Anglo-Chinese Junior College

JC2 Biology Preliminary Examination
Higher 2

A Methodist Institution
(Founded 1886)

CANDIDATE NAME		FORM CLASS	
TUTORIAL CLASS		INDEX NUMBER	

BIOLOGY **9744/04**

Paper 4 Practical 06 August 2024
2 hours 30 minutes

Candidates answer on the Question Paper.
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your Name, Class and Index number in the spaces on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate in the boxes provided.
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.

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 Examiners' use	
1	/ 12
2	/ 23
3	/ 20
Total	/ 55

Answer **all** questions.

1 In some countries, certain plants have seasonal growth. During the summer plants transport sucrose from the leaves to store it as starch in the roots.

Table 1.1 summarises the changes in the leaves and roots during the year.

Table 1.1

season	leaves	roots
summer	leaves synthesise sucrose	sucrose stored as starch
winter	no leaves	starch stored
spring	leaves growing	starch converted to glucose

You are required to identify the source of four plant extracts. These have been taken from

- a root in winter
- a root in spring
- phloem sap from the stem in summer
- phloem sap from the stem in winter

(a) Use the information in Table 1.1 to predict which substances you would expect to be present in each of the four plant extracts, then complete Table 1.2.

Key: ✓ (tick) substance present in plant extract
✗ (cross) substance absent from plant extract

Table 1.2

source of plant extract	substances present in each of the plant extracts		
	starch	sucrose	glucose
root in winter	✓	✗	✗
root in spring	✓ **or** ✗	✗	✓
phloem sap from the stem in summer	✗	✓	✗
phloem sap from the stem in winter	✗	✗	✗

[2]

1m for first two rows, 1m for last two rows;

One sample was taken from each of the four plant extracts shown in Table 1.2.

You are required to identify from which plant extract each of the four samples **S1**, **S2**, **S3** and **S4** was taken.

You are provided with:

- Benedict's solution, in a container labelled **B**
- iodine solution, in a container labelled **I**
- hydrochloric acid, in a container labelled **H**
- sodium hydrogencarbonate powder, in a container labelled **S**
- a source of heated water.

Hydrochloric acid H and sodium hydrogencarbonate S are irritants, Benedict's solution B is harmful and iodine solution I is a stain. Suitable eye protection should be worn. If any of these reagents come into contact with your skin, wash off immediately under cold water.

Do not carry out any tests until you have read the instructions on pages 3 to 5.

(b) Describe the two tests that show that starch and glucose are present in a plant extract.

test for starch:

On a white tile, place 3 drops of sample, then add 1 drop of iodine; OR
To 1 cm^3 of sample, add 3 drop of iodine;
…… [1]

test for glucose:

1. **To 2 cm^3 of sample, add 2 cm^3 of Benedict's solution (volume must be 1:1);**
2. **Heat in a boiling water bath for 2 minutes (min 1 minute, max 3 minutes);**

…… [2]

[Turn over

For the test for sucrose, refer to steps **1** to **9**.

1 Before starting the test for sucrose, perform the test for glucose you have described in **(b)**.

(c) Explain why the test for glucose must be conducted before the test for sucrose can be carried out.

1. **The test for sucrose / non-reducing sugar test involves <u>hydrolysis</u> of sucrose / non-reducing sugar to form glucose / reducing sugar;**
2. **Hence necessary to test for the absence of glucose / reducing sugar at the start;**
   OR
3. **The test for sucrose / non-reducing sugar will eventually still involve a test for glucose / reducing sugar / Benedict's test;**
4. **Hence necessary to test for the absence of glucose / reducing sugar at the start;**

……..... [2]

2 To start the test for sucrose, put 2.0 cm^3 of a fresh sample into a test-tube.

3 Put 2.0 cm^3 of **H** into the same test-tube.

4 Shake the test-tube gently to mix the contents.

5 Put the test-tube in a boiling water-bath. Leave the test-tube for 2 minutes.

6 After 2 minutes, remove the test-tube from the water-bath and put it in a test-tube rack.

7 Leave the test-tube to cool for a further 3 minutes.

8 After 3 minutes, put a small amount of **S** into the test-tube. The mixture will fizz and rise up the test-tube. Continue to add small amounts of **S** until there is no more fizzing.

Note: there may be a little of S left in the bottom of the test-tube. This will not affect the results.

9 Perform the test for glucose you have described in **(b)** on the solution from step **8**.

Proceed as follows.
Use the beaker labelled **hot water** to collect approximately 400 cm^3 of hot water from where it is provided in the laboratory. Heat the water to boiling, if needed.

Select the appropriate reagents from those provided and carry out suitable tests to identify the samples **S1**, **S2**, **S3** and **S4**.

(d) Record your observations in an appropriate table. You do **not** need to make conclusions on the presence or absence of each substance in the samples tested.

Sample	Observations		
	Test for starch / Iodine test	**Test for glucose / Reducing sugar test**	**Test for sucrose / Non-reducing sugar test**
S1	The mixture remained yellow-brown	The mixture remained blue	The mixture remained blue
S2	The mixture turned blue-black	The mixture remained blue	The mixture remained blue
S3	The mixture remained yellow-brown	The mixture remained blue	Brick-red ppt is formed
S4	The mixture turned blue-black	Brick-red ppt is formed	Brown/Brick-red ppt is formed

1. **Appropriate table layout (sample on leftmost column);**
2. **Appropriate column headings (heading for 'sample/extract/plant extract');**
3. **Observations (correct phrasing of observation with good details);** **R! ticks/crosses**
4. **Complete set of results + correct trend;**

[4]

(e) Complete Table 1.3 to match the samples, **S1**, **S2**, **S3** and **S4**, with each plant extract.

Table 1.3

source of plant extract	sample
a root in winter	**S2**
a root in spring	**S4**
phloem sap from the stem in summer	**S3**
phloem sap from the stem in winter	**S1**

[1]

[Total: 12]

[Turn over

2 Yeast contains an enzyme that will break down hydrogen peroxide into oxygen and water. The loss of mass resulting from the release of oxygen can be measured.

