UNITED STATES
COURT OF FEDERAL CLAIMS

IN RE: CLAIMS FOR VACCINE INJURIES RESULTING IN AUTISM SPECTRUM DISORDER, OR A SIMILAR NEURODEVELOPMENTAL DISORDER	)	
FRED AND MYLINDA KING, PARENTS OF JORDAN KING, A MINOR, Petitioners,	)	
v.	)	Docket No.: 03-584V
SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.	)	
GEORGE AND VICTORIA MEAD, PARENTS OF WILLIAM P. MEAD, A MINOR, Petitioners,	)	
v.	)	Docket No.: 03-215V
SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.	)	

CONDENSED TRANSCRIPT WITH KEYWORD INDEX
REVISED AND CORRECTED COPY

Pages: 351 through 662/760

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Courtroom 402
National Courts Building
717 Madison Place NW
Washington, D.C.

Tuesday,
May 13, 2008

The parties met, pursuant to notice of the Court, at 9:00 a.m.

BEFORE: HONORABLE GEORGE HASTINGS
HONORABLE PATRICIA CAMPBELL-SMITH
HONORABLE DENISE VOWELL
Special Masters

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C O N T E N T S

E X H I B I T S

For the Petitioners:	IDENTIFIED	RECEIVED
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P R O C E E D I N G S

(9:00 a.m.)

SPECIAL MASTER VOWELL: Please be seated. All right. We will go back on the record in the hearing today. I'll be presiding today.

Dr. Aposhian remains at the witness stand. I understand we have the Intertel operator so our folks at home can hear us. We're all set with that.

All right. Dr. Aposhian, I will remind you you are still under oath. And you may proceed.

Whereupon,

VASKEN APOSHIAN

having been previously duly sworn, was recalled as a witness herein and was further examined and testified as follows:

MS. RENZI: Good morning, Special Masters. Good morning, Dr. Aposhian.

THE WITNESS: Good morning.

FURTHER CROSS-EXAMINATION

BY MS. RENZI:

Q I want to continue asking you about the report that you filed in this case. Do you have that with you?

A I don't have that.

Q Well, we'll give you a copy.

A Thank you. Thank you very much.

Q Now, Dr. Aposhian, it's unclear from your report whether it's the ethyl mercury in the thimerosal-containing vaccines or if it's the resulting inorganic mercury that causes autism. Which one are you causally implicating?

A Thimerosal is in the vaccine that is injected into a child. All the scientific literature indicates the thimerosal is quickly converted to ethyl mercury. The ethyl mercury is then quickly distributed to the tissues, crosses the blood-brain barrier, gets to the brain, and there it is deethylinated to mercuric mercury. That is the metabolism of, the metabolic root of what happens once you give thimerosal.

Now, whether it's thimerosal per se or ethyl mercury per se that you're asking a question about, I'll ask you to be a little more specific in your question.

Q I'm asking you, it's your opinion that thimerosal-containing vaccines are causally related to autism. Is that correct?

A That is my opinion, yes.

Q And I'm asking you whether it is the ethyl mercury component or the inorganic mercury component

once that ethyl mercury deethylates that is the cause of autism.

A I don't think ethyl mercury per se stays in the brain long enough to have an effect that we have yet measured. I think it is the mercuric mercury that is the culprit. It is the mercuric mercury that remains in the brain almost forever, and has very definite toxic effects in the brain.

Q And what's the basis for your conclusion that the mercuric mercury has toxic effects in the brain?

A The scientific literature. If you read the scientific -- I mean, I quoted a paper in which they injected mercuric mercury directly into the brain. Can we go perhaps -- let's go back to that, actually. If you have copies of those slides. I don't have -- do you have copies of my slides? You were given the sheet yesterday with the copies of --

Q Which slide are you referring to?

A Pardon?

Q Which slide are you referring to?

A Well, I'd like to see the copy so I can give you a number.

Q Oh, you don't have the slides with you.

A If you'd give me a minute, I'll bring it up

on my computer. I certainly do have the slides. Sunday. Here they are. Let's see.

(Pause.)

Q Doctor, do you know --

A Slide No. 68. I'm sorry I'm slow, there are many slides.

SPECIAL MASTER VOWELL: I'm sorry, Dr. Aposhian, which slide number did you say that was?

THE WITNESS: Slide No. 68.

SPECIAL MASTER VOWELL: Sixty-eight, and this is on --

THE WITNESS: Six-eight.

SPECIAL MASTER VOWELL: This is on Petitioner's Trial Exhibit 2.

THE WITNESS: The paper entitled "Gaugher, et al, Identity of Ultra-Structural Effects of Mercuro-Chloride and Methyl Mercury After Inter-Cerebral Injection."

So among other things, they injected directly into the brain methyl-mercury -- I'm sorry, mercuric chloride. And found, thus, in spite of their distinctive clinical syndromes, these two classes of mercury compounds -- namely, mercuric chloride and methyl mercury -- are capable of inducing neuronal necrosis.

BY MS. RENZI:

Q And that's the basis for your opinion that inorganic mercury causes autism?

A I didn't say -- I don't think that was the question you originally asked me. You asked me what was the evidence for, I thought you asked me what is the evidence that mercuric chloride does damage to the brain.

Q I asked that question. I also asked the basis for your opinion that the mercuric chloride is causally related to autism.

A In my scientific opinion, it does.

Q What is the basis for your opinion?

A Based on papers like this, and a vast variety of evidence that indicates that the mercuric ion has a high affinity for sulphydryl groups, and will tie up the active centers of enzymes, not only in the tissues, but in the brain as well, and inhibit those enzymes.

The thioredoxin system, which was maybe Slide 10 or 11 if we want to go back to that --

Q No, that's fine.

A Is it necessary?

Q No, thank you.

A Okay. Is a good indication. The latest

paper of nanomolar amounts of mercury of mercuric chloride, nanomolar amounts -- those are very small amounts, those concentrations -- are inhibiting this purified thioredoxin system. And so, yes.

Q And that's an in vitro study. I mean, an in vitro study, correct?

A That's an in vitro. But it's an in vitro study in which we don't have to worry about competing enzyme reactions, we don't have to worry about the breakdown of substances, or the formation of inhibitory substances for other reactions. It's a very purified DNA-recombinant-synthesized enzyme. It's the purest kind of system that you can find in biochemistry.

Q And from that in vitro study, you conclude that inorganic mercury from thimerosal --

A I'm sorry, I can't hear you.

Q And from that in vitro study, you conclude that thimerosal-containing vaccines, the inorganic mercury, causes autism?

A Well, that's not what you asked me originally on that. What I'm saying is there is a body of information, published information, that indicates that mercuric ion is, has a high susceptibility, a great affinity for the active

centers of essential enzymes. And if those enzymes are inhibited, you're going to have problems.

Q And how does that cause autism?

A Well, since, in the brain, there are the centers, actually the control of movements, there are the controls of thought -- the brain controls our body and everything that we do about it. And once you begin inhibiting critical enzymes, inhibiting critical proteins in the brain with an inhibitor such as mercuric chloride, you are going to have problems.

There is no question we all have a certain amount of mercuric chloride in our brains. But I think, and I'll have to think one more -- yes. I think if you look at the autopsy data, you will find that mercuric chloride remains in the brains of those autistic children.

Q Doctor, is it your opinion that all forms of mercury exposure, both prenatal and postnatal, lead to autism? Cause autism?

A Perhaps we can go back to -- no, let's go on to the other slide.

Q And Doctor, I am assuming what you have on your computer is this slide? Is the testimony from --

A If you have a copy, it would be much easier for me to go through them. I put them in my suitcase,

which --

Q I only have my one copy, I'm sorry.

A Okay. But anyway, let me -- so you don't see this, either. Anyway, there is a diagram in the slides that were handed out to you anyway showing that ethyl mercury, that thimerosal is converted to ethyl mercury. And through various kinds of metabolism, it ends up in the brain, causing encephalopathy. It causes autism.

Yes, I think ethyl mercury will be, is metabolized, and mercuric mercury -- thank you very much -- and ethyl mercury and mercuric chloride itself are very harmful to the brain.

Q Oh, so it's both the ethyl mercury and the mercuric chloride? And the mercuric --

A The ethyl mercury is going to be there for a short period of time. And it's just --

Q Does that do any damage?

A Pardon?

Q Does the ethyl mercury do any damage that contributes, or causes autism? Just the ethyl mercury.

A The ethyl mercury is the source of the mercuric ion that resides in the brain after ethyl mercury has been metabolized in the brain. And it is

the mercuric mercury that remains in the brain, and probably has the long-term effect.

I think the scientific literature, a great deal of it supports that hypothesis.

Q Okay. My next question was, then, do you think it's both prenatal exposures to mercury and post-natal exposure to mercury that cause autism?

A I think it depends on the individual. It depends on what the diagnosis is going to be. I think, as many people, I think one of the best people that I know of is Professor Ellen Silbergeld at Hopkins, who got the MacArthur Award, the Genius Award, the only toxicologist, male or female, who had ever gotten that award, told me quite some time ago that in her opinion, the thimerosal and ethyl mercury will trigger a response.

There is already, in a pregnant woman, mercuric mercury to some extent. And methyl mercury to some extent. And there is no such thing that we know of as a mercury-free human being.

And so there can be an effect prenatally, there can be an effect post-natally. It's going to depend on the concentration and the species of mercury that you're talking about. Is that an answer to your question? Is that suitable?

Q What kind of mercury are in-utero infants normally exposed to? What species?

A The in-utero infant, as you call her or him, is exposed to what the mother has been exposed to. The mother may have been exposed to methyl mercury from fish that she ate, and that methyl mercury and various forms of it can be stored in the woman's body. The recommendation that many of us have, and the recommendation I think that many countries now have -- Sweden, Norway, and we're trying to get it through the FDA in this country at the present time -- is that women of child-bearing age and pregnant women should not eat fish that contain a great deal of methyl mercury.

So one source of the mercury in that infant would be the methyl mercury that comes from the mother.

Another source of mercury in that infant in utero would be the mercury that comes from any amalgams that she may have in her mouth. Those are -- the third source, of course, would be if the mother has had a vaccination of some kind. I don't remember which vaccine it is, but certainly some women do get a vaccine sometimes during pregnancy, or if not before. And this mercury is stored in a woman's body.

And so when the child, when conception occurs, and during the maturation of the embryo, there is mercury passing from the mother in the blood to the infant. Three possibilities of virus sources. One is fish, the second is amalgam, and third is vaccination.

Q So does the methyl mercury consumed by the mother and passed on to the infant, does that contribute to autism?

A You're asking whether methyl mercury contributes to autism?

Q Prenatal exposure to methyl mercury. Or prenatal exposure to dental and --

SPECIAL MASTER VOWELL: Could you talk in the microphone, please?

BY MS. RENZI:

Q I'm asking if the prenatal exposures to mercury, you said methyl mercury through fish consumption, through dental amalgams, do those contribute or cause autism? That prenatal exposure.

A I said that an infant, in utero, would be exposed to the mercury that's in the mother. The mercury in the mother could come from fish, amalgams, or vaccinations.

Q I understand that. But does it cause or contribute to the autism? If a child develops autism,

can you look back to the methyl mercury exposures and prenatal exposures?

A Okay. It would be very reasonable to believe, as some of us do, that the methyl mercury in the mother will eventually be converted in the child's brain, to eventually, some of that methyl mercury would get into the child's brain.

And through these various sources, when the child is born and in early childhood, there will be an accumulation of mercury in that child. And there is a prevailing thought by many, that many people have, that the vaccines could be the trigger, what pushes the toxicity of the mercury over the threshold to cause autism. That is one of the theories.

Q So is it your opinion that the exposure to methyl mercury can cause autism?

A Again, we're getting into terms that you've got to be more specific about. The child is exposed to thimerosal. The thimerosal is metabolized to ethyl mercury. One might say, as far as the true definition of exposure, the child is not exposed to ethyl mercury. The child has thimerosal converted to ethyl mercury in the body, and that ethyl mercury then travels to the brain and to other tissues and is deethylated to get mercuric mercury.

So if you ask me is a child exposed to ethyl mercury, I would say we're dealing with semantics.

Q You know, it might be helpful if we put up the chart that you had up yesterday about different roots, different exposures to mercury.

A Is this the one that you want?

Q Yes.

SPECIAL MASTER VOWELL: And we are on, what page is this?

MS. RENZI: Slide 23.

SPECIAL MASTER VOWELL: Okay, slide 23, Petitioner's Trial Exhibit 2.

THE WITNESS: It's entitled, "Influence of the mother and other sources for mercury exposure of infants." Is that the one you want?

MS. RENZI: Yes.

THE WITNESS: I'm sorry, I don't know your name.

MS. RENZI: My name is Ms. Renzi, Linda Renzi.

THE WITNESS: Pardon?

MS. RENZI: Linda Renzi.

THE WITNESS: Thank you, thank you, ma'am.

MS. RENZI: Sure.

//

BY MS. RENZI:

Q Now, Dr. Aposhian, from your chart and from your testimony yesterday, we get methyl mercury from the mother through fish consumption, through chicken consumption, and we get inorganic mercury from dental amalgams, and then if the mother has had a thimerosal-containing vaccine, the child is exposed to ethyl mercury. The mother. Is that correct?

So in utero, the child would be exposed to methyl mercury from fish, chicken, inorganic from amalgams.

A Yes.

Q And if the mother has a vaccine, ethyl mercury.

A Yes. If the mother has -- yes, yes. Ethyl mercury, yes.

Q And post-natally, the child is exposed to methyl mercury from breast milk, methyl mercury from fish consumption, methyl mercury from chicken. Dental amalgams I guess if the child would be old enough to have fillings.

A Or from the mother.

Q Or from the mother.

A Via breast milk.

Q And then thimerosal-containing vaccines.

Now, what is the basis for your opinion, given all the methyl mercury exposure, that the child has, both in utero and post-natally, that it's a vaccine, 12.5 micrograms of ethyl mercury that tipped that child, triggered that child to have autism?

A Well, it also could be 187.5 micrograms of mercury. That is what a child gets after a set of vaccinations. So there's a big difference from 187 as compared to 12.5. However, you could say that 12.5 chronically, over a period of time, might also cause such effects.

What we are pointing out, what we have proposed as a theory, not only by me, by many other people, is that one possibility for the cause or the etiology of autism is that the vaccine is enough to exceed the threshold of what some children may have, the amount of mercury that some children may have in their brain.

Q So are you saying today you need 187.5 micrograms --

A What about that --

Q -- of ethyl mercury? Are you saying that you need the full vaccine?

A I'm not saying how much you need. All we're saying is there's a good possibility that that amount

of mercury given in vaccines could trigger the cause of autism.

But please remember, there is a tremendous variation, as I hope we showed you in the data yesterday, there's a variation, a variability in how children respond to the same amounts of vaccine. Some had eight times greater, I think, or five to eight times greater amount of mercury in the blood; others had almost no mercury in the blood at a given time.

Q If you have so much exposure from methyl mercury in utero and post-natally --

A I'm not certain, you're saying so much exposure. It depends on the person's diet. It depends on the woman, whether a pregnant woman is going to eat tuna steaks, it depends on whether she's going to eat a tunafish salad sandwich every day for lunch, as they used to do in the past. Most of the women at our university -- we're not an Ivy League school, our tuition is among the lowest, we get young women who don't have very much money, and they have to be very careful in what they eat. And when they come to us to begin with, they're usually eating tunafish sandwiches, of which we recommend they do not.

