



CANDIDATE
NAME

ANSWERS

CIVICS
GROUP

2

4

-

REGISTRATION
NUMBER

BIOLOGY

9744/04

Paper 4 Practical

26 August 2025

2 hours 30 minutes

Candidates answer on the Question Paper.

READ THESE INSTRUCTIONS FIRST

Write your name, civics group and registration number 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 stapler, paper clips, highlighters, glue or correction fluid/tape.

Answer **all** questions in the spaces provided in 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.

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

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	55

This document consists of **17** printed pages.

- 1 The enzyme amylase catalyses the hydrolysis (breakdown) of starch to reducing sugars, as shown in Fig. 1.1.

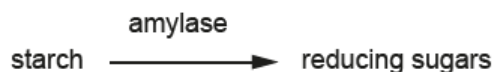


Fig. 1.1

A student investigated the effect of temperature on the hydrolysis of starch by the enzyme amylase, which has an optimal temperature close to 40°C.

The student carried out the following steps:

- step 1: put 2 cm³ of amylase into a test-tube
- step 2: put 18 cm³ of starch solution into a different test-tube
- step 3: put the test-tubes into a water-bath at 10°C
- step 4: left the test-tubes for 3 minutes
- step 5: put the starch solution into the test-tube containing the amylase
- step 6: left the test-tube for 2 minutes
- step 7: immediately determined the relative amount of reducing sugars in the test-tube
- repeated steps 1 to 7 at 40°C and at 100°C.

You are provided with three beakers, **S1**, **S2** and **S3**. These three beakers contain the same products of the reaction involving starch and amylase as the test-tubes prepared by the student in step 6. Each beaker contains the products of the reaction produced at one of the three temperatures tested.

You will determine:

- the relative amount of reducing sugar present in each beaker
- the presence or absence of starch in each beaker
- which of the beakers **S1**, **S2** and **S3** contain products of the amylase-catalysed reaction carried out at 10°C, 40°C and 100°C respectively.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume / cm ³
S1	products of the reaction between starch and amylase carried out at a particular temperature	none	20
S2	products of the reaction between starch and amylase carried out at a particular temperature	none	20
S3	products of the reaction between starch and amylase carried out at a particular temperature	none	20
U	products of the reaction between starch and amylase at an unknown temperature	none	20
B	Benedict's solution	irritant	30
iodine	iodine solution	irritant	15

If any solution comes into contact with your skin, wash off immediately with cold water.

It is recommended that you wear suitable eye protection.

To investigate the effect of temperature on the hydrolysis of starch by the enzyme amylase you will need to:

- carry out the test for reducing sugars on **S1**, **S2** and **S3**
- determine the relative amount of reducing sugar in each solution
- carry out the test for starch on **S1**, **S2**, and **S3**
- determine the presence or absence of starch in each solution.

- (a) (i) Explain why the student left the test-tubes in the water bath for 3 minutes before adding the starch solution. [1]

To allow for equilibration, whereby contents of the test-tubes reach the temperature of the water bath.

To determine the relative amount of reducing sugar in each solution, the time to the first colour change will be measured using 5 cm³ of each solution.

- (ii) Describe how you will carry out the test for reducing sugars. [1]

Add same volume / 5 cm³ of Benedict's solution with solution to be tested, and heat in boiling water bath for 5 minutes.

- (iii) State how you will determine which solution has the **highest amount** of reducing sugar present. [1]

Solution with the highest amount of reducing sugar will have the shortest time for first colour change.

- (iv) State the independent variable in this investigation. [1]
- temperature

Carry out step 1 to step 5.

- step 1 Label one test-tube, **S1**.
 step 2 Put 5 cm³ of **S1** into the test-tube.
 step 3 Repeat step 1 and step 2 for **S2** and **S3**.
 step 4 Carry out the test for reducing sugars as you described in (a)(ii).

If the **time taken to the first colour change** is longer than 120 seconds then record as 'more than 120'.

- step 5 Record in (a)(v) the time taken to the first colour change for each solution.

Carry out step 6 to step 11 to test for the presence of starch.

- step 6 Label a clean test-tube, **S1**.
 step 7 Put 5 cm³ of **S1** into the test-tube.
 step 8 Repeat step 6 and step 7 for **S2** and **S3**.
 step 9 Put 2 drops of iodine solution into each test-tube. Shake gently to mix.
 If any starch is present the solution will change to a blue-black colour.
 step 10 Observe the colour in each test-tube.
 step 11 Record in (a)(v) the colour for each solution.

(v) Record your results in an appropriate table.

[4]

Solution	Time taken for first colour change for reducing sugar test / s	Colour observed for starch test
S1		
S2		
S3		

1. **Heading** with appropriate units (time taken for first colour change for reducing sugar test / s, colour observed for starch test)
R: units in body of table
2. Correct **trend** for results of reducing sugar test
- Time taken: $S3 < S1 < S2$
- S2 needs to be recorded as "more than 120" (R: > 120)
3. **Precision** of timing: to whole number in seconds
4. **Colour** correctly described for each sample for starch test;
negative test: yellow-brown / brown (S3) (R: orange)
positive test: blue-black (S1 and S2)

Note:

S1 = 0.5% starch and 1% glucose

S2 = 1% starch

S3 = 10% glucose

(vi) Complete Table 1.2 using your results in (a)(v).

