H

RAFFLES INSTITUTION
2024 Year 6 Preliminary Examination

Higher 2

CANDIDATE NAME											
CIVICS GROUP	2	4	S	0	3		INDEX NUMBER						

BIOLOGY

9744/04

Paper 4 Practical

15th 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 index number, CT group and name 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 a 2B pencil for any diagrams or graphs.
Do not use staples, paper clips, highlighters, 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, hand in the Question Paper containing your answers.
The number of marks is given in brackets [ ] at the end of each question or part of the question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	

This document consists of **19** printed pages and 1 blank page.

Raffles Institution
Internal Examination

Answer **all** questions.

1 You are advised to read the whole of the question before starting the practical work, as you will need to make decisions about how to obtain high quality results using the apparatus and materials provided.

You are going to investigate the clotting of milk.

Young mammals are fed on milk. To enable efficient digestion of milk it is clotted in the stomach using the protease enzyme, rennin. Rennin converts the soluble protein caseinogen in the milk to an insoluble form called casein.

The manufacture of cheese also requires the clotting of milk. This can be done in two ways by:

- acidifying the milk
- using the enzyme, rennin

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard
M	100% milk	none
E	1% rennin	harmful irritant
A	1 mol dm^{-3} hydrochloric acid	corrosive

If **E** or **A** comes into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

In **Part 1** you will observe the clotting of milk using two methods:

Method 1 involves the use of **A** and **Method 2** involves the use of **E**.

In **Part 2** you will investigate the effect of various temperatures on the clotting activity of **E**.

Part 1 – observing clotting

Method 1 – using acid

1. Add 5 cm^3 of **M** to a test-tube.

2. Add 1 cm^3 of **A** into the same test-tube.

3. Put a bung into the test-tube and mix the contents by inverting the test-tube three times.

4. Tilt the tube and rotate it slowly such that the contents touch all of the internal glass surfaces as shown in Fig. 1.1. Do **not** shake the tube.

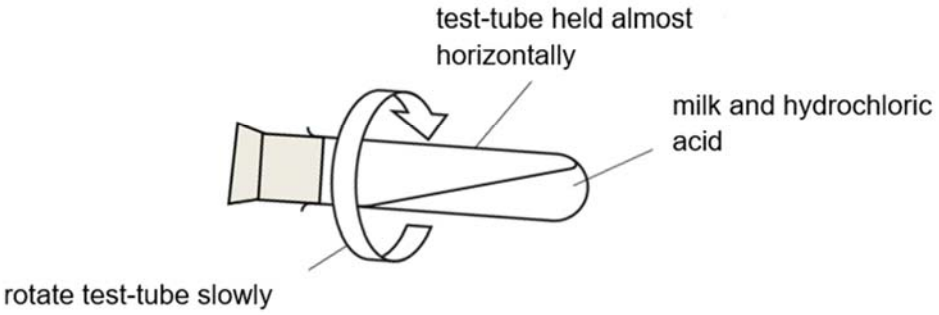


Fig. 1.1

(a) Record your observations. [2]

1. larger **white / creamy** solid precipitate in supernatant/ liquid;
   A: white clotted milk A: white clumps A: white suspension
2. small white / creamy specks **stick to wall** of test-tube ;
3. supernatant / liquid, becomes less opaque/ becomes translucent/ turns clearer;
   R: white liquid or solution / clear solution

5. Keep the test-tube for later reference.

Method 2 – using rennin

1. Prepare and maintain a water-bath at 55 °C.

2. Put 5 cm^3 of **M** into a test-tube.

3. Put 1 cm^3 of **E** solution into a separate test-tube.

4. Place both test-tubes in the 55 °C water bath for 3 minutes. It is important to maintain the temperature of the water bath during the entire 3 minutes.

5. Add the 5 cm^3 of **M** into the test-tube containing **E**.

6. Put a bung into the test-tube and mix the contents by inverting the test-tube three times and immediately start the stopwatch.

7. Tilt the tube and rotate it slowly such that the contents touch all of the internal glass surfaces as shown in Fig. 1.1. Do **not** shake the tube.

8. Continue to rotate the tube and record the time taken for clotted milk particles to **first start to form** on the walls of the tube as shown in Fig. 1.2.

