



Mission 10

Chemical Tests for Life—Tests for Carbohydrates and Proteins as Signs of Life

Overview

In mission 10, students test for carbohydrates and proteins in four soil samples. They may also test samples from their own bodies and from other organic and inorganic materials. They ponder the nature of positive-negative tests and the implications of negative test results, which could mean two things: that life was not present or that life may have been present but was not detected. Mission 10.1 focuses on carbohydrates; mission 10.2 focuses on proteins.

Notes

In mission 9, students discovered one way to test for life in the soil of another planet. Can we also detect life by detecting the chemicals that life-forms on other planets are made of? All life-forms need energy in an easily accessible form and information” that tells them how to live. There are only a few atoms that can form complicated molecules to carry out these functions. Carbon is one such atom. Silicon may be another. All life-forms on Earth use the same types of carbon-based molecules: carbohydrates (sugars and starches), proteins, lipids (fats and oils), and nucleic acids (DNA and RNA). These molecules are made of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and a few other atoms, including phosphorus (P) and sulfur (S), all of which are common everywhere in the universe.

Mission 10.1 Materials

For a Class of 30

- Butcher Paper
- Samples of organic material (e.g., potato, dried leaves, wool, sawdust, human hair, human nails, skin cells, beef jerky, feathers)
- Samples of inorganic material (e.g., plastic, sand, steel wool, plaster, salt)
- Large beaker
- Heat-resistant pad (asbestos recommended)
- Sugar
- Concentrated hydrochloric or sulfuric acid
- Cold cookie sheet or equivalent
- Baking soda
- Iodine

- Box of cornstarch
- 3 cups clean or sterile fine-grained sand
- 4 seltzer tablets (Alka Seltzer works well)
- 8 1/4-ounce packages of yeast
- 4 tablespoons of dried potato flakes
- 4 soil-sample bowls for the soil
- Stick-on labels or a grease pen

For Each Team

- 4 50-ml beakers
- Mortar and pestle
- Dropper bottle of iodine
- 4 soil-sample carrying dishes for the soil
- “Testing for Carbohydrates” directions
- *(optional)* Bunsen burner and/or matches
- *(optional)* Tongs
- *(optional)* Mirrors or watch glasses
- *(optional)* Toothpicks
- *(optional)* Nail files
- *(optional)* Scissors
- *(optional)* Distilled water
- *(optional)* Test tubes

For Each Student

- “Testing for Carbohydrates” worksheet
- Safety glasses
- Pencil

Getting Ready

1. Make a large data table on butcher paper for the carbohydrate (iodine) test. Write team numbers 1-15 along the top and materials to be tested down the left side so that teams can Mark ‘+’ for a positive result or “-” for a negative result for each material tested.
2. Make four soil samples as follows. (These amounts make enough for 15 teams.) The first two soil sample mixtures will be made again in mission 12 (make enough for both missions now and/or save any leftovers from this mission). All four sample mixtures will be used again in mission 10.2.

Earth Sample # 3-Crush the seltzer Tablets into fine pieces using the back of a spoon to press on the packets before they are opened. Open the packets and mix the seltzer

with tablespoons of the sand. Put the mixture into a bowl and label it “Earth Sample # 3.” (Add 1/4 of a seltzer tablet and 1 tablespoon of sand for each extra team.)

Earth Sample # 4-Mix the yeast with 16 tablespoons of the sand. Put the mixture into a bowl and label it “Earth Sample # 4.”

Earth Sample # 5-Mix the dried potato flakes with 16 tablespoons of the sand. Put the mixture into a bowl and label it “Earth Sample # 5.”

Earth Sample # 6-Put 16 tablespoons of pure sand into a bowl and label it “Earth Sample # 6.” This will be the control. Microwave this sample to be sure that it is sterile.

3. Copy the “Testing for Carbohydrates” directions for each team and the “Testing for Carbohydrates” worksheet for each student.

Classroom Action

1. **Lecture.** Review safety procedures. Your school probably has a printed set of specific procedures. Ask students to secure long, loose hair and floppy sleeves with rubber bands.
2. **Demonstration.** Put some sugar (a carbohydrate) into a beaker. Slowly add concentrated hydrochloric or sulfuric acid. At some point, there will be a puff of steam (water vapor) and the sugar will bubble up and turn into a lump of carbon. (Pouring baking soda over it will neutralize it for safe disposal.) Hold a cold cookie sheet over the beaker while you pour the acid. This will let the water condense onto the cookie sheet.

