Howard Schachman

University of California Professor of Molecular Biology: Discussions of His Research Over His Scientific Career From the 1940s Until 2010

Interviews conducted by
Sondra Schlesinger
(2007-2010)

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Howard at the ultracentrifuge in 1966

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INTRODUCTION
by
Sondra Schlesinger

Howard's career at the University of California has spanned over 60 years—a period that has seen many changes in the world and also in Howard's field of biochemistry. The field of molecular biology did not even exist when Howard arrived at Berkeley. In 1948, the year that Howard joined the faculty at Berkeley, biologists had not yet recognized that DNA was the genetic material. O. T. Avery, C. M. McLeod and M. McCarthy published their landmark discovery in 1944 showing that it was DNA, not protein, that was responsible for bacterial transformation. Those results did not convince everyone and the oft-cited Hershey-Chase experiment showing that only the DNA of the bacteriophage need enter the bacterial cell for phage replication was not published until 1952. In the early 1940s, although most scientists agreed that proteins consisted of amino acids linked together in a linear array, how they were synthesized and their three-dimensional complexities were unimagined.

The Bancroft Library at the University of California had recognized Howard's contributions to the University and several years ago had carried out extensive interviews with him covering his political activities focusing mainly on issues on the Berkeley campus. But Howard's most important contributions are in the field of science and this book represents an attempt to record some aspects of these contributions. Howard and I discussed his research trying to put his thinking and

discoveries in an historical context.

When Howard first arrived in Berkeley his focus of research was on the virus, tobacco mosaic virus (TMV)—and our delving into the area of virology was of particular interest to me because of my long term research interests in that field. Although the presence of nucleic acid in TMV had been detected in the 1930s, it was not until the experiments of Fraenkel-Conrat and of Gierer and Schramm in 1956 that demonstrated that it was the RNA in the virus particle that carried the genetic information. At this stage of Howard's research he was not thinking about genetics—that does come much later—but was devoting his attention to establishing the structure and uniformity of virus particles, particularly of TMV but also of papilloma virus. Could they be considered as molecules or as some advocated were they organisms?

Throughout our discussions Howard emphasized how his interest and expertise in the ultracentrifuge informed his research. In 2000, in an essay for the *Annual Reviews of Biochemistry*, he described his "love affair with the ultracentrifuge." That "love affair" was not monogamous and as he discusses in this book he often brought the use of the ultracentrifuge to collaborations with colleagues at Berkeley and in many other universities and research units. His contributions to the use of the ultracentrifuge in biological research also led him away from virology. Howard (and the ultracentrifuge) played an essential role in discovering the existence of ribosomes, a crucial step in deciphering how proteins are synthesized. In the 1960s he began a long-term collaboration with John Gerhart and an even longer involvement with the enzyme aspartate transcarbamylase (ATCase). His research on this subject converted him from being essentially a physical biochemist to one who embraced the areas of molecular biology and genetics. It also consumed a large fraction of our conversations. Howard's interest in virology had overlapped with my interest in that subject, then as we moved into his research on ATCase we moved into an area of biochemistry that coincided with some of the interests of Milton Schlesinger (my husband) and because of this relationship I have a special interest in this subject as well.

Although research scientists like to look to the future, to think about what we don't know, what we might discover, Howard and I wanted to record our conversations because we thought that it is also valuable to look back, to appreciate how far we've come. The conversations that Howard and I had over the course of several years have tried to provide that perspective.

This oral history on Howard Schachman's scientific career joins a previous oral history focusing on his contributions on behalf of free speech, academic freedom, and free scientific inquiry. Both oral histories and video clips can be found on the website of the Regional Oral History Office at http://bancroft.berkeley.edu/ROHO/collections/subjectarea/univ_hist/fac_adm_reg.html. The Howard K. Schachman papers relating to his teaching, research, and political activities are in the Bancroft Library.

Acknowledgements

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Professor Howard Schachman Oral History Discussions with Sondra Schlesinger

Interview #1: Taped on October 18 and 22, 2007, January 16, 2008
Begin Audio File 1 Oct 2007 01-16-2008.mp3

Schlesinger: Howard, I know you went to MIT, but I think you studied chemical engineering. So let's discuss the history of how you went from chemical engineering to biology. And I'll interrupt as you go along.

01-00:00:20
Schachman: Well, I graduated from MIT in 1939. It was the preeminent school in the United States for chemical engineering students. And despite the fact that I had very good grades and came from such an eminent place, I couldn't get a job—primarily because I was Jewish. I tried hundreds of places, literally, over a hundred.

Schlesinger: Had anybody told you that this was going to be a problem?

01-00:00:46
Schachman: No, I didn't realize it would be a problem. I even had connections with some vice presidents of major companies or something of that sort. Invariably, I brought the issue up. Why can't you employ me? It turns out that, oh, they're not prejudiced at all and they pointed out all the Jews that they had working for them. Well, the truth of the matter is if you were a distinguished scientist with a record of accomplishment, you'd have no problem getting a job in one of these companies. But if it was a run of the mill college graduate that they were looking for, they discriminated against Jewish kids. And therefore, it was extremely difficult. So after looking and looking and looking, I finally found a job at a paint company, which was miserable. I also found a job with a company that made alcohol. They made alcohol for commercial purposes and also for drinking. And this meant changing over at night from one function to the other function. It was a major operation because you couldn't contaminate the alcohol producing equipment that was used for commercial purposes with that used for oral consumption. Neither of those jobs was very satisfactory. There was no way that I was using my talents or education at all. Then one evening, I went to a lecture at the Franklin Institute in Philadelphia, and heard a talk on the physical chemistry of tobacco mosaic virus by Max Lauffer.

Schlesinger: Was there some particular reason why you decided to go to that lecture?

01-00:02:17

Schachman: Well, I was still looking for jobs. I had written all sorts of fan mail to various companies, trying to impress big shots—I wrote to "commanders in chief" [laughs] of the companies and people of that sort—they didn't call them CEOs in those days. I was using every "trick" I could think of to find contacts to get a decent job. I saw a notice in the newspaper that this talk was going to be given. I lived in Philadelphia. And so I went and heard the lecture. It was a beautiful lecture, very inspiring.

Schlesinger: What did you know about viruses at that time?

01-00:02:57

Schachman: I knew nothing. I knew a little bit about the centrifuge. Max Lauffer had done some very beautiful work on ultracentrifugal analysis of tobacco mosaic virus. My undergraduate thesis was on the Sharples centrifuge, so I knew what a centrifuge was, but I certainly knew nothing about the analytical ultracentrifuge, as built by Svedberg and his disciples. So I came home that night and sat down and wrote a fan letter to Max Lauffer, at the Rockefeller Institute for Medical Research, Princeton, New Jersey, and told him how much I loved the lecture, and asked, "How do you get a job of that type? I would love to do that kind of science." Instead of throwing the letter in the wastepaper basket, he was kind enough to answer me in a very nice way. He informed me that you can't get a job doing that kind of research without a PhD, so I would have to go to graduate school to get a PhD before I could ever get his kind of job. "But in the meantime," he wrote, "We have an opening for a technician. If you'd be interested, we'll be happy to interview you." Obviously, I was interested. I got on the train and went from Philadelphia to Princeton Junction; and from Princeton Junction, I was able to get to the Rockefeller Institute for Medical Research, which is out in the country. It's about five miles from Princeton University itself. And I had a wonderful interview that ultimately led to my becoming a technician in Lauffer's lab. Lauffer was sort of the senior postdoc in Wendell Stanley's laboratory. So that's my first connection with Wendell Stanley.

Schlesinger: And that whole laboratory was working on tobacco mosaic virus?

01-00:04:34
Schachman: Almost exclusively, at that time, on tobacco mosaic virus. Stanley had already achieved international acclaim for his crystallization of TMV (tobacco mosaic virus).

Schlesinger: Had he already won the Nobel Prize?

01-00:04:47
Schachman: No, not yet. He didn't win it until I got in there! [Schlesinger laughs] The interview was interesting, because in the course of waiting for one interview after another, I sat in the library and I heard comments by somebody who turns out to have been a very distinguished scientist, in a sort of derisive imitation of a Jewish accent. He was imitating Moses Kunitz, who was in that same institute at Princeton, in a different department. Kunitz was an Eastern European Jew, who spoke the way Eastern Europeans speak, which was characteristic of many Jewish people. I was somewhat perturbed by that. Then a little later, when I came back and Max Lauffer continued the interview, he asked me my religion. And my comment to him was, "Not you, too." And he smiled. He didn't take offense at that. [laughs] I was so perturbed by the idea that everybody was probing my religion and my ancestry, my grandparents, et cetera. So he indicated that they just wanted it for the record, that there would be no prejudice. It turned out there certainly was no prejudice and I got the job and I thrived. They were extremely good to me and encouraged me to go on to get a PhD, which is another part of the story.

Schlesinger: I think most of this has been discussed before, so let's just stick to the science. I think, if I remember correctly, you had told me something about introducing the use of the centrifuge or introducing the use of the Sharples centrifuge in that laboratory. Is that correct?

01-00:06:35
Schachman: That's correct. Yes, much of my time was spent isolating tobacco mosaic virus, which was done by loading ten or twelve plastic tubes, I don't remember the details, into a preparative rotor. You had to cap each one, and then you would spin the rotor in a vacuum centrifuge, a preparative high-speed centrifuge. And the virus would be spun down to the bottom of the tubes. Then you throw away the supernatant, re-suspend the pellet, and do it several times. That was the method of purification. It was a beautiful technique. It was labor intensive; you had to do a lot of work. I had already had experience with the Sharples centrifuge, which was a continuous flow tubular centrifuge, through which liquid flowed. It was like a milk

separator. Liquid would enter the bottom, and a supernatant would exit at the top. It generated centrifugal fields almost as great as the centrifugal fields that one used in the preparative centrifuge routinely used in Stanley's lab. So one day I suggested to Stanley that you might be able to flow this virus suspension through a Sharples centrifuge and be able to isolate much larger quantities of tobacco mosaic virus with much less labor. It'd be much easier. So he said, "That would be interesting to try." I said, "Well, I knew the Sharples people" that was because during part of my undergraduate thesis I had some contact with them. Since I still lived in Philadelphia and was commuting to Princeton, I took some extract—plant juice, as it was called—about a liter of it, from the lab home with me one night to Philadelphia. I had made an appointment with people at the Sharples Company, and they allowed me to use their Sharples centrifuge. They were very co-operative. Passing the liquid through the centrifuge operating at about 50,000 revolutions per minute resulted in the virus being "pelleted" onto a plastic liner, which I then brought back to Princeton with me. And of course, I scraped it off with a spatula, and there was the TMV.

Schlesinger: Actually, I'm amazed that TMV, as a single particle, would centrifuge in the Sharples.

01-00:08:28

Schachman: Well, the centrifugal force was 62,000 times gravity. That was almost comparable to the preparative centrifuges we were using at that time. If you flowed the liquid at about fifteen or twenty milliliters per minute, that would mean the solution would stay in the rotor for sufficient time to permit the particles to be forced to the outside of the rotor. It was about twenty inches long and about two inches in diameter. So it would take quite a while for liquid that was entering in the bottom to become the supernatant and to exit at the top. During which time, particles would be subjected to a large centrifugal field. Maybe the yield was only 60 or 70 percent. I never determined how much. But it was very exciting at that time. And immediately, Stanley went and ordered a Sharples centrifuge, and we switched over to that method of purification.

Schlesinger: At the time that you were a technician, were you involved in understanding any of the biology of tobacco mosaic virus?

01-00:09:23

Schachman: Well, there were two major classes of virus research at that time, the one in Stanley's lab, which was the chemistry and

physics of viruses; and the other one was the work on bacteriophage in the Delbruck laboratory at Caltech, which was much more biological. Both of these institutions flourished and gorgeous research was coming out of both. But what we did at Princeton in Stanley's lab was mainly chemistry and physics. He wanted to prove that viruses were particles, or molecules, if you want to use that phrase. And that orientation was very different from understanding the biology and the life cycle of a virus. All that was discovered primarily with bacteriophage, rather than plant viruses.

Schlesinger: At the time that you were working with tobacco mosaic virus, did people know that there was nucleic acid inside?

01-00:10:17
Schachman: No. That was an observation that was missed in the Stanley laboratory and was discovered in England by Bawden and, in particular, by Pirie. This issue became the subject of considerable controversy because they claimed that Stanley's work had not really led to the isolation of the true virus. The truth of the matter is that the phosphorous analysis that he did, did not show the presence of 5% RNA in tobacco mosaic virus. So that was discovered in England.

Schlesinger: But people knew that bacteriophage contained nucleic acid.

01-00:10:51
Schachman: Not at that time.

Schlesinger: Okay. So actually, at that time, people didn't know that nucleic acid was the genetic material.

01-00:10:58
Schachman: Oh, absolutely right. They did not know. Except for the old experiments on transforming principle that were in the literature, but were not being interpreted properly.

Schlesinger: Well, what year are we talking about now?

01-00:11:08
Schachman: Well, we're talking about the late 1930s and early 1940s.

Schlesinger: And the Avery, McCarty, MacLeod experiments. They published their paper in 1944, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types" in the *Journal of Experimental Medicine*—so it was about the same time.

Schachman: Right.

Schlesinger: You were describing your work as a technician.

Schachman: Your asking about my role as a technician provides a great opportunity to comment a little on the culture and practices of the renowned Rockefeller Institute for Medical Research in the 1940s. Its principal laboratories were in New York City with three additional groups located in the country about 5 miles from Princeton, New Jersey. Two of the departments were there because they needed more space than was available in New York; they were the Departments of Plant and Animal Pathology where important research on viruses was being conducted. The third group headed by John Northrop was involved with pioneering studies on enzymes; it was located there because Northrop didn't like the big city.

Most of my activity as a technician for Stanley and Lauffer was running the air-driven ultracentrifuge and determining sedimentation coefficients of TMV and other viruses as well as intermediates in the denaturation process. When Stanley received a government contract to purify and study influenza virus with the long-term aim to produce a vaccine, the pressure to do sedimentation velocity experiments increased tremendously. The ultracentrifuge we were using had been built in the shops of the Rockefeller Institute in New York under the guidance of Ed Pickels about whom I will have a lot to say later.

Viruses are so large that relatively low speeds (20,000 rpm) suffice to cause the particles to traverse the cell in about one hour. I did many experiments every day; reading the plates and calculating sedimentation coefficients became a major chore. In order to reduce the burden and avoid calculation errors, I devised an alignment chart that was analogous to the slide rules that were in common use for mathematical calculations. It was simple to design and construct, based on my knowledge as an undergraduate at MIT. With it, I could produce results very rapidly. Max Lauffer thought the work should be the subject of a publication. But at the Rockefeller Institute of Medical Research, technicians did not publish papers. After some deliberation between Max Lauffer and Wendell Stanley about circumventing policy at the Institute, Max proposed writing a joint paper (Schachman and Lauffer) for submission to the *Journal of Biological Chemistry*. I, of course, was thrilled at the idea, and Max wrote the paper in a way that I could not have done. It then went to the higher-ups at the Institute where the idea of a joint

paper was rejected. They apparently did not like the idea of having one of their principal scientists appear in print as a co-author along with a technician. The plan was aborted, and I was given the option of publishing it alone as long as a note was attached indicating that I was a Technical Assistant. The paper, “An Alignment Chart for the Computation of Ultracentrifugation Results”, did appear in the *Journal of Biological Chemistry*, 143, 395-402, (1942) with my acknowledgement to Max Lauffer. There was a prominent footnote alerting the reader to my status. This problem of publishing reared its ugly head again several years later when I was assisting Lauffer in his studies on the denaturation of TMV. The method being used was very tedious with the potential of large experimental errors, so I initiated some studies on my own based on the disappearance of the turbidity of the solutions as the large virus particles were degraded. Lauffer became very intrigued by the approach and adopted it for the ongoing research. He did a great job and this work was important. When the paper was being written, the issue of authorship was discussed briefly; this time the paper by Lauffer, as the sole author, acknowledged my contribution. I was ecstatic that the technique I had devised proved so useful in a very significant paper.

The Rockefeller Institute had Neanderthal policies at that time; long lab coats were provided for the PhDs and short ones for the technicians. Eating facilities were relatively grandiose for the scientific staff and virtually non-existent for technicians; Max used to kid me that his dog would be more welcomed in the Clubhouse than I would be. Technicians also had to punch a time clock upon arriving and departing. Once again I caused some controversy because I was sleeping on a folding cot in the lab. My home then was in Philadelphia and, because of gas rationing during the war, I spent many nights at the lab in Princeton. It didn’t make sense to punch the clock since I was not leaving and actually doing experiments late in the evening. Both Lauffer and Stanley came to my defense, and my refusal was tolerated despite the objections of an administrator in charge of personnel. I want to add that they were remarkably patient and supportive throughout my struggles. Moreover, my career in science and education would not have occurred without their incredible encouragement and support.

Stemming from my original discussions with Max Lauffer, I wanted to obtain a PhD while working as a technician in his lab. He suggested I try Rutgers University that was not too far away, but I didn’t consider it prestigious enough. Princeton was not interested in part-time students, so I proposed that I spend

summers at the Harvard Medical School where there was a Department of Physical Chemistry with a distinguished group of protein chemists headed by Edwin J. Cohn. Of course, I had an ulterior motive; my girlfriend from my MIT days lived in the Boston area. Through the intervention of Stanley and Lauffer, the Harvard group went along with that arrangement and I was given time off to take courses at Harvard during the summers. As I recall, Stanley forwarded my paychecks from Rockefeller. The first summer was devoted to the famous Physiology course at the Harvard Medical School. In the second summer I enrolled in the Organic Chemistry course taught by Bartlett on the Cambridge campus. After the first of the 2 six-week sessions, Stanley suggested that I return to the Institute because the research on influenza virus was going full blast and there was a need for lots of ultracentrifuge experiments. For me, leaving with only half the course requirement fulfilled was devastating. Bartlett was unsympathetic, but the young faculty member who was going to teach the second half, Robert B. Woodward, was very encouraging and offered to send me brief outlines of his lectures and counseled me to obtain more detailed notes from others who had taken the course previously. He indicated that I could return to Cambridge for the final exam and do all of the lab experiments at the Rockefeller Institute. Stanley kindly gave me permission to take off for one week prior to the final exam in the summer school, so I returned to Cambridge where I studied day and night with considerable help from Woodward and a good friend who was getting his PhD in organic chemistry from MIT. Along with the regular class, I took the final in which I did very well and expected credit for the entire course. But Bartlett was very upset because that young kid didn't follow his advice to come back another summer, and a long battle ensued over my getting credit for the course in Organic Chemistry. I lost the battle despite Woodward's great efforts. As a result Harvard has been deprived of listing me as a PhD. Those two summers had a lasting impact, however. The romance flourished and the girlfriend, Ethel Lazarus, became my wife several years later while we were both serving in the military forces.

In the meantime enrolling in Princeton became possible because there was a shortage of graduate students and they were eager to please Stanley; it was easy to take courses at the university since I was away from my responsibilities as a technician for only a few hours each day and my research was done in the Institute laboratory. More on that will be discussed later. Obtaining a PhD while working as a technician at the Rockefeller Institute was very rare. Moses Kunitz, as an assistant to Jacques Loeb in the New York lab, did it and he

subsequently encouraged his technician while in the Princeton lab to obtain her degree at Rutgers. So I was the third.

But my work at Princeton was interrupted by my going into the navy. When I came back, Lauffer had already left the Rockefeller Institute to go to the University of Pittsburgh, and I sort of became the only physical chemical person in Stanley's laboratory. And I worked in collaboration with Walter Kauzmann who had just become a young faculty member at Princeton. My research on tobacco mosaic virus represented my PhD thesis. I would commute back and forth the five miles from Princeton University to the Rockefeller Institute laboratory, where I did all the research. So I managed to get my PhD that way, with terrific help and enthusiastic cooperation from Walter Kauzmann. I used to claim I was his first graduate student. He denies that, which tells me something about him. By that time, Stanley was negotiating with the University of California to become the Chair of Biochemistry and the Director of the (about to be created) Virus Laboratory. Though I had no knowledge of it, he was planning on taking me with him as a young faculty member. So all of a sudden I arrive in Berkeley, California as an Instructor in Biochemistry, even though I never had a biochemistry course in my life.

Schlesinger: And just remind us of the date.

01-00:13:09

Schachman: This would be 1948. Stanley moved here in 1948. And a few months after his arrival, I left Princeton. I was the last one to leave. There were three of us. Stanley, then Arthur Knight and I were the three people who moved to Berkeley from Princeton.

Schlesinger: Did either you or Stanley talk about the fact that you didn't know any biochemistry?

01-00:13:32

Schachman: No, not really. By that time, I had published a paper on tobacco mosaic virus and a few other things so there was really no great difficulty. When I came here I audited courses in biochemistry and read a lot. And the Princeton PhD program was really very good, because that's how I taught myself a lot of biochemistry. Not only did you have to defend your thesis, you were required to submit twelve propositions that you had to be able defend in front of a faculty group. Since I wanted to learn some biochemistry, I made them all very biochemical. So for example, I read a book on immunology at the time, and designed a proposal for separating isotopes, or d and l isomers by

immunochemical techniques. And the people at Princeton who were on the faculty examining me grew very intrigued, because they had never heard about this kind of approach. It was very useful for me. I read a little bit about enzymes. So essentially, my biochemistry was self-taught, until I arrived here. Then I sat in on courses by Barker and others who were teaching fantastic courses on metabolism. So I gradually picked up biochemistry in Berkeley.

Schlesinger: When you came here as a faculty member, were you beginning to teach biochemistry?

01-00:14:53
Schachman: I began teaching physical biochemistry.

Schlesinger: And what did that encompass?

01-00:14:59
Schachman: That encompassed, for example, the ultracentrifuge, the use of the ultracentrifuge for studying macromolecules. I was lucky enough to have a student who was just arriving in Berkeley, who went around looking for labs to work in. And he showed **impeccable** judgment by choosing me to work with.

Schlesinger: This is Bill Harrington?

01-00:15:18
Schachman: This was Bill Harrington! We became fast friends and began working on the degradation of tobacco mosaic virus.

Schlesinger: So that's the question I wanted to ask you. Was the project that you chose the degradation of TMV?

01-00:15:28
Schachman: That's right. We were sort of trying to probe into structure.

Schlesinger: And now at this point, you knew that tobacco mosaic virus had nucleic acid.

01-00:15:37
Schachman: Yes, by that time it was well known, right.

Schlesinger: But was it known that the nucleic acid was the genetic material?

01-00:15:52
Schachman: It hadn't been proven yet, no. No, that work was ensuing, basically. The famous Hershey-Chase experiment that showed that when phage infected bacteria only the DNA was injected into the bacteria was in 1952. That would be the first real

example. And then later, Fraenkel-Conrat, in Berkeley, and, independently, Schramm in Germany, showed the same thing more or less was true with the plant viruses.

Schlesinger: The experiment that Fraenkel-Conrat did—was that the experiment when he reconstructed the tobacco mosaic virus?

01-00:16:23

Schachman: Well, he had different strains of TMV, so therefore, he was able to reconstitute the RNA from one strain with the protein coat of the other one. He wound up getting the protein coat in the progeny that was characteristic of the RNA, rather than of the protein coat that he used. So that was the beginning.

Schlesinger: Was that done when you were here?

01-00:16:47

Schachman: Yes.

Schlesinger: I remember reading that he didn't believe it at first, or he was very disappointed. Do you remember what people's reactions were?

01-00:16:56

Schachman: Well, I think everybody was quite excited about it. And then he was involved in further experiments. I did a lot of the service work for various people, in order to help them in their research. Generally they were colleagues of mine in the department. Fraenkel-Conrat and Robley Williams were working on this problem. They took the TMV apart and isolated the RNA, and then they mixed it back with protein from the TMV, and they reconstituted intact virus. There was a green house on the roof of the Virus Laboratory, and they ran upstairs to test its biological activity and discovered that it was infectious. So they talked about regenerating active material ("Creating Life"). The truth of the matter was that the RNA itself was active, but they didn't know that. When they assayed the RNA in the greenhouse on the roof, the RNA did not appear to be active, primarily because it was already hydrolyzed. It was inherently not very stable. There is ribonuclease on our fingertips and probably in most of the equipment used in the manipulations. So if you protected it as soon as you took the virus apart and mixed it again with protein, it looked like you were reconstituting life. They asked me, Fraenkel-Conrat in particular, to be a co-author of the paper, and I just felt that I had done some service work characterizing his RNA preparations, his protein preparations, and the reconstituted virus. I declined to be an author of the paper. So it was published as Fraenkel-Conrat and Williams.

(My contribution was acknowledged by them.) The paper attracted enormous acclaim in the newspapers as "reconstituting life", when in fact, that wasn't the case at all.

Schlesinger: Well, let's go back to your own research, then.

01-00:18:30

Schachman: My own research involved Bill Harrington and me working side by side, independently. In those days, faculty members worked with their own hands, and I did for many, many years. So I was working on the degradation of tobacco mosaic virus with detergents. He was working on the alkaline degradation of tobacco mosaic virus.

01-00:19:52

Schachman: He was seeing intermediates in the alkaline degradation, because ultimately, if you had the pH high enough, the virus would degrade into very low molecular weight protein, on the order of 100,000 or thereabouts. If you used excess detergent, there was massive degradation of the virus and there would be no virus left at all. But we saw particles by the treatment with sodium dodecyl sulfate that were about two-thirds the length of the normal rods that we started with. Bill was seeing intermediates at mild pHs. So I began doing reconstitution experiments, where I would mix what I felt were partially degraded material with normal virus. And every time I did this, I saw much more of the partially degraded material than of the intact virus. We couldn't understand that. The question that immediately arose was: is some of the detergent sticking to the partially degraded material? Then when I mixed it with normal tobacco mosaic virus, it was coming off and degrading that. That seemed like a very implausible explanation. So the more we thought about it, the more we were convinced that we were looking at an ultracentrifuge anomaly. At that particular time, there were big arguments going on in the literature about the analysis of serum by ultracentrifuge techniques. More albumin always showed up in the ultracentrifuge than the faster moving globulin. Two people in England, Johnston and Ogston—Ogston was a very brilliant physical chemist—began to look into this anomaly. They finally decided that it had something to do with the ultracentrifuge. It had nothing to do with serum. When you ran mixtures, ordinarily, the area under each of the boundaries produced by the schlieren optical system would give you the amount of the material that was in the mixture. But it turns out that if the faster moving material was viscous, it would cause an anomaly and you saw more of the slower component than you expected. The reason for this is very simple in principle,

although it was complicated for the people originally to work it out. When molecules move in the ultracentrifuge, they move through solvent. If you have slow and fast molecules moving side by side and the fast molecules cause a large increase in the viscosity of the solution, the slow molecules would move slower in the presence of the fast molecules than they would move by themselves. As a consequence of this, in a conventional ultracentrifuge experiment, where you start with a homogeneous solution of two components, the fast ones move ahead, but the slow ones are moving more slowly in the presence of the fast ones than they are moving when the fast ones are no longer there, and they pile up at the back. And therefore, you wind up seeing more of a slower component than was really present in the original mixture.

Schlesinger: Did you say it's because this faster component actually increases the viscosity of this solution?

01-00:22:49

Schachman: This is especially true if the fast component is a viscous component. In the case of tobacco mosaic virus, with this long, rod-like shape, it caused a large increase in the viscosity of the solvent. So therefore, smaller particles would, in its presence, move much more slowly in the presence of those rods than they would when they're by themselves. So that was the reason why we were seeing more of the slower component. Johnston and Ogston figured out the mathematics of this and they wrote this paper in the *Biochemical Journal*. And the paper was critical of the Svedberg Laboratory, which was the pioneer laboratory in the world on the analysis of serum. Many people treated the Johnston and Ogston paper with great skepticism. In fact, they had trouble getting it published in the *Biochemical Journal*. Moreover, it was very difficult to read because the legends to the figures were mixed in print; and therefore, figure one had the legend for figure two, or one-B had it for one-A. It was unbelievable. And Bill and I fought our way through this thing, finally rectified it. And then we realized we had a wonderful problem, a much more exaggerated phenomenon, because we were using tobacco mosaic virus as the fast component, and it was much more viscous than globulin was. So the albumin:globulin ratio was wrong, to be sure; but it was only a few percent wrong. In contrast, in our case, you could get huge amounts of errors because of this viscosity of the faster component. So it was obvious to us immediately that what we needed to do was measure how fast does a slow molecule move in the presence of a fast molecule? Well, this is not easy to do because you can't do it by conventional techniques. So

the answer is very simple. You do a layering experiment. You take a solution of fast and slow components, and you load that halfway through the cell. So the cell is filled from the bottom to halfway up with that mixture. And then you layer on top of that, a solution of only the fast component, at the same concentration as it is in the mixture. Now the ultracentrifuge has a boundary. The boundary is due to the slow component; but fast molecules are in front of it and fast molecules are behind it. So the movement of the boundary only tells you the sedimentation rate of the slow component in the presence of the fast. And that's what you needed to put in the Johnston-Ogston equations to check the anomaly.

01-00:25:13 So we would do the experiments. We'd get down on our knees. We had the ultracentrifuge cells in a little holder. We took a syringe needle and filled the cell halfway filled with TMV and the partially degraded material. And then we would layer on top of that the intact TMV solution. Thus we had the boundary we wanted. We put the cell in the rotor. The rotor was in our arms. Unfortunately, however, we had to turn the rotor through ninety degrees to attach it to the shaft in the vacuum chamber. We did that very quickly. We bypassed the whole machinery. We had this rotor spinning even before the vacuum chamber was closed. And we got some results, but it was quite clear this was an inadequate technique. So that's when I picked up the phone, called Ed Pickels, and I said, "Gee, Ed, I've got a wonderful problem, where I need a cell that allows layering to go on while the centrifuge is spinning."

Schlesinger: Now you have to tell me something about Ed Pickels.

01-00:26:05

Schachman: Ed Pickels had been a student of J.W. Beams at the University of Virginia. They published some of the original papers on air driven ultracentrifuges. This was gorgeous work, in the physics department. Beams was interested in spinning things. And Pickels was one of his best graduate students. After Ed Pickels received his PhD, he joined the Rockefeller Foundation laboratories. Those labs were at the Rockefeller Institute for Medical Research in New York City. They had a wonderful machine shop and, under Pickels' initiative, they built air driven ultracentrifuges. They were not for commercial purposes, but they were in competition, in principle, with the Svedberg oil turbine ultracentrifuges, which were much, much more complicated. And when I was a technician in Lauffer's laboratory and Stanley's lab at the Rockefeller Institute labs in Princeton, I used an air driven ultracentrifuge that was built in the

laboratories of Ed Pickels, in New York City. Oncley had one at the Harvard Medical School. There were about five such air drive ultracentrifuges in the country, compared to roughly twelve or so oil turbine ultracentrifuges. So Pickels was obviously interested in building centrifuges, even though he published papers on viruses and chlorophyll containing proteins and so on. When I was looking around to try to get an ultracentrifuge built for the United States Navy, long before I got my PhD, I was on a trip to the San Francisco area where I met a man by the name of Hanafin who said he'd like to hire me to build an ultracentrifuge. And I told him I couldn't build an ultracentrifuge. I was in a Navy uniform. I had just gotten married, and was on my honeymoon with my wife. And I said, "The guy you should contact is Ed Pickels." And he contacted Ed Pickels, and Pickels joined Morris Hanafin, with two other men, and they started a company called the Specialized Instrument Corporation, which started building the Model E, the famous Model E.

Schlesinger: Is that where the word Spinco comes from?

01-00:28:10

Schachman: That's the word—Spinco, Specialized Instrument Corporation. And they thrived very well. Soon the demand for their centrifuges exceeded their capacity to build them. It was sort of a garage type industry. People in their garages would construct cells, some would build rotors, some would build parts for the optical system. They never had a plant where everything was built in one location. Their small plant was used to assemble all the parts. Ultimately, they knew they needed much more capital, so they sold themselves out to the Beckman Instrument Corporation, and it became the Spinco Division of Beckman Instruments. There they were, in Belmont, California. It was long before they moved to Palo Alto.

So I called Pickels on the telephone and I told him what the problem was. He got excited. Bill and I got in the car, we drove down to Belmont where we discussed a new type of ultracentrifuge cell. What we needed was a cup with a very small hole in it that would fit into the top of the centrifuge cell. The upper solution would be placed in the cup containing such a tiny pinhole that capillarity would prevent liquid from leaking through it. The lower, more dense solution of the two components was in the bottom of the cell. Under the influence of a centrifugal field, the liquid in the cup would be forced through the hole in the cup, thereby forming a boundary in the spinning rotor. And we began joint experiments with Pickels. Everything he constructed was given to me and we tried it in our laboratory.

And the next thing you know, we had a functioning synthetic boundary cell. It was very exciting. I think they patented it, although I knew nothing about patents at that time. We published a paper—I think it was Pickels, Harrington and Schachman I can't remember the order—on the synthetic boundary cell. Bill and I were then able to go back to our anomaly and check the one number that was needed in the Johnston and Ogston equation to prove that we could account for the anomaly in the analysis of mixtures. So then the whole mixture problem was clarified.

Schlesinger: Did you ever get a comment from Johnston and Ogston about this?

01-00:30:04

Schachman: I don't recall whether I did or not. Ogston was a very strange guy. I tried to visit him years later. My first trip overseas was, in fact, to give a talk in Stockholm, at a meeting on macromolecules. I carried with me—it was so new—the synthetic boundary cell that was built in Palo Alto. I think I stopped in England to see if I could visit him. He had left Oxford—I think that's where he was at that time—and was somewhere in London, they told me, playing the piano. Ogston was a very brilliant, creative physical chemist, but in England he could satisfy his tastes and do whatever he wanted. He'd left his university for a while to indulge in playing the piano. I remember taking this centrifuge cell with me to Uppsala on a visit. I wanted to meet Svedberg. I never met Svedberg, but I did spend considerable time with Pedersen who was a colleague of Svedberg's and a co-author of the famous book, *The Ultracentrifuge*, by Svedberg and Pedersen, which I called the Bible. They were thrilled at looking at this cell. It was a very fascinating cell and it had a tremendous impact for quite a while on the field. It was a revolutionary development. But like most revolutionary developments, they have a short half-life.

Schlesinger: Okay, so now we're ready to talk about how this helped you in your research.

01-00:31:41

Schachman: Well, for example, with a cell like this, you could take something as small as sucrose and it was inconceivable at that time that anybody could measure the sedimentation coefficient of sucrose because it has a molecular weight of only 342. We could fill the synthetic boundary cell, half full with sucrose solution, and place water in the cup. So when the rotor started to accelerate in the centrifuge, we formed a gorgeous boundary of sucrose. And

therefore, you could watch that boundary and how it spread with time—that was due to diffusion—and it would move. It would move a trace amount, but it would certainly move, and you could measure the sedimentation coefficient of sucrose very easily. So it became quite clear that the cell had expanded the opportunity to use the ultracentrifuge for smaller and smaller molecules. In those days, there were practically no experiments being done by sedimentation equilibrium. So this was a fascinating example of the potential of the synthetic boundary cell. Another illustration of its use was for determining the size of insulin, a very small protein, of the order of 12,000 molecular weight. We were able to measure the sedimentation coefficient of insulin by using the synthetic boundary cell. So that was fine. But then you ask yourself, how about another application? What would happen if you layered a solution of three milligrams per ml on top of a solution of six milligrams per ml, of the same material? That would give you what I call the differential boundary. What would be the movement of that differential boundary? So it turns out you use the transport equations, the same sort of equations that were used by Johnston and Ogston, and you measured a new sedimentation rate. The movement of that boundary would give you the change in sedimentation coefficient as a function of concentration. So it became another application.

Schlesinger: Why does the sedimentation coefficient change as a function of concentration?

01-00:33:39

Schachman: Again, because of viscosity. At higher concentrations, especially of more elongated macromolecules, the solutions become more viscous, and the molecules “know” about the presence of neighboring molecules. If you ran, for example, calf thymus DNA at one milligram per ml, the sedimentation coefficient would be five Svedbergs (S) or something of that sort. [The term Svedberg was introduced as the unit of sedimentation velocity; it corresponds to 1×10^{-13} seconds]. If you ran DNA at great dilution such as one microgram per ml, the sedimentation coefficient would be 20S. So there was a tremendous concentration effect, because the presence of other DNA molecules slowed down each individual DNA molecule. And that’s essentially what this layering technique could tell us.