For reducing sugar content use only the words: **none**, **low**, **medium** or **high**.

For presence of starch use only the words: **present** or **absent**.

You may use each word once, more than once or not at all.

Table 1.2

	reducing sugar content	presence of starch
S1	low / medium	present
S2	none	present
S3	high	absent

1. correct trend for reducing sugar based on candidate's results;
2. correct results for starch based on candidate's results;

[2]

- (vii) Explain which of the beakers **S1**, **S2** and **S3** contain products of the amylase-catalysed reaction carried out at 100°C. [3]

1. **S2** contained products of the amylase-catalysed reaction carried out at 100°C;
2. First colour change was not observed for S2
(A: S2 did not show colour change) for the reducing sugar test. This showed that none of the starch was hydrolysed to form reducing sugars.
OR
Presence of starch and absence of reducing sugar showed that starch was not hydrolysed to form reducing sugars.
3. This is because at 100°C, amylase was denatured and did not hydrolyse starch;

U contains the products of the reaction involving starch and amylase solution in a water-bath at an unknown temperature.

You will need to:

- carry out the test for reducing sugars and the test for starch on **U**
- compare the results for **U** with the results for **S1**, **S2** and **S3**
- estimate the temperature of the water-bath used for **U**.

Carry out step 12 and step 13.

step 12 Repeat the test for reducing sugars and the test for starch on **U**.

step 13 Record your results in (a)(viii).

- (viii) Record the result of the test for reducing sugars for **U**.

time taken to first colour change s

Record the result of the test for starch for **U**.

colour [1]

a **time** (reducing sugar) and a **colour** (starch) for **U** ;

Note:

- Time taken for **U** needs to be more than **S3** but less than **S1** (mark according to student's earlier results)
- Blue-black for starch test of **U**

(Note: **U** contains 0.5% starch and 3% glucose)

- (ix) Use your results in (a)(v) and (a)(viii) to estimate the temperature of the water-bath used for **U**.

temperature of water-bath °C

[1]

Lower than 40°C but higher than 0°C

- can ECF from viii - correct estimate for **U** based on candidate's results
- can accept using inequality signs

- (x) Describe how the student could obtain a **quantitative** estimate of the concentration of reducing sugar in a solution. [4]

Any four from:

1. Create at least five known concentrations of reducing sugar;
2. Conduct Benedict's test for known concentrations of reducing sugar as well as solution with unknown concentration of reducing sugar;
Note to marker: Accept "reducing sugar test"
3. Record time for first colour change for all solutions during Benedict's test;
4. Draw a graph of known concentrations of reducing sugar against time to first colour change;
+
compare time for unknown sample with times for known samples of reducing sugar to obtain estimate;

OR

5. Create at least five known concentrations of reducing sugar;
6. Conduct Benedict's test for known concentrations of reducing sugar as well as solution with unknown concentration of reducing sugar;
7. After Benedict's test, filter the mixtures, dry and weigh the precipitate for each mixture.
8. Compare weight of precipitate for unknown sample with that for known samples of reducing sugar to obtain estimate;

R: mention of colorimeter / comparison with colour chart

[Total: 19]

- 2 There are a variety of methods used to estimate the water potential of plant tissue.

A student decided to estimate the water potential of potato tissue using the method described.

- Five different concentrations of sucrose solution were used.
- The masses of five potato pieces were measured before and after soaking in these different sucrose solutions.
- The percentage change in mass was then calculated for each potato piece.
- The procedure was repeated three times.
- The mean percentage change in mass as a result of soaking was then calculated for each concentration of sucrose. The concentration of sucrose solution that would result in no change in mass of the potato pieces indicates the water potential of the potato tissue.

Table 2.1 shows the results of this investigation.

Table 2.1

sucrose concentration / mol dm ⁻³	trial	initial mass / g	final mass / g	change in mass / g	percentage change in mass / %	mean percentage change in mass / %
0.0	1	2.4	2.5	0.1	+4.2	+8.2
	2	2.5	2.8	0.3	+12.0	
	3	2.4	2.6	0.2	+8.3	
0.2	1	2.4	2.5	0.1	+4.2	
	2	2.4	2.5	0.1	+4.2	
	3	2.3	2.4	0.1		
0.4	1	2.5	2.5	0.0	+0.0	+1.3
	2	2.5	2.6	0.1	+4.0	
	3	2.5	2.5	0.0	+0.0	
0.6	1	2.6	2.5	-0.1	-3.8	-6.7
	2	2.5	2.3	-0.2	-8.0	
	3	2.4	2.2	-0.2	-8.3	
0.8	1	2.4	2.2	-0.2	-8.3	-9.9
	2	2.4	2.2	-0.2	-8.3	
	3	2.3	2.0	-0.3	-13.0	

- (a) (i) Use the data in Table 2.1 to calculate the percentage change in mass of the potato piece soaked in 0.2 mol dm⁻³ sucrose solution in trial 3.