Ask students if the escaping vapor is composed of water molecules. Can we be sure just by looking? How *can* we be sure? (*We could test the condensed liquid as was done in mission 7, “Water!”*) Ask students if the black matter is carbon. How can we be sure? (*We could test it.*) Ask students what might happen if they added heat energy instead of chemical energy to a carbohydrate sample. Using a Bunsen burner or matches, char several organic and inorganic samples in front of the class. Have students observe the charring process carefully. If you wish the class to try this on their own, show them how to safely char powdered substances in test tubes. Always handle hot test tubes with tongs, and always wear safety glasses.

3. **Discussion.** Invite the class to brainstorm about what kinds of things will char over a flame, then about what things won't char. Make two lists on butcher paper. Ask students to discover the major single difference between the two lists of items. Lead the class to the conclusion that items on the first list are living (or were once living) organic materials (*e.g.*, wood, toast, rice). Items on the second list are nonliving, inorganic materials (*e.g.*, metals, stones, plastics, glass). Describe to students the charring process: 1) the temperature is raised very high, 2) a lot of steam (water

vapor) escapes, and 3) charred residue is left behind. Ask students what steam is. *Water vapor.*) What the black charred matter is. *(Carbon. Before burning, water and carbon were joined together in a carbohydrate molecule. The fire caused a chemical reaction in which the water molecules escaped, leaving the carbon atoms behind. Before the reaction, the carbohydrate often looks very different.)*

4. **Optional Activity.** Flame test for carbohydrates. Ask students to test some different samples of your choice to see whether or not they are organic materials. They may heat them up in test tubes or hold them directly over a flame. (If you feel this cannot be done safely in the classroom, do a demonstration instead.) Each test should be considered “positive” (+) if carbon forms and steam is given off, and “negative” (-) if this does not happen.
5. **Discussion.** Starch is a complex carbohydrate. Though simple carbohydrates such as glucose can form from nonliving reactions, starch is too complex to form in that way. The presence of starch is good evidence that life is, or was, present, because it must have been produced by a living organism. Ask students how living organisms can make compounds that are not found in nature (produced abiotically). *(They use organic catalysts called enzymes to instigate chemical reactions.)*
6. **Activity.** Divide the class into teams of two students each. Hand out the “Testing for Carbohydrates” directions to each team and the “Testing for Carbohydrates” worksheet to each student. Ask students to determine what a positive test for starch would look like by adding a drop or two of iodine to one teaspoon of cornstarch into a small test-tube. Students rinse all test tubes with water (distilled preferred).

POSITIVE: A change from yellow-brown to blue-black is a positive (+) result, meaning that starch is present. This indicates that life is, or was, present in the sample. In this case, the corn was alive.

NEGATIVE: No color change is a negative (-) result. It means that starch is not present, or not detectable for some reason (sample may not be ground up well enough for the iodine to reach the starch or the concentration of iodine may be too low).

“Soil samples.” Students use their four carrying dishes to obtain one teaspoon. or less of each of the four soil samples. Students should put each soil sample into a separate test tube, label the four test tubes, add a few drops of iodine to each test-tube, and look for a color change. Teams should record all their results on the class chart.

7. **Discussion.** Ask students which soil samples contained life (either alive or once was alive). *(Earth Sample # 5.)* Ask students which soil samples did not contain life. *3, # 4, and # 6, because these samples gave negative test results.)*

Now tell students what was in each soil sample. They may be surprised by the yeast, a life-form they did not detect! Note that a negative (-) result does not mean that there is no life; it only means that there is no starch. Life may still be present, because not

all living things produce or store starch. But life may also be absent. A negative result is inconclusive.

8. **Activity.** Have students test other samples of your (or their) choice for the presence of starch. A slice of raw potato gives an excellent reaction. Some samples may need to be ground using the mortar and pestle before iodine is used. Be sure to clean test tubes between samples.