You will investigate the effects of the concentration of hydrogen peroxide on the rate of enzymatic activity.

You are provided with:

- Yeast cell suspension, in a container labelled **Y**
- 1.0% hydrogen peroxide solution, in a container labelled **P**
- Distilled water, in a container labelled **W**

Hydrogen peroxide solution P is an irritant and is harmful. Suitable eye protection should be worn. If P comes into contact with your skin, wash them off immediately under cold water.

Each time that you take a sample of yeast cell suspension Y, you should make sure that it is mixed thoroughly by stirring it with a glass rod. You should also collect Y from below the surface, so as to minimise the volume of froth collected.

(a) (i) You will carry out proportional dilutions of the 1.0% hydrogen peroxide solution **P** to obtain a range of concentrations in which the concentration of hydrogen peroxide is reduced by 0.2% between each successive dilution.

You will prepare 10.0 cm^3 of each concentration, using **P** and **W**.

Using a table in the space below, show how you will prepare the different concentrations of hydrogen peroxide solution. One of the concentrations should include 1.0% hydrogen peroxide.

concentration of hydrogen peroxide / %	**volume of P / cm^3**	**volume of W / cm^3**
1.0	**10.0**	**0.0**
0.8	**8.0**	**2.0**
0.6	**6.0**	**4.0**
0.4	**4.0**	**6.0**
0.2	**2.0**	**8.0**

1. **Appropriate table layout (final H_2O_2 concentration on leftmost column) + correct column heading;**
2. **5 correct concentrations of H_2O_2 given;**
3. **Correct volumes of P and W, both to 1 dp;**

[3]

Read steps 1–6 before starting the investigation. Proceed as follows.

1 Prepare the concentrations of hydrogen peroxide solution according to the table in **(a)(i)**, in the vials provided. Label the vials where appropriate.

2 Use the electronic mass balance to weigh the mass of the reaction mixture in subsequent steps.

3 Put 5.0 cm^3 of yeast suspension **Y** into the vial containing 1.0% hydrogen peroxide solution. Start timing immediately and proceed to step **4** without delay.

4 Weigh the mass of the reaction mixture, including the weight of the vial, using the set-up shown in Fig. 2.1.

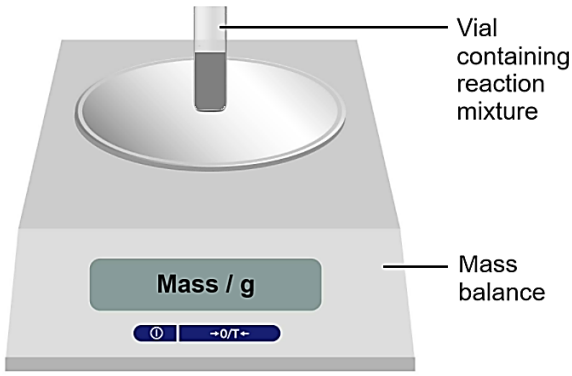


Fig. 2.1

5 After five minutes, weigh the mass of the reaction mixture in the vial again.

6 Repeat steps **3 – 5** with each of the other concentrations of hydrogen peroxide that you prepared in step **1**. You do **not** need to wait to complete the measurement for one concentration of hydrogen peroxide before starting on the next concentration.

(ii) Record your results in Table 2.1.
Calculate the change in mass and the percentage change in mass. No workings are required.

Table 2.1

concentration of hydrogen peroxide / %	initial mass of reaction mixture / g	final mass of reaction mixture / g	change in mass / g	percentage change in mass
1.0	**20.27**	**20.20**	**– 0.07**	**– 0.35**
0.8	**20.65**	**20.60**	**– 0.05**	**– 0.24**
0.6	**20.40**	**20.36**	**– 0.04**	**– 0.20**
0.4	**20.61**	**20.59**	**– 0.02**	**– 0.097**
0.2	**20.34**	**20.33**	**– 0.01**	**– 0.049**

1. **All initial masses and final masses recorded to 2dp;**
2. **All change in masses correctly calculated and recorded to 2dp;**
3. **All percentage change in mass correctly calculated and recorded to 1 or 2sf;**
4. **Correct trend (highest percentage change for 1.0% or 0.8% + lowest percentage change for 0.2% or 0.4%);**

[4]

[Turn over

(iii) Suggest a suitable control for this experiment to show that it is an enzyme that catalyses the break down of hydrogen peroxide.

Adding 5.0 cm^3 of boiled and cooled Y / yeast suspension to hydrogen peroxide instead; R! distilled water

…… [1]

(iv) Other than the lack of a suitable control, describe **two** modifications to this method that would increase the confidence in your results.

1. **Maintain the temperature at 37°C (A! 30 to 40°C) using a thermostatically controlled water bath / using a mix of cold and hot water and measuring the temperature using a thermometer;**
2. **Maintaining the pH of the reaction mixture by adding 1 cm^3 of buffer solution;**
3. **Repeating the experiment at least two more times and obtaining a mean / average OR to allow identification of anomalies;**
4. **Repeating the experiment using higher concentrations of hydrogen peroxide (to obtain a more significant change in the mass of the reaction mixture);**

R! Use of smaller concentration intervals

R! Use of vol-related apparatus with finer precision

max 2m

…… [2]

(b) A student wanted to determine the Michaelis-Menten constant (K_m) for the enzyme-catalysed break down of hydrogen peroxide by the enzyme found in yeast, enzyme **Y**.

K_m is the substrate concentration at which the reaction rate is 50% of the maximum rate of reaction (V_{max}).

K_m gives an indication of the affinity an enzyme has for its substrate.

The student measured the initial rate of reaction at different concentrations of hydrogen peroxide. The results are shown in Fig. 2.2.

