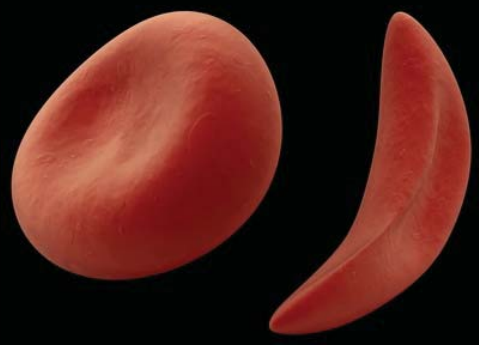
Sickle Cell Anemia: A Fictional Reconstruction*



by
Debra Stamper
Department of Biology
King's College

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 next page).

Johns Hopkins University School of Medicine
720 Rutland Avenue
Baltimore, MD 21205-2196

Dr. William Castle
Harvard Medical School
25 Shattuck St.
Boston, MA 02115

Dear Dr. Castle,

I am writing to you in hopes of securing a research position in your lab. I am completing my last year of medical school at Johns Hopkins and would be available to move in August.

Two years ago I saw my first case of sickle cell anemia. I was so intrigued by the case that I decided to see if I could investigate more of the physiological mechanisms that lead to the symptoms these individuals display. Recently I compared the transmission spectrum of blood obtained from a normal patient with that of a patient with sickle cell anemia. My data indicate that there is a difference in what wavelengths of light are optimally transmitted through the blood of sickle-celled individuals.

I read your journal article in which you reported that sickled cells flow through the cardiovascular system at a slower velocity than normal shaped red blood cells. I do hope that you still have an interest in sickle cell anemia. I feel that I would make a positive addition to your lab. I am most interested in being able to investigate whether this difference in light transmission could account for the physiological complications seen with this disease.

Please feel free to contact me at your earliest convenience.

Sincerely yours,

Irving Sherman

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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.

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Sincerely yours,

Irving Sherman

"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."

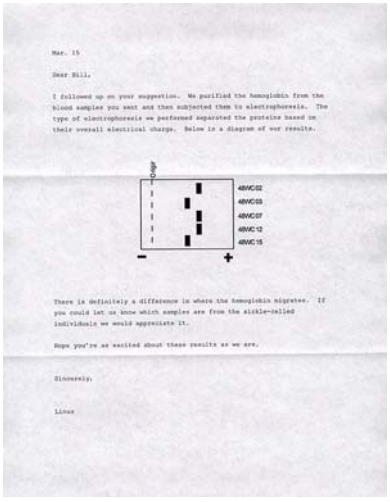
Dr. Castle remembered the letter from Irving Sherman. "Linus, have you given any thought to studying hemoglobin from individuals suffering from different forms of anemia?"

"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 next page.)



Mar. 15

Dear Bill,

I followed up on your suggestion. We purified the hemoglobin from the blood samples you sent and then subjected them to electrophoresis. The type of electrophoresis we performed separated the proteins based on their overall electrical charge. Below is a diagram of our results.

There is definitely a difference in where the hemoglobin migrates. If you could let us know which samples are from the sickle-celled individuals we would appreciate it.

Hope you're as excited about these results as we are.

Sincerely,

Linus

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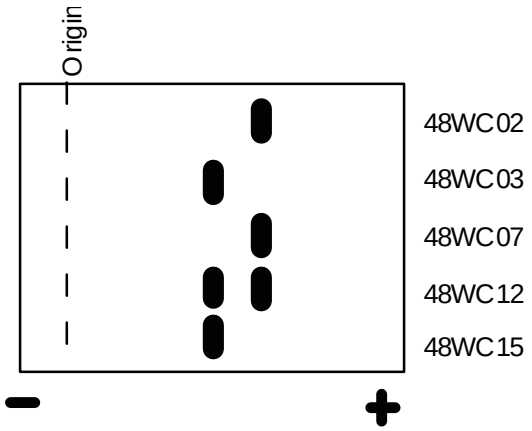

There is definitely a difference in where the hemoglobin migrates. If you could let us know which samples are from the sickle-celled individuals we would appreciate it.

Hope you're as excited about these results as we are.

Sincerely,

Linus

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

1. Why did Dr. Castle not tell Dr. Pauling initially which samples came from the sickle-celled individuals?
2. From these results, what level(s) of protein structure of the hemoglobin is altered in the sickled-cell condition? Explain the basis for your answer.
3. Are Linus Pauling's results supported by Vernon Ingram's results? *Hint:* Compare the molecular composition of the different amino acids implicated.