Mission 10.2

Materials

For a Class of 30

- 5 grams of Ninhydrin powder (see “Ordering Information,” in appendix)
- Cooked chicken breast (or light-colored lunch meat)
- Butcher paper
- Samples of organic material (*e.g.*, potato, dried leaves, wool, sawdust, human hair, human nails, skin cells, beef jerky, feathers)
- Samples of inorganic material (*e.g.*, plastic, sand, steel wool, plaster, salt)

For Each Team

- 4 test tubes
- Hot water baths (60-70° C recommended)
 - Ideal lab equipment
 - Beaker (about 150 ml)
 - Water (distilled)
 - Thermometer
 - Bunsen burner
 - Ring stand and rack (optional) Any container with heated water poured into it
- Dropper bottle of Ninhydrin solution
- Tweezer⁰
- 4 soil-sample carrying dishes for the soil
- (optional) Mortar and pestles
- (optional) Toothpicks
- (optional) Nail files (optional) Scissors
- “Testing for Proteins” directions

For Each Student

- “Testing for Proteins” worksheet
- “A Biochemical Search for Life” worksheet
- Safety glasses
- Pencil

Getting Ready

1. Make a large data table on butcher paper for the protein (Ninhydrin) test. Write team numbers 1-15 along the top and materials to be tested down the left side so that teams can mark '+' for a positive result or '-' for a negative result for each material tested.
2. Dissolve the Ninhydrin powder into the water to form a saturated solution. Students will need easy access to the Ninhydrin solution. If possible, provide 15 dropper bottles or small labeled beakers of Ninhydrin solution at a central station.
3. Copy the "Testing for Proteins" directions for each team and the worksheets "Testing for Proteins" and "A Biochemical Search for Life" for each student.

Classroom Action

1. **Discussion.** Brainstorm as a class or in teams about proteins. What are they for? Why do we need them? (*Structure and function of the human body.*) Where do we get them? *We eat food containing protein.*) Can you "uncook" an egg? Why not? (*The proteins have been tangled and bent by exposure to heat; there is no way to fix this. Proteins are long, fragile molecules.*)

Talk with students about amino acids. There are 20 or so common amino acids, including glycine and alanine. All living things that we know of have proteins that are made from combinations of these same amino acid building blocks, which are linked by peptide bonds in long chains, but in different numbers and sequences. (You may want to do the "Going Further 'Exploring Combinations' " activity now.) Proteins are complex molecules. Though amino acids can form from nonliving reactions, proteins are too complex to form and persist in most natural situations. The presence of proteins is also good evidence that life is, or was, present, because they must have been produced by a living organism.

2. **Activity.** Reassemble the class into the previous teams of two students. Hand out the Student Fact sheet: "Biochemistry of Proteins" and the Student Worksheet: "Testing for Proteins." Tell students that they will be testing samples for the presence of proteins. They determine what a positive test for protein would look like by adding a drop or two of Ninhydrin solution to one teaspoon or less of a known protein, chicken muscle. The chicken muscle should be cooked, since raw chicken may contain *Salmonella*. Light-colored lunch meat may be substituted.

Students add enough of the Ninhydrin solution to cover the chicken, then set the test tube in a hot water bath (about 60° C; cooler temperatures will produce slower reactions, about 5-10 minutes; hot tap water should. This reaction takes one to two minutes.

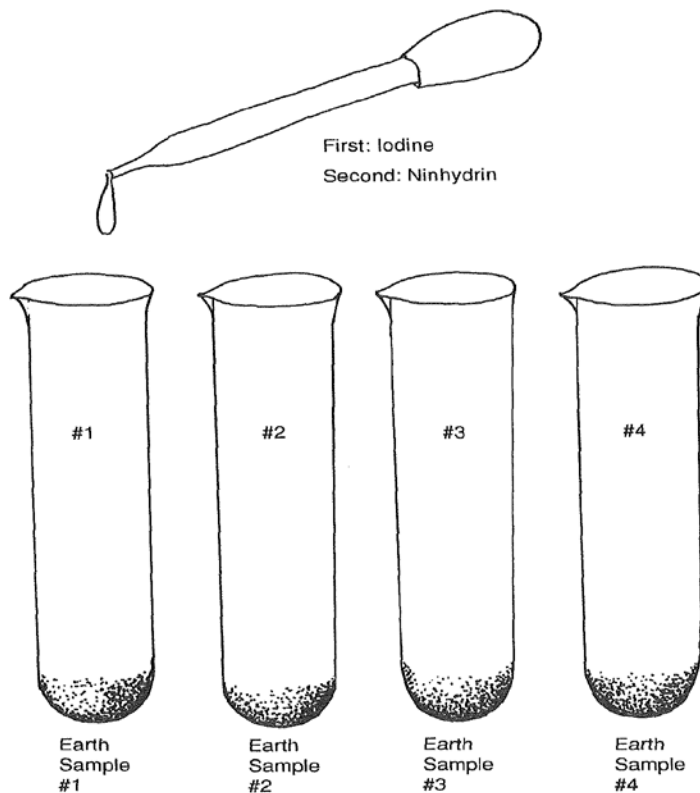
Teacher's Note: Caution! Have students avoid touching the Ninhydrin solution; it will identify the proteins in their skin by turning it blue! Have students rinse all the mortar and pestles and test with water (distilled preferred).

