FOURTH FORM BIOLOGY

THE SPRING TERM PART I

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Experimental Design

Experimentation in science is a disciplined and controlled way of asking and answering questions about the world in an unbiased manner. Observations about the natural world allow us to ask focused questions, which can lead to **hypotheses** to explain these observations. A hypothesis is a statement that seeks to make predictions that can then be tested using experiments.

A hypothesis is a statement about nature that can be tested by experiments or by new observations.

The results of an experiment may support the original hypothesis, or it may lead us to reject the hypothesis. Note, however, that it cannot *prove* that a hypothesis is correct. Further support for the hypothesis may come from other experiments.

One of the most powerful types of experiment in science is the **controlled experiment**. In a controlled experiment the researcher sets up several groups to be tested, keeping the conditions and setup as similar as possible from one group to the next. Then, the researcher deliberately changes one factor (a **variable**) in one group (the **experimental group**) that they hypothesise might have some kind of effect. In the other group, the factor is not altered, and this is referred to as the **control group**.

Variables

A variable is a factor or condition that is manipulated or measured in an experiment. Examples could include temperature, pH, concentration of a particular chemical or drug, light intensity, time, mass, height, volume of oxygen. We can consider three types of variables:

1. Independent Variable

The independent variable is the factor that you are investigating in the experiment, and is the one which is set at pre-determined levels in the experiment. For example, you may investigate the rate of respiration of an organism at different temperatures (e.g. at 20, 30, 40, 50, 60°C) – in this case the temperature is the independent variable. If you were investigating whether a new kind of drug has any effect on an illness, the drug would be the independent variable (i.e. patients in the experimental group receive the drug, those patients in the control group do not receive the drug).

2. Dependent Variable

This is the variable that the experimenter measures – for example, volume of carbon dioxide produced, number of patients recovering from an illness, the number of colonies of bacteria that grow.

3. Controlled Variable

All other variables in an experiment need to be kept constant between the test group and the control group. These are referred to as **controlled variables**. For example, when comparing the growth of bacteria from two different sources the temperature would be kept constant so that any differences in the growth rates would not be due to temperature. Controlling all other variables is critical to the validity of an experiment because it allows you to demonstrate that any differences you observe between the test and the control group can be ascribed to the factor you are investigating, and not to another factor. Controlling all other variables is the only way to ensure that the experiment is a **fair test** of the variable that is supposedly being studied.

Some variables, such as temperature, are relatively easy to keep constant. Others can be much harder to actively keep constant, and these are sometimes called *uncontrolled variables*. These are variable that you hope are constant in the experiment, but you don't actively do anything to keep them constant. In a well-designed experiment uncontrolled variables would be kept to a minimum so that they do not affect the results.

Reliability

Even in a carefully designed experiment, it is possible that the result you obtain is anomalous – something went wrong by chance that you were unaware of and that if you were to repeat the experiment you would get a different result. Repeat measurements must be taken in order to make sure that the results are **reliable**. A reliable set of data would give you approximately the same results for each repeat, and we can only see how reliable the data are if you have several or many repeats. Only if the experiment is repeated several times and you get consistent results can you assume that the result is reliable. A minimum of five repeats is usually considered adequate, unless you are sampling something which varies greatly in a large population.

By repeating an experiment you are able to identify **anomalous** results. These are results that are clearly different from the others that you obtain and can be considered outliers. We might then exclude an anomalous result when calculating the mean of a set of data.

Accuracy

An accurate measurement would give you a 'true' reading of whatever has happened in your experiment. It really has two aspects: **what** you decide to measure and **how** you go about measuring it.

If you wanted to measure the rate of growth of a plant and decided to do this by measuring the change in length of a single leaf on the plant over time, this is unlikely to give an accurate measure of the plant's overall growth – since the leaf may soon reach a maximum size, and whilst the plant is producing more leaves, you are recording no growth.

