Faculty of Science

Laboratory Manual

Biochemistry

Bachelor of Biotechnology (Hons.)

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Biochemistry

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')
- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful

- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

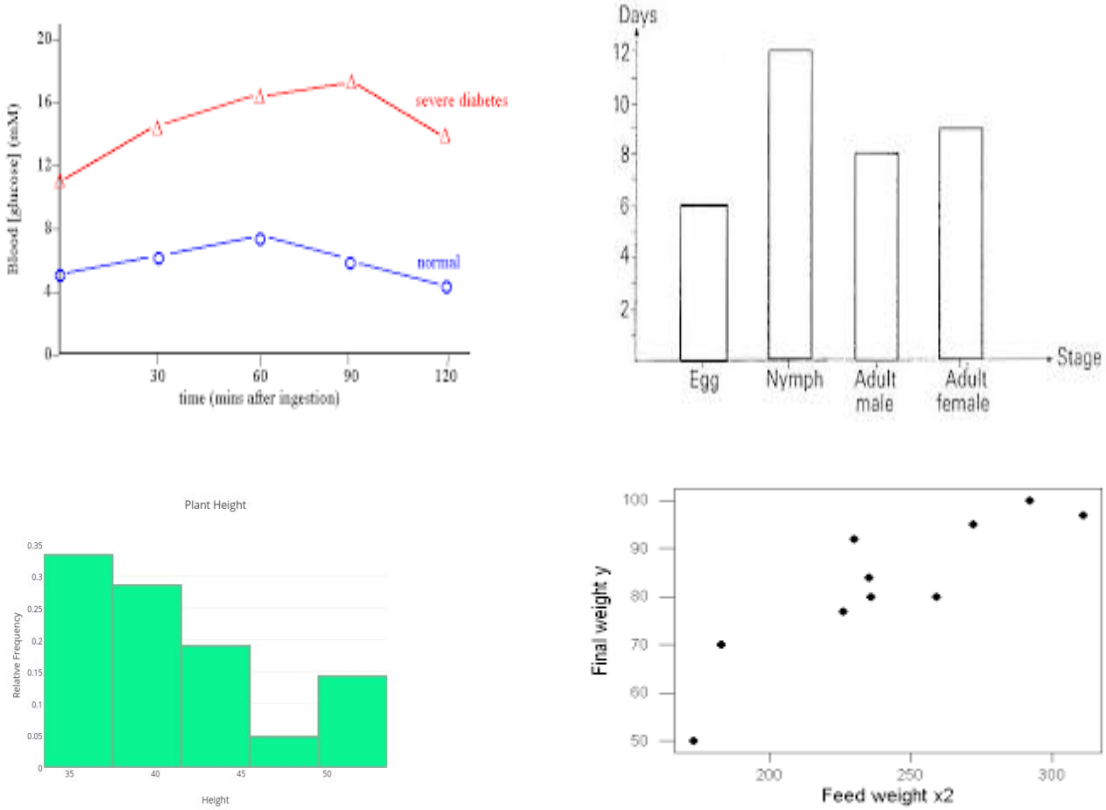

Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.
- Cite sources to substantiate background information.

- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1

Title: The effect of pH on enzyme activity

Objective:

After completing the practical, you will be able:

1. To determine the effect of pH on enzyme activity

Introduction:

Enzymes are proteinaceous catalysts, which speed up the rate of a biochemical reaction. They reduce the activation energy that is essential for starting any type of chemical reaction. With a low energy requirement for activation, the reaction takes place faster. The overall performance of an enzyme depends on various factors, such as temperature, pH, cofactors, activators, and inhibitors.

The rate of a chemical reaction and/or the enzyme activity is greatly influenced by the structure of the enzyme. Or in other words, a change in the structure of the enzyme affects the rate of reaction. When pH of a particular medium changes, it leads to an alteration in the shape of the enzyme. Not only enzymes, the pH level may also affect the charge and shape of the substrate. Within a narrow pH range, changes in the structural shapes of the enzymes and substrates may be reversible. But for a significant change in pH levels, the enzyme and the substrate may undergo denaturation. In such cases, they cannot identify each other. Consequently, there will be no reaction. This is why pH is a determining factor of enzyme activity.

