

## **Dr. Bruce Chesebro Interview**

### **Office of NIH History** ***Oral History Program***

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MP: So I wonder if you can start off just by telling me a little bit about how you got interested in science and how you came to the Rocky Mountain Laboratories.

BC: Well I got interested in science as an undergrad working in a virology lab at Caltech.

MP: Oh okay.

BC: And I worked on SV40 virus and polyoma virus in DeIVeccio's lab and it was – I worked with some post-docs. It was a really good experience. It made me want to go to med school and when I went to medical school I just slowly evolved – it wasn't a straight line but I slowly evolved an interest in viruses and infectious diseases. I wouldn't say it was really strong in the beginning. I had already done that and I thought I wanted to do something different but in the end I came back to thinking that was really neat and I really like virology especially and immunology and I spent two summers in Sweden. Well I spent one summer in Sweden and then I spent one year and a half in Sweden in a virology immunology lab; the first summer studying polio virus and antibodies and the second summer – the second year and a half studying antibodies attached to viruses and as a way to get the structure of antibodies. So we actually did electron microscopy of virus antibody complexes and we could see big, large molecular weight antibodies attached to polioviruses, and then we were – the timing was really kind of amazing – we were in Sweden in Stockholm and Pharmacia, the company, was in Uppsala developing Sephadex sepharose. And so we had some of the first sepharose that came out.

MP: Can you explain what sepharose is?

BC: Well those are gels, agarose gels, that can separate proteins based on size, but previously Sephadex could only separate up to 200,000 molecular weight and sepharose could separate up to 1 million molecular weight, and we were interested in IgM immunoglobulin and it was about 1 million molecular weight. From serum we were able to get the IgM pure on sepharose columns that nobody had ever had pure. Of course, we had sort of purified it by binding specific antibodies to polio and looking at them in the electron microscope. Now we had test tubes full of pure antibodies without any viruses and we looked at them in the electron microscope and we were the first guys to see these starfish like molecules that were circular pentamers of IgM, and that was a really exciting time because immunology in that time was all about the structure of immunoglobulin. Nobody knew anything about cells and it was just, just at that time that cellular immunity started to become the next field of immunology but before that it was all the structure of immunoglobulins. And so we had the structure at a different level than the biochemist. We had it sort of at this visual level where you could really see it and it was really exciting to put that together. So it was a pretty exciting time. We had some very noticed papers in *Science* and the *Journal of Experimental Medicine* from this kind of obscure lab at the Karolinska Institute in Sweden, my boss and another Swedish med student and me as a Harvard med student got a lot of notice.

MP: And who was your boss there?

BC: Svehag was his last name, S.E. Svehag. He just retired last year from his being professor.

MP: Have you kept in touch with him?

BC: Yeah I went over and gave a talk at his retirement symposium so I have kept in touch with him.

MP: That's great.

BC: Then I came back I was an intern at Stanford for one year and then I worked in an immunology lab for on year at Stanford with Hugh McDevitt. But all along I'd been applying to go to NIH because the alternative was to go into the army. The Vietnam War was a huge issue at that time and I knew I wanted to do research so I had a good chance of getting into NIH and I had this contact from my time in Sweden with Henry Metzger at NIH who had been the guy who did all the biochemistry on IgM. And so he selected me for his lab.

MP: And what year was that?

BC: It was around 1970.

MP: '70, okay.

BC: So I was in his lab from '70 to '72 and did a lot of biochemistry on immunoglobulin molecules and their combining sites.

MP: And where was his lab at?

BC: It was in Building 10.

MP: Building 10, okay.

BC: He was in NIAMD, the arthritis institute. Henry also just retired last year.

MP: So that's how you came into NIH and you came in as you as public health service at that time.

BC: Yeah, I was a commissioned officer at that time and I stayed in it since until very recently when I switched over as I was telling you. So then when I was looking for jobs at the end of this two years of military service obligation. I did look at some jobs at the NIH and elsewhere, but I also looked out here at the Rocky Mountain lab because I wanted to go back to infectious diseases and immunology. I thought I could work on that here pretty well because they had a lot of infectious diseases, but I did get the job finally, and I started working on a mouse retrovirus.

MP: And who was here? Who was here that you were interested in working with or did you...?

BC: I really got the opportunity to start my own lab and that's why I took it. I was at that point starting my own lab. So I didn't come here –

MP: But what about – why Rocky Mountain? Like why – I mean did you like the scenery or did – I mean were there other things that drew you to this area?

BC: I liked the idea of living in the Rocky Mountain West, and I'd never lived in a rural environment so that was a complete experiment in living. I thought I would try it out and they have really good animal facilities here – at the time we had really good animal facilities, better than NIH because the NIH animal facilities were all – a lot of them often off campus and very small and we had big animal facilities and they were all right next door to us. So if you wanted to do animal work this was a much better place than NIH.

MP: And what kind of animals did they have here at the time?

BC: Well they had a lot of different kinds but I was mostly interested in mice and inbred mouse lines.

MP: And there was a good mouse colony here?

BC: No, we just had good facilities and we bought the mice from Jackson lab. In fact RML – this is a bit of a diversion – but RML had no inbred mouse lines here. They only had out bred mice and everything I wanted to do had to do with inbred mice. That was the whole thing of genetic control of the immune response and I wanted to study genetic control of the immune response to a virus, a retrovirus. I knew that Frank Lilly had shown a Friend virus the H2 or MHC gene of mice influenced ability to recover from Friend virus. I thought it might be just like I had studied at Stanford where MHC or H2 genes controlled immune responses to synthetic peptides. It turns out that is the central key in immunology. Several people got the Nobel Prize, some years ago, showing that the MHC is so important in regulating the recognition of antigens. In addition, that whole story was started by McDevitt and Benacerraf at the level of immune responses and then by the other people who studied VHLA and H2 genes in mice – in humans and mice. So I sort of focused on that and started working on Friend virus. I was trying to figure out why mice recovered and didn't recover. I did everything with inbred mice and I sort of directly followed the work of Frank Lilly who was at Einstein, Albert Einstein Medical Center in New York, and Frank helped me immensely by conversations on the phone and sending me virus – he wondered what the hell I was up to, but he was so nice. I mean I was a complete unknown and he was helping me indirectly. I mean he was a very, very generous guy.

MP: That's great.

BC: Yeah, he was a wonderful guy.

MP: So Friend virus was how you got into retroviruses?

BC: Yeah, and I worked on Friend virus for a lot of years. We still work on an aspect of it, but the main – it is probably not worth going into it now, it was unique because it caused a disease in adult immunocompetent mice and it was very fast, this disease. And so if the mice had a good immune response to it they could turn it around and make it go away and that was pretty dramatic to see that and that was what we studied.

MP: Oh that is neat.

BC: It's been a very good model. A number of people have kept on it and I sort of turned over that part of the project to Ken Hansen's group, who is a guy who was working with me for a number of years, and he's extended it very nicely in the last seven or eight years. But I switched over to looking at the brain

disease aspects of those retroviruses and whether they could cause any brain diseases. Other people had already shown that they could and I just stumbled across my own virus strains derived from the Friend virus system that caused some unique brain diseases and started working on those and they're more models for HIV brain disease. They have minimum pathology with severe clinical deficit and they infect microglia, and all of that is very similar to HIV dementia.

MP: How did the advent of HIV affect your work?

BC: Well, not as much as you'd think.

MP: So not as much of an effect as you would have thought?

BC: Well it was a huge political deal and it was so much involved in the political –

MP: I would imagine it would change the resources?

