result of Tateishi's data, but we wanted to make a better measurement and address this question particularly.

Then, are the filters removing infectivity at random or are they selecting a more attentive population. Finally, we want to ask the question how dependent is filtration efficiency on the spike preparation? So, this is what we prepared. Also we have two spikes.

One is the brain homogenate spike from 263K hamster strain scrapie, then the PrP fibrils purified from the same models, the hamster model, and during the purification we used 1 percent sarkosyl. But I want to clarify that this sarkosyl was removed at the last step of the purification, so there was no sarkosyl in our experiment.

Both spikes were treated with solvent detergent before adding to factor 8 ---- in dilution from 1 to 100. So, our

vehicle was factor 8 eluate, and the spike were treated with solvent detergent. However, this solution was thinned, diluted 1 to 100. So, there was no detergent, really, in our experiment. But I want to emphasize that both preparations were exposed to detergents, and in that case we measured the titers by the end point titration.

This is the experimental configuration on a study that we did. This is the brain of homogenous spike, the purified fibril spike. They are identical, in terms of configuration. We spiked the material here, the first stage, and then filter on the -- the first filter was 75 nanometer. Then the filtrate went through the first 35 nanometer. The filter of this the second 35 nanometer and so on. So, we spiked only once. There was no spiking in the middle.

We also put two filters. There's

millimeter in tandem, and this is because we wanted to address the question of what is the mechanism of these filters, and this point was mentioned yesterday by Bob Rowher. So in this particular case, what we wanted to learn -- if the first filter is selected, then what it goes through -- what passes through the first filter has a size that's less than 35 nanometers, so it would not be stopped. It would not be removed by the second 35 nanometer filter.

On the other hand, if this filter removes infectivity by stochastic or random event, then there's going to be some particles that pass through the filter that are larger than 35 and that will be stopped by the second 35 millimeter filter.

This is the filter configuration. This is where the challenge material is placed to pump the liquid through the filter. This is the Planova nanofilter, and then during filtration we closed this

outlet. We then look at pressure and make sure that we don't exceed the pressure for each filter. During filtration we collect the filtrate on this tube here.

This slide shows the results of this experiment, and this first line here corresponds to the infections dose per millimeter that used to challenge each filter. This is the recovery, and this is the log of retention.

So, for brain homogenate spike, we have a first filter remove four logs of infectivity. The first 35 nanometer filter moved almost nothing. Another thing, the second 35 nanometer removed more than the first one.

As I already explained to you, this obviously was an unexpected result and it's not clear at this point why we see such a phenomenon. It's possible that there is some other mechanism going on that we don't have control over.

The second 35 nanometer filter was only 20 infections units per liter. We challenged the first 15 nanometer filter and we saw no infectivity in the 15 nanometer filtrate. So this corresponds to less than the 6 infectious units per milliliter, and this corresponds to more than .5 logs of retention for that filter.

This is the result of purified fibrils. The first 75 nanometer filter removed 3 logs or 3.1 logs of infectivity; nothing from the first 35 nanometer filter, and more on the second 35 nanometer filter. Again, this is the same thing I just talked about for brain homogenate. Then the first 15 nanometer filter removed more than 2 logs of infectivity.

So, if we compare now the data from the two spikes, where we used -- the first 75 nanometer filter removed more -- one or more of the brain spikes compared to the fibril spike, indicating that the

infectivity distribution in the spike fibril is more toward the smaller size, compared to brain.

In the 35 nanometer filter we show no difference basically, and also we -- in the 15 nanometer filter we saw there was a -- first of all -- a complete removal of infectivity for most of them. For this fibril here, we saw more than 2 logs of infectivity removal, and of course the first thing we wanted to know is: Is this the limit of the removal or this is the limit of our detection assay. But if we could challenge the same filter with more infectivity, would we find more removal or not?

So, the conclusion at this point is that the majority of removal was by the 75 nanometer filter, and this filter removed selectively. That's because whatever passed the 75 nanometer filter was not blocked by the 35 nanometer filter,

which means that it was smaller than 35 nanometer.