It depends on who, if the person you're talking about is, what their diet is, as far as how

much methyl mercury gets into that person.

Q Well, that's actually my next question, then. If there is a high consumption of methyl mercury by women, pregnant women, such as in the Seychelles, is there a higher rate of autism in the Seychelles, where there is a large fish consumption?

A That's a very good question. Because again, as in the Iraqi study, when the Seychelle Island study began, no one even thought about studying, about testing the population for autism. It's my understanding in conversations I've had with Dr. Clarkson that they are looking into that now.

But let us also state that the Seychelle Islands may not be an example of what happens in the rest of the world. The Seychelle Islands, their diet, being a tropical country, is very high in citrus fruit. And it has been shown by a superb young woman epidemiologist in Montreal doing a study in Brazil that the diet is important as far as the toxic effects of methyl mercury.

The women that ate citrus fruit did not have as many toxic signs of methyl mercury. So it's a very complex phenomena, and a complex question that you're asking.

Q Now, have you read the report of Dr.

Clarkson?

A Of who?

Q Have you read the expert report submitted by Respondent of Dr. Clarkson?

A Yes, absolutely, I did.

Q And do you know what he said about autism in the Seychelles? I have a quote for you.

A You can quote. I don't have it with me. I do --

Q It's on your screen.

A Oh, fine. It's one of the things that I did underline when I read it about a couple weeks ago. And what does he say?

Q He says -- this is at Respondent's Exhibit K at page 6 and 7. He said, "In some 30 years of detailed pediatrics, in neuron physiological tests on large cohorts of these infants who have continuously elevated mercury blood levels, I have found no evidence of an increased prevalence in autism.

"Admittedly, we did not specifically look for autistic children. But many of the neurocognitive tests we carried out, none of which uncovered neurological deficits would surely have detected such cases."

A Is this published? Is that what you just

said?

Q Pardon me?

A Did you say --

Q I said this is his opinion in his report, about finding, whether they found autistic signs or symptoms in the Seychelles.

A So one could say that the Seychelle Islands population may not be typical of the way people react to methyl mercury, because the Faroe Islands say something entirely different.

Not only are they genetically different, the seafood they eat, I think most people will agree, are different, and their diet is certainly different.

I don't argue with Dr. Clarkson at all. I have great faith in what he says. If he says that they don't find it in the Seychelle Islands, I have no reason to disagree with that.

Q Now, I recall from your testimony in Cedillo, you said there is no citrus in the Faroe Islands, is that correct? To your recollection.

A When I visited the Faroe Islands, we had an international meeting on methyl mercury there, I think about the year 2000, I don't remember the exact year. To try to find, to buy an orange there, it was very unusual. There are almost no trees left on the Faroe

Islands. Okay? So I doubt that these people have as much citrus in their diet as they do in the Seychelle Islands.

Q And do you know whether there is a higher rate of autism in the Faroe Islands?

A I have no idea.

Q Would you be surprised if there wasn't?

A I'd have to stop and think about that. It's a very important question, and I just would not like to make a snap judgment like that.

Q I want to refer now to page 9 of your report.

(Pause.)

A I'm all thumbs, my apologies. I have page 9 now.

Q Okay, you have it. You have a comment on the top of page 9 of your expert report that says, referring to the articles authored by Dr. Clarkson and Dr. Magos, that those articles should be viewed, and I'll quote, "cautiously, as current scientific investigations may render some of their conclusions false, inaccurate, and outdated." And you were referring to the review articles of Dr. Magos and Dr. Clarkson.

A May I make a comment?

Q Yes.

A I'd like to apologize to Dr. Magos and Dr. Clarkson. I don't quite understand how that got in. I have a great deal of respect for them.

However, times do change. I think both their papers are very deficient in the idea of hypersusceptibility and polymorphism. This is not meant with any disrespect, and I hope it does not harm my friendship with Dr. Clarkson, who I have a great deal of admiration for. But their papers tend to be deficient in the genetic aspects of mercury toxicity.

Q Were you aware, Dr. Aposhian, that 47 out of your 54 peer-reviewed articles regarding mercury refer to or rely on reports or articles by Dr. Clarkson or Dr. Magos?

A Say that again?

Q About 84 percent of the articles --

A I have a great deal of respect for them. I don't know what your question is, ma'am.

Q My question is, you don't believe now that their review articles --

A I don't say that.

Q -- should be viewed cautiously.

A I think all review articles should be reviewed cautiously. My review articles, they are

some of the most quoted ones in the world on arsenic that were recently published, I tell my students they should be viewed cautiously.

We have an exercise in graduate school where we give students a paper and say find out what is wrong with this. We teach our students to be skeptical. No paper is perfect.

However, I do want to apologize to Dr. Clarkson and Dr. Magos for this statement that is in here. I meant no offense, and how it got in there is difficult for me to understand. "They must be viewed cautiously, as current scientific investigation may render some of their conclusions false, inaccurate, or outdated." I absolutely retract that statement, and I apologize.

However, I do think that no review article is perfect, including my own, as well as Clarkson's and Magos's. But no disrespect is intended to these two fine gentlemen and scientists.

Q Doctor, before this trial started, did you discuss any of the mercury parts of this case with any of the other experts? With any other of Petitioner's experts? Did you talk to Dr. Kinsbourne?

A Did I discuss this report?

Q This report or your testimony yesterday.

A Now, can we do it one at a time?
Q Yes.
A Is the first question, did I discuss this report with any expert?
Q Yes.
A Yes, I did.
Q Who did you discuss your report with?
A I discussed this, I needed some help in deciding whether we can make certain assumptions, with Professor Dean Carter of the University of Arizona.
Q I'm sorry, I'll clarify this. With any experts that are participating in the litigation today?
A Absolutely not that I can recall. I'm trying to think what mercury experts you -- our attorneys are fine gentlemen. They know mercury. I wouldn't call them scientific experts. I'm thinking about Dr. Gerth, who I know is a superb scientist. I haven't even had a conference with him about this. Our neurologist I just saw again for the first time since the Cedillo trial yesterday.

So I guess the answer to your question is no, I have not discussed this with anyone else that's connected with this trial.
Q Your testimony yesterday, did you discuss

that with any of the experts participating in this trial today?

A Absolutely not.

Q I'd like to go back to your report now.

A Sure.

Q On page 14 of your report.

A I'm on page 14.

Q Okay. You state that there is increasing evidence for neuroinflammatory events being involved with the development of autism. Is that correct?

A That's what it states here.

Q And you cite Pardo, which is Petitioner's Master List 72.

A You're speaking down to the desk. I'm sorry.

Q I'm sorry, Petitioner's Master List 72, just to clarify where this is in the record, for the record. You quote the Pardo paper, or you cite to the Pardo paper, correct?

A I didn't hear that, I'm sorry.

Q The Pardo paper, P-A-R-D-O. Is that what you are relying on for your statement?

A That's one of them. There are a number of papers from the Zimmerman group in particular, from Hopkins, that are superb papers dealing with the

neuroinflammatory events involved in the development of autism.

Q Well, I'd just like to ask you about the article in your report, the Pardo report. The Pardo paper.

A Can I have that paper, since I haven't seen it for a while?

Q Sure.

A Because he has a number of papers, and I want to be certain we're talking about the right one. Thank you. "Neuroinflammation in Autism," by Pardo, Vargas, and Zimmerman.

Q Okay. Did that paper implicate ethyl mercury causing the neuroinflammation they report?

A This review paper I haven't read probably for two or three months. And let's see what their diagram here is.

Certainly, if you look at figure 4, you can certainly include ethyl mercury and environmental effects. "We hypothesize that --" in the conclusions they state, "We hypothesize that environmental factors, for example neurotoxins," which would be mercury compounds, "infections, maternal infections, and presence of genetic susceptibility and the immunogenetic background of the host influences the

development of abnormalities," et cetera, et cetera, "for the generation of autistic symptoms."

Q And are they referring to vaccines, thimerosal-containing vaccines in that article?

A Do they mention vaccine?

Q Yes.

A I would have to read the paper now very carefully. Perhaps if someone has a computer, one might --

Q Well, let's assume that they don't.

A Pardon me?

Q Let's assume that they don't, okay?

A That they don't?

Q That they do not, yes. How do you tie the neuroinflammation to your belief that thimerosal-containing vaccines cause autism?

A My first inclination would be to leave that to our neurologist, who will be testifying later on today.

Q So you have no opinion on that.

A I'm not a neuro -- I don't have an opinion that I can give you just like that, since this is a court of law. I would have to go over the slides I presented yesterday, which do offer a great deal about neuroinflammation, before I could really make a

statement that would be truthful.

And so if you want to give me time to go back over these slides, which we have right here --

Q You gave your testimony yesterday, but without reviewing your slides you have no cogent opinions to give me at this moment?

A I have an opinion, but it's an opinion that I don't want to share with you, because I'm not certain that I, I want to tell the truth. And I'm not positive that if I give you something quickly off my mind, that it will be based on scientific fact.

We, in science, are not known for making rapid decisions. We have very simple minds that have to go in a logical way.

Q Well, we'll move on. I will not ask you to review your slide presentation from yesterday.

A All right, thank you very much.

Q I'd like to discuss Pink's Disease, Pink Disease.

A All right. You must notice, however, that we took everything out of the slides. There is no, there is hardly any mention of Pink Disease in the presentation I made yesterday, number one.

Number two, in the Cedillo trial, I mentioned Pink Disease as an example of how

conservative and reluctant the medical establishment was to make, to declare that the mercurous mercury or teething powder was its cause. And that is an example of a disease that was stopped by government regulation, not by good scientific cause.

Q But in Cedillo we also discussed whether this was a dose-related phenomenon --

A Could you talk in the microphone? I'm sorry.

Q In Cedillo we also discussed, if you recall, whether this was a dose-related phenomenon or an example of hypersusceptibility. Do you recall that?

A I recall that, and I recall all the respondents making a big thing of it, and the respondents in this trial. And I -- I was just told to speak into the microphone myself.

And I certainly did emphasize hypersusceptibility during the Cedillo trial, and I probably emphasized it too much.

However, I believe scientifically that there was a large element of hypersusceptibility in those children with Pink Disease. Unfortunately, I don't think anyone can prove it one way or another, because the literature is very deficient about Pink Disease during the years that it was a disease affecting our

children.

Q But you would agree that the mercury urine levels taken from children with Pink Disease was elevated.

A In some of them there was a variation. In some of them there was an elevation.

Q Which could be a dose-response phenomenon.

A Which could be a dose response.

Q On page 8 of your report, and I believe in your testimony yesterday, you discussed porphyrins.

A Page 8 of the report.

Q Page 8 of your report, you discuss porphyrins.

A Yes, I have page 8.

Q Do you consider yourself an expert on urinary porphyrins?

A Do I think I'm an expert on --

Q Urinary porphyrins.

A I'm not an expert on urinary porphyrins.

Q Can a urinary porphyrin profile, specifically the presence of elevated precoporphyrins, precoporphyrin levels, can that be used to diagnose mercury toxicity?

A Certainly the porphyrin profile changes in people who have been exposed to mercury. There is a

correlation between the amount of mercury exposure of an individual, especially dentists, but not only dentists, and how the porphyrin urinary profile changes.

Q Can it be used to diagnose mercury toxicity?

A It's used by some people.

Q Who is it used by?

A By a large number of people, of physicians who treat autistic children. It also is in the scientific literature, Woods especially, and in most of the new current books it's cited as a way, as one of the changes that occur when people are exposed to mercury.

Now, whether someone wants to use that as a diagnostic tool or biomarker is, of course, up to the individual physician.

Q Do you know if it's accepted in the general medical community as a way to diagnose mercury toxicity?

A I don't think the general medical community, probably with the exception of one or two medical toxicologists, know anything about heavy metal toxicity. I don't think most medical schools teach anything about mercury or heavy metal toxicity to medical students. So that when they get out, very few

of them know about metal toxicity. I think that has been a complaint by many organizations, and many national meetings have been held to try to remedy this situation.

Q Now, porphyrin profiles can't be found in medical textbooks?

A Oh, yes.

Q To diagnose mercury toxicity?

A Again, what I've stated is it depends on who is making, on what the physician making a diagnosis or treating the patient wants to do. There are certainly places in the world now that you can send the urine to have the porphyrin profile done.

Q Can you name one or two?

A There's one at Paris. I think it's the Institute of, I want to say the Pasteur Institute. I don't know what the institute is, but I can, I think the paper is quoted here.

Q That's fine. It's Dr. Nataf's.

A You can also send to the University of Washington in Seattle, and Jim Woods will be very glad to do it for you. He has done, we have published with him, in fact, he had done some for us.

Q Is it your opinion that the unique porphyrin profiles can be used as a biomarker to diagnose

mercury, autism due to mercury toxicity?

A You're asking me a question that really belongs, should be asked of a physician. I don't do diagnosis. I'm not a physician. I'm a research investigator. I use various tools at my disposal. But I do not diagnose humans. That is an MD's responsibility.

Q So an elevated precoporphyrin level, you don't know if that could be used to diagnose autism.

A I'm just trying to give you the most truthful answer that I know. I know of people who use it, who use urinary porphyrin profiles as a biomarker, as a potential biomarker, one of many biomarkers, of questionable use for autism. But that doesn't mean that I approve or disapprove. I just haven't really, I haven't written a paper on the use of urinary porphyrins as a diagnostic tool. I guess that's the best way of putting it.

Q Now, you just referred to James Wood and the article that looked at the dentists with chronic exposure to mercury vapor, correct? That's the article you were referring to?

A That's one of the papers. There were papers from Paris that showed, I think, that some of the porphyrins were elevated or changed in autistic

children.

Q We'll go to that. Let's just, can we just focus on the Woods article right now?

A Sure, okay.

Q And that measured, those were dentists working with mercury vapor, is that correct?

A The dentists were exposed to mercury vapor, among other things, as a normal individual would be exposed to mercury and methyl mercury in the diet primarily.

Q And did Woods, he didn't look, though, to exposure to ethyl mercury, did he?

A He does not mention, Woods does not mention ethyl mercury in his paper. However, I think he may be doing work along those lines at the present time. But I know of no paper -- and I could be wrong -- I know of no paper with Jim Woods's name on it that deals with ethyl mercury.

Q And he didn't look at an autistic population.

A I don't know whether he did or not. I know of no paper that he did.

Q Well, I assume that none of the dentists were autistic.

A I'm not sure that's a good assumption.

There are some very highly performing people, as you well know, that have Asperger's.

Q But he didn't identify any of the --

A He did not identify any.

Q -- dentists as having autism. Did Dr. Woods, and you said he -- extrapolate then -- you said he didn't use or talk about ethyl mercury in any of his papers. So we can conclude that he didn't extrapolate that his study can be used to demonstrate that thimerosal-containing vaccines can cause mercury toxicity.

A I don't think he's been interested at all in the vaccine. I don't know of any paper by Jim Woods that deals with vaccines. Again, I could be wrong. I don't know of any.

Q Okay. The second study you refer to is then the Nataf study? Dr. Nataf in Paris?

A Yes, yes.

Q Is Nataf important to your opinion about porphyrin profiles?

