H

RAFFLES INSTITUTION
2023 Year 6 Preliminary Examination

Higher 2

CANDIDATE NAME

CIVICS GROUP | 2 | 3 | S | 0 | 3 | |

INDEX NUMBER | | | | | | | |

BIOLOGY

9744/03

Paper 3 Long Structured and Free-response Questions

14th Sept 2023

2 hours

Candidates answer on the Question Paper.

Additional Materials: Writing paper.

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group & name in the spaces at the top of this page.
Write in dark blue or black pen.
You may use a 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 writing paper provided.

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, **hand in your essay question SEPARATELY.**

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

For Examiner's Use	
Section A	
1	**/ 28**
2	**/ 12**
3	**/ 10**
Section B	
4 or 5	**/ 25**
Total	**/ 75**

This document consists of **13** printed pages.

Raffles Institution
Internal Examination

Section A

Answer **all** the questions in this section.

1 Immunofluorescence is a technique that scientists use to visualize the distribution and morphological appearance of specific proteins within cells.

The indirect immunofluorescence technique involves:

1. The production of specific antibodies that bind to a specific antigen. These are known as primary antibodies.
2. The addition of the primary antibodies to a preparation of cells or tissues fixed on glass slides.
3. The addition of secondary antibodies labeled with fluorescence dye called FITC which will bind to the primary antibodies.
4. Visualization under a fluorescence microscope. Under the light of excitation wavelength, 450nm, FITC will fluoresce.

Fig. 1.1 shows the principles of the indirect method of immunofluorescence.

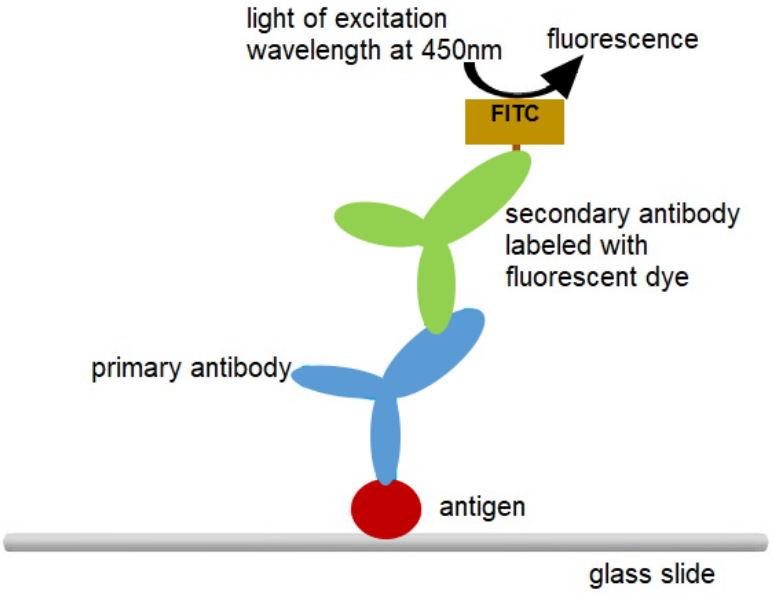

Fig. 1.1

Immunofluorescence was used to study cells undergoing cell division. Fig. 1.2 shows a micrograph of a cell. The lighter area shows the position of the fluorescence.

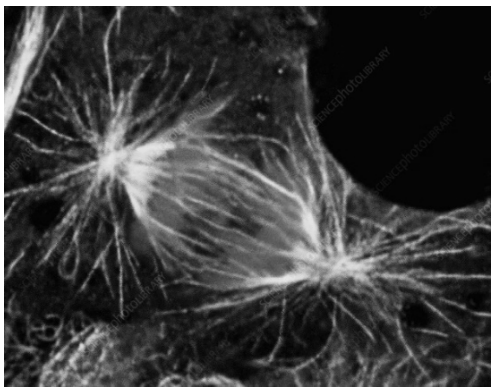

Fig. 1.2

(a) (i) Identify the cell structures that fluoresced in Fig. 1.2 and name the specific antigen (protein X) that the primary antibodies bind to. [2]

cell structure	
specific antigen (protein X)	

(ii) Identify the stage of mitotic division that the cell in Fig. 1.2 is in and describe the events that occur at this stage.