BC: Not as – regarding the resources, NIH had a very progressive and far thinking attitude about it. They didn't say – they wanted to put money into AIDS related research and they considered all retrovirus research AIDS related. This was an extremely smart thing for the leaders of NIH to do, because there was a tremendous amount of spin off into many, many fields: cancer, immunology, health of babies and pediatrics. You know what I mean, just everything that relates to AIDS. AIDS can touch just so many different kinds of systems. It is not just an immune system disease. It causes a brain disease. It affects the GI tract. You know it causes cancer. I mean – so AIDS – in order to really corral all of those aspects of AIDS and be able to treat them and manage them you really have to think of this very multi-system disease and attack it on all fronts, and NIH's idea was, "Okay, attack on all fronts. We've got people working on many of these fronts. Let's have them all go to work," and this has really been an important thing to do. I think in contrast to something that's gone on in the last few years where the idea was to work on biodefense; the NIH has not been allowed to have as broad a view of biodefense although they have been able to say, "Okay we'll work on biodefense and emerging diseases,". Which is good, but they haven't had as open a view as to what might be relevant to biodefense and it seems to me that that hasn't been as good of a way to spend the public's money because you can't predict what is going to be necessary for biodefense. You have to just guess.

MP: So is it more narrowly defined?

BC: It is much more narrow.

MP: What you can work on that qualifies?

BC: That qualifies for biodefense and since certain moneys are restricted to being only biodefense monies it is restricted how you can do it.

MP: I see, so it creates more bureaucracy?

BC: It creates restrictions and it channels the money more in certain ways that those aren't necessarily going to be the most productive ways. It is a different philosophy. If you think that you know enough you really should channel the money in a special way then that is a good idea, but if you don't think you know enough, and I think that is the answer, we don't know enough as to how to channel the money,

you had better use a broad channeling mechanism. And I think that has been a bit of a difficulty because the higher up in the bureaucracy you go the more tricky it has been to get people to accept a broad channeling like this. And nobody knows who is right about this, but it is just a general philosophy. Since I've seen both ways working – and by the way NIH had a history of doing this through AIDS – the way they did it for AIDS decades ago, four decades ago, during the war on cancer and the preceding explosion of cancer research that occurred in the '60s and '70s. That was handled with a very, very broad approach as to what is important for cancer and a lot of immunology was funded by cancer research. Well, cancer if you could treat it with immunology, and you can in some cases, it is really important to have done that and not only that but the immunology research that came out of that cancer funding then has been applied to all kinds of other kinds of research, non-cancer. So it has had enormous benefit. As long as the money is being spent on something productive and interesting to the general picture of biomedical research it is probably good. It was sort of hard for the political system to understand that targeted research isn't always as profitable as just stimulating research in general. The political system needs a target and this is the way politics work. That's not bad. That's just life. The public needs to know that we're doing something about something they're worried about. So that's the way we work and so it's not particular to any particular administration, or any particular politicians or any particular government employees it is just a continual adjusting process. I think overall NIH has done a really good job of it.

MP: Of promoting?

BC: Of promoting a broad prospective and having a lot of different things happen. For instance the progress in HIV virology could never have occurred – in the short few years it took to clone the virus and understand, get all its proteins and understand how it replicated, if we hadn't had the preceding 30 to 40 years of retroviral research. And so the HIV wasn't the first virus cloned, retrovirus cloned, so it was easy for people to switch into HIV and to work on it. But I didn't switch right away, but I did eventually. I did switch and I sort of kept HIV going because I was interested in viral assays. Then also these murine retroviral brain diseases and then I sort of tried to look at HIV brain disease because that seemed to be a niche that fit with the murine model and then you could compare the mice to the humans and you could only do certain experiments retrospectively in humans. In mice you could do it prospectively and so we've been trying to do that pretty much since then and I found it to be a really easy thing to integrate with scrapie research, because that is another infectious disease of the brain.

MP: So tell me how you first got involved with scrapie research.

BC: Scrapie was worked on here as you know by Hadlow and his assistant Rick Race and other people who were here at the time. So there was for many years, since the '50s, and so there was an experience here working with live animals with scrapie. The animal handlers knew how to handle the mice. We had the mouse house that was specifically designed to permit scrapie in it, and people had experience doing pathology on it and handling biochemistry in the labs. And so what I got into was in collaboration with Ashley Haase who is at Minnesota. The idea of using the newly developing technology of cloning to try and find out what sequences were over expressed in mice that had scrapie. And so we made messenger RNA from scrapie mouse brain and normal mouse brain and we were going to try and say, "Well which genes are overexpressed?" A little bit like people are doing with microarrays today. We were doing this in the '80s and trying to do it by differential hybridization and differential cloning. At and right at the time we had these libraries just about made, or at least these nucleic acid preps ready, the partial amino acid sequence of prion protein was published by Lee Hood and Stan Prusiner, and so we made an

oligonucleotide that matched those amino acids that were published. It was only about twenty and we screened our library for that. We got a full-length clone of PrP.

MP: In both – you got a full-length clone?

BC: We got a cDNA.

MP: The cDNA.

BC: Yeah.

MP: Before they did?

BC: Yeah pretty much. I think that Weisman had cloned a similar clone from hamster brain and, but we published pretty simultaneously and in the end we both had – we had the mouse clone. Theirs wasn't full. They had the hamster clone. Theirs wasn't full length, which is not a big deal, because they were easily able to extend it to full length. Once you have one you can get DNA clone; it's very stable, you can see the whole thing. But in fact we gave them our clone, our mouse clones, so they could do it easily in mice. But we had this clone and it put us right up with them instantly as an example of how the technology can work. They were way ahead and we were down here and all of a sudden we were within a month or so we could do everything they could.

MP: And why was that?

BC: Well once you have the clone, you can get the sequence. You can make any mutants you want. You can do what – you can express it in vitro. We had to learn to do all of that but in theory it catches you up. So everything you do with this clone is new and everybody is trying to do the same thing so you're actually working in parallel like this instead of they're always three years ahead of you.

MP: Right.

BC: Yeah it's amazing.

MP: I mean to sort of start out at that point, basically.

BC: Yeah it was pretty amazing; it was really good for us.

MP: And tell me about those early studies. I mean what do you think – I mean when you were able to pull – so the clone was something that was being produced in mice whether they were infected with scrapie or not.

MP: The RNA.

BC: The RNA was expressed in normal mice at the same level as in scrapie mice brain. And so we knew it wasn't the infectious agent. The RNA, that wasn't it. So something else had to be going on and then we found it in all of these tissue culture cell lines. In fact we finally only in screening about 20 of them, this was one of Byron Caughey's first projects because he was a post doc with me, we only found a couple

of the cell lines in our incubator that were negative for PrP expression. So all of the common cell lines that people were working with were all PrP positive and if you look at the cell surface most of them express it on the cell surface. That's kind of interesting when you start worrying about issues relating to biohazards and whether PrP expression is bad everybody has been working with it ever since they've been working with cells because it is on so many cells. It is not obvious.

MP: It's on cells whether or not you think you're working with scrapie.

BC: Almost every cell culture that we looked at was positive. Not every one. It is not a required gene, but it almost has every cell culture you looked at was PrP positive.

MP: Right and what were negative?

BC: Well I don't think one can generalize about what were negative, but we found some myeloid cells that were negative. We found some that were positive. We found some lymphoid cells that were negative and we found some that were positive.

MP: I see so it was more complicated.

BC: It was just sort of random who wasn't expressing it and who was and I don't really know why. You can find tissues that are higher and tissues that are lower and I don't think in a sense we don't know anything – we don't know very much about the normal function of PrP in the brain or in non-brain non-neural tissues it is really hard to say why it would be on one cell and not on another. There're a lot yet to be discovered even though we've had the gene cloned for almost twenty years. It is kind of amazing. We don't really understand it's normal function.