A Again, it would help me if you talked in the microphone. Could you move the microphone over towards you? I'm sorry.

Q How important to you is the Nataf study, in your opinion?

A It shows that autistic children, I think, I've forgotten what the number is. I don't have the paper in front of me.

As I remember it, I want to say probably 120 children, they studied 120 autistic children, I think, and gave them I think DMSA, also. And if I remember correctly, I haven't read that paper for at least six months, but if I remember correctly, the porphyrin profile in the urine went back to normal after they gave the chelating agent to bring the mercury up.

Q Did that study measure levels of mercury in either urine or blood of the subjects?

A I haven't read that paper in six months, so I'd have to see a copy of it to know what they did.

Q Would you like to see a copy of that paper?

A Thank you.

(Pause.)

A They do porphyrin levels, porphyrin levels. They did chelation studies. I see nothing in any figures, I don't know about the text, but I see nothing in any of the figures that they followed mercury levels in the urine. But again, I think the implication is that DMSA would bring out lead and/or mercury in the urine.

I don't know, I don't see in the abstract

either that urine mercuries were studied, but let's see in the methods. The impression I have is that they probably did not do urinary mercury levels, but I have not studied the paper closely. That's the impression I have.

Q Well, without knowing these levels, the mercury blood and urine levels, how does the Nataf study demonstrate an association between mercury toxicity and porphyrin profiles in autistic children?

A They studied autistic children. They gave DMSA, all right? DMSA we know mobilizes and increases the excretions of mercury. And so it's a supposition on their part that these children, when they were given DMSA, not only had a change in the coporphyrin excretions, but also had a change in mercury. That is an assumption on their part.

Q The porphyrin studies by Woods and Nataf were renal porphyrins, correct?

A Were?

Q Renal. It's kidney, urine porphyrins, correct?

A Again, I --

Q The profiles studied in the Nataf and the Woods papers are renal porphyrins, correct?

A Are urinary. You said renal.

Q Urinary porphyrins, correct?

A Urinary. That's the problem, I couldn't connect that word with --

Q Now, Doctor, yesterday during your direct testimony Mr. Williams asked you if the urinary profiles was one, a semi-quote, I'm not sure since I don't have the transcript.

Mr. Williams asked you if this was one genetic marker to this efflux problem. And you responded yes. And can you explain how a pattern of kidney porphyrins has any relation to a biomarker for mercury efflux?

A I would like to know what my direct quote was. I wish you --

Q Your direct quote was "yes."

A -- would give me the direct quotation, in the context that I made it.

Q I believe you said yes to the question if this was one genetic marker, referring to porphyrins, one genetic marker to an efflux problem.

A To an efflux problem.

Q Yes.

A Yes. One would expect that if mercury were accumulating in the tissues, that the porphyrin profile would change. Does that answer your question?

Q Is it a genetic biomarker for mercury efflux?

A It probably would be, because of the dentists. It was clearly shown that 15 percent of the dentists had a different urinary porphyrin profile because of the difference in the way they metabolized porphyrins.

Q Do urinary porphyrin profiles tell you anything about the presence of levels of mercury in the brain?

A In the brain?

Q Yes.

A I don't, I don't know.

Q And on page 8 of your report you also describe a polymorphism that causes elevated --

A Can I get to page 8 first, please?

Q Sure.

A Thank you. What part of page 8 are we dealing with?

Q Pardon me?

A What part of page 8?

Q The CPOX-4 polymorphism.

A Are we talking about the first major paragraph? The urinary porphyrin profile was found in 85 percent of the dentists, with 15 had an atypical

porphyragenic response? Is that what we're talking about?

Q Yes. Yes.

A And what about that, please?

Q Are you referring to the CPOX-4 polymorphism?

A We are referring to what Jim Woods I guess would call the changes in the metabolism of coporphyrins.

Q Do you know whether that's a CPOX-4 polymorphism?

A I don't have the metabolic shot in front of me. And with the long names of porphyrins, coporphyrins and all, I really would rather have a metabolic chart in front of me so I can trace the metabolic pathways before I come up with a word that --

Q It's your opinion that there is a genetically susceptible population who, in response to ethyl mercury exposure, develop autism, correct?

A It's my thinking that there is a population that, when exposed to the thimerosal vaccines, will develop, some of them will develop autism.

Q In the genetically susceptible population that you believe exists, those are the children that

cannot excrete mercury? Is that correct?

A I would not say they cannot excrete mercury. I would say they cannot excrete mercury, as much mercury as is normal, and as much mercury as they should excrete if they did not, if they were normal.

Q And you call this a mercury efflux disorder?

A We call it a mercury efflux disorder, similar to the copper efflux disorder known as Wilson's Disease.

Q And this genetically susceptible population, we don't know the rate, whether its efflux is reduced by 50 percent, 25 percent, the excretion of mercury. We don't know.

A We don't know that, because there has not been enough research yet. The idea of a mercury efflux disorder was first presented at the IOM Symposium, which I think was in the year 2004. It takes time to do such experiments.

Q And does the inability to excrete mercury cause a form of mercury toxicity that results in autism?

A Let's go over that sentence very slowly now. Would you please repeat it slowly, section by section?

Q Does the buildup of mercury, because a child cannot excrete the mercury, does that result in a

mercury toxicity that leads to autism?

A In my opinion, yes.

Q And what is the basis for your opinion?

A We would have to go back and look at the, all right, the basis, number one, the experiments by Holmes and Haley, Haley being at Chemistry at Kentucky. The experiments by Bradstreet that show that if you give the DMSA or mercury-mobilizing agent, more mercury comes out of these kids, as compared to controls.

You must mention the Adams study, in which baby teeth were used as an indication of the mercury content of the tissues.

Q And Doctor, I don't mean to interrupt, we'll go over the studies later. I'm not asking the basis for your belief that there is a mercury efflux; we'll get into that later.

But does that result in a toxicity that causes autism? Is there a toxic --

A I think we're talking about the same thing. Or if we're not, I don't understand why we're not.

We believe, I believe that mercury builds up in the tissues, and that mercury level in the brain, because of the metabolism of ethyl mercury to mercuric mercury, causes, is one of the causes, one of the

causes of autism.

Again, you must realize the autism spectrum of disorders are a broad band of diseases. They are all different diseases. Different kids with so-called autism react differently to given therapies.

Now, if you're asking me whether I think mercury toxicity in the cells is one of the causes of autism, there is no question in my mind that it is one of the causes of autism. And the addition, the injection of a thimerosal-containing vaccine can be the trigger, making that child go over the threshold of a disease process. Does that answer your question?

Q I'm not sure. Do you agree with Dr. Deth and Dr. Mumper that most children with autism suffer from mercury toxicity?

A Yes. Well, let's say, see, science is quantitative. Science is numbers. I would hate in this sense to use an adjective, "most." I would say a great many. Even that's bad. A certain percentage of children with autism in my opinion clearly suffer from mercury toxicity. What that percentage is, I don't know.

Q Is your genetically, the genetically susceptible population that you believe exists, are they unable to excrete all forms of mercury? Or is it

just ethyl mercury?

A I really don't know, because that study hasn't been done.

Q But it's your belief that there is a mercury efflux disorder. Is it a mercury efflux disorder or an ethyl mercury efflux disorder?

A There's a mercury efflux disorder that they cannot get mercury out of their cells to any great extent.

Q So you think it's all mercury; methyl, ethyl.

A It can be anything. But the ethyl mercury, we must understand, when it gets into the brain is deethylated. Because it's very, very toxic mercuric mercury, which, when injected directly into the brain, as you pointed out earlier in the slide that you brought up, has very toxic, causes neuronal necrosis.

Q Methyl mercury demethylates into mercuric mercury in the brain as well, correct?

A Pardon?

Q Methyl mercury demethylates --

A Yes.

Q -- into mercuric mercury in the brain, as well.

A Yes.

Q Thank you.

A But it's not the methyl -- more of it is excreted. It's the excreted part, it's removed more rapidly from the brain, so that the amount of mercuric mercury formed from a given dose of methyl mercury is less than, the percentage is less than the conversion of ethyl mercury to mercuric.

Q Excuse me, did you say methyl mercury is excreted faster than ethyl mercury?

A No, I thought I said -- let me reword it to be certain -- that methyl mercury is removed from the brain faster than ethyl mercury is removed from the brain. This is based on the infant monkey studies of Burbacher, where speciation was done, and it was clearly shown that of the two, of the total mercury remaining in the brain, the animals getting ethyl mercury had a higher percentage of inorganic or mercuric mercury.

Q Is mercury efflux a lifelong condition?

A We don't know that much about it. I would expect so. We don't know.

Q You don't know.

A I guess that's the best way of putting it; we don't know.

Q And is the only outcome of mercury efflux

disorder autism?

A Again, as it took them 100 years to show that Pink Disease was due to mercurous mercury in the teething powder, it's probably going to take the medical community another 50 or 100 years to come to any conclusion about the mercury efflux disorder.

And no disrespect is meant to the medical establishment. I think it's clearly accepted that the American medical establishment, one of its strengths actually is its conservatism.

Q So in the mercury efflux disorder, you're not removing mercury from the tissues as well as the brain, is that correct?

A Yes. It's probably a matter of degree. There's more mercury accumulation in the brain because the mercuric mercury cannot get out of the brain. What mercuric mercury is bound to also cannot get out of the brain as easily as mercuric mercury gets out of tissues.

The greatest concentration of mercuric mercury in the body usually is in the kidney.

Q Assuming that mercury efflux disorder is all mercury, and assuming it's a lifelong condition, what mechanism do you know of that would just cause autism without resulting in clinical signs of mercury

toxicity?

A Well, I think if you talk -- I'm not a clinician, as you well know, I'm not an MD, as you asked me many times. I'm not a neurologist, as you well know.

But if you talk to many of the physicians who treat and diagnose autistic children, they will say that they see very similar signs of, they see very similar -- they have similar diagnostic evidence for mercury toxicity in some of their autistic children.

I'm not a clinician. I don't want to make that statement. I'm telling you what I am told.

Q Can you reconcile this position with the one you took in -- do you remember you spoke at the 2004 Institute of Medicine? Where you said that the signs and symptoms of mercury poisoning are so indefinite and non-specific that you can come to any conclusions that you want.

So is that different than your opinion today, that --

A Can I read this?

Q Sure.

A It's four years ago, you know.

(Pause.)

A There is a term that we've used, that I

thought we used here, called micromercurialism, all right? And it's usually meant to be a, people that have been exposed to what is normally considered below-toxic amounts of mercury, but will have signs, some signs, some non-specific signs if you will, of mercury toxicity. And the term micromercurialism I think is certainly in the literature.

And so what should have been said here, and what I'm saying is the signs and symptoms of micromercurialism or micromercury poisoning are so indefinite and so non-specific that you can come to any conclusion you want.

And to some extent, that's also true with anything but extremely severe mercury toxicity. Many of the signs of mercury toxicity are signs and symptoms seen in other diseases. Many people -- that's one reason why a good clinician, I'm sure you'll agree, will want a mercury determination done on the blood, and/or on the urine, before the term "mercury intoxication" is made. Most of the board-certified clinical toxicologists that I know of would make that statement.

Q If a person had efflux disorder, the genetically susceptible population has an efflux disorder, can they experience mercury toxicity from

eating fish? Too much fish?

A Probably. Yes, I would say they could. Again, depending on how much fish they eat, and how old they are, and what their genetic predisposition is.

Q Is there any evidence that such a thing is occurring? Mercury toxicity from fish consumption, or children nursing? Breastmilk with methyl mercury in it?

A The first question is, is there any evidence of mercury toxicity from fish eating?

Q Yes. In this country.

A Absolutely. In the Philippines, in Brazil, many of these people, there are huge amounts of scientific literature that show that people who eat fish, especially near gold-mining, where mercury is used to amalgamate mercury, the mercury gets into the water. The fish consume that water. There is a tremendous amount of literature.

In fact, that's the Minamata story, also, where, in Minamata, these people had, the factory dumped mercury into the river; the river empties into Minimata Bay. The fishermen, the cats, the birds ate this, ate these fish, and they got sick. There's no question about that.

Q There is no question about that, but those were huge doses of methyl mercury.

A Of course. But you didn't mention huge doses.

Q I'm saying we have a genetically susceptible population who cannot efflux mercury. They eat tunafish, cans of tunafish. Can they suffer mercury toxicity?

A Yes, they could. If they are hypersusceptible, or if they have a genetic predisposition, they certainly could.

Q And is there any evidence of mercury toxicity due to a lack of excretion of mercury that results in mercury toxicity from tunafish consumption?

A There certainly is a paper that one of the respondents has criticized from, I think it's the University of California. A physician that's a woman who showed that many of her patients who were eating high levels of tuna steaks and other things, and other high-priced fish, had health complaints. And when she recommended that they stop eating fish for six months, the complaints disappeared.

The criticism of these papers is that she did not determine mercury.

Q And that's the Hightower study, correct?

A Thank you very much, the Hightower study.
Q So we don't know if these people were genetically susceptible because of efflux.
A That's correct.
Q So that paper doesn't demonstrate an efflux disorder.
A But you asked me whether I knew of any, I thought you asked me did I know of any case where eating tunafish or fish could cause mercury toxicity.
Q Due to an efflux disorder. So you would expect --
A Due to an efflux disorder, we have no evidence in those cases that they had efflux disorder.
Q Now, in your testimony yesterday and in your report, you talk about a spectrum, a band, of autism. And I think we can pull it up. It's certainly not as colorful as the one you had yesterday, but we have it on the screen here.
Can you identify where, on this band, children who develop autism from thimerosal-containing vaccines would fall?
A We don't know the various, the various kinds of autisms. The various severities of autism have not been quantifiably presented by the medical community. So there is no way that I could fill in the bands

between Asperger's, which is high-functioning ASD, or autism, which is the most critical cases where they are severely affected.

My point is there is nothing that I know of that we can put in the middle. That I know of. There are probably physicians that could put some things in there, but they would not give us very many bands. That's why it's called the autism spectrum disorder.

Q Is there any evidence that there is more mercury in the brains of autistic children, compared to non-autistic children?

A I thought there was a paper that I quoted in my presentation yesterday that showed that autistic children had a high amount of mercury in their brains. I'd have to go through my -- will you give me a minute to go --

Q You don't know if off the top of your head?

A Pardon?

Q I mean, you gave this, you gave your testimony yesterday; you don't recall what article you're referring to?

A I think there are 135 or something slides. I certainly could not quote every one of those slides to you.

Q Would this finding be critical to your

opinion?

A I don't understand your question.

Q Is this article critical to your opinion?

A It is one of a series of articles that I think is very important -- but again, I would like to look at it on my slide set before I made a statement about it, because it has been a very traumatic couple of days, as I'm sure you realize.

Q I won't have you do that. We'll just move on.

A All right.

Q Is mercury efflux disorder recognized in the general medical community?

A I would say I don't know. I do know it's recognized in a large number of physicians who treat autism.

Q Does Dr. Mumper recognize it?

A Pardon?

Q Does Dr. Mumper recognize mercury efflux disorder?

A You'd have to ask her.

Q Okay.