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(iii) The secondary antibodies are goat antibodies that bind to the primary antibodies which are mouse antibodies.

With reference to Fig. 1.1, state the name of the specific site that the secondary goat antibodies bind to and explain why the secondary goat antibodies do not bind to other sites.

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(b) To produce primary mouse antibodies which bind to protein X, the following steps are carried out.

Step 1	produce protein X
Step 2	inject protein X into a mouse
Step 3	harvest plasma cells from the mouse
Step 4	fuse plasma cells with tumour cells to produce an 'immortal' hybrid cell that produces antibodies
Step 5	harvest specific antibodies

Fig.1.3 illustrates how the primary mouse antibodies are produced.

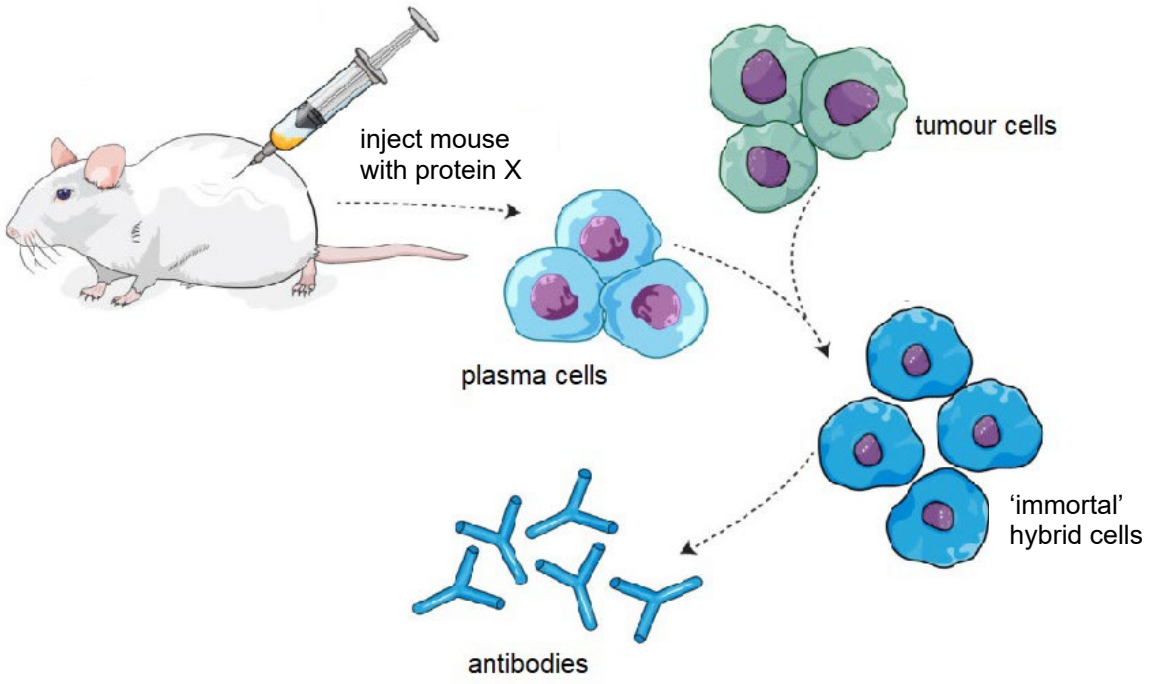

Fig. 1.3

(i) Explain why plasma cells alone cannot be used directly for the long term production of antibodies.

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(ii) The Hayflick limit is the number of times a normal somatic cell population will divide before cell division stops. In order for the 'immortal' hybrid cells in Fig. 1.3 to divide indefinitely, the cells need to overcome the Hayflick limit.

Explain how the fusion of the plasma cells with the tumour cells enable the 'immortal' hybrid cells to overcome the Hayflick limit.

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(c) In order for a mouse to produce primary mouse antibodies, protein X needs to be injected into it. Dr N, a relatively inexperienced scientist, was tasked to use genetic engineering to produce purified protein X. This was Dr N's experimental approach.