A better variable to measure, potentially giving a more accurate measure of growth, would be the overall gain in mass of the plant. So you use a potted plant, but use a pair of old bathroom scales to weigh it – you might get a set of measurements which all varied slightly over time but they would not be accurate since the scales are not sensitive enough to detect the small change in the mass of the plant.

Data Presentation in Biology

Tables

Numerical or descriptive data are usually recorded in a table. Below is a good example of a table.

Hydrochloric Acid Concentration /%	Time taken for block to turn colourless /s	Rate of Diffusion /s^{-1}
10	102	0.0098
25	82	0.012
50	55	0.018
75	39	0.026
100	26	0.038

Note the following points:

- Independent variable is in the first column.
- Dependent variable is in the column(s) to the right.
- The table has lines drawn and a border (use a ruler and pencil for this).
- Each column is headed with an informative description **units.**
- Units are separated from column heading using a solidus (/).
- **No units are put in the body of the table**, only in the column headings.
- All data are presented to the same number of significant figures (or to the same number of decimal places).
- Calculated data (in this case, rate) is given in the right hand column.
- **Never use fractions** – always convert to a decimal.

Drawing Graphs

The type of graph used (e.g. bar chart, histogram, line graph, pie chart or scattergram) should be appropriate to the data collected.

The graph should be of an appropriate size to make good use of the paper [i.e. at least 50% of the graph paper].

Line graphs

Line graphs are used to show relationships in data which are not immediately apparent from tables. The term graph applies to the whole representation. The term **curve** should be used to describe both **curves** and **straight lines** which are used to show trends.

The independent variable should be plotted on the horizontal axis (x) and the dependent variable plotted on the vertical axis (y). Line graphs are used to plot data where both the dependent and independent variables are numerical (e.g. time and acid concentration, as in the table above).

- Use a ruler and pencil to draw in the two axes.
- You will have to choose a sensible scale for the x- and y-axes. Choose one that makes it easy to plot the points.
- Chose a scale that is **uniform** and easy to plot – i.e. 2 large squares represents an interval of 10°C all the way along the axis; don't use fractions. Each axis should be scaled using multiples of 1, 2, 5 or 10 for each 20 mm square on the grid. This makes it easy for you to plot and extract data. Do not use multiples of 3 as this makes it harder to plot intermediate values accurately.
- Use at least half the grid provided, do not make the graph too small.
- The origin should be indicated with a 0; the data should be examined critically to establish whether it is necessary to start the scale(s) at zero. If not, you may have a **displaced origin** (i.e. it does not start at zero) for one or both axes, but this must be made obvious by labelling the displaced origin very clearly.
- Label the axes, and always include units (in pen).
- Plot your points (in pencil) using an x to mark each point; avoid using dots as these get obliterated when you draw a line through them.
- Use a ruler and pencil to join a straight line point-to-point; unless otherwise instructed, do not draw lines of best fit or smooth curves with biological data. This indicates uncertainty about the results for values of the independent variable between those plotted.
- Do not extrapolate lines either side of the outermost points you have plotted unless asked to do so.
- If you do include a title, write an *informative* title – do not just say "Graph of rate versus temperature" as this is obvious from the axis labels.

After plotting the points you need to decide if any of them are anomalous. Ask yourself the question 'do they fit the trend?'. But what is the trend? You should know something about the theory behind the investigation so you should be aware of the likely trend. If you think one or more of the results are anomalous, then it is a good idea to ring them. Put a circle on the graph away from the line and put a key to state that the circled point(s) represent anomalous result(s).

