How-To-Do-It

Mystery of the Crooked Cell

An Investigation & Laboratory Activity About Sickle-Cell Anemia

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In the spring of 1992, we joined a team of scientists and educators to create laboratory activities for a new program called "CityLab" located at the Boston University School of Medicine. CityLab is a biotechnology learning laboratory for middle and high school teachers and students funded by the National Institutes of Health and the Howard Hughes Medical Institute. The program is designed to provide laboratory facilities and curriculum in biotechnology unavailable to most school systems. We sought to create meaningful investigations for students and teachers at CityLab, one that would engage students and encourage them to build ideas and concepts based on their prior experiences and knowledge. It was decided that each activity would be built around themes introduced through a scientific question presented as a mystery. Each mystery would lead the students on a thoughtful investigation so that the laboratory experience becomes a tool to solve a problem or acquire needed information. The first unit focuses on sickle-cell anemia, one of the first diseases identified to be at the molecular level. The lesson that follows has been used and refined over the past five years with more than 3,000 students and 200 teachers.

Background

Sickle-cell anemia is a genetic disease that affects the hemoglobin molecule of red blood cells. The hemoglobin molecule is comprised of four polypeptide chains. The most common type of hemoglobin, hemoglobin A, consists of two alpha chains and two beta chains. In sickle-cell anemia, a single amino acid substitution in the beta chain from glutamate to valine causes the molecule to form insoluble chains when the oxygen concentration of the blood is low. These insoluble chains distort the cell, giving it the characteristic sickle shape. The irregularly shaped blood cells lead to a cascade of symptoms. The sickled blood cells become hard and inflexible. Blood no longer flows freely through the capillaries, causing pain and swelling. The sickle-shaped red blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin). Jaundice often results when the liver cannot metabolize bilirubin fast enough.

A sickling episode, or crisis, can be brought on by infection, dehydration, overexertion, high altitude, chills or cold weather. Sometimes there is no apparent precipitating factor. People with sickle-cell disease are susceptible to fevers and infections. There is no cure for sickle-cell anemia. Hydration, bed rest, painkillers, and antibiotics are often prescribed. Recent research has focused on re-expressing the fetal hemoglobin gene. After birth, the gene for fetal hemoglobin turns off while the gene for adult hemoglobin becomes activated. If the gene for fetal hemoglobin could be turned on again, it may compensate for the diseased hemoglobin and provide relief for people with sickle-cell anemia.

This lesson is organized into two parts: a pre-lab and a laboratory investigation. During the pre-lab, students visit learning stations and acquire clues about a mystery disease (sickle-cell anemia). Each station challenges the students to explore different aspects of sickle-cell anemia. Working in groups, students manipulate models and gather data to construct an explanation of how sickle-cell anemia affects the patient at the molecular level. Following the pre-lab, students enter the laboratory where they apply the concepts acquired in the pre-lab to test a fictional patient for the presence of sickle-cell hemoglobin using gel electrophoresis.

Pre-Lab

The purpose of the pre-lab is to explore the connection of hemoglobin to the symptoms exhibited in sickle-cell anemia. It provides students with the opportunity to construct ideas and concepts about the mechanism of the disease based on their prior experience. The objectives of the pre-lab are as follows:

- Observe prepared normal and sickle-cell slides.
- Manipulate models of blood cells to gather data and make inferences about sickle-cell anemia.
- Analyze an inheritance pattern using a pedigree.
- Work cooperatively to explain the symptoms exhibited in sickle-cell anemia.
- Construct an explanation of the mechanism of the disease.

Pre-Lab Materials

- Four microscopes with 1000X capability
- Prepared slides of sickle-cell blood and normal blood (Figures 1a & 1b).
- Prepared slides are available from several biological supply companies; one model of a red blood cell with normal hemoglobin and one model of a red blood cell with affected hemoglobin (Figures 2a & 2b)
- One capillary model with several models of round and sickled red blood cells (Figure 3a)
- Newsprint
- Markers

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Figure 1a. Prepared slide of normal blood magnified 1000× for Station A.

