NANYANG JUNIOR COLLEGE
PRELIMINARY EXAMINATIONS
Higher 2

CANDIDATE NAME **ANSWERS**

CLASS

BIOLOGY

9744/04

Paper 4 Practical

24 August 2023

Candidates answer on the Question Paper

Additional Materials: As listed in the Confidential Instructions

2 hour 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name and CT 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, highlighters, glue or correction fluid.
DO NOT WRITE IN ANY BARCODES.

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.

Shift
Laboratory

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

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

For Examiner's Use	
1	
2	
Total	

This document consists of **16** printed pages and **0** blank pages.

[Turn over

Answer **all** the questions.

1 Some fruit contains large quantities of ascorbic acid (vitamin C). Ascorbic acid can be absorbed through the partially permeable wall of the gut.

You will investigate the rate at which ascorbic acid from a 2 gdm^{-3} ascorbic acid solution diffuses across a partially permeable membrane. Dialysis (Visking) tubing acts as a partially permeable membrane.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	quantity
A	2 gdm^{-3} ascorbic acid solution	irritant	80cm^3
W	distilled water	none	200cm^3
D	dialysis tubing in distilled water	none	20cm
I	iodine solution	irritant	25cm^3
S	starch solution	none	20cm^3

It is recommended that you wear suitable eye protection.

You will need to:

- allow ascorbic acid to diffuse out of the dialysis tubing into the distilled water surrounding the dialysis tubing
- estimate the concentration of ascorbic acid that has diffused out of the dialysis tubing.

Carry out step 1 to step 27.

step 1 Tie a knot in the dialysis tubing as close as possible to one end, so that the end is sealed.

step 2 To open the other end, wet the dialysis tubing and rub the tubing gently between your fingers and thumb.

step 3 Put 10 cm^3 of ascorbic acid solution A into the open end of the dialysis tubing.

step 4 Rinse the outside of the dialysis tubing by dipping it in the container of water labelled **D**.

step 5 Carefully place the filled dialysis tubing bag into the large test-tube and secure position using an elastic band, as shown in Fig. 1.1.

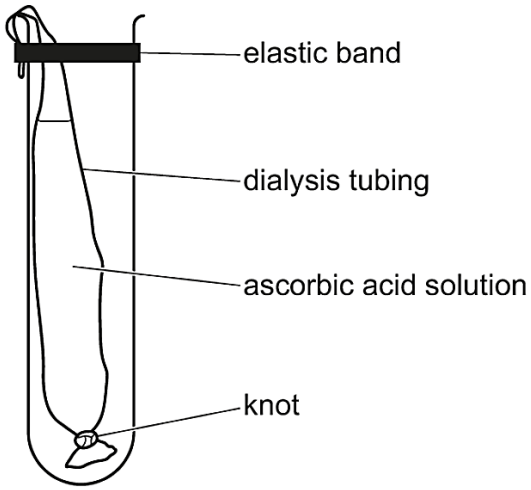


Fig. 1.1

You will need to fill the large test-tube with a known volume of distilled water so that it just covers the liquid in the dialysis tubing.

step 6 Use a syringe to add the distilled water to the large test-tube and record in **(a)(i)** the volume you added.

(a) (i) State the volume of distilled water you added.

volume of water ..... cm^3 between 15 and 30cm^3 [1]

step 7 Start timing and leave the dialysis tubing bag in the distilled water for at least 20 minutes.

While you are waiting continue with this question.

You will need to dilute the 2 gdm^{-3} ascorbic acid solution, **A**, to provide a range of known concentrations.

You will need to make up 20 cm^3 of each concentration of ascorbic acid.

Table 1.2 shows how to make up two of the concentrations you should use.

(ii) Decide which other concentrations of ascorbic acid to make and complete Table 1.2.

Table 1.2

concentration of ascorbic acid / gdm^{-3}	volume of ascorbic acid solution / cm^3	volume of distilled water / cm^3
0.0	0.0	20.0
0.5	5.0	15.0
1.0	10.0	10.0
1.5	15.0	5.0
2.0	20.0	0.0

[3]

1 shows three other concentrations of ascorbic acid and in descending order ; @0.4, 0.8, 1.2, 1.6
2 correct volumes of ascorbic acid and water to make 20 cm^3 and correct concentration ;
3 precision (1dp)

step 8 Using the beakers provided, make up the concentrations of ascorbic acid stated in Table 1.2.

step 9 Put 1 cm^3 of starch solution **S** into a clean test-tube.

step 10 Put 5 cm^3 of the 0 gdm^{-3} ascorbic acid solution (distilled water) into the same test tube. Shake gently to mix.

step 11 Fill a syringe with 2 cm^3 iodine solution **I**.

step 12 Add one drop of **I** to the mixture of **S** and ascorbic acid solution, as shown in Fig. 1.2. Mix gently.