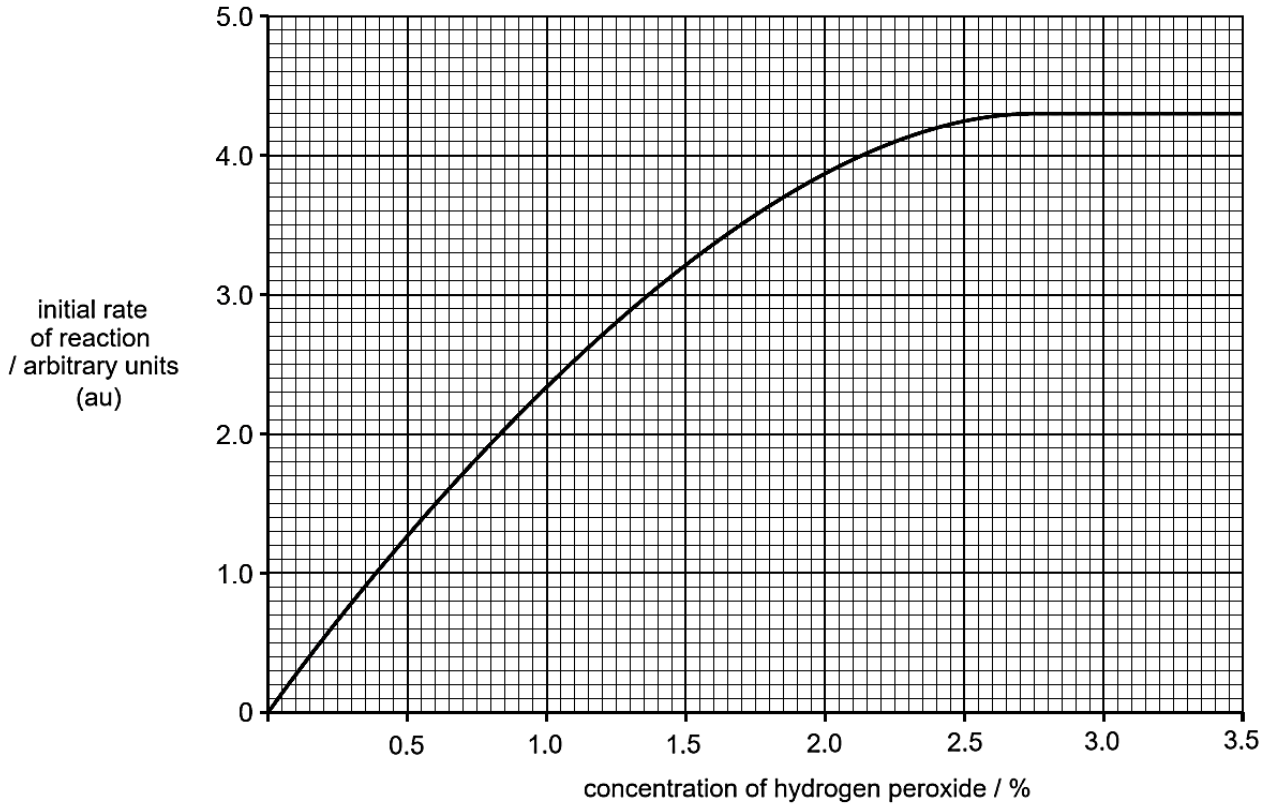


Fig. 2.2

(i) Use the graph in Fig. 2.2 to estimate the Michaelis-Menten constant (K_m).

Show your working on the graph and in the space below.

1. **reads off V_{max} correctly from the graph at <u>4.3 au</u>;**
2. **calculates ½ V_{max} correctly at <u>2.15 au</u>;**
3. **reads off the value for K_m (in %) correctly from the graph at <u>0.9%</u>; A! 2d.p**

K_m = ... [3]

[Turn over

(ii) The K_m value for another enzyme, **Z**, is 2.5%.

State which enzyme, **Z** or **Y**, has a **lower affinity** for its substrate.

Give a reason for your answer.

enzyme **Z;**

reason **K_m value for enzyme Z is higher / indicating a greater concentration of substrate is needed to reach a reaction rate that is 50% of V_{max};**

…… [2]

(c) Living, respiring yeast cells also contain enzymes which reduce methylene blue, a blue dye commonly used for the staining of biological samples, turning it colourless. In non-respiring cells, the reduction of methylene blue does not occur.

It is thought that respiration in yeast cells is inhibited by a high concentration of sodium chloride solution in the immediate environment.

The half maximal inhibitory concentration (IC_{50}) is a measure of the concentration of a particular inhibitory substance that is needed to inhibit a given biological process by 50%.

Plan an investigation, based on observing the colour of yeast cells mixed with methylene blue, to measure the IC_{50} value of sodium chloride solution on the respiration of yeast.

You have been provided with the following which you must use:

- prepared sodium chloride solutions with concentrations ranging from 0.1% to 2.0%
- yeast cell suspension, containing glucose as the respiratory substrate
- methylene blue solution
- microscope with an eyepiece graticule.

You may select from the following apparatus and plan to use appropriate additional apparatus:

- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.
- syringes and Pasteur pipettes
- timer, e.g. stopwatch
- microscope slides and cover slips.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone using it
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed.

You can consider all steps in the procedure to be low risk and there is therefore no need to include reference to any safety measures in your plan.

…….. [8]

<table>
<tr><td>1</td><td>Independent variable</td><td><u>State what the independent variable</u> is and use at least five different variables with <u>regular</u> intervals.

The <u>independent variable</u> is the concentration of sodium chloride. Use sodium chloride solutions of 0.4, 0.8, 1.2, 1.6 and 2.0%.</td></tr>
<tr><td>2</td><td>Dependent variable</td><td><u>State what is the dependent variable</u>.