Each and every enzyme is characterized by an optimum pH. At this specific pH level, a particular enzyme catalyzes the reaction at the fastest rate than at any other pH level. For example, the enzyme pepsin (a protease enzyme) is most active at an acidic pH, whereas the enzyme trypsin (another protease enzyme) performs best at a slightly alkaline pH. Thus, the optimum pH of an enzyme is different from that of another enzyme. When study pH, it is clearly defined as the measurement for the acidic or alkaline nature of a solution. To be more precise, pH indicates the concentration of dissolved hydrogen ions (H+) in the particular solution. An increase or decrease in the pH changes the ion concentration in the solution. These ions alter the structure of the enzymes and at times the substrate, either due to formation of additional bonds or breakage of already existing bonds. Ultimately, the chemical makeup of the enzyme and substrate are changed. Also, the active site of the enzyme is changed, after which the substrate can no longer identify the enzyme.

The optimum pH confers the required ionization of the enzyme, the substrate and the enzyme substrate complex for maximum reaction velocity. The nature of the ionisable amino acid side chains (from amino acids like glutamic acid, aspartic acid, glutamine, asparagines, lysine, arginine and histidine) in the amphoteric protein must be of considerable importance in this respect. The type of ionization required for maximum activity promotes the maximum binding of the substrate and enzyme and yet facilitate the maximum rate of breakdown of the enzyme and product.

The principle of the reaction of glucose oxidase in this experiment is as follows:

1) β-D-glucose $+O_2 + H_2O \longrightarrow$ D-gluconic acid $+ H_2O_2$

2) H_2O_2 +D-Dianisidine $\longrightarrow$O-Dianisidine H_2O

Materials:

Reagents

-0.2M citric acid
-0.4M Na_2HPO_4
-0.052% glucose-oxidase (GOD), 0.008% peroxidase solution (POD) (30 ml)
-0.1% glucose solution (5 ml)
-0.25% dianisidine solution (5 ml)

Apparatus:

-12 test tubes
-2 X 10 ml graduated pipettes
-3 X 5 ml graduated pipettes
-3 X 1 ml graduated pipettes
-3 X 100 ml graduated beaker
-2 X 25 ml beaker
-Stop watch
-2 X test tube rack
-Spectrophotometer and cuvettes

Methods:

1. Make up buffers in 6 test tubes as shown in the following table:

pH	0.4 M Na_2HPO_4/ ml	0.2 M citric acid/ ml
3	2.1	7.9
4	3.9	6.1
5	5.2	4.8
6	6.3	3.7
7	8.2	1.8
8	9.7	0.3

2. In a test tube place 2.5 ml of buffer solution pH 3, 2.5 ml of glucose oxidase-peroxidase enzyme solution, and 0.2 ml of dianisidine solution.
3. Mix the reagents well then place 2.6 ml (half of the mixture) into cuvette, follow with 0.1 ml of distilled water. This mixture will use as the blank for spectrophotometer at 450 nm.
4. The enzyme activity can be measured by using all reagents as above (2.6 ml) but substituting 0.1 ml of glucose solution for H_2O.
5. Record the increase in optical density every 15 seconds for 5 minutes.
6. Repeat the procedure with the other buffer solutions. All readings must be in duplication.

Calculations:

Calculate the zero order rate constant at each pH, and then plot the rate constants against the pH.

Practical 2
Title: Macromolecules

Objectives:

After completing the practical, you will be able:

1. To define monosaccharide, disaccharide, and polysaccharide and give examples of each
2. To name the monosaccharide components of sucrose and starch
3. To describe the test that indicates the presence of most small sugars
4. To describe the test that indicates the presence of starch
5. To define hydrolysis and give an example of the hydrolysis of carbohydrates

Introduction:

Living organisms are composed of molecules that come in diverse shapes and sizes and serve a variety of purposes. Some molecules form of the structure of an organism's body, for example, the cellulose that makes up the cell walls in plants, the proteins and phospholipids that comprise cell membranes, and the fibers that make up animal muscles.