MP: Why do you think that is?

BC: It is hard to figure out normal functions. There's a lot of genes like this. It is easy to clone genes. It is hard to figure out their functions. If they don't have an obvious problem when you delete them you make knock out mice, then how do you exactly figure out what is going on? Maybe they do something but when they are gone something else replaces them so it is a redundant function. That's what everybody says because that has been shown for certain genes, but for PrP we don't know the answer, so it is not at all clear.

MP: And what kind of technologies were you using in these early days? I mean you mentioned cloning was something that you were doing and then when you went to check the cell lines was that more cloning and pulling out – or you were looking at mRNA expression in these?

BC: We did northern blots.

MP: Northern blots.

BC: So it was cloning. It was like – it was molecular biology and...yeah I would say that and then we – then we spent a lot of effort in those early years working with Rick Race to try to infect cell lines.

MP: Oh, tell me about that.

- BC: That was a big deal because everybody wanted to get – it is always a good idea with infectious diseases to have them growing in cell cultures not just in live animals, because you can manipulate cell cultures in much more detail. So Rick Race actually started that experiment and did serial passages of cells that he attempted to infect by putting brain homogenates on them, and he got some to keep the infection long term. They all kept the infection for three or four passages, but we thought it was just carry over, not necessarily replicating infection. We thought it was just material that stuck to the cells and didn't go away, but finally you kind of dilute that out and then he had some that went for 20 or 30 or 40 passages and we knew that we were way past the dilution endpoint of the stuff that we'd put in, if they were still positive. Then he actually made clones of those cells and found in screening them all in mice that two of them were carrying an agent and so we have used those for most of the studies since then.
- MP: So two of the clones from the original studies. Why was it so difficult though to get the agent, because I know that other people have been working on this for some time. They have been trying to grow – trying to keep the infection going in tissue culture and unable to do it.
- BC: It doesn't spread very well from cell to cell in the cell lines that we were using. And so when you put material in there it isn't – it didn't work the way some infectious agents work, viruses that you infect one cell and then it makes so much virus that it starts spreading through the culture. This was making too small an amount of infectivity to spread through the culture, or it was in a form that it didn't spread through the culture. It was too cell associated. We actually don't know the answer.
- MP: I don't understand how do you then overcome that difficulty.
- BC: Well, we just lucked out. We tried to put a lot in so that it didn't have to spread and then tried to get cells that were positive to grow in separate cultures where they wouldn't be taken over by cells that were negative. That's always been the problem, to keep the cells that are positive from dying off and having cells that mutate to some negative state, through some way we don't have them take over and it is a huge problem even today. If you change the fetal calf serum source then sometimes the scrapie cells don't grow as well as the uninfected cells that inevitably seem to appear.
- MP: And do you know why that is? How there are these little things that make a big difference in terms of the way that you culture the cells.
- BC: Now other labs have figured out that you can find more susceptible target cells in the N2A cultures and they've approached it a different way both Prusiner's lab and Weisman's lab and also a laboratory in France did this, and they approached it trying to find cells that would be more susceptible. So they just made a lot of clones, I don't know exactly how they did this in each of these labs, but some of them have just made a lot of clones and then tried to infect each of the clones. This is the way Weissmann did it and he found some clones that couldn't be infected at all and some that could be infected very well and it spread through really fast. So it must be with some cells – these N2A cells are quite heterogeneous to begin with, they are polyploid, so they aren't easy – they're definitely not a single entity.
- MP: So it is always a matter you don't really know what the conditions are that allow certain cells that create greater susceptibility within certain cells, but it is a matter really of carefully selecting and maintaining those cells.

- BC: But it is a matter of randomly selecting because we don't know what we're selecting for and then just have a collection of selected cells and see if you can find ones that are better, and that's what seems to be the easiest way to go.
- MP: And the other problem, I think I remember with Weissmann, is that the cells that he has selected for their susceptibility to a certain agent – I don't remember if it is 263K.
- BC: It was Chandler.
- MP: Chandler, but then the agent is not as susceptible to some of the other agents, right? I mean you couldn't get any other agents to work and what about with the SN2A cells?
- BC: Well we put Chandler on them, but we do know if you start over again you can put 22L – the 22L worked better than Chandler and then Sue Priola found that 22L went in those cells and also in some fibroblast that express PrP. That was extending it even further saying, "Look it doesn't even need to be in normal derived cells, these fibroblasts work here." And so it starts to get more and more general, which was surprising; because in the beginning nothing worked so everybody thought it is very specific, but then as we know more what to do then it has gotten more and more broad. You can infect more and more kinds of cells and I don't know if we understand why even now.
- MP: And then what are these cells useful for? Can you give an idea of some of the uses to which your lab has used this?
- BC: The cells.
- MP: The cells.
- BC: Well they allow you to study – if you can put the agent into cells, and different kinds of cells, or you can put genes in those cells, you can study with genes like foreign PrP genes can block the infection. So Sue Priola and I did that; we put hamster PrP into the cells expressing mouse PrP and found that it actually shut down the ability to generate the PrP res from the mouse. In some cases the mutations competed and in some they didn't really compete they just kind of shut it off, and so there seem to be two different things going on depending on how many mutations there were between the mouse PrP and the gene we were putting in from the hamster. So, we could map that to certain amino acids even. Say, okay, you need – if there is a difference of amino acids 138 that's really important and that's the main difference that causes this to shut down. So you could do experiments like that that you couldn't do otherwise and the other thing you can do in these kinds of cells is that you can do drug treatment experiments to see what makes this agent go away. What kind of drugs can you use. Byron Caughey and people working with him such as Yong-Sun Kim and also Remi Demaimay who came from Dormor's [spelled phonetically] lab and worked with me, did drug studies and treatment experiments in these cells and that you can do them faster than in mice. Now they've got the drug screening assay that is being used by J. Francisco in these same cells.
- MP: So you have a tissue culture – I'm probably jumping ahead a little here, but you mentioned the cell free system. So the cell free system, the tissue culture and then a transgenic animal, you know, making these kind of manipulations in a mouse?

BC: Yes.

MP: How do those different levels of modeling interact with each other and how do you decide which level to do a certain experiment?

BC: Well, it depends what the question is. If people want to just simplify it as much as possible, they've tried to go to the cell free in vitro conversion and hope they're only considering two proteins interacting, of course they really have many proteins in the PrP res preps, but not as many as in the whole cell. So they're partly simplifying and they can manipulate the source of the PrP sens. You can put molecules in there. So there's a lot of molecules from different species. You can study species specificity and species restriction, which is a huge general issue in the PSE field. You can study it in self-reconversion. You can study it in a tissue culture cells, which is what I was just talking about, or you can study it in hamsters versus mice or you can study it in transgenic mice, expressing hamster PrP. So you can manipulate the system a lot of different ways and ask more bigger or smaller questions that relate to molecules or to tissues or to cultures. It just allows you to the bigger the question the broader the question, i.e. what's going on in the brain the more complex it is because the brain is a complex place of which we understand relatively little so we don't really know what all the possible molecules are that could be interacting there. Whereas in tissue culture we're only working with a small number of cell types in this kind of tissue culture so things are simpler.

MP: So you can have from the very simple, like the cell free system where you're saying it really is more complicated than it looks because you do have more proteins in the solution.

BC: You have other molecules besides proteins, yeah. But yeah, so the systems increase in complexity but they also increase in relevance. So you're getting a tradeoff between how relevant you want to be to the final thing; which is disease in humans or primates or animals. So you're back down to humans, primates, mice, for example, and maybe what we do in mice isn't relevant to primates or humans or deer but you try to figure out how to make those jumps and make sure that what you're studying in transgenic mice is relevant.