Show your working clearly.

Percentage change in mass = $0.1/2.3 \times 100\% = +4.3\%$ (to 1 d.p)

percentage change in mass =+4.3%.....

[1]

Note:

- Working shown clearly
- units and plus sign must be shown in final answer
- final answer to 1 d.p (A: 3 s.f, +4.35%)

- (ii) Use the data in Table 2.1 to calculate the **mean** percentage change in mass of the potato pieces soaked in the 0.2 mol dm⁻³ sucrose solution.

Show your working clearly.

$(4.2\% + 4.2\% + 4.3\%) / 3 = +4.2\%$

mean percentage change in mass =+4.2%.....

[1]

Note:

- Working shown clearly
- units and plus sign must be shown in final answer (only penalise once for lack of plus sign and/or units)
- final answer to 1 d.p (A: 3 s.f, +4.23%)

(iii) Plot a graph of the **mean** data in Table 2.1 on the grid in Fig. 2.1.

Use a sharp pencil.

Graph of mean percentage change in mass / % against sucrose concentration / mol dm⁻³

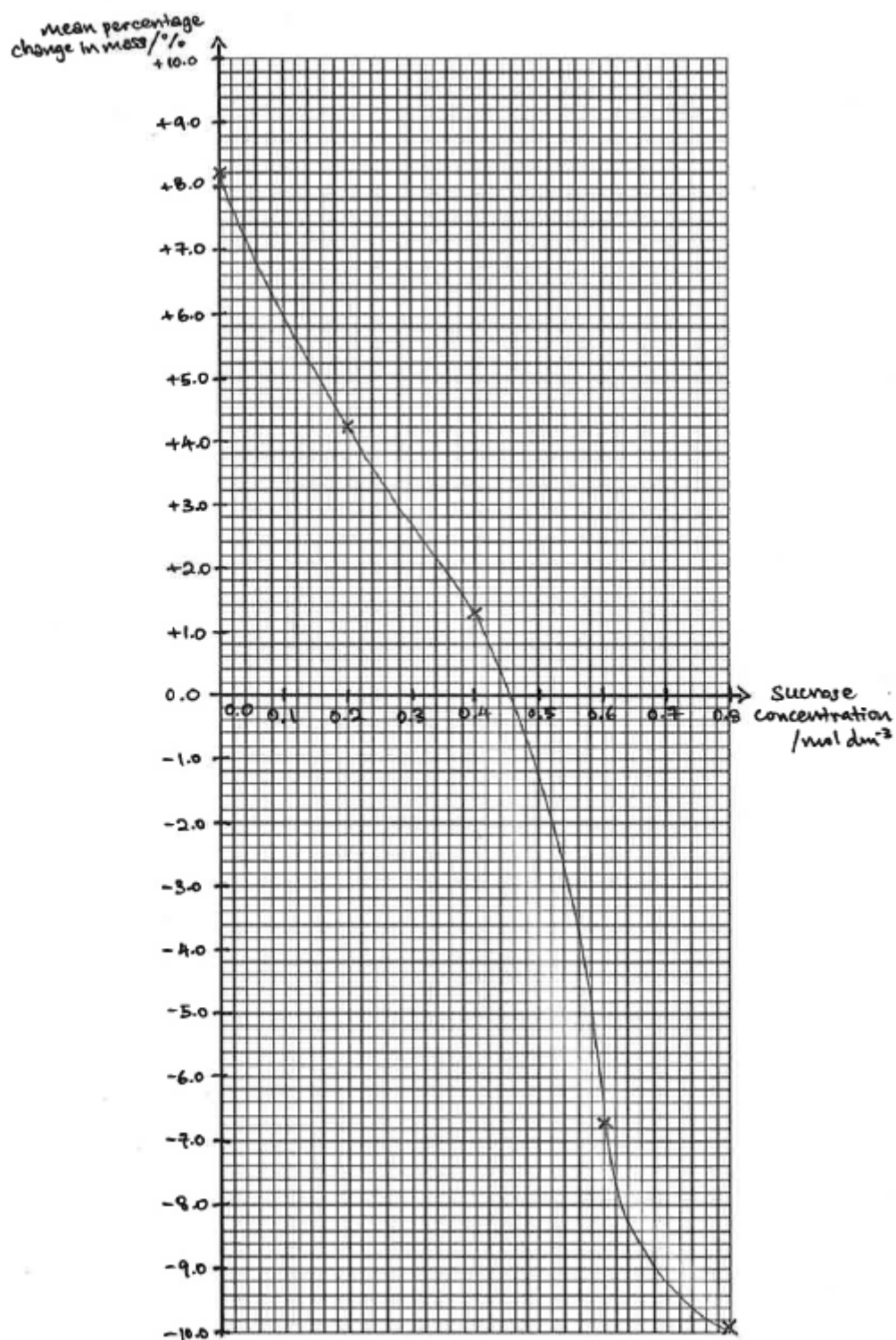


Fig. 2.1

[4]