This is a point I have already mentioned. The second 35 nanometer filter removes more than the first. The fibril preparation should ---- less retention than brain homogenate, and this is for the first 75 nanometer filter.

Also, the other conclusion is that the 15 nanometer filter retains more than 2 logs of infectious 35 nanometer filter. This is something, again, that Dr. Rowher was talking about yesterday that we think that the appropriate spike for a small filter like 15 nanometer filter. It's really a pre-filter, some sort of pre-filter or what we call a condition filtrate, so that you remove all the large particles and you challenge that filter with particle size that are compatible with filter pore size.

Also here the detergent may facilitate filtration infectivity. That is

because I said our samples, even though the experiment did not contain detergent, but the spikes were exposed to detergent.

In summary, now, the spike modality can affect clearance potential, as I showed you for the two different spikes. The effectiveness of nonfiltration can be strongly dependent upon process parameters. This is what I've been mentioning about the presence of the treatment of the spike with detergent we might have found a completely different results if we had not treated the spike with detergent.

So, the idea here is that the result of nanofiltration depends exclusively -- in the context that nanofiltration is done with a specific of process parameters they apply to the spike, and to the vehicle, too. So, this of course, is strongly cautious against over interpretation of removal studies and also extrapolation of data from one study to

another.

It's also suggested that ---- of multiple removal step can always be assumed, and the serial tandem processing is an excellent means to reveal a selective removal.

Okay, we're going back to our model here, our pictorial model. What we found at this point in this study is that we have not found in any infectivity on the 15 nanometer filter, so we can exclude -- at least in that study will not support this model here, the single protein model. It also doesn't support any of -- there is a particle, a TSE agent different from fibrils that can be filtered, that has a filtration size less than 15 nanometers.

But it appears, at this point, to say ---- fibrils and the ---- has reduced the fibrils to a different -- to a distribution infectivity. So, some are removed by the 75 nanometers; very few are

removed by the 35; and all of them are removed by the 15 nanometers.

As I said earlier, what we wanted to know is if we challenge the 15 nanometer filter with more infectivity, something that has already gone -- something like five or six logs of infectivity that have already been filtered to a 35 nanometer filter, so it's been conditioned, do we still find a complete removal of infectivity?

We have done already the studies, but the studies are almost complete but we're still missing a piece of the puzzle, so I cannot talk to you about these studies at this moment. But what we have done to address the unanswered question that came from the first study is what is the limited size of TSE agent? So, we challenged the 15 nanometer filter with titers of around 6 or 7 logs of infectivity that have already been conditioned on the 35 nanometer filter.

Also, the other question was:

What is the maximum pore size capable of removing infectivity? Well, when we started the study, we thought it was something between 35 nanometers and 50 nanometers; so we tested some filters that had the pore size between these two values.

Finally, we explored the effect of surfactant and like detergent on the filterability of TSE. And we think we're going to get this completely studied in a short time, and we hope that we can present this at another time.

Thank you.

DR. BOLTON: Thank you, Dr. Gregori. Questions.

DR. McCULLOUGH: I would just say excellent design of your study and formulas and the hypothesis and very interesting results. Just commending you.

DR. GREGORI: Thank you.

DR. BOLTON: I have a question actually. When you say you have purified

fibrils, was that material treated with proteinase-K?

DR. GREGORI: This one in particular were, yes. They were --

DR. BOLTON: So, you did have fibrils there. I just want to point out that there are alternate methods for purifying TRP which do not use proteinase-K digestion and those do not produce fibrils; they produce protein globules, and they will have different physical properties that may or may not filter in the same way.

I think -- a major point that you made I think is very important to emphasize, and it's been done in your study and in previous studies by Dr. Rowher and in my laboratory and others, that it is indeed true that the exact biochemical milieu is very important in how the agent acts in filtration and centrifugation and many other kinds of biophysical conditions. So, it's very difficult -- and we'll be going

forward -- very difficult to extrapolate from any particular study designed in a certain way to other conditions that may apply.

