Laboratory Exercise #4: Biologically Important Molecules

Introduction:

The major groups of biologically important molecules are: Carbohydrates, Lipids, Proteins and Nucleic Acids (DNA and RNA). Each group of molecules provides important functions for the living cell. All of these molecular types are considered organic compounds – compounds containing at least carbon atoms and hydrogen atoms. In general, these four groups of molecules are characterized as molecules that are large in size (high molecular weight).

The large molecules of each group (Carbohydrates, Lipids, Proteins and Nucleic Acids) are formed by joining one or more smaller “building block” molecules together in a process called a dehydration synthesis reaction. It is called dehydration synthesis because when two of the “building block” molecules are united chemically (synthesis), a molecule of water is always formed as one of the chemical products (the reacting “building blocks” were dehydrated). Since each group of biologically important molecule uses this process of dehydration synthesis to construct larger molecules, the process of dehydration synthesis should be remembered as an important biochemical process which is used to build big molecules in all living things.

Conversely, when living cells are required to break down large Carbohydrates, Lipids, Proteins or Nucleic Acids, a chemical reaction which is the reverse of dehydration synthesis occurs. This “breaking down” reaction is called hydrolysis. During hydrolysis reactions, water is added chemically to a large molecule in such a way that the large molecule starts breaking up into the smaller types of molecules that were originally used to construct it. Hydrolysis reactions are used by all living things when living things find it necessary to break down or degrade larger molecules. Therefore, this chemical process called hydrolysis should also be remembered.

During this laboratory session, you will learn about the structure and properties of Carbohydrates, Lipids and Proteins and how to test for the presence of these organic molecules. Nucleic Acids will not be studied in this lab. However, you will get a chance to work with DNA in a future lab.

Lab Safety:

Eye safety:
1. You must wear goggles while performing the chemical tests.
2. Know the location and use of the emergency eye wash station.
3. Be aware that other students are conducting their own tests at the lab bench. Be mindful of what’s going on around you.
General:
1. Supply or Stock solutions must be kept “pure”. Never pour extra fluid or chemicals back into a stock bottle.
2. Handle glassware and reagents carefully.
3. Broken glassware must be disposed of properly.
4. Follow directions carefully. Never improvise when conducting chemical reactions – the results could prove dangerous.
5. Never ingest any chemicals or foodstuffs used in the laboratory exercise.
6. When transporting test tubes, always carry them in a test tube rack.
7. When dispensing chemicals from dropping bottles, first loosen the dropper from the bottle by gentle twisting and then continue the procedure.
8. Use caution when a water bath is being used. The hot plate is hot and so is the water!
9. Have your instructor demonstrate how to finger-vortex a test tube.
10. Because of the class size and the limited number of test tubes, each group will have to wash and rinse the test tubes for reuse. Rinse well and turn the tubes over in a test tube rack to drain. (Place some paper towel under the rack).

When testing the available foods:
The different groups in lab should try to divide up the testing responsibilities so that as many of the foods available can be tested using:
1. Benedict’s Test
2. Lugol’s Test
3. Grease spot test
4. Biuret test

Lab Part 1: Carbohydrates
Background:

Carbohydrates are the main type of molecule used by most organisms when energy needs to be provided for living processes or for energy storage. They are also important structural components for many organisms, especially plants (cell walls). The building block (monomer) of carbohydrates is a small molecule called sugar. The simplest sugars are called simple sugars or monosaccharides. They have the molecular formula \((\text{CH}_2\text{O})_n\) where \(n\) may equal any number from 3 to 8. For simplicity, we will assume \(n=6\). This would make the molecular formula for most simple sugars \(\text{C}_6\text{H}_{12}\text{O}_6\). Some examples of simple sugars are: glucose, fructose and galactose. Most cells use glucose as the preferred molecule to provide the energy to make ATP.

Disaccharides are formed by the linking of two monosaccharides together by dehydration synthesis:
C₆H₁₂O₆ + C₆H₁₂O₆ → C₁₂H₂₂O₁₁ + H₂O. Some examples of disaccharides are maltose (malt sugar), sucrose (table sugar) and lactose (milk sugar). These disaccharides are formed as follows:

- glucose + glucose → maltose + water
- glucose + fructose → sucrose + water
- glucose + galactose → lactose + water

Polysaccharides are formed by linking many monosaccharides together by a series of dehydration synthesis reactions. Polysaccharides are used as energy storage molecules. Plants store energy as the polysaccharide called starch. Vertebrate animals, including humans, produce the polysaccharide called glycogen which is stored in the liver and muscle tissue. Plant cell walls contain the polysaccharide called cellulose. Fungal cell walls and the exoskeleton of arthropods contain the polysaccharide called chitin.

