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Oral History Project

Interview with Dr. Alan Schechter

Conducted on November 18, 2019 by Kenneth Durr

KD: This is an interview with Dr. Alan Schechter for the NIDDK Oral History Project. Today is November 18, 2019 and I'm Kenneth Durr. Dr. Schechter, thank you for taking the time to talk today.

AS: You're welcome.

KD: Particularly coming straight back from the west coast as you did, let's talk about the east coast. You grew up in Brooklyn, I understand.

AS: Yes. As my wife would say, much of America was cloned in Brooklyn.

KD: Where did you go to school?

AS: I went to the public schools in Brooklyn and probably the most significant one was Midwood High School, which had a very good academic reputation, perhaps not as much as Stuyvesant and Bronx Science, but was almost comparable to those schools.

KD: Did you get interested in science at that time?

AS: I think earlier. I remember in the second grade doing a scrapbook which I called "Science and Nature." My parents wanted me to change it to "Science and Industry," but I thought that science really was the discussion of nature, not industry. I think from then on, I always had a more than average interest in natural science.

KD: It sounds like they were interested in making sure you got a job.

AS: Yes. Probably yes, as most parents would be.

KD: You went to Cornell.

AS: Yes.

KD: What did you major in?

AS: I majored in what was called Zoology but was really beginning to become broader in focus, as the biology program was then divided into Zoology and Botany. I also took almost enough courses to major in chemistry, but at that point a chemistry major required a knowledge of German, which I did not wish to study, so I stayed with Zoology. That turned to be very fortunate because several professors in the Zoology department were extremely good and were extremely supportive of undergraduate students working with them in the laboratory, and I did so, and that event probably had the largest effect on my own career.

KD: What kind of work were you able to do?

AS: I was working on insect physiology. The professor whose course on cell biology I took in my third year was named Howard Schneiderman and he was interested in insect physiology and metamorphosis in insects, the moth going to a cocoon and then the caterpillar and then the butterfly. I worked with him and with several graduate students for two summers and during my senior year on studying physiological processes in the caterpillar of the silkworm.

In particular I had a project on the respiration of the silkworms. The respiration had been noted some years earlier to be discontinuous, and I worked to try to explain by measurements of oxygen and carbon dioxide levels why this discontinuity occurred in the

insect respiration. It was related to the larger project going on in the laboratory of studying metamorphosis phenomena in insects, in general.

KD: Were you sure that you wanted to continue in research after undergrad?

AS: Yes. By my third year I was debating between going for a doctoral degree in a biological or chemical science or going to medical school. I think probably, of course, most influencing my ultimate decision was that my mother had developed breast cancer during my first year in college and she became very ill during the summer after my junior year, and died after surgery. That event, and clearly her desires, tipped me into pursuing medicine rather than a degree in science per se.

KD: Did you see yourself becoming a physician at that point?

AS: Yes, but I think from the very beginning, unlike most students at that era I was very interested in the scientific studies of medicine rather than practicing primarily.

KD: You went to Columbia.

AS: Yes.

KD: Who did you work with there as you began to work on your research in medicine?

AS: My major mentor there, for reseach beginning in my third year was I. B. Weinstein, who later became head of the Cancer Center at Columbia. I might mention why I chose Columbia as I was accepted to virtually every medical school to which I applied. I chose Columbia because it had listed in the catalog an M.D.-Ph.D. program in biophysics. During my first year I went to the physics department to discuss enrolling in such a program and I got the feeling that they had not seen a student in some years or decades, so that turned me off. Then I approached the biochemistry department at Columbia about working in one of their labs, and again, they did not seem very enthusiastic. After my

second year I did a summer externship at Harvard Medical School with Mahlon Hoagland whose work I had read about in *Scientific American*. Then when I came back to Columbia for the beginning of my third year, I started attending the weekly seminars at the Francis Delafield Cancer Hospital where many of the research-oriented Columbia faculty in Medicine were then stationed.

In one of the first sessions in September in 1961, I met Bernie Weinstein who had just come to Columbia from working at MIT, after getting a medical degree. I had a conversation after a seminar and he immediately invited me to join his laboratory, and that was really the beginning of my longer medical career. I was very fortunate, between Schneiderman at Cornell and Weinstein at Columbia, that doors opened to allow me to pursue both academic studies and somewhat free-wheeling research.

KD: The M.D.-Ph.D. would have been pretty unusual at that time.

AS: Yes, it was an unusual path then. In some ways I think my first years at the NIH working in Anfinsen's laboratory fulfilled that both in terms of the rigor of science being done, and the FAES courses and seminars for associates created by Anfinsen and others at NIH in the early 1960', but I never actually got the degree. As you said, it was not a common pathway at that point.

KD: Your junior year you were working Weinstein?

AS: Yes, in free-time during my junior year and during all electives in my senior year. As a result, when I graduated I had had very few clinical experiences or electives since I worked in that laboratory for much of my junior and senior years in medical school. However, I was an author on two papers based on those studies, one in the *Proceedings of the National Academy of Sciences* and one in *Science*. So it turned out to be a very fruitful experience.

In fact to relate that more to NIH, Weinstein and I went in the fall of that year to a New York Academy of Sciences meeting at which Marshall Nirenberg spoke for the first time in the U.S. about the work he was doing here at NIH on the genetic code. We immediately picked up on that and pursued those studies in mammalian systems (rat liver) unlike the work here with Marshall who was focusing on bacterial systems. It's that work, which for the first time demonstrated the universality of the genetic code (as least for some codons) and later (with Paul Marks) the interactions of these messenger RNAs with polyribosomes, that led to those two "major" publications within the next two years.

KD: How did you get involved with hemoglobin?

AS: On the one hand there were a number of staff members at Columbia, in particular Helen Ranney, at that point an Associate Professor of Hematology, who was working on hemoglobin diseases, and Paul Marks, who was also a rising star at Columbia, also working on globin synthesis. And during what was my fourth year, some of the faculty in Medicine had arranged a weekly seminar on the new genetics to which I as a student was allowed to participate in and included Vernon Ingram, Max Perutz, John Kendrew, and others as visiting discussants.