1. **Scale** on x-axis:
 0.2 mol dm^{-3} to 2cm OR 0.1 mol dm^{-3} to 1cm
 +
 scale on y-axis:
 2% to 2cm OR 1% to 1cm;
R: x-axis at the bottom of the graph paper
2. **Axis**
 label on x-axis:
 sucrose concentration / mol dm^{-3}
 +
 label on y-axis:
 mean percentage change in mass / %;
3. **Plots**:
 correct plotting of all five points using small crosses;
4. **Line**:
 all points joined using best fit curve
NOTE: do not reject point to point joining
 +
 no **Extrapolation**;

- (iv) Use your graph in Fig. 2.1 to estimate the concentration of sucrose solution that would result in **no** change in mass of the potato pieces.

concentration of sucrose solution = mol dm^{-3}
 [1]

correct estimate from the graph – i.e. intersection of graph with x-axis;

The student decided to use another method to estimate the water potential of potato tissue. This method involved soaking potato tissue in different concentrations of sucrose solution, and then measuring the change in density of the sucrose solution for each concentration of sucrose solution that the potato tissue was soaked in. The more water present in a sucrose solution, the less dense it is.

For each concentration of sucrose solution, some of it was removed prior to soaking the potato tissue and coloured with a dye. To test if there is a change in density of a sucrose solution after the potato tissue has been soaked in it, a drop of the respective coloured sucrose solution will be used as shown in Fig. 2.2.

Fig. 2.2. also shows the three possible outcomes that the student would see for each concentration of sucrose tested.

- 1 The coloured sucrose drop will rise if the drop is less dense than the sucrose solution in the test-tube.
- 2 The coloured sucrose drop will remain where it is released if the drop is similar in density to the sucrose solution in the test-tube.
- 3 The coloured sucrose drop will sink if the drop is more dense than the sucrose solution in the test-tube.

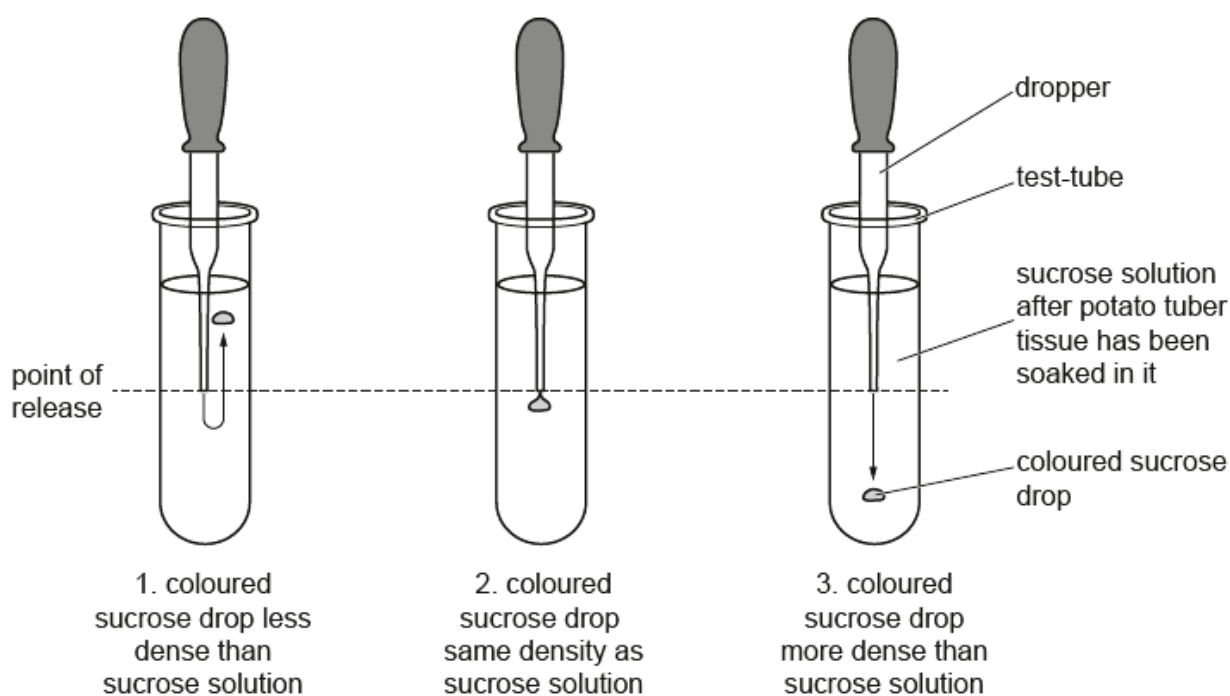


Fig. 2.2

- (b) Plan an investigation to estimate the water potential of potato tissue by measuring the change in density of sucrose solution.