Test for Simple Sugars:

We will use a chemical reagent called Benedict’s reagent to test for the presence of simple sugars. When a solution containing Benedict’s reagent and a simple sugar is heated it will change color from the original blue color of Benedict’s reagent to green, orange and finally brick-red. The brick-red color is a positive test for a monosaccharide. Most disaccharides and polysaccharides should give a negative reaction (no brick-red color development).

Procedure for test of the presence of monosaccharides with Benedict’s reagent:

1. Set up a row of 6 test tubes in a test tube rack. Label the first tube #1, the second tube #2 and so on with a grease marking pencil. Make sure the out side of the tube is clean and dry before you try to mark on it.
2. Add 2 mL (40 drops or one dropper full) of the sample to be tested as follows:
   a. Tube #1 – water
   b. Tube #2 – Glucose
   c. Tube #3 – Starch
   d. Tube #4 – Food Choice (your choice-record name in the data table)
   e. Tube #5 – Food Choice (your choice-record name in the data table)
   f. Tube #6 – Food Choice (your choice-record name in the data table)
3. Add 2 mL of Benedict’s reagent to each of the 6 tubes
4. Gently vortex using your fingers.
5. Determine the color of the contents of each tube right after you vortex but before heating. Record this color as the original color in the appropriate spot in the data table labeled “Results of Tests for Carbohydrates” under the Benedict’s Test heading.
6. Now, using a test tube holder, place all 6 tubes in a beaker of boiling water which is on the hot plate for about two minutes. Be careful not to burn yourself or spill the hot water!

7. Using a test tube holder, carefully remove the tubes from the hot water bath and place them in the test tube rack to cool for 1-2 minutes.

8. Observe each tube carefully and record the color of each tube after boiling in the appropriate place in the “Results for Tests for Carbohydrate” data table under the Benedict’s Test heading.

Test for Starch: Lugol’s Iodine Reagent (I₂KI)

Since polysaccharides don’t react with Benedict’s reagent, we need a different test to detect the presence of polysaccharides. Lugol’s iodine reagent changes from a yellowish-brown color to blue-black color in the presence of starch. Monosaccharides and disaccharides do not react with Lugol’s iodine.

**Procedure for Starch (polysaccharide) Test**

1. Set up another row of 6 clean test tubes. They do not have to be completely dry except where you are trying to mark them. Label them 1-6 as you did in the preceding test.

2. Add 20 drops of the same samples to the same numbered tubes as you did in the previous test.

3. Record the color of each tube in the “Original Color” column under Lugol’s Test in the Tests for Carbohydrates data table.

4. Add 2-3 drops of Lugol’s reagent to each of the 6 tubes.

5. Vortex gently by hand.

6. Observe the tubes and record these results in the “Final Color” column under Lugol’s Test in the Tests for Carbohydrates data table.

Lab. Part #2: Lipids

**Background:**

Lipids are organic molecules that are insoluble in water and other polar solvents. However, lipids are soluble in nonpolar solvents such as chloroform, benzene and ether. Lipids include fats and oils (important as energy storage compounds), phospholipids and glycolipids (part of the structure of cell membranes), waxes (protective surface coatings on many plants and animals) and steroids (found in most cell membranes and many hormones). Oils are liquid at room temperature and fats are solid. Both are triglycerides formed by combining 3 fatty acid molecules with 1 molecule of glycerol by the process of dehydration synthesis. The next page shows a typical fatty acid and glycerol. Also show is a typical fat that would be formed by the dehydration synthesis of 3 fatty acids uniting with one glycerol molecule. The resulting water molecules formed by this reaction are also shown. Some fatty acids have some double covalent bonds in the carbon “back bone” such as the one shown. This type of fatty acid is referred to as unsaturated. Fatty acids that have no such double bonds (like those used to form the typical triglyceride on the bottom of the next page) are called saturated.
linolenic acid, an omega-3 fatty acid
(the omega carbon atom is shown in blue)

Glycerol

Tristearin

This is a typical triglyceride
Tests for Lipids

I. Grease spot test for lipids:
   You perform this test every time you buy muffins of doughnuts in a paper bag. Lipids make unglazed paper translucent (almost clear).
   1. On 8 separate pieces of unglazed paper: place one drop of water, mineral oil, corn oil, olive oil, starch solution and 3 different foods of your choice on separate pieces of the unglazed paper.
   2. With a pencil, draw a circle around the spot and write the name of the sample on the piece of paper.
   3. Allow the spot to dry thoroughly. Go ahead with the rest of the lipid tests in the next section that follows and then come back (in about 30 minutes) and analyze your results for this test!
   4. Hold the paper in front of a light source and observe the spots.
   5. Record your results in the “Grease Spot Test” column in the Tests for Lipids data table. Use the words – Translucent / not translucent to record your observations.