Step A	harvest total chromosomal DNA from a human cell
Step B	design PCR primers to amplify the coding region of the protein X gene
Step C	perform PCR on the coding region of protein X
Step D	insert the coding region of protein X into a bacteria plasmid
Step E	insert the recombinant plasmid containing the coding region of protein X into *Escherichia coli* bacteria by a process called transformation

Fig. 1.4 below summarises Dr N's approach.

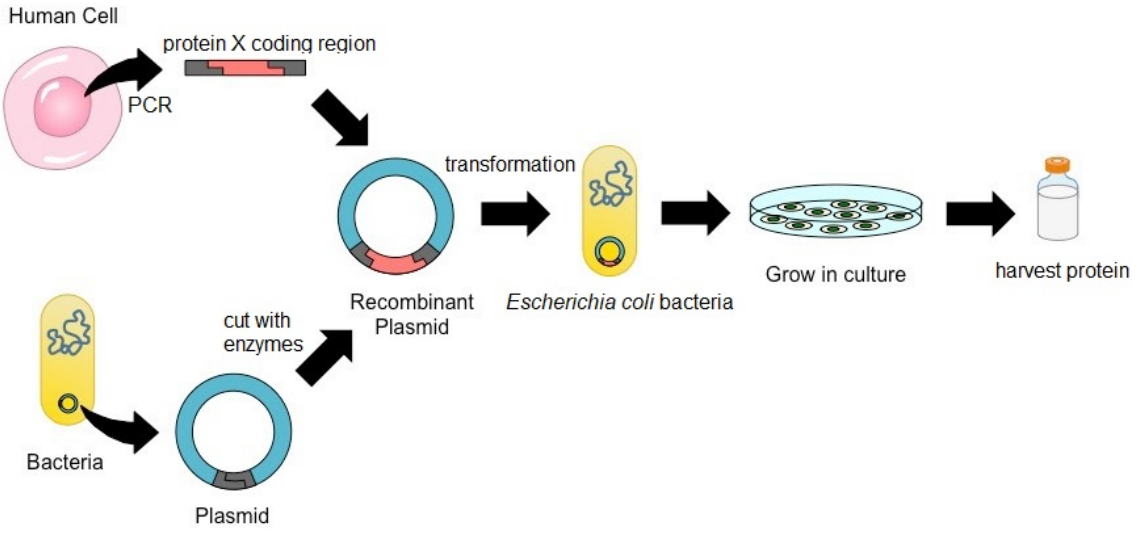

Fig. 1.4

Dr N obtained the nucleotide sequence of the coding region of protein X gene from his supervisor, Prof S.

The first and last 20 nucleotides of the template DNA strand are shown below.

3' GTACAGTTAA CATTGTCACG ……TGATAAAAGA GACATTTCAA 5'

(i) Using the information above, help Dr N to design a 10 nucleotide long 'forward' and 10 nucleotide long 'reverse' primer that can be used to amplify the coding region of the protein X gene.

State the sequences of the respective primers in the table below.

type of primer	sequences of primer
'forward'	
'reverse'	

[2]

Dr N was tasked to set the PCR conditions to amplify the coding region of protein X.
His proposal is shown below:

stage	temperature	duration
1	65^{o}C	30s
2	72^{o}C	60s
3	95^{o}C	90s

Repeat stages 1, 2 and 3 sequentially for 30 cycles.

(ii) Comment if Dr N's proposed PCR conditions will successfully produce the desired PCR product.

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(d) In a parallel experiment, Prof S, successfully amplified the coding region of protein X. This was inserted into a bacterial plasmid and the resultant recombinant plasmid was then introduced into a bacterium, *Escherichia coli*, as shown in Fig. 1.4.

Prof S successfully produced polypeptides using this approach.

(i) Name the non-coding sequence that must be present in the bacteria plasmid to enable the expression of protein X.

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(ii) Further analysis shows that the polypeptide produced is much longer than expected.

Suggest an explanation for this observation.

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(iii) Explain why this polypeptide can still be injected into the mouse to produce the correct primary antibodies as shown in Fig. 1.3.

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[Total: 28]

2 Fig. 2.1 shows the life cycle of SARS-CoV-2, which begins with the binding of the Receptor-Binding Domain (RBD) in the S1 subunit of the spike protein to Angiotensin Converting Enzyme 2 (ACE2).

