**Sickle Cell Anemia: A Fictional Reconstruction\***

**NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE**

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*\* Disclaimer:* This case is a work of fiction that refers to real events and people. All of the discoveries mentioned in Section 1 were made by the individuals they are attributed to, as were the observations made by Dr. Vernon Hahn described in Section 2. The time between discoveries has been dramatically condensed, however. Every effort has been made to present the scientific considerations concisely and accurately. Any errors should be attributed to the author and not the original investigators.

Part I – The Inquiry Begins

It was a brisk fall day in Boston—the type of day that Dr. William Castle preferred to start with a cup of coffee while he caught up on his correspondence, which often appeared to be an endless task. As a faculty member of Harvard Medical School, he had always received a fair amount of inquiries, but after he had published his data indicating that pernicious anemia was due to a vitamin B12 deficiency, the amount of mail he received was sometimes overwhelming.

Sitting in his reclining chair he sorted through the large pile that had accumulated. He began to meticulously segregate it into smaller piles he would open in a prescribed order. Usually the delegation of a particular envelope was a relatively easy choice. There were a few that caused him to pause for a moment, such as the one he was currently holding. The return address indicated it was from an Irving Sherman at Johns Hopkins University. Since it was from someone he had never heard of, he was inclined to place it in the pile to be opened later. But on this day he decided to take another sip of coffee and see what Mr. Sherman had to say (see Irving Sherman’s letter attached).

 “Well, this may have some merit,” Dr. Castle mused to himself. He recognized that these results indicated it was likely that there was a difference in one or more molecules found either in the blood or within the red blood cells. Since sickle cell anemia was named due to the change in the shape of the red blood cells, the molecule involved was most likely within the red blood cells. Dr. Castle placed the letter in a conspicuous place on his desk so that he would remember to send a reply to the ambitious student.

A few months later, Dr. Castle found himself taking a train to a conference. As usual, he had brought a manuscript to work on. As he glanced around the car he was surprised to see Linus Pauling sitting a few rows in front of him. Dr. Pauling was a renowned chemist who had been doing some innovative research on elucidating the structure of different proteins. Having met Dr. Pauling at a previous conference, Dr. Castle decided to strike up a conversation.

“Well, Linus, this is a surprise.” “Hello, Bill. Where are you heading?” “Oh, I’ve been invited to give the plenary talk at this symposium on the physiology of red blood cells down in Atlanta.” “What a coincidence. I’m heading to the same meeting to present some of my recent data on hemoglobin.”

A surprised look crossed Dr. Castle’s face. “I had no idea that hemoglobin had caught your attention.” “Yes, I’ve been working on it for the last year or so. It appears to have a number of interesting structural properties.”

Dr. Castle remembered the letter from Irving Sherman. “Linus, have you given any thought to studying hemoglobin from individuals suffering from different forms of anemia?”

“I’m not very familiar with anemia. I had thought that anemia was a condition in which the body simply made fewer red blood cells. Is there any indication that there is a difference in the hemoglobin?”

“Until a few months ago I would have told you no, but a young medical student recently mentioned that he has observed that blood from individuals with sickle cell anemia transmits light differently than normal blood. I was thinking that this may be due to some difference in the structure of their hemoglobin.”

“It definitely seems worthwhile looking into. If you could send me some blood samples, I could try a couple things when I get back to the lab and let you know what I find out.”

A few weeks later, one of the letters in his stack of mail caught Dr. Castle’s eye. “Ah, let’s see what Linus has found.” (See Linus Pauling’s letter attached.)

Eagerly Dr. Castle reached for his lab notebook and quickly looked to see which samples came from the individuals with sickle cell anemia. “Jim, come and look at these results Linus Pauling just sent to me,” he called to his research assistant. He handed the letter to Jim and said, “The samples labeled 48WC03 and 48WC15 are from some patients who have been diagnosed with sickle cell anemia. The others are the controls.”

“This is interesting,” exclaimed Jim. “It seems to fit nicely with what I have recently learned. I was at a meeting last week and heard a talk by Vernon Ingram. He used different enzymes to cleave the hemoglobin from sickle celled and normal individuals. He found that all the fragments generated were identical except for one. After analyzing that fragment, he determined that the only difference between normal and sickle-cell hemoglobin is the amount of glutamic acid and valine residues. Hemoglobin from sickle-celled individuals contains more valine.”

*Questions – Complete in your packet*

1. From Irving Sherman’s letter, what did Dr. Castle report about blood cell function?

2. What did Irving Sherman’s data indicate?

3. Why did Dr. Castle not tell Dr. Pauling initially which samples came from the sickle-celled individuals?

4. From Linus Pauling’s results, what level of protein structure of the hemoglobin is altered in the sickled-cell condition – primary, secondary, tertiary, or quaternary level? Explain the basis for your answer.

5. Are Linus Pauling’s results supported by Vernon Ingram’s results? *Hint:* Compare the molecular composition of the different amino acids implicated.

 

glutamic acid valine

*Part I References*

Bloom, Miriam. *Understanding Sickle Cell Disease.* Jackson: University Press of Mississippi, 1995. Todd, James Campbell, and Arthur Hawley Sanford. *Clinical Diagnosis by Laboratory Methods*, 14th ed. Edited by I. Davidson and J.B. Henry. Philadelphia: W.B. Saunders Company, 1969, 227–237.