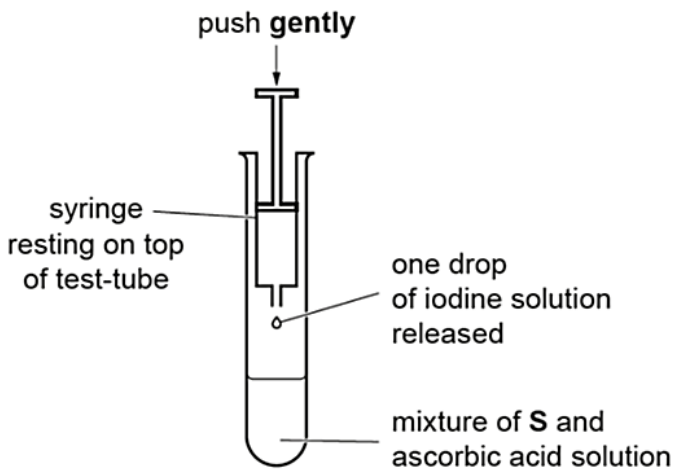


Fig. 1.2

step 13 You should see a blue colour. This is the end-point you are looking for when you test the remaining ascorbic acid solutions (step 21).

step 14 Record the volume of iodine solution you have added.

step 15 Put 1 cm^3 of starch solution **S** into a clean test-tube.

step 16 Put 5 cm^3 of the lowest concentration of the remaining ascorbic acid solutions into the same test-tube. Shake gently to mix.

step 17 Fill the syringe containing iodine solution **I** to the 2 cm^3 level again.

step 18 Add one drop of **I** to the mixture of **S** and ascorbic acid solution as shown in Fig. 1.2. Mix well.

step 19 Continue adding drops one at a time, mixing after each drop, until you see a blue colour.

step 20 As soon as you see a blue colour, wait 10 seconds. If the blue colour disappears then add another drop.

step 21 Continue adding drops until the mixture stays blue for at least 10 seconds. This is the end-point.

step 22 Record the volume of iodine solution you have added.

step 23 Repeat step 15 to step 22 with the other concentrations of ascorbic acid you prepared in step 8.

Record your results in **(a)(iii)**.

(iii) Record your results in an appropriate table.

1 heading for independent variable: concentration of ascorbic acid **and** g dm^{-3};
2 heading dependent variable: volume **and** iodine **and** cm^3;
3 results for all concentrations, including 0.0 g dm^{-3}
4 greatest volume of iodine for highest concentration of ascorbic acid ;
5 records results to appropriate accuracy (1 or 2dp to 0.05)

If table border is not drawn, overall minus 1m

[5]

step 24 Remove the dialysis tubing from the large test-tube and place it in the beaker labelled **For waste**. Stop timing and record the time that the dialysis tubing has been left in the distilled water (time from step 7 to step 24).

time = ……

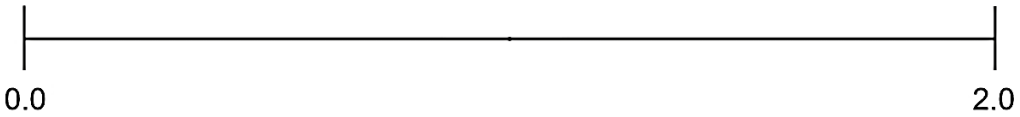
step 25 Pour the solution from the large test-tube into a small beaker.

step 26 Use a syringe to remove a 5 cm^3 sample of the solution from the small beaker.

step 27 Repeat step 15 to step 22 using this sample. Record your result in **(a)(iv)**.

(iv) Record the volume of iodine added. ……

(v) Complete Fig. 1.3 to show the positions of each of the concentrations of ascorbic acid, recorded in **(a)(iii)**.



concentration of ascorbic acid / gdm^{-3}

Fig. 1.3

[1]

1 correctly labels scale bar with different concentrations of ascorbic acid, at equally spaced intervals;

(vi) Use your results in **(a)(iii)** and **(a)(iv)** to estimate the concentration of ascorbic acid in the solution outside the dialysis tubing bag.