There is also a wide array of molecules that perform all the functions of life. For example, enzymes catalyse the chemical reactions necessary for biological processes; absorb light so that you can see with your eyes.

In this laboratory, you will study three classes of the largest biological molecules, called macromolecules: carbohydrates, lipids and proteins.

Part 1: Carbohydrates

Most carbohydrates contain only carbon, oxygen and hydrogen. The simplest forms of carbohydrate molecules are the monosaccharides (single sugar). One of the most important monosaccharides is glucose, the end of product of photosynthesis in plants. It is also the molecule that is metabolized to produce another molecule, ATP, whose energy can be used for cellular work. There are many other common monosaccharides, including fructose, galactose, and ribose.

Some disaccharides (double sugars) are also common. A disaccharide is simply two molecules, lactose (milk sugar) consists of glucose and galactose, and sucrose (table sugar) consists of glucose and fructose. Can you discern a rule used in naming sugars?

Carbohydrates are also found in the form of polysaccharides (many sugars), which are long chains of monosaccharide subunits linked together. Starch, a polysaccharide composed of only glucose subunits, is an especially abundant component of plants. Most of the carbohydrates we eat are derived from plants.

Starch is the plant's way of storing the glucose it makes photosynthesis. When you eat starch, you are consuming food reserves that the plant has stored for its own use. The starch of potatoes and root vegetables, for example, would be used the next spring for the plant's renewed growth after the winter die-back.

Animal stores glucose in glycogen, which is another form of polysaccharide. Although starch and glycogen. Although starch and glycogen are both composed of glucose subunits, the glucose

molecules are bonded together in different ways, so these polysaccharides are not identical. Glucose subunits are bonded together in a third way in the polysaccharide cellulose. While starch and glycogen are meant to be metabolized for energy, cellulose, which is the most abundant carbohydrate in the world, is a structural molecule that is designed not be metabolized. Cellulose makes up the cell walls of plants and is a primary component of dietary fiber. For most animals it is completely indigestible. Those that can digest it, such as termites and cows, do so only with the assistance of organism such as bacteria, fungi, or protistans.

Most disaccharides and polysaccharides can be broken down into their component monosaccharides by a process called hydrolysis, which is accomplished in organisms by digestive enzymes. This process is important in seeds. If the seed's food resource is starch, it must be able to convert to the starch to glucose. The glucose is then used to generate ATP, which in turn is used to provide the growing plant embryo with energy for metabolic work. Hydrolysis of starch begins when the seed takes up water and begins to germinate.

A chemical hydrolysis can be done in the laboratory by heating the molecules with acid in the presence of water. You will perform a chemical hydrolysis in this exercise.

Investigation 1: Monosaccharides and Disaccharides

You will use Benedict's reagent as a general test for small sugars. When this reagent is mixed with a solution containing single or double sugars and then heated, a coloured precipitate (solid material) forms. The precipitate may be yellow, green, orange, or red. If no monosaccharide or disaccharide is present, the reaction mixture remains clear. However, Benedict's reagent does not react with all small sugars. For example, sucrose gives a negative Benedict's reaction.

Glucose will be used to demonstrate a positive Benedict's test. What should be used as a negative control for this test?

Materials:

- Benedict's reagent
- 1% solutions of glucose, xylose, arabinose, fructose, lactose, sucrose, galactose, and starch
- Test tube
- Beaker
- Hot plate

Methods

1. Make a boiling water bath by filling a beaker about half full of water and heating it on a hot plate. You will need to use this water bath in several activities.
2. Place in a test tube 1 ml of a 1% glucose solution and 5 ml of Benedict's solution.
3. Prepare a control.
4. Place the two tubes in boiling water for 2-3 minutes.
5. Observe the colour of the solution and note whether a precipitate has formed.
   Note: a change in colour of the solution is not indicative of a positive reaction. A precipitate must appear.

6. Repeat the test with 1% solutions of xylose, arabinose, fructose, lactose, sucrose, galactose, and starch.
7. Record all of your results in a table.

Investigation 2: Starch

Starch is tested by using iodine reagent. A dark blue colour indicates the presence of starch.

