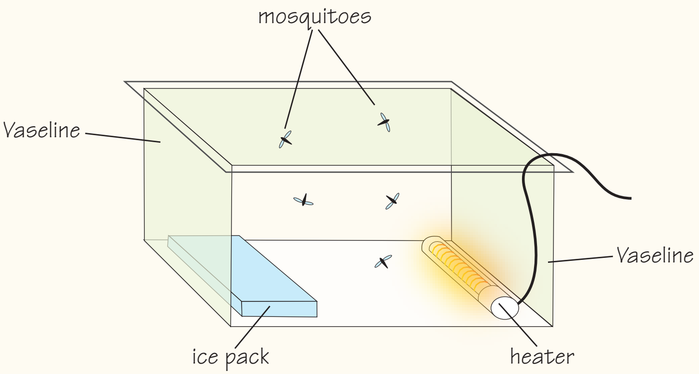
Activity 2A: Experimental design and interpretation

You are unlikely to be familiar with the investigations given in the questions but they give contexts that may be used to give experience with planning, interpreting and reporting. They can be used for practice in identifying variables (dependent, independent, others that need controlling). Diagrams, tables and graphs can be used as examples of 'best practice'. Look for errors in the experimental procedures described, and be able to suggest improvements to ensure that the results are valid and reliable. Practise writing aims, hypotheses, methods, conclusions.

1. A Level 2 Biology student was asked to design an investigation to find out if mosquitoes were attracted to their mammal host by sensing temperature changes. The student's method follows.

> An aquarium was set up as shown in the following diagram. Vaseline was smeared on the end walls to trap the mosquitoes. A heater was placed at one end and an ice pack at the other. Five mosquitoes were put in the aquarium and left for an hour. The investigation was repeated 10 times, and in order to have a good control, it was conducted five times in the light and five times in the dark.



a. Evaluate the method – i.e. is it accurate and detailed enough to allow a valid conclusion to be reached? Could it be repeated by another person?

b. Write a step-by-step method, taking into account any changes and additions that you would make as a result of your evaluation.

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2. Following are three hypotheses.
   a. The length of daylight determines flowering in petunias.
   b. During growth, the increase in the mass of roots in petunias matches the increase in the mass of their stems and shoots.

   c. The smellier the bait used in fishing, the quicker fish will be attracted to the bait.

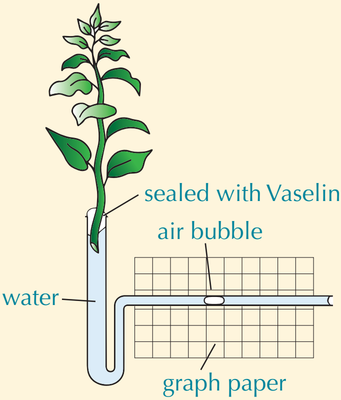
Take *each* hypothesis in turn and design an investigation to test it. It should be possible for a Level 2 student to carry out the investigation in a standard laboratory/horticulture area. Write a step-by-step method for each investigation. There is no need to give any expected results or to write a conclusion.

3. The diagram below shows two different 'methods' (***Method A*** and ***Method B***) by which the rate of transpiration of a plant may be measured.

Method A

Bubble potometer

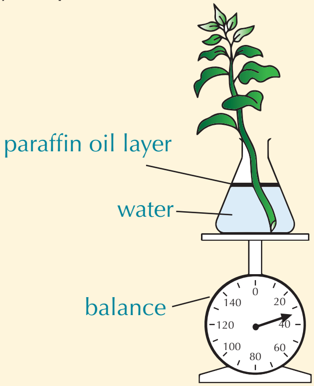
A bubble potometer measures the rate at which a bubble moves along a piece of glass tubing as water is lost by transpiration.



Method B

Weight potometer

The weight is taken at the start of the experiment and again at the conclusion. The difference in weight is a measure of the amount of water lost by the plant.



