OLIVER SMITHIES INTERVIEW

Session 1: October 27, 2005

1. Flying

NC: It is October 27th, 2005. I'm Nathaniel Comfort and this is an oral history interview with Oliver Smithies. We are in his laboratory at the University of North Carolina.

Dr. Smithies, we're going to begin chronologically, but I wanted to first begin with a question about flying.

SMITHIES: Oh yes.

NC: I wanted to ask—you have a number of references to flying, and you use flying metaphors sometimes in your writing. When did you begin flying? Do you have your own airplane? Tell us about flying a little bit.

SMITHIES: Well, those are two different questions. I always wanted to fly since I can remember as a child, when I was probably about a fourteen-year-old, something like that, reading a lot about flying and about soaring, and thinking it would be something neat to do. I remember in my home county of Yorkshire, in England, when I was about that age, going and investigating what was going on, and meeting a pilot who had a broken arm or something like that, and his glider wasn't in good shape. But he was telling me about it and how enjoyable it was, etc., etc.

But that was just at the beginning of World War II, so all of those sorts of activities stopped, so I didn't do anything about it. Then later on I thought it was not possible to be a pilot and be color blind, and I'm color blind, so I didn't do anything more about it. Then I think it was—I don't remember the exact time, although my log books would tell me, but it's probably about forty years ago now, I had a rather unhappy time in my marital relationships and was taking some time off from being together with my wife and began to think about flying, and realized that I could fly gliders without having a color-vision problem.

I also found out fairly quickly that it was possible to fly a regular airplane with a color-vision problem, although I realized later there would be limitations, so I began to take lessons more or less at the same time in gliding and in a regular airplane, and I eventually managed to get my licenses in those.

No, I've got the history wrong when I think about it. That's not quite the right time. I first began shortly after I was in Wisconsin. I moved to Wisconsin in, oh, let's see, about 1960, and shortly after being in Wisconsin I was still working with collaborators in Toronto, and I went to Toronto to learn a new technique from one of my old collaborators, or my old friends, too, in Toronto. I went there to learn this technique, and I found that I just didn't like it, and I didn't feel that I wanted to spend a lot of time in the laboratory doing something that I didn't like doing, so I decided not to bother to learn that particular technique.

Then for some reason or other I thought I would go down to Toronto Island Airport

and see if I could get some flying lessons. So I went down to Toronto Island Airport and I took some flying lessons there. Then when I came back to Wisconsin I also went to the local airport there, not the one in Madison, Wisconsin, but the one in a small, not-quite suburb, but nearby town, Middleton, Wisconsin, and I took some flying lessons there, and flew for a little time, not very well, but I began to get going.

I did manage to get as far as to where I was flying solo in a small airplane. The person who was my instructor at that time was called Field Morey, F-i-e-l-d M-o-r-e-y. His name was Field because he was born on an airfield, and his father was one of the early pioneers of aviation in the United States, dating back to the days of Lindbergh and so on. And this young man, he was a young man at the time, he gave me some lessons and I managed to go solo.

Then my wife and I tried again in our marriage, and she didn't really like me to go on flying, and so I didn't do anything more about it. It was then only about fifteen years later—or whatever the time is, I don't remember the dates exactly—that I then—my wife and I finally decided it wouldn't work after quite a long more time, and I began to think what would I do with my time now I didn't have to mow lawns and things, and that's when I took up flying again.

I went to the little airport, Morey Airport. It's called Morey Airport after the father, originally. I went to Morey Airport and took my log book there and said, "I used to fly with Field Morey, and maybe I could fly with him again."

Field Morey looked at my log book and said, "Yeah, we can fly together." And that began a long friendship which still exists between the two of us, and he was my instructor and is my instructor. In fact, next month I'm going to go flying with him and a friend, and we're going to do a trip of about a couple of thousand miles around a whole number of airports in the western U.S., practicing instrument approaches. But anyway, that was the beginning of my flying again.

Then ever since I've gone on and continued to fly. Probably I've flown more than four thousand five hundred hours as a private pilot, which if you think about it is ten hours a day every day for more than a year, so a substantial part of my leisure time has been spent flying, and I fly pretty well, try to fly every week still. I flew this last weekend, and I have the enjoyable thing of being able to say that I flew both my airplanes—I have two airplanes—I flew both my airplanes on my eightieth birthday, and there aren't very many people who can say that, and so that's rather nice. So it's been very much a part of my life.

If you look behind you, you'll see all sorts of reminders of it there, on the top left there. That's the aircraft that I keep at Raleigh-Durham [Airport]. It's a Cessna 182. It's a very safe and versatile airplane, a single-pilot airplane. It can carry four people. The one, if you look over in this direction, that's the motor glider [glider/airplane combo] that I have, which I keep at Chapel Hill Airport here, and which is just a fun machine. It's not really a terribly good airplane. It's not a terribly good glider, but it's a lot of fun, and that I fly as well.

NC: So that you can fly up on your own power, and glide down?

SMITHIES: Yes. It has the advantage over a regular glider of, that you don't need

anybody to help, and you don't need another aircraft, so you can go and fly it any time. It'll take off on its own power, and then when you find an up current, you can cut the engine off and feather the propeller and be a glider from then. But being a compromise between an airplane and a glider, it doesn't do either very well. But still, it's a fun machine. So you'll see that many of my notebooks have little notes about I did something flying on that particular day, or whatever.

2. Childhood and Education

NC: All right. With that I'd like to now back up, and could you tell us, just start with when and where you were born, and tell us about your family background and childhood.

SMITHIES: Yes. I was born in Halifax, Yorkshire, England, in 1925, June 23rd, and I'm a twin. I was the older of the two by four hours, and my brother is still alive. His name is Roger William Smithies, Roger, and we're fraternal twins, so we have many differences. But, of course then, being brothers we have many similarities. We have lots of things in common. I just came back from having several days with him, and my wife was with me, my wife Nobuyo, my second wife [Dr. Nobuyo Maeda], at a celebration that he had arranged to bring all the family members together to celebrate our joint eightieth birthday, so I see him quite often.

So I was born in Halifax and at that time we lived in a row of houses called Woodhall Crescent. It was actually just a row of houses, it wasn't really a crescent, on, I think the road was called Huddersfield Road; I've forgotten the name of the road. So those houses are still there. They were owned by the town council, I think, would be called council houses. My parents rented a house there, and we lived there from the time I remember. I'm not sure whether we were living there when I was born. Now, I don't really know that. Lived there until I was eleven.

My father was an insurance salesman. He worked for the Canadian company, some life assurance company of Canada. My mother was a teacher of English at the local technical college. There were, in a sense, three levels of education going on for children in Halifax at that time. There were the elementary schools, which you could stay at that school until you were fourteen or whatever, and then you left school.

Or you could go, after having been in the elementary school, which we call a primary school here, then you'd go to a high school, or in our case the school was called a grammar school. It was a rather distinguished one we'll talk about later. Or you could go to a technical college, or what was called a technical college, but it was at the level still of—you didn't get a degree yet, that sort of thing. I might have that a little bit wrong. I have it wrong. The technical college was more advanced, but it wouldn't lead to an academic degree. It would lead to various certificates in engineering or whatever it might be. That's what—my brother ended up going to the technical college later, after the high school, and I ended up going to Oxford University.

So my mother taught English there, English literature and English composition, you might say. She was very fond of the language, and we learned much from her as a result of that. I think much of my enjoyment of words and writing, which I really do enjoy when I do it, comes from her. Getting the words just right, and enjoying doing them is quite important, and if you look around here, you can see that I've just been doing some writing, and I've got a dictionary here, and a thesaurus, and a practical English usage

book, and these are my constant companions when I'm writing, because I can't find exactly the right word I want. My notes, when I'm writing, are really terrible scrawls. They'll be written on top of three or four layers of corrections on the same page. I still don't type, and so I don't use a computer when I'm composing or when I'm writing. And that was from her.

My father was, though he was an insurance salesman, he was a very practical person. We had an automobile, which at the time was rather uncommon; most people didn't have cars. Now, remember we're talking about 1935, that sort of a time in England; well, anyway, around that time, before '35. We had a car but it was not a very good car, and it was constantly breaking down, and there was a lot of repair work. I remember helping him when I was probably about seven, helping him to repair the engine and things like that. We'd have to replace the valves and didn't have the money to buy decent valves, so you had to just pick amongst your collection of worn-out valves, which is the best one to use and that sort of thing.

So I got from him, I think, a great deal of what you might call the practical skills, and maybe the hand skills came from him, if you want to think of some coming from one parent and the other. But obviously, it's a mixture. But I learned a lot from him about making things work, and his father, my grandfather on his side, was a very practical man. I, for example, remember very well even to this day him teaching me how to straighten a bent nail, because he couldn't bear to throw things away, and I'm the same if I see something. I'll see a piece of good wire in the road when I'm walking; I'll pick it up, because it could be useful for something. And I'll see a good screw, I'll pick a good screw up, but we would also pick up bent nails. At that time we were at that sort of level, and he showed me how to straighten nails and things. So those are my two parents.

My father was a quite successful insurance salesman, but he always sold at what he felt was the level that a customer could take, not trying to oversell, so he was a great friend of farmers and small people. He just sold small insurance policies to them. I think he had a great belief in the value of an insurance policy, which I later came to doubt very much, but he honestly thought they were very good, and he helped people and became friends of the families that he helped, or to whom he sold insurance. I think much of the kindness, if I have any, towards other people comes from his example. So I sometimes say I got some of my brains from my mother, and any kindness I might have from my father, and I enjoyed both of them very much.

So that was when I was between the ages of when I can remember, until I was eleven, we lived at that house on Woodhall Crescent. It was about a mile, I would say, a mile away from the local school, which was just a country school, really. It was a village. The village was called Copley, C-o-p-l-e-y, and that was an interesting village because it was one of the industrial villages made, you might say almost developed by a factory. It was a cotton mill down in the valley. It's a pretty valley, and down in the bottom of the valley was this cotton factory, and around it were a lot of houses built at the time of the factory, sometime in the nineteenth century.

At that time they were considered quite good houses, but they didn't have any internal toilets, they didn't have water inside, and they just had outside toilets. It was quite primitive, we would say now, but in their day they were progressive. And the little village, then, had this school, the elementary school, which was not particularly a good school, and that's where we would walk to every morning, and, of course, come home at

noon for our meals. In Yorkshire at that time, and probably still, the midday meal is the main meal of the day, and we would come home for dinner, as we called it then, in the middle of the day.

So I went to that school until I was eleven. I don't know whether you want me to tell you about the teachers and things, or whether this is the right time to tell you. I could go on for hours on this topic. You're going to have to stop me; it's not the other way around.

NC: What were your favorite subjects in school?

SMITHIES: I don't really remember very much about what I learned in the elementary school, except I think I must have enjoyed math rather, because I don't know that I was ever taught it well by the school, in the school. The headmaster was not very good. There was a senior teacher, a man whose name was Nethergate, N-e-t-h-e-r-g-a-t-e, as I remember it. I think I've got his name right. Nethergate, who was an excellent teacher. I remember him as my main teacher in that school, but I don't remember what I was taught particularly. I remember learning decimals with my father. My father taught me decimals on the condensation on the wall of the bathroom, because I was in having a bath. I must have been six or seven or something like that, and the steam on the wall makes a misty surface, and I remember him teaching me decimals on the bathroom wall. I still have a vision of that. I don't know how accurate this is, but it's my recollection of learning from him about decimals and things, which seemed neat.

So I did quite a lot of—I made things. I had access to my grandfather. My mother's father lived, oh, only ten or so houses away from us in the same row of houses. His name was Ben Sykes, S-y-k-e-s, and my grandmother's name was Elizabeth, I think. I think she was always called Lizzie, but I don't remember. She was just called Nannan. You know how children have different names for grandparents, and ours was Nannan. She was Nannan Sykes and he was Grandpa Sykes. I don't think we called him Grandpa Sykes; I can't remember what we called him. But anyway, they were nearby.

So he had a collection of books that were, oh, something like bound magazine articles, The Boys Journal or some such thing, and it had various projects in there that you could read about. I remember reading about making what many kids tried to make, a string communications system, where we had a microphone-cum-speaker at one end of a piece of string, and go to another one on the other end.

That particular recipe or that particular plan required that you got a pig's bladder. You made a hole in a piece of wood, of maybe nine inches or so, or ten inches in diameter, and you nailed this pig's bladder, a sheet of the bladder onto the surface, and then you stretched it with a weight and let it dry, and it would make a cone then that was quite good. I made one of these, and my friend who lived nearby made another, and I remember quite vividly his was quite poor. Mine was rather elegant, it really was. But we never did learn the principle that that type of microphone-telephone system only works when the string is tight. I don't think we ever learned that principle, so it never worked.

But I used to make things, and I think from a very early age, as a result of reading some sort of comic thing in this book, in this collection of books or some other sort of thing, I knew what I wanted to be. I wanted to be an inventor. That was what I wanted to be. I didn't know what it was to be a scientist, but I wanted to be an inventor, and that's what I've been ever since, really. All my science has been invention, if you like, not just

discovery. A big part of it has been inventing new techniques, and finding things out with the new techniques, and some good science as well, but invention has been a big part of it, tool making if you like. So I knew from an early date that I wanted to do that.

NC: I do want to come back to the idea of tool making.

SMITHIES: Let me just—I think we didn't get far enough on that to realize the connection that happened in that part of England, well, throughout England at that time, that when you were eleven, so remember, I'm at the elementary school with my brother, and a sister five years younger was then also at the school—then at age eleven you had an exam which was an intelligence test. I don't know whether they were experimenting with this, or whether they really knew if it was good, but anyway they had what they called an intelligence test, and that would determine which high school you could go to, whether you would go to the best one, the grammar school, or you'd go to the next one down, and I don't remember the names of all the others.

So at eleven, I took that exam and I got admitted to the grammar school, or offered it, but my twin brother didn't. There were not many places for the whole town. There were only sixty places in a year in that school, and the town was a town of about a hundred thousand people, so it was very limited access to that more advanced education. And so I didn't want to go. I said, "I'm not going to go if he can't go." But some student who was just ahead of him, evidently, on the list dropped out and he did get to go on, so we both went.

And so we changed to that school—at about the same time we moved our house from down in the valley near Copley to up on top of the hill, as it were, on the flatter part above the valley. There's a street called Dudwell Lane, and we lived at 33 Dudwell Lane, then, for the rest of my time in Halifax. That was about probably a mile and a half from the grammar school, and it was called Heath Grammar School, H-e-a-t-h Grammar School. It was a very old school, I mean, in terms of history. It was an Elizabethan grammar school. Queen Elizabeth [Elizabeth I] started some of these schools, and this was started in 1596, or something like that, so it had been a good school for a long time, enough that the street on which it was built was called the Free School Lane, because the school was free, so it was on Free School Lane.

The teachers were very high-caliber teachers. Many of them had degrees from Oxford University or comparable universities. They were first-class teachers. The classes were small. There were thirty in a class, and two parallel streams. I was put into the—and my brother, the both of us were put into the lowest stream. There were two streams that they had separated people according to what they thought their abilities would be, and one was a bit more advanced than the other. I was put into the lower one, but I think I was top of the class so much that they just moved me into the other stream, the A one. I've forgotten whether it was called 3A, 3B, or whatever it might be, and I moved into that. My brother stayed in the other stream.

In that stream, then, we had a pretty rigorous education. We did several languages. We did French from the beginning and Latin from the beginning, and later on did some Greek and some German, and then we did math. The math was good math, right up to differential and integral calculus before you're seventeen, you know, so that's pretty good. In fact, I never took any more math, never did any university math or anything. I didn't need to do any.

I think probably the math was one of the subjects I enjoyed very much. The teacher's name was Brown. We used to call him Oddy Brown. He was a rather strange man, and he wasn't a good disciplinarian, and in general the students didn't like him. His classes were disorganized. He would ask, for example, for a logarithm from the class, and they would give him the wrong logarithm deliberately, and that sort of thing, so there wasn't a good interaction. But he knew math very well, and I learned from him to love calculus and understand it well. I think I was one of the few students who really enjoyed what he had to say. But he had a Ph.D., I don't know from which U.S. university, but he had a Ph.D. from the States, and he really was a mathematician.

But the other teachers were good. Now, I had a good chemistry teacher and his name was Phoenix, like the bird. We had lots of practical chemistry. I mean, you had good labs and the freedom to do many things. So that went on for about, let's see, it would be four years there, so from eleven to twelve, thirteen, fourteen, fifteen, by the time I was fifteen or so, sixteen maybe.

Then you moved into what was called the sixth form, and that was only for students who wanted to go on to the university and tended to be very small, and I decided to do the physical sciences, physics, chemistry, and mathematics, in the sixth form. I think maybe there were only three of us, or two or three of us, and even less than that, and you more or less had to teach yourself. I had a grand time studying for the exams for entrance into the universities. I read a lot and enjoyed the various physical equations that one needed to understand optics or heat or whatever it might be.

I had the notebook, which unfortunately I haven't been able to find. I don't know that it exists anymore. But I wrote down the derivations of many formulas, and I would always pick the best derivations of the formulas. I couldn't remember the formulas very well, but I always remembered the derivations of the formulas, so I got to enjoy that side of things, and I did the experiments, some of which were pretty atrocious, with organic chemistry, reading about "this is one of the most disgusting-smelling chemicals known to humankind." So then I would make that one. [laughter]

And various, I'm afraid, explosives which I made, so that Phoenix, cleaning up one day after some mess I'd left, the bench crackled under his hand. I'd been making nitrogen tri-iodide, which when it dries is really quite sensitive to explosions. They're not very big explosions, and this crackling. So he griped, "That Smithies again!"

Actually, I made some of that and brought it home, and put it on—wet, it was reasonably stable. When it got dry it would get very unstable, sufficiently so that although I put it on top of a bookshelf in my home, my father walked by and it exploded from just his walking by, not very violently. There wasn't very much of it. That was the sort of chemistry that you learned.

But I got a scholarship to Oxford. So that's two years in the sixth form, and then one applied for scholarships, so that means I'm seventeen now, round about seventeen, seventeen or eighteen. Then I went to Oxford and applied for a scholarship. I didn't know anything about Oxford, really, and asking, well, what should you apply for? Well, my teachers were pretty good, and they said, "Well, why don't you try for the best college, best academic college?" The best college was Balliol, B-a-l-l-i-o-l. Balliol College, Oxford, is still recognized as probably academically, the best college of Oxford, which

has many colleges, you know.

I applied for that, and I got the best scholarship that they had, so that was rather neat. I remember getting a telegram about that, very excited, in which they made a comment that something or other. They said, “Mathematics very promising for a person so young,” which for me was amazing, because I remember the math exam quite well. There were maybe about fifteen questions and I didn’t answer any of them. I couldn’t answer any of them. I mean, I couldn’t get to the end of any of the questions that I remember.

I remember one in particular that was—I still remember it, because it’s a very interesting question. It was—now, remember this is probably 1939, 1940, ’41. Yes, ’42 it would be, wouldn’t it? England’s at war then, so we had lots of experience with fighter aircraft, and fascinated by those, of course. The problem was: a Spitfire weighs so much; it has eight machine guns, which I knew, of course; and the machine guns fire bullets of this mass at so-and-such a velocity, and at how many per second; and how much does it slow down when it fires its guns, was the question. And you had to work out how much it slowed down.

I did this and I got 150 miles an hour. I know that’s wrong, and did it again. I wrote these down and I tried several ways, and I always came up with the wrong answer, and I wrote in my paper that, “I’ve done this several times, and I can’t see where my mistake is, but this answer is obviously ridiculous. It’ll probably slow down about thirty miles an hour.” I think that that’s where they got the idea of math very promising for a person so young, because it showed that you used common sense as well as mathematics in thinking about it. But I don’t remember being able to answer any of the other questions, and that one I struggled with and didn’t get right.

So I got a scholarship to Oxford, and that’s when I—that was after the first semester, as it were, the first term as we called it, in the scholarship period of one’s education there. So I talked about four or five years in the ordinary part of the school, and then in the sixth form two years in which one did advanced things, and which I mentioned physics, chemistry, and mathematics, no longer any of the arts at that point. And then you applied for a scholarship, so then that made three years, but that’s the time when I was alone in that third year, because there wasn’t anybody else left, when the experiments were done, when they exploded.

Then I had another two semesters as it were to just play around, and so I just went on doing those sorts of things for a couple of semesters before I then went to start college in the following September, I suppose it would be. So it goes on, and one can go on indefinitely with it.

NC: So how did the war affect your education and your career plans?

SMITHIES: To me it’s rather mysterious, because there was, obviously, there was general conscription. I mean, there was a draft throughout the whole country. But they had various sorts of, I don’t know quite what the word was anymore, of deferments. You could be deferred. That was one possibility. Your service in the army could be deferred to complete your education, because the experience from World War I had taught the British government that you’re better to let a person make a contribution that they can make academically, rather than immediately put everybody into the same level of armed service, because they’ll make a bigger contribution as what used to be called a boffin, as

a scientist or whatever it might be, or even if you were an M.D. or if you were going into medical school, it's much better to let you complete your medical training and then serve in the medical corps of the army, so that was deferment. Then after you got your degree and after you'd served some period in the army, then you would go on with your life, as it were.

There was also a different form, and I don't know quite why I got into it. I'd never had anything to do with applying. You didn't apply for these things. But I was directed, you might say, rather than deferred, to do my studies, so I never was in the army. I wasn't drafted, because I was, in this sense, directed. There was some sort of a board that met, of which I think Sandy [Alexander] Ogston, who later became my major professor for my degree, and had been partly responsible for my getting into Balliol College initially, because he was on the Admissions Committee, you might say—I think they were part of a board, and they would review students' progress and say, "Yes, this student ought to go on doing what he's doing, because that's the best thing to use him for." So I never did serve in the army or anything, but we had the equivalent of ROTC. It's ROTC here, isn't it? **endnote 1** Yes. I sometimes forget which country I'm talking about—the equivalent of ROTC there, until we learned [to use] rifles and how to shoot a Bren gun, **endnote 2** and then, of course, did fire watching for incendiary bombs at night. We even had that back in high school, because back in high school, I was still in high school during the beginning of the war, and there were fire bombs, incendiary bombs dropped on my town.

It was too far north for the German Air Force to reach much, and we had very little damage in my hometown, and Oxford escaped damage, too. It didn't get much. I think it might have had a few incendiary bombs, but nothing that caused any real damage.

So in that sense, there wasn't any impact. But there was a big impact on the number of people who were in college. Therefore, classes were very small. My whole college was only ninety people, about, at that time. That was the whole college. And there were only—well, I think I was the only student in my year, the only medical student in my year in that college, and I had two friends who were the year or two years before me in the college, in the medical side of it. I've probably got that wrong. I really forget exactly how many medical students there were, but there weren't very many, anyway, so that meant exposure to our teachers was very direct, to our professors. We had close contact with them, and I'm sure as a result of that our education didn't suffer, and might have even gained from that. So the impact of the war, in that sense, was quite small.

Of course, my father had to go into the—well, he didn't have to go in, but he volunteered to join the armed services. He was overage for conscription, but he had this idea that "if I serve, my boys won't have to serve." I don't know where he got the idea from but that was his thinking. He served in the armed forces and he didn't get injured, but he certainly had a lot of problems as a result of it, because he got diphtheria and malaria, and he was in India and Burma, and his health was markedly affected.

NC: In your Lasker Award interview with Raju Kucherlapati—

SMITHIES: Yes.

NC: —you said you were a medical school dropout.

SMITHIES: Well, that's true, because when I went to the college I essentially think I got

my scholarship as a result of my physics, which was really quite good. I mean, I understood physics very well, and the math thing, and chemistry was not as good. When I went there they asked me what I wanted to do, because you more or less had a choice when you went to the university, and I said I couldn't quite decide whether I wanted to be a physicist or an M.D., and I don't know why I wanted to be an M.D.

I don't have any recollection of wanting to cure people or anything, but I must have had somewhere this sort of feeling, and I decided to enter medical school, which surprised them, I think, somewhat. But I did enter medical school, and then Sandy Ogston was my tutor at the time. I'll explain a little bit about that later, because the tutorial system in Oxford is very much a part of how I learned science and how I have tried to teach it.

Let's see, the med school dropout thing, yes. The first two years were essentially getting a bachelor's degree, and you did anatomy, and anatomy really was anatomy in those days. You did dissections of cadavers and you would dissect—well, you would have one semester, for example, entirely on the arm, another semester entirely on the leg, another semester on the thorax and abdomen, the head and neck, and so it was very detailed in anatomical dissections, and you were expected to learn anatomy this way.

I actually quite enjoyed it. I couldn't remember stuff very well, and I didn't always work hard enough. I remember one professor, a woman, who was—I should tell you about her a bit later—[Dr.] Alice Carleton. She was a gorgon of a woman, and she died her hair pale blue. It was white and it was dyed pale blue, and she was cross-eyed. But she was a rigorous teacher of anatomy. I remember her one time looking at what I'd been doing and saying, "This is terrible, Smithies." Some sort of score of what—"You can do better than this, can't you?"

[In cringing voice] "Yes, Miss Carleton. Yes, Miss Carleton." [laughs]

"Well, go ahead and do it," or words to that effect. So I was doing anatomy, and she—I might as well tell you this story anyway, because it's a good story. She would go around the class, you know, pointing to people and asking questions, boom, boom, boom, boom, down the row of the class. And if the person didn't answer, she'd just move to the next one, just point to the next one.

I remember very distinctly her asking a question to which I knew the answer, and I didn't know she was looking at me, because of her crossed eyes. So I didn't answer, and then she said, "Next," and the person next to me answered.

And I said, "Look, I could have answered that," you know, that sort of expression. And she said, "You might well wake up, Smithies."

But then it came around the next time in the same class, and she asked a question. This time I didn't know the answer, so I remember those incidents rather well. But physiology was also a part of what you learned, and organic chemistry, so you were doing three subjects really, physiology, anatomy, and organic chemistry, and I did those. You did two years and you got your degree. It was a bachelor's degree and was not what's called an honors degree. You didn't have any grade, as it were, attached to it. And you could then stay on an extra year if you wished, which a few people did.

The honors school then of animal physiology it was, was you could take a year—I think it was a year—to get an honors degree. I don't have it here but I have it in the corridor, a picture of the final class of that, and there were probably twenty students, so that it was very small, and again you have this marvelous teaching.

During that period it became clear to me that I didn't want to go on with medical school; I wanted to do research. I was somewhat torn about doing neurophysiology. I was fascinated by many experiments which were done in nerve conduction at that time, and it was an excellent school of neurophysiology, so-called Sherrington School in Oxford, and I was thinking about doing that. Then Ogston came back from—this was still during the war. He came back and began to teach his style of physical chemistry combined with biological problems, which nowadays we call molecular biology, but there wasn't a name for it then.

It was called physical biochemistry, if anything, and I found I liked this very much, what he was—the way he taught and the things he talked about where you used physical chemical principles to investigate or describe what's happening in biological processes, like say the energy metabolism, of how is energy generated in the body, etc. I liked this a lot, and decided that I would then do a Ph.D. with him in this subject.

But I felt that I didn't have enough chemistry then, so I got an honors degree. I got a first-class honors degree, which was fairly distinguished, and you pretty well had to have—to become a professor in one of the major universities, you have to have first-class honors. I decided then to do a Ph.D., or a D.Phil. [Doctor of Philosophy] as it was called in Oxford, with Sandy Ogston. But I wanted to do—it was in the biochemistry department and he was a chemist, so I decided that I needed to do chemistry. So I dropped out of med school at that time.

That's when I dropped out of med school, after the third year, then changing from being a—after that physiology degree, normally I would have gone and done clinical work then in a London hospital, for example. But that's the point when I then joined the chemistry undergraduate curriculum. I joined it as a second year, because I'd already done organic chemistry and I could miss that. So I joined on the second year and did the second and third year of the chemistry degree, and took another degree at the end of that in chemistry. But you couldn't get an honors degree at that point, because of the regulations, the university regulations. You could only have so many years to do this, and it was called overstanding, so I was overstanding, so I didn't get a first-class honors or a second-class honors, or whatever in chemistry, but I did chemistry.

