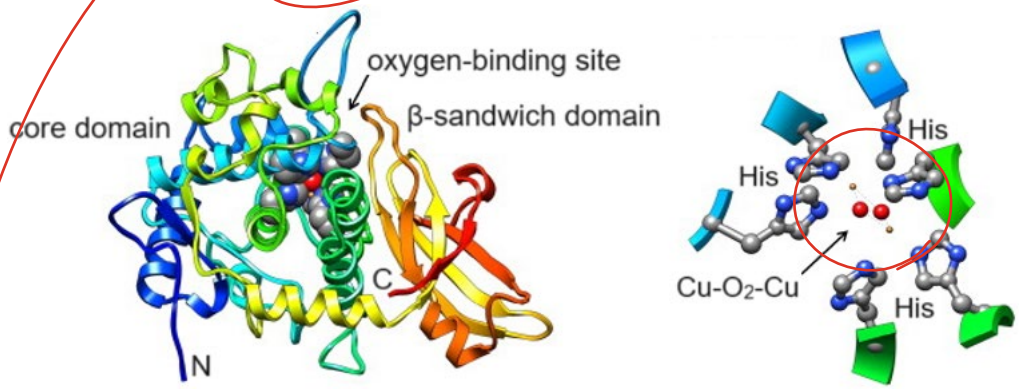


Fig. 1.1

(b) With reference to the information provided,

(i) state the highest level of organisation of haemocyanin. [1]

- Quaternary structure;

(ii) explain how the oxygen binding site of each haemocyanin subunit is formed. [2]

- **Extensive folding** of the polypeptide to bring the **six histidine residues** close together in the **correct orientation**;

- Forming a **specific shape** of the oxygen binding site that allows **two copper ions** to be bound;

(c) The amino acid sequences of a portion of the core domain of the haemocyanin subunit from four different invertebrate species have been compared. Fig. 1.2 shows the results.

species 1	LGGASKPKKAASKAPTKKPKATPVKKAKKKLAATPKKAAPGGTAAPGGTAACHA	0
species 2	LHGASKAKKAAPKAPTKKPKATGGKKAKKKLAATPKKAAPLGTAAPGGTAACVA	7
species 3	LGGASKPKKAASKAPTKKPKATPVKKAKPKLAATPKKAAPGGTAAPGGTAACHA	1
species 4	LGGASKPKGAASKAPTKKPKATPVKKAKKKSAATPKKAPPGGTAAHGGTAACHA	4

Fig. 1.2

it explains that species change over time and share a common ancestor through a process called natural selection

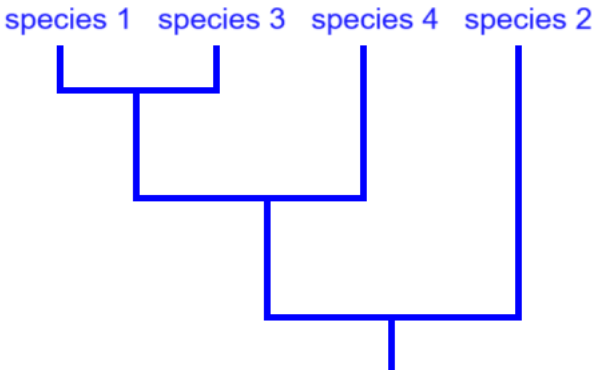
Using information in Fig. 1.2,

(i) Explain how the results shown support Darwin's theory of evolution. [3]

- Ref. to **descent with modifications**;
- All four species have haemocyanin (homologous protein), thus have **descended from a common ancestor with haemocyanin**;
- The haemocyanin of each species **accumulated mutations independently** after the four species diverged from the common ancestor;

(ii) Draw a phylogenetic tree that represents the evolutionary history of the four invertebrate species. [2]

- Species 1 and 3 are most closely related. [diff by 0,1 amino acid]
- Species 4 differs slightly is closely related to species 1 and 3. [4 amino acids]
- Species 2 shows more differences, so it is the most distantly related. [7 amino acids]
- Start drawing from those most closed related



7 different amino acids

(iii) Recent studies have concluded that species 1 and 2 diverged 2.6 million years ago.

Calculate the mutation rate of haemocyanin. Show your working in the space below. Leave your answer in 3 significant figures.

..... x 10^{-6} mutations per year [1]

- 7 mutations / 2.6 million years = 2.69 x 10^{-6} mutations per year (3 s.f.);

(iv) With reference to the genetic code, suggest how the answer in **(c)(iii)** may be an underestimate. [2]

universal? degeneracy? which more relevant here?

$\frac{7}{2.6 \times 10^{6}} = \frac{7}{2.6} \times 10^{-6} = 2.69 \times 10^{-6}$

- Ref. to **degeneracy** of the genetic code and silent mutations;
- **Total number of mutations may be higher** than 7 as some **mutations may not result in changes in the amino acid sequence**;

(d) Haemocyanin has demonstrated anti-cancer properties as it has been shown to stimulate apoptosis in tumour cells in experiments, reducing the mass of tumours in mouse models, as well as the number of viable cells in cancer cell cultures.

