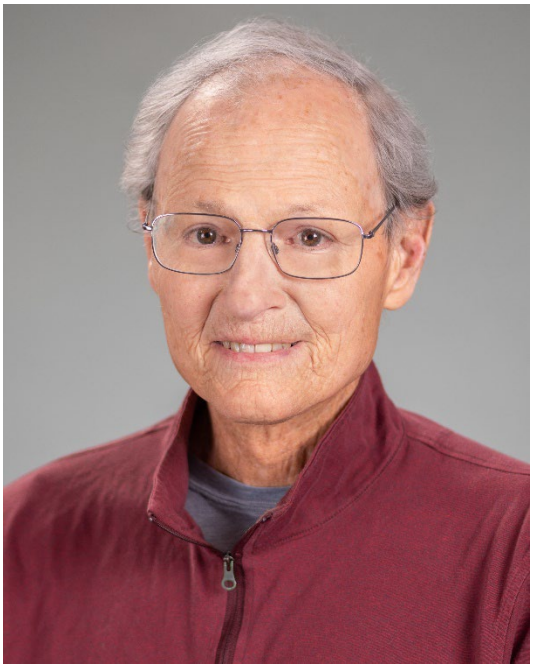
Dr. Bruce Chesebro



Dr. Bruce Cheseboro, May 2022

This is an oral history with Dr. Bruce Wilcox Chesebro on October 24, 2022, about his career at the Rocky Mountain Laboratories of the National Institute of Allergy and Infectious Diseases (NIAID). The interview is being done over Zoom. The interviewer is Dr. Victoria A. Harden, Founding Director, Emerita, Office of NIH History and Stetten Museum, National Institutes of Health.

Harden: Dr. Chesebro, would you please state your full name, that you know that this interview is being recorded and that you give permission for the recording?

Chesebro: My name is Bruce Wilcox Chesebro, and I know this interview is being recorded, and I give permission.

Harden: Thank you. Let's start at the beginning. You were born March 19th, 1942, in Los Angeles, California, as the eldest child of Marvin Marsh Chesebro, who worked as an attorney, and Genevieve Wilcox Chesebro, a homemaker. Would you tell me about your childhood and your education just through high school, especially about anyone in your family or school who was important in encouraging you towards medicine or science?

Chesebro: I went to a Catholic school for the first to sixth grade, St. Brendan School in Los Angeles. And then I went to a prep school called Harvard School in North Hollywood, California. And then I attended California Institute of Technology in Pasadena, California.

Harden: But let's focus on your life through high school right now. Was there anybody in your family or perhaps teachers who nudged you to go into medicine or science?

Chesebro: No. I got the idea to go into medicine and research science at the next stage at Caltech [California Institute of Technology].

Harden: What did you enjoy growing up? Were there sports?

Chesebro: I enjoyed a lot of sports. Surfing and basketball were the two things I liked the most.

Harden: What year did you graduate from high school?

Chesebro: 1959.

Harden: Did you skip a grade?

Chesebro: No, I started early.

Harden: Let's move along then to Caltech, where you were a chemistry major. Tell me about your undergraduate training and in particular any professors with whom you worked.

Chesebro: I did the routine courses for being a combination chemistry and biology major at Caltech. That was my first exposure to biology, and I liked it a lot. It was a lot different from the typical, traditional biology where you learn a species and how to categorize species, etc. It was molecular—this was at the dawn of the cracking of the genetic code. Every week there'd be a new amino acid code worked out. It was extremely exciting to hear all that going on.

In my first year of biology, which was my second year at Caltech, I heard many really interesting talks from many different professors, including [Dr.] Renato Dulbecco. I ended up wrangling a job in the laboratory of Dr. Dulbecco, who was a virologist at Caltech. He assigned me to work with one of his postdocs. Dr. Dulbecco is famous for in applying the bacteriophage plaque assay to animal viruses. He got a Nobel Prize for that some years later. The man I actually worked with was [Dr.] Klaus Bayreuther, who was from Germany. We worked on polyomavirus. I was super excited about that because we studied growing viruses in tissue culture cells. At about that time I realized that I might want to go to medical school.

Harden: Why medical school and not just get a Ph.D. in virology?

Chesebro: That's an interesting question. At Caltech, everybody pushed you to stay in research and not to go to medical school. There weren't any clinicians I was exposed to at Caltech, so maybe I was just a contrarian, or maybe I wanted to see how all of this research information applied to real humans. So, I chose to apply to medical schools, and I got into Harvard Medical School. That was an offer I couldn't refuse.

Harden: Before we move on to Harvard, tell me about your extracurricular activities at Caltech. You continued your interest in water sports, I believe.

Chesebro: Yes, I was really interested in swimming and water polo. I did that every year at Caltech, and it was a major success for me. I set several school records, even though I had never been a good swimmer competitively in high school sports. I did know how to surf, and so I knew how to swim pretty well, but I'd never been into competitive swimming. However, when I tried competitive swimming and water polo, they were really the perfect match for me. My main extracurricular activity was doing those two sports.

Harden: I noted that you were inducted into the Caltech Sports Hall of Honor in 2021.

Chesebro: Yes, I was inducted into it in 2015 and then again in 2021 for the swim team.

Harden: For two different things?

Chesebro: Well, in 2015, it was an individual honor, just for me. The one in 2021 was for the whole team, which that year won the conference title. They made a group induction. They had put it off for a year because of COVID, but I have a big plaque, saying "Caltech Sports Hall of Honor." In 2015, I went down to Caltech and gave a little talk, and it was fun. It brought back a lot of memories.

Harden: In the fall of 1963, you began your medical education at Harvard Medical School. Why did you decide on Harvard, and how different did you find it from Caltech and Southern California in general?

Chesebro: Very different. Really different. It took a lot of getting used to. Harvard Medical School was a lot about memorizing facts and following rigid curriculum, whereas Caltech had been about understanding facts. All the tests that Caltech were open book. You didn't have to memorize anything. It was all about thinking, and medical school was not.

Harden: Was there someone that nudged you towards Harvard or did you just pick it and apply?

Chesebro: I picked it and applied there and other places, too, but I can't remember where.

Harden: Were there particular members of the Harvard Medical School faculty that influenced you towards a career in research?

Chesebro: Not really. What influenced me was that when I was at Caltech, I had spent one summer in Germany just trying to learn German, and I had a great time doing that. And then I did the same thing the next summer in France—no science again. I really was excited about living in another culture and trying to learn a language.

But somehow I did get hooked up with two professors at Harvard who were very friendly, [Dr.] Torsten [Nils] Wiesel—he was the main one—and Dr. David H. Hubel. They were neurophysiologists and later won a Nobel Prize for vision neurophysiology. I didn't work on anything with them. I just talked with Torsten Wiesel because he was Swedish, and I wanted to spend the summer in Sweden. He introduced me to a virology lab in Stockholm where I was able to spend the summer. That lab was under the direction of Professor Sven Gard. He assigned me to [Dr.] Sven-Eric Svehag, who was then an assistant professor. Dr. Svehag became a lifelong friend. He was a virologist-immunologist, and I did a summer project on immunology with him. Then, when I finished med school, I went back to Sweden and spent another year and a half there.

Harden: In 1966, you married Joan Burgess [Dr. Joan Burgess Chesebro], a music teacher. Can you tell me a bit about her? Where did she grow up and what were her interests?

Chesebro: Joan grew up in Providence, Rhode Island, and I met her in medical school. She was a classmate in my class. So she was also a doctor. She came to Sweden with me and worked in the Pathology Department at Karolinska Hospital in Stockholm.