That was pretty important because I now had two skills that aren't so often combined. I had the first-class education in anatomy and physiology, an introduction to the workings of the body, you might say, and confidence in physiology, combined with confidence in chemistry. So I had a chemical education, as it were, which made me feel comfortable in either field, and I've used both ever since, sort of without thinking. I'd even go from one to the other with no difficulty, and that's proved to be very helpful in the work I've done, so that's the med school dropout, and at the same time shows how the combination that resulted from that has been quite powerful for me.

3. Doctoral thesis; Postdoc at the University of Wisconsin

NC: Okay. And from there you went to Wisconsin. Now, how did you choose?

SMITHIES: Well, then I did a rather undistinguished Ph.D. with Sandy Ogston. I enjoyed it a lot. I devised a method of—I think I should tell you about this. I devised a method of measuring osmotic pressures. Osmotic pressure is the pressure that you need to put on a solution to prevent water getting into it.

That's a rather difficult way of saying it, but if you have a membrane, for example, through which a protein cannot pass, and you have a protein solution on one side of the membrane, and you have water on the other side of the membrane, if you think about it, there isn't as much space for the water where the protein is. So the concentration of water on the side of the membrane where the protein is, is less than the concentration of water when it's pure water. So the pure water tries to diffuse from where it's the high concentration to the low concentration, going through the membrane, and the protein can't get through, because it's impermeable to protein, and so there's a tendency for the water to go through, to try to dilute the protein solution.

But you can stop that by applying a pressure, and the pressure that's needed on the side of the membrane where the protein is, to prevent water coming through, is the osmotic pressure, and it can be used to study interactions between molecules and molecular weight. I was given a problem of understanding something which would seem extremely strange to a person now. I was looking at the problem of the inter-conversion of the serum protein albumin into globulin. There were only two serum proteins known at that time, albumin and globulin, and some of the ultra-centrifuge studies which were done at that time to measure molecular weights had been done by Ogston. He had got evidence of a rather strange behavior of these proteins in ultra-centrifuge; it appeared that the low molecular weight substance changed into the high molecular substance, so there was some sort of—or vice versa, I don't remember which direction it was. But albumin was converting into globulin, and I was going to do osmotic pressure measurements to try to sort this out.

That's completely fallacious, of course, this conversion, and Ogston did solve it with another student. The other student has had a big influence on that one. His name was Johnny Johnston, and between them they solved that anomaly, and it was always called the Johnston-Ogston effect afterwards, that they understood why there was this apparent conversion, which I can explain at some other time. It's related to what happens in traffic, and how if you have a lot of cars there, they can't go so fast. You can understand it in terms of how cars behave on the road.

But I was investigating this problem, so I had to measure osmotic pressures, and I went to Cambridge with Sandy to a scientific meeting. There was a scientist there who was making measurements of osmotic pressure, but he was using very simple apparatus and getting a lot of results. But I thought it was extremely crude, more or less like measuring with a ruler how high the water goes in a capillary. That was the basic thing. Of course, he didn't just use a ruler, but it seemed very crude. As a matter of fact, he got lots of results.

I didn't get many results with my method, but I spent, then, two years developing an extremely precise method for measuring osmotic pressures, so this was my first tool, if

you like. I made this machine, this apparatus for measuring osmotic pressures, and Sandy Ogston used to worry about me, saying, “Oh, have you got any results yet, Oliver?”

I said, “Don’t worry, Sandy. It’s going to work. It’s going to work.” I was quite sure I was going to make it work. It took me about two years to make it work, but it was extraordinarily precise, so precise that my data, which I can show you in a thesis later when we’re doing the visual one, if you like, that I couldn’t put the experimental points on the same graph as the line. I had to interrupt the line to put my experimental points on there, they were so close to a theoretically smooth curve.

NC: Wow.

SMITHIES: And you had to control temperature to a thousandth of a degree in order to make this machine work, and I had to devise the method of controlling the water-bath temperature to about a thousandth of a degree. It used a light bulb. This light bulb would flash off and on as it heated the water or didn’t heat the water, and that was left on my bench when I left the lab, and “Batty” [Baruch S.] Blumberg took over my bench. **endnote 3** He was another Sandy Ogston student. He got the Nobel Prize later for his discovery of the Hepatitis B virus. But he inherited my bench, and it’s always said, I don’t know with complete truth, that in a fit of rage he destroyed my water bath, because he couldn’t bear this flashing light. I’m sure he did disassemble it, but I don’t think it was quite a fit of rage.

But anyway, this was a machine that I made, and so I was now a physical chemist, you see, working with biological substances. That’s, then, the status I’m in. I’ve measured the osmotic pressures very precisely and developed a lot of theoretical equations which, when I came to write them up for publication, proved to have a fallacy in the middle of the derivations, so they never got published. But my thesis committee didn’t see the fallacy either, so it didn’t make any difference. But it was massive calculations, seven-figure calculations with mechanical calculators that would drop digits.

But anyway, so I was a physical chemist then, working on biological principles, and there was another graduate student working in the laboratory, Buzz Baldwin, who was a Wisconsin student. He was a Rhodes scholar and so he was a very smart guy. He was there and we were talking about where I should do a postdoc. Sandy thought I ought to go somewhere, maybe go to the States and do a postdoc. I mean, I wasn’t very keen on this. I didn’t really like the idea of going to America. I didn’t much care for America, from what I knew, I was completely prejudiced, and so all the more reason, he felt, that I should go. He suggested that it would be a good idea.

So we thought about various places. I think there was an opportunity in Yale, and then because of Buzz Baldwin, there was an opportunity in Wisconsin. The laboratory in Yale, I think, was Edsall’s laboratory, and it was known to be a huge place with a lot of postdocs. **endnote 4** I think that people felt it wasn’t the sort of environment that I was used to, or liked. It was not as traditionally the type of academic environment that would be in England. It was much more what would now be a big research endeavor, in those days, and they thought I wouldn’t be so happy there, and they suggested I went to Wisconsin. So I went and did the postdoc in Wisconsin.

NC: How did you choose Jack Williams for your advisor there?

SMITHIES: Well, that was because he was the, Jack Williams was the advisor of Buzz Baldwin. We had no particular thought about what to do when I went there, and I remember they were having problems with isolating the protein that I had done all my osmotic pressure measurements with, which was called beta-lactoglobulin. They had lots of it. They purified it all right, but it was an oil, an oily syrup as it were, that they couldn't crystallize it. I made my reputation by bringing it up to room temperature and having it in a glass beaker and stirring the side of the glass beaker with a glass rod, and this will make very fine fragments of glass. You don't see them, but you're actually making tiny fragments of glass which have very smooth surfaces, and they catalyze crystallization so it crystallized overnight, and my reputation was made from then on. I could crystallize lactoglobulin.

So I started to study lactoglobulin, which was the protein then that I'd been working on in Oxford, and began to look at different methods of studying it. I don't know why I thought it was not pure, but for some reason or other—so I'm not quite sure I had some idea that it was not pure, but I used several different methods to try to establish whether we could measure whether it was one substance or a mixture of substances.

Unknown to me at the time, and not solved while I was working on it, it was a mixture, but it was a genetic mixture. That's to say there were two forms of lactoglobulin. It's a milk protein made, obviously, in cows, and some cows make one slight variant of the protein versus the other, so there's some amino acid substitution or something like that. So it really was a mixture of two things. And when I tried it with the method of electrophoresis that was available at that time, which was called Tiselius electrophoresis—we'd had one of those machines in Oxford. We'd had two complicated machines.

Now, I'm a little wrong. There was an ultra-centrifuge. In those days ultra-centrifuges were extremely complicated machines and very expensive. There were only maybe three in the world. It was developed in Sweden, Uppsala, by [Theodor] Svedberg, and we had a Svedberg ultra-centrifuge which I wouldn't have anything to do with in Oxford. **endnote 5** It seemed to me a big, complicated machine, and you didn't get much information out of it. But I knew about it.

Then they had a machine for measuring diffusion constants, which was complicated and stretched more or less across a couple of rooms. And when I got to Wisconsin, they had also a centrifuge, Svedberg ultra-centrifuge, so that was one of the few that were in existence, and they had a machine, an apparatus called an electrophoresis apparatus, the Tiselius electrophoresis apparatus. He got the Nobel Prize for electrophoresis.

NC: He was a student of Svedberg, [Arne] Tiselius.

SMITHIES: Yes, he was a student.

NC: And this machine was enormous.

SMITHIES: It was, yes. It would stretch across two rooms, again. They had the same principle as the diffusion machine that I was used to in Oxford, and it took a lot of protein to do it. I mean, you would have to have about half a gram of a protein to get one electrophoresis run.

NC: The proteins are in free solution.

SMITHIES: Yes, they were in free solution. The method was relatively straightforward. You would put concentrated solution in a chamber, which you could look at optically, into what you might call a U-tube, and then you would have two boundaries. You see, the protein solution is heavier, and so the protein solution will be on the two arms of the U-tube, and there would be a boundary between the protein solution and the free—on the water or the aqueous electrolyte solution above it. So you had the one boundary on the left-hand side of the apparatus, and another boundary on the right.

Then you would pass an electric current, or apply an electrical potential difference between the two sides of the U-tube. One side will be positive and one will be negative. So on the side that was positive, negative ions would migrate upwards, so that was the ascending boundary. And on the other side the negative ions, now, if they're trying to go round the U-tube up the other side, they would go down, so that was the descending boundary of the Tiselius electrophoresis, and it was called boundary electrophoresis, because you were doing the electrophoresis by looking at the proteins on the boundaries.

It was a complicated machine and I found that I got different results on this ascending boundary and the descending boundary, so it's obviously got anomalies of some sort. I mean, I could see there was a mixture of two substances, but it might be two-to-one on one side and one-to-seven on the other side, or whatever it was.

It was obviously not right. And it was very sensitive to what the electrolytes were, how much salt you had there, for example. So I was unsatisfied with that method of determining whether my protein solution was pure, and the ultra-centrifuge failed to show any difference at all, because as it happens the genetic variants don't differ in molecular mass, so that they didn't have any difference in ultra-centrifuge. That gave a beautiful symmetrical peak, so that wasn't any help.

I did some immunological measurements with a guy there who knew something about how to make antibodies, and couldn't really make much sense out of that, and we killed some rabbits, which we didn't want to do particularly, but they easily, when handled incorrectly, can break their neck by their own physical exertion, and I remember one of them dying that way and not liking it. So we did antibody things and they weren't much good, and I did some solubility measurements, precipitation, and they were very interesting.

I could really demonstrate two components quite well by increasing the concentration of ammonium sulfate, which was used to precipitate proteins in those days. As you increase the concentration of ammonium sulfate slowly, you reach the concentration where something will begin to precipitate, and you can measure that, and then as the concentration increased, maybe the second component will precipitate, and I could get two components very nicely.

But also, there was a very strange thing, too, because if you waited a little while—this protein was very pure, remember—it would begin to crystallize. When it crystallized the solubility altered very markedly. Then I could see a third component come out of the other two; the evidence was there, which was really the crystallization. It would move

through the whole thing, and eventually everything would have very low solubility and everything would have crystallized, so that wasn't very good either.

So I had four methods of measuring protein purity, none of which were very good, and that's the paper I wrote out of my postdoctoral period. I don't think anybody, well, I don't know anybody who ever quoted it. My paper on osmotic pressures I'm sure nobody ever quoted. Nobody ever used the method again, I never used the method again, so that died a natural death, and the other one was instrumental in what I later did, the protein purity one with Jack Williams.

I don't think Jack had very much to do with what was going on, what I did, as opposed to it was more or less me doing what I wanted. But they had a good lab and they taught me rigorous physical chemistry, and you could learn a lot of physical chemistry and talk to physical chemists and be comfortable with the theoretical part of that there, so I know I increased my feeling of physical chemistry a lot by being there, though I don't remember any particular thing I learned.

NC: It was an interesting choice of a laboratory. According to the historian Lily Kay, Williams' most important contribution to chemistry lay in his invention of scientific laboratory apparatus, so was he a toolmaker as well? **endnote 6** Were there other tool-makers there?

SMITHIES: No, I never had any interaction with that part of Jack Williams. I remember him as a very modest man, and rather self-effacing. I don't have a very strong recollection of him, really, actually, so that's what I mean. It wasn't the interaction with him. It was Bob [Robert A.] Alberty. Alberty was a pretty famous physical chemist came out of there, later did quite a few things. A-l-b-e-r-t-y, Bob Alberty, and he was doing some pretty rigorous work, and they were doing marvelous measurements of diffusion constants of proteins, very high-precision measurements. I can't remember the name of the person who did that there, but I admired him a great deal.

So it was excellent physical chemistry going on in the place, no question at all. And, of course, I learned what it is to be in the United States, and I got over my prejudices slowly, and I met a Wisconsin girl, and, of course, that means that then biology takes over, as it were. She didn't want to move to U.K., and so I decided to stay in North America.

I couldn't stay in the U.S. because of my visa. My visa was definitely limited to the time I was there, and so I went and found a job in Canada, and then later she joined me there.

NC: Let's talk a little bit more about electrophoresis. It was a technique that was going through rapid evolution in the early fifties.

Zone electrophoresis was coming in, which I don't really understand very well—
SMITHIES: That's right.

NC: There were a lot of new approaches to it.

SMITHIES: Well, let me explain the difference between zone electrophoresis and boundary, because as I explained the boundary electrophoresis then, you had your

protein solution and separated it from liquid, and you had the ascending and descending boundaries. So you have a mixture, if you think about it—let's think of three proteins, let's say A, B, and C, and let's imagine that A goes faster than B, and that B goes faster than C when an electric current is applied, an electrical potential, which is to say, so that in the ascending boundary, initially there's A plus B plus C.

But A will migrate faster, so there will be pure A will come out at the beginning, and A will be going faster. And it will form a boundary with now B is also moving. The B isn't moving as fast, so you'll now, as you go down from the leading front, the next front that you see is now, you've got A up pure. The next one will be B, B plus A, and the next one below that, because B is faster than C—there'll be three boundaries, and the bottom one will be where C has got to. So A has gone fastest. It's pure, ahead. Then B is there, so it's now A plus B, and then going down again, then you get to the point where C is, and now you have A plus B plus C. So you have three boundaries, and the concentration difference across the boundary will tell you how much A there is, how much B there is, and how much C there is.

NC: The only one where you get a pure substance is A.

SMITHIES: But you only get a pure substance of A, yes. On the descending boundary the opposite will happen. You will have C will be the only pure one, because it's left behind. But there were many anomalies of the boundaries. The boundaries were full of anomalies, and that's why I could get these different results.

Well, now, zone electrophoresis, which Tiselius and his collaborators invented also—

NC: [Henry G.] Kunkel, right? **endnote 7**

SMITHIES: Kunkel, yes, mainly Kunkel. Well, I don't know mainly Kunkel, but Kunkel was the person who did it. They used electrophoresis with zone electrophoresis, in which they put, instead of having a complete—

4. Research in Toronto and the development of starch gel electrophoresis

SMITHIES: Well, now, I'll backtrack. So they would have some supporting material, and into which they could introduce your sample, A plus B plus C as a little pile, you might say, a little—you put it into a hole in the supporting material.

Now when you separate it, now when you find the electrical potential, A would get ahead, but it would really get ahead as a complete little package now, because A isn't just moving faster. So you would have A plus B plus C, and very soon you would have A alone and B alone and C alone, in the order like you if had a mixture of three, a group of people standing at a starting line of a race. You have A plus B plus C and they run, and A will be ahead and B will be next and C will be next, and they'll all be pure.

But if you had a mixture of A plus B plus C going back, you would have exactly the same as in the boundary. The A people would be ahead, and then the B people would be there, and then the A plus B plus C people would be there, and you wouldn't have any purity. So you have to have a defined zone, a zone instead of a complete solution.

NC: And what is the medium that this is moving through?

SMITHIES: Well, they'd use several different sorts of medium. They used tiny particles of polyvinyl chloride, PVC, as a medium, because proteins didn't stick to it. They also used starch grains, and they used starch grains which, you know, the ordinary powdered starch that you probably don't see very much. Anyone who does cookery will get cornstarch, and that's starch grain. That's very fine starch grains in corn. Potato starch is a bit coarser. But anyway, you can take a starch like that and you can suspend it in water or you can add water to it, and it doesn't dissolve because the grains stay as grains, and so you can make, in a sense, a sand pie, a wet sand pie, but instead of having sand it's starch. So the starch grains are supporting free liquid around the grains, and that was the material that Kunkel used. And if it's at that point, I can tell you how I began to learn about that.

NC: Yes. So the Kunkel and Slater 1952 paper on starch electrophoresis, that's with starch grains. **endnote 8**

SMITHIES: That's correct.

NC: They never clearly say starch grains. They just say they're using starch.

SMITHIES: Yes. It was starch grains. It was powdered starch.

NC: Okay. So when you went to Toronto and visited with Andrew Sass-Kortsak, he was using Kunkel and Slater's method.

SMITHIES: Well, we ought to backtrack a little bit, because the chain of events is not really quite as simple as that. First of all, what was I working on when I went to Toronto? Well, I went there and through a contact in Wisconsin I got in contact with—his name won't come back for the moment, but he was a distinguished biochemist in Toronto, and he in turn got me in touch with a man called David Scott, David A. Scott.

David Scott was by then a white-haired oldish gentleman who was a fellow of the Royal Society. He'd been pretty important in the early days of insulin. In particular, he was the person who first learned to crystallize insulin as the zinc salt, so zinc insulin was crystalline, and you'll see here a zinc insulin crystal. This is from him. He gave me this picture. It was one of his favorite pictures of zinc insulin crystals. Those are still used for helping diabetics, as far as I know, because they're very low solubility, and so they dissolve very slowly, and so a suspension of very fine crystals of zinc insulin releases slowly. It gives long-release insulin, which is desirable for helping diabetics.

But he had worked with insulin, and he was now working almost by himself in a small laboratory, about 200 square feet, I would say, more or less by himself. He had one—no, I don't think he even had a technician anymore. But he would do an experiment in the morning, and I always said, and it wasn't far wrong, that he did the same experiment every day. He was trying to solve a problem and it needed the same method. He was looking for something to which insulin bound in blood. He never found it, but that was the experiment he was into.

He would work in the morning and then have his assays done in the afternoon. He would go play golf in the afternoon with his friend who was called Fraser, Dr. Fraser. He called me Dr. Smithies. He said, "Well, Dr. Smithies, I'd like to have you come here, and

you can work on anything you like, as long as it has something to do with insulin." So I thought that was interesting, and so I began to review the literature on insulin that I could find.

In those days, of course, we wouldn't have computer searches and things of that sort, so you could go to the library and you could get an Index Medicus. I think it's called the Index Medicus, and that listed all of the articles in any year, and of course categorized them by topic, to some degree, and would give you the title; no abstracts, you could get the title. I still have my notebook up there, which I'll show you later if you want, in which I collected all these references to insulin.

See, now we're talking about 1950-something or other, '53 perhaps. Yes, I think it's 1953, and insulin was discovered in the 1920s, so there was quite a literature. I reviewed this literature and learned how they made insulin, because Toronto was where insulin was discovered, of course, and the laboratory that David Scott was in was called Connaught Medical Research Laboratory. **endnote 9** It was, if you like, a two-headed monster, not quite a monster, but it was two-headed.

Part of its activity was in making insulin and making biological products. It had been formed in World War I to manufacture biological products not readily available in Canada, and to carry out medical research, and those were its missions. It was also part of another department. It wasn't pathology; I don't remember what the department was. But then as a result of that, insulin was being made there, and all the assays for insulin were going on, which is what David Scott used then when he wanted assays.

But I became familiar with how they made insulin, and it was a horrendous process. They would take pancreas from—frozen pancreas, usually, maybe fresh, I don't know; I think most of it was frozen, and would extract it with acid alcohol. I mean, incredibly harsh method in which you extracted the protein that we're going to use with alcohol laced with hydrochloric acid. I mean, for all my instinct of making proteins and handling them, I said, this is desperate.

So I became somewhat convinced that what was called insulin must be a degradation product of something else. I read what had been done by [Frederick] Banting and [Charles] Best during their work on discovering insulin, and they had started with the pancreas and they'd tried—they thought that that was where the important substance came from. So their idea, initially, was to destroy the exocrine function of the pancreas.

The exocrine function makes pancreatic enzymes that are used then to digest your food, so that the pancreas secretes trypsinogen and chyminogen, and a whole bunch of different precursors of enzymes that are very, very digestive. When they're activated, and they often activate themselves once they've started, then they would dissolve any protein, or nearly any protein. So their idea was that we can't get this material out of the pancreas unless we get rid of that function, so if we tie off the pancreatic duct, the duct from the pancreas which leads to the intestine, then that will cause the pancreas, part of the pancreas that makes the enzymes to destroy it, and we were able to isolate the active principle from what's left.

They did those experiments but the animals died. They got infections. They had all sorts of problems and it didn't work, and eventually they began to try to make extracts of the straight pancreas. Then they discovered this type of crude extraction that was

powerful enough to prevent the proteolytic enzymes, the enzymes which digest protein, from working, because hydrochloric acid will prevent those proteins from working. So it didn't self-digest when it was extracted with acid, and the alcohol helped, because by having the alcohol there, a lot of proteins weren't soluble in the presence of alcohol, so it extracted only a minority of the components as this **endnote 10**acid-alcohol extract. Then it was purified in various ways which I never knew about in Toronto, and they made, eventually, crystalline.

But I thought, as I say, that this was rather crude, so I wanted to go back and look for a precursor. So we decided, David Scott and I, that that would be my project, to look for a precursor of insulin. Of course, there is a precursor, there's pro-insulin. I never discovered it, but that was what I was aiming to do. And I realized that you have to have a better method of establishing the purity in my substances, so I heard about this electrophoresis.

One form of the zone electrophoresis was called filter-paper electrophoresis, and in that you soaked a piece of what amounted to blotting paper, like a piece of blotting paper, or a paper towel in a solution. You put a spot of your protein mixture onto the paper towel, if you like, and you pass the current between it, and then the components in the spot would separate A, B, C, instead of mixtures, because it was zone, and you could see them, and you could stain them with a protein stain.

I heard that, because of David Scott playing golf with Fraser, I heard that Fraser had a person working with him as just a technician who was doing the electrophoresis with filter paper. So I went down and looked at this electrophoresis with filter paper and I began to—I think I went down at that point. I might not have, though. I might have tried it by myself first. Perhaps I did, because I tried to do filter-paper electrophoresis and it didn't work very well. Yes, it was by myself at first.

It didn't work well, and insulin would stick to the paper terribly. I mean, it wouldn't migrate as a nice spot. It would, in a sense, unroll like a carpet. So you put pure insulin on there, and as it bound to the paper it would move and leave behind a trail of stuck insulin, and it would stop moving when it ran out, until you would get this unrolled carpet, and the more protein you put on, the further the carpet would extend, but it would still be a carpet.

And I was trying to find a way of preventing insulin from sticking to the paper. I knew I had to have a method like filter-paper electrophoresis, because it used a very much smaller amount of protein than the Tiselius machine, and it would separate the things into pure components instead of this impossible mixture of the boundary. So it was clear in my mind that I had to have zone electrophoresis, and the filter paper was giving me trouble.

I tried adding various chemicals to stop the sticking. I added—I have my notebook there, because again, that's an important page in my notebook where I'd been using positive ions like sodium. I tried all the ions, sodium, potassium, rubidium, cesium, or whatever it might be, and then I tried divalent ions, calcium, and trivalent ions, and then I tried the amino acids and various things to try to get this stuff not to stick, and didn't succeed.

Then I heard that they were using this zone electrophoresis in the hospital, and they

were using starch grains, and I thought I'd go and look at it. I went over and somehow got an introduction to Sass-Kortsak. Andrew Sass-Kortsak was using this method, the Kunkel method, and it was quite laborious, because you would make a tray into which you could pour this slurry of starch and electrolyte and salt solution and let it settle. Then you'd get this damp mass of starch grains, as I say, like a sand pie.

So the protein would migrate around the grains in the free solution, so it was really free-solution electrophoresis, but in a supporting matrix that was inert, because you would use PVC, which is completely insoluble to—I mean, which liquid couldn't get in at all. But when you did this, my recollection is the machine they operated was something like about a foot and a half, or thirty centimeters or so long, and fifteen centimeters wide, and maybe a centimeter deep. Then you could therefore do a relatively large preparation. You could put, of course, a lot of material into a slot in this starch slurry, and do the separation.

But then to find the protein, you couldn't see it, so you had to cut the damp starch slurry into the best you could do for slices, and do a protein determination on each slice. That meant one electrophoresis would take you fifty-to-a-hundred protein determinations in order to get a plot of where the substances were, and that required a technician, obviously. You couldn't be spending all your time doing it. I didn't have a technician. I didn't even have a glass washer, a dish washer I mean. I'd use the same apparatus all the time and washed it myself, and didn't have any helpers, so I knew I couldn't do that.

But I also knew, then, you see, that you could stain a protein, and then you could see it without all this protein determination. But obviously, starch grains are incompatible with a protein stain, because it will fall apart. So I thought I have to make the starch, therefore, into a solid, and I remembered from my childhood helping my mother—I have a strong vision of this in my head, because I remember it quite well; it was when we were living in Dudwell Lane and I was probably thirteen or fourteen, something like that—helping her to do the laundry. And she would cook starch up, starch powder, and make a sort of semi-gelatin-like material, and dip my father's shirt collars into this and then iron them. Then when it was left around, it would set into a jelly, I'd noticed.

So I thought, well, if I take the starch and cook it, I can make it into a jelly, and then I can stain the jelly, because that will stain without falling apart. So this was the idea. It was a time saver, and I could do the experiments then and use the advantage of starch without having to do all this protein determination all the way along. So this was on a Saturday morning. I used to do work on weekends just the same, and I remember thinking, well, I'll go back and find this.

So I went back to the lab, to Connaught Labs. I'd been working there in the lab a while at that point, and was a bachelor, so I was used to working any hours I liked, and I'd work into the evenings often. I knew all of the chemical supply places in the whole building, and I didn't think it was at all wrong to go and help myself when I needed a chemical. So I looked around and surely enough I found some starch in one of these chemical storerooms, and it was starch according to Lintner [brand name for a soluble potato starch]. I didn't know what that meant.

I took this starch and I tried cooking it up and yes, surely enough it went into a sort of solution and it would set into a gel, but it would only set into a gel when it was rather high concentration. So by the time you had about between 10 and 15 percent of solid starch

in your solution and you cooked it up, it made a very gooey mix, and that set into a firm gel. So I made these gels out of this starch, and they were therefore concentrated gel, which I had no idea what this would mean, except it let me stain them.

I put a sample of insulin into—I made a cut with a razor in this starch gel and put insulin in and passed the current, and impatient, I probably ran it for half an hour or something like that. We can see in my notebook. And then stained it, and oh, the insulin was beautiful. Now it migrated beautifully, clean, as a beautiful zone, so all of the adsorption properties of the paper had disappeared, and I could use it for that. So I now had a method for doing things, and I used this method for a while.

By that time I had a good friend, Gordon Dixon, who was a graduate student. He was from England originally, and he was working in the laboratory of the person whose name I can't immediately remember, who had got me to Toronto in the first place. So he was studying enzymes, Gordon Dixon, and he had some evidence of some cabbage enzymes that he was looking at, that it was a mixture, but he couldn't prove it. So I said to him, "Oh, well, Gordon, well, let's do it with this new starch-gel method."

So we put the protein solution into the starch gel and we ran it, and we didn't have any way of staining for the enzyme, so we still had to cut it into slices. We cut it into slices and each slice then was cut into three or four pieces. One piece was used to estimate enzyme A, one enzyme B, one enzyme C, one enzyme D, or whatever it might be, and so with this we could see the separation of the proteins, and it gave a beautiful separation and proved quite conclusively that he did have a mixture, and what they were, and it solved his Ph.D. problem, and we published a paper together on this, the first paper that I published on starch-gel electrophoresis, which was not really describing much about it, because it was mainly in relation to the enzyme. **endnote 11**

NC: This is the February 1955 paper in *Nature*. **endnote 12**

SMITHIES: That would be about '55, yes. And then about that time I just was curious one time, and thought I'd put some serum on there.