(b) Time taken for the milk to clot. .....7.....s [1]

A:7-78s

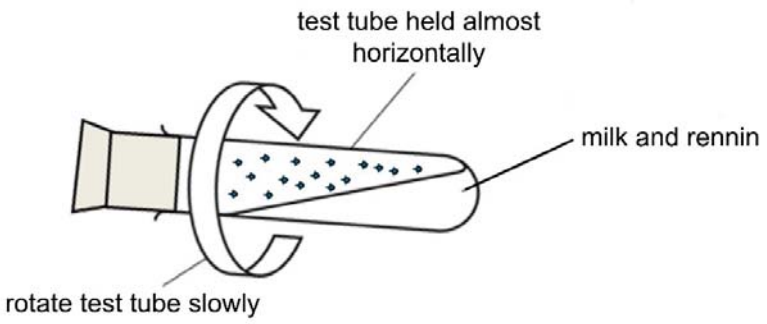


Fig. 1.2

9. Keep the test-tube for later reference.

(c) Suggest the advantage of adding the milk to the rennin solution rather than adding the rennin solution to the milk. [1]

1. the volume remaining in the milk test-tube is a lower proportion of the total volume / ORA **;**
2. smaller percentage error;

(d) Use the universal indicator paper to test the pH of:

- milk
- milk with acid (use the test-tube from Method 1 step 5)
- milk with rennin solution (use the test-tube from Method 2 step 9)
- rennin solution

Record your results in the space below and explain the advantage of carrying out this test before investigating the effect of concentration of rennin on the clotting of milk.[2]

pH of milk:……7……..……..

pH of milk with acid:……2……..……

pH of milk with rennin:……7……..

pH of rennin: ……6…….

1mark;

A: 8, 2, 8, 7/6

Explanation:

Since the pH of the milk and renin is the same as the pH of milk, clotting is not due to the ***acidity**** of rennin but due to its enzymatic activity;

(e) Caseinogen molecules are hydrophilic and therefore soluble in water. When formed, they are incorporated, along with certain other molecules and ions, into spherical structures called micelles. Each micelle has a hydrophobic core and a stabilising outer layer of hydrophilic caseinogen molecules.

During clotting the micelles are broken open and their contents clump together to form clots.

Suggest why the contents tend to clump together.[1]

1. the hydrophobic contents come together due to hydrophobic interactions and are shielded from water by clumping **;**

(f) Suggest why the time taken for clotting was different in the two methods.[3]

1. Clotting was faster with the acid/ in Method 1;
2. [Method 1] H^+ ions / protons which interact with charged or ionisable amino acid ***side chains / R groups**** on protein, disrupting the (hydrogen and ionic) bonds in the protein, leading to denaturation / clotting ;
3. [Method 2] Rennin activity relies on (time needed for) successful effective collisions between substrate and active site / forming enzyme-substrate complex with caseinogen
4. Larger number of protons compared to enzymes;

Part 2 – investigating the effect of temperature on the rate of clotting of milk

(g) Carry out the steps in **Method 2** for all 3 temperatures at 35^0C, 45^0C, and 55^0C..

Record your results for all 3 temperatures in a suitable table in the space below to show the effect of temperature on the rate of clotting of milk.

If the end-point has not been reached by 180 seconds, stop timing and record this as 'more than 180'.[4]

Table showing the effect of different temperatures on rate of clotting of milk

temperature /°C	time taken for little specks of clotted milk particles to first start to form on the walls of the test-tube / s	rate of clotting of milk / s^{-1}
55	33	0.030
45	82	0.012
35	169	0.0059

1. **H** - correct headings with units. Rate column, with units, needs to be present;
2. **P 1** - time recorded in whole numbers (no units in cell);
3. **P 2** - rate in 2 sig fig (based on least precise raw data) with correct calculation (no working shown in cell + no units in cell);
4. **T** - correct trend i.e. as temperature increases the rate of clotting increases;

(h) The descriptions of precautions that should have been taken when conducting the experiment are in the table below.

Complete table 1.1 by writing explanations for the descriptions of the precautions that should have been taken to ensure high quality results. [3]

Table 1.2

	description of precaution	explanation
1	All test tubes and syringes were labelled clearly.	This was done to avoid misidentification/ contamination/ mix-up.
2	The experiment was carried out on one tube at a time.	This was done to ensure accurate measurement of time taken for clotting to occur/ rate of clotting.
3	Syringes of appropriate volume were used.	This was done to reduce percentage error/ maximise precision/ reduce uncertainty to increase accuracy.

(i) Complete Table 1.3 by describing an improvement to reduce the effect of each error that may have affected your results. [3]

Table 1.3

sources of error	improvement
No replicates were done.	Three replicates could have been conducted to identify anomalies;
Judging the end point or when little specks of clotted milk particles to first start to form on the walls of the test-tube was difficult.	Observe against a black background so as to see the specks better/ A video of each tube could have been taken and playback to check that the same end point is reached each time/ Fix time to allow clotting and filter to obtain dry mass of milk clot.
Measurement of volumes could be inaccurate due to the volume of syringes used.	Micropipettes could have been used; R: measuring cylinder/ burette due to small volume used in this experiment.
It was difficult to maintain temperature of water bath during equilibration time.	A ***thermostatically controlled**** water bath could have been used;

(j) A scientist carried out an investigation into the effect of enzyme concentration on the coagulation of milk. The scientist calculated the activity of the enzyme for each concentration of enzyme.