Harden: On your Basic Information Form, you identified her as a music teacher. How did she shift from being a doctor to being a music teacher?

Chesebro: That's a later part of the story. She had been a good musician all her life, playing various instruments. We came back to the United States in 1970, 1972, and I was a Research Associate in Bethesda in [Dr.] Henry Metzger's lab in NIAMD [National Institute of Arthritis and Metabolic Diseases], while Joan was a radiology resident at Georgetown University Hospital. We had a baby in Sweden, and after she had the baby, she didn't work in the Karolinska Institute anymore. Instead, she was a full-time mom.

Harden: May I ask, was that was her choice? Or did they prevent her from working after the baby arrived?

Chesebro: That was her choice. She's gone back and forth from being a mom to being a researcher or a clinician a couple of times. And she's always preferred being a mom.

Harden: You and Joan had three children, Annmarie, Eric, and Brian, even as you were juggling all sorts of education and training work. How did you manage what is today called work-life balance?

Chesebro: It was pretty easy for me. I just did what I always do, and I didn't have to change my career as much because I was just an "assistant parent." Joan was the vital parent. She was the mom who took care of the kids and that was much more important than what I was doing. I loved the kids. I loved being a father. She took some time off from working outside the home. She didn't take time off when we were in Washington, D.C. By then it was convenient to be a resident and work as a mom because the kids were old enough to go to daycare and be cared for there. But when we moved to Montana, things were very different.

Harden: Right now, I want to drop back and ask you to tell me more about what you were doing in Sweden with your research. Harvard supported you with a special research stipend, I believe, and I want to understand how what you were doing in Sweden fit in with what Harvard wanted you to do to finish your medical degree.

Chesebro: They gave me a special award when I came home to graduate, which was a monetary award, but while I was in Sweden, I was paid by Swedish government as a lab technician. What Harvard did was give me time off.

There was another person who influenced me at Harvard. I'd almost forgotten about him. [Dr.] Phin Cohen at Harvard was a hematologist who taught both my wife and me in the physical diagnosis part of introduction to medicine. He was a wonderful teacher and a really good friend to us. He told me not to go back to Sweden because there were better researchers at Harvard. However, I'd been there once, and I liked it a lot, so I wanted to go back. Maybe I didn't know any better, but I did like it in Sweden. So I told Phin, "Well, I'm going anyway." And then I found out that Phin was taking a year off to go to Holland to do his research. He was interested in freezing blood platelets and other blood cells. And he went to Holland where they were experts in lipid metabolism and membrane freezing. So he was telling me not to go to Europe, yet he was going to Europe because he thought he had found a really good lab to go to. He also liked living the life of a European. It was a funny arrangement, and I was always teasing him about this in later years. He was a wonderful person.

Harden: When Harvard gave you the Borden Award for undergraduate research, it was noted that it was a "special field." Was that field immunology, or was it virology, or even something else?

Chesebro: It was a combination of the two. It wasn't virology, but it was immunology. What we worked on was purifying the immunoglobulin IgM, the largest sized immunoglobulin subclass. It's called IgM for macroglobulin. At the time, nobody had any idea what the overall structure of antibody molecules was. Sven-Eric Svehag had figured out that you could see these molecules if you mixed antisera with very clean viruses and then pelleted the viruses. The antibody would be stuck to them, and then you could look at them with an electron microscope. Several labs around the world did that, including at NIAID.

[Dr.] Albert Z. Kapikian did that with another classmate of mine, Ray Dolin [Dr. Raphael Dolin]. And they discovered the Norwalk virus.

We worked on that technique before they did. I think there was also a lab in England working on it, and I forget where else, but my boss [Sven-Eric Svehag] actually was working on it using poliovirus before my arrival. I devised a new way to purify IgM in particular and to look at it without the virus, so it wasn't encumbered. You could see just the antibody molecules and you could see that they looked like little spiders—they're really very interesting. When a single molecule grabs onto a virus, you can see that it looks like a spider sitting on a virus.

Harden: Very Interesting!

Chesebro: Each IgM molecules has 10 legs, and they can stick out and grab the next virus with these additional legs. This was really important for the fixation of complement when IgM bound to red blood cells or other cell types. People didn't know much about immunoglobulin ultrastructure at this time, and they were very interested in the structure and functions of antibodies. There was no cellular immunology at that time. It was just prior to the discovery of how cells contribute to immunology.

I was so excited by finding something so unique and interesting to so many other people that it just made me want to do this forever. That's what happened. So, it was quite a change to go back to medicine. I went from having the time of my life working on this electron microscopic study of IgM to going back and being a medical intern at Stanford University.

Harden: You moved back from Harvard to California, so you were back on the West Coast, doing your internship at Stanford. Tell me about being an intern. It was all clinical, right?

Chesebro: Yes, it was. I really didn't like it much at all. First of all, I'd forgotten how to be a doctor after being away from medicine for a couple of years. I hadn't embedded it totally into my brain, so I wasn't particularly good at it. I needed a lot of supervision because I was scared that I didn't know enough. But other people thought I was doing fine. I got through it all right. But after I finished being an intern, I looked for an escape route.

In the second year of the residency at Stanford, our time was divided into various sub-specialties, and we did three months of rotation in each of four areas. I took one rotation in rheumatology. During this time, I became friends with [Dr.] Hugh McDevitt, who was Professor of Medicine and Microbiology. And then I went into Hugh McDevitt's lab full time for the rest of the year.

Harden: So you didn't have to do all four rotations?

Chesebro: No, they just let me do research.

Harden: Somebody had identified you as a really promising medical researcher by that time.

Chesebro: Yes. That's what had happened. They thought I was going to do well in research, and I was ahead of everybody else in terms of papers. And then during the time I was in Hugh McDevitt's lab, I applied to the NIH. It was the time of the doctor draft for the Vietnam War, and I applied to be a Research Associate at NIH and got selected by Henry Metzger in the Arthritis Institute [then called the

National Institute of Arthritis and Metabolic Diseases, NIAMD], now known as the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Harden: Hold that thought. I'm going to come back to that in some detail, but I want you to tell me more about Dr. McDevitt and what you did in his lab. I think that you were working on genetically controlled immune responses in mice. Is that close?

Chesebro: Yes.

Harden: Okay. Tell me about it.

Chesebro: At that time there were several laboratories studying how mice controlled immune responses to specific antigens. The laboratories of Hugh McDevitt at Stanford and of [Dr.] Baruj Benacerraf at NYU [New York University] were among the leaders. They were trying to figure out what controls responses to different antigens. McDevitt had noted that the transplantation antigen genes, H2 [major histocompatibility complex] in mice, influenced these responses. Other people found that in humans the HLA [human leukocyte antigen] genes had this effect. At Stanford, Dr. McDevitt's group was trying to figure out how this worked mechanistically. We made chimeric mice by combining immune system cells from different mice. and using these chimeric mice we tried to figure out which cell types could mediate a response to a specific antigen molecule. This was very difficult because the transplantation antigens were linked to the effect we were studying. You couldn't just transfer cells from one mouse to another because the transplantation antigens were a barrier for grafting of the cells.

I was there almost a year, and we wrote a paper about this work. Later this field became very important to the understanding of cellular immunology and the collaborations of B and T lymphocytes in immune responses. Dr. Benacerraf and two other geneticists, one at the Jackson Lab and one in Paris, France, got the Nobel Prize for this approach to immunology. Unfortunately, McDevitt was not included on this Nobel Prize.

