AS Biology Practical
Assessment Booklet
INDEX

1. FOOD TESTS

Carry out practical work to detect the presence of carbohydrate and proteins using biochemical tests:

- iodine test;
- Benedict’s test;
- glucose specific tests;
- Biuret test

2. CHROMATOGRAPHY

Carry out practical work to identify amino acids using paper chromatography:

- prepare, run and develop the chromatogram;
- calculate Rf values.

3. ENZYME ACTIVITY

Carry out practical work:

- investigating factors, such as temperature, pH, substrate concentration and enzyme concentration that affect enzyme activity;
- illustrating enzyme immobilisation;
- using a colorimeter to follow the course of a starch–amylase catalysed reaction (or other appropriate reaction).

4. Homogenisation and Centrifugation

Carry out practical work to demonstrate knowledge and understanding of the use of homogenisation and centrifugation;

5. MICROSCOPES

Carry out practical work:

- examining photomicrographs and electron micrographs;
- recognising cell structures from photomicrographs and electron micrographs;
- drawing individual cells or cell sections;
- staining tissues to aid observation when using a microscope (for example using iodine or methylene blue);
- calculating true size (in μm) and magnification, including using scale bars; and
- using a graticule and stage micrometer to measure cell length.
6. Measuring Water and Solute Potential

Carry out practical work to include:

- measuring the average water potential of cells in a plant tissue: – using a weighing method for a potato or other suitable tissue; – calculating the percentage change in mass; and – determining the average water potential from a graph of percentage change in mass against solute potential of immersing solution;

- measuring the average solute potential of cells at incipient plasmolysis by: – using onion epidermis or other suitable tissue; – calculating percentage plasmolysis; and – determining the average solute potential from a graph of percentage plasmolysis against solute potential of the immersing solution (at 50% plasmolysis the average pressure potential is zero);

7. OBSERVING MITOSIS

Carry out practical work:

- preparing and staining root tip squashes;

- recognising chromosomes at different stages of cell division;

- identifying the stages of mitosis; and

- examining prepared slides or photographs of the processes of mitosis and meiosis and identifying the structures visible and the different stages;

8. Ileum and Mesophyll Leaf

Carry out practical work to include:

- examining stained sections of the ileum using the light microscope and electron micrographs or photomicrographs to identify the villi (and associated blood capillaries and lacteals), crypts of Lieberkühn (and Paneth cells), mucosa, columnar epithelium, goblet cells, muscularis mucosa, submucosa, muscularis externa and serosa;

- examining sections of a mesophytic leaf using the light microscope or photographs to identify the epidermal layers, waxy cuticles, palisade mesophyll, chloroplasts, spongy mesophyll, vascular tissue (xylem and phloem), and guard cells and stomata; and

- making accurate drawings of sections of the ileum and the leaf to show the tissue layers and drawing block diagrams of tissues within the ileum and the leaf.

9. RESPIROMETER

Carry out practical work to include understanding how to use a simple respirometer to:

- measure O2 consumption (with KOH present); and

- measure the net difference between CO2 production and O2 consumption (with no KOH present) and so determine CO2 production.
10. BLOOD VESSEL SLIDES AND HEART

Carry out practical work:

• examining prepared slides and/or photographs of blood vessels (in section) to distinguish between arteries, veins and capillaries; and

• dissecting the mammalian heart to identify heart chambers, AV valves, semilunar valves, chordae tendinae, papillary muscles, interventricular septum and major blood vessels: vena cavae, pulmonary artery and aorta;

11. BLOOD CELL IDENTIFICATION

Carry out practical work examining stained blood films using light microscopes and/or photomicrographs to identify red blood cells, polymorphs, monocytes, lymphocytes and platelets;

12. ECOLOGICAL SAMPLING

Carry out practical work to include the qualitative and quantitative techniques used to investigate the distribution and relative abundance of plants and animals in a habitat:

• sampling procedures to include: – random sampling; – line transect; and – belt transect;

• sampling devices, including quadrats, pin frames, pitfall traps, sweep nets and pooters;

• estimating species abundance, density, frequency and percentage cover; and

• appreciating and, where possible, measuring the biotic and abiotic factors that may be influencing the distribution of organisms.
1. FOOD TESTS

Carry out practical work to detect the presence of carbohydrate and proteins using biochemical tests:
• iodine test;
• Benedict’s test;
• glucose specific tests;
• Biuret test

1. Starch (iodine test). Add a few drops of iodine/potassium iodide solution to the sample. A blue-black colour indicates the presence of starch as a starch-polyiodide complex is formed.