I should also mention something else that we didn't go into yet. The cell biology course that I took with Schneiderman in my third year at Cornell actually turned out to be an introduction to the very new field of molecular biology, although it did not have the name then. I became very interested in this work and actually heard Francis Crick talk on the Cornell campus. Thus I learned a lot about what was new and exciting in molecular biology and I think that influenced my interests and goals, even as compared to my fellow students at Cornell and Columbia.

Also in my fourth year I became an instructor in the cell biology course (I think the youngest instructor (age 19) on the Cornell faculty at that point) and thus continued to learn more about the new field of molecular biology. So in my fourth year of medical school, when the senior faculty started a weekly "molecular medicine" seminar and they

invited distinguished scientists to come to Columbia to give talks and for discussions, I was not out of place. In particular, I got to meet some of the outstanding people at the time, perhaps of all times, in the field of hemoglobin research.

Though I did not immediately continue working on hemoglobin, the experience of working with or having met Bernard Weinstein, Paul Marks, Helen Ranney, Vernon Ingram, Max Perutz and others, certainly influenced my later decisions about my research career.

KD: At some point then you had to get some clinical experience in.

AS: Yes. After four years of medical school I applied for internships. One of the more interesting ones in New York was that at Albert Einstein. Irving London had, just a few years before, created an academically oriented program on the Medical services of the new medical school, Albert Einstein School of Medicine in the Bronx, New York. I became one of 18 interns there and spent two years taking care of patients in a city hospital environment, which involved usually five or six admissions every day. This was in a hospital program of being on call every other night and every other weekend, a system which was later considered not appropriate and not good for either the patients or the doctors, but we survived this "tests of fire" and I think felt the better for it.

KD: Was there any opportunity to do clinical research at that point?

AS: No. On the one hand the faculty all had research projects, often in their laboratories rather than patient-focused, but they clearly needed "bodies' taking care of patients, and so research by the house staff was not encouraged at all, even in the few electives. Although I did discuss once or twice doing some projects during my two years at Einstein, I was a little disappointed in the response. At every experience up until then I had been able to find research electives, but in a city hospital the need for staff to take care of patients at the door was too great.

KD: At this point—you finished up to about '63-'64?

AS: Yes. I graduated from medical school in '63. The internship and what was then called the first year of residency went from July 1st, 1963 to June 30th, 1965.

KD: So you're looking at opportunities where to go next. Tell me about where you looked and what brought you here.

AS: That process I myself find an amusing story I was quite busy as an intern, as I alluded to before, so much so that some days I lost track of whether it was day or night. Those years were really quite stressful.

Once, coming into work one morning I met a co-intern named Paul Davis who I'm still quite close to, who asked me as I was walking through the emergency room, had I applied to NIH. And I said, "No. Is there an application process now?" He said, "Yes. It ends in two weeks. If you want to apply you had better get your application in." Although I knew a bit about the NIH process, somehow I had missed the dates of the opening and closing of this application process.

I think perhaps this was because the senior person at Einstein, Irving London, was away doing a sabbatical in France and nobody in the system happened to mention it to the interns. I don't remember how Paul Davis heard about it. I immediately went to the pay phone and made a long-distance call to NIH, which probably cost me 50 cents. I asked for the application forms, which they sent me "special delivery," and I spent the next couple of nights filling them out. So I got my application in for the Associate Program just a couple of days before the deadline, and I would have missed it completely that year except for that chance conversation.

KD: Why was there so much interest to get to NIH?

AS: Then it was very explicit that if you did not go to the Public Health Service program at NIH you were almost certainly to be drafted into the military. Although the Vietnam war was still not full blown, that was a possibility or just doing other military service.

Also by that time, I would say that after the opening of the Clinical Center in 1953, the reputation of the NIH as being an important place for doing clinical research or medically- related research rapidly increased. That was not true before 1953, before the hospital opened. So although the people I knew about at NIH were doing very basic research like studying fire-flies, lightning bugs, and things like that, these projects seemed to me of little medical relevance. However by the late 1950s and early 1960s, the NIH was beginning to have a reputation for important clinically-related research. In addition it offered a possibility to work here for two years and thus avoid the draft or the necessity of applying for the Berry Program to finish a clinical training program and then go into the service.

KD: The process is you're applying to the PHS.

AS: Yes.

KD: What led you to NIAMD or whatever it was called at that point?

AS: After I submitted my application, in which I probably said that I was interested in practically every project that they had listed on many pages, I was invited to come here for two days, a Monday and Tuesday, and interview. I was on call that weekend. At 6:00 on Sunday after not having slept for two days, Dr. Paul Davis came and covered for me while I drove down here. By the time I got to Baltimore I couldn't go any further and I pulled into a motel and slept the night and got up at five a.m. and continued to Bethesda, unfortunately getting stuck in traffic on the East-West Highway and missing my first two or three interviews.

They had arranged for me to have interviews every 30 minutes from eight a.m. to five or 5:30 p.m., both Monday and Tuesday. So I had about 30 or more interviews during the two days, perhaps in large part because I had already published two major papers and that probably was a bit unusual for the candidates. In some ways I think I had full choice among many different programs because of my background, i.e., there was a virtual smorgasbord laid out for me during those two days.

KD: It sounds like a grueling kind of a smorgasbord.

AS: Yes it was. In fact by the afternoon the first day I could hardly remember my own name. I was answering the same questions over and over again. It was quite exhausting.

KD: Did any of the interviews stand out in particular?

AS: Yes, many. There were many interesting projects, but I also knew, on the one hand, of Marshall Nirenberg's work because our own work was derivative of that work and I obviously knew that field very well. But also Dr. Anfinsen's work—he had come to Columbia to participate in a symposium at Columbia, which I attended. I had spent the day hearing most of the papera and I was very impressed by his talk. In addition I've always been interested in books in science, and I had known of his book the *Molecular Basis of Evolution,* which was published, I thinkx, in 1959. So between having heard him speak and meeting him, and having read his book that was, for me, a very major draw to his laboratory.

KD: Give me the lay of the land when you showed up at NIAMD and what you found.

AS: I should correct one thing is that I was initially accepted into the general NIAMD program for research associates. I think at that point each institute would appoint four individuals as so-called research associates, in contrast to the clinical associates who had explicit clinical responsibilities. The research associates only had clinical responsibilities if they wished, and also did not actually choose the laboratory among the many in

NIAMD or the other institutes until just before they came here when they basically had a choice of any laboratory in the Institute.