You must plan to use:

- 1 mol dm⁻³ sucrose solution
- potato tissue
- methylene blue

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

- normal laboratory glassware, e.g. test-tubes, beakers, dropper, glass rods etc.
- syringes
- scalpel, cork borer, white tile

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 variable that you will need to control
- use the correct technical and scientific terms
- include reference to safety measures to minimise any risks associated with the proposed experiment

[9]

1. Use minimum of **5 different concentrations of sucrose + examples** (e.g. 0.2, 0.4, 0.6, 0.8, 1 mol dm⁻³, or 0.2, 0.3, 0.4, 0.5, 0.6 mol dm⁻³)
2. Mention of **simple dilution** for creating the stipulated sucrose concentrations
3. **Dilution table** to show how sucrose solutions are made
A: serial dilution in place of simple dilution
4. Mention of **colouring** a certain volume of the respective concentrations of sucrose solutions with **methylene blue**.

5. **Constant variables** (max 2m, 1m for each constant variable):
 Note: need to specify exact values for constant variable (temperature, volume, time)
- Temperature + method to maintain same temperature when potato is immersed in sucrose solution
 - Surface area of potato pieces + method
 (A: dimension / size + example of dimension/size given)
 (I: mass)
 - Potato tissue obtained from same potato for the entire experiment
 - Volume of sucrose solution used for soaking potato tissue + allows all potato pieces to be fully submerged in sucrose solution
 - Potato pieces are removed from sucrose solution after same time of immersing in sucrose solution
6. Replicates:
3 replicates per sucrose concentration to **check for anomalous results**
7. Control:
Keeping all other variables constant, replace potato tissue with glass cubes of same dimensions.
 Note of marker: accept no potato in setup
 R: wrap potato in clingwrap
8. To show that sucrose solutions of equal concentrations will have same density and coloured drop will remain at where it is released
9. If one of the tested sucrose concentrations has the same density as the coloured drop (A: if the coloured drop stays at the point of release), the potato tissue has the same water potential as this sucrose solution
10. **Repeat the experiment two more times** to **check for reproducibility of the results**
11. If none of the tested sucrose concentrations has the same density as the coloured drop (A: if the coloured drop only sinks/floats all tested sucrose concentration), the potato tissue does not have the same water potential as any of the sucrose solutions
12. Idea of repeating with new concentrations of sucrose to get closer to the sucrose solution that is equivalent to the water potential of the potato tissue
13. Data table

14. Risk and precaution
 e.g. methylene blue is a skin irritant, wear gloves and goggles when handling it.

[Total: 16]

- 3 Starch grains are present in plant cells. The starch grains have different sizes and shapes depending on the type of plant.

As the starch grains get larger, patterns form on the surface of the starch grains. These patterns can be observed using a microscope.

You are provided with a sample plant **C**.

You will need to:

- prepare a microscope slide of starch grains from **C**.
- observe the starch grains present on the microscope slide.
- draw **two** starch grains.

Carry out step 1 to 10.

- | | |
|--------|--|
| step 1 | Label one clean and dry microscope slide, C . Put the slide onto a paper towel. |
| step 2 | Put sample C onto a white tile. |
| | Note: |
| | Do not use the seed coat of sample C in the preparation of the microscope slide. |
| step 3 | Cut a thin slice (approximately 1 mm) from the end of sample C . |
| step 4 | Cut this slice into smaller pieces. |
| step 5 | Put two drops of distilled water onto these small pieces. |
| step 6 | Use a Pasteur pipette to transfer drops of the liquid from around the small pieces prepared in step 5 onto the slide labelled C . |
| step 7 | Put a coverslip over the liquid on slide C . |
| step 8 | Use the microscope to observe the starch grains on slide C . |

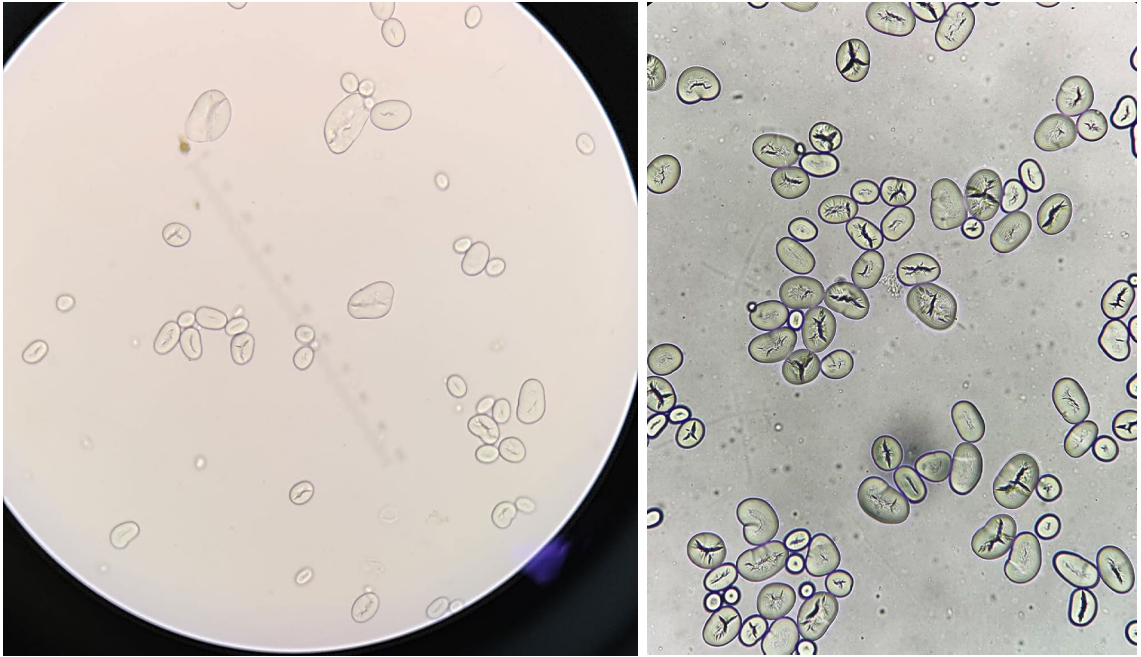
You may need to reduce the amount of light entering the microscope and will need to adjust the fine focus to observe the surface of the starch grains clearly.