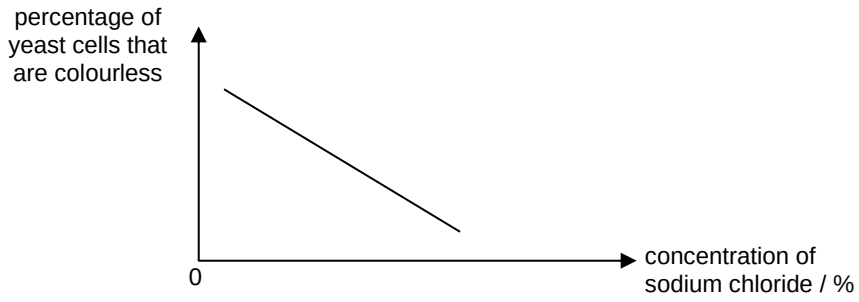
The <u>dependent variable</u> is the percentage of yeast cells that turn colourless, which is used to measure the rate of respiration.</td></tr>
<tr><td>3
+
4
+
5</td><td>Dependent variable</td><td>Specifies method of measuring / monitoring DV

3. Counting number of yeast cells that are colourless over the total number of yeast cells
4. Within the field of view of the <u>microscope</u> using an appropriate objective (need to specify how the area to be observed can be fixed)
5. Calculate percentage of cells that turn colourless</td></tr>
<tr><td>6</td><td>Controlled variables</td><td>Identifies at least TWO variables to control

1. Volume of yeast suspension and volume of sodium chloride solution (to mix in test-tube/beaker)
2. Volume of pH buffer
3. Volume of mixture of yeast+sodium chloride and volume of methylene blue (to put on glass slide)
4. Temperature of incubation of yeast+sodium chloride mixture
5. Length of time of incubation of yeast+sodium chloride mixture
6. Length of time left for staining by methylene blue</td></tr>
<tr><td>7
+
8</td><td>Controlled variables</td><td>Describes how two identified variables are controlled. (Must specify appropriate value)

1. Using a <u>syringe/measuring cylinder</u>, add 5 cm^3 of yeast suspension to 5 cm^3 of sodium chloride solution in a test-tube / beaker;
2. Using a <u>syringe/measuring cylinder,</u> add 1cm^3 of <u>pH buffer</u> to the mixture of yeast and sodium chloride solution;
3. Using a <u>Pasteur pipette/dropper</u>, add one drop of the mixture of yeast and sodium chloride to one drop of methylene blue on a glass slide;
4. Maintain the temperature at 37^oC (A! 30 to 40^oC) using a <u>thermostatically controlled water bath</u> / using a mix of cold and hot water and measuring the temperature using a <u>thermometer</u>;
5. Start the <u>stopwatch</u> and let the reaction mixture incubate for 5 minutes;
6. Start the <u>stopwatch</u> and let the methylene blue stain for 5 minutes;</td></tr>
<tr><td>9</td><td>Recording of results</td><td>Shows how results are to be presented in the form of a table with independent variables in appropriate columns/ rows and appropriate units for headings
R! “Table” without lines and/ or borders

<table>
<tr><th>concentration of sodium chloride / %</th><th>Number of colourless yeast cells</th><th>Total number of yeast cells</th><th>Percentage of yeast cells that are colourless</th></tr>
<tr><td>0.4</td><td></td><td></td><td></td></tr>
<tr><td>0.8</td><td></td><td></td><td></td></tr>
<tr><td>1.2</td><td></td><td></td><td></td></tr>
<tr><td>1.6</td><td></td><td></td><td></td></tr>
<tr><td>2.0</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
</table>

10	**Recording of results**	**Shows graph with appropriate axes labels and units, line/ curve with expected trend drawn** 
11	**Calculation of IC_{50}**	**Estimating the IC_{50} value by reading off the graph drawn where the percentage of yeast cells turning colourless is 50%.**
12	**Control**	**Describe a suitable control** Conduct the (negative) control experiment using specified volume of distilled water instead of sodium chloride. (This shows that the inhibition of yeast cell respiration is due to sodium chloride.)
13	**Reliability**	**Reference to repeating at least two more time with different experimental subjects** Repeat the entire experiment at least twice using fresh yeast suspension and sodium chloride solutions, and calculate the mean/average of the results OR to allow identification of anomalies
14	**Accuracy**	**Reference to relevant method to increase the accuracy of results** Repeating experiment with smaller intervals of sodium chloride concentrations so as to obtain a better estimate of the IC_{50}.
15	**Validity (other considerations)**	**Other considerations to improve validity of experiment** • Mixing the solutions with a glass rod on the glass slide / AVP • Before withdrawing yeast suspension, make sure to mix thoroughly by stirring it with a glass rod / collect yeast suspension from below the surface, so as to minimise the volume of froth collected. • Lower coverslip gently at an angle / tap after placing the coverslip to minimize trapping of air bubbles under the coverslip

[Total: 23]

3 During this question you will require access to a microscope and slide **L1**.

L1 is a slide of a stained transverse section through a leaf of a land plant that is affected by a fungal infection. The fungal infection affects the upper leaf surface of this plant.

(a) (i) Use your microscope to observe the different tissues in the region of slide **L1** shown by the darkly shaded area in Fig. 3.1. The observed area should include at least **one** vascular bundle and be **affected by fungal infection**.

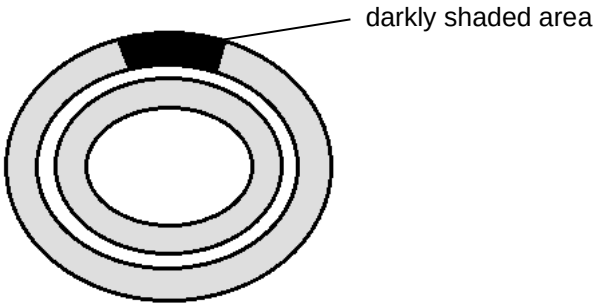
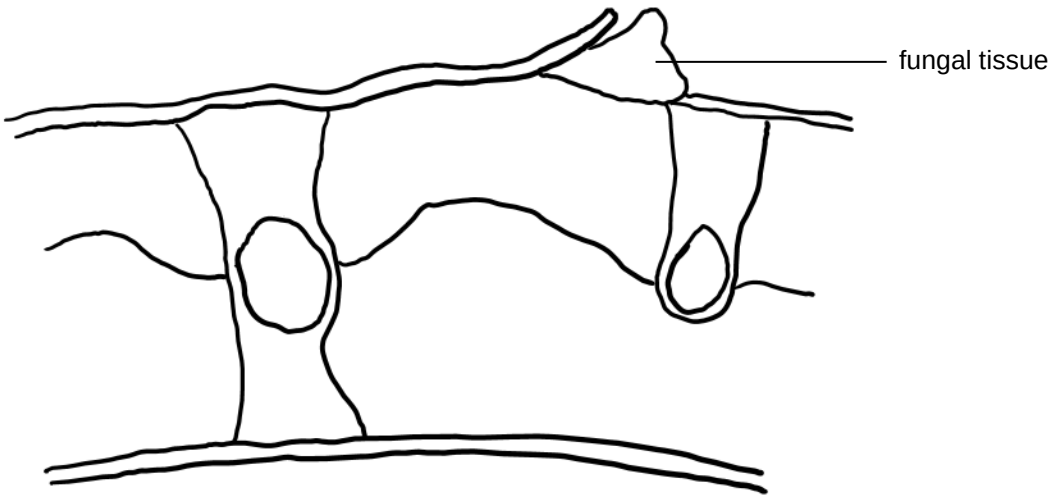