Harden: The Nobel Archives are not opened until 50 years after a prize is awarded. And we'll all likely be dead before we can find out about the Nobel committee's deliberations.

Chesebro: Well, I know that the Nobel Prizes are not immune to political influence.

Harden: Absolutely true.

Chesebro: And that's not surprising. But in this case, many people called Hugh McDevitt and said, "You should have gotten a Nobel Prize along with those other researchers." They recognized his enormous contribution to this work. In contrast, my contribution was extremely small, but it did have an inspirational effect on my attitude towards research. I loved it.

Harden: As you were finishing up your work at Stanford, the Vietnam War was going full blast, and you were a doctor who would be drafted unless there was an alternative way to discharge your military service. With your record in research, being a Research Associate at NIH was one of those ways.

Chesebro: That's right.

Harden: When I was reading some of your papers, I saw that you already had some connection with Henry Metzger. He gave you material for you to use, I believe.

Chesebro: Yes, that's right. There was a very important connection with Dr. Metzger. When I was in Sweden, we were purifying IgM. These large molecules of about 1 million daltons molecular weight are very hard to purify from normal rabbit blood or human blood or mouse blood because the concentration is so low in blood. Dr. Metzger had been studying IgM in his lab at a biochemical level to see what its properties were. He had used patients who had a kind of leukemia in which the leukemia cells produce IgM in tremendous quantities. This disease is called Waldenstrom's macroglobulinemia. It's a disease of plasma cells, which are the cells that make antibodies. They are the neoplastic cells involved. Dr. Metzger had a large amount of purified IgM, and he sent us some to look at with the electron microscope. This material was much cleaner than ours, and it helped us immensely to sort out the structure.

Harden: I would bet that Dr. Metzger had already identified you as a good candidate to come to his lab because, as I think I told you, he was on the Advisory Committee for the Office of NIH History and Stetten Museum, and I got to know him very well over the years.

Chesebro: Oh, I didn't know that.

Harden: Also, he was very close to “Hans” Stetten [Dr. DeWitt Stetten, Jr.], whom you may be old enough to remember.

Chesebro: Yes, I do remember him.

Harden: They were both paragons of basic research at a time when there was considerable pressure to do more applied research. This is why I said that I suspect Dr. Metzger had identified you quickly as somebody who was committed to basic research.

Chesebro: Dr. Metzger, as you know then, was an M.D., but he did basic research. A lot of M.D.s were pressured into doing clinical research. I was a Research Associate in the Associates Training Program. I had no clinical duties at NIH. I just had to be in the lab working with Dr. Metzger.

Harden: Tell me what you did with him.

Chesebro: First of all, as you probably know, Dr. Metzger was a wonderful person and a very smart man. We obtained mouse myeloma cells from mice which were induced by [Dr.] Michael Potter by injecting BALB/c mice intraperitoneally with mineral oil. We then grew these cells in vivo and purified the immunoglobulins which they produced. We had immunoglobulins of many different classes, including IgA and IgM that were hard to purify from normal mice. Dr. Metzger decided that we should try a technique that he had done when he was a postdoc. We used affinity labeling of the antigen combining site itself to see what parts of the polypeptide chains in the IgM were actually nearest to the binding site.

We used myeloma proteins that bound to the moiety phosphorylcholine and labeled them with an analog of phosphorylcholine that was very chemically reactive with a variety of amino acid residues. When we mixed this analog with the purified myeloma, the concentration of the phosphorylcholine analog became high in the combining site regions, so that there was enhanced labeling the antibody

protein near the combining site Our results showed that the combining site was in the same parts of the immunoglobulin molecule as people had found with another ligand-antibody combination that was completely different than phosphorylcholine. Subsequently sequencing that showed that the regions around these pockets turned out to be the hypervariable regions of immunoglobulin molecules which generated the much of the diversity of the binding specificities encoded in all immunoglobulin molecules.

Harden: When you completed your time with Dr. Metzger, you moved to the Rocky Mountain Laboratory—it was still RML singular in 1972 [This facility was known as the Rocky Mountain Laboratory (singular) from 1931 until 1982, when its name became plural: Rocky Mountain Laboratories.]. You were jumping institutes from what is now NIDDK to NIAID, and going to Montana—a young man who grew up in the big city suddenly moving to a very small town in Montana. Tell me who recruited you? How did the process unfold? Why did you decide to accept the position in Hamilton?

Chesebro: Well, that is good question. It was a more personal issue: I liked outdoors. I liked fly fishing for trout, and I liked skiing in the mountains and hiking in the mountains. I'd done a lot of that during high school and college, and I missed that when I lived in the east. Also, I was from Los Angeles and missed the west. I had visited the Rocky Mountain Lab when I drove from Stanford to Bethesda. I gave a seminar there and met some of the people, and there were quite a number of virologists there at the time. I was thinking about going back into virology, because that was one of the areas I loved the most when I first started in Dulbecco's lab and also in Svehag's lab.

Harden: In 1972, RML was a very different organization than it became later. Tell me who was there when you first arrived and whether there was someone who specifically recruited you.

Chesebro: Well, I invited myself. They didn't recruit me. They were very unaggressive about recruiting anybody in those days at RML. But on my way driving from Stanford to NIH, my wife and our daughter—we only had one child at that time—detoured up through Montana, and I gave a talk at RML. I contacted Jack Muñoz [Dr. John J. Muñoz], a research doctor who was on the editorial list for the *Journal of Immunology*. He was the only person I knew at RML. He worked on pertussis and its powerful toxin, pertussigen, which influenced the immune system in many ways.

Harden: I remember meeting and interviewing with Dr. Muñoz in the 1980s.

Chesebro: He was a very friendly, nice person who left me with a good impression of the hospitality and the collegiality of the staff at RML. And I thought, wow, I can work at an NIH-level lab with very good scientists and be near the mountains and the rivers if I go to Rocky Mountain Lab. So it just seemed like an unbelievably interesting possibility, and I thought I needed to try it. I'd never lived in a small town, and neither had my wife.

Harden: And how did you both adjust to Hamilton?

Chesebro: It was easy. We loved it.

Harden: I take it she likes to ski and do other outdoor activities, also.

Chesebro: She didn't like to fish that much, but she likes to ski and hike, and she likes the outdoors. Neither of us are very good hikers now due to advancing age. The other benefit of living in Hamilton, Montana, for both of us is that Hamilton is a really great place to raise a family.

Harden: I've heard that before from other RML scientists. And what did you think about the Hamilton schools?

Chesebro: They were good. The important thing was that Hamilton was not an impoverished small town. Some small Montana towns lack strong employment opportunities and have been shrinking over the last decades. But Hamilton has been growing, and it's partly because NIH invested so much in the Rocky Mountain Lab and brought so many people there. The U.S. Forest Service has also a pretty big footprint in all of Western Montana, including Hamilton. Those are high-paying federal jobs. In addition, there are many jobs associated with the schools that provide a stronger tax base for the schools and the community. So it's a good place to live, and the schools are good.

Here's one example of how welcoming RML was to me. When I came to the Rocky Mountain Lab on my first day of employment, the Director of the lab, Herb Stoenner [Dr. Herbert G. Stoenner] came up to me and said, "I want to take you around to meet all the people who work here." And he introduced me to every person he could find on the campus. That seemed unusual to me, and of course I didn't remember them all, but they all knew me from that moment on because I was just one person they had to think about. So, from that day on, from the electricians and plumbers to the heads of groups and postdocs, from the young people to the old people, all of them knew me by name. It was a smart thing for him to do because it preserved a certain kind of culture at the lab that doesn't exist anymore. New people come and go all the time now. I don't know their names. It's just a little too big to do individual introductions now.