You will use a solution of potato starch to demonstrate a positive test. What negative control should be used for this test?

Materials:

1. Starch solution
2. Iodine reagent
3. Test tubes

Methods:

1. Prepare a starch solution by mixing thoroughly 2 g of starch with 10ml of water and then pouring this mixture into 200 ml of boiling water.
2. Get two test tubes and label them 1 and 2.
3. Put a few ml of the starch solution in Tube 1.This is the positive control.
4. Tube 2 is the negative control. What substance goes in it? How much should be used?
5. Put a few drops of iodine reagent into each tube.
6. Record the observations.

Investigation 3: Hydrolysis of Carbohydrates

As discussed earlier, disaccharides are composed of two monosaccharides linked together. Polysaccharides are long chains of monosaccharides. The bonds joining these subunits can be broken in a process called hydrolysis. In this procedure, you will hydrolyze sucrose and starch by heating them with acid.

What monosaccharides will result from the hydrolysis of sucrose?

What monosaccharide will result from the hydrolysis of starch?

The hydrolysis reactions will be carried out in two large test tubes. One contains sucrose and hydrochloric acid (HCL) and the other contains starch and HCL. You will sample the sucrose tube twice: once before the hydrolysis has begun and again after 3 minutes. You will take 6 samples from the starch tube: 2 before the hydrolysis has been done, 2 after 5 minutes of hydrolysis, and 2 after 15 minutes. Two samples are needed at each time so that one can be tested for small sugars (Benedict's test) and one can be tested for starch (iodine test).

Materials:

- Starch solution
- Sucrose solution
- 2N HCl solution
- Benedict's reagent
- Iodine reagent
- Test tubes

- Pipettes
- Water bath

Methods:

1. Get eight test tubes and label them 1 through 8. Line up the test tubes in order in a test tube rack.
2. Get two large test tubes and label them starch and sucrose. Use an empty beaker as a testtube holder if the test tubes don't fit in the rack.
3. Pipette 6 ml starch solution and 3 ml 2N HCL into the tube labelled starch.
   Caution: HCL is a strong acid. Handle it with caution.
4. Pipette 5 ml sucrose solution and 1 ml 2N HCl into the tube labelled sucrose.
5. Swirl each tube gently to mix the contents.

Sampling

6. Draw 1 ml of solution from the sucrose tube and put it in Tube 3. (Skip Tube 2 for now.)
7. Using a different pipette, draw 1 ml of solution from the starch tube and put it in Tube 3. (Skip Tube 2 for now.)
8. Draw an additional ml of solution from the starch tube and put it in Tube 4.
9. Place the extra-large starch and sucrose tubes in your boiling water bath. Note the time.
10. After 2 or 3 minutes, draw 1 ml of solution from the sucrose tube and put it in Tube 2.
11. You are now finished with the sucrose solution. You may remove it from the water bath.
12. After 5 minutes, draw 1 m of solution from the starch tube and put it in Tube 5.
13. Put a second ml of starch solution in Tube 6.
14. Wait 10 more minutes and then repeat steps 11 and 12, putting the solution in Tube 7 and 8.

Testing for Starch and Sugar

15. Add 5 ml of Benedict's reagent to Tubes 1,2,3,5 and 7. Place these tubes in the boiling water bath for 5 minutes.
16. Add 3 or 4 drops of iodine reagent to Tubes 4, 6 and 8.
17. Remove the tubes from the water bath for 5 minutes for them to cool. Record the results in the following table:

	Tube Number							
	Sucrose		Starch					
	1	2	3	4	5	6	7	8
Time (min)	0	2-3	0	0	5	5	15	15
Benedict's reagent								
Iodine reagent								

Interpretation of Results:

Explain the results you obtained using the Benedict's test on the sucrose solution.
Explain the results you obtained using the iodine reagent test with starch.
Explain the results you obtained using the Benedict's test with starch.
Why does hydrolysis of starch take longer than hydrolysis of sucrose?

Part 2: Proteins

A protein's structure is determined by the amino acid subunits that make up the molecule. Although there only 20 different naturally occurring amino acids, each protein molecule has a unique sequence. The amino acids are interact with each other to help shape of the molecule.