Cancer formation involves the accumulation of different mutations. Some mutations are described as dominant mutations, while others are described as recessive mutations.

(i) Outline a type of mutation in cancer formation that is described as dominant. [1]

- Gain-of-function mutation of proto-oncogenes to oncogenes;

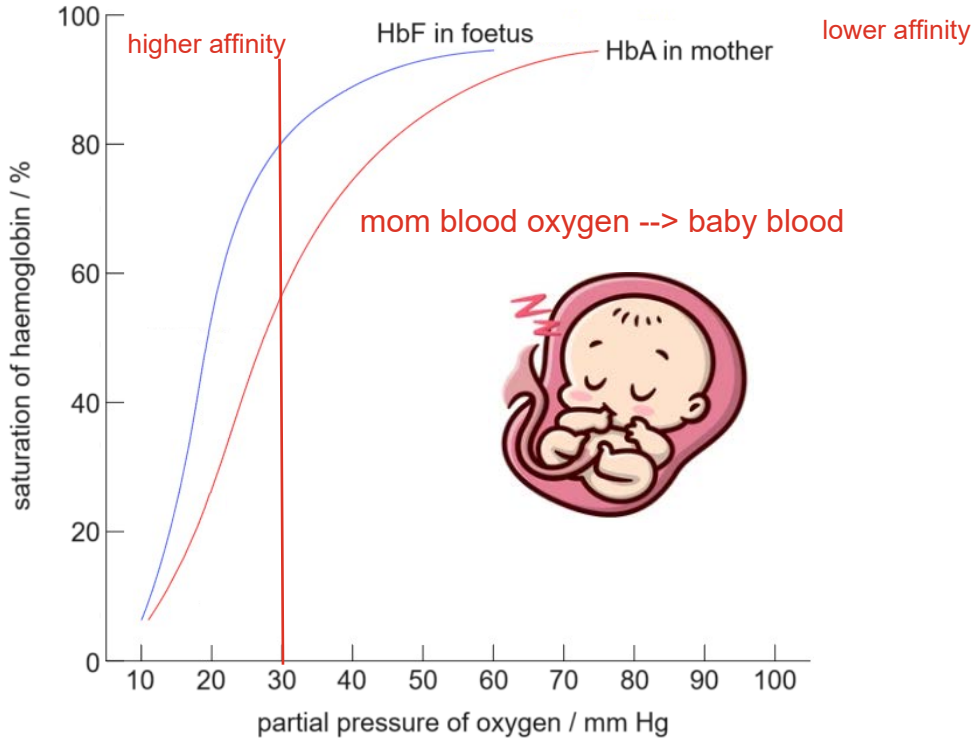
(ii) Explain why this type of mutation is described as dominant. [2]

to decide if is dominant, need to see normal

- **Heterozygotes** with 1 mutated allele and 1 normal allele have **higher level of stimulation of cell cycle**;
- When both mutated and normal alleles are expressed, heterozygotes have **more proteins / higher protein activities** that stimulate the cell cycle;
- Mutation results in **over-expression** / expression of **constitutively active proteins**;

(e) In humans, the protein that transports oxygen is haemoglobin. Haemoglobin A (HbA) is the most abundant type of haemoglobin in adults, making up 95-98% of total haemoglobin. A variant of haemoglobin known as foetal haemoglobin (HbF) is found in human foetus. It is produced in the foetus at around six weeks of pregnancy, and its levels remains high after birth until the baby is roughly two to four months old.

Fig. 1.3 shows the affinity of the two types of haemoglobin.



indicate concentration of oxygen

Fig. 1.3

With reference to Fig. 1.3,

(i) compare the affinity of the two types of haemoglobin at 30 mm Hg of oxygen. [1]

- HbA – 56-58% saturation vs. HbF – 80-81% saturation; accept a value from these range of values

(ii) suggest an advantage of the foetus expressing HbF. [1]

- Able to pull oxygen from HbA in mother's blood / red blood cells to HbF to be transported to tissues in the foetus;

(f) HbA is made up of two α-chains and two β-chains, while HbF is made up of two α-chains and two γ-chains. After birth, the expression of the γ-chains is switched off, and the baby's body then begins to express the β-chains.

mutation beta chain

The regulation of expression of the γ-chains involves two proteins, BCL11a and ZBTB7a. They bind to promoter sequence of the γ-chain during the transition from foetal to adult haemoglobins. Disrupting the binding of these proteins is shown to increase HbF levels and is being developed as a therapeutic strategy for genetic blood disorders like sickle cell disease and β-thalassemia.