Harden: When you started at RML, one of your first projects was on Aleutian disease of mink. Would you tell me about that work?

Chesebro: Well, that project just fell into my lab and Marshall Bloom's [Dr. Marshall E. Bloom] lab. Marshall was a beginning researcher, and he was working for Jack Muñoz. I was starting my own lab and working in Jack Muñoz's section. Joan and I were actually living in Marshall's house because our house wasn't ready yet. He and his wife let my daughter and my wife and me live in this big house that they had rented. We got to be good friends.

But anyway, the Aleutian disease of mink project just tumbled into our laps. We were thinking about Aleutian mink disease virus, and people in Canada had examined it with a special technique called counter-immunoelectrophoresis, where you electrophorese the immunoserum from an infected mink against purified virus on a slide in an agar matrix. With this method, instead of letting the virus and antibodies find each other by diffusion, you put them in an electric field so that everything moves faster, and big things like viruses can move more rapidly to be detected by the antibodies from the immune serum. The Canadians had looked at the precipitates from this reaction with an electron microscope and seen clumps of antivirus antibody complexes, but they didn't know that what they were seeing. To me they appeared to be virus-antibody complexes, similar to what we'd seen with poliovirus. We thought, “Whoa, that's the virus. That's not some other artifact. That's the virus itself we're looking at.”

I think these researchers in Canada had some idea that it had physical properties that gave it a buoyant density that suggested it might be a virus, a picornavirus or a parvovirus.

We got tissue from mink that had been infected by Bill Hadlow [Dr. William J. Hadlow], and we extracted the virus, and we ran it in a special CsCl gradient for purifying parvoviruses virus. With this method we detected a visible band containing DNA that was consistent with a tiny virus that had a high density. We looked at it in the electron microscope, and it looked similar to what was seen by the Canadian researchers. And then we did something that could not easily be done by most other labs. This was to do a quantitative infectivity titration of the samples of our gradient in real live mink! This was similar to a quantitative LD50 assay. Normally, in mice that would not be a difficult experiment. But ADV [Aleutian disease virus] infects mink, not mice, so doing this experiment in mink required the availability of animal facilities to follow numerous mink for several months after inoculation. The results showed that the quantity of live infectious virus peaked in the samples that contained the particles that we could see in the electron microscope. This virus appeared to be the virus that caused Aleutian disease in mink.

The Aleutian mink disease virus is a parvovirus, and it turns out that parvoviruses are interesting. In humans, I think the most commonly known human parvovirus is B19 parvovirus, which is involved in hematology. It can cause a severe anemia in patients with sickle cell disease. It can cause birth defects because it can be transferred from mothers to fetuses, and the fetuses then can get heart defects. It's a very important clinical virus if a fetus is infected early in pregnancy. It also causes a disease in humans called erythema infectiosum. They used to call it Fifth disease. It's a minor clinical disease if you catch it as a young child. You get a rash and a fever, and it goes away a couple days later. But if you have sickle cell anemia as a trait or the genetic makeup that gives you a full-blown sickle cell anemia and you get infected with B19 parvovirus, it's a devastating disease.

That's parvovirus, and I didn't ever do anything on B19 parvovirus. The reason I mention it is that most of the molecular attributes that apply to ADV, Aleutian disease virus, also apply to B19 parvovirus. So ADV is a wonderful model for B 19 parvovirus, even though ADV only affects mink.

Harden: In 1979, RML was completely reorganized administratively. NIAID Director [Dr.] Richard M. Krause and Director of Intramural Research Ken Sell [Dr. Kenneth W. Sell] were behind the reorganization. Dr. Herb Stoenner was retired. And you in 1978 had been named Acting Chief of the Laboratory of Persistent Viral Diseases. In 1979, you were chosen as the full Chief. John Swanson [Dr. John L. Swanson] was brought in as Chief of a new Laboratory of Microbial Structure and Function. All of these changes from the historic field work at RML to a molecular biological focus caused huge controversy. Would you to tell me about this major shift in research priorities and how it all went down.

Chesebro: It was a little bit rocky for people who lost power and slots. And for people who gained power and slots, they ended up with more resources. In terms of deciding who deserved what and whether this was fair or not, it’s not too easy to say. I mean, they tried to judge this on current productivity, not on history. For example, both Dr. Muñoz and Dr. Hadlow, who are now deceased, lost a lot of positions, even though both were very intelligent scientists in their different fields.

Everybody had their own style. Swanson's style of how to lead a lab was totally different from mine. Mine was more traditional. We had PIs [Principal Investigators], and people under them, and they had technicians, and they also had postdocs or grad students. In Swanson’s lab, everybody was independent, and they had to sink or swim on their own. He let everybody do what they wanted to do and hoped that everybody would do something good. People had a lot more independence in his lab. And he himself worked at the bench every single day as if he were a postdoc. That's what he liked to do.

As for me, I also liked to work at the bench. However, in recent years did I stop working at the bench. But I still organized the lab into groups where there were PIs, and technicians and graduate students. It was much more traditional in my lab.

Harden: Following up on your newly named lab, did you get to name it the Laboratory of Persistent Viral Diseases?

Chesebro: No, I did not. That was handed to me saying, "Here's this new lab. We've got permission to name it this." I remember distinctly Ken Sell telling me that they had to jump through a lot of hoops to get that name approved by the higher authorities, so they didn't want to change it. They made it very clear that although I could do a lot of things, I couldn't change that name.

Harden: I wonder why.

Chesebro: I think there was a political issue of making sure the name of a lab didn't look like the name of another lab whose work was already being done somewhere else.

Harden: Yes. I know, for example, that both NIAID and NCI had Laboratories of Immunology, but they worked on different problems, even though that might not have been clear to non-scientist politicians.

Chesebro: In the name “Persistent Viral Diseases,” the key word was “persistent.”

Harden: And how did you define persistent?

Chesebro: We didn't define it, but in general we used it to mean more chronic virus infections, including the so-called “slow viruses.” Friend murine retrovirus was a persistent virus, and other people were studying Aleutian disease of mink as a persistent virus. But the people studying Aleutian disease such as Dr. Hadlow weren’t even in our lab actually. [Dr.] John Coe was an immunologist, and he didn’t even work on viruses.

Harden: Your next line of research, I believe, was on Friend virus leukemia. You did a lot of work on that virus. Please tell me about it.

Chesebro: Working on Friend virus leukemia combined my interest in virology with my interest from working with Hugh McDevitt about host genes that influence the immunology of disease. I was also influenced in this work by [Dr.] Frank Lilly, a professor at Albert Einstein College of Medicine in New York. Frank Lilly was the head of the genetics department, ultimately, but when he was a postdoc and young researcher, he identified several host genes that influenced susceptibility to the Friend leukemia virus. I took up that project with his help. He was incredibly generous in giving me advice and donating the virus strains. I worked on the Friend virus system for over 20 years. We found three genes that when combined had a very strong influence on the ability of mice to recover from Friend leukemia.

Harden: I believe that Friend virus was also used as a model for resistance to HIV infection, correct?

Chesebro: Yes, and I remember at an HIV meeting saying, "By analogy with Friend virus, there are going to be people, they may be rare, but there are going to be people who probably have the genes that will allow them to recover from HIV.”

Harden: Wow.

Chesebro: And it turned out that there were.

Harden: Yes.

Chesebro: But I remember one researcher telling me I was crazy.