So the actual choice of Anfinsen, although that probably was the reason I chose NIAMD, did not occur until some months after my initial interviews. Once I had made that decision, presumably in the spring of 1965, I then showed up, and as usual Dr. Anfinsen hardly remembered who I was or why I was there, but things worked out okay.

KD: Did he give you work to do right away?

AS: No. Ironically, at that time two groups had split off from him. He had left NIH in 1961 and had returned in 1963. He had gone to Harvard Medical School but was not happy there and came back. When he came back, he also recruited back to NIH two of his physician associates who had participated in his initial studies on refolding of proteins, Dr. Charles Epstein and Robert Goldberger, and they each, by that time in 1965, had their own sections. And he with great generosity, probably compared to how most people would act now, encouraged the associates who were coming to NIH in 1964 and 1965, and even a year or two later, to work with either Dr. Goldberg or Dr. Epstein rather than he himself, and he suggested I work with Dr. Epstein. So my first two years were with Charles Epstein. Although when Dr. Epstein left to go to San Francisco 1967, I then switched to working full time with Dr. Anfinsen himself.

KD: Tell me about Dr. Epstein and what you were studying there.

AS: Charlie had been involved in some of the key work on protein folding and then went off to the University of Washington in Seattle to work with Arno Motulsky to train in medical genetics. When he back to NIH he wanted to continue both his basic research and to start a clinical genetics program at NIH. Ironically, the powers that be at NIH never really encouraged him to do the clinical genetics and ultimately, he left to go to the University of California at San Francisco in order to establish clinical genetics.

But during the two years that I worked with him he was both teaching clinical genetics in the FAES night school and beginning to envision programs that would test genetic theories, such as the effects of polyploidy on gene expression in liver cells. That was one project that he encouraged me to work on. However, I had still been focused more on the protein refolding studies of Chris himself, and Charlie gave me the option of continuing on a project related to protein folding or moving into the gene expression studies, and I chose the former. Perhaps in retrospect that was a mistake, gene expression has indeed turned out to be a robust area for many decades. My orientation at that point was and has continued for many years, although not entirely now, to be related to protein structure-function.

KD: Protein folding under Dr. Anfinsen was also pretty robust at this point.

AS: Yes. And all the outstanding individuals throughout the world who were interested in protein folding regularly came through the laboratory, gave seminars, and perhaps one half of the post-doctoral fellows and the projects were all related to protein folding. I chose a project a little bit related to my own interest in hemoglobin - to study the folding of myoglobin, which was a single chain hemeprotein quite similar in structure to one of the chains of the hemoglobin tetramer. My project was to study the effect of the heme group on the folding of the myoglobin, what's called the apomyoglobin, the protein without the heme, and my studies for those two years focused on that little niche of protein folding.

KD: Did you make a determination? Did you come up with some good results in that niche?

AS: Interestingly I think that the major determination I made was that the project was ill-defined because it turned out that the heme molecules, that we were adding to the protein easily aggregates in solution, and to do sophisticated thermodynamics and kinetics we really needed to know the effective molecular weight and the effect of concentration of the individual heme groups that we were studying, but the kinetics and the thermodynamics were complicated because of the aggregation process.

We did get some results and we had one major paper in the new *Journal of Molecular Biology* showing that the protein flickered among conformations and adding the heme group appeared to stabilize the folded form. So the heme didn't really catalyze the folding, but actually stabilized intrinsically the fluctuating protein. That's a model I think that has been quite useful in general. But the more sophisticated analyses we tried to do were not feasible because of the complexity of the chemistry. However, the project and courses I took to learn more of the chemistry made me a much better laboratory investigator.

KD: When you moved over to work with Dr. Anfinsen himself did you change your emphasis?

AS: Yes. Instead of continuing on myoglobin and heme proteins, he had, a year or two before begun to work with a protein called Staphylococcal nuclease. His initial work was largely done with bovine pancreatic ribonuclease, and a few other proteins, but mostly ribonuclease. That protein has four disulfide links and the initial studies were basically studying the breaking and formation of the disulfide bonds related to the folding process.

But by the time I came, Dr. Anfinsen had reformulated the goals of the folding work with the idea of doing total chemical synthesis for proteins. This was catalyzed in part by the work at Rockefeller University by Merrifield who was developing so-called solid-state methods of synthesizing large peptides. Anfinsen, who was a master of moving into new fields, had gone back to the books, journals and meetings to learn the chemistry for organic syntheses of peptides and proteins. At this point he had reformulated his goals, i,e., if his ideas about protein folding were correct, chemically synthesizing a large protein and having it fold up spontaneously in solution would now be a practical goal based both upon the theoretical work and the chemical syntheses being developed.

KD: Why would he want to do the total synthesis?

AS: Until his work on protein folding, for which he shared or basically won a Nobel Prize, nobody conceptualized that this was a relevant question. It was always thought that the

protein would only fold correctly in the cell when there were enzymes or other structures catalyzing the correct three-dimensional structure. But if his model and work was correct then in solution one could get a protein to fold.

He wanted a protein which did not have the disulfide bonds because he realized the disulfide bonds made the process more complicated. So he switched when he came back to NIH, from ribonuclease to Staphylococcal nuclease and I joined that project, which lasted for half a dozen years, of studying overall many aspects of the folding of nuclease as well as parallel chemical syntheses of peptides from the Staphylococcal nuclease.

KD: Did you achieve the chemical synthesis?

AS: Probably not, in reality. The large peptides were synthesized, and they could complement—if you cleaved the nuclease into a couple of large peptides you could replace one of the peptides and get activity. But the total synthesis of all, I think it's 149 amino acids, was never really accomplished. It was too long for the methods then or subsequently I think. There were one or two claims, not from our laboratory but others, suggesting that they had gotten some activity, with staphylococcal nuclease and other long proteins but these were not very impressive.

