Samuel Charache

Interviewer: Dr. Valerie L. Williams

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Williams: We are at the Johns Hopkins School of Medicine. Thank you for meeting with me today. Now, today, as I mentioned before, I'd like to begin to thread together the fetal hemoglobin story. So I thought it might be most useful if we just go through the papers chronologically on the research on this topic, and you just let me know what I'm missing, if there are any details that I should be picking up on that I'm not. Also, something about the significance of the research for that time period. I have papers that were or were not significant. I'm going to sit next to you so we can share information. Now, my understanding is that the first significant paper, the first paper that really linked fetal hemoglobin to sickle cell disease, was the paper in 1948 by Dr. Janet Watson, where she made a clinical observation that newborn infants did not have the manifestations of sickle cell disease. So I guess my first question is, is that where it sort of started, the hypothesis that linked fetal hemoglobin to sickle cell disease, and was this a very significant paper for that time period?

Charache: Yeah. I think it was a very significant paper because she synthesized the fact that there weren't many sickle cells there and that they had the fetal hemoglobin and that they didn't begin to get sick until the fetal hemoglobin went away. At least I think that's in this particular paper.

Williams: Right. I've marked some of her results, and she basically did say that the newborns, I

think, lived with their sickle cells, longer. There were two results from that one.

Charache: Did she actually measure their red-cell survival?

Williams: I may be thinking of another one.

Charache: Now, that was Fisher and Singer, I think.

Williams: Yeah. I'm ahead of myself. You're right. That was just the synthesis of some information. You're correct. She looked through the literature and sort of made this observation. Okay.

Charache: And, as we mentioned -- I think we mentioned earlier -- is that this is a paper that has pretty much been neglected, a purely clinical observation that had the kernel of the thing right there.

Williams: Right. This is particularly interesting because it almost fits the model of an observation or something that began at the bedside, and then what seems to follow is more bench research. Is that correct?

Charache: No. The bench research has really been mostly in the past 10, maybe 15 years. But in the beginning, it was a fascinating meld of people seeing interesting things in the clinic and people in the laboratory seeing things that probably had something to do with it, but they weren't quite sure what.

Williams: I see. Interesting. Okay. So it's this meld of observation and laboratory work, and the struggle was to begin to connect these two.

Charache: Yes, exactly.

Williams: Okay, that was in 1948. The next significant paper, at least the next paper that I have -- you'll speak to its significance -- is the paper from Singer and Chernoff, and this one is

more a characterization of fetal hemoglobin and its relationship to hemoglobin S. And I think their idea is formation specific for hemoglobin S, and did F hemoglobin show these characteristics as well?

Charache: Well, as the tactoids formed, actually, I had thought it was Harris who was the first one who showed the tactoids.

Williams: He did.

Charache: But because saying the tactoids formed immediately told you something about the organization of the molecules, because tactoids meant linear arrays. And then -- well, maybe you'll be getting to it, Sherman's paper, where he showed that they were birefringent which, again, went along with this idea of the parallel arrays.

Williams: That's correct, right, and I have those papers as well. I think here, this was just a characterization of -- what I've highlighted is just a characterization of, if there is a relationship between, as they refer to them, type S and type F hemoglobin, and, if so, what they were. Now, the work of Singer, was that significant?

Charache: Yeah. I don't think of this really being one of the landmark papers, but I think it was Singer and his wife.

Williams: Right, Lily.

Charache: Who did mixtures of hemoglobin. By this time, they knew that the sickle hemoglobin gelled when it got deoxygenated, which was another key observation. But the Singers, as I remember it, were the ones who made mixtures of fetal hemoglobin and sickle hemoglobin and showed that the fetal hemoglobin somehow or other interfered with the polymerization.

Williams: Right.

Charache: Well, I don't know if they said polymerization. That's what it was.

Williams: They said gelling.

Charache: But polymerization goes one step beyond.

Williams: You're right.

Charache: They didn't really know what was going on.

Williams: That's right. I think at that point, it was minimum gelation concentration.

Charache: Yeah.

Williams: That was the terminology that was used. You're correct. And I actually have that paper. I didn't put that one in this binder because it didn't speak as specifically to fetal hemoglobin as this one did. But I think you're right. Or you said, it's really the landmark paper.

Charache: And then somewhat after that, but not a long while after it, Anthony Allison did some studies in the *Biochemical Journal* where he got much more sophisticated, because he was the one who got the idea for using the viscometer to look at the changes before gelation actually occurred. That is, it was a much more sensitive thing. I remember when I was doing these experiments, you never knew really when it gelled. All you could do was hold the vessel upside down, and if it didn't pour out, then it had gelled. But using the viscometer, it gave you a real handle.

Williams: Now, was that the falling-ball viscometer?

Charache: Yeah.

Williams: Okay.

Charache: And that's what started me off. Taking off from what Allison did.

Williams: Okay, so prior to that, you were literally blowing, I believe, nitrogen, deoxygenating the blood samples. I can't remember this. I read it somewhere.

Charache: To do the studies, before Allison, you would deoxygenate them, say, with nitrogen and then see what happened. And one of the things people did was see how long it took for things to gel. The endpoints were so vague, you couldn't make much out of it. But then, I forget who did what, but using gases of different mixtures of oxygen and nitrogen and showing that it wasn't an all-or-none thing.

Williams: I do remember reading about that procedure, which was prior to or perhaps there was some overlap with using the fall-ball viscometer.

Charache: And then somewhere along the line here, somebody deduced at least the fact that fetal hemoglobin was different; that is, it wasn't only electrophoretically and biochemically different; that is, with the alkali resistance, which people knew about, but the fact that it had a gamma chain instead of a beta chain. And people later on used that to try to make an explanation for how it would work.

Williams: Right. And I guess that came a little bit later.

Charache: Yeah.

Williams: I think part of what they were explaining here is that even though you might have an electrophoretic sample of hemoglobin S, there was another component of it that they noticed. That is, there's a 5 to 20 percent.

Charache: Yes, but their technique wasn't terribly sensitive.

Williams: I see.

Charache: Well, maybe they could pick up 5 percent, but they were doing well to see that sickle trait had two bands, not one.

Williams: Okay, right. Because what they would do, apparently, is look for the alkali resistance to determine whether or not the hemoglobin S was there or not. But they couldn't for sure say it was there just based on electrophoresis. So the Singers, the Carl and Lily Singer paper, you stated it being the landmark paper. And it's also interesting that Singer and Fisher, in either of these papers, they never cite Watson even though a lot of their conclusions are saying similar things.

Charache: I suspect they just didn't know.

Williams: Okay.

Charache: I mean, we didn't have fancy bibliographic programs and so on.

Williams: No Pubmed, Medline searches. So these are -- this is still Singer and all the studies on the abnormal hemoglobin. And just to talk about this research, because this seems to be key research in the '50s, what was really, in your mind, the significance of just this plethora of papers coming out during this time that identified all the different hemoglobins? What -- was there a burning question that people were trying to answer in doing this?

Charache: Not really yet, because it was interesting. All right? But the fact that having enough fetal hemoglobin could make a clinical different, other than Watson's paper. That didn't emerge till later.

Williams: Okay. That's so interesting. Even though it was stated in her, even though the hypothesis was stated.

Charache: Yeah, but the actual clinical evidence of it didn't show till later.

Williams: I see. So the next paper I have here is from John Jackson. Now, he did a very interesting study where he linked, I think, well, he tried to evaluate semi-quantitatively the effect of fetal hemoglobin on the clinical severity of sickle cell.

Charache: Right.

Williams: And that seemed to be a burning question: how can we understand the range of clinical symptoms? Now, was this a key paper.

Charache: Nobody paid much attention to it.

Williams: Really?

Charache: But it was, really. He didn't grasp as well, but he got the general idea. What year is that?

Williams: This is 1961.

Charache: Yeah, because like, here's this paper.

Williams: Oh.

Charache: And there were actually a few papers by these people, and they had actually picked up hereditary persistence of fetal hemoglobin in Africa.

Williams: Oh, really?

Charache: I mean, I don't know if they actually called it that or not, but they noticed that there were people who seemed to have sickle cell anemia but who weren't that sick and who had a lot of fetal hemoglobin.

Williams: In Africa.

Charache: Yeah.

Williams: As opposed to the Mediterranean.

Charache: Right. But these were primarily clinical papers. You see here, he's got this picture

where he's got the A.

Williams: The A, the S, and the C.

Charache: I don't know if they actually picked up the fact that there was a lot of fetal hemoglobin, usually with a varying amount of persistent fetal hemoglobin, and it was this variable amount of the persistent fetal hemoglobin that probably was making the difference.