Harden: In 1988, you published a paper on MHC [major histocompatibility complex] recombinant mice in which you emphasized the contribution of [Dr.] Jack H. Stimpfling of the McLaughlin Research Institute in Great Falls, Montana, who developed and shared a panel of such mice. Why were these mice so important and why did you think you should single out this man for praise?

Chesebro: Well, Jack Stimpfling worked in a one-man lab in a grocery store when he started off, and [Dr.] Kim J. Hasenkrug, who spent much of his career at RML, started his research in Jack Stimpfling's lab. I first learned about Jack Stimpfling from Hugh McDevitt, who was my mentor at Stanford. Jack Stimpfling was well known to Hugh, and to all the immunologists who were interested in effects of transplantation antigen genes on immunology. Earlier Jack had decided to select recombinant mice that separated different pieces of the transplantation locus known as H2 in mice and about which we talked about earlier. He selected these animals by red cell typing using hemagglutination tests.

When people got interested in cellular immunology and the role of MHC in immunology, Jack Stimpfling had this array of mice with all these recombinations between various alleles and segments of genes of various strains of mice. They were an unbelievably valuable resource for solving the issues related to cellular immunology in the 1980s. To make matters even better, he shared the mice with everybody. If you put him on a paper as a co-author, he'd say, “Thank you very much.” But he didn't require anything. He just wanted the mice to go out to good laboratories and get used in interesting experiments.

He was a figure whom everybody in cellular immunology knew. They might not have known him personally, but they certainly knew about his mice. I knew about him from working with McDevitt, and when we found that certain mice could recover from Friend virus, one of the main ideas I had was to test Jack's mice and see where the recovery gene mapped in H2.

Harden: It was generous of him to do that.

Chesebro: This project started with recombinant mice from Dr. Stimpfling. The results identified mouse major histocompatibility complex (MHC or H2 genes) capable of helping mice recover from FV leukemia, and this work turned into turned into a long series of experiments showing that both CD4-positive helper T lymphocytes and CD8-positive cytotoxic T lymphocytes, as well as virus neutralizing antibodies were all required to facilitate spontaneous recovery from FV [Friend virus] leukemia by mice. Initially, this work was done by numerous postdoctoral fellows and laboratory technicians including Kathy Wehrly (retired); Jane Nishio, RML lab technician (deceased); William Britt (Dr. William J. Britt], former postdoctoral fellow; and Leonard Evans [Dr. Leonard H. Evans], RML Principal Investigator (deceased).

In addition, these same individuals plus others participated in generating some of the earliest monoclonal antibodies reactive with FV and other murine retroviruses which opened the door to studying how passive antibody administration influenced FV disease. These antibodies were also used

extensively by laboratories using retroviruses as delivery vehicles in gene transfer experiments in animals and humans.

Furthermore, these monoclonal antibodies led to the development of a quantitative in vitro test for counting live murine retroviruses particles. This assay known as a the “focal immunoassay” could detect focal areas of retrovirus infection on cell monolayers adherent to plastic dishes. It was similar to previous plaque assays for cytotoxic viruses such as poliovirus, but did not require any cell killing effect, just viral protein expression. The monoclonal antibodies were used to detect the expressed viral proteins in a very sensitive manner, which had not been possible previously.

Additional immunity experiments were facilitated by a collaboration with the lab of Dr. Bernard Moss at NIAID where the FV envelope gene and other structural protein genes were expressed in vaccinia viruses which were used to immunize mice against challenge with live FV. [Dr.] Patricia L. Earl from NIH, as well as Drs. Richard Morrison, Professor of Microbiology at the University of Alabama; Kim Hasenkrug, recently retired from LPVD; and Dr. Masaaki Miyazawa, Professor of Immunology at Osaka University, Japan, were among those mainly responsible for leading these experiments.

Two other series of experiments involving pathogenesis of mouse retroviruses were done starting around 1986. The first was the discovery of a new pathogenic effect induced by the Friend helper retrovirus (F-MuLV). The second was the cloning of the envelope of polytropic F-MuLV variant which induced rapid onset of neurological disease in mice.

Previously, most retroviruses were believed to be pathogenic due to induction of cancers of various tissues. In the case of Friend helper virus, the cells target appeared to be multiple lineages of bone marrow cells, and infection by this virus induced a leukemia in immature nucleated erythrocytes which did not survive normally and led to severe and anemia and death of the animal. However, together with [Dr.] Marc Sitbon, a postdoctoral fellow from France, in 1986, we discovered that early after virus infection at age 20-25 days of age, the mice were quite capable of producing mature erythrocytes. However, these cells were recognized as abnormal and removed from the circulation by the spleen and liver because they produced virus. Thus, this was a hemolytic mechanism of anemia secondary to retrovirus infection and was one of the first examples that retroviruses could have a pathogenic effect in vivo which was non-neoplastic. Subsequently molecular cloning experiments were able to identify two amino acid residues in the viral envelope gene which were vital to this phenomenon, but the exact mechanism of action of these residues remains unclear.

In a second series of experiments by my group and the group of [Dr.] John L. Portis at RML, mouse retroviruses which caused two different types of non-neoplastic brain disease after infection of baby mice were studied. As in the case of hemolytic anemia that I mentioned, the viral envelope genes were also critical in the induction of these two diseases. For example, viruses with a special ecotropic envelope, studied by Portis and colleagues, caused disease with spongiform degeneration of brainstem similar to prion diseases. However, no abnormal prion protein was found in these mice. In the other model, studied in my group, viruses with a special polytropic envelope gene caused a brain disease with infection of microglial cells and induction of abnormal cytokine and chemokine responses. In this model there was no spongiform degeneration, and the mechanism of neuronal damage was not clear.

We were also interested in the effects of retroviral envelope variation with regard to clinical neurological aspects of HIV. Only a subset of HIV patients develop clinical brain symptoms during their illness. However, these can be very distressing as they often lead to severe nerve pain as well as signs of

dementia. Together with colleagues from the Department of Neurology at Johns Hopkins School of Medicine, [Dr.] Christopher Power, a neurology fellow in my laboratory, compared the predominant HIV envelope protein sequences in groups of HIV patients with and without signs of dementia. Although this was only a small patient group, the results suggested that sequences in certain regions of envelope differed markedly in patients with dementia compared to those without dementia. Later experiments done by Dr. Power in his laboratory in Canada confirmed these findings and suggested that some types of HIV envelope protein appeared to be neurotoxic which might explain these clinical findings.

Harden: I was curious about your other research relating to HIV/AIDS. What else can you tell me?

Chesebro: HIV became a virus of intense interest in 1984 when it was demonstrated as the cause of HIV/AIDS. At this time, there was no numerical assay available for measuring live HIV. Instead, people were using endpoint dilution assays with titer given as the highest dilution capable of infecting a tube of growing lymphocytes. Such assays were sensitive at measuring live viruses but were not as accurate as would be desirable for many tests. Therefore, in my laboratory, Kathy Wehrly, the head laboratory technician, and I put a lot of effort into finding and developing tissue culture cell lines which grew attached to plastic dishes and were susceptible to HIV infection. These cells were the well-known human cell clone, HeLa, in which [Dr.] Daniel R. Littman at New York University expressed human CD4, a known receptor for HIV. We found that these cells were susceptible to many lab-adapted HIV strains, and at high dilutions the infections remained separately localized in the tissue culture monolayer allowing enumeration by staining with anti-HIV antibodies as was done in the murine retrovirus focal immunoassay described above.