- | | |
|---------|--|
| step 9 | Select two starch grains on slide C that show distinct circular patterns on their surface. |
| step 10 | Make a large drawing of these two starch grains in (a)(i) . |

- (a) (i)** Make a large drawing of **two** starch grains from slide **C**

Use a sharp pencil.

[4]



1. uses most of the available space and lines continuous, thin and sharp ;
2. draws two starch grains from slide **C** ;
3. all the starch grains have surface patterns ;
4. for sample **C** shows the correct pattern on at least one starch grain;

(ii) Suggest why the starch grains get smaller as plants germinate.

[1]

starch broken down to glucose / to release energy;

A: "convert" starch to glucose

R:

- starch is used during germination
- plants can photosynthesise and therefore do not need to store starch
- starch is broken down to sucrose

- (b) Fig. 3.1 is a photomicrograph showing the epidermis of a plant.

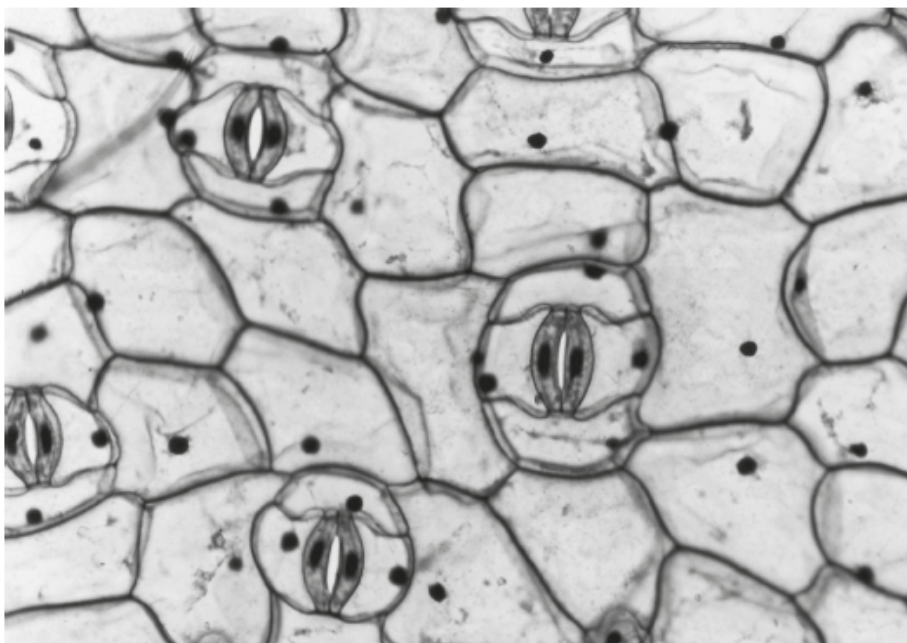


Fig. 3.1

The actual area of the epidermis visible in the photomicrograph in Fig. 3.1 is 0.04 mm^2 .

The leaf from which Fig. 3.1 is taken has a **total surface area** of 2000 mm^2 .

- (i) Use Fig. 3.1 to estimate the total number of stomata on the leaf.

Show your working.

$$\text{Total number of stomata} = 4 \times (2000 / 0.04) = 200,000$$

Total number of stomata =

[2]

1. counts number of stomata in the photomicrograph in Fig. 2.2 = 4; (R: 5)

2. shows 2000 divided by 0.04

AND correctly calculates number of stomata by **multiplying number of stomata** in section by 50 000 = 200 000;

- (ii) One way to improve the accuracy of the estimate of the total number of stomata on a leaf is to use a photomicrograph with a larger area.

State one other way to improve the accuracy of the estimate of the total number of stomata on a leaf. [1]

repeat / sample more areas;

A: count number of stomata on both upper and lower epidermis

R: count stomata in areas with complete stomata

- (c) K1 is a slide of a stained transverse section through a plant root.

- (i) Draw a large plan diagram of the vascular tissue region of the root on K1, as indicated by the shaded area in Fig. 3.2.

Use a sharp pencil.

Use **one** ruled label line and label to identify the xylem tissue.