The results are shown in Table 1.4.

Table 1.4

enzyme concentration /%	activity of enzyme /arbitrary units (au)
0.05	19
0.10	34
0.15	50
0.20	65
0.30	96

(k) Plot a graph of the data in Table 1.4 on the grid in Fig. 1.3.

Use a sharp pencil.

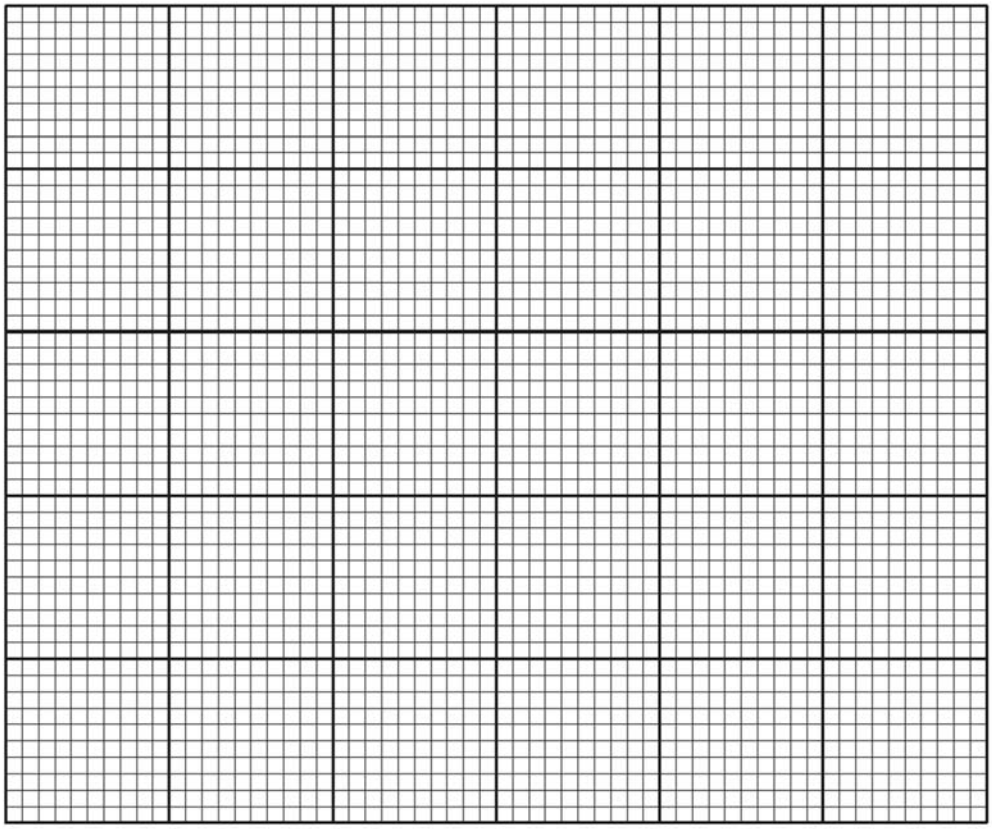


Fig. 1.3 [3]