Figure 1b. Prepared slide of sickle-cell blood magnified 1000× for Station A.
Pre-Lab Engagement (10 to 15 minutes)

Organize students in groups of four. Have each team read a description of the patient who came to Dr. Herrick, a Chicago physician, in 1904 (Figure 4). The essential question is, “What is the mechanism of the disease?” Instruct the students to make observations and gather clues about the condition described in the patient scenario. Ask the students to identify and underline any clues in the description that may help them determine the effect of the disease on the patient. When they are finished, invite a student from each team to write two clues on the board. Discuss the clues as a class. Ask for clarification or expansion of ideas where appropriate. Encourage the students to think freely and make connections based on the evidence given in the patient description as well as on their own experience. The discussion usually leads to many good ideas about the mechanism of the disease. However, the students soon determine that they need to explore the disease in greater depth in order to substantiate their ideas and gain a deeper understanding of the disease.

Pre-Lab Exploration (40 to 50 minutes)

To assist the students in their investigation, set up the four stations described below. Each station is comprised of manipulatives that in some way model or illustrate concepts relating to the mechanism of the disease. Give each team descriptions of the stations (Figures 5a–5d) that include directives which encourage exploration. Urge the students to gather observations that may yield insights to the mechanism of the disease. It is helpful to assign the roles of “reader” and “recorder” at each station to facilitate cooperation among team members. Rotate each team through Stations A, B, C, and D, allowing about 10 to 15 minutes per station.

Set up each station as follows:

Station A: Four microscopes with prepared slides of sickle-cell blood and normal blood (Figures 1a & 1b).
Station B: Y connectors, models of normal and sickled red blood cells (Figure 3a). We use Model Magic™ to make the cells.
Station C: Models of red blood cells made from snap-lock beads (available from Fisher Price) and balloons. One model of a cell with normal hemoglobin and one of a cell with...
sickle-cell hemoglobin in red blood cells (Figure 2b).

Station D: Pedigree and pedigree symbol key (Figure 5d).

Pre-Lab Explanation
(20 to 30 minutes)

Collect and display the data and ideas collected by the class by putting four pieces of newsprint around the room and labeling them A, B, C and D respectively. Each team writes its observations for each station on the newsprint, using a different colored marker for each team. If an idea or observation is already written by another group, they need not repeat it. In this manner, all the observations are recorded and each group is required to read the observations of the other groups.

At this junction we find it helpful to ask the students to consider the information the class has collected at the stations and to reflect individually in writing on the essential question, “What is the mechanism of the disease?” Individual reflection gives each student time to collect and organize his/her thoughts in preparation for group discussion.

After individual reflection, ask the members of each team to regroup and synthesize an explanation for the mechanism of the disease. Next, we ask teams to present their explanations to the entire class. We encourage students to be creative in their presentations by giving them the option to present verbally, in writing, with diagrams or concept maps, or by using role play. Students often generate many ideas and interesting topics for discussion. Encourage the students to debate their ideas and consider them in light of the observations they made. We have found that the discussion frequently becomes lively with considerable student-student dialogue. Challenge and elaborate on students’ ideas to lead them to discover the following points:

1. The blood cells are irregularly shaped.
2. The irregular shape of the RBCs interferes with their ability to flow through the blood pathways.
3. The hemoglobin units connect to each other when oxygen concentrations in the blood are low, resulting in abnormally shaped blood cells.
4. The condition is inherited.

Refer to the stations to assist the students’ discovery of the above points. Demonstrate the cause of sickling using the models from Station C (Figure 2c).
Patient Description

In 1904, a student from the West Indies came to a Chicago physician, Dr. James Herrick, with a puzzling condition. Below is a summary of some of the observations Dr. Herrick made. Your job is to learn more about this condition and to find out how the disease is affecting this person’s body. Read the description below and underline the information that you think may provide important clues that will help you understand the disease.

The patient reports feeling well most of the time. But he also reports odd recurring events. For instance, one day after a short swim he became so tired that he could hardly move. He became short of breath and complained of pain in his joints and muscles, especially the arms and legs. He felt unusually weak and required bed rest lasting a few weeks. These symptoms occurred repeatedly during his youth. He also had frequent fevers and infections.

The patient complained of fatigue and soreness in the joints. Upon inspection, the whites of his eyes had a yellowish tint. He complained of pain in the left abdominal area, which was tender to the touch.

A family history reveals that he has two brothers and three sisters. None of them has this problem. His uncle and his grandmother used to have similar attacks. His grandmother died a young woman. His parents do not have this condition.

Figure 4. Patient description.