There’s another problem that was potentially solvable by this technique and it turned out to be a very, very useful issue in ultracentrifuge practice. The big question that you just raised is: why do molecules sediment more slowly when they’re

concentrated? Well, there are two reasons: one is the one I gave you, the viscosity of the solution is greater at the higher concentration and the sedimenting molecules “sense” the presence of their neighbors. But there is another one. When you force molecules down to the bottom of the cell, if the cell is closed, liquid must be flowing back. Therefore, the question comes up: what is the backward flow of liquid in an ultracentrifuge? Could you use that information to determine the volume of molecules that were being sedimented? Max Lauffer had proposed in a paper what he called a new method for determining hydrodynamic volumes. He would say, “Why don’t we make a boundary of something like sucrose, in the presence of tobacco mosaic virus?” So I’ve got tobacco mosaic virus and sucrose in the bottom of the cell; I layer on top of it tobacco mosaic virus. I now have a sucrose boundary. As the virus particles sediment to the bottom, there must be some backward flow of liquid and the movement of sucrose, which has very close to zero sedimentation rate, should provide a measure of that backward flow. Indeed, you could do such an experiment in this new cell without any difficulty at all. It proved easy to measure the backward movement of the sucrose boundary, and you would say that every time I sediment so many milligrams per ml of tobacco mosaic virus, I must transport backward so much water. And Lauffer thought that such experiments would be a tool for measuring hydration. It turns out, although he titled his paper as a new method, as in most scientific research, there are very few that are new. Because in 1907 or thereabouts, G.N. Lewis, a very famous physical chemist from the University of California here in Berkeley had published a method of determining the hydration of ions through their movement in an electric field. The technique wasn’t available, but he visualized the experiment. And many years later, Longsworth actually did the experiments and found out that it was much more complicated than had been visualized. It depended on the nature of the material that you’re using to measure the backward flow. Different results were obtained with sucrose or D_2O or albumin or some other molecule. But nevertheless, the technique was fantastic. So I had a student (Bob Hersh) work on this problem for his PhD thesis. We wrote a very lovely, complicated paper that had very little applicability to anybody but a couple of specialists, on backward flow in the ultracentrifuge and a method to determine hydration—except we pointed out you couldn’t really determine hydration. It was much more complicated than that. So I published probably about four papers on the application of the synthetic boundary cell. And people were buying it. Spinco loved it because they were selling

ultracentrifuges because of the new synthetic boundary cell. So that really finished that aspect, so why don't I rest for a while now?

Schlesinger: Except that you haven't told me how this has really helped you in understanding tobacco mosaic virus.

01-00:37:36
Schachman: It had nothing to do with understanding tobacco mosaic virus. After that, it explained that anomaly to us. And so my research divided into two parts: one was to continue to work on the structure of tobacco mosaic virus with Bill Harrington and by myself, and also the ultracentrifuge became a tool that we began to use.

Schlesinger: Okay, Howard, let's go on with the ultracentrifuge and your love affair, as you say, with the ultracentrifuge.

01-00:38:07
Schachman: Well, in those days, measurements of sedimentation coefficients in the ultracentrifuge, in conjunction with either diffusion measurements or viscosity measurements, were used to calculate the size and shape of macromolecules. And it was a major triumph, as a matter of fact, when Lauffer did that for tobacco mosaic virus, because he predicted that the virus was a rod-like particle. This was long before—or not long before, but it was before—the electron microscope was available. When Stanley went to the laboratories of the RCA in Princeton, where they had constructed an electron microscope, they saw their first pictures of tobacco mosaic virus, by electron microscopy. It was a rod and the physical chemistry done by using data from the ultracentrifuge represented a colossal triumph.

Schlesinger: I think that was first done in Ann Arbor.

01-00:38:49
Schachman: Well, there were pictures done in Ann Arbor. That might've been true. And of course, that's where Robley Williams came from.

Schlesinger: I know. And Milt (Schlesinger) claimed that he was the one who destroyed that microscope. [laughs]

01-00:38:57
Schachman: Is that right?

Schlesinger: many years later.

01-00:39:01
Schachman: Oh, that's interesting. During the early period of my career in Berkeley, I had another graduate student in the lab, Ping-Yao Cheng, a very talented Chinese graduate student.

Schlesinger: Was he from China, or was he an American Chinese?

01-00:39:16
Schachman: He came from mainland China.

Schlesinger: And that was when?

01-00:39:19
Schachman: So this would be about 1950, thereabouts.

Schlesinger: I wonder how he got out.

01-00:39:28
Schachman: He might have been from a very wealthy family, I'm not sure. There was an interesting political issue, which I can tell you about later, that's relevant to Cheng. We began to work on bushy stunt virus. Bushy stunt virus was a virus discovered in England, and it stunts the growth of tomato plants. It's a very serious problem for agriculture. Stanley brought a leaf in from England, despite the risk that it would cause problems in the state of California with regard to agriculture. The situation with tobacco mosaic virus is very different, since plants seemed to grow very well even though they were infected with TMV. But on the other hand, the bushy stunt virus could really wipe out tomato crops, and there was great concern about it. So we had to be much more careful in handling bushy stunt virus. It was a much more spherical virus. Again, there were no pictures at that particular time.

Schlesinger: But tomato bushy stunt virus, that is the one for which Bernal, Fankuchen and Riley were able to obtain some information from X-ray pictures of powdered crystals in 1938 (!) but obviously at that time could not really interpret anything about the structure.

01-00:40:25
Schachman: That might very well be. And of course, that's the one that Steve Harrison ultimately determined the structure of—a magnificent contribution to crystallography and to virology. So I was interested in the size and the shape of bushy stunt virus. And we knew from its hydrodynamic properties, the dependence of sedimentation coefficient on concentration and viscosity, that it was much more spherical, unlike TMV, which was rod-like. When you used the equations, almost all the equations said that the molecules or the macromolecules must be large compared

to the solvent molecules. So the question comes up, what is large? We didn't know. So bushy stunt virus had a molecular weight on the order of ten million or thereabout, I can't remember exactly. And that seems very large compared to water with a molecular weight of eighteen. But on the other hand, Einstein didn't tell us exactly, for his viscosity equation, or Stokes, for his falling ball experiment, how much larger it had to be. So we decided we would test the applicability of Stokes' law and the Einstein viscosity equation, for these particles. So I went to Robley Williams, who was a colleague at that time, who was then using polystyrene latex particles in the electron microscope, to calibrate microscopes and determine magnification factors. And he was doing a lot of work on it. These are remarkable particles. These are fantastically homogeneous. It was an accident at the Dow Chemical Company that made these particles, because when they tried to reproduce it later to make uniform particles, they had great difficulty—although ultimately, they overcame that difficulty. So Robley gave me some polystyrene latex particles. And I said, "We'll measure the sedimentation coefficient." Their sedimentation coefficient was on the order of 2500S, compared to 130S for bushy stunt virus and 190S for tobacco mosaic virus, and 0.2S for sucrose. So that was fine. It was very easy to measure the sedimentation coefficient. But if I want to test Stokes law, I need to know their density. We knew that it couldn't be very dense, so we took H_2O-D_2O mixtures. D_2O has a density of 1.1 g/ml. So you could sediment the latex particles in increasing concentrations of D_2O. We did separate experiments with 10% D_2O, 20% D_2O, 30% D_2O and so on. And ultimately, you had enough D_2O so that the particles floated instead of sedimenting. If you plotted the sedimentation coefficient versus the density of the H_2O-D_2O medium, you could, by interpolation, not extrapolation, determine exactly where they wouldn't sediment at all. So we could measure the density as very close to 1.05 g/ml.

Schlesinger: They were really very light.

01-00:43:15

Schachman: Absolutely. This technique permits measurements to about 4 significant figures. They were very light, you're absolutely right. That's a very crucial part, because it was mostly detergent that wrapped around the polystyrene that caused their density to be very low. So they were nice round, homogeneous particles. And we could then check Stokes law and see whether that matched the results from the electron microscope, because Robley Williams had told me exactly how large they were in his

microscope. Then we began to use the same particles for testing the Einstein viscosity equation. So we were able to prove experimentally that the intrinsic viscosity (2.5) that Einstein had predicted for spherical particles was correct. Whereas we were always getting 4 to 5 for bushy stunt virus, when we did the measurement with polystyrene latex particles, we obtained 2.50. So we wrote a paper in the *Journal of Polymer Science*, proving that in fact, with polystyrene latex particles, both the Einstein equation gave the right result, and the Stokes law also yielded the right result. Therefore, the explanation for bushy stunt virus was that the particles were hydrated, and they had a viscosity much higher, twice the viscosity you would've gotten if they were anhydrous particles. Now, that's interesting in and of itself. Because when Einstein wrote his paper in 1905 on viscosity, he was dealing purely with theoretical considerations—pure hydrodynamics. He then asks in that paper, "Well, do I know whether it satisfies experimental data?" He looked in the literature, and the only thing he could find was data on sugar (he certainly did not know at that time that sugar was sucrose). Nobody knew the molecular weight of sucrose. There were still arguments about the existence of molecules. And when he did that, he found out that the experimental viscosity of the sugar solutions was about twice the viscosity that he had predicted. Now, a theoretician with less confidence would've said, there's something wrong with the theory; but he had a lot of confidence. He proposed—unbelievable in 1905—that the particles in solution occupied a larger volume than the anhydrous sucrose molecules—or sugar molecules, he called them—because they were hydrated. It was unbelievable prescience. Most people would've said, "Gee, he added a fudge factor; he had to fix the experimental data to match the theory." [laughs] But the truth of the matter was, he made a prediction or a speculation as to why the experiments didn't fit the theory or the theory didn't fit the experiments, and his speculation was absolutely right. And it was validated many years later.

Schlesinger: But he was still alive at that time. I don't know if he still remembered or cared.

01-00:45:50

Schachman: No, he didn't care, I'm sure. So that was a nice piece of work that Cheng and I did and we were very happy with. But that was interesting in and of itself, because as I've already told you, if you get the D_2O concentrated enough, you could get the material to go backward. So then we say, why don't we get the D_2O concentration just enough so that they are still sedimenting, but very slowly. That was okay. The sedimenting boundary

showed up very clearly. But you had to operate the ultracentrifuge at 60,000 RPM to do this, because you had made the difference in density between the particles and the medium very, very small. Close to zero! But now the compressibility of liquid at the bottom of the cell made it more dense, and the density of the water:D_2O became higher than the density of the polystyrene latex particles. So therefore, in one experiment, particles at the top of the cell were sedimenting, and particles at the bottom of the cell were floating. And then you ask, well, that's very simple; we'll now calculate the floatation rate and see if it matches with the theoretical equation. It didn't match! Why didn't it match? Because the particles themselves were being compressed! So in this second paper with Ping-Yao Cheng we had essentially described a compressibility-measuring instrument. Namely, we could measure the compressibility of particles at the bottom of the cell, compared to the top of the cell, because of the centrifugal field. You will remember that at 60,000 RPM, the centrifugal field is 300,000 times gravity and the pressure is about 250 to 300 atmospheres. So under those pressures, particles as well as liquids were being compressed. So it became an interesting problem. But it was a forerunner of something that happened much later, that you could see particles moving centrifugally and centripetally in the same experiment. This became very relevant to DNA and the Meselson-Stahl experiment many, many years later, with cesium chloride gradients.

Schlesinger: But where did you publish this paper?

01-00:47:58

Schachman: We published one in the *Journal of the American Chemical Society*, and the other paper was in the *Journal of Polymer Science*. And those were the journals I would use in those days.

Schlesinger: I was going to say they would not be so interesting to biologists.

01-00:48:10

Schachman: The biologists would never know. I used to claim that that they were among the most interesting papers I have ever published. But, of all the papers I published, they did the least for my reputation. Nobody could care less. It was a fascinating experience, because in one week, we had done the whole experiment. We calculated all the parameters we needed, and we were able to show what was going on in a week of experimentation. You could predict before you started the experiment that this is what you are going to find and this is the

kind of result you ought to get. And it was fun, but as I say, nobody could care about that because it was so abstract a concept. And I'm sure it's gotten practically no recognition anywhere, even though I'm thrilled by the experiment. So that was that.

Schlesinger: It is January 21, 2008 And Howard, I know you wanted to add something about synthetic boundary cells so why don't you go ahead and tell me.

01-00:49:19

Schachman: Right. At the time we wrote up the paper on the synthetic boundary cell that was the work of Ed Pickels, Bill Harrington and myself, I became aware of the fact that such a cell had already been built by a man by the name of Gerson Kegeles, who at that time was at NIH. He was a sensational physical chemist and a wonderful person. And he had this idea to build such a cell based on the backward flow paper that he had read by Max Lauffer—very different from the motivation that led us to the design and construction of our synthetic boundary cell. Kegeles (Keg to me and all his other friends) left NIH and went to Clark University, a very small school, and when he went to Clark University, he immediately wrote a grant application that went to the National Science Foundation. NIH, at that time, was not funding very much research yet, and NSF was the logical repository for funds. The proposal went to the Biochemistry Panel, of which H. A. Barker, who was a senior colleague and a wonderful biochemist on the Berkeley campus, was a member of that panel.

I was on another panel, the Molecular Biology Panel, as a young faculty member. There were very distinguished senior scientists on it, and I was thrilled to have this opportunity to work with men who were my heroes in science. Barker got this proposal. He came to me and he said, "Gee, Howard, I got a crazy proposal here from a young guy who is asking for enough money to buy an ultracentrifuge," which in those days, cost about $25,000. That was a big grant for NSF. And he said, "Would you mind looking at it?" It's perfectly legitimate for people who recognize problems in evaluating grant proposals to talk to one another confidentially. He knew that I was on another panel of the NSF, so it was perfectly appropriate to get my opinion. I read the proposal by Kegeles. It was terrific. He was asking for funds to purchase an ultracentrifuge based upon this one idea. And I recommended enthusiastically that NSF fund it, so Barker would write up his evaluation. Because not only was

the guy terrific, it was a very creative idea and he needed an ultracentrifuge. And he got the ultracentrifuge at Clark University. So when I was about to publish, I remembered all this. So I immediately sat down, I wrote a letter to Kegeles—there was no email in those days—[laughs] and told him, “Hey, Keg, I’m getting ready to publish this paper. Here’s a copy of it. I recall that you had already had this bright idea a long time ago, though your cell was very different and your motivation was very different. But I would hate to see you devoid of the recognition for the priority that you had for this, and I want you to go ahead and publish it.” He wrote me back a very sweet note. It said, “Howard, I have no data, compared to what you have, and I have no basis for publishing anything.” I responded, “But you’ve got to publish something.” So he put together a picture of a boundary that he’d formed, and a very small write up, and he got the note published. I felt terrific. It was my first, personal experience dealing with ethical behavior in science, and I’m very proud of what happened in those days. And this interview gives me an opportunity to put this story on the record.

Schlesinger: Okay, Howard, now I think we’re ready to turn to a subject that I enjoy talking about, and that’s virology.

01-00:52:49

Schachman: Okay, right. Well, it’s obvious by that time, from our discussion so far, that my research was divided into two parts. One was working on the ultracentrifuge, which was motivated by a problem I had over tobacco mosaic virus, and the other part was studying viruses per se. And in the course of my activities as a young faculty member, I would do research, experiments for various colleagues. So Arthur Knight was studying rabbit papilloma virus. And every once in a while, he’d give me a preparation to look at, and I would do a few sedimentation velocity experiments and give him the results. He was interested in seeing how homogeneous it was. So I began to become interested in rabbit papilloma virus, especially since J.W. Beard, who was a distinguished virologist at Duke University, had published two papers on rabbit papilloma virus. The first one was with Hans Neurath, a very distinguished physical chemist. They talked about the size and shape, they did diffusion measurements and all sorts of very sophisticated—in those days, sophisticated—ultracentrifuge experiments and they published a paper on the size and shape of rabbit papilloma virus. A couple years later, Beard comes along with a second paper. Neurath was not on this paper. And this paper raised many issues over the fact that the virus in these preparations varied tremendously in sedimentation coefficient. It bothered

him no end! By that time, he was doing sedimentation experiments in various solvents and was trying to measure hydration. He came to the conclusion that his previous paper was all wrong! He wrote, “If this papilloma virus is assumed to be molecular, variation in experimental data is difficult to explain. On the contrary, variation among living organisms is to be expected.” That’s his language. And then he goes ahead and says, “The conclusion can not be evaded, however, that the properties and behavior of the papilloma virus revealed in molecular micrographs and in the present work are much more closely similar to those of the viruses mentioned above—vaccinia virus, influenza virus—and to living matter in general than to the characteristics expected of molecules.” So it was a very blistering criticism.

Schlesinger: Where was that published?

01-00:55:03

Schachman: This was published in the *Journal of Biological Chemistry*. He was a wonderful guy. As I used to say, his pen dripped blood, because he was a very critical individual, but he was as critical of his own work as he was of somebody else’s work. Rabbit papilloma virus is obtained by trappers in Kansas and in other parts of the country where they trap rabbits. When they see a rabbit that has a bunch of warts on the skin, they skin the rabbit, and they take the skin with the warts on it and they drop it into a bottle provided by scientists in various universities. These bottles contain glycerol as a preservative. Then they ship these bottles, sell them, to various laboratories like Beard’s laboratory in Duke or Knight’s laboratory in Berkeley, California. So I went to Knight when I saw the second paper and I said, “Gee, Art, when you isolate rabbit papilloma virus do you use more than one bottle?” He said, “Of course.” He said, “There’s very little virus in these warts, I need many bottles to give me a preparation with a couple of milligrams per ml.” I said, “So therefore, not only do you have many bottles, but you will have many skins from many rabbits.” He said, “Right.” So I said, “Well, how, then, can one talk about the virus being an organism and varying in size?” I began to study it by myself, in some detail, with materials supplied by Art Knight. And I found the virus preparation had impurities in it. The more I centrifuged the preparations to remove the impurity, the higher the sedimentation coefficient went.

Schlesinger: So was this preparative centrifugation?

01-00:56:43

Schachman: Yes! To purify it more I would put the preparation in a preparative centrifuge, spin down the virus and throw away the supernatant, which had the slow moving components in it.

Schlesinger: But you pelleted the virus, you didn't do something that we would do now like a sucrose gradient?

01-00:57:00

Schachman: Yes, I centrifuged the virus to the bottom of the tubes as a pellet, and then resuspended it, and repeated that procedure several times in a preparative centrifuge. It was an unsophisticated technique. This was in the 1950s, [chuckles] early 1950s. And the more I purified the virus, the higher the sedimentation coefficient became. So I sat down and wrote a paper by myself. My conclusion in my paper was very, very different from Beard's. And let me see if I can find what I said at the end of the paper. It's published, of course, in the *Journal of the American Chemical Society*, which is not a very significant journal for papers in virology, so virologists wouldn't ordinarily see it. So I wrote:

> "Thus it appears that most of the preparations of rabbit papilloma virus studied thus far contain virus particles of essentially uniform physical properties and variable amounts of an impurity, rather than virus particles which varied in their physical properties from preparation to preparation."

This was rather significant, because there were still ongoing arguments about the nature of viruses. And the classic joke phrase that came from Tom Rivers, whom you know about, was that it's clear from Stanley's work that we can differentiate between "a virus being an organule versus a molecism." So I published this paper in the *Journal of the American Chemical Society*, and I received a very sweet note from Beard, essentially saying, what a fool he was [laughs] to publish what he did. And he was very happy to have it straightened out. So he was not a man that bore resentment. He was perfectly willing to acknowledge his own mistakes. And I never referred to any mistakes on his part, in my paper. So that was my first experience. This problem came back again—

Schlesinger: Let me just ask one question. Were the impurities degraded virus, or just junk?

01-00:58:56
Schachman: We don't know. They're just tissue components. See, when you sediment something essentially through solutions of nucleic acids, they're very viscous.

Schlesinger: Right.

01-00:59:09
Schachman: So if you have a lot of them present, it will slow down the virus like crazy. And therefore, you get a very low sedimentation coefficient, 260S. When you start removing that viscous, slow component, you're sedimenting the virus by itself now, it went way up to 295. And that was really the explanation for it. It had nothing to do with the virus changing. It was really the dangers of running materials that are impure. So the problem came home again many years later, with—not many years, several years later, with tobacco mosaic virus. That was even more complicated. And we're getting out of time scale, but that's okay; it's relevant to put them together.

Schlesinger: Okay, but then tell us what many years later is; what are the dates?

01-00:59:52
Schachman: Oh, probably 1955, '56, something like that.

Schlesinger: But these papers were published when?

01-00:59:56
Schachman: This paper was published in 1951. And then I published later papers in around '56, '57, on the homogeneity of tobacco mosaic virus. As I've indicated earlier, when you have long, elongated particles, the sedimentation coefficient varies tremendously with concentration. So you have to do experiments at various concentrations, and then take your data and extrapolate the sedimentation coefficient versus concentration curve to go to infinite dilution. And because of the dependence of sedimentation coefficient on concentration, the boundaries become artificially sharp. That's perfectly obvious, if you think about it. Let us imagine I put three milligrams per ml of tobacco mosaic virus into the ultracentrifuge cell. The front end of the boundary is at three milligrams per ml. The back end of the boundary is at a tenth or a fraction of a milligram per ml. So if molecules go faster at a tenth of a milligram per ml than they do at three milligrams per ml, the particles that fall behind because of diffusion don't really fall behind, because they sediment faster and catch up again. And it's what I call the artificial sharpening. Now, there were a lot of people who were

skeptical about artificial sharpening, so I wrote a paper by myself, again, on this subject. I did the experiment with a solution of about three milligrams per ml of TMV, and I ran the centrifuge at a speed appropriate to bring the boundary out about one-third of the way through the cell. This is about 20,000 RPM. I then slowed down the centrifuge to about 5,000 RPM, at which time the movement of the boundary would be very, very slight because the centrifugal field was much lower. That allowed the boundary to spread because of diffusion. After a prolonged time at the lower speed I turned the centrifuge back to 20,000 RPM. By the time the boundary moved a few millimeters, it had already sharpened. So you could demonstrate sharpening by eye, as well as conceptually. And this becomes very important later with DNA, which has a colossal dependence of sedimentation coefficient on concentration. So I knew there was artificial sharpening, and I knew that the boundaries of tobacco mosaic virus would be very sharp, and it would be misleading. They could have broken particles in it and you would never know. But by that time, Bill Harrington and I and Trautman and Schumaker had already exhaustively studied the Johnston-Ogston effect. So I knew that I could use the Johnston-Ogston effect to magnify materials at the bottom. During that period, Robley Williams and I used to have wonderful discussions about the relative merits and deficiencies of our respective techniques and tools. I would point out the potential defects of using electron microscopy, and he would be critical of interpreting data from the ultracentrifuge. At that time, he was a relatively new professor but more distinguished [laughs] and older than I. He had been newly appointed to the faculty, recruited from Michigan, and was doing superb, ingenious electron microscopy. I would argue with him that all these broken rods you see in the electron micrographs were because they broke during the drying process. They were not present in solution, according to me. And he said, “Well, you—

[End Audio File 1]

Begin Audio File 2 01-21-2008.mp3

02-00:00:10

Schachman: So Robley and I used to have a wonderful time arguing about the relative merits of his technique versus my technique and the

perils of his and mine. He was a superb electron microscopist and he was very upset about the people who were publishing what they wanted to see, rather than what was present. He used to complain about it bitterly and I would complain similarly about abuses in the interpretation of ultracentrifuge experiments. So we used to bet on experiments. I said, “I can detect 1% of broken rods in a preparation of TMV by exploiting the Johnston-Ogston effect. Therefore, there couldn’t be all the broken rods that you see in solution. They must’ve been artifacts that stemmed from the drying process.” So he did some absolutely magnificent experiments in which he sprayed microdrops with polystyrene latex particles in them—they would have very few particles in a drop—and he would see eight particles that were 3,000 angstroms long, then a particle that was three-quarters of that, and one that would be a one-quarter length, another particle would be two-thirds of 3,000 angstroms and a one-third piece. So he was able to show that when he had the broken rods, they all added up to an integral number of intact rods. So I think we published simultaneously, in two separate papers, that the ultracentrifuge showed that TMV particles were homogeneous, and the electron microscope showed that the broken particles were artifacts that occurred during the drying process. That took care of the old argument about tobacco mosaic virus being very heterogeneous with some people saying that the particles varied in length. One expert who was very critical of Wendell Stanley said that the molecular weight of tobacco mosaic virus lies somewhere between zero and infinity. And I used to joke that he’s the only man who hasn’t had to retract his conclusion. But the point is that TMV is homogeneous, just as rabbit papilloma virus is homogeneous. That put to rest the argument that these viruses were growing and were the equivalent of organisms. That takes care of that phase of my work.

02-00:02:12

So in the course of my using the centrifuge to help investigators, I called Art Pardee and Roger Stanier one day and I said, “Hey, tell me, you guys, what is the nature of the milieu of *E. coli*? How are particles or molecules organized in *E. coli*?” Nobody knew. So I said, “Gee, why not let the ultracentrifuge provide its vision of an extract of *E. coli*.” So they began grinding up *E. coli* with alumina in a mortar and pestle.

Schlesinger: So this is now in the late fifties, is that right?

02-00:02:47

Schachman: In the early fifties.

02-00:02:50
Schachman: So we took extracts of *E. coli* and put them in the ultracentrifuge. As soon as I did, I saw two very sharp boundaries, one of about 30 Svedbergs, one of 50 Svedbergs and then trailing behind, a very, very sharp boundary which was clearly identifiable as DNA. So Pardee, Stanier and I got together and we did an exhaustive study on what I called the macromolecular organization of *E. coli.*

Schlesinger: This was before ribosomes were known?

02-00:03:18
Schachman: This is the discovery of ribosomes! That's right. The only evidence available at that time was some pictures by electron microscopy and a paper by Luria. But the real demonstration of ribosomes, essentially, was that paper by Schachman, Pardee and Stanier.

Schlesinger: At this point, people didn't know how proteins were being synthesized.

02-00:03:40
Schachman: Absolutely right, nothing was known. Right.

Schlesinger: And when you were doing the studies in the ultracentrifuge, you also didn't know what these particles were composed of, because you weren't looking at optical density.

02-00:03:54
Schachman: Right, just schlieren optics, and therefore, we were observing refractive index gradients. Changes in refractive index are relatively speaking independent of chemical composition. Proteins, nucleic acids, salts and sugars give approximately the same increase on a weight basis.

Schlesinger: So all you knew was that they were particles, but you didn't know what they contained.

02-00:04:02
Schachman: Right. But Pardee then began to do the chemistry, I was doing the physical chemistry, and Stanier was doing the microbiology. So we immediately found out that the particles were ribonucleoproteins, and we then knew where most of the RNA was. We knew there was also some RNA in small molecules and that turned out later to be the tRNAs. Then we considered the DNA; we recognized it as a very sharp boundary. A few years later, Chao, a postdoc in my laboratory, began studying yeast. And it turned out we discovered the magnesium effect. The 30S and 50S went to 80S, when you added the magnesium

ion. So that was a major discovery of the magnesium effect on these particles.

Schlesinger: And that was done in yeast?

02-00:04:47
Schachman: That was done in yeast.

Schlesinger: And then did you go back and look in *E. coli*?

02-00:04:49
Schachman: No, we didn't. We knew these particles had to be involved in protein synthesis. I tried to interest one of my good friends in studying protein synthesis. He was a postdoc in the lab, Ken Paigen, who went on and became a distinguished scientist in other fields. He had come from Borsook's lab at Caltech, so he knew about protein synthesis. But nobody knew about ribosomes yet. I said, "Hey, Ken, why don't you go work on this?" But he didn't. And I didn't, either. It would've consumed the rest of my life to work on ribosomes. And it's ironic, because the people who work on ribosomes and who did all this gorgeous structure work have no idea that ribosomes were discovered [chuckles] by ultracentrifugal analysis of *E. coli* extracts.

Schlesinger: And I'm trying to remember, who were the first persons to show that ribosomes were involved in protein synthesis?

02-00:05;37
Schachman: I don't really know. Mary Petermann did experiments on animal cells, and found very similar particles in animal cells, but I'm not sure who then picked it up and found that they were involved in synthesizing proteins.

> In an article entitled Proteins in Five Dimensions published in *Protein Science* (3:1136-1139, 1994) Art Pardee wrote the following: "Ribosomes were isolated in 1952 by Mary Hamilton and Mary Petermann in animal cells and by Howard Schachman, myself and Roger Stanier in bacteria. Discovery of these multiprotein complexes brought the realization that there are organized, biochemically active entities larger than individual proteins but smaller than mitochondria and chloroplasts then visualized in the microscope. Paul Zamecnik and Mahlon Hoagland's group showed ribosomes to be machines for protein synthesis."

Schachman: Jim Watson got very interested. Watson thought this was a major discovery, and he then worked with Tissiéres on these particles.

So one day, Roger Stanier—he studied a whole bunch of organisms and we were covering the waterfront. He was the microbiologist. One day he said, "Well, let's look at a photosynthetic organism." He brought me an extract of *rhodospirillum rubrum* and it's dark purple. You couldn't see through it. So I said, "Roger, where are the chromophoric groups?" He said, "Well, they're probably attached as chlorophyll-like compounds to small molecules." I said, "Well, then I won't be able to see the particles as they sediment through the cell, because the solution is so opaque. But that's okay. I'll spin down the particles in a preparative centrifuge, if that happens, and then we'll re-suspend them and I'll be able to see whether we have the same 30 and 50S particles in *rhodospirillum rubrum*." As the ultracentrifuge rotor was accelerating, I was watching through the viewer in the optical system and I saw the color boundary, the opacity, disappear. It was migrating much faster than 30S and 50S ribosomes. It was quite clear; I could follow that and I did follow it, and I got a sedimentation coefficient of about 200S. These were isolatable, and we isolated them. We called them chromatophores. So we showed that the pigments in this organism were not attached to small molecules; they were built into another particle and a chromatophore in microorganisms is equivalent to the chloroplast in higher plants. So these studies led to two major papers, one with Pardee and Stanier in *Archives of Biochemistry*; and the other was a paper in *Nature*, on the chromatophores. They both, of course, generated a huge amount of work afterwards. I always joke about this because I wasn't interested in that type of research. In these investigations, it was biology that was interesting, whereas in my compressibility paper with polystyrene latex particles I was interesting and had performed a fascinating experiment [laughs] and the sample was uninteresting.

That paper with Pardee and Stanier, of which I was the senior author, was obviously the one that got me promoted to tenure on the Berkeley campus.

Schlesinger: What volume could you put in the ultracentrifuge?

02-00:08:16

Schachman: About three-tenths of a milliliter. So it didn't require much material. And you could operate at a few milligrams per ml.

Schlesinger: But you couldn't use it preparatively, then. What did you do to isolate the chromatophores?

02-00:08:34

Schachman: To isolate them, then we went to the preparative centrifuge and spun them down, and threw away the supernatant, which now contained the ribosomes. We could pick the appropriate speed just to spin down the chromatophores. Chromatophores were comparable in size to tobacco mosaic virus, the sedimentation rate for the virus is about 200 Svedbergs. So Stanier was ecstatic about all this. The papers attracted a huge amount of attention among microbiologists. We had found that *E. coli* wasn't just a soup containing a bunch of protein molecules; instead it had organized particles within it. I was annoyed because I felt—even though I was not biologically oriented myself— that we ought to find out how the DNA is organized. I didn't believe it was a free molecule. When you work with DNA in milligrams per ml in the centrifuge, it's a vertical line. The boundary is so hyper-sharpened by the phenomenon that I told you about a little while ago, that we didn't know whether there was a nucleus. I was equipped to ask the question: is there a nucleus in *E. coli* that contains the DNA? But I never followed that up.

It was a very fascinating period. But by that time, I was intrigued by the artificial sharpening phenomenon, and I became interested in DNA. This is before the Watson and Crick structure came out. And I had a postdoc—

Schlesinger: You became interested in DNA because you saw it in the *E. coli* extracts?

02-00:10:16

Schachman: Right. And it was a vertical line, and people were talking about how homogeneous it was. And nearly all the DNA studies in those days were performed with calf thymus DNA. So it was a major extraction process to get the DNA out of the calf thymus.

Schlesinger: And I think the calf thymus DNA was not as large as was later found with bacterial or viral DNA and it was very heterogeneous.

02-00:10:34

Schachman: But we didn't know it was heterogeneous at that time. In fact, Ogston, who was a brilliant physical chemist, remarked how homogeneous it was because it gave you a vertical line boundary in the ultracentrifuge. By that time, I already knew that that could be an artifact, because of the artificial sharpening phenomenon in sedimentation velocity experiments. So I picked

up the telephone and I said to Ed Pickels—for many years I called him in Palo Alto, in those days, he was still in Belmont, California. I'm almost sure that Spinco wasn't part yet of Beckman. I said, "Hey, Ed, I've got this phenomenon. I want to work with DNA and milligrams per ml just gives you such artificial sharpening in the ultracentrifuge that it's hopeless; I'll never get anywhere doing physical chemically with that. We have to work in micrograms per ml. Fortunately, the ultraviolet absorbance is such for DNA that you could work in micrograms. But that means I need an absorption optical system. So would you go back to the drawing boards and get the design out of Svedberg's book?" It was already there. The absorption optical system had been built by Svedberg originally, and then discarded because it was laborious to turn the pictures into diagrams of concentration versus distance. "Would you rejuvenate that system and see what happens?" He already had anticipated the idea of having an extra optical system. So he had holes in the vacuum chamber, above and below, and he had just opaque metal plates covering the holes.

Schlesinger: You say this is before Watson and Crick, so this is before '53.

02-00:12:03

Schachman: That's right. So he said, "Okay, I'll put one together." He used an additional light source that emitted ultraviolet light, a bromine-chlorine filter, quartz windows at the top and bottom of the vacuum chamber and sensitive photographic film. He threw together an absorption optical system in no time flat. And I got it, the first one! It was experimental. So I began to study DNA, calf thymus DNA, at great dilution. And we did physical chemistry. Arthur Peacocke was here, a postdoc from England. He was a physical chemist.

He was working in my lab as a postdoc. He's an interesting person and I'll tell you about him in a second. So we published a paper on the physical chemistry of DNA as relatively elongated stiff rods. If you work out the theory for sedimentation velocity experiments, you find that for long rods the sedimentation coefficient is essentially independent of length; it provides a measure of the diameter of the rods. We actually calculated the diameter, even though we didn't think they were necessarily straight long rods. But even if they were bent, it wouldn't have mattered that much. It turned out that thickness we calculated was much too large for a single stranded molecule, but we had no idea of what DNA looked like in those days. So we published a paper in *BBA* on the physical properties of DNA. By then, I

began to push the absorption optical system, and was able to show that the DNA was unbelievably heterogeneous.

Schlesinger: Is this calf thymus DNA?

Schachman: Yes, we were working almost exclusively on calf thymus DNA.

Schlesinger: But this was after you did the *E. coli* work, though.

02-00:13:42

Schachman: Yes. And I should've switched over, and I didn't. That's exactly right. I wasn't thinking in terms of biological ideas. So at that time, shortly after we published the paper on the thickness of the DNA, the Watson and Crick proposal came out. And I said, "Gee, that's interesting. If it's a double stranded molecule, we ought to be able to prove that by the ultracentrifuge, because if you use deoxyribonuclease to cleave it, you would have to cleave both strands." We thought the enzyme cleaved only one bond at a time. You have to cleave it in two places, one at one strand and a second opposite or near opposite in the other strand, and then it will fall apart. I remember Stanier being very upset that I was doing these experiments, because he said, "The Watson and Crick idea is so terrific." I said, "Of course, it's terrific. I'm not disputing that. But I would like to get evidence in solution that would support it, or raise other serious questions." So Chuck Dekker and I began to work on DNA together. He was a nucleic acid expert, a colleague of mine in the department, and a very good friend. And we had a wonderful time together. We wrote a paper, which I'll talk about in a second. But before I forget Peacocke, let me point out about Peacocke.

02-00:15:08

Peacocke then went back to England, where he was doing physical chemistry at some university. He then became very interested in the church and he became a minister. He began giving lectures on religion and things of that sort. He had been a good scientist but he gave up science completely. About five years ago, Peacocke won the Templeton Award. The Templeton is equivalent to the [chuckles] Nobel Prize, and it's for the combination of religion and science. Peacocke came to Berkeley and gave a talk. I visited him in England, and he came with his wife to the hotel, and we had a wonderful time. But he gave up science and became a leading minister and won a prize worth almost a million dollars. [laughs]

Schlesinger: But he must still have some relation to science, if he won the Templeton Prize.