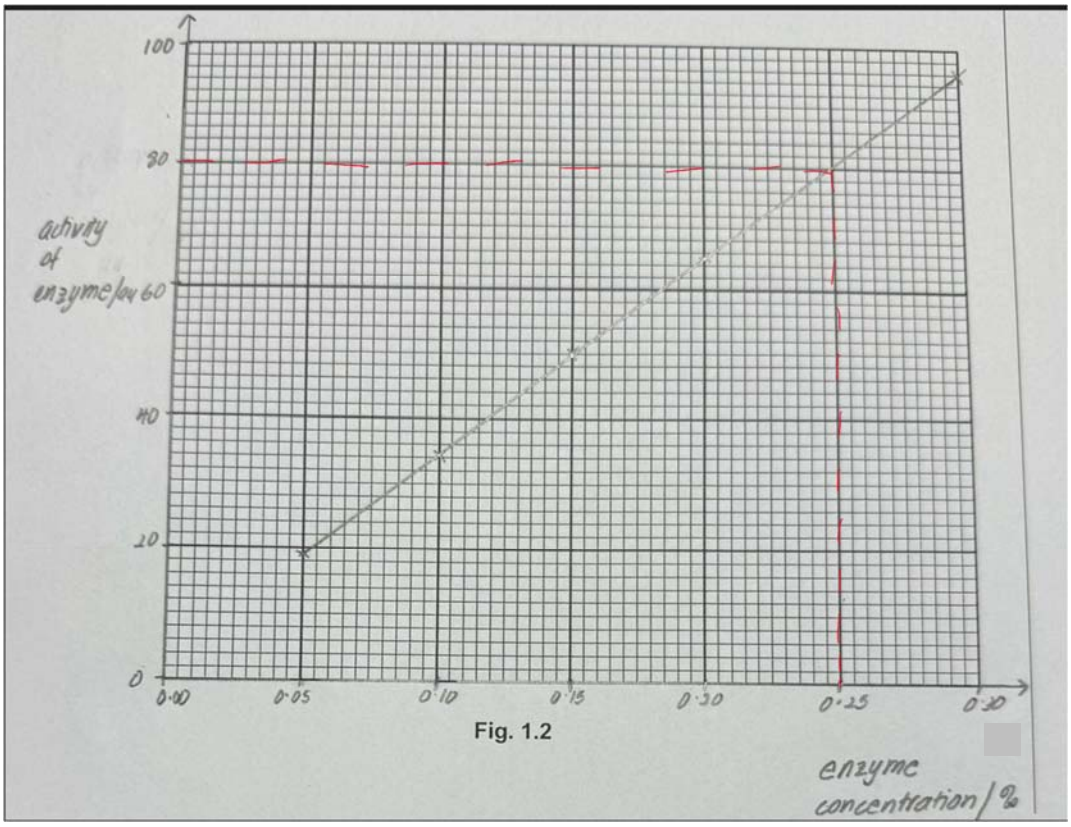


Fig. 1.2

1. **S**cale : 2/3 of graph
2. **A**xes : correct with units (enzyme concentration/% must be on x-axis)
3. **P**ts : correctly plotted using X and **L**ine : passes all pts

(I) Use your graph to determine the activity of the enzyme when the enzyme concentration is 0.25%.

Show on your graph how you determined this value.
Need to show dotted lines on the graph.

activity of enzyme : ……80……[1]

[Total: 24]

2 Yeast cells contain enzymes that catalyse respiratory reactions. Different sugars have different effects on the respiratory reactions in yeast.

Two students had a discussion on whether galactose or sucrose will produce a faster respiratory reaction with yeast.

Jason claimed that galactose would produce a faster reaction, while **Kate** claimed that it should be sucrose. The two students decided to investigate.

One way to investigate the progress of enzyme-catalysed respiratory reactions in yeast is to test for the release of carbon dioxide.

A sample of yeast cells, **Y**, can be added into a piece of Visking tubing, **V**, which can then be placed inside a beaker containing 100 cm^3 distilled water, as shown in Fig. 2.1.

The Visking tubing membrane can contain between 10.0 cm^3 to 12.0 cm^3 of liquid. It is selectively permeable, hence any carbon dioxide molecules produced by the yeast cells when sugars are added will diffuse through the membrane of the tubing into the water surrounding the Visking tubing.

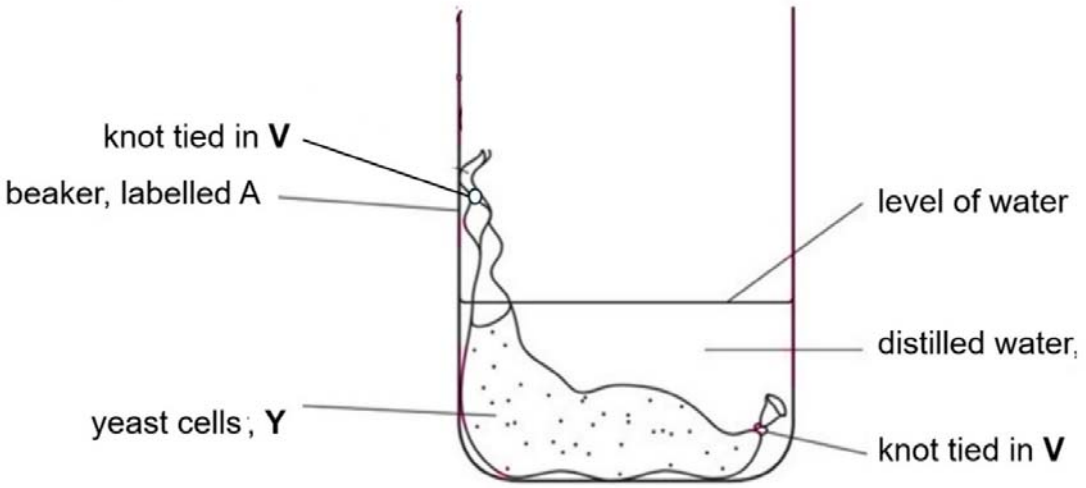
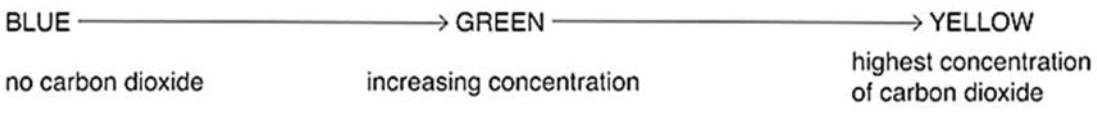