A I know that I've been invited to various think tanks that deal with autism, that are by invitation only, that have anywhere from 20 to 100

people. And during those talks, people refer to mercury efflux disorder. Based on the evidence that I have pointed out, as far as the Adams paper, the Bradstreet paper, and the Holmes paper. And their confirmation by the MIT Group.

Q What think tanks were you invited to? Do you have --

A There was one think tank, a very, very interesting one. There is a small reservation owned by, formerly owned by the RCA Company that was used for worldwide transmission, north of San Francisco about 45 miles. It's owned by a foundation whose name begins with C, and I just don't remember which one.

At that time they brought about 20 people together. They brought two couples with autistic children; they brought four scientists together, of which I was one; they brought some MDs. So it was a small group of people, and mercury efflux, that's one of the meetings that you're asking, a think tank.

The other think tank is by the Autism Research Institute. That's held about twice a year.

Q Is that a part of, DAN is part --

A DAN is, excuse me, DAN is part of that group. And it's a very large group. At meetings there are probably 6,000 people, not at the think

tank, that show up at a DAN meeting. It has been a tremendous help to the autism community, the community with autistic children, because established medicine for many years would not do anything about treating autism.

Autism is now considered by many people to be a recoverable disease, by many physicians. Certainly not by many establishment physicians. But there are certified cases of recoverable disease.

What is sad is that everyone agrees that the more money the parents have, or had, the more likely their child was to recover. And that's because the family could spend all sorts of money in trying every single kind of treatment. It's almost un-democratic.

Q Your hypothesis that thimerosal-containing vaccines, your hypothesis that thimerosal-containing vaccines cause autism. Is that limited to just clearly regressive autism?

A I have not thought about that at all, and I would not want to venture such an important opinion without thinking about it more carefully.

Q You've never thought about this before?

A Your question?

Q Yes.

A No, I have not.

Q Could you think of a mechanism by which mercury efflux disorder could cause only clearly regressive autism?

A Again, I would want to sit and think about it before I made such a statement. I think it still could be an accumulation of mercury at a given time, plus a genetic hypersusceptibility to the mercury. That's about all I feel safe saying at the present time, because this is a court of law and I must tell the truth.

Q You testified at Cedillo and in your report, you have it in your report, that there are hypotheses that there is a specific window of development; that the reason -- and your second hypothesis is the reason not all children who receive vaccinations develop autism is because not every child gets vaccinated at exactly the same point in time. Is that your hypothesis?

A It is part of my hypothesis to explain why more children don't get -- if you look at the story of thalidomide, which is a known, which most -- do you know the thalidomide story?

Q Yes, I do.

A Okay. It is probably one of the worst teratogenic episodes that the human population has

been exposed to. But not every child whose mother took thalidomide during her pregnancy got, got the problem, got the thalidomide, the monster formation, born without arms, born without wrists, et cetera.

There is a window for all teratogenic agents. And there is a tremendous amount of literature about this. And that window is usually very narrow, relatively narrow.

And so it is very reasonable to me that one of the reasons, not the only reason, that children, that all the children getting thimerosal did not develop autism.

Q Now, thalidomide was a prenatal exposure, correct?

A Thalidomide was a prenatal exposure.

Q So the window was prenatally.

A Yes. But there are other teratogenic agents known that have an effect on the window post-natally.

Q And what are those?

A I think, I'm not positive, valproic acid may be one. But the literature, if you pick up any book, and I'm not even sure about valproic acid being post-natally. But if you pick up any toxicology textbook, there's usually a chapter on teratology, and lists agents that are affected prenatally and post-natally.

Q And you just listed prenatal ones in your report, for example.

A Pardon?

Q You just listed prenatal exposures in your report, for example.

A That's right.

Q Is the window, does it apply only to a genetically susceptible population? Or is it just a timing issue?

A I think it's both. But there is no question that in teratology, there has to be a predisposition to that effect of the metal. There has to be a predisposition.

And so time, and since that predisposition to an agent also has a window, there has to be a time element there, also.

Q And is that predisposition mercury efflux, and then the window?

A That predisposition would be that one, that the child would be deficient in the mechanism for bringing mercury out of the cells; just as the Wilson's Disease patients don't have the gene for the copper transport protein that brings copper out of the cells.

We use Wilson's Disease, or hepatolenticular

degeneration as it's called, as an example of an efflux disorder, which is accepted by the medical community. The conservative ones, also.

Q But if there's a very narrow window, how is there an accumulation? So you're accumulating mercury, and then there's --

A Oh, be careful.

Q -- a very narrow window?

A Be careful. We're not talking about accumulation in that window. We're talking about an effect in that window.

In other words, when my arm -- and think of me as an embryo, if you will.

Q I'll try.

A When the DNA tells my arm to begin developing, well, the arm just doesn't shoot out. The first thing that happens is something, a bud is going to occur here, and there's going to be some kind of a hormone, or something that will come in and say hey, make that arm a little longer. And ah, then all of a sudden we have an elbow. And then again another.

So this process can take maybe a week or a month. But each part of it, each part of it is a window. Are you with me? So that that window can be very narrow.

Maybe thalidomide had no effect way out here when my fingernail was formed, but thalidomide may have an effect up here when my shoulder is being formed.

So the window, the window is when some particular morphological event is occurring, so that the arm will continue to develop. And that development, if you have a teratogen around, is based on a number of things. It's based on the width of the window, the time element, the concentration of the teratogen, and the predisposition of the mother and the predisposition of the embryo, through the toxic effects of that teratogen.

Q So dose is important. Because I thought from your report --

A Dose is one of the important factors.

Q Okay. With autism, are you talking about a neurodevelopmental window?

A I'm trying to look at the whole picture. There certainly is part of the development of autism, as far as a neurodevelopmental window. Which neurodevelopmental window that is, we don't know. If we knew, we would know the cause of autism.

Q So if I put up William Mead's vaccination schedule, for instance, one of the Petitioners in this

case, can you tell me where the neurodevelopmental window would be?

A You must remember that a child's brain does not stop maturing, does not stop being formed until at least puberty. So to ask anyone what window is going on at this time is almost an impossible question.

At each of these times, that child is maturing, that child is developing. His brain is developing. Now, what brain function is developing at 2.5 months of age, or how many brain functions are developing at 2.5 months of age, I would leave that to a neurologist, or a developmental neurologist, say.

Q So you can't tell me what's going on in the brain when that window is open.

A I can't tell you because I don't know which window you're talking about. We have many windows.

Q The window, the window that when they receive the thimerosal-containing vaccine, it leads to autism. That window.

A If we knew that, we could cure autism. We don't know it. No one knows it.

Q And we don't know what part of the brain the window, we don't know whether it's during the development or what part of the brain --

A Well, we probably will know it soon because

of a paper that Burbacher may have impressed, or at least he's about to submit, whereby he has used a technique to not only determine the concentration of mercury in the brain of infant monkeys who got thimerosal, but also is able to localize, visually localize where the mercury is in various parts of the brain, in certain sections of the brain. That may be a very valuable tool to tell us what's going on.

But at the present, we don't know.

Q Now, you said these windows are very narrow, correct?

A It depends on which window you're talking about, and it depends on the definition of "narrow." Some are narrow, some are narrower. They're not large.

Q Well, it's your hypothesis, so why don't you tell me? I mean, tell me when the window is, how long it lasts, and how it leads to autism.

A It took the thalidomide people almost four years to come up with the window for thalidomide. We have not, I don't know of anyone that is looking for that window at the present time, because the federal government, the NIH in particular, has been very reluctant to support research on autism.

When you stop and think that more money is

spent trying to keep a man like me alive, an old man, with research on cancer and high blood pressure, millions are spent by the National Institutes of Health. Whereas when I die, it's not going to cost very many people anything.

But when a child gets autism, it's going to cost society at least, what was the figure, $3 million or $300 million. And very little money in comparison in this country is spent on research to help the children, in particular to find out the cause of autism. And this is where my faith in democracy was restored, because we would not have autism research going on in the National Institutes of Health today if the parents of autistic children did not go to Congressman Burton, and a whole series of congressional hearings were set up, and pressure was put on the National Institutes of Health to do autism research.

It's very difficult for me to say that. I'm a child of the NIH. My complete education and research have been supported by the National Institutes of Health. But it's the one time that I've been ashamed of the National Institutes of Health, that they did not support decent research on autism.

Q I'm going to go back to your windows.

A All right.

Q During this window of vulnerability, does any, is it just mercury that can have an effect on the development of this child? Or is it any --

A Again, we don't know, because we don't know what mercury -- I'm sorry, we don't know what window we're really talking about. But usually, usually windows are susceptible to many agents.

Like the thalidomide window has been proposed by some people as being the autism window. But I don't think the medical community accepts that as a whole.

Q Now, in Cedillo you said your narrow window was a hypothesis. Has anything come out, any peer-reviewed articles, that is this anything more today than a hypothesis than it was in Cedillo?

A A hypothesis as to the mercury efflux? Or the hypothesis --

Q The window of susceptibility.

A Window of susceptibility.

Q The timing issue.

A I think I would guess that most clinicians would agree that any kind of a toxin given during the child's development pre- or post-natally would have a potential window of effects. I don't think, well, I

don't think any embryologist would disagree with that, and I don't think any pathologist would disagree about that. And as far as medical toxicologists, I was certainly not one to put words in their mouth.

Q But your window is not for, your window is for 12.5 micrograms, or 25.5 micrograms. We're not talking toxic doses if they're in a particular window; we're talking about micrograms, is that correct?

A We're talking about micrograms that are being given to a child who may have large accumulations of mercury in his or her tissues already.

Q Because of mercury efflux disorder.

A Pardon?

Q Because of mercury efflux.

A Because of exposure via the mother, transfer of mercury through the placenta, and because he or she has an efflux disorder.

Q On page 24 and 25 of your report --

A I have so much paper here.

Q I'm just going to get a sip of water.

A Are you going to show it on here, or no?

Q No.

A Okay, I have page 24 now. I have page 24.

Q Okay.

MS. RENZI: Special Master, I have maybe 35 more minutes of questions. Do you want me to carry on and finish it up?

SPECIAL MASTER VOWELL: I'd say go ahead.

MS. RENZI: Okay.

SPECIAL MASTER VOWELL: Does anyone need a break at this point? Dr. Aposhian, could you use a break?

THE WITNESS: I'd love to have five minutes just to collect my thoughts, but I don't need anything more than that. But it's not necessary, Special Master.

SPECIAL MASTER VOWELL: Let's go ahead and take our mid-morning break, then. We'll resume then at about 10 minutes to 11:00.

(Whereupon, a short recess was taken.)

SPECIAL MASTER VOWELL: Please be seated. All right, we're back on the record in the Theory II and the King and Mead proceedings.

Ms. Renzi, you may proceed. Dr. Aposhian is back on the witness stand. And once again, I remind you, sir, that you're still under oath. I remind you that you are still under oath.

THE WITNESS: Thank you, ma'am.

//

BY MS. RENZI:

Q Dr. Aposhian, on pages 24 and 25 of your report.

A Yes, ma'am.

Q You list six pieces of evidence that you say if taken alone leave some doubt, but if taken together implicate thimerosal as the etiology of some autism spectrum disorders. Do you agree with that?

A Yes, ma'am.

Q I'd like to go through those six pillars. And I know you've discussed this yesterday, and we'll discuss it today.

The first one is the Adams study, the tooth study. And I believe you wrote and testified that this study found that teeth from autistic children contained more mercury than those of non-autistic children, correct?

A Yes.

Q And that demonstrated that autistic children have a higher body burden of mercury than non-autistic children. Is that an accurate description?

A I have the statement here, just that Adams demonstrated that teeth from autistic children contained more mercury than those of non-autistic children.

Q Is it your opinion that that demonstrates a higher body burden of mercury in autistic children, compared to non-autistic children?

A Do I say here that it does?

Q I'm asking if that's your opinion, can you conclude that.

A Oh. The teeth have been used as -- the answer is yes.

Q Can you cite to any peer-reviewed articles that demonstrate that tooth mercury concentrations reflect mercury body burden?

A Would you repeat the question, please? Talk into the microphone, please.

Q Can you cite to any peer-reviewed articles that demonstrate that tooth mercury concentrations reflect mercury body burden?

A I can just state that there is evidence that lead, zinc, and other metals, including mercury, which are increased in teeth, are a reflection of the amount of mercury in the body and the other tissues. This has been clearly shown for lead by Needleman in his very classic studies on lead, and it's been shown by other people, too. The teeth have been used as a marker.

Q For mercury body burden?

A For mercury in this case Adams has done.

Q You testified in Cedillo that teeth are not excretory organs, correct? Are teeth excretory organs?

A Did I say it was an excretory organ?

Q You said it was not.

A Yeah, I haven't thought about it. It's considered to be a tissue, an organ, that is, that takes up phosphate, it takes up calcium and many other things.

Q Now, you stated in your testimony today, and I believe yesterday, that all papers are subject to review and criticism. You said all papers, any peer-reviewed article, you show them to your students and you get criticisms of all papers that are peer reviewed. Is that correct?

A Yes.

Q What are your criticisms of the Adams study?

A Of the --

Q Adams study.

A I think most people -- well, probably the, I don't have the paper in front of me, probably the number of controls that he got could have been increased. That's probably true with many studies, that the number of autistic, the number of teeth, the

number of autistic children that contributed teeth and the number of control children that contributed teeth is probably small.

Q Do you recall how many controls or --

A I don't have the paper in front of me. I read over 100, I read many papers. And there's certain things that I know I can just go back and look at.

Q Would you like us to hand you the Adams study?

A Pardon? Okay, thank you.

(Pause.)

A N was 15 with autism spectrum disorder, and N was 11 typically developing children.

Q Were the levels of mercury found in the autistic, in the teeth of the autistic children, were they indicative of mercury toxicity?

A I don't think there have been enough studies performed to have used teeth as an indication of mercury toxicity, or teeth as a biomarker. I don't know of any studies that have taken teeth from severely mercury-toxic people, and examined for mercury.

Q Well, what was the average mercury level of the teeth in the autistic children, reported by Adams?

And you can see table 2 on page 4, if that's helpful.

A For autistic children it was 0.15.

Q Is that indicative of mercury toxicity?

A I don't know. But it is more than what's in the controls.

Q Do you know whether mercury levels vary depending upon the tooth? Whether it's an incisor, a canine, a molar?

A I don't know that.

Q Would you expect mercury concentrations to vary depending upon the sex of a child?

A Yes.

Q Do you know whether the Adams study controlled for gender?

A I don't know where -- usually there are more autistic boys than there are autistic girls. And I would just have to see where, number of male and female, there were 81 percent males in the autistics, and 45 percent in the control.

Q So he didn't control for gender.

A No, he doesn't control for gender.

Q Does lead concentration affect mercury tooth concentration?

A What?

Q Does lead concentration in the tooth affect

its mercury concentration?

A Does blood --

Q Lead. Does lead.

A Does lead.

Q Yes. Does lead tooth concentration affect mercury tooth concentration?

A You're asking me whether lead concentration affects mercury concentration in the teeth?

Q In a tooth, yes.

A I don't know.

Q Do you know what type of mercury was measured in the teeth? Was it ethyl mercury or inorganic mercury?

A I don't know whether he did speciation or not. Let's see.

(Pause.)

A I don't think they did speciation. I think he's doing total mercury.

Q Do levels of mercury in baby teeth reflect anything, tell you anything about the levels of mercury in the blood?