2. Reducing Sugars (Benedict’s test). All monosaccharides and most disaccharides (except sucrose) are called reducing sugars because they will reduce ions like Cu2+. Add a few mL of Benedict’s reagent (which is a copper (II) sulphate solution) to the sample. Shake, and heat for a few minutes at 95°C in a water bath. A coloured precipitate of copper (I) oxide indicates reducing sugar. The colour and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue colour means no reducing sugar, a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present.

Non-reducing Sugars (Benedict’s test). Sucrose is called a non-reducing sugar because it does not reduce copper sulphate, so there is no direct test for sucrose. However, if it is first hydrolysed to its constituent monosaccharides (glucose and fructose), it will then give a positive Benedict’s test. So sucrose is the only sugar that will give a negative Benedict’s test before hydrolysis and a positive test afterwards. First test a sample for reducing sugars, to see if there are any present before hydrolysis. Then, using a separate sample, boil the test solution with dilute hydrochloric acid for a few minutes to hydrolyse the glycosidic bond. Neutralise the solution by gently adding small amounts of solid sodium hydrogen carbonate until it stops fizzing, then test as before for reducing sugars.

3. Clinistix - used to detect glucose

Clinistix strips are impregnated with chemicals that change colour in the presence of glucose. Clinistix is specific (unlike Benedict's test). Strips turn purple/blue if glucose is present. Can be a way for doctors to detect the presence of glucose in the urine (symptom of diabetes)

4. Protein (biuret test).

Add a few mL of biuret solution to the sample. Shake, and the solution turns lilac-purple, indicating protein. The colour is due to a complex between nitrogen atoms in the peptide chain and Cu^{2+} ions, so this is really a test for peptide bonds.

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2. CHROMATOGRAPHY

Carry out practical work to identify amino acids using paper chromatography:

- prepare, run and develop the chromatogram;
- calculate Rf values.

Amino acids have no colour. Therefore all of these procedures need to be carried out "blind", and the results will be seen when a revealing agent (ninhydrin) is sprayed on the resulting chromatogram.

You are provided with a number of solutions of amino acids, and solution X (a mixture of 2 amino acids). Do not use the same pipette for more than one liquid.

Chromatography paper must not be touched with the hands (at the bottom, at least). Use plastic/rubber gloves, and work on a clean surface (e.g. inside page of pad of paper).

Cut a suitable length of chromatography paper (slightly longer than the glass chamber) and mark it with a pencil line about 1.5 cm from the bottom. Again using a pencil, put 3 marks on the line forming crosses, the outer ones labelled with (3 letter codes for) the amino acids you are going to use, and X in the middle. Put your name at the top.

Using 3 different pipettes, place a drop of each amino acid, and the mixture X, at the appropriate positions on the line. If you have time, gently warm the paper and repeat the spotting process (on the same positions) to raise the concentration of the amino acids, but ensure that the paper is completely dry before proceeding.

Partially fold the paper in half lengthways, to remove its tendency to curl up.

In a fume cupboard or a secluded (well ventilated?) area of the lab:

Using a funnel, pour a small amount of chromatography solvent (butanol/ethanoic acid) into the glass chamber (to about 1 cm depth). Place on the lid to allow the atmosphere to become saturated with vapour. Leave the chamber on the bench in its final position, so that it does not splash up; bring the paper to meet it.

Line up the paper with the (outside of the) chamber, and either fold over the top or cut it so that it will fit. The solvent should touch the lower part of the paper but not cover the drops on the line.

Attach the paper to the lid, and then place the lid on. The paper should not touch the sides of the chamber.

The solvent should gradually rise up the paper, passing the line and heading upwards.

After about 3 hours, the liquid should have risen about three-quarters of the height of the paper. If the solvent nears the top of the paper, proceed immediately to the next stage.

Remove the paper from the apparatus, and use a pencil to mark the position of the solvent front. Up to 5 pieces of chromatography paper can be placed across a clean A4 sheet of paper, stapled at the top of the chromatograms.

Place the chromatograms into an oven at about 45 C to dry.

Either in a fume cupboard or outside on a still day, spray ninhydrin evenly over the paper.

Care: ninhydrin is dissolved in an inflammable solvent.

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Return the chromatograms to the oven to develop the colour. Spots should be visible as purplish smears on the paper. The image of the spots may be enhanced if the sheet is photocopied on a darker than normal setting.

**Calculation of Rf values**

Measure the distance from the start line to the solvent front and to the front of each spot.

For each spot, calculate the Rf value (Rf means *relative to front*):

\[
\text{Rf} = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}}
\]

Compare the values you obtain with reference Rf values. Different solvents and different types or makes of chromatography papers will give slightly different results.