MP: In terms of how do you make the decisions – let's say like a transgenic mouse system with humanized – human prion protein, that expresses human prion protein, versus like a primate; if you're trying to model as closely as possible the human prion diseases which system would you choose to do your experiment in and why?

BC: Well I think you'd better ask John Collinge that question not me because we don't do that. We just haven't considered that that was our role to do that. We don't have access to human CJD or BSC samples, and so we have stayed away from directly modeling the human system. The closest thing we've come to doing that is to see whether it infects primates or monkeys, but aside from that we haven't worked with any human transgenic mice, human PrP transgenic mice. So the PrP transgenic mice that we've made have been to ask much more fundamental questions like does the PrP need to be on astrocytes or neurons in order to cause the disease, or both, and we're finding that in some cases either is okay and in some cases you need both. So that's really an interesting development and it doesn't really have anything to do with species specificity.

MP: So when you say in some cases you mean depending on strain or the prion agent?

BC: No our newest data says that, and in fact you heard [unintelligible] talk about it, although it wasn't this morning, by Lisa, in the three transgenic mice that express on neurons only, astrocytes only or on multiple cell types when, you inoculate them in the eye only the one that expresses on multiple cell types gets retinal degeneration.

MP: Okay.

BC: But they all get brain degeneration. So, in fact, they all die of scrapie, but in only one case is the retina damaged really severely so that it is actually totally wiped out, there is no retina left. You could see it on the slide this morning, and so that doesn't happen in the other two mice and so we don't really understand why the cell killing effects of the scrapie infection don't work in the retinas of the other mice when the brain cells of those very same mice are killed. We don't know why the nerve cells in their retina aren't killed the same way the nerve cells in their brains are killed. So this opens up a whole new question of how do cells interact to cause damage? How does scrapie interact with cells to cause damage and are there different kinds of drugs you need to treat? Are there different kinds of mechanisms going on that make the damage happen. And so I'm not sure where we'll go with this, but there are a lot of different questions you can ask from this, but none of it has really to do with the direct question of species barriers.

MP: It's more about modeling pathogenesis right?

BC: Yeah.

MP: Because – what you can do with these models is you can manipulate where the protein is expressed and so you can figure out what sites are necessary or how deleting it from certain sites affects the course of disease.

BC: Yeah.

MP: Right. And I thought it was interesting there was one study that you published fairly recently that the PrP was only expressed on the astrocytes.

BC: Right.

MP: And not on the neurons and the neurons were still succumbing to death. I mean they were still degenerating in the way that they would as if they had been producing PrP right?

BC: Right.

MP: Can you talk a little bit about that and the significance of that?

BC: Yeah I think it means that there is an indirect way that neurons can be damaged by scrapie infection where the PrP – the normal PrP and the abnormal PrP are made on astrocytes and somehow that triggers a response by the astrocytes to damage the neurons. That response could be the PrP peptides or the PrP aggregates that are abnormal actually swim over and damage the neurons, or it could be independent of PrP peptides or aggregates. It could be that the astrocytes don't do something that they need to do to keep the neurons happy, like scavenge glutamate for example, or they actually – it could be more positive. The astrocytes might release a toxin that is not PrP, like a chemokine or a cytokine, that

damages the nerve cells and says to them that they can't function properly anymore, tells them to die or something like that. There is probably also a direct mechanisms of damage of neurons when the PrP is expressed on neurons and the PrP res is formed on neurons. It shows, and I would say that our studies together with those of Collinge and Mallucci, which were published in *Science* last year.

MP: Yes.

BC: Demonstrating two different mechanisms, and perhaps the neuronal mechanism, the direct mechanism, is more damaging than the indirect mechanism and that's why it seemed to take a long time to damage by the indirect mechanism whereas the neuronal mechanism might be faster. In our NSE transgenic mice the mice that expressed PrP on neurons died pretty fast, whereas our GFAP transgenic mice, that express only on astrocytes, died pretty slowly.

MP: The difference in the results?

BC: It could be levels of PrP, but it could also be cell type, and it could be functionally very different on the different cell types.

MP: But it could be the fact that you're looking at different time courses. So for instance with Mallucci, the Collinge results where they saw the protection when the neuron stopped producing PrP-C and then they saw sort of a reversal of the pathology. You think that the reason maybe they saw that was they didn't see damage that you would project because it was a short time course?

BC: I'm not that comfortable commenting on their paper on tape.

MP: Oh, I'm sorry.

BC: I don't really think that's the subject.

MP: Okay.

BC: It's such a technically complicated thing and I'm not sure that their interpretation of their results is the only interpretation. And so it's beyond the scope of thinking about it, but I think that their results agree with ours in the sense that if the PrP is on neurons you're probably going to have a different mechanism of pathogenesis and I guess what is surprising, I will say this about their results, is that they didn't see the pathogenesis that we detected when it is on the astrocytes because they have some data in their paper saying that PrP is in on astrocytes and that it isn't damaging, and it's perhaps they didn't wait long enough or perhaps their system is different in some other ways – they used different agents than we did, different strains, maybe their strain doesn't work when it is only on the astrocytes, I don't know. The pathology that they saw was minimal and very transient and it's hard to interpret whether it was really a significant amount of pathology because basically the mice didn't die. And so what then do you think – how do you interpret that? How do you distinguish that from just a low expresser? We have plenty of transgenic mice that are lower expressers that don't die. We don't know how to interpret that and they made a pretty elegant interpretation because it was a pretty elegant construct but it's hard to be sure that they had really full-fledged pathology going on for this transient time. It is a weakness of the system, but it is still a very interesting paper.

MP: I'm sorry I jumped ahead a little bit and if you need to go just let me know. I can always stop by again maybe tomorrow or something if you have time, but can you talk a little bit about the development of the cell free system? Like where the idea for that came from.

BC: You know I think that you should talk to Byron.

MP: Byron.

BC: I can tell you about it. I helped him.

MP: That's fine, but he was the one who worked on this?

BC: He did and Dave Kocisko.

MP: And he was a post-doc at the time?

BC: No, no.

MP: He was already running his own lab?

BC: The guy who really worked on this was Dave Kocisko.

MP: Okay.

BC: Who is downstairs working on drugs now and that was his Ph.D. project and he was a student of Peter Lansbury. I'm going to give you the historical part. The science you get from Byron. So, Dave Kocisko was Peter Lansbury's grad-student from MIT and Lansbury came up with this idea of interacting with Byron, and who came up with the idea to do this cell free conversion. You would ask Byron that. I don't know if that was Lansbury's idea or Byron's idea or Dave's idea, but the three of them collaborated to do this and my contribution was to give them our clones that expressed PrP so they could get a lot of GPI negative PrP which worked really well in this assay. And so – and they could radiolabel it off these clones, but then they ended up –

MP: So that was the trick, using GPI negative material?

BC: No, that wasn't the only trick that's just what they happened to use. They could get a lot of it. We thought it was going to be a really important trick, but in fact it isn't a really important trick. You could use GPI positive and you get it right out of – and we thought it was going to work really great because you could S3-35 label the cells and get secreted PrP, but it didn't label and secrete very well. It secreted but it wasn't labeled so well and they found the better stuff was inside the cells and then it didn't matter if it was secretable. And so, in the end, a lot of different combinations and permutations worked fine and they were able to do this with PrP immunoprecipitation from many different tissue culture lines that they labeled. And so it wasn't restricted to anything in particular that we came up with.

MP: So the main variation?

BC: Yeah, the main variation is having a really good PrP res. The main variable to make this thing work is to have good PrP res preps and the PrP res preps have continued to be – if you don't have a good prep it won't work.

MP: Okay.