Fig. 2.1

The students found out that mixing the yeast cell suspension with any sugars in a 1:1 volume ratio would result in immediate activation of the yeast.

To test for the release of carbon dioxide, a sample of the water (1.0 cm^3) surrounding the Visking tubing can be taken and added to two drops of an indicator, **B**. As the concentration of carbon dioxide increases, **B** changes from blue to green to yellow.



(a) Explain why the indicator changes from blue to yellow.

……

……[1]

1. Carbon dioxide dissolves to form (carbonic) ***acid**** (A: solution becomes acidic);

The students also found that the difference in the results of the yeast cells with the different sugars can be determined between 3 to 10 min.

To follow the progress of this reaction you will need to take samples from the water surrounding the Visking tubing at different times up to a suitable time. If the colour change to yellow takes more than 10 min, record as "more than 600 seconds".

You are provided with the following which you **must** use:

labelled	contents	hazard
Y	yeast cells in sucrose solution	none
B	bromothymol blue indicator solution	harmful
W	to collect distilled water from teacher's bench	none
S	sucrose solution	none
G	galactose solution	none
V	two 15 cm length of Visking tubing in a container containing distilled water	none

You are also provided with the following which you may use:

	contents	number of items
1	pasteur pipette	2
2	10 cm^3 syringes	4
3	1 cm^3 syringe	4
4	spotting tile	1
5	glass rod	1
6	100 cm^3 beakers	2
7	paper towels	8
8	glass marker pen	1
9	stopwatch	1
10	suitable eye protection	1

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

(b) State:

the time interval when you will remove the samples ……

the total volume that you will put into **V** …… [1]

1. Time interval: 10 s to 60 s
2. **V**: 10.0 cm^3 to 12.0 cm^3

(c) Describe the steps that you will take to investigate whether **Jason** or **Kate** is correct, including steps to ensure reliability of results. You are not required to plan for replicates. [6]

The procedure is as follow:

1. Prepare the spotting tile by using paster pipette to add 2 drops of the indicator B into each well. Use a marker to label timing on the well.

2. Pour 80 cm^3 of water into a 100 cm^3 beaker.

3. Tie one end of the Visking tubing. Use 10.0 cm^3 syringes, mix 5.0 cm^3 yeast + 5.0 cm^3 S (or G) to add into Visking tubing (**A**: 6.0 cm3 yeast + 6.0 cm^3 S / G). Tie the other end of the Visking tubing. (Total volume should not exceed 12.0 cm^3)

4. Place the Visking tubing into the beaker of water prepared in step 2, checking that the yeast cells **Y** are under the water level by using glass rod to push down gently. Start the stopwatch immediately.

5. After a (stated) fixed interval (**A**: 10s – 60s as stated in (a) from 3 min), use 1.0 cm^3 syringes to remove 1.0 cm^3 water outside the Visking tubing and add to well of spotting tile.

6. Note time taken for colour of **B to turn yellow** (or until 600 s is up) using the **stopwatch**.

7. **Repeat** steps 1 to 4 **for the other sugar,** and note the time for colour of B to turn yellow.

8. **Precautions:**

a. Wash the outside of Visking tubing with distilled water before immersing into 100 cm^3 water in beaker.

b. Use of **glass rod**: Yeast is stirred before using/ Water outside Visking tubing is stirred at regular interval or before testing for CO_2/ yeast and S or G are stirred in a beaker using glass rod, and use a syringe to)

c. **Equilibration** to allow **rate of respiration to stabilise.**

(d) Record your results in an appropriate format. [3]

Solution	Time for **B** to change from blue to yellow/ s
S	180
G	240

1. Correct heading - solution & time
2. Correct unit – time must be in seconds.
3. Time recording in whole numbers

(e) State which student is correct, and suggest why the sugar stated by the student results in a faster rate of reaction. [2]

Student Jason / Kate – as long as choice of student follows results obtained in (d)