A method called semi-synthesis in which you divided a protein into a few large fragments and then stitched together the large fragments has continued and has evolved greatly to accomplish the original goal. In fact we had a symposium here just a year ago to honor Dr. Anfinsen in conjunction with the exhibit highlighting his work that's on the first floor of this building. And one of the speakers from the University of Chicago has pioneered the semi-synthesis and gotten a great deal further, but recombinant DNA methods and synthesizing proteins in mammalian cells, bacterial cells, or yeast cells, won the day over the solid phase chemical synthesis, I believe, although perhaps some people would disagree with my interpretation.

KD: Describe the lab and the development of Dr. Anfinsen's lab during these years: how he mentored people, who some of the folks you worked with were, and the general atmosphere.

AS: His research group was never larger than five or six individuals. Perhaps one technician and three or four post-doctoral fellows of whom half or more were physicians; he was very preferential in getting physicians as trainees. Physicians flocked to him on the one hand but I think in many ways he was more successful with them and I would argue. although perhaps I am somewhat self-serving in saying this, in training physicians for later careers than in training Ph.D. individuals.

The mentoring was always that each person had his or her own project and there would be a weekly meeting to discuss results, and then every two, three, or four months, a more formal seminar to present the results before the group as a whole. People were encouraged to submit abstracts to the meetings and then write papers.

I think in terms of mentoring perhaps what's somewhat different from what I perceive now is the smaller size of the group, but also individuals were encouraged to go to other seminars. We had a weekly seminar with an outside speaker from other labs at NIH or even from outside of NIH to come and talk about his or her own work, which might be peripheral to the central focus of the laboratory. But we were all strongly encouraged to hear these seminars. We were also encouraged to take courses and to broaden our knowledge.

I think the idea was to have people have a two or three-year experience which would then allow them to run their own laboratory, not to be hands as part of a larger project. That has been a major change in mentoring, which I think was much better in the past, at least for some labs, than it is often the case now.

KD: An approach like that presupposes there's going to be a lot of growth, and everybody's going to get—

AS: Yes. You hit upon one thing is that each person will be a PI and now perhaps one of the reasons for the change is that not everybody can establish their own independent laboratory now.

KD: Who were some of your colleagues? Who came up with you in that lab?

AS: Pedro Cuatrecasas, who became a leading professor at Hopkins and then the Vice President for Research of several major pharmaceutical companies. Bob Canfield, who became a Professor of Medicine at Columbia. Ed Haber, who became head of Cardiology at Mass General and then Vice President of a pharmaceutical company. Those of my era, included Henry Epstein, chair of Anatomy and Cell Biology in Galveston; David Sachs, who became Professor of Surgery at Mass General and pioneered the studies of use of organ transplant from the pig; Bruce Furie, who has been an important person in blood clotting; David Ontjes, chair of Medicine at the University of North Carolina, etc. There were many other individuals, the ones I'm mentioning were largely among the physician groups, including for example Irwin Chaiken, head of a protein chemistry laboratory in Philadelphia.

KD: What's it like to work in the lab of a Nobel Prize winner when that happens?

AS: The work that I was doing with Anfinsen was largely before the Nobel Prize. He got the Nobel Prize in 1972, and most of my work directly with him was from 1967 to 1972, but her focused on our work together in his Nobel Prize lecture – which worried me a bit as we had not completed snd published that work to ascertain fully its validity.
But even afterwards there was little change except the first year or two Anfinsen was preoccupied with invitations and being on committees, and the like. He had always been very active in human rights, activities for the National Academy of Sciences and other organizations, signing letters and petitions; he was one of the founders of the Federation of American Scientists, which had a very important role in the Nuclear Test Ban Treaty, signed during the Kennedy Administration. And he was not intimidated by NIH rules of

limiting signing petitions or letters, and he remained very active in all this before and after the Nobel Prize. There was ironically little day to day change after the first year or so.

One of the stories I still enjoy telling is the following: After the Nobel Prize, he changed fields and began to work on interferon - expecting that it would be more effective in cancer therapy than it actually has turned out to be. But in order to prepare the interferon they grew yeast in large batches and people would say to me they had seen Dr. Anfinsen wheeling two large carboys of yeast from Building 10 over to Building 6 - many hundreds of yards - where the fermenter was, by himself - up and down the hills. It's not something one ordinarily expected to see a Nobel Prize winner do on the NIH campus.

KD: So he's willing to do his own work.

AS: Yes. For example, he made contacts and interactions with other scientists very much younger than he was. He was not ashamed to go to seek help from others, which is not often the case.

KD: Were you able to get any clinical experience during the 1960s?

AS: Probably less than I should have had. On the one hand my wife became a leading hematologist in the Washington area, becoming Head of Hematology at the Washington VA Hospital. Through going to meetings and interacting with her colleagues I kept up to some extent, reading the *New England Journal of Medicine* and *Lancet*, and a few other journals as well as seminars and general lectures.

My one attempt at actually doing clinical work myself was, after Dr. Epstein left, I helped Elizabeth Neufeld, John Mulvihill and a few others, set up an Inter-Institute medical genetics program at the Clinical Center. This is something that Charlie Epstein had wanted to do but had not really been successful at because of the bureaucracy. For ten or more years we ran an Inter-institute medical genetics program and I helped as an

organizer, although others became more involved in recruiting the fellows. We did also have a successful yearly Board Review course. We had a weekly clinic, and once a year I would act as the attending physician for the month at the discussion of the patients, after the morning clinic.

I never during that time had prime responsibility for the patients or was actually "hands-on" with the patients. It wasn't until some years later (in the late 1970s) when I became explicitly involved in the sickle cell disease program that I had actually had more direct clinical responsibility. However, between reading the journals, going to the Grand Rounds and other conferences, and participating for more than a decade in the medical genetics program I was able to remain involved in clinical research. Ironically once I focused on sickle cell disease, I moved more into Hematology than Medical Genetics as the former became the "home" of most sickle cell presentations by about 1980.

KD: As far as your career was concerned, what was the next step in 1972? Did you become a lab chief?

AS: In 1972, I had become a section head. After Dr. Anfinsen's Nobel Prize I had a strong feeling I should then choose another area of research. It had been fun for five years working with him, but I thought all things being considered I should do something different.