POSITIVE. A change to blue is a positive (+) result, meaning that protein is present. This indicates that life is, or was, present in the sample. In this case, the chicken was alive.

NEGATIVE. No color change is a negative (-) result. It means that protein is not present, or not detectable for some reason.

“Soil Samples.” Students use their four carrying dishes to obtain one teaspoon or less of each of the four soil samples. Students should put each soil sample into a separate test-tube, label the four test tubes as shown in figure 10.1, cover the soil with Ninhydrin solution, put the four test tubes in the hot water bath, and look for a color change. Students should test all four soil samples. Teams should record all their results on the class chart.

Figure 10.1—Experimental Setup.



Teacher's Note: *Test results* Earth Sample # 3 (seltzer tablets; no life) (-) Earth Sample # 4 (yeast; life) (+) Earth Sample # 5 (potato flakes; life) (+) Earth Sample # 6 (only sand; no life) (+)

3. **Discussion.** Ask students which soil samples contained life according to this test. (*Earth Samples # 4 and # 5; This test does show the living yeast.*) Ask students which soil samples did not contain life. (*Earth Samples # 3 and # 6; the same as mission 10.1.*) But what if these soil samples were from Mars, and no one knew what was in them? Could there be undetected life? Note that a negative (-) result does not mean that there is no life; it only means that there is no protein. Life may still have been present. Perhaps the delicate proteins were destroyed before the test was done. Perhaps alien life would not have proteins. Perhaps the proteins are isolated in some kind of inorganic shell. In those cases, life would still be present; a negative result is inconclusive.
4. **Activity.** Have students test other samples of your choice for the presence of protein, a sign of life. They may collect organic samples from themselves. Students can gently scrape some cells from inside their cheeks, produce some spit (saliva contains proteins), file their nails, and cut up a little bit of hair. It is important to grind up things like hair in a mortar and pestle to free the proteins so that they can react. (Some reactions are slower, and dark hair may not show.)
5. **Discussion.** Give teams a chance to share what they found or learned. There are likely to be items that some teams marked '+' while others marked them '-'. Discuss what this could mean, and the notion of "majority vote." Scientists never use a majority vote. Instead, they require that the results of an experiment be reproducible and corroborated by subsequent investigation. However, the interpretations of results are sometimes debatable. What is the reliability of this test? How might it be possible to get a "false-positive" test? (*Contamination, etc.*) How good would these tests be in determining if there was life on Venus or on Mars?
6. **Activity:** Hand out "A Biochemical Search for Life" worksheet to each student and have students solve these problems either in class or as homework.

Going Further

Activity: Grease Stains

Lipids (fats and oils) are complex organic Molecules that are characteristic of life. An easy way to recognize a lipid is by the grease stain that it makes on paper. Have students test various substances for lipid content. Include high-lipid substances such as vegetable oil, butter, and french fries. Ask students if they can figure out a way to test for lipids in an alien soil or atmospheric sample.

Activity: Exploring Combinations

Ask students to make words using the letters in protein. Give students points for each word (and bonus points for longer words). Decide whether they may use the same letters twice or not. Ask students to do this as a homework assignment or as an in-class team-brainstorming contest.

Tell students that proteins form in the same way: different sequences of amino acids make different proteins (the same way ten and net are different words). Proteins are made from combining about 20 different kinds of commonly found amino acid building blocks into long chains. Typical proteins are hundreds of amino acids long.

Ask students how many different words can be made with 26 letters. Just look at a dictionary! How many different proteins could be made with 20 amino acids?