[5]

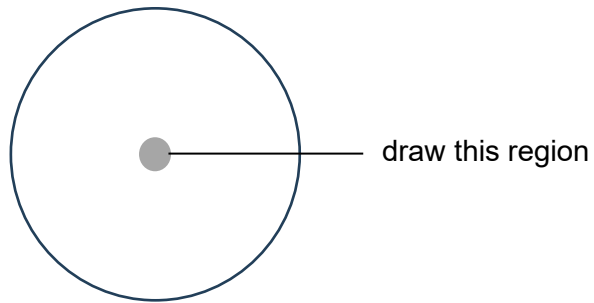
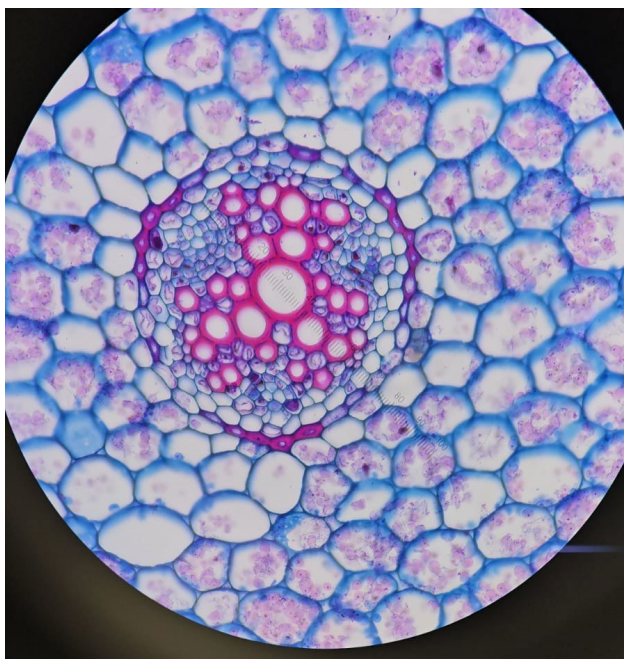
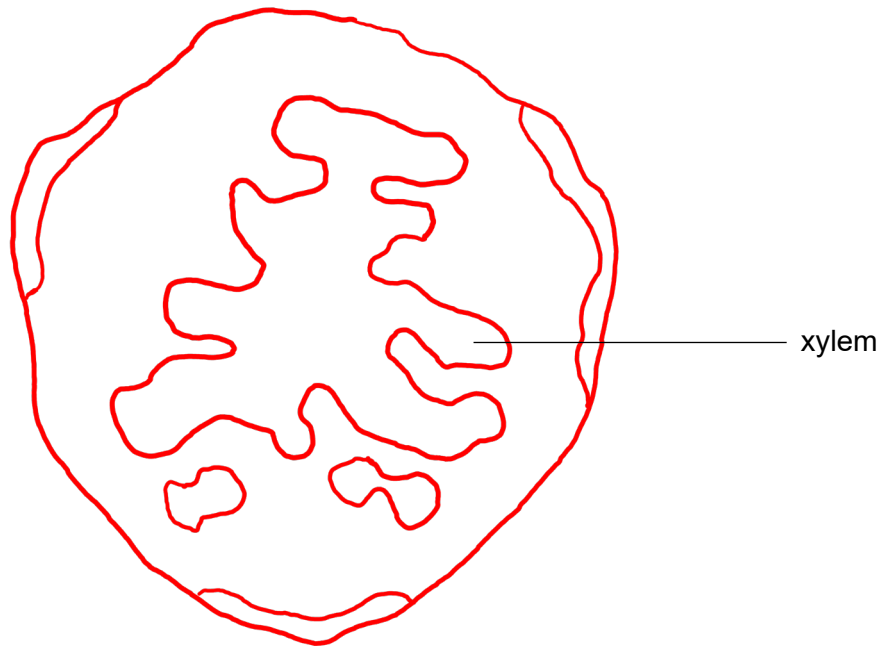


Fig. 3.2



Note: this picture was taken using 40X objective lens

Plan drawing	
S	Smooth and continuous pencil drawing Size of drawing – suitable size (e.g. occupy at least half of the given space) Similarity of drawing to specimen observed • <u>circular</u> region is drawn for the outer boundary (R: bound by double lines) • phloem tissue not marked for • pink suberin at endodermis not marked for, but if drawn, needs to be accurately represented as discontinuous regions at the periphery.
T	Title Plan drawing of vascular tissue viewed at x40 objective lens / x400 magnification R: “viewed at x10 objective lens / 100x magnification”
A	Annotation / label xylem tissue correctly labelled + label lines are straight (drawn with a ruler) Actual dimensions in µm. (e.g. diameter of vascular tissue = 200 µm) A: 150 µm to 250 µm
M	Magnification of drawing = drawing length / actual length (Both should be in µm) = _____ X (whole no.) (R: 3 s.f)
P	Proportionate drawing Proportion of xylem tissue relative to entire vascular tissue is appropriately represented / accurate shape of xylem tissue region (R: too regular a shape for xylem tissue region) No individual cells should be drawn.