Williams: I see, okay.

Charache: But this didn't get a whole lot of publicity.

Williams: That is amazing.

Charache: Because what these guys did was really the same thing as this.

Williams: Right.

Charache: The people in Africa with a lot less to work with other than the patients.

Williams: And a lot earlier. I mean, this is 1955.

Charache: Yeah.

Williams: To some extent it had been established before Jackson. Here, they were more groundbreaking, I would imagine.

Charache: Lehman, do you know about him?

Williams: I've seen the name, yes.

Charache: But a most remarkable guy, because in a way, he was the one who got me involved in abnormal hemoglobins.

Williams: Oh, really?

Charache: Because I went to hear a lecture of his when I was a medical student, and he was talking about looking for hemoglobins off on some island somewhere and was sailing across in

some kind of a crazy boat, and I thought to myself, you know, that's not so bad. Here's somebody who's using medicine to get to go to some interesting places.

Williams: How funny.

Charache: And I eventually got to meet him, and he took me out. It's the only time I've ever sat at the head table at a university in Cambridge.

Williams: Oh, really?

Charache: But he took me to lunch there and was I impressed!

Williams: Oh, that's so funny.

Charache: But he's a marvelous guy and quite a scientist.

Williams: Okay. So that's the truth, the island.

Charache: Well, I wanted to travel then.

Williams: So, were you disappointed when you had to use the falling-ball viscometer.

Charache: No. It was all sort of in the back of the head. This was the road to utopia.

Williams: I see, with your magnet. Right? I thought that was so funny from the previous interview. So, Jackson's paper?

Charache: Now, what year is that again?

Williams: This is '61.

Charache: Yeah. Because, you see, here, in '60, is this.

Williams: Oh. I looked for that one. Okay, all right. Yes.

Charache: And here is the lady who got hit by the car.

Williams: Oh.

Charache: Now, they don't mention anything about this. The whole pickup was serendipitous.

Williams: Yes. So this connects from the previous interview.

Charache: Right.

Williams: Oh, that's wonderful.

Charache: This was in the days when you had to have naked pictures of people.

Williams: Yeah, I see that, right. Those bites are bad.

Charache: That was really the beginning. They called it hereditary persistence of fetal hemoglobin because Eddington and Lehman had pretty much called it that and they reference them, I'm pretty sure.

Williams: Now, just to clarify, was this the first paper . . .

Charache: These are some of the really key references.

Williams: Well, I'll have to get a copy of that because I've seen this paper referenced, and there's the one that I have.

Charache: And that was really the beginning of a whole bunch of papers from Hopkins about hereditary persistence.

Williams: Just to be clear, so hereditary persistence is also characterized by the distribution of fetal hemoglobin?

Charache: And the key discovery at that point was Klaus Betke, who found the differential elution. It was in an acid buffer, and if you dipped the slide in and you did it just exactly right, the hemoglobin, the adult hemoglobin would elute out, leaving the fetal hemoglobin behind.

Williams: Oh, I see.

Charache: You couldn't distinguish them within the cells, and we had a medical student who came to spend first just an elective, then stayed, Marguerite (Peggy) Shephard. She used this

technique to show that in hereditary persistence of fetal hemoglobin, all the cells were protected.

Williams: Meaning all the cells contained fetal hemoglobin.

Charache: That's right.

Williams: Okay.

Charache: And she used to make pictures and then use a densitometer to measure the density of these things, because that was really the only way you could do it. Well, then, later, Boyer and Dover got much fancier ways of doing it.

Williams: Okay, all right. So that's the distinguishing characteristic.

Charache: Because that -- the real argument had always been, why is it that some children who have a high fetal hemoglobin do get sick? What she was able to do was get somebody with hereditary persistence and one of these kids who had just about the same amount of fetal hemoglobin and looked at the distribution of the fetal hemoglobin in their red cells and showed that the sick kid had heterocellular distribution, that is, not all the cells had any; whereas in hereditary persistence, they all did.

Williams: They all had some. And did they all have the same percentage?

Charache: Nobody could tell at that point. It only came later with Dover and Boyer, where they could actually quantitate how much was in it.

Williams: Okay. But the first distinction was to (a) determine that there was a difference in the distribution; that is, in some cases, every cell had fetal hemoglobin, and in another case, the hetero, some cells do and others do not have fetal hemoglobin.

Charache: So that if you measured the average, the average fetal hemoglobin in the two samples

could be the same, say 20 percent, but in hereditary persistence of fetal hemoglobin, at least in principle, all the cells would have 20 percent fetal hemoglobin, whereas in the heterocellular distribution, some might have 2 percent and some might have 40 percent.

Williams: Okay. And the key here is that, for those who had heterocellular, that meant that their cells were not protected from irreversibly sickled cells. Right? I mean, that . . .

Charache: Don't say that.

Williams: Should we not go there?

Charache: Don't get into irreversibly sickled cells. Nobody understands them yet.

Williams: Really?

Charache: Other than the fact that they have very short survival.

Williams: I see.

Charache: Among people with heterocellular persistence of fetal hemoglobin, those that had more fetal hemoglobin did better than those that had less, and that was really this paper, discussing Saudi Arabian's heterocellular fetal hemoglobin did have a milder course.

Williams: Right. And now we are jumping ahead. Because I have this paper and I do want to talk about that one. But this is important because, you know, this is putting a lot of things together for me, as you can tell. Well, let's just continue. So I don't have that paper. I do need to get that one.

In 1964, your paper with Dr. Conley, "Rate of Sickling of Red Cells during Deoxygenation," was a key paper, because here, in my mind, this was the first time that it was quantitatively identified the amount of fetal hemoglobin needed to prevent sickling.

Charache: Well, Allison had pretty much said, done it. Oh, he had curves that showed this sort of thing.

Williams: Okay. So your statement, as little as 30 percent hemoglobin of each red cell prevents sickling under physiological conditions.

Charache: But that was from the study. That's what people were saying.

Williams: All right, okay.

Charache: But what Allison showed and which I tried to show, and I think did, was that not all hemoglobins were the same.

Williams: Right.

Charache: And we had the simple-minded idea that it was just a case of dilution; that is, that there was this minimum gelling concentration, and let's say you had to have 20 grams per deciliter of deoxyhemoglobin, and if you had some other kind of hemoglobin there, it would dilute the sickle hemoglobin and it wouldn't gel as well. But it didn't work that way. It did pretty much with fetal hemoglobin. But with hemoglobin A and hemoglobin C, that if all you looked at was the percent of sickle hemoglobin in whatever you were studying, they actually reduced the amount of deoxygenated sickle hemoglobin that you needed for the gel to form or the cell to sickle or whatever, and this wasn't original with me at all, but maybe we got a little bit better numbers to show it. With this crazy system we had, and, again, we weren't making really good measurements of the rate of deoxygenation, but deoxygenating blood samples the same way each time, you showed that the ones that had the lowest minimum gelling concentration were the ones that sickled the fastest. And you could interpret that to mean that that took less

deoxygenation to get to this critical concentration.

Williams: I see.

Charache: Now, Alan Schechter showed years later that this whole thing is much more complicated than we ever dreamed.

Williams: Right. Okay. And this sort of speaks to, like you said, all of the information that was sort of coming out just from studying the abnormal hemoglobin, how the mixture of them affected various phenomenon.

Charache: And then there was this wonderful guy, Elijah Morton, who lived out on the edge of town, and he had sickle hemoglobin and hemoglobin J. And he was -- he behaved clinically like someone with sickle trait.

Williams: Really?

Charache: And we were able to show that his red cells and what-all behaved just like sickle trait. In other words, whatever it was that made the hemoglobin J didn't differ in some critical manner from hemoglobin A as far as this gelling phenomenon was concerned, or by now we knew it was polymerization.

Williams: So these were key studies. And like you mentioned before, it wasn't that you were necessarily trying to answer specific questions with these studies.

Charache: Well, there was a specific question. I mean, why are some people sick and others weren't? But it never got more focused than that, other than the fact that the fetal hemoglobin did have something to do with it and that hemoglobin C was not the same as hemoglobin A as far as this interaction, is what we called it.

Williams: I saw this terminology called the experiments of nature. I don't remember where I saw

this, but that was almost a description of how one researcher viewed the time period when there were many different people coming in that they could actually view the manifestations.

