

NANYANG JUNIOR COLLEGE
PRELIMINARY EXAMINATIONS
Higher 2

CANDIDATE
NAME

CLASS

BIOLOGY

9744/04

Paper 4 Practical

28 August 2025

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.

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 Examiner's Use	
1	
2	
Total	

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

[Turn over

Answer **all** the questions.

- 1** Yeast cells contain enzymes that break down sugars to release energy. In these reactions carbon dioxide is released. Some of this carbon dioxide dissolves in water forming carbonic acid.

The progress of these enzyme-catalysed reactions can be followed by measuring the time taken for an indicator to change colour as carbonic acid is formed. You will investigate the effect of changing the concentration of yeast suspension on the time taken for the indicator to change colour.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume/ cm ³
Y	10% yeast suspension	none	100
H	hydrogencarbonate indicator solution	none	50
W	distilled water	none	100

Note that it is normal for **Y** to froth over time and the froth may overflow from the container.

If any solution comes into contact with your skin, wash off immediately under cold water. It is recommended that you wear suitable eye protection.

Read step 1 to step 4.

- (a) (i)** Using Table 1.1 **and** step 1 to step 4, assess the risk of this procedure as **low**, **medium** or **high**. Give **one** reason for your answer.

risk

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reason

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..... [1]

The hydrogencarbonate indicator solution, **H**, will change colour from red-brown to yellow as carbon dioxide passes through it.

To help you identify the end-point (yellow) carry out step 1 to step 4.

- Put 5cm³ of **H** into a test-tube.
- Put a drinking straw into this test-tube.
- Gently breathe **out** through the straw so that you are blowing air bubbles through **H**.
- Stop blowing when the colour changes to yellow. This is the end-point.

Fig. 1.1 shows the apparatus you will set up for the investigation.

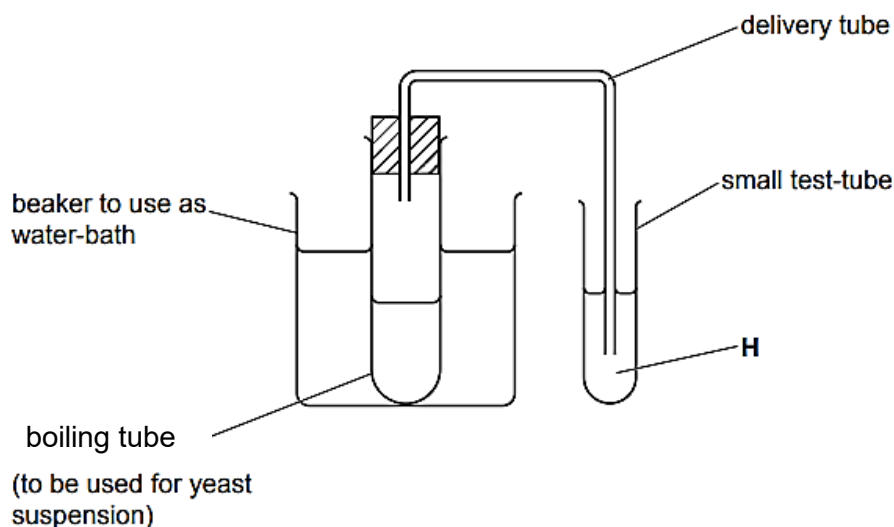


Fig. 1.1

- 10 Use a glass rod to stir the 10% yeast suspension, then pour the prepared 20cm³ of the 10% yeast suspension into the boiling tube.
- 11 Put the boiling tube in the water-bath.
- 12 Put the bung in the boiling tube, and make sure it covers the mouth of the boiling tube tightly. Ensure the end of the delivery tube is in **H**, as shown in Fig. 1.1.
- 13 Start timing.
- 14 Stop timing when the end-point is reached.

The test-tube from step 4 can be used to confirm the end-point.

Record the result in **(a)(iii)**. If the time taken is more than 240 seconds, record the result as 'more than 240'.