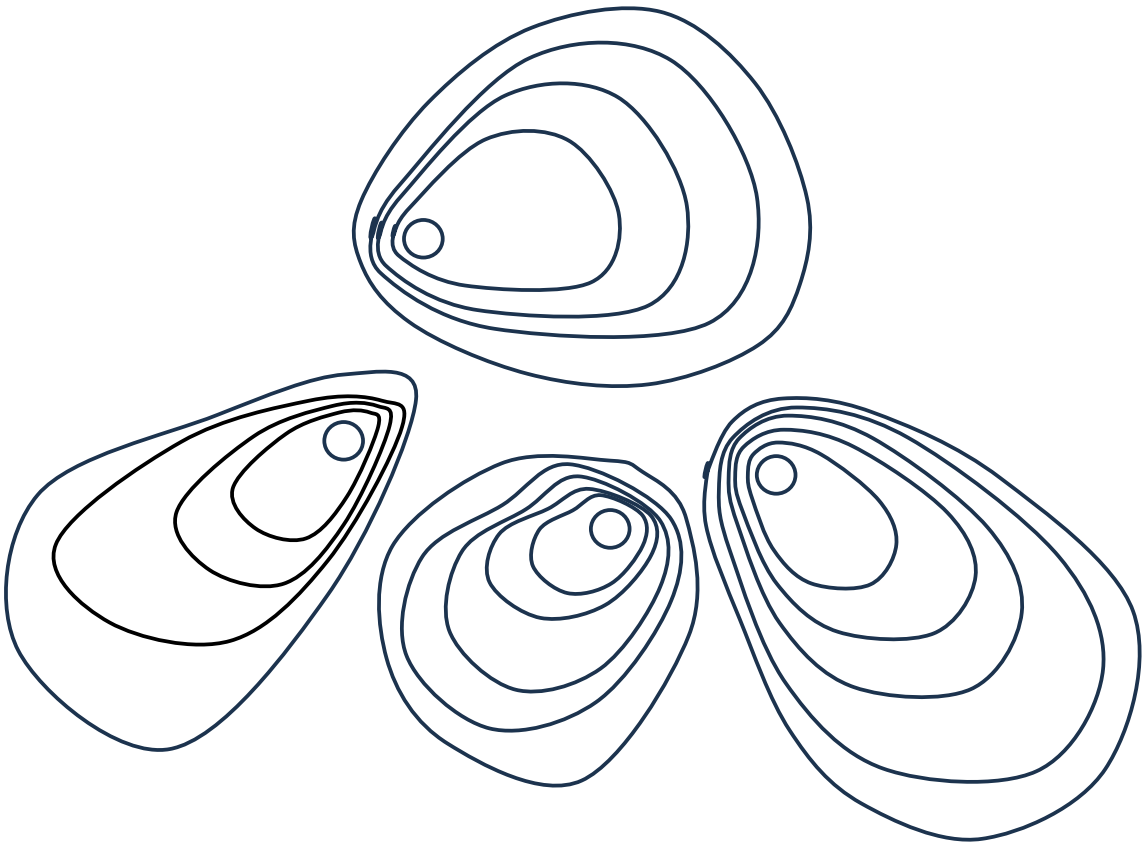
1. (Jason) Galactose is monosaccharide and can be taken up by cell easily/ respire directly;
2. Sucrose is disaccharide and will need to be hydrolysed to monosaccharides;
3. (Kate) sucrose is hydrolysed to glucose and fructose;
4. to give twice the concentration of monosaccharides; [Total: 13]

3 Starch grains from different types of plant differ in size and shape. Some starch grains have rings on their surfaces.

You will now observe and draw starch grains from one type of plant.

1. Put one **clean** and **dry** microscope slide onto a paper towel.
2. Using a pipette, put a few drops of **P** onto the slide. **P** is a suspension of starch grains.
3. Cover the drops of **S** on the slide with a coverslip and use a paper towel to remove any excess suspension.
4. Use the microscope to find and observe the starch grains on the slide.
   You may need to reduce the amount of light entering the microscope and adjust the fine focus to observe the starch grains clearly.
5. Select, from a single field of view, **four** starch grains that show different sizes and features.

(a) Make a large drawing of the **four** starch grains that you have selected.



1 2/3 size of page, and lines continuous, thin and sharp;
2 draws only four starch grains;
3 shows correct details of surface markings;
4 draws single line around each grain and all four must be correct shape;

[4]

(b) Remove the slide from the microscope and place it on a paper towel.

Fig. 3.1 shows some different types of starch grains and patterns on the surface of the starch grains.