The blockage created by the sickled cells is illustrated in Station B (Figure 3b), while the prepared blood slides at Station A indicate anemia and irregularly shaped red blood cells (Figure 1b). The family history suggests the possibility that the condition is inherited. At this point, the students are usually curious about the name of the disease. Let them generate their own name for the condition based on their understanding of it and emphasize that their name is just as valid as the name given by Dr. Herrick. He based the name on his observations of sickle-shaped cells and the decrease in the number of red blood cells or anemia.

Assessment

Ask the students to make a concept map depicting what they learned today about sickle-cell anemia. An example is given in Figure 6.

Developing the Concept of Hemoglobin Electrophoresis

The purpose of the laboratory component is to apply the concepts developed in the pre-lab to a clinical test for sickle-cell anemia using gel electrophoresis. The objectives of the laboratory component are to perform gel electrophoresis to distinguish normal hemoglobin from sickle-cell hemoglobin, to interpret the results of gel electrophoresis, and to demonstrate the concept and process of gel electrophoresis.

Before proceeding with the laboratory investigation, it is necessary to make a logical connection to the concepts developed in the pre-lab. In doing so, the laboratory component becomes a tool in the continuum of an ongoing problem rather than an isolated end in itself. The transitional activity that follows links the pre-lab concepts to the ensuing laboratory investigation.

Transition: Creating a Need for the Laboratory Investigation

With the understanding of sickle-cell anemia generated by the pre-lab, ask the students to consider ways to test for the disease. A common response is to examine the blood and look for signs of anemia or sickled cells. Anemia, however, is not unique to sickle-cell anemia, nor are the blood cells necessarily sickled unless the patient is in crisis. Furthermore, thalassemic blood samples frequently look very similar to sickle-cell blood samples (thalassemic is a hemoglobin disorder associated with the defective synthesis of hemoglobin). Since hemoglobin is the molecule affected by the disease, the conclusion is to observe the diseased or affected hemoglobin for characteristics that would distinguish it from normal hemoglobin.

Developing the Concept for the Test

The next goal is to help the students realize the conceptual basis of the test that distinguishes normal hemoglobin from affected hemoglobin. Raise the question by holding up a tube containing a sample of hemoglobin and ask whether they can identify it as normal or abnormal. (We use red food coloring and water to create a light rust color simulates the color of both normal and affected hemoglobin for this demonstration.) The students realize that they first need to see what a normal hemoglobin sample looks like in order to identify whether the unknown is normal. Place control samples of normal hemoglobin and abnormal hemoglobin next to the unknown. Again ask whether they can identify which sample is normal and which is affected by visually comparing the three samples of hemoglobin. The samples look exactly alike. Therefore, a tool is needed to distinguish hemoglobin samples that look identical but have different properties. The tool, electrophoresis, becomes the laboratory component of the investigation.

Electrophoresis Role Play

A role play is used to demonstrate the theory behind electrophoresis. Have two groups of three students come to the front of the room. Each group represents a hemoglobin protein and each person represents an amino acid. Note that both molecules have the same number of amino acids and are therefore the same size. Give each student a card with a number representing a charge of −1 or 0. To one group, assign two −1 charges and one 0 charge. To the other group, give two people 0 charges and one person a −1 charge. Consequently, one group
Station A

Station guide sheet

Recorder ____________________________

Reader ____________________________

At this station you will use a microscope to observe blood samples magnified 1000×. The slide marked P represents the patient’s blood sample. The slide marked N represents the normal blood sample.

Describe (in writing or pictures) the differences you see between the two blood samples.

Figure 5a. Station A guide sheet.
Station B

Station guide sheet

Recorder

Reader

The tubing at this station represents the pathways of blood in the body. Models representing the patient’s red blood cells are also given. Red blood cells must flow freely through the body in order for the blood to do its job of delivering oxygen and picking up wastes. Use these models to show the effect the patient’s red blood cells will have on the flow of blood.

Figure 5b. Station B guide sheet.
At this station you will be given two sets of models. Each model represents a blood cell. One model represents a patient’s blood cell and is labeled “P.” The other model represents a normal blood cell and is labeled “N.” The pieces inside the blood cells represent blood proteins called hemoglobin. Hemoglobin is the oxygen-carrying component of blood. This model uses only a few pieces to represent the millions of hemoglobin units found in real blood cells. Scientists have discovered that abnormal hemoglobin units connect with one another when oxygen levels in the blood are low.