02-00:16:02

Schachman: Fortunately, he was a minister who believed in evolution and in science, so he would link science and religion without saying they're incompatible one with the other. So that's why he won that prize. That prize has been won by a lot of very distinguished guys, but most of them are purely religion people, rather than a combination of science. I think Freeman Dyson, a distinguished physicist at the Institute of Advance Study, won it a few years ago.

02-00:16:29

Schachman: Dekker and I began to work on the enzymatic breakdown of DNA. Dekker knew about the work of Gulland and his collaborators. Gulland was a biochemist in England who was doing titration experiments. All this is with calf thymus DNA, of roughly five million molecular weight, by the time it is purified. Gulland had concluded, based on his titration experiments, that there were a significant number of mono-esterified phosphate groups in DNA molecules of molecular weight 5 million. In other words, there had to be phosphate groups at the end of a chain, or at the end of branches or something of that sort. Those data from a very reputable scientist were in the literature and were totally ignored by Watson and Crick. Either they ignored it because they didn't know about it, or they ignored it because their intuition told them something was fishy. This is a fascinating aspect of science—when do you ignore data? But Dekker and I used that information because we considered it reliable. We said to ourselves, how could you have a double stranded structure that would have only two ends and still find many phosphate groups that were only mono-esterified?

In a continuous chain every one of the phosphates had to be di-esterified, so if you want to accept the results from Gulland's laboratory, you have to account for many ends. One possibility was there were branches. Such a structure would require that there be tertiary phosphoryl linkages and that seemed highly unlikely. So we said, "Why don't we see what the molecular weight would be when we heat denatured the DNA." I was doing viscosity and sedimentation experiments. Dekker was doing all sorts of phosphate titration experiments. We were doing it with our own four hands. We came to the conclusion that when you heated DNA of five-million molecular weight, instead of it going to half of that, two-and-a-half-million, it went down to 50,000. Our data were compatible with the Watson-Crick structure that the DNA was double stranded, but that it had nicks all over the DNA strands and those nicks were responsible for the fact that it would fall apart into small pieces.

Verne Schumaker came into my lab at that time. He had been working next door in the Donner Lab, and he became a postdoc. I had a student by the name of Glenn Richards and I said, “Gee, if this is true, we ought to be able to measure the number of strands by looking at the number of hits necessary with nuclease to cause it to fall apart.” This would be almost like using radiation to determine the number of hits on a double stranded structure. So Verne Schumaker played with the theory. He was a good mathematician and we then studied the kinetics of the digestion of DNA by deoxyribonuclease using sedimentation and viscosity. By that time, they (Richards and Schumaker) were doing a lot of the experimental work, instead of me doing all the experimental work with Chuck Dekker. We calculated the number of strands from these studies of the kinetics. It was quite clear that we needed more than one hit to break the DNA, so it couldn’t have been a single stranded molecule. But when we did it, we came out with 1.5 strands. I joked that put us halfway between Watson and Crick, and that’s not a very good place to be. But how do you get 1.5 instead of two? If you already have preexisting breaks, then hitting at one strand somewhere near a preexisting break on the other strand would cause the molecule to fall apart. In that case you would not require two attacks. According to the theoretical equations, the numbers of breaks in a five million molecular weight DNA would’ve been only seven in order to account for going from 2.0 down to 1.5. So we published that. Paul Doty did the same type of experiments with Charlie Thomas and they got similar kinds of results. When Dekker and I wrote the paper we talked about a molecule with interrupted strands and said, “It is conceivable that the calf thymus DNA has already been exposed to nuclease during the isolation procedure, so it could’ve had cleaved strands before we began the studies.” And of course, that turned out to be the explanation. We weren’t using *E. coli* or phage DNA at that time. All the later studies were performed with viral or bacteria DNA where the isolation procedure is much more direct and the purified DNA had not been so exposed to nucleases. As a result the two strands had much fewer (if any) breaks and the double stranded structure has survived. Also the DNA molecules from bacteria or phages are much larger than those we studied from calf thymus.

I’ve used this story when I’ve talked to Congressional people about how people’s intuition—like Watson and Crick’s, or Pauling’s on the alpha helix—would be to ignore some data because it’s too complicated and can’t fit it into a picture; build a simple model first, and then modify with more information later. It’s a very useful pedagogical discussion.

Schlesinger: You'd use it with Congressional people to say that it's okay to ignore data?

02-00:21:16

Schachman: That's right, over the ethical issue. When the word misrepresentation came up, I used to tell them about Watson and Crick leaving out this information because their intuition told them the DNA preparations were not good, and therefore, including these data will only complicate the story; let's build a general picture first, and if we have to, we'll modify it later. Pauling, for example, used the wrong enthalpy for a hydrogen bond, when he talked about the alpha helix. If he had used the one that was correct, he would've gotten into unbelievable complications. But his intuition told him, start with a simple story first, and then we'll modify it as it goes on. Congressmen were very impressed. I said, "The word misrepresentation or selection of data can not be part of your definition of misconduct." And I was able to win that point.

Schlesinger: That's a nice story.

02-00:22:06

Schachman: As a matter of fact, I was at a meeting in a room at NIH with, I don't know how many, thirty lawyers, and James Wyngaarden (a previous Director of the NIH). This meeting dealt with the definition of misconduct in science because the lawyers did not want to use the word fraud. Wyngaarden and I were the only scientists in the room. The rest of them were lawyers for different agencies of government and chief counsels of major universities. Wyngaarden said, "Gee, I want to add to what Howard has just said." And he started to talk about his experience on sabbatical sitting in Monod's laboratory in Paris, where they were talking about some very complicated enzymes, and Monod said, "Leave that out. We can't possibly understand that now. Let's understand this much first."

Schlesinger: It's interesting because I was just about to say that I remember that when Monod and Jacob came up with their models that they had to leave out a lot of data. Eventually, some of those data turned out to be important, but it would not have been possible for them to come up with their theory of enzyme induction and repression if they had originally started taking all of the available information into account.

02-00:22:56

Schachman: Absolutely. That's a very important part of science. And it's a part that people, who are not scientists, do not really understand. So it was fascinating to see Wyngaarden jump into

that argument very, very quickly, by bringing up his experience at the Pasteur Institute.

Schlesinger: I think we probably all could remember examples in our own labs.

02-00:23:13

Schachman: I'm sure. In every field of science, I'm sure that's true. Well, Einstein oversimplified like crazy in his theory of the viscosity of solutions. And Stokes for his equation of falling objects. They all do. Any conceptual theory was based on a simple model. You start with large solid spheres in a medium that was composed of smaller molecules.

So that was a major sojourn for me into the DNA field. It was partly based upon the fact that we started with the ultracentrifuge and went to absorption optics, and then we went to determine whether this molecule is double stranded or not. It also got us into the ribosome field, and it got us into viruses. So I was having a wonderful time combining physical chemical studies with the ultracentrifuge, along with its application of the ultracentrifuge, to a variety of biological problems.

We were using the absorption optical system extensively for a lot of the work, especially on *E. coli* extracts and studying DNA. I remember Spinco was selling them, not like hotcakes, but the optical system had so many advantages for certain types of research that people would buy an ultracentrifuge because of the optical system. I remember some of my friends calling me up complaining bitterly it wasn't very useful. So I said, "What are you using it for?" They would say, "Well, we're using it for proteins." I said, "Well, you're crazy. Schlieren optics are much better for proteins. The absorption system is useful for nucleic acids, where you have huge extinction coefficients."

There were certain people who were very skeptical about our ability to work at micrograms per ml, even though we could see, with the absorption optics, micrograms per ml, because they said convection would cause trouble. What they neglected was that when you run things at 300,000 x gravity, i.e. 60,000 RPM, the ions in the salt solutions would redistribute through the cell and you would have a density gradient due to the buffer. And the density gradient would be sufficient such that you couldn't stir things from the bottom to the top. So DNA boundaries at one microgram per ml would still be very stable, not because the DNA was intrinsically causing enough of a density change, but because of the salt gradient. You would get a gradient of the

order of 0.01 grams per ml. It was really a significant gradient. Vern Schumaker and I wrote a little paper on the use of the absorption optical system. We showed that you could have a stable boundary of cytochrome c, for example. There, we used the heme absorption, which is, again, intense, almost like purines and pyrimidines and we were able to get boundaries of cytochrome c at extremely low concentrations. We could study hemoglobin at micrograms per ml again, by using the heme absorption. This work got me involved with some of the arguments over hemoglobin. It became such a mess that I decided to let my postdoc, George Kellett, publish it by himself, because I really didn't want to get into an area where I had no competence. I really was watching these people fight with one another rather than fight over the data. George liked that sort of combat; I didn't. So there was my sojourn with absorption optics.

But then one day I said to myself, "This is impossible. Interpreting absorption optical patterns is a lot of work. You needed a densitometer. You can't see the boundaries while the ultracentrifuge is operating, because there was no visual technique for looking at a solution that was water clear. It was inflexible, it was inaccurate, it was labor intensive, and we ought to do something about that. So we needed to build a photoelectric absorption scanning apparatus." So I went to Beckman about that.

Schlesinger: Your friend, or is this somebody else now?

02-00:27:20

Schachman: They were Beckman by that time, and Pickels was a major force in the Spinco division of the company. They were heavily involved already in building the amino acid analyzer, according to the design of Moore and Stein at the Rockefeller Institute. So I was able to make connections with a very good electronics man who worked up on the hill, in the radiation lab here in Berkeley. Stanley pulled some strings to allow us to get this guy to work for us, without paying an exorbitant overhead cost to the Lawrence Radiation lab. The Rad lab was already charging the federal government for overhead, and they were quadrupling the overhead they charged [chuckles] the Berkeley campus for overhead. So I didn't have nearly enough money in my grants to do this, but Stanley was able to straighten this out. So this fellow Ken Lamers began to work in my lab, almost full-time. By that time, we had several centrifuges.

02-00:28:21 So about two years of hard work went on, with the lab completely torn apart, with this guy coming into the lab and doing the electronics. I had Sue Hanlon as a graduate student. She worked very hard on this and we wound up with a single beam photoelectric scanning optical system, which would automatically make traces of the change in absorbance as a function of distance in the cell, and it would be printed out on a graph paper, right off the centrifuge. So you really knew what was going on as the machine was running. The trouble with single beam is that if the lenses got dirty, you would have all sorts of trouble. So it was quite obvious that the single beam optical system was an intermediate step. What you needed was the equivalent of a double beam spectrophotometer. And therefore, we had to get a double beam instrument. So again, Ken Lamers began to work in the laboratory. Another couple of years went by, and ultimately, we wound up with a wonderful photoelectric scanning apparatus that corrected for dirt on the lenses and things of that sort, changes in intensity of the light source. So we were very happy. We began using it for a whole variety of experiments. For example, Izchak Steinberg, a very brilliant young physical chemist from the Weitzmann Institute, a student of Ephraim Katzir's, came to work as a postdoc. He and I began to work on the interaction of methyl orange with serum albumin. So we were essentially bypassing, as I like to joke, equilibrium dialysis. With that technique one used cheap dialysis tubing that cost fifteen cents, [chuckles] and we were using a $50,000 instrument (the price of the ultracentrifuge had indeed increased) to do similar experiments. But it had certain advantages. So we were able to show that you could study interacting systems by changing wavelengths. And by that time, in collaboration with Spinco, we put a monochromator on the bottom of the ultracentrifuge, lifted the ultracentrifuge up on blocks and mounted the monochromator on it. So that phase of our research became a very vigorous activity.

Schlesinger: But this was just a model system.

02-00:30:18
Schachman: We were using it as a model system, right. Because the data were already there, from Klotz's lab at Northwestern, about this interaction and so we wanted to see if we got the same results. I remember Paul Berg sending a postdoc from his lab to study the interaction of tRNA with the synthetase and things of that sort. So it was a very exciting period. At that time, I also became interested in enzymes and this, ultimately, led to my going to St. Louis to work with Arthur Kornberg. But I used to ask the

question: what happens to the enzyme when it reacts with the ATP? Does the enzyme change its shape?

Schlesinger: Are you talking about a specific enzyme now?

02-00:31:02

Schachman: Hexokinase, let's say. And we worked on hexokinase for quite a while. Does hexokinase change its shape? Forget the glucose. So we're not going to worry about the actual enzymatic reaction, we're just going to worry about the change in the enzyme itself, as a consequence of interacting with the donor, ultimately, ATP. So there's no way of knowing that. You could do two experiments—one of hexokinase by itself and another of hexokinase plus ATP. But the change in the sedimentation coefficient of hexokinase as a result of interacting with ATP would be so small that the experimental error in each of the two measurements would obscure that result; you would never know anything. So what you needed, therefore, was an optical system that measured the difference directly. This is classic physical chemistry; differential methods always dominate if you have to look for small changes. So I said, "How do you do that?" Well, the answer to that is the Rayleigh interferometer. The Rayleigh interferometer compares one cell with another cell. There were already double sector cells being used in the ultracentrifuge, so why don't we put hexokinase in one side of the cell and hexokinase plus ATP in the other side of cell and if the boundaries move at separate rates, we'll get a difference boundary. Well, all you needed now was to make the Rayleigh interferometer. So again, [laughs] I got in touch with Ed Pickels at Beckman, and I told him what the idea was. And in no time flat, we got a cardboard mask, essentially, or very cheap little things that you cut with scissors, practically, and we were working on a Rayleigh interferometer. Well, when I say we were working on a Rayleigh interferometer, it took another half a dozen years, plus about four papers, for us to get one that really worked, which is now the method of choice in the existing commercial ultracentrifuge. So the two sets of experiments, the absorption optics with the scanner, and the Rayleigh interferometer, became the dominant optical systems. There are no ultracentrifuges in existence now that work with Schlieren optics. [laughs] So both of these ideas led to the development of these two approaches. We began doing a huge amount of work on changes in sedimentation coefficient, and studies of that particular type, which led, ultimately, to the experiments on aspartate transcarbamylase.

02-00:33:22

But before I go to aspartate transcarbamylase I ought to tell you a little bit about one other ultracentrifuge experiment that has nothing to do with us. When Svedberg started his lab, they were working with absorption optics. Tiselius was in his laboratory, and they got the idea that they ought to have a partition cell for the ultracentrifuge. When the experiment is done, they could remove the upper supernatant liquid in a nice, clean fashion. In that way, they would be separating the liquid in the top from that in the bottom part of the cell. So they put a tiny, little platform in a conventional ultracentrifuge cell, with a bunch of holes in it, and would lay a piece of filter paper on that. If you wanted to do something like radioactive labeling, for example, you could determine whether the radioactive material stayed in the supernatant or went to the bottom, or how much was left, and so forth and so on.

Arthur Kornberg, with whom I had been very friendly, began to work on DNA synthesis and I knew about it. I would talk to him occasionally—he had essentially just reached a level of purification of the enzyme that he was synthesizing precipitable material presumed to be DNA. It was precipitable ^{32}P from the ^{32}P labeled deoxyribonucleotide triphosphates. He used precipitation as the only technique he had available.

Schlesinger: This is acid precipitation.

02-00:34:48

Schachman: Acid precipitation – to prove that he made a polymer. So one day I said to him, “Arthur, I can tell you the sedimentation coefficient of your ^{32}P if you want.” He said, “How can you do that?” So I told him about this partition cell, which you bought from Spinco. It was readily available. In fact, there was another form of it built by David Yphantis and David Waugh at MIT, which was very clever. The partition cell of each type was readily available. So Arthur said, “Well, I’ll send you some extract. Can you tell me the sedimentation coefficient?” So we spun it down and I measured the ^{32}P in the supernatant. And it was quite clear he was getting ^{32}P to sediment at about 20 Svedbergs. All this was calf thymus DNA primed, in those days.

Schlesinger: But Howard, did this mean that when you were measuring ^{32}P, you would have to fractionate the samples?

02-00:35:44

Schachman: That was essentially a fractionation device. It could’ve been done by sucrose gradients.

Schlesinger: Well, we're going to come to that soon.

02-00:35:50

Schachman: That's right, but it was done originally by me and I was able to tell him very quickly that the sedimentation coefficient of their synthesized, precipitable material was about 20 Svedbergs, which was comparable to the size of the calf thymus DNA. At that time, 99.99999% [chuckles] of what he sent me was calf thymus DNA, and he had just had a trace of ^{32}P, but the ^{32}P had the sedimentation rate of the bulk material. So of course, he was ecstatic with joy. And that led to further discussions and a decision that I would go to his laboratory on my sabbatical leave, which is what I did.

I was interested in protein chemistry and I was going to study thermophilic organisms, and why were enzymes in thermophilic organisms much more stable than the enzymes from mesophiles. So that's essentially what happened. I worked it out and planned my sabbatical to go with Arthur Kornberg. My friends were shocked because Berkeley professors went to Cambridge, England or to Oxford or Copenhagen or Stockholm, but they don't go to St. Louis. But as I told them, St. Louis, in terms of biochemistry, was the Paris of the Middle West. So I then went to Arthur's laboratory.

Schlesinger: Before we go to that, the story with Matt Meselson and cesium chloride, is that before or after your sabbatical?

02-00:37:17

Schachman: It's probably before but there were discussions even while I was on sabbatical.

Schlesinger: So I thought that since we've heard about it from Matt's point of view, why don't you tell us that story?

02-00:37:43

Schachman: Well, Vern Schumaker was in the lab with me, and the Watson and Crick story came out. We were working with absorption optics and we knew a lot about sedimentation. We had done the double strand stuff, and we knew about the number of breaks so the idea came up: why can't we see if we could check the idea of the two strands separating, and then the daughter molecules having one strand from the parents? There was a post-doc by the name of John Smith, who had come from Markham and Smith's laboratory in England. He was a postdoc in the Virus lab, not in my lab, but down the hall in the Stanley Building. We began talking about checking the idea of the replication of DNA. We knew that if you wanted to fractionate the DNA, you had to

make one strand dense. We would have to make heavy DNA. So we could replace essentially all the thymine with bromouracil. So we could grow *E. coli* with bromouracil. And then we knew that we had heavy DNA. If we wanted to tell the difference between heavy DNA and light DNA, we needed to raise the density of the solvent. DNA has a density of around 1.7 or 1.9, maybe even 2.0. And the difference between it and water is significant. If you just raise the density a little bit, you're still not going to have enough to play with. But if you raise the density of the water to 1.6, then it should be possible to determine the difference between molecules of 1.9 and 1.8 in density. If the solvent has a density of 1.6 then the buoyant density differences become 0.3 and 0.2. That is clearly preferable to working with a solvent of density 1.0 where the buoyant density differences would be 0.9 and 0.8. We knew enough about doing that. We're sitting in the lab, discussing all this with Vern Schumaker and Sid Katz, who was a postdoc of mine at the time, called from across the room: "Add cesium chloride to the water because it's very soluble and you can raise the density way up with cesium chloride." So immediately, we began doing experiments with cesium chloride. We were beginning to make some progress but the cesium chloride itself was absorbing ultraviolet light because of some impurities that we didn't understand. We used thin cells to overcome that obstacle. Somewhat about that time, I found out that Matt Meselson was doing exactly the same kind of experiments. He was using rubidium chloride and I told him about cesium chloride. So he switched completely to cesium chloride. He then did this magnificent piece of work with Frank Stahl, and wrote me a note and said, "Howard, we ought to publish it together." We had nothing to speak of, other than very sloppy experiments, representing little progress. We were still using bromouracil. He had already switched to ^{15}N, because the experiments were working so well. So I said, "You can thank me for the idea, and go ahead and publish." So he went ahead and thanked me, and that was the end of it. The problem, however, continued in another vein, because he had worked with Jerry Vinograd. Not only did Meselson and Stahl write this unbelievably beautiful paper, they also worked with Vinograd to write a paper on determining molecular weights by the width of the bands in the density gradient experiments. I used to argue with Matt strenuously that that is invalid because multi-component systems have to be treated by much more sophisticated equations than they were using. For example, if I sediment a virus particle in D_2O, the virus particle doesn't know the difference between D_2O and H_2O. If there's a hydration

layer, the D_2O can get into the hydration layer as well as the H_2O, and it will have no effect whatsoever. If you now switch from D_2O to sucrose, then you have to ask the question: Does the sucrose get into the hydration layer inside the virus particle, or the pores of the virus particle, as much as water? If not, you're going to get a fictitious sort of density. If you now go from sucrose to something much bigger, like serum albumin, you're going to have the same kind of problem. So I said, "When you sediment DNA in cesium chloride/ water mixtures, these are 60% cesium chloride. You have to worry about whether the DNA is hydrated with pure water or it's a mixture of cesium chloride and water. Therefore, since you don't know this, you can't very well talk about this molecular weight. It took years of periodic intervals when I would meet with Matt to convince him that, in fact, they oversimplified. It was a good idea to oversimplify, but nevertheless, this method of determining molecular weights disappeared from the scientific literature.

So Matt and I meet every once in ten years or something of the sort and we refresh our memories about old times. But their paper was a fabulous paper.

Schlesinger: I think we should just add here, for the people who read this at some point, that Larry (Fredric Lawrence) Holmes, a historian of science at Yale, had written a book, which he called *Meselson, Stahl, and the Replication of DNA A History of The Most Beautiful Experiment in Biology* (Yale University Press, 2001) about the Meselson-Stahl experiment.

02-00:43:17

Schachman: Right. And we actually started it earlier, but didn't get anywhere. And they did a beautiful job. My contribution was telling him about cesium chloride.

Schlesinger: Well, it's an important contribution.

02-00:43:28

Schachman: Right. One of the sad stories is that Sid Katz was quite a neurotic physical chemist, and he had a major breakdown. He actually thought he deserved some credit for their magnificent work. Obviously that was unjustified. I used to say that coming up with ideas is easy, it is the execution that represents significant contributions. Sid wrote letters of complaint and wound up in an institution. I was approached one time by somebody— I think it was a rabbi who went to visit the institution—to try and get Katz's name in the literature as having a major contribution. All he did was suggest cesium chloride to

me, and I passed that information on to Meselson and Stahl. But the execution of the experiment was strictly theirs, and they deserved full credit for it, and got full credit for it. It's a beautiful experiment.

Schlesinger: That is the end of our discussion on January 21.

[End Audio File 2]

The following insert is a summary that Howard wrote about his years working with the ultracentrifuge. He wanted to provide more details about the scientific contributions that the ultracentrifuge made (with his help!) and also to acknowledge the many friends and colleagues with whom he interacted.

As you can readily see from the interviews thus far, I had a relatively long love affair with the ultracentrifuge. It lasted about 20 years and provided about 25 great graduate students and post-docs with an opportunity to do some innovative research. It is fascinating to recall that our entering into this type of research stemmed from a biophysical problem—determining the subunit structure of tobacco mosaic virus. Once we had some new tools like the synthetic boundary cell or the photoelectric scanning absorption optical system we looked for applications and found lots of biochemical problems that could be attacked. So our research agenda weaved back and forth from developing tools and techniques for ultracentrifuge investigations and applying that instrument for studies of biological systems. Friends and colleagues throughout the country approached me with potential applications of these techniques in their own research. As a consequence I had some wonderful visitors like Martin Kamen, Seymour Cohen, Gordon Tomkins, Irving Klotz, Fred Karush, Manuel Morales and Pete von Hippel, all of whom spent considerable time in the lab.

In addition to problems undertaken with visitors, I also did what I considered "service" work for colleagues in the university and especially for those in the Virus Laboratory. Wendell Stanley was excited about the possibility of purifying and characterizing poliovirus and he supported enthusiastically the efforts of Howard Bachrach, Carleton Schwerdt and Fred Schaffer who used a combination of chemical techniques and preparative ultracentrifugation to purify the virus. My role was merely to examine their preparations in the Model E analytical ultracentrifuge. I have vivid recollections of worries about the possibility of the ultracentrifuge cell leaking and poliovirus being sprayed into the laboratory. Cell leaks were rare, but they did occur, and we devised some traps in case there was a leak. Fortunately, there were none. One paper by Schwerdt and Schaffer in the *Annals of the New York Academy of Sciences* (61, 740 , 1955) contains considerable ultracentrifuge data that we obtained in the Model E, including sedimentation velocity patterns of the purified virus and a plot of the corrected sedimentation coefficient versus the density of H_2O-D_2O mixtures. Extrapolation of the data yielded a value for the apparent density of the virus that

was clearly greater than that of pure proteins. These results indicated, for the first time, that the virus contained significant amounts of nucleic acid.

Before leaving my discussion of our ultracentrifuge research, I would like to mention one small study that we found particularly satisfying. I have already referred to sedimentation experiments in which the solution density was increased by a third component like sucrose, cesium chloride, or D_2O. We already knew about the risks of preferential interactions in multi-component systems and realized that using D_2O to increase the density was preferable theoretically over any other reagent. But one can only get to 1.10 g/ml with D_2O, and we desired greater densities. One evening I was at a party along with David Samuel from the Weizmann Institute who was doing post-doctoral research in Calvin's lab. Most research using ^{18}O as a label was performed with $H_2{}^{18}O$ obtained from Israel and David Samuel was an expert in its production. I probed about the technique and asked if in the process of enriching the water for ^{18}O by repeated distillation, didn't they also increase the deuterium concentration. The answer was obvious; they were making $D_2{}^{18}O$ and then they performed back exchange experiments to replace the deuterium to make $H_2{}^{18}O$. I asked David if he could give me some of the "intermediate", $D_2{}^{18}O$, which would have a density of 1.20 g/ml. Much to my pleasure, David sent me a precious, small sample of pure $D_2{}^{18}O$ that Stuart Edelstein and I used in an exciting (to us) study showing that one can determine both molecular weights and partial specific volumes in two sedimentation equilibrium experiments—one with ordinary water and the other with the heavy water. As papers go, that one received considerable attention from the ultracentrifuge community.

Begin Audio File 3 01-22-2008.mp3

Schlesinger: It is now January 22, 2008 and we're going to begin to talk about Howard's sabbatical. This is tape number three.

03-00:00:22

Schachman: Well, as I had indicated earlier, I had already been doing experiments with Arthur Kornberg. We knew each other since World War II, 1945, when Arthur left NIH to take a sabbatical because at that time, he was doing nutrition research. He got permission at NIH, which is almost unprecedented, it probably was unprecedented, to take a leave to go work with somebody else. He went to New York to work with Ochoa. And the second year after that, his leave was extended and he went to St. Louis to work with Carl Cori. I had heard he was going away. I didn't know him then, but I had heard about it and I approached him. It turned out I sublet his apartment in Bethesda. As I've said on numerous occasions, I knew Arthur long before everybody recognized how important he was, because to me, he was very

important; [laughs] he had an apartment for rent in the Washington area during World War II, and such apartments were very, very rare. So we moved to into the Kornberg apartment. He had no children at that time.

Schlesinger: Where were you coming from?

03-00:01:31

Schachman: I was in the Navy. I was across the street at the Naval Medical Research Institute, in the back of the National Naval Medical Center. I had just gotten married. So the first apartment that Ethel and I had together was actually the Kornberg sublet. Then I was in touch with him off and on over the years and then when he began to do the DNA synthesis, somehow or other we began talking, and I indicated I could contribute a little bit by using the ultracentrifuge. These were methods that were foreign to him and I think I indicated already that I had done something with ^{32}P. So by that time, I was sort of wrapping up a lot of my work on ultracentrifuge techniques. We had written a series of papers, and still had more to publish on the photoelectric scanning absorption optical system and its application; and also had made a fairly good start already on the Rayleigh interferometer for measuring small changes in sedimentation coefficient. I had a bunch of wonderful students. I wasn't interested in going overseas because I would lose track of them. So the issue came up of going to what I called the Paris of the Middle West. By the time I got to Arthur's lab there was a hurricane coming to St. Louis. I remember vividly calling from the highway and Arthur said, "Come to the house." I said, "Gee, we're tired of driving. I think I'll stay here on the highway. We'll stop at a motel." And it's a good thing I did, because the hurricane [chuckles] more or less broke down trees a block or two away from his house.

Schlesinger: Was it a hurricane or a tornado?

03-00:03:07

Schachman: Tornado, I guess, is what you call it in the Middle West; I don't remember. So I'll change it to tornado. It was ferocious. There was a lot of damage done in the area of University City, where Arthur lived. So the next day we drove there, and then we did move into the Kornberg house.

By that time, we had two kids and they had three kids. When I got there, Arthur was already in the middle of a major clash over the publication of his papers in the *Journal of Biological Chemistry*. And it was fairly clear from reading their reviews that

one of the referees was Erwin Chargaff, who was a stickler for detail. Arthur and Bob Lehman had published a paper called "The Enzymatic Synthesis of DNA", in *BBA*. It was based upon a few counts being precipitable by acid. So Arthur asked my opinion. By this time, he had written these very extensive, not yet published papers, because he now had net synthesis, and I had given him information about the size of the DNA. So he asked me what I thought and I said, "Well, I wouldn't have called the original paper 'The Enzymatic Synthesis of DNA,' but I wouldn't back off now." So Arthur had a terrific battle. The battle was ultimately resolved when John Edsall became the editor of the *JBC*. Edsall, being a marvelous diplomat and very sensible, was able to soften all the language and get Arthur to resubmit the papers, because Arthur was about to withdraw them. The language that is used today is that Arthur's papers were rejected. I don't remember them being rejected; but I do remember it was a very contentious issue, and he was extremely upset. When I got there, I remember it so well that one of the first nights we were there, there was a party somewhere. Gerty Cori was there, and she was already pretty sick. She grabbed hold of me—she was a combative individual. [laughs] I was shocked. She began blasting the people in Seattle that were working on phosphorylase, "It was their enzyme, why did Fischer and Krebs move into this field?" So I was aware of how contentious biochemistry can be.

I came there with a station wagon loaded on the top with all sorts of reprints, because one of my tasks for that year was going to be to finish up a review for *the Advances in Protein Chemistry* on the ultracentrifuge that I had been asked to write and agreed to write. In addition, I knew that one didn't spend a summer in St. Louis if one could avoid it. So therefore, what we were going to do is leave our material there and then drive on to Woods Hole, where I had been teaching. I taught about seven summers in Woods Hole.

Schlesinger: In which course?

03-00:06:14

Schachman: The physiology course. It was traditional in those days to have a physical biochemist teach as one of the six instructors in the course. The students were sensational. I've had unbelievably wonderful students. It was one of the most challenging teaching experiences of my life. As a matter of fact, I think I drove with—I can't remember whether it was that summer or the following summer—Paul Berg and Millie Berg also came along. Jerry Hurwitz, as a matter of fact, from the Kornberg lab at that time,

was a TA for me in succeeding summers. So Woods Hole is one of the great treasures of my experience as a faculty member in Berkeley, by getting a sabbatical leave and going there. So I spent three months at Woods Hole doing interesting experiments and teaching a course.

Schlesinger: Did they have an ultracentrifuge?

03-00:07:08

Schachman: Oh, they had an ultracentrifuge. Oh, yes. I had it doctored up with all sorts of special gadgets that appealed to me, and it was very exciting. As a matter of fact, I remember one of Matt Meselson's students took the course, and she was terrified because Matt wouldn't let her anywhere near the ultracentrifuge in Pasadena, but when I was doing the experiment, they (the students) ran the ultracentrifuge. They were shocked that they were allowed to manipulate this [chuckles] very complicated machine. But there were really very few ways that they could do damage to it. It was a wonderful experience because we would set up an experiment that would run in the ultracentrifuge—it's one of the few times I ever used the automatic clock for continuing and then turning off the machine. We would then go down to the beach and go swimming. When we came back, the centrifuge had finished the run and it turned itself off. So it was great fun.

So I went back in the fall to St. Louis. The big problem was to set up the physical chemical lab. Arthur had already ordered a Model E ultracentrifuge, and he'd already ordered a Model H electrophoresis apparatus from Beckman, the Spinco division of Beckman. There was an old men's room, a big, gigantic men's room that was then converted into a physical chemistry lab. So I used to joke that I spent the whole of my sabbatical year in the men's room. St. Louis was unbelievable at that time, because we were in an old hospital building that you probably know very well. [laughs]

Schlesinger: Same building that we were in when we first went there.

03-00:08:37

Schachman: When you got above the floor where patients were, you came to the Microbiology Department. And the Microbiology Department had Paul Berg, Jerry Hurwitz, Dale Kaiser, Dave Hogness and Mel Cohn. It was a big open space and in the big open space were cabinets galore with all the glassware. There were a bunch of small rooms, not very many, the order of six small laboratories. Any time you needed something, you had to go out

in the hall to get it. The Biogen to grow cells was out in the hall, for example. The distilled water was out in the hall. So you bumped into people all the time. There were unbelievable dialogues. You couldn't do an experiment without all the other people in the department knowing about the experiment because there were discussions going on all the time. I have never seen anything like it. And unfortunately, I'm afraid that that style of a small department is disappearing from the scene. So I began immediately. I had brought a bunch of viscometers with me and set up a constant temperature water bath. And my question to Arthur was, "Why is there so much degradation of this product? There must be a nuclease in your preparation. Why don't I study the degradation of DNA caused by the nuclease in what you call the polymerase preparation, which makes DNA?" One of the first experiments I proposed doing was to leave out one of the nucleoside triphosphates. He said, "Why don't you leave them all out?" I said, "Well, to reproduce the conditions as much as possible. You claim, and the evidence showed, that you needed four deoxyribonucleoside triphosphates. I'll leave out dGTP, because that was the hardest one to make." The reason Kornberg's lab was so fantastic was that they made all their own substrates. It was an enzyme laboratory par excellence. Their refrigerator had all the substrates they needed, and they worked very hard to get the enzymes necessary to prepare the substrates. So with some reluctance—he was not too happy about that—I set up an experiment with three deoxyribonucleoside triphosphates, and I would watch the viscosity go down due to the nuclease. My attitude was to get rid of the nuclease. He was not as focused on that as I was, but that's because I was a physical chemist, and he was interested in the product, and therefore, it was a very different orientation.

The viscosity kept going down and down and down, and we continued the experiment. That was the beauty of the viscometer, you just leave it in the water bath and you don't have to have an assay of thirty minutes and then precipitate the mixture so it is destroyed and you see what you've got. I continued the experiment. As I recall, we went out for a beer, a bunch of us, and came back, and all of a sudden the viscosity was sky high—very, very high and kept going up and up and up. It finally reached a maximum, turned around and went back to zero. So that meant we made a polymer and then the polymer, whatever we made, disintegrated. The next morning we talked about it, and Arthur was not happy. You don't do enzymological experiments that are six hours in duration. The water bath was at thirty-seven degrees; we were running it for a

long period of time. Bacteria grow very well at that temperature. Maybe bacteria grew, and that clogged up the viscometer. Then a bacteriophage [laughs] came along and lysed the bacteria and you lost the viscosity again. So the moral of the story was: just repeat it; let's see what happens. So we repeated it. It was almost a dead ringer for what happened the day before, except this time, we had a pipette with citrate in our hands, and as soon as the viscosity reached the maximum and began to go down, we dropped citrate into the viscometer and the viscosity remained constant.

Schlesinger: That's an inhibitor of the nuclease?

03-00:12:27

Schachman: Right. Ties up the nuclease, that's exactly right because it was probably magnesium dependent. We now had something in the viscometer that was a viscous polymer. The next day we set up the same experiment, except now we added an aliquot of this to the original reaction mixture, thinking if there were a primer that was necessary, this would eliminate that long six-hour lag period. And sure enough, viscosity took off very, very quickly. So within a few days, we knew that we had made a polymer, and it was a polymer that didn't have four bases in it, it either had two or three, or one, [chuckles] but it wasn't typical DNA. So from that point of view, Arthur Kornberg was very unhappy, because at that time, the difference between the DNA polymerase that Arthur was working on and the Ochoa enzyme was that one (the DNA polymerase) will require four bases and the other (an RNA polymerase) would require only one. So Arthur was unhappy that this main characteristic of enzymatic synthesis of DNA had been eliminated. Moreover, we could easily demonstrate that we could boil the thymus DNA and do the same experiment. So you didn't need a rigid template. So two major ideas went down the drain very, very quickly. By that time, John Josse was in the lab; Charlie Radding was coming soon, I think, or maybe was already there. And the question is, what did we make? And soon enough it was clear we made a polymer of deoxyadenylate-deoxythymidylate. There was no cytosine in the product and no guanine, because we had left that one out. John Josse had worked on this marvelous technique for transfer of ^{32}P. So you put the phosphorous on the dATP and hydrolyze it in such a way to find out what the next base was. It turned out quantitatively, 100%, whatever went on as A wound up on T and therefore, it was clear that this was a copolymer of deoxyadenylate-thymidylate in alternating sequence. So we began to characterize those. It was a very, very exciting time. Now, all this period, the sociology of the

Kornberg lab was very appealing to me. Arthur used to have group meetings at eight o'clock in the morning and they would discuss what they would do that day. It was almost like a quarterback organizing the team.