Show this estimate on Fig. 1.3 by placing the letter **U** in the correct position along the line. [1]

estimates the correct concentration of sample by placing U in the correct position on the line ; (accept intermediate value)

(vii) Calculate the **rate** of diffusion of ascorbic acid out of the dialysis tubing.

Show your working.

1 shows division by, time correctly
2 (correct) answer and units g dm^{-3} min^{-1} / g dm^{-3} s^{-1}

@ g min^{-1} or g s^{-1} as units

rate of diffusion = ... [2]

(viii) Identify **two** significant sources of error when finding the concentration of ascorbic acid in the sample from the large test-tube.

any two from:

1 **difficult to read scale / see meniscus on syringe** because iodine solution is **too dark** ;

2 volume of **each drop of iodine being too large** such that when whole drops of iodine are added, it may have **exceeded the end-point;** (Idea that: excess iodine may have been added)

3 Volume of iodine required for sample U **may not match** any of those in the standard / idea that range that the reading fall within is large.

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[2]

(ix) Explain **two** improvements to the investigation that would increase confidence in the estimation of ascorbic acid in the sample outside the dialysis tubing bag.

any two from:

1(A) repeat two more times (replicates) to achieve **three readings** and find **mean**

1(B) increase **reliability** and reproducibility of results;

2(A) use of, burette / syringe, with narrow divisions

2(B) **increase precision / accuracy** of results ;

3(A) use **white card / tile** behind tube

3(B) **better visualise** and determine when the end-point occurs ;

4(A) find **mass of iodine** added instead of volume / use **diluted iodine with lighter colour**

4(B) overcome issue of **error in reading off scale of syringe** due to dark coloured iodine;

5(A) **Increase number of concentrations** within same range

5(B) Allow **more accurate matching** of concentrations to standards

6(A) plot a **graph with volume of iodine against ascorbic acid concentration** using **best-fit line**. **Read-off the ascorbic acid concentration** from this graph using volume of iodine used for U.

6(B) provide a **more accurate estimation** of the ascorbic acid concentration.

.....

.....

[4]

(b) Iodine solution (iodine in potassium iodide solution) turns blue-black when starch is present in plant tissues.

However, as ascorbic acid is also found in plant tissues, some scientists investigated the effect of testing for starch with iodine solution when there was ascorbic acid present.

The concentration of ascorbic acid was 0.0001 moldm^{-3} and the concentration of starch solution was standardised.

The percentage of starch which reacted with the iodine solution was measured.