Bar Charts and Histograms

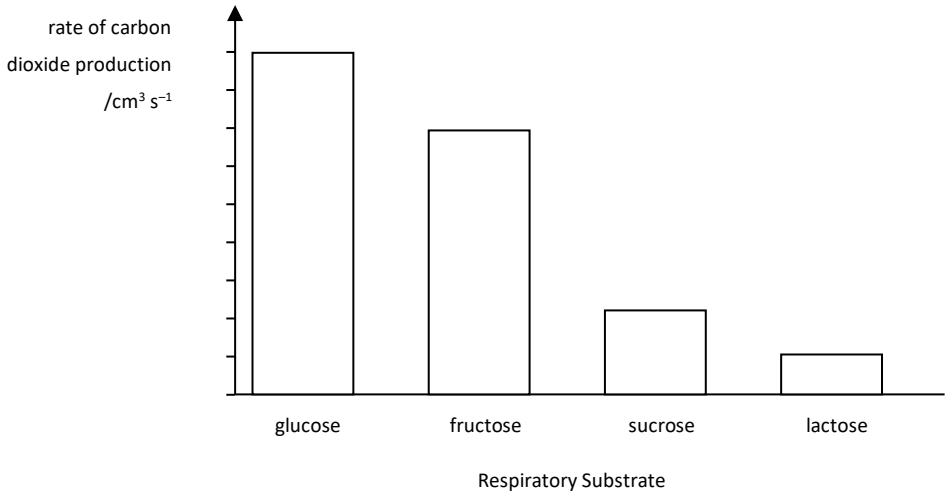
These are used when the independent variable on the x-axis is discrete, i.e. whole numbers or categories and the data under consideration deal with frequencies.

Bar Charts

Bar charts should be used if the **independent** variable is **qualitative** (i.e. non-numerical). If you are investigating the rate of respiration of yeast when given different substrates, the independent variable is the type of substrate, e.g. glucose, maltose, sucrose, etc. In this case there is no continuous scale for the independent variable and a bar chart is the appropriate way to present the results. The dependent variable is continuous as it is the rate of respiration and would be measured in units such as 'rate of carbon dioxide production/$cm^3\ s^{-1}$'.

Rules for drawing bar charts:

- Use most of the grid provided, do not make the chart too small.
- Draw the chart in pencil.
- Bar charts can be made of lines, or more usually, blocks of equal width. There must be space between the lines or bars; they do not touch.
- The intervals between the blocks on the x-axis should be equidistant.
- The y-axis should be properly scaled with equidistant intervals; the scale should usually start at 0 and this should be written at the base of the axis.
- If all the numbers are large a displaced origin may be used but the start number should be clear at the base of the y-axis.
- The y-axis should be labelled with the headings and units taken from the table of results.
- The lines or blocks should be arranged in the same order as in the table of results.
- Each block should be identified; there is no need to shade the blocks or colour code them.



Histograms

Do not confuse bar charts with histograms. A histogram is drawn for **continuous data** that is subdivided into classes. A good example is collecting data on continuous variables, such as measurements or mass. Sometimes the intervals can be whole numbers, for example the numbers of seeds in fruits.

Histograms are used when the **independent** variable is **numerical** and the data are **continuous**. The raw data needs to be organised into classes. The number and range of classes will largely depend on the type and nature of the data.

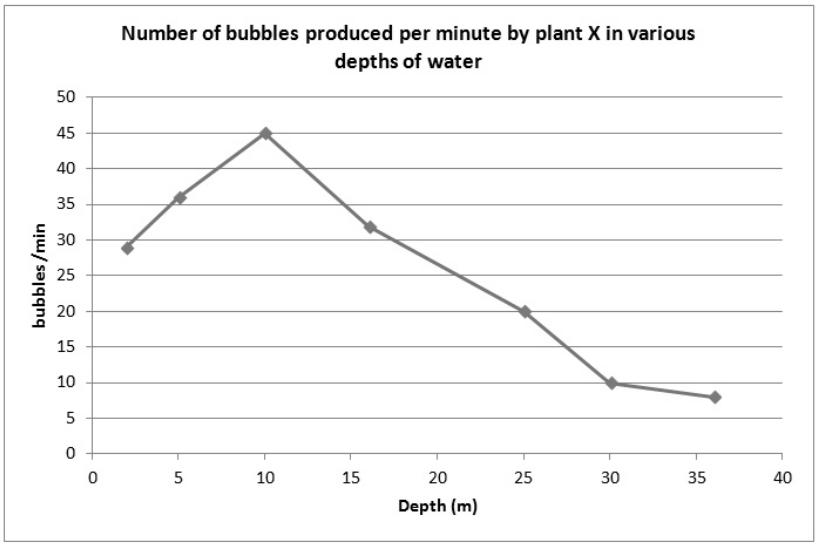
- Use most of the grid provided, do not make the histogram too small.
- Draw the histogram in pencil.
- The x-axis represents the independent variable and is continuous; it should be labelled clearly with an appropriate scale.
- The blocks should be drawn **touching.**
- The area of each block is proportional to the size of the class; it is usual to have similar sized classes so the widths of the blocks are all the same.
- The blocks should be labelled, e.g. '3.0 to 3.9' which means that 3.0 is included in this class, but 4.0 is not. 4.0 will be included in the next class: 4.0 to 4.9.
- The y-axis represents the number or frequency and should be properly scaled with equidistant intervals; it should be labelled with appropriate units.