I'd probably been seeing—I knew by then, and I don't know why I knew—oh, I knew because of Andrew Sass-Kortsak's work, probably, and maybe from some reading—by then the complexity of plasma proteins had increased, and now there were five known. There was albumin, alpha-1, alpha-2, beta, and gamma globulin, so there were five known proteins in plasma at that time, and you could separate them by zone electrophoresis, I think also by Tiselius, but you could certainly separate them by zone electrophoresis.

For curiosity, I just put some plasma, or serum into my machine and ran it, and my god, when I came back, I could count twelve different spots, twelve different bands instead of five.

NC: Just out of curiosity you threw some serum in there.

SMITHIES: Yes. I think it was just curiosity. I can look at my notebook. There wasn't any particular reason to do this except curiosity, I think. But then I realized I've got something very important, because now I could see that they were much more complicated, and so I didn't do any more experiments on insulin at all. That day I stopped them and I just

began the others. David Scott didn't mind. He was very kind. But we were good friends by then, and he now called me Oliver, and I called him—I still called him Dr. Scott, but he called me Oliver, as a result of an incident we might have time to talk about, when he converted from Doctor Smithies to Oliver.

But anyway, this was obviously promising, but it took me a while to get it reproducible, couldn't get the method reproducible very well. It took me probably six months to get the method so I could get the same result every time, and I was just about ready to publish it. I'd used, of course, I'd used my own blood for the electrophoresis. Relatively easily I could go downstairs and there was a physician in the department who would draw a sample for me, and I could use that for a while. And I persuaded Gordon Dixon to give me some, and George Connell was another graduate student in that other laboratory in biochemistry, so I'd use theirs, and we always got more or less the same results with the three of us.

Then I was just about ready to finish and I got a sample of a blood from—oh, I've got to look up this guy's name. I can't go on saying "unknown person." What was his name? He worked a lot on starch. He knew quite a bit about starch. Well anyway, we'll have to go on. That's one of the problems of getting older. I always had a problem with names, it's got worse.

But I got a sample from one of his technicians, who was a girl—a woman, probably; she was an adult—a woman in his lab, and I ran her sample—very, very different, many extra bands. My notebook says "many extra bands," or "many extra components." She had a whole bunch of things that were different, but this is the first time that I knew that I'd run a sample from a woman. Actually, I had run some others, but not known it, because George Connell's sister worked in the [Toronto] Hospital for Sick Children, and I got some samples from her there, based on not knowing that they were from her.

But anyway, I thought that this was a male-female difference, and I couldn't do many of these electrophoreses per day, so I just could do two. I'd get somebody to give me, a man and a woman to give me a sample. I would run them each day, and every time the men were the same and the women were the same, so I called one Type M and the other Type F. I found there were two types of F, slightly different, so I called one F1 and one F2.

Then the day came when they were the other way around, and I thought, well, I'd just muddled the samples. So I ran them again, and no, I hadn't muddled the samples. So it became pretty obvious that it wasn't a difference due to sex, because I'd called my fianceé in Wisconsin and told her I had a new method of telling men from women, but it turned out not to be the case. So at that stage then, thinking about it, it seemed to me it was likely to be genetic. I talked to the guy in—it's important to get his name. I'll have to find his name. I told this guy and he said, "Well, there's a geneticist in the Hospital for Sick Children that you could talk to."

NC: And that was Norma Ford Walker.

SMITHIES: Norma Ford Walker. By now I had the proportions of these types. What I called M would be roughly something like half the sample—it wasn't really, but roughly a half, and roughly twice as many were F1, and the same number of F2. I had this thought that they might be homozygotes, heterozygotes, and homozygotes of Mendelian

inheritance. **endnote 13** I went to talk to Norma about this, and she had a clinic in the Hospital for Sick Children, a genetic clinic, very advanced, if you think about it, at the time. I mean, how many people were doing genetic counseling in the 1950s?

NC: Only a few.

SMITHIES: Very few. She was a student of a different Fraser, Clarke Fraser from McGill [University], or had been associated with him, anyway. **endnote 14** She was an excellent geneticist, and she was looking at cystic fibrosis, among other things. She would have a family clinic, as it were, on Saturday morning and the families would come in and she would talk to them.

It was a rather depressing occupation in those days, because you couldn't do much other than tell a person that you have a one-in-four chance of having it again. If you have another child, for example, there's a one-in-four chance of Down's, which she was also interested in, and you couldn't do anything about it. You couldn't do prenatal diagnosis. They didn't know about trisomy 21 at that time, and there was no cure for cystic fibrosis, so it was pretty depressing. I remember seeing this little girl, a beautiful little four or five, three or four-year-old young girl, happy little child with cystic fibrosis. You knew she wasn't going to live, and couldn't do anything about it.

She was also interested in Down's Syndrome, which at that time was called Mongolism. She worked out some statistical methods of trying to get a clear knowledge about Mongolism and about trisomy 21 as it later turned out, from fingerprints and palm prints. **endnote 15** They were abnormal, and then the facial characteristics. She would give each one a score, as it were. There'd be a score for the fingerprint and the palm print, and a score for the facial features and so on, and then by multiplying these together she was able to get a fairly precise diagnosis of this really is a child with Mongolism, as it was called, or not. So she was a good geneticist.

So she had these families, and they used to take blood samples and have the blood groups determined. I think it was partly in order to determine whether—I don't know exactly why she was doing it, but she obviously knew all about paternity things. Bruce Chown in Winnipeg did the blood grouping for her. C-h-o-w-n, yes, Bruce Chown, and we would send the samples there. I would get some residual sample from them, you see, so I could get the plasma, and then I could do the family with my method, to see whether my genetic model was right. And that went fine.

But she used to tell—well, that's a separate story. But anyway, then about the fourth or fifth time I came back and it didn't fit. My prediction was wrong about what the samples were, and Norma said, "Well, I'm afraid that's the end of your hypothesis, Oliver."

And I said, "No, I don't think so, Norma." I said, "Let's just wait. Let's wait for Bruce Chown's results." And when Bruce Chown's results came it was clear that it was non-paternity, so, in fact, my result had shown the non-paternity, which made us even more sure that we were right. So we'd didn't do more than—I think we did about seventeen families altogether, and then Norma and I published a paper on inherited differences in the normal plasma proteins, which was the first difference in proteins, of normal proteins physically, that was determined to be genetic. **endnote 16**

The only other genetic variation that was being—well, maybe there were two. There was a genetic difference due to sickle-cell anemia; the hemoglobins were known. And [Pierre] Grabar and [Curtis A.] Williams were finding some differences, immunological differences in immunoglobulins. **endnote 17**

NC: I don't know that paper. Who were the authors?

SMITHIES: Well, Grabar and Williams had—they did agar gel immuno-electrophoresis. No, I'm not sure whether it was Grabar, but anyway it was the IGG differences that were discovered about that time, immunological differences. I think it was IGG. So when I say it was the first, it was certainly the first physical demonstration. So then I became sure now that this was correct.

NC: Was this your first exposure to genetics?

SMITHIES: Yes. I'd learned genetics with—no, it wasn't my first exposure. I'd been exposed in Oxford, but the geneticist there I remember only vaguely, because it was so dull I didn't go to any of his lectures after a couple of times, talking about butterflies or something. It seemed very dull and had nothing to do with reality, and so this was my first real beginning. So I, in a sense, learned my genetics as a molecular genetics, not as a geneticist going molecular. I was a molecular person going genetic.

NC: Right. And you were—

SMITHIES: That probably had something to do with it never seemed very difficult to me, particularly, as a topic.

NC: You were one of the first people who went that route.

SMITHIES: Well, it just grewed, as it were, like Topsy. So, yes, I think I would probably be called one of the first molecular geneticists. And, in fact, a whole cottage industry then sprang up, because lots of people looked for genetic differences with this method, this electrophoresis, and there was a whole journal devoted to it. Biochemical Genetics it was called. It's still published, but it's become a very obscure journal and a very minor journal. It never became very important. But at the time my electrophoresis method was the most widely quoted method in science that had ever been published, in biological sciences, except for the one on how to determine protein concentration. **endnote 18**

NC: So the reception was strong and immediate.

SMITHIES: Well, I went back to Wisconsin because my fianceé was there, and I used to go back by bus fairly regularly, a twenty-four-hour bus ride. I would visit the physical chemists, and I showed them my results, and "Oh, they said, "it's all artifact." They just couldn't believe these beautifully sharp bands. And they talked about what's called Liesegang rings, which you can form artifactually, and precipitating crystals, which give you beautiful series of bands. You see, I got this lovely series of bands.

But I could disprove this immediately. I said, "Well, it's rather interesting my artifact is inherited," and so it was obviously not an artifact. So they were convinced by that, and there was no difficulty convincing other people about the inheritance, because the data was so—they were rock solid and very easy to understand, and simple, and you could

understand it. So we published in *Nature*, and then I published the method in a separate paper, and then went from there.

NC: Was it picked up rapidly by people who knew?

SMITHIES: Oh yes. People began to use it, and we used to teach people who would come. I made some very great friends with that. I went to the Federation meetings at that time [FASEB; Federation of Associate Societies of Experimental Biology]. They were held always in Atlantic City, and they asked me, or I arranged, I don't remember which, to have what would be now called a poster session, or a booth as it were. I had my apparatus there and my results, and I didn't know any better; I sat with the thing the whole meeting. I didn't do anything else except there at the booth, as it were. But people came.

Eloise Giblett, G-i-b-l-e-t-t, was one of the people who came, and she was from, I think, Seattle, Washington. **endnote 19** She was a blood-group worker and she was very fascinated by this, and became one of the first people to do a lot of studies with it. And other people came, and it really didn't have any difficulty being accepted.

I had some difficulty making it reproducible later, because I ran out of the starch that I had got from a company, oh, something like Hartz or a local Toronto company, and it turned out they got it from Merck, and they wouldn't tell me, or they couldn't tell me what batch numbers they had, so they wouldn't tell me what batch number they had sold, and so I couldn't trace the good batch number back, and I didn't know what it was exactly.

Then I found out the starch was—not just immediately at that time; I probably already knew—that it was hydrolyzed starch, partly hydrolyzed, so it was called starch according to Lintner. Lintner had developed this method of partially hydrolyzing starch with hydrochloric acid, which made it easier to go into solution, and yet still didn't prevent it from being a good starch to be used in cooking and to make a jelly with, for example, or whatever. So there was a big industry that used the Lintner process.

Merck, I couldn't get to the bottom of it with them, although eventually I found out where they got the native starch from, and so I went to this—there was a starch supplier who imported starch in Toronto—again, I don't remember his name, but I probably have a record of it—who imported it or who sold a lot of starch. And initially he wouldn't tell me either where the starch had come from, because I tried some new batches and they didn't work. Then I think he said it came from Holland.

It came from Holland because Canada had had a potato blight and they couldn't sell their Canadian starch. A lot of potatoes are grown in Canada, Prince Edward Island and New Brunswick and so on, and I think I'd tried making the starch from these and had been unsuccessful.

So I got—it's a bit murky in my memory, but I think I got the starch from this—no, I remember. I got then BDH, British Drug Houses, sold native, uncooked potato starch, untreated potato starch. So I got BDH starch and then I learned to make my type of starch from it, by hydrolyzing it with hydrochloric acid in the right way. I had to work out the conditions but they weren't difficult. Then I used BDH starch for quite a time, and then that changed in some way.

Then through BDH, I eventually got to this supplier in Toronto, and then he told me the starch came from somewhere. Meanwhile I'd been going there and been isolating starch from all the potatoes I could find. I would go to a grocery store and I'd buy Prince Edward's. I'd buy Idaho potatoes. I'd buy this and would make starch from it, and it was fairly easy to make, make starch from all these different potatoes. Idaho starch, I remember vividly, wasn't very good for this particular base. I don't like Idaho potatoes either, to eat, very much. They don't have much taste and they're too starchy.

Anyway, I got all these different starches, but eventually, then, through this man found out it came from Holland. The reason there was a difference is—it took quite a long time to work out, but starch actually is a polysaccharide, many chains of carbohydrate molecules in a string. But it comes in two forms. It comes as a linear polymer, that's to say A, A, A, A, A, A, going on indefinitely. That's called amylose. But it also has another form in which it branches, so it'll go A, A,A, and then there'll be a branch, and now there'll be two or three, so it's a branched polysaccharide. One is called amylose and the other is called amylopectin, I think is my recollection. The proportion of amylose to amylopectin is probably potato-strain dependent, and it affects the property of the starch gel that you can make after you've hydrolyzed it, so some will be too sticky and some won't be strong enough, and the right proportion controls some of that, and I learned some about that.

Then it became clear that the method was so much used that people were asking me to supply them with starch, that Connaught Laboratories decided to make it and sell it, because, remember, they were a supply company, a drug supply company, a pharmaceutical company. So they decided to make it themselves, and I taught the guy who was in charge of that side of it to make it, only I modified the procedure, because my procedure was using acetone and alcohol. It was all right on a small scale, but it was completely hopeless on a big scale, because the acetone is very inflammable and it's expensive, and so I developed a method of hydrolyzing it just adding hydrochloric acid to water and stirring it, and then taught him how to wash it.

Then I went away to a meeting or on vacation, and the guy in charge was making the first batch. So I taught them to wash it with tap water and then wash it with distilled water afterwards. But he thought, oh, the distilled water is too expensive to do, so he just gave more tap water washings, and I was quite annoyed with him for that. But eventually they got it working, and then they sold that for many, many years. Connaught Laboratories sold that starch. I think they made several millions out of it.

We never patented the method. I didn't know much about patent laws then, and the starch was something that had already been described, but, of course, the starch electrophoresis had not been described. It would have been patentable, but I didn't patent it. In fact, I've patented almost nothing I've ever done. So then they made it commercially and that went on for a long time.

And when I eventually moved from Toronto back to Wisconsin, then they started to ask me to buy the starch from them. This is pretty disgraceful, so I said, oh well; I had the lab tech, a friend, Otto Hiller, H-i-l-l-e-r; his daughter is still my lab tech now. It's a second generation, or line, our lab tech. But he was a great friend, and he'd been making the apparatus for sale, because it was made out of plastic. He made the apparatus and people could buy it from him.

NC: How much did it cost?

SMITHIES: Oh, I don't know. It would have been relatively cheap, a couple of hundred dollars maybe, maybe less. I know it was cheap. It didn't cost much. He made beautiful equipment. I still have some of his apparatus around that he made, various parts of it. That's a whole other story, which is interesting, about computers. He and I did a lot of computer work, building our own.

So I said to Otto, "Well, let's do it ourselves." We thought about it and decided, well, why are we importing this starch from Holland and then processing it ourselves? Why don't we get them to process it? They already know how to do that, because they were making Lintner starch. So we arranged for—we would buy a truckload. I mean it was that sort of quantity. We'd buy a truckload of starch and they would process it, and we would test it, and if it wasn't any good we'd reject it. And so they would use it or sell it or whatever. We had some deal. I don't remember all the details of it, but we would get samples from them of a lot that they'd process for us, according to our specifications, and if it was all right we would get it.

So it would come in a truckload at a time, and so Otto then would repackage it and sell it at that time. So it was a much more efficient method than the Connaught method, and it was priced less, and so we undercut them and made our own. The motivation wasn't profit. The motivation was I was annoyed at the thought that they would ask me to buy the starch I'd developed for them.

NC: But then you sold it to other sites.

SMITHIES: Well, I didn't, Otto did. I didn't. It was his business. I don't think I—no, I didn't get any royalties or anything. I think we had some deal or other, but I don't remember what it was.

NC: Well, we're getting kind of close. I want to give you a break.

SMITHIES: Yes, I think we should stop now for a while.

NC: Could I ask one last question before we stop, because it kind of ties right into this period?

SMITHIES: Yes.

NC: And that is, it sort of passes for common knowledge among people who look at the early history of human genetics that the DNA double helix in 1953 had very little impact on human genetics. Most of those people at looking at cytogenetics and, you know, chromosomes and so forth. Did the double helix have any impact on you in your work in immunogenetics or biochemical genetics?

SMITHIES: I think it's rather difficult for me to answer that. I don't think I know the answer. I know that what had the impact was the beginning of thinking about the genetic code, not particularly the structure of DNA. I went to meetings, you see, at that time, and when people—see, my electrophoretic discoveries were quite unique and it was at the cutting edge of science. So I was invited to Cold Spring Harbor and places like that when geneticists would meet and talk about molecular things, and begin to think about what it

meant in terms of genetic code.

I remember going to meetings where [Francis] Crick was there and we would talk about these things. I can probably find some things related to that. But it was all—I understood what I was trying to do. I was trying to make my complicated inheritance pattern make sense, because my patterns were much more complicated than the patterns seen in, say, sickle-cell hemoglobin, where [Linus] Pauling and [Harvey A.] Itano had shown that you had one sort of hemoglobin with one gene, and a slightly different hemoglobin with the other, and if you were heterozygous you had both. **endnote 20**

Well, mine wasn't like that at all, because I'd one band in the homozygote and a highly complex series of what looked like polymers in the other homozygote, and the heterozygote was polymers, too, but different polymers. And I was fairly sure that this meant that there was some sort of polymerization. It was obvious that there were polymers and I could show anyway that their molecular weights increased, the big ones. So I was at that time working very hard to make it simpler, doing chemical tests.

But I went to these meetings and there was, it was sort of seamless, in a way, the transition for me to molecular [genetics]. You see, I was a chemist, and so I went and wanted the chemical solution, so I was expecting a simpler solution that would fit Mendelian genetics, because I knew the principles of genetics, and didn't think it would be any—it would just be a matter of solving it. It was a very interesting solution and quite unexpected solution, which led to all sorts of other things that we'd better talk about at another time.

But the actual double helix there was not really—I think it's true to say it was not important. But as soon as DNA began, people began to think about the linearity of the genetic code, then that was the—we would be talking about [sickle cell], we would be talking about hemoglobin, you see, and Vernon Ingram then showed there was one amino acid difference, and then that made it pretty clear that there ought to be a difference in coding. **endnote 21** I think you'd see my—here it is. This is—that was when the genetic code was first published, and it's a valine to glutamic acid substitution in hemoglobin, and so it's just one base-pair change from here to here.

And that's what we thought we might have in our system, only we—and I'll talk a bit later—it was much more complicated than that. That's why I got some edges on homologous recombination as a result of that. Quite a lot of my early thoughts at that time had been on, as we'll talk about later, on homologous recombination.

NC: Yes, sort of stemming out of thinking about the genetic code.

SMITHIES: No, out of the differences that turned out in the protein that I'd been studying, which was haptoglobin [hemoglobin-binding protein]. It turned out that the genetic difference—a guy in Sweden wrote to me after I'd published, because I'd published that it was a hemoglobin-binding protein, and he said, "Oh, you don't know, but that's haptoglobin," and he referred me to the French workers, who was Jayle, Max Ferdinand Jayle. I never met him. He was blinded when working out a method to assay that protein. He made ethyl peroxide and it exploded and blinded him. So there were many historical things.

NC: All right. Well, let's take a break and we'll come back and pick up with haptoglobin, Wisconsin, and so forth afterward.

SMITHIES: All right.

Session 2: October 27, 2005

5. Department of Medical Genetics, University of Wisconsin; Teaching Methods

NC: All right. We are back with Oliver Smithies. It's now almost three o'clock on October 27th, 2005, and we are continuing this oral history.

We left off before lunch, we were talking about starch gel electrophoresis and you were just moving into haptoglobins, and you were just about to return to Wisconsin as an assistant professor.

SMITHIES: Right. And I remembered the name of the professor whose name I—it was ["Charlie"] Haynes.

NC: H-a-y—

SMITHIES: Well, that I didn't have time to finish looking up when I got interrupted, but I think I can find it fairly easily. But it's Haynes anyway; I think it's H-a-y-n-e-s.

NC: And he was at Wisconsin?

SMITHIES: No. He was in Toronto and he was a professor of biochemistry. Yes, he was in Toronto, and he was the major professor of Gordon Dixon and George Connell, whom we probably will talk about at some stage. I think he probably was the—I know he was the person who suggested I meet Norma Ford Walker, and he might actually have been the first person to suggest that, well, maybe what I had was the homozygote, the heterozygote, and the other homozygote. I have a vague idea that he actually suggested that to me at the time.

NC: You started off thinking that was a two-allele system.

SMITHIES: Well, I didn't think it was two alleles at all. I thought it was just male and female, so I initially I thought I could tell males and females, and then I got the three types. I don't remember exactly. It was just a fair amount of interaction with people at that time. It was almost immediate that the thought came that it was three, that those were the three forms. But we used to call it Type 1—M, F1 and F2, for Female 1 and Female 2. But we knew they weren't females by then, but we'd got the old notation, and only later on did we change the names to be more sensible.

NC: Type 1, Type 2 fast and slow.

SMITHIES: Yes. Then we called one gene 1 and the other gene 2, and because the heterozygote was more like one of the homozygotes than the other, we called the first one (1,1), and the other homozygote (2,2), and the heterozygote (2,1) rather than (1,2), because it looked more like 2, but it was the heterozygote.

NC: And eventually you realized that was three alleles at one locus rather than two different loci.

SMITHIES: No, I never did think it was two loci. No.

NC: No?

SMITHIES: Okay, let's go back to that. I thought it was male and female, and then thought it was genetic, and then came to the thought that what we called M was one of the homozygotes, and what we called F2 was the other homozygote, and F1 was the heterozygote, but all at one locus, all at one locus.

NC: All right.

SMITHIES: But the gene turned out to be complicated. The difference turned out to be not a simple difference. It was a complicated difference. That's what we should talk a little about; if you want the history of my involvement with homologous recombination, that part has to be explained.

NC: I do. Before we go on with that I'd like to get you into Wisconsin.

SMITHIES: Yes, okay. So then, after being in Toronto for several years, about six or seven years, then I married the girl from Wisconsin and she came to Toronto. She wasn't happy in Toronto.

NC: Was she a scientist?

SMITHIES: Yes, she was a Ph.D., and she's still alive. She's a Ph.D., and her name was Lois Kitze, K-i-t-z-e. She was a Ph.D. and worked with viruses, but in Toronto she wasn't happy. So we decided—by then I could go back to the States. I think the requisite time had passed, or whatever. And so by then I was a geneticist, you see, because I'd changed from being a physical chemist to a molecular biologist/molecular geneticist. I'd done good work, I mean, it was well recognized at that point, and so I didn't have any difficulty getting a job in Wisconsin, so I went back to Wisconsin and became—I don't remember whether I was an associate professor; I think probably associate professor in Wisconsin, in the Department of Genetics, which was where [James F.] Crow was, James Crow. Jim Crow was the chairman at the time, and Josh Lederberg had just left. Josh Lederberg was in medical genetics.

There were two departments, medical genetics and genetics at Wisconsin, and I think I had a joint appointment between the two, as far as I remember. **endnote 22** But anyway, I joined that department as a result of this and became a geneticist. Jim Crow then suggested that I help him as a T.A., just an ordinary teaching assistant in his beginning genetics course, as a way of making sure that I was—I think it was his way, sad to say this, but I think it was his way of getting me up to speed. So I was just an ordinary T.A. for his course.

NC: Was it for medical students?

SMITHIES: I think it was a general course. I think it was not for medical students

particularly. It was a course in genetics, a very famous course. He wrote these notes, genetic notes, which are still to this day considered very good. He's a marvelous writer and a marvelous teacher altogether, an absolutely incredible writer. There are some things he's written that in ten or fifteen words it conveys a whole situation.

But anyway, as a result of that I did the T.A. there, and I remember constantly going up to these geneticists and saying, "You know, all you do is when you don't understand it, you just invent a name for it, and then you think you understand it." And they kept—all the time there were things like "lack of penetrance" and, oh, I don't remember some of the others, "reduced expressivity," and words of that sort, which meant it just didn't get expressed or it doesn't turn up right. And I mean they're only rewording it.

NC: Like the dormitive principle. **endnote 23**

SMITHIES: And it was just by rewording it made them, or I felt that it made them feel to understand it, and so I used to tease them about this, and we would have constant talks about it.

NC: So this was a very exciting time in that department. Crow expanded the department very rapidly.

SMITHIES: Yes.

NC: This was the time of Charlie Cotterman and Klaus Patau and Marsden and—

SMITHIES: Yes, and [Robert] DeMars.

NC: DeMars, that's who I meant, not Marsden, Robert DeMars. Tell me about that time, and those people, and the dynamic in the department.

SMITHIES: Well, initially I was assigned to a place in physiological genetics, in physiological chemistry—

NC: In the medical school?

SMITHIES: In the medical school, because they didn't have, yet, space for me. So I worked in a teaching laboratory there. But I'm a messy scientist in my experiments, on the outside, my experiments are always dirty and messy, or messy anyway, and I always say, the inside of my test tubes are clean, but the outside I don't promise. And this upset the person there, the chairman of that department, Phil Cohen, who was chairman of, I think it was physiological chemistry. You know, it was biochemistry and physiological chemistry, which was the same thing in the medical school, and biochemistry was biochemistry in the College of Agriculture.

So I was a guest, in a sense, and I would sort of move down the bays of the teaching lab. As it got a bit messy I would move to the next bay. He was trying to teach med techs on the other side, and he thought this was a terrible example, so he tried to get my space remodeled very quickly. He got my space remodeled, and then I moved into the basement of—I don't actually know which building it was, but I was in the basement for a while. Then they had a new building, the Genetics Building it was called, and then I got onto, I think it was the fourth floor. I think it was four. My recollection is 406; it might have

been 306.

But on that floor, then, was shared by several other scientists, and Bob DeMars was one, and the geneticist who worked a great deal with ribosomal RNA. I've forgotten his name for the moment, a Japanese—[Masayasu] Nomura. Nomura was also on that floor, and we shared a cold room. I know Nomura was very fussy and clean, and he used to get very fed up with my sharing this cold room with him. You know, it was really a pain for him. We became good friends; he just didn't like the way I did science, in that sense.

But anyway, we shared that and there certainly was a good interaction. The department in general, scientifically interacted very well. It was very bad at interacting with each other in other senses. The Department of Genetics in the College of Agriculture was always fighting with the medical geneticists. The so-called geneticists in the College of Agriculture looked down on the medical genetics people. For example, they used to talk about John Opitz, who was really a distinguished diagnostician of childhood abnormalities, and they used to just tease him that all he did was look at funny babies, you know. But he was very skillful and he was good at it.

But as a result of that, the two departments didn't get along, and they used to fight continuously, and I remember trying to get rid of that fighting by suggesting, "Why don't we just unite as one department?" We couldn't do that. It couldn't be done because they were in two different colleges. The School of Medicine and the College of Agriculture were different parts of the university, so they eventually joined as a laboratory of genetics with one chairman.

But then subsequently they still—this fighting continued all the time I was there, and it ended up with—had quite a lot to do with my moving from there, because they would look for an appointment in medical genetics, which a person with some interest in human genetics would be appropriate, and then they would fill it, because they had a big revolt from the genetics side, they would fill it with somebody who had nothing to do with human genetics, and then promise to do—the next one will be all right. But it wasn't and so that sort of thing was going on. They fought a lot, and it was most unpleasant.

I used to sometimes come home and say, "I don't know whether I want to be a member of this department," because of that. But scientifically it was very good.

NC: In what ways did this come out? Was it at faculty meetings?

SMITHIES: Yes. All these were in faculty meetings, often in struggles for space, or what are we going to do—appointments probably was the biggest thing. It really wasn't a very pleasant environment for me, that part of it. The science was good.

NC: Most of the people in medical genetics had joint appointments in genetics?

SMITHIES: No. I think rather few had joint appointments. They mostly were in genetics. It was a considerably bigger department, and the medical genetics, there were a few—Klaus Patau in medical genetics, as I remember. I was, and Eeva Patau [Eeva Therman, Klaus' wife] probably was, and John Opitz was, and DeMars. But I don't remember all of the way they were divided.