One or both of the spots from solution X may be at the same level as another (known) amino acid alongside it. This should assist in identification.
3. ENZYME ACTIVITY

Carry out practical work:

- investigating factors, such as temperature, pH, substrate concentration and enzyme concentration that affect enzyme activity;
- illustrating enzyme immobilisation;
- using a colorimeter to follow the course of a starch–amylase catalysed reaction (or other appropriate reaction).

USING A COLORIMTER: Investigate how enzyme concentration affects the initial rate of an enzyme-controlled reaction

PROCEDURE

Milk protein (casein) is broken down by protease enzymes such as trypsin. The opaque white colour of the milk is replaced by a clear solution. Light passes more easily through the final solution and so the reaction can be monitored using a colorimeter (see diagram) or light sensor.

1. Plan how you will dilute the 1% trypsin stock solution with distilled water to produce additional test solutions of 0.2%, 0.4%, 0.6% and 0.8%. Aim to produce 10 cm³ of each concentration. Once checked, make up the solutions as planned.

2. Place 2 cm³ of trypsin solution and 2 cm³ of distilled water into a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.

3. Measure 2 cm³ of milk suspension into a second cuvette.

4. Add 2 cm³ of trypsin solution to the milk in the cuvette. Working quickly, mix and place the solution into the colorimeter and start the stop clock.

5. Measure absorbance immediately and then at 15 second intervals (or more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.

6. Rinse the cuvette with distilled water and repeat for each concentration.

![Diagram]

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ANALYSIS OF RESULTS

1. Record your results in a suitable table.

2. Plot a graph of absorbance against time. It should be possible to plot each concentration as a different line on the same axes.

3. Use the graph to determine the initial rate of reaction for each concentration. Do this by drawing a tangent to the initial part of each curve and calculating the gradient of each line.

4. Draw a second graph to show the initial rate of reaction against the concentration of the enzyme.

5. Write a short conclusion to describe and explain the result of this investigation.

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</table>
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Better milk for cats: immobilised lactase used to make lactose-reduced milk

Method

1. Mix the enzyme with the sodium alginate solution, then draw it up into a 10 ml syringe.
2. Add the alginate-enzyme mixture a drop at a time from the syringe to the calcium chloride solution and observe the formation of small beads. Do not allow the tip of the syringe to come into contact with the calcium chloride solution, as this will cause the alginate to harden, blocking the outlet. The beads, which contain the enzyme immobilised in a matrix of calcium alginate, should be allowed to harden for a few minutes.
3. Attach a short length of tubing to the tip of a syringe barrel. Place a small disc of nylon gauze inside the barrel, to prevent the beads from blocking the syringe outlet.
4. Separate the beads of immobilised enzyme from the liquid with the tea strainer.
5. Carefully tip the beads into the syringe barrel.
6. Close the tubing on the syringe barrel using a tubing clip.
7. Test the milk before treatment using the glucose test strips, to ensure that it does not contain any glucose.
8. Pour a small volume of milk over the enzyme beads, then undo the clip and allow the treated milk to run into a small beaker.
9. Test the milk leaving the column using the glucose test strips. If necessary, return the treated milk to the column until the desired concentration of glucose is achieved.
4. Homogenisation and Centrifugation

*Carry out practical work to demonstrate knowledge and understanding of the use of homogenisation and centrifugation;*

**Homogenization:**
The suspended cells are then disrupted by the process of homogenization.

It is usually done by:
(i) Grinding
(ii) High Pressure (French Press or Nitrogen Bomb),
(iii) Osmotic shock,
(iv) Sonication (ultrasonic vibrations).

Grinding is done by pestle and mortar or potter homogenizer (a high-speed blender). The latter consists of two cylinders separated by a narrow gap.

The shearing force produced by the movement of cylinders causes the rupture of cells. Ultrasonic waves are produced by piezoelectric crystal. They are transmitted to a steel rod placed in the suspension containing cells. Ultrasonic waves produce vibrations which rupture the cells. The liquid containing suspension of cell organelles and ether constituents is called homogenate. Sugar or sucrose solution preserves the cell organelles and prevents their clumping.

**BREAKING CELLS AND TISSUES**

The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called **homogenization**, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

1. **Break cells with high-frequency sound.**
2. **Use a mild detergent to make holes in the plasma membrane.**
3. **Force cells through a small hole using high pressure.**
4. **Shear cells between a close-fitting rotating plunger and the thick walls of a glass vessel.**

The resulting thick soup (called a homogenate or extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all of the membrane-enclosed organelles.