DR. GREGORI: Yes, that's exactly my point.

DR. BOLTON: Steve.

DR. DeARMOND: Although the study was well designed and looks very nice, it's really difficult to interpret because in the one you start with a homogenate, which theoretically is the native state of whatever the agent is -- we presume it's prion protein.

DR. GREGORI: Yes.

DR. DeARMOND: The other is highly processed prion protein because the fibrils require proteinase-K digestion plus detergent to form them. So, it's a different situation, and in both cases there's going to be a whole range of sizes of molecules from individual molecules that

can pass through that in the native state would be about 30, 35 kilodaltons. But they should pass the 35 filter.

But the majority seem to be dimers or polymers, and we think even a hexamer is the basic unit, the basic natural unit of the infectious agent. So, this is a very complicated thing to interpret.

But the question I wanted to ask is: The reduction that you're getting in infectivity titers by the time you get beyond the 15 nanometer is putting it in terms of the other chemicals ways -- sodium hydroxide denaturation and heating -- are you getting similar -- it looks like the decrease in titer is similar to treating sodium hydroxide and heat. By the time you get to the 35, is it the 35 or at the 15 nanometer pore?

DR. GREGORI: I'm not sure I understand actually your question.

DR. DeARMOND: So you've got a

marked reduction --

DR. GREGORI: I'm sorry, we were not doing activation. Those are not inactivation studies.

DR. DeARMOND: But you end up arriving at inactivation.

DR. BOLTON: Steve, I think the answer to the question is yes.

DR. DeARMOND: Thank you.

DR. BOLTON: Because by the time they'd gotten past the 15 nanometer filter, they had no measurable infectivity, at least in the amounts that they tested.

DR. GREGORI: Yes.

DR. BOLTON: I don't know how many animals you inoculated with what volume, but it was below the level of detection within the assay parameters.

DR. DeARMOND: Then the 35 nanometer, was that comparable to standard denaturation?

DR. BOLTON: Well, we consider one

normal sodium hydroxide for an hour or more to be essentially terminal activation, so I'm not sure --

DR. GREGORI: I still don't understand why we're considering removal same as an inactivation.

DR. DeARMOND: I was comparing reduction in infectivity titer using this technique -- thinking of industry. So industry could use this technique to reduce infectivity titer to close to zero, without having to use sodium hydroxide and heat. So, I was going to the practical side.

DR. GREGORI: Oh, okay.

DR. BOLTON: Only if the method is applicable to their process.

Sue?

DR. CERVENAKOVA: All right.

DR. PRIOLA: Two quick questions. One: 15 nanometers was the size you gave as the minimal unit size. How many PrP molecules is that?

DR. GREGORI: How many what?

DR. PRIOLA: How many PrP single molecules --

DR. GREGORI: Fifteen nanometers?

DR. PRIOLA: Yes.

DR. GREGORI: I don't know. I would say --

DR. DeARMOND: I think that's less than the size of a single one. I think about 50, 60 nanometers go to a dimer, which is about 60, 70 kilodaltons. I think that's what Tikvah Albert came up with, a cross-sectional diameter of the minimal diameter of the infectious agent.

DR. BOLTON: It's much more complicated.

DR. GREGORI: I really don't know this question.

DR. PRIOLA: That's fine. I was just wondering if -- the second thing was more of a hypothetical thing. Have you ever thought of spiking with something other than

material from a clinical animal, taking into consideration what you said and what Dr. Rowher said yesterday that the nature of the spike is important, and it may be that the nature of the infectious aggregate, if it is in fact PrP SE, can differ in pre-clinical versus clinical animals. It may be smaller in a pre-clinical animal, for example.

DR. GREGORI: Right, yeah, I understand the question. No, we have not done that because usually these studies we go for the highest titer we can achieve to show removal. And so we have never tried, as far as I know, we never tried the same studies with pre-clinical brains.

DR. PRIOLA: Yeah, it's more a hypothetical, scientific question than a practical application.