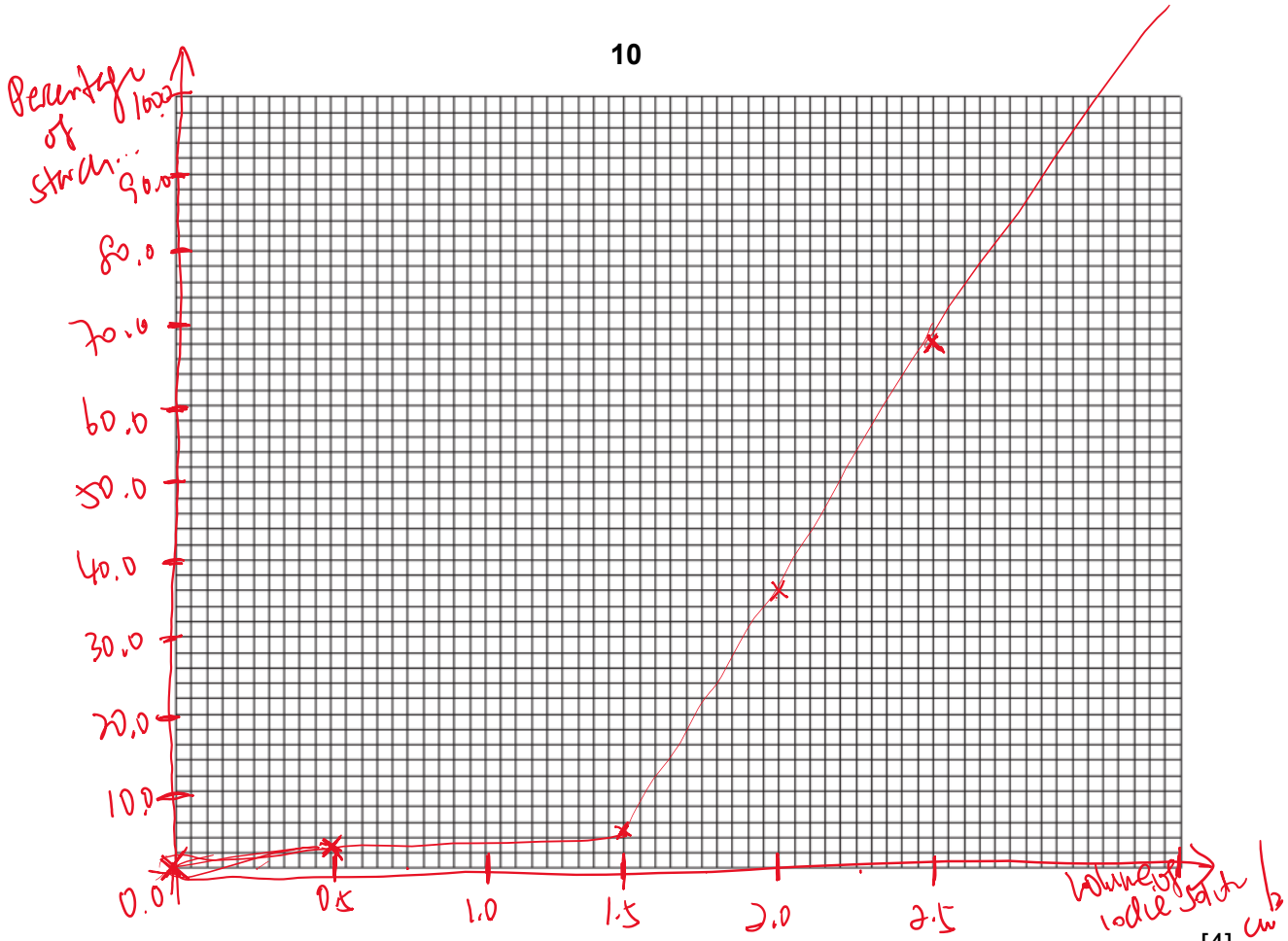
The results are shown in Table 1.3.

Table 1.3

volume of iodine solution / cm^3	percentage of starch which reacted with iodine solution
0.0	0.0
0.5	2.0
1.5	5.0
2.0	36.0
2.5	68.0

(i) Plot a graph of the data shown in Table 1.3.

You will need to consider the answer to (b)(ii) before you plot your graph.



[4]

X axis: volume of iodine / cm^3 + y axis: percentage of starch reacted (with iodine solution) OR Amount / Concentration of starch reacted / % + origin labelled clearly ;

(xaxis) 0.5 to 2cm labelled each 2cm except origin and 3.0 / 3.5 + (yaxis) 20 to 2cm labelled each 2cm except origin and 100;

- Scale needs to be **marked out** at **regular intervals**
- Awkward scale will affect accuracy of plot points

Correct plotting of five points as small cross or dot in circle or cross + marked clearly;
Rej if additional plot point marked out at y=100

ruled sharp lines exactly point-to-point or ruled line of best fit + sharp smooth line (accept point to point ruled line or best fit curve);

(ii) Estimate the volume of iodine solution needed for 100% of the starch to be reacted. Show on your graph how you obtained the volume of iodine solution.

volume of iodine solution = ... cm^3 [2]

show on graph (ii) value with extrapolation
accurately read off graph

(iii) In the presence of ascorbic acid, iodine is immediately reduced to iodide ions (colourless). Explain how the presence of ascorbic acid may affect the use of iodine solution as a test for the presence of starch in different plant tissues.

Idea of too much ascorbic acid then iodine may not turn blue-black

Or

Idea of having to add more iodine in order to observe colour

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Need to know how much ascorbic acid in plant tissues to make test accurate / need to react ALL ascorbic acid to know conc of starch

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[2]

(c) Antibiotic resistance in bacteria is a global problem that has caused scientists to research into antibacterial substances other than antibiotics. Ascorbic acid has properties that make it a good antibacterial substance. For example, ascorbic acid has low pH and also strong antioxidant activity which can inhibit bacterial growth.

A student decided to carry out an investigation to determine the effect of ascorbic acid on the bacterium, *Bacillus subtilis* which respires aerobically.

The student wanted to find the lowest concentration of ascorbic acid that would inhibit the growth of *B. subtilis*.

The student carried out a trial experiment. A sample of fresh broth culture was transferred to a culture tube and incubated for 24 hours.

Fig. 1.3 summarises the results of the trial experiment.