Use these models to investigate what happens to the shape of red blood cells with abnormal hemoglobin when the oxygen levels in the blood are low. Record your results below.
Based on the family history given below, how do you think the patient got the disease? Record your answer on the back of this page.

Key to symbols

- male
- female
- deceased
- affected male
- affected female
- offspring
- parents

Figure 5d. Station D guide sheet.
has a net charge of \(-2\) and the other group has a net charge of \(-1\). Point out that the difference in overall charge between the two molecules cannot actually be seen with the naked eye. However, the charge difference does make the hemoglobin react differently in an electric field. We illustrate this concept by telling the class to imagine the classroom as an electrical field with the positive pole at the back of the room and the negative pole at the front of room. In an electrical field, the negatively charged hemoglobin molecules migrate toward the positive pole. The group with a net charge of \(-2\) will move more quickly because it has a greater negative charge drawing it toward the positive pole. Pretend to turn on the electricity and have the two groups of students migrate as the molecules would. The groups can be distinguished by their different rates of migration with respect to their net negative charge (Figure 7). To check student understanding, have them predict and demonstrate the migration if the molecules both had a charge of \(-2\).

**The Laboratory Investigation: Protein Electrophoresis**

(Solution & sample preparations are given in Figure 8.)

The laboratory component incorporates the same concept as described above. Each student receives three samples of hemoglobin: 1) the patient, 2) normal hemoglobin and 3) sickle-cell hemoglobin. The patient sample may represent sickle-cell hemoglobin, normal hemoglobin, or both in the case of a carrier. The samples of hemoglobin are put into an electrical field and the rates of migration are compared. Procedures vary depending on the electrophoresis system used. General instructions are as follows:

1. **Prepare a 1.3% agarose gel.**

Dissolve by heating the appropriate amount of agarose in the electrophoresis buffer described in Figure 8 and cast the gel. Introduce the function of the gel by comparing it to a track that provides a matrix for the migration of the hemoglobin. The wells act as a starting gate while the gel itself holds the hemoglobin and helps us visualize it.

2. **Prepare the gel electrophoresis box.**

- Orient the gels in the electrophoresis box with the wells at the negative pole.
- Slowly pour the hemoglobin electrophoresis buffer into the electrophoresis box. Fill the electrophoresis box until the gels are covered with a 2 to 3 mm layer of buffer.

3. **Load the samples.**

Put 15\(\mu\)l of the patient sample, 15\(\mu\)l of a normal hemoglobin, and 15\(\mu\)l of sickle-cell hemoglobin into separate wells. The preparation of the samples is described in Figure 8. You can make the patient sample normal, sickled or carrier for each gel. Create a mixture of patient samples for each class so students can compare results.

4. **Electrophorese.**

Connect the cables and run the gels at 100 volts until the bromophenol blue dye has migrated about 30 mm from the wells.

**Interpretation**

Results will vary. Some patient samples will display two bands representa-
### Preparation of Hemoglobin Samples

I. Prepare 500 ml of 1.5M Tris, pH = 9.2, and filter sterile

II. Make hemoglobin stock solution:
   1. Add 250 μl of 1.5M Tris to 5 mg of hemoglobin A.
   2. Add 250 μl of 1.5M Tris to 5 mg of hemoglobin S.

III. Prepare 40 ml of 2X sample buffer:
   8 ml glycerol
   4 ml 1.5M Tris
   28 ml deionized water
   0.01gm Bromophenol blue

IV. Prepare hemoglobin A sample:
   2.25 μl hemoglobin A stock
   6.75 μl 1.5M Tris
   9.00 μl 2X sample buffer

V. Prepare hemoglobin S sample:
   2.25 μl hemoglobin S stock
   6.75 μl 1.5M Tris
   9.00 μl 2X sample buffer

VI. Prepare carrier sample:
   1.125 μl hemoglobin A stock
   1.125 μl hemoglobin S stock
   6.75 μl 1.5M Tris
   9.00 μl 2X sample buffer

### Preparation of Hemoglobin Electrophoresis Buffer

Dissolve 8 g Tris and 3.6 g Glycine in 300 ml of deionized water. Bring total volume to 500 ml.