Schlesinger: But it wasn't just Arthur's group, was it?

03-00:14:57

Schachman: It was just Arthur's group. Paul Berg was not in it and Jerry Hurwitz was not in it. It was Julius Adler, Bob Lehman, John Josse, when he came, and Ernie Simms and Sylvie Kornberg. I was, at that time, still writing my review. I would stay up probably till three o'clock every morning in the apartment, typing away on my review. Whatever I wrote at night, Ethel would retype the next day and then after that, I would bring it in, and Arthur was very kind; he had his secretary rearrange that. Then I took that, finally, and sent it back to Berkeley, to my secretary back home. So it went through a series of stages, with me correcting things. But Arthur knew I was staying up till roughly three o'clock in the morning, so he was very kind. This is almost unprecedented for him. He changed the meeting time from eight o'clock till around nine-thirty. So I was very lucky. Herman Eisen lived a few blocks away from where I had rented an apartment in University City.

Schlesinger: So Herman had already come.

03-00:16:01

Schachman: Oh, yes, Herman was already there. We were already good friends from Woods Hole. Also I had begun collaborating a little bit with Fred Karush and Herman, using the ultracentrifuge for some antibody problems. I would go with Herman almost every day, to work. Either he would drive or I would drive. I would get there around nine-thirty, and then Arthur would have a meeting, and we would discuss who did what. It was so different an environment from what I was used to as a faculty member. First of all, Arthur's people were all postdocs, with one exception, Steve Zimmerman who came into the lab as a graduate student. There were very few students. It was a medical school environment, so it was very different from Berkeley. So I was astonished at the way we would sit and discuss things. For example, one day Arthur said, "Howard," at one of the meetings, "How would you like to work on the kinase to make dGTP?" Maurice Bessman was working on it, and he wasn't getting anywhere. I can assure you, that didn't sit well with Maurice Bessman, because he was being replaced [laughs] by Schachman. And I got the enzyme out by sheer luck. It might've

been the bacteria that day. It wasn't that I had better technique. On the contrary, compared to those enzymology guys, [laughs] I was a physical chemist who pipetted with great precision and very slowly. They were turning out data left and right. Till I finally figured out, this isn't physical chemistry, this is sloppy enzymology; why don't you cut the tip off the pipette and don't worry about accuracy? So it was a learning experience par excellence.

Schlesinger: I can only comment that you must have not interacted with Ollie Lowry, who was very precise in all of the kinds of work he did. He must have been in Pharmacology then.

03-00:17:58

Schachman: That's right. He was still around. Oh, he was a major figure at Wash U at that time. So no, I didn't know that he had the opposite point of view. But I remember we set up assays, and they would take a ten ml pipette, and they would pipette in one ml in each of ten tubes. I, on the other hand, took a one ml pipette and refilled it ten times and that was stupid. I soon learned, hey, there's no value in being precise here. You just want to get roughly one ml plus or minus a tenth, and you don't do that the way I did it. So I switched very quickly and soon learned their techniques. But it was clear to me that Arthur was interested in results and he didn't like to waste time. If things weren't working, try something else. One day I got in a long discussion with him about Steve Zimmerman, who was a graduate student. I indicated my philosophy that students have to fail, that they have to work on a problem for a couple of months until they finally bail themselves out. Then they feel a certain degree of triumph over that, and they've also learned how to analyze their own deficiencies and what's wrong with their experimental technique and approaches. He couldn't accept that way of doing research. You needed to get results. So if you're not getting results today this way, I'll come in and help you out and we'll do something else. So it was very, very different.

This was illustrated one day by a long walk I took—because you're right near the park, at Wash U. You know this better than I. I took a long walk with Julius Adler, who was getting ready to leave for his first academic position. Julius, who's a sensational scientist, expressed a certain degree of concern over whether he would be able to make it in the outside world, as an independent scientist. So I said, "Julius, you're the co-author of one of the greatest papers that's been published in the last decade in biochemistry. Why should you be concerned?" He

said, “But it’s not my work. This is all done by Arthur and an organized team.” It was quite clear this method did not lead to an exorbitant amount of self-confidence in the individuals because Arthur was in there pulling strings. But he was very tolerant. I used to talk about the difference between his philosophy and my philosophy. And the answer was, don’t second guess success. [laughs] His was fantastically successful, and mine was moderately successful, so you use whatever you want to use. But there was a fundamental difference. In his lab there were mostly postdocs and in mine the lab was full of graduate students. So by the end of that sabbatical year, we had pretty much proven that we had deoxyadenylate and thymidylate as a copolymer, and you didn’t need native DNA to begin with. It became obvious this was the so-called de novo synthesis of a copolymer. And one of the questions came up—

Schlesinger: And I didn’t think you needed a primer.

03-00:20:59

Schachman: You didn’t need a primer. So that was one of the key questions, is it a de novo synthesis? So the answer that we provided at that particular time was to take an exorbitant amount of polymerase, thinking it may already contain the primer in it. We’d boil it to denature the polymerase, and add that to a mixture of the two triphosphates with a fresh aliquot of polymerase and it turned out that we had the same long lag before synthesis occurred. In other words the large amount of boiled polymerase did not contain anything to accelerate synthesis. So we had the feeling that this was the de novo synthesis. I think subsequently to that, Arthur and his colleagues—I haven’t kept up with it—have demonstrated that it’s not de novo; there seems to be some small oligonucleotides containing alternate A and T. But it was easy to comprehend that A and T could form an alternating sequence, and it could form a hairpin on itself. So it was a sensational year. I then went back—

Schlesinger: Before you go back—because they also were able to synthesize a GC polymer, but it was not the same. Was that also done when you were there?

03-00:22:07

Schachman: No. [laughs] As a matter of fact, it wasn’t done. We tried innumerable times. I tried with a viscometer, because I had this wonderful simple assay that was much better than the precipitation assay they were using. We never got anywhere. A

couple of years later, the Kornberg group had already moved to Stanford, and I was still going back and forth, driving from Berkeley to Stanford to do experiments and to work on the paper with them. Bob Lehman, I think, wanted to prepare some material, a large amount of DNA, for a lab course that they were teaching, because they were using polymerase in the lab course. They threw in a huge amount of polymerase and a huge amount of 4 deoxyribonucleotide triphosphates, and they got—Well, the tube turned solid because it made so much viscous DNA. It turned [laughs] out to be dGC. It was C in one chain and G in the other chain. Everybody was shocked. So we then can make the dGC, but it was not the alternating sequence, the way dAT was. So the surprises were legion in that particular field.

So at the end of the nine months in St. Louis, I then went back to Woods Hole. I think Jerry Hurwitz came with me, sort of as a TA, even though he was not part of the Kornberg group. He was an independent faculty member. I brought with me polymerase, and the students in my lab course at Woods Hole were synthesizing DNA within a year of the discovery of DNA polymerase by Arthur Kornberg. It was a very exciting period.

Schlesinger: Was that before the medical students at Washington U were synthesizing DNA?

03-00:23:53

Schachman: Probably. So it was absolutely sensational. Then on the trip home—we were driving, of course—there was a biophysics meeting in Colorado, and the whole Kornberg family was there. I remember we stopped in Colorado and saw them all, and then we came back to Berkeley. And that was the end of my sabbatical.

Schlesinger: And what year are we now?

03-00:24:17

Schachman: ‘56, ‘57, something like that. It took a couple years before they moved here. I remember when we decided we’d write the paper, I sat at a tape recorder and dictated the paper. Mary Abbott, who was my secretary, typed the paper, and it was a pretty well organized paper. Arthur was shocked at the fact that I’d dictated this over a machine. The paper that was published [laughs] bore no relationship whatsoever to what I dictated; but it was still coherent and it had all the material in it.

By then, the lab in Stanford hired Buzz Baldwin. He was a young physical chemist who had spent a lot of time with the

ultracentrifuge in Madison, Wisconsin and then had got a degree in Cambridge, England—or Oxford, I guess—with Ogston. Arthur hired him as a young assistant professor. He more or less moved into that arena, and I left DNA completely. So it was a year full of experiences on the sociology of science, the role of education, the nature of education, the self-confidence of workers, an incredibly wonderful collaboration, and the development of wonderful friendships. My two kids and Ethel had a fantastic time. We had picnics with the Kornbergs, we went to baseball games—Arthur loved baseball games—we went to basketball games, the three Kornberg boys and the two Schachman boys. We've been friends now for sixty-two years. So that is essentially the end of my St. Louis story.

Schlesinger: That's a nice St. Louis story.

Schlesinger: Howard, before we move into the next phase of research, because you told me that you were going to talk about your decision between working on nucleic acids or proteins, and that brought to mind this slide that is infamous that you show, of funding for research for proteins and nucleic acids. So why don't you tell me about that?

03-00:26:22

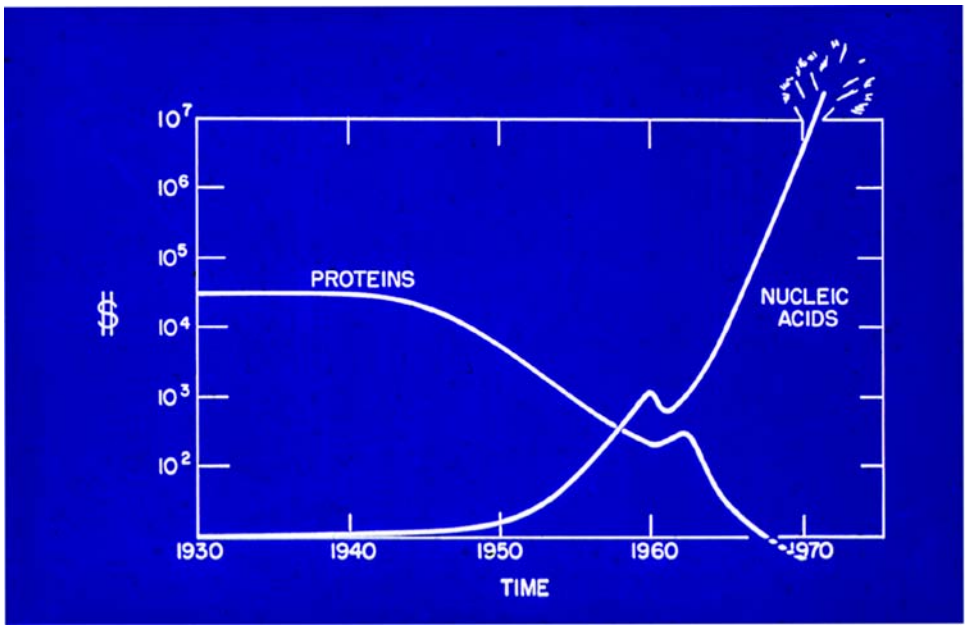
Schachman: There'd been a tradition at the Gordon Conferences for many, many years, after the lobster party on Thursday night, to have some comedian get up there and give a talk. And for years, Linderstrøm-Lang, who was one of the great people in all of protein chemistry, from the Carlsberg Laboratory in Copenhagen, would perform. He was an artist, he was a raconteur, as well as being an unbelievably brilliant theoretician and experimentalist. He would give magnificent talks. He once gave a talk called "The Thermodynamics of the Male Housefly," which was sensational. People had been drinking a lot of beer or a lot of wine, and the audience would be receptive to somebody taking the heat off all the competitive science that had been going on from Monday to Thursday. It was unbelievably well received. In fact, it was a shame, because the talk was fabulous and Academic Press heard about the talk and they then got it printed somehow. They printed it and made it available, I think, as Christmas cards or something of the sort, called "The Thermodynamics of the Male Housefly." Unfortunately, when you read it, it bore no relationship whatsoever to having heard it with Linderstrøm-Lang's own Danish accent. So I then learned the difference between giving a talk, where the audience is receptive because they've been loosened up by alcohol, and printing something that falls flat. So

when Lang stopped coming to the Gordon Conferences, the man who took his place was Reinhold Benesch. He was a very good protein chemist who had made a living on the Jewish stage in London before he became involved with protein chemistry. He then moved to the United States. He was incredibly funny, but his humor was very different from Lang's. His humor was in recalling an infinite collection of stories and jokes and being able to tell them in some appropriate style. So he would give talks. And then one year it fell on me. I was asked to do this on a Thursday night. So by that time, the grant program was in trouble, and I decided I'd give a talk on a new enzyme, which I had just been working on, called "Money Transferase."

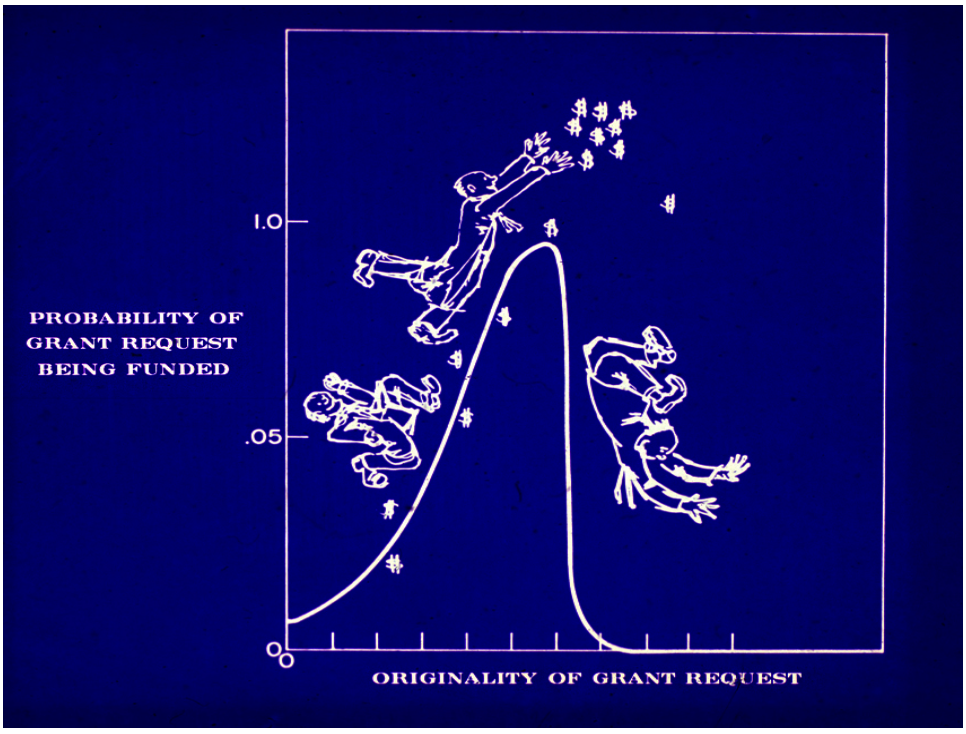
Schlesinger: You don't remember what year this is?

03-00:28:43

Schachman: I could find out. It'd be the fifties – the fifties, definitely. So I talked about the preparation, the assay, and the role of alcohol as a co-factor in the activity of money transferase, I talked about Las Vegas and things of that sort. Where you did the experiments would have an influence on money transferase. In the course of it, I talked about the plight of funding research. And then I talked about where the money from the NIH was going to go and I had this bright idea of publishing a slide called *The Average Size of a Grant in $ versus Time, for both Proteins and Nucleic Acids*. As I showed in the slide, protein chemists in the forties were getting about $30,000 a year with only a relatively slight increase with time whereas nucleic acid workers got what they deserved—namely nothing. Then all of a sudden in 1953, which was, of course, coincident with the proposal of the Watson-Crick structure, the slide showed a tremendous burst of activity for nucleic acids with all the dollar bills going up this huge, steep slope, and the dollars for proteins, concomitantly, went down. Then around 1960 or thereabout, I showed a dip in the nucleic acids curve and a rise in the protein curve. And then all of a sudden the trends became obvious; proteins went toward zero and nucleic acids would go toward infinity. When I joked about what was the blip, I said, "Well, protein synthesis was discovered and the protein chemists thought they had renewed life." But the truth of the matter is, protein synthesis was a nucleic acid field, [laughs] not a protein field, and that accounted for the temporary blip. Here is that slide:



And here is another slide that is also one of my favorites: It is relevant even now, because so many research workers complain that their grant application were not funded, because the proposal was too original!



So that was the issue. I joked about that when I got back to Berkeley, because I had Earle Stellwagen as a graduate student at that time, working on aldolase. He had done magnificent work as a graduate student showing you could denature aldolase and tear it apart into single chains and then reconstitute aldolase. So it was essentially raising the Anfinsen experiment to a higher level, because Anfinsen had refolded ribonuclease. We had no disulfide bonds in aldolase. But it was the reconstitution of enzymatic activity, and we were quite excited about it, so I decided I would stay with protein chemistry for a while.

At about that time, John Gerhart was a colleague of Earle Stellwagen's. He was a graduate student with Art Pardee. He knew Earle very well. When Pardee moved to Princeton, John finished up his PhD research at Princeton, but he got his PhD through the Berkeley Department of Biochemistry. We knew how terrific he was, so we hired John Gerhart. I think we might've been called the Department of Virology at that time; I'm not sure because sometime around then we changed the name again to the Molecular Biology Department. John Gerhart became an assistant professor in our department, without ever having post-doctorate training. So he came into the lab, and he knew me very well because he had been a friend of Stellwagen's before he went to Princeton. The Princeton experience was only about a year or two, I can't remember how long it was. He came to me and said, "Gee, you've got all these fantastic techniques for measuring small changes in the sedimentation coefficient and also absorption optics in the ultracentrifuge. Can you help me? Because all I've got so far is kinetic observations." The interpretation of Gerhart and Pardee in their beautiful paper on the allosteric behavior of aspartate transcarbamylase (ATCase) was clearly based exclusively on enzyme kinetics. So I said, "Sure." He said he knew the enzyme would dissociate. So we put it in urea, and of course, it fell apart. Its sedimentation coefficient was 11.7S.

Schlesinger: This is the active enzyme.

03-00:33:02

Schachman: That's the active enzyme. A sedimentation coefficient of 11.7 corresponds to a molecular weight of about 300,000. When you put the enzyme in urea, the molecular weight drops to the order of 20- or 30,000. So that's not going to be very, very useful. But he had already described the treatment of the enzyme in three ways. One involved silver ions, or mercuric ions, I guess; another one was heat denaturation; and the third one was low concentrations of urea. So I said to John, "But you've already

published a fascinating paper on the desensitization of the enzyme. When you heated it, the enzyme lost its cooperativity and it lost its sensitivity to CTP and ATP. (CTP was the inhibitor of the enzyme; ATP was the activator of the enzyme.) And when you treat it with mercurials, again, you did not destroy—that's *very* unusual—you did not destroy enzyme activity, but you dissociated the enzyme activity from the regulatory role." So he uncoupled the two phenomena of enzyme activity and regulatory properties. So I said, "Why don't we look at this in the ultracentrifuge with the mercurial, parahydroxymercuric benzoate?" This ordinarily inactivates enzymes by binding to sulfhydryl groups. But it didn't inactivate ATCase even though this enzyme had lots of sulfhydryl groups.

We put this protein with a beautiful (11.7S), very sharp boundary in the ultracentrifuge and followed the experiment using schlieren optics. As soon as we started adding mecurial to it, we found immediately two components instead of the parent molecules; and one had a sedimentation coefficient of about 2.8S, and the other one was about 5.8S. So I said to John, "Well, it's obvious you've got one component as the regulatory subunit and you've got another one as the catalytic subunit. Why don't we go ahead and publish?" This has been a long-standing joke between us. John's response was fairly obvious. He said, "But we don't know which is which." To which I responded, "But John, if we guess that the leading one is the catalytic subunit, we have a 50:50 chance of getting it right, which is probably better than we would've gotten if we did the appropriate experiment." So, because John was a young, serious, conscientious and terrific scientist, we did a sucrose gradient experiment. And in no time flat, it turned out the 5.8S component was the catalytic subunit and the other material was the regulatory subunit. You could then reconstitute the enzyme by removing the mercury by adding mercaptoethanol. It became clear immediately that aspartate transcarbamylase was composed of discrete subunits, that one was responsible for catalysis, and the other for regulation of enzyme activity. This was an explanation for the allosteric behavior of ATCase. At about that time, Monod visited Berkeley. Now, Monod had already been working on the paper with Changeux and Wyman. This unbelievably magnificent paper in 1965, on the nature of allosteric proteins, was using hemoglobin as a model. Changeux was Monod's graduate student, and he was working on threonine deaminase as another allosteric enzyme. But Monod, when he saw our results, just went absolutely out of his mind with enthusiasm. I remember Gerhart and I would sit in the coffee room in Stanley Hall, the smaller Stanley Hall in those

days, and Monod would be at the blackboard. He was just bubbling over with enthusiasm because it seemed so obvious that having two different types of subunits was the way to have allosteric control. Well, it turns out that the example of aspartate transcarbamylase was not typical. There are dozens of other enzymes [laughs] that don't operate by that mechanism.

Schlesinger: But Howard, as we said earlier—yesterday, I believe—one of the things that Monod was able to do was to select data that he wanted.

03-00:36:38

Schachman: Oh, yes, absolutely. He was very good at that. He was a conceptualizer par excellence. I remember people being unhappy about both Monod and Crick because they would change their minds. My attitude was, these guys are thinkers, they're not experimentalists. I can't change my data. If my data turned out to be incorrect in a subsequent investigation, I'd feel terrible about it. But if their ideas turn out to require revision, just modify them. I think that's part of the nature of that type of approach. I was not such a conceptualizer. These guys were—I don't call them theoreticians because theoreticians usually, in my opinion, deal with mathematics and things of that sort.

Schlesinger: Yes, they don't need data. [chuckles]

03-00:37:18

Schachman: Right, so it was fantastic. So then Monod asked if Changeux could come and work with us, because he was getting nowhere with threonine deaminase, which wasn't nearly as good an enzyme as John Gerhart's aspartate transcarbamylase. So that started Gerhart and me off on a major path, which then consumed the next thirty-five years of my life, working on aspartate transcarbamylase. Fifteen or twenty students have gotten their PhDs working on that enzyme, and it became the paradigm of protein chemistry. It involved protein engineering, it involved cloning, it involved DNA sequencing. It broadened my approaches to science enormously. And I still am fooling with it, to a small extent, by collaboration with some other people.

Schlesinger: Did you interact with Harvard at all, with Don Wiley and Lipscomb? (These are the scientists who did the X-ray structure of aspartate transcarbamylase.)

03-00:38:15

Schachman: Well, that's an interesting question. I certainly did and unfavorably. Not with Don Wiley, because my interactions with him were extremely favorable. In the course of the research,

Gerhart and I were making a lot of progress. Down the hall, Robley Williams was doing electron microscopy.

Schlesinger: Was the aspartate transcarbamylase coming from *E. coli* or—?

03-00:38:33

Schachman: *E. coli*, always from *E. coli*. At that time, Lipscomb, a superb crystallographer at Harvard who was Don Wiley's mentor, asked Gerhart for some crystals. John Gerhart already had crystals. So the crystals were sent to Harvard and Lipscomb began to work on aspartate transcarbamylase. But unlike other people who worked on aspartate transcarbamylase, like George Stark, who was then at Stanford, where there was a free exchange and wonderful exchange of scientific information, Lipscomb was incredibly competitive and secretive. So we never got anywhere with him. Originally, Tom Steitz had been working on the structure of aspartate transcarbamylase as a graduate student of Lipscomb. He published that it had 2-2 symmetry. By then, Changeux was in my lab and Changeux was not a very thorough experimentalist; a very bright guy, very enthusiastic, but he believed in symmetry because that's where the Monod idea came from. A tetramer was just so appealing. So we published a dumb paper that the enzyme was composed of two regulatory subunits and two catalytic subunits. After all, 2-2 symmetry fit that picture. So the Lipscomb laboratory had sloppy crystallography data, and apparently Steitz had made a mistake in not correcting for hydration. We had inadequate physical chemical data. But everybody agreed, so we also published, and we were all wrong on it being a simple tetramer. At that time, Klaus Weber had come to Harvard. He began to work on the sequence of the regulatory chains, and was able to show beyond a shadow of a doubt, there had to be six of them.

Schlesinger: Six regulatory chains?

03-00:40:35

Schachman: Six regulatory chains. And then later, we showed that there were two catalytic trimers, so it became six of each. And I'll go into that in greater detail next time. So I guess that's a good place to stop.

Schlesinger: This is the end of our taping for January 22, 2008.

[End Audio File 3]

Interview #3: March 17, 2008, March 19, 2008
Begin Audio File 4 03-17-2008.mp3

Schlesinger: March 17, and this is tape 4. Howard, let's go back to the ultracentrifuge, because you told me that you wanted to talk a little about one of the people that worked in your lab at the time.

04-00:00:26
Schachman: Yes. In the fifties, I had another graduate student, whose name at that time was Ann Forman, a very beautiful young woman, who was actually the runner up to the homecoming queen contest on the Berkeley campus. So that gives you some indication of what she must've looked like. She'd been working her way through school and she needed a job. She worked for some company, Baxter-somebody. I used to joke with her about that. Was that a cosmetic outfit? But in any case, Ann began to work in my lab as a dishwasher. She was very talented and an extremely interesting young woman who was interested in science. During the course of working in my lab as an undergraduate and later as a graduate student, she started a romance with a graduate student who was working for his PhD with Hassid, William Zev Hassid, who was in the same building. Vic Ginsburg was his name. That romance blossomed and he was still working, so Ann decided she would stay in the lab. She got her Bachelors degree, did some undergraduate honors with me, and then continued for a Masters degree and did some very beautiful work on an interesting problem. At that time, Linderstrøm-Lang, who's one of the great men in the history of protein chemistry, from Copenhagen, had talked and written about different mechanisms of the break down of proteins by enzymes. He described the one by one mechanism in which the proteolysis leads to the rupture of all the bonds in one molecule before the enzyme attacks another protein molecule. This is the one-by-one or all-or-none mechanism where one sees fully digested molecules and native molecules during the course of the reaction. No intermediates are detected. The alternative is the zipper mechanism, where the enzyme gradually works its way toward final products and intermediate breakdown products are observed.. So Ann began working on two problems. One was the digestion of insulin by chymotrypsin, and the other one was the breakdown of ribonuclease by pepsin. These were both interesting proteins and we used the ultracentrifuge and viscometry along with protein chemistry to follow the kinetics.

Schlesinger: Did you tell me the date?

04-00:02:24

Schachman: This would be in the late fifties, because the papers were finally published in 1960. She was doing magnificent work. In the meantime, Vic was going along slowly for his PhD. So Ann became interested in the possibility of continuing and getting a PhD. At that time, the graduate advisor was a very good friend of mine, but he had Neanderthal views about women in science. His view was that women change their names and they drop out. It's terrible to let limited spots in a graduate program be given to women, because they won't finish, and some man was therefore deprived of this opportunity. So like a fool, he said to Ann, "You're much too beautiful. Why don't you go home and have babies?" She got absolutely disgusted—for good reason—and finished up with two magnificent papers in the *JBC*, on these two proteins. In addition, she did some work—

Schlesinger: Finished up her PhD or her Masters?

04-00:03:24

Schachman: Her Masters degree, because she quit after the Masters. She also worked on the Archibald Method, which was an interesting method proposed by some mathematician in Nova Scotia, on the problem of sedimentation equilibrium, which I didn't talk about when I talked about the ultracentrifuge. It had a heyday for it that lasted about a year of excitement; and then, like every other new development in science, [chuckles] its peak passed. But we wrote a nice paper, with another technician of mine. Ann was the senior author of that paper. So she had a very productive period with me. Vic finished and was then going to postdoc with Herman Kalckar at NIH. They got married; I went to their wedding, the Ginsburg's wedding. Herman was going to hire Ann and I said, "Don't hire Ann, because Ann is a very organized, systematic worker, and Vic works in spurts. He'll play cards for twenty-four straight hours, not thinking about science, and then he'll have a good idea and do some very creative work. They should not be side by side in the same laboratory."

Ann went, with a Masters degree, to NIH. Bernie Horecker hired her in his lab. I remember him distinctly calling me up complaining about Ann, that she was obstinate and so forth and so on. So I said, "Well, Bernie, what are you doing and what is she doing?" He said that he wanted a technician who'd put a tenth of an ml of this solution in a tube, and seven-tenths of an ml of another liquid in the tube, followed by two-tenths of an ml of still another reagent. It adds up to one ml, and we give her written instructions. I said, "Bernie, she's extremely creative. Leave her alone. Give her a problem, let her go into the library

and read [chuckles] and come back and work on your problem. And give her another enzyme that you're interested in." So of course, that's what he did. She thrived. By that time, Bill Harrington was at NIH, also. She began to work with Bill, and especially with Bill Carroll. She published a magnificent paper with Bill Carroll, on the two state denaturation of proteins, which was part of the PhD that she obtained while working at NIH, either at Georgetown or George Washington, I never remember which school. Then she went on to a forty-year career as an independent scientist, in Earl Stadtman's department at NIH. Earl just died about a month and a half ago, and Ann died suddenly about two weeks ago. That's what reminded me of this story. So she's had a fantastic career and it illustrates the prejudice that existed at that particular time. The memo that was sent around the NIH campus talked about the Ginsburg husband-wife relationship as one of the original groups at NIH, where the husband and the wife both worked in separate places on the NIH campus, just like Earl Stadtman and Terry Stadtman were independent scientists on the NIH campus.

Schlesinger: Well, I think there were many examples there.

04-00:07:20

Schachman: That's right; NIH was a forefront place. Because in those days, of course, universities had nepotism rules, you couldn't hire a wife and a husband in the same department and it was very difficult to find a second, separate department for one of the spouses. So that's why I wanted to get the Ann Ginsburg story in. She made really significant contributions on glutamine synthetase for years. About fifteen years ago, I went on a sabbatical to NIH as a Fogarty Scholar, as part of the Fogarty program. I worked with Ann. She was, by that time, an expert in calorimetry and we published a very interesting paper on aspartate transcarbamylase. So I spent a lot of time with her.

Schlesinger: Okay, Howard. Let's return to aspartate transcarbamylase now. We'll go a few years into the future. In January, we left things where you described the enzyme as having twelve chains.

04-00:08:29

Schachman: Right, the enzyme was composed of six catalytic chains and six regulatory chains. And it turns out, after a lot of hard work, all of those working on ATCase were in agreement that the catalytic chains were organized as two trimers and the regulatory chains were organized as three dimers. Establishing that was, in itself, somewhat difficult, and three different groups (including mine) had it wrong at first. I've already mentioned that Klaus Weber

had shown there had to be six regulatory chains, and that forced us to reevaluate the so-called tetramer view, which was rushed into print and had some sloppy data. Then there was the mistake in interpreting the crystallography that Tom Steitz had made. By that time, we began working on the catalytic subunits. One of my students had worked on aldolase earlier, and he began using hybridization as a technique to study protein oligomers. The way the technique works is as follows: If you have a dimer, and if you have two types of dimers, and you take them apart into monomers, and then you re-associate them, you will wind up with the original two dimers plus the hybrid dimer. So therefore, there will be three species present. And if you have electrophoresis and the two parent molecules differ in mobility, then you will see the intermediate as well as the two parent molecules. Actually, Milt (Schlesinger) had used this technique.

Schlesinger: I was just going to say that.

04-00:10:03

Schachman: Right, takes you back to Milt Schlesinger's work with Cy Levinthal.

Schlesinger: It was intracistronic complementation that had confused people, because they didn't understand how you could have two proteins coded by the same gene (cistron) that had mutations so that both were inactive and then end up with an active enzyme. Alkaline phosphatase is a dimer and what Milt showed was that two mutationally-inactive dimers could be disassociated and the reassociated hybrid dimer was enzymatically active if the mutations in the two inactive dimers were in different parts (now we would say domains) of the polypeptide chain.

04-00:10:29

Schachman: That's right. In those days, we weren't doing site-directed mutagenesis, so if you wanted to do these experiments *in vitro*, you had to do it by chemical modification. What Ted Meighen did in my lab with aldolase was to succinylate one of the samples with succinic anhydride. That meant we changed a significant number of amino groups into succinyl derivatives. So that put a carboxyl group on the protein instead of positive amino group. And therefore, the mobility was altered. You could then take the succinylated protein and the native (unmodified) protein, put them in 8M urea and if the proteins were reconstitutable, which many of them were, you would then wind up with a hybrid set.

Schlesinger: And was the succinyl derivative active?

04-00:11:09

Schachman: Frequently not. But we didn't care at that time. We were interested only in the structural aspect. If you over succinylated, you would electrostatically blow the molecule apart. So you had to do very limited modifications of amino groups. But the technique worked beautifully on aldolase, and we were able to demonstrate that aldolase was a tetramer. So we immediately applied this to the catalytic subunit, and sure enough—

Schlesinger: Of aspartate transcarbamylase?

04-00:11:37

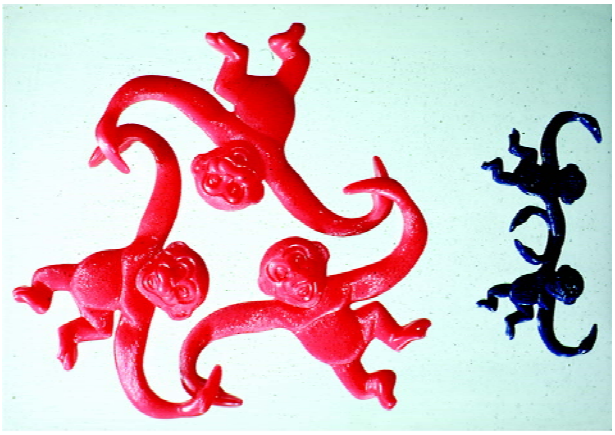
Schachman: Of aspartate transcarbamylase, and there was no question that the catalytic subunit was a trimer, because we wound up with a four member hybrid set. It was very clearly established. Independently of that, George Stark—with whom we used to share group meetings, he was in Palo Alto at that time, on the Stanford faculty—was doing cross-linking. And he was able to show by cross-linking experiments that the catalytic subunit had to be a trimer. By that time, Don Wiley had shown there was 2:3 symmetry; so therefore, we had trimers instead of tetramers. Since the evidence indicated that the regulatory chains in solution existed as dimers, we adopted the view that ATCase was composed of two catalytic trimers and three regulatory dimers. Now, at that particular time, I had a student by the name of Cohlberg who was a very nice kid, now on the faculty at Cal State somewhere in Southern California. He began working on cross-linking the dimers. We asked a simple question. Because the dissociation of ATCase that Gerhart and I had done produced trimers and dimers, did that mean that within the intact enzyme the chains existed as trimers and dimers? Trimers are very stable, because to break a chain out of a trimer, you had to break bonds between A and B, as well as A and C. And that makes it much more stable than the dimer. In a dimer, there is only one set of bonds linking the monomers. So an A-B dimer can come apart into A and B very easily. So I kept asking a question. Because we isolated dimers, does that mean there were dimers as part of the ATCase molecule *in vivo*? Or did single chains come out and then dimerize when they came out? So I said, "Well, we know enough about cross-linking. Why don't we cross-link the chains?" So we cross-linked the regulatory dimers—

Schlesinger: *in vivo*?

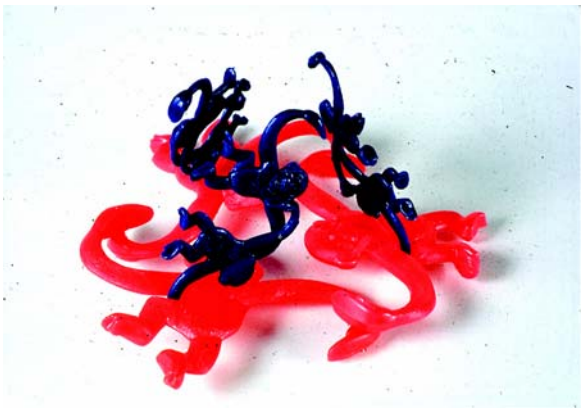
04-00:13:26

Schachman: No, *in vitro*, with dimethyl suberimidate, a standard cross-linking reagent that we just picked up out of the literature. We then asked the question: would the cross-linked regulatory dimers reassociate with catalytic trimers to form ATCase that would be active? It turned out we got beautiful ATCase from the cross-linked material. Not only that, we were able to study kinetics. By that time another terrific graduate student, Mark Bothwell, was working on the problem. We were able to show that the cross-linked regulatory dimers reacted with catalytic trimers just as well as un-cross-linked ones, kinetically. So there was no question in our mind that the regulatory dimer was an endogenous piece. Now, that was interesting because at that time, Lipscomb had been flirting with the idea that the two catalytic trimers were like a sandwich, and there were three regulatory chains at the top and three regulatory chains at the bottom, rather than it being a hamburger, in which the meat in the middle was, in fact, the three regulatory dimers. So it turns out by doing that experiment, we were able to use monkey models, which became very popular, and we were able to demonstrate that the two trimers were held together by regulatory dimers. And so the monkey models became a part of the literature at that time. My Harvey lecture had pictures of the monkey model assembly of ATCase.