Describing and Explaining Graphs

Questions in exams will often ask you to **describe** or **explain** results from experiments. Describe and explain mean different things: describe means you should succinctly put the results into words by

describing the main trends in the data. Explain means you should account for the trends you see using correct scientific theory.

Below is an example:



Description: *as the depth of water increases from 2.5 m to 10 m the number of bubbles increases from 29 to 45 bubbles per minute; at depths greater than 10 m the number of bubbles produced per minute decreases.*

Explanation: *at depths greater than 10 m the intensity of light decreases and this causes the rate of photosynthesis to decrease, and so less oxygen is produced. At shallow depths, the plant is partly exposed to the air and dries out, thus reducing the rate of photosynthesis.*

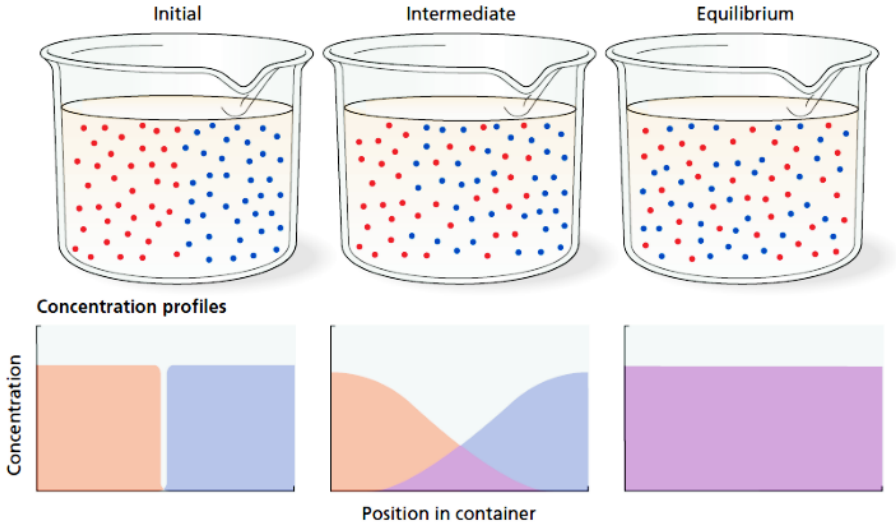
In the description there is no reference to the biology of the plant or any explanations of why the rate of bubble production changes. It only includes the general trends seen in the graph. If the graph had two lines, for example referring to two different species of plant, then you could **compare** and **contrast** the graphs – highlighting the ways in which the two plants show the same pattern (compare = similarities) or ways in which they differ (= contrast). For example, one may have a steeper curve than then other, or reach a maximum at a greater depth, or may have a lower rate at some or all depths.