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The investigation can serve as the centerpiece for a variety of topics to be explored further:

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### Central Dogma

Using the amino acid sequence of the affected and normal hemoglobin, the students can identify the mutation from glutamate to valine, which results in the change in net negative charge of the affected hemoglobin. Working back through the central dogma, they can identify the point mutation in the DNA resulting in the amino acid alteration.

### Inheritance

Genotypes can be derived from the phenotypic results expressed on the gel, and the probability of inheriting sickle-cell anemia can be predicted given the genotypes of the parents (Figure 9).

### Random Mutation/Selective Pressure

Interestingly, plasmodium did not infect humans 10,000 years ago. It was an avian pathogen. However, a mutation in the plasmodium enabled it to jump species and infect humans. We ask the students whether they think anyone could have had sickle-cell anemia in tropical areas. People of African, Asian and Hispanic-Caribbean descent have a higher incidence of sickle-cell anemia. Selective pressure for the allele results from its ability to decrease the mortality rate of people infected with malaria. Malaria is caused by a protocyst in the genus Plasmodium, which is transmitted to human hosts by mosquitoes. Plasmodia infect red blood cells where they multiply and eventually rupture the cell. Cells with sickle-cell hemoglobin are less susceptible to infection by Plasmodia. Therefore carriers (heterozygotes) benefit from the presence of sickle-cell hemoglobin, while remaining largely asymptomatic (some heterozygous individuals may show mild symptoms) with respect to sickle-cell anemia.

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**Figure 9.** Photograph of hemoglobin gel electrophoresis results. Lanes 1 & 3—normal hemoglobin; Lane 4—affected hemoglobin; Lane 5—both normal and affected hemoglobin characteristic of a carrier. Genotypes indicated: Lanes 1 & 3—homozygous normal, AA; Lane 4—homozygous affected, SS; Lane 5—heterozygous, AS.
anemia prior to 10,000 years ago. It often leads to a discussion concerning random mutations and the distinction between a Lamarckian and Darwinian perspective on evolution.

Treatments

We sometimes ask the students how they would treat sickle-cell anemia based on their knowledge of the disease. Bone marrow transplants, blood transfusions, and gene therapy are often mentioned. Recently attention has been given to turning on the fetal hemoglobin gene, which is turned off shortly after birth. Hydroxyurea, which has been used to treat cancer and blood disorders, has been found to stimulate the production of fetal hemoglobin.

The Mystery of the Crooked Cell is one of several activities offered at CityLab which uses problem-based, discovery-oriented experiences to introduce students to techniques and concepts in molecular biology.

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Suggested Readings


Classroom Technology Reviews

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This series of videos is designed for use in grades 6 to 9 to introduce the major concepts in each of the following systems: respiratory, digestive, circulatory, and skeletal and muscle. The blackline masters accompanying each topic include a video-based quiz, a list of a dozen or so terms to be defined, a diagram of the relevant system to be labeled, a classroom activity, and a final quiz on the topic.

Although each video is introduced by a film clip showing up-to-date looking teens participating in an activity related to the topic and contains an occasional glimpse of these same students throughout, the remainder is more reminiscent of a filmstrip than a video. The narration is read rather quickly, with a new vocabulary term appearing for as little as 15 to 20 seconds in some cases. For the most part, a large amount of the factual material is presented in a strictly lecture format not likely to appeal to the intended audience. Unless it were used interactively with the teacher stopping the tape frequently to discuss and review material presented, students will find themselves trying to listen, write, process and remember too many facts in a short time period.

Most of the material is factually correct, although simplified for the target audience, the statement from the respiratory system that “blood which contains carbon dioxide . . . will appear blue” is not correct. Additionally, it would be more accurate to state that nutrients, hormones and minerals are “dissolved” in the plasma, not “floating” in it. The terms weigh and weight are used in place of mass.

The rational treatment of the dangers of smoking in the respiratory system video is done well. The approach gives youngsters credit for knowing that cigarettes are dangerous, while emphasizing the need to consider respiratory health in the same way we think of our other health needs. Students may find the nicely illustrated functioning of the vocal cords interesting. This video also has a nice demonstration of diaphragm function the students can do themselves. The muscular and skeletal system video includes some X-rays of skeletal injuries students of this age will like!

The blackline masters provide a convenient way of rounding out a lesson, but with rare exception, they are at the recall level and provide little that is interesting for students with nothing new, even to a relatively inexperienced science teacher. Writing the definitions

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