Here is a picture of the monkey showing one catalytic trimer and one regulatory dimer.



And we wound up with two trimers cross-linked, essentially, by three regulatory dimers and that became accepted widely. Here on the right is the monkey version. (Only one of the trimers is shown.)



Then Lipscomb, along with the people who followed Don Wiley in this research, came out with high resolution X-ray structures and the structural aspect of ATCase was essentially completed.

04-00:15:46 So the next aspect of our research was, in fact, the overall study of the conformational changes in the enzymes resulting from interacting with substrates and analogs. This was the other aspect of John Gerhart's original approach to me. Not only did he want to study the subunit composition by dissociation with mercurials or other techniques, but he also wanted to study the change in the enzyme as a result of interaction with CTP and ATP as well as the substrates. The reason he asked me to do the latter was because we had been working on this difference sedimentation velocity technique. The concept started out with Rayleigh optics, where you put protein by itself in one limb of a double sector cell, and protein plus a ligand in the other limb, and let the optical system subtract the patterns. If the ligand slowed down the material or speeded up the material, you'd get a difference sedimentation pattern, and you'd be able to measure a 1% change in sedimentation coefficient, to about 1% accuracy. Well, when we tried this with ATCase and carbamylphosphate and succinate, the change was *enormous*, so we didn't need that technique. Gerhart and I then began to follow up the change in the sedimentation coefficient as a function of the ligands binding to it.

Schlesinger: When you said you didn't need the technique, what technique did you use, then?

04-00:17:00

Schachman: We then used the simple method of having two centrifuge cells in the two holes in the rotor. In ordinary sedimentation velocity experiments, one of the holes in the rotor is occupied by a counterbalance cell that is opaque. For these experiments we wanted to obtain two sedimentation velocity patterns in the same experiment. So one of the cells had wedged windows, which displaced the pattern on the photographic plate. In that way we could see the schlieren pattern from each cell simultaneously and you could measure the sedimentation coefficient of the enzyme in the absence and presence of ligands in the same run. The main reason you do that is because the biggest error in the determination of sedimentation coefficients is attributable to uncertainty in the viscosity of the solvent, which is dependent on the temperature. And even though there's a temperature-measuring device in the ultracentrifuge, it's not that accurate. So if you do two successive runs and you're off by one degree in temperature that leads to 2% uncertainty in viscosity. If you are trying to measure a 2% change in the sedimentation coefficient, you would never know whether you had it. But if you put the two cells in the same rotor at the same time, then the temperature variation disappears because it's all at the same temperature and, therefore, you can easily see one boundary moving 2% faster or 2% slower. And sure enough, with carbamylphosphate and succinate, succinate being an analog of aspartate, it turned out that although the enzyme bound ligands leading to an increase in both the molecular weight and the density of the protein, the liganded protein sedimented more slowly.

Schlesinger: But you didn't really increase the molecular weight of the protein that much.

04-00:18:18

Schachman: No, but enough that you'd expect a slight increase in sedimentation coefficient. Instead of that, we found a 3% decrease in sedimentation coefficient. So the only way you could account for that is either an elongation of the molecule or a swelling of the molecule. We attributed it to a swelling.

Schlesinger: Was that just a guess?

04-00:18:38

Schachman: Yes, that's right. You can't prove by this one measurement alone which it is. But you can say there's a global conformational change, which became the focal point of years of activity in our laboratory. We wanted to link this

conformational change to the binding of ligands. So that effort becomes a whole new story.

Schlesinger: And if you just added succinate by itself, would you get the change?

04-00:19:01

Schachman: No. It's a two-substrate enzyme, and carbamyl phosphate is a real substrate, and succinate was an analog of aspartate, so there was no reaction.

Schlesinger: Right. But you needed both ligands to get the change.

04-00:19:13

Schachman: That's right. Precisely.

04-00:19:14

Schachman: Then some years later, George Stark, a superb enzymologist who was interested in the catalytic mechanism, did some great work with Kim Collins. They constructed what they thought would be a transition state analog. They wanted to determine the nature of the molecule representing the transition between carbamylphosphate and aspartate on the path to form carbamylaspartate. And they synthesized a compound called N-(phosphonacetyl)-l-aspartate (PALA). It was an elegant synthesis, and the compound would bind like crazy to ATCase. Very soon it was used to replace carbamyl phosphate and succinate as the ligand of choice for all types of binding studies. As years go on, it turned out not to be a transition state analog, but it is certainly a bi-substrate analog. The affinity of ATCase for PALA is almost in the nanomolar region. We replaced carbamyl phosphate, and succinate in all our subsequent research on studying conformational changes with PALA. It was a tremendous inhibitor for ATCase and George Stark thought it might be useful in studies of cancer. It was a wonderful inhibitor. The Cancer Institute got very excited because it would stop DNA synthesis like crazy because it interfered with the production of dCTP.

Schlesinger: And then what happened?

04-00:20:41

Schachman: It turned out not to be useful in the cancer field. They made a pound of it at the Cancer Institute. I think they contracted with the Stanford Research Institute to synthesize this material. And we were using it in microgram amounts, so we still have some in my refrigerator here. It's been used extensively for years.

Schlesinger: Well, why didn't it work?

04-00:21:04
Schachman: We don't know. Probably there's an alternative pathway for making dCTP. I'm not sure.

Schlesinger: Either that or it had other effects.

04-00:22:10
Schachman: It wasn't toxic, to my knowledge, but I've forgotten. George Stark became interested in ATCase from other organisms and ATCase from higher organisms turns out not to be a single enzyme; it's not allosteric, it's not a hexamer, it doesn't have regulatory chains. It is a trifunctional enzyme, with the enzyme carbamylphosphate synthetase and a third enzyme (I think it is dihydroorotase). There are three enzymes all linked together in one big protein.

Schlesinger: Do you know where the evolutionary transition in the structure of the enzyme is?

04-00:21:42
Schachman: I don't think that's ever been studied, but I don't know.

Schlesinger: It might be known.

04-00:21:46
Schachman: In yeast, it's a complicated trifunctional enzyme, I'm pretty sure.

Schlesinger: So this catalytic and regulatory subunit division is only in bacteria?

04-00:21:56
Schachman: In *E. coli* and a bunch of other bacteria. So they all use different mechanisms, obviously.

Schlesinger: This is March 19, 2008 and we are continuing with Howard's interview.

04-00:22:30
Schachman: Well, last time, we talked about the overall structure of aspartate transcarbamylase and how various groups—the crystallographers with 2:3 symmetry, cross-linking experiments done by George Stark, our hybridization experiments all led to the conclusion that the molecule was composed of two catalytic trimers and three regulatory dimers. So there were six chains for catalysis and six chains for binding the regulatory molecules such as dCTP and ATP. I'd indicated, also, that we'd done some experiments on cross-linking. We were very concerned

when we saw regulatory dimers, because regulatory dimers existed in a dynamic equilibrium with monomers.

Schlesinger: For this protein, or in general?

04-00:23:28

Schachman: For this protein. And it may be true in general, because dimers frequently are less stable than trimers, for example. I haven't mentioned it earlier, but when we started trying to get the *E. coli* to overproduce ATCase, we discovered—and we weren't alone in this; George Stark also saw it, and Lipscomb's lab also saw it—that we wound up with what looked like an impurity. We went after that impurity with great effort. Yang actually purified it, and it turned out to be an ATCase-like molecule lacking one regulatory dimer. So essentially, it contained six catalytic chains and four regulatory chains, which we diagnosed as two trimers and two regulatory dimers, rather than three.

Schlesinger: And did it have the same kind of activity?

04-00:24:25

Schachman: We purified it, it had activity, it was allosteric. We also discovered that at low ionic strength it was not as stable as the intact enzyme. When we tried to take pictures of it, in collaboration with Robley Williams we found out that it was much less stable than the holo-enzyme and if you placed it under conditions in which the bonds between the regulatory and catalytic chains were weaker, it would then undergo an equilibrium transformation to give holoenzyme and dissociated subunits.

Schlesinger: When you said take pictures, I assume you meant electron micrographs.

04-00:24:57

Schachman: Yes, Robley Williams was taking electron micrographs on material that we were providing. So we were collaborating, in some respects, with Robley, although we never published with him; we published side by side with him. It was at that period of time we discovered that zinc was an important component. I sent material, actually, to Bert Vallee to find out what was going on. And of course, he calls me up and says, "It was zinc." I said, "But Bert, you *always* find zinc." [Schlesinger laughs] Everything he did contained zinc. So it was a zinc-containing enzyme, except that the zinc was in the regulatory chains, and it was responsible for holding them together. Vallee had been studying zinc-containing enzymes where the zinc was needed for catalytic activity. So when we isolated the regulatory dimers, we

found out that they were in dynamic equilibrium with monomers. We immediately added zinc and that stabilized them as dimers, rather than as monomers. So zinc was an important constituent and it bound to the sulfhydryl groups of the regulatory chains. In retrospect, that discovery provided an explanation for the earlier experiments of Gerhart. When John Gerhart had done his original desensitization experiments, he had used a mercurial. The mercurial drove the zinc out, and the mercurial bound to the sulfhydryl groups of the regulatory chains, and the enzyme fell apart. He obtained catalytic trimers, which were active, the enzyme lost its allosteric properties, and he didn't understand it. It all fit together when we began working in collaboration with one another. So we know now we have zinc-containing regulatory dimers. As I indicated at the end of the last session, Cohlberg, Bothwell, and Nagel began cross-linking the regulatory dimers using typical cross-linking reagents, so that we could answer the question as to whether the dimers were an endogenous part of ATCase, rather than an artifact of monomers assembling after the molecule was dissociated. Whitehead had already published a postulated model.

Schlesinger: Who is Whitehead?

04-00:26:52

Schachman: Somebody who worked on ATCase a little bit and I think Lipscomb had bought into that discussion that the regulatory chains were on the top and the bottom, rather than acting as cross-links between two trimers. I remember vividly an experience with Jeff Cohlberg, my student. We were in a meeting somewhere, probably in San Francisco, and Lipscomb was there. In those days, we were still in open discussions with Lipscomb, even though he was a relatively secretive guy. I told him we had done this cross-linking experiment with the regulatory subunits and they assembled magnificently. We did competition experiments; they were just as good in assembly as uncross-linked regulatory dimers. So he looked at me and listened very carefully, Cohlberg standing by my side. Lipscomb listened and said, "That's interesting," and didn't say another word. Cohlberg and I walked away and Cohlberg said, "Why didn't he communicate?" I said, "Jeff, that's the last time I will talk to Lipscomb about our research, because it's got to be a two-way street." And it clearly wasn't a two-way street.

So we now know we have regulatory dimers serving essentially as "cross-links", between two trimers and I referred earlier to the monkey models. The Cohlberg, Pigiet (and Schachman) paper actually had a wooden model, in which we had two trimers with

heterologous binding domains cross-linked by wooden sticks, essentially, of regulatory dimers. All we had at that time were Robley Williams' electron micrographs and our stoichiometry. And that began, then, to be the accepted model which all of us bought into.

So then the question came up immediately thereafter, how do you assemble this enzyme? So there are two aspects of assembly. One aspect, of course, is the *in vivo* assembly, which is obviously the much more important part. But you couldn't do that until you started to have genes and you could do all sorts of experiments with separate subunits and having the bacteria produce either catalytic trimers alone or regulatory dimers alone, if they could. Or you could study the *in vitro* assembly. Since we had the trimers and the dimers apart from one another, we could then study the process. I had a bunch of wonderful students. One of them was Drusilla Burns. She was interested in the problem of how do trimers form. So it took two aspects, and two nice *JBC* papers with her. One aspect was dissociating the trimers in urea or guanidine hydrochloride, typical denaturing agents that everybody had been using for decades in protein chemistry. These reagents lead to the formation of unfolded polypeptide chains. Then the assembly process that you could visualize is that they must fold first, and then associate and we would study that. The slow step, presumably, would be the folding step, and you ought to be able to get first order kinetics because the folding of a chain is a slow process, and that's not dependent upon concentration. So Drusilla did a beautiful piece of work on that, using all kinds of tools that typical protein chemists use. And sure enough, we worked out the kinetics of that process. It was first order, and we felt terrific about it. You get very high yields, incidentally, of active catalytic trimers. The reconstitution process is close to 100%. It's remarkable.

Schlesinger: So you just get rid of the urea by dialysis?

04-00:30:30

Schachman: Right. Or by rapid dilution. And the nice part about that kind of experiment is you can have a mixture containing succinylated catalytic subunits in urea. Then you wind up with hybrids. So you could do all sorts of manipulations. And it's simple, straightforward protein chemistry, because the tools were all available—optical rotation and we used, of course, sedimentation to prove that we had trimers, and then we used enzyme activity. So that was a wonderful study, and it was

published in *JBC*. But the big question was then, what if you had the folded monomers? How would you be able to study them?

Schlesinger: You mean study them as monomers, is that what you mean?

04-00:31:08

Schachman: Well, as monomers, and also their assembly into active trimers without going through the folding step. Because we were hypothesizing that folding was the first process, and that once you folded a monomer, it associated rapidly with two other monomers and you wound up with catalytic trimers. It turns out there were other reagents available, sodium perchlorate and sodium thiocyanate, which were known to dissociate oligomers but presumably, did not go into the backbone and unfold the chains. So sure enough, Drusilla was able to take sodium thiocyanate and sodium perchlorate—they'd been studied by lots of other workers with other programs—

Schlesinger: How do they work?

04-00:31:46

Schachman: Presumably, it works by disrupting some hydrophobic interaction. It was not nearly as well known as urea breaking the organization of the backbone in the chains. Sure enough, the assembly this time was second order, because the slow step is two monomers coming together, and as soon as the two monomers get together, a third one goes in. So she published that as a separate paper in *JBC*, and we felt pretty confident we understood the assembly process and that would be analogous to *in vivo* assembly. We would then postulate that *in vivo* you made catalytic chains, the catalytic chains fold, then the folding is followed by association and you wind up with trimers and presumably, then they interacted with regulatory dimers. But the process, of course, was speculative at that particular stage of our knowledge. Nonetheless we felt great about our progress.

Then we asked the question, well, supposing we now have trimers and dimers; how about the assembly of ATCase oligomers. Now, this is obviously much more complicated. I had another wonderful graduate student named Mark Bothwell, who is now a professor at the University of Washington up in Seattle. He began to work on that process and we wound up with a model involving ten different reactions, some of them were reversible and some not. For example, if you take a catalytic trimer and postulate that one regulatory dimer goes on, that's a reversible process because you only have one bond between a catalytic chain and a regulatory chain. Then a second one goes

on; that's also a reversible process. Then a third one goes on. Each of these reactions is reversible because they involve single bonds between catalytic and regulatory chains. The best way to visualize how this scheme was developed is to look at this diagram summarizing the 10 different reactions, those that are reversible and those that are not reversible.

Here on the right are the 10 reactions indicating the steps that are reversible and those that are not. As soon as you start putting trimers onto them, you begin constructing a stable product. If, for example, you have two catalytic trimers linked by two regulatory dimers, that intermediate is stable because you have to break two bonds to take it apart.

$$C + R \rightleftharpoons CR \quad (1)$$
$$CR + R \rightleftharpoons CR_2 \quad (2)$$
$$CR_2 + R \rightleftharpoons CR_3 \quad (3)$$
$$CR + C \rightleftharpoons C_2R \quad (4)$$
$$C_2R + R \rightarrow C_2R_2 \quad (5)$$
$$C_2R_2 + R \rightarrow C_2R_3 \quad (6)$$
$$CR_2 + C \rightarrow C_2R_2 \quad (7)$$
$$CR + CR \rightarrow C_2R_2 \quad (8)$$
$$CR_3 + C \rightarrow C_2R_3 \quad (9)$$
$$CR_2 + CR \rightarrow C_2R_3 \quad (10)$$

Schlesinger: Say it again so we get it clear.

04-00:34:23
Schachman: So we have a catalytic and a regulatory chain binding.

Schlesinger: One and one.

04-00:34:27
Schachman: One and one. Now you have two possibilities at that particular stage. You can bind another regulatory subunit; and that would give you—

Schlesinger: Two regulatory subunits.

04-00:34:38
Schachman: On one catalytic subunit. That is correct, but note that there are only single bonds involved in each of those interactions and the reactions would be considered as reversible. Similarly you could visualize having a second catalytic subunit binding to the other half of a regulatory dimer to yield an intermediate with two catalytic trimers cross-linked by one regulatory dimer. That complex would be unstable because each of the bonds between the regulatory dimers and the two catalytic trimers would be reversible. So through Bothwell's research, we wound up with a map that we published (See the figure on page 95).

That was all speculative. That was paper and pencil work. The big problem was, how do you demonstrate this? Mark Bothwell went ahead with gorgeous techniques—stop flow types of experiments that were relatively crude but would work. They were not stop flow experiments in the formal sense, but Bothwell did use "chase" additions to search for intermediates. We worked out a scheme illustrating all the reactions that presumably occurred. We could see some intermediates. C_2R_2, for example, appeared as a relatively stable intermediate.

Schlesinger: With stop flow were you just looking for enzymatic activity? What were you assaying?

04-00:35:55

Schachman: Bothwell did electrophoresis experiments and looked at the species. You couldn't do sedimentation analysis on this system, so all studies involved electrophoresis. In some of the "chase" experiments he used succinylated proteins so as to alter the charge. In this way we wound up with a scheme that allowed us to talk about how ATCase was assembled *in vitro* from catalytic trimers and regulatory dimers. So basically, that study consumed a significant amount of years of work, involving three graduate students.

Schlesinger: And you're now in the seventies, 1970s?

04-00:36:28

Schachman: This is all done in the seventies, '74. In fact, the Burns' papers were not published until the eighties, early eighties, but there were some in the late seventies, early eighties. It was an exciting period. We knew about zinc then, we knew about how assembly of trimers would occur, we knew about hybridization experiments. And the next question after that is, what's happening *in vivo*? Obviously, the only way you're going to get there is if you could get the genes out. The gene that makes catalytic trimers was already known; it's called *pyrB*. But nobody knew the location of the gene encoding regulatory chains. Lo and behold, my lab started to work on genetics—far from my field. I had a wonderful graduate student, Duane Jenness, who began to explore this. His research dealt with *pyrB* mutations as suppressors of arginine auxotrophy. This research was way outside my area of competence, but Duane was terrific and he received help from John Roth, a faculty member in the department. What little I know was learned from Duane and his research led us later into studies of interallelic complementation. It was very easy for us to ask where is the gene encoding the regulatory chains by looking at the DNA. But assessing the

presence or absence of regulatory chains in bacteria was not nearly so direct. Studying mutants with deletions of *pyrB* DNA was no problem because it codes for a protein that has catalytic activity. If such activity is present then you know something about the presence of *pyrB.* If you have enough DNA sequence following that region containing *pyrB*, you may be able to find a region of DNA responsible for the regulatory subunits. There was evidence from the work of others that the two genes were in close proximity and perhaps in a singe transcriptional unit.

Schlesinger: Well, they could know from genetics if it was an operon.

04-00:37:44

Schachman: Right. That's absolutely correct. We were not certain as to whether the two genes were part of an operon. But we also knew that Klaus Weber had published the polypeptide sequence of the regulatory chain. So once we got a large fragment of DNA that contained the gene coding for the catalytic chains, we found some additional DNA, which corresponded to the gene for the amino terminus of the regulatory chains. There was a fifteen-unit intercistronic region between them. We then knew that we had an operon and that the two contiguous *pyrB* and *pyrI* genes constituted a single transcriptional unit encoding the catalytic and regulatory chains of ATCase. We were, I think, the first to publish on the structure of the *pyrB-pyrI* operon.

Schlesinger: It was a different way of identifying an operon than had been done before when people did it by genetics.

04-00:38:21

Schachman: That's right. That's right. So that led to fascinating experiments that were, of course, foreign to my laboratory. My student, Dave Pauza learned how to do DNA sequencing. If my memory serves me correctly, he went down to Arthur Kornberg's lab. Dave Pauza, a very bright guy, but tended to be sloppy and very dogmatic, he knew all the answers in advance.

Schlesinger: He was a student in your lab?

04-00:38:44

Schachman: He was a student in my lab. And he was the one who began the work on the nucleotide sequence of the DNA responsible for both the catalytic and regulatory chains. So sure enough, he came up with the evidence that there was an intercistronic region. The DNA fragment he had isolated contained the coding region for the beginning of the regulatory chains. So we were very happy. But Dave tended to be in a big hurry and tended to

be sloppy. And I actually went in the lab myself to learn how to do sequencing. I did a couple of experiments, but—

Schlesinger: You were doing Sanger or Maxam-Gilbert?

Schachman I think it was Maxam-Gilbert, but this ought to be checked since right now I am not certain.

Schlesinger You would have remembered if you ever did Maxam-Gilbert; it was very hard.

Schachman: It was hard, especially for me. I was sitting in the lab pipetting, because I was suspicious and I didn't want to be wrong. Dave knew I was skeptical. At one time, we were nervous about how many nucleotides there were between the two gene coding sections. So I convinced myself there were fifteen nucleotides in the intercistronic region. And I convinced myself that we knew the sequence. But we then went ahead and got the whole sequence.

Schlesinger: Of the regulatory subunit gene?

Schachman: The structure of the whole operon containing the genes for both the catalytic and regulatory chains. We were about to publish it. By that time, a variety of people in the lab had worked on the problem. Pauza obviously should've been considered as the first author, but a lot of people in my lab were very unhappy; they felt that others contributed enormously. I remember talking to one of my postdocs, and I told him how perplexed I was about how to deal with authorship for this publication. He said, "Well, I came from a lab where the problem was solved in a case like yours where the professor was skeptical about the first author, and others would've been extremely unhappy if he had been the first author, because they contributed enormously, as well." Then he said, "So the professor put his own name first." That would have been very rare, in my case; I hadn't published a paper with my name first on a paper in years and years. But I thought that was an interesting idea and I actually did [laughs] some of the experiments, but a trivial amount. So we published the paper and the authors were Schachman, et al. with Dave Pauza as the second author.

It's probably the only paper in which my name is first author. All the papers with John Gerhart were Gerhart and Schachman. It was especially interesting because we had this horrible unpleasant sort of competition, even though we worked in different areas, with the Lipscomb lab. When Lipscomb had to

refer to this work for the amino acid sequence of the chains in ATCase, he had to refer to Schachman, et al. So it gave me a great deal of pleasure that in this particular case, my name was first rather than last.

Schlesinger: So was there a lot of genetics, or just sequencing, in the paper with you as the first author?

Schachman: Well, then Mike Syvanen was another one of my graduate students. He began working with John Roth who's on the floor down below us, a superb geneticist. And they published some stuff on that. So I learned very little genetics in the process.

Schlesinger: That's what I was wondering, if you were going to be first author of a paper that had a lot of bacteria genetics.

Schachman: No, no, by no means. And by that time, of course, we then began our cloning operation and we began doing site-directed mutagenesis. We learned about Michael Smith, who had developed that gorgeous technique up in British Columbia, for which he won the Nobel Prize. We used the technique as described by Zoller and Smith in a review article.

So we began doing site directed mutagenesis and conducted a series of wonderful experiments with mutants. Incidentally, that's when the structure work became important, because we began asking questions about the active site of the enzyme. I had a student, Ellen Robey, who is now a full professor in this department here in Berkeley. She's in the immunology section. She went to work with Richard Axel and became interested in immunology. Ellen began working on modifications of the enzyme that affected catalytic activity. She started with chemical modification and then began to do site-directed mutagenesis. Obviously it was necessary and useful for us to know the detailed structure. Lipscomb was publishing structures "left and right" on ATCase, but they were not deposited in the Brookhaven bank, despite admonitions about the practice of publishing detailed stereo-diagrams without releasing the coordinates. In one particular case, when the pressures began to be enormous to deposit your coordinates at the time the paper was submitted for publication, he actually deposited and then withdrew them. In those days there were footnotes in papers indicating that the coordinates had been deposited, or the coordinates are being deposited, or the coordinates will be deposited. In this particular case dealing with ATCase, the coordinates had been deposited, but by the time I searched the Brookhaven bank to get them, they were withdrawn. So it was

quite clear that Lipscomb did not want us to have the coordinates. So Ellen Robey was a bright young student. She decided she didn't know how much of this apparent conflict was due to Schachman and how much to Lipscomb and the two just not getting along with one another. She wrote Lipscomb a letter and told him that she was coming to Boston. She was going with her boyfriend, to Europe, they planned to stop in Boston, and she's working on the activity of ATCase. She would like to visit him and see the structure and learn something about the coordinates. So she went. She was an attractive young woman and Lipscomb entertained her royally. He was very gracious to her there, and promised her that he would send the coordinates and they would be in Berkeley when she returned from Europe. Ellen came back from Europe and, of course, wanted to know where the coordinates were. I said, "They're still coming." [They laugh.] She told me the story of her visit. So we never did get the coordinates from him. Ultimately, this is the problem I worked on when I became ombudsman at NIH and Harold Varmus said, "Howard, why don't you tackle this problem?" Do you know that story?

Schlesinger: Some of it, but the Bancroft doesn't know the story.

04-00:45:25

Schachman: Well, that was a fascinating period. I think it was Alex Vlodower, a very good crystallographer at one of the government labs, who began pressuring Harold Varmus to do something about the fact that NIH was supporting all this research in crystallography leading to the publishing of all these gorgeous structures without the coordinates being available. Obviously this hindered further investigations. So Harold turned to me and said, "I am, of course, *very* well aware of the problem. Howard, how would you like to work on this problem?" I said, "Sure." I immediately formulated a letter that I sent to two crystallographers whom I knew very well—one was Brian Matthews in Oregon; the other was Fred Richards at Yale. I said, "Gee, I'm interested now in pressuring the crystallographers to do something about getting the coordinates deposited. Varmus will not operate on his own initiative as 'big government'. But if Varmus is pressured from superb people from the outside to do something about it, he will then be responsive and apply pressure on the journals to do something." It was the journals that were ultimately responsible for this problem, and they weren't doing anything, because they were in competition. Each of them wanted the crystallography papers. So you had Ben Lewin at *Cell*, you had *Nature*, you had *Science* all publishing papers, and no coordinates. Fred Richards was a

great believer in deposition. Brian Matthews originally was one of those who wasn't depositing because he felt, gee, I'm doing all this hard work to get a structure; I want to be able to exploit it. But as time went on, he began to see that he had twenty wonderful structures and he wanted to make them available to the world. So Brian and Fred both cooperated with me and we wrote a nice letter that they liked very much. Their participation was crucial because they were crystallographers and I wasn't. I mailed the letters then to about twenty well-known crystallographers throughout the country asking if they would join in this effort. Oh, and this'll interest you no end. One of the people, of course, I sent it to was Don Wiley at Harvard. Don Wiley immediately— in fact, he called me on the telephone, as I recall—said, "Are you sending it to the Colonel?" The Colonel, of course, among the cognoscenti was Lipscomb. He knew darn well that Lipscomb was the major culprit in this field—although not alone; there were about four or five major figures who were publishing detailed structures and not disclosing the coordinates. So I laughed and said, "No." He said, "But you ought to send it to Steve (Harrison)." I hadn't sent it to Steve. I had sent it to Max Perutz who was a vigorous advocate that coordinates be deposited at the time of publication of the structure. Max Perutz put our letter on the bulletin board in Cambridge, England. I began getting letters from people in Cambridge, England who wanted their names on the letter; that's how enthusiastic he was. So I wrote to Steve. Steve was traveling. I think he was in Europe at the time. This may be particularly interesting to you because you're a good friend of Steve Harrison's. He had a very unpleasant experience with the Brookhaven Bank. They were apparently very bureaucratic and they caused a lot of grief to those people who were making depositions. He was very angry. I was surprised how angry Steve was and his anger came out with criticism of the idea. He didn't want to go to the Brookhaven Bank. I think he wanted the coordinates published, but not with them. Of course, there was no other vehicle. There was something in Britain, but I didn't know anything about the British thing. In any case, I didn't put Steve's name on the letter; I had about twenty names and I gave them all to Harold. I wrote a formal letter to Harold Varmus and said, "These twenty people all subscribe to this letter asking you to go after *Science,* and all the other journals, to make sure that the coordinates are deposited." Varmus wrote back, because we had this nice relationship, and he said, "Howard, that's wonderful, but I need permission from each of these authors." I had to go back to each one of these guys another time and ask them for permission to transmit this to Harold

Varmus. So I got that. Varmus wanted a copy of the list of journals that I would send it to—*JMB*, for example. Somewhere along the line, somebody suggested, hey, you don't have Aaron Klug. Aaron Klug was a big shot in *Journal of Molecular Biology*. Aaron didn't like the letter, so he never signed it. He indicated you ought to have a time period. The crystallographers were debating, should you have a year after you published it? Some of the crystallographers were having industrial concerns. They wanted to make drugs for their structure. Some of them were not sure of their structure, but they didn't say that in the paper. They didn't want you to have their coordinates too soon because Karplus, who's a theoretician, would come along and he would prove that your structure was wrong because he would do energy minimization. And the third reason people didn't want it was because they wanted to make mutants, and then you would scoop them in making a mutant that they might've made. So there were a variety of reasons why some of the crystallographers, who had worked *extremely* hard to get their structures, and sometimes several years to get a structure, didn't want them released immediately. Anyway, a significant number of them signed the letter, and then Varmus wrote to the journals. The journals then decided they had to worry about the competition problem. So finally, the editor of *Science*—I guess it was Floyd Bloom at that time—and the editor of *Nature*, who I've forgotten who it was at that particular time—

Schlesinger: It was probably John Maddox.

04-00:50:49

Schachman: I think it was after John Maddox, I'm not sure. It might've been John Maddox. They convened a meeting, and as I like to say, "They got Henry Kissinger, and they met somewhere around a table." But they didn't include [laughs] Ben Lewin who was furious that *Cell* was not part of the big three, it was the big two. In any case, they finally agreed on some new rules requiring deposition of the coordinates for publication of structures in their journals, although they never really enforced their rules at that particular time. So at that stage, Varmus had done his job, but we were still having the problem; coordinates were not being deposited. So I said I was going to go visit the people at Howard Hughes, because among the culprits were three superb Howard Hughes people, the most outstanding of whom was Tom Steitz. Tom had published the structure of hexokinase years ago. He said, in retrospect, that he wished he had made the coordinates available, because he's lost them and he doesn't have them anymore. But he's done other ones subsequently and was not making them available. So Marvin Cassman, who was director

of NIGMS, National Institute of General Medical Sciences, and was a former postdoc of mine, and with whom I interacted a great deal as ombudsman at NIH, said, “Howard, I’d like to go with you.” So I said, “Fine.” So we made an appointment to go to Howard Hughes. Purnell Choppin was head of Howard Hughes at the time, but we saw the next in command.

Schlesinger: Max Cowan?

Schachman: Max Cowan! So he sits there and listens. “Oh, that’s terrible publishing all these papers and not making the data available. Are we involved?” I said, “Yes, among the top five are Howard Hughes Investigators.” He responded, “Oh, gee, that’s terrible. We ought to do something about it.” Then Marvin and I left. We’re standing outside the wonderful establishment in Bethesda, [laughs] where Howard Hughes had its headquarters, and Marvin says, “Did we get anywhere?” I said, “No, we’ve been hoodwinked.” He says, “I think I’m going to start something new.” I said, “What’s something new?” He said, “We will not fund somebody who has published a three-dimensional structure with stereo diagrams in a journal and has not deposited their coordinates in the Brookhaven Bank.” So I wrote to Harold immediately and said, “Marvin is going to withhold money on renewals of grant applications.” And Varmus said, “Okay, I’ll adopt that as NIH policy.” And then all of a sudden, we realized that the way to solve an ethical problem was with money. [They laugh] If you withhold the money, then they’ll publish their coordinates. And so that issue was more or less resolved.

[End of Interview]

Begin Audio File 5
(This was labeled audio file 1 but it is the fifth file)
schachman_howard_5_08-20-22-08.mp3

05-00:00:01
Schlesinger: It is August 20, Wednesday, 2008 and we’re going to continue the history with Howard Schachman. Okay, Howard, let’s start.

05-00:00:14
Schachman: Well aspartate transcarbamylase or ATCase as it’s abbreviated has proven to be a fantastically interesting enzyme because it’s complex enough to have many facets and it’s simple enough to be fathomable. There are a variety of experiments that I’m extremely proud of that were done by students in my lab which

required a new approach. I don't like to use the word new because every time you use the word new you find a Faraday or a Maxwell or somebody had done it in 1900. So you shouldn't use "novel" or "new" in describing approaches.

05-00:00:55
Schlesinger: Or unique.

05-00:00:56
Schachman: Or unique, right. So one aspect was as you remember there were two catalytic trimers, crosslinked by three regulatory dimers. Trimers themselves were relatively unusual but it gives you an opportunity to investigate the whole idea of: Why oligomers? Originally we started out talking about proteins that were single chains and then Svedberg and his colleagues in the thirties using the ultracentrifuge found out that many proteins were multiples of smaller units and he proposed overly simplistic ideas, which turned out to be erroneous. But oligomers certainly outnumber monomers and the big question is: Why oligomers? Why do you need a dimer? Why do you need the trimer? Why in the case of ATCase do you need a hexamer? There are a whole variety of reasons. One is that the monomer itself may not be soluble and therefore when it is built into a higher order polymer like a trimer, some hydrophobic regions would be buried and then the protein would be soluble. Another one is a monomer won't fold properly without interaction with two other monomers if it's going to be a trimer. So this whole issue came up as to why there are oligomers. We had an opportunity to study both dimers in the case of the regulatory subunits and trimers in the case of the catalytic subunits. So the one idea that has intrigued protein chemists was that for an oligomer, it was the interface between the various chains that was involved in catalysis. Based on that premise, crystallographers had looked for ligands that bind and sure enough they found that ligands bind at the interface. So they proposed that there were two parts to an active site, one came from one monomer, and the other part came from the other monomer. That was a very appealing idea. We thought, gee we have a wonderful opportunity to test this with the catalytic trimers of ATCase.

05-00:03:02
Schlesinger: Howard, what was the date that you're talking about now?

05-00:03:05
Schachman: We're talking about the 1980s.

Milt (Schlesinger) had worked on oligomers and I had worked on aldolase as an oligomer and hemoglobin has been known for decades to be a tetramer.

By this time site direct mutagenesis was developing so we could do things that one couldn't do in the forties or fifties or sixties. That was when we went back and attacked this problem. Two students, Ellen Robey, who's now a Professor of Immunology at Berkeley (and who was introduced in the previous tape), and Susan Wente, who's now Chair of the Department of Cell and Developmental Biology at Vanderbilt were the two students, Wente followed Robey, and we thought we would use the idea of hybridization, which again was something Milt had worked on.

05-00:03:54
Schlesinger: Right.

05-00:03:55
Schachman: And I had worked on with aldolase with other people. We wanted to see whether we could make hybrid trimers that would be active from inactive parental monomers. So we took the trimers, we modified in one case a lysine residue. In another case we modified a serine residue and in a third case we modified a histidine residue.

05-00:04:22
Schlesinger: But now you're modifying by chemical methods, not by mutagenesis.

05-00:04:26
Schachman: We started with chemistry; that was what Robey did. By the time Susan took over the problem, we did site direct mutagenesis. So we made specific modifications. We made three different mutants—a lysine mutant, at lysine-84, a histidine at 134, and then a serine at 52. So we had three different mutants, all of which were essentially dead. We then asked the question, if we took two of the three (we'll make the pairs with all three of them ultimately) and if we could take them apart and then allow the monomers to associate at random would we be able to regenerate active enzymes? So if you dissociate two trimers, you should wind up with a hybrid set composed of four species. You should be able to fractionate them—two of the extremes will be the two parents back again and the others will be the hybrids in which you have two chains of one and one chain of the other and vice versa for the other hybrid.

05-00:05:30
Schlesinger: How did you separate them?

05-00:05:32
Schachman: So that's the key. We found out that low ionic strength pyrophosphate buffer caused the trimers to dissociate into monomers. It is also possible to dissociate the trimers with urea. Fortunately with ATCase we could put the catalytic trimers in urea and by removing the urea reconstitute the enzymes with very, very good yields. We were lucky. Our yields were almost 100 percent.