References

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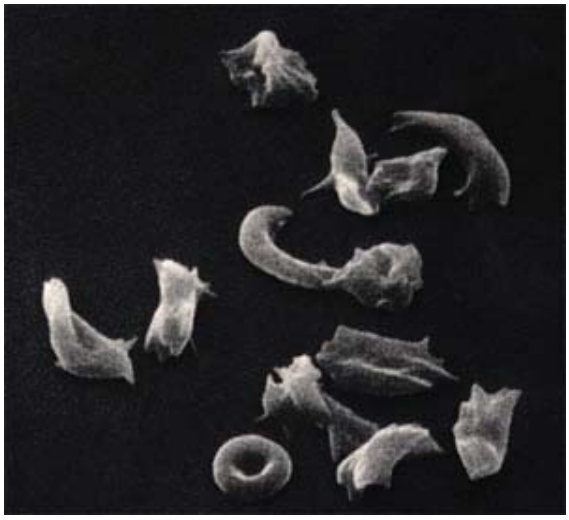
Part II – Normal Functioning



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."

Questions

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.

Image credit: Electron micrograph by Patricia N. Farnsworth, Department of Pharmacology and Physiology, New Jersey Medical School. Used with permission.

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

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

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

Part V – Throwing Water at the Problem

"So, John, are you ready to set up some experiments?"

For a moment John couldn't believe he had heard Dr. Castle correctly. "Sure am," he responded enthusiastically.

"Good. I would like you to test how different solutions alter the rate at which red blood cells sickle. Why don't you start with these solutions that I have listed here. Let me know what the results are and be prepared to give a physiological explanation for your results by tomorrow afternoon."

As quickly as he had entered the room, Dr. Castle was out the door. The only evidence of his presence was the scrap of paper that John held in his hand. Although the writing was difficult to make out, it appeared that he was to test a 500 mM solution of sucrose, a 300 mM solution of NaCl, a 100 mM solution of NaCl, and a saline solution.

Irving Sherman, the postdoc who was supervising his work, had overheard the conversation. "So, what does Bill have you testing?"

John handed him the list.

"Hmm…I see he has you testing the effect of osmosis on the rate of sickling. Do you know what a saline solution is composed of?"

"Isn't it just an isotonic solution of sodium chloride?" responded John.

"Yep. Well, I see you have everything you need to get started. Let me know if you run into any problems."

John proceeded to make the different solutions. He then placed 4 ml of each solution into a different test tube. After checking to insure that all the tubes had the same volume, he added 1 ml of blood that had come from a patient with sickle cell anemia to each tube. He then measured how much time elapsed before the cells at the bottom of the tube started to sickle. After repeating this process four times, the average time for sickling to occur was:

500 mM sucrose	4 min 18 sec
300 mM NaCl	2 min 11 sec
100 mM NaCl	21 min 24 sec
saline	7 min 35 sec

Questions

1. Calculate the osmolarity of each of the solutions tested.
2. Determine the tonicity of each of the solutions tested.
3. Compare the solute and water concentration of each solution to the solute and water concentration found within the red blood cells.
4. Describe what should happen to the red blood cells after they were placed into each of the solutions. Include in your answer the movement of water and any possible changes to the shape of the cell. Rank the cells from each solution in terms of their volume (starting with the largest cells to the smallest cells).
5. Assuming that hemoglobin (Hb) does not leave the cell and that all red blood cells start with the same amount of Hb molecules, compare the concentration of hemoglobin found within the cells that will change after they have been placed in each of the test solutions.
6. What conclusions (if any) can you draw from these results about the influence of the concentration of hemoglobin on the sickling of the cells?
7. Read the following and then answer the questions below:

Vaso-occlusion is a condition that occurs when a blood vessel becomes damaged or blocked, resulting in the diminished passage of blood (as well as the cellular and molecular components contained within it). Individuals with sickle cell anemia will have many episodes of vaso-occlusion throughout their life. Whenever the red blood cells start to sickle, they can then block small blood vessels, resulting in severe pain.

Peter Belles is a new intern at St. Luke's Hospital and has a patient who has previously been diagnosed with sickle cell anemia. The patient is complaining of severe joint pain and Peter's diagnosis is that he is suffering from a vaso-occlusion episode. Since Peter learned about sickle cell anemia in his general biology course, he elects to try to treat the patient without conferring with the attending physician (probably not the most advisable situation). He feels confident that he can reverse the sickling by infusing the patient with the correct osmolar solution.

Which of the following solutions do you think Peter is most likely to try? Be sure to give the basis for your answer.

a. saline solution
b. 100 mM NaCl
c. distilled water

8. For each of the above solutions, list any possible side effects that may occur after the patient has been infused.