Charache: That's just what it was. I mean, the clinic was leading the lab at that point, without any question. I mean, the clinic people were seeing all these interesting things, and going to the lab and saying, "What can we do that would at least come out the same way as what we saw in the clinic?" which is how the gelling experiments and all of that fit in. They, I don't think, led to any critical insights other than, I mean, we named our paper "The Kinetics of Sickling," which was only because we were doing these things with the deoxygenation of blood. Well, later on when people got to the time, the kinetics became very important.

Williams: That, I think, was later.

Charache: That's a long time later.

Williams: Right.

Charache: But that was really . . . Other than the fact that my boss, Dr. Conley, in that paper -- and we put it in the paper -- came up with the idea that since some cells sickled faster than others, if there was some way to speed up the circulation so that red cells wouldn't stay as long in deoxygenated tissue beds, and he said, "Well, you can do this with thyroid hormone, but, of course, that would have other things that would be deleterious." But to my knowledge, that was the first . . . Conley's idea that how long the cells passed through a deoxygenated bed, and Alan Schechter and Bill Eaton seized upon that. I don't know if they knew it then, but it was the same idea as what Conley had.

Williams: This research is fascinating, you know, how it ebbs and flows. I mean, themes picked up later on, but they're mentioned earlier. I mean, that's why the history is so fascinating. So that was 1964, and at least at this point, I believe people, researchers began to mention increasing the synthesis of fetal hemoglobin as a desirous thing to be able to do, except we don't know how to do that, and at this point, of course, people understood that fetal hemoglobin had alpha and gamma chains. This is 1967. This is your paper on abnormal hemoglobin. And I more or less read this just to review what was known about the abnormal that there was not a particular emphasis on fetal hemoglobin in this paper.

Charache: No. That was a review paper.

Williams: Okay. So this is 1967.

Charache: And if you want to talk about irreversibly sickled cells, it's got nothing to do with that.

Williams: I just mentioned that because I had been reading it, and one explanation . .

Charache: Well, at one point we thought they were going to be the key, but they weren't.

Williams: Right. And that was a little bit later.

Charache: Yeah.

Williams: Yeah. I was sort of jumping ahead by mentioning it because that obviously was the paper that I had just finished reading.

Charache: It was a later paper. Because this was about the point where the fellow who was here, Jim Wheeler, he got into the hereditary persistence of fetal hemoglobin, and then he moved on and I picked up. And we were studying -- this was about the point where the kid with homozygous hereditary persistence of fetal hemoglobin showed up. And the big argument was, was hereditary persistence of fetal hemoglobin thalassemia or not?

Williams: Oh, yes.

Charache: And here was the homozygote that was not anemic. And I used to go up and, well, I used to have to bribe him. He was about seven or eight years old, and money wasn't that important yet, so I had to bring him presents to get blood.

Williams: The candy?

Charache: Well, it was watches, all sorts of things.

Williams: Oh, really?

Charache: Well, his blood was . . . I mean, George Dover, until just a few years ago, was still getting blood out of William, who's now in his forties, living down in Virginia.

Williams: Oh, how is he?

Charache: Well, he's one of the few people in this country who has only fetal hemoglobin in his red cells.

Williams: Really?

Charache: But he was a big help in all of this.

Williams: Okay.

Charache: And his family.

Williams: Now, what I don't have a good understanding of is the thalassemia when questions about sort of the hereditary persistence of fetal hemoglobin in thalessemia, where that overlap began to occur and how that was played out.

Charache: Well, people with thalassemia have increased fetal hemoglobin.

Williams: That's right.

Charache: And people with delta beta thalassemia, where they don't really have the gene, they've

had a big deletion, they make a lot of fetal hemoglobin. And the question was, how do they really differ from hereditary persistence of fetal hemoglobin? And later on, it turned out it all had to do with, at least in the deletion mutants, how much got deleted and where exactly the deletion was and so on.

Williams: Right, the locus of deletion.

Charache: And I can't speak authoritatively on that part.

Williams: That's sufficient. There were questions that were raised in some of the papers about thalassemia, and I never understood.

Charache: There is sickle beta thalassemia, which is a very -- it's clinically much the same as hemoglobin SC disease, but in sickle beta thalassemia, there's 70 percent of S in the hemolysis A and in SC disease there's only 50 percent, which was the clinical demonstration of the fact that C interacted, whatever that meant, better than A did with the sickle hemoglobin.

Williams: Okay, all right.

Charache: And then, as people looked harder, there were all sorts of variants of sickle beta thalassemia where there was more and less hemoglobin A, and that gave a whole new set of reagents to play with and clinical situations.

Williams: I see, right. Now, of all the abnormal hemoglobin, were there "stars"? I mean, were there different variants that people paid more attention to others for various reasons?

Charache: Yeah. I think one of the ones that was a star was hemoglobin S, was hemoglobin O Arab.

Williams: Okay. I've seen that one.

Charache: Because it had the sickle mutation and another mutation; that is, two mutations in the

same beta chain. And it looked like, on electrophoresis, it looked more like SC disease. But clinically, it was more severe than SC disease. In fact, it can be worse than sickle cell anemia. But the whole thing was, what did that other mutation do to make these cells behave differently? Well, first was the distinction that it wasn't SC disease, because if you looked at it on agar instead of on cellulose acetate, you could see that it was something different.

Williams: Okay.

Charache: But it was a very interesting hemoglobin. And then there was another one, which was hemoglobin S Memphis.

Williams: I've never seen that one.

Charache: And hemoglobin S Memphis, nobody else anywhere has ever discovered it again, and there is considerable question, maybe it was a mistake.

Williams: I see.

Charache: But hemoglobin S Memphis was another one of these things with two mutations. And then another star -- I mean, we're jumping around in time -- but another, to me, real star was hemoglobin S Antilles.

Williams: I've not heard of that one.

Charache: I mean, this is one that looks like sickle trait, but there are two mutations in this molecule, and that makes it gel more, so that people with S Antilles really have something like sickle cell anemia, and that -- they put the S Antilles gene into some of these transgenic mice to make the mice that are sort of like sickle cell anemia. So the S Antilles was another winner.

Williams: Okay.

Charache: And then there are a couple more like that, but it's the same general idea.

Williams: You know, as I was reading through these and I said, wow, you people categorize all the different hemoglobins in terms of, "Oh, this is a real winner," and "This is a star" because of their exotic properties, so to speak, or if they were unique in the fact of how they interact with hemoglobin S.

Charache: Well, I think none of these things become unique or become stars unless the person who's got them is not what you thought he would be.

Williams: I see.

Charache: That is, either he's sicker or he's less sick than you would have anticipated. And then you say, "Ah-ha, this is a very interesting hemoglobin. Let's get to work on it."

Williams: I see, okay. So that explains a lot. I'm not going to go into this paper on the O_2 dissociation curve in sickle cell anemia. I didn't read it very much. I don't know if you're familiar with it.

Charache: Yeah. That nobody knew quite what to make of that paper.

Williams: Oh, okay.

Charache: Yes, there was no question that their observations were correct, and it had some very practical consequences because it meant that at a given oxygen pressure, sickle cells would be more desaturated, i.e., would sickle more readily than a normal cell than you'd expect them to.

Williams: I see. Okay.

Charache: Okay? And again, later on was part of the explanation for why people with sickle cell

anemia are anemic, that because their red cells gave off oxygen more easily, they didn't need as much hemoglobin. That was only a small part of their anemia, but that fit into the picture.

Williams: Okay.

Charache: And then, a long time later, the Benesch's, Reinhold and Ruth, showed that 2,3 DPG had something to do with it.

Williams: Right.

Charache: But it was the Benesch's who showed that it really was the same phenomenon as polymerization that just shifted the dissociation curve because it was essentially the polymerized hemoglobin was being removed from the reaction system and pulling the reaction toward deoxygenation. So that's why it happened.

Williams: Right, right. And I do remember, again, a lot of the papers from the Benesch's, and Dr. Rainey, in fact, talked about that a bit, the DPG. So the paper that I have, obviously, this hereditary persistence of fetal hemoglobin, this was 1969, was then a review more than . . . It wasn't like the original paper that was published.

Charache: No.

Williams: Nothing in here. Now, this is all that I have, at least for this. I think this is '49 to '69, sort of the first 20 years. I don't know if there's anything else.

Charache: Well, one of the Saudi Arabians comes to the picture.

Williams: Well, that's next. I have that, and that's the '70s.

Charache: That's '78, in the '70s. Somewhere along in there, Murayama came into the picture.

Williams: Yeah. But, you know, like I said, I have that in a different binder.

Charache: It didn't have to do with fetal hemoglobin. It had to do with what was going on in the red cells.

Williams: Right, right. Yeah. That was '66 that he first proposed the mechanism of polymerization, saying that there was a site on the beta 6 valine that could interact.