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a. For each of these 'methods':
   i. identify the dependent variable
   ii. identify any other variables that might need to be controlled, and say how this might be done
   iii. explain why the test tube has been sealed with Vaseline (***Method A***) and the water in the flask covered with oil (***Method B***).

b. A student used ***Method A*** to compare the transpiration rate of a leafy *Coprosma* stem in still air and in windy conditions. The results follow.

Time (10-min intervals)	**Distance moved by bubble** (in mm)	
	Still conditions	**Windy conditions**
0	0	0
10	5	5
20	10	11
30	12	14
40	15	17
50	17	19
60	17	21
70	17	23
80	17	24

   i. Graph the results.
   ii. Write a conclusion.
   iii. Suggest ways in which the experiment might have been improved (e.g., 'Was a valid range for the independent variable used?' 'Did the experiment go on for long enough?').

4. A student wanted to investigate osmosis in potato cells. She obtained three pieces of potato. The first piece (*Sample A*) was placed in distilled water, the second piece (*Sample B*) was boiled then placed in distilled water, the third piece (*Sample C*) was placed in concentrated sugar solution. The graph shows the changes in weight of each of the three samples over 3 hours.

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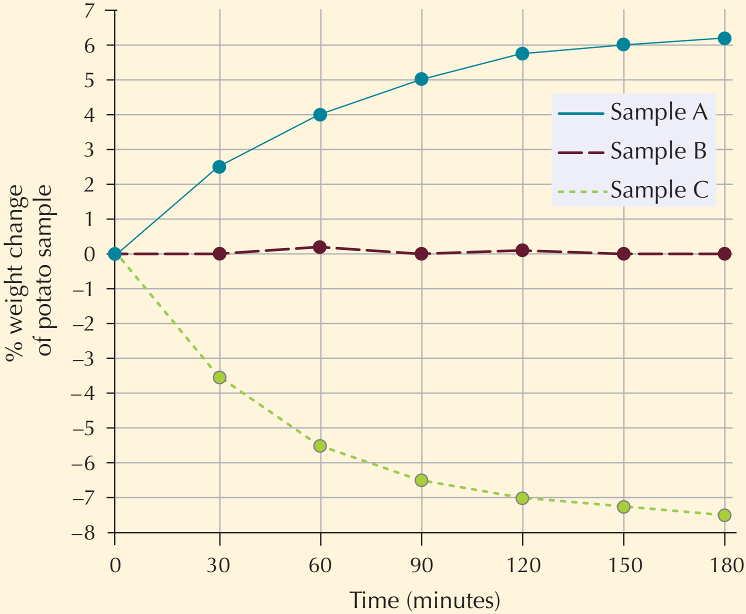
Graph of changes in weight of potato samples in different solutions



a. Design a fair test for this investigation, and then write a step-by-step method for carrying it out.

b. Using your knowledge of the concepts of osmosis (studied in AS91156 (Biology 2.4) – see pages 161–167), write a discussion of the results obtained.

5. A student wanted to investigate the effect of changing pH on the action of the enzyme pepsin (pepsin is a digestive enzyme that acts on protein in food; egg white is a good source of protein). The student used 2 g of egg white in 10 mL of pepsin solution for each pH value, weighing the egg white before and after it had been placed in the pepsin.

The graph shows the changes in the weight of the egg white after it had been exposed to the action of pepsin for 24 hours.