When carefully conducted, homogenization leaves most of the membrane-enclosed organelles intact.
Centrifugation:
The separation (fractionation) of various components of the homogenate is carried out by a series of centrifugations in an instrument called ultracentrifuge. The ultracentrifuge has a metal rotor containing cylindrical holes to accommodate centrifuge tubes and a motor that spin the rotor at high speed to generate centrifugal forces. Theodor Svedberg (1926) first developed the ultracentrifuge which he used to estimate the molecular weight of hemoglobin. Present day ultracentrifuge rotate at speeds up to 80,000 rpm (rpm= rotations per minute) and generates a gravitational pull of about 500,000 g, so that even small molecules like t-RNA, enzymes can sediment and separate from other components. The chamber of ultracentrifuge is kept in a high vacuum to reduce friction, prevent heating and maintain the sample at 0-4°C. During centrifugation, the rate at which each component settle down depends on its size and shape.
5. MICROSCOPES

Carry out practical work:
• examining photomicrographs and electron micrographs;
• recognising cell structures from photomicrographs and electron micrographs;
• drawing individual cells or cell sections;
• staining tissues to aid observation when using a microscope (for example using iodine or methylene blue);
• calculating true size (in μm) and magnification, including using scale bars; and
• using a graticule and stage micrometer to measure cell length.

Observing Onion Cells

Method:
1) Collect all your apparatus (carrying the microscope carefully).
2) Using tweezers carefully peel off a thin layer of epidermis from the onion.
3) Lay the membrane on the microscope slide in a single flat layer.
4) Place a very small drop of iodine on to the membrane.
5) Carefully lower a cover slip on top of the membrane (make sure there are no air bubbles).
6) Place the slide on the stage of the microscope.
7) Make sure the lowest objective lens is over the specimen.
8) Carefully use the course focusing knob to lower the objective lens to just above the slide.
9) Look through the eye piece and carefully use the fine focusing knob to focus the image.
Observing Cheek Cells

Method:

1. Gently scrape the inner side of the cheek using a toothpick, which will collect some cheek cells.
2. Place the cells on a glass slide that has water on it.
3. Mix the water and the cheek cells using a needle and spread them.
4. Take a few drops of Methylene blue solution using a dropper and add this to the mixture on the slide.
5. After 2-3 minutes remove any excess water and stain from the slide using a blotting paper.
6. Take a few drops of glycerine using a dropper and add this to the test mixture.
7. Take a clean cover slip and lower it carefully on the mixture with the aid of a needle.
8. Using a brush and needle, press the cover slip gently to spread the epithelial cells.
9. Remove any extra liquid around the cover slip using a blotting paper.
10. Place this glass side on the stage of the compound microscope and view it.
Magnification and Scale

Cells are extremely small but knowing the sizes of objects viewed under the microscope can be really useful. For example, a plant scientist might want to compare the relative sizes of pollen grains from plants in the same genus to identify to help identify different species.

With a compound microscope, the magnification is the product of both lenses, so if microscope has a 10x eyepiece and an 40x objective, the total magnification is 400x.

Magnification is defined as the ratio of the size of the image to the size of the object.

\[
\text{Magnification} = \frac{\text{Size of Image}}{\text{Actual size of object}}
\]

The relationship between these three values can be shown using the equation triangle to the right, which offers a quick way of rearranging the values in order to derive related formulas. Ask your teacher to demonstrate how to use it if you are not familiar with it.

Printed images of structures seen with a microscope usually show a scale bar or give the magnification or both, so that the size of an object can be calculated. For example, the magnification of the micrograph shown below is given as 230x.

![Light micrograph of a section through the cortex of a kidney (230x)](image)

In the micrograph above, you can see a spherical structure called a glomerulus. In the image, the glomerulus is approximately 35 mm across. You can check this using a ruler. Thus:

\[
\text{Actual size of glomerulus} = \frac{\text{Size of Image}}{\text{Magnification}}
\]

\[
= \frac{35 \text{ mm}}{230x}
\]

\[
= 0.15 \text{ mm}
\]

In most micrographs, most measurements are expressed in micrometers. A micrometer (µm) is 10⁻⁶ mm, so 1 mm is 1000 µm. So the diameter of the glomerulus = 0.15 x 1000 = 150 µm.

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Magnification and Scale

Because cells are small, they are viewed through lenses and microscopes. Photographs and diagrams often have scale bars to show the degree of magnification of the image. This image shows a red blood cell. The scale bar shows 2 μm, which represents the actual size of the bar. From this, you can calculate both the size of the cell and the magnification of the image.

Magnification of the image

Use a ruler to measure the length of the scale bar in the upper-right corner of the micrograph. This is 9 mm. Convert this number to micrometers. This is equal to 9,000 μm. Thus:

\[
\text{Magnification} = \frac{\text{Size of image (scale bar)}}{\text{Actual size of object (scale bar)}} = \frac{9,000 \, \mu\text{m}}{2 \, \mu\text{m}} = 4,500x
\]

Size of the cell

Use a ruler to measure the diameter of the red blood cell in the center of the above micrograph. This is 30 mm. Convert this number to micrometers. This is equal to 30,000 μm. We already know the magnification is 4,500x.