- 15 Put the contents of the boiling tube into the container labelled **For waste**.
- 16 Rinse the boiling tube. You may use water from the beaker labelled **For washing**.
- 17 Put another test-tube of **H**, prepared in step 7, into the apparatus shown in Fig. 1.1.
- 18 Repeat step 10 to step 17 for the other concentrations of yeast suspension you prepared in step 6.

(iii) Record your results in an appropriate table.

[4]

(iv) Describe **two** significant sources of error in this investigation.

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[2]

(b) Baker's yeast, *Saccharomyces cerevisiae*, has two metabolic pathways to produce ATP, aerobic respiration and fermentation. Oxygen is **not** used in fermentation.

These pathways are summarised in Fig. 1.2.

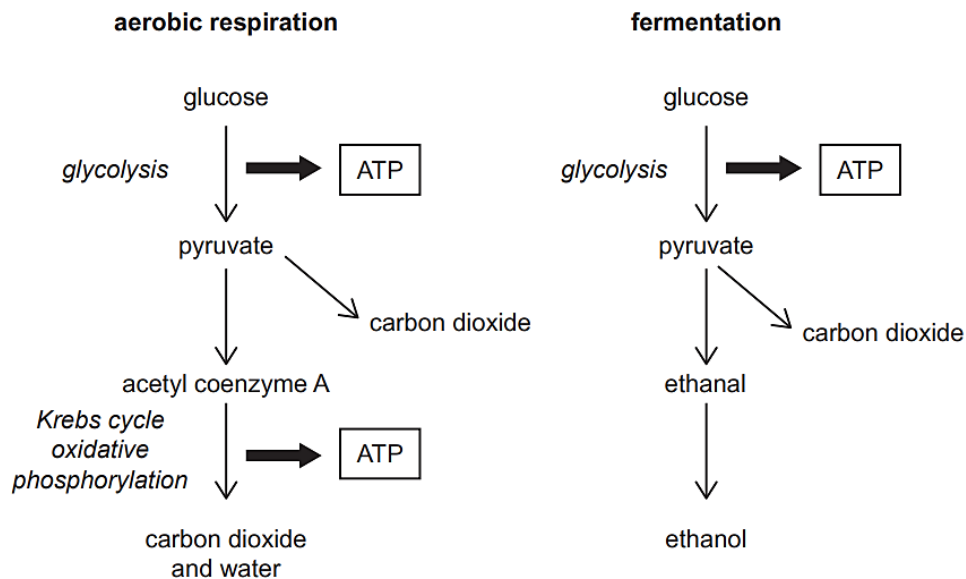


Fig. 1.2

Some students were researching details of the respiration of yeast. In their research, the students found information about the Crabtree effect. This effect occurs when yeast is kept in high concentrations of glucose. The yeast gains sufficient ATP from substrate-level phosphorylation during glycolysis. Yeast does not carry out the Krebs cycle or oxidative phosphorylation and therefore does not use oxygen.

The students wanted to investigate the effect of temperature on the activity of yeast and decided to use yeast solutions in which the yeast obtained all its ATP by fermentation.

- The students made a suspension of yeast from 1g of dried yeast and 25cm³ of water.
- The suspension was left for 2 hours at 20°C.
- After 2 hours, 25cm³ of glucose solution was added to the yeast suspension and the mixture was stirred.

The students measured the volume of carbon dioxide produced by yeast fermentation.

Plan an investigation to find the temperature at which yeast carries out fermentation at its maximum rate.

You can assume that you have access to all standard laboratory equipment.

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 independent variable and dependent 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 do **not** need to include details of how to make the yeast suspension or reference to any safety measures in your plan.

space for diagram

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- (c) A student carried out an investigation into the effect of temperature on the activity of the enzymes in Y.

The student immobilised the yeast cells in alginate beads which were then dropped into a solution of the substrate at different temperatures.

As carbon dioxide collected in the beads, they rose to the surface of the solution of the substrate as shown in Fig. 1.3.