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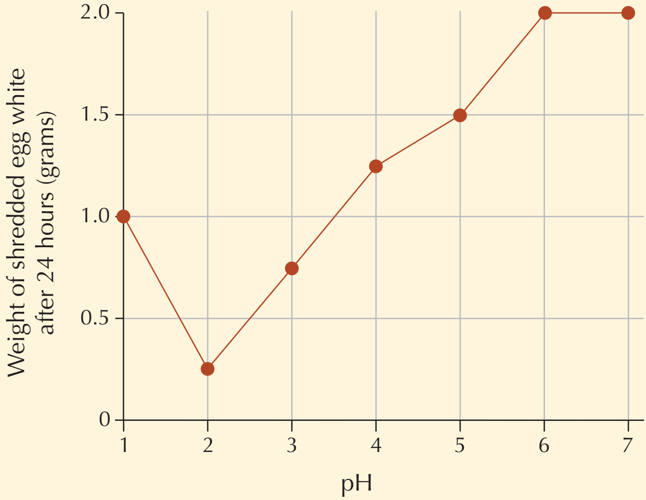
Graph showing the influence of pH of the action of pepsin on egg white at body temperature



a. Design a fair test for this investigation and then write a step-by-step method for carrying it out.

b. Using your knowledge of the concepts of enzyme activity (studied in AS91156 (Biology 2.4) – see pages 173–179), write a discussion of the results obtained.

Activity 2A answers: Experimental design and interpretation

Aspects of certain answers are not definitive (e.g. the methods given are examples of those that might be used to test the hypothesis given). Other methods of testing may be equally suitable; the details of the method given (e.g. quantities/times/trials) may all be changed to suit the circumstances.

1. a. The investigation as given would not be accurate enough to allow a valid conclusion to be made or for the investigation to be repeated by another person.

- The Vaseline will trap any mosquito that lands on it by chance and cannot be taken as an indicator of temperature attraction.
- The heater and ice pack will give temperature differences, but there has been no attempt to measure the temperature in the aquarium or to relate it to the temperature of any mammal host.
- The ice pack will melt and spread water along the floor, which will not only affect the temperature gradient but will also provide a safety hazard with the heater.
- The size/dimensions of the aquarium have not been given, so the set-up cannot be duplicated.
- The time allowed for one trial (one hour) is much too long to be realistic – mosquitoes find their host very quickly.
- The control does not relate to the investigation. If temperature difference is being tested, then the control would be an aquarium with uniform temperature

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throughout to see if the mosquitoes were attracted to any particular end/area – if they were found to prefer one area per se, this would mean the results were biased from the very start.

- To better test the hypothesis, the body temperature of the host needs to be known and this generated (by a heater) in the centre of a suitable container. As the distance from the heater increases, it will represent the temperature gradient from the host. If the mosquitoes fly towards the heater consistently, then they are probably being attracted by temperature.

b. Method: This would need trialling and changing as required.

- Set up a large transparent plastic container 1 m square. Include a large, clear, labelled diagram of the final set-up used.
- In the middle of the container, place a suitable heat source set at the temperature of the host (e.g. 37 °C for humans). The heat source would need to be tested, e.g. heating coil/beaker of hot water/low wattage bulb.
- Cover the heat source (safely) with cloth/fur so that it resembles more the host's surface and will act to control other variables such as light/steam/humidity. It is essential to control all other variables so that only the temperature changes.
- Measure the temperature of the heat source and maintain it at the set temperature throughout the trials. You must state how this is done. Measure the temperature gradient from the heat source to the walls of the container. The temperature gradient is the independent variable.
- Release 10 mosquitoes into the container at a time and after 1 minute record how many have landed on the heat source. The number of mosquitoes landing on the heat source is the dependent variable.
- Repeat 10 times with different mosquitoes each time. This ensures reliable results.

2. In all the investigations, the suggested method would need trialling, then changing as required.

a. Method

- Select 300 petunia seedlings of the same variety. The plants should be the same age/size/stage of growth.
- Grow the seedlings under the same conditions (e.g. same container/soil, same amounts of water/nutrients, exposed to the same light intensity and same temperature).
- Divide the plants into 6 groups of 50 plants each. When the seedlings are mature, vary the hours of light that each group is exposed to. The hours of light is the independent variable, keeping all other variables the same.

  Group 1 – 24 hours light.