Rearrange the magnification equation to find the actual length of the red blood cell.

\[
\text{Actual size of object (cell)} = \frac{\text{Size of image (cell)}}{\text{Magnification}} = \frac{30,000 \, \mu\text{m}}{4500x} = 6.7 \, \mu\text{m}
\]

Drawing a scale bar

Assume you made a micrograph of a deer tick. You observed the tick under a stereoscope at 20x magnification and know the tick’s actual length is 2.0 mm. You want to draw a scale bar representing an actual distance of 1 mm. How long should the drawing of the scale bar be?

Use the formula:

\[
\text{Size of image (scale bar)} = \text{Magnification} \times \text{Actual size of object (scale bar)} = 20x \times 1 \, \text{mm} = 20 \, \text{mm in length}
\]
**Eyepiece graticule**

The eyepiece graticule is a glass disc fitted into the eyepiece of the microscope. These can be fitted to existing eyepieces or eyepieces can be purchased with graticules already fitted. The disc is marked with a fine scale from 0 to 100. The absolute size of the scale is not important as this is what will be calibrated.

![Eyepiece graticule scale](image)

**Stage micrometer**

The stage micrometer is used to calibrate the eyepiece graticule. A stage micrometer consists of a microscope slide on which is engraved a fine and accurate scale. Because the scale has to be accurately produced to give reference dimensions, stage micrometers are much more expensive than eyepiece graticules.

**Method 1**

When carrying out calibration, each objective lens has to be separately calibrated. This will result in separate calibration factors for each objective.

Start with the lowest power objective on the microscope. The scale on the stage micrometer is aligned with the scale of the eyepiece graticule and then a reading is taken from the scales. These readings are then used to calculate the calibration factor for the objective lens in use. The following example shows how to calibrate the graticule for the x40 objective lens:

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Reading from the two scales we find 100 divisions on the eyepiece graticule equals 25.9 divisions on the stage micrometer.

For the particular stage micrometer we are using 100 divisions = 1 mm (this information is marked on the stage micrometer).

- Each division is 1/100 mm = 10 µm
- 100 eyepiece div = 25.9*10 µm
- 1 eyepiece div = 259/100 µm = 2.59 µm

Repeat the calibration for the remaining objectives.

Using the calibrated graticule

Looking at a pollen grain using the x40 objective lens, we measure the diameter of the grain using the eyepiece graticule. As an example, the grain measures 12 divisions on the graticule. As each division corresponds to 2.59 µm, the pollen grain is 12*2.59 = 31 µm in diameter.

Method 2

Calibrating the eyepiece graticule Calibrating the eyepiece graticule is done using a stage micrometer, which is a slide with an accurate linear scale engraved on it. This slide is placed on the microscope stage. Looking down the microscope, both scales can be seen at the same time. This allows the size of the divisions on the eyepiece graticule to be measured. One large division on the stage micrometer is 1000 µm (1 mm). Each large division is divided into 10 smaller divisions, each 100 µm (0.1 mm) wide.

1. Place the stage micrometer on the microscope stage.

2. Line up the divisions on the eyepiece graticule with those of the micrometer as shown in Figure 1.

3. Work out the length of one eyepiece graticule unit in micrometres as shown in Figure 1.

4. Repeat for each of the objective lenses on the microscope.
Using the eyepiece graticule to measure the length of objects

1 Place a slide on the microscope stage.

2 Measure the length of the specimen (e.g. the length of one cell) in eyepiece graticule units using the eyepiece graticule scale.

3 Calculate the length of the specimen in m by multiplying the length in eyepiece graticule units by the calibration value for 1 unit (1333 μm in the example on p. 1).
6. Measuring Water and Solute Potential

Carry out practical work to include:

• measuring the average water potential of cells in a plant tissue: – using a weighing method for a potato or other suitable tissue; – calculating the percentage change in mass; and – determining the average water potential from a graph of percentage change in mass against solute potential of immersing solution;

• measuring the average solute potential of cells at incipient plasmolysis by: – using onion epidermis or other suitable tissue; – calculating percentage plasmolysis; and – determining the average solute potential from a graph of percentage plasmolysis against solute potential of the immersing solution (at 50% plasmolysis the average pressure potential is zero);

Measuring the Water Potential

of a Potato Cell

Instructions

Prepare 10cm³ of each of 0.8M, 0.4M, 0.2M, 0.15M and 0.075M sucrose solutions, each in a separate tube. Be careful to mix the water and the more dense strong sugar solutions thoroughly. Add a sixth tube of water to complete the series.