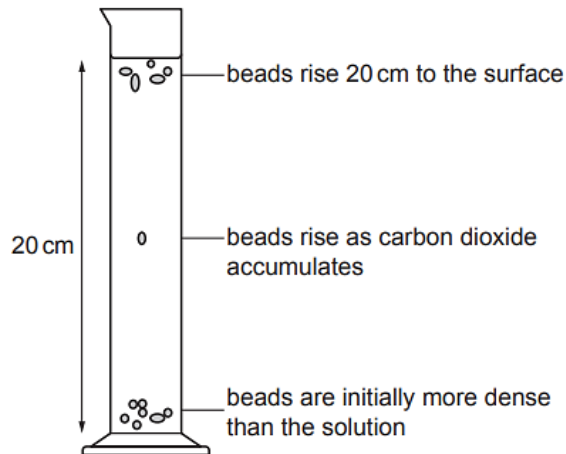


Fig. 1.3

The student measured the time taken for 5 beads to rise to the surface, a distance of 20cm. The processed results are shown in Table 1.3.

Table 1.3

temperature / °C	mean time to rise / seconds
10	72
25	19
40	11
50	36
55	109

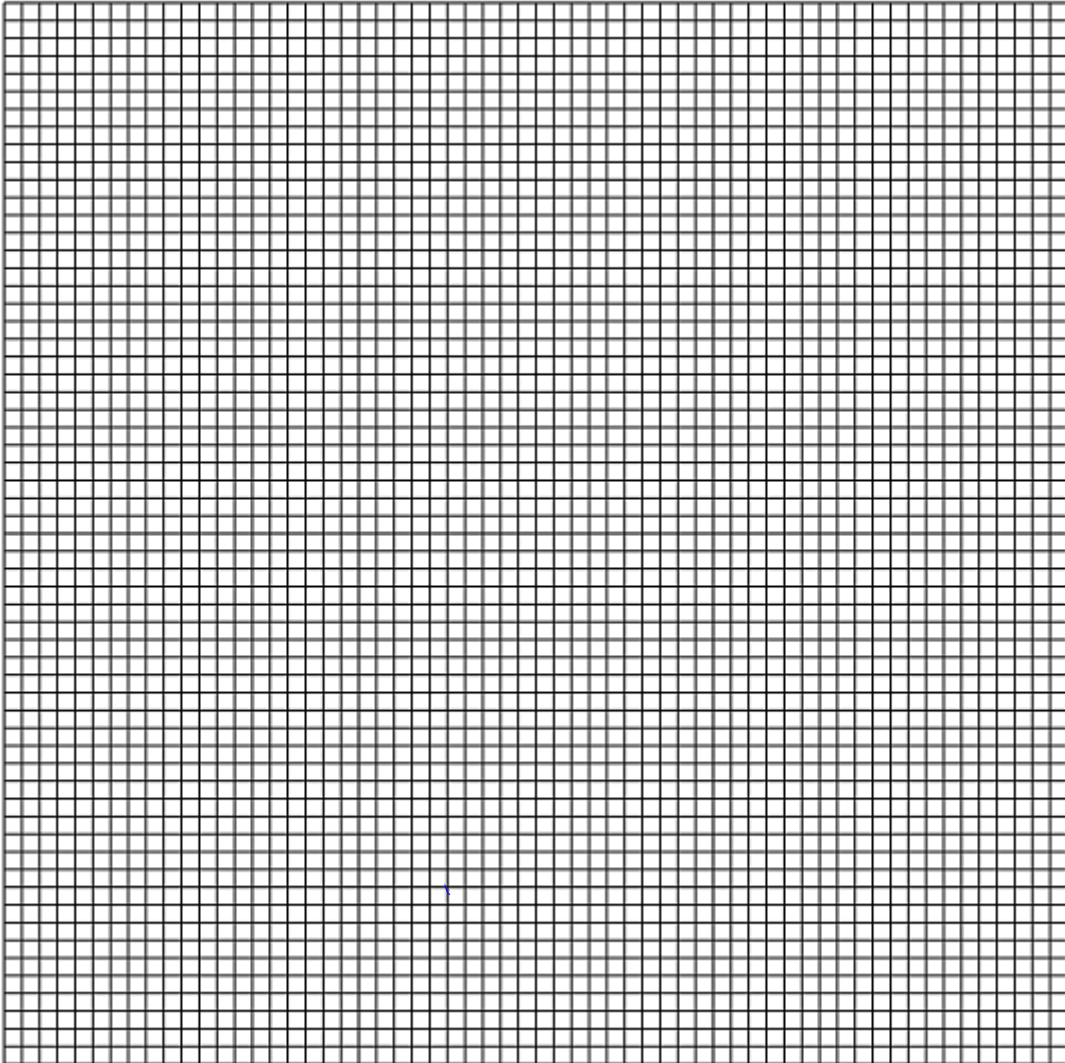
- (i) Identify the temperature at which the beads rise the fastest.°C