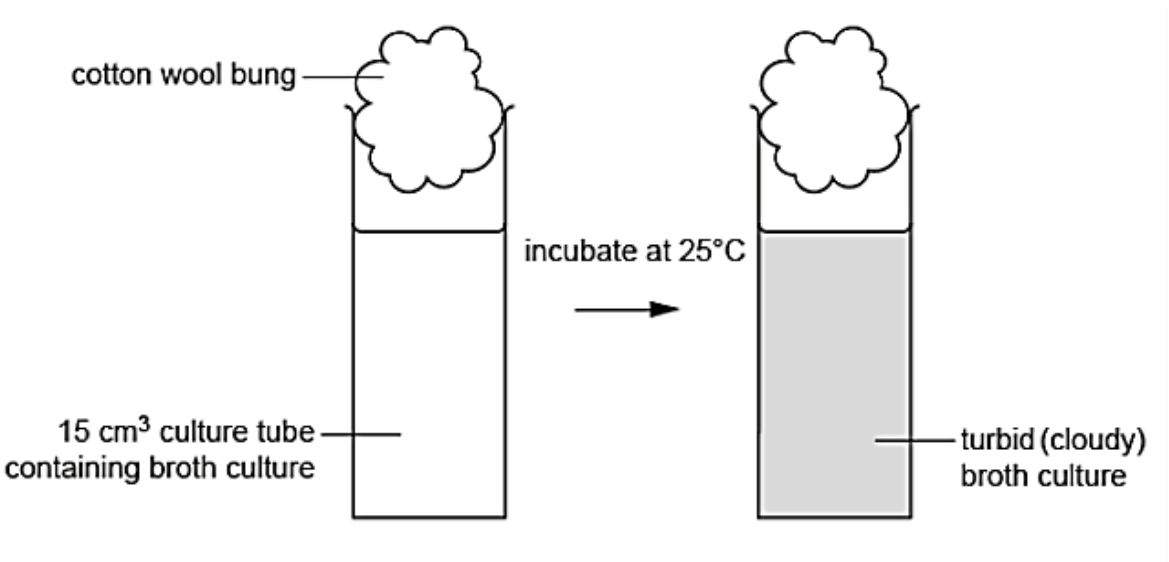

Fig. 1.3

The student decided that turbidity of the broth culture is a measure of bacterial population growth (bacterial growth).

Describe how the student could determine the lowest concentration of ascorbic acid that would inhibit the growth of *B. subtilis*.

- Details on dilution to obtain the different concentrations of ascorbic acid are **not** required.
- Details of using aseptic technique (techniques to prevent contamination of the student, the environment or other people) are **not** required.

In addition to normal laboratory apparatus and materials, the student was provided with:

- fresh broth culture of *B. subtilis*
- 20 gdm^{-3} ascorbic acid solution
- 15cm^3 flat-bottomed glass culture tubes with sterile cotton wool bungs
- A choice of graduated pipettes to measure volumes accurately: 0.2 cm^3, 2.0 cm^3, 10.0 cm^3, 25.0 cm^3
- incubator
- colourimeter

Your planned method should

- have a clear and helpful structure so that the method described could be repeated by anyone reading it
- identify the independent and dependent variables
- identify the variables that you will need to control
- use the correct technical and scientific terms
- involve the use of the apparatus and materials provided

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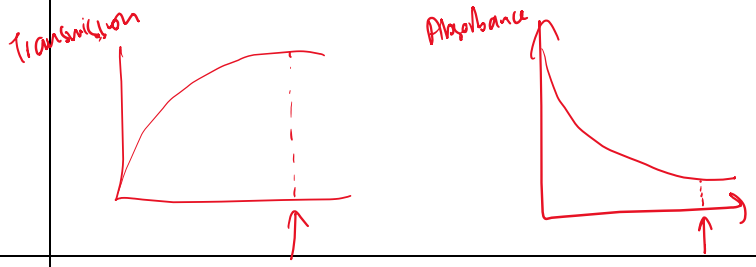
[6]

[Total: 33]