I first chose to work on protein nucleic acid interactions in part because through Dr. Goldberger the laboratory had an interest in histidyl-tRNA synthetase and there had been several investigators from Naples who had worked in the laboratory, who had gone back to Naples, and were encouraging me to come there on a sabbatical. So I decided in early 1972 to go to Naples with my family, which we did for three and a half months in late 1972. There I worked with several Naples groups, especially that of Francesco DeLorenzo, on the histidyl-tRNA synthetase and then bring that project back to NIH to continue working to examine the general nature of interactions between tRNA and a

protein. Many people felt that protein nucleic acid interactions were a prime new area, and there were methods for isolating tRNAs which were just developed.

On the surface this seemed like a good project. The irony was that Dr. Anfinsen's group would still work on Staphylococcal nuclease, which easily breaks up tRNA. So I was trying to work with tiny amounts of tRNA in a laboratory, in which everything was contaminated with nucleases and our yields were very, very low. After six months or a year of doing this I decided that I could not stay in the same laboratory and do a project with tRNA, so I began to look for other areas of research.

KD: Tell me about the process of looking for other areas of research.

AS: I don't think it was very organized. I had recruited a post-doctoral fellow who was interested in complement from the American Red Cross laboratory in Bethesda, and I had thoughts of working on the protein chemistry of the complement proteins and started a project with that one person. However, I found that area very much dominated by a few laboratories and not as open as protein synthesis and protein folding and other areas had been. Perhaps most important, a summer student came to the laboratory one year and her not well thought-out project opened up a new area for us.

I had been aware of the possibility of doing prenatal diagnosis of abnormal hemoglobins with specific antibodies, and I had also—from the work that I had been doing with Drs. Anfinsen and David Sachs in particular—been using the Merrifield method to synthesize short peptides from Staphyloccal nuclease. At this point, I realized that we might use short peptides from hemoglobin to isoloate specific antibodies to sickle hemoglobin, for example. The initial project was just to have a summer student do that synthesis of the peptide and try to pull out sickle hemoglobin-specific antibodies from an anti-hemoglobin antiserum. So that was our foray into hemoglobin—for not more than a summer student (who later she had a good career in medicine, I should mention).

Also by complete chance on a Friday, at about 4:30 pm a few months later, I received a telephone call from an administrator, Dr. John Hercules, in the National Heart, Lung and Blood Institute, who had been hired a few months before to administer the Sickle Cell Research Program, to be told that he was going on Monday and Tuesday to a site visit at Columbia University. The visit was to evaluate Cyrus Levinthal's project on the structure of the sickle hemoglobin polymer, and the person who was supposed to be the protein chemist for that review had cancelled and I was asked if I would come instead and I said sure.

So on early Monday morning I met Dr. Hercules and several other people, took the train up to New York and spent the next two days hearing about sickle cell disease research at Columbia. Then Tuesday night coming back from New York it occurred to me on the train that the peptides that we were synthesizing to isolate what we called monospecific antibodies might also inhibit the aggregation of the sickle hemoglobin inside the red cell.

I came back to NIH and within a day or two had convinced my several post-doctoral fellows, I think then Neal Young, Jurrien Dean, perhaps Bruce Furie at that moment, that we should start working on trying to develop a treatmdent for sickle cell disease using small peptides as competitive inhibitors of the aggregation process. So suddenly related to that excursion to New York, what I had heard and the realization that we already had hemoglobin peptides from the antibody project, I was suddenly working on sickle cell disease, without really "asking" anyone and also that the disease was now thought of as the focus of a different Institute than the one I worked in.

KD: So you just picked up the lab and did a pivot at that point?

AS: Yes. That's always been one of the great strengths at NIH. One doesn't ask anybody. One just starts working on something and then six months or a year, or two years later, one has to make a report on that. That's the sequence of how that happened.

KD: You said somebody called you from the Heart Institute.

AS: President Nixon had announced a year or two before, in the State of the Union Address, that there would be a national program at the NIH in sickle cell disease research. It was clear that ultimately it would be either funded by my Institute, NIAMD, or the "Heart Institute," NHLBI. The Heart Institute opted to take up that program; our Institute unfortunately did not do so. NHLBI had begun to hire administrators in the extramural program to give contracts and grants in the field of sickle cell. So for the next 20 years I became very close to those administrators and have interacted with them as a consultant to a much greater degree than I have ever interacted with my own Institute's extramural program.

KD: Tell me about the research as you began to scale it up and find things out.

AS: The first thing about the peptides, that became an area of intense effort for us, and I made one significant mistake at the beginning. I was so sure of our idea that I tended to keep secret initially what we were doing—that I've learned, that's almost never a good idea. As a result, we wasted time in using assays and approaches that were not optimal, and then when we were more open with what we were trying to do, we became aware of better assays and moved the project ahead rapidly. We also learned at that time that several other laboratories, including in particular Alex Rich at MIT, were working simultaneously on just the same idea that we were working on.

Again, a theme that I've mentioned several times this morning is the irony was that the peptides never worked very well for several reasons. On an obvious level, they're difficult to get into the red cell and we knew that would be a problem but we thought that if they worked, we would try later on to optimize their transport. But more importantly we learned from the work of Allen Minton here in our institute that the hemoglobin inside the red cell is so concentrated, so non-ideal in its physical chemistry, that if you add peptides in large amounts you use-up free water and actually tend to salt out the hemoglobin. Even if your peptide is on the one hand preventing the aggregation by stereospecific means, it's also tending to promote the aggregation by using up free water.

Thus we and the others working on this project came to realize over three or four years that the idea probably would never actually work in practice. But it began to make us think about the basic biophysics of hemoglobin. This was a focus that Bill Eaton and Jim Hofrichter were working on in the Laboratory of Chemical Physics of NIAMD, as well as Allen Minton in another laboratory in our Institute as well as other groups around the country.

Related to my own previous background using NMR and other physical techniques to study protein folding in my work with Dr. Anfinsen, I knew the NMR people at NIH fairly well. Through a discussion with one of them, Dennis Torchia, we hit upon the use of a method called solid state NMR, which was developed largely at MIT, to examine the intra-cellular aggregation of sickle hemoglobin inside sickle red cells. Starting in 1979 and continuing for the next four or five years, that was our main focus of work to understand better what happens inside the red cell.