Fig. 3.1

Draw a large plan diagram of the part of the leaf shown by the shaded area in Fig. 3.1.

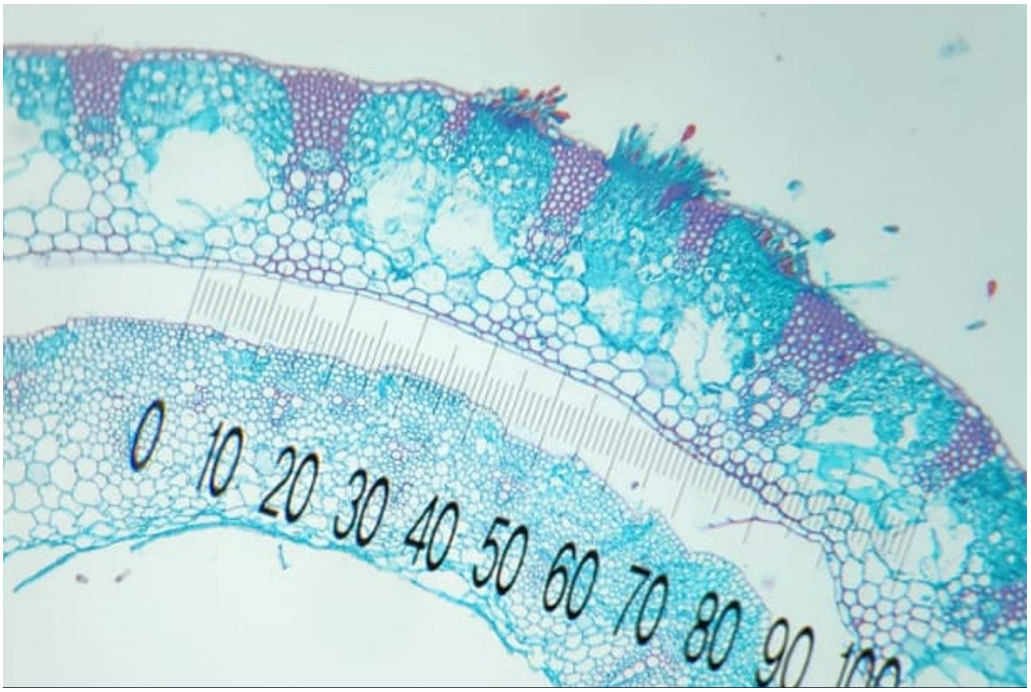
A plan diagram shows the arrangement of different tissues, including their correct shapes and proportions. No cells should be drawn.

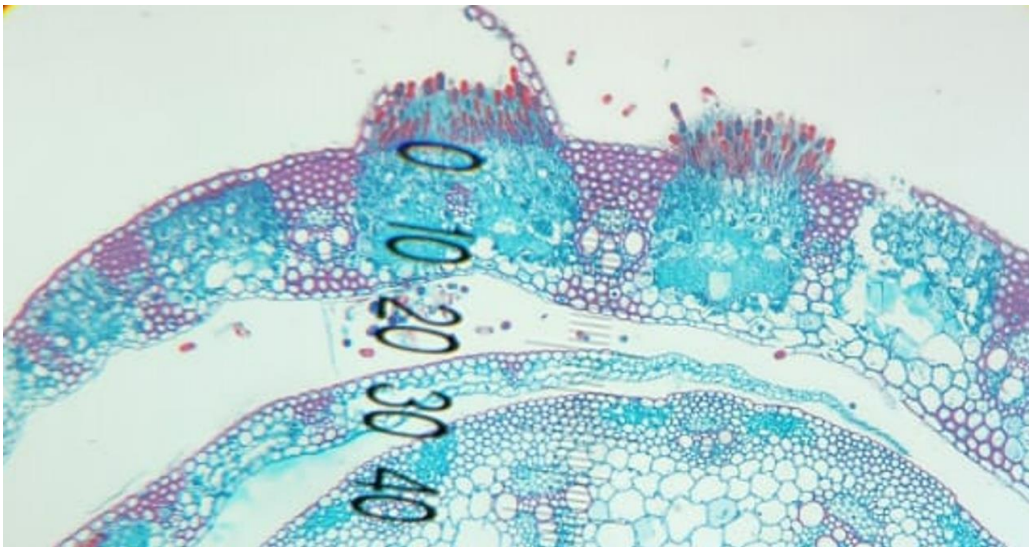
Your drawing should also include any fungal tissue observed. Use **one** ruled label line and label to identify the fungal tissue.