Procedure:	**IV**	5 concentrations of ascorbic solution (4.0, 8.0, 12.0, 16.0, 20.0gdm-3) + standard dilution using stock solution
	DV1	turbidity by measuring percentage light transmission / absorbance using colourimeter
	CV1-4 (2m max)	Constant variables and describe how two identified variables are kept constant (with apparatus);: e.g. CV1: volume of ascorbic acid / volume of culture broth + using syringe / graduated pipettes CV2: mixing of ascorbic acid and culture broth + using stirring rod CV3: temperature → state a reasonable temp / temp range (10 – 50 degrees) + incubator CV4: reaction time / incubation time → fixed time (12 – 48 hours) + stopwatch / timer / clock
	T	Recording: Show how results are to be presented in the form of a table with independent (ascorbic acid concentration / %) and dependent variables (percentage light transmission) in appropriate columns; Table with correct headings and units for independent and dependent variables
	G	Graph of the mean percentage light transmission against concentration of ascorbic acid / gdm-3 
	R	Reliability: Reference to repeating at least 3 readings a total of 3 readings to calculate mean
	C	Control experiment: refers to control experiment without ascorbic acid; *Replace ascorbic acid with nutrient medium (reject distilled water)*
	Con	Conclusion: The lowest concentration of ascorbic acid that would inhibit the growth of bacteria is the concentration where the broth is clear / clearest

2 **L1** is a slide of a stained transverse section through a plant root.

You are not expected to be familiar with this specimen.

(a) **(i)** Draw a large plan diagram of the region of the root section shown by the shaded area in Fig. 2.1.

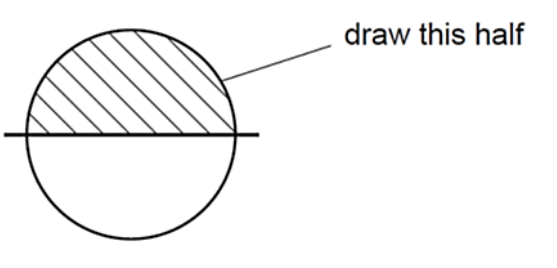
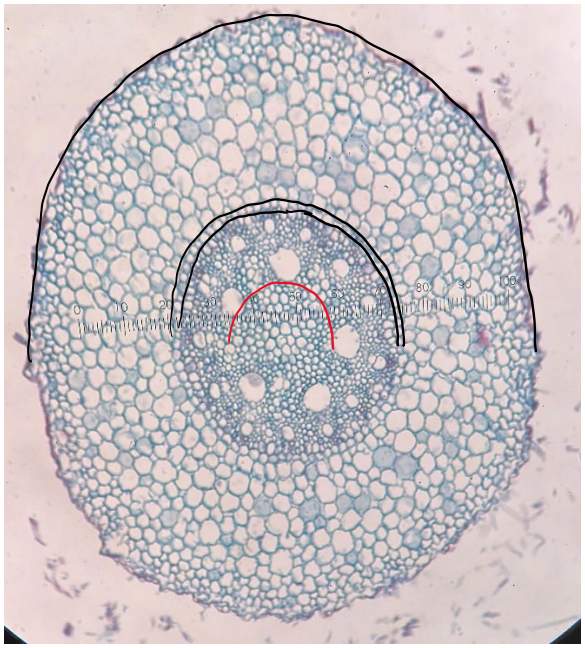
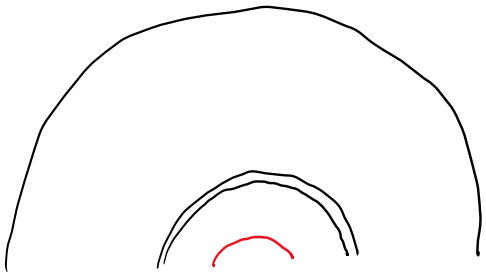


Fig. 2.1

D - minimum size (at least ½ of space) + no shading + no cells + half of the root drawn;
L - at least 3 layers drawn (refer to black lines);
VB - Vascular bundle layer drawn (refer to red line)
P - correct proportion of the tissues (diameter of the central region to the total diameter should be 1/3 to ½)





[4]