Edelstein, Stuart J. *The Sickled Cell, From Myth to Molecules*. Cambridge: Harvard University Press, 1986. Stryer, Lubert. *Biochemistry*, 2nd ed. San Francisco: W.H. Freeman & Company, 1981, 57–102.

Part II – Normal Functioning

It was during the hot, humid days of August that Irving Sherman, fresh out of medical school, arrived in Boston. It seemed almost unbelievable that he owed his being in Boston to the short letter he had written last October. But here he was, continuing the research he had begun at Johns Hopkins Medical School. The major difference was that now he would be working with one of the premiere experts on different forms of anemia, Dr. William Castle.

But he had more mundane matters on his mind. Today he was starting a new graduate student in the lab. As he crossed the Charles River on his way to the lab, located at Massachusetts General Hospital, he pondered the best way of bringing the newcomer up to speed.

Upon entering the lab he was pleased to see that the new graduate student, John Brockley, had already arrived and was talking to Dr. Castle.

“Ah, Irving, you’re just in time. This is John Brockley. I’m sure you’re both anxious to get started, so I’ll leave you two to get acquainted.”

“So, John, did Bill tell you what project you will be working on here?”

“He mentioned that I would be testing out some different treatments for sickle cell anemia.”

“Do you know much about sickle cell anemia?”

“Just what I learned in my biology classes. I know that it is a disease seen in individuals from African descent and was named for the change in the shape of the red blood cells, but that’s about all.”

“Well, I think the best thing for you to do is to familiarize yourself with the normal functioning of red blood cells and sickled cells. Then we can have you start reviewing some of the previous experiments that have already been done. Here’s a short list of some pertinent questions you should be able to answer.”

The following is a short excerpt from p952 in the “Dragonfly” textbook Biology (Miller and Levine).

Red Blood Cells The most numerous cells in the blood are the red blood cells, or erythrocytes *(eh-RITH-roh-syts*). Red blood cells transport oxygen. They get their color from hemoglobin. Hemoglobin is the iron-containing protein that binds to oxygen in the lungs and transports it to tissues throughout the body where the oxygen is released.

Red blood cells, like those shown in *Figure 37-8*, are shaped like disks that are thinner in the center than along the edges. These cells are produced from cells in red bone marrow. As these cells gradually become filled with hemoglobin, their nuclei and other organelles are forced out. Thus, mature red blood cells do not have nuclei. Red blood cells circulate for an average of 120 days before they are worn out from squeezing through narrow capillaries. Old red blood cells are destroyed in the liver and spleen.

*Questions – Complete in your packet*

1. Red blood cells found in the plasma of mammals do not contain a nucleus. List all the possible benefits and limitations imposed on these cells by not having a nucleus.

2. Predict how the sickling of red blood cells could impair their functioning.

3. Predict how the average life span of a cell located in the brain differs from the average life span of a red blood cell. Provide a basis, based on cellular features, for your prediction.

4. It has been observed (using an electron microscope) that when the red blood cells are sickled there are little spikes that puncture the plasma membrane. Predict how this will affect the functioning of sickled cells and their life span.

Part III – Starting at the Bottom

“First he had me reading about red blood cells and now I’ve got to read through these articles describing previous findings. What I really want to do is get started on my own experiments,” lamented John to Christine, a fellow graduate student who was working in a different lab at Harvard.

“I know the feeling. It seems everyone goes through the same process. So what’s in those articles?”

“The first one is a report from E. Vernon Hahn. I guess he was some kind of a surgeon practicing in Indianapolis. He states that if you take a tube of blood from a sickle-cell patient and allow it to sit undisturbed for a while, the cells at the bottom of the tube will become sickled while those at the top of the tube retain their normal shape.”

“I wonder why only the bottom cells sickled?” posed Christine. “Do you suppose that the cells that sickle are heavier?”

“I’m not sure, but I don’t think that weight is the critical issue. After he shook up the tube, the sickled cells returned to their normal shape.”

“You’re telling me that cells can return to their normal shape after they have sickled? I always thought it was a permanent change,” Christine said.

“That’s what I had thought. Actually, that may be a good thing. If this shape change is reversible, then maybe we have a better hope of finding an effective treatment.”

*Questions - Complete in Packet*

1. How was the environment of the blood different at the top of the tube versus the bottom of the tube? Parameters to consider should include such things as: density of cells; concentration of nutrients, waste products, gases; pressure differences; and possible temperature differences.

2. How would shaking the tube alter the environment of the tube? Consider what would happen to the concentration of different molecules.

3. What environmental factor do you believe is responsible for causing the cells to sickle?

4. How would the repeated sickling and unsickling of the cells affect the average life span of red blood cells?

Part IV – Ghosts

“So, has anyone determined if low oxygen is the factor that is causing the cells to sickle?” Christine inquired.

“Actually, Dr. Hahn rigged up an apparatus that allowed him to pass different gas mixtures over red blood cells,” replied John. “He observed that as he lowered the percentage of oxygen present in the gas, more and more cells would sickle. Then, as he increased the oxygen concentration, they returned to their normal shape. He also tested this using red blood cell ghosts.”

“Ghosts?” interrupted Christine.

“Apparently he was able to remove the hemoglobin from the red blood cells. After he exposed these ghosts to very low oxygen concentrations they still retained their normal shape and showed no signs of sickling.”

“That’s interesting, but I don’t understand why he had to do the experiment with the ghosts.”

*Question – Complete in Packet*

1. Why did Dr. Hahn need to test the ghosts?