  Group 2 – 20 hours of light / 4 hours of dark.

  Group 3 – 16 hours of light / 8 hours of dark.

  Group 4 – 12 hours of dark / 12 hours of light.

  Group 5 – 8 hours of light / 16 hours of dark.

  Group 6 – 4 hours of light / 20 hours of dark.

- Keep the plants in the light/dark regime given above until flowering occurs in any/all of the groups. Record the number of plants that flower in each group. The number of plants that flower is the dependent variable.

b. Method

- Select 300 petunia seedlings of the same variety. The plants should be the same age/size/stage of growth.
- Grow the seedlings under the same conditions, e.g. same container/soil, same amounts of water/nutrients, exposed to the same light intensity and same temperature.
- Divide the plants into 6 groups of 50 plants each. When the seedlings are 4 weeks old, dig up the plants in the first group. Remove the roots from the plants, wash off any soil and dry them. Put the roots and the stems/shoots separately in a desiccator/oven and dry them out completely to get their dry mass ('biomass'). Record the biomass of the roots, then that of the stems/shoots. The biomass of the roots and the biomass of the stems/shoots is the dependent variable – the biomass is a measure of the growth of the parts of the plant.
- Every 4 weeks, repeat the experiment for the remaining groups of plants.
- Compare the biomass of the roots with that of the stems/shoots for all the plants.

c. Method

- Select samples of standard fish bait – same kind of bait, same size and shape/volume.
- The smell of the bait samples needs to be different. The different smell of the bait samples is the independent variable – the measure of smell would be qualitative not quantitative. This could be done by using bait of different ages, e.g. keep one in the freezer (would need defrosting before use); one in the fridge, one left at room temperature for a day, another for two days, etc. A range of five different levels of smell could be used.
- Set up a large aquarium (e.g. 5 m long and 1 m wide and 1 m deep made out of plastic draped in a wooden frame). Stock with 10 fish of the same species/size/age/sex. Feed them the same food and amounts, but deprive them of food for 24 hours before the trials.
- The five samples need to be lowered into the tank at the same time at 1 m distances along the tank and you need to record how many fish go to each bait during a set time interval (e.g. 1 minute, 5 minutes…). The number of fish that go to each bait is the dependent variable.
- Repeat test with the samples of smelly bait at the same time the next day, but alter the order of the baits in the tank.
- Repeat as above until five trials have been done.
- Repeat the investigation with other types of bait.
- Repeat the investigation with other species of fish.

3. a. i. ***Method A*** – the dependent variable is the rate of movement of the air bubble (e.g. cm per minute); ***Method B*** – the dependent variable is the change in weight of the plant (e.g. g × 10).

ii. Other variables that need to be controlled include the air temperature, humidity, air movement, light intensity. A laboratory at room temperature (e.g. 18 °C) in daylight in still air would provide reasonable conditions for the investigation, provided the factors (air temperature, humidity, air movement, light intensity) are measured and each trial is done under the same conditions.

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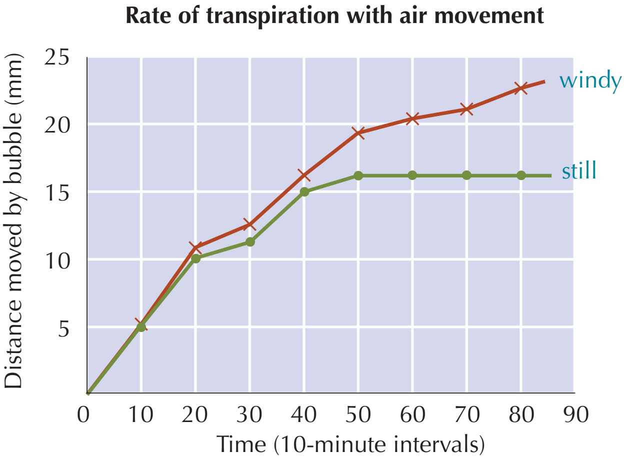
iii. To stop evaporation of the water in the container would cause an error in the measurements. It is essential that water drawn up from the container is to replace that lost in transpiration only.

b. i.



ii. The rate of transpiration increases as air movement increases.

iii. For the conclusion to be valid, a range of independent variables should have been used, not just 'still' and 'windy' conditions – i.e. the air movement should have been varied from 'still' (zero movement) to strong wind with about three or four other air movements in between. The amount of air movement should be measured using an appropriate meter/scale.