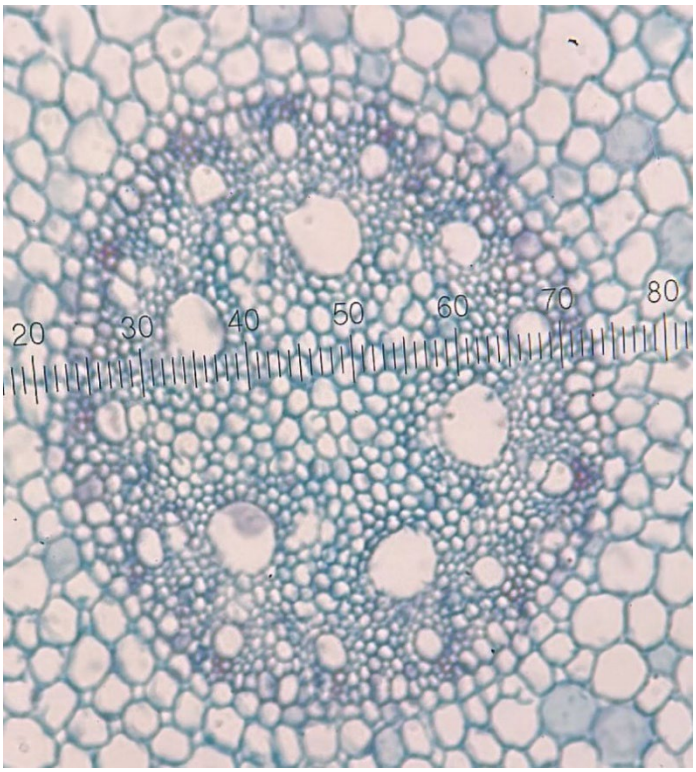
(ii) Observe the cells in the central portion (pith) of the root in **L1**.

Select a group of four adjacent cells that make up this tissue.

Each cell must touch at least **two** of the other cells.

- Make a large drawing of this group of **four** cells.
- Use **one** ruled label line and label to identify the cell wall of **one** cell.

D - minimum size and all lines continuous, thin and sharp and no shading ;
C - draws only four whole cells and each cell touches at least two other cells ;
CW - each cell to have double-lined cell wall with consistent thickness + three lines where cells touch ;
S - correct shape of cells;
L - label line and label to one cell wall ;



[5]

(b) Fig. 2.2 is a photomicrograph of a stained transverse section through a root of a different type of plant.

You are not expected to be familiar with this specimen.

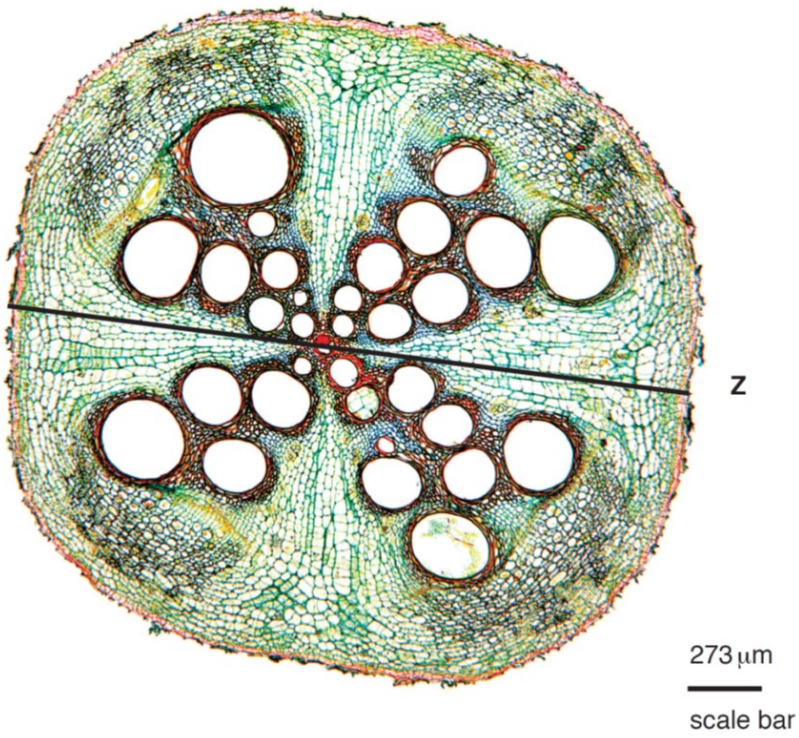


Fig. 2.2

annotates 3 correct features on Fig 2.2 using labels Q, R and S with appropriate differences as annotations; ; ;
e.g.
Fig 2.2 xylem tissue arranged in a cross shape, L1 arranged in a ring
Fig 2.2 has varying sizes of xylem vessels, L1 has uniform sizes of xylem vessels
Fig 2.2 has absence of pith or central region, L1 has large pith
Fig 2.2 has a cortex that takes up a smaller proportion of the root, while L1 has a cortex that takes up a larger proportion of the root (accept comparison on proportion of vascular bundle to root)
Fig 2.2 has a angular / squarish shape, L1 is round / oval
Cells in the cortex of Fig. 2.2 are elongated while cells in the cortex of L1 are more circular/round;

(i) Annotate Fig. 2.2 to describe **three** observable differences between the root in Fig. 2.2 and the root on **L1**.