(i) State the level of control of gene expression involving BCL11a and ZBTB7a proteins. [1]

- Transcriptional control;

(ii) Suggest a structural similarity between BCL11a protein and a general transcription factor. [1]

not active site not sequence

- **DNA binding site** complementary to the **shape of the promoter sequence** (TATA box);

(iii) Suggest how BCL11a and ZBTB7a proteins regulate the expression of the γ-chains. [1]

- **Block binding of general transcription factors and RNA polymerase** to the promoter, **prevent formation of transcription initiation complex**;

(iv) Outline how cells can reduce the intracellular concentration of BCL11a and ZBTB7a proteins after they are synthesised. [2]

control at post-translation

- **Addition of ubiquitin** to N-terminus of the proteins;
- Which mark them for **degradation by proteasomes**;

(g) Fig. 1.4 shows the effect of oxygen level on the affinity HbA to oxygen in normal conditions (solid line), as well as the effects of different conditions (dotted lines).

higher affinity

lower affinity

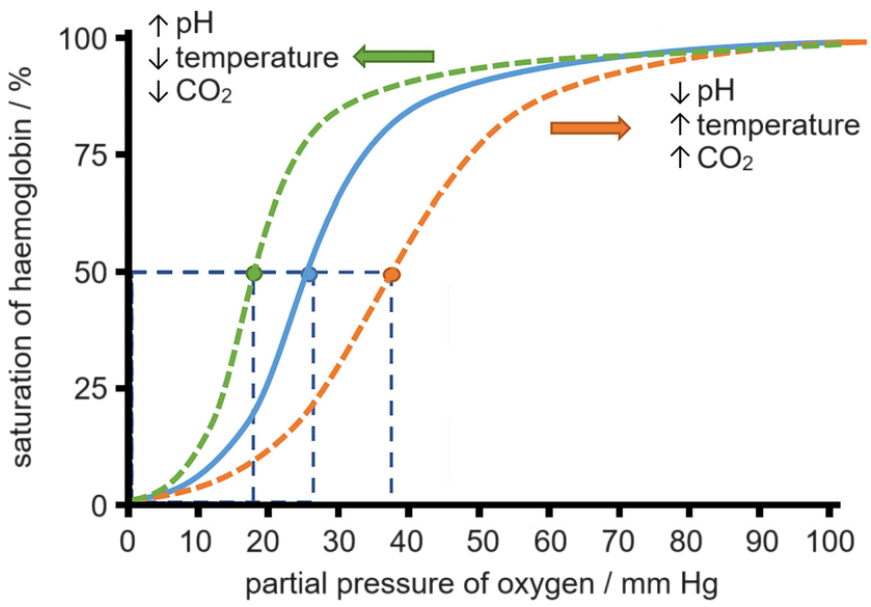


Fig. 1.4

With reference to Fig. 1.4,

solid line graph

(i) explain the effect of increasing oxygen level on oxygen affinity of HbA in normal conditions. [4]

- Slow increase in low oxygen level + Q.V.;
- Fast increase as oxygen level increases + Q.V.;
- Slow increase and plateau at high oxygen level + Q.V.;

- At low oxygen level, HbA is in inactive state – slow increase;
- Cooperativity and conformation change to active state with high affinity – fast increase;
- Saturated at high oxygen level – plateau;

meaning high CO2, see right side dotted line graph

(ii) suggest how oxygen is transported to tissues undergoing high level of activities. [2]

- Ref. to high level of CO_2 released by these tissues due to high level of respiration, reducing the pH in the blood near the tissues;
- (higher local temperature?)
- Lower oxygen affinity of HbA results in release of oxygen near these tissues;

[Total: 30]

2 From the 1920s until the 1970s, insulin obtained from the bodies of animals was used to treat diabetes. Since the late 1970s, insulin has been produced by genetic engineering.

The production of human insulin by genetic engineering involves the use of plasmids and the bacterium *Escherichia coli*.

Multiple copies of a gene that codes for the human insulin polypeptide are mixed with cut plasmids.

During the process, only some of the plasmids that are taken up by host bacteria will lead to the expression of human insulin.

Fig. 2.1 shows:

- a cut plasmid and the gene coding for the insulin polypeptide
- three different plasmids that have been formed as part of the genetic engineering process.