1) Label each solution.

2) Cut up about 10g potato tissue into small cubes about 2mm square. Weigh accurately about 0.75g potato tissue into each empty tube. Blot the tissue before weighing and be sure the balance is carefully zeroed. Record the weight in each tube accurately.

3) When all the weighings are complete, cover the tissue in each tube with the corresponding solution (5cm³ is sufficient) and note the time. ie. cover the tissue in the 0.2M tube with 0.2M sucrose etc. Note how the potato looks when this is complete.

4) Leave the tubes for 15 minutes, swirling occasionally. (Time to make a diagram of the technique so far.)

5) Remove the tissue from each tube, blot it on a paper towel and weigh each amount separately; making sure that the balance is carefully zeroed before each weighing. (Six weighings, one from each tube.)

6) Make a table of Initial weight; Final weight; and Final weight/Initial weight for each concentration.

7) Plot a graph of Final weight/Initial weight against concentration.

8) Using your graph, predict the concentration of sucrose which is in equilibrium with the potato tissue and hence calculate the Water Potential of the tissue in MPa by consulting
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<th>0.2</th>
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\[
\text{percentage change in mass} = \frac{(\text{end mass} - \text{start mass})}{\text{start mass}} \times 100
\]
Observing osmosis, plasmoylsis and turgor in plant cells

EXPERIMENT 1

a Get a single layer of plant cells. If you are using red onion, cut a 1 cm square from a fleshy piece of onion and then peel off a single layer of the red cells. If you are using rhubarb, peel a piece from the epidermis. If you are using toadflax peel a piece of the lower epidermis of a leaf.

b Place the strip on a slide and cover it with a drop or two of distilled water. Add a cover slip.

c Look at the cells through a microscope. Start with the low power lens. Draw and label 3 plant cells.

d Take another strip of cells from your plant material. This time mount the cells on a slide with 5% sodium chloride solution.

e Examine the cells through the microscope. Draw and label 3 plant cells.

f After a few minutes draw out the sodium chloride solution with a piece of filter paper placed at the edge of the coverslip. Replace it with distilled water added at the other side of the coverslip.

EXPERIMENT 2

To find the $\psi_s$ of a tissue by the method of limiting plasmolysis.

Thin sections of a suitable tissue are placed in different sucrose solutions. The solution that causes the cells to reach incipient plasmolysis can be regarded as having the same solute potential as the cell sap. Since cells plasmolyse at different rates,
incipient plasmolysis is regarded as the condition in which half the cells are visibly plasmolysed.

**Method**

1. Cut six 5 mm by 2 mm pieces of onion epidermis and place in distilled water in a petri dish to make sure the cells do not plasmolyse.
2. Label 5 petri dishes 1-5
3. Measure out 10 cm$^3$ of each of the following sucrose solutions and place in the petri dishes:

   - Tube 1 - 0.3 M
   - Tube 2 - 0.35 M
   - Tube 3 - 0.4 M
   - Tube 4 - 0.5 M
   - Tube 5 - 1.0 M

4. Add one piece of onion to each petri dish, shake gently and leave for 30 min.  
   *(Meanwhile, do experiment 1)*
5. Remove each piece of onion and mount it on a microscope slide in a drop of the solution in which it has been.  Observe under L.P.
6. Count all the cells in the field of view and count all the cells that have plasmolyed.  
   Repeat for a total of three fields of view.  Now work out a percentage of plasmolyed cells for this solution.
7. Plot a graph of percentage plasmolysis against molarity of sucrose solution.
8. From the graph, read off the molarity of sucrose that would cause 50% plasmolysis.
9. Consult the table below of solute potentials of sucrose solutions for different molarities.  The solute potential of the sucrose solution giving 50% plasmolysis will be equivalent to the solute potential $\psi_s$ of the cell sap of the onion.
Table of solute potentials of sucrose solutions (at $20^\circ$C)

<table>
<thead>
<tr>
<th>Concentration of sucrose solution (molarity)</th>
<th>Solute potential Kpa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>-130</td>
</tr>
<tr>
<td>0.10</td>
<td>-260</td>
</tr>
<tr>
<td>0.15</td>
<td>-410</td>
</tr>
<tr>
<td>0.20</td>
<td>-540</td>
</tr>
<tr>
<td>0.25</td>
<td>-680</td>
</tr>
<tr>
<td>0.30</td>
<td>-820</td>
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<tr>
<td>0.35</td>
<td>-970</td>
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<tr>
<td>0.40</td>
<td>-1120</td>
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<tr>
<td>0.45</td>
<td>-1280</td>
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<tr>
<td>0.50</td>
<td>-1450</td>
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<tr>
<td>0.55</td>
<td>-1620</td>
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<td>0.60</td>
<td>-1800</td>
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<tr>
<td>0.65</td>
<td>-1980</td>
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<tr>
<td>0.70</td>
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<tr>
<td>0.75</td>
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<tr>
<td>0.80</td>
<td>-2580</td>
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<tr>
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<td>-2790</td>
</tr>
<tr>
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<td>-3010</td>
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<tr>
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<tr>
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<td>-3510</td>
</tr>
<tr>
<td>1.50</td>
<td>-6670</td>
</tr>
<tr>
<td>2.00</td>
<td>-11810</td>
</tr>
</tbody>
</table>
7. OBSERVING MITOSIS