- Draw label lines to **three** features of the root in Fig. 2.2 that are different from the root on **L1**.
- Label one line **Q**, label one line **R** and label one line **S**.
- Next to each letter, describe how each feature of the root in Fig. 2.2 differs from the root on **L1**.

[3]

(ii) Use the scale bar and the line **Z** on Fig. 2.2 to calculate the actual width of the root.

Show all the steps in your working and use appropriate units.

Length of line Z = 9.1cm;
Length of scale bar = 1.0cm;

Actual width of root = 9.1/1.0 x 273;
= 2484.3 µm
= 0.25 cm;

actual width of the root = …… cm [4]

(c) A student investigated the effect of salt stress on the growth and development of plants. The length of the roots of the plant, *Arabidopsis thaliana*, are measured 3 days after growing them in two different culture mediums. Culture medium **A** contains 1.0% sodium chloride while culture medium **B** does not contain sodium chloride. All other variables were kept constant.

Table 2.1 shows the results of the investigation.

Table 2.1

Root	Length of roots / cm	
	Culture medium **A**	Culture medium **B**
1	7.5	9.8
2	9.4	8.5
3	7.9	8.4
4	7.6	7.8
5	8.4	10.1
6	8.4	10.5
7	8.5	11.4
8	7.8	9.2
9	7.6	11.2
10	8.6	8.8
mean ($\bar{x}$)	8.2	9.6
standard deviation (s)	0.60	1.23
variance (s^2)	0.36	1.51 (2dp)

(i) Complete Table 2.1 by calculating the variance (s^2) for the root lengths of *Arabidopsis* in each of the two culture mediums. [1]

(ii) A *t*-test can be used to determine whether there is any significant difference between the root lengths of *Arabidopsis* in culture medium **A** and root lengths of *Arabidopsis* in culture medium **B**.

Calculate the value of *t* and the number of degrees of freedom, using these formulae:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

Show your working.

Degrees of freedom = 10 + 10 – 2
= **18**

$$t = \frac{|8.2 - 9.6|}{\sqrt{\left(\frac{0.36}{10}\right) + \left(\frac{1.5129}{10}\right)}}$$

= **3.23 (2dp)**

value of *t* = …3.23 ……

number of degrees of freedom = …18……
[2]

(iii) For this *t*-test, the student proposed the null hypothesis:

there is no difference between the root lengths of Arabidopsis from culture medium **A** and the root lengths of Arabidopsis in culture medium **B**.

Table 2.2 shows the critical values for *t* at several different probabilities and degrees of freedom.

Table 2.2

degrees of freedom	probability, *p*				
	0.5	0.1	0.05	0.01	0.001
1	1.00	6.31	12.71	63.66	636.62
2	0.82	2.92	4.30	9.92	31.60
3	0.76	2.35	3.18	5.84	12.92
4	0.74	2.13	2.78	4.60	8.61
5	0.73	2.02	2.57	4.03	6.87
6	0.72	1.94	2.45	3.71	5.96
7	0.71	1.89	2.36	3.50	5.41
8	0.71	1.86	2.31	3.36	5.04
9	0.70	1.83	2.26	3.25	4.78
10	0.70	1.81	2.23	3.17	4.59
11	0.70	1.80	2.20	3.11	4.44
12	0.70	1.78	2.18	3.05	4.32
13	0.69	1.77	2.16	3.01	4.22
14	0.69	1.76	2.14	2.98	4.14
15	0.69	1.75	2.13	2.95	4.07
16	0.69	1.75	2.12	2.92	4.01
17	0.69	1.74	2.11	2.90	3.97
18	0.69	1.73	2.10	2.88	3.92
19	0.69	1.73	2.09	2.86	3.88
20	0.69	1.72	2.09	2.85	3.85

3.23

Use Table 2.2 and your answers to **(c)(ii)** to decide whether the null hypothesis suggested by the student should be accepted or rejected.

Explain your answer.

accept or reject null hypothesis

reject null hypothesis

explanation

At 5% significance value, calculated t-value 3.23 is **larger than critical t-value of 2.10**,

The calculated t-value has a probability of **0.01<p<0.001**;

Addition of **sodium chloride resulted in slower growth of roots**;

Ignore: Difference between mean root lengths in culture medium A and B is **significantly different** and **not due to chance**; (already written in the question)

[3]

[Total: 22]