Diffusion

Cells need to exchange various chemicals with their environment, such as oxygen and carbon dioxide. The cell surface membrane is selectively permeable, which means that some molecules are able to freely cross the membrane, whereas it may be impermeable to other molecules. There are three main ways that molecules can cross the cell surface membrane: **diffusion**, **active transport** and **osmosis**. Molecules such as carbon dioxide and oxygen are able to freely cross a cell surface membrane by diffusion.

Diffusion is the net movement of particles from a region of high concentration to a region of lower concentration down a concentration gradient.

Molecules in **solution** or in the **gaseous phase** are not static; they have kinetic energy and are in continuous motion. This random motion causes the net movement of molecules down a concentration gradient. Diffusion only occurs in a liquid or a gas medium; in a solid the molecules do not have sufficient energy to freely move around. In the diagram below, the concentration of two molecules changes over time so that they are equally distributed in the solution – an equilibrium has been reached.



At equilibrium there is still movement of particles between the two regions, but there is no **net** movement of particles – on average the number of particles moving one way is balanced by the number moving the other way; the concentration in the two regions is the same.

Note that we always describe diffusion as occurring *down* a concentration gradient, not "along" or "across" a gradient.

Factors Affecting the Rate of Diffusion

Temperature

Raising the temperature gives the molecules more kinetic energy, and thus they spread out from the area of high concentration at a faster rate.

Surface area

The larger the surface area separating two regions, the faster the rate of diffusion.

Concentration gradient

The greater the difference in concentration between two areas, the faster the rate of diffusion.

Distance

The shorter the distance separating the two areas, the faster the rate of diffusion. The term "*short diffusion path*" is often used to describe adaptations in organisms that minimise the distance over which a molecule has to diffuse in order to speed up the rate of diffusion.

Note that we always refer to the *rate* of diffusion increasing or decreasing; avoid using the phrases *more efficient diffusion* or *easier diffusion* as these are not very precise terms; also, we do not talk about *more diffusion* occurring, only about the *rate of diffusion* increasing.

Osmosis

Consider a beaker of water into which a cube of sugar (sucrose) is placed. The sucrose will dissolve in the water, and over time the sucrose molecules will diffuse in the water until the solution has a uniform concentration. In this example, we are only really concerned with the sucrose molecules diffusing once they have dissolved, not the water molecules.

However, now consider two compartments separated by a partially permeable membrane which both contain sucrose solutions of two *different* concentrations. A partially-permeable membrane is one which allows small molecules (such as water) to pass through it, but does not allow larger molecules to pass through (such as sucrose molecules). It is a kind of 'molecular sieve'.

The sucrose molecules are unable to simply diffuse between the two compartments to equalise their concentration in each compartment because of the membrane. What about the water molecules? When solute molecules dissolve in water, they interact with the water molecules. Each sucrose molecule attracts a shell of water molecules around it, which are now unable to diffuse freely across the membrane. This reduces the number of free water molecules in the solution that are able to diffuse, effectively reducing the concentration of water molecules in that volume of space.

The movement of water across cell membranes occurs by a special kind of diffusion called **osmosis**.

Osmosis is the net movement of water molecules across a partially-permeable membrane down a water potential gradient.

Although the principles of diffusion listed above still apply to osmosis, biologists use a different set of terms to describe the movement of water molecules. When considering the diffusion of oxygen into a cell, for example, we only need to take into account the concentration of oxygen inside and outside of the cell. However, with osmosis we need to consider the solutes and water molecules inside and outside the cell membrane, as these will determine whether there will be a net movement of water into or out of the cell across the membrane.