Similar to the influenza virus, SARS-CoV-2 infects the surface epithelial cells of the respiratory tract.

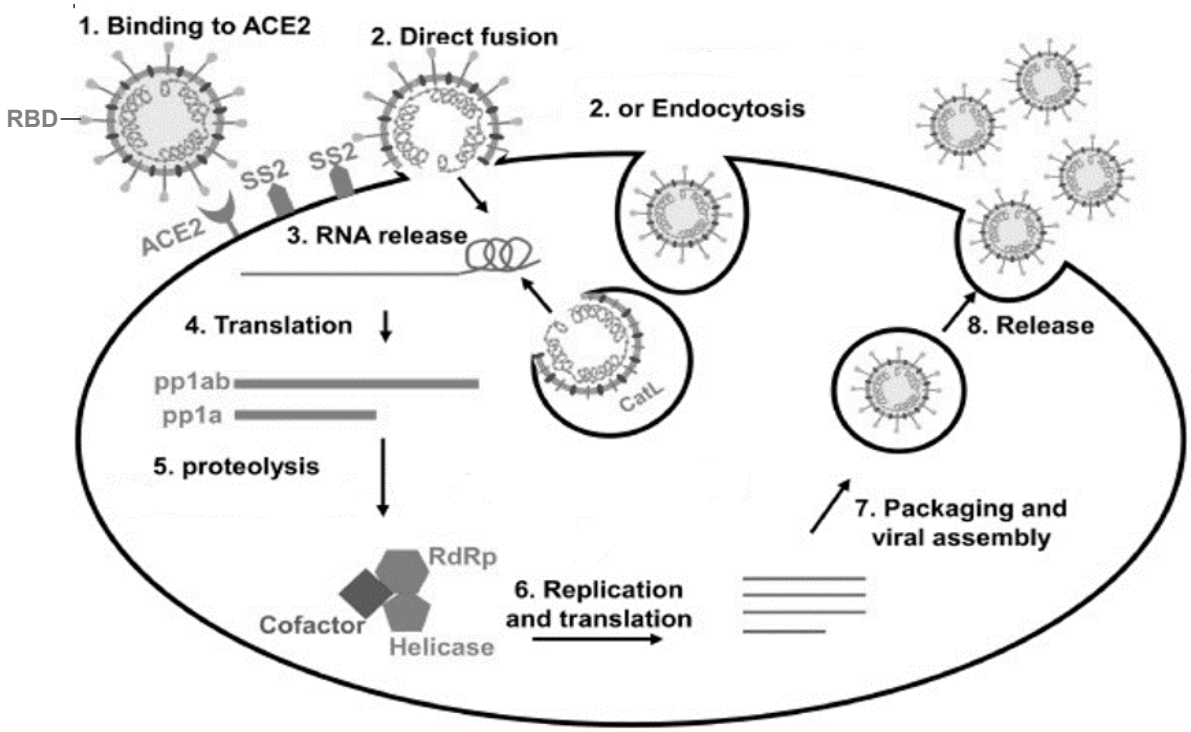

Fig. 2.1

(a) Explain why both the SARS-CoV-2 and the influenza viruses recognize the surface epithelial cells of the respiratory tract.

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(b) Describe two differences between the replication cycle of SARS-CoV-2 and influenza viruses.

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(c) Describe a strategy that can target a named protein which can be used to block key steps of the life cycle of SARS-CoV-2.

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(d) Vaccine preventable diseases (VPDs) including measles and mumps have been re-emerging in countries despite sustained high vaccine coverage.

The durability of vaccine-induced immunity compared to immunity induced by natural infection has been studied by a group of scientists and their results are shown in Fig. 2.2.

The incidences of the infections before the vaccine era and during the vaccine era was recorded.

The amount of antibodies (Ab titre) and proportion of cases by age were also monitored and recorded.

Repeat exposures during community outbreaks served as natural asymptomatic boosting events.