The investigation should have continued for a longer time, because the rate of transpiration for the plant in windy conditions was still increasing/had not stabilised.

4. a. **Method**

- Three similar-sized potato slices were cut from the same potato. The weight of each slice was recorded, in grams.
- One slice (sample B) was boiled in a beaker of water for 5 minutes to damage the membrane around its cells; the other two slices (samples A and C) were untreated.
- Sample A was placed in a 1 L beaker containing 500 mL of distilled water.
- Sample B was placed in a 1 L beaker containing 500 mL of distilled water.
- Sample C was placed in a 1 L beaker containing 500 mL of distilled water with 5 g of salt (NaCl) dissolved in it.
- The three beakers were left in the same place in the laboratory at room temperature for 180 minutes.
- Every 30 minutes, the slices were removed from the water, quickly blotted dry (to remove excess water), weighed, then returned to the beaker.
- At the end of the experiment, the % weight change for each slice was calculated.

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b. Discussion

Osmosis is the passage of water across a semi-permeable membrane. The net movement of water across the membrane is from an area of high water potential to an area of low water potential. When the potato cells are placed in distilled water, water potential is much higher outside the cells. Therefore, water moves in across the membrane and the cells gain weight. When the potato cells are placed in a (strong) salt solution, there is lower water potential in the salt solution than in the cells. Therefore, water moves out of the cells across the membrane and the cells lose weight.

When the water potential becomes equal on either side of the membrane, osmosis still occurs *but* there is no net movement of water in or out of the cell (the solutions inside and outside the cell are isotonic).

When the membrane is destroyed (e.g. by boiling), osmosis no longer occurs and there is no net loss or gain of water by the cells as a concentration gradient no longer exists.

5. a. Aim: To investigate the effect of pH on the action of the enzyme pepsin.

Method

- Solid egg white was cut into seven equal-sized cubes and weighed. The cubes were trimmed and reweighed until each was 2 g.
- 10 mL of a solution of the enzyme pepsin was put in each of the seven test tubes.
- Stock solutions of known pH (1, 2, 3, 4, 5, 6, 7) were obtained, and 1 mL of each pH solution was added to each test tube. The pH of each tube was checked using universal indicator paper.
- The tubes were placed in a water bath set at 37 °C and left to adjust to the temperature, which was checked inside each tube with a thermometer.
- When the tubes were at 37 °C, a cube of egg white was added to the solution in each.
- The tubes were left in the water bath for 24 hours, then the remaining egg white was removed, blotted dry (to remove excess fluid) then reweighed.

b. Discussion

The enzyme pepsin is found in the human stomach where it breaks down ('digests') protein. Egg white is mainly protein, therefore is broken down by pepsin. As the egg white is broken down, it loses weight, as the large protein molecules become smaller (soluble) amino acids. Enzyme action is influenced by pH. Pepsin is found in the stomach and the digestive juices there are (strongly) acidic so have a low pH. It is therefore expected that pepsin would have a preferred pH corresponding to high acidity (i.e. pH 1–3). This is borne out by this experiment, with maximum digestion occurring at a pH of 2, which corresponds to the conditions inside the stomach. The pHs of 1 and 3 are next most effective at bringing about digestion, but the activity of pepsin declines steadily as the pH increases above 3, with enzyme activity ceasing at pH 6, as seen with no egg white being digested.

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