NC: But those of you in medical genetics had joint appointments in genetics?

SMITHIES: Some did, not all.

NC: Not all?

SMITHIES: No.

NC: Okay. What about the medical genetics group as a whole? Did you interact socially? Did you have journal clubs or happy hours or things like that?

SMITHIES: No, I don't remember anything of that sort of interaction particularly. Maybe I wasn't a very—I'm not an unsocial person. We just didn't seem to do many things together in non-work time. My non-work time was spent looking after the garden or whatever it might be. Actually, I worked most of the time, I think probably, which I still do, so that's what I did. I didn't ever feel that the people were antisocial or anything of that sort. That wasn't a part of it.

But later on in Wisconsin, and then I had friends—well, my closest friend for a long time was Otto Hiller, who was my technician in Toronto. He was actually helping David Scott, whom we talked about. And I had another friend there whom I would talk to from time to time—name won't come back immediately—and he suggested I really ought to have a technician. "I don't want a technician," I said to him. "I don't feel that I need a technician." He said, "Well, why don't you try somebody half time anyway."

So then Otto, who was helping Dr. Scott, became my half-time technician, and very quickly—well, we were good friends, and very quickly he became, then, a full-time technician. We published papers together, and when I moved to Wisconsin he moved with me to Wisconsin. But he and I then were good enough friends that we would spend our Saturday afternoons making computer things, trying to build a computer out of pieces. There weren't any kits for a computer, but we tried to build it, starting with the actual chips themselves, and tried to design it. We didn't succeed, but we tried, and that sort of thing. So Otto and I used to spend Saturday afternoon almost invariably together, messing around in that sort of way.

NC: Did you have heavy teaching responsibilities?

SMITHIES: Well, I had teaching responsibilities. I managed to get them arranged in a way that they weren't oppressive, because I got them all concentrated. So I said, "I'd rather teach solidly for three months than have it scattered throughout the year, because I can do better if I do one of those things at once, because there's always this conflict. I'm always wanting to be doing the work in the lab, and it's going to mess me up." So I managed to do it in that sort of way, though it was very extensive.

I taught a course with Bob DeMars. It was probably the best course that I was ever involved in. I don't know whether it was originally his course. I think it might have been, because we had some conflict later on about this. But anyway, it was a molecular genetics course and we each taught a part of it. Then I used the method that I'd been taught in Oxford to teach this course, which I can explain in a little while, and it was a very demanding course. The students were expected to work very, very hard, and I worked very hard at it. People who took it often told me later how important it was for

them. So that was my teaching responsibility, primarily, so it was teaching graduate students, and, of course then, in the lab.

Maybe it is the time to tell you how I used to teach it, because in Oxford the teaching is done in several levels, you might say. There are more than twenty individual colleges, and these are places where people live, and they have a community. They have fellows, in other words professors that work together, but they won't be the same discipline. I mean, there'll be a theologian might be there, or a chemist might be there, or whatever, within a college.

Then there will be tutors within the college, so the people who are responsible for the training of individual students. A tutor may have, oh, five or ten, I don't know what the number would be now, but when I was there, it might be three or four students that are his primary responsibility. They were males in my college at that time; there weren't any females. It was still segregated. The male and female colleges were separate, and our faculty was all male, so that's why I say he.

Your tutor would meet with you once a week, and in that time you would read an essay to him on a topic of his choice. So it might be—I remember once being given, "Write an essay on pain." That was the total topic, was pain, and you were expected—the scholastic standard was very high, and you were expected to spend maybe half a day looking at what was in textbooks, and maybe another half day or perhaps a day looking at what you might find in reviews, and then all the rest had to be from original papers. You weren't expected to do anything from anybody else's writing. You had to go to the original literature, and that made you always aware of what one might call it in quotes "real science," rather than somebody's abstraction of it, although a written paper is an abstraction already.

But you learned to learn from that, and teach that way, and so it was very powerful, so these weekly essays or these tutorial periods could be quite stressful to some people. I know one, my friend Geoffrey Thomas, the CGAT, C.G.A. Thomas, **endnote 24** used to be terrified of his weekly session with his tutor at that time, and I was with the same person, who was called David Whitteridge, and he was an exceedingly bright guy, but had a quite sharp tongue. He would terrify Geoffrey Thomas, who would sit for the day before, smoking cigarettes so the whole room was full of smoke, and he might be drinking sherry and he would be miserable.

But I enjoyed seeing Whitteridge in these weekly things. I remember writing something for him, you know, a term test. We always had exams. Every semester you had exams, and my college was rather smart. They made the exams at the beginning of the semester, not the end of the semester, so that meant you had to have learned again and revised again all of the material that you'd done, so that you were really being examined on your cumulative knowledge, not on what you'd just learned. It was very smart. So they'd have these exams at the beginning of each semester, and not at the end. So it wasn't an exam of what you'd just done; it was an exam of your total.

But anyway, I was writing one of these for him, and I remember getting it back and it said "diffuse, undisciplined, and at times inaccurate." That was written all across the top. He was like that, and the tutorials are like that. So my first chemistry, one of my first chemistry tutorials was with R.P. Bell. He was a physical chemist, a first-class physical chemist. They were all fellows of the Royal Society and distinguished scientists. He gave

me a topic to write on. “Write me an essay on the Pauli Exclusion Principle in the Periodic Table,” which is a marvelous topic, because the exclusion principle being that you can’t have two electrons with the same quantum numbers, and out of that comes the whole periodic table.

It’s a beautiful topic and I had a glorious time with it for a week, and came to him with my essay. We didn’t get down past halfway down the first page, because there was something I didn’t understand or couldn’t justify, and more or less the whole of that hour was spent drilling in the idea that you never, ever write down something you don’t understand and that you can’t justify. And that was the sort of scholastic level it was at.

So the whole university had that sort of level, and that’s why it produced a lot of, and still produces a lot of distinguished people, because that method of teaching is incredibly powerful, but it’s also very expensive, in the sense that you can have one hour with your professor once a week, and he can’t manage, I mean, he can’t manage fifty students. He can’t do fifty in a week. He could do maybe—maybe a very energetic one would have fifteen, perhaps. So you’re taking one-fifteenth of the time of a professor to do that. But anyway, that was the sort of teaching that was going on there.

I don’t know how we got onto that one. [laughs]

NC: It was your teaching style.

SMITHIES: Oh yes, I’ll finish that story then. So then I used to teach, but I wouldn’t give them anything other than original papers to read. I would print for them, Xerox for them in those days, Xerox for them maybe five papers, and say, “Now, these are what you have to read before we have the meeting.” Then we would have the meeting. Then I’d go over them and teach people to read underneath what’s written in the paper, because if you read the introduction you can find how the person now justifies why they did the work, but almost always that’s not really why they did the work. The real reason that they did work was maybe accidental, or curiosity, or many other reasons.

But when you write a scientific paper it has to look good, and so people write as if it was all planned, and a lot of it isn’t. So I would teach them to read in that way, and understand what the individual knew before he started, or she started the work, and what observation did they make that let them make a big leap, because these were always key papers. I mean, this wasn’t just run-of-the-mill papers. If you’re going to pick five for a topic, they’re going to have to be good.

6. Teaching and Students at Wisconsin

SMITHIES: As I say, it might be transcription, total subject. You have to pick five papers to let people understand transcription, so as a result, of that you would teach people to learn what it was that people knew when they started, and why they saw it. Often enough it was a very good observation that a person made, or they invented a new method for an old problem, and I used to then teach them, “Never try to solve a new problem with a new method. Try to solve a new problem with an old method, or an old problem with a new method, but if you try to do a new problem with a new method, you won’t know what’s gone wrong. You won’t know whether it’s your method that’s gone wrong, or whether whatever it was you were thinking is wrong. So you try not to do that; that combination is a dangerous combination.”

NC: You've got two unknowns.

SMITHIES: Two unknowns at the same time, and that sort of thing, and teach them—I remember when Rodney Porter got the Nobel Prize for his work on gamma globulin structure, antibody structure. He discovered that there were two parts to an antibody. There was a constant part, and there was a variable part, which is very important to understanding how the thing worked.

And just as it happened, just before he got the Nobel Prize, I'd been purifying bovine gamma globulin. I got it pretty pure, and I used his procedure in order to cut the constant part of the antibody from the non-constant part. The constant part he called FC later on, for constant, but in fact that C wasn't constant originally. It was crystalline. What happened is I saw—I came in in the morning after this thing had been cooking; it was full of crystals. My preparation was just crystals, crystals of protein, and so then I knew what had led Porter to that observation. Almost certainly, it crystallized for him, and when he saw that crystallization and then realized that, that meant part of it must be constant now, and it crystallized.

I wrote to him and said I thought it was—I never got a reply. But the power of making an observation, and then often it's an accidental observation that's made, and then people can make a connection that wasn't there—

NC: I'd like to ask one more thing about—

SMITHIES: —so that was the way I would teach that course. I'm going to go on, because it is rather important, I think, in terms of how we should teach. There's no reason not to use this system, because I've used it on, quote, "lay audiences." I mean, that's to say, people who are not scientists.

So I was asked, for example, to take part here in North Carolina on what was called a mini-med school. The mini-med school consisted of maybe, I don't remember the number, but let's say twenty lectures total, given over a course of ten weeks or so. People who were a secretary or a doctor or anybody could come to these things, and you were supposed to teach them your part of genetics in one lecture. I decided to do the history of genetics, molecular genetics, and I taught them entirely from original papers. You could do it just as well.

So that it came to, "Well, let's start with hemoglobin, see. Now, sickle-cell hemoglobin—." What you do is you find the paper, the first paper, if you translate the title, which is obviously written in scientific language, but all it's saying is, funny-shaped red cells in a person with anemia, and that's the whole topic, and there's a picture of them. That was the discovery of sickle-cell anemia, and that you can teach a person easily by showing them the original paper.

Then I'm showing them the original paper of Neel, Jim Neel's working out the pedigree—you can show them that. **endnote 25** It's very easy. The pedigree is of a simple pedigree. It's very easy for them to—an ordinary person, if they're helped, can understand an original paper. And so I took them all the way through. I'm showed them Avery, MacLeod, and McCarty's experiment for DNA. **endnote 26** I showed them Vernon Ingram's spots for sickle-cell hemoglobin, I mean, all the way through, right from

beginning to end with original papers.

That's part of what we do wrong in our teaching. We don't teach at a level that students can handle if it's taught right. It means that people take digested science, and they don't—it's like reading Reader's Digest and never having read an original book by somebody. It's not the same thing. Anyway, so that style I've used ever since. It worked. I won't let my postdocs or my students talk about a compound unless they can put the formula on the board. They have to put the formula on the board.

So just a new topic, writing about, here comes this thing, the T-PLL [T-cell prolymphocytic leukemia]. It took me twenty minutes to find the formula of it, but once I've seen the formula I can understand what that molecule is doing. I couldn't understand it before. It's just a name. What's a name? A name doesn't tell you anything. You have to know what it is. So you have to teach people that sort of level of understanding that I was taught, and that's what I got from my university.

NC: I'm wondering if you can give us a virtual tour of your laboratory at Wisconsin in the mid-sixties. Walk us in on a typical Monday morning, and point out the equipment.

SMITHIES: You mean the actual laboratory in which I worked, or the total laboratory?

NC: Your laboratory, yes.

SMITHIES: No, I think I probably can't. I don't have a very good visual memory of it. I remember it was—well, I can probably say a little bit about it. There was a rather long room with a bench running down the middle, the usual sort of thing as I remember it, and some benches down the side, and at the end coming from the corridor, at the opposite end were the windows, but they were in two rooms, for two rooms that were about 200 square feet apiece, something like that, one of which was an office, and I don't remember what we used the other for. But eventually we put the computers in my office. I didn't bother to use the office, didn't like the office anyway. But I don't remember very much about what it looked like, particularly.

NC: What kind of instruments would you have? Who would be doing things in the lab?

SMITHIES: Well, it wasn't very highly instrumented. It depended on the time. Initially I was doing—when I went there, I was still doing a lot of working on the structure of haptoglobin, and so most of my experiments were done with starch gel electrophoresis, and enzymes and on things of that sort, so I imagine that that's what it was. We needed a spectrophotometer. We had a centrifuge. There were also centrifuges in the building, but not used very much. It was not heavily instrumented. I mean, this lab is not very heavily instrumented if you look around. I mean, even now my centrifuges and electrophoretic power supply and a PCR machine, and the spectrophotometer I don't even use anymore. I just look at the gel and estimate how much I got by just looking. I don't bother to measure things that carefully. I don't need to for most of the work I do. You can do it by just looking at it and like a pinch of salt, just this and that and the other.

NC: Did you have graduate students?

SMITHIES: Yes, I had graduate students and I had a postdoc. I had a number of graduate students, and we did protein chemistry together initially. I'm not very good at remembering exactly what happened all the time in that, for some reason or other. I don't know, it just seemed like it was a good time and we did good science, and I know these people are still my close friends, and that's it, but don't remember exactly what we did together.

NC: Who were some of your students?

SMITHIES: Oh, I think my first student was—well, he eventually had a company here in North Carolina. Well, my first graduate student, and postdoc at the same time, was Walter Nance. He was a graduate student because he was doing a Ph.D., and he was a postdoc because he was already an M.D. He later became the president of the Genetic Society of America, so he is a distinguished individual. **endnote 27**

NC: We'll be interviewing him.

SMITHIES: You will, yes, okay. You'll get some good history from him. But he was my first—he says he's my first graduate student. I'm not absolutely sure he's right. I think there might have been one other, but he thinks he was.

NC: How did he come to you?

SMITHIES: Crow suggested I ought to have a student and suggested him, I think. I was always used to working by myself, you see. I'd worked on most of my science. All my science in Toronto was done by myself with Otto. And I had a man who became a very close friend, David Poulik, with whom we had—he got me going on quite a few things in relation to interpreting what could be seen on starch gel with what had been seen on filter-paper electrophoresis, and it was out of that came two-dimensional electrophoresis, and so he and I did a lot of work together on that. We were good friends, too.

NC: Now, did Nance have a similar experimental style to you?

SMITHIES: No, I don't think so.

NC: How did you work together?

SMITHIES: He did a lot of starch-gel electrophoresis, because he was interested in the haptoglobin polymorphism, in understanding it, and did quite a bit in relation to population studies, as I remember. His thesis turned out to be understanding what happens—I think it was; I don't exactly remember now, but it's up there on a shelf—but it was in relation to what happens to the haptoglobin genes once they're there, as it were, because one of them is a duplication, a partial duplication. We haven't talked about that, but it's a partial duplication of the other gene, of the other alternative of the other allele, and so all sorts of things happen as a result of that, which is where the homologous and the non-homologous recombination comes in, which we should talk about at some stage.

NC: Yes. I do want to get to that.

SMITHIES: So I can't give you a good picture of the lab, then, at that level. I'm not very

helpful.

NC: But did Nance work in a similar way as you did, just sort of working in the lab? You called yourself a messy scientist. Was he messy, too?

SMITHIES: Well, I think he was much tidier than I was. But no, I don't remember how he worked, particularly. I had some pretty messy ones, my graduate students. I had one during the Vietnam War period—was it John Gilman? Anyway, his name was Gilman; I think it was John. And he was terribly messy. He would do all experiments as sloppy as it's possible to do them and get a result, but he would get the results. He solved problems in silly ways and not in others. I mean, instead of purifying the protein and doing sequences—we were doing amino acid sequences at the time—he would do three at once and deduce a sequence from a mixture of three, which he could just about do, and that sort of thing. But he was very messy.

There was an incident which I remember well. The student protests were large in Wisconsin. I remember coming across him one day and saying, "What are you doing, John?"

He said, "I'm making a bomb."

I said, "Well, I'd rather you didn't make a bomb in my lab." [laughs] "Please don't do it." I don't know if he was really making a bomb. That's my recollection. He was probably making something that was obnoxious, but that was John Gilman. He was messy.

And then I had another student, Vicky Valancius, and she couldn't do experiments very well, but she was a very good thinker, and she devised all sorts of neat experiments to show that what was happening—and this was now in the DNA days—what was happening in our DNA work was what would be expected from what was known in yeast. She did some very—wrote a very pretty paper. It was quite instructive. But she was not a good experimentalist. It was borderline science always with her, experimentally. So the ability to do good experiments and the ability to think aren't always in the same person.

7. Haptoglobins, globin genes, homologous and non-homologous recombination

NC: All right. Let's talk about haptoglobins. We started on that. We got through the starch gels. You had three different types, and they turned out to be haptoglobins.

SMITHIES: Yes. So then the question was how to make them make sense, so I spent a lot of time doing what I used to call Saturday morning experiments. On Saturday morning you can do an experiment that is crazy. It doesn't have to be terribly logical, and you don't have to measure anything, and you can just use any chemical you feel like and do anything, because you shouldn't be there on Saturday morning anyway. So Saturday morning experiments are just play experiments.

So I tried to think of as many possible ways of separating, making one of the haptoglobins, which was complicated, simple. In order to explain this, you have to understand that this was a hemoglobin-binding protein, so in whole plasma you could easily see it by just adding hemoglobin and looking for where the hemoglobin ran, and that would be hemoglobin haptoglobin, if you added it to plasma. It wouldn't be pure hemoglobin, so you could tell where haptoglobin was without purifying it.

So you could see in a Type-1, what we called the (1,1) haptoglobin, you would get a single band that was fairly easy to see, which was easy to see. If you had a Type-2 individual, which was the other homozygote, then you would get multiple bands, thirteen or fourteen bands in a series which were clearly polymeric. So my thought was that there must be some way of converting the polymeric structure into something that was simple, because it had to be underlying [the underlying structure]. The genetics were simple, so it really had to be simple if we could find out how to do it.

So I spent a lot of time trying different chemicals. I remember one time I tried phenol, and it dissolved my apparatus. [laughter] I mean, literally, my starch-gel trays were dissolved by the phenol and I couldn't use it anymore, that particular tray. I tried urea. I got the idea of urea from Gordon Dixon, who was then, by that time had moved away, I think, or no, I think he'd come back from doing a postdoc. Anyway, I used alkaline urea to try to dissociate polypeptide chains, which is a method that [Christian] Anfinsen had used to solve the disulfide structure of ribonuclease, and to show that the protein would refold completely without any help, just from its amino acid sequence, for which he got the Nobel Prize. **endnote 28** It was beautiful work.

I tried alkaline urea and I could get rid of all the polymers, but all I got was really smeary stuff and it didn't look very good. But messing around, and eventually I tried a whole bunch of different things, and I found that if I'd use the alkaline-urea method to break the disulfide bonds and therefore dissociate the polymers into monomers, that this also unfolds the protein, that that in itself was messy, because they could still interact and make a mess, as it were, because there was nothing holding them stable.

But if I blocked the S-H groups, which were made from the disulfide bonds, with a compound which makes an irreversible blockage, which is iodoacetamide, that I could make the pattern a bit better, but I still couldn't understand it until I tried running gels in urea, which Gordon suggested. Rather than making gels out of 8-molar urea, he found he could do that, Gordon Dixon. Then when I changed the pH, I found that when I used an acid gel with protein that had been reduced with mercaptoethanol in the presence of urea, and had had its S-H groups then blocked with iodoacetamide to make it stable, that when that was run in an acid urea gel I got very pretty patterns, very easy to understand.

That gel was then used by Gerry [Gerald] Edelman to solve the problem of light chain and heavy chain of myeloma proteins. He showed that by doing what I'd been doing on haptoglobin, if he did that with myeloma proteins and ran them in the gel that I had used, which Dave Poulik taught him, then he could see that the light chains of the immunoglobulin were Bence-Jones proteins in the urea, and he then solved the availability of haptoglobin, or at least—I mean of immunoglobulin, for which he got the Nobel Prize. I was always a bit mad with him, though, because he never acknowledged properly that the method was mine, that method, and I don't think he even gave a reference to me. But I don't think we'll put that in the final one. **endnote 29**

But anyway, so this is a good method for separating proteins, polypeptide chains now, in a way that you could see them and get clear bands. What I found was very surprising, that my haptoglobin (1,1) gave me two bands, and it had been one band before, and the (2,2) gave me three bands, and the heterozygote gave me—no, I've got it wrong. It was obvious there were two chains when I did this, that there was a constant

chain, some sort of which we call beta, which all three genetic types shared, and that ran slowly in the gel. Then there was what we called the light chain of the haptoglobin, and they turned out to be correct, that with the (1,1) haptoglobin had two bands, and in the (2,2) haptoglobin had one heavy band that was slower, and in the heterozygote there were three bands. **endnote 30**

So that was what the situation was when I came to Wisconsin, back from Toronto to Wisconsin, and we were trying to solve that problem. We couldn't understand why there were three bands in the heterozygote and two in one homozygote and one in the other homozygote, and it was very much heavier, and heavier staining was the one in the homozygote, the (2,2). So (2,2) was giving a heavy band and a heavy band, and (1,1) was giving it a fainter band and a fainter band, and the heterozygote was giving the fainter, fainter, and the heavy, so there were three bands.

Then George Connell, who was doing by then, had come back too and was part of our team, he had worked out how to purify haptoglobin in large quantities. I'd worked out how to make it in smaller quantities, and he scaled it up so that we could do these studies, because in those days it took a lot more protein. Then we thought, well, maybe there was proteolysis going on, and that the two bands that came in the homozygote 1 were because the molecules are being cut by a proteolytic enzyme into two pieces, and so there was some bond that was susceptible. So we decided to make the haptoglobin from plasma instead of from serum, which we'd used mainly before, and George did a rather horrendous experiment, because he used a nerve-gas poison, which was a very good inhibitor of proteolytic enzymes, and prepared haptoglobin from a unit of blood that he got.

NC: Was it nitrogen mustard?

SMITHIES: No, it was di-isopropyl fluorophosphonate is my recollection.

NC: Di-isopropyl fluorophosphonate?

SMITHIES: —fluorophosphonate is what I remember, DEPC, which I think is a neurotoxin. **endnote 31** Anyway, it's nasty stuff. But he worked with this, and then a very strange thing happened. Suddenly now we found that we got only one band in the (1,1), and some other individuals get two bands, and some other (1,1) individuals gave only the slow band. So we realized there was another genetic difference underneath the one that we'd understood, so that the 1 could exist in two forms, what we called 1F in the end, for fast, and 1S for slow, and 2 was the other alternative, so you could now have altogether six genotypes.

You could have a 1F,1F. You could have a 1F, 1S, and you could have a 1S, 1S, so that's three genotypes corresponding to what we used to call (1,1). And then the heterozygotes were now simpler, because the heterozygotes only had two bands. They either had the fast haptoglobin band, 1F and the 2, or they had the 1S and the 2, but they never had three bands, because we'd used mixtures of plasma. So now we sorted that out. George Connell sorted that out, really, so we now knew that we had three possible alleles, not two possible alleles.

Then Gordon Dixon began to do—and I learned how to purify the, and I showed them how to purify the, worked out a method of how to purify the light chain without the heavy

chain being there, so we could easily get the one that carried the genetic information. And Gordon Dixon started to do fingerprints from them. That was the method that had been used, and that was still the only protein for which this had been solved.

That was what Vernon Ingram used to solve the hemoglobin one. What he did was treat the protein with a proteolytic enzyme that broke it into peptides, and then ran a two-dimensional chromatogram, one way was electrophoresis and the other way was chromatography, and you would get a—when you stained it, it looked like somebody put their fingerprints all over a piece of blotting paper, so that's why they were called fingerprints, and you could see, then, the peptides.

We did this, and Gordon got these peptides, and they were very difficult to understand, the fingerprints, because when we looked at an individual who was 1, if they were 1F there was one band, or there was one spot there which we called the 1F spot, and if you were a 1S that spot was slightly changed in position because of amino acid changes, and there was the 1S spot, so that was perfectly okay. There was 1F and there was 1S and the heterozygotes had both. That was understandable. But when we did the (2,2) individuals they had 1F and 1S spots, so they looked like another mixture again. It was crazy. Both of them were there.

Then we noticed another peptide that was there in the 2, and we used to call that then—it was unique to the 2—we called that the 2 peptide, so there was the 2 peptide, and then there was one called the 1 peptide in the 2 position, and we had all sorts of nomenclatures for this, because we were completely confused.

Then I had to prepare for a talk; I think it was a meeting in—I have an idea that it was in Japan, but I'm not sure. Anyway, I was preparing to go to a scientific meeting, and so I got the peptides map of Gordon's and was looking at them very carefully, and then I noticed that there were some quantitative differences as well. So there were two other peptides, which changed the intensity in the 2 and gave this extra peptide, so 2 had 1F, 1S, and two other peptides which were changed in the amount, and the amounts were all wrong. 1F and 1S, it was a complete mess, and we kept trying to think, well, what are we doing wrong?

Then I was arranging for Gordon to do—then he did some amino acid analysis. You couldn't do peptide sequences in those days, easily. He got some amino acid compositions, and we found out with what we called the 2 peptide, the 2 spot, had some of the amino acids that were present in the two other spots, which were varying in amount. It was all completely crazy, and we couldn't believe our results. Then I remember going back to Toronto to talk to these guys about it, and we had a meeting. I think Gordon went out of the room for a moment, or one of them went out of the room, and I said, "It still looks like a mixture. Well, why don't we believe our results?"

And suddenly it clicked that it was two genes, both genes that fused into one gene, you see, so the 1F gene and the 1S gene at the genetic level had fused into one gene, so that now everything suddenly made sense, because there would be a junction peptide, which is what we called the 2 peptide; there would be the 1F peptide and the 1S peptide, because they were here and here, but there would be half the relative amount, because you would only get 1F, one per double amount of protein, and 1S per double, so they were reduced in level. They were down and the two N peptides, which were the N and C, they also were down in amount relative to the total, because there was only on

N terminus and one C terminus per double length of molecule, and where those two peptides had now disappeared, they'd disappeared into the junction peptide. So it suddenly made sense. So I go back to Wisconsin and I go to Jim Crow and I said, "Jim," I said, "I've got this situation. I've got a gene 1F and I've got a gene 1S, and I've got a fusion. I've got a gene that's the two together. How can that happen?"

"Oh," he said, "that's been known for a long time." And he pointed out what had happened in Drosophila with the bar-eyed locus. Well, now that's also a complicated story which is not immediately understandable. [Alfred H.] Sturtevant and [Calvin] Bridges worked it out, but they didn't get it quite right. They, by looking at the chromosome, they could see that the Bar-eyed, which was the color of the eye in the fly—a normal eye of a Drosophila is uniformly red and a nice oval shape. But the Bar-eye, all the color part is sort of squished in from the side; it makes a long line.

The Bar-eyed mutation had arisen once by a man called [S. C.] Tice, discovered it in 1915 or something like that. **endnote 32** He did the original once, so it was a very rare event. But Sturtevant in the 1920s had studied it and found out that if he took a Bar-eyed homozygous female, that sometimes the offspring would revert to the wild type. They would become normal. Or they would get worse, and he called it ultrabar, so that by crossing two bars you could end up with getting a normal out, or something worse. **endnote 33**

Then Bridges didn't get it quite right, but nearly right, saw that it was really related to a duplication, that the chromosome was doubled in size in the bar locus, so you went from one patch of chromosome that was single, to two, and that was the bar. That was very rare. That only occurred once. Tice saw that. But once you got this, then the subsequent event was not very common, but common enough that you could see it many times, as it would go from two back to one, or up to three, and that was called unequal crossing over. So you went from two to three or back to one, and it was predictable.

Well, I realized that that wasn't quite right. What Jim Crow told me wasn't right, it wasn't quite right, that my haptoglobin was the production of bar, not the subsequent changes of going back to infrabar, etc. It's a little bit complicated. So haptoglobin, that 2 gene only arose once, almost certainly. But once it was there, then it could do very strange things, because the 2 could go back to 1, or go to 3, if this was right, if it was behaving like the bar locus.