Charache: And I don't remember because along in there, Perutz got the molecular structure.

Williams: Right, of hemoglobin.

Charache: Then he got sickle hemoglobin from us, and he and Wishner got the structure of the polymer, which then explained all kinds of things because of the way things fit together.

Williams: And that was the '70s, and I do have that. So let's go to '1970, then. Now, one of the first papers I have -- this is from Bertles and Rabinowitz and Döbler, and this was *Hemoglobin Interaction: Modification of Solid Phase Competition.*

Charache: They got some of the best electron micrographs of what these things actually looked at. It was Johanna Döbler.

Williams: Johanna Döbler.

Charache: She was an x-ray electron microscopist.

Williams: Right. This paper actually explained the well-known lack of interaction of hemoglobin F with S during the sickling phenomenon, and that was based on some of their electron microscope studies here, that they could now talk about.

Charache: And who was the second author on that?

Williams: Roseanne Rabinowitz.

Charache: Yeah, because Jacobson [sp.] hadn't gone into it.

Williams: Bertles and Rabinowitz.

Charache: Yeah. I don't know who Rabinowitz was.

Williams: There were other papers from Bertles.

Charache: Well, you keep getting back to irreversibly sickled cells. But he went down to Jamaica and did some good studies with Paul Milner on dense cells, the irreversibly sickled cells.

Williams: Okay. So this was kind of, I thought, somewhat of a key paper because now here was structural information that could explain what people were seeing in test tubes in terms of the interaction. So you would agree with that. Now, the next one is very interesting. Now, this is where the studies with the Arabs. This is the Arabs in Kuwait with unusually high level of [unintelligible].

Charache: This one I didn't know.

Williams: This was the first, I think that actually documented this interaction. And it's not cited as widely as the other one, which I have as well, but it, I believe, is the earliest, and it was in 1970. And it's just a documentation. They went through here and reported patients' HbS percentages and sort of started this whole trend. And then there was this following one by Gilby.

Charache: Yeah. Now, he was one of the guys in Bahrain. There were this bunch of them on the, what is it, the eastern side of Saudi Arabia, and there was Gilby and there was Perrine. Susan Perrine is Richard Perrine's daughter, and she today is all into the butyrate therapy, so she picked it up and ran with it.

Williams: Right. Now, how did this happen? I mean, was this pure serendipity, that people in Saudi Arabia were basically noticing the people?

Charache: These guys were running a clinic in Bahrain, and they saw people with sickle cell anemia

who weren't terribly sick.

Williams: Right. But the whole issue since sickle cell anemia, did that spring from the U.S., would you say? I mean, why would they . . .

Charache: They were just curious physicians who were seeing something that they couldn't explain . . .

Williams: I see, okay.

Charache: . . . and looked at the fetal hemoglobin, because by now people knew fetal hemoglobin had to do with something.

Williams: I see.

Charache: But this . . .

TAPE 1, SIDE B

Charache: One of the questions that got raised about this was that, just about this time, Graham Serjeant [sp.] in Jamaica was reporting on the fact that sickle cell anemia was milder in Jamaica than it was in the United States, and people who went down to Jamaica became convinced that the reason it was milder was that the patients couldn't get to see a doctor, and they were so used to having so much trouble that they just didn't complain.

Williams: Oh, how funny.

Charache: And this, then, was raised about Saudi Arabia: Are these Arabs out in the middle of the desert, if they can get a glass of water, they think they're doing very well? Well, that turned out not to be the case, because people went -- I went, among others -- to visit, and saw for ourselves that this really was a milder disease -- not a non-disease. They did have problems. But not as much as people in the United States.

Williams: Now, when people talked about clinical severity, by this time was there a standard metric system?

Charache: There absolutely was not, and as far as I'm concerned, there still is not.

Williams: Okay.

Charache: There are people who claim have severity scales, but I think that because it's so subjective both on the physician's point of view and the patient's point of view as to how much pain is terrible pain and how much disability is disability, that . . . I mean, you know, just having nothing to do with sickle cell anemia, you'll see one person with a wooden leg and he's walking down the street quite happily, and the other one is homebound, can't get out.

Williams: Right.

Charache: Well, the same thing's true with sickle cell anemia. And trying to make a functional measurement of how severe is severe, I mean, I think everybody agrees that somebody who's had a stroke, a stroke is a stroke, and if you've got aseptic necrosis of your femur, that's bad. But there are degrees and degrees, and I don't think there's any absolutely reliable gauge of severity. It was just sort of an overall concept. But that's why people wanted to go and look for themselves, because Graham Roger Serjeant said his patients weren't all that sick, but Graham's criterion for what was sick was different from what we had here in this country; whereas Pembrey and Gilbey and those people were much more in their -- they looked at patients much more like we did.

Williams: So initially, was there just skepticism?

Charache: There was skepticism, but it dissolved. And the real question was, what was this? I

mean, was this some funny kind of thalassemia, because at this point people knew that there was a lot of alpha thalassemia in the Middle East, and was this some funny interaction with alpha thalassemia? Well, it probably wasn't. And then, once the idea of haplotypes came up, this was a new haplotype.

Williams: Right. I was always struck by this because, I mean, this is a major, at least in the history of fetal hemoglobin research, this is a major event. The idea that, when people found out that there was a mild version of sickle cell disease, it was almost -- it changed the landscape of the research in a sense.

Charache: Well, yeah. Later on, when the haplotype business came out, they showed that, for instance, people with the Senegal haplotype had more fetal hemoglobin than the other two. But it wasn't that much more. This was enough more that you couldn't blink at it. I mean, it was for real. And so it was enough more fetal hemoglobin to make people sit up and pay interest.

Williams: Yeah, okay, because I wondered about that because then, after that, that's sort of, for the early '70s, almost the rage in fetal hemoglobin research.

Charache: And then they discovered that people in India have the same thing. And the idea was that the Indians had brought slaves from Arabia to India, or maybe it went the other way, but that turned out to be the same haplotype in India as in Saudi Arabia.

Williams: Right. I do remember reading. And I think -- now, this is a review. I don't know if this was in '72, Cooper and Goodwin.

Charache: I don't even know that.

Williams: I remember them just because of my connection. I mean, I picked it up just because of

my connection there. But that was a review of what the state of the art was.

Charache: But now Clegg, Weatherall, and Mayer in it, and they're looking harder.

Williams: This is '72.

Charache: To find out what it is that's different. And I don't know if . . .

Williams: Let me see.

Charache: Yeah, but this one, I guess they didn't look to try to figure out why. But these are very . . . Nobody knew who Perrine and what-all were. These were just guys who were hired out to go work there.

Williams: And this is the one that actually has the Jamaican and Saudi Arabian comparison here, and so maybe that was the first time.

Charache: Well, people had been talking about it for a while.

Williams: Okay, then. The protective effect is well documented. Oh. And so the key question here is why this population can't produce such high levels of fetal hemoglobin in the presence of the sickle cell gene as compared with other affected groups. So that was -- I think this was the question that they raised.

Charache: But then later on, the same bunch did the alpha thalassemia bit, looking to see if that could be what it was.

Williams: Okay. I believe their hypothesis here was that the Jamaican population had hemoglobin like that found in adults; Saudi Arabian sicklers had fetal hemoglobin similar to that found in newborns, that that was the difference they found between the two groups, the composition.

Charache: Well, you see, here, they're looking at G gamma and A gamma.

Williams: Right.

Charache: This -- we haven't gotten to that point yet.

Williams: Oh, okay.

Charache: People discovered that there were two major kinds of gamma chains, one that had alanine and one . . .

Williams: That had glycine.

Charache: And people started looking to see whether those relationships were what were making the difference. I don't think today people think that the G gamma-A gamma ratio is what's determining clinical severity, but it could very well be related to what was wrong with these people.

Williams: I see.

Charache: That is why they had the increased fetal hemoglobin.

Williams: Okay. Then I must be missing the research data that talked about that.

Charache: That's Weiss and Schroeder.

Williams: Okay. I see their names. Okay. I need to get that.

Charache: And then they came up with, there was also a third kind that had three anine, but that was really just a subset of one of the others. But the G gamma-A gamma ratio, it turns out that led to the discovery that there are two gamma-chain genes, that they're duplicated, and that various mutations, the lesions, what-all, lop off various pieces of this, and that determines, has something to do with the ratio.

Williams: I see. Okay, then. So then I would say I'll need to go back, then, and try to fill that in, because I'm not sure if that's early '70s or they were looking at that ratio. This is 1972.