A In the blood?

Q In the circulating blood.

A I don't know. I think it would depend on the particular genetic composition of the child, but I

don't know the answer to your question.

Q Do mercury levels in the teeth tell you anything about the amounts of inorganic mercury in the brain?

A I don't know whether there has been a correlated study along those lines. I don't think anyone has done the study.

Q The second piece of your pillar is the Holmes study.

A Yes.

Q And that's on page 24 and 25 of your report, if that's helpful, in the Holmes study's Petitioner's Master List 237. And the Holmes study found that hair from autistic children contained less mercury than the controls, is that correct?

A That's correct, as I said in the report.

Q And then from that, do you conclude from this study that autistic children cannot excrete mercury?

A That autistic children, I'm not sure what sentence you're talking about now.

Q I'm asking if it's your opinion, based on that study.

A Yeah. I think this is one of the examples of there's less mercury being excreted into the hair

in autistic children, which is an indication of more of it being, more of it staying in the tissues of autistic children, as compared to controls.

Q What percentage of mercury gets excreted through the hair? What percentage of mercury --

A A very small percent.

Q What percentage gets excreted through feces? Through the feces or through urine. Can you break that down?

A The feces is a major source of, is a major route of the excretion of mercury.

Q How about urine?

A Urine is a route, if you use -- well, first of all, you can use urine as a measure of mercury exposure, of how much mercury is in the body. You can increase the mercury excretion in the urine by giving a chelating agent, like DMSA.

Q Were measurements of either feces or urine, mercury measurements taken in either feces or urine in the Holmes study?

A No.

Q Did the Holmes study attempt to determine or control for mercury exposure in their test subjects before they took hair samples?

A I'm sorry.

Q Did they control for --

A Excuse me, ma'am. But if you would talk directly into the microphone, it would be a big help to me.

Q Did the Holmes study attempt to determine or control for mercury exposure in their test subjects, before taking hair samples?

A They just took hair samples, as far as I know. Again, I read that paper a number of times after it was published. I don't remember whether they did any interviews or anything else about mercury.

Q What studies have confirmed the Holmes study?

A A study from the MIT Group, which had a small number of children, but used an entirely different technique, is an example of a confirmation of the Holmes study.

Q And is that also known as the Hughes study HU? Is that the Hughes study, HU 2003?

A Yes. Yes, it is.

Q How many autistic children were included in that study?

A It was either two or three. It was a small number. I don't -- it's a small number.

Q And did the author of that paper control for

dietary intake of mercury?

A They state quite clearly that they did not.

Q And isn't it true that two of the three autistic subjects had undergone heavy metal detoxification prior to being tested?

A If the paper says so, that's true.

Q And wouldn't dietary intake and mercury detoxification, wouldn't they be factors that would affect levels of mercury found in the hair?

A It would cause more mercury to be found in the hair if the intoxication was for a lengthy period of time. But the hair, it depends on how the hair analysis was done; whether it was done in segments to correlate with time, or it was a complete hair sample.

Q Are you aware of any papers that dispute the findings of Holmes?

A We have quoted the Ip paper as being incorrect. And we have quoted other papers. We have, there are indications that people believe the Holmes paper makes sense now, especially after the reanalysis by, I want to say DeSoto is her name, I think it's DeSoto. It's from yesterday's, it's in the record.

Q Now, the Ip study did not find a significant difference in mercury hair levels between autistics and non-autistics, is that correct?

A The Ip study, I want to be certain we are quoting it correctly. But essentially, that's correct as I recall it.

But the point is that the DeSoto people pointed out they reanalyzed the data originally reported by Ip, et al, in 2004, and found the original p values were in error, and that a significant relation does exist between, they did blood levels, and the diagnosis in autism spectrum disorder.

Moreover, the hair sample analysis results offer some support for the idea that persons with autism may be less efficient and more variable in eliminating mercury from the blood.

Q Did the DeSoto article criticize the findings of the Ip hair study, or just the blood findings? Did they find the hair measures to be incorrect?

A They criticized the blood levels.

Q I've put up on the screen what DeSoto said about the hair levels.

A You're not talking into the microphone, I'm sorry. Forgive me.

Q I've put up on the screen what DeSoto said about the hair studies in the Ip. It's on the screen, sir.

A Oh.

Q It says there was no difference in the mean hair levels. This is essentially the same result as reported in the initial, the original article.

So the DeSoto study doesn't dispute the hair findings of Ip, is that correct?

A I would have to read the paper again very carefully. This statement implies that. I just don't remember this statement, per se.

However, could you go on to say what the "however" is? However, given that hair levels would normally expect to be higher occurring, it might be surprising that blood levels could predict an autism spectrum disorder, but that hair and mercury levels could not. Indeed, hair and mercury levels for the whole sample were correlated.

Q Are you aware of the Fido article of 2005? F-I-D-O. And that's Respondent's Master List Article 138. Are you familiar with that article?

A Can you expand for me? Oh, this is the article from Quake. And so I don't know how good these investigators are. There is no American investigator associated with this study. I don't know whether they used the proper techniques. I don't know. I'm not willing to make, to give an opinion on

this paper.

Q But did it dispute the findings of the Holmes study?

A Pardon?

Q Did it dispute the findings in the Holmes study?

A They dispute the findings in the Holmes study. Could you give me the general reference, what -- that's what I thought, okay. I'm ready to go on.

Q How about the Kern study, 2007? And that's Respondent's Master List No. 274. Did the Kern study dispute the findings of the Holmes study?

A I think it does dispute it.

Q And how about Adams 2006, Respondent's Master List 2?

A Well, let's read that abstract.

(Pause.)

A I don't see in this particular abstract the word "mercury," though I could miss it very, very easily. They have done iodine levels, chromium levels, potassium levels, zinc, lithium. I don't see in the abstract the word "mercury." Again, I could be wrong. So what is the point of this article? Why are you bringing it up now?

Q Does it confirm the Holmes study, then?

A It doesn't try to confirm the Holmes study. As far as I can see, this article does not, as far as the abstract is concerned, does not mention mercury analysis of hair.

Q Are you familiar with the rest of -- I won't ask you to read the article, Doctor, so we will move on.

A I'm just reading the abstract.

Q Okay. We'll move on. I want to turn to chelation now, which you use as -- chelation.

A Yes, what about it?

Q These are the third and fourth pillars of your six pillars.

A This is the Bradstreet report? Is that what you're referring to?

Q We'll start with the Bradstreet report. And that report stated that autistic children treated with DSMA excreted more mercury than controls. Is that correct?

A Yes. They were comparing controls with autistic children.

Q What is DSMA? What is DSMA? DMSA, excuse me.

A DMSA is dimercaptosuccinic acid. It is a

water-soluble, relatively non-toxic chelating agent that mobilizes metals, such as lead, arsenic, and mercury. It is approved by the Federal Drug Administration for the treatment of children with bloodlead levels of 45 micrograms per deciliter of blood or more.

But it also has an off-label use for treating mercury intoxication and arsenic intoxication, because its safety has been proven in children.

Q And it's fair to say that you performed a significant number of chelation studies, is that correct?

A Bradstreet?

Q No, you.

A Yes, that's correct.

Q And you've published several peer-reviewed articles on chelation, is that correct?

A Many.

Q Do you know how many articles you published on DMSA chelation?

A I have anywhere from five to 10. I don't --

Q That's okay if you don't know, but several.

A I just don't judge my productivity by numbers. I judge my productivity by quality of the

papers in first-class peer-reviewed journals.

Q Have you ever published a peer-reviewed experimental study on chelation where you did not get both pre- and post-chelation measurements?

A In most of our papers, I don't remember exactly how many, I think in most, but I also have shortcomings, as almost most beginning investigators do when they enter a new field. We have usually always insisted on doing pre and post.

Now, whether any of our early papers did not do pre-urinary mercury levels or heavy metal levels, I just don't remember.

Q And you testified in Cedillo that you always try to get a baseline; it's the proper way of doing a test. Isn't that correct?

A It depends on what the purpose of the experiment or the study is. It depends on how easy it is to get patients. It's very difficult to just make a statement with no reservations. And if I said that at the time, then you have the quote that I said it.

But let me say that we usually insist on doing pre and post, but we can understand with autistic children, to those of us who have worked with autistic children, how difficult it is to get a blood sample and a urine sample. And to try to get two in

the same day is, makes it very difficult to do the experiments.

Q So the Bradstreet study didn't take pre-chelation mercury levels, is that correct?

A They did chelation. And they compared not pre and post, but they compared a group of autistic children with a group of control children.

Q Did the Bradstreet study control for dietary intake of mercury?

A I don't think so, but I don't recall. My impression is they did not. It's very difficult, especially with autistic children, to have that kind of control.

Q Could dietary intake affect post-chelation urine mercury levels?

A Absolutely. If, if there was a great deal of mercury-containing foods in the diet.

Q Your fourth pillar is -- and it's on page 25 of your report -- you state, "The most beneficial treatment for autism as reported by parents of autistic children was chelation therapy." And that's the fourth pillar of your six pillars. Do you recall that?

A I recall something like that. Where is this on the --

Q Page 25.
A Page 25. Yes, I see it now. Yes.
Q And you rely on the 2006 Autism Research Institute consensus paper addressing chelation, is that correct?
A Yes, yes. What was the question about that?
Q You rely on the consensus paper from the Autism Research Institute.
A Essentially, I also rely, to some extent I also relied a great deal on what parents told me at these think-tank meetings, or the meetings associated with a think tank. Although I realize the shortcomings supposedly of taking parental views. It's not a controlled clinical trial.
Q Is the consensus paper, it's not a peer-reviewed paper, it's just a consensus paper issued by the --
A It depends on how you define peer review. That consensus paper was given to 100, at least 100 think tank members, of which maybe, at least, my guess is at least 20 peer reviewed it. I did not peer-review it because I thought it would be a conflict of interest, because of my interest in DMSA.
Q But it wasn't peer reviewed by a journal editor or --

A It was not published in a journal, per se.
Q Can you cite to any peer-reviewed journal articles that demonstrate chelation improves the neurological manifestations of autism?
A Improves --
Q The neurological manifestations of autism.
A I don't know whether -- no, Bradstreet was short term. I know of no good paper that proves it. The NIH started such a study, but has, for a variety of reasons, abandoned the study.
Q Have any of the authors on the consensus paper done studies upon which to base their opinion? Or peer-reviewed studies upon which to base their opinions that chelation is useful for the treatment of autism?
A I honestly don't know. I just don't have the names of the reviewers before me.
Q Do you remember that Dr. Mumper was one of the authors on that paper?
A I just don't remember.
Q Do you know whether Dr. Mumper performs chelation? Do you know whether Dr. Mumper performs chelation?
A Why don't you ask her? Because she would be a witness. She is a very reliable physician, and I

would hate to put, I would hate to misquote her.

Q Okay.

A I think I know what she does, but I would think since she is going to be here, an expert witness here, I would rather not make that statement.

Q Would you agree that by the time most autistic children are chelated, the chelator is removing inorganic mercury from the body?

A When most autistic children have been chelated, what was the rest of it?

Q Is it removing inorganic mercury from the body?

A Oh. May I rephrase the question? Are you asking me whether DMSA will stimulate the excretion of inorganic mercury and/or methyl or ethyl mercury? Is that what you're asking me?

Q I'm asking you that by the time children undergo chelation therapy, which is after the vaccination schedule has been administered, is that usually correct?

A I'm sorry, I don't understand your question.

Q Okay. I will ask you, what does DMSA remove from the body? What does it chelate? What kinds of mercury does it chelate? We'll go with your question.

A Oh, fine. You're asking what DMSA chelates.

Q Yes.
A All right.
Q In mercury.
A That's fine. We're just talking about mercury.
Q Yes.
A And let's be very specific about this. DMSA will chelate, by definition chelate means forms a ring, with mercuric mercury. It will not chelate methyl mercury. But it will cause the increased excretion of methyl mercury, because DMSA will tie up two individual molecules of methyl mercury, and will not form the chelate. So that's why many of us prefer to use the term "metal mobilizing agent" rather than "chelating agent." Is that clear, ma'am?
Q Yes.
A Thank you.
Q When you chelate autistic children, what are you removing from the body? Is it mostly inorganic mercury?
A I think it's, I don't know whether anyone has actually done that study. But in animal studies that we and other people have done, I think we've done anyway, and certainly Clarkson has done both human and others, one would expect that both organic mercury and

inorganic mercury would be, the excretion of both of them would be increased in the urine.

Q What did Bradstreet measure in the urine when he chelated?

A I think he did total mercury. I'm not positive, but my guess is that since the laboratory did not do speciation, my guess is he did total mercury. But I don't know.

Q And where is most of that mercury coming from when you chelate?

A Where does mercury come from when you give DMSA?

Q Yes.

A The majority of the mercury would come from the kidney. But from animal experiments that we've done, we also get mercury from other tissues in the body.

Q Now, you testified earlier that once inorganic mercury is in the brain, it stays there for a very long time. Is that correct?

A That is what Vahter and other people have published.

Q And you have also performed chelation studies on animals to determine whether chelation removes mercury from the brain. Is that correct?

A We have performed chelation studies in animals that have been exposed to mercury vapor, and have been able to show that DMSA did not remove mercury from the brain. That's your question, and that's the answer.

Q It did not remove inorganic mercury.

A It did not, we did not do speciation, if I remember. We did total mercury.

Q And the paper, the consensus paper from the Autism Research Institute also says chelation does not lower brain mercury levels, is that correct?

A That's not correct if you just say the word "chelation." In studies that we are now writing up, we clearly show that a chelating agent called Depenicillamine, that Clarkson used in Iraq also for treatment of humans, that Depenicillamine does decrease brain mercury.

Q What kind of mercury in the brain, inorganic or organic?

A I'm trying to think. We have done speciation. And right off -- it will reduce both. But it reduces one of them much more than the other. And I think it reduces the organic mercury in the brain much, brings much more organic mercury out than it does inorganic. Although it brings some inorganic

mercury out. That's Depenicillamine.

Q And what paper is that? What are you citing? What is the basis for that?

A That's studies that we have been doing for the last two years in my own laboratory, and we are in the process of writing it for publication now.

Q Does DMSA remove inorganic mercury from the brain?

A DMSA will not remove any metal from the brain. But penicillamine will.

Q So if it's your hypothesis that autism is caused by inorganic mercury building up in the brain, how is chelation beneficial if it doesn't alter brain inorganic mercury levels?

A Well, if there's mercury in the intestines, if there's mercury in other tissues in the body, the mercury levels can interrupt or inhibit the function of certain enzymes in those tissues. No one claims that we are only just dealing with the brain when you do chelation work, because most people know, most clinicians that use it know that if they give DMSA, it's not going to affect brain mercury.

And also, you must keep in mind that the damage to the brain probably has been, has been made, and it may not be reversed by the mercury.

Q So how does it improved neurological function?

A Did I say it improved neurological function? Did I say DMSA improves neurological function?

Q Do you believe it does?

A Pardon?

Q Do you believe it does? Do you believe chelation with DMSA can improve neurological function in autistic children?

A Did I say that?

Q I'm asking you for your opinion.

A Oh. I think you should ask Dr. Mumper that, because she has much more experience using chelating agents and dealing with autistic children.