[Turn over

1.	**Accuracy**	**Show all the structures that can be seen in the defined part (2 epidermal layers + 2 mesophyll layers + vascular bundle)**
2.	**Accuracy**	**Correct shape and proportions of tissue types (spongy mesophyll layer slightly thicker than palisade mesophyll layer)** ***R! individual cells/ individual xylem vessel drawn***
3.	**Clarity**	**Use of sharp pencil to draw clear, single, continuous, neat lines**
4.	**Scale**	**Use at least $2/3$ of space provided**
5.	**Label**	**a) Fungal tissue of correct proportion drawn at the upper epidermal layer** **b) Clear, straight line with label for 'fungal tissue'**





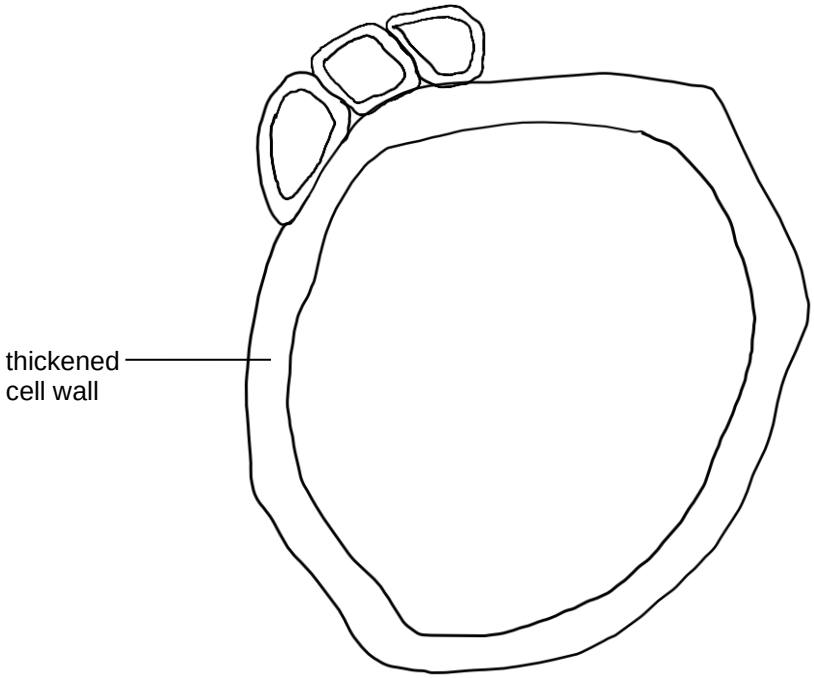
[5]

(ii) Observe **one** vascular bundle of the section on **L1**.

Select **one** large xylem vessel element and **three** adjacent smaller cells.

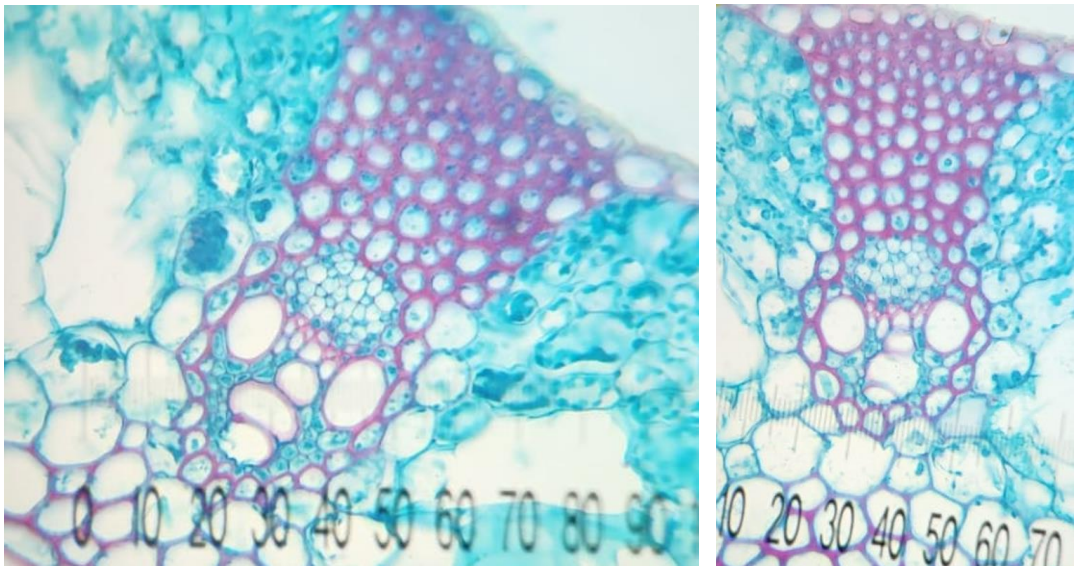
Each smaller cell must touch the large xylem vessel element and at least one of the other smaller cells.

Make a large, **labelled** drawing of these **four** cells.



1.	**Accuracy**	**a) 4 irregularly shaped cells (1 large + 3 smaller cells)** **b) Each smaller cell touching the large cell and one other cell**
2.	**Accuracy**	**Shows thick cell wall in at least the large cell** **R! chloroplasts drawn in smaller cells**
3.	**Clarity**	**Use of sharp pencil to draw clear, single, continuous, neat lines;**
4.	**Scale**	**Use at least $2/3$ of space provided**
5.	**Label**	**a) Clear straight label lines** **b) Labels for: (thickened) cell wall** **R! cytoplasm label for large xylem vessel**

[Turn over



[5]

(iii) Fig. 3.2 is a photomicrograph of a stained transverse section of part of a leaf from a different species of plant.

Observe the photomicrograph in Fig. 3.2 and the section on **L1** to identify differences between them.

Fig. 3.2 has been annotated to describe **one** of these differences. A label line has been used to indicate the feature that is different.

Complete Fig. 3.2 by:

- identifying **and** annotating **two** more differences between the micrograph in Fig. 3.2 and the section on **L1**
- using a label line to identify the feature that is different.