The bond between amino acids in a protein is a peptide bond and is identified by a Biuret test. Specifically, peptide bond in proteins complex with Cu^{2+} in Biuret reagent and produce a violet colour; therefore, free amino acids do not react positively.

Biuret reagent is a 1% solution of $CuSO_4$ (copper sulfate). A violet colour is a positive test for the presence of protein; the intensity of colour relates to the number of peptide bonds that react.

You will use a solution of egg albumin (a protein extracted from egg whites) to demonstrate a positive Biuret test. What negative control should be used for this test?

Materials:

- 1% egg albumin
- Concentrated KOH
- 0.5% $CuSO_4$
- Test tubes

Methods:

1. Get two test tubes and label them 1 and 2.
2. Put 3 ml of 1% egg albumin into Tube 1.
3. Tube 2 is the control. What substance goes in it? How much should be used?
4. Add an equal volume of concentrated KOH (20%) to both tubes. Mix thoroughly.
5. Slowly add 1 ml of 0.5% $CuSO_4$. Mix.
6. After 2 minutes, record the colour in each tube.

Part 3: Lipids

Lipids are oily or greasy compounds insoluble in water, but dissolvable in nonpolar solvents such as ether or chloroform. The lipids we will consider in this laboratory are fats and oils, which are generally used as storage molecules in both plants and animals.

Lipids provide long-term energy storage in cells and are very diverse. Lipid digestion occurs primarily in the small intestine where bile produced by the liver breaks lipid globules into smaller droplets, and then pancreatic enzymes break large lipid molecules into smaller components for absorption.

You will use the paper test to indicate the presence of lipids in various foods. Although this test is not sophisticate, it is quick and convenient.

Materials:

- Brown paper
- Vegetable oil
- Water

Methods

1. Get a small square of brown paper. Write ‘oil’ on one half and ‘water’ on the other.
2. Put a tiny drop of vegetable oil on the half of the paper labelled oil. Rub it gently with your fingertip.
3. As a negative control, put a tiny drop of water on half of the paper labelled water. Rub it gently with a different fingertip to avoid contamination.
4. Allow the spots to dry. This may take a while.
5. When the spots are dry, hold the paper up to the light.
6. Record your observations.

Practical 3
Title: Macromolecules in food

Objective:
After completing the practical, you will be able:
1. To describe the methods to test the presence of carbohydrates, starch, protein and lipid in a sample.

Introduction:

Macromolecules are very large molecules, formed of smaller subunits.

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen. Your body uses carbohydrates (carbs) to give you energy and helps keep everything going. Your body can use carbohydrates immediately or store it in your liver and muscles for when it is needed. In general, the smaller carbohydrate compounds are commonly referred to as simple sugars, which are found naturally in foods such as fruits, vegetables, milk, and milk products. These simple sugars are broken down quickly and give you short term energy. Starch is a complex carbohydrate. Starch must be broken down through digestion before your body can use it as an energy source. Quite a few foods contain starch and dietary fiber such as breads, cereals, and vegetables.

Proteins are part of every cell, tissue, and organ in our bodies. These body proteins are constantly being broken down and replaced. The protein in the foods we eat is digested into amino acids that are later used to replace these proteins in our bodies. Protein is mainly found in the following foods: meats, poultry, and fish, legumes (dry beans and peas), tofu, eggs, nuts and seeds, milk and milk products, and grains. Most adults in the United States get more than enough protein to meet their needs. It is rare for someone who is healthy and eating a varied diet to not get enough protein. There are 20 different amino acids that join together to make all types of protein. Our bodies cannot make some of these amino acids so these are known as essential amino acids – it is essential that our diet provide these. A complete protein source is one that provides all of the essential amino acids, for example; meat, poultry, fish, milk, eggs, and cheese are considered complete protein sources. An incomplete protein source is one that is low in one or more of the essential amino acids.