Q But it's one of your pillars. I mean, it's one of the six pillars that you say leads you to believe that vaccines cause autism.

A Do I say that DMSA cured autism? No, I don't say that in those six pillars.

Q You say that chelation was beneficial.

A I said that parents believe it's beneficial to the, when DMSA was given to their children. A long list, a questionnaire was given to parents by the Autism Research Institute. And a long list of possible, vitamin B-6, thyroxin, a whole bunch of

things.

What was consistent to almost 68 percent or whatever figure it is that I quote was that parents came up with DMSA. Now, no question this is not a controlled epidemiologically suitable clinical trial. We are just reporting what was said.

Q I know you said that chelation isn't FDA approved; it has a --

A I didn't say that. Pardon?

Q It has an off label? You said it has an off-label use for the --

A I said not chelation, I said DMSA.

Q DSMA. DMSA.

A DMSA has an off-label use for treating mercury intoxication, or to mobilizing mercury, to mobilizing, immobilizing arsenic. It has been used and published in peer-reviewed medical journals for those purposes.

And the FDA approves a drug now for efficacy and safety. So off-label use usually means that the physician knows it's safe to use; the physician may not know how effective it will be in off-label use.

Q Do you disagree, then, with the Institute of Medicine's 2004 conclusion -- and we can put that up.

(Pause.)

Q That because it is unlikely to remove mercury from the brain, chelation is useful only for immediately after exposure, and before damage has occurred?

A I don't see anything about the brain here. It says because chelation therapy has potential serious risks, but now you've changed it. We may not be looking at the same thing, ma'am. Okay, here we are now.

Q "Because it is unlikely to remove mercury from the brain, chelation is useful only immediately after exposure, and before damage has occurred. Moreover, chelation therapy has serious risks. For example, some chelation therapies might cause the release of mercury from soft tissues stored, thus leading to increased exposure of the nervous system to mercury.

"Because chelation therapy has potentially serious risks, the committee recommends that it be used only in carefully controlled research settings with appropriate oversight by the Institutional Review Boards protecting the interests of children who participate."

Do you agree or disagree with that statement?

A I don't completely agree with it. I am governed by an Institution Review Board in all the human experiments I do. And I have had a very firm connection with established medicine.

However, when I see that someone improves, even though I'm not convinced that the improvement may be due to the drug that he's getting, or she's getting, or the placebo effect; if the child gets better, in the case of someone in my own family that, when something was given, the person got better; and so I don't give a darn whether it was recommended or not recommended by established medicine.

So if we can go through these sentences one by one, I'll point out to you some of the problems with the sentences. And there are just three or four sentences.

"Because it is unlikely to remove mercury from the brain, chelation is useful only immediately after exposure, and before damage has occurred."

Now, first of all, there are now chelating agents, we have known -- we've had chelation, chelating agents that will remove mercury from the brain. British antileurocyte, the name for, the official name for dimercaprol. It will remove mercury from the brain.

But when it's first given during the first couple of days, it will cause a redistribution of mercury. It will take mercury from the tissues, and that chelate, the true chelate, will be moved up the brain and across the blood-brain barrier.

So British antileurocyte dimercaprol is no longer recommended for the treatment of heavy metal poisoning, because for the first couple of days it will increase the levels. All right? So there is, but in the long run the brain mercury level does go down. But you can do some damage. So the first sentence isn't completely truthful, all right?

"But moreover, chelation therapy has serious risks." That's absolutely correct.

"For example, some chelation therapies might cause the release of mercury from soft tissue stores, thus leading to increased exposure of the nervous system to mercury." That statement is also correct.

"Because chelation therapy has potential serious risks, the committee recommends that it be used in only carefully controlled research settings, with appropriate oversight by Institution Research Boards protecting the interests of children who participate."

I cannot disagree with that as an academic.

However, when I hear and meet parents who say I got my child back with tremendous improvement if they are given DMSA, even though there is no controlled study, I can't ignore that. And I can't ignore the fact that some people, some children are given DMSA, and it did not help them.

So I don't know what you're asking me now about this. As a research person, I certainly agree with the last statement. But as a, but I'm not treating a human being; I'm doing research. Dr. Mumper could probably, Dr. Mumper could probably address that more clearly.

Q The fifth pillar for arguing thimerosal-containing vaccines cause autism is the Hornig study. Do you still rely on the Hornig study as one of your pillars?

A That's a very difficult question now, because some people have claimed that they can't repeat it. But according to the grapevine, she is now coming up with another study that will. So I just, at the present time I have no firm opinion.

Q And you are referring to the Berman study, which is Respondent's Master List 42? They tried to replicate the Hornig study, and could not? Is that correct?

A Yes, yes.
Q Okay.
A And I think I have it in the list of slides that we showed, actually.
(Pause.)
A What do you want to ask about the Berman study?
Q I have no questions on the Berman study, other than it tried to replicate the Hornig study and could not. Is that a fair assessment of that, of the Berman study?
A But there is also criticism of the Berman study.
Q Okay. I'm not going to ask you about the criticisms, unless --
A You're not going to ask?
Q No.
A All right, thank you.
Q Your sixth pillar, you state -- and that's on page 25 of your report, the sixth pillar.
A Page 25.
Q That is that there's evidence of post-natal loss of brain cells in autism, particularly in the cerebellum. What's the basis for that statement?
A I thought it was, that's mentioned here, the

portion is 203 at page 584 and reference cited there. But that's all I know at this present time.

Q Do you know whether that article discusses the possibility of thimerosal as a cause of autism?

A I don't frankly even remember reading the article. I don't know when I read it, so I just have to familiarize myself with the title of the article, and maybe I can answer your question.

I don't recall thimerosal as being part of that article, but I'm not certain.

Q If we take out the Hornig study, which you're not sure about any more, how many of these pillars do you need? You said taken individually it doesn't matter, but if you take them altogether it shows the vaccines, thimerosal-containing vaccines can cause autism.

What if we take away two pillars? What three studies or two studies -- how many pillars do you need, I guess?

A As many as I can find.

Q What if we only had one pillar?

A It would depend on the quality of the paper, and whether I believe what they did, and whether I have any confidence in what the investigator has done in the past. And what the peer-reviewed critiques

are.

Q Well, you said taken alone, you really couldn't draw that conclusion. But taken together, you could.

A In these papers. I certainly feel better after having more than one pillar, as you put it.

Q How about two?

A I don't understand the purpose of the question. The more we have, the better off we are. The more evidence we have, the more convincing it is. If you have one piece of evidence, it's one piece. If you have two pieces of evidence, then it should be twice as good.

Q Did you put these pillars in order of what you think are the most important studies that show thimerosal-containing vaccines show autism? Is there a particular order?

A I really put them in the order of their importance as to the connection of mercury with autism. I did not have in mind in any of these the use of thimerosal in vaccines at the time, when I put these in order.

Q Dr. Aposhian, yesterday in your testimony, and I only have a black-and-white copy, but as I recall you stated that the parts --

A Give me the slide number?
Q All of your slides. It's a general question.
A Oh.
Q You said some of it was written in blue, and some of it was written in red.
A Yes.
Q What did the blue represent, again?
A The blue was either a direct quotation or what, if you read the article, you could understand the author was saying. It was in some cases my abstract of that article, but it was what the author was saying. That was the blue.
The red was my expert opinion. And I tried to keep those separate, as best as I could.
Q You wrote your report in August of 2007, is that correct?
A If that's what the date is.
Q And I know you testified yesterday that following the completion of your report, that you had health problems in your family that sort of distracted you from focusing on this litigation. Is that correct?
A That's correct.
Q When did you start refocusing on this

litigation?

A The word "focus" is not a good term.

Q Okay. When did you --

A I've always thought about it.

Q When did you begin working on this case, towards litigation? Reworking on this case.

A I just don't recall. You know, my guess is it was always in my mind, and I was always thinking about it. One just doesn't put things out of their mind. Anyway, it's not possible for me to put something completely out of my mind.

Q But when did you actually, you don't recall when you actually started working on it again preparing for your testimony?

A I don't recall. We have the figures. If it's really important, I have an invoice I can check. Would you like that?

Q Well, was it a month ago? Two weeks ago?

A I would guess at least probably, my guess is December, January, something around that time. Since this is a court of law, I don't want to say something that's not --

Q I'm not asking for an exact date, so we don't need to look at your invoice. I was just wondering.

A Okay. All right.
Q So you think December, January some time.
A That's what my guess is, but I really don't know.
Q Okay. And in your testimony yesterday, you cited to quite a few articles that weren't in your initial report, is that correct?
A Did I --
Q You referred to articles that were not in your initial report, is that correct?
A Yes, that's correct.
Q And one of those articles, for example, you relied on was Dicicco-Bloom, et al. It's an article from 2006. And we'll show you your slide.
A Yes, yes.
Q What is that article about?
A Can you put it back on?
Q Well, if you testified to it yesterday, let's see what you can --
A But can you just have the article up for me to look at? May I ask the courtesy of --
Q Well, we'll put it up. But what I'd like to ask you first, what do you recall about that article before we put it up?
A I recall it being a very good and clear

article that I emailed to a number of people, recommending that they should read it. Some of my associates at the University of Arizona and at other universities.

It explained a great deal about autism in relatively simple language, that I and many people who are not MDs might understand. That's what I remember about the article. And that's why I have slides which quote them directly. And if I can go there, I think that's, you have -- thank you, sir. I now have it in front of me.

SPECIAL MASTER VOWELL: And we're referring to slide 77.

THE WITNESS: Thank you very much, Special Master.

BY MS. RENZI:

Q How did you find this article?

A Can I find 77 first?

Q Sure, sure. I'm sorry. It's right up on the screen.

A I still would prefer seeing my own slide, thank you. Yes, okay. What is your question, please?

Q How did you find out about this article? I mean, it obviously was published prior to you writing your report, but how did you find this article

subsequent to writing your report?

A Let me tell you how I usually work, so that we can be clear, and so I don't have to, you know, say I don't know or something.

One reads an article. And as one reads the article, one reads a statement with a reference. And if that statement is important, then one wants to go back and check that reference to see whether what the person is quoting in the article is really correct.

And so I suspect that in this case, the Dicicco-Bloom article I picked up because I read some article, and suddenly "The Development of Neurobiology of Autism Spectrum Disorder" appeared in the reference list. And therefore I went to my abstracting service, which is Hightower Press I think, Highwire Press that Stanford University puts out, and immediately got the article. Does that answer your question?

Q Yes. Now, you didn't think this article was important when you wrote your initial report, is that correct?

A I'm not even certain I knew it existed. I don't know whether -- you're implying that it's not in my initial report, so I would suspect I didn't even know it existed. I had less than a month to put this report together, when I usually take much more time to

very thoroughly and carefully do things.

Q I understand that, and that's not what I'm asking. I'm just asking why, subsequent to your report, you found this article important to rely on.

A Because neurobiology is basic to autism spectrum disorders. Developmental neurobiology is even more basic. And so the title intrigued me. And I read it to learn more about developmental and neurobiology of autism spectrum disorders. I don't know everything. And the more I can learn by reading good, peer-reviewed scientific articles, the better off my knowledge is.

Q Did you find this article before or after you read Dr. Kinsbourne's report?

A Before I read --

Q Dr. Kinsbourne's report.

A I don't remember, to tell you the truth. I don't know. I was very impressed by Dr. Kinsbourne's report. Is this in his report?

Q Well, we can ask Dr. Kinsbourne about his report. I don't know. Actually, I just want to know why you relied on it, if you relied on it post seeing Dr. Kinsbourne's report.

A The answer is, I don't know, but I doubt it. I try to stay independent of other people's reports in

a given case.

Q Did you create all the slides for your presentation yesterday yourself?

A As far as I know, I did. The only time I used some help from my young students was if I could not transfer a picture, a photograph from a journal to PowerPoint, which sometimes is very difficult for me. And so then I'd call one of my students on the telephone, and tell her -- her name is Emily Goldberg -- and she does it, so it's nice.

Q So you wrote all of the parts in blue yourself?

A Absolutely. Absolutely. I type with my fingers; it's not the easiest thing.

Q No, the only reason I was asking, and we'll pull up a slide, which is slide 4.

A I'm sorry. I think this slide was put together from material that I gave the law office.

Q Okay. So the fact that you refer to your laboratory as his laboratory, those were, okay. So those were preexisting slides that you put in.

A I think so.

MS. RENZI: Okay. I have no further questions.

THE WITNESS: Thank you, Ms. Renzi.

SPECIAL MASTER VOWELL: Do we have questions now, or do we want to wait? I have a couple of questions, Dr. Aposhian.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: Dr. Aposhian, are you aware of any estimates of the average daily intake -- and I hesitate to use that word -- of mercury in any, of all species in humans?

THE WITNESS: Yes, ma'am. I'm sorry I didn't show that, because I had gathered that I should not repeat very much from the Cedillo trial.

SPECIAL MASTER VOWELL: This is a separate theory, a separate case, Doctor.

THE WITNESS: Pardon?

SPECIAL MASTER VOWELL: This is a separate theory, a separate case. We're not incorporating your testimony from Cedillo in this case, that I'm aware of.

THE WITNESS: I'm sorry, I did not know that. In the Cedillo slides -- and if you want, I can bring it up, because I think I have that talk here -- there is -- and it's published in the, I think it's called the Toxicology of Methyl Mercury, the National Research Council Publication 2007, in the year 2000, I think, and in the World Health Organization.

There is a wonderful table that lists the species of mercury with the intake of each one for the general population, and also the retention.

The greatest exposure of the general population to mercury in general, without speciation now, is the mercury from amalgams. Of course, that exposure is mercury vapor, okay?

And of that, if I remember correctly, it's in the ballpark of six to 10 micrograms per day. Of course, depending on how many amalgams you have and that sort of thing, but that's the average. It's a wonderful table.

And if the, if our lawyers will remind me, or if you'll give me your email, I will be happy to email it to you. It's also, it's published in the article, in the toxicology chapter that I wrote for the NRC Monograph.

SPECIAL MASTER VOWELL: So it's available, and we could obtain it.

THE WITNESS: Pardon?

SPECIAL MASTER VOWELL: So it is available, and we could obtain it.

THE WITNESS: Absolutely. And would you like more information now?

SPECIAL MASTER VOWELL: Certainly.

THE WITNESS: Okay. Of all the species of mercury, the organic mercury they're talking now about is methyl mercury, that's the far right-hand side; that of all these exposures of mercury, we retain most of the methyl mercury that we're exposed to, all right? And the inorganic mercury, the mercuric mercury if you will, as far as the general population is concerned, you're exposed to a little bit of it in food, but it's generally considered to be of very low value.

So to summarize, the greatest exposure of the general population to mercury is via dental amalgam mercury; that the methyl mercury, of them all methyl mercury is retained the most. It's on order of I think six to 20 micrograms per day that you retain. And the inorganic mercury is sort of insignificant.

SPECIAL MASTER VOWELL: All right. Now, if we're dealing with children from birth to age three, understanding that those children receive some exposure to mercury from the dental amalgams of their mother, is there a different table or a different assessment of an average daily exposure?

THE WITNESS: No, ma'am, there is not. There is not one that I know of, because this question came up in our NRC meetings.

The closest thing you can find is the Seychelle Islands and the Faroe Islands and the New Zealand studies, but it's not in table form at all. It just gives an idea about low exposure of children to methyl mercury in fish or in whales. But we don't have a table like we have for the general population.