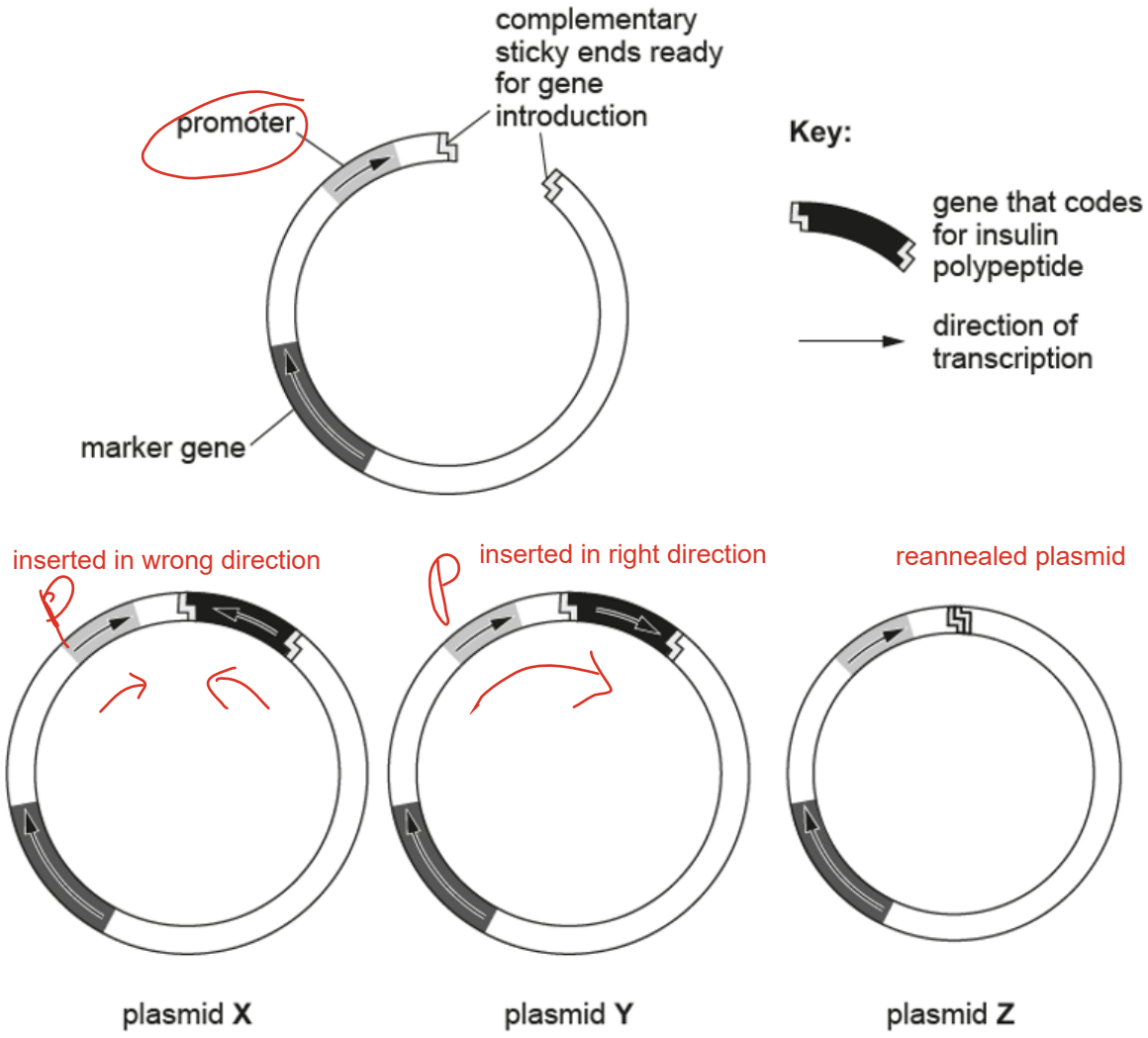


Fig. 2.1

(a) With reference to Fig. 2.1,

(i) explain why a promoter is often present on the plasmid. [1]

- For RNA polymerase to bind so that the gene is expressed (activated, transcribed);

(b) Suggest the advantages of using genetic engineering to produce human insulin compared to insulin obtained from animals. [2]

write from GE pov, not from animal

1. provides a reliable and consistent supply, not dependent on animal slaughter/ ref. to insulin obtained from the bodies of animals could lead to shortages;
2. avoids potential allergic reactions to animal insulin / ref. to slight differences in the human and animal insulin molecule;
3. reduces the risk of transmitting animal pathogens/ diseases;
4. avoids ethical concerns some people might have about using animal products;
5. lower cost of large-scale production of insulin compared to animal-sourced insulin, potentially making treatment more accessible;