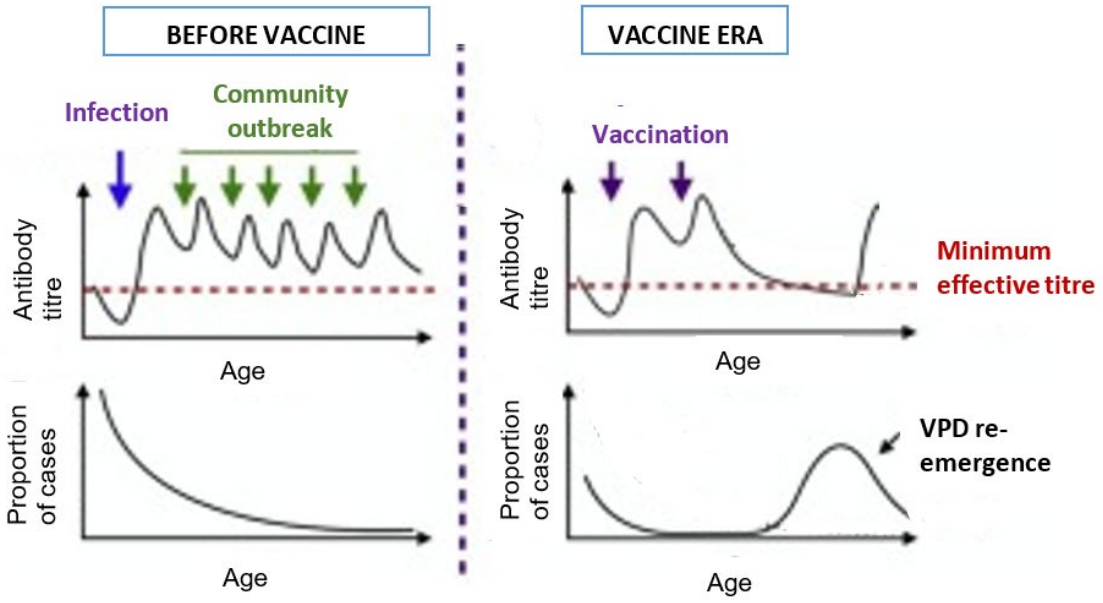

Fig. 2.2

(i) Describe the differences in the proportion of cases against age before and after vaccination was introduced.

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……[2]

For Examiner's Use

(ii) Explain how vaccination causes a rise in antibody titre to a level similar to that produced during an infection.

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(iii) Suggest how the vaccination programme can be modified to prevent the re-emergence. of VPD.

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[Total: 12]

3 Long periods of studying or other kinds of stressful mental activity cause the buildup of adenosine molecules in brain tissue.

Adenosine is a ligand that binds to a G protein-coupled receptor on brain cells, activating a G protein by replacing its bound GDP with a GTP. A subunit of the G protein then binds to and activates adenylyl cyclase (AC), a membrane-bound effector protein. Adenylyl cyclase then catalyzes the conversion of ATP to cyclic AMP (cAMP), a second messenger.