BC: And if you do have a good prep it will and we sort of defined preps on how good they are based on how they work. So...

BC: I see.

MP: So before you had to optimize the prep to get it to work and now you can tell if a prep has been optimized by whether or not it works in the system. It has become a criterion for evaluating the prep?

BC: Yeah. I would say that that's true. And so Byron's technician Greg is an expert at making preps that work, but when we switched to a new system like Deer that's been hard.

MP: Really?

BC: So it was hard to get PrP from Deer that worked in the assay, but Greg Raymond did get it, and then when we tried to use it on other assays with cells like Sue's assay it doesn't work very well in Sue's assay on cells. And we don't know if it is because the prep isn't good or if the cells aren't good.

MP: But, so, depending on the species and depending on where the PrP is coming from it might require slight in the prep, like the actual prep of the material?

BC: I don't think we know why it doesn't work.

MP: Okay.

BC: I mean your guess is as good as mine on that. We really don't know.

MP: Okay, I'm really interested.

BC: We thought it would work easier in the cells than in the cell free because it requires less purity, but maybe it requires higher concentrations. You're going have to ask Sue this question because Sue knows much more about cell overlay assay than I do and she and her post-doc developed that. So it's a question – I do know that you don't need it as pure to make that work as you do for the cell free conversion, but I don't know why she thinks it fails when it doesn't work.

MP: Right I see, what she thinks those variables are. So I'm interested because one of the things I'm interested in with my dissertation is the relationship... When it was published, that was from 1998, when that was the original you showed that there were some mice that were propagating this where previously it had been thought, based on Kimberlin's work, that this was not – 263K was not propagating through this. So, I wonder if you can talk about – go back to the beginning of that and how you figure out – like, were you surprised by those results, what were you expecting, how would you design the experiment? And, then, where that carried you after that because it was such an interesting result.

BC: Well, the experiment was designed by Rick Race.

MP: Oh, okay.

BC: And he was just seeing what happened. I don't think he really thought that this would work, and you'll have to ask him what he was thinking –

MP: Okay, I will, definitely.

BC: Because I'd like to hear his answer, too, because I got involved in it pretty late and I thought it was really exciting.

MP: So what did you think – okay, let me rephrase that...

BC: That's why I got involved.

MP: Let me ask you a question – what did you think when Rick Race presented you with these results?

BC: I can tell you that because I can remember when he presented. He presented it at a small scrapie meeting and nobody asked any questions. Nobody thought it was interesting. I went up to him afterwards and I said, "You know, this is fantastically interesting, Rick, and if you write this up correctly you can get a *Nature* paper out of it." So, I think that I recognized, even more than anybody else, that this was going to be really interesting to a lot of people.

MP: So no one there saw it?

BC: No one saw it.

MP: Did he see it – did Rick see it?

BC: I don't think he did.

MP: Or did you have to convince him?

BC: I had to convince him a little bit. That's what you have to ask him. I mean, he might have thought it but he didn't push it; he didn't sell it saying, "Look, this is really interesting guys. What aren't you asking me any questions or why aren't you excited about it?" But I got him pretty revved up because I was really excited. I said, "You know, this is showing that clinical disease readout isn't telling you about replication." That means that all kinds of situations where we're crossing species with agents or feed in nature could be, actually, replication and lead to adaptation. Then we started thinking about looking for adaptation and I got involved in it then in the subsequent passages and stuff, in advising him and helping him write it up, but he did it all.

MP: And what were the initial results that you saw?

BC: He saw titering; he did it by infectivity and titering; in the first year he found no agent, but in the second year he found a lot in both brain and spleen.

MP: And this is intra-cerebral inoculation? For an entire year he wasn't finding anything, and then all of a sudden...

BC: He didn't look really early but, you can see it in that '98 paper. It was low – maybe he found a little bit. When we went back and looked carefully by titering it accurately, we found agent at all time points, but in very little amounts. So, right after inoculation we found some, which was probably the inoculate at 2 hours, but then at 5 days or 20 days it was very much lower, hundreds of fold lower, or maybe thousands of folds lower, I can't remember the exact numbers, and then, slowly, starting at 250 days or 400 days it started to come up. So right around the end of the first year we could see it coming up again when we started quantitating it, and then it came up higher and higher. So, it acted like a virus. I notice you have "eclipse phase" written down there. It isn't a true eclipse where there's no virus during the time the virus is actually disassembling.

MP: So that's what an eclipse phase is in viruses.

BC: Yeah.

MP: It's when the virus disassembles. There's actually no activity in the presence.

BC: There's none there. And then, suddenly, it appears again, and that's why they call them the eclipse. But, a lot of viruses don't really disappear because it's not really that precise. Bacteriophages were found to do this and came from that area. Some of them go in, they inject their DNA and then they're gone, you can't find them, and then suddenly they start appearing again. Of course, the time is 20 minutes. I mean, it's a fast deal. With a bacteriophage we're talking about 2 years, so it's a completely different thing. With hearty agents that don't self-destruct on infection or exposure to tissue you don't see things disappear completely, you just see things go way down, and then they come back up again. So if you can show a curve that goes back down and then comes back up, you're showing elimination and then subsequent re-replication, and that's what we were trying to show.

MP: So how did – what was the reaction when you published that? Did you get phone calls? I mean, like, just from reading that and being familiar with the field it seems like such a big result. This open questions of are we generating new strains, sometimes possibly more propensity towards the host, sometimes less sometimes...this sort of strange thing going on and, of course, the relevance to that for especially the UK.

BC: I need to look up that paper and see what we said.

MP: I have it.

BC: I'm thinking about the early one, the *Nature* scientific communication because in that paper we don't really say that the agent replicated because we didn't have titers, that is what sticks in my mind. We say that the agent persisted, and we were very careful to not say that it replicated because we really didn't have titers to say it with. You see, we have all these times, these incubation periods, and of course the

incubation period goes down suggesting the amount is going up, but all of the mice are dying at all the time points from 200 up to 782.

MP: So since you don't have titers –

BC: We suggested it – we might have suggested that it might be replicating, but we didn't say that this data proves it. But, still, people – the fact that it persisted made people really interested, and it was quoted in a lot of newspapers and it got a lot of press, but I must say that when I presented the titered data in 1999 in Tubing.

MP: In Tubing [spelled phonetically]?

BC: The big meeting in Tübingen. We really knew it was replicated, but John Collinge, unbeknownst to me, when he saw this set up some of the same experiments and he published in, about a year after the Tübingen meeting [1999], that it replicated based on analyzing one mouse at an early time point and one mouse at a late time point and titring them and seeing a difference.

MP: I see.

BC: And he managed to get an enormous amount of publicity for that titration experiment, to the extent that we had trouble publishing our subsequent papers.

MP: Really!

BC: Yes. This paper was rejected by *Nature*, *Nature Medicine* and one other journal – *EMBO Journal*, I believe. I'm not sure which ones, but it was rejected by several journals because they only were interested in the headline "It Replicates". He had shown, I think inadequately, because he'd only analyzed two mice, one low and one high. One early and one late is what I mean, and he had all his three mice, one non-injected, that's a negative control, I don't count that one, but one early was inoculated and one late and he said, "Early it's low, late it's high, it must have replicated." Well, in this paper we analyze bazillion mice.

MP: So many more. And you have curves.

BC: Yeah, so, we think we did a much more thorough job of it, but I must say that our data agree completely with Collinge's two mice. Bbecause he analyzed one at this time point and one late, and so he kind of stole our thunder on the replication point. Maybe we were too conservative and we should have said, "This data suggests very strongly replication because this is 313, this is 168, and that's a huge difference and therefore there's a lot more later than there was earlier and therefore it must have replicated." We didn't feel that on the basis of a single mouse, mouse #1 who was the donor for this guy, that we could say that since mouse #1 and mouse #6 are statistically different that we can trust it. Mouse #1 might be an outlier and all the other mice look kind of the same, we just didn't trust an outlier.