For this temperature calculate the rate at which these beads rise 20cm.

rate = [2]

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

Use a sharp pencil for drawing graphs.



[4]

(iii) Explain the shape of the graph:

between 10°C and 40°C:

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between 40°C and 55°C:

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[3]

- (d) Ethanol is a renewable alternative to fossil fuels and is increasingly used as a biofuel or blended fuel in transportation.

Recent research has demonstrated that immobilised yeast cells can produce greater quantities of ethanol than free yeast cells under the same fermentation conditions. Immobilisation typically involves enclosing yeast in a matrix, such as calcium alginate beads, which allows substrates like glucose to diffuse in while ethanol and carbon dioxide diffuse out.

A student conducted an experiment to compare the percentage ethanol yield between free yeast and immobilised yeast over 6 days of fermentation. He carried out a statistical test to determine whether there was a significant difference between these means.

- (i) State a statistical test that could have been used to determine whether the difference in percentage ethanol yield between free yeast and immobilized yeast is significant.

.....
[1]

- (ii) A summary of the student's results is shown in Table 1.4.

Table 1.4

percentage ethanol yield on day 6 of fermentation		significance of difference
immobilised yeast	free yeast	
82.73	68.79	$p < 0.05$

Comment on what these results show and suggest an explanation for any pattern.

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[3]

[Total: 29]

2 Methylene blue stains dead cells blue.

Living cells are **not** stained blue so they will appear white or clear.

You are provided with:

- methylene blue solution, **M**, (handle carefully as it will stain your skin)
- suspensions of yeast cells, labelled **S1**, **S2** and **S3**.

Each suspension, **S1**, **S2** and **S3** has been heated for ten minutes at 45°C or 80°C or 100°C.

You are required to:

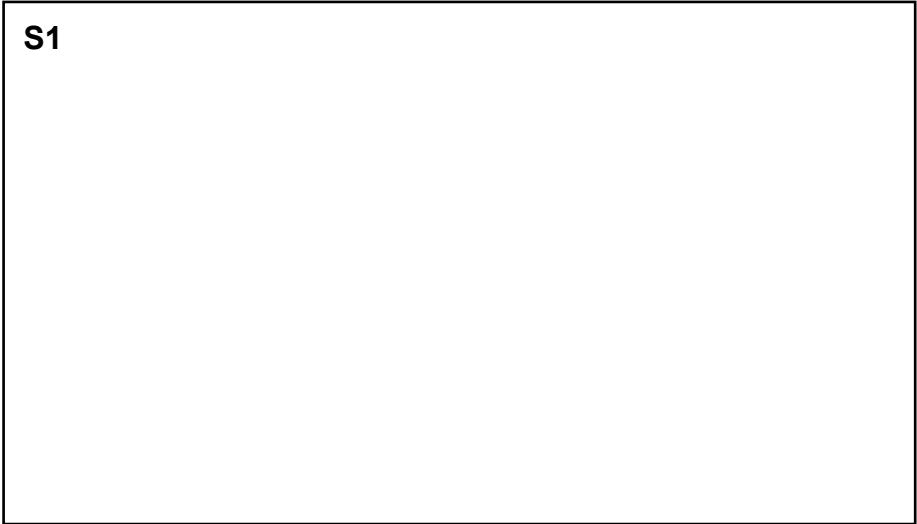
- use the microscope to observe the colour of the yeast cells from **S1**, **S2** and **S3**, after **M** has been added
- record your observations by using annotated drawings of three yeast cells from each of **S1**, **S2** and **S3**
- identify the temperature at which each of **S1**, **S2** and **S3** was heated.