The term lipid is sometimes used as a synonym for fats, but this is technically incorrect. True fats are composed of triglycerides. Moreover, triglycerides that are solid at room temperature are called “fats” while those that remain liquid at room temperature are called “oils.” Triglycerides are also composed of carbon, hydrogen and oxygen atoms, but in different ratios than in carbohydrates.
Triglycerides have long chains of carbon and hydrogen bonds, which creates the hydrophobic (“water fearing”) tail. This property prevents triglycerides from mixing readily with water and causes them to separate relatively easily in solution.

Most of the fat that you eat should come from unsaturated sources: polyunsaturated fats and monounsaturated fats. A fat is unsaturated if it contains at least one double bond. In general, nuts, vegetable oils, and fish are sources of unsaturated fats. Saturated fats are oftentimes the "solid" fats, but other saturated fats can be more difficult to see in the foods we consume. A fat is saturated if it consists of only single bonds. In general, saturated fat can be found in the following foods: high-fat cheeses, high-fat cuts of meat, whole-fat milk and cream, butter, ice cream and palm and coconut oils.

Methods

You have learned the methods to test the presence of carbohydrates, starch, protein and lipid in a sample. Now it is time to put them into good use.

You will be given a few samples. Your job is to test the presence of carbohydrate, starch, protein and lipid in each sample.
Record your observations in a table.

Samples:

Pears
Potatoes
Soft drink
Cream cracker
Onions
Instant noodles
Soy beans
Sago

Practical 4

Title: Estimation of total serum proteins and serum albumin

Objective:

After completing the practical, you will be able:

1. To estimate the total serum protein and total serum albumin using photometric method (spectrophotometer)

Introduction

Principle of Lowry test is based on reaction between peptide nitrogen with the Copper (II) ions under alkaline condition with the oxidation of aromatic protein residues. Reduction of Folin-Ciocalteay phosphomolyb diphostungstic acid to heteropolymolybdenum blue by copper catalysed oxidation of acids.

The protein molecule has a large complex structure, each molecule is made up of smaller units called amino acids. Amino acids are the building blocks of the protein molecule. Amino acids are relatively small molecule. They are water-soluble and consequently can easily diffuse through the walls of the intestine. There are 20 common amino acids. However there are eight amino acids which the body cannot manufacture successfully. The body needs these hence they are known as the essential amino acids. They are obtained by eating foods that have these amino acids. The eight essential amino acids are valine, lysine, leucine, isoleucine, methionine, phenylalanine, tryptophan, and threonine.

Lowry protein assay is a biochemical assay for determining the total level of protein in a solution. This method is highly sensitive to low concentration of protein. The total protein concentration is exhibited by color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. This method is sensitive to pH changes, therefore it need to be maintain at 10-10.5; unlike alkaline conditions the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue.

Biuret method has been used to measure total protein in serum. The formation of a Cuprum-protein complex requires two peptide bonds and produces colored product which is measured by absorption spectroscopy at 540-560 nm.

$$\text{Protein (sample/standard)} + \text{cuprum} \xrightarrow{\text{pH 12}} \text{cuprum-protein complex (coloured product)}$$

2 main components that measured in total protein are:

I. Albumin
II. Globulin

Low protein is primarily caused by glomelular nephritis, nephritic syndrome, protein malabsorption and digestion and cirrhosis of liver. High concentration of protein mainly due: multiple myeloma, bone marrow disorder, amyloidosis, chronic inflammatory condition and HIV/AIDS.

Reagent & Equipment

- Protein kit (Biuret reagent and protein standard)
- Albumin kit (BCG reagent and albumin standard)
- Spectrophotometer
- Cuvette
- Micropipette

Methods

Prepare 3 cuvettes:

I. Blank
II. Sample A
III. Sample B

Pipette into cuvette	Microlitre (µl)
Reagent	1000 µl
Sample/standard	20 µl
Mix and incubate for 5 minutes and read the absorbance (using spectrophotometer) of all cuvettes at 560 nm against reagent blank	

- Wave length: 540 nm
- Incubation: 5 minutes
- Standard concentration: 4g/dl
- Temperature: 37°C

Calculation

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standards}} \times \text{Concentration of standard} = \text{Total protein (g/dl)}$$

Expected value:

=6.2 to 8.5 g/dl

Notes

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