Biological Molecules lab

The molecules that make up all living things fall into four groups (Table 1). With the exception of lipids (fats) these molecules are called macromolecules, and they are made up of repeating monomers that are held together by strong covalent bonds. Use your textbook and/or notes to complete Table 1.

Table 1: Molecules of life and some of their properties

Type of molecule	Name of monomer	Name of polymer	Examples	Functions in living things
Carbohydrates: Sugars Monosaccharides Disaccharides	XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX	XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX	• *glucose*	
Carbohydrates: complex carbohydrates	*monosaccharide*			
Proteins				• *multiple*
Nucleic acids* *we'll cover Friday	*nucleotide s (G, A, T, C and G, A, U, C)*	*Nucleic acid*	• *DNA* • *RNA*	*Store and transmit hereditary informatio*
Lipids	XXXXXXXXXX XXXXXXXXXX	XXXXXXXXXX XXXXXXXXXX		• *Energy storage*

We eat all types of macromolecules, which are broken down into their monomers (and often even smaller) in our digestive tract. We either use these monomers to build our own macromolecules, or break them down further to release energy. Proteins serve a host of other functions in our bodies.

In today's lab, your group is given an unknown food to test for the presence of various biological molecules. Your mystery food, different from what the other groups' food is, may be one of the following: potato juice, onion juice, nonfat milk, full fat milk, fat-free yogurt, full fat yogurt, egg whites, avocado, nuts, egg yolk, Sprite, part of the instructor's lunch…

The testing involves applying a number of different types of **indicators.** Indicators are compounds that change in a specific way in the presence of specific types of molecules. Often there is a color change, but there may be other changes.

Your job is to use the tests to decide if your unknown contains:

1. Lipids
2. Starch
3. Monosaccharides
4. Protein

Some foods may have more than one of the above. The samples are in liquid form to make them easier to test.

Controls

How will you know what color the indicators are supposed to change to? The best way is to run tests on **controls** alongside your unknown. Controls are substances whose composition is known. A **positive control** should contain only the substance we are testing for. For example: if we were testing for the presence of alcohol, vodka would be a better choice for a positive control than a margarita. There are many other components in a margarita that might react with the indicator, potentially giving a false result.

In addition to a positive control, a good experiment includes a **negative control**: a substance known to *not* contain the thing you are testing for.

Running positive and negative controls establishes standards against which you can compare your unknowns. In other words, positive and negative controls show you what a positive result looks like, and what a negative result looks like.

Since you are conducting multiple tests (monosaccharides, complex carbohydrates, proteins, and lipids), each of the tests needs its own controls.

From the list below, select a positive and a negative control to use for each of the tests. Record your choice in Table 2.

Controls to chose from:

Distilled water
Amino acid solution
Protein solution
Glucose solution
Starch solution
Vegetable oil solution

Table 2: Positive and Negative controls chosen for each test

Testing reagent	Substance being tested	Positive control	Negative control
Sudan III *OR* brown paper	Lipids		
IKI (Iodine)	Starch		
Benedict's	Monosaccharides		
Biuret	Protein		

*******DO NOT PROCEED UNTIL YOU'VE CLEARED CONTROLS WITH THE INSTRUCTOR!

For each procedure, you will run 3 test tubes: your unknown, a positive control and a negative control.

Table 3: Results from Procedures 1-4 (Write a +/-, plus a description of the color)

Test	Procedure number	Unknown	Positive control	Negative control
Lipid (Sudan III or grease spot):	1a or 1b			
Protein (Biuret)	2			
Monosaccharides (Benedicts)	3			
Starch (Iodine)	4			

Procedure 1a: Lipid Test (Sudan III) – ask instructor which test you are using

Sudan III is an indicator that stains lipids red. You will not use test tubes for this procedure.

1. Using pencil, draw 3 circles ~1 cm in diameter and 2 cm apart on 1 sheet of filter paper. Number circles 1-3.
2. Add 1-2 drops of each substance to the corresponding circle on the filter paper.
3. Allow paper to sit for 10 minutes so each substance can penetrate the paper.
4. Place filter paper into a beaker containing Sudan III. **Caution:** Use care when handling Sudan III to avoid staining hands or clothing.
5. Leave the paper in for 5 minutes. Sudan III will stain lipids bright red. Usually the brighter the color, the more lipids are present.
6. Using forceps, remove the filter paper and place it in the beaker of water for 1 minute.
7. Identify your positive control and your negative control. Did they react as expected (i.e. the positive control changed color with the indicator, and the negative control did not?)
8. Compare the unknown sample to your positive and negative controls.
9. In the Table 3, write a "+" if your substance turned red, or a "-" if the substance did not turn red. Record results for your controls too

Procedure 1b: Alternate Lipid Test (Grease Spot)

1. Label 3 squares of brown paper as follows: unknown, positive control, negative control.
2. Place a drop of each substance on the appropriate square.
3. Allow the substances to dry.
4. The positive control should leave a grease-spot on the paper. You should be able to read print through the spot. On the other hand, you should not be able to see anything through the negative control spot after it has dried.
5. Compare the unknown sample to the controls and record your results in Table 3.