BC: Right. You wanted to have a more thorough investigation.

BC: We wanted to have more mice. We wanted to be able to reproduce that early mouse, but apparently that wasn't necessary for Collinge to get his paper in *PNAS*, and it wasn't necessary for the entire media community to think that this was really important.

MP: Right, and to be convinced that it was already done.

BC: That's okay, but what really bothered me was that the scientists who reviewed our paper didn't look into that subtlety and didn't realize that his data was pretty weak and pretty preliminary, about as preliminary as this – our '98 paper – and, yet – and so they just said it's not worth publishing in this fancy journal. And so it was hard, so we ended up going into a more – a journal like *Journal of Virology*, which is a really high quality journal, but not as glitzy. And so that's part of the politics.

MP: Well, you mention CWD and the issue with people getting this disease or other animals in nature but you do mention that this presents this sort of issue, this concern, about having these reservoirs around and even if they are not causing disease in animals, the potential is that they could. Or you mention that the feed with BSE – just because chickens and pigs don't get it, it doesn't mean that eventually, through many passages, there could be a new strain produced. Did you ever get any calls from the USDA or the FDA, the people who manage animals or deer about how to use this thing?

BC: Not really. We got calls from the media not about this paper, nor about the Collinge paper, but we started to be recognized because of this earliest paper as somebody you call to ask the questions.

MP: Right.

BC: So Rick and I had lots of questions, and so did Byron and Sue, about species specificity because all of our work related to species specificity. So we got calls about that. The media doesn't respond to papers in *Journal of Virology*. What you have to do – if you want the media involved you have to call up the media or have a press conference and make a big deal out of it. On the other hand, if you publish a paper in *Science* or *Nature* and they write reviews about it, they call you, you don't have to call them. So it depends on whether your goal is to alert the media or not. And same with the papers in *PNAS*. Even Collinge's paper in *PNAS* would not have been picked up by the media had he not orchestrated a big media campaign, and he really did a lot to do that because this is his way of presenting the data in a broader forum, and whether that's good or bad depends on who you are and where you are.

MP: I guess one of the things I'm thinking about is the issue of the relationship of science to policy, to disease management policy, and do you see the media as one way that scientists can influence disease management policy if an article gets a lot of press and if people are forced to deal with it, or do you see other ways that your results can have an effect on the way that policy is generated? Do you see what I'm saying?

BC: Sure, absolutely. Well, there's no doubt that the media influences science policy and so articles do attract the attention of people who know nothing about science but who know about politics and care a lot about health policy so, it is a very good way to reach politicians, or their staffers, on topics. And they can't read papers in *Nature* or *Science* or *Journal of Virology*. There is no way they're going to pick them up, so the media translates them for them, and so this is very important – it shouldn't be the only way that people make policy because there are intermediate – there are high-level government scientists in between the politicians and the people in the labs that make the policy and should be sort of guiding it

along scientific lines not sensationalistic lines, and so you need some balance here because the media will always take the sensational first, but they'll also go with something interesting, they're not immune to interest, and they know that the readers will be interested in certain things and they don't all have to be sensational, so it just kind of depends on the time and the place and the reporter and how that person pitches it, but clearly science policy is not a linearly developing matter; it zigs and zags all over the place.

MP: Right, there are all kinds of different inputs and outputs

BC: Yeah and we, as the scientists, don't direct science policy; we influence it. But the public influences it, the media influences it, world affairs influence it – it's just really a complicated process because it's all tied up in whether the public's money should be spent over here or over there and I think we just have to realize that that's the way our system works and it has its pluses and minuses but mostly it works pretty well.

MP: Have you ever had a desire to have your research used more at high level – I mean, do you know what I mean?

BC: Well, everybody's had – all researchers are egotistical in the sense that they want to publish their research, they don't want to keep it for themselves, they want people to look at it and say it's great. And to have people use it more. Sure, everybody wants people to use it more, but how you define more depends on who you are and what level you're happy with, it just depends on how you want to run your life. It gets to be very personal.

MP: You aren't going to Capitol Hill.

BC: If you want to be a kind of lobbyist! Yeah, I had an experience like this on this topic a number of years ago because we developed a plaque assay for HIV because I was interested in plaque assays and developed some for murine retroviruses. So, we put CD4 into hela cells and we developed an immune-plaque assay that shows these positive cells. These are hela cells expressing the HIV reaction, and here are all the negative hela cell.

MP: Right, I see.

BC: And if you dilute this out you get them separated and you can count them and so we tried to get that to be patented and then used by a lot of people in the HIV field. I realized, kind of partway through that, we did get them patented, but to get the people in the HIV field to use it was not sufficient just to talk about it at meetings and make the reagents and cells available to a lot of people. We were not trying to make money off of this from scientists, but to get the US Government to patent it because they thought this was a good idea but they really weren't trying to make a lot of money they were just trying to get people to use it. I realized that I, personally, or somebody working directly with me, was going to have to make a full-time campaign to sell the assay and to advertise the assay so that many researchers would see how easy it was to use and they would want to use it, and that became something I didn't want to do. I'm not a marketing person and I don't really want to put my time into marketing, whereas I think it would have been effective had I done it because it was an easy assay and we could have gotten somebody – some company, perhaps, to package the reagents. We gave all the reagents to the AIDS repository and they sent them out so people could do the assay easily but it was so hard to convince the

community to use it. They would much rather use a commercial assay that was way harder to use – the plate assay where you do P24s at various times and you don't have any idea how much virus you're really making, but you just – and these assays were extremely expensive. We even developed a cheap P24 assay, and we gave that to the AIDS repository, and relatively few people use it even though it's 1% the cost of a regular assay.

MP: And you think that's because of marketing because those companies are able to market their kits and make sure people know about it, it's easy to order, it's easy to find? Yeah, I can see that.

BC: It's all about marketing because scientists don't want to do any troubleshooting. They think the company has done it all for them, they don't realize that maybe they have and maybe they haven't, and so they're afraid to venture past this other point. And the less comfortable they are in the lab the more they are afraid to venture past that point. Some people are perfectly comfortable venturing out beyond and troubleshooting, and they're the people who take your assay and improve it and then it develops a new life and better and better and better. But, a lot of people don't want to do that anymore. That used to be the way people worked with assays in the past. They would take assays and always improve them so there was a continual evolution, but then it got to the point where, no, you buy it all by kits and nobody knows how to develop an assay they just do what the company says.

MP: Do you think that's a problem? I mean, I used to work in the lab and it always used to strike me that there was this big, black box because sometimes they wouldn't even tell you what the reagents were and if you're doing an experiment and results come out a certain way you don't know if it's because of something you did or something in the kit that's reacting with something and – I mean, how do you feel about that sort of reliance on this kit-based science culture?

BC: I think it's terrible. I think it's nice that they package kits and get people started and doing things, and I think it's very bad when they don't tell you everything that's in the kit. I mean, we've had that experience ourselves but sometimes it isn't worth our time to really figure out how to get around using their kit, and it's often a very good place to start, but not always. I mean, I have a long – I could talk for hours on insight to hybridization to detect chemokines and cytokines in the brain, and we worked very hard to get that to work, and we worked with companies and we worked with labs, and nothing ever worked until we finally stumbled on a combination of methods that did work.

MP: A combination of kit-based methods?