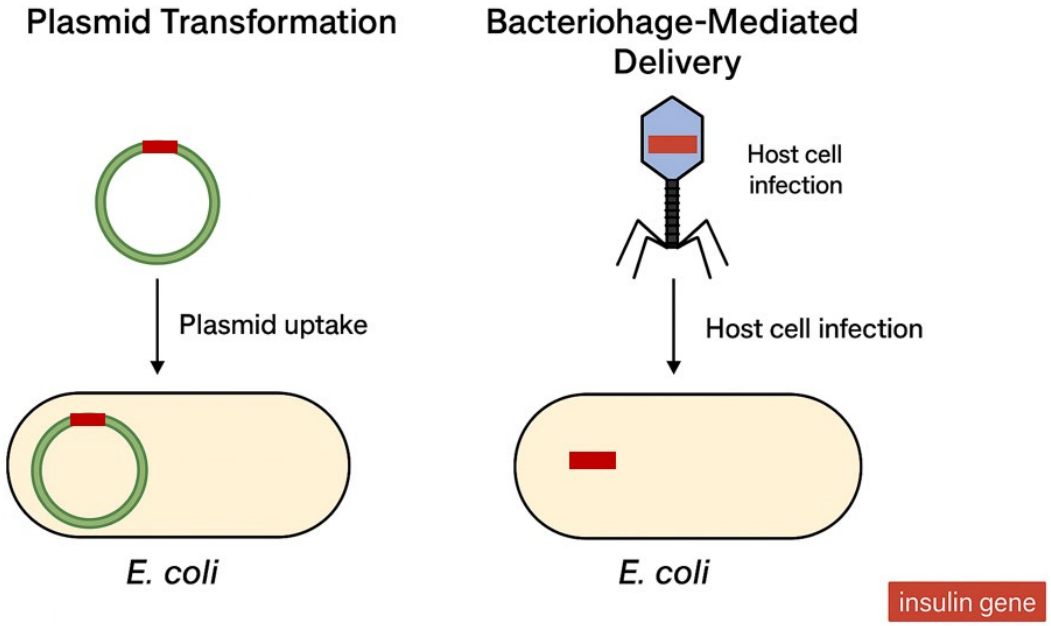
(c) During early research, when the plasmid was first introduced into the *E .coli,* non-functional human insulin was produced.

Explain why there was no functional insulin and suggest what improvement was needed in order for the gene to be expressed in *E. coli*. [3]

1. Human insulin gene is eukaryotic in origin;
2. Bacteria protein synthesis machinery does not have post-transcriptional modification (accept no RNA splicing / bacteria lack spliceosomes and cannot remove introns);
3. Obtain the mRNA for human insulin to form the complementary DNA which is then inserted into the plasmid;

(d) In addition to plasmid vectors, the insulin gene may be packaged into bacteriophage particles for delivery into *E. coli*.

Fig. 2.2 shows direct plasmid uptake compared to bacteriophage-mediated delivery.



label

Fig. 2.2

1M 3M

(i) State whether a virulent or temperate phage would be used and explain how it could be engineered to deliver the insulin gene into bacteria. [4]

1. Temperate phage;
2. Phage carrying the human insulin gene attaches to specific receptors on *E. coli* cell surface;
3. Phage penetrates the bacterial cell wall by contracting the contractile sheath of the tail, which drives the hollow tube of the tail into the host bacterium
4. Human insulin gene packaged in phage capsid is injected into bacterial cytoplasm;
5. Human insulin gene can be integrated into the bacterial chromosome to enable expression of the insulin gene;
6. Phage will not cause the death of host cell;
7. Bacteria reproduce asexually by binary fission / can be cultured in large quantities, leading to a high yield and a continuous, abundant supply of insulin;

[Total: 10]

3 Bees are well-known as the main pollinator of the world, estimated to contribute to the pollination of 75% of all major food crops.

There have been warnings about the impacts of climate change on bees. The Intergovernmental Panel on Climate Change (IPCC) reported a projection of 3°C rise in surface air temperature by 2100 under intermediate greenhouse gas emission scenario SSP2-4.5. The surface air temperature is projected to increase by 6°C under the worst case-emission scenario SSP5-8.5.

(a) Explain how greenhouse gas emission leads to rise in surface air temperature. [1]

traps heat in the form of infrared radiation released by earth and reflect it back to earth, resulting in enhanced greenhouse and rise in temperature;

During winter (December to February), there are little flowers available for bees to forage, hence they undergo an adaptation of hibernation referred to as 'overwintering'. During late fall (November), queen bees stop giving birth to forager bees and begin raising winter bees, which have larger body fat content. Once temperatures drop below approximately 10°C, the bees hibernate through winter and minimise heat loss by clustering inside their hive in a massive huddle. In early spring (march), these bees emerge from their colony in search for food.

To investigate the effects of climate change on the emergence of bees after overwintering, scientists subjected *Osmia bicornis*, a species of mason bee, to 3 different conditions:

- control – based on the daily temperatures reported for that day (used as the baseline for the other 2 conditions)
- SSP2-4.5 – 3°C higher than the baseline
- SSP5-8.5 – 6°C higher than the baseline

The results of the investigation are shown in Table. 3.1. The emergence timing is defined as the number of ordinal days since 1st of January. The weight loss is defined as the percent of body mass lost relative to the starting body mass measured upon hatching from their cocoons. Bees of similar starting body mass were selected for this investigation.