Charache: If they're talking about G gamma and A gamma . . .

Williams: I guess we could look at their reference for it. Here is where they found it here. Schroeder. There it is.

Charache: Well, that's probably it.

Williams: So that's '68.

Charache: In around there.

Williams: Okay. All right. Let me just mark that. So that was '72. This is just a follow-on, almost like an abstract. I'm not sure why I picked up on that, just to find the reference. I found this. I'm not sure -- this goes back to the, I guess the different amino-acid substitutions here for the glycine, right, the gamma thalassemia.

Charache: I don't think this is any kind of a landmark.

Williams: Okay, all right. These are just editorials that I picked up, but maybe this is where I heard they were irreversibly sickled cell and sickle cell disease, fetal hemoglobin.

Charache: Well…

Williams: And this is now '74.

Charache: Somebody at this point did this acid elution trick and were able to show that the irreversibly sickled cells had less fetal hemoglobin in them.

Williams: Right, right.

Charache: So that whether or not a cell became irreversibly sickled was governed in good part by whether it had the fetal hemoglobin. But what the significance of the irreversibly sickled cells was not clear. Nobody really has been able to relate that to whatever they think severity is.

Williams: I see.

Charache: Serjeant showed that it is related to the severity of the hemolytic anemia and that in general these irreversibly sickled cells, the dense cells, because they are dense, they have a shorter survival. And the fact that they were dense, that led to the whole current business about Gyorgy Gárdos's channel and so on.

Williams: Right, okay. Well, it may have been here that I first saw this, and they mentioned Bertles and Milner.

Charache: Yeah. That was the study when Bertles went down to Jamaica, where Milner was working.

Williams: Right.

Charache: And that's a very good paper.

Williams: Okay. So that would be a paper to include in that period. This was in *Electron Microscopy*, '69, Bertles and Döbler, reversible and irreversible sickling. So you would put this in the category of trying to make a link to clinical severity.

Charache: Yeah. The whole thing is -- that's what it is.

Williams: Okay, all right. What about this one by Claus Betka [sp], estimation?

Charache: Well, that's where he . . .

Williams: Fifty-nine.

Charache: And, see, here's what I was just talking about where Serjeant showed that the ISC was a determinant of hemolysis, and I think most people would agree with that much.

Williams: Okay, then, okay. That was in a letter. Now, regulation of fetal hemoglobin production. This is '74.

Charache: Pedalion [sp.], yeah. He actually is still on the faculty here even though he works in Philadelphia.

Williams: That's what I thought, right.

Charache: But he knew about the haplotypes at this point. Or did he? Maybe not.

Williams: I don't remember that,

Charache: They only got into it when Antonarakis came by. Because as he got interested in sickle cell anemia and then Nagel and Dominique Labie did the haplotype business, and then this Greek guy who was working with Kazazian , Stelios Antonarakis did some really nice studies on the haplotypes and how they all fit together.

Williams: Now, did this produce information here that was . . . Do you know?

Charache: I don't think so. I think that more holds things together.

Williams: And that was my take on it. I mean, this was data on gamma-chain synthesis, which I think . . .

Charache: You know, this is, I think, a good review, but I don't think there's anything new.

Williams: Okay, all right. Some of these papers do a good job, like you say, of almost saying what the state of the art of the research is at this time.

Charache: And you need those kinds of papers because it gets to be so diffuse that the youngster doesn't know what to do.

Williams: Well, right, and certainly there have been points where I got there. So sometimes I'm not sure this is a state-of-the-art paper or if this is necessarily explaining new information, but my impression was that this was more of a review, and this, of course, is like a review paper. And I haven't gone through it in enough detail to talk about it at this point. I just

wanted to find out from your thoughts if this was necessarily a big paper when it came out.

Charache: It's too long.

Williams: It's too long.

Charache: Really good papers are never that long.

Williams: Okay. But . . .

Charache: And where was it published? Do you know?

Williams: I keep that always in the back. This was *Seminars on Hematology*.

Charache: Yeah. See, those are review articles.

Williams: Okay, all right, then. Now, this was Bertles, 1974.

Charache: And that looks like the New York Academy of Sciences, and they also are rarely anything new.

Williams: Okay, then. And you're probably right. How much . . . Oh, okay. He was talking about . . .

Charache: The ISCs don't have the fetal hemoglobin.

Williams: Mm-hmm. You know, sickled cells have such a variety of shapes. How did people definitively characterize . . .

Charache: Well, those are irreversibly sickled cells, which are not the same as the sickle cells in a sickle-cell prep. If you want to think of it that these are like the sickled cells that have had all the little fine projections knocked off in the circulation as they go banging around one into the other. I mean, that's not what they are.

Williams: Right, but all the spikes and some of the formations.

Charache: Yeah. And the other part of it is that Sam showed that if you take all the hemoglobin out of these cells, they still stay that shape, that it isn't the hemoglobin that's in them that's making them have that shape. And, in fact, when they're oxygenated, that's the definition of an irreversibly sickled cell, that it's an oxygenated cell that still looks like that.

Williams: So at this point . . .

Charache: So that the membrane has been . . .

Williams: Rigidified.

Charache: Yeah.

Williams: This is in Annals, New York Academy.

Charache: This was a landmark paper.

Williams: I thought so.

Charache: Because she had more information about kids than anybody, a big population of patients that had been studied carefully, and she became, almost overnight, the guru of pediatric sickle cell anemia when it came to clinical things.

Williams: Okay.

Charache: A very smart lady, well organized. She had -- I remember she'd always introduce the biostatistician when she gave a paper because she thought that the biostatistician had played such an important role.

Williams: Okay, in this. But this was before the group from NHLI, I guess at the time, put together their natural history study. Right? The cooperative. I mean, they did . . .

Charache: Yeah. I think so.

Williams: They also did a longitudinal. This is ‘75, so this . . .

Charache: My guess is that Darlene was part of the CSSCD. But this is Darlene all by herself.

Williams: Okay, yeah. I wondered if that . . . I think it predates it, but I didn’t know if there might have been some connection.

Charache: Other than maybe Darlene showing them you could do it.

Williams: Right. Now, why had it taken so long to do a natural history study? Because I understand that they almost provided the baseline for understanding the clinical course of the disease to do a natural history.

Charache: What do you mean? The CSSCD?

Williams: Well, just natural history studies in general.

Charache: Well, it’s a hard job, and it takes a tremendous amount of organization and a good bit of money. But you’ve got to get a bunch of doctors all to agree to collect the same information. That’s not an easy job under any circumstances.

Williams: All right, then.

Charache: And this was before the days of the great big cooperative studies that go on today.

Williams: Okay, then. So this was a major undertaking.

Charache: Oh, that was Darlene all by herself with the people in her own lab, and it really was a terrific job.

Williams: Okay. And like I said, I’m sure . . .

Charache: And I think was acclaimed by everybody. I mean, this is a review paper, but there were papers before that.

Williams: Right. Let’s see. Because, like I said, I know that the big cooperative study was done, I

believe, in ‘78. Let’s see how it was referenced. This is the first one, Powers.

Charache: That’s with Schroeder.

Williams: Okay. So the impact of this paper was to . . .

Charache: Just give a corpus of information to look back at these things and say this kid is like kids in Los Angeles or this kid isn’t.

Williams: Okay, okay. Like I said, this one I know is fairly important in that it predated the . . .

Charache: It’s just that I think it was probably the largest number of kids that had been closely followed anywhere at that point.

Williams: Okay, all right, then.

Charache: Did you come across Dr. Diggs in your studies?

Williams: Oh, sure, Lemuel Diggs.

Charache: Because he did studies where he’d have 20 or 30 patients, and when he was doing it, that was the best there was.

Williams: I see, okay.

Charache: But this was head and shoulders over Diggs.

Williams: Okay. And that she did this alone is what’s so amazing. I mean, this is a single-author paper.

Charache: Oh, no. This is a remarkable lady.

Williams: Yeah. I mean, that is -- I kind of marvel at that, too.

Charache: This one, “Crisis in Sickle Cell Anemia,” that was one of the really good papers. But Diggs was quite . . . And this one, “Sickle Cell Anemia in the Home Environment.”

Williams: Oh, with Flowers.

Charache: That was a natural-history kind of thing.

Williams: Okay. That was ‘71, okay, and the crisis of sickle cell. Oh, that’s ‘56, so that would go back.

Charache: Diggs was a pathologist, but he had a real clinical interest, and . . .

Williams: Now, at this point would you still say that the clinical observations were leading to basic research at this point?

Charache: Yeah, I think so.