II. Solubility in Polar and Nonpolar solvents:
   Lipids are insoluble in polar solvents and soluble in nonpolar solvents. For this test, the polar solvent is water; the nonpolar solvent is mineral oil (a mixture of hydrocarbons).
   1. Set up two rows of 8 clean test tubes in a test tube rack. Number the tubes in each row 1-8 with a grease marking pencil.
   2. Add 1 mL (~20 drops) of water (a polar solvent) to each of the 8 tubes in one row. This row will be used first and then saved for the Sudan Red Test.
   3. Add 1 mL (~20 drops) of mineral oil (a non-polar solvent) to each of the 8 tubes in the other row. This row will be used after the first row has been used.
   4. Add the following to the row of test tubes that contain water:
      a. Tube #1 – add 1 mL of water
      b. Tube #2 – add 1 mL of mineral oil
      c. Tube #3 – add 1 mL of corn oil
      d. Tube #4 – add 1 mL of Olive oil
      e. Tube #5 – add 1 mL of starch solution
      f. Tube #6 – add 1 mL of the first food used in the grease spot test
      g. Tube #7 - add 1 mL of the second food used in the grease spot test
      h. Tube #8 - add 1 mL of the third food used in the grease spot test
5. Vortex each tube fairly vigorously by hand.
6. Wait 5 minutes
7. Examine each tube carefully. Has the sample dissolved in the water or do you see two separate layers?
8. Record your observations in the appropriate place in the Tests for Lipids data table. If soluble record as (+) ; if insoluble record as (-).
9. Save these first 8 tubes for the next Sudan Red Test for lipids!!
10. Repeat steps 4-8 for the second row of 8 test tubes that contain mineral oil as the solvent. Don’t forget to record your results in the Tests for Lipid data table.
11. Wash and rinse the test tubes.

Sudan Red Test: Sudan Red is a lipid soluble dye. When it is added to a mixture of lipids and water, the dye will preferentially stain the lipid containing layer coloring it red. As with all chemical reagents, do not get Sudan Red on your skin. Wash immediately if you do.

1. Add 5-7 drops of Sudan Red dye to each tube that you saved from the solubility exercise you just completed.
2. Vortex each tube by hand.
3. Wait 3-10 minutes.
4. Examine each tube carefully. Where is the red color found?
5. Record your observations in the appropriate place in the Tests for Lipids data table. Write water if the dye remains in the water. Write lipid if the dye is concentrated in the lipid
6. Clean and rinse the test tubes.

Lab Part III. Proteins

Proteins are complex, highly specialized molecules composed of the atoms C, H, O, N and usually S. The monomers for protein synthesis are called amino acids. There are 20 different amino acids that are commonly used to build proteins. With few exceptions, all living things use these same amino acids to build their protein molecules. The following diagram represents the structure of a typical amino acid. The center of the molecule is the alpha carbon which is bonded to four different groups: (1) an amino group [NH₂] which attracts a proton at neutral pH to become [NH₃⁺], (2) a carboxyl group [COOH] which loses a proton at neutral pH to become [COO⁻], a hydrogen atom [H] and (4) the “R” group (also called the side chain). The different amino acids have different R groups – otherwise the twenty amino acids have identical structures. The diagram below illustrates the amino acid Alanine. If the R group was changed to just a H atom, the amino acid would be glycine.
Proteins are built by linking amino acids sequentially by dehydration synthesis. This is shown on the next page using the amino acids glycine, alanine and cysteine.

Notice that dehydration synthesis is the same process that is used to unite the monomers in carbohydrates and lipids. This holds true for nucleic acids as well.

Proteins have important roles in living organisms. Proteins can serve as antibodies, hormones, receptors, enzymes, to provide support, to regulate gene expression, contractile structures (muscle and cilia/flagella) and transport such as hemoglobin.

**Tests for Proteins and Amino acids**

**Biuret test for Proteins:** Biuret reagent is a light blue solution which must turn purple when mixed with a solution to indicate the presence of proteins.
**Procedure:**

1. Label a set of 8 clean test tubes numbering them 1-8 with a grease marking pencil.
2. Add 2 mL (40 drops) of sample as follows:
   a. Test Tube #1 – Water
   b. Test Tube #2 – Albumin
   c. Test Tube #3 – Alanine
   d. Test Tube #4 – Glucose
   e. Test Tube #5 – Food of Choice
   f. Test Tube #6 – Food of Choice
   g. Test Tube #7 – Food of Choice
   h. Test Tube #8 – Food of Choice
3. Add 2 mL of biuret reagent to each tube.
4. Vortex each tube by hand.
5. Wait 2-3 minutes.
6. Examine each tube carefully. Note the color.
7. Record your color observations under the Biuret heading in the Results for Proteins and Amino Acids Data Table. When answering the question “Is protein present”, respond “yes” or “no”.