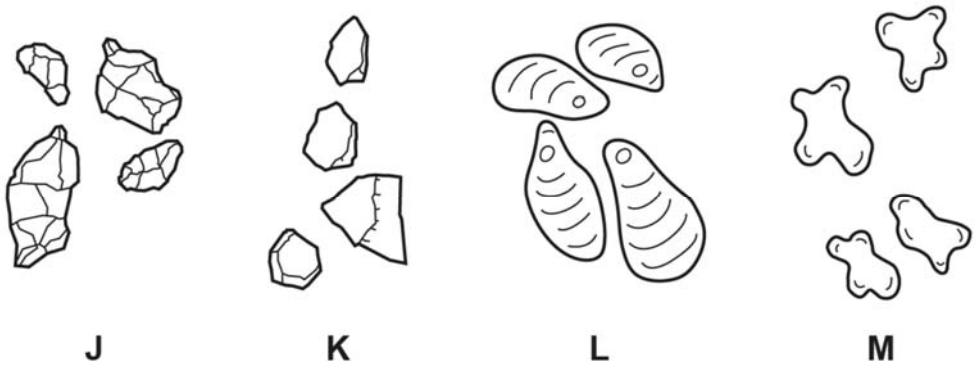


Fig. 3.1

(i) Use the diagrams in Fig. 3.1 to identify which of the starch grains, **J**, **K**, **L** or **M**, matches most closely the starch grains drawn in **(a)**.

answer .....L..... [1]

A student calibrated the eyepiece graticule in a light microscope using a stage micrometer scale.

The calibration was:

1 eyepiece graticule unit = 16 μm

The student used the microscope to observe and draw three starch grains to the same scale. The student drew a line across the length of each drawing of a starch grain. The student's drawings are shown in Fig. 3.2.

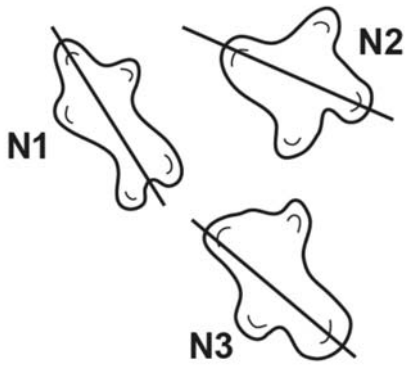


Fig. 3.2

(ii) When viewed using the microscope, the student found that starch grain **N1** measured 4 eyepiece graticule units along the position of the line drawn in Fig. 3.2.

Calculate the mean **actual** length of the three starch grains in Fig. 3.2.

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

Length of N1 = 23mm, N2 = 22mm, N3 = 25mm

Mean actual length of starch grains = $\frac{25 + 22 + 23}{3 \times 23} \times 4 \times 16 = 64.9um$

1. Correct measurement – 22 mm, 23 mm, and 25 mm;
2. Correct working;
3. Correct final answer based on student's measurements;

mean actual length = ...64.9 um.....

[3]

(c) **J1** is a slide of a stained transverse section through a part of a plant.

Draw a large plan diagram of the region of the root on slide **J1** indicated by the shaded area in Fig. 3.3. Use a sharp pencil.

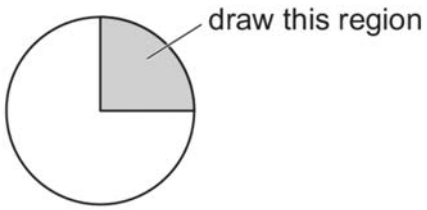
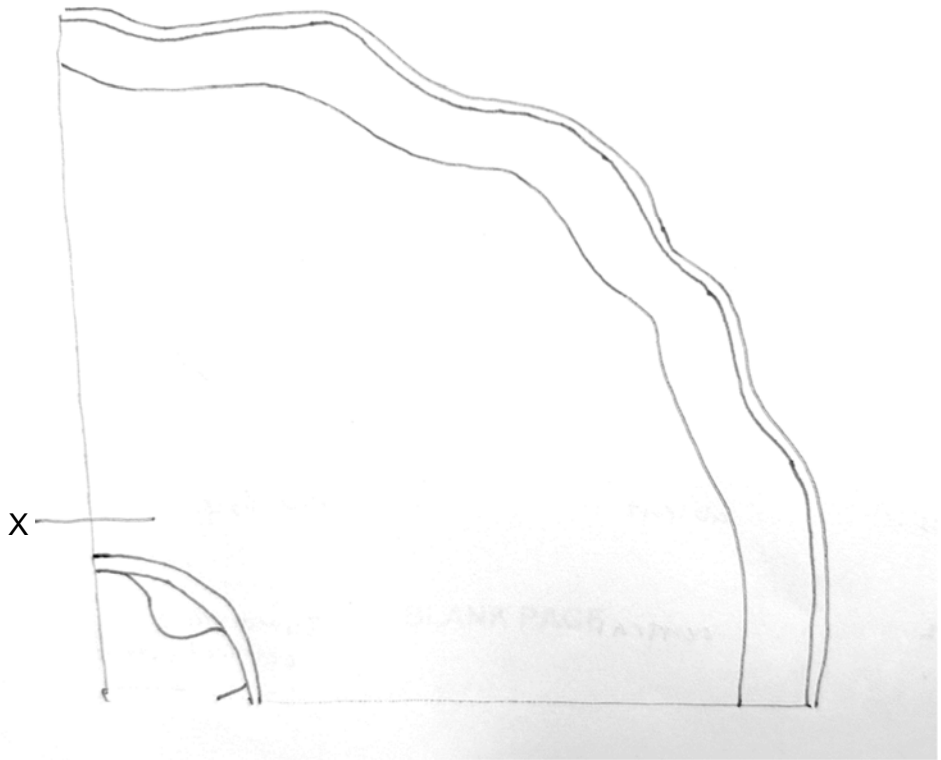