1. Label three microscope slides **S1**, **S2** and **S3**.
2. Place **one small drop** of **S1** onto slide S1 and add **one small drop** of **M**. Mix carefully using a glass rod. (If **M** comes into contact with your skin rinse with cold water.)
3. Repeat step 2 with **S2** and **S3**.
4. Leave for five minutes.
5. Add a coverslip to each slide.
6. Use the paper towel to dry off any excess liquid around the coverslip.
7. Use the microscope to observe the yeast cells on each slide, then select cells which you can draw and annotate to describe the effect of the methylene blue, **M**.

(a) (i) Prepare the space below and record your observations by:

- making drawings of **three** cells from **each** of the slides in the boxes provided
- annotating your drawings to describe the effect of methylene blue, **M** on the cells.

S1



S2**S3**

[4]

- (ii) Use your observations to identify the temperature that was used to heat each of the suspensions **S1**, **S2** and **S3**.

Complete the table.

suspension	temperature / °C
S1	
S2	
S3	

[1]

- (iii) Explain how you identified the yeast cells that had been heated at 100°C.

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[1]

- (iv) A student was provided with a suspension of yeast cells which had been heated at a temperature between 45°C and 80°C.

Describe how you could modify this investigation to provide quantitative measurements that can be used to estimate this temperature.

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[3]

Fig. 2.1 is a photomicrograph of yeast cells.

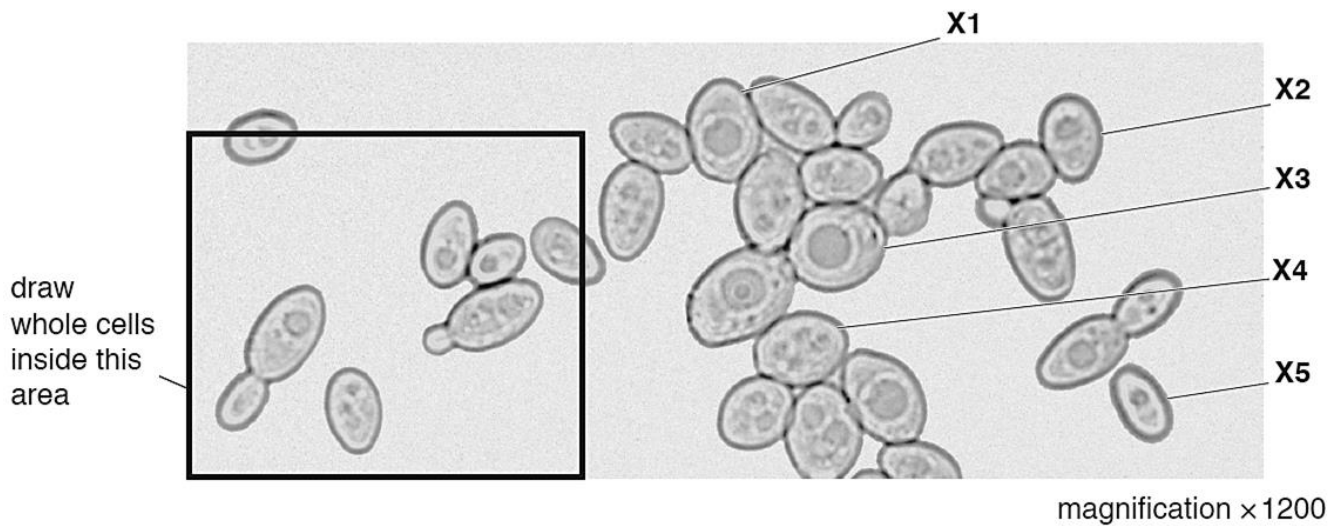


Fig. 2.1

- (b) (i)** Make a large drawing of the whole cells shown in the area on Fig. 2.1.