SPECIAL MASTER VOWELL: All right. You also refer in your testimony to some of the findings in the brains of primates and the brains of autopsied autistic subjects. Are there any studies you are aware of that measure the brain mercury level of, in autopsy, of autistic subjects?

THE WITNESS: Oh, yes. Let me be sure I have the question right. You're asking me are there any studies that have measured the mercury levels in the brains of autistic children.

SPECIAL MASTER VOWELL: That is correct.

THE WITNESS: Yes, there are.

SPECIAL MASTER VOWELL: Or adults. Autistic children or adults.

THE WITNESS: Of autistics, yes. Yes, there are such studies.

SPECIAL MASTER VOWELL: And did you cite any of them, either in your presentation or your paper?

THE WITNESS: I thought I did, but again,

the cross-examination was so good, my thoughts were not quite concentrated. But let me very quickly, if I can.

(Pause.)

THE WITNESS: I think there's one from, I'm positive there is one from Hopkins, if I can put my finger on it. Ah.

I'm not positive, but I would suggest that one look at a paper by Vargas, et al. I'm not positive. That would be, it's slide 79.

SPECIAL MASTER VOWELL: Thank you, I will look at that.

THE WITNESS: If it's not that one, and if I find one, I'll try to tell --

SPECIAL MASTER VOWELL: Bring it up to the attorneys, and they can bring it up to us at a later time.

THE WITNESS: Thank you. My apologies for not being able to come up with it right away.

SPECIAL MASTER VOWELL: That's not a problem, no one expects that. I'm just trying to get the questions answered while we have you here.

THE WITNESS: All right.

SPECIAL MASTER VOWELL: You also mentioned yesterday, in talking about thimerosal and ethyl

mercury, you seem to equate thimerosal with merthiolate. Was I misunderstanding what you said, or is that correct?

THE WITNESS: I think that's correct.

SPECIAL MASTER VOWELL: And when we're talking about merthiolate, we're talking about that topical antiseptic that you and I were probably painted with as children, but --

THE WITNESS: Did you say anesthetic?

SPECIAL MASTER VOWELL: Antiseptic.

THE WITNESS: Yes, antiseptic. Yes, yes.

SPECIAL MASTER VOWELL: Yes. It certainly wasn't an anesthetic, as I recall it.

THE WITNESS: That's correct. And that has been prohibited now.

SPECIAL MASTER VOWELL: Prohibited now. But at the time you and I were children, and some of the people over 40 in this room, it was fairly common in use as an antiseptic.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay. And that would also refer to mercurochrome.

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: I think chrome was another formulation of that.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: And then finally, I got a bit confused during the cross-examination, so let me make sure I understand what you're saying.

Thimerosal is injected. It is converted by the body rapidly to ethyl mercury, and then the ethyl mercury at some point is, and I have forgotten the term, but it is converted to mercuric mercury.

THE WITNESS: It enters the tissues.

SPECIAL MASTER VOWELL: It enters tissue.

THE WITNESS: And then it's deethylated.

SPECIAL MASTER VOWELL: Deethylated.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay, deethylated as opposed to demethylated, which is what happens with methyl mercury.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay. It is deethylated to mercuric mercury.

THE WITNESS: Mercuric mercury.

SPECIAL MASTER VOWELL: And that is the form of mercury that persists in the brain.

THE WITNESS: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay. And it doesn't matter whether it comes from ethyl or methyl

mercury.

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: Except that more ethyl mercury is converted to mercuric mercury in the brain.

THE WITNESS: A greater percent.

SPECIAL MASTER VOWELL: A greater percentage, all right.

THE WITNESS: I'm sorry, let's be careful. There is a greater percentage of the total mercury --

SPECIAL MASTER VOWELL: Correct.

THE WITNESS: -- that becomes mercuric mercury in the case of ethyl mercury exposure.

SPECIAL MASTER VOWELL: All right. Now, my question is, is it mercuric mercury in the brain that you are contending is what causes autism? The mercuric mercury in the brain? Is that correct?

THE WITNESS: Yes. Of course, we cannot rule out that some of the organic mercury is also doing it, but the mercuric mercury is what stays there, and stays there for a long time. And the mercuric mercury has an extremely high affinity for the enzymes of the brain, the have sulphydryl group.

The methyl mercury is too big to get into certain enzymes that have an SH.

SPECIAL MASTER VOWELL: But once it's demethylated, it becomes mercuric mercury, it doesn't matter how it got there.

THE WITNESS: Yeah. Once it becomes demethylated, then it can go in as a mercuric ion and inhibit an enzyme.

SPECIAL MASTER VOWELL: All right. Those are my questions. Any redirect, Mr. Williams?

MR. WILLIAMS: Yes.

REDIRECT EXAMINATION

BY MR. WILLIAMS:

Q Dr. Aposhian, I want to start by going over the DeSoto criticism of the Ip study, and the analysis of the hair data from that study.

You were shown on cross the first DeSoto paper criticizing Ip, but you weren't shown the second DeSoto paper that responded to Dr. Aschner's letter about the hair analysis? Do you recall that?

Let me put that up. This is Petitioner's Exhibit 612.

THE WITNESS: Can you make it bigger for me, Scott?

MR. WILLIAMS: Show the title first, Scott.

BY MR. WILLIAMS:

Q Just to remind the Special Masters, Ip did a

study comparing the blood levels and the hair levels of mercury in autistic children and controls. And the first paper published by Ip said there was no difference. And it was interpreted as therefore a negative study on whether there was this efflux disorder, correct?

A True.

Q But then DeSoto and colleague published a reanalysis of the Ip data that concluded there was a statistical significant difference between the autistic children, the blood levels in the autistic children of mercury, and the blood in the controls. That was where it was higher in the autistics, right?

A Yes, sir.

Q And then Dr. Aschner wrote a letter to the editor, which is what we have up on the screen here now, and I want to show what his criticism was of the DeSoto --

MR. MATANOSKI: Actually, counsel?

MR. WILLIAMS: Yes?

MR. MATANOSKI: Are you going to pose a question? I mean, I understand background, but it's going on quite -- do you have a question to pose to the witness?

MR. WILLIAMS: Yes. I can ask it.

SPECIAL MASTER VOWELL: Let's direct your question, queries, up here, okay? Rather than engaging in colloquy among counsel. Please address your inquiries to the Bench, or your objections to the Bench.

Mr. Williams, I do think you need to get to a question, however.

MR. WILLIAMS: Okay.

BY MR. WILLIAMS:

Q Did Dr. Aschner write a letter to the editor of the Journal criticizing the DeSoto reanalysis of the Ip data?

A Yes, sir.

Q And I have highlighted a section that I believe summarizes -- let me ask you. Does this highlighted portion here, does that summarize what Dr. Aschner is criticizing DeSoto's reanalysis for?

A That's not necessarily so.

Q No, I'm just asking you, is this what Dr. Aschner was arguing?

A Yes.

Q Yes. And then DeSoto and Hitlan, did they write a response to Aschner's letter, that starts on the second column of this same paper?

A Yes, they wrote an answer. Sorry.

Q Okay. Now, if we go to the second page of their reanalysis, I've highlighted some sections that I want to ask you about. The first one is, it says here that a case in point of apparent misunderstanding is this letter by Aschner. And they quote his criticisms. And then they say, "We believe that it should be clear that our conclusion was not related to the hair analysis, and the statement by Aschner appears to reflect a misunderstanding of our article."

Now, toward the bottom of this page they start reanalyzing the hair analysis data, and that's what I want to ask you about. It says, there's a paragraph that says, the hair analysis data," I'm going to pull it up here.

Do you see where they say, "The hair analysis data is in fact interesting, but is of secondary importance. That said, because it was brought up in Aschner's critique, we address the rest of his criticism."

And it goes on. They reiterate his criticism, which is, can you explain what his criticism was again, about the chelation therapy?

A Yes. He says the chelation therapy and changes in the diet in fish consumption, both most likely to occur in the ASD group supposedly, in the

two months preceding the mercury analysis, are likely to affect blood, but not hair mercury samples.

Q Okay. Then on the start of the next column, on the second half of this page, would you read what they say there about whether they agree with Aschner's critique or not?

A "To some, chelation among autistic patients could, as Aschner suggests, cause a correlation between blood and hair to be different in the autistic group, compared in controls. So do we agree with Aschner's critique? No."

Q And then I've highlighted a section just a little bit lower on that same page, where they start to explain why they disagree with Aschner. Could you read that? And then I may have some questions to ask you about that.

A "In other words, the autistic sample has lower hair levels of mercury than their blood levels would predict, and not the higher levels, as would be the case if they had undergone successful chelation therapy. They are consistent with the idea that the autistic sample might perhaps be worse at ridding the body of circulating mercury, and not consistent with the idea that the autistic group might have recently experienced a high level of mercury removal from blood

circulation in controls."

Q So what are they saying here now, about the hair levels and the chelation criticism of Dr. Aschner?

A They are not agreeing with Aschner's criticisms, and they say they are consistent with the idea that autistics might perhaps be not good at ridding the body, or may have an efflux, if you will, disorder.

Q So is it fair to say that the current state of back-and-forth about Ip's data on hair, that DeSoto and Kaplan interpret the hair analysis to be consistent with your theory that autistic children have a mercury efflux disorder, that you can see in their hair analysis?

A It is consistent.

Q Now I want to turn to the teeth paper by Adams.

A Uh-huh.

Q This is Petitioners' Exhibit 138. You were asked quite a few questions about this this morning. What I want to go to, what you were asked is whether this is indicative of body burden; whether the additional -- just to summarize, the tooth study found that in autistic children, there was more mercury in

their teeth than in the controls. Is that right?

A Correct.

Q Okay. And you were asked whether that was indicative of a body burden or not, right?

A Correct.

Q Let's see what the authors say about that. If you look at the second page, middle of the second, first column, I have highlighted a sentence about that. What do the authors of this paper say about whether this reflects a body burden or not?

A "A decreased ability to excrete mercury should result in a higher body burden."

Q And then on the right-hand side of this paper, this right-hand column, would you read what was highlighted there about baby teeth studies?

A "Baby teeth are formed in utero, and during the first few years of life, so they provide a measure of cumulative exposure during that critical period of development. Previous studies have demonstrated that mercury can be reliably measured in teeth."

Q And do you agree with those statements?

A Absolutely. Yes.

Q And then finally, over in their discussion section on page 1049 of the article, the first sentence of the discussion, I'll ask you to read that,

please, once we blow it up here.

A "The two- to threefold-higher level of mercury in the baby teeth of children with autism is important, because it strongly suggests that they had a higher body burden of mercury during several years of prenatal/infant development."

Q And do you agree with that interpretation of the data?

A I do agree.

Q Then on the last page of the article, start with the paragraph on the bottom left. Would you read that, please? And then I'll ask you some questions about it.

A "It is interesting to note that the median mercury level in control teeth was 50 parts per billion, which is similar to the level of mercury, 40 to 50 parts per billion, found by Burbacher, et al, 2005, in the brains of infant monkeys following dosing of the monkeys with thimerosal in a manner designed to mimic the U.S. childhood vaccination schedule.

"If baby teeth levels correlate with brain levels, then this suggests children with autism in this small study had median brain levels of mercury in the range of 140 parts per billion, which is approaching the range of what has previously been

calculated as necessary to result in mercury-induced neurological disorders by Takeuchi and Eto, 1975."

Q In fact, I want to show you the very next sentence of this, too, that I didn't highlight, but it's the last sentence of that paragraph. If you could just pull that up, Scott. If you'd just read one more sentence, please.

A "They found that levels of 260 to 630 parts per billion were able to induce Minimata Disease, which was a severe form of mercury poisoning."

Q All right. And again, do you agree with this interpretation of their teeth data and Burbacher's data?

A I do, yes.

Q And then their conclusion, this two-sentence conclusion of the paper, I'll have you read that.

A "The results of this small study suggest that children with autism have a higher body burden of mercury, probably due to a decreased ability to excrete mercury that is likely in part due to a high usage of oral antibiotics."

Q And do you agree with that statement?

A Yes, I do, sir.

Q Now, you were also asked some questions about the 2004 IOM report. And I just want to show

you some, the references to this 2004 IOM report for a moment.

Could you blow the references up, Scott? Thank you.

First of all, do you note that the IOM committee cites you as one of the authorities they have consulted in writing this report? Is that your name there?

A That is my name, sir.

Q Okay. But then let's look to see if this IOM 2004 report cites any of the adult monkey studies that were published back in the mid-1990s, that we spent quite a bit of time with yesterday.

A The Charleston --

Q The Charleston and Vahter. Let's see if there's any Charleston papers in the citations to this 2004 IOM report.

Would you pull up the Cs, Scott? We lost the citation page. Will you blow up the difference between, yes, that part.

So there's a Chin and Akomi, but there is no Charleston paper cited, right?

A That's correct.

Q Okay. Let's go to the V section of the citations. Let's see if any of the Vahter papers are

cited. You need to show the one above that, Scott.

So we have Verstraeten, Verstraeten, Verstraeten. We have Vericcio. We have Vulman. But there are no Vahter papers cited there, are there?

A There are none, sir.

Q Do you know how the IOM austere committee could have overlooked those adult monkey studies from the mid-1990s?

A Do you want my opinion, sir?

Q Yes.

A The problem with that committee was there was no toxicologist, no biochemist, no physiologist, no one who deals with toxicity, per se. This committee was almost completely made up of epidemiologists who study, or vaccine people. There is a gross, in all the IOM reports that have been published about vaccine safety, there have been no toxicologists, certainly in this one and the one that I attended that committee. No toxicologists who would be an expert, or no biochemists who would be an expert on how methyl mercury and thimerosal and inorganic mercury would affect a child.

Q Now, I'm going to represent to you that there is no discussion of neuroinflammation in that 2004 IOM report, in relation to autism. Would that

surprise you?

A I have not read the complete report, but that is a shock to me, really. Because, it's just a shock. The Zimmerman work is going to be classic. And I would suspect as he continues, he'll probably have a Nobel Prize.

Q Now, I want to show and go to your report for a moment, the one from last year, and look quickly just to see some of the topics that you had in your report, that you have not been cross-examined about.

Let's turn to page 13. Do you see the topic? Just pull up the bottom paragraph there, Scott, no. 5, the brain and mercury.

Now, here you're analyzing the Burbaches and Pichichero numbers. If we go to the top of the next page of your report, explain what you were doing there in these calculations.

A We were trying to estimate -- and it is an estimate -- of the amount of mercury in the brains of these children. And we had to, it was a very tricky thing, so tricky that I had to go ask another toxicologist who just got a prize from our society, whether it made sense to him what I was doing. Because of the assumptions that were being made.

Essentially what we were trying to do was to

find out how much mercury was in the brain after the thimerosal vaccinations.

Q And my recollection is, you weren't cross-examined about this analysis at all. Is that your memory, also?

A That's correct.

Q Now, also on this page, same page 14, do you have a section of your report entitled, "Neuroinflammation in Autism?"

A Yes, sir.

Q And you cite the Pardo paper, the review at 2005, at the bottom of that?

A Yes.

Q And then on the next page, you talked about the Connors twin study on terbutaline?

A Yes.