Research: Vegetarians and Ecology

Students have probably heard that they need meat or dairy products to get enough protein in their diet. Ask them if they believe this. Explain to them that these foods do not have a monopoly on proteins, and that proteins are found in all living cells, both animal and plant. It is possible and feasible for our bodies to make all the protein we need from a variety of vegetable foods (which includes grains). Some vegetarians believe that eating vegetables is better than eating meat because it cuts out so much unnecessary cholesterol from our diet, and because raising so much livestock is such a drain on our environment. Cattle raising can lead to destruction of the rain forest, overgrazing, erosion, loss of critical habitat for endangered species, and even global warming (from methane production)! Of course, some beef can be produced at sustainable levels using ecologically sound practices.

Research: Nutrition and Proteins

Discuss amino acids with students. A protein that contains all the essential amino acids that a body needs is called a complete protein. A protein that does not contain all the essential amino acids is called an *incomplete* protein. Beef, pork, and chicken contain complete proteins. Corn and beans contain incomplete proteins, if a person ate only corn, or only beans, she or he would become ill because some amino acids would be missing. However, if a person ate corn and beans in one meal, she or he could stay healthy because corn has the amino acids that beans are missing and beans have the amino acids that corn is missing.

Chemical Tests for Life

Tests for Carbohydrates and Proteins as Signs of Life

Testing for Carbohydrates--Teacher's Key

Table 10.1-Carbohydrates Test Results, Teacher's Key (Answer to #1 on page 188).

Sample	Is Iodine Test + or - ? Describe Color Change	Is Starch Present	Was Life Present?
Cornstarch	+ From yellow brown to blue-black	Yes	Yes (Corn)
"Earth Sample #3"	- No color change	No	No (Seltzet)
"Earth Sample #4"	- No color change	No*	Yes (Yeast)
"Earth Sample #5"	+ From clear to blue	Yes	Yes (Potato)
"Earth Sample #6"	- No color change	No	No (Sand)

*Students will say "No" because they did not detect any starch. Discuss this!

- In a "negative" iodine test there is no color change. A negative result means that starch is not present. However, life may be present.
- In a "positive" iodine test, the iodine turns color from yellow-brown to blue-black. A positive result means that starch is present. This means that life is (or was) present.
- An exobiologist would care about starch because it would indicate the presence of life, because only living things produce starch.

Testing for Proteins-Teacher's Key

Table 10.2-Protein Test Results, Teacher's Key (Answer to #1 on page 191).

Sample	Is Ninhydrin Test + or - ? Describe Color Change	Is Protein Present	Was Life Present?
Chicken Muscle	+ From clear to blue	Yes	Yes (Chicken)
"Earth Sample #3"	- No color change	No	No (Seltzet)
"Earth Sample #4"	- From clear to blue	Yes*	Yes (Yeast)
"Earth Sample #5"	+ From clear to blue	Yes	Yes (Potato)
"Earth Sample #6"	- No color change	No	No (Sand)

- In a "negative" Ninhydrin test there is no color change. A negative result means that protein is not present.

3. In a “positive” Ninhydrin test the color changes from clear to blue. A positive result means that protein is present.
4. Earth Sample # 4 seemed to have no life (based upon the negative test for starch), but the protein test was positive, so there must be some life there (there are yeast cells present).

Chemical Tests for Life

Tests for Carbohydrates and Proteins as Signs of Life

A Biochemical Search for Life-Teacher's Key

1. Based upon these data, there is life in the Mariner Valley! Although the carbohydrate tests were negative, the positive result for protein must be interpreted as indicating life. (Alternative possibilities include a contamination of the sample with protein brought from Earth, or some alien geochemistry that produces proteins without life.) The char test for carbon may be negative if the protein in the soil is too scarce to be revealed by this test.
2. Based upon these data, there is no life in the Rhea Mons area. All the tests were negative. (One could still argue that alien biochemistry would be too different for this to be a conclusive result, or that the random sample missed collecting life.)
3. The iodine turns blue-black, indicating a positive reaction; there is starch in this glob! The Ninhydrin solution does not change color, indicating a negative reaction; there is no protein in this glob! However, one positive is enough to indicate that the glob is (or was) alive. Or at least that parts of it are (were) alive. (It may be forgotten junk food; it may be a pretty pink mold.)