Williams: Mid-‘70s?, okay. Because at some point, the tide began to turn, and I want to be sort of cognizant of the interplay between basic and clinical research, the balance of it, and when and how it began to shift, because I think that’s an important part that I’d like to capture.

Charache: Somewhere along in there, people began looking at red-cell membranes in sickle cell anemia. I think it was probably later. This was Rubin, who was out in Oakland, where he’s looking at the lipids in the sickle cell membrane and showing that they were different and that they had been, he said, flipped. I forget exactly what got flipped. But if you’re trying to look at when did people start going back to the laboratory, Rubin was one of the first, I think, who did that.

Williams: Right. They were just staying as closely connected to what they were seeing in the clinics and then taking it back to try to understand it.

Charache: Yeah.

Williams: Okay. Now, my next paper, “Deletion of Beta Globin Structured”

Charache: Yeah. Well, now, this explained what that was.

Williams: Mm-hmm. And this was considered major or . . .

Charache: Oh, yeah, because nobody knew what it was, and here it just wasn't there.

Williams: Yeah.

Charache: I don't know. I've got my name on that. I don't know how that could be.

Williams: This might have been -- let's see, deletion. Well, you're all through here, so . . .

Charache: Yeah, but I don't know -- I certainly didn't have much to do with that.

Williams: Oh. So this is Wheeler that you were talking about here.

Charache: Yeah.

Williams: Okay. *Nature*, 1975. Is that right?

Charache: I think that must have been a courtesy that I gave them.

Williams: Okay. So, tell me, though, a little bit about Dorsey. And as far as I remember, this is their first entrance into -- this is Y.W. Kan. I don't know when he got into the prenatal diagnosis business.

Williams: I see.

Charache: Those he had worked with Heisman [sp.], and then there was some kind of a falling out, and she went across the country to get as far away from him as she could and was working with Kan, and they were some of the first to do practical prenatal diagnosis. And Kan had been interested . . . I mean, I knew Kan from a long time back, and he'd been interested in hemoglobin and red cells for a long time, but he was much more of a biochemist, not the clinician, even though he'd been clinically trained.

Williams: The other thing that's significant about this time period is that this is really when Heart, Lung and Blood begins to really formulate their sickle cell branch, they start to give these grants.

Charache: That's right. That's got a whole lot to do with it becoming scientific, I mean, because they put a lot of money into what was supposed to be science that never really panned out. But you had to plant the seed somehow and get people to get the idea that this might be a career worth following, because prior to that, sickle cell anemia, as such, was a subset of abnormal hemoglobins, which were a curiosity and no more than that.

Williams: Exactly, exactly, right. I mean, what you start to notice, or at least I did in doing the literature, is now there's just a different group of researchers.

Charache: And they smelled the money.

Williams: Yeah. Bringing their talents or skills and expertise to the question, whereas before, you notice there was a lot of similar researchers working around the same . . .

Charache: You know, people like Darlene Powers were in it for the love of it.

Williams: I see, mm-hmm.

Charache: Y.W. Cahn [sp.], he was not a Johnny-come-lately either. I mean, he'd been an addict for a while. But then other people who made tremendously significant contributions came only because they discovered they could make a living that way.

Williams: And like you said, as well they could.

Charache: All the stuff that I did myself, that was all under Conley's original grant, Pathogenesis of Hemoglobinopathies. It had nothing to do with the name of the grant. It had nothing to do with sickle cell anemia.

Williams: Yeah. And if I remember correctly, those were all AM grants from Arthritis and Metabolic Diseases, if I remember. There were a few that you actually . . .

Charache: No. Heart and Lung, I think, didn't get into it until the sickle cell anemia prevention.

Williams: Yeah. Here's one. Right. So that's AM.

Charache: Yeah.

Williams: I don't know what the other ones are, HE and OG. I don't know that. But I do recognize that one. So, right, You have to look at these papers in the context of what also is happening within the funding stream, so to speak. Now, these are some letters to editors, and I copied these just to hear what the dialogue that's going on.

Charache: Now, that's Morrie Steinberg.

Williams: Right. So now he's saying do not change during the painful crisis. Was there some -- did they hear . . .

Charache: Yeah, because if the irreversibly sickled cells had something to do with the sickle cell crisis, all right, and they were the ones that have the least fetal hemoglobin in them, that then you might think that the level of fetal hemoglobin would rise with a sickle cell crisis. In other words, all the low-F cells got knocked off, leaving the high-F cells behind.

Williams: Oh, I see.

Charache: And that's not what happened. Or at least he didn't think so.

Williams: That's an interesting hypothesis. Okay.

Charache: I think that was the argument. Yeah. I mean, here he says, "Erythrocytes with low levels of hemoglobin F sickle more readily and are trapped and destroyed, but other people had shown a fall in hemoglobin F levels. Therefore, we studied in prospective fashion the variability."

Williams: The concentrations of the course in sickle cell disease. Was this a big debate during that time, what happens during . . .

Charache: Yeah, yeah, I think so. There were some papers came out of Albert Einstein that were looking at the same kind of question, you know, what happened during a sickle cell crisis.

Williams: That's Nagel.

Charache: Yeah. And this was Henny Billett. I think, was the first one.

Williams: Okay. Now, this is Graham Serjeant. This must be another. This is clinics and hematology. This is probably a review. That's '75.

Charache: You have to include Graham Serjeant in whatever.

Williams: Oh, I know.

Charache: I mean, Graham Serjeant is one of the phenomena . . .

Williams: Yeah, of this whole field. Right. What about Johnson? What about Watson in 1949? Again, I think this was some documentation that sort of missed her first statement here.

Charache: If you ever get a chance, you should try to meet Graham Serjeant.

Williams: Oh, really?

Charache: Graham Serjeant is John Bull himself.

Williams: Oh, really?

Charache: He looks like John Bull and he acts like John Bull and he's funny.

Williams: Is he?

Charache: And prejudiced. I mean, he's _____.

Williams: Really? I wonder if he's going to be . . .

Charache: I mean prejudiced towards his own views, not toward black or white or green or blue.

Williams: Oh, okay. I'll look -- I wonder if he's going to be at the conference.

Charache: He might be. You never can tell.

Williams: Okay, okay. Well, part of why meeting with you is so I can start to identify people that I should be speaking with.

Charache: Talking to Graham Serjeant is a phenomenon. Don't say I didn't warn you.

Williams: Okay, all right.

Charache: You'll probably wonder what you walked into if you talk to him.

Williams: Okay. This is another -- I don't know if this is anything interesting, hemoglobin F. Strauss. What is he saying?

Charache: Somebody wanted to write a letter.

Williams: Right. Heterocellular 1977.

Charache: That's Serjeant.

Williams: Okay. And I don't remember here. But this is the rapid hemolysis, elevation of HBF carriers.

Charache Yeah. About that time, the George Dover from here, working with Boyer, worked up these techniques for F cells and F reticulocytes and got interested in inheritance of fetal hemoglobin levels and actually went down to Jamaica and studied Serjeant's patients.

Williams: Okay. So this is late '70s, and here I think I may be a bit sketchy, but it seems that, again, this whole idea of the actual impact of fetal hemoglobin on the cells, what are the clinical manifestations. I'm trying to think of other characteristics that came out during this time period on HBF, but it seems like this begins to define where it's headed, I would say, during this time period.

Charache: I don't think so. I don't think anyone had a clue where anything was going. I don't think that anyone ever dreamed you could really do anything about it until Joe DeSimone did

the azacytidine.

Williams: I mean, people allude to the fact that this could offer a clue for therapy, but . . .

Charache: But nobody had a clue what to do. I mean, not . . . Well, I think I mentioned before the idea of using gonadotrophic hormones, but other than that, I don't think anyone had any idea.

Williams: That was Hoofnagle.

Charache: Well, no. That was probably Alter [sp] gave Hoofnagle the idea.

Williams: Okay, okay.

Charache: Hoofnagle did it.

Williams: Right. Were there other . . . I mean, were people trying to increase the synthesis of fetal hemoglobin?

Charache: Nobody had any idea how to.

Williams: Uh-huh. So it was really sort of stuck at this being able to make these clinical observations.

Charache: Yeah, that's right. And, I mean, that's why DiSimone's, the azacytadine thing, even though the hypothesis was probably totally wrong, it was the first logical approach to stimulating fetal hemoglobin production, and he was going to de-repress the gamma G, and it was just an incredible jump. And then doing it to show that it worked. I mean, you could get a good idea and not do anything about it, but he did both.

Williams: Okay. I mean, like I said, I know that during the '70s sort of the other rational approach, if you will, to sickle cell, urea cyanate trials and carbamylation, were all going on sort of in this other world of research.