Fig. 3.2 Upper epidermis is continuous and not disrupted

L1 Upper epidermis is disrupted by fungal tissue

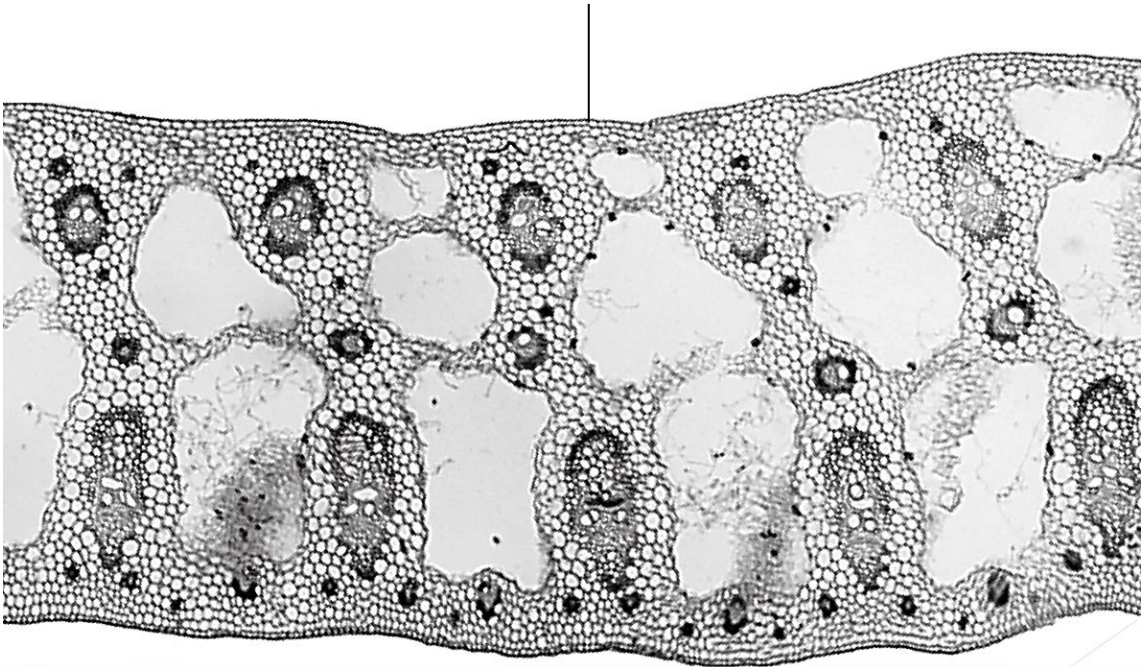


Fig 3.2

Any of the two comparisons between Fig. 3.2 vs L1;;

1. **Mesophyll layers: No clear distinction of palisade and spongy mesophyll layers vs clear upper layer of palisade mesophyll and lower layer of spongy mesophyll;**
2. **Arrangement (or number) of vascular bundles: Vascular bundle arranged in two/three rows vs vascular bundle arranged in a single row;**
3. **Size of vascular bundles: Vascular bundle is small relative to the leaf section/does not span the leaf section vs vascular bundle is large relative to the leaf section/may span the leaf section;**
4. **Arrangement (or number) of air spaces: Two/three rows of air spaces vs single row of air spaces;**
5. **Size of air spaces: Air spaces are large relative to the leaf section/may span the leaf section vs air spaces are small relative to the leaf section/does not span the leaf section;**

6. **Label line correctly drawn to feature being compared; *compulsory MP** [3]

[Turn over

(b) A scientist investigated changes in the mean width of stomata in the leaves of a plant growing in hot, dry conditions. The scientist measured the widths of stomata at different times of day, from 02:00 hours to 22:00 hours. Fig. 3.3 shows where the scientist measured the width of each stoma.

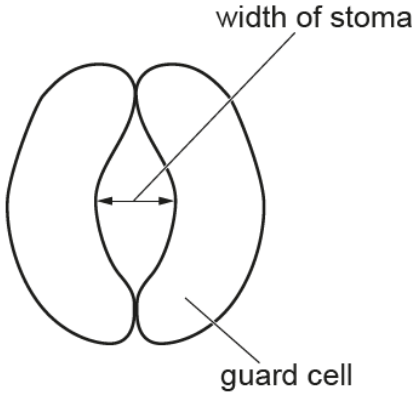


Fig. 3.3

The scientist calculated the mean width of stomata for each time of day. The results are shown in Table 3.1.

Table 3.1

time of day / hours	mean width of stomata / arbitrary units (au)
02:00	86
04:00	36
07:00	4
15:00	2
22:00	95

For Examiner Use

(i) On Fig. 3.4, plot a graph of the data shown in Table 3.1.