Dr. Eaton and his colleagues were studying hemoglobin solutions; Dr. Ronald Nagel and others at Albert Einstein Medical College were studying the behavior of sickle red cells. We were trying to work out something bridging those two areas of research, the solution studies and the entirely cellular studies, by using NMR methods for examining what was happening to the hemoglobin inside the red cell. That became our real entree to the sickle hemoglobin field where we made, I think our most important contributions.

KD: This contribution is showing what the sickling mechanism was?

AS: Yes, in a way. I should mention here that most of this work was done with Dr. Constance Noguchi who was trained in physics at George Washington University, and came to these projects of using NMR, with a sophisticated physical background and mathematical abilities.

The main thing that we showed using the NMR techniques was that aggregation of the hemoglobin occurred at very very high oxygen saturation values. As soon as you remove oxygen from a sickle cell, you get intracellular aggregates and polymer, and this occurs way before any sickling occurs. So we have spent the last 20 or 30 years developing models for sickle cell pathophysiology based upon the amount and properties of the intracellular hemoglobin aggregates and not on cell sickling per se. That has allowed us to develop assays that correlate with the severity of the disease to have a sophisticated way of evaluating potential therapies and to understand many other aspects related to the pathophysiology, and even clinical aspects, of this disease.

KD: Did your work lead to the fetal hemoglobin?

AS: It didn't lead to it. In fact a pediatrician named Janet Watson at Downstate Medical College in Brooklyn, in 1948, first observed that newborns did not manifest the disease and correlated that with an initially (first six months of life) unusual hemoglobin (fetal hemoglobin). By the '50s and '60s, the Drs. Singer in Chicago and many others had studied the effect of this fetal hemoglobin on both the so-called "gelation" of sickle hemoglobin and with clinical manifestations.

Our work allowed an explanation of the effect of different levels of fetal hemoglobin on intracellular aggregation of the sickle hemoglobin molecule. So we could predict how much fetal hemoglobin was necessary to get different levels of clinical benefit.

We were also very interested and involved from early with the use of drugs, initially a drug called 5- azacytidine and later hydroxyurea, which had been shown by others to increase fetal hemoglobin. We were in direct collaboration with Dr. Nienhuis here at NIH and others around the country or the world in evaluating how much benefit there might be from different levels of fetal hemoglobin achieved with these different therapeutic approaches.

KD: Griffin Rodgers was working in your lab at this point?

AS: Griffin came I think in 1982 or 1983 as a post-doctoral fellow with me. I've tried to also emphasize as much as possible having physician investigators to work with. During his first two years here, he did some projects related to our basic work on intracellular aggregation or polymerization of sickle hemoglobin, which we had started in 1979.

By 1985, I think, the work with hydroxyurea both in Boston at Children's Hospital and in several other places had advanced sufficiently that in consultation with the group in the Heart Institute, Arthur Nienhuis and Tim Ley, we thought it was time to do a systematic study using the Clinical Center resources of ten or a dozen patients to see what benefit we could achieve in the patients.

One of the things we realized was that the Boston group, who were the first to observe an effect of hydroxyurea in some cancer patients and in animal models, probably used too high doses when they treated the first sickle cell patients and did not get an effect, and were getting marrow suppression, if I remember that correctly. And we developed protocols here, especially with Griffin Rodgers' lead, starting with low doses and titrating the dose up stepwise, which we could do because the patients were here for long periods. I think ultimately there were ten patients studied here, each was hospitalized for about three months. We were sure of compliance and we also could follow fetal hemoglobin and other parameters for several weeks in detail, then do a stepwise increase in the dose and look at the effects.

We contributed much to understanding the dose response and the time course of the response, and then the effects on the intracellular behavior of the hemoglobin, which facilitated a later multi-center study of hydroxyurea whose results were reported in 1995, which showed that there was a significant clinical benefit.

KD: This was an opportunity for you to get back into the clinical side of things in a big way.

AS: Yes, precisely. The thing that made Dr. Rodgers coming to the lab so beneficial was that it was the ideal time for us to be involved in clinical work, and we fortunately also had the interest and cooperation of the NHLBI Hematology Branch. So between Dr. Rodgers based in our laboratory and the clinical staff in the Hematology Branch in NHLBI (who were really more interested in thalassemia than in sickle cell disease, but were willing to work on sickle cell disease), we were able to get therapeutic studies started—perhaps about 1984. It was at that point that I really got involved myself, and I was a co-investigator in all the clinical protocols; I would meet most of the patients, although I was not directly responsible for their care.

KD: Did you bring in any outside help when you were doing your clinical study?

AS: To an extent, for example, in measuring "F-cells" we collaborated with George Dover and Sam Charache at Johns Hopkins. They had a laboratory that measured not only fetal hemoglobin, but so-called "F-cells," which were the percent of red cells that have fetal hemoglobin detectable by our antibody methods. We could measure the fetal hemoglobin per se in the clinical laboratory, the cells, but the "F-cell" measurements were done at Johns Hopkins. Over the years we had many collaborators, but the basic clinical work was done by our groups here at NIH.

KD: Did you remain involved with the big clinical study in the mid-'90s?

AS: You refer to the the multi-center study of hydroxyurera. Dr. Rodgers and I wrote the editorial in the *New England Journal of Medicine* at the time of the publication of the results, but the study was organized by the Sickle Cell Branch of NHLBI and we were not one of the centers involved in the study per se. However, I did attend many of the meetings for planning the study. For example, the initiation of the study was delayed for about two years because people could not agree upon the definition of pain in painful crises.

There was a physician whose name was Dr. Payne on the committee, if I remember correctly, who always disagreed with the definition that the sickle cell people wanted to use. There were many meetings that I was at when we argued endlessly about how to define a painful crisis. I was involved in all that, but we were not one of the centers that contributed data.

KD: When did the nitric oxide angle come in? You alluded to it earlier that you have been continuing your research in this area. This is a separate avenue.

AS: There is a continuity, perhaps not as much continuity in some of the other transitions I mentioned, in that on the basis of the multi-center hydroxyurea study, the FDA approved the use of hydroxyurea to treat severe adult sickle cell patients in 1998. By 1998, we also knew and realized that not all patients responded to hydroxyurea, and even those who did respond to hydroxyurea did not get complete amelioration of their clinical manifestations. I wrote some reviews and did some clinical studies in which we pointed this out in the mid and late 1990s but the workers in the field were reluctant to accept this conclusion at the time (but not now).