Q And later I'll show that you went into the animal model. In fact, we can jump to that right here in just a second.

No one asked you any cross-examination questions about the Connors paper or terbutaline model, did they?

A That's correct. No one asked me about terbutaline. Very important paper, very important concept. These are papers coming from mostly the

Johns Hopkins Group, and they've done very, very good work.

MR. MATANOSKI: Your Honor, at this time I will properly address my observation to you.

Typically, redirect is about the matters that have been gone over on cross. Just because we haven't referred to a matter in the report doesn't mean we don't believe it's important, or that we won't cover it.

I'm not going to object to Mr. Williams going through parts of the report we didn't cover, but perhaps Dr. Aposhian didn't cover yesterday, because it would be enlightening to us about what his report, what parts of his report are important to his opinion.

However, if this is just argument about what Respondent didn't ask in cross, then this is not the time to argue one's case.

SPECIAL MASTER VOWELL: Mr. Williams?

MR. WILLIAMS: I think it's proper in redirect to point out areas of his testimony in his report which were ignored by the cross. That's what I'm trying to do.

SPECIAL MASTER VOWELL: Okay. Remember that you're not pitching this to a jury; you're pitching it to three people who have read these reports.

MR. WILLIAMS: Okay.

SPECIAL MASTER HASTINGS: And you can also argue this on brief, too.

MR. WILLIAMS: Okay. Then I'll turn to one last topic, and that is the Pardo paper itself that you cited in your report, and which you were asked some questions about on cross. It's Petitioner's Exhibit 72. Let's show the title first, please. Scott.

BY MR. WILLIAMS:

Q This is the 2005 Pardo review paper, right?

A Yes. A very good paper.

Q The first thing I want to ask you about is on page 4 of this exhibit, where there is a section of the paper called, "Infections and Autism."

Could you read the first sentence of that section, please?

A "Infections have been associated with autism in small numbers of children, and include prenatal rubella (Chess, Fernandez, and Corns, 1978), and cytomegalovirus (Sweetner, et al, 2003, Yamashita, et al, 2003) and post-natal herpes encephalitis (Long, Bean, and Brown, 1981).

Q Now, but when they cite post-natal herpes encephalitis as a cause of autism here, is that

consistent with your conception that there are post-natal insults that can lead to neuroinflammation in autism?

A Yes, sir.

Q Then on page 6 of the Pardo review, it's the very middle of this paragraph, Scott, here, with all the figures. Pull that up.

Let me read this to you, because it's hard. Does it say in here that neuroglial activation in autism may be part of responses that result from disturbances of neuroglial function, or neuronal neuroglial interactions during brain development, and secondary extrinsic effects resulting from unknown factors that disturb post-natal CNS development? Does the paper say that?

A That it does say. Yes, sir.

Q And is that consistent with your general theory that it's the inorganic mercury post-natally inducing neuroinflammation leading to autism?

A Yes, sir.

Q And then at the very bottom of this column, and the very top of the next column, does this paper also say that a potential explanation of the CNS dysfunction --

A I don't have that yet, sir.

Q -- in neuroinflammation is extrinsic etiological factors, non-genetic neurotoxic or environmental, involved in the pathogenesis of autism that can produce neuronal and cortical abnormalities to which neuroglial reactions are only secondary responses? Do you see that?

A Yes, I see it now. I'm reading it slowly because it just didn't come up until just a few seconds ago. Yes, I see that, sir.

Q And is that consistent with your notion that the extrinsic factor of inorganic mercury in the brain can lead to these problems?

A Yes, sir.

Q Then finally, on the last page of the paper, in their conclusions, if you blow up just the top half of that conclusions paragraph, Scott -- this is on page 9 of the exhibit. You have part of it highlighted. It says -- well, let me ask you to read what I've highlighted there, Doctor.

A And you have to give me more of a view, Scott, please. I need the complete sentence. Thank you. Yes, we have just about it, yes.

"We hypothesize that environmental factors -- for example, neurotoxins, infections, maternal infections, in presence of genetic

susceptibility and immunogenetic background of the host influences the development of abnormalities in cortical organization and neuronal circuitry and neuroinflammatory changes responsible for the generation of autistic symptoms."

Q And when they say that neurotoxins could be responsible for the generation of autistic symptoms, is that consistent with your notion that inorganic mercury is the cause, in some cases?

A Yes, sir.

Q And then let's look at figure 4 quickly, the last thing I want to ask you about. Let's blow up figure 4 that they just referred to.

Over on the left, they have a balloon there for environmental toxins.

A Yes, sir, I have it.

Q Do you see that?

A Yes.

Q And what does that, what does the arrow point to from the environmental toxins that goes around the top, over to the middle?

A To the central nervous system, neuronal organization synapse in neural transmitters, neuroglial activation, and you can go on to CNS, cytokines, et cetera.

Q All right. And then at the very bottom of this diagram, over in the right-hand corner, do you see where it says "autistic phenotype?"

A Yes.

Q What's the first word under "autistic phenotype?"

A "Regression."

MR. WILLIAMS: Thank you. That's all I have.

MS. RENZI: Just a couple questions. Could we pull that slide back up, please?

RECROSS-EXAMINATION

BY MS. RENZI:

Q Dr. Aposhian, I just have a couple of questions.

A Yes, ma'am.

Q In 2004, you presented to the IOM, is that correct? They invited you to present? The 2004 IOM.

A I think that. I don't keep date straight, but I think it was 2004.

Q And you presented your theory that autism was caused by a mercury efflux disorder. Is that a fair summary of your contention?

A I think that's quite correct.

Q And you stated today you were very surprised

that the Charleston and the Vahter paper were not presented to that IOM, is that correct?

A It was not listed as one of the articles, one of the papers in the bibliography that was shown.

Q So you didn't present the Charleston article in 2004.

A You must realize that I was told I could speak for a short period of time. And I thought it was much more important to bring up the Wilson's Disease model than to do a complete survey of all the work that many good people had done.

Q And you didn't present the Vahter article, either, did you?

A The Vahter article?

Q Vahter.

A Vahter? No. She's a very good friend of mine, I know her work. We just didn't have time to go into all of that.

Q You just presented your theory on mercury efflux, is that correct?

A I don't know. I'm sure there was introductory material and other theories. But the major point was to present the mercury efflux theory.

Q Did you discuss neuroinflammation with the IOM?

A I did not, because my knowledge of neuroinflammation at that time was practically non-existent.

Q Did the IOM, didn't the IOM reject your hypothesis, that autism was caused by a mercury efflux disorder?

A I really don't know. I don't know that.

Q Okay. I just want to return to this chart for a minute that Mr. Williams asked you to look at. It's on your screen, that chart.

A Yes.

Q And they call this "Hypothetical Interactions of Environmental and Genetic Factors." So how do post-natal insults lead to neuroinflammation? What's the mechanism? What's the process?

A How does post-natal --

Q Insult.

A -- insult, an environmental insult --

Q Lead to neuroinflammation.

A I would much rather have our neurologist answer that later on.

Q So you don't know.

A I think I know, but since it's a Court of law I just don't want to make a mistake in saying what

I think, and so I would rather pass it on to the neurologist.

Q But you offered your opinion today that you agree with this Pardo hypothesis, that's correct? And you just accept it because it's --

A I agree with the Pardo hypothesis.

Q But you don't understand why.

A That doesn't mean I have to be an expert in every single part of the hypothesis.

Q But do you have an understanding of the mechanism of how this occurs that you can accept this hypothesis?

A In my own mind, I have such a mechanism, which I'm not confident in presenting in public.

Q So it's not one you could articulate to the Court at this moment.

A Pardon?

Q It's not one you could articulate to the Court today.

A It's not one that I can be absolutely 100-percent certain that I would be giving the correct information at this time.

MS. RENZI: Thank you. I have no further questions.

SPECIAL MASTER VOWELL: All right. It would

appear to be an appropriate time, I understand Dr. Aposhian needs to leave this afternoon, so we would excuse him.

Mr. Matanoski, does the government wish a sort of caveat in terms of further cross-examination of Dr. Aposhian?

MR. WILLIAMS: In the future?

SPECIAL MASTER VOWELL: In the future.

MR. WILLIAMS: Yes, ma'am. With the caveat that we may not be asking for that.

SPECIAL MASTER VOWELL: I understand. And this had to do with an off-the-record discussion that we'll put in the record at some point, but a discussion that we held before we began this morning, concerning Dr. Aposhian needs to leave today. There may be more cross-examination of him later based on the matters in his testimony that were not included in this report. Have I correctly stated that, Mr. Matanoski?

MR. MATANOSKI: That's correct, ma'am.

SPECIAL MASTER VOWELL: And Mr. Powers, you all did not object to that.

MR. POWERS: That's correct, Special Master.

SPECIAL MASTER VOWELL: All right. Then Dr. Aposhian, you are excused at this point, with the

understanding that there may be questions for you at a future time.

THE WITNESS: Thank you.

(Witness excused.)

SPECIAL MASTER VOWELL: And it would appear to be a good time to take our lunch break. Do we want to discuss the plan for proceeding this afternoon before we all break?

MR. WILLIAMS: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay. So what's the plan from Petitioners on what we intend to do this afternoon?

MR. WILLIAMS: This afternoon, just looking at the expected length of testimony, and without really knowing the expected length of cross, but anticipating fairly extensive cross of Dr. Deth, who is our next witness, my best guess is that Dr. Deth will take the balance of the afternoon today. And that we would therefore shift our schedules to have Dr. Kinsbourne take the witness stand Wednesday morning.

I expect he would be done before the lunch break on Wednesday, and Mylinda King and George Mead would be available. We anticipate completing both direct and cross in that remaining afternoon session.

That's what we would propose.

And we would still be able to get Dr. Mumper. That would leave all day Thursday for Dr. Mumper, and I anticipate we would have no problem being done with Dr. Mumper in that one day.

SPECIAL MASTER VOWELL: And we still then have Friday available for overflow, should cross or directs take longer than expected.

MR. WILLIAMS: That's correct, Special Master. So that's what we would propose as a schedule, actually for today and running through the rest of the week.

SPECIAL MASTER VOWELL: Okay. So we will plan on taking up Dr. Deth after lunch. So let's reconvene then at 1:25, 1:30.

MR. WILLIAMS: Thank you. It's such an easier number, what's the difference, Special Master?

SPECIAL MASTER VOWELL: Fair enough, 1:30. Thank you.

(Whereupon, at 12:25 p.m., the hearing in the above-entitled matter was recessed, to reconvene at 1:35 p.m. this same day, Tuesday, May 13, 2008.)

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A F T E R N O O N S E S S I O N

(1:35 p.m.)

SPECIAL MASTER VOWELL: We are proceeding in the current, in the Theory II Omnibus proceeding, and in the Mead and King cases.

Call your next witness.

MR. WILLIAMS: Thank you. We call Dr. Richard Deth.

SPECIAL MASTER VOWELL: Dr. Deth, please have a seat there. And to the extent you can keep your chair over to your right-hand side, that would be helpful so that we all can see you. And then if you could sit down and then raise your right hand.

Whereupon,

RICHARD DETH, MD

having been duly sworn, was called as a witness and was examined and testified as follows:

SPECIAL MASTER VOWELL: Thank you.

DIRECT EXAMINATION

BY MR. WILLIAMS:

Q Dr. Deth, would you tell us what is your present position?

A I am currently a professor of pharmacology at Northeastern University, in the Department of Pharmaceutical Sciences, located in Boston,

Massachusetts.

Q And how long have you been at Northeastern?

A I have been there a long time. I believe it's been, let's see, it will be 32 years this coming September.

Q And would you just summarize your education for us, please?

A Sure. I have a, my original Bachelor's Degree is in pharmacy, so I am actually a pharmacist. I received that pharmacy degree from the University of Buffalo School of Pharmacy in 1970. And in 1975 I completed my PhD in pharmacology. I received that degree from the University of Miami in Florida, and then went ahead to do a post-doc, a post-doctoral fellowship in Belgium, in the University of Louvain in Belgium.

I returned briefly to Florida, to Miami, but then took a faculty position in 1976 at Northeastern, where I am now.

Q And you've been there ever since.

A I have.

Q Now, do you have a laboratory in which you do research?

A That's right. Throughout this period of 30-several years, I have maintained a lab. I originally

did cardiovascular research, where I was studying contractions of blood vessels, things relating to hypertension. And then that moved to studies of receptors: the molecules that respond to neurotransmitters.

As we studied the receptors in these blood-vessel preparation, we ultimately discovered a process that relates very much to the autism and the issue at hand today.

Q Was your cardiovascular research funded by NIH?

A It was. I had cardiovascular grants from the National Institute of Heart, Blood, and Lung for almost 15 years. I had also from the American Heart Association, grant support.

Q Now, do you have students that you both teach and supervise in research?

A Certainly. One of the pleasures of being a university professor is to be able to participate in the development of the young scientists. And in fact, I have two PhD students now in the lab; they'll be the 14th and 15th doctoral students that will have been trained in my lab. The previous 13 have graduated and gone on to different careers. And there is also a mix of undergraduates and other students, as well.

Q And have you published some of your original research in the peer-reviewed literature?

A I have, and I certainly make an effort on a continual basis to do that. I have I guess just short of 70 publications at the moment.

Q Have you also written some book chapters?

A I've written several book chapters; several more relating to autism are in press at the moment.

Q And you also had one book you've written.

A An important thing for me was several years ago now, about five years ago, I wrote a book called The Molecular Origins of Human Attention, the Dopamine-Folate Connection. And the work that prompted me to write that book and the content of that book are again closely related to the issues at hand today.

Q Then how long have you been doing research related to autism?

A Well, the key event that brought us in this direction was in about 1998. And this will come out by a matter of course as I review some of our work, but it involves discovery about a dopamine receptor in 1998. We discovered a new signaling activity of one of these receptors. And that signaling activity prompted us to, it prompted me to make the decision to

pursue that, and to eventually leave behind the cardiovascular work, and bringing us into the field of neuroscience and neuropharmacology.

And so I guess that wasn't an immediate decision by any means to pursue autism. It was only when that line of work became coincident with some of the theories and concerns about autism that it really became autism-related research. Now, that, I would suppose, is about five years now.

Q And you've prepared some slides to illustrate your points today?

A I certainly have, that's correct.

Q Let's turn to slide 2, please. And would you explain what this slide depicts?

A Yes. Thank you for the opportunity. And this slide says we're here to discuss thimerosal actions, especially in the brain, where our work has greatest reference. I thought it would be a good introduction to the previous testimony, Dr. Aposhian in particular.

But this slide just serves to outline how the thimerosal, or in fact the organic mercury gets to the brain, and some of the critical things that it does once it gets there.

And so I've tried to depict in this slide

here the molecular structure -- and you'll pardon, this has got a lot of science in it, and I hope you can gather what you can from it -- but it would be the actual chemical formula of thimerosal, with the ethyl mercury being attached to a sulphur on the carrier, which is thiosalicylate.

And in fact, the mercury is released from that sulphur carrier to release the ethyl mercury that we talked much about here. And the released ethyl mercury then has different fates or possibilities, and excretion from the body, and detoxification directly is one of those possible fates.

But alternatively, because of the ethyl group's ability to make the mercury atom more easily penetrant of the blood-brain barrier, the ethyl mercury can cross this normally sufficient barrier