Fig. 3.3 is a photomicrograph of a stained transverse section of a stem from a different plant.

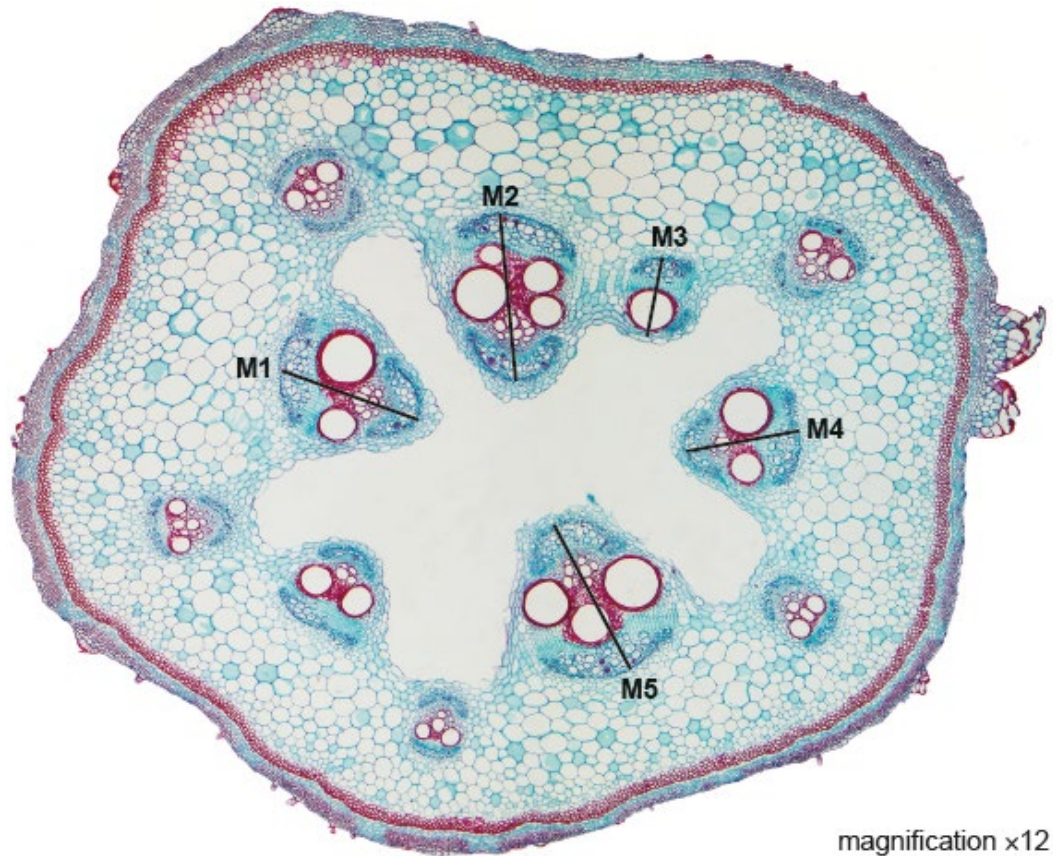


Fig. 3.3

- (ii) Measure the length of the vascular bundles using the lines **M1**, **M2**, **M3**, **M4** and **M5** in Fig. 3.3 **and** calculate the mean actual length of the vascular bundles.

Show your working.

$$\mathbf{M1 = 19\ mm} \quad \mathbf{M2 = 22\ mm} \quad \mathbf{M3 = 10 / 11\ mm} \quad \mathbf{M4 = 15\ mm} \quad \mathbf{M5 = 21 / 22\ mm}$$

Mean actual length [4]

1. states five measurements for length of vascular bundles and units (A: mm or cm);
2. shows five measurements added together and divided by 5 (to calculate a mean);
3. shows value divided by 12;
4. answer given to nearest $1\mu\text{m}$;

- (iii) A student suggested that the mean actual length of the vascular bundles calculated in (c)(ii) was not accurate for the whole plant.

Describe **two** modifications to the method used in (c)(ii) that would allow a more accurate mean length of the vascular bundles for the whole plant to be calculated.

[2]

any two:

1. measure all the vascular bundles in Fig. 3.3
2. larger magnification to make it easier to measure
3. take more sections of stem from the same plant (A: measure vascular bundles from other parts of the plant)
(note to marker: accept "do more replicates")

R: use eyepiece graticule to measure actual length

- (iv) State one observable difference between the vascular tissue seen in K1 and the stem shown in Fig. 3.3. [1]

any one:

1. There is only one vascular tissue region in K1 whereas there are 11 (A: multiple / many / numerous) vascular bundles in the stem shown in Fig. 3.3.
2. The vascular tissue shown in K1 is circular in shape but each vascular bundle in Fig. 3.3 is an irregular shape. (R: curved triangle / has a pointy end)
3. The size of the vascular tissue region in K1 is smaller than a single vascular bundle in Fig. 3.3.

R:

- Mention of cortex present in Fig. 3.3
- Vascular bundles separated by a gap/space vs no gap/space in K1

[Total: 20]