• preparing and staining root tip squashes;
• recognising chromosomes at different stages of cell division;
• identifying the stages of mitosis; and
• examining prepared slides or photographs of the processes of mitosis and meiosis and identifying the structures visible and the different stages;

Investigating mitosis in allium root tip squash

Method

a Cut off 1-2 cm of the root tips. Put in a small volume of ethanoic acid on a watchglass (or other shallow dish) for 10 minutes.
b Meanwhile, heat 10-25 cm³ of 1 M hydrochloric acid to 60 °C in a water bath.
c Wash the root tips in cold water for 4-5 minutes and dry on filter paper.
d Use a mounted needle to transfer the root tips to the hot hydrochloric acid (see b) and leave for 5 minutes.
e Wash the root tips again in cold water for 4-5 minutes and dry on filter paper.
f Use the mounted needle to remove two root tips onto a clean microscope slide.
g Cut each about 2 mm from the growing root tip. Discard the rest, but keep the tips (Note 2).
h Add a small drop of stain and leave for 2 minutes (Note 3 and Note 5).
i Break up the tissue with a mounted needle.
j Cover with a coverslip and squash (Note 6) using method A or method B below.

Method A Place the slide and coverslip on a double layer of paper towel and fold the paper over the coverslip. Make certain that the slide is on a flat surface and squash down on the coverslip with a strong vertical pressure, using your thumb. Do not twist or roll the thumb from side to side

Method B Tap the coverslip about 20 times by dropping a wooden mounted needle or a pencil, blunt end down, from a height of about 5 cm onto the middle of the coverslip.

k View the root tips under a microscope (x400 magnification) and look for the chromosomes within cells which are actively dividing.

l Locate the meristematic zone, which has small, apparently ‘square’ cells with nuclei which are large relative to the whole cell area.
m If cells are overlapping, squash the slide again. Avoid moving the coverslip from side to side.

n Make sketches of (or take photographs of) cells that show any of the stages of mitosis.
o If available, compare the allium meristem tissue with that from other species.

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8. Ileum and Mesophyll Leaf

Carry out practical work to include:

- examining stained sections of the ileum using the light microscope and electron micrographs or photomicrographs to identify the villi (and associated blood capillaries and lacteals), crypts of Lieberkühn (and Paneth cells), mucosa, columnar epithelium, goblet cells, muscularis mucosa, submucosa, muscularis externa and serosa;

- examining sections of a mesophytic leaf using the light microscope or photographs to identify the epidermal layers, waxy cuticles, palisade mesophyll, chloroplasts, spongy mesophyll, vascular tissue (xylem and phloem), and guard cells and stomata; and

- making accurate drawings of sections of the ileum and the leaf to show the tissue layers and drawing block diagrams of tissues within the ileum and the leaf.
9. RESPIROMETER

Carry out practical work to include understanding how to use a simple respirometer to:

- measure O2 consumption (with KOH present); and
- measure the net difference between CO2 production and O2 consumption (with no KOH present) and so determine CO2 production.

Measuring the Rate of Metabolism

a Use a funnel to pour 5 cm³ of potassium hydroxide solution (Note 3) into each respirometer vessel. Make sure none of the potassium hydroxide touches the sides of the vessels.
b Add small rolls of filter paper to act as wicks.
c Fill the basket or cage with respiring material and put it into a vessel B. Make sure that the seeds or invertebrates are not touching the potassium hydroxide or the wick. Add water to vessel A to match the volume of respiring material in vessel B (see diagram).
d Fit vessel A with a bung holding two connecting tubes – one with a screwclip on flexible tubing. Alternatively, fit a bung with a 3-way tap connected to the same items.
e Fit vessel B with a bung holding a 1 cm³ syringe and a connecting tube as shown in the diagram. Alternatively, fit a bung with a 3-way tap connected to the syringe and tube.
f Draw a suitable manometric fluid (Note 3) into the manometer U-tube. The fluid must be free of bubbles and come to about the middle of the scale on each side.
g Open the screw clip and remove the syringe, then connect the manometer U-tube. Check the apparatus is airtight (Note 1).
h If using a water bath, put the apparatus in the water now and leave to equilibrate for 5 minutes.
i Set the piston of the syringe at about the 0.5 cm³ mark and insert the syringe as shown. Close the screw clip. Use the syringe to adjust the manometer so that the fluid levels are the same on both sides.
j Record the exact position of the syringe piston, the position of the menisci on both sides of the manometer, and the time.
k Record new positions of the manometer fluid at four-minute intervals. When it nears the end of the scale on one side, restore it to its original position and note the new position of the syringe piston.
l Plot a graph of meniscus level against time.
A. Potassium hydroxide solution plus water to equal the volume of the seeds in the other tube.