Later, Emily Platt [Dr. Emily J. Platt] and [Dr.] David Kabat at the Oregon Health Sciences University added an additional HIV co-receptor, CCR5, to these HeLa-CD4 cells, and we found these cells functioned in focal immunoassays using most HIV strains obtained directly from humans. Jane Nishio, Kathy Wehrly, [Dr.] Brandon Walter and others in our lab at RML used these cells to identify the V2 and V3 regions of the HIV envelope protein as important in determining permissiveness for HIV infection of T lymphocytes and macrophages. We and many other groups studied this phenomenon over multiple years and found that in most individual patients there was a progression from early macrophage tropic viruses detected early after infection to more broadly lymphocyte tropic viruses which appeared later in disease. Apparently, the macrophage-tropic viruses were better at spread between individuals, but during disease progression the viruses with broad tropism spread more within each patient and were probably responsible for the lymphocyte killing and immunosuppression associated with severe clinical disease.

Another area of research combined our studies of HIV and murine retroviruses. As I said before, some years earlier, we identified a mouse non-H-2 gene, Rfv-3, which was required for successful production of FV neutralizing antibodies during FV infection. However, we were not able to determine the mechanism of action of this gene. However, a few years later, we did identify the chromosomal location of the Rfv-3 gene in mice. Subsequently, in [Dr.] Warner C. Greene's group in San Francisco, [Dr.] Mario L. Santiago was studying genes causing resistance of mice to HIV infection and he cloned a mouse gene known as APOBEC3G, which appeared to make mice quite resistant to HIV even when they should have been susceptible as they expressed the main HIV receptor human CD4. Mapping experiments for APOBEC3G showed that this gene was localized close to Rfv-3 in a single mouse chromosome and suggested that these two genes might be identical. Additional experiments by Drs. Santiago and Hasenkrug showed that this was true. Their results indicated that Rfv-3 was capable of influencing the modification of RNA residues in retroviral genomes to perturb the viral infection and also the immune

response to the virus. Although some details are still unresolved, this was a most interesting blending of viral immunity events in two separate virus systems.

Harden: Now let's turn then to your prion research. You'd known Dr. Hadlow from the time you arrived at RML, and you knew about his interest as a pathologist in scrapie. In 1982, when [Dr.] Stanley B. Prusiner] published that prions were a new type of infectious agent, you were not convinced. You published a paper in 1985 in *Nature,* and an *NIH Record* article based on that paper stated that you and your colleagues at RML "believe that the real agent is more likely a small virus particle, but many more questions must be answered before any agent can be definitely identified." Would you begin telling me about how your work started and developed during this early period?

Chesebro: Yes. Dr. Hadlow was always a friend and a colleague, but not a scientific collaborator. He was a pathologist, and I was an immunologist/virologist with an interest in pathogenesis of viral diseases.

Harden: Do you know how he thought about scrapie and what cause of it was?

Chesebro: Dr. Hadlow was the person who suggested that CJD [Creutzfeldt-Jakob Disease] in humans was an infectious disease just as scrapie was in sheep. This idea was based on the similarities in the neuropathology of CJD in humans and scrapie in sheep. But Hadlow did not know what the moiety was that spread the infection. He presumed it was a virus, but he did not carry out experiments to test this possibility.

Harden: I see.

Chesebro: I think it was during the RML reorganization that I got hooked up with Dr. Hadlow's main assistant veterinarian, [Dr.] Richard E. Race, who knew how to take care of the scrapie mice and how to inject mice with scrapie and follow the disease in the mice—what their symptoms were and so forth. We decided it would be a good idea to try to figure out whether some kind of virus caused scrapie. And so we started working on it.

I cannot remember what our initial ideas were, but what happened was that I began collaborating with another colleague, [Dr.] Ashley T. Haase, a professor of microbiology at University of Minnesota in Minneapolis for many years. He worked on visna virus but was interested in viruses of the brain in general. He wanted to do something about scrapie and figure it out at the molecular level. He knew more about DNA and RNA than I did, and he came to this project planning to use cloning, because cloning had just become very popular. He was in contact with some of the people who had the early unpublished protocols for how to clone DNA and RNA molecules. It was his idea that we would purify scrapie agents as well as we could and then make a DNA or cDNA library and try and analyze it through different approaches to see if we could figure out what the scrapie agent was.

So the early work was a collaboration primarily involving Ashley and Rick Race and me. We were able to make a cDNA library from scrapie-infected mouse brain, but we didn't know how to screen it at first. Then it turned out that Dr. Stanley Prusiner at UCSF in California had concentrated the infectivity in scrapie brain. He had gotten a partial 20 amino acid sequence of a major protein in these samples. We made oligonucleotide probes that matched those 20 amino acid polypeptides. We screened our library, made from the RNA of scrapie brain, and we got some hits, and it was the luckiest thing in the world. We got partial clones of prion protein. They weren't intact, but when we sequenced them, we could put

them together, and we had the coding sequence for the entire protein which became known as “prion protein” or “PrP”.

Next, we searched brains of scrapie-infected and uninfected mice and normal mice, and we were surprised to find that the amount of PrP RNA in the brains of scrapie mice and normal mice was exactly the same. So PrP was a normal host protein. That finding was sort of a bombshell because if prion protein (PrP) was mainly a component of the scrapie infectivity, it should have been increased in the scrapie-infected mice. Then Stan Prusiner noted that the PrP protein material in the scrapie brain was aggregated, and resistant to digestion by proteinase, and that's how he purified it. So, he suggested that the agent was a protein-resistant molecule that wasn't a virus. This idea had also been suggested in 1967 by J.S. Griffith [Dr. J. S. Griffith] in *Nature,* but it received little support at that time. However, with Prusiner’s support this interpretation became the predominant idea in the field.

However, we and a few other research groups continued to believe that the scrapie agent might be a virus which induced this proteinase resistant PrP form, and we all continued to work on this problem for quite a few years. About five or six years ago, we came up with an interesting experiment in my laboratory in which [Dr.] Mikael Klingeborn*,* a post-doctoral fellow from Sweden, showed that scrapie infectivity could be propagated in test tubes containing normal PrP but lacking any live cells. For this experiment, we used a technique known as protein misfolding cyclical amplification (PMCA). At that point I thought, wow, scrapie infectivity actually increases when you break apart the PrP aggregates and let them seed the misfolding of new aggregates from normal PrP added to the mixture. Although not all the details are clear, it is now fairly well accepted that the scrapie agent must propagate by protein seeded polymerization and not as a virus requiring live cells for replication.

In the ensuing years, our lab carried out numerous experiments studying the pathogenesis of prion infection in live animals and cell cultures. In early experiments, Richard Race was able isolate scrapie-infected clones of certain tissue culture lines which allowed in vitro study of the infection in live cells. Early work by [Dr.] Byron W. Caughey, when he was a fellow in my lab, showed using pulse-labelling with radioactive methionine of these infected cells that the disease -associated PrP isoform was labeled very slowly and had an extremely slow turnover in scrapie-infected cells. This differed dramatically from the normally occurring PrP isoform found in most cells, which was labeled rapidly in methionine pulsed cells and had a short half-life. These results suggested that the disease associated PrP might be generated by later slower events after standard protein synthesis, which turned out to be the seeded polymerization concept proposed by Byron Caughey and Peter Lansbury [Dr. Peter T. Lansbury*,* Jr.].