On your drawing, use a label line and label to show one observable feature of these cells which identify them as being similar to animal cells.

[5]

- (ii) Use the magnification to calculate the mean maximum actual length, in μm , of yeast cells, **X1**, **X2**, **X3**, **X4** and **X5**.

You may lose marks if you do not show your working or if you do not use the appropriate units.

..... μm [4]

- (c) Rhizophagy, derived from "rhizo" (root) and "phagy" (eating), refers to a recently discovered nutritional symbiosis where plants actively take up microbes such as yeast into their roots, extract nutrients from them, and then release them back into the soil. This cycle is thought to be a way for plants to obtain essential nutrients, particularly micronutrients, from soil microbes.

Yeast is a waste product of the brewing industry and can be used as biofertilizer. The addition of live or dead yeast to fertilised soil has been shown to improve plant growth and health of crops like corn (*Zea mays*), sugarcane (*Saccharum officinarum*) and tomato (*Solanum lycopersicum*).

K1 is a slide of a stained transverse section through a *Zea mays* root.

You are not expected to be familiar with this specimen.

Use a sharp pencil for drawing.

You are expected to draw the correct shape and proportions of the different tissues.

- (i) Draw a large plan diagram of the whole root.

- (ii) Fig. 2.2 is a photomicrograph of a stained transverse section through the root of a *Solanum lycopersicum* tomato plant. You are not expected to be familiar with this specimen.

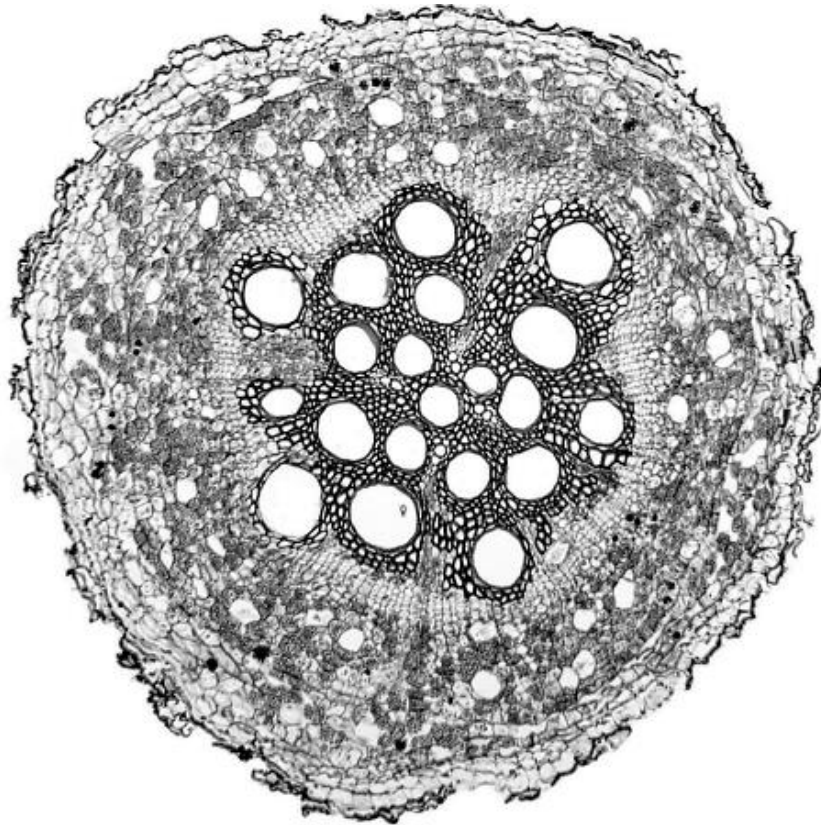


Fig. 2.2

Prepare an appropriate table so that it is suitable for you to record the observable differences between the root on **K1** and the root in **Fig. 2.2**.

Record the observable differences in your table.

[4]
[Total: 26]