Charache: That's right. And they were all based on sticking something alien into the sickle cell one way or another.

Williams: Right.

Charache: And it didn't work too well.

Williams: Right. So this was kind of an impasse almost for both groups. I mean, they were jumping up against these toxic effects. That was the extracorporeal carbamylation. Again, that world was going from the bench, I would say, to the bedside; that is, trying things out in test tubes and saying, "Let's try it with a patient." And here even . . .

Charache: Well, I'm trying to remember. I guess I told you before why Cerami ever thought of cyanate, but even that, I mean, that was a remarkable idea.

Williams: Oh, it was.

Charache: Because that came from the urea, and the urea came from Murayama. But the cyanate, that was, again, very smart guy.

Williams: Right. And like I said, it's interesting when you separate it in this way because you can, like I said, track what's going on down these different roads and start to see where each of them began to sort of stop and falter, if you will, rather than moving forward. So, all right. Well, now we start to make major strides. Let me see, chapter three. This is the '78 through '84 period. And I guess as I'm talking to you, I'm trying to think in my mind how would I characterize research during different time periods. Now, this was the natural history study. This is the one that you pointed to in Saudi Arabs. This is '78. And this was considered another landmark?

Charache: Yeah.

Williams: Okay.

Charache: Especially . . . I mean, not so much a landmark, but sort of corroborating what people thought ought to be the case, that if you could find a bunch of people who had higher fetal hemoglobin, they ought to have a milder disease.

Williams: So this was sort of validating current information. And I don't remember, again, if there were any new pieces of information that came out of this one. I don't think I looked at this one.

Charache: Other than the fact that here were these people and they had a milder disease.

Williams: Right. Okay, all right.

Charache: Not no disease, again, but milder disease.

Williams: Milder, sure. Okay. And you have that one.Now, this is one of the first, I think, discussions about therapy now, and this is where I saw the term experiments of nature. Who is this? Let's see. I have to go all the way back. This is *The Lancet*. Oh, a commentary for *The Lancet*, I guess, like an editorial. Okay. But this was the first time that I think people maybe were more serious about trying to look at this as a _____.

Charache: And this is important because they had recognized that people with thalassemia who had more fetal hemoglobin also had a milder disease.

Williams: Oh, okay.

Charache: But the reasons were totally different.

Williams: Okay. And here he says again, "Virtually nothing is known about the genetic regulatory mechanisms involved in the control of the switch from fetal to adult." So that was sort of a major difficulty in terms of therapy. And none of the small laboratory animals has fetal

hemoglobin, so it's been difficult to find a model to study it. They seem to be expressed *in vitro* and colonies grown from adult bone marrow.

Charache: Yeah. This was going on at that point where people were able to grow these colonies -- I'd forgotten that -- because you could do things with these colonies that would make them make more and less fetal hemoglobin.

Williams: Oh, really?

Charache: As I remember it. I never really got into that. But it seemed to me that you could manipulate the colonies to make them do things. I'm not sure.

Williams: Okay. Well, I will look that up and see if that, if there's anything else _____. This is just a very short piece. "Why does a Saudi sickler produce large amounts of HBF and has a mild disease whereas an African sickler produces very little and has a severe disease. The possible switch from fetal to adult production is exciting."

Charache: See, they're talking about the switch.

Williams: Right, from fetal to adult.

Charache: But *the switch*, as if this were a switch.

Williams: I see, yeah, right

Charache: Something you could turn.

Williams: Manipulate like a key.

Charache: Yeah.

Williams: Right. We just have to find the key.

Charache: I mean, that's sort of a conceptual framework.

Williams: Right, that people operated under.

Charache: Once you can talk about it, you can say, "Well, what can we do to make it switch over?"

Williams: Mm-hmm. So that was an interesting conceptual framework. Okay. That was *The Lancet.* Okay. Now, this I think -- this was '78 and this started. Right?

Charache: You bet.

Williams: Was this a landmark paper?

Charache: That's right it was.

Williams: So, yeah. This was the baboons. Right?

Charache: Right.

Williams: It's DiSimone. Now, one thing that we haven't talked about, and that is the conferences. Were there conferences that introduced these ideas, that provided . . .

Charache: Not this.

Williams: Okay. Fertile ground for these ideas? No.

Charache: I mean, DiSimone must have gone to some meeting somewhere where they were talking about methylation and repression of globin, repression of gene function. I mean, he is a Ph.D. and he'd probably go on to some scientific meeting and heard this story about methylation. And somewhere he learned azacytadine inhibited methylase or methyl whatever and got the idea that if you could inhibit the methylation of the gamma gene, that it wouldn't be repressed, and you'd make more fetal hemoglobin.

Williams: Okay. Now, the one thing that I'm a bit naive about, and maybe you can -- what is the link to erythropoietin? What am I missing?

Charache: I know what it was. I think it was George Dover here who was one of the first to show this in a kid with sickle cell anemia who had gotten chemotherapy, and as a result, all his

blood cells got turned off. And then the effect of the chemotherapy wore off, and there was a big burst of red-cell production. And with the burst of red-cell production, the kid made more fetal hemoglobin, which then came back down when he sort of settled down, as if erythropoietin had stimulated the fetal hemoglobin production.

Williams: The more recent information I'm not as up on, but this is so helpful to me. So this was the big paper, and this was done with Paul Heller. What's the connection between DiSimone and Paul Heller?

Charache: Paul Heller was a hemoglobin type from way back, and he was working at the University of Illinois, and I think Joe was in his division. And Paul was the clinician, and Joe must have gotten the idea and talked about it with Paul, and Paul encouraged him. As I think I told you, Joe had his hands on the baboons.

Williams: Right. He had the baboon colony. He inherited it.

Charache: That's right.

Williams: Right, right, okay. So that's '78 .

Charache: And that really turns everything on.

Williams: Okay. So, the next -- this is another question about a lead, fetal hemoglobin, a therapeutic lead. And I think it's more of a review. This is from a British medical journal. Let's see, who was this? Author not available. Okay. So this is another commentary from a British medical journal.

Charache: But I guess the idea is that since there are two conditions where they both have increased fetal hemoglobin, and you could find out. Or you might carry it from one to the other.

Williams: Right, being able to make use of genes that are normally switched off during

development is novel. He said this could be maintained after birth even at low level

Charache: And here's Dover.

Williams: Right, individual variation of production in survival of F cells. So this is the one that you referenced before with Boyer.

Charache: Yeah.

Williams: Okay, all right. This was another big or . . .

Charache: Yeah. I think their technique . . . I mean, they provided nomenclature. I mean, they were the ones who came up with heterocellular and I forget what all the cells the same is called. But the fact, they made it much more precise that there were these F cells and there were F retics, and you could identify them and you could measure how much there was in them with all these wonderful antibody techniques. They were terribly cumbersome, still are as far as I know. But to me, that was a real landmark paper.

Williams: And what's interesting to me is we talked that landmark papers can be landmarks for different reasons. I mean, like you said, some can simply clarify existing knowledge.

Charache: And I'd say that's what this was, that there were no new concepts here, but for once, instead of just talking about it, they could measure it.

Williams: Right, right.

Charache: And they could measure F cells in normal people and show that normal people had some of them, not very many.

Williams: Yes. Okay. We're closing in. We're going to 1980. We've got 20 more years to go. But this is that paper. So that's '78.Would Pembry, Serjeant, Richard Perrine, Weatherall.

Charache: Yeah.

Williams: This seems like a duplication of an earlier paper.

Charache: Now, I don't think of this as being anything in particular.

Williams: All right, then.

Charache: I'm sure it has something useful in it, but . . .

Williams: Okay. Well, and I can't . . .

Charache: This may be the one where they looked to see if it was alpha thalessemia. I mean, these look like globin chromatograms. Yeah.

Williams: Globin-chain synthesis.

Charache: Yeah. I bet that they were looking to see if there was something alpha thalessemia-ish about it.

Williams: Okay. Well, I'll look at that. This is a long paper, so there must be something in this.

Charache: Right, weigh it.

Williams: That's the determining factor: how much do the papers weigh?

Charache: All right. Now, this must be Steinberg and Hebbel. No, it's Schechter and Bonds. Oh, no. They're determining severity.

Williams: Yeah. What determines severity. I don't remember here. And this is another one of those editorials, so I'd have to look at this again and see what they're saying.

Charache: But they never tried to make a severity index, to my knowledge.