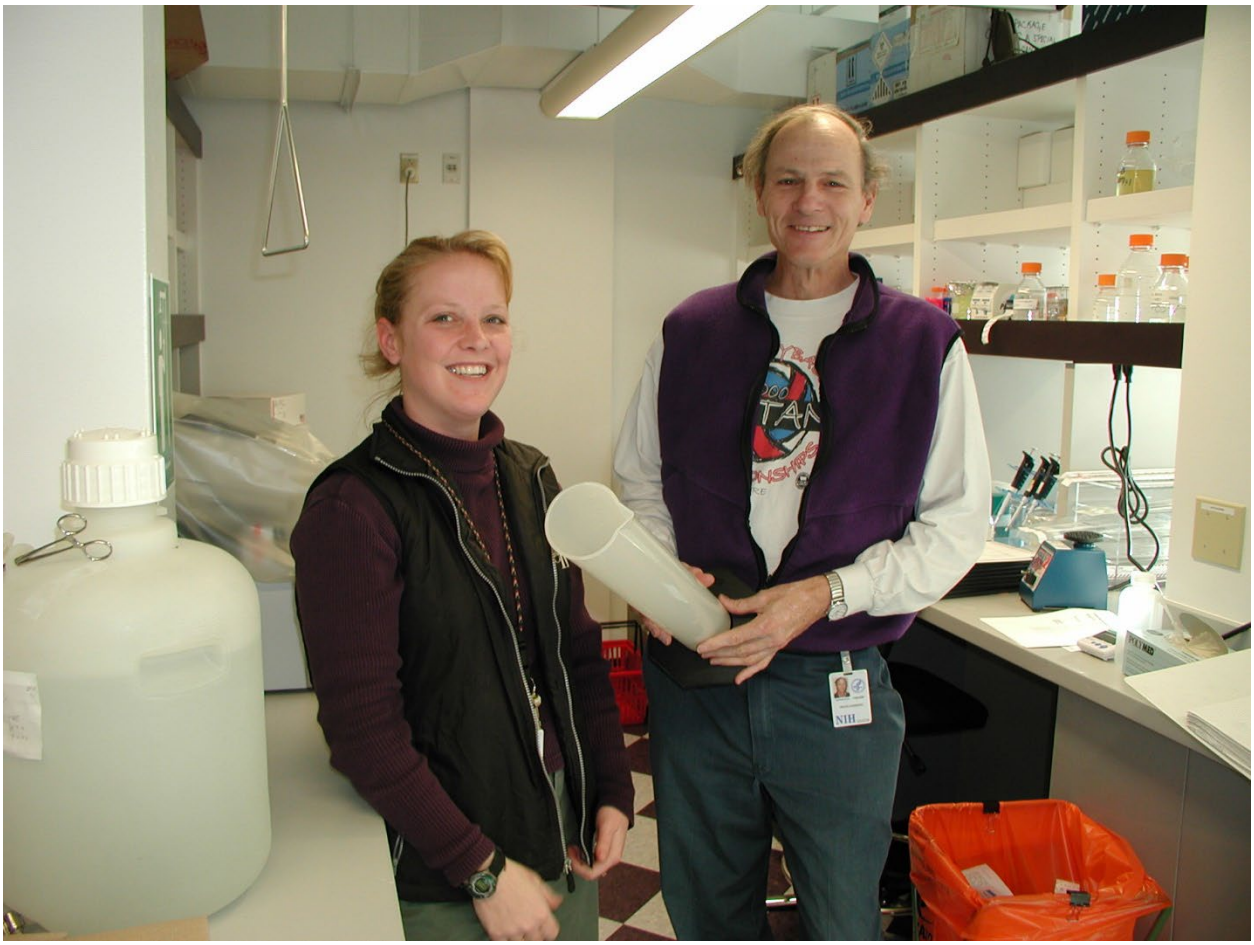
Prion agents have been well-known to show species-specific behavior. For example, sheep scrapie does not appear to transmit to human, but is capable of being transmitted to mice. The basis for this specificity turns out to be related in large part to the amino acid sequence similarities of the PrP molecule between species. Some decades ago, various sheep scrapie isolates were adapted to mice by forced in vivo passage, and after repeated passage in mice, these isolates adapted strongly to this new species host. However, the mechanism of this adaptation process was not clear. Other experiments in our group led by Richard Race showed that forced passage of hamster scrapie to normally resistant mice induced no clinical disease. However, a long-term carrier state was established, and when a very low-grade replication was demonstrated, this result opened the possibility of adaptation of hamster prions to mice. This experiment was noteworthy as it suggested that cross-species feeding of prion containing tissues in agricultural situations could give rise to new prion agents which might be capable to spread to new species including humans. This led to laws in the USA and elsewhere prohibiting feeding of brain, spinal cord, and other tissues which replicate prions to high levels.

In order to investigate the mechanisms involved in cross- species prion infection, [Dr.] Suzette A. Priola and others in our group used mouse cell cultures chronically infected with mouse scrapie. Gene transfer technology was used to transfer expression of PrP from hamster to these mouse cells, to show that presence of hamster PrP could dramatically reduce infection by the original mouse prions. These experiments were extended to show that only 1-2 particular amino acid residues from hamster PrP were required for this interference. We hypothesized that these differences decreased the structural interactions required for prion replication and cross-species passage in vivo. Recent structural experiments of PrPSc from various species by Dr. Caughey's group appears to provide structural evidence to support this prediction.

Expression of PrP was also shown to be able to induce organ or cell-type specific prion infection in vivo in transgenic mice. Using PrP null mice, hamster PrP was expressed in transgenic mice using constructs restricted to expression in certain cell types. These mice were generated in collaboration with Michael Oldstone [Dr. Michael B. A. Oldstone] at the Scripps Research Institute in La Jolla, California. Infection of mice with hamster PrP expressed in neurons only appeared to induce a similar disease pattern to infection of mice with hamster PrP driven by with the original prion promoter. PrP expression in astrocytes also led to susceptibility to hamster scrapie but the disease was much slower for unknown reasons. These data suggested that neurons were the main cell infected by prions which gave rise to typical prion disease.

PrP is known to be anchored to the cellular plasma membrane by a Glyco Phosphatidyl Inositol (GPI) linkage. This structure is utilized by a variety of proteins, but its biological role in the PrP system is not known. To test the role of this PrP linkage in prion disease we generated a transgene that produced only GPI-anchorless PrP, and mice expressing only this PrP isoform were generated in the Oldstone laboratory. Interestingly, after scrapie inoculation, these mice produced large amounts of disease associated PrPSc and high amounts of infectivity in a variety of tissues not known for high prion infection. However, the clinical disease seen was much less aggressive than normal, Initially, we thought there was no clinical disease at all, but creation of mice which were homozygous for the transgene led to consistent detection of prion-like neurological signs at late timepoints. Histopathology of these mice showed perivascular deposition of abnormal PrP usually with typical amyloid properties in numerous tissues including brain, retina, brown and white fat, heart, gastrointestinal track, lymphoid organs. These experiments were done in collaboration with [Dr.] Martin Jeffrey, [Dr.]Brent Race, [Dr.] Rachel A. Lacasse, Michael Oldstone, and others. This was new and interesting information, but most important for prion disease pathogenesis was the lack of typical spongiform pathology and prion disease signs, indicating that the GPI link of PrP was in fact required for the generation of prion disease pathogenesis. This raised the question of whether the agent produced in transgenic mice producing only anchorless PrP was nonpathogenic or, alternatively, were the brain cells in these mice resistant to the pathogenic process? Based on brain grafting experiments done in our laboratory by Rachel Lacasse the latter explanation appeared to be the correct answer.