Table 3.1

	Control			SSP2-4.5			SSP5-8.5		
Variable [unit]	n	Mean	Std. error	n	Mean	Std. error	n	Mean	Std. error
Emergence timing [ordinal days]	166	103	0.51	159	85.7	0.82	156	63.9	1
Mean temperature at emergence [°C]	166	16.4	0.1	159	16.3	0.2	156	16	0.2
Mean weight loss at emergence [%]	166	9.43	0.18	159	11	0.24	156	13.5	0.26

(b) With reference to Table 3.1, discuss the impact of a 3°C rise in temperature on the population of bees. [4]

data
- rise in temperature resulted in the **mean emergence timing being shifted earlier** + from 103 ordinal days in control set up to 85.7 ordinal days in SSP2-4.5;
- (-) flowers may not yet be available for forage resulting in starvation and reduction in bee population size;
- (+) should the start of overwintering remain unchanged, the bees have longer duration of actively foraging for food, enabling the bee population to grow more in the year following emergence;

data
- rise in temperature resulted in an **increase in the mean weight loss** + from 9.43% in control set up to 11% in SSP2-4.5;
- (-) the bees that emerged from overwintering may be weaker to forage for food, resulting in decrease in bee population size;

(c) Explain how the results highlight the importance of immediate efforts to reduce greenhouse gas emissions from human activities. [3]

More immediate efforts to reduce greenhouse gas emissions will **delay** the increase greenhouse gas emissions hence slow down the rate of temperature increase;

data Larger rise in temperature of 6°C in SSP5-8.5 results in **greater extent of mean weight loss / more significant shift of emergence timing earlier**, as compared to a smaller rise in temperature of 3°C in SSP2-4.5;

[importance of delaying] Any 1:
- To enable more time for organisms / bees to adapt to the environmental changes, hence reducing the rate of species extinction;
- To enable more time for innovations that increases resilience to food security;

(d) Although many crops rely on insect pollination, only about 25% of global production does, as major staples like cereals do not depend on insects. Hence, should pollinators like bees become extinct, crop yield is expected to decline only by 5-8%.

self pollination, same flower, or flowers in the same plant

Explain why it is still important to protect the bees from effects of climate change. [2]

data, first sentence

Bees are responsible for the pollination of 75% of food crops, hence their extinction would reduce crop diversity;
which reduces nutritional quality of our diets / affects our health when some nutrients are lacking;

Bees are producers of honey;
Source of food for humans and some species of animals / medicinal effects / source of income for some / AVP;

[Total: 10]

ESQ instructions

4 **(a)** Describe how insulin regulates blood glucose level. [13]

A. Stage 1: ligand-receptor interaction

1. In response to a **rise in blood glucose level above the norm of 100mg / 100cm^3 of blood ;**
2. **Insulin** is released from the **β cells of the islets of Langerhans in the pancreas** into the bloodstream and is transported around the body ;
3. Insulin binds specifically to extracellular **ligand binding site on RTK** (**insulin receptor**) present on target cells (**muscle and liver cells**) ;
4. The binding of insulin causes **conformational change** to the receptor, **activating it** ;
5. The **tyrosine kinase** region at the intracellular domain of **each polypeptide** is then activated ;
6. Each tyrosine kinase catalyses the **phosphorylation of the tyrosine residues** on the intracellular domain **of the other polypeptide** / ref. to **cross-phosphorylation** ;

B. Stage 2: signal transduction

7. Specific **relay proteins** recognise the activated insulin receptor **and binds to phosphorylated tyrosine residues** in the intracellular domain of the insulin receptor ;
8. The binding causes a **conformation change and activation of the relay proteins** ;
9. The activated relay proteins leave the receptor and **bind to other downstream proteins or kinases**, **activating them** ;
10. Activation of a kinase triggers a **phosphorylation cascade**, leading to **signal amplification** ;
11. **Different activated relay proteins activate different downstream proteins or kinases**, thus triggering **different signal transduction pathways** and **different cellular responses due to the activation of one RTK** ;

C. Stage 3: cellular response

12. A signal transduction pathway is activated that causes **vesicles in the cytoplasm that contain glucose carriers to move and fuse to the cell surface membrane** ;
13. Increase in glucose carriers on the cell surface membrane of liver and muscle cells leads to **increase rate of glucose uptake into cells via facilitated diffusion** ;
14. **Activates glucokinase** to phosphorylate glucose to glucose-6-phosphate, the first step of both **glycogen synthesis** and **glycolysis** ;
15. **Stimulates glycogenesis** (glycogen synthesis) by activating the enzymes involved in glycogen synthesis e.g. **glycogen synthetase** which polymerises phosphorylated glucose to glycogen in liver and muscle cells ;
16. **Inhibits glycogenolysis** (glycogen breakdown) by inhibiting **glycogen phosphorylase** which catalyses the breakdown of glycogen to glucose in liver and muscle cells ;
17. **Inhibits gluconeogenesis** (making glucose from non-carbohydrate sources) by **inhibiting the breakdown of amino acids and lipids** ;
18. Other effects of insulin – increase rate of **protein and fat synthesis in liver cells**, increase rate of DNA and ATP synthesis, stimulate cell division, etc.
19. Overall, **these cellular responses help bring blood glucose level back to the norm of 100 mg / 100 cm^3 of blood**.
20. Once the norm blood glucose level is reached, **negative feedback** mechanism will **prevent further release of insulin from the β cells** ;

QWC: All 3 stages with at least 2 points from each.