DR. BOLTON: I have a comment, actually. In your study where you seem to get higher removal rates in the second 35

nanometer filter, were those samples along the line adjusted for constant protein concentration? I forget if there was supplemental protein added to the material to begin with.

DR. PRIOLA: No. These brains -- the homogenate -- the spike where in factorate monoclonal antibody eluate. That's constant as the vehicle. So, that's the concentration of that protein supposed to be -- is not removed by these filters. So that would be the background of the studies.

DR. BOLTON: The question would be whether or not you're getting just adsorption to the filter and that could still, I suppose, happen in the presence of the antibody, but it sort of depends on what the protein concentration is.

DR. DeARMOND: Just one other comment on size. If the prion protein, of course, in the native state, is

triglycosylated -- or diglycosylated, mono, and no glycosylation, and the sugars account for about 35 percent of the volume of the molecule. So, it's possible that the majority are going to be stuck at the 35, but the mono and then finally the non-glycosylated forms could get through multiple filters, and they could be a smaller proportion that would get through. So, it's a very complicated -- that's why the interpretation is extremely complicated here.

DR. BOLTON: I hope you're not confusing kilodaltons with nanometers, because the size --

DR. DeARMOND: I'm trying to remember the sizes of the molecule, and you're right, I can't do it.

DR. BOLTON: Dimers and trimers are going to be much, much smaller than 15 nanometers as a cross-sectional. So, certainly -- now, I just wrote something on

this, so I should remember it but I don't. But the size of a tetramer or hexamer is going to be much smaller than a 15 nanometer pore size, even smaller than 9 nanometer pore size.

A shell of PrP of about 35 or 40 subunits might be in that range of 12 to 15 nanometers. Other questions or comments? Ah, one more.

DR. ESHKOL: Maybe just to remind the audience.

DR. BOLTON: Would you introduce yourself?

DR. ESHKOL: Aliza Eshkol from Geneva ----. I presented yesterday, data on the validation of the ---- production, the possible validations, which included not only the filtrations, and we have never been able to remove by 20 nanometer filters more than 2 logs of infectivity.

DR. BOLTON: Again, I think that's important to point out, that the different

milieu or the different pre-treatment makes these studies very complicated to interpret.

At this point, just before we -- well, let me ask again, are there any other comments or questions from the committee?

Dr. Belay has reminded me that there is in fact a single variant CJD case that resides in the United States, and he would like to bring the committee up to date on that case. Dr. Belay?

DR. BELAY: Yes. I think everybody's aware of this case because it's been in the media, widely reported. This is a person who was born in England and moved to the United States as a teenager and has been residing or living in the United States since then. The patient is still alive and receiving medical care in the United States.

Initially, there was a question on whether or not this case should be assigned to the U.S. or to the U.K. The working group had already made a decision that vCDJ

cases should be assigned by the country in which they had illness onset or they resided at the time of illness onset. Because this patient was a U.S. resident, it's now being called a U.S. case.

DR. BOLTON: Could you give us when she was a teenager, in terms of the epidemic, the BSE epidemic?

DR. BELAY: She was born and raised in the United Kingdom throughout the 1980s. As you recall, this is a time period where BSE was rising and no preventive measures were instituted to prevent export to humans.

DR. BOLTON: When did she move to the U.S?

DR. BELAY: Early 1990s.

DR. BOLTON: Any other questions or discussion? Well, I would entertain a motion to adjourn then.

One thing I would like to say, actually, before we do that. It's clear

that we have seen maybe the very early defects of the guidance implemented in January. I think that we need to get an update on that at our meeting in October, so that we have a little bit better picture of what effects that may have had, so I would just ask David and FEA to arrange to have that done.

DR. SCOTT: We plan to do that and I want to thank all our speakers today for all of their efforts in updating us on the blood supply and also for the scientific updates, which were very informative and useful. We will update you at the next meeting.

DR. BOLTON: Thank you, Dot, I was remiss in thanking everyone. Thank you all, all the committee members, the members of the public for attending this meeting.

(Whereupon, at 12:32 p.m., the PROCEEDINGS were adjourned.)

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