05-00:06:00
Schlesinger: Did you dissociate the trimers independently and then mix them?

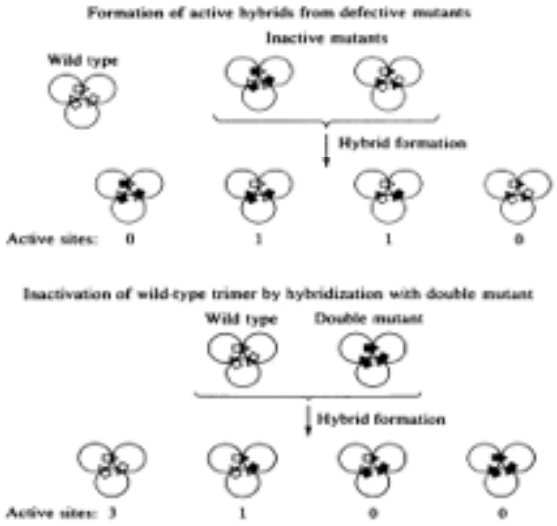
05-00:06:07
Schachman: No, we made a mixture of the two mutant trimers and put them in dilute urea or in pyrophosphate to dissociate them. Then when we removed the urea (or the pyrophosphate) they associated together at random and we got a four-membered hybrid set.

05-00:06:20
Schlesinger: Okay but then how do you separate the four different trimers?

05-00:06:22
Schachman: All right, now, we got terrific reactivation, so that was already an indication that we were generating active sites by the scrambling process. But we wanted to prove it by isolating each one. So in that particular case, we used a technique we had been working on for a long time. I had a wonderful postdoc from England by the name of Ian Gibbons who found that you could modify reversibly the lysine residues of proteins with tetrahydrophthalic anhydride. At a pH of about 8, you could modify the charge by converting positive groups to negative charges, and by lowering the pH to about 6 you could get the tetrahydrophthaloyl groups back off again thereby regenerating the original protein molecules. For example, you can take the catalytic trimer and modify it with excess tetrahydrophthalic anhydride and in the process it becomes inactive, because of the very large net negative charge the trimers come apart into monomers. But you can reform the trimers by changing the pH to about 6. Under these conditions the tetrahydrophthaloyl groups come off and you get back native wild type material. So in this particular case where we wanted to make hybrids, we would modify one of the mutants with a relatively low amount of tetrahydrophthalic anhydride and then form the hybrid set. For these experiments, we used lower levels of tetrahydrophthalic anhydride so that there was no dissociation of the trimers; however the modification was sufficient to alter the net charge.

We then mixed the modified (tetrahydrophthaloylated) mutant with the other unmodified mutant and formed the hybrid set either with low levels of urea or pyrophosphate. In this way we have a mixture of proteins with different amounts of charged groups on the trimers. We then would fractionate them on an ion exchange column and get all four species purified. Finally we would take the tetrahydrophthaloyl groups back off again and regenerate the hybrids that we wanted. In this particular case, what Susan Wente was able to do was to purify each of the two hybrids in the different sets, and she found that they had 32 percent and 34 percent of the activity of wild-type trimers. This showed that each had one active site per trimer instead of three active sites per trimer. She did that with all three pairs and they all worked out extremely well. So the scheme is shown in this beautiful chart and the residues that were involved are shown in the figure.

Hybridization Scheme to Demonstrate Shared Active Sites



She actually did an additional experiment with a double mutant (also shown in this figure). In that case we modified both parts of the interface and we mixed that double mutant trimer with wild-type trimers. In this particular case we're inactivating the wild type by negative complementation rather than getting positive complementation. She purified that hybrids and sure enough found one active site and one PALA binding site; i.e. one active site that bound substrate.

So this was actually one of the nicest experiments I think we have ever done and I was very proud of this research. It involved years of work with two different graduate students, each of whom made enormous contributions and it built on techniques that we had been working on to modify proteins to change their charge so we could fractionate mixtures. Milt and I were doing hybrids about the same time as a matter of fact.

05-00:09:12
Schlesinger: They separated the alkaline phosphatase hybrids by labeling one of the mutants with heavy isotopes. [Fan, D.P., Schlesinger, M. J., Torriani, A, Barrett, K.J., and Levinthal, C. “Isolation and characterization of complementation products of Escherichia coli alkaline phosphatase” *J Mol Biol*. 15, 32-48 (1966)]

05-00:09:17
Schachman: That’s much harder because you had to separate the hybrids by density centrifugation.

05-00:09:20
Schlesinger: Well you could make a lot of alkaline phosphatase in bacteria.

05-00:09:23
Schachman: Right.

05-00:09:25
Schlesinger: But the idea of negative complementation has also been carried through to today. When people do studies in cultured cells where they inactivate some pathway by putting in an inactive polypeptide that then will lead to the oligomer formed being inactive. It is referred to as dominant negative.

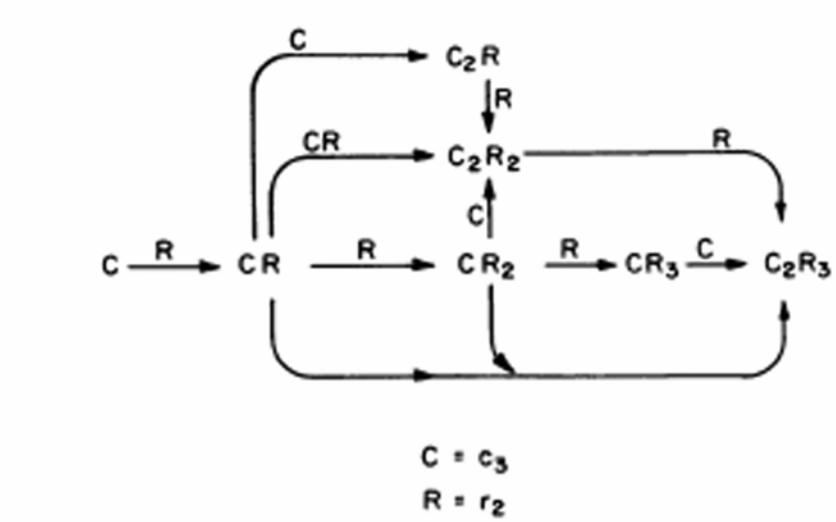
05-00:09:39
Schachman: Right and this became rather interesting because complementation was a field that belonged to the geneticists. Protein chemists never used the word complementation and here we were doing positive complementation and then we did negative complementation and we did it at the structure level. From my point of view it was a very satisfying experience to bring these basic ideas into the field of protein chemistry.

05-00:10:01
Schlesinger: Well I think biochemists may have had a hard time understanding geneticists when they talked about complementation. But as soon as they could relate it to proteins it became very clear what was going on.

05-00:10:11
Schachman: That’s right. So that was an experiment that made us very happy.

Okay so in continuing in that same vein since we were talking about trimers and dimers, trimers of course are intrinsically extremely stable because as I illustrated with my monkey models (as seen in the earlier figure)—the one monkey has a left hand linked to a right hand of another monkey and the other monkey has his left to a right hand and when you make a closed circle out of three monkeys, you have to break two bonds or two sets of bonds to pull out a monkey. Whereas in a dimer, you have one set of interactions between one monkey and the other as shown in my slide of the blue monkeys. So dimers come apart very easily whereas trimers are very much more stable. We had some wonderful discussions, I remember, with George Stark who was worrying about the trimers coming apart and he found that at great dilution he lost activity. He called me, I remember—we had a very good relationship; he was at Stanford in those days—he would call me up and we had group meetings where we shared ideas and he called up and said, “Howard is it possible for the trimers to come apart?” I said, “of course it’s possible but I don’t think that’s what’s going on.” I said, “if you lost activity at great dilution, I have a feeling it’s because the protein was absorbed on the glass walls. It is known that you lose ribonuclease activity when you go to extreme dilution and it’s a monomer, it doesn’t come apart. In any case, let me push the ultracentrifuge a little bit harder to see if I can go even down to the very low microgram level to see if there’s dissociation.” We had been working with ultraviolet optics and were able to push the wavelength down to 2180 angstroms. So instead of using milligrams per ml of protein we could use micrograms and sure enough the trimers were stable. So when trimers were studied at extremely low concentrations there was absorption on the micropipettes or the glass walls. In summary, trimers were very stable. But the dimers on the other hand were in dynamic equilibrium with monomers and one of the key questions was: When we isolated the dimers from ATCase, were they dimers in the ATCase molecule or were they monomers that associated to dimers when we isolated them? So it was an endogenous versus exogenous sort of relationship problem. What one of my students did was to cross-link the dimers with dimethylsuberimidate, a well-known cross-linking reagent other people had discovered. Now we had dimers that could not come apart. We then asked the question: Would those cross-linked dimers re-associate with catalytic trimers to form ATCase-like molecules? And they did. Then we said, well maybe it’s still artifactual. So we then did a competition experiment in which we used wild-type dimers that were not cross-linked in competition with cross-linked dimers and they

competed equally. Therefore we were convinced that the dimers were in fact the cross-links, so to speak, between two catalytic trimers. So we built this monkey model of ATCase in which we had one group of trimers crossed with blue dimers hanging off of it and then another catalytic trimer on the bottom and Mark Bothwell decided to work on the assembly of ATCase in the test tube and he designed a beautiful diagram that Lehninger “fell in love with” and put it into his famous Biochemistry textbook as shown here.



Scheme for the assembly of ATCase from subunits. Only the association reactions are illustrated in the various pathways even though some of the reactions are probably reversible (See figure on page 80).

I described previously the assembly process that Mark Bothwell outlined that has about ten different reactions in it, some of which are reversible and some of which are irreversible because a catalytic trimer has three binding sites for a regulatory dimer and you can then postulate all sorts of reactions, some of which are irreversible because if you get two links together, you can’t get it apart anymore but if you hang one regulatory dimer onto a catalytic trimer that’s reversible because that has only one binding site.

Many of these reactions in this group Bothwell was able to identify so we worked out the scheme for assembly and we hoped that that was relevant to the idea of assembly *in vivo*. We’ll come back to that at a later stage after we got into cloning and we were able to isolate the two separate genes.

So there were two major aspects then to our study on ATCase which intrigued us very much and I found it was an opportunity to do experiments that were not mainstream experiments, which I've always enjoyed watching and having my students do. So the third big experiment that we wanted to see if we could do was performed by Yang.

05-00:15:41
Schlesinger: So let's just go back and make sure that I understand what the three experiments were. The first big experiment was the one where you were looking at hybrids.

05-00:15:50
Schachman: Right. It's the one we talked about today, of course.

05-00:15:53
Schlesinger: Right and the second is—

05-00:15:55
Schachman: Assembly and its mechanism. They are not in a consecutive order. These experiments were done independently by different people.

05-00:16:01
Schlesinger: Because you said "the third big experiment that you wanted to do" I thought it should be clear what the first two were.

05-00:16:06
Schachman: Right. Right. It's one that I am still very proud of. There's something fascinating about one's recollection in doing oral histories of science. When you are asked the questions, "What was the best thing that you've ever done? What are you happiest about in your career?" And at one stage I commented the one thing that got me promoted to tenure, for example, wasn't because I was interesting or imaginative, it was because the cell was interesting. I went to Roger Stanier and Art Pardee and I said, "Gee, what's it look like inside *E. coli*?" And they said they don't know, so I said, "Well, why don't we break open some extracts and see what happens" and that's how we discovered ribosomes. Well, what was interesting there was *E. coli*, not Schachman or Pardee or Stanier. On the other hand I did an ultracentrifuge experiment with a student way back—Cheng—and there I had an idea that if you built up the density of the solvent so it was very close to the density of the particles that were sedimenting, you would be able to see sedimentation at the top and because of compression in the ultracentrifuge cell, you could see flotation at the bottom. So in one day or one week basically Cheng was able to take polystyrene latex particles, put them in H_2O/D_2O mixtures so that the H_2O/D_2O was just a little

bit less dense than the particles; the particles were sedimenting at the top of the cell but at the bottom of the cell the compressibility of water—H_2O/D_2O—was so great that the particles floated at the bottom. We were able to get sedimentation and flotation in the same experiment and moreover the flotation rate didn't make sense in terms of the density of the particles unless the particles themselves were being compressed. So we wound up essentially with a compressibility machine. [P.Y. Cheng and H. K. Schachman "The effect of pressure on sedimentation, and compressibility measurements in the ultracentrifuge." *J. Am. Chem. Soc.* 77 1498-1501(1955)].

I used to joke about this because it was an experiment that I found very exciting and satisfying intellectually but it didn't amount to a row of beans; there were six people in the world who read the paper [laughter] and it's never been quoted by anybody. So the same thing is true here. We do experiments that sometimes satisfy us and other experiments that are perfectly obvious and the result is important in terms of understanding some very basic phenomenon. So that's why the active site experiment satisfies me and the assembly experiments satisfy me whereas doing site direct mutagenesis—there are 150 amino acids you can modify.

05-00:18:48
Schlesinger: Right, and people do that.

05-00:18:50
Schachman: And there are people who are doing it.

So the next experiment that I would like to cite which I would call, among the creative experiments from our laboratory, was one done by Ying Yang that I started to refer to before I gave that autobiographical comment. We asked a simple question: The Monod, Wyman, Changeux model describes a concerted transition whereby the molecules are converted from the T-state to the R-state. That would mean that all of the chains undergo a conformational change. So we asked: If we bound ligands to one catalytic trimer, would we be able to demonstrate a conformational change in the other catalytic trimer? Answering that question required a variety of things. It required the ability to build a proper hybrid, the ability to wipe out the active sites on one trimer and the ability also to add a chromophore on that inactive trimer which would be a signaling device for a conformational change. So Yang satisfied all three requirements by protein engineering experiments. She wiped out the active

site; at that time I think we did it by pyridoxylation, but I don't remember for sure. She also added a chromophore, a nitrotyrosyl group that Marc Kirschner had worked on earlier in my lab. It was a very sensitive chromophore for monitoring conformational changes in wild-type trimers and intact ATCase. As a control for the next step, Yang demonstrated that the addition of active site ligands had no effect on the chromophore in these modified trimers. She used those doubly modified catalytic trimers along with wild-type trimers and regulatory dimers to construct a hybrid set of ATCase-like molecules. Before the final construction of the hybrid set, she tetrahydrophthaloylated the modified, inactive trimers so she could separate the members of that hybrid set (as I described above). This was really engineering since the work involved triply modified trimers. In this way Yang purified the one hybrid that had a wild-type catalytic trimer at the top, if you like, and the colored inactive trimer at the bottom. She then added a ligand that binds to the active sites in the upper trimer and found a significant change in the absorption spectrum of the chromophores located on the lower inactive trimer. This complicated experiment proved that there was "cross-talk" between one trimer and the other trimer. This "cross-talk" was mediated through the global conformational change in the whole molecule; moreover by doing sedimentation titrations and spectrophotometric titrations of the color change we were able to demonstrate they went in parallel. When the molecule swells due to the conformational change in the quaternary structure—the global transition—there are local changes in the chains even though they do not bind the ligands. So that was again a major experiment; it took several years for Yang to do all that research and she did a magnificent piece of work in accomplishing each step in this process. So among the experiments that we've done on ATCase, that one ranks very high in my recollection of things that were created.

But before we stop, I want to say something about our *in vivo* assembly experiments. As I had indicated, one of the beautiful pieces of work in my lab on ATCase was that performed by Mark Bothwell who studied the assembly problem and devised the scheme shown earlier for the various reactions possibly involved in the formation of the holoenzyme from catalytic trimers and regulatory dimers. But all of the conclusions were based on test tube experiments along with speculations based on computer simulations. Could we learn anything about the *in vivo* assembly process? Certainly not at the time that Bothwell was in the lab; but some years later we had the genes available as the *pyrB-pyrI* operon. So we began, (when I say, "we", it

means Ying Yang and a wonderful undergraduate student, Lisa Ryner, who then went on to a very successful graduate career) making deletions in order to have *pyrB* separate from *pyrI.* Therefore, we could use the normal promoter and control features to over-produce catalytic chains by themselves, on the one hand, and similarly we had cells producing regulatory chains without making catalytic chains. In one of the first experiments, we had both genes present in the cell with one on the chromosome and the other on an episome. We had two different messenger RNAs instead of a bicistronic messenger. It did not matter. The cells with unlinked genes produced large amount of ATCase. Moreover, Ryner was able to manipulate conditions so as to over-produce catalytic or regulatory chains and detect the results through gel electrophoresis. The results were directly in accord with the scheme Bothwell devised. In other experiments, strains were constructed that possessed either *pyrB* or *pyrI*, but not both. In that way, it was possible to demonstrate that cells containing *pyrB* produced catalytic trimers. Alternatively, the strain containing *pyrI* made large quantities of regulatory dimers that were readily detected by adding pure catalytic trimers to the extracts thereby converting them into the holoenzyme, ATCase. These limited *in vivo* experiments provide strong support that each gene in the operon through transcription and translation produces unfolded polypeptide chains that, in the case of the catalytic chains, assemble into trimers and, for the regulatory chains, dimers result. Having the genes on an operon is a great advantage in producing the appropriate amounts of the two types of chains and varying their amounts depending upon physiological conditions. The results from the *in vivo* experiments were directly in accord with the scheme devised earlier by Bothwell.

05-00:22:11
Schlesinger: Okay. So are we going to stop now?

05-00:22:13
Schachman: Yes.

05-00:22:53
Schlesinger: This is Friday, August 22, 2008 and we're going to continue with ATCase and you're going to tell me something about allostery.

05-00:23:06
Schachman: Correct. I thought I would start with the focus of many years of research in our lab, which was provoked by John Gerhart whom I have talked about before. He was terrific in doing experiments with his hands as well as having a first-class mind. Moreover, he was very thorough, careful and rigorous. So he came to me

because he kept getting invitations to give talks and he had very little data other than the work he had already published with Pardee. That important research demonstrated that ATCase showed a sigmoidal dependence of enzyme activity on the concentration of aspartate in the presence of carbamylphosphate. Furthermore, the curve was shifted to the right by CTP because CTP was an inhibitor, and the curve was shifted to the left by ATP because it was an activator. The Gerhart-Pardee paper attracted an enormous amount of attention. Their findings were directly analogous to the sigmoidal binding of oxygen to hemoglobin where the Bohr effect shifts the curve one direction and you can shift it in the other direction by changing pH or by adding certain reagents. The parallel to hemoglobin was shown in another way. When John Gerhart treated the enzyme in a variety of ways that I'll talk about in a few minutes, he lost all of that sigmoidal dependence and he also lost the CTP and ATP inhibition and activation. He had accomplished something akin to the formation of myoglobin as contrasted to hemoglobin, respectively, just as myoglobin no longer is like hemoglobin.

05-00:25:24

Schlesinger: I have two questions. One is to tell me the date and also to ask was this work on ATCase the first example of an enzyme showing this kind of effect?

05-00:25:35

Schachman: It was among the first. Umbarger had reported similar types of results and also the group at the Pasteur Institute had analogous findings.

05-00:26:09

Schlesinger: I don't remember if we talked about this on the tape or whether we just talked about it but let's continue.

05-00:26:15

Schachman: Right, so it may be repetitious, that's true. The Gerhart paper with Pardee was in 1962. [Gerhart, J.C. and Pardee, A. B. "The enzymology of control by feedback inhibition." *J Biol Chem.*, 237,891-896 (1962)] The famous Monod, Wyman, Changeux model paper ["On the Nature of Allosteric Transitions: a Plausible Model." Monod J., Wyman J., and Changeux, J.P. *J Mol Biol.* 12,88-118 (1965)] was a classic. It impressed me to no end. The principle was very simple. They argued that an enzyme or hemoglobin—a protein—can exist in two conformational forms – one was called T for taut, compact or constrained and the other was designated R for relaxed, swollen and more flexible. They visualized a dynamic equilibrium

between the two different conformations. They also considered the proteins as oligomers containing multiple sites. So when you add substrate or a ligand like oxygen to hemoglobin, it binds at one site and promotes the transition from the T form to the R state. Therefore the other sites are activated and you get cooperative binding. If you have CTP, which binds preferably to the T state, then you stabilize the T state relative to R and therefore it's an inhibitor. ATP on the other hand presumably binds preferentially to the R state and therefore it's an activator. So these are linked functions; they are chemical equilibrium reactions that are linked to one another, and therefore they are perfectly straightforward. It was very attractive to me and it became the focus of our ideas and research. We wanted to test it and we said, let's see whether that simple model is sufficient to handle all the experimental data for ATCase and we'll keep designing more sophisticated experiments as we get more and more tools to see whether we can show that you need something beyond those simple concepts. So that became a focus of roughly twenty years of research in the lab, doing all sorts of novel, crazy experiments.

So the first question that came up was: Is there a global change in ATCase as well as a local change in conformation and can you differentiate them? Well when John Gerhart came to me with the problem, he said you've just built this tool to measure small changes in sedimentation coefficient, maybe that will help us. So I put the ATCase in the centrifuge with the substrate analogs; one was carbamylphosphate, a true substrate, and instead of aspartate, we used succinate. And sure enough, when we added succinate the sedimentation coefficient changed but it went down! Since the binding of ligands increased the weight and density of the protein, the decrease in the sedimentation rate meant that the enzyme had undergone a global conformational change such as an increase in asymmetry or swelling of the molecule. We preferred swelling of the molecule. So that was the first evidence of a global conformational change. To measure local conformational changes, we needed to put chromophores on individual polypeptide chains and look at the change in spectrum and I have already talked about that.

05-00:29:40
Schlesinger: Couldn't you use circular dichroism?

05-00:29:41
Schachman: You can use circular dichroism and in fact we did. Other people did as well. Circular dichroism monitors local changes in the

backbone structure of individual polypeptide chains and there is some ambiguity in interpreting results. We wanted to use specific chromophoric groups on certain residues.

05-00:30:03
Schlesinger: When you say talk about chromophores—couldn't you just get away with using tryptophan?

05-00:30:08
Schachman: You could use tryptophan fluorescence. We actually tried that; it wasn't a very good tool. At a later time Marc Kirschner used chemical modification to introduce specific chromophores onto ATCase. He nitrated a few tyrosine groups and that became a very beautiful, visible probe. You could then use a spectrophotometer with visible light and watch the change in spectrum resulting from the addition of ligands.

05-00:30:30
Schlesinger: And did it affect the enzymatic activity?

05-00:30:33
Schachman: No, very little. Now let me return to the issue of allostery. John Gerhart had already shown that the enzyme treated with mercurials or silver ions lost its sigmoidality—its cooperativity—and it also lost its sensitivity to CTP and ATP. In the language of Monod et al, the cooperativity is called the homotropic effect and the inhibition or activation is designated as the heterotropic effect. Allostery is manifested by both homotropic and heterotropic effects. As I've indicated in a previous discussion, examination of mercurial treated ATCase in the ultracentrifuge revealed that the molecule had dissociated and we observed two boundaries instead of one. The faster moving species turned out to be the catalytic subunits and the other one turned out to be regulatory subunits. That discovery led to a major activity characterizing each of the two types of subunits and led us to develop a model. Lipscomb's lab did the X-ray diffraction to determine the three-dimensional structure. So our focus in the study of allostery was on the global conformational change.

I described before that George Stark and Kim Collins had designed a compound called PALA (N-(phosphonacetyl)-L-aspartate), which looks like the sum of the two substrates, carbamylphosphate and aspartate having a little bit or a lot of each of the two substrates in it. They considered it to be the transition state analog on the catalytic pathway to products of the reaction. We don't think that's an appropriate name; we called it a bisubstrate analog, but it proved to serve as a marvelous tool and it changed the nature of our research. It was

beautiful from my point of view and a couple of my students worked on this extensively. Because it bound so beautifully to ATCase, it was to us the equivalent of oxygen to hemoglobin. We could now get rid of enzyme kinetics and two substrates and we could study the binding of PALA to ATCase, which is directly equivalent to the binding of oxygen to hemoglobin. The only trouble was that it bound so tightly that the only way you could measure the extent of binding was to work at trace concentrations of ATCase. Therefore you had to make radioactive PALA. So two of my students worked on that problem. Jim Newell, together with David Markby, worked very hard and they learned how to synthesize labeled PALA from George Stark.

05-00:33:48
Schlesinger: Labeled phosphate?

05-00:33:49
Schachman: No, it was labeled with tritium. So we then worked with very dilute solutions of ATCase and PALA and we could do equilibrium dialysis essentially under those conditions. Sure enough we were able to show the sigmoidal binding of PALA to ATCase and also CTP inhibited the binding and ATP activated the binding. So we now had a new tool to work with in our lab.

05-00:34:24
Schlesinger: And you had to go away from using the ultracentrifuge.

05-00:34:26
Schachman: That's right, we didn't use the ultracentrifuge at all for that purpose. Okay, let me take a little rest. Can I do that?

05-00:34:34
Schlesinger: Sure.

05-00:34:43
Schlesinger: Okay, we're ready to move on.

05-00:34:47
Schachman: All right, so from our point of view the model was extremely attractive and linked functions were a well-established concept in interpreting chemical equilibria. As a consequence we treated the CTP and ATP effects, which became the focal point of considerable argument over the years as a part of the overall picture dealing with the T to R transition. There seemed to be a consensus of agreement among workers in different labs that the substrate promoted conformational change. But, there was vigorous disagreement about whether CTP and ATP effects could be interpreted by the same model. As a slight digression, I

might point out that there is another area of some controversy. My old friend and colleague, Dan Koshland, who has just passed away, had proposed a model for allostery that was independent of the Monod, Wyman and Changeux model and was built on Pauling's old papers on hemoglobin. Dan had for years used the phrase "induced fit" and he coined that phrase because he was unhappy with the lock-and-key model of Ehrlich of way back. That was a step forward to be sure but when you say "induced fit," it looks like you can't get to the second conformation unless you push it, induce it. I had always preferred the phrase "ligand-promoted." In my view, a protein molecule is breathing and therefore it can exist in the two or more forms and you don't induce it into a second form, you pull it by an equilibrium when you add substrates which bind preferentially to one of the different conformations. So that became a focus of considerable contention between Dan and me because I preferred the Monod model, which says you can be in the R state but the equilibrium may be very unfavorable and therefore you have to pull it. When we got into the quantitative aspects of ATCase, we used the Monod model to fit experimental data on the change in the sedimentation coefficient, the change in enzyme activity and the change in the reactivity of sulfhydryl groups with the mercurial. Using that model and accompanying theoretical equations, we reached the conclusion that the equilibrium constant favoring the T conformation over the R conformation was between 250-1000 to 1. Therefore ATCase molecules existed primarily in the T state. If you added CTP, more molecules would be in the T state because the equilibrium constant would increase perhaps from 1000 to 10,000. You wouldn't see this by any change in the physical properties because the bulk of the molecules were already in the T state. Some of the workers who did not accept our view argued that since they could not detect a conformational change upon the addition of CTP, the heterotropic effect must operate by a mechanism other than that involved when substrates bind at the active sites. According to them, the Monod model could not account for the heterotropic effect. I used to argue, wild-type ATCase in the absence of ligands is already in the T state so adding something to increase that amount will not be observable. Similarly, though ATP would promote it toward the R state, if you changed it from a thousand to one to 500 to one, you still won't be able to see it. Therefore the absence of detectable changes in ATCase caused by the addition of CTP and ATP is not because they are separate phenomena, it's because the equilibrium was too heavily in favor of the T state and the only way ultimately you're ever

going to see this—this now gets into more modern work—is to change the equilibrium of ATCase so it's close to unity. So now it's half T, half R. If CTP has an effect, it will become 70% T and if ATP has an effect, it will be 70% R. So we then went to mutants and this is a much later experiment but Ed Eisenstein and David Markby did site directed mutagenesis on residues affecting the interface between T and R states and we isolated several mutants. One mutant had the properties we wanted. Note that we did not have any structural information on the mutant. It was clear from enzymological and physical chemical measurements that it had much less cooperativity than the wild-type enzyme. When we analyzed the various types of data, we concluded that the T/R ratio was about 2. When you added CTP that ratio increased to about 10 or higher and the ratio was decreased significantly when ATP was added.

05-00:39:09
Schlesinger: That must have been really satisfying.

05-00:39:11
Schachman: For us it was extremely satisfying. So we were convinced by that experiment that the model accounted for everything that you wanted to know about from T to R and you didn't need any special hypothesis—such as CTP and ATP had a separate phenomenological effect. As far as we were concerned, that ended that debate but other people said, well it's a mutant; it's not wild-type enzyme and they didn't see this as a phenomenon. So that brings me to a much later experiment, which I'll talk about later because I haven't thought about it in great detail.

05-00:39:45
Schlesinger: I might just add that in viruses, people realized that the virus was breathing because monoclonal antibodies were interacting with an intact particle even though the X-ray structure showed that the region that the monoclonal antibody interacted with was buried. And so the only way you could explain that would be if the virus particle was actually breathing so the idea that proteins were breathing and there was an equilibrium, I think is now accepted but it took a lot of experiments.

05-00:40:22
Schachman: Oh, that's a very good point because I used to argue that you can't trust results from crystallography on inert crystals of proteins. I used to say, "Who wants to work on dead enzymes?" In fact Fred Richards, the crystallographer at Yale, was one of the first ones to push this subject and ask: Are the crystals reflective of the true structure in solution? So he then did absolutely gorgeous work. He built crystal columns and would

pass substrates down the columns to see whether the substrates were converted to products; were the enzymes in the crystal active? Some enzymes were active and some were not active and this was beautiful—he had to correct for diffusion on the surface. It was a magnificent piece of research but most people don't do this so when you crystallize an enzyme, you may very well, by forces of crystallization and the solvent that you use, promote a new conformation which is not active. So that's why I always used to argue that NMR would be preferable to crystallography except that NMR wouldn't work with big molecules until relatively recently and we resorted to the crystalline structures. So whereas the Lipscomb group was doing chemistry, focused strictly on their crystalline structures, we on the other hand focused primarily on solution so we had arguments—the CTP, ATP effect was a focus of considerable friction in the ATCase community and I feel convinced that the outcome supported our view but obviously other people have a different opinion. All right let me stop for a minute.

(pause from 42:15 to 42:22)

05-00:42:43
Schlesinger: Okay.

05-00:42:45
Schachman: All right we're back again to the model and the model basically said there are two states—T and R, so we logically asked the question, can we demonstrate that there are no intermediates? Can we demonstrate that it's a concerted transition with no individual molecules having intermediate structures. Such a concerted transition means that if you are halfway through the transition, you have 50% of the molecules in the T state and 50% of the molecules in the R state and not individual molecules that are 50% T and 50% R. So that was a logical problem for the ultracentrifuge. When Stark had made PALA for us, we had discovered that four PALAs—and don't forget there are six binding sites—four PALAs were sufficient to convert the molecules completely from the T state to completely in the R state. So that said, gee that's wonderful. If you have two PALAs per enzyme molecule, you should be about halfway through the transition. So that provided an opportunity for the ultracentrifuge to make a real contribution to this field.

05-00:43:33
Schlesinger: So the question that you're asking is when you're talking about two PALAs, you have to ask the question whether that means that all the molecules have two or whether some molecules have three and four?

05-00:43:45

Schachman: Right. And with allosteric enzymes exhibiting cooperativity that's what's going to happen. But you have to ask a further question then. If the average sedimentation coefficient changed—it's about a 3.5 percent change in the sedimentation coefficient in going from T to R; remember it's a decrease in sedimentation because the molecule swells. So the question is if you're halfway through the 3.5 percent—1.75 percent—do you have molecules in the T state and molecules in the R state or are all the molecules halfway between T and R? So we said, gee the way to do that is to look at boundary spreading in the ultracentrifuge. Because PALA binds so tightly, that was the beauty of this bisubstrate ligand that Collins and Stark had made, it binds so tightly we knew it wouldn't come off at a decent rate and therefore from the ultracentrifuge point-of-view, we could separate molecules in the T and R in the centrifuge. Don't forget 3.5 percent is not much of a change in sedimentation coefficient but it ought to produce enough boundary spreading for us to be able to tell whether the boundary was broader because we had some T molecules and some R molecules. If all of the molecules are halfway between the two conformations the boundary won't be broadened at all because there would not be two separate species migrating in the cell; it would be even narrower. So Bill Werner, a student of mine began doing these ultracentrifuge experiments. We found when we had enough PALA to reduce the sedimentation coefficient about halfway through the transition the boundary was broader. We wanted to check our technique because we weren't 100 percent sure we could measure boundary spreading that well. So we had a wonderful way of doing that—we had a mutant of ATCase that had been discovered very early, long before site directed mutagenesis. Gerhart had picked up that mutant. It was discovered somewhere else and we had worked up the enzyme and purified it. That molecule was dead as a doornail and would not bind PALA so it was fantastic; it existed exclusively in the compact form. So we said to ourselves, gee, why don't we mix that with some molecules that are wild type containing bound PALA. We would make a fifty-fifty mixture and then we know we'll have a collection of T state and R state molecules. We will then do the boundary spreading experiment on that known mixture. Sure enough, the boundary spreading on that mixture was precisely equal to the boundary spreading in the experiment on ATCase that contained 2 PALAs per ATCase molecule. So we were convinced from this elaborate sedimentation velocity experiment that there were no intermediates. When there is sufficient PALA to convert the ATCase half way through the transition, one has a mixture of

50% T state molecules and 50% R state molecules. By playing around with that technique, we were able to estimate that the number of intermediates could not be as much as 5%. So from our point of view it was T to R. So that was one very detailed set of experiments; again I'm summarizing in a few minutes what took a couple of years for Bill Werner to do—it was his PhD thesis.

05-00:47:12
Schlesinger: But I was going to say it's always an interesting question to ask students about, distinguishing between whether 50 percent inhibition means that all of the molecules are inhibited 50% percent or half of the molecules are inhibited 100% and the others not inhibited.

05-00:47:25
Schachman: Absolutely. It's a very fundamental question, which permeates all the biological phenomenon.

05-00:47:30
Schlesinger: Absolutely. For all of our experiments I should say.

05-00:47:33
Schachman: That's exactly right and as you know the folding problem in monomeric proteins was: Was it either folded or unfolded when one is halfway through the transition? Is it because half of the molecules are already folded completely or are all of the molecules partially folded? The two-state theory became a very important part of protein science. Charlie Tanford worked on that problem and Harold Scheraga. Of course as increasingly powerful tools were developed like NMR, you find out that for what you used to call two- state, you can actually see some intermediates so it was quite clear on the way there are intermediates but in equilibrium conditions, it's either folded or unfolded.

All right, so now let me go to another approach. I had a fascinating graduate student who actually had been an undergraduate at Harvard, had worked in Lipscomb's lab; he had done some work with Lipscomb and especially with one of Lipscomb's postdocs. He came to Berkeley and wanted to work on ATCase. Very few graduate students have ever come to my lab and told me what they want to do in the way of a research problem but Jeff Foote told me what he wanted to do and he was very clever. So I said, well let's go ahead and do it. So this was a problem that he initiated, not that I initiated. He had studied ATCase in the backward direction when he was still at Lipscomb's place. In the backward direction—that is instead of

going from carbamylphosphate plus aspartate to give carbamylaspartate plus phosphate, he wanted to mix carbamylaspartate and phosphate with the enzyme and have the enzyme go backwards. Instead of phosphate, he used arsenate so he could study the back reaction. It turns out ATCase is an incredibly poor enzyme in the back reaction whereas the catalytic subunit is a very good enzyme in the back reaction. So the question immediately posed was, why? And the answer could easily be that carbamylaspartate and arsenate or carbamylaspartate and phosphate cannot promote the T to R transition. Under those circumstances the molecules of enzyme, as far as the substrates for the back reaction are concerned, remain in the T state that has very little enzyme activity. It is important to recognize that the activity of wild-type ATCase for the physiological reaction on the pathway to pyrimidines is attributable to its conversion to the R state. A catalytic subunit doesn't have this attribute and it is effective in catalyzing both the forward and the back reaction. It is not as good in the back direction as in the forward direction but, on the other hand, it works. So therefore, intact ATCase was an unbelievably poor enzyme whereas the catalytic subunit was quite good. So we said, gee if you start adding PALA to this, as soon as you add a little bit of PALA to the mixture of ATCase, carbamylaspartate and arsenate, the PALA will activate the enzyme and all of a sudden the activity should go way up.

05-00:50:38
Schlesinger: In the reverse direction?