In other words, it's easy to understand. [Smithies was using hand signs, then sketches, through tracks 13 and 14] The chromosome normally would line up perfectly, so 2 will line up with 2. But because this is identical to this, nearly, sometimes it will line up—this will align with this, so (1,1), (1,1), here like that. I can't do three with my hands. Maybe a bit of paper will do it. You see then, if you think of the original gene being, let's call the original gene A, and then that's 1, and then the 2 was A,A—this is 2—and so A,A, if A,A lines up with A,A, and you get a crossing over, you get nothing new, always produces A.

But if A,A lines up incorrectly, it can line up like this, and still A is opposite A, so if this crosses over, now it isn't symmetrical, because this gives you a 3 and this gives you 1. That's what happens in the bar locus, and this is what we predicted therefore was happening, and would happen in haptoglobin. And so we looked for the 3, and we found

it. We could find the 3 in any population we looked at, if we looked at enough people. So this reaction of going from 2 to 3 is what you call homologous recombination, because this sequence is the same as this sequence.

Going from this to this was non-homologous, because it was a breakage of this chromosome somewhere here, and a rare event, and broke with another one like it and these two joined together, but there was nothing, there was no particular reason why that would happen. It was a very rare accident, chromosomal breakage in these. So this was, in going from gene 1 to gene 2, is rare.

NC: Are these on different—

SMITHIES: Well, they're on different chromosomes. There must have been, because you can't—I mean, it was a meiosis event, in other words, during germ-line formation.

NC: But it's like the two chromosomes 1. It's not chromosome 1 and chromosome 2.

SMITHIES: Yes, oh yes. Well, there was only one. At that point there was only a 1 chromosome. There wasn't—the 2 gene didn't exist. So this was the original haptoglobin locus.

NC: But the chromosomes are homologous. What I get confused about is the different senses of homologous.

SMITHIES: Yes.

NC: These are homologous chromosomes, but the crossover event is in non-homologous regions.

SMITHIES: Yes, that's correct. So let me just a bit—use a few more letters. So let's say we do A, B, C, D, E, F, G, H, I, J, and we'll say that this is haptoglobin.

NC: Okay.

SMITHIES: So this is homologous and this is homologous. So if this breaks here, and this one breaks here, so D, E, F, G, H, I, J, A, B, C, this is non-homologous. There's nothing in there that's the same as this. But if that joins to that, now I will get A, B, C, D, E, F, E, F, G, H, I, J, and this is the 2 gene, you see, where this is duplicated now. You see? But that is a non-homologous event, going from a gene that has—because there's nothing in this sequence like this sequence, so it's illegitimate, if you like. It's an illegitimate crossover. People used to use that word, too. It means there's nothing about that that causes it to happen. It's just a very rare event that a chromosome breaks and heals itself with some other chromosome that broke, so it's rare. So the haptoglobin, from generating the haptoglobin 2 gene therefore only occurred once. Generating the bar duplication, Tice's observation, only occurred once, because it's a very rare thing for chromosomes to break like that. It's quite artificial. There's nothing causing it to happen except some sort of maybe a cosmic ray or something hit it. Who knows.

But that's a very rare event, and that's the same thing that happened to go from wild type to the bar locus, which Tice observed, rare. But once you've got the bar, because bar is really a duplication—

NC: Once you have D, E, F, E, F—

SMITHIES: —now this event is common, relatively common.

NC: Yes, I've got you.

SMITHIES: Usually it will line up correctly, A, B, C, D, E, F, E, F, G, H, I if it's a homozygote, it'll cross over anyway and it doesn't make anything new. But occasionally that will line up with this. It's homologous.

NC: Right. The two Es will line up—

SMITHIES: Exactly.

NC: —but they're different Es. They're not in the same position.

SMITHIES: Yes, they're duplicated.

NC: Yes, yes, okay.

SMITHIES: So that's what I call unequal but homologous crossing over, so it was unequal but homologous.

NC: They're always homologous chromosomes, between which the crossing over is occurring.

SMITHIES: Yes. But so this is a non-homologous recombination that led to that. This is homologous. So I knew that this happened fairly frequently.

NC: Now I understand it.

SMITHIES: So that's very important to know, because that was the initial thing that made me understand that homologous recombination is a very predictable event. Non-homologous recombination is not predictable, but this is predictable.

NC: All right.

SMITHIES: Now then, later on, then, as we moved into DNA—I might as well finish this story, because it's scientifically consecutive—later on we moved into doing DNA sequences, and began to study what happens in hemoglobin. No, that's not quite correct. This event also was described, this unequal but homologous was also described in hemoglobin genes by Baglioni, who understood it because I'd talked about haptoglobin at a meeting, and then he realized that what he saw was happening was similar.

So unequal but homologous crossing over was understood at the level of hemoglobin genes, globin genes, and the more you looked at hemoglobin genes the more you could see examples of this, because the beta globin locus in humans has five different copies of the beta globin gene as G-gamma; no, sorry, it starts with epsilon. It starts with epsilon chain, which is the embryonic hemoglobin chain at that time.

Then there were two fetal chains, which are called gamma, and one is called G-gamma and one is called A-gamma, because they differ in one amino acid, but they're very similar, because G and A, that's the only change between the two. Then there is the adult hemoglobin. There are two adult hemoglobins. There's a delta chain, which is a very minor chain, and the beta chain. So there's a string of genes, all of which are similar but not identical. And so every now and then they undergo that sort of unequal crossing over, partly homologous, not perfect homology, but they'll find a little section that's identical and crossover. **endnote 34**

So one of the famous examples was Lepore hemoglobin. Lepore hemoglobin is made when the delta gene crosses over with the beta gene, and you need a little diagram for that one, too. [Smithies diagram] So here's delta and here's beta, not that far apart. And occasionally, delta would line up with beta like this, and this is what Baglioni described.

They will cross over, so that out of this you get a chromosome now which has a delta-beta gene, and this is called Lepore hemoglobin, and is a result of this sort of event. It's an anemia, because the delta is a minor hemoglobin that isn't very strongly expressed, and so this now how a minor promoter driving the adult hemoglobin, and it doesn't make enough, so this is an anemia. I've forgotten what it's called. It's called Lepore hemoglobin, anyway, but it leads to anemia.

But my students began to study this and found several things of interest. That found that this event happens more than once, because it happened more than once, so the Lepore gene has arisen in many different populations throughout the world. And if you got the Lepore gene that has arisen in Turkey and one that's arisen in Pakistan, shall we say, or one that's arisen in Africa, then the crossing over wasn't always in the same place. So sometimes it would be like delta-beta, and sometimes it will be delta-beta that would be different. But they always crossed over in places where the sequence was identical, the crossing overs. **endnote 35**

So I began to understand more about what controlled homologous recombination. Then another thing came up, and then, well, Nobuyo [Smithies' wife] had a good one on this. Haptoglobin locus was here and she found a haptoglobin-related gene nearby, which was a duplication, but it wasn't quite the same gene anymore, and she found individuals who had four or five copies of this because of unequal crossing over. It's very common. I mean, it's common relative to mutations; not that common.

Then the two globin genes here, we had G-gamma and A-gamma, and I got involved in cloning DNA at that point. The DNA, it just became possible to clone DNA—

NC: This is in the seventies now.

SMITHIES: —in the seventies, yes. Fred Blattner was a master—he was in the Genetics Department, and still is—an absolute master at phage genetics, bacteriophage genetics. **endnote 36**

NC: At Wisconsin?

SMITHIES: In Wisconsin, yes. And also understood bacterial genetics a lot. You will remember the history of cloning, of DNA cloning, that's not to say cellular, individual

cloning, that people were worried that it might cause a problem, so there was a moratorium, and it was decided that it wouldn't be permitted until you had a very safe system, so that if your bacteria got spilled, it wouldn't replicate. So you had to get very much handicapped bacteria, or very much handicapped bacteriophages. **endnote 37**

Fred decided to do it in bacteriophage Lambda, and we made more—and I had a year's sabbatical at that time, which I did in Wisconsin. I just stopped doing any teaching and any committee work and just worked with Fred, and learned to handle DNA. He knew more about DNA, much, than I did. Learned to handle DNA with Fred, and learned the genetics of bacteriophage. He made a very safe vector out of this.

So we had a bacteriophage and there's a marvelous set of histories about that that we won't have time to go into, which was going to Washington, convincing the committee that you now make safer—no committee would ever accept what you did without upping the ante. **endnote 38** They always would say, "We want it safer," because they were scared to rubber-stamp it, so they wouldn't ever let it stop. So they said, "We want another thousand-fold worth." So they got it ten to the minus thirteen [10-13] or something like that, relative to wild type before they would accept it.

But anyway, as a result of this, I mean that's to say that maybe something like that—that the chance of your bacteriophage surviving relative to the wild type was only one chance in less than a million million, extremely small. So to do these, we had to have bacteria plates which had millions of bacteria colonies on them, phage colonies, and that 10-13 is an exaggeration. It wasn't that—but it was anyway rare.

So then we were ready to go when it was decided it was now safe enough, and so we decided to try to clone one of the hemoglobin genes. Tom Maniatis said, "Clone the beta gene as a cDNA [complementary DNA], not as the actual gene." So we decided to try to see what we could get out, and quite unintentionally what we isolated was one of the fetal genes. We didn't know what it was, but we'd isolated this. It was obviously a bacteriophage now which would hybridize to the beta-globin probe, which was from Maniatis, and meanwhile, Maniatis isolated the actual beta-globin gene, not the cDNA now, because the gene is longer than the cDNA. You've got the whole gene.

And we isolated it, and I've forgotten the name of the person who isolated delta, and we all combined together and published one glorious paper of the isolation of all these genes, of the globin genes, and how the locus was put together. **endnote 39** It was a nice one. But then as a result of that, we got both of these genes, and then we found that this gene had transferred sequence to that at the DNA level, so that was one of my postdocs. This name retrieval is hopeless for me anymore. [the postdoc was Jerry L. Slightom, who is now Chief Operating Officer of AureoGen, a biotech company] Anyway, I'll get it back. It's annoying. That means that part of the tape I won't let you have.

NC: We can fill it in.

SMITHIES: Yes, we can fill it in later with the name, because I know him so well. Anyway, he and I were part of that publication, and we realized that the G-gamma gene and the A-gamma gene had exchanged a huge block, so inside this gene was another piece of sequence that had come from here. So this homologous exchange is going on all the time, and so this is what made me very aware—these things are what made me very aware of homologous recombination, the haptoglobin, initially non-homologous,

then once it had deviated, lots of homologous recombination you could easily find, then the Lepore hemoglobin, which was recombination happening in sequences that were identical, but in different places on the chromosome, and then the G-gamma and A-gamma, they were exchanging sequences. So it was obvious that if you had a piece of DNA, it would exchange sequences with another piece of DNA if there were regions of identity between them.

NC: So the non-homologous recombination event that led to the type-2 haptoglobin—

SMITHIES: Is a very rare—

NC: —is a rare event. But that's one of the conditions that can lead to regular homologous unequal recombination—

SMITHIES: Yes, it's sequence identity.

NC: —and so that led you to recognizing that as one mechanism for leading to homologous recombination.

SMITHIES: No. I think I just knew by then that if you got homologous—homologous recombination will happen wherever there are sequences that are alike.

NC: It could happen due to all sorts of DNA conditions, and non-homologous is only one of those.

SMITHIES: Non-homologous is completely chance.

NC: Yes. Right.

SMITHIES: And it won't have any rules, or very few rules. Maybe there are some rules, but it isn't a regular kind. Homologous recombination will occur the longer the sequence is that's identical. So if you have a huge long piece of DNA that's identical with another huge long piece nearby, it will cross over often, unequally, because there's a big target. But if there's only a little bit that's homologous it will be rarer, but it will happen. And so wherever two pieces of DNA have a sequence in common, there is an opportunity for crossing over. It may not be—I mean, chromosomal-wise it's not homologous, but at the DNA level it will be homologous.

NC: Now, there are people working in non-human systems that had been aware of some of this for quite a while, and you cite some of them in your 1964 Cold Spring Harbor Symposium article. **endnote 40** You cite the maize geneticist, John Laughnan. You cite Ed Lewis. So is this another case of the sort of Bar gene, where you're tapping into the older literature on other organisms then?

SMITHIES: Oh yes. I mean, that way, now not at the level—I'm not really talking yet about DNA, when you're back in the 1960 experiments. You're talking about experiments where we're still thinking about genes as entities. We're not really thinking about nucleotide sequences, and we aren't using DNA. We're only using whole chromosomes and looking for what happens. So what I was saying at that point was that the idea of gene duplication, that was Ed Lewis that worked out—he called them pseudogenes, I think, at that time. That was Ed Lewis' finding, and then the bar locus,

then, was Bridges and Sturtevant and those people who understood all that. So, yes, I mean, that was the literature I was referring to, and that was part of being able to just show it was just that homologous recombination and non-homologous recombinations were just part of my being, you might say. I didn't need to be thinking consciously about them. They'd be automatically in your head, as it were.

NC: Were you aware of Barbara McClintock's work in the 1930s on unequal crossing over?

SMITHIES: Yes. I never understood it. It was too difficult for me. I couldn't understand what Barbara had been doing. I didn't know what that was.

NC: Because she worked with unequal crossing over.

SMITHIES: Yes, I know, but I didn't understand what she was doing. Her papers were very difficult, and corn genetics wasn't easy, and I think I never made the effort to understand it at the time.

NC: Okay.

SMITHIES: But her work didn't impinge on mine, really, in that sense, in the timeframe of science.

NC: Okay. I wanted to ask a little bit more about that Cold Spring Harbor meeting. That was a big meeting among the Cold Spring Harbor Symposia, and it was interesting in that series because it brought in a much higher fraction of medical people than typically go to Cold Spring Harbor meetings. And you, I think, organized an ad hoc session on proteins.

SMITHIES: I can't remember what we did in that meeting. I really would have to look at it to see what that meeting was. It's up there if you can get it. It's right in the 1964 *Cold Spring Harbor [Proceedings]*, those big journals there. Can you see 1964? Can you get at it? Yes, just pull that one out and bring it. [pause] I guess I don't remember what was going on at that one. Hemoglobins, yes, inactivation. And I had a paper in here, did I?

NC: Yes. It says in the introduction that you organized a special ad hoc session.

SMITHIES: Let me just see what it was. I don't remember what I did in that one. Well, I'm referred to it in a lot of places, but 309. [looking at p. 310 of Smithies, Chromosomal rearrangement and protein structure, *Cold Spring Harbor Symposia in Quantitative Biology* 1964; 29:309-319] Oh yes, chromosome rearrangement, yes. You see, and I had unequal but homologous crossing over and a lot of things of that sort, yes. Yes. So now what was your question?

NC: I was just wondering about this ad hoc session.

SMITHIES: See, there's this business that I explained to you. Now you can see it, the N and the C and the F, and the N, F, and then the junction between the two. Then you can see why N will be half the amount per milligram of protein, compared with the N here, because there's one per one length and one per two lengths here, why the Cs will be down, and why the Fs are stronger in this one next to it than they ever are here, because

they're at half level. When you put the same amount of total material on this will be half the concentration that it would be if you put the same amount of material—so these two were low, this was extra, these two were low, and that was this quantitative business that I saw was funny, and this was the junction.

NC: And the J—this is Figure 1 from the Cold Spring Harbor 1964 paper—and the J comes from, is it asparigine, alanine, tyrosine?

SMITHIES: Well, I don't remember those, at least not very well. You see, this is all we knew about. We didn't really know much about them.

NC: I'm just thinking if you lined these up this way, at the alanines, you would get alanine, asparagine, tyrosine, the crossover between alanines.

SMITHIES: No, it's a crossover somewhere near—well, let me just see how I do it here. I drew dotted lines to show where the crossover had occurred. So the crossover was somewhere—maybe near the alanine. Yes, you're right. It's near the alanine. But this eliminates something, you see. It isn't a complete duplication, because it crosses over part way. Anyway, Nobuyo, my wife, later solved this at the DNA level, and she found that it was exactly what we predicted. She did this at the DNA level and proved that this is what, in fact, had happened. But yes, so that was what I was talking about at that time.

Here's the haptoglobin going to the triple one. There's haptoglobin going to triple, and this is going to—that's the Lepore hemoglobin, and that's the anti-Lepore, as it was called. So anti-Lepore still kept these both, and that's Lepore. And then these were the different points of crossing over, you see, because you got Lepore, and all these different Lepores, and they crossed over in different places, or maybe I was predicting that they would happen. This was then how we thought that might happen, and so on.

NC: Tell me what else you remember from that meeting. Cold Spring Harbor meetings can be very intense socially.

SMITHIES: Well, they were a lot of fun. I had a good time. What did it say about [long pause]—"some additional work shall be presented." I don't know what it was that I organized. No, I don't remember. But it was a pretty peaceful meeting. I don't remember anything at this meeting. That were some that weren't so peaceful, but this was peaceful.

NC: What was the tenor of the meeting? Where did it seem like human genetics stood in 1964, as a discipline?

SMITHIES: Well, I think you're asking me things that I'm not good at remembering. I don't remember a lot of ordinary things, you might say, in life, unfortunately. Walter Nance put it right to me. He said, "How come, Oliver, that you can remember an experiment that I did three years ago, and you can't remember the name of that person who came here only two weeks ago?" So one's memory is selective, and I find I don't remember many of these things. I don't remember a lot of things that happened.

Nobuyo will tell me, "Well, we've been here before. We did this." And I just don't remember it very well. I think my mind must remember other things, and you only have a limited amount of memory, two gigabytes, as it were. [laughter] You parcel it out. So I

don't remember very much about it, no. I can't help you on that one.

NC: Okay.

SMITHIES: I probably won't be able to tell you a lot of that, of the general status of things, but some I might. You know, it depends whether it registered, as it were.

NC: All right.

8. Polymerase Chain Reaction; Ethics of Genetic Information

SMITHIES: I remember when the antibody variability of the joining, you know, of the FC, of the joining of the constant region with the variable-region genes was described, I was very excited. And I remember the PCR [polymerase chain reaction], when Kary Mullis described PCR and those things. Those are very strong memories.

NC: What kind of impact did that have on you, if you remember PCR?

SMITHIES: Oh, I remember it very well, and then thinking, when we were wanting to find ways of doing gene targeting, and somebody at that time, I think it was, let's see, who was it? I can't remember his name, unfortunately. But somebody else suggested to me that we might be able to do a screening for determining whether a colony had got recombinant by PCR, but at that time I hadn't thought about it and I couldn't see that you could do it for what I wanted.

But later on then, I worked with Hyung-Suk Kim here, and we realized we could do PCR if we made one of the arms of homology short enough, that we could do PCR across the arm. The original one that I was trying to do was—the first experiment on homologous recombination, the arms were long and I couldn't have detected it by PCR because it would have required amplifying a piece of DNA four or five kilobases, which was quite impossible at that time by PCR. But so I just thought it was very neat. I just thought it was beautiful.

But later on, then, when we were realizing that we could do PCR to find homologous recombinants in doing gene targeting, then I remembered, of course, PCR, and by then one of my students had gone to work with, I guess it was Genentech, wasn't it, where—no, it wasn't Genentech. It was Cetus, wasn't it? Cetus, went to work at Cetus. So I called him up and said—

NC: This was in the late seventies?

SMITHIES: Yes, it would have been then. Said, "I'd like to do some of this PCR." I thought maybe we should collaborate with the people in Cetus. So I initiated some correspondence with them, and they said they would send me some of the Taq polymerase, which at that time was very, very scarce, and they would also give me some details about some sort of a cycler machine, because Kary Mullis at Cold Spring Harbor had already said that it could be done by a temperature thing, so he'd talked about that.

So I didn't hear from them for many months and I got fed up with this, and I decided—I was in California—that I would go and see them. So I went to Cetus and you

signed the usual statements about that you won't do anything, you won't say [anything about] any of their work, and anything you do that comes out of their work they own, you know. It's a type of document. I signed a document like that and went to talk to them, and they showed me a PCR machine that they'd made, I think with a Peltier factor for heating. **endnote 41** So that wasn't anything. I mean, the Peltier part was new, but the machine wasn't new. The principle had already been stated. I talked with them a bit, and then nothing more happened for a long time.

So then we decided to make the Taq polymerase ourselves, because since they weren't sending it, and the person who first isolated Thermophilus aquaticus is a professor at Wisconsin, so he had the original bug. **endnote 42** So we got the original bug from him, and my postdoc, one of my postdocs at the time began to start to isolate the Thermophilus aquaticus, this Taq protein directly from this bug. Meanwhile we heard some rumor that some company in Milwaukee that was about sixty miles away was making it, and we managed to get in touch with them, and they said, "Yes, we are making it," and they would like us to try using it, and we sort of went and set up a deal with them. We would test their Taq polymerase whenever they had it, and that lasted maybe ten or fifteen years. They supplied all our Taq polymerase free, and we did tests for them on whether this batch was good, or that batch was good.

But anyway, they sent us Taq polymerase, and then we made our own machine, our own—I can show you the remains of the original one. It's outside in the corridor there—which was just changing the temperature of water bath by pumping from one water bath to another. I mean, the hot-water bath will pump through your chamber and then it would switch, and then it would switch to a lower temperature, and we had three temperatures, I think. Originally we started with tiny little water baths like that, but it didn't work.

So anyway, we got it to work eventually, and there's a story behind that. But so the PCR eventually worked and became a very vital tool for detecting homologous recombination. We published a paper on it that was almost not accepted, because by then people knew PCR, but I don't think they'd understood the need to do certain things for homologous gene targeting, and so it was a useful paper. **endnote 43**

Then a company started to want to make our machine in Wisconsin, so we gave them the information and they started, but they were very sloppy and they made very poor product initially. Later made a better one, but by then it was very late and it was never got onto the market or anything, so I still have the remains of their machines now, which I still use, but they never sold any, I don't think, or maybe a few, a half dozen or something.

But then I got a letter from Cetus at one time saying that, "Oh, we're going to sue you because now you're making this machine. You broke all these agreements."

And I said, "No. But if you remember I wrote to you after I got back from seeing you, and said that this is what I had learned when I was at Cetus, and all of it was already in the public domain," and if they thought there was anything that I had learned that was not in the public domain they should let me know, and they never responded to that. So I produced this letter and said, "Look. I told you what I've learned, and it wasn't anything that wasn't in the public domain, and I proceeded to use it then. And you had the chance to say it was," I said, so that closed that one. That was a rather nice incident which I rather enjoyed at the time. [laughter]

There's one other like that I didn't tell you about. You can use it in a different part of your history. That was with Norma Ford Walker, because you remember I told you we sent the blood samples to Bruce Chown to have the red cell typing done, and Norma used to give the results to the family, just for curiosity's sake. You know, it was bait, like, "Oh, we'll give you all your blood groups if you'll let us take a little blood sample from you."

But that was before understanding the dangers of that sort of thing in terms of what happens, so this was in relation to Rh particularly. She was very interested in why some individuals, some babies have a problem with Rh incompatibilities, and others didn't, and I was trying to see if haptoglobin had anything to do with it. Maybe there had to be a discrepancy in the haptoglobin before the baby would get Rh disease. It had nothing to do with that, and we didn't solve it, but that's why we were doing Rh in the families.

So she sent it to one family, and then the young woman whose baby was involved, they used to do exchange transfusion. I used to see them do these things. This is a little thing about the size of a rabbit, because they were nearly always premature, and they would be exchanging the blood with a big syringe, and pull out blood and put in another blood for the babies. They had to do exchange transfusions, because they were so loaded with, what's that yellow compound that you get from—bilirubin, bilirubin from hemoglobin backed up.

But anyway, as a result of that, one of the young mothers came back and said, "I don't understand what's happening in my family, because I'm Rh negative and my mother's Rh negative, and my father's Rh negative, but my brother's Rh positive." Clang, clang. It was obvious what was wrong; it was non-paternity. And so what are you going to do?

You can't—so, "Well," we said, "we'll inquire from Dr. Chown." And so we wrote to Dr. Chown and said—I don't think it was exactly as simple as the description I've given, but it was more or less that.

And then Bruce Chown wrote back a letter which to this day I wish I'd kept, because in those days there wasn't any Xeroxing, so it was a carbon copy on very thin, that very thin—

NC: Onionskin.

SMITHIES: —copy paper, and I got this with a rather poor imprint, impression of him—he went into a long description to this family about the inheritance of the blood group types, and hemoglobin A, and AB blood groups, and the O blood group. And there was one—I think it's called the Bombay phenotype, or something like that, where it typed like an O, but it was really plus, or vice versa, and so he made it so confusing that nobody could possibly follow it. And said, "So that might be the explanation for your result." So this made the family happy.

And then at the bottom in handwriting he wrote, "And you know, Oliver, it might even be true." [laughter] But anyway, that was one of the histories of—well, that's the way people learned. It wasn't so much freedom of information as you shouldn't give information that can be harmful to people, or you have to be very cautious that the

information you give may hurt people, when it's intended to help them.

NC: It's a very different standard than is used in genetic counseling today.

SMITHIES: Well, it wasn't really there wasn't a standard. The occasions didn't arise, you see. This was the beginning of this sort of work, and nobody would have had any experience with those things. It wasn't a different standard. I mean, the ethical standards were just the same. It was knowing what you should do to prevent mistakes happening. It wasn't known. I mean, nobody would have done this deliberately or carelessly. It was just that nobody had yet talked about that.

And I've been to meetings where they've talked about that and been very annoyed about them more recently, maybe not very recently, perhaps five years, ten years ago, talking about the ethics of communicating to a family that they have a problem that other members of the family ought to know about, in order to be protected, or to be helped. And some ethicists saying that under no circumstances should you go and give this information to those people, and I had a lot of difficulty with it at the time, thinking that common sense says that you should. I mean, even though it wasn't asked for, but it should if that information will help them.

But it's become very complicated, and I misrepresent that case, I'm sure. It wasn't exactly that, but it was something of that sort, so I've seen some of those discussions take place. It's not an easy matter.

9. Homologous recombination, gene targeting, gene therapy, and knockout mice

NC: Homologous recombination is one of your best known inventions or discoveries.

SMITHIES: Yes, yes.

NC: And in 2001, I think it was, you shared the Lasker Award with Mario Capecchi and Martin Evans for the development of knockout mice.

One of your original suggestions for an application of homologous recombination was actually as a strategy for gene therapy, I think.

SMITHIES: Yes, that was always my motivation.

NC: So how did that evolution take place?

SMITHIES: Well, I think it's fairly straightforward. The original experiment that I—or I'll backtrack a little bit, because remember now we'd been cloning DNA, so we had normal globin gene—

SMITHIES: —in a test tube.

NC: This is the early 1980s now.

SMITHIES: Yes. So we'd cloned and sequenced DNA, so we hadn't any problem with getting normal DNA, clones of normal DNA. We knew about homologous recombination, so I kept thinking it should be possible to correct a faulty gene by using DNA with the

correct sequence in it, but I couldn't see how to show that that was possible, because it was likely to be a rare sort of event.

Then in this course I was teaching—I told you about the molecular genetics course—there was a paper came out. The dates would be off a little bit, but it came out like April 2nd, something like that.

NC: This is the Wigler and Axel paper? **endnote 44**

SMITHIES: Yes. This paper was a paper in which a very elegant technique, but difficult technique, was used to find something that was rare. What they did was to transfer RNA from one strain of E. coli that had a mutated transfer RNA, so this transfer-RNA gene would replace a stop codon with, I don't remember, let's say with tyrosine. I don't know what it was. But in other words, it would suppress a stop mutation, because it was mistaken and it would replace the stop with something. So if there was a mutation which had a stop codon, then, it could be suppressed by this piece of RNA, so they would call it suppressor RNA, because it would suppress a stop codon mutation. So with Fred we'd been doing a lot of work with stop codons, because in order to make the bacteriophage Lambda subject to death if it was spilt, as it were, to use it for the safety vectors, we put in mutations, or Fred put in mutations in the various spaces which were either amber or ochre or whatever, that were stop codons, so that they were chain-termination mutations and the bacteriophage couldn't grow.