(b) Glucose serves as the primary respiratory substrate within cells. It undergoes a series of chemical reactions, primarily in the cytoplasm and mitochondria, to generate ATP, the cell's main energy currency. This process, known as cellular respiration, involves both anaerobic (without oxygen) and aerobic (with oxygen) stages.

respiratory enzymes

Discuss, with named examples, the importance of conformation in respiration. [12]

A. General Concept

1. Conformation refers to the specific 3D shape of a protein, determined by its primary sequence and stabilised by interactions (H-bonds, ionic bonds, hydrophobic interactions, disulfide bonds) ;
2. Loss of native conformation (due to high temperature, pH changes, inhibitors) disrupts respiratory processes (e.g. enzyme's active sites, electron flow, proton pumping, and ATP yield) ;
3. Competitive inhibitors (e.g., malonate) mimic substrate conformation to block enzyme active sites (succinate dehydrogenase) ;
4. Non-competitive inhibitors (e.g., cyanide) bind to a site other than the active site of cytochrome oxidase, altering enzyme conformation and stopping electron transfer to oxygen ;

B. Glycolysis (cytoplasm)

3. **Glucose transporters (GLUT)** – conformation enables specific binding and facilitated diffusion of glucose into the cell ;
4. **Glucokinase/hexokinase** – active site conformation allows binding of glucose and correct positioning for phosphate transfer from ATP ;
5. **Phosphofructokinase (PFK)** – allosteric regulation where binding of allosteric inhibitor, ATP alters enzyme conformation, reducing glycolytic rate ;
6. Correct conformation of **dehydrogenases** allows NAD^+ binding for dehydrogenation, producing NADH ;
7. Specific **enzymes act as catalysts** in **substrate level phosphorylation** that produces ATP by transferring a phosphate group directly from a high-energy substrate molecule to ADP ;

C. Link Reaction and Krebs Cycle (mitochondrial matrix)

8. **Pyruvate transport proteins** – conformation enables specific recognition and transport of pyruvate into the mitochondrial matrix ;
9. In Link reaction, multi-enzyme complex where conformations **facilitate sequential reactions** (decarboxylation, oxidation, CoA attachment) ;
10. Ref. to conformation of first enzyme in Krebs cycle / citrate synthase allows **induced fit with acetyl-CoA and oxaloacetate**, preventing premature reaction ;
11. **Dehydrogenases** (e.g., isocitrate dehydrogenase, succinate dehydrogenase) have conformations positioning substrate and NAD^+/FAD for efficient electron transfer ;
12. **Decarboxylase enzymes**' active sites orient substrates for CO_2 release ;

D. Oxidative Phosphorylation (inner mitochondrial membrane)

13. **Electron carriers** (e.g., cytochromes, Fe–S proteins) have conformations positioning prosthetic groups (heme, Fe–S clusters) for **efficient electron transfer** along the ETC ;
14. Proton pumping proteins undergo **conformational changes** that move protons across the membrane, establishing a proton motive force ;
15. **ATP synthase** – proton flow through channel, inducing conformational changes in enzyme's active sites (binding change mechanism) for ATP synthesis ;
16. Correct conformation of oxygen-binding site in the last electron acceptor / cytochrome oxidase enables oxygen reduction to water ;

E. Anaerobic Respiration

17. In **mammalian muscle**, lactate dehydrogenase has a conformation allowing reduction of pyruvate to lactate, regenerating NAD^+ for glycolysis ;
18. In **yeast**, alcohol dehydrogenase conformation enables conversion of ethanal to ethanol, also regenerating NAD^+ ;
19. Maintenance of enzyme conformation under anaerobic conditions is essential for continued ATP production via glycolysis (small yield) ;

QWC: At least one point from **4** out of 5 areas (**A-E**)

5 **(a)** Describe how the genomes of viruses are inherited through the reproductive cycle of a lambda phage. [13]

A. Attachment and Entry

1. **Adsorption** — λ phage recognises and binds to a **specific receptor** on the **cell surface** of the host **bacterium** via its base plate and **tail fibre**.
2. **Injection of DNA** — phage contracts tail sheath to inject **linear double-stranded DNA** into the bacterial cytoplasm, leaving the capsid outside.