Another interesting question was to determine the normal role of PrP in animals which were not infected with prions. This question was approached using several lines of mice depleted for expression of PrP. The most important line was the knockout made in the 129/Ola mouse strain by [Dr.] Jean Manson and coworkers in Edinburgh, Scotland. These mice did not have changes in the chromosome in the sequences flanking the PrP KO locus, so the changes observed could be attributed to the deleted PrP gene without complication known to occur in the other PrP knockout lines used by other groups.



Dr. Bruce Chesebro and postdoctoral fellow Dr. Rachel LaCasse, undated photo.

Except for their total resistance to prion infection, there were surprising few defects in PrP null mice. The main defect we uncovered in PrP null mice was a defect in spatial visual memory. This is similar to the ability to remember where the exit door is located after one has entered a large room or theater. In the case of mice, they are trained to locate a food delivery lever based on the visible landmarks in a room rather than a colored light over the lever in their cage. This work was done in collaboration with [Dr.] Jose R. Criado and Michael Oldstone at the Scripps Research Institute in LaJolla, CA.
Harden: In your bibliography there are several experiments about the possible ability of the chronic wasting disease (CWD) in the deer family known as cervid prion disease to infect humans. Please tell me about them.

Chesebro: Ever since the discovery of CWD in captive mule deer in Colorado by [Dr.] Beth S. Williams, people have been concerned that this disease might also spread to humans. Furthermore, there is a high level of exposure of humans via hunting or animal husbandry to deer and other CWD susceptible cervids. There also an issue of possible infection in North American native populations in the USA and Canada who depend on consumption of cervids as their main source of protein.

Laboratory testing of CWD infection in human tissue culture cells did not show positive susceptibility, but these tests were not completely reliable due to lack of sufficient controls. Therefore, about ten years ago, the RML prion group consisting of Richard Race, Brent Race and myself, plus numerous

technical assistants, began a series of live animal tests using CWD from cervid brain (deer and elk) to attempt to infect nonhuman primates, Cynomolgus macaques, by intracerebral or oral routes. These animals were followed for up to 13 years at RML, and no convincing evidence of CWD infection was detected. Data collected involved clinical observations, histopathological analysis and biochemical tests for the aggregated disease-associated form of prion protein.

In this work, age-matched primate uninoculated control animals were used, and occasional areas of abnormal PrP staining were seen in both the CWD-infected and non-infected animals, but these data did not constitute evidence for primate infection by CWD since these abnormalities appeared to be age-related artifacts as they were found in uninfected old animals. Other groups, notably in Canada, have claimed to obtain successful infection of similar primates, but these experiments lacked age-matched controls and the putative positive staining they reported as evidence for CWD infection was similar to what we saw in uninoculated control animals. Thus, because of the genetic similarity between humans and Cynomolgus macaques, these experiments suggested that humans are likely to be resistant to CWD infection. However, humans might differ in subtle ways from Cynomolgus macaque monkeys and long-term evaluation of humans known to consume cervid meat will be important to continue to rule out this possibility.

Harden: You have published about drugs that are partially effective in scrapie infected mice and that household bleach will decontaminate steel wires but not solid brain matter. That work struck me as being much more clinical than your laboratory work.

Chesebro: In truth, the main motivator for that research and the person who led these experiments was Brent Race, Richard Race's son. He's an excellent research scientist and veterinarian. He's been working with me for 10 or 15 years, and he's interested in the practical aspects of controlling the infection and decontamination. He works also on the cervid prion disease.

Harden: Have you done other experiments to study the pathogenesis of prion disease in animals?

Chesebro: Prion diseases have always been thought to be primarily a disease due to infection of neurons. However, the areas in brain with prion-induced neuronal damage also invariably show strong activation of astroglia and microglia, and roles of these cells in prion disease remains unclear. Therefore, in recent years, we have investigated the role of glial cells in prion disease pathogenesis. One of the most surprising results of these studies involved the depletion of microglia over the entire disease course by using a drug called PLX 5622. This study was initially conceived and led by [Dr.] James A. Carroll at RML. Mice fed this drug have very few microglia detectable in brain and other tissues as long as the drug treatment is continued. We expected that, if microglia contributed to prion induced brain damage by releasing reactive oxygen species and other toxic molecules, that drug treatment of mice to remove microglia might ameliorate the disease. In fact, we observed exactly the opposite results in our experiments. Drug-treated mice without microglia died 3-4 weeks earlier than untreated mice! This happened with all three scrapie strains we tested. Since then, we have been trying figure out how microglia contribute a beneficial effect for the animals infected with prions. So far, our experiments have not provided any strong leads to explain this interesting phenomenon.

Harden: What other prion pathogenesis experiments are you currently pursuing?

Chesebro: For the past several years we have been interested in studying prion infection of retinal tissue in vivo. We started this project nearly 20 years ago, when [Dr.] Lisa A. Kercher was a postdoctoral

fellow in my lab. Dr. Kercher's work showed that in normal mice photoreceptors were the main cells infected and killed by prions and that these cells underwent a cell death process known as apoptosis. In addition, her experiments using transgenic mice expressing hamster PrP only in neurons or only in astroglia showed that prion infection could be diverted to these cell types and still cause a fatal destructive retinal disease.

More recently James F. Striebel, a senior technician in the laboratory, has followed retinal prion infection using high resolution confocal microscopy with immunofluorescence staining to simultaneously mark both PrP aggregates and specific subcellular structures in photoreceptor cells. In these experiments, the first infected cells were cone cells followed shortly thereafter by the rod cells. Using high power magnification, in both these cell types, PrP was found in the region of the cilia which connect the inner and outer segments of the cells. The results suggested that cell damage might be due to obstruction of these cilia by abnormal disease associated PrP aggregates. Another site of accumulation of abnormal PrP was in the ribbon synapses where the rods and cones are connected to the bipolar neurons. These connections are quite unusual, and they might be damaged by local accumulation of PrP aggregates. Current experiments are attempting to work out these details.

Harden: You have never left RML since you arrived nearly 50 years ago, and I can't believe that you haven't been offered many enticing offers to go to academia. What has held you in Hamilton and at NIAID? What has NIAID done for you despite all the paperwork and the ethics rules and everything else that a federal employee has to put up with?

Chesebro: There are lots of things. It's a great environment. The facilities were always really good. They have upgraded them even more now. But over the years, the funding situation was such that we didn't have to apply for grants. We had a steady stream of money coming in to do experiments. We didn't have to do all that hard work that people in academia had to do to raise money to run their labs. That was the single most important thing.

Another important thing for me was the high-quality animal facilities, because we did all of our work in animals. NIAID had really good animal facilities here in Montana, much better than in Bethesda.

From purely scientific point of view, the great advantage is not having to apply for grants.

Harden: That's the single response I get to this question from every Intramural scientist I have ever interviewed.

Chesebro: Also, we had more space per researcher in Montana, but if I'd been in Bethesda, I would've been hurting for space.

Harden: NIAID was a poor institute until AIDS appeared. Once it started getting AIDS funding, it was easier for NIAID to support all kinds of research, I believe.

Chesebro: That's right. I remember that distinctly. But even before AIDS appeared, I was able to recruit people here who worked in academia and were getting their grants from NIAID. They were more than happy to come here and not have to apply for their NIAID grants anymore.

Harden: When did you actually step down as chief of the Laboratory Persistent Viral Diseases—I have seen two different dates.

Chesebro: I stepped down October 1st of 2021.

Harden: We've come to the end of my list of questions. Is there anything else you want to talk about before we stop?

Chesebro: I don't think so.

Harden: Thank you very much for a wonderful oral history.