I was aware that the use of hydroxyurea was not an end in itself to the search for a therapy for sickle cell disease. In fact, Dr. Rodgers and I were trying to tell the community this and a lot of people were skeptical and thought that if we just use more hydroxyurea that everybody would benefit, or that the levels of fetal hemoglobin were enough to get major benefit. We published one review article in 1989 in the *New England Journal of Medicine*, in which we said that you really needed 25 or 30 percent fetal hemoglobin to get improvement in most of the symptoms. Most of the patients were getting at most 15 or 20 percent fetal hemoglobin with hydroxyurea, so we knew that one needed other modalities of treatment.

In 1997 or 1998, I was asked to referee a paper in the *Journal of Clinical Investigation* about using inhaled nitric oxide to treat sickle cell disease. Although I recommended that

they not publish the paper, the Journal accepted it, but asked me to write the editorial, and I did write the accompanying editorial in which I hinted that I was skeptical of the results.

Just about that time a physician named Mark Gladwin had joined the NIH Critical Care Medicine Department, and he had experience in inhaled nitric oxide and also had an interest in sickle cell disease. He went to see Dr. Rodgers about working with him on the sickle cell project and Griffin Rodgers mentioned that I had been reading about and thinking about nitric oxide recently and that Gladwin should speak to me, which he did. This was another transition without prior approval. Mark and I immediately started to try to repeat the *Journal of Clinical Investigation* studies of having normal individuals and sickle cell individuals inhale nitric oxide, and we quickly established that the work reported in the paper could not be true.

At that time there was also a group at Duke University under Jonathan Stamler claiming that hemoglobin binds nitric oxide in a unique way, which allows it to be transported from one part of the body to another. So Mark Gladwin and I, even though we showed that the nitric oxide inhalation did not effectively treat sickle cell disease, began to wonder whether the Duke University model was correct, and we spent the next three or four years working together testing that hypothesis, primarily in normal volunteers. Ultimately, we concluded the Stamler hypothesis was wrong, but that nitric oxide could indeed be transported in the body in the form of nitrite ions, and that hemoglobin and other hemeproteins could reduce nitrite ions to nitric oxide, and that was the mechanism that the Duke group was actually seeing.

In the early part of the 2000s, the first decade of this century, we began to publish papers on this new mechanism for producing nitric oxide in the body, and that has led us now for 20 or 21 years into a study of nitric oxide in the body, both as a potential therapy for what various cardiovascular diseases (including sickle cell disease to the extent that its pathology is from impaired blood flow) but also more generally as an important signaling molecule.

KD: Explain that concept. How does that work?

AS: The signaling molecule? The main aspect is that nitric oxide—and this goes back to its discovery in 1985 as the fourth important biologic gas—that nitric oxide activates soluble guanylate cyclase to make cyclic GMP, and the cyclic GMP affects calcium levels in cells, and this changes the behavior of cells. The most important change is that it relaxes smooth muscle, lowers blood pressure, and increases blood flow. Also, nitric oxide is produced by virtually every cell in the mammal, in all animals, probably most plants, too, and it's a ubiquitous signaling molecular, which is effective in nanomolar or picomolar levels, and so had not been discovered to be biologically important until 1985.

After 1985, most work in the field, between 1985 and 2000, was based on nitric oxide being produced by enzymatic effects on arginine, oxidizing arginine to citruline, and characterizing the three classes of nitric oxide synthase enzymes: eNOS, iNOS and nNOS. Our work, confirmed and extended by others, has shown that reduction of nitrite ions (NO_2^-) to NO and also of nitrate NO_3^- to nitrite and then to NO is probably as important or more important in the body than the nitric oxide synthase mechanisms, especially under conditions of hypoxia.

I think this work started with sickle cell disease, and another irony, probably for reasons I could discuss with you, will not be very effective in treating sickle cell disease, but did allow us to open a door into a whole aspect of nitric oxide physiology and pathophysiology.

KD: You're essentially exploring the potentialities of this fourth important biological gas.

AS: Yes.

KD: What do you see down the road with this kind of research?

AS: The two areas that we have concentrated on in the last five or six years are showing that nitric oxide produced in the blood by red cells acting on nitrite ions affects blood clotting, and perhaps even more exciting, we have in the last three years shown that all skeletal muscles have very high levels of nitrate ions. Probably most of the nitrate in the body is stored in muscle and that with exercise that nitrate in the muscle can be reduced to nitrite and then NO and affects blood flow to the muscle. It's been known for 140 years that with exercise blood flow to muscles can increase 20 or 50-fold or even more, but nobody had really known the mechanism of that in detail until now.

One of the ironies, and I've used that term a number of times this morning, one of the ironies is that our work so far has been much more interested in sports medicine than among people studying muscle diseases. And the sports medicine people are very interested in nitrate and its related compounds improving athletic performance. Probably the major way that this occurs is the nitrate, which can be obtained in the diet, in foods, in vegetables or through beet juice or enzymatically, is stored in the muscle. Then when one runs, reductive mechanisms occur, which reduce that nitrate eventually to nitric oxide and increase blood flow to the muscle. It's a big thing in the sports medicine field right now.

KD: It's easy to see why.

AS: Yes. You say where are we going? I'm not sure yet that there will be specific therapies related to it, but I think it's opening a whole new aspect of physiology and pathophysiology, and it came about from looking for a new modality to complement the hydroxyurea in the treatment of the sickle cell patients. We were realizing that the hydroxyurea would not be curative. We thought that nitric oxide inhalation or being given NO some other way like a nitric oxide donor compound might have clinical benefit. I add that very recently we have extended our studies of how NO is generated to mechanisms in the normal mammalian eye which are based on these reductive pathways.

KD: Let's shift gears. Somewhere along the road NIAMD becomes NIDDK. Tell me about how the Institute itself helped further your research, how you worked under the different scientific directors. I want to get a sense for the development of the institution.

AS: I think from when I first came in 1965, NIAMD and its various names, now NIDDK, has always been considered scientifically one of the best institutes; perhaps with the "Heart Institute" (now NHLBI), among the strongest, especially in basic research, and that reputation I think still continues. It's also had, for the most part, the fewest administrative problems or reorganizations and things like that, and difficulties with Institute directors or Scientific directors that make staff unhappy and cause people to leave. I can't imagine a better place within NIH, or in general to work, than this Institute has been in these 55 years.