But if it got the suppressor gene it could grow, because it would suppress the ochre and amber. So we used a strain of bacteria which had the suppressor gene to enable the bacteriophage, which had the ochre and amber mutations, to grow. It would grow because the tRNA gene, the suppressor tRNA gene in the E. coli would mistakenly correct the errors in the phage, so the phage could grow when it was grown in a bacteria, which had to be specifically a bacterium that was a suppressor, a mutant form of the RNA. So only certain combinations would work, and that was part of the way of getting the safety into the system, that a rather rare form of bacteria could make a rather rare form of the phage grow only when they got together.

So Wigler's paper used that suppressor RNA to isolate the gene that was a gene coding for a transforming gene. It had been worked out at that time that DNA from some tumors could transfer the immortal phenotype. It could transform. In other words, you could take a tumor and you could make DNA from it, and you could treat a normal cell with it, and here and there—a whole bunch of normal cells—and here and there a colony would now grow that had been transformed into replicating rapidly.

So it was known that there were genes that caused—that were transforming genes, and that's what Wigler was trying to identify. So he cut the DNA up from a tumor and clamped onto the end of it some suppressor sequence, just by joining it by ligation, and then he retransformed with that. Then he got colonies transformed. Then he isolated the DNA from those colonies and did some various tricks with bacteriophage that let him pull out all of the pieces of DNA, or many of the pieces of DNA that had the suppressor gene.

But somewhere in there, maybe a piece of DNA suppressor gene got next to the transforming one, so if he kept selecting first the suppressor and then the transformation, suppressor, etc., by repeating the cycle enough times, eventually he got to the point

where the only thing left was the suppressor gene attached to the transforming one, so he isolated this gene by that procedure. So by combining the selection of the transforming principle with the selection due to suppressor gene, he could find when these two things came together.

So he devised a method that would let you find two things coming together which were very rare. So I realized that I could do that for what I wanted to do. I could use a suppressor gene on my incoming DNA. It wouldn't be on the target, and I would get recombination. Then I would look—and I would isolate DNA and put it into Lambda phage, and I would only isolate the DNA that had the suppressor gene, so now I'm only getting back DNA which has somehow got the suppressor gene in it.

But then I would block these colonies with a hemoglobin probe, and only the colonies that showed hemoglobin would light up. So now, then, I would get very rarely, I was hoping, a colony which I knew had the suppressor gene, because it grew, and lit up with hemoglobin. But that part of hemoglobin wasn't on my incoming DNA. The suppressor gene was on the incoming DNA. The probe was on the target, and they would only come together if it had been homologous recombination. I could predict what size it would be, everything, and so we did this experiment over the course of a long period of time. It took three years to make it work, but that was the thing. **endnote 45**

So I was trying to learn how to modify hemoglobin, you see, so my target was the hemoglobin gene, and my first practical experiment, which Nobuyo was the person who saw we could do it, was to correct a mutation in the HPRT gene [hypoxanthine-guanine phosphoribosyl transferase], which we could correct by homologous recombination in ES [embryonic stem] cells, and that was the first experiment that worked for us in ES, it was correcting the gene, not knocking the gene out. And that worked. It worked the first time, actually. It was one of those experiments you did it once and it worked. I didn't do it, Tom Doetschman did it, but Nobuyo had made the constructs for some other purpose. **endnote 46** He did the experiment and it just worked first time, so we got homologous recombination, we got gene targeting to work in one go, once we did that system. But that was, again, gene correction.

Now Mario Capecchi, I think very smartly, realized that more useful in a general field, now, in genetics as a whole, as distinct from in gene therapy, that the ability to knock out a gene is more useful, because if you have to make any—if you've got a whole bunch of genes and you want to understand what they're doing, the simplest thing you can do is to knock them out and see what happens. That's probably the simplest generic change that you can make that will give you information, and so he developed a method to knock out the genes, so knocking out the genes was not something that I tried to do. I wasn't particularly interested in knocking out genes. We, of course, used it. But I was interested in correcting genes. My motivation was correction, and Mario started to do gene knockout, so I would say that knockout is Mario's development, not mine.

NC: How closely did you work with Capecchi?

SMITHIES: Oh, we didn't work closely at all. We just talked to each other when we were at meetings. We had no problems. We were friendly and we would talk about what we were doing. He was not a very talkative person, just by nature, nothing to do with being secretive.

NC: But he came up with that idea independently, just from casual conversations and reading your publications.

SMITHIES: Well, he did research in my publications. He knew about—you see, it was two years after my first publication that the ES cell work became—so after the first publication of homologous recombination, nobody could repeat it until two years later, and he did the knockout and we did the correction of the HPRT gene, both within, I mean they were published within ten days of each other. And we each didn't know what the other had ready to publish. We just were publishing, and it turned out that within, I mean, a matter of a couple of weeks, we were together.

NC: What I don't yet understand is how much he drew on your homologous recombination and the gene therapy idea.

SMITHIES: Well, I don't know the answer to that. I think the only person who can tell you that is probably Mario, but I know he knew about my work because it had been published for two years, so it was well and clearly understood that you could get homologous recombination. But he'd been thinking about it before my paper came out, I know. Other people had been thinking about it as well. There was quite a lot of activity going on.

But there was a general—I think it's fair to say [Raju] Kucherlapati feels that, and he's a neutral observer in many ways—he thinks that people at the time just didn't think it would ever work with mammalian genome, because it was too complicated and too big. It might work in yeast. It was known to work in yeast, but it was sort of thought, well, it probably won't work. You see, the method with the suppressor gene and the bacteriophage, blotting them with—

[Interruption. Side conversation not transcribed.]

SMITHIES: So I think we were talking about Mario, the difference between homologous and non—between gene knockout and gene correction, if I remember rightly. I'd been always interested in the idea of correcting a gene, and that was what my original paper was about. But I think many, at least certainly Mario, realized that it's more useful in many ways, in a general scientific way, to knock out a gene than it is to be correcting one, because if you have many genes and you don't know what they do, probably the single simplest thing that you can do, which is generic and can be used with any gene, is to knock it out and see what happens. So those were the commonest mutations which would be used by geneticists to solve gene functions, and so he worked on that.

But it was actually a couple of years after my first paper before either he or I or anyone else was able to get gene targeting to work again, so that was two years later. I think you were interested in knowing how much he knew about my work and how much I knew about his, and whether we talked to each other, and the answer is we always had an easy time talking to each other.

He's not a very talkative individual, so I don't think he would spontaneously come and tell you about what he's doing, but if you met him at a meeting, and we would meet at meetings, I'd say, "What are you doing, Mario?" and he would tell you, and I would tell him, and we never had any problem with being competitive in that sense. It was never antagonistic at all. He'd been thinking about doing gene targeting before my paper came

out, I'm sure, but he knew, of course, of my paper when it did come out.

So we all knew it would work, but it took a long time to make it work again. At that time it was only possible when we decided to go for an easier target, a gene that you could select directly, whether it had been corrected or knocked out, and the HPRT gene was a good target for that, because you could use selective conditions which would tell you that it had been knocked out, which is what Mario Capecchi did, or you could use selective conditions that would tell you when you'd corrected it, which is what we had done, and our two papers came out within a couple of weeks of each other, not planned in any way, it just happened, so that we both made it work again about two years later.

NC: Would you still like to try to get gene therapy to work? Do you think that's a worthwhile—

SMITHIES: Oh yes. We just sent a paper off to PNAS [Proceedings of the National Academy of Sciences] on trying to improve the methods of using homologous recombination for gene therapy, but it still remains, you might say, a holy grail, because the frequency is low. But we have improved methods of finding homologous recombination that don't require drugs and that can be done with fluorescent-colored genes, and the way you can use fluorescent-activated self-sorting fact sorting to improve it, but it's still not really anything like practical.

I think it's only—for a while yet, until somebody thinks out how to do it better, it'll only be practical for correcting things that you can grow from colonies, really, so stem cells that you can grow from a colony, then you can use it for, but if you can't isolate a colony it won't be nearly so practical. It's not impossible. I still think it can be used, but it's difficult.

NC: An example of the kind of diseases it could treat?

SMITHIES: Oh, I mean everybody knows the sorts of diseases that are relatively easy, the ones that are in bone marrow, anything that where, because if you can transfer the bone marrow stem cell, which you can do, if you can transfer the bone marrow stem cell, you can correct sickle-cell anemia or thalassemia. The thalassemias are a very common form of anemia in the Mediterranean originally, but it's very common in China and India, because they're related to malaria, just like sickle-cell anemia is related to malaria. So those are single-gene defects and they would be relatively easy to correct.

But it isn't worth trying to correct them until you can make the procedure succeed without introducing problems, and at the moment the problems of the bone marrow transplant are still so severe that you can't use that method unless it's a type of mutation that's going to be killing [the patient], or almost close to killing. So the very severe thalassemias are treated by bone marrow transplantation, and they will be targets at the current time for therapy by homologous recombination.

But there aren't any obvious other targets that are easy. I mean, if your brain problem is with some of the inherited differences such as Goucher's disease and things, then getting the correction into the brain is not easy, and so it's still a difficult problem. Every now and then I think I've probably done what I can do to it, and [can leave it] for the next generation to solve that one.

10. Leaving Wisconsin, coming to the University of North Carolina, complex disease

NC: Well, we're coming to the late eighties, which begins to bring us up to UNC.

SMITHIES: Yes.

NC: You had been at Wisconsin for more than thirty years.

SMITHIES: Twenty-five or so, between twenty-five and thirty, I don't remember exactly.

NC: Subtracting out your sojourn to Toronto, it's more. So it's a long time to spend in one place. What brought you to North Carolina?

SMITHIES: Well, it was very straightforward. My first marriage was, by mutual consent, dissolved, oh, I don't remember the exact date, but it's something like twenty-five years ago now. We decided we'd tried long enough to make it work. It didn't really work and we should have quit much earlier.

But then eventually I met Nobuyo and we got along so well that we wanted to go on living together and become married eventually, we didn't get married right away, but we wanted to be together. So she was a postdoc and she was at a stage when it was necessary for her to find a job in her own right.

NC: Postdoc in your lab or in another lab?

SMITHIES: At that point—she was originally a postdoc in another lab, and she came to my lab quite by accident, and that's another story, as it were, which I don't know whether you want or not. She was an excellent scientist, a very good molecular biologist with a flair for understanding molecular evolution.

She'd trained with Walter Fitch, who was one of the leading molecular evolutionist persons in the world at that time, and she'd come from a laboratory in Japan where they'd been doing molecular evolution, and so her initial work with me was related to molecular evolution. **endnote 47** But she got more and more interested in DNA work, and continued molecular evolution, and as I mentioned earlier, she solved the problem of haptoglobin at the DNA level.

But then looking for a job, this was part of the time when there was the problem between the two departments in Wisconsin. She applied for a job in the medical genetics department, for which I thought she was extremely suited, and I think many other people thought she was. But eventually that job was co-opted into a non-human genetics job by the other part of the department, and that was a bit of a last straw, as it were. I don't remember exactly how it happened, but nobody really—well, I do know what happened in some ways.

Then we decided Nobuyo should look for a job somewhere and I would go along. I could probably get a job in the same place, but we wanted her to get the job first so it wouldn't be on my coattails, because she didn't want that and I didn't want that. So she applied for several jobs and she was offered the interviews at least, and maybe even job offers, I'm not sure which stage it was at, in three different places.

One was in Davis, California, one was in University of Illinois in Chicago, and one was here. I went to all three places. I wrote to people here and I wrote to people in the other places, saying, “Are you interested in the possibility of my coming to you?” And I went out to Davis, and they really weren’t terribly keen on my coming. They had just finished losing a rather temperamental National Academy member. They were glad to be free from any more troubles with people who were prima donnas, and they thought maybe I’d be a prima donna, so they weren’t very keen. So they didn’t make it terribly attractive for me.

NC: Who was that?

SMITHIES: No, I don’t remember who the person was that they lost. That saves any embarrassment; I don’t know who it was. And I found the place—one of the criteria was whether the flying was decent, and the flying was possible, not terribly good, but it was possible there. There was gliding available not too far away, but they weren’t very keen on me.

When I went to Chicago they were keen on me and the science was nice, but the flying there was pretty desperate, because then you’re right underneath the inverted wedding cake of protected air space for O’Hare and Midway, so flying a glider there is pretty hopeless. You’ve got to go quite a long way before you can get free air space that you can mess around in, and so I wasn’t terribly keen on that.

And here I liked it. I already had contacts with people here. I liked the people. I liked the department, and Nobuyo was offered a job here, and it was particularly impressive that the chairman at the time, Joe Grisham, was known very much, and said this, that he very much wanted to make women have equal status in his department, and there were already several women who were on the faculty, which was not that common. He tried to abolish the differences between M.D.s and Ph.D.s in terms of pay.

He couldn’t do that. He made them sort of equal status in rank, but the pay differences were beyond him, but that was not a consideration anyway.

But it showed that this was a nice chairman, nice department, so we decided we’d come here, and Wisconsin never made any serious effort to keep me. I always felt a bit unhappy about that, that I never had anybody come and ask me what would have kept me, or anything like that. So we came here and that was the reason. It was just because we could work together. We couldn’t go on working together in Wisconsin without Nobuyo getting a proper position.

NC: Who was the chair at that time?

SMITHIES: I think it was—it wasn’t Jim Crow anymore. It was a plant geneticist—his name won’t come back to me.

NC: It wasn’t [Royal Alexander] Brink anymore?

SMITHIES: No, no, that was before. Nelson, I think Nelson was the chairman, Oliver Nelson. I don’t know that he knew, or it might not have been Oliver; I think it was Oliver Nelson, but I don’t know that it was his fault, really. But somehow I never seemed to

be—I didn't feel to have been ever approached to see what would make me stay, because it was very easy. All they had to do was make sure that there was a proper appointment for Nobuyo. It would have been extremely simple. So it could have been done. I know Crow afterwards was annoyed for years about it. He said, "You would never have left if I'd been chairman."

And that's why I came here. It was a simple reason. I've been very happy here. The department's an excellent department and there's a wide variety of knowledge, and the things I got interested in more and more were complex human diseases, which means you have to understand pathology, and people could teach me pathology here.

NC: Reading your papers, you seem to have become more disease-oriented since then.

SMITHIES: Oh yes, when I became here I became very interested in complex diseases, yes. But I always wanted to look at the common ones. I've never been very interested in looking at rare diseases, even though they might be simpler. So the sickle-cell anemia is the most common single-gene defect in the human population that we would be exposed to, and the same way the thalassemias are very common; and cystic fibrosis, which was the first gene we tried to isolate a knockout for here, when I came here, is the most common in the Caucasian population of the single-gene defects, so these are the common ones.

But when you think about them, they're very rare compared with atherosclerosis or hypertension or obesity, and Nobuyo made enormous contributions to understanding atherosclerosis, because she developed a mouse model of atherosclerosis where nobody thought it was possible to do it in the mouse. They said it won't ever happen. They don't live long enough, and they have very high good cholesterol, high HDL, and very low bad cholesterol, LDL, and so they'll never get atherosclerosis.

But she did it. It was done independently of my work, in the Rockefeller University, but her animal is the one that of all the animals that the Jackson Laboratories sell, apparently that's the one that they sold more than any of, of the mutant animals. So that worked well, and her example of working with a complex disease encouraged me to think about moving to others, especially when I had somebody that came to see me who wanted to come and work with me, who was an M.D. and had been working in things related to hypertension, and that got me changed to working in complicated diseases.

NC: So how has the environment here, besides Nobuyo, pushed you in that direction of complex diseases? I'm talking about meetings in hallways, or seminars, or happy hours, something like that.

SMITHIES: No, I don't think so. I don't think particularly. It's just been that there's plenty of support if you wanted to find out how to interpret this or that, or whatever was going on.

NC: So you don't see that it's particularly being at North Carolina that has pushed you in that direction?

SMITHIES: No, no. I think the pushing in that direction really hadn't anything to do with me being here particularly. It was a very good place to do it, but the actual moving was an accident. John Krege came to see me. He was a resident. Krege, K-r-e-g-e. He was a

young resident and he came, and I've written about that history, but it's entertaining. He came and said he wanted to work with me in the summer, and I've always been skeptical about residents. They say, "I'd like to spend half time next summer with you, and you teach me molecular genetics."

And I'd say, "Okay, and then in the next summer I'll come and spend half time with you, and you teach me surgery." And then they get the message that it's going to take a while. He said he'd work with me quite a time, and then I asked him what—he wanted to come for a long time. He didn't want to come just one summer.

Then I asked him what he'd been working on, and he said he'd been working on eclampsia, on pre-eclampsia, pre-eclampsia, and I didn't even know what it was. He explained to me that it was hypertension associated with pregnancy. It was fairly common and very serious and very damaging, and it could only be stopped by terminating the pregnancy, really. And I said, "Oh, well, I've just got interested—." I'd just read two articles on the genes controlling blood pressure. Two articles had come out in mapping, in using—I think they were both in rats, if I remember rightly. Yes, in spontaneously hypertensive rats; they'd tried to map genes which controlled the hypertension.

Two groups independently did it, and they both came out with a region which included the ACE gene, the angiotensin-converting enzyme gene. I knew a little bit about that, because I was taking medication for ACE inhibitors. I was taking the ACE inhibitor because I had hypertension if I didn't, and so I knew a little bit about that. So I said to John Krege, "Why don't we see what we can do, if we can model this disease in humans? So let's knock out that gene for the ACE gene and see what happens."

He couldn't join me until sometime later in the year, and I said, "Well, I'll do the gene construct and make it, and then when you come here, you can do the targeting and study it." So we carried out that bargain, and then in the meantime I taught myself something about hypertension and about the genes controlling hypertension, and got more and more interested in it.

Then almost exactly a year later, another paper came out which changed my way of thinking very greatly, and that was a paper from Salt Lake City and Paris by [Xavier] Jeunemaitre, was the person in Salt Lake City, and Pierre Corvol in Paris, and a whole bunch of other people. **endnote 48** What they'd shown was that if you look in a human population you can find a polymorphism in one of the genes in the renin-angiotensin system that differs in individuals who are hypertensive. In particular, the gene is the gene coding for angiotensinogen [the precursor molecule for angiotensin, an oligopeptide that raises blood pressure]. That's a protein made in—and I taught myself—that's a protein made in the liver and it's circulating in the bloodstream, which is acted on by an enzyme made in the kidney. Angiotensinogen is made in the liver. The protein made in the kidney, renin, is an enzyme, and it cuts off ten amino acids from the end of angiotensinogen and makes an inactive peptide, angiotensin-1, and that's converted by the angiotensin-converting enzyme into an eight-amino-acid peptide, which raises blood pressure very considerably. So angiotensin-converting enzyme takes an inactive peptide and makes a blood-pressure-raising peptide, and so that's the situation in that.

But what Jeunemaitre and company found was that a difference in the gene coding for angiotensinogen seemed to be related to blood pressure, and they got correlations,

but they couldn't establish causation in the human situation, so I thought, well, I'll try to replicate their work in the mouse. What they primarily described was that there was a difference in an amino acid at position 235, a methionine-to-threonine difference, and that the people who had the hypertension were more likely to have methionine at that position.

So I thought, well, I'll change that—no, it's the other way around. It was the threonine had the hypertension. I thought, I'll change methionine in the mouse to threonine and see if it increases the blood pressure. But I couldn't do that, it turned out, because the mouse doesn't have methionine there. So then I read the paper more carefully, and they were cautious about that. I don't think they really thought the methionine-threonine difference was important.

But what they did see was the difference in the amount of angiotensinogen in the individuals who had the higher blood pressure. They had a higher level of angiotensinogen, so that got me interested in genetic changes which alter the level of a protein, not the amino acid sequence. So you don't change the protein, but we change it quantitatively. When you think about it, that's what most differences are between humans. It's not that we have, one of us has a nose and the other [doesn't[, it's how long is the nose, or how tall are we, or how short we are. We have all the same limbs and it's just the proportions that change. So most of the variation of humans is not qualitative absence of something or presence of something. It's just a quantitative difference.

So the quantitative differences began to fascinate me, and I thought that the more and more likely that these are the differences which are important in the common differences between humans, but there are likely to be many of them, so a lot of small differences instead of one or two big differences. So I began to change the amount of [angiotensinogen], with the protein, and that's when I really changed, so it was probably 1993 when I began to do the quantitative work. That's when much of my motivations had—although I've changed recently.

11. Tool making, patenting, invention and discovery

NC: Well, we've been going for more than two hours. If you have the stamina for a couple more minutes I wanted to ask just a couple of quick questions—

SMITHIES: Yes.

NC: —about tool making, because it's such an important theme in your work, and we can sort of finish up with that.

SMITHIES: Yes.

NC: You've said on a number of occasions that you consider yourself a toolmaker, and we've talked about a number of your inventions and tools and so forth. And so, okay, I'm persuaded that you're a toolmaker. But you're not just a toolmaker, because you've also—

SMITHIES: I've used the tools I've made.

NC: —you're also interested in the sort of high concept. You're interested in evolution

and gene therapy and so forth.

SMITHIES: Well, more often it was, in a sense it was that the tool making was driven by the other, not the other way round.

NC: This is what I want to get at.

SMITHIES: That is to say that I would invent a tool to solve a problem that I couldn't solve with the existing tools. I would put it that way round, so I can just show you an example here which I think is still lying here, that is a tool that is one of my inventions going back to Wisconsin. [rummaging in drawer] Here it is. This is an Eppendorf pipette invented many years ago for pipetting one milliliter—it's fastened together so I can't press it down, but it goes in and out and pipettes one milliliter very accurately, repetitively. That tool I invented in Wisconsin in 1950-whatever, and you know the Eppendorf pipette's used all the time. Of course I never patented it and nobody else ever used it, and I never published it.

NC: And when did you invent this?

SMITHIES: That tool was invented in, oh, probably 1956 or something like that; no, no, earlier than that, 1953.

NC: This goes to 1953, about?

SMITHIES: Yes. This is made in—

NC: You press on—

SMITHIES: I'll show it to you. I can take it apart and we can put it back together.

NC: It's a brass rod, sort of a tube, pen-shaped object, this little screw coming out from it—

SMITHIES: Well, this is just to hold it together, because it doesn't hold together anymore. Take a moment to just get it—

NC: Perhaps tomorrow we can get a picture of this.

SMITHIES: Oh yes, we should get a picture of this tomorrow, and some other tools. I still have my power supply from the early days of starch gel. This piece of rubber tubing has got so perished that it won't hold the pipette anymore, but we can fasten it on, you see, so that—

NC: Okay, yes.

SMITHIES: There weren't any O-rings. O-rings hadn't been invented yet, so we couldn't use O-rings, and this was a piece of Teflon, only it was polished steel inside, and so it was airtight and it would go in and you could get a milliliter of fluid, for my solubility measurements that I described earlier. I needed to set up many tubes, and I needed accurate volumes, and this would deliver accurately a one-milliliter.

NC: And it still works.

SMITHIES: It probably still works. It probably won't, because it's leaking too much. But you can get the idea. Everything is leaking, because that wouldn't actually be tight, and I don't have any rubber tubing here anymore.

NC: It'd probably work if you had another piece of rubber tubing.

SMITHIES: It's still—there's nothing wrong with it. It was calibrated and then sealed with—it was very accurate. I did my measurements with that, so that's one of the early tools. That gave you the answer to your question.

NC: So the idea comes first—the problem comes first…

SMITHIES: The problem comes first and then the tool is invented to solve the problem.

NC: But then, in many cases, the tool has turned out to have unexpected properties.

SMITHIES: Well, no, I don't know that that's true. I think it's turned out to be useful to many people, which is different.

NC: Well, didn't starch-gel electrophoresis have the unexpected property of being much more—

SMITHIES: Well, yes. No, it wasn't invented as a tool to do anything. It was just a convenience. That one had more accidental aspects to it, because I hadn't any idea that when I invented that, as it were, that it would lead to molecular separations, size separations, because the concentrated gel makes molecules, then the big molecules are slowed down more than small molecules, relative to what's in water. So you get an element of size separation on top of the electrical separation, which is where it becomes powerful, and I had no idea that that was there.

NC: How could you?

SMITHIES: So that was an accidental finding, I mean an accidental property of the system. So that tool, although it was invented—it wasn't really invented to solve a problem. It was just a way to prevent having to do repetitive protein determination. So that part of it, its useful properties in that case were completely accidental, and so in one sense the word—it was not a tool invented; it was a tool discovered. Do you understand the difference? I didn't invent it. I found that I had a tool that I didn't know was good, so that's a bit different.

NC: Well, I think that's a property of many good inventions. I mean PCR also had all sorts of unexpected properties.

SMITHIES: Oh no, I don't think it did. I don't think that's true. I don't really agree with that. I think it was that Kary Mullis saw what he could do immediately. As soon as he thought of it, he knew what he could do. Now, it was applied to many problems, but it always did the same thing. It never does anything different except amplifies DNA, and what you can do when you've amplified DNA is just the same as what you can do with starch-gel electrophoresis. You can do many things with it, or gene targeting. It's always

gene targeting, but you can do many things with it. But that's not quite the same thing.

NC: That's what I mean. That's what I'm getting at. You mentioned earlier this morning that you had patented almost nothing. You certainly have had a number of patentable inventions.

SMITHIES: Yes, I think I probably have.

NC: What is your attitude about patenting?

SMITHIES: Well, my idea at the time was that, I think it was governed by my first experience in Wisconsin. If you remember, I mentioned that when I moved to Wisconsin they asked me to—to get the starch I had to buy it again, and I decided to make it a new way. So the new way I made it was not the way that it had originally been made, and I talked to a person in Wisconsin who had a lot of experience with patenting. It's taking me a moment to get his name. He discovered warfarin, you know, Coumadin, the anti-coagulant. **endnote 49**

NC: I can't remember either. [Long pause while Smithies types on computer.] We're Googling "warfarin inventor." We can find it out.

SMITHIES: It's important for me to remember. Anyway, I met him and we were talking about it, and I said, "Do you think it would be a good idea if I patented this starch? I think I could patent this new form of it."

And he said, "Well, if you take my advice, Oliver, you won't." He said, "I've had nothing but pain out of having patented warfarin, and so much time wasted in dealing with it and with all of the paperwork and everything. You'll be better off not to." And so I never did, and I've never really felt the need to. I think there is a mistake in not patenting anymore, because I think often enough people won't use it. It won't be developed commercially unless it's patented, because nobody will put money into it unless they can get money out, and they can't get money out easily if it's generally available to everybody. So they do better if something is patented, but it takes a lot of effort. There are several things that I've done that I know I could have patented, but I've been a bit lazy with it.

NC: Did the Bayh-Dole Act of 1981, I think, that authorized patenting of biological substances, did that have any impact on you?

SMITHIES: No, not really. I hardly know the name of the act, but I've always thought there were some big mistakes made in the patenting laws, and they probably will eventually be corrected. But patenting of something that already exists, rather than the usage, seems to me not to be a sensible thing. I mean, just it's like finding a gene that's already there. That doesn't seem to me to be patentable. Finding a use for it and doing something with it, that's a different matter, but just finding it—I think it's been a mistake of patent law.

NC: Okay, last question. If you had to pick the five most important inventions in human genetics, before the Genome Project, what would they be?

SMITHIES: I'm not probably a good person to ask that question. Inventions, you really

mean inventions, or do you mean discoveries?

NC: I mean inventions. If you'd rather change it to discoveries you can, but see if you can do inventions.

SMITHIES: No, I can think of inventions. Well, I'm sure that starch-gel electrophoresis would be one of them. That's pretty fair, because it was the second-most-quoted paper in scientific literature for quite a long time. The only other one that beat it was Lowry's method for determining protein concentrations. So it set up a whole thing. Or cytogenetic things, or the ability to spread chromosomes and count chromosomes, that's a method, really. That was very important, because it let people find chromosomal abnormalities and understand trisomy-X or trisomy-21 better.

Well, of course, hybridization too, but you're saying before the Genome Project. Well, the ability to do in-situ hybridization on chromosomes with colored markers I think would be one that's very high on my list. We've put PCR in already. There's no question about that. So we've got, how many have we got now?

NC: There's four.

SMITHIES: We've got four, probably. Human genetics. Well, of course, the various uses of the microscope, but those were already—they're sort of general, and they're not particularly genetic. Cloning, of course, is obvious, I mean gene cloning. Yes, I mean that comes ahead of some of those others that are down, and so there's DNA sequencing, of course. That I think you include when you talk about Genome Project; you mean that also, the DNA sequencing.