05-00:50:39
Schachman: In the reverse direction; we were adding substrates for the reverse reaction. But don't forget PALA is an inhibitor! So we had an apparent paradox that an inhibitor is now activating the enzyme and, if you add trace amounts of PALA, you could get the enzyme increasing in activity 40 to a 100-fold. So therefore we said to ourselves, gee, this is incredible. The enzyme was so poor in the back reaction that we have to use milligrams per ml of enzyme rather than micrograms per ml and therefore we had high concentrations of PALA. Now since we knew something about the binding of PALA, any PALA that we add is 100 percent bound. So if we add one hundredth of a PALA per enzyme molecule what happens? The enzyme activity increases. Now we go to five hundredths, it goes up some more and ultimately you get to about three PALAs per ATCase—the enzyme is forty times more active than it was before. But don't forget we've wiped out three sites of the six because the PALA is bound. Now we add more PALA and the enzyme activity goes

back down to zero and we could show that it takes, roughly speaking, six PALAs; it actually took 5.7 PALAs per ATCase to inactivate it completely. So that activation was because PALA was promoting the transition from the T state to the R state. So now we go back to 1/100 of a PALA, 1/200 of a PALA, 1/300 of a PALA, and ask how much more activity are we getting as a result of PALA binding and since we know the low activity of the T state and we could tell what the activity was in the R state, we could calculate how many sites we were creating with one PALA molecule. Under certain conditions that number turned out to be 4.7. The maximum value would have been 5.0. Thus the binding of PALA to 1 site was actually responsible for the remaining 5 sites (actually 4.7) in that molecule being converted from the T state to the R state. The conclusion that one PALA can lead to the conversion of 5 sites from the T state to the R state is powerful evidence supporting our view that the allosteric transition is concerted

05-00:52:52
Schlesinger: And one would be enough to convert it to the R state?

05-00:52:55
Schachman: To the complete R state. So that was the other piece of evidence that we published.

05-00:53:01
Schlesinger: And that was his PhD thesis?

05-00:53:02
Schachman: That was Foote's PhD thesis and that's the subject that apparently David Wemmer came to me just the day before yesterday to talk about. Just after you left he asked me if I have a copy of that paper. Apparently John Kuriyan had communicated with him about it by e-mail and they wanted to put this in their book—it's in textbooks. It's a very beautiful piece of work that Foote did. He was an extremely creative scientist.

05-00:53:29
Schlesinger: And what happened to him?

05-00:53:31
Schachman: He's a strange guy. Oh you'll love this. He flunked his prelims in the department; my colleagues were going to throw him out because he was bizarre. He made of fetish of being bizarre. He would write me letters, "Dear Schachman;" since then he still writes me "Dear Schachman." Never called me Professor Schachman or Dr. Schachman; never called me Howard—just Schachman. He got a job at the Hutch in Seattle and—who's the person at the Hutch that won the Nobel Prize?

05-00:54:05
Schlesinger: Lee Hartwell.

05-00:54:06
Schachman: Lee Hartwell thought he was fabulous, which indeed he is. He's unbelievably creative but strange and a lot of people don't like him. He makes a fetish really of being strange and I think annoying other people. I am very fond of him so we've had a wonderful relationship. He married a graduate student who worked in our department with Steve Beckendorf and they have a child. Ethel and I have visited him in Seattle and recently I learned that he didn't get tenure. He wrote a couple of very provocative commentaries in PNAS with Herman Eisen. Oh you want to look that up. Foote and Eisen wrote a very fascinating paper—I don't think he's ever met Herman but Herman heard about him from me; and I think I might have told him to write to Eisen and they published these papers within the past five years. (in 1995 and 2000). Oh that will be a nice link for you for the history that you are doing with Herman Eisen.

All right, we can turn that off now and off anyway – a good breaking point.

[End Audio File 5(This was labeled Audio File 1.)]

Begin Audio File 6 (This was labeled Audio File 2.)schachman_howard_6_01-23-09.mp3

06-00:00:02
Schlesinger: January 23, 2009. This is Tape 6 for Howard Schachman. Okay Howard, we're going to continue with ATCase.

06-00:00:16
Schachman: Correct.

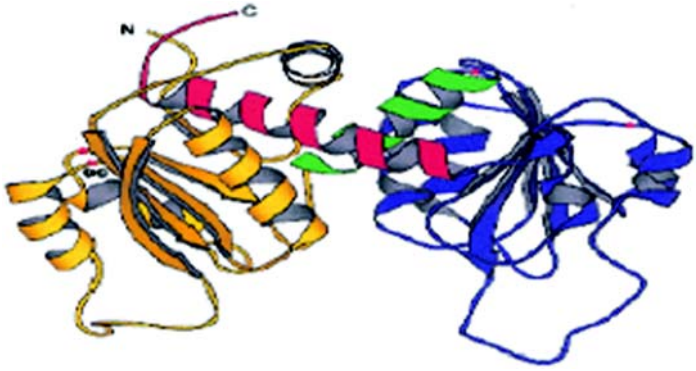
06-00:00:17
Schlesinger: And why don't you just begin.

06-00:00:19
Schachman: Well in previous discussions, we spent a long time on the assembly of the intact enzyme comprised of six catalytic chains and six regulatory chains composed as two catalytic trimers and three regulatory dimers. Now I want to go back and reduce it to a smaller scale and talk about the trimers themselves. Trimers are relatively rare but they do exist. Oligomers of even numbers are much more common than oligomers of odd numbers. There are very few pentamers; there are very few heptomers for

example. I don't know of any nonomers—with nine chains. So we had spent some time years earlier—and I might have mentioned it in previous discussions about the assembly of the trimers and I had this wonderful student, Drusilla Burns, who did a beautiful job on the assembly of the trimers themselves. By this time, you doubtless have noticed that I had many wonderful students!

You can take the trimers apart, as you well know, with urea in which case the chains become unfolded. Then you have to dilute the urea and some proteins assemble very beautifully and catalytic trimers assemble with almost 100 percent yield. So when you assemble from unfolded polypeptide chains produced by 6M urea, then the slow step in the process is in fact the folding of individual chains. Once they are folded they assemble relatively rapidly. We asked the question, could you also get chains that were single but folded? And it turns out there are reagents which dissociate proteins through hydrophobic interactions more than through backbone interactions and one of these was potassium thiocyanate, as I recall. In that case we got folded monomers; the assembly process in this case is much faster and in fact it turns out to be second order because two individual chains have to assemble. As soon as you get a dimer, a third chain comes along very quickly—so you never see dimers. And Drusilla did a beautiful job in two successive papers showing the assembly from either the unfolded chains or from the folded chains. So that got us focused, and we went back to the single chains and asked the question, what about the single chains themselves? And it turns out when people talk about refolding of polypeptide chains from urea solutions, for example, they always ask the question, what's the relevance to the biological cell? Does the cell form proteins the way you refold polypeptides in a test tube? Are you wasting your time studying the *in vitro* folding of intact polypeptide chains? Perhaps folding is occurring as the polypeptide chain is being synthesized. For proteins that are folded into a single compact domain, it seems plausible, and even likely, that the folding process is contemporaneous with the growth of the polypeptide chain. It is important, to recognize, therefore, that the crystallography studies showed that the individual catalytic chains in ATCase are folded into two clearly identifiable domains. This is illustrated here by the picture of a single chain taken from the x-ray structure of intact ATCase deduced in Lipscomb's lab.

One of the fascinating features about the two domains is that the chain starts with the N-terminus and folds like crazy for about 150 amino acids (the N-terminal domain is in yellow). It then goes through a long helix (green) and starts the second C-terminal domain (blue); but it doesn't stop in the second domain that presumably is an independently folding unit. Instead there is a crossover helix (red) from the second domain back to the first domain. As a result, the C-terminus is very close to the N-terminus as you can see on the right.



Seems like a dumb way to fold a protein; I mean it is not the way I would expect "GOD" to have planned it. So we asked very simple questions; could you make individual domains? Could you cut back on the C-chain? How much of the C-chain do you need? And it turns out you can cut off about five amino acids with DNA manipulation.

06-00:04:06
Schlesinger: So this was done by genetic engineering?

06-00:04:10
Schachman: Genetic work. That is correct; this research occurred long after Burns had left the lab, and we had been working with the DNA encoding the two types of chains. It turned out, as shown by Cynthia Peterson, with five amino acids cut off from the C-terminus, you can still get beautiful ATCase even though five amino acids are missing. Once you start cutting residues in the helix, which is the crossover helix, then you no longer can get folding and assembly and you can't make ATCase. So that got us focused once again on the whole assembly process and mainly on the *in vivo* mechanism. Can we separate the domains and study them independently? We spent a lot of time on cleaving the individual pieces in various locations.

06-00:04:45
Schlesinger: Cleaving by using a protease?

06-00:04:47
Schachman: Both proteolysis experiments and later using genetics and DNA. It's much easier to do with DNA than with a protease because proteases do not provide the specificity to cleave at only one

place. With DNA manipulation, you can cleave it anywhere you want. But, we did some proteolysis experiments first, to see where they would take us. Ying Yang started this project, and a post-doc, Vince Powers, extended the research. Although they digested the catalytic trimer with chymotrypsin, fully expecting based on the previous work of others, to cause a loss of activity and the formation of separate proteolytic fragments, they still had full activity. This unexpected observation led to physical chemical and mass spectrometry experiments as well as amino terminal sequence analyses. These studies showed that the trimers were intact and that only a single peptide bond between Tyr 240 and Ala 241 had been cleaved in each chain. This cleavage site was in a surface loop and. therefore, susceptible to proteolysis. Apparently the resulting fragments remained as a complex as a result of non-covalent interactions, almost as if the polypeptide chains had not been cleaved. Hence we had active trimers with a single bond cleaved in each of the chains. Electrophoresis in polyacrylamide gels containing SDS revealed two bands with the larger component corresponding to a 26 kDa fragment and the smaller one represented by an 8 kDa peptide. Placing the proteolyzed trimers in urea caused their dissociation, unfolding of the chains and separation of the fragmented polypeptides. We were now in the position to ask the important question. Could they interact appropriately and then associate to form trimers? Removal of the urea led to the formation of active catalytic trimers, thereby demonstrating that the 70- and 240-amino acid fragments were capable of folding, docking and associating into active trimers in good yield. These trimers when mixed with regulatory dimers formed ATCase having high enzyme activity but no regulatory properties. Sedimentation velocity experiments indicate that these reconstituted ATCase molecules were in the swollen (or R-state), so we could account for the lack of allosteric properties.

We were then in the position to go back to *E. coli* and asked the question: What would happen if we had two gene fragments that could be co-expressed in the same cells or could be expressed independently in different cells? Ying Yang did all these experiments and showed clearly that if both gene fragments were expressed in the same cell, the resulting polypeptides did fold, dock and associate into active catalytic trimers. It is of interest that the genetic constructs led to some overlap in the polypeptide fragments; the N-terminal peptide contained residues 1-242 and the C-terminal peptide was from 235 to 310. Despite the overlapping residues, assembly was very efficient. The final stage of that research was to produce the fragments in separate cells. Ying found that she could make separate

extracts that contained “junk” (i.e., the peptides we were looking for were in the insoluble fraction of the individual cell extracts). Mixing the “junk” from separate extracts containing the two different gene products, adding 6.5 M urea to dissolve the “hoped-for” protein, and then diluting the urea led to active catalytic trimers composed of cleaved polypeptide chains with an 8-amino acid redundancy.

06-00:05:28
Schlesinger: So *in vitro* when you do the folding of the fragments separately and then put them together you actually had enzymatic activity?

06-00:05:34
Schachman: That’s right. And also by using the DNA; so we could do it either way, *in vivo* or *in vitro*. So we began studying—

06-00:05:42
Schlesinger: Oh you didn’t finish your sentence—did it also work *in vivo*?

06-00:05:45
Schachman: That’s right.

06-00:05:46
Schlesinger: You could separate the two domains.

06-00:05:47
Schachman: We have to be careful here in using the term “domain”! The polypeptide fragments that we used in these complementation experiments do not correspond to the N- and C-terminal structural domains observed by x-ray diffraction studies. It is important to recognize that the proteolysis experiments revealed a susceptible peptide bond. If we constructed each gene fragment that coded for the corresponding polypeptide fragment and then inserted them separately into different cells, we obtained ”junk”; we could take the “junk” from two extracts, mix the together, dissolve it in urea, then take the urea away and we would get active material back. Or if we put the two gene fragments in the same cell, at the same time we got ATCase. So everything was working beautifully meaning these fragments were very smart; but one polypeptide contained 240 amino acid residues, representing the major part of the chain. Any attempt to isolate the N-terminal domain and the C-terminal domain as separate pieces was doomed to fail because we had two crossovers. That means we have to break the chain in two places. The best way to see that is to look at the structure—one of the crossover helices is in green and other in red. The red one is the C-terminal helix going from the blue domain back into the yellow domain so that the C-terminus is very near the N-terminus. Although we spent a lot of time with fragments and

found the research very interesting, we were still far way from isolating the individual structural domains.

This issue of isolating the individual structural domains arose in another context that is worth discussing for a moment. One of my students, Lauren Murata was investigating ornithine transcarbamylase (OTCase) using a homology-based approach to determine its tertiary structure based on our knowledge of ATCase. A variety of studies including *in vitro* complementation experiments with two inactive mutant forms leading to reactivation supported the view that the structures of the two enzymes were similar. At the time she was doing that research, a paper by Houghton, O'Donovan and Wild with the title "Reconstruction of an enzyme by domain substitution effectively switches substrate specificity" appeared in *Nature*, [338, 17 (1989)]. That research dealt with OTCase and ATCase, and the authors claim that they "were able to switch one of their amino-acid specific equatorial domains to produce a viable chimeric enzyme." This clearly was an important area of research and it seemed critical for us to evaluate further their conclusion that, "The formation of this active chimeric enzyme shows that by exchanging protein domains between two functionally divergent enzymes we have achieved a switching in substrate specificity." As is often the case with papers in *Nature*, the essential documentation was virtually non-existent. So Murata moved into that area and did extensive research [Murata, L.B. and Schachman, H.K., *Protein Science*, 5, 719 (1996)] leading us to the conclusion that "none of the chimeric proteins exhibited *in vivo* activity and all were insoluble when over-expressed." It is worth mentioning also that attempts to make hybrid trimers composed of catalytic chains from ATCase and OTCase were also unsuccessful. I knew the two senior authors of the Houghton paper and wrote to them about our interest and requesting further documentation. There was no answer. Moreover, there was no follow up of the work. You can rest assured that if we had made that discovery, there would have been a flood of further papers. Now you can see why I am so interested in "domain switching" and the fact that there are two cross-over helices between the domains in ATCase.

Now, having discussed the structure of the catalytic chains let me turn to the logical questions that I raised with Ying one day: What could we do about the observation that the N- and C-termini are so near each other in the structure? Why is the chain constructed that way? Why can't we link the ends and introduce new N- and C- termini somewhere else in the polypeptide chain? Can we do it? Where can the new termini be located?

Will we be able to make ATCase with new N- and C-termini and will it be allosteric? If the existing termini are too far apart to link in a peptide bond directly, we have two choices—you add a linker to give you a little bit more flexibility at one end so it can flip around, or alternatively cut the C-terminal region back a little bit so as to make it even closer to the N-terminal residue. And so Ying did that with a couple of other people in the lab, and we then wound up with circularly permuted polypeptide chains.

06-00:07:25
Schlesinger: You're going a little bit fast although I think I know what you did. You made the cDNA, put it back into *E. coli* and then expressed it.

06-00:07:34
Schachman: That's exactly right.

06-00:07:35
Schlesinger: Okay.

06-00:07:35
Schachman: So that got us into an entirely new type of research; we drifted far away from protein chemistry. Starting with the *pyrB-pyrI* operon and using various genetic manipulations including site directed mutagenesis, ligases, restriction enzymes, and PCR, Ying constructed different plasmids containing two DNA fragments that encode different parts of the catalytic chain. One, for example, would encode from residue 235 to 310, and the other would encode the N-terminal region from 1 to 242. These were then used to construct a DNA molecule that encoded a continuous polypeptide chain having residue 235 as the N-terminus. It also contained the six amino acid linker between residue 310 and 1 as well as a partially redundant sequence of eight amino acids at the C-terminus. This construct and another slightly different one were used by Ying for the *in vivo* synthesis of active catalytic trimers composed of circularly permuted polypeptide chains. The purified trimers then were converted to holoenzymes by the addition of regulatory dimers. Although these ATCase molecules were active catalytically, they were not allosteric.

06-00:08:13
Schlesinger: But you have to have a start codon.

06-00:08:17
Schachman: Of, course; Ying made the appropriate genetic constructs with start and stop codons, a good promoter and other parts needed to satisfy the requirements of protein synthesis—no question about that. So we wound up doing circular permutation

experiments. At that time, there were only a few proteins in which the polypeptide chains had been rearranged to create new N- and C-termini and, if memory serves me correctly, all of them were monomeric proteins. To me this was an exciting area because it seemed to provide an additional tool for studying the *in vivo* folding of nascent, growing polypeptide chains. Why was our first product not allosteric? Was it due to the new N- and C-termini or the elimination of those in the wild-type enzyme? So that was the beginning of a concentrated research effort in our lab. By this time, I was no longer accepting graduate students and all the work was done by post-docs who usually stay in the lab only a few years.

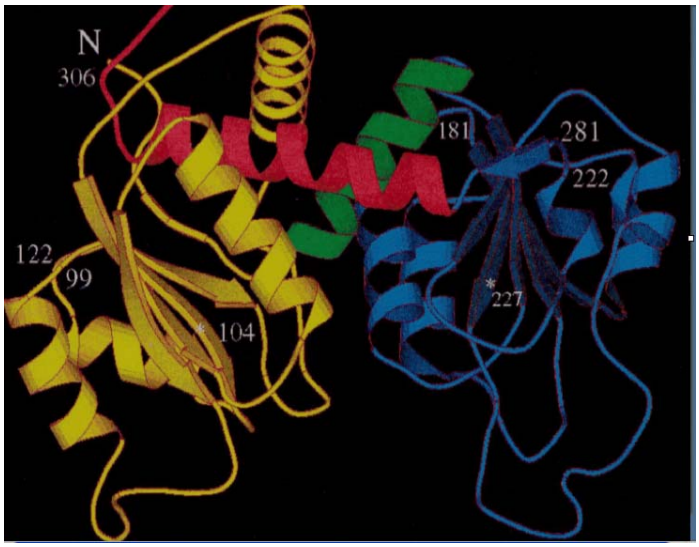
Schlesinger: Did you look for other ways to make the circularly permuted polypeptide chains? The procedure you described seemed pretty much brute force.

Schachman: You are right. It was cumbersome. When we started, we had little experience with the tools of genetic manipulation. But it did not take long for us to realize that there were much more efficient ways to accomplish our goals. Basically, all one needed was circular DNA that contained the entire coding region for the polypeptide chain. Then one could cut the DNA at specific places by using various restriction enzymes and employing PCR. In this way one would make linear DNA capable of encoding the complete polypeptide chain with new N- and C-termini. We decided where the termini would be. Zhang undertook this research by constructing *pyrB* tandem genes because we thought it would be easier to circularize than a shorter single *pyrB* gene. After the tandem construct was circularized, he used restriction enzymes to linearize it, incorporated the components needed for *in vivo* protein synthesis, and also included the *pyrI* gene so that the cells would make ATCase containing the circularly permuted catalytic chains and wild-type regulatory chains.

Schlesinger: How did you decide where to place the N- and C-termini?

Schachman: It was somewhat arbitrary and involved guesses as to what might work. We wanted starts and stops in each of the structural domains, and speculated that the new termini would have to be in flexible regions of the chain rather than in structured regions like alpha helices or beta sheets.

As you can see, in this diagram, which is like the previous ribbon diagram on page 113, but here I've added the numbers to illustrate the residues where we could start the chain at various places such as residues, 99, 104, 122, 181, 222, 227, and 281.



Schlesinger: Did you purify these proteins and characterize them?

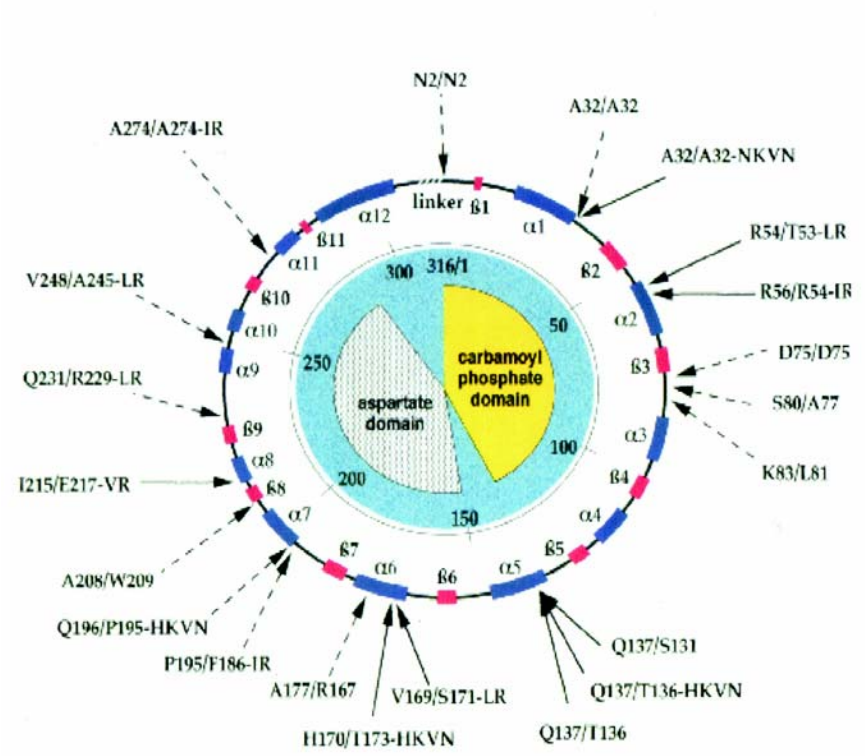
Schachman: Three of the variants that Ni constructed, with N-termini at positions 122, 222, and 281, were purified and were shown to have kinetic and physical properties characteristic of wild-type ATCase. Not only did they exhibit both homotropic and heterotropic effects, but also the addition of PALA promoted a global conformational change as revealed by the decrease in the sedimentation coefficient. Our first goal was reached. We could have N-termini widely dispersed throughout the catalytic chains and in either of the two structural domains.

This research was then extended by Ni [Ni Xinhai and Schachman, H. K., *Protein Science* 10, 519 (2001)]. Ni not only constructed ATCase containing circularly permuted catalytic chains but he also had the cells make analogous molecules composed of fragmented chains. All of his constructs had the allosteric properties of wild-type ATCase. The circularly permuted chains had N-termini at positions, 99, 122, 181, and 212 and the fragmented chains had interruptions between 98 and 99, 121 and 122, 180 and 181, and finally between 221 and 222. Docking of the fragments obviously occurred efficiently *in vivo*. These studies show conclusively that the continuity of the polypeptide chain within structural domains is not essential for the assembly, activity and allosteric properties of ATCase.

Our success up to that point led us to ask more questions. We had selected as potential termini unfolded, flexible regions in each of the domains. Would it be possible to have termini within

organized regions of secondary structure like alpha helices or beta pleated sheets? To me it seemed unlikely that we could have breaks in the chain within those secondary structure elements. But it was worth a try. By then we realized that we would have to make over 300 variants in order to determine whether nearly every amino acid in the chain could serve as an N-terminus. We would really become boring protein engineers like some of the people who were modifying innumerable residues in proteins by site-directed mutagenesis.

Just about that time (1993), a new post-doc, Roney Graf, arrived from Switzerland. He had never done this kind of work before and I said, “hey Roney, why don’t you try to have *E.coli* make circularly permuted catalytic polypeptide chains and incorporate them into ATCase-like molecules.” Can new termini be introduced almost everywhere in the polypeptide chain? Where can such termini not be tolerated? Graf did a great job solving innumerable technical problems and developed a technique whereby *E. coli* produced a collection of random ATCase-like molecules. So he devised a general technique that has been unbelievably interesting. Colonies expressing detectable amounts of active circularly permuted proteins were identified by an immunoblot technique or by their ability to grow in the absence of pyrimidines in the growth medium. Sequence determinations of the positive clones revealed a variety of unexpected circular permutations. Some had N- and C-termini within alpha helices, and most were located in the C-terminal domain with only a few in the N-terminal domain. Subsequently Beernink and Yang [Beernink, P. T., Yang, Y. R., Graf, R., King, D. S., Shah, S. S., Schachman, H. K., *Protein Science*, 10, 528 (2001)] extended this research significantly and found that E. Coli made many more ATCase molecules containing circularly permuted catalytic chains in both domains. The technique designed by Graf led to all sorts of insertions and deletions with the result that the termini were frequently unexpected. Nonetheless, we were able to study nine variants.



06-00:10:48

Schlesinger: Did you make any ATCases that were better than the original?

06-00:10:52

Schachman: No. That's right. That's a good question—can we make them better? And that work would have been followed up if my lab had continued to function for the next few years. So a couple of students and Alex Ni came along and he did some very pretty work with some of the samples they produced by this random method and we had a lot of fun doing that. So basically that sort of was the culmination of our major activity except for the work I told you a little bit about with NMR which has been done with Louis Kay in Toronto, subsequent to my closing my own lab. So basically I think we've covered nearly everything that we need to cover. [End Audio File 6]

Interview #_: March 8, 2010
Begin Audio File 7 03-08-2010.mp3

07-00:00:14

Schlesinger: Howard, before we turn to the NMR studies, let's go back to some of the work that you did with Tom Alber using x-ray diffraction.

07-00:00:34

Schachman: OK. As you have gathered from our past conversations, we didn't have a very good relationship about exchange of information with the Lipscomb laboratory and for years this was a very frustrating experience because he was just so secretive about his research.

It's rather interesting, because I had a student, Hiromi Komiya, who transferred to my laboratory after not doing well with her former professor and she was obviously well suited for looking at data and computational work. And in the course of conversations with her, I suggested she look into X-ray work and I co-opted Sung-Hou Kim, who's here in the chemistry department, onto this problem. She began to work with Sung-Hou Kim on a mutant that we were working on. But somehow or other, Sung-Hou never really got turned on by ATCase, and she wrote her PhD thesis. It was a beautiful piece of work. It never got published in the real sense of the word, and she got her degree with me and I was very happy. She moved on. She then went to work, actually, with Doug Rees, at Caltech, who found her a wonderful person for the computational aspects in his own lab. So my suspicion about her talent turned out to be correct.

In any case, Sung-Hou never followed up the work and it was too bad. Some years later, Tom Alber joined the faculty and Tom did get interested in ATCase. He had a post-doc by the name of Endrizzi, and I had a brand new post-doc, Peter Beernink and they became very good friends. Beernink became excited about the idea of learning crystallography, which was wonderful for me. We were right next door to one another in the old Stanley Hall. So a wonderful relationship developed between Beernink, Endrizzi, Alber and Schachman, and we decided to work on the catalytic subunit. Interestingly enough, there was a void in all the ATCase literature. Namely, nobody

had studied the catalytic subunit because presumably it was very difficult to crystallize. Beernink got crystals that were beautiful very, very early and with Endrizzi's help he learned a lot about crystallography. We published a lovely paper in 1999 on the crystal structure of the catalytic subunit.

07-00:03:11

Schlesinger: So all of the work that Lipscomb was done with the—

07-00:03:11

Schachman: All holoenzyme. Right. So this was a major void in the situation because we would love to know more details about the pure catalytic subunit. Why is the catalytic subunit much more active than the unliganded enzyme, or the T state of the enzyme? So we would expect the catalytic subunit to look like the catalytic subunit within the whole enzyme in the R-state. When you did the crystallography, you found out just the opposite was true—that the crystal structure of the pure catalytic subunit was much more similar to the catalytic subunit within the holoenzyme in the T-state. So this was a paradox. It obviously had a lot of flexibility. It wasn't completely symmetrical in a three-fold sense. So it became a subject of considerable interest and they did a wonderful job.

07-00:04:05

Schlesinger: Give me the dates of this so that people will know—

07-00:04:09

Schachman: So this is May of 1999 it was published.

07-00:04:10

Schlesinger: Oh, okay. But when was the work done? Just around that time?

07-00:04:15

Schachman: Oh, probably '97, '98.

07-00:04:17

Schlesinger: Okay. I mentioned it just so that people would sort of know what other people were doing at that time with the enzyme.

07-00:04:

Schachman: Right, okay. So that was actually a very satisfying piece of work. But the next question is how do you relate that to the holoenzyme and to the binding of ligands? So the next step in this study, after publishing that, was to go ahead and study the complex of PALA with the pure catalytic subunit and relate that to the PALA binding in the holoenzyme. That was also a nice piece of work that they did. It became very clear that when you talk about PALA binding and when you talk about R-state, you have a wonderful problem, because PALA is like a disulfide cross-link. The two domains in the catalytic subunit, pure catalytic subunit, close their hinge angle enormously when PALA is bound. It's like a disulfide cross-link except it's a noncovalent bisubstrate analog. So therefore, when you look at ATCase, the holoenzyme, with PALA bound to it, you're not looking only at the R state, you're looking at an R state, which has a ligand that causes a loop closure in the catalytic subunit. So the whole issue became much more complex and much more interesting than we had ever anticipated. We then began to ask more and more questions, which I'll come back to at a later time. What is really the R-state in the enzyme? And you can't talk about the PALA liganded enzyme as R-state because part of the effect of PALA is to close the loop, as well as to promote the transition from T to R. So that work still remains open and I'll come back to it in a little while in terms of the NMR at the very end.

07-00:06:10

Schlesinger: Howard, did anybody ever look at *E. coli* in which the bacteria didn't have a regulatory subunit? Did the bacteria care?

07-00:06:20

Schachman: Well, we made strains of bacteria that were devoid of the catalytic subunit but not just the regulatory subunit. We never did cellular physiology. It's a very good question. We talked about that because you ask your question—how important is feedback inhibition for *E. coli*? And the answer is we don't know and it's too bad people haven't studied it. Because other strains or other organisms don't use feedback inhibition with the ATCase except in pyrimidine biosynthesis. So it's never been resolved.

Related to this area of understanding what the R state of the enzyme was another piece of work done in the laboratory

initiated by David Markby a terrific graduate student, and it was then followed up by another graduate student, Bin-Bing Zhou. I'll give you the reprint of this. Well, David Markby asked a very simple question, “You spend a lot of time on domains of the catalytic subunit. Why don’t we consider the domains in the regulatory subunit?” As you remember, the regulatory subunit is a dimer. Part of the regulatory chain associates with another regulatory chain for the lower half of the molecule. The other part of the regulatory chain binds through a catalytic subunit and they’re seen structurally in the Lipscomb structures. So there are two domains to a regulatory chain: one for binding to itself and the other one for binding to the catalytic subunit. So we asked, “Why don’t we see if we can make one of the peptides, one domain, by itself genetically?” So we did the typical genetic manipulations, with a linker so we could find it, and then we put this genetic construct for about a seventy amino acid polypeptide into *E. coli* and asked it to make this peptide. Now, the question is how do you find the peptide? You find the peptide by then adding a huge amount of catalytic subunit and hope it will bind to that peptide and then look for a shift in the gel pattern, and that’s exactly how we did it. We purified the seventy amino acid peptide and studied that in some detail and how it interacted with pure catalytic subunit.

07-00:08:51

Schlesinger: But you didn’t have any microbiologists who wanted to know whether in fact it could still function in the bacteria, right?

07-00:08:56

Schachman: That’s right. That’s exactly right. So David started this work with Bin-Bing and then Bin-Bing followed up and it became Zhou’s Ph.D. thesis, and the effects on the catalytic subunit are profound. There’s a terrific change in the catalytic mechanism and we think that this is a very wonderful example of transduction, of a small polypeptide, seventy amino acids, binding to a trimer of three chains, each of 30,000. So that means we have roughly a hundred thousand molecular weight proteins binding three seventy amino acid peptides. And the change in the catalytic properties of that complex relative to the pure catalytic subunit is just like you’re converting the molecule to the R-state. And we have lots of data that were published and it became a very interesting story and that’s what I’m hoping will

be followed up ultimately by NMR, by Lewis Kay and his colleagues. So that will get me into the third area of science.

07-00:10:05

Schlesinger: What was the first area?

07-00:10:08

Schachman: The first area was the crystallography of the catalytic subunit. The second area is this work on the small peptide binding and now this is the third area. And these are all the most recent work that we have done. And I think I might have mentioned it, but if I didn't I'll mention it now. One day I got a phone call from my good friend Cyril Kay in Canada—we might have been friends for fifty years or thereabouts—saying "Howard, Lewis, who's a superb NMR specialist at Toronto is looking for an interesting protein to work on that's big because that's the challenge for NMR spectroscopy. And I wondered if it's okay with you if I suggest ATCase to him." I said, "Yes, I would love it, because I'd love to see ATCase in good hands and I'm a great believer in NMR. So by all means." So Lewis Kay was going to a meeting in San Francisco.

07-00:11:03

Schlesinger: Wait. Lewis Kay and Cyril Kay—

07-00:11:04

Schachman: Father and son. So Lewis Kay was at Toronto and establishing a wonderful reputation already and he was going to a meeting in San Francisco. So I went to San Francisco. We got together. We spent a lot of time there and we agreed that this would be the way to work and I said, "Ying, in my lab", the lab was still functioning in those days, "has a sister in Toronto. She would be thrilled to go visit in Toronto." So Ying went to Toronto for two weeks and worked in his lab, brought all the strains there, outlined all the problems to Lewis Kay and his post-doc, whose name is Algirdas Velyvis. And we then began having conference calls on the telephone and the work went on for about five years before Lewis and Velyvis were ready to publish a paper. And then they finally published a paper on a solution NMR study about the active site in ATCase and the role of nucleotides. So the problem was beautifully handled by them. I was an innocent

partner. All we did was ask questions and criticize and ask more questions when we got answers and they did all the beautiful work. But we were very thrilled with the result.

07-00:12:28

Schlesinger: Howard, let me just stop for one minute, because I am not all that familiar with NMR myself. When you were asking questions, how much of the actual data did you look at and evaluate?

07-00:12:40

Schachman: I can't. I mean, very little, very little. It's very difficult. It's very difficult even to follow their figures and the way they publish data is so different from anything I'm used to that I'm totally unable to criticize and reevaluate the work.

It's sure based on trust. It's a good example of a collaboration and if it turns out that they were wrong or they were faking the data, I would be a co-author of wrong or fake papers and that's the penalty of a collaboration that is so involving diverse fields that were so diverse that I couldn't understand them.

Lewis Kay and his colleagues were experts in incorporating the appropriate isotopic labels that permitted them to probe structural details and changes in conformation. Much of their work was focused on methyl groups and they were able to observe 27 resonances originating from the 27 isoleucine residues in ATCase. So the most important thing was they began to study the unliganded enzyme, which was what we called the T state. They then began a little bit of work, and they got one set of signals indicating to them that all the chains were the same. So the symmetry was very nice. Beautiful example of labeling techniques that are very sophisticated. They used all sorts of microbiological methods to get deuterium into certain residues or whatever you need, ^{15}N, things of that sort, so you know what you're looking at—side-chains of valine, leucine, and isoleucine.

07-00:14:10

Schlesinger: But in all of those measurements, all of the valine, for example, would be labeled, it'd be almost impossible to just label one region.

07-00:14:22

Schachman: That is correct, they were labeling all of the residues corresponding to a particular amino acid. They knew how to identify the resonances attributable to the methyl groups in the side chains of isoleucine residues. So you can spot them immediately, even though other things are labeled as well, you know where they will wind up in the nuclear magnetic resonance field. They can identify them very easily. And I cannot really go into detail unless I pore through the paper much more thoroughly. And they were convinced that the chains were all very similar, so that the whole identity problem and symmetry problem were essentially solved. There was very little R-state because they saw one set of resonances. We had also given them the double mutant that Jim Newell had made in our laboratory, which we had talked about earlier, as a model of the R-state. So they had that, as well. They were able then to look at that molecule by NMR. In addition, they could tell whether the isoleucine resonances were attributable to the regulatory subunit or catalytic subunit by reassembling ATCase molecules from separately labeled subunits. So they knew that when they looked at a resonance from an isoleucine side chain it was a catalytic isoleucine side chain, not a regulatory isoleucine, because they had built hybrids, from the various unlabeled and labeled subunits. By using our double mutant they were able then to obtain high-resolution NMR patterns of ATCase corresponding to the R-state. They now had essentially an R state model and a T state model. They then used carbamoyl phosphate or N-phosphonoacetamide as ligands that would bind and shift the allosteric equilibrium slightly toward the R state. When they did that, they saw resonances that resembled those observed with the double mutant. Now for the first time, with ATCase in the presence of phosphonacetamide they detected resonances that could be attributed to both the T and the R states. These observations meant that the interconversion between the T and R states was sufficiently slow relative to the NMR time scale that both forms were seen in a single spectrum.