Williams: Let's see. Do I have that one? I have some. This is another review from of treatment of established disease lags behind. There's no effective long-term therapy, and two potentially effective forms of therapy for beta thalassemia compromise. Azacytidine is known to be carcinogenic, caution is urged. I guess what I don't know . . . So there were

trials with azacytidine, patient trials?

Charache: Oh, yeah. We did some. I think we did the first.

Williams: Okay.

Charache: In patients.

Williams: In patients. And they were carcinogenic?

Charache: It's a carcinogenic drug so that nobody was very happy using it. But the patients we had were so sick that even though they knew what the risk was, they thought it was worth the chance.

Williams: Okay. So I don't have . . . I'm sure you published that. Yeah, here it is. I have to get that, "Treatment of Sickle Cell Anemia with 5-azacytadine Results in . . ." That's you. Dover, Smith, Talbot. Yeah, so that would be another one just to follow up on the azacytidine story.

Charache: Yeah.

Williams: Okay. I need to get that one. David Nathan . . . Oh, that's interesting. I don't have that. "Augmentation of Fetal Hemoglobin Production in Anemic Monkeys by Hydroxyurea."

Charache: Yeah. Now, that was -- who was that? That was . . .

Williams: It was Nathan. That's the only name I recognize.

Charache: Yeah. No, but I think I told you the story that Nathan had had a patient who made more fetal hemoglobin when he got hydroxyurea.

Williams: I don't think he told me that one.

Charache: Yeah. He talked about this at a conference.

Williams: Okay, all right.

Charache: And Dover and I took the idea and came home with it. But Nathan was already working on this himself, first with the monkeys, and then Platt gave it to patients.

Williams: Okay, that's right.

Charache: But this was Nathan's clinical observation that led to all of this.

Williams: Okay, all right. We've got another significant clinical . . . I mean, that's why this is such a rich story, because you really see this clinical research, clinical observation informing what's happening in the lab.

Charache: Yeah, absolutely.

Williams: And you don't see that, again, with this other research trajectory, which really begins with people making observations and testing and then saying let's try it on patients. I see a distinction.

Charache: Well, I don't know.

Williams: I thought that a lot of the modifications for hemoglobin and interaction with hemoglobin was really based on observations that people made in test tubes.

Charache: I don't think so.

Williams: You don't think so?

Charache: No. I think part of it is that hemoglobin is so easy to get a hold of that if somebody's anemic, you say, "Well, there must be something wrong with his blood or his bone marrow," and you can get both of them and look at them. I mean, it's not easy to take a piece of somebody's thyroid gland or his spleen or his stomach or something, but, I mean, blood is so easily biopsied that it facilitates the leap from one to the other.

Williams: Interesting, mm-hmm. I've not heard that before. That's very interesting.

Charache: Yeah. That's, to me, always been the great disappointment why dermatology hasn't gotten further than it is. It's easy to get skin.

Williams: Right, right. Well, there was a recent article in *Science* by Jonathan Rees, "Clinical Sciences and the New Complexity," and he actually argues that some of the biggest leaps in dermatology were made from clinical observation and not interesting observations at the bench. And he cites acne as almost the case for this, and he says basically the idea that retinol A -- I think that's the treatment -- was almost, it was just a clinical observation from patients that had been using sun lamps. Is that right? Yeah, I think so. I think that's what he said. But they could not have predicted it. There was nothing from their research . . .

Charache: Yeah. But science is full of that, isn't it?

Williams: I suppose, but he says we don't always need to have a fundamental understanding of a mechanism of. . .

Charache: I would absolutely agree.

Williams: In order to treat it.

Charache: Right. Science . . . I mean, chance favors the prepared mind.

Williams: Right, right.

Charache: I mean, if somebody sees something in a patient and he says, "My, look at that! Why?" And if you can get his blood and look at it, that helps.

Williams: That helps, yeah. Well, but to the extent, you know . . . I mean, I guess one of the tracks that I want to study in this research is that really it seems that when Pauling came forward with this idea of a molecular disease, everything turned toward a rational approach,

meaning we know the structure, we know what we need to do, and let's base a body of research on a rational approach. It seems to me that fetal hemoglobin is almost the opposite.

Charache: There's nothing to do with that. Well, but it wasn't that that wasn't a good idea. It turned out to be so much harder than anyone could ever dream of.

Williams: But that's the problem, I think, in sort of funding research or thinking that research that's based on a fundamental understanding is a sure shot.

Charache: Well, but that's a hard one to sell to Congress. All right? I mean, if you're saying let's give a bag of gold to a whole bunch of bright young people, like the MacArthur grants, and let them just do something with it, Congress can't do that.

Williams: I see.

Charache: Their constituents will say, "What kind of jerk is this that we elected? He's giving away money for nothing." And so the Congress has to try to balance stimulating science for science's sake, and applied science.

Williams: I suppose, I guess -- I mean, I don't think you . . . Well, maybe you could have predicted fetal hemoglobin, but Janet Watson's observation was way before she had any understanding of fetal hemoglobin. It was like you said. It was just a smart person saying, "Well, wait a minute. This is a genetic disease. Newborns ought to show some symptoms. I mean, there's no reason that they shouldn't." But it's sort of not necessarily based on a rational understanding of it at all.

Charache: But it comes later.

Williams: Yeah. Yes, I know. I suppose you're right. I mean . . .

Charache: I mean, science I don't think progresses logically.

Williams: Even though . . .

Charache It's punctuated with all these smart guys who get a different synthesis of things.

Williams: Of information.

Charache: And if you have a patient to show you or you get the astrophysicist and he sees something up in the sky that doesn't fit and he says, "Ah-ha!" well, that's just like seeing the patient that doesn't fit.

Williams: Right, right, right. So . . .

Charache: But you've got to have the astrophysicist who's trained to recognize whatever he's seeing, and so you've got to have the physician scientist, too.

Williams: Right, right. And they're a critical piece of this, that it also seems to me that there are no more or that the mechanism for producing these physician scientists is just sort of drying up. It's very hard, with managed care, for people to be seeing patients and also participating in laboratory research.

Charache: Well, I don't think that's necessary.

Williams: You don't think?

Charache: No.

Williams: Good physician scientists or . . .

Charache: I don't think it's that so much as the fact that the place where the physician scientists are is the medical schools.

Williams: Right.

Charache: If you're saying managed care is making life hard for the medical schools, yeah, then I'll

say yes. But, I mean, you've got to have the medical schools.

Williams: Oh, no. I mean, I agree. It's . . .

Charache: With whatever that involves, I mean, because it's basic science, it's clinical science, it's the whole thing.

Williams: No. I think we're saying the same thing there. I mean, I think that the academic medical centers are facing troubles or under pressure, under stress as a result of the managed care.

Charache: Oh, sure.

Williams: And so I think the physicians have been even pressed harder in having to make choices.

Charache: Things . . . I mean, this is -- you could get off on this talk all day.

Williams: Right.

Charache: But, I mean, this recent business with the Privacy Act, far be it for me to say that Mr. Bush has done anything good, but the change between the way that thing was promulgated and the way it ended up, it's better for science than it would have been before, maybe some patients' rights not so good. I don't know.

Williams: Right, right. Well, I only bring this up just to notice that some of the earlier researchers that I spoke with, yourself included, there was a very close relationship between the patient and the laboratory, and that, of course, is because you were literally seeing your subjects of study.

Charache: Sure.

Williams: And what I find more interesting now is that you really start to see this gap between that. I mean, there's not the researcher that sees the patient necessarily and then walks back to the laboratory and tries to understand.

Charache: Well, because he probably doesn't know how. But if he's got a buddy that he knows who's interested in this sort of thing, I mean, I'm over here and there's somebody in the biophysics department across -- this is hypothetical -- but there's somebody in biophysics who's interested, let's say, in blood flow, and I say, "Here's a very interesting problem in blood flow," and . . . Well, actually, that is what happened. I was here, and a friend of mine was over in biophysics, and he was interested in modeling things. And I was filtering red cells through little filters, and the question was, what could you tell from these results? Well, he modeled what was going on and eventually came up with a model that potentially explained what we were seeing.

Williams: I see.

Charache: And things like that, or the whole business with endothelial adhesion in sickle cell anemia, people knew for years that sickle cells were sticky, but the thought that they'd stick to endothelium, Hebbel must have talked to somebody who was growing endothelial cells over a glass of beer or something or other, and he said, "Well, what would happen if we . . ."

Williams: And history.

Charache: We'd better get out of here.

Williams: All right, then. Well, I think that's it

Charache: That's a grand whole book all by itself.

Williams: Well, like I said -- we'll go ahead and shut this off.

END OF INTERVIEW