NC: I meant pre-1990.

SMITHIES: Well, then, DNA sequencing would be—protein sequencing would be, you see, too, first, but it just gets outdated. So protein sequencing and DNA sequencing were extremely important for genetics, more so than that in-situ hybridization. I'd take that one out of there. And then I mean the Southern blotting one has been so marvelously productive, but it's not in the same category as doing DNA sequencing and protein sequencing, nor is—starch gel isn't in that category. Those are really, they're both—Fred Sanger, you know.

NC: All right. This has been a long day, but really a fun one.

SMITHIES: Okay.

NC: Thank you very much.

SMITHIES: Just hold a minute. I've got to find the name of that person [Link] and put it on tape.

[End of Session 2]

Session 3: October 28, 2005

12. Religious upbringing, music

NC: It is October 28th, 2005. I'm Nathaniel Comfort and I'm with Oliver Smithies in his laboratory.

SMITHIES: And by the way, the real pronunciation of my name is Smith-eez, not Smith-ees. It's Smith-eez. It's a soft th.

NC: Thank you. And we are continuing the oral history interview that we began yesterday. I had a few follow-up questions that we didn't get in touch on yesterday. Going back to your childhood and your family, was your family religiously observant, and did you absorb much of that? What was your religious upbringing?

SMITHIES: Well, they were Church of England, which was pretty standard in England at that time, probably is still. So I went to church with them and I was a chorister. All our family had always been singers, and I was a chorister, a choir boy from eleven till—well, no, from earlier than eleven. It's earlier than that. It was from probably about six until my voice broke, I suppose, which would be fourteen or so. So I went to church with them. I didn't think very much about it one way or another, went to Sunday school, just that was part of my upbringing.

I felt myself a member of the Church of England until I was in college about maybe two or three years, and then I began to not feel that I believed what was being said, and so I stopped. I used to go to the chapel services in the college, which would begin at ten o'clock at night as I remember, and I may not be right, but ten o'clock at night. There was also a sort of curfew for students who lived in colleges. It was considered they ought to be home by that time, and it was not any curfew of any, you know, not a governmental curfew or anything. But you were supposed to be home at that time, and the college doors would lock, and if you were late, you would have to climb over the walls and get in surreptitiously. But if you got in in time, then the evening chapel service would be in—[Christopher] Longuet-Higgins, who later became a very distinguished theoretical chemist, and did a lot of work in artificial intelligence, and was elected to the Royal Society and all those sorts of things—he was the organist there.

NC: Who was this again?

SMITHIES: Longuet-Higgins, Christopher Longuet-Higgins. I'll give you a spelling later. It's a complicated spelling. He was the—it was called the organ scholar, some person who was a good musician, who would be given a scholarship because he was good at the organ, and also because he would look after the choir. So he was the organist, organ scholar, and he played the organ.

There was a good organ in the chapel. I used to love going to hear the organ, which he would play a voluntary before and a voluntary afterwards, and so I got later on I just would go for the organ. I mean, I would be there for the rest of it, but I wouldn't be listening till the organ. He had a choir which I joined, which was the college choir, recruiting some women from some other colleges, presumably.

[Interruption. Side conversation not transcribed.]

SMITHIES: I'll go back and say it. So he was the organ scholar, and then he also looked

after a choir, and he recruited women from some of the women's colleges, so we would perform various works. By then I was a tenor, though a not very good tenor. In fact, I remember going to him one time saying, "Christopher, I think I ought to drop out. I can't keep up with this. I don't seem to be able to keep my part." You see, this was a transition from being a treble in a choir to being a tenor, which is, then, an inner line, and it's a little bit more difficult to handle.

"Oh," he said, "don't worry about that, Oliver. One day you'll be a good member." And I did, in fact, turn out to be a good choral member as a tenor. But my religious things were not really part of it, although we did a lot of religious work. I remember we did the Bach Magnificat, and we were doing a performance of it and Longuet-Higgins started it off and it sort of fell apart in the first three or four measures. He just stopped it and started it again. He was really a pretty confident guy. And we did things like that.

Then later I joined the Bach choir, the Oxford Bach choir, and continued to sing in the Oxford Bach choir. There's a rather enjoyable story, which I perhaps should tell, about my entry into that choir, because, see, you had to go and have an audition to get into the Bach choir, the Oxford Bach choir.

I was a little nervous, as I mentioned, about being a tenor, and so I didn't try until later on, not right away when I went to the university.

So I went for this audition and Sir Hugh Allen was the director of the choir, and he was a notoriously sharp-tongued person. I went into this huge great room, and there in some far corner, it seemed like, was Sir Hugh Allen in front of a grand piano, and I walked over there. "Ah," he said, "your name is Smithies. Are you from Balliol [College]?"

"Yes, sir."

He said, "This isn't your first year, is it?"

And I said, "No, sir."

He said, "Do you know how I know?"

I said, "Yes, sir. My tie's been washed." See, he had to have looked at my tie in order to find out that I was from Balliol, because he hadn't been given that information, so I knew that he must have looked at my tie, and I knew it had been washed, which would tell him that I wasn't a beginning student. And so that little interchange took place extremely rapidly, in a few seconds, so then obviously I was on good terms with him.

And then he said, "Well, Smithies," he said, "sing your lowest note."

There was a secretary there keeping notes, and she reached over rather hesitantly. "Excuse me, Sir Hugh, but this gentleman is a tenor."

"Oh," he said, "in that case sing your highest note." So I started to go up. He said, "Stop! Stop! You'll blow your head off." And I got into the choir. But anyway, that's a nice little story. There are a lot of these. I have many of them. But that tells you about my religious affiliation, which is not very strong, and I must say I'm not even an agnostic. I'm just an atheist in real life.

NC: And that was since your Oxford days?

SMITHIES: Pretty well, yes.

NC: Pretty well, okay.

SMITHIES: I have my own thing, which I don't say to people who are religious, because I don't want to disturb their beliefs, but I'll say it for the record, that I think humans invented God because they couldn't understand probability. And if you think about it you can see what I mean. They couldn't understand that things are by chance, some things. I mean, I was talking about my losing a very close friend, and wife of an old postdoc of mine, only about ten days ago in an automobile accident, just hit from behind. She was at a stop sign and somebody barreled into her at a hundred miles an hour, and instantaneously killed her—then that's what I mean by chance, that if you're religious then you have to find explanation, and I don't think there are any. So that's just my feeling about it.

It doesn't mean you don't think some of the religious teachings are good. Many of the teachings are fine. But it's interesting how the teachings, the religious teachings in so many of the different religions—now, they're many quite related religions in many ways, but they all teach basically the same thing. They teach you to be good to each other, to help each other, and not to do things that are, you might say antisocial, but are labeled not antisocial, they're labeled as religious laws, as it were, or rules. And then they fight over the little frills all the time. Ridiculous.

[Tape recorder turned off.]

13. Science vs. medicine, MDs vs. PhDs, Professional societies; Views on genetics and race

NC: Okay. Now I wanted to ask you about M.D.'s and Ph.D.'s. You have moved back and forth between the medical world and the science world—

SMITHIES: Yes, and I've taught in both.

NC: —and you've taught in both. So I wondered how you perceive the differences between the medical and the scientific communities, and how the relationship between science and medicine has changed in your career.

SMITHIES: Well, I think perhaps I have a little simile, you might say, or whatever, that to me explains the difference between the two communities, and that is that an M.D. has to take fifteen—now less than fifteen—minutes to decide what's wrong with a person and to make recommendations, whereas a Ph.D. will take fifteen years to do the same thing, and that's the difference between the two philosophies.

One has to handle the fact that you have to make decisions with insufficient knowledge, based many times only on other people's experiences, which you have to know, therefore, rather than on many rational things, whereas the Ph.D. is taught to examine every little assumption that's made and try to check everything out before making a decision. And it takes a long time, and it's very laborious. So the two

educations are as a result of that quite different, and therefore the ways of thinking and solving problems are different.

It's why the person who has an M.D. and a Ph.D.—now I'm not going to say an M.D./Ph.D., because I don't think that's the same thing—a person who has an M.D. degree and a Ph.D. degree, or has a Ph.D. degree and then got an M.D. degree, is a rather special person, because they understand both worlds well, if they were well trained, and therefore they're particularly precious. The person who is a Ph.D., such as myself, and does medical things, unless he's lucky, and I'm lucky because I'm a med-school dropout—he doesn't have, often, or she doesn't have the background that makes that side of things comfortable and familiar, and have a good basis on which to make judgments. So that's the problem when a Ph.D. is working on a medical problem.

But the problem the other way is when an M.D. is working on basic problems, he tends, or she tends to think that the solutions can be obtained quickly, and just by comparison with what's been done before. So it becomes a different type of investigation, I think, in the two. Now, I can't document these thoughts, but I think they probably have some basis for being real.

I've found that teaching—I haven't done all that much teaching of students who are working for an M.D., but I've had a little, and I've found that you can find a way to accomplish the Ph.D. type of teaching with an M.D. audience, because they're so focused on getting on with seeing patients that they have no patience with the little things that have to be thought about often. So I've tried to find good, basic science papers, you might say, or topics in which it's clear to the student the medical relevance, so that it helps to get that connection made.

Then in going the other way, of course, then you try to make sure that, in teaching a Ph.D. student about, let's say you're teaching about hemoglobin and the synthesis of hemoglobin, which can be taught at a completely molecular level, molecular biology and genetics without any reference, or very little reference, to patients. Then you try to put them in touch with some major scientist who has written on thalassemias, for example, beautifully. I have a book there that was a gift from David Weatherall, who is a very famous M.D. who, I don't know whether he has a Ph.D. or not, but he's written on—

NC: I think he does. We'll be talking to him.

SMITHIES: Yes, but he's worked for many years on the hemoglobinopathies and thalassemias, and I have a book from him which he signed and sent to me, because I'd had his book and I let students read it to get the feeling. Then they can feel from that book what it is when you're dealing with these apparently theoretical things, in relation to people, little comments like you never let a resident near a vein on a thalassemic patient, because he'll mess it up, and they're the lifeline to that patient. So you have to put somebody who really knows how to insert an intravenous needle or whatever, and he'd mention things like that in the book, and lets the Ph.D. see the humanitarian problems that they wouldn't otherwise see when they can get exposure like that.

Well, his book was so popular that I gave it around, but it got, as often books when you lend them, never returned. And I didn't know who'd got it. Oh, I wrote this thing to David Weatherall saying, "Where can I get your book, David?"

And he said, “Well, it isn’t in print anymore, but I have a few copies left,” and so he sent me that one copy and signed it, and I have that still.

NC: Okay. We talked a fair amount yesterday about people that you’ve worked with, people in your department at Michigan and so forth. Who did you consider your closest colleagues at other schools during the fifties and sixties particularly? You know, was it Arno Motulsky’s group at Washington, Victor McKusick’s, or Barton Childs at Hopkins, Jim Neel at Michigan? Who were your closest colleagues?

SMITHIES: Are you talking about the time when I was doing starch-gel things?

NC: Yes, in the fifties and sixties.

SMITHIES: Yes, that would be in the fifties and sixties. I don’t remember having a feeling of that sort for anybody in particular. I mean, I knew all of these people that you mentioned and was very comfortable talking to any of them, and all of them, if I had a problem. I would call them up or whatever and talk to them. There wasn’t any one particular one, but I mean, Arno Motulsky I’ve known very well for a long time. Barton Childs I knew, and Victor McKusick, I went and gave a talk once in his department. I think it was somewhere over Thanksgiving if I remember, or it was some holiday or other, I know, because he was editing one of the volumes of his famous book on the Mendelian traits. I don’t remember the exact title of it—

NC: *Mendelian Inheritance in Man*?

SMITHIES: Yes, *Mendelian Inheritance in Man*, and he asked me to do some reading for him. I remember feeling a bit abandoned, given this task to read it, but I know Victor very well and admire him and his work enormously. So he’s a person, and then Clarke Fraser in McGill, so all that community, but just there was no particular one, but no barriers anywhere that I had. I had easy relationships with all of them.

NC: I wanted to ask a little bit about your professional affiliations. Were you involved in the American Society for Human Genetics in the fifties and sixties?

SMITHIES: Well, I was more involved with the Genetics Society of America.

NC: In the seventies, yes. That was my next question.

SMITHIES: In the seventies. Yes, okay. Not particularly. I mean, I went to their meetings and I talked at them, but I don’t remember being particularly active. I don’t think I was particularly active. I just went to meetings and if I was invited gave a talk or sometimes might have submitted one, but I usually gave talks when I was invited to give talks. I seem to have had enough invitations that I haven’t had to ask to go to meetings and talk, anytime that I remember.

NC: Then in the 1970s, you were vice president, in 1974, and then president in 1975 of the Genetics Society of America. Why the GSA? I think of them as mainly doing organismal genetics, not so much human genetics.

SMITHIES: Well, no, it’s really the total subject in genetics, I think the Genetics Society of America would feel, and I feel. At one time it almost—I don’t know whether it’s right to

say that the human geneticists were just part of that, of the Genetics Society, and then later separated. But don't really—I'm not very good at the history of those things, and my memories of them are poor and inaccurate.

I remember the time of being the president of the Genetics Society, and the vice president. It was a particularly miserable time for me, because it happened that it was in the early days of my having been diagnosed as having hypertension, and the medications for it at the time weren't very widespread. Now, I was on reserpine, which is notoriously bad for some individuals in terms of depression. In fact, my father was treated for hypertension. He had to quit taking reserpine because it made him suicidal, I think, almost, or feel that way, and I was desperately depressed at that time with that drug, and not realizing what it was. But I had all sorts of incidents that told me how severe the depression was. It's very severe, until I stopped using it, and that was in that period of time.

It's rather entertaining. We were talking about—I mentioned that one of my dear friends was killed a couple of weeks ago, so we were talking about her. Her name is Harriet Rappaport, and she was the wife of my postdoc, Byron Ballou, and we've been close friends for—we were working it out—some thirty-five years. I first really met her at a Genetics Society of America meeting in California, the meeting at which I was elected to be vice president, because they always elected you as vice president, which was, in a sense, president-elect at that time. I don't know whether they still do.

I met her as a result of going on a wine-tasting party with Byron, and she invited me to go in the car with them. I didn't have any other particular friends at the meeting, and they knew me. I spoke to her [and] at some later stage in the thing, I spilled a glass of sherry all down her front, and that was our introduction, but we became close friends for many years.

But they pointed out to me that that was also the time when we realized—I don't know that the Genetics Society of America knows this, but I wasn't a member when they elected me vice president, and I quick joined, because I hadn't ever bothered to become a member. I'd gone to their meetings but I hadn't been a member, so I'm afraid I'm not a very active participant in societies. It's not an act of dislike. It's just a laziness, I think, on my part.

The Genetics Society of America had a pretty tough time then. It was facing the problems of intelligence and the inheritance of intelligence, and the problems that were—is Stockley his name? I've not got his name right, one of the inventors of the transistor.

NC: [William] Shockley.

SMITHIES: Shockley, yes. Shockley had just put out this statement about—some particularly obnoxious statement; he often made them at that time—in relation to racial stereotyping. And the Society, at the meeting—I think by then I was the president—voted to say more or less that intelligence was not inherited, I think, or it wasn't quite as blatantly wrong as that, but it was trying to rectify this problem, in a way.

Then I sent the recommendation out to the rest of the Society. There was some stipulation that if some big thing was ruled at the meeting, it would be sent to the

members, and the members then responded, and made very wise—all sorts of wise statements were made about—but really, it's very difficult to make a statement like that, that measurement of intelligence depends on what sort of methods you use to measure intelligence, and if they're wrong—

SMITHIES: —focused on a particular type of knowledge, on particular experience, then it will bias the measurement, or bias the score by the measurement. It becomes quite ridiculous in the end, because if you think about at that time anyway, the exposure—we're talking about the 1970s or so. What year is it, is it that sort of time?

NC: '74, '75, yeah.

SMITHIES: Somewhere around then, but I don't remember the exact date. But then the environment of, for example, a black kid growing up wouldn't have the same environment to begin with, and wouldn't be exposed to books in the same way, wouldn't be exposed to mathematical thought. They're exposed to music.

So where do you find—black kids come along and they become marvelous inventors of music, and jazz, and all the glorious things related to that side of intelligence, because everybody would agree that music is intelligence, if you want to, so there you see it, and they're not so good at the other side, because they didn't get exposed to it as children.

So it made this point, the community of geneticists out there, sitting in a more calm atmosphere than an annual meeting, with a Shockley document in front of them, were able to make these criticisms, and the result was we couldn't come up with any good statement, and it was a very lukewarm statement which eventually passed, which I took no pride in, but there wasn't anything much we could do, I felt at the time. And I wasn't helped, as I say, by being in this reserpine trough, as it were, so it was a rather painful experience, but I learned things from it, of course.

14. Eureka moments; science applied to medical problems; model systems and human variability

NC: Okay. I wanted to also move forward in your science, and talk about your work on kidney function. That was a delightful paper that you sent us on why the kidney glomerulus does not clog. **endnote 50** It seems almost like you're thinking of, it's not electrophoresis, but it's the movement of particles through gelatinous kinds of things.

SMITHIES: Well, really, the history on it is different from what you might see, because it's impossible to write that sort of a history in a scientific paper, but really, when I talk about it in a lecture, I'm able to give the history, and to me it's a very enjoyable history. And that is, that I went to a meeting in Stockholm—I think it was arranged by Karl Tryggvason, but I don't remember whether he was the organizer of it.

But anyway, he was one of the speakers, and I was talking about my work in relation to hypertension and the various experiments we'd done with mice. But I spoke towards the end of the morning, and he spoke at the beginning of the morning, and he talked about the kidney glomerulus, and how in the kidney glomerulus the separation of large molecules from small molecules occurs during the passage of fluid from the blood to the urine side of the glomerulus. It's the ultrafiltrate at that time, because there's a huge volume of fluid goes in, goes out through the glomerulus, the order of 150 liters a day,

but only a liter of it comes out as urine, so the ultrafiltrate is a huge volume, and most of water and most of the other things in it are reabsorbed.

But anyway, he was talking about this, and he was talking about the effect of genetic variance of some proteins that affected the cells which are downstream of the glomerular basement membrane. Just to try to say it in a few words so that it will be helpful for people, the capillaries in the kidney glomerulus where this process occurs are relatively high pressure, and so liquid passes through the endothelial cells that are the immediate lining of the capillaries, and then encounter the basement membrane of the kidney, of that part, which is called the glomerular basement membrane, which is a continuous sheet of material. Then underneath—

[Brief interruption]

SMITHIES: —and a sheet of material, which is a sheet of material, the basement membrane is a sheet of material, then the basement membrane is supported by cells which are called podocytes, which form an interlocking network rather like the fingers of two hands interlocking, and the space between these fingers is called the slit diaphragm. Karl had been looking at mutations which affected the proteins in this slit diaphragm and had found that when that protein was missing, one of the proteins there was missing, that the individuals or the mice had a very high excretion of albumin. He explained this in terms of the current hypothesis of glomerular function, which has been in existence probably for twenty or thirty years, and is in all of the textbooks, and that is that the glomerular basement membrane is a coarse filter, and that this slit diaphragm, which is then downstream of it, is a fine molecular sieve.

I listened to this and I thought, he can't be right. This can't be right. It would clog if that happens, because there's nothing washing out the material, the molecules which are being held back by this slit diaphragm, because the basement membrane is between them and the bloodstream, so there's no flow there, no tangential flow, if you like. So I listened to him and then I went at the intermission, or probably at lunch I think, I don't remember which, and I said to him, "Karl, why doesn't it clog?"

And he said, "That's a good question."

And then I said, "Oh, I know why it doesn't clog. That's not how it works." Because it came to me that I knew that it wasn't right, and I knew how it did work, and then I proceeded over the course of the rest of the meeting to try to persuade other people that I was right. I knew how it worked, and I'm still quite sure that it's correct. I knew how it worked because I'd written the memoirs for my professor, Sandy Ogston, my tutor and later my thesis advisor.

He'd written to me at one stage when he was about seventy or seventy-five or something like that, saying, "When I die, Oliver, I'd like you to write my memoirs for the Royal Society." The Royal Society requires a memoir for each of its members, and usually that's only written by a member of the Royal Society, a fellow of the Royal Society. I wasn't a fellow of the Royal Society, but nonetheless he asked me if I would.

And I said, "Of course I will, Sandy, but there's no assurance that you will die before me." And then so when he did die, and he did die before me, because I'm still here, I took on this task. By then I was a fellow of the Royal Society, or a foreign fellow of the

Royal Society, foreign member I think it is, to be correct—but anyway, in writing his memoirs, I got all his papers, all his publications, and I printed all his papers out and I read them all, essentially. This is my usual method of learning something; you read everything.

So I read all his papers. I mean, I don't remember how many there were; eight hundred, something like that. But I was guided mainly by some abstractions of it that he'd written himself where he was rather proud of one paper that he'd written on the behavior of large molecules in gels, and he'd derived an elegant formula, extremely simple formula to explain the partition, the distribution if you like, of large molecules in a gel, compared with their distribution in free solution.

He worked out a formula which was related to the radius of the large molecule, big R, and the radius of the fiber, little r, and the number of fibers per square centimeter in a cross section, and the formula was very simple. It said that the fraction of space available to a large molecule is equal to—and he used exponentials, and that takes a little bit of understanding, but—the fraction of space is e to the minus Pi (p), big R plus little r squared times n [e– p(R+r)2n].

As I got familiar with this formula later on, I realized that it's quite easy to see that formula in physical terms and to explain it in physical terms. It's just that a big molecule will meet a fiber, and therefore it can't get closer—the center of the big molecule can't get closer to the center of the little fiber than the sum of the two radiuses. It's a physical impossibility, so that the space into which it cannot get is a circle with radius big R plus little r, and that's p(R+r) squared. And obviously, if there were one fiber per centimeter it'll have a small effect, but if there are n fibers per square centimeter, then there will be more space excluded, so that's where the n comes from. So the formula is really very rational and very simple and elegant.

Elegant things are simple. This is very elegant, and I loved it, and it had been tested very well. It had been tested by Ogston himself and his student, and then by many other people over—I think the formula was written in 1953 or something like that, don't remember the exact date—been tested over fifty years, and it's beautifully accurate. It will predict the space gloriously.

And I realized that that's what's happening in the basement membrane, that the space in the basement membrane is controlled, for different molecules of different sizes, by this formula. Therefore I went and came back here to North Carolina, and went to see my nephrologist friends in this department; it's a pathology department, and people who are used to doing the topology of the kidney, and the whole university has had a tradition for many years of being very strongly involved in the physiology of the kidney. Some of the most important work in the earlier stages of working things out in the kidney went on here in North Carolina, so I had people I could go to. So they referred me—Bill Arendhorst referred me to a collection of papers, oh, of something in the 1970s or thereabouts, which had a lot of data in them. I was able to find a paper there which compiled data on the concentration of molecules downstream of the glomerulus, compared with the concentration in the plasma, in relation to their sizes.

So I took these data and I applied Ogston's equation [e– p(R+r)2n] to them, and I found that Ogston's equation fit them beautifully, a glorious straight line. So it showed that there wasn't anything—for example, albumin fit on this line perfectly, and so people

had been making up all sorts of, in my mind, crazy ideas to explain why albumin was very low. It isn't. It fits exactly on the line of all the other molecules, and so there was nothing special about albumin. You didn't have to invent a slit diaphragm, which you will find in the literature, which is supposed to have pores in it which are just a little bit bigger than albumin, or a little bit smaller, I don't remember which way, so that it accounts for why albumin doesn't get through, or only a very little bit. And these were imaginary pores. They don't exist, and they've been in the literature for thirty years or whatever it is.

So anyway, then I came back with that, and then I sent a paper, a very short paper to Nature Medicine I think it was, probably, one of the Nature papers, a 500-word statement of this idea, and they didn't even review it. They sent it back. "We aren't interested in hypotheses. We're interested only in facts," or words to that effect, a very silly response. But anyway, it didn't worry me at all, because I just laughed. I've had many letters of that type in my experience.

So I sat around and spent the next six months really going deep into the topic, and understanding better, and then finding other things that came out of that, and it still continues to grow. And then I wrote this paper that you were referring to, after six months. But it's got a lot better since then, because a number of other things I've realized from—I'll backtrack a little. I had to make some computer simulations of the system in order to convince myself it was right, and I've done that over, perhaps now over more than ten years.

When I'm investigating a new subject or a new topic, or trying to make sense of one that I've already been working on I find that rather simple computer simulations are extremely valuable. They're valuable not because they explain the results or anything, but they're valuable because they make you think very carefully about the variables, because you can't write a simulation without deciding what are the important variables. If you can make it work, then that probably means that you've identified the important variables correctly.

Then I found in developing that hypothesis, the computer simulation of that paper in PNAS, that I had to make some assumptions regarding the behavior of albumin after it got through the basement membrane that were different from what people had been saying. Then I've since found that the evidence for what I had to postulate in the computer simulations has gotten better and better, so I'm very convinced that it's correct. We're doing experiments to try to check it, and that's one fairly substantial part of the work I'm doing now.

But it's been a very enjoyable paper. That was one of the—you were asking me a little bit have there been any eureka moments in one's life, of a sense. That was definitely a eureka moment, because just talking to Karl in that intermission and suddenly realizing that I knew it was wrong, but I knew the explanation. You know, it's a five-second connection between the two, of asking the question and suddenly realizing the answer.

Ogston had the same experience himself. He got elected to the Royal Society as a result of it. There'd been a difficulty—with [Hans] Krebs of the Krebs cycle—with the acceptance of that, because it required at one stage going from a molecule which had no asymmetric carbons in it. In other words, going from optically, a not-optically-active

compound, to an optically active one, and yet the product was always levorotatory, let's say, rather than dextrorotatory [rotating the plane of polarization of light to the left rather that to the right], and nobody believed that you could go from a molecule with no asymmetric carbons in it to a molecule with asymmetric carbons, without getting an equal mixture of the two, because all of the synthetic chemistry methods at that time, when you carried out a reaction like that in a test tube, you would always get the mixture of the two isomers, enantiomers [mirror-image molecules], I suppose, is their name.

And Ogston realized that that wasn't true if—if the symmetrical molecule bound to the enzyme in three places with three groups, for example, a carboxyl group or whatever it was. I don't remember what they were, but as long as it bound with three places. And the reaction, say, for example, there were two carboxyl groups and one had to be changed to a different form, then to get optically active only one of them—let's say, the left-hand one of the two would have to have the reaction. But if the active side of the enzyme was underneath the left one, it would always produce that isomer, so it was called the three-point attachment then.

And he realized it in—it only took a few seconds, and he wrote the paper in—he said it took him fifteen minutes to do it, to think of it, and he wrote the paper in half a day, or something like that, and it was maybe half a page long, and sent it to Nature and it was published, and it was correct. He was always rather embarrassed by it. You see, it was too easy, as it were, and he shouldn't have been made a fuss of because he'd had that thought, but that's quite untrue, quite unfair, because it's those insights which people have that are often a big step forward.

Anyway, Krebs wrote to him with great delight when he saw the paper, and I had a little bit similar experience with mine, because I got an e-mail from somebody who was working on pre-eclampsia. Ananth [Karumanchi] is his first name, and I've always had a bit of a problem with his second name. But Ananth sent this e-mail saying, "Oh, I read your paper and I'm so delighted with the effect," or in other words, and later on, he said he almost fell out of his chair when he read it, because he could understand his results now. Now he understood his results, and these are in relation to pre-eclampsia.

I think we talked about that earlier. That was what John Krege had been studying, and so now I'm working on pre-eclampsia. I didn't even understand what it was when I first heard about it, but as a result of Ananth's response to that, realizing that it can be explained very easily—explained is the wrong word—that it's an obvious consequence of the way I was thinking.

NC: It strikes me as an example of what you were saying before about the scientific approach to a medical problem.