Procedure 2: Protein Test

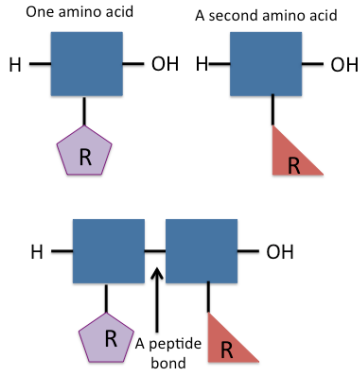

Figure 1: At the top of the figure there are 2, un-bound amino acids (no peptide bonds). The lower image shows the 2 amino acids joined together in a strong covalent **peptide bond**. Proteins are made of long chains of amino acids connected with peptide bonds.

Biuret Reagent is protein indicator that starts out blue but then turns violet in the presence of a **peptide bond**. Note: Benedict's solution also starts out blue, so *read the label*!

1. Label 3 test tubes. Add 3 mL of unknown to #1, positive control to #2, and negative control to #3.
2. Add 1 mL of 2.5% sodium hydroxide (NaOH) to each of the 3 test tubes. Biuret's Reagent only works in the presence of a strong base. **Caution:** if you splash NaOH on your skin, wash it off immediately with water.
3. Add 5 drops of Biuret Reagent to each test tube, swirling *gently* after each addition.
4. Identify your positive negative controls. Did they react as expected?
5. Compare the unknown sample to your positive and negative controls.
6. In the Table 3, write a "+" if the unknown turned violet, or a "-" if the substance did not turn violet. Record results for your controls too.

Procedure 3: Reducing Sugar (monosaccharide) Test

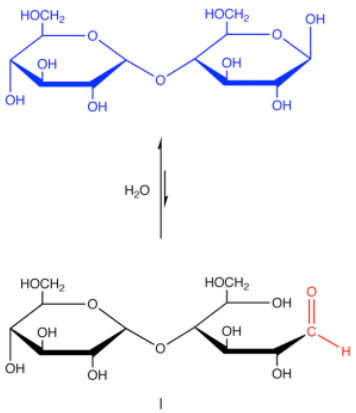

Figure 2: a reducing sugar. Monosaccharides can change between ring form and linear form in water, and can be reduced by having a hydrogen atom added to the oxygen in the ring. Here, a disaccharide (maltose), shows one sugar ring can open up and a hydrogen atom be added—reducing the sugar. Long chains of monosaccharides bound together (polysaccharides) and some disaccharides cannot do this.

Benedict's reagent is an indicator for monosaccharides (and a few disaccharides). In the presence of monosaccharides, it starts out blue and changes to a green and eventually reddish orange when heat is applied.

1. Label 3 test tubes with numbers near the top of the tubes to ensure they don't come off in the hot water bath. Add 3 mL of unknown to #1, positive control to #2, and negative control to #3.
2. Add 10 drops of Benedict's Solution to each test tube, swirling *gently* after each addition.
3. Place the test tubes in the hot water bath for 3-5 minutes. Remove the test tubes using test tube holders. **Caution:** It is HOT!
4. Examine your positive control and your negative control.
5. Compare the unknown sample to your positive and negative controls.
6. In the Table 3, write a "+" if your substance turned green or orange, or a "-" if the substance did not turn green or orange. Record results for the controls too.
7. Dump all liquids down the sink when instructed.

Procedure 4: Test for Starch (a complex carbohydrate)

Figure 3: Starch consists of a chain of glucose molecules that forms a helix

Potassium Iodine (IKI) is an indicator for starch. It is brown but interacts with the specific 3-dimensional shape of starch to become blue/black.

1. Label 3 test tubes. Add 3 mL of unknown to #1, positive control to #2, and negative control to #3.
2. Add 5 drops of Potassium Iodine to each test tube swirling *gently* after each addition.
3. Identify your positive control and your negative control.
4. Compare the unknown sample to your positive and negative controls.
5. In the Table 3, write a "+" if your substance turned blue/black, or a "-" if the substance did not turn blue/black. Record results for your controls too.

Procedure 5: Synthesis

Write your group's results on the white board and copy other groups' results into table 4.

Table 4. Class results

Lab group number	Lipid	Protein	Monosaccharide	Starch	Food prediction
1					
2					
3					
4					
5					

Questions

1. Were you able to determine your unknown?

2. What evidence do you have to support this guess?

3. What is the function of your group's food in the organism it comes from?

4. Compare the results of the potato juice to the onion juice. What does it tell you about how each plant stores energy?