However, of course there have been changes, not all which I am positive about. I think that when I came, I would guess that NIAMD was about 60 percent basic research and 40 percent clinical research. That perhaps was the opposite of the Heart Institute, which was I would guess, very approximately 40 percent basic and 60 percent clinical. I think in the intervening 50-some odd years NIAMD has gradually gone to being about 95 percent basic and five percent clinical. I think that's happened in much of NIH. Going back to the mid-1990s I've written and spoken against this trend of deemphasizing the clinical studies and putting so much focus on the basic research.

KD: What do you think is behind that?

AS: Good question. I think clinical studies are more expensive. With the increased concerns about the ethics of clinical experimentation and the FDA rules not only are the studies themselves more expensive, but the related reviews have become much more difficult and tedious, and now perhaps only investigators in other countries or the pharmaceutical industry can afford to do these kinds of studies.

I think also clinical research is much harder than basic research. Many people would argue the opposite, but I think that to do good clinical research you have to have both a laboratory which can pioneer new techniques as well as hypotheses about the use of those techniques and access to patients. Again, there's a chance that your hypothesis will turn out to be wrong. In that case people will be very skeptical.

I think in basic research nobody knows whether the results are going to lead to other things. Everybody says, good, it's interesting to know how C. elegans does this or that but I am often skeptical as to whether or not this particular studies will lead to general biological principles; this can only be known 20 or 30 years later.

Thus, I think clinical research is really muchg more difficult and expensive than most so-clalled basic research and as a result of that clinical research has now more and more been done only with the pharmaceutical industry, which then have the prices at the other end to justify the initial expenditures, or in other countries.

KD: What's wrong with leaving the clinical side to industry?

AS: I think for example, just a day or two ago there was a newspaper report that stenting is not better than drug treatment of coronary artery disease. I meant to look up who funded that study and hope it was an NIH study, as I think it's a very important study. It was reported on major news programs and in the major newspapers. But that study could have been done ten or 20 years before now if there was the will to do so.

These are the kinds of studies that only an organization like the NIH can do, that looks across not at one specific product, but looks across at the bigger questions of what's the optimal treatment for a condition. It's something even today with all the difficulties and expenses of doing clinical studies, the NIH can and should continue to do.

KD: This is one very important transition or change that you've seen in NIDDK. Anything else that has changed over the years or remains the same in this Institute, in your eyes?

AS: I think what I alluded to before, that the support and the freedom has remained quite robust and very positive. I'm quite happy about the overall situation. As with all organizations, the amount of bureaucracy has increased, and the number of mandatory courses and the like have proliferated, and many people get frustrated by this. I think to some extent that's also true of our colleagues in the universities. That is the bureaucratic aspects have changed, but not the intellectual freedom and the support for good research.

KD: Somewhere along the road you went from being mentored to being a mentor. When did you become Lab Chief?

AS: When Dr. Anfinsen retired in 1981 at the age of 65 and he moved to Israel initially for one year, but then came back and became a professor at Johns Hopkins, I became the Acting Lab Chief. And after the bureaucratic needs for having a formal search and all that sort of thing, I became the official Lab Chief about a year later. I've been the Lab Chief now for 38 years, perhaps longer than some people think is ideal.

KD: What have you applied in your mentoring of scientists coming up? What have you applied that you've learned earlier and what sorts of things have you developed on your own?

AS: I think I have tried to model myself on the way that Anfinsen did, the best I could. I think I've encouraged post-doctoral fellows to take courses, to go to seminars, to formulate their own research; they're not "given" a project to do, but what they do is discussed between them and their mentors. They're encouraged to collaborate with others either in the lab or outside the lab with the expectation that each of them will have later an independent career.

I don't know to what extent I've been successful or not. I think the post-doctoral fellows are more than hands for a couple of years. I've also never wanted to have a group of more than five or six at most. I don't understand and I don't think it's a good idea to have labs

with 10 or 15 or 20 people. I think that there are many reasons for this, including that the questions being asked tend to get more and more focused and reductionist and all the staff tend to lose the big picture.

KD: You're looking to keep the ability to have a personal relationship with the people in your lab.

AS: Absolutely, yes.

KD: Anything else we should talk about that we haven't hit on?

AS: The other thing that you do have on your list of questions: I've had at the very beginning an interest in the history of medical research. At the same time, my first year here I was taking Earl Stadtman's and Roy Vagelos's course in biochemistry, which everybody thought was a very good thing to do. I took a one-term course in medical history and everybody thought this was bizarre to do. I still remember that reaction.

I had always thought that to understand where research is in the context of decades or half centuries and not just the last year or two of papers. This is very important in designing one's own research, as well as to understand the successes and important failures of an organization like the NIH.

I helped, in 1985, to create what became the Office of NIH History, and I've been involved with it one way or other going back further—indeed for almost 45 years—and I am still informally active with that organization. I'm very sad and unhappy that this Office is not as robust as it was when Victoria Harden was the NIH historian—until she retired in 2005 because of the illness of her husband.

I think NIH could and should do much more in preserving its history, such as oral histories like we are sitting here doing right now, but also collecting documents and encouraging people to write articles and books about various aspects of NIH history.

There have been major failures as well as successes in the NIH program. Unless one understands both aspects of that one can't really know where things could or might go.

KD: You did your part there. I think you stepped in for a while when Vickie Harden retired.

AS: I was the Acting Director for two years because although they had a commitment at that point, which they no longer have, of recruiting a new historian, it became clear that they were not going to have a new director of the History Office for a couple years. I had been chair of the Advisory Committee for more than a decade before that. From 2005 to 2007, I spent about15 to 20 percent of my time running the History office. I might have considered doing it on a part-time basis for longer, but they did hire a historian at the point who became available because of the hurricane in Louisiana, but he became ill and retired a few years later, and NIH never replaced him, unfortunately.

KD: As a historian who has worked with the NIH Office of History, I've appreciated the work you've done and thanks for this interview. It's been great.

AS: Thank you very much. I appreciated your outline of the questions to be covered; also I appreciate the thought and effort you put into looking into my career.

KD: Thank you.