BC: Well, yeah, a combination of kit-based and non-kit-based methods and some of the parts of the assay were important to use the kits, some of them had some mystery reagents in them like the hybridization buffer, and we never went through the trouble of getting that commercial kit hybridization buffer out of the assay, we just used it even though they wouldn't tell us exactly what was in it because it worked and there were so many variables in the process we didn't want to troubleshoot another ten variables.

MP: Right, so just to save time because there's only, as a scientist you're trying to do all these things and there's only so much that you can do.

BC: Well, I found it crazy that they didn't tell us because we were buying their kit and we needed their anti digoxin immunoassay kit and their digoxin precursors that were nucleotide labeled to tag our RNA molecules with digoxin, or digoxigen – “dige” – and we were buying all the stuff and this hybridization

buffer was almost like free – it came in the kit – but we would like to know what it is and they didn't want to tell us. I just didn't understand that, when we were good customers.

MP: Right. It wasn't like you were going to start making your own.

BC: Yeah, exactly.

MP: You just wanted to know.

BC: I've also had experiences with companies where you get in a situation like that and you explain that you need some information they find a way to give it to you.

MP: Okay.

BC: I've had them do that.

MP: Even though they're not supposed to.

BC: Well, they don't tell you what they're not supposed to. They never do that – they know that or they'll lose their job, but they tell you more information than they'll give normally up to the point where you get enough information to know – like they won't tell you the exact concentration of something, but they'll say it's between this and this. Because they'll say, "The exact number is a proprietary secret, but we're allowed to tell you that it's between this and this." That's good enough. Sometimes that's all I need, to know it's between this and this and I really don't need to know the exact number.

MP: Because that can help you figure out what effect it might be having.

BC: Yeah, and I don't want to stop buying it from them anyway because it's much easier to buy it from them and they know that, so I don't want to stop that, I just want to know how it might affect something else I'm doing, and they've helped me by doing that. But you have to move up the ladder to get a higher-level technical representative to agree to tell you that. You can't go – the first technical rep has more restricted information they can give and as you work your way up you get to somebody who actually has the power to give you more information without jeopardizing their job.

MP: More discretion.

BC: Yeah, more discretion. And, I mean, that is a pretty good way to handle it. If they need to keep this a secret a certain way, then that's a pretty good way to handle it. So I'm not unhappy with that.

MP: Right. So I wanted to go back to that original question. I sort of wanted to talk to you about the evolution of...

BC: You've got about – let's go for just under ten more minutes. I want to leave at ten of 12:00.

MP: Okay, well then let me ask you this. This is the question that I brought up at the beginning about how you feel like your background and the perspective that you get from working with retroviruses and these

different agents influences the way that you think about TSE, sort of your views on the nature of the agent, that kind of thing.

BC: Well, it's very influential for sure, because I consider myself an infectious disease person who sort of specialized in virology and immunology of viruses. I think that the TSE field mostly had, in the earlier years, people who thought of themselves as infectious disease people who worked with microbes of some sort, and the other kinds of people who were in this area were pathologists. So, you had these two kinds of people. Now, that's not the case. There are a lot of people who have not worked in infectious diseases who are in the field, people who work on proteins and protein aggregation or molecular biology, and they have a very different perspective on the agent. I think that is the reason for my prejudice that we haven't ruled out a virus because it's so hard to rule out a virus and all of the phenomenon can be explained by a virus and nobody's looking, so that's a problem, that the field is being warped in its perspective by the background of the people who are in it now. So you have an infectious disease field where nobody knows – where there are very few people who are specialists in infectious disease. There are more specialists in neurodegeneration there than are pathologists, there are molecular biologists, but there really aren't too many people who really know about infectious diseases and who think about them as infectious agents and titering them, quantitating them – this is kind of the key.

MP: So the issue of titering, there are a few people who point out, for instance, when it comes to figuring out whether something is an infectious agent versus a toxin, right, or something that is just toxic to cells it is important to do things like titering to figure out – so can you explain that? What does titering tell you that other methods don't necessarily tell you?

BC: Well, that's about quantitative biology and that comes from my background – I can tell you about it. At Cal Tech the whole biology department was focused on quantitative biology, so when they worked on phage they counted the phage. They didn't count them under the microscope they counted them by plaque assays, and they did growth curves that showed eclipse phases that we were talking about, and the people working on neurospora and gene splitting and chromosome analysis. The stuff that they work on to do neurospora genetics is all based on quantitative genetics. So, quantitative microbial genetics and titering all go together to measure how much you have under difference circumstances at different times, and that tells you a lot about whether the agent is replicating or not replicating, where it's replicating and it can tell you, in the end, how it's replicating. So, all of that's pretty important, and that's my prejudice.

MP: Do you feel like that gets left out of a lot of work today because you don't always see titering, you don't always see these sort of type of things?

BC: Yeah, I think it's a weakness. I mean, there are a bunch of labs who still do it.

MP: Oh, sure.

BC: But a lot of people want to extrapolate just from incubation rate to titer, and that's fine. I was uncomfortable doing that here because of my sort of prejudice, but you could have said, "Okay, this mouse at 313 days is really different from this mouse at 128 days and therefore these must be replication."

MP: Right, and if you'd been someone who really had a lot of faith in the incubation time assay, or in the incubation period assay, you might have been willing to extrapolate to that.

BC: I think I would have been willing to extrapolate if I'd had three mice at 204 days instead of one. I didn't think one mouse was enough.

MP: Right, right. So it was the issue of the numbers.

BC: Numbers again, so that's another aspect of quantitation.

MP: Exactly.

BC: So, I'm really biased by numbers, but a lot of people use numbers. The Collinge experiment was an unusual experiment in that they didn't use any numbers for a big conclusion. I don't know why they didn't. It would seem if they were really planning on doing that experiment prospectively they would have set up groups of four or five mice and titered them all, but maybe they didn't do it that way. Maybe they just grabbed some mice off the shelf from an experiment they had going and they were able to get this information from them. I don't know. But I think that it does affect how people think about the field. To be a virologist gives you a perspective that viruses are hard to find and hard to count if you don't know what they are.

MP: So if you don't like a sequence or something?

BC: You need a sequence or an antibody. You need a reagent that detects the virus specifically. You need a pathological effect in a tissue culture cell line that you can dilute out and see like this, or an antigen that you can dilute out and see like this or a sequence that you can dilute out and see when does it go away. And if you don't know what you're looking for you can't figure it out. That's the problem with finding the viruses – the hepatitis viruses over the years, there's still more to be found and they can't really find them all because there are not that many people who have the unusual ones and there's no easy way to titer them and there's no easy way to put them in cultures so you can't figure out what to do next. And it's the reason it's so difficult to study the viruses that cause gastroenteritis in humans, and Norwalk agents and those viruses, because they're really hard to grow in vitro and so no one knows how many different ones there are. Same for the cold viruses; they're really hard. There are so many of them you can't keep them straight, and each one requires a different culture system so you've got this mess on your hands, you don't know if you're looking at adenoviruses or corona viruses or rhinoviruses and there are a bazillion strains of rhinoviruses. So, all of a sudden, the ability to quantitate them and measure them and everything, by antibodies, and measure them specifically, is a huge problem for a lot of human viruses that don't easily grow in cultures.

MP: What do you think about the various different ideas about what kind of a, if it were a virus, like the virino hypothesis, Alan Dickinson's idea, or Charles Weissmann has been talking a lot about the short nerve theory – you know, like the small interfering RNAs, maybe it's something like that.

BC: I've never heard him talk about that, that's very interesting.

MP: It's something he's brought up a couple of times.