I realize that this is a complicated story so let me try to summarize even though it will be repetitive. By using the double mutant they were able to see resonances corresponding to what they called the R-state of the enzyme. By using unliganded wild type enzyme, they have resonances that correspond to residues

in the T-state of the enzyme. Then they used N-phosphonoacetamide, which would perturb the equilibrium of wild type enzyme just a little bit, which other people claimed had no effect but the ultracentrifuge showed that carbamoyl phosphate alone had a very small effect nothing like carbamoyl phosphate and succinate. So by using N-phosphonoacetamide or carbamoyl phosphate—carbamoyl phosphate is bad because it hydrolyzes very easily. So N-phosphonoacetamide was used as an alternative to it. They were able to see, in addition to the predominant resonances that corresponded to the T-state, some new ones now for the first time—remember that this is wild type ATCase—that corresponded to the resonances they are already seeing for the double mutant which they knew was a model of R-state. So now they were able to see T and R on the same picture. That means there was slow exchange. On the NMR time scale, going from T to R or R to T is slow enough that you can see them both, because if it was very rapid you would see some intermediate. So this was fantastic. And now they're able to prove that there are both R and T state molecules represented in the NMR spectrum. Now you can add CTP and/or ATP and see what happens. When you add CTP, you're getting more molecules going into the T-state or the fraction of molecules in the R-state decreases. When they added ATP, the T state was diminished and one observes increases corresponding to the R state. So they were able to prove what we had already been saying, that CTP and ATP do perturb the T to R equilibrium and it was a complete validation of everything we had been saying with a double mutant. And now this work has been extended enormously by Lewis Kay and Velyvis and I'm hoping that they're moving toward going to the peptide-protein interaction. That will be sort of a culmination of what I would like to call the R-state of the enzyme. We'll see how that works out.

Although this was the end of our discussion Howard did want to add a last comment on ATCase.

My last post-doc, Feruz Kurbanov, was an extremely capable person interested in learning crystallography which, of course, involved more collaboration with Tom Alber and his group. Potentially, one of the most attractive targets for study was the double mutant formed by Jim Newell which we termed an R–state model because of its physical and enzymological

properties. Our detailed ultracentrifuge studies demonstrated that the unliganded molecules were swollen with a sedimentation coefficient very similar to that of PALA-liganded wild-type enzyme. Kurbanov's first diffraction studies led to a swollen structure, leaving us ecstatic about the agreement. But, as is often the case, further diffraction studies revealed several distinct structures one of which was clearly compact and analogous to unliganded wild-type ATCase. I was convinced that the various conformations seen by x-ray diffraction were the result of intermolecular forces between molecules that arose in the crystallization process. To me it was clear from extensive sedimentation velocity experiments that all the molecules in solution were swollen, and I attributed the compact structures Kurbanov observed in some studies as the result of an artifact due to crystallization. Obviously, artifact was the wrong term, because the structures were real. Tom and I had difficulty in rationalizing the different points of view and writing the paper proved a very formidable task. Delay after delay ensued and the fairly good draft is still sitting in my files unpublished. All of this relevant to the discussion of the NMR studies in Kay's laboratory. There is no doubt from the NMR experiments on the double mutant that the molecules are predominantly in one conformation, thereby validating the conclusion from the sedimentation studies. Alber and I have discussed this issue over the past few years, and he recognizes the power of the NMR experiments and their support of the view that in solution the double mutant molecules are all swollen. Just recently we talked about resuscitating the manuscript by agreeing that the crystallization process might have led to the formation of the various structures some of which were compact. I hope that we can arrive at a satisfactory paper, because I have terrible guilt feelings that the beautiful work by Kurbanov was never published through no fault of his own.

End Audio file 7

Interview #_ July 19, 2010
Begin Audio File 8 07-19-2010.mp3

[Note: The next section of this transcript has been sealed by the narrator until July 19, 2020.]

Schlesinger: Howard, you gave me in your CV a long list of awards and honors, but rather than discuss all of them I'd like to hear from you the ones that were a surprise and the ones that you were most proud of. So I'll open it up and let you talk and maybe I'll ask some questions. But really, let's talk about the ones that were a surprise and then the ones that, in a sense, you feel the most proud that you received.

08-00:44:57

Schachman: Okay. Well, as you can gather, I was very lucky with wonderful people in my lab, students and post-docs, over many, many years. Over fifty PhDs were granted in my lab and maybe a hundred or something like that postdocs spent time with me. And moreover, I was very, very fortunate with a group of fantastic technicians. I hate to use the word technician in that sense. But my first technician obviously dates way back to 1949 and her name was Alice Schwartz. Her husband and she started the Bio-Rad Lab. So to some extent, when Dave Schwartz, who was a chemistry student and wanted to go into business, thought, "Gee, Schachman's getting lots of requests for ultracentrifuge experiments. So why don't we see if we can run the ultracentrifuge for people, because he gets requests from oil companies and people for whom he doesn't want to run the ultracentrifuge." So he went down to Spinco, with whom he knew I had very good relationships and the head of Spinco at that time was perfectly happy to see if he could sell more ultracentrifuges, so he said, "You can come down whenever you want to Belmont." It was before they moved to Palo Alto. "And run the ultracentrifuge in my laboratory for whatever samples you want." So Dave was able to get requests from Shell Oil, from Standard Oil, all sorts of very unusual requests to run ultracentrifuge experiments that formerly had come to me and I was not particularly interested in doing any of those. And if he couldn't understand how to run them, he would discuss them with either me or with Bill Harrington, with whom he was also very friendly. And we would help him interpret results. As I recall, he offered me ten dollars an hour or something for working for him. I can't remember the numbers.

But Alice helped him a lot. Then he also knew that we were getting requests throughout the country from people who

wanted to grow viruses and Alice was an expert in this. She knew the plant greenhouse on the top floor and on the Oxford track there was a plot of land, which was used to infect tobacco plants. And, again, he got into business selling tobacco mosaic virus. And gradually Dave Schwartz built a billion dollar empire called Bio-Rad Labs based largely on the work his wife had done with me.

She was replaced by Jean Miller who was a fantastic technician, and then ultimately my super duper technician was Ying Yang who worked for me for over thirty years, whom I've already described in several places in my oral history and was worth at least five post-docs at one time.

So by 1962, I already had received my first award. It was from the E.H. Sargent & Company and it was an award for chemical instrumentation (1962). And that was based almost exclusively on my ultracentrifuge work and I was very, very happy about it. And then a short time after that I got the John Scott Award of the City of Philadelphia (1964), which is a very prestigious award that is given out.

08-00:48:28
Schlesinger: There's no connection between that award and the fact that you were from Philadelphia?

08-00:48:31
Schachman: No, absolutely none. As a matter of fact, Bruce Ames won that award several years ago. I've forgotten how long ago. But it's quite a distinguished award. And part of that comes from the fact that Stanley would nominate you for these kinds of things and his nomination meant an awful lot. Well, then one day a very fascinating conversation occurred which will interest you because you know him very well. Herman Kalckar, whose ability to communicate was virtually zero, called me on the telephone and started mumbling in his characteristic way. As I like to say, people tell me when he spoke Danish, his native tongue, he was even more difficult to understand than when he spoke English. So I went home that night. I said to Ethel, "Ethel, Herman Kalckar called today." She said, "Did you understand him?" I said, "Well, yes. Either I won a prize or I'm going to be on the committee to pick winners of a prize." She said, "What will you do?" I said, "Oh, I'll have to wait until I get something in the mail because I certainly didn't understand him." So it turned out, some weeks later, I was notified from the Massachusetts General Hospital that I was the winner of the Warren Triennial prize (1965), which is given as its name implies every three

years. It's a very prestigious award and, of course, I was ecstatic about that. And it was wonderful because I was able to solve the problem of Herman Kalckar's conversation without telling him, "I don't know what you're talking about."

So then later I won the Merck Award (1986) and a couple of others. So I was richly rewarded and I was very, very pleased about it. And that essentially kept me going for quite awhile. I was elected to the National Academy of Sciences (1968), the American Academy of Arts & Sciences (1966). I got a couple of honorary degrees. By 1987, I had already begun to become active in the issues of science policy. In 1987, I became President of the American Society for Biochemistry and Molecular Biology. (At the time it was the American Society of Biological Chemists.)

And, Sondra, you will appreciate this because this also involves a fascinating story. The first time I was asked to run for President—they wanted two candidates—I was told that the other person being nominated was Mildred Cohn. I was crazy about Mildred Cohn. She was a fabulous woman who died just relatively recently at the age of 96. Mildred and I knew each other very, very well and I knew her reputation. I knew there wasn't a chance in the world that I would be elected in a ballot that had her name versus my name on it. And sure enough, I got a phone call one day from the President saying that Mildred won the election and I was ecstatic with joy.

So that was Mildred Cohn. Then later, whoever the nominating committee was called me again. "Would I be a candidate for the Presidency?" So this time I was told that the other nominee was Alton Meister. Alton Meister was a very distinguished biochemist and the biochemists at that time were much more interested in enzymes than they were in physical biochemistry. So he was much better known than I was and I was confident that Al Meister would be elected, there were some problems about the election with ballots and so forth and so on. But ultimately Al Meister did become elected as President.

Some years later I get a phone call from the chairman of the nominating committee, and as I recall, it was Lubert Stryer at that time at Stanford University. And Lubert said, "Howard, we want you to be a candidate for President." I said, "Hey, Lubert. Have you ever heard of Harold Stassen? I'll be the Harold Stassen of the Biochemistry Society." Well, the young people

don’t know who Harold Stassen was but he had run repeatedly for the Presidency of the United States as a Republican candidate and, of course, never got elected. So here I was the third time. This time I didn’t know who the other candidate would be and it turned out that it was Terry Stadtman. Terry Stadtman was not nearly as well known as I was, and, of course, my name had been on the ballot for two previous years. So I became elected President of the American Society of Biological Chemists (ASBC) in 1987. By that time there was under discussion a change in name to American Society for Biochemistry and Molecular Biology (ASBMB), but it had not yet occurred. As I remember, Paul Berg had been the President of ASBC and I was already active in public affairs in the Society. One of the issues at the time was the possibility of a change in the name of the society because of the widespread acceptance of the new field of molecular biology. Should ASBC expand into this area or let a new society arise with the mission of molecular biology

08-00:54:19
Schlesinger: Was there much discussion about the name change?

08-00:54:20
Schachman: Indeed, the change in the name of the society became a hot issue. And that’s interesting in and of itself. You’ll be fascinated to hear this. Because I realized molecular biology was starting to grow already. It was 1980s and I knew that if we didn’t do something there would be a new society called the American Society for Molecular Biology. So I advocated changing the name. Arthur Kornberg and all sorts of friends of mine were blistering in their criticism that I would weaken biochemistry by incorporating the name molecular biology into the same title. So there was a mail ballot on this and I remember an enzymologist at Wisconsin wrote why we should not change the name and I wrote the piece as to why we should change the name to include molecular biology. It was overwhelmingly adopted as a name change. So it became ASBMB and when I became President—I can’t remember which I became President of. But ultimately it turned out what Lubert Stryer didn’t tell me was that the President-elect of the Society would automatically become President of FASEB, the Federation for American Societies of Experimental Biology the following year. Subsequently they have changed their procedures so now they elect people from the various branches.

So that really created a major problem because when I became President I became heavily involved in traveling back and forth

to Washington because of all sorts of controversies. For example, I testified many times before committees of Congress on different subjects involving misconduct in science, formulating policies acceptable to the academic communities, on overhead, on regulatory processes and things of that sort and it was a lot of fun.

08-00:56:23
Schlesinger: Howard, when you started to testify before Congress, did somebody work with you and rehearse you or help you in that?

08-00:56:31
Schachman: Absolutely.

08-00:56:33
Schlesinger: Was that from the Society?

08-00:56:35
Schachman: And it was very, very useful. We had a public affairs officer in the Biochemistry Society, ASBMB, Pete Farnham, who was very good. And he would help me organize. For example, I became probably the national expert on indirect costs, largely through his tutelage. I have files, drawers, that are still filled with papers on indirect cost. I knew a lot about it. I knew a lot about how the rules were being violated. I testified, along with Presidents of universities, in front of Congressional committees. The members in Congress were very sympathetic to me and extremely critical of them. The university administrators wanted indirect costs and they didn't want to pay any attention to rules and regulations. They used them in a relatively ruthless fashion and I was arguing they should be used to aid and abet the research for which the money was given. That language persisted throughout all of my testimony: To aid and abet the research for which the money was given. They didn't want to do that. I remember vividly a wonderful conversation with one of the more friendly university presidents, the President of Johns Hopkins. And, "Howard," he said, "do you mind if we use the money for physics?" I said, "Bill, no, I do mind when you use the money for physics." I said, "If biochemistry at Johns Hopkins has grants and has indirect costs money, I don't want it to be exclusively used for the professors who wrote the grants. You can fix the elevator in the building. You can fix the dark rooms. But it should be used for the biochemistry department, not for the physics department and let the department of AEC, Atomic Energy Commission, fix the physics department." So he said, "I guess you're right." But, as you probably know, that became a major focus and Don Kennedy, who was one of the Presidents—

I remember a meeting at which there must have been thirty university Presidents in a room and me. And I was fighting all of them over how indirect cost money should be allocated. Don Kennedy ultimately lost his job as President of Stanford because he got in conflict with Congressman Dingell over indirect costs. And some of the things they did were terrible. Don Kennedy's explanation for that was the comptroller did it. All you had to do was send the comptroller in those days to Baghdad. That was before Baghdad became an international property. But on the other hand, he didn't do that and he defended what I consider to be totally illegitimate use of indirect cost money. And I'm a believer in indirect cost.

So the big issue ultimately was the fraud issue. When the fraud issue arose, the executive director of FASEB called me on the telephone and said, "Howard, there's going to be a meeting here in Washington."

08-00:59:40
Schlesinger: So, Howard, before you go on. The fraud issue, was there one example that brought it to public attention?

08-00:59:50
Schachman: Yes. Congressman Gore in the early eighties was beginning to have hearings on fraud in science. So it became a big issue. Some of those cases were absolutely horrible stories. Moreover, the top officials in the scientific community behaved stupidly, in my opinion, on that. For example, Phil Handler said, "Science is self-correcting, so leave us alone." The Director of NIH said, "We're taking care of these things." Well, the Congressman didn't believe that and there was no evidence that it was true. Science is self-correcting but it's only the important science that's self-correcting. The unimportant science goes on and on and on and even some of the important reports are still fraudulent. Most of those cases, compared to the more recent ones are trivial but on the other hand, Congressman Gore, who was a young congressman at that time on the Oversight Committee, who was very annoyed, as were many others, at the arrogance of the scientific establishment.

08-00:60:57
Schlesinger: So this was now in the late eighties already or this was the early eighties?

08-00:61:02
Schachman: No, this is probably early eighties. Don Fredrickson I think was—

08-00:61:05
Schlesinger: So this was before you were President of the—

08-00:61:07
Schachman: That's right. So that had already arisen. So therefore, the AAAS decided to have a meeting to decide—

08-00:61:14
Schlesinger: So you got involved with this because of your activities in the Biochemistry Society?

08-00:61:21
Schachman: That's right.

08-00:61:22
Schlesinger: Not in AAAS?

08-00:61:24
Schachman: No. Well, AAAS organized some of this. I got involved by getting a phone call that the AAAS decided to have a meeting and notified the Executive Director of FASEB inviting the organization to participate. He called me and offered to go, but I indicated that the subject bordered on my interest in academic freedom. So I decided to fly across the country to attend the meeting organized, as I remember, by Robert Rosenzweig, who had been a provost at Stanford, who was very active I think in the AAAS at that particular time. And it was a meeting with, I remember, Al Teich being there. And by and large, most of the people in that room, I would say there were twenty in the room, were non-scientists. The other person representing our society with me was Bernie Davis. And Bernie Davis was a very strong-minded guy.

08-00:62:40
Schlesinger: And not very diplomatic.

08-00:62:41
Schachman: That's a good phrase. Not very diplomatic but a wonderful guy with whom I agreed nearly all the time. But I didn't like his style. And it became a good cop/bad cop thing. Bernie would say things that would really antagonize everybody. You could just look in the room and see they were getting angry at him. And then I would come along in a very mild way and soften what he said and as a result of that Al Teich liked me and began asking me to come to more meetings. I have been to innumerable retreats over this particular issue, because by that time the language of fraud was being opposed by the attorneys and they wanted to change it to misconduct in science. I was concerned, as was Bernie, about what is misconduct in science. We knew what fraud was. But the government officials didn't like the word

fraud because many of them were attorneys and fraud had to prove that somebody got hurt by the activity and they had to prove intent and so forth. There were four criteria for fraud. Can't remember all four of them at the particular moment. But somebody being damaged by it, as in a fraud in the bank, for example, that's easily established. But fraud hurting somebody from a paper that was mistaken or wrong or faked, that was harder to establish. So they didn't like that, so they wanted language that was all encompassing. And that's when I became involved. So as a consequence, I got involved, as I've already indicated, in innumerable retreats, one of which occurred at NIH, which I described to you earlier in our discussions, but I want to expand that a bit more here.

Jim Wyngaarden was Director of NIH. I went to Wyngaarden's conference room. I knew him, not well, and I would say there were thirty lawyers in the room and Wyngaarden, who was a very distinguished biomedical scientist, and me. So there were only two people in the room representing the field of science. The lawyers began sprouting all sorts of phrases. Misrepresentation, deception, selection of data as parts of the definition of misconduct in science. And that's when I became extremely alarmed and I came up with the phrase: fabrication, falsification, and plagiarism. We fought like crazy over this idea of misrepresentation. They wanted to expand the definition so that everything under the kitchen sink should be included under misconduct in science. So I began reciting stories from science. I recited the Watson and Crick story. I said when Watson and Crick published their paper, there were data in the literature presented by a very distinguished English laboratory headed by Gulland, showing that there were monoesterified phosphate groups, loads of them, in a DNA molecule. That meant there were chain ends and therefore a double stranded structure could not possibly encompass those data. Either they decided the data were not worthwhile or they didn't know about the data. Whatever it was, their intuition led them away from it and they didn't include it at all. I then recited a story about Pauling on the enthalpy of a hydrogen bond. I said he was the only man in the world smart enough to know that the number he was using in building his alpha helix was only appropriate in the absence of water and he should have used another one for water and he didn't do it and nobody knew anything about it. These people built models simplifying the evidence that's available, throwing away stuff that was in conflict with it because they didn't know how to encompass it and that's how science progresses. And then the next day somebody comes along and says, "But you

forgot this," and they add it, they modify the structure. Some of the officials were charmed by the examples.

And then Wyngaarden described his experience on a sabbatical leave in Monod's laboratory, how Monod silenced everybody when he wanted to bring in another enzyme. He said, "It's too complicated. Forget it."

So the issue then became the fight over the definition of scientific misconduct and I remember leaving the Wyngaarden meeting feeling very good. He felt terrific because we had argued for the restricted definition of fabrication, falsification and plagiarism and we had fought very strenuously against the phrase that some of the lawyers wanted called, "other practices that seriously deviate from those that are commonly accepted within the scientific community." That's all in quotes. So he was ecstatic with joy. I went back to California and a couple of days later I got a phone call from Jim Wyngaarden saying, "Howard, we lost the battle downtown." Downtown, as far as NIH is concerned, meant the Department of Health and Human Services or the public health policy group downtown. They changed what had happened at NIH to include the phrase, "Those practices that seriously deviate." So we were still back in the same old battle. And that battle went on for almost ten to fifteen years to get rid of that simple phrase, "Those practices which seriously deviate."

At that time also they had a system called the ALERT, in capital letters. A-L-E-R-T, in which the Public Health Service would list on the ALERT the names of people against whom allegations had been made, even though those cases hadn't been adjudicated. It was absurd to think that you could do something like that but there were all sorts of people who couldn't get grants and who were having trouble even with job changes because they were listed on the ALERT, because an allegation had been raised even though they were totally innocent of the charges for which they were being charged. So I would say that I was probably the national leader in fighting this issue of what is the definition of misconduct in science and I was extremely pleased one day when I got a phone call that FASEB had decided to give me the Public Service Award for all my activities in this public arena. FASEB Public Service Awards were almost invariably, before that and since then, given to people in the Congress. As I like to say, awards for public service or public affairs by professional societies in the Washington area are given to people from whom you want something rather than for

what they have already done. So I was the only scientist to my knowledge that has ever gotten this award.

Then in 1994 Harold Varmus became the Director of the National Institutes of Health. I remember vividly, I happened to be in Washington for some other purpose, I went into his office to congratulate him because we had worked very hard privately as a society on that issue of who should be Director of NIH. And he knew I was active in that. So he said, “Gee, I’m really worried about this job.” His phrase was beautiful. “Up until now I’ve spent all my life with people under thirty-five and now I think I’m going to not see any people under thirty-five.” And I’m wondering what’s going on around the world and I would love to have somebody travel around the various campuses and talk to the people at the universities to see what they think of NIH. What do you think of that idea?” I said, “It’s a terrific idea if you get the right kind of person.” I left and that was the end of the conversation. I get home and the phone starts ringing from all sorts of friends of mine. “I hear you’re going to work for Harold.” And that was all news to me. So he obviously wanted to get my reaction to the idea without telling me that he was thinking of me. He figured I was probably over the hill by that time scientifically, and I might as well retire and come to work at NIH. So I picked up the telephone and called him. He said, “Why don’t you come to Washington? Come over the weekend so we can talk at home and we’ll talk about the position.” So I went back to Washington and we talked about it and then I said, “Look, I’m going to Baylor University to give a big lecture. And I’ll tell you what I’ll do. We’ll do a trial experiment. You have connections at Southwestern Medical School, with Brown and Goldstein, your wonderful colleagues, distinguished scientists. I’ll be willing to spend a couple of days independent of my giving seminars at Baylor to do this at Southwestern.” So he said, “Terrific, you arrange your trip to Baylor and let me know your schedule so that I can make the other contacts.”

So I called some of my friends in that area and said, “Listen, I’m coming in but I don’t know the people at Southwestern,” I knew Brown and Goldstein but I didn’t know them well, “and I would love to know whom I can trust and whom I can’t trust. So can we have dinner together the night before so you can fill me in on the politics of the school and the people I should listen to, the people I should ignore.” So it was very useful. So by the time I got to Southwestern, I was really very well prepared.

When I got there, it was extremely well organized. I met with graduate students, I met with young postdocs, I met with more

senior postdocs, I met with young faculty, senior faculty, administration, deans, everybody. It was about two or three days. And I remember vividly. This was a trip that I had planned in advance and Ethel was with me. One morning the phone rings in the motel and its Harold Varmus on the telephone. How he knew where I was, I do not know, because he obviously had to call Berkeley. I hadn't given him an itinerary. I didn't tell him where I was staying. He was flying in an airplane and he called me to find out. And he had already talked to some people about the previous day. He wanted to talk to me about it. So he was obviously very interested in finding out whether this vehicle was very good.

And it was terrific. There was no question. Just this one visit told me immediately that people would talk to me even though many of them didn't know me. Some knew my name as a scientist. But they would talk to me because they thought I wasn't NIH, but on the other hand, I had a voice and I could get to the ear of the director of NIH. So, for example, I was at a meeting with graduate students and most of them were ecstatic with joy. There was one kid who was miserable. I said, "Why are you so unhappy?" He said, "Well, my Professor just lost his grant so I'm going to be without money." So I said, "What are you going to do about it?" He said, "I'm going to look for a Hughes Professor." I said, "Do you care what he works on?" He said, "Hell, no, I don't care what he works on, I just know he'll be able to pay me." So the following night I had dinner with the Dean and with the President and I told them the story. And, of course, they were shocked. "This can't happen in our institution. We support our students." Well, the truth of the matter is they didn't support their students and that is a very poor way for students to choose a professor and choose a laboratory in which to work. So it's the kind of story that you would never get under ordinary circumstances but I got it because I was meeting with a bunch of kids who trusted me.

Two terrific young faculty members were there, for example, and they've both become very successful since. This is now twenty, thirty years ago. And they asked me should they apply for R25's? It was a grant that you got for five years. It was $350,000 for five years versus R01. They were much easier to get, they were created for young people who were starting their careers. So they asked me what they should do. I said, "Do you have to pay part of your salary?" And, of course, the answer was, "Yes, we have to pay part of our salary." I said, "If you're getting $75,000 a year and you have to pay part of your salary, you're not going to have anything left over for your lab." So I

said, “You’re good enough that you ought to be able to get an R01. I would apply for an R01.” Well, that was the kind of information that I could provide to them and I could then take back to Washington and tell Harold about.

I visited over fifty institutions in my capacity there and invariably I was able to come back with ideas that I would tell Harold Varmus about. These included criteria for evaluating a grant, search committees, things of that sort. It was a wonderful vehicle, which unfortunately has not been continued, because they would talk to a non-NIH person in ways that were much more candid than to an NIH person.

08-00:76:45
Schlesinger: Howard, let’s end by your reciting to me the newest award that didn’t go to you but which some of your research led to, and that was the awarding of membership in the Royal Society.

08-00:77:00
Schachman: Oh, well, I also have to give you a couple of science awards as a consequence of my more political activities..

08-00:77:04
Schlesinger: Okay. We have time for that. I only want to go into the ones that were very special to you.

08-00:77:12
Schachman: Okay.

08-00:77:12
Schlesinger: Okay, so go ahead. You can do that first.

08-00:77:20
Schachman: So by that time, as you can see, I was very deeply involved in looking for alternative funding strategies, reducing bureaucratic burdens, improving the quality of peer review and ensuring the integrity of research and public policy affecting science became a major activity. So I was very pleased in 2001 when the American Association for the Advancement of Science conferred on me the Scientific Freedom and Responsibility Award for all my activities. And that same year, ASBMB established the Howard K. Schachman Public Service Award given to public officials for their contributions to the advancement of science.

08-00:78:04
Schlesinger: That’s really very nice because that goes on forever.

08-00:78:10

Schachman: That's right. So the award is given every year and it's usually given to a congressman or a senator. I mean, people like Specter and Harkin got it one year. Ruth Kirschstein got it another year. A Republican Congressman, Mike Castle, who is trying to make a comeback in the Republican Party, got it relatively recently. So it's a nice award. I frequently get invited to come to the award ceremonies but I haven't been there for years. In Berkeley, the University of California awarded the Berkeley Citation for Distinguished Achievement and Notable Service in 1993 and I was appointed a Faculty Research Lecturer in '94. So I've had more than my share of awards and very pleased about it. In fact, in 2008, I received the Carl Brändén Award for Scientific Research and Public Service and also was named Distinguished Emeritus of the year by the University of California Berkeley Emeriti Association. So I'm very pleased that some of the awards that I received have come from my science policy work, which has become a major part of my more recent activities.

As you know, at the end of my discussion of my science I described the work on NMR that was being done by Louis Kay, the son of a very good friend of mine, in collaboration with us. He was just elected a week ago—the induction ceremony occurs in London—to the Royal Society. The citation to that included his work on ATCase so I feel to some extent very pleased. Even though he's done all the work on that, I've raised issues and asked questions and he answered them. And that concludes the awards.

[End of interview]

Howard K. Schachman

Education:

BS 1939 Massachusetts Institute of Technology, Chemical Engineering

PhD 1948 Princeton University, Physical Chemistry

Professional Experience:

Instructor Department of Biochemistry and The Virus Laboratory University of California at Berkeley	1948-1950
Assistant/Assoc. Professor Department of Biochemistry University of California, Berkeley	1950-1959
Assistant/Assoc. Res. Biochemist The Virus Laboratory University of California, Berkeley	1950-1959
Professor/Res. Biochemist Dept of Biochemistry and Virology and The Virus Laboratory University of California, Berkeley	1959-1964
Chairman Department of Molecular Biology University of California at Berkeley	1969-1976
Director The Virus Laboratory University of California at Berkeley	1969-1976
Professor/Res.Biochemist Department of Molecular Biology, Department of Biochemistry and The Virus Laboratory University of California, Berkeley (The departments of Biochemistry and Molecular Biology were disestablished in July 1989, in connection with reorganization of biological sciences at Berkeley)	1964-1989

Professor/Research Biochemist Division of Biochemistry 1989-1994

and Molecular Biology (BMB), (Emeritus, 1991)

Department of Molecular and Cell Biology (MCB)

Present Position:

Professor of the Graduate School/and The Virus Laboratory, 1994-

Research Biochemist, University of California at Berkeley

Honors and Awards:

Guggenheim Fellowship, 1957-58; California Section Award, American Chemical Society, 1958; E. H. Sargent & Co. Award for Chemical Instrumentation, American Chemical Society, 1962; John Scott Award of the City of Philadelphia, 1964; Warren Triennial Prize of the Massachusetts General Hospital, 1965; American Academy of Arts and Sciences, 1966; Riley Lecturer, Notre Dame University, 1967; National Academy of Sciences, 1968; National Sigma Xi Lecturer, 1969; Harvey Lecturer, 1974; Honorary Doctor of Science Degree, Northwestern University, 1974; Carter-Wallace Lecturer, Princeton University, 1976; Gardiner Memorial Lecturer, New Mexico State University, 1976; Jesse W. Beams Memorial Lecturer, University of Virginia, 1978; Scholar in Residence, Fogarty International Center, National Institutes of Health, 1977, 1982, 1983; American Society of Biological Chemists - Merck Award, 1986; Bernard Axelrod Lecturer, Purdue University, 1986; Behring Diagnostics Lecturer, University of California, San Diego, 1987; President, American Society for Biochemistry and Molecular Biology, 1987; President, Federation of American Societies for Experimental Biology, 1988; William Lloyd Evans Award Lecturer, Ohio State University, 1988; Doctor of Medicine (Honorary), University of Naples, 1990; Alexander von Humboldt Award, 1990; Berkeley Citation for Distinguished Achievement and Notable Service, University of California, Berkeley, 1993; Carl and Gerty Cori Lecturer, Washington University School of Medicine, 1993; Faculty Research Lecturer, University of California at Berkeley, 1994; Herbert A. Sober Award of the American Society for Biochemistry and Molecular Biology, 1994; Public Service Award of the Federation of American Societies for Experimental Biology, 1994; Carl Cori Lecturer, Massachusetts General Hospital and Harvard Medical School, 1994; Foreign Member, Accademia Nazionale Dei Lincei, 1996; Alberta Heritage Foundation for Medical Research Visiting Professorship, 1996; Theodor Svedberg Award, 1998; Burroughs Wellcome Fund Lecturer, 1999; Scientific Freedom and Responsibility Award of the American Association for the Advancement of Science, 2001; Establishment of the Howard K. Schachman Public Service Award by the American Society for Biochemistry and Molecular Biology, 2001; Walter C. Mackenzie Lecturer,

University of Alberta School of Medicine, Edmonton, Canada, 2001; Lovelace Memorial Lecturer in Ethics, University of Maryland Biotechnology Institute, 2006. Carl Brändén Award for Scientific Research and Public Service from the Protein Society, 2008, Distinguished Emeritus of the Year from University of California Berkeley Emeriti Association, 2008.

Public Advisory Committees:

Member of Study Sections and Panels of NIH and NSF in 1960's and 1970's;

Advisory Committee to Director of NSF, 1970's; Board of Scientific Counselors,

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases,

National Institutes of Health, 1983-87; Board of Scientific Consultants of the

Memorial Sloan-Kettering Cancer Center, New York, 1988-97; Board of Scientific Counselors, NCI, Division of Cancer Biology and Diagnosis, 1989-92; Member of Scientific Council and Scientific Advisory Board of Stazione Zoologica, Naples, Italy, 1988--; Special Advisor to Director of the National Institutes of Health and Ombudsman in the Basic Sciences, 1994-2002; Member, Committee on Scientific Freedom and Responsibility of the American Association for the Advancement of Science, 1998-2004. California, San Diego, 1987; President, American Society for Biochemistry and Molecular Biology, 1987; President, Federation of American Societies for Experimental Biology, 1988; William Lloyd Evans Award Lecturer, Ohio State University, 1988; Doctor of Medicine (Honorary), University of Naples, 1990; Alexander von Humboldt Award, 1990; Berkeley Citation for Distinguished Achievement and Notable Service, University of California, Berkeley, 1993; Carl and Gerty Cori Lecturer, Washington University School of Medicine, 1993; Faculty Research Lecturer, University of California at Berkeley, 1994; Herbert A. Sober Award of the American Society for Biochemistry and Molecular Biology, 1994; Public Service Award of the Federation of American Societies for Experimental Biology, 1994; Carl Cori Lecturer, Massachusetts General Hospital and Harvard Medical School, 1994; Foreign Member, Accademia Nazionale Dei Lincei, 1996; Alberta Heritage Foundation for Medical Research Visiting Professorship, 1996; Theodor Svedberg Award, 1998; Burroughs Wellcome Fund Lecturer, 1999; Scientific Freedom and Responsibility Award of the American Association for the Advancement of Science, 2001; Establishment of the Howard K. Schachman Public Service Award by the American Society for Biochemistry

and Molecular Biology, 2001; Walter C. Mackenzie Lecturer, University of Alberta

School of Medicine, Edmonton, Canada, 2001; Lovelace Memorial Lecturer in

Ethics, University of Maryland Biotechnology Institute, 2006. Carl Brändén Award for Scientific Research and Public Service from the Protein Society, 2008, Distinguished Emeritus of the Year from University of California Berkeley Emeriti Association, 2008.

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National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases,

National Institutes of Health, 1983-87; Board of Scientific Consultants of the

Memorial Sloan-Kettering Cancer Center, New York, 1988-97; Board of Scientific Counselors, NCI, Division of Cancer Biology and Diagnosis, 1989-92; Member of Scientific Council and Scientific Advisory Board of Stazione Zoologica, Naples, Italy, 1988; Special Advisor to Director of the National Institutes of Health and Ombudsman in the Basic Sciences, 1994-2002; Member, Committee on Scientific Freedom and Responsibility of the American Association for the Advancement of Science, 1998-2004.

Publications of Howard K. Schachman

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Sondra Schlesinger - a brief sketch

Sondra Schlesinger received her Ph.D. in biochemistry from the University of Michigan in 1960. She and her husband (Milton Schlesinger) then went off for a year of postdoctoral training with E. B. Chain at the Istituto Superiore di Sanita in Rome, Italy where they learned to appreciate and enjoy the archaeology, culture and cuisine of that country. They both continued their postdoctoral training for another three years at the Massachusetts Institute of Technology where Sondra worked on enzyme induction and regulation in bacteria in the laboratory of Boris Magasanik. In 1964 they moved to Washington University where they both became assistant professors in the Department of Microbiology. Sondra initially continued her research in the field of microbial genetics and physiology, but in the early 1970s her scientific interests switched to the field of animal virology and she directed her research to the structure and replication of RNA animal viruses. Most of her research was concerned with enveloped RNA viruses, in particular, the alphavirus, Sindbis virus, that caused cytopathic infections in cultured cells,. Her most recent work with this virus involved identifying regulatory sequences in the virus genome essential for replication of the viral RNA and for packaging of the genomic RNA to form the icosahedral nucleocapsid of the virus. Her research also included studies that led to the development of the virus as an expression vector for the synthesis of heterologous proteins. Vectors derived from Sindbis virus and other alphaviruses are being used to introduce new genes as RNA into cells. They have the potential of being used for vaccines in humans and have become a valuable tool for neurobiologists as these vector particles can infect neurons in culture.

In the 1990s during the time that Sondra was President of the American Society for Virology she began to carry out oral histories with several virologists including Bernie Fields (at the time he was Chairman of the Department of Microbiology at Harvard Medical School) and David Baltimore (Nobel prize winner and past President of the California Institute of Technology). She made her first attempt, however, with her mentor Boris Magasanik and that was a valuable lesson because the first time she forgot to turn on the tape recorder. Then under the auspices of a grant from the Alfred P. Sloan Foundation she established a web site on the history of structural virology that consists of a description of the history and conversations with several structural virologists including Don Wiley, Steve Harrison, and Michael Rossmann and their discussions can be seen at http://virologyhistory.wustl.edu.

After both Sondra and Milton became professors emeriti and had closed their laboratories they moved to Berkeley. Because of their friendship with Howard Schachman and Sondra's interest in history of science she and Howard agreed to carry out an oral history focusing on Howard's scientific accomplishments. This was encouraged and aided financially by the Bancroft Library**.**