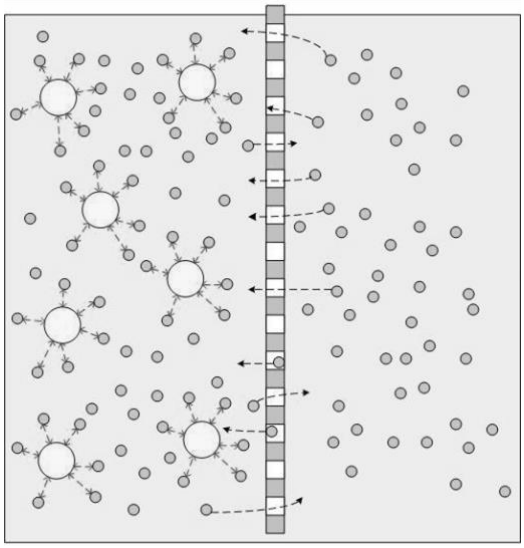
We can, therefore, refer not to the concentration of water molecules, but to the **water potential** of a solution. Water potential measures the concentration of free water molecules in a solution. It is a measure of the tendency for water molecules to move by osmosis across a partially-permeable membrane.

By convention, the water potential of pure water is 0 (zero). As you start to dissolve solutes in pure water, the water potential becomes lower (more negative). Thus, as solute concentration increases, the water potential decreases. Since water molecules move across a partially-permeable membrane from a more dilute solution to a less dilute solution, this can be described in terms of water potential as water molecules moving from a solution with a higher water potential to one of lower (more negative) water potential.

Osmosis, like diffusion, is a passive process, and relies only on the kinetic energy of the water molecules. It only refers to the movement of water molecules across a partially permeable membrane.

Consider two regions, separated by a partially permeable membrane, as in the diagram below. In the right compartment is pure water and in the left are dissolved sucrose molecules. Some of the water molecules are attracted to the sucrose molecules. Note the following points:

- The water potential of the pure water is zero. The water potential of the sucrose solution is less than zero.
- A water potential gradient therefore exists between the two compartments.
- Water molecules move by osmosis from the region of higher water potential (right hand compartment) to the region of lower water potential (left hand compartment), down the water potential gradient.
- The sucrose molecules cannot move between the compartments because the membrane is impermeable to a large molecule like sucrose.



Osmosis in Animal Cells

The movement of water across membranes has great significance for cells since this will affect the volume of the cell – if a cell is placed in a solution of higher water potential than that of the cytoplasm then water will enter and the cell will swell up. Likewise, a cell may shrink in volume if placed in a solution of lower (more negative) water potential.

These changes can be readily observed using red blood cells. When placed in a concentrated salt solution they lose water by osmosis and shrink, the cell membrane crinkling around the remaining cytoplasm. For red blood cells, the term **crenated** is used to describe this phenomenon. If blood cells are placed in distilled water, water rapidly moves into the cells and the cells burst (**lyse**; **lysis**). Therefore, for animal

cells to retain their size and shape, they must exist in an environment that has the same water potential as their cytoplasm, or to deploy specific mechanisms (such as a **contractile vacuole** in *Paramecium,* a spherical sac which collects water and periodically discharges it to the outside) to remove excess water from the cell.

Osmosis in Plant Cells

Plant cells generally have a water potential that is markedly lower (more negative) than their surroundings. The solutes are located in the vacuole, which is surrounded by the tonoplast membrane, and the cytoplasm. As described above, this may lead to an influx of water down a water potential gradient, leading to the volume of the vacuole and cytoplasm increasing. However, in contrast to animal cells, the plant cell does not burst. This is because plant cells (as well as fungal and bacteria cells) are surrounded by a cell wall which, when fully stretched, will resist any further expansion of the cell.

As water flows into the cell, the pressure inside the cell increases, which pushes the cell contents against the cell wall; this is known as **turgor pressure**. When turgor pressure is at its maximum, the cell is said to be **turgid**. Turgor pressure plays an important role in supporting plants and maintaining their shape. Turgor pressure is responsible for holding the stems of non-woody plants upright, and for holding leaves in a flat, horizontal position.

If plant cells lose water, the volume of the vacuole and cytoplasm is reduced and the pressure exerted against the cell wall falls. The cell is now referred to as being **flaccid**. If more water is lost from the cell, the cell surface membrane is pulled away from the cell wall, leaving a gap between the two structures. This is termed **plasmolysis** and the cell is said to be **plasmolysed**.