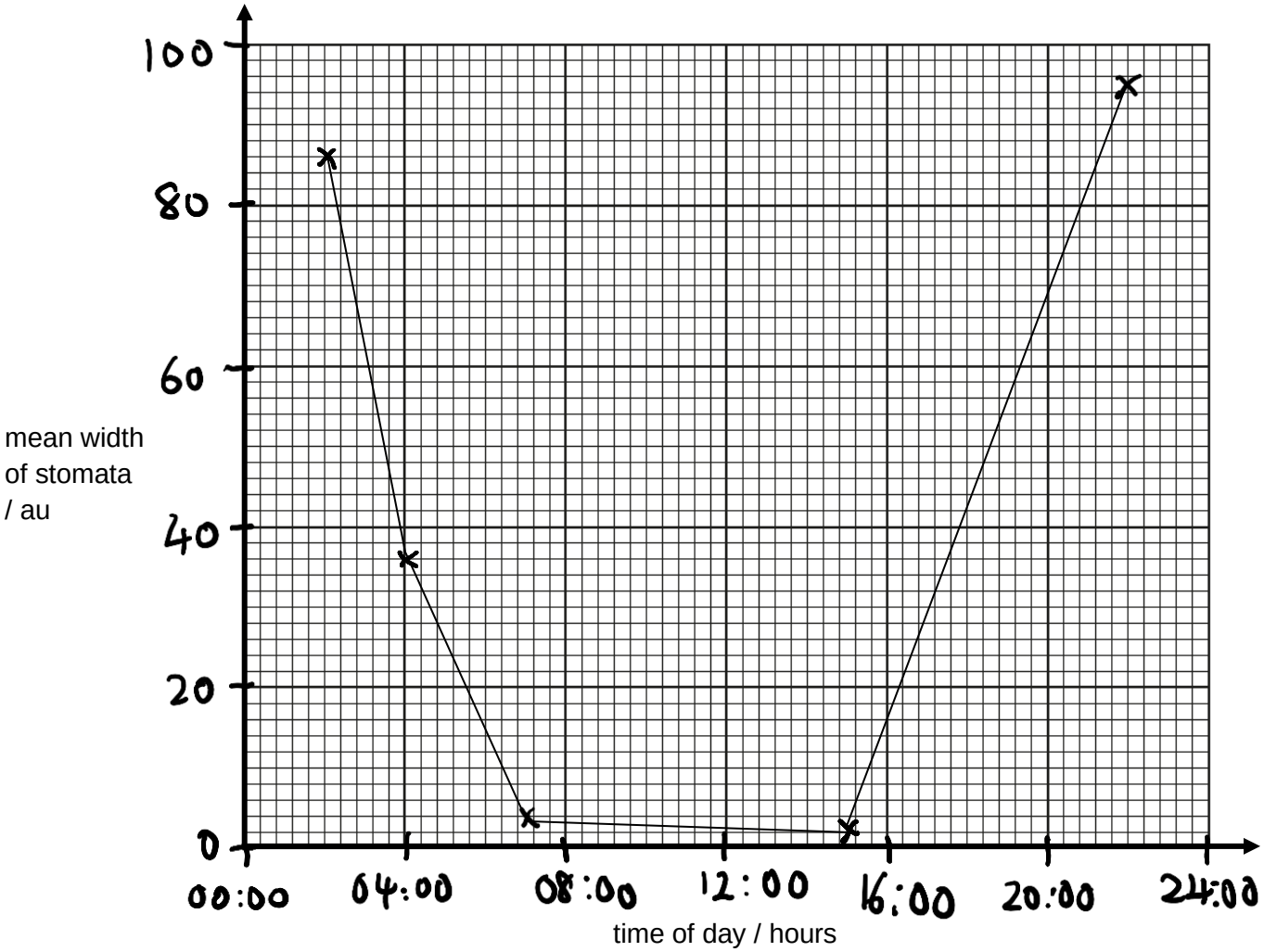


Fig. 3.4

1. **Appropriate axes labels with units (time must be on x-axis);**
2. **Axes scaled appropriately so that graph takes up at least 50% of the grid on both axes and divisions are equidistant; A! intervals of 4h or 5h on x-axis**
   R! zero time represented as '0' instead of '00:00'
   R! 25:00 if using 5h on x-axis
3. **All points correctly plotted;**
4. **Dot-to-dot plot or line of best fit, with no extrapolation beyond extreme measured data;**

[4]

[Turn over

(ii) Fig. 3.5 shows a stage micrometer scale that is being used to calibrate an eyepiece graticule.

The length of one division on this stage micrometer is **0.02 mm**.

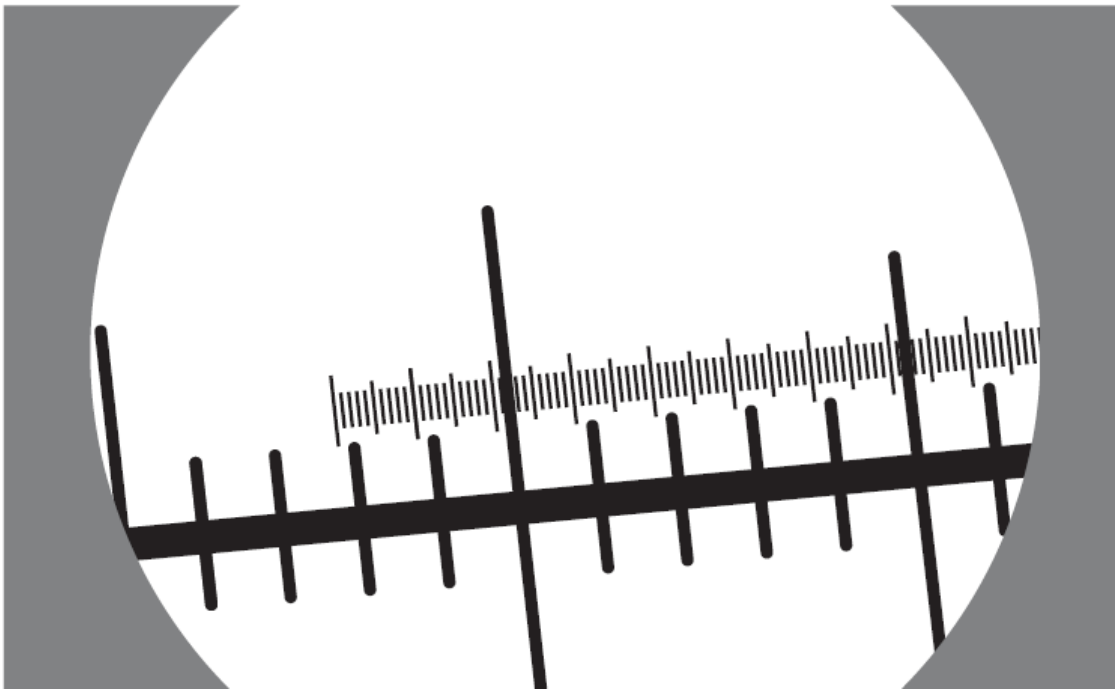


Fig. 3.5

Using the eyepiece graticule shown in Fig 3.5, the width of a guard cell measures **3 eyepiece graticule divisions**.

Calculate the actual width, in micrometres (μm), of this guard cell.

Show all the steps in your calculation, including the appropriate units.

1. **Calibrating eyepiece graticule using stage micrometer:**
   5 stage micrometer divisions = 50 eyepiece graticule divisions = 0.02 x 5 mm
   A! ratio of 1 stage micrometer division : 10 eyepiece graticule divisions

2. **Calculating length of one eyepiece graticule division:**
   1 eyepiece graticule division = 0.02 x 5 ÷ 50 mm = 0.002 mm = 2 μm

3. **Calculating actual width of guard cell + conversion into μm (can be done in earlier steps):**
   3 eyepiece graticule divisions = 3 x 2 μm = 6 μm (1sf) A! 2sf

R! if no description given for working step

actual width of guard cell = …… μm
[3]

[Total: 20]