BC: Well that's a very virological idea because. Because short interfering particles could encode the key elements for the disease, but be able to replicate on their own without a helper. That's exactly what happens with Friend virus. Where we started this conversation, there's a defective virus that can't replicate on its own, it's the key virus that causes the neoplasia, the erythroleukemia, the splenomegaly, and the helper virus is absolutely essential but doesn't cause the disease on its own. So to get the disease in adult mice, which is what we were studying, you need these two viruses and they have to be together. The helper virus will cause disease, sort of similar but not identical, but only in baby mice. And so it's a slightly different kind of disease, too, and out of studying that we got into the brain diseases, but this defective helper relationship is very common in virology. So, it's not just defective interfering particles because we don't have to interfere, but they can just be defective.

MP: So if there were two parts required and one of them were common enough that even if one of the required – like, let's say there were two required parts and one was small and was sticking with the prion protein aggregates and another one was common enough that it was around, then you could potentially have this purified protein which would include some nucleic acid and still produce the disease?

BC: Yeah, or it could be inducible. That's, I think, one of the other issues. Any of these situations where people are putting things, brain extracts, from one mouse into the other – if you have a mouse, you could be inducing the helper effect that's necessary, or you could be inducing the important effect. Maybe the agent is really there in the genes and you just have to get it to come out. After all, that's happened with lots of agents, so you don't really know if that's the case or not, and it applies to a lot of these transgenic mouse experiments, where the agent will only transfer to another transgenic mouse that has the same gene but at low expression level. They're there, but the transmission might just be turning up the transgene and that doesn't sound like scrapie because scrapie transmits at very low concentrations to normal mice, and so what Stan Prusiner is about to publish in *Science* that he's been talking about at these meetings, the in vitro fibrils don't transmit disease to normal mice.

MP: They don't.

BC: They only transmit something into abnormal mice, transgenic mice.

MP: Then can transmit to more normal mice.

BC: Now this is a step in the puzzle –

MP: How would you explain that?

BC: Well, something is being induced in the other mice or the other alternative is, and he would choose this one, that the other ones are more sensitive and so he's able to amplify. He's made so little stuff that he needs a very sensitive mouse to get it to amplify at all, and in normal mice it won't amplify because he has so little stuff. So, either he's made the wrong stuff or he has so little.

MP: Which one is it is still the question.

BC: Yeah. And the question is: Are these mice, because they have 16 times as much expression, but it's a deleted protein that they're expressing, does that make them 1000 times more susceptible or only 16 times more susceptible? And I don't think he knows; he can find that out.

MP: By titering.

BC: Yeah, by titering scrapie lesion into them and seeing how the titers are and if he can show that the titered regular scrapie agent was 10 times higher in his transgenic mice than in the other guys' it wouldn't surprise me, he might find it 100 times higher, and then he would know that, okay, either his agent is wrong or – and it only works in this kind of mouse – or he's really got so little that it won't go in this mouse, but could also find that out by titering into this mouse, which he hasn't done either, at least from what he's told in his talks.

MP: So, if he were to titer it would be more convincing to you. If you were to see some titer it would be much more convincing.

BC: Oh yeah. But he probably can't titer because I think he's suggested in comments to Byron that they can't dilute it so the titer is – he has no titer.

MP: So it's not able to titrate.

BC: It's titer is 1 instead of 1,000,000, you know? It's one unit.

MP: That's awful!

BC: Well, that doesn't mean it's irrelevant.

MP: Yeah, it doesn't mean it's irrelevant but it becomes much harder to justify, right?

BC: It doesn't suggest that you've generated very much infectious agent if you believe that. I still think that it's more likely that you've generated some – that you've induced the infectious agent in the first mouse, not in the tissue culture, and that's the big difference, after all. If you don't have the agent in this protein-only fibril prep then you haven't made the protein-only agent.

MP: Right.

BC: And it's interesting that they're regarding it as a big breakthrough when, of course, Stan Prusiner has been sort of claiming that he's proven the protein-only theory long ago, but he's still trying to prove it. Apparently he really doesn't believe that he's proved it a long time ago so he has to keep proving it, and I agree with him.

MP: Just one quick question, I know you have to go, so remember when we were at the meeting at San Moritz, the presentation mentioned other studies like this where they've taken the APP transgenic mice that produced the precursor to A $\beta$  protein and they would inject a seed molecule. They could show that with Alzheimer's and you've written in some of your papers there's always this comparison made between TSEs and these other neurodegenerative diseases have amyloid forming and polymerization but, yet, these other ones aren't transmissible. Can you justify that or, in the light of that kind of data, how do you explain that data or what's going on there.

BC: Well, basically, all of those systems – it gets back to the titer question. They're all able to induce very local pathology when you put a bunch of fibrils in the brain. I do believe that the conversion process that we see in cell-free conversion and tissue culture cells happens in vivo and does allow fibril genesis and PrP conversion without fibrils, maybe, to occur and spread locally and maybe even progress through the nervous system. I think they're modeling that. I don't think they're modeling infectious disease because their titer is so low. They don't have any titer. So, it's not like scrapie; they have to put in a massive amount of it; they can't dilute it 1 to a 1,000,000 the way we can. So that's a completely different kind of thing, but I think that phenomenon also occurs in scrapie, and I think its PrP conversion is very analogous to that phenomenon.

MP: So you need both in a sense. You think there are a couple of things going on in scrapie – that there's an infectious agent and this replication going on, but that there's also this propagation of this amyloid PrP via this mechanism that we've seen of polymerization.

BC: And that follows all the rules that occur in all the amyloid diseases, and I don't think we know that they're the same in the sense that the infectivity is that same mechanism. It isn't convincing to me that these high titer inoculate amyloid into brains of certain kinds of mice is mimicking an infectious disease. That gets to the whole point of what you think an infectious disease really is and whether it really replicates in terms of logarithmic expansion, hundreds of folds expansion of the amount of stuff of agent that's there or not. I mean, not every agent has to replicate to that level to be defined as an agent, but it's also defined a lot of other ways; like amyloid diseases. No amyloid disease is contagious. So a lot of infectious diseases are defined as contagious diseases. So, that's completely missing from any animal model, any amyloid model except the amyloid models that are induced by actual inflammatory infectious diseases like amyloidosis induced by TB or malaria or leprosy.

MP: I was going to bring that up.

BC: So, there you've got this agent that induces the amyloidosis. Well, scrapie could be doing that.

MP: So, in that case, just going back, then, to the cell-free system, is that then a model that would then, in your point of view, not be considered a model for infection but more of a model for the polymerization process that occurs.

BC: Because Byron and I have talked about this many, many times, and that hasn't stopped me and Byron from collaborating to try and show that in the cell free conversion experiment we can generate lots of infectious agent. That's been a goal of Byron's, and I participated in it, because if we could show in vitro that we could generate 1000 fold more infectivity than we put into the two I would start believing that this wasn't a virus because that would be a really good piece of information, but we have never been able to do that.

MP: What fold are you able to do usually?

BC: Three fold.

MP: Three fold. Okay.

BC: Three to four fold. That's what Soto could get reproducibly, and that's what we got using this system, not 20 fold the way he reported. So nobody has gotten 20 fold except Soto, but he didn't really titer, he just got amounts of PrP res and he said, "Oh, this ought to work." But nobody else got 20 fold at the level of PrP res.

MP: Okay.

BC: So, everybody else gets fivefold or three fold or something. So that's the problem. I don't really have a closed mind but we might not be able to do this better and get it to work and Prusiner's trying to do the same thing.

MP: You're still trying.

BC: Prusiner's trying to do the same thing so there's certainly a competition to get it, and his approach is a little different from ours, but I think we would want to titer it. Therefore, Byron's first question to Prusiner was, "What's the titer of the material?"

MP: I remember.

BC: So that's why. That's why that's so important. Well, I've got to leave.

MP: All right. Excellent.

BC: We can talk again if you want.