B. Early Decision: Lytic vs Lysogenic Pathway

3. **Circularisation** — injected linear DNA circularises ;
4. The next step depends on the reproductive mode: either lytic or lysogenic cycle ;
5. In the **lytic pathway**, viral genomes are inherited by **direct replication and packaging** into progeny virions ;
6. In the **lysogenic pathway**, viral genomes are inherited **as part of the host genome** during bacterial binary fission, ensuring persistence without immediate destruction of the host ;
7. Environmental cues (e.g., nutrient availability, host stress) influence pathway choice ;

C. Lytic Cycle (Genome Inheritance via Direct Replication and Packaging)

8. **Replication** – the phage uses the host's DNA replication machinery to **replicate its viral genome** ;
9. **Transcription/Translation** – the host's protein synthesis machinery is used to **synthesise viral proteins** and structural components ;
10. **Assembly** – the phage protein components, e.g. capsomeres, assemble around the viral genome to form **mature phage particles** ;
11. **Cell lysis** – phage-encoded **lysozyme** breaks bacterial cell wall, releasing progeny phages, each carrying a complete λ genome ;

D. Lysogenic Cycle (Genome Inheritance via Prophage State)

12. **Integration** – **viral DNA integrates into a specific site on the host bacterial chromosome** ;
13. **Prophage** – integrated phage DNA has a gene coding for a **repressor protein** that prevents the transcription of most of the other prophage genes ;
14. **Replication** – phage genome is replicated **passively** along with host DNA during bacterial cell division ;
15. **Vertical transmission** – daughter cells inherit both bacterial and λ genomes as part of the same DNA molecule ;

E. Induction and Return to Lytic Cycle

16. **Induction trigger** — **stress** (e.g., ionising radiation, UV radiation, toxic chemicals, lack of nutrients, etc.) causes certain proteases synthesis which **hydrolyses the repressor**, resulting in the **expression of the prophage genes** and start of the lytic cycle ;
17. **Excision** – the prophage exits the host bacterial chromosome ;
18. **Resumption of lytic cycle** – excised DNA undergoes replication, packaging, and cell lysis as above.

QWC: At least one point from **4** out of 5 areas (**A-E**)

(b) Proteins perform a vast range of functions in living cells – from catalysing metabolic reactions to providing structural support. To become functional, a protein must be synthesised accurately, folded correctly, and sometimes modified after translation. chemical groups

Transcription and Translation Post Translation in rER lumen Post Translation in Golgi

Discuss, with named examples, the involvement of covalent bonds in the synthesis of a functional protein. [12]

phosphodiester bond peptidebonds 3D conformation, hydrogen b, ionic b, hydrophobic interactions and disulphide b

A. Transcription (DNA → mRNA)

1. **Phosphodiester bonds** form between the 3′ hydroxyl group of one ribonucleotide and the 5′ phosphate group of the next during transcription ;
2. Catalysed by **RNA polymerase**, these covalent bonds link ribonucleotides to form the **sugar–phosphate backbone** of mRNA ;
3. Bonds are essential for mRNA stability and integrity, enabling accurate transfer of genetic information to the ribosome ;

B. Translation (Primary Structure Formation)

4. **Peptide bonds** formed between amino acids during **translation** in ribosomes via condensation reactions ;
5. Catalysed by **peptidyl transferase** (rRNA catalytic activity) in the large ribosomal subunit ;
6. Peptide bond involves covalent linkage between the **carboxyl group** of one amino acid and the **amino group** of another, determining the **primary structure** of the protein.

C. Post-Translational Folding & Stabilisation

7. **Disulfide bonds** form between cysteine residues, stabilising the **tertiary structure** (and quaternary if applicable) ;
8. Example: **Insulin** / antibody has inter- and intrachain disulfide bonds that stabilise its functional conformation ;
9. Disulfide bonds form in the lumen of rough ER (or Golgi), catalysed by an enzyme ;

D. Post-Translational Covalent Modifications

10. **Glycosylation** - covalent attachment of oligosaccharides to amino acid residues ; Example: glycoproteins like **erythropoietin** require glycosylation for stability and activity ;
11. **Phosphorylation** - covalent addition of phosphate groups (via phosphoester bonds) to amino acid residues.
12. Example: phosphorylation of **protein kinases** regulates enzyme activity ;
13. **Lipidation** - covalent attachment of lipid groups for membrane targeting ;
14. **Ubiquitination** - covalent attachment of ubiquitin to tag proteins for degradation by the proteasome ;

E. Multi-Subunit Assembly / Cofactor/Prosthetic Group Attachment and Functional Implications

15. Covalent bonds (e.g., interchain disulfide bonds) may link polypeptide chains in quaternary structures ;
16. Example: **antibodies** have heavy and light chains covalently linked by disulfide bonds ;
17. Some proteins require covalent bonding to a prosthetic group for functionality.
18. Covalent bonds provide **structural integrity** that resists denaturation under physiological conditions ;
19. Covalent modifications fine-tune **protein activity**, **localisation**, and **lifespan**, ensuring precise regulation in response to cellular signals ;

QWC: At least one point from **4** out of 5 areas (**A-E**)