B. Capillary U-tube containing coloured oil.

B. Plastic or metal cage containing seeds.

B. Filter paper rolled to form a wick.

Potassium hydroxide solution.
10. BLOOD VESSEL SLIDES AND HEART

Carry out practical work:

• examining prepared slides and/or photographs of blood vessels (in section) to distinguish between arteries, veins and capillaries; and

• dissecting the mammalian heart to identify heart chambers, AV valves, semilunar valves, chordae tendineae, papillary muscles, interventricular septum and major blood vessels: vena cavae, pulmonary artery and aorta;

* The capillary is a significantly smaller structure and thus is shown at a substantially higher magnification than the artery and vein.
11. BLOOD CELL IDENTIFICATION

Carry out practical work examining stained blood films using light microscopes and/or photomicrographs to identify red blood cells, polymorphs, monocytes, lymphocytes and platelets.

- Red Blood
- Polymorph
- Monocyte
- Lymphocyte
- Platelets
12. ECOLOGICAL SAMPLING

Carry out practical work to include the qualitative and quantitative techniques used to investigate the distribution and relative abundance of plants and animals in a habitat:

• sampling procedures to include: – random sampling; – line transect; and – belt transect;
• sampling devices, including quadrats, pin frames, pitfall traps, sweep nets and pooters;
• estimating species abundance, density, frequency and percentage cover; and
• appreciating and, where possible, measuring the biotic and abiotic factors that may be influencing the distribution of organisms.

Biotic sampling technique A: Quadrat

1 To find the distribution of plants in an area, use quadrats to take random samples across the area. Random sampling avoids bias. One way to do this is to use two long measuring tapes to create ‘X’ and ‘Y’ axes in the area. Then use a table of random numbers as coordinates to select positions in the grid to place each quadrat, as shown in the diagram. Place enough quadrats to be a representative sample.

2 Identify (and note) all the plant species in each quadrat and estimate each species’ percentage cover.

3 For each species, show how many quadrats it appears in (frequency), and calculate its average percentage cover. To calculate average percentage cover for each species: • add together all the percentage cover values for one species • divide by the number of quadrats.

4 Present a summary of the data as a bar chart or table with the species in alphabetical order.

5 Compare your data with another survey. Compare the list of species, the number of species, the frequency of occurrence of each plant species, and the percentage cover of each species.
**Biotic sampling technique B: Point quadrat frame**

1 To find out the distribution of plants in an area, choose a line through your environment at random and start with the point frame at one end. For each species, record how many pins are touching an example of the species. If a plant is touching any single pin in two places, count that as 1 pin touched.

2 Add up the numbers of pins touched for each species of plant and record the data.

3 Move the point frame along the line, taking samples at one metre intervals.

4 Compare samples to see how the frequency of each species changes along the line. Look to see if each species occurs all along the line, or if some occur in clusters.

5 Present a summary of the results by listing all the species in alphabetical order. Show in a table the frequency of each species, by showing the total number of pins touched. In addition show the distribution of species along the sampling line.

6 Compare your data with another survey. Compare the number of species you find, the range of species you find and the frequency of occurrence of each plant species. Also compare how the species are distributed along the sampling line.
**Biotic sampling technique C: Continuous line transect**

1. To find out the distribution of plants along a transect, place a measuring tape in a straight line along the area to be sampled.

2. Work from one end of the tape to the other. Note the plant species which are touching one edge of the tape. Record the plant species and its distance along the tape (e.g. grass 10 cm-21 cm).

3. Present the results in a chart like the one below, showing the distribution of each species along the transect. Show the species in the chart in the order you found them as you worked along the transect.

4. Compare your results with a later survey, or one from another area, by comparing which species are present, and their pattern of distribution along the line.

(Based on data from [www.countrysideinfo.co.uk/wetland_survey/trans1a.htm](http://www.countrysideinfo.co.uk/wetland_survey/trans1a.htm))
Also appreciate the difference between line and belt transects:

Pitfall Trap

Pooter

Sweep Net