**Ninhydrin Test for Amino Acids:** Because individual amino acid molecules contain a free amino group, they are readily detected with the ninhydrin reagent which reacts chemically with free amino groups to form a purple or violet color.

**Procedure:**

1. Obtain 8 small pieces of filter paper and put a drop of each sample that was used in the previous biuret test in the center of the filter paper.
2. Draw a circle around each spot with a pencil and write the name of the sample next to the spot.
3. Allow all of the spots to dry thoroughly. You may facilitate this process if a hair dryer or drying oven is available.
4. When the spots are dry, ask your lab instructor to put a drop of ninhydrin reagent on each spot.
5. Wait at least 20 minutes.
6. Observe each spot carefully.
7. Record your observations in the appropriate places in the Results for Proteins and Amino Acids data table.
Possible Food Choices:

The following are some of the foods that may be available:
1. Skim Milk
2. Whole Milk
3. 7 UP soda
4. Diet 7 UP
5. Egg White
6. Egg Yolk
7. Potato
8. Navy Beans
9. Onion

Again, the different groups in lab should try to divide up the testing responsibilities so that as many of the foods available can be tested using:
5. Benedict’s Test
6. Lugol’s Test
7. Grease spot test
8. Biuret test
### Part I  Data Table

(The following data tables may contain extra categories - disregard if necessary)

#### Results of Tests for Carbohydrates

<table>
<thead>
<tr>
<th>Tube Contents</th>
<th>Benedict's Test</th>
<th>Lugol’s Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original Color before boiling</td>
<td>Final color after boiling</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>_______</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>_______</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>_______</td>
<td></td>
</tr>
</tbody>
</table>

### Part II  Data Table

#### Results of Tests for Lipids

<table>
<thead>
<tr>
<th>Tube contents</th>
<th>Solubility Test</th>
<th>Sudan Red Test</th>
<th>Grease spot Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Is it soluble in a polar (water) solvent? How many layers?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Is it soluble in a non-polar (oil) solvent? How many layers</td>
<td></td>
<td></td>
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<tr>
<td>Water</td>
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<td></td>
<td></td>
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<tr>
<td>Mineral Oil</td>
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<td></td>
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<tr>
<td>Corn Oil</td>
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<td></td>
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<tr>
<td>Olive Oil</td>
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<td></td>
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<tr>
<td>Starch</td>
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<td></td>
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<tr>
<td>Food</td>
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<td></td>
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<tr>
<td>Food</td>
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<tr>
<td>Food</td>
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</tr>
</tbody>
</table>
Part III  Data Table

Results of Tests for Proteins and Amino Acids

<table>
<thead>
<tr>
<th>Tube Contents</th>
<th>Biuret Reaction</th>
<th>Ninhydrin Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color after 2-3 minutes</td>
<td>Is Protein Present?</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
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<tr>
<td>Alanine</td>
<td></td>
<td></td>
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<tr>
<td>glucose</td>
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<tr>
<td>Food - ______</td>
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<td>Food - ______</td>
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</tbody>
</table>

When you have finished all your work:
1. Wash, rinse and air dry all the test tubes you used in test tube racks.
2. Return all the reagents and other equipment to the proper places.
3. Make sure that your laboratory work area surface has been cleaned
4. Make sure that all group members have all the test data for the known samples and unknown samples
5. Make sure that you wash your hands thoroughly before leaving
6. Make sure that all hot plates are turned off
Writing Assignment – Biologically Important Molecules

Please answer these questions in Google Docs. You must attach your data tables or you can turn those in at the beginning of lab next week.

Please write in prose. Do not number your answers. Rather, embed your answers in a series of paragraphs.

- Discuss the results for the carbohydrates tests.
  - What are carbohydrates
  - What 6 items did you test
  - What did the Benedict's test tell you about each
  - What did the Lugol’s test tell you about each
  - Are the tests consistent? Explain
  - Be sure to thoroughly explain all of your conclusions

- Discuss your results for the lipids tests
  - What are lipids
  - What 8 items did you test
  - What did the solubility test tell you about each
  - What did the Sudan red test tell you about each
  - What did the grease spot test tell you about each
  - Are the tests consistent? Explain
  - Be sure to thoroughly explain all of your conclusions

- Discuss your results for the proteins and amino acids tests
  - What are proteins
  - What 8 items did you test
  - What did the Biuret test tell you about each
  - What did the ninhydrin reaction tell you about each
  - Are the tests consistent? Explain
  - Be sure to thoroughly explain all of your conclusions

- How are carbohydrates, lipids and proteins similar? How do they differ?