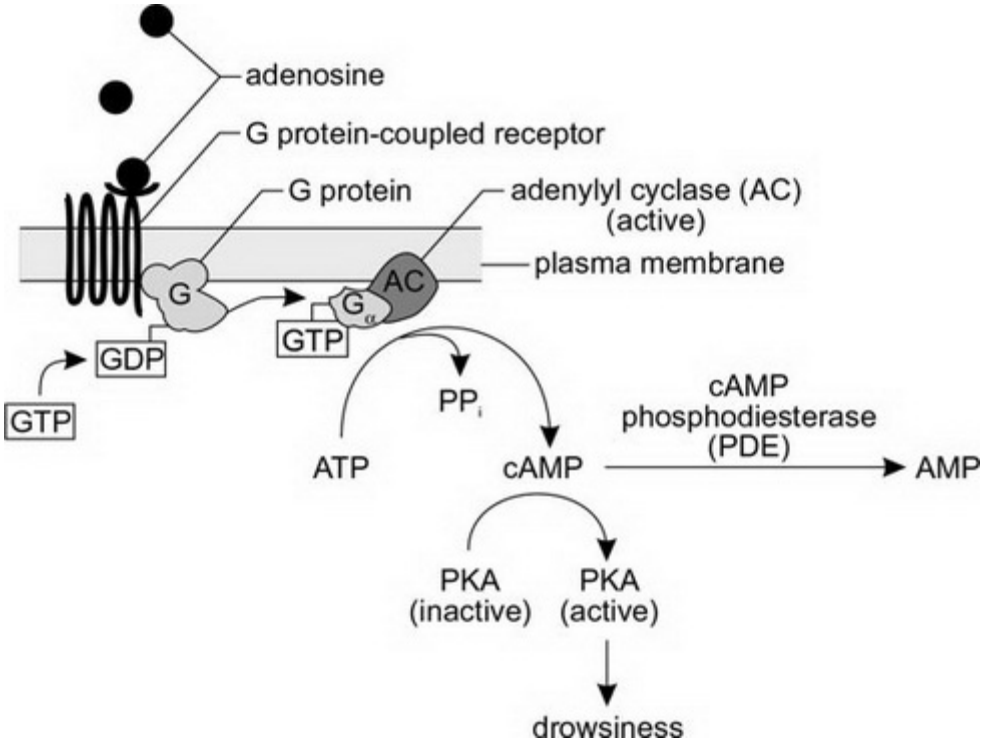

Fig. 3.1

(a) Describe how the structure of adenylyl cyclase relates to its function.

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(b) With reference to Fig. 3.1, identify a stage where signal amplification is observed and explain how the signal is amplified in this stage.

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The cellular response to cAMP varies widely in different types of cells. In brain cells and other cells of the central nervous system, cAMP activates a Protein Kinase A (PKA), which slows brain activity and causes drowsiness.

Normally, cAMP concentrations in the cell are kept low by the enzyme cAMP phosphodiesterase (PDE), converting cAMP to regular AMP (not cyclic). But high levels of cAMP can be attained during periods of mental fatigue or other kinds of stress.

Caffeine is an adenosine signaling antagonist, blocking the effect of adenosine.

Fig. 3.2 shows the structures of adenosine and caffeine.

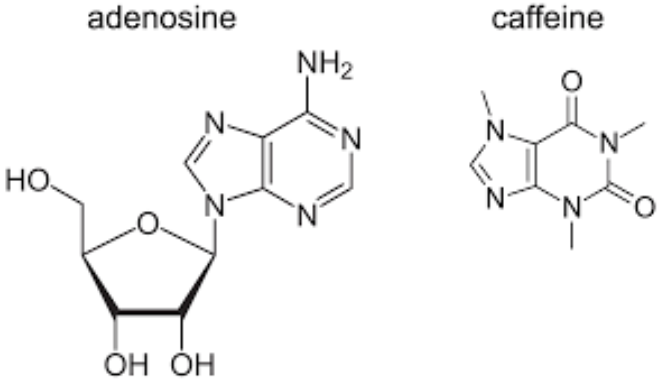

Fig. 3.2

(c) With reference to Fig. 3.1 and Fig. 3.2, explain the effect of consuming several caffeinated drinks on an individual.

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(d) Drugs known as cAMP phosphodiesterase inhibitors are used in the management and treatment of chronic obstructive pulmonary disease, psoriasis, psoriatic arthritis and erectile dysfunction.

With reference to Fig. 3.1, discuss the impact of treatment with cAMP phosphodiesterase inhibitors on an individual who

- suffers from psoriasis;
- is highly stressed; and
- does not consume anything with caffeine.

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[Total:10]

Section B

Answer **one** question in this section.

Write your answers on the separate answer paper provided.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in sections **(a)** and **(b)**, as indicated in the question.

4 **(a)** Discuss whether you agree or disagree that the impacts of human activity on climate change will lead to the greater spread of mosquitoes.

Comment if this matter requires both urgent and global action. [15]

(b) Explain why, in a mammalian cell, glucose is a better respiratory substrate compared to triglycerides and why triglycerides are suitable as storage molecules. [10]

[Total: 25]

5 **(a)** The fluid mosaic hypothesis for the plasma membrane was formulated by Singer and Nicolson in the early 1970s and is universally accepted.

With named examples, discuss the significance of the properties of fluid mosaic model in living organisms. [15]

(b) Outline how phenotypic variation is brought about in named animal viruses. [10]

[Total: 25]

End of Paper