SMITHIES: Yes, that's right. I think it bridges those two things rather nicely, and you see, that's what Ogston had had as his guiding principle all of his life, that he used his physical chemical acumen, you might say, to try to think about biological problems. He always wanted to have, if you like, a physical-chemical explanation of them, and this is a physical-chemical explanation of how the glomerulus does what it does, and then the interaction with reality, you might say, through patients. I don't know that it—by no means would I say it explains everything, not at all, but it's a paper I'm actually quite proud of, of all my papers. It would be one I would put quite high amongst my hierarchy of what I like that I've done.

NC: Okay. Model systems. You've worked with many different model systems through your career. Do you choose the organism to fit the problem, or the other way around? Is the organism a sort of a tool for you?

SMITHIES: I think we're really talking about two sorts of models. I just meant computer models, computer simulation, but I think your question is really more about the animal model.

NC: Yes.

SMITHIES: The answer is, I have at various times thought of making them as models of disease. For example, the cystic fibrosis one, which is the first one that Bev Koller did in my lab. In the very early days of the gene targeting, we decided we'd make a model of cystic fibrosis, because just then Francis Collins and other people had identified the gene responsible, the defect in which causes cystic fibrosis. So we set out with that definitely to make a model. I also tried to make some models of thalassemias in animals, and so those were models to replicate diseases, and most of those were knockout.

NC: I just mean more generally using different organisms in your experiments.

SMITHIES: Let me perhaps finish this thought first before we go on to that, because then later I began to be more, if you might say more interested in the problem rather than in the model, in making a model of disease, I was more interested in what was lying behind the disease, rather than making a model of the disease. So that causes you to do different sorts of experiments. You do different experiments if you want to model a disease from the experiments you do if you want to establish the cause. They aren't necessarily the same thing, if you want to find the detailed mechanisms.

I got more and more interested in—I've been interested in human variability ever since the haptoglobin days, because that was a normal gene. It had no effect that anybody has been able to detect in relation to selection. There is a possible selection, but it's a very obscure one and only like in situations where there's insufficient iron in the diet, and it might have a protective effect to have that polymorphism.

But anyway, I'd been interested in human variability, so human variability, and particularly the variability that occurs in people who are not in any way sick in the ordinary sense of the word, but that how various combinations of perfectly normal variations may be rather harmful, whereas other combinations of these very normal variations may be very beneficial, if you happen to have one deck of cards, you might say, versus another deck of cards, one hand of cards versus another. So I got more interested in making experiments with animal models that would help give that type of information, rather than replicate a particular disease, although they tend to go together, of course, because if you're getting at the cause of the disease, then you're also often generating a model of the disease at the same time, or a subset of the possible model.

But I've enjoyed working with mice particularly as the experimental subject, because it has become increasingly obvious, and I think most people would agree on this now, that many of the genes that are shared between humans and mice, and other mammals in general, there's an enormous overlap of similarity between them, and much of the differences are really quite small between the different mammals. I mean, I've often

made comments, for example, that the difference between male and female is greater than the difference between a male mouse and a human mouse (sic), and it's gone on for a long time as the difference between—

NC: Human male.

SMITHIES: No, the difference between a human male mouse, I mean a human male and a male mouse is less than the difference between a human male and a human female, and that's because the history of the difference between sexes is much older than the difference between the different mammals. The male and femaleness and how it's worked out in mammals goes back to the very beginning of mammals, and that's been evolving all of this period of time, so there's an enormous amount of difference between them which we don't know, most of which we don't know.

We know the obvious differences. We know the obvious anatomical differences. We know the development of the genitalia. We don't know all that much about the difference in the brains, and there probably are differences in male and female brains, and this is nice. It's a source of great joy rather than a source of anything to moan, "Dear, oh dear, we can't talk about that." So there are differences between us, and I don't know how we got onto this stuff, but my wife gets very cross with me when I say that I think that there's more difference between—females and males are different species.

NC: Well, you only say that because you're a man. [laughs]

SMITHIES: Well, I don't know. I think a woman could say it in the same way, just the same way.

NC: Tell me a little bit more about genetic variability and human variability. You came into human genetics at a time when a certain sector of the field was beginning to rediscover older ideas about human variability and genetic individuality and so forth. Can you reconstruct for me how you became interested in that idea, and whether there were any papers that you read or anything?

SMITHIES: Well, I think maybe the part of the variability that I got interested in with the accidental discovery of the haptoglobin differences was in a sense a molecular difference, so that I was more interested initially in molecular differences, rather than in anything more obvious or general that is clearly there.

There was already a huge background of knowledge of that type, although the details weren't worked out, in the blood groups, because the blood groups were already very well—many, many blood groups were well known at the time I got involved, which was in the mid-1950s, so there were beautiful textbooks, and experiments related to human variability at a molecular level in the blood-group antigens. But when I say molecular, I'm saying something that wasn't quite correct, because, in fact, the molecular basis of the differences were not known. The fact that there were differences in blood groups was known.

But it was quite some time later before what the actual molecular differences associated with the blood groups were, so that the discovery by Vernon Ingram, or the elucidation by Vernon Ingram of the molecular difference between the two hemoglobins, hemoglobin-A and hemoglobin-S, was the first, you might say, chemical description of

the human variability. But that was also a variation which it took quite a long time before people had realized, and Tony [Anthony C.] Allison was responsible for that, that really it was a beneficial mutation, the sickle-cell mutation, but beneficial under certain environmental circumstances, being malaria particularly. **endnote 51** So it's beneficial under those circumstances, but you only see the harmful effects of the homozygosity for it if you're in a non-malarial area.

So the question always was whether these molecular variations that one was seeing and people were beginning to discover more and more, how many of these are determined by some hidden advantage of one or other of the genotypes that we don't know. For example, it's been postulated, and I think with some likelihood of being right, and some experiments help to confirm it, that the cystic fibrosis mutation is very protective against cholera when heterozygous, against the loss of fluid that occurs, and the huge dehydration that occurs, which kills. **endnote 52**

But that's, again, one of these obviously homozygously damaging mutations, whereas the ones that—the blood groups and the haptoglobins and the things that have been discovered since in that way of human variability have such minor immediate effects that they have to be regarded as being, in quotes, "normal" variations, rather than abnormal. But perhaps that definition is really artificial, and it's just the frequency that you're talking about. If it's a high-frequency thing, it's unlikely to be very deleterious. That doesn't mean there aren't some circumstances where it might be helpful, and some circumstances where it might be detrimental.

But there weren't any particular—you were asking me about any papers or anything. There wasn't any particular paper or anything. It was just a natural outcome of finding the haptoglobin one.

NC: Okay. Arno Motulsky talks about rediscovering Archibald Garrod's papers, and, of course, Barton Childs is a big Garrodian. Were you influenced by Garrod? Did you read his papers?

SMITHIES: Well, that's the phenylketonuria one, is Garrod? Does that—

NC: No, alkaptonuria, the inborn errors of metabolism.

SMITHIES: Well, I think it did include—didn't it include—

NC: Not PKU. It was alkaptonuria—

SMITHIES: Who did that?

NC: That was [Asbjorn] Folling.

SMITHIES: No, somebody else's. I had the lecture. I don't remember. There was a Royal Society lecture given almost entirely on phenylketonuria by one of the early—I thought it was—it wasn't Garrod? **endnote 53**

NC: No.

SMITHIES: I don't know who, but it isn't the person you mentioned.

NC: Asbjorn Folling is the person who discovered the mutation in the thirties.

SMITHIES: Which is the one that's the phenylalanine-tyrosine one? The tyrosine-hydroxylase.

NC: So PKU and alkaptonuria are in the same metabolic pathway—

SMITHIES: As the tyrosine—

NC: And each other; it's the tyrosine pathway.

SMITHIES: Who did the tyrosine-hydroxylase then?

NC: I'm not sure.

SMITHIES: No. Anyway, you asked whether I was aware of that paper. Well, there was a Croonian lecture, a Royal Society Croonian lecture— **endnote 54**

NC: The inborn errors—

SMITHIES: Inborn errors of metabolism; who was that by?

NC: That was Garrod.

SMITHIES: That's the one I'm talking about, and he does talk about phenylketonuria in that one, I think, but maybe I've got it wrong. It doesn't matter anyway. The lecture, that Croonian lecture, I gave that lecture to medical students. You know how we were talking about how to use original papers. I gave that lecture to medical students verbatim, except I used modern idiosyncratic phraseology. I mean, I didn't use an archaic word when there was a modern word. But I didn't use any other data, and I gave the whole lecture and explained this just exactly from that lecture, and they enjoyed the lecture.

Then I asked them at the end if anybody thought there was anything strange about it, and I think one student—after I gave them the answer—I think nobody actually said there was anything strange about it. They thought it was a good lecture, you know, and they enjoyed it. But then when I told them, well, I'd really given this one just to let them see, then somebody said, "Well, I did think it was strange that they didn't use radioactive isotopes to prove it," because they got to that answer without the use of—he got to that answer without any radioactive isotopes. So the answer is, yes, of course those papers were influential, but they were influential to me more in the teaching than in the doing.

NC: Very good, thank you.

SMITHIES: That is a Croonian lecture by Garrod. That's the one we're talking about, isn't it?

NC: Yes, yes. It was a set of lectures, about three or four of them.

SMITHIES: Yes, they were for the Royal Society, that's right. It's one of those that I read.

NC: They're masterpieces.

SMITHIES: Yes, they're beautiful, absolutely.

NC: Okay. That is almost all I had.

SMITHIES: I have a couple more eurekas for you that you wanted.

NC: Sure.

SMITHIES: You mentioned that. One was about the two-dimensional electrophoresis, and that was very much a eureka event, because I'd been working with David Poulik, whom I mentioned earlier.

NC: That's P-o-u-l-i-k.

SMITHIES: Yes, that's right. We always called him Dave, but his first name was Miroslav and he was from Czechoslovakia. He'd been beautifully recognized relatively recently with an honorary degree from Czechoslovakia, which is, of course, where Mendel came from.

NC: But he wasn't in Brno?

SMITHIES: Yes. That's exactly where Miroslav Poulik was given an honorary degree, by the University of Brno. So that's that connection, because they recognized his contribution. But anyway, I was working with him to understand the relationship between the known, the already described plasma proteins, which were at that time albumin, alpha-1, alpha-2, beta, and gamma globulins. There were five so-called, five proteins in plasma. And what was I seeing that was different?

So we would run an electrophoresis by the standard paper electrophoresis, which would give me the five bands, and then I would cut out a band and put it into the starch gel and compare it with the plasma run in the other half of the gel. So half of the gel would have the band from the filter paper, and the other half would have plasma, and I would run the two in parallel and see what bands were there. You can see these. You would take maybe ten or fifteen different cuts from the filter paper and do ten or fifteen gels to compare it.

It was quite laborious and not very efficient, and I couldn't identify the—a couple of bands that I had in my starch-gel electrophoresis would be run earlier than albumin, pre-albumins I called them. I couldn't find out where they were in the ordinary pattern. I thought they must be related to albumin. So I then thought, well, I'll cut the albumin out; instead of cutting it out at right angles to the direction, I will cut the strip of paper in the same direction as the filter paper. I can show you the gel later. It's a page in my notebook.

I turned it around and I saw the pattern, and then I suddenly realized, my god, well how stupid it is to cut all these bands. All you've got to do is turn the sample 90 degrees and run it in two dimensions, and you get all of the answer in one experiment instead of every little strip, because you've got the two systems working at right angles to each other, and they have different properties, because one of them is entirely separating by,

in this case by—entirely is not quite the right word, but separating mainly by charge in the aqueous solution in the filter paper, because aqueous—the actual electrophoresis is occurring in the fluid around the filter paper. It doesn't go through the fibers of the filter paper.

And so that's an unimpeded electrophoresis, you might say, whereas the electrophoresis in the starch is very much impeded by a size effect of the same sort that Ogston described in gel penetration. It also occurs in gel migration. In parentheses, his equations to do that weren't quite as good. It's more complicated.

But anyway, the separation in the starch then is a different type of separation. It still uses charge, but it now imposes on top of that the huge effect of molecular size, so there were two different categories of separation, and I realized that that was a very powerful tool, and I published a paper on it; it's a very short paper in Nature on two-dimensional electrophoresis. **endnote 55** That was the first electrophoresis of two dimensions, so that was another of my invented tools.

But that was a eureka moment, because I remember exactly where I was standing when I suddenly realized, oh my gosh, that all I've got to do is turn the thing around for all of it, and I can do the whole thing in one experiment, just standing near the door near a centrifuge and the idea came.

I had another eureka idea, since we're talking about them, with the haptoglobin. I mentioned—I think I told you about that one, where I went back to Toronto—I was now in Wisconsin—went back to Toronto to talk to George Connell and Gordon Dixon about our results. We were trying to solve this, and then suddenly realizing the explanation. That was, again, a eureka moment. So there are a few of them. So I have at least three that I've had of that sort, the kidney one we were talking about, the two-dimensional electrophoresis, and the haptoglobin solutions.

The gene targeting was not a eureka one at all. That was a long progression of trying to think how to do it, but it perhaps was eureka in another sense. I suddenly realized how I could do it, how I could make it work, but it didn't have that same momentary impact that the others did, you know, where you can say one half minute before you didn't have the idea, and then a half minute later, the idea was complete.

NC: Okay. Last question. Looking back over all of your inventions, which one is your favorite, and why?

SMITHIES: Oh, I think it has to be starch-gel electrophoresis, because that's an invention. You see, I'm not calling gene targeting an invention, a tool. Well, let's back up. Did you say inventions or tools? Ask me the question again.

NC: I said invention.

SMITHIES: Yes, right. Well, so I don't know then whether one would categorize them in that way. I think the more important advance, if you like, was the gene targeting, but that was not in a way an invention. That was a thought of how to do something that was worthwhile, and doing it. And it took three years to make it work, from the conception to the idea, so it wasn't a trivial thing. The starch gel was an accidental thing, so in some ways it was a finding and not an invention.

So I think when I look back at it, maybe I didn't have any inventions, but I've made tools and things. I've used things that I've thought of, or I've thought of new things and invented tools, if you like. But it's difficult to say, then, which is my favorite. Those two, the starch gel, if we call those inventions, the starch gel and the gene targeting are my favorite inventions.

NC: Okay. Thank you very much, Dr. Smithies. It's really been fascinating and fun.

SMITHIES: Well, I hope it will be helpful. And we have a few things that I can show you. Some of the original starch gels are still in existence, and some early apparatus and so on. We can look at it sometime.

NC: I hope to get those on the videotape. Thank you.

END OF INTERVIEW

ENDNOTES

1. Reserve Officers' Training Corps
2. A type of light machine gun. See http://en.wikipedia.org/wiki/Bren
3. Baruch S. Blumberg shared the 1976 Nobel Prize in Physiology or Medicine with Carleton Gajdusek, for their discoveries concerning "new mechanisms for the origin and dissemination of infectious diseases." See http://nobelprize.org/nobel_prizes/medicine/laureates/1976/blumberg-autobio.html.
4. John Edsall was at Harvard, not Yale. See "Eloges," Joseph S. Fruton, Isis, 94 (2003), 106–108. DOI: 10.1086/376101. Available at: http://www.journals.uchicago.edu/ISIS/journal/issues/v94n1/940105/940105.web.pdf.
5. On the origins of the Tiselius electrophoresis apparatus, see Kay, L. E. "Laboratory Technology and Biological Knowledge: The Tiselius Electrophoresis Apparatus, 1930-1945." *Hist Philos Life Sci* 10, no. 1 (1988): 51-72. On the Svedberg ultracentrifuge, see Creager, Angela N. H. *The Life of a Virus: Tobacco Mosaic Virus as an Experimental Model, 1930-1965*. Chicago: University of Chicago Press, 2002, chap. 4.
6. Referring to Kay, "Laboratory technology and biological knowledge."
7. Henry G. Kunkel, 1916-1983. See his National Academy of Sciences Biographical Memoir, http://books.nap.edu/html/biomems/hkunkel.pd.
8. Kunkel, H. G., and R. J. Slater. "Zone electrophoresis in a starch supporting medium." *Proc Soc Exp Biol Med* 80, no. 1 (1952): 42-4.
9. See Bliss, Michael. *Discovery of Insulin.* Chicago: University of Chicago Press, 1982, on the role of Connaught laboratories in the insulin story.
10. This is quite an accurate and succinct description of the initial Banting and Best approach.
11. NOT in fact the 1955 *Nature* paper, but rather: Dixon, G. H., and O. Smithies. "Zone electrophoresis of cabbage enzymes in starch gels." *Biochim Biophys Acta* 23, no. 1 (1957): 198-9.
12. Referring to Smithies, O., and N. F. Walker. "Genetic control of some serum proteins in normal humans." *Nature* 176, no. 4496 (1955): 1265-6, but incorrectly.

13. I.e., homozygous dominant, heterozygous, and homozygous recessive.
14. I am not aware of any direct link between Ford Walker and Fraser; Ford Walker was trained and worked in Toronto.
15. This is known as dermatoglyphics. For more on Norma Ford Walker's dermatoglyphic research, see Miller, Fiona Alice. "The importance of being marginal: Norma Ford Walker and a Canadian school of medical genetics." Am J Med Genet 115, no. 2 (2002): 102-10; Leeming, William. "The early history of medical genetics in Canada." Social History of Medicine 17, no. 3 (2004): 481-500.
16. Smithies, O., and N. F. Walker. "Genetic control of some serum proteins in normal humans." Nature 176, no. 4496 (1955): 1265-6.
17. Williams, C. A., Jr., and P. Grabar. "Immunoelectrophoretic studies on serum proteins. I. The antigens of human serum." J Immunol 74, no. 2 (1955): 158-68; "II. Immune sera: antibody distribution." J Immunol 74, no. 6 (1955): 397-403; "III. Human gamma globulin." J Immunol 74, no. 6 (1955): 404-10.
18. Lowry, O. H., et al. "Protein measurement with the Folin phenol reagent." J Biol Chem 193, no. 1 (1951): 265-75.
19. See Giblett, E. R. "Back to the beginnings: an autobiography." Transfus Med Rev 20, no. 4 (2006): 318-21.
20. Pauling, Linus, et al. "Sickle cell anemia: a molecular disease." Science 110 (1949): 543-48. http://links.jstor.org/sici?sici=0036-8075%2819491125%293%3A110%3A2865%3C543%3ASCAAMD%3E2.0.CO%3B2-C.
21. Ingram, Vernon M. "Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin." Nature 180 (1957): 326-28. http://www.nature.com/nature/journal/v180/n4581/pdf/180326a0.pdf
22. Probably everyone in the medical genetics department had a joint appointment in genetics, but Smithies' principal appointment was in medical genetics, at the medical school.
23. "Dormitive principle" refers to a definition masquerading as an explanation. From Gregory Bateson: "A common form of empty explanation is the appeal to what I have called "dormitive principles", borrowing the word dormitive from Molière. There is a coda in dog Latin to Molière's *Le Malade Imaginaire*, and in this coda, we see on the stage a medieval oral doctoral examination. The examiners ask the candidate why opium puts people to sleep. The candidate triumphantly answers, "Because, learned doctors, it contains a dormitive principle." Bateson, Gregory. *Mind and nature : a necessary unity*. (1979); paperback edition, Toronto ; New York: Bantam Books, 1988, p. 95.
24. A pun on the initials of the bases in DNA: Cytosine, Guanine, Adenine, and Thymine.
25. Almost certainly referring to: Neel, James V. "The inheritance of sickle cell anemia." *Science* 110 (1949): 64. "http://links.jstor.org/sici?sici=0036-8075%2819490715%293%3A110%3A2846%3C64%3ATIOSCA%3E2.0.CO%3B2-%23.
26. Avery, Oswald T., Colin M. MacLeod, and Maclyn McCarty. "Studies on the chemical nature of the substance inducing transformation of Pneumococcal types." *Journal of Experimental Medicine* 79 (1944): 137-58.
27. See Nance interview for more on the Smithies-Nance relationship.
28. See the Anfinson exhibit and papers at http://profiles.nlm.nih.gov/KK/.
29. "The Covalent Structure of an Entire ?G Immunoglobulin Molecule," Gerald M. Edelman; Bruce A. Cunningham; W. Einar Gall; Paul D. Gottlieb; Urs

Rutishauser; Myron J. Waxdal, Proceedings of the National Academy of Sciences of the United States of America, Vol. 63, No. 1. (May 15, 1969), pp. 78-85. http://links.jstor.org/sici?sici=0027-8424%2819690515%2963%3A1%3C78%3ATCSOAE%3E2.0.CO%3B2-Y. See also: “The 1972 Nobel Prize for Physiology or Medicine.” John J. Cebra, Science, New Series, Vol. 178, No. 4059. (Oct. 27, 1972), pp. 384-386; http://links.jstor.org/sici?sici=0036-8075%2819721027%293%3A178%3A4059%3C384%3AT1NPFP%3E2.0.CO%3B2-6

30. The publications resulting from this work and what Smithies describes below are: Connell, G. E., and O. Smithies. "Human haptoglobins: estimation and purification." Biochem J 72, no. 1 (1959): 115-21; Connell, G. E., G. H. Dixon, and O. Smithies. "Subdivision of the three common haptoglobin types based on 'hidden' differences." *Nature* 193 (1962): 505-6; Smithies, O., G. E. Connell, and G. H. Dixon. "Chromosomal rearrangements and the evolution of haptoglobin genes." *Nature* 196 (1962): 232-6 http://www.nature.com/nature/journal/v196/n4851/pdf/196232a0.pdf; ———. "Inheritance of haptoglobin subtypes." Am J Hum Genet 14 (1962): 14-21; Smithies, O., G. E. Connell, and G. H. Dixon. "Gene action in the human haptoglobins. I. Dissociation into constituent polypeptide chains." J Mol Biol 21, no. 2 (1966): 213-24; Connell, G. E., O. Smithies, and G. H. Dixon. "Gene action in the human haptoglobins. II. Isolation and physical characterization of alpha polypeptide chains." *J Mol Biol* 21, no. 2 (1966): 225-9.
31. Note: DEPC is diethyl pyrocarbonate, a nuclease inhibitor; Smithies either meant to say diethyl pyrocarbonate or “DFP” here. Both are neurotoxins and enzyme inhibitors and their papers do not mention either compound.
32. Tice, S.C. "A New Sex-Linked Character in Drosophila." *Biological Bulletin* 26 (1914): 221-30.
33. Sturtevant, Alfred H. "The effects of unequal crossing over at the Bar locus in Drosophila." *Genetics* 10 (1925): 117-47.
34. For discussion and illustration of the structure and evolution of the globin gene family, see Efstratiadis, A., et al. "The structure and evolution of the human beta-globin gene family." Cell 21, no. 3 (1980): 653-68. http://download.cell.com/pdfs/0092-8674/PII0092867480904298.pdf
35. On Lepore hemoglobin, see: Baglioni, C. "The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion." *Proc Natl Acad Sci U S A* 48 (1962): 1880-6; Labie, D. "Genetics and structure of hemoglobin lepore." *Folia Haematol Int Mag Klin Morphol Blutforsch* 90, no. 2 (1968): 110-6.
36. Blattner (Ph.D. Johns Hopkins, 1968) is now the Oliver Smithies Professor at Wisconsin (http://www.genetics.wisc.edu/faculty/profile.php?id=92).
37. On the recombinant DNA controversy and moratorium, see: Watson, James D., and John Tooze. The DNA Story: A Documentary History of Gene Cloning. San Francisco: W. H. Freeman, 1981; Krimsky, Sheldon. Genetic Alchemy: The Social History of the Recombinant DNA Controversy. Cambridge, Mass.: MIT Press, 1982; Wright, Susan. "Recombinant DNA technology and its social transformation, 1972-1982." Osiris Second Series, Vol. 2 (1986): 303-60 http://links.jstor.org/sici?sici=0369-7827%281986%292%3A2%3C303%3ARDTAIS%3E2.0.CO%3B2-1.
38. I.e., the Recombinant DNA Advisory Committee, or RAC.
39. Efstradiatis et al., “Structure and evolution of the human beta-globin gene family” (1980).

40. Smithies, O. "Chromosomal Rearrangements and Protein Structure." *Cold Spring Harb Symp Quant Biol* 29 (1964): 309-19.
41. The Peltier effect, described in the 1930s, relates heat transfer to electric voltage.
42. Actually, they were from Indiana University. Original description of T. aquaticus is: Brock, T. D., and H. Freeze. "Thermus aquaticus gen. n. and sp. n., a nonsporulating extreme thermophile." *J Bacteriol* 98, no. 1 (1969): 289-97. http://jb.asm.org/cgi/reprint/98/1/289
43. Kim, H. S., and O. Smithies. "Recombinant fragment assay for gene targetting based on the polymerase chain reaction." *Nucleic Acids Res* 16, no. 18 (1988): 8887-903.
44. Wigler, M., S. Silverstein, L. S. Lee, et al. "Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells." *Cell* 11, no. 1 (1977): 223-32
45. It is striking how this “experiment” is really in some ways a description of the development of a tool. Smithies knows from first principles that it ought to work; the question is how to adjust the system to get the desired result.
46. Doetschman, T., et al. "Targetted correction of a mutant HPRT gene in mouse embryonic stem cells." *Nature* 330, no. 6148 (1987): 576-8. http://www.nature.com/nature/journal/v330/n6148/pdf/330576a0.pdf
47. See Fitch’s home page at the University of California, Irvine: http://www.faculty.uci.edu/scripts/UCIFacultyProfiles/DetailDept.CFM?ID=2117.
48. Jeunemaitre, X., et al. "Molecular basis of human hypertension: role of angiotensinogen." *Cell* 71, no. 1 (1992): 169-80.
49. Warfarin was invented by Karl Paul Link at the University of Wisconsin. It is called “warfarin” after “WARF,” the Wisconsin Alumni Research Foundation, a university fund for professors’ research. See Last, J. A. "The missing link: the story of Karl Paul Link." Toxicol Sci 66, no. 1 (2002): 4-6. http://toxsci.oxfordjournals.org/cgi/content/full/66/1/4
50. Smithies, O. "Why the kidney glomerulus does not clog: a gel permeation/diffusion hypothesis of renal function." Proc Natl Acad Sci U S A 100, no. 7 (2003): 4108-13. http://links.jstor.org/sici?sici=0027-8424%2820030401%29100%3A7%3C4108%3AWTKGDN%3E2.0.CO%3B2-V
51. See, for example, Allison, Anthony C. "Protection afforded by sickle-cell trait against subtertian malaria infection." *British Medical Journal* 1 (1954): 290-94.
52. See, for example, Bijman, J., H. De Jonge, and J. Wine. "Cystic fibrosis advantage." Nature 336, no. 6198 (1988): 430; Rodman, D. M., and S. Zamudio. "The cystic fibrosis heterozygote--advantage in surviving cholera?" Med Hypotheses 36, no. 3 (1991): 253-8; Fontelo, P. "Protection against cholera." *Science* 267, no. 5197 (1995): 440; Lewis, L. G., and M. B. Cohen. "A selective advantage for cystic fibrosis carriers." *J Pediatr Gastroenterol Nutr* 21, no. 1 (1995): 117-8.
53. He may be confusing Garrod’s Croonian lecture, below, with Lionel Penrose’s Galton lecture: Penrose, Lionel S. "Phenylketonuria: a problem in eugenics." Lancet 247, no. 6409 (1946): 949-53. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T1B-49KCJ2R-10V-1&_cdi=4886&_user=10&_orig=browse&_coverDate=06%2F29%2F1946&_sk=997523590&view=c&wchp=dGLzVzz-zSkWb&md5=e4f6e9117025a7e3cac7e214179c6c2d&ie=/sdarticle.pdf
54. Garrod, Archibald Edward. Inborn errors of metabolism: The Croonian Lectures delivered before the Royal College of Physicians of London in June 1908.

London: H. Frowde and Hodder & Stoughton, 1909. The four diseases Garrod discuss are alkaptonuria, cystinuria, pentosuria, and albinism.
55. Smithies, O., and M. D. Poulik. "Two-dimensional electrophoresis of serum proteins." *Nature* 177, no. 4518 (1956): 1033.