Fig. 3.3

Use **one** ruled label line and label, with the letter **X**, the tissue that contains most of the starch grains.



1. **S**cale: at least 2/3 of the space provided

2. **P**roportion & **L**ines: thickness of each layer correctly represented AND smooth thin lines
3. **O**bservation: draws at least 4 layers - epidermis, cortex, vascular tissue, xylem; xylem must be cross/star shaped (R: xylem shows 2 layers; I: shoots and air spaces)
4. **P**lan diagram (no cells, no shading) + correct quadrant
5. Label **X** correctly (only cortex. R: phloem)

[5]

Fig. 3.4 is a photomicrograph of a transverse section through a stem of a different type of plant.

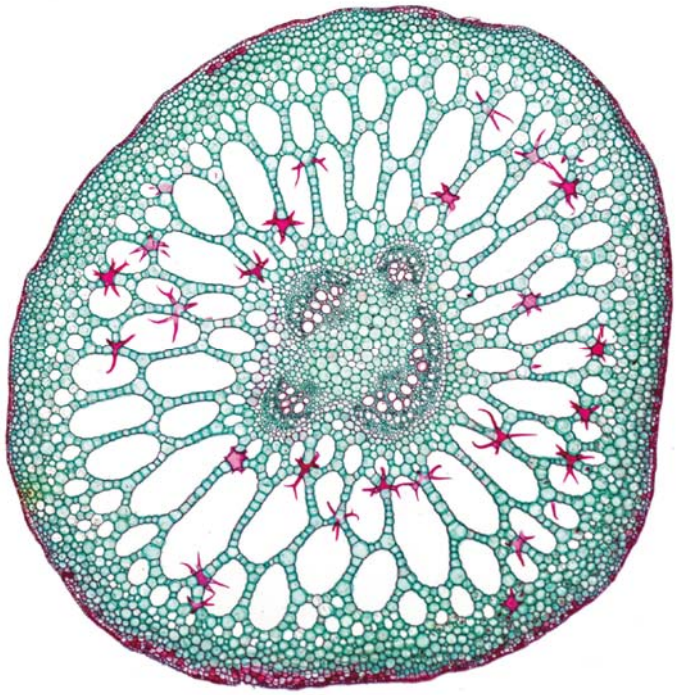


Fig. 3.4

Identify **two** observable differences, other than size and colour, between the specimen on slide **J1** and the plant part in Fig. 3.4.

1. Table with ruled lines

*any **two** from:*

Pt.	feature	slide J1	Fig. 2.2
2.	Arrangement of vascular tissue A: shape of xylem tissue	Radiating from centre OR Cross/star shape A: one central region A: cross/star shape	4 vascular bundles arranged in a ring A: Four separate regions A: semi-circular A: disc/lens shaped
3a.	presence of central pith	no central pith	presence of central pith
3b.	tissue at the centre	vascular tissue	non-vascular tissue/undifferentiated cells
4.	Star-shaped structures A: sclereids / AW	absent	present

	A: spikey/spiney R: hair-like structures		
5.	air spaces diff J1 samples may have what appears to look like air spaces (likely to be broken tissue)	absent A: fewer/irregular shape	present A: more/regular shape
6.	AVP	described	described

[3]

Suggest a habitat where the plant in Fig. 3.4 might grow and explain using one observable feature shown in Fig. 3.4, which enables it to live in this habitat.

Habitat

.....

explanation

.....

.....[2]

Aquatic;
air spaces + idea of buoyancy (R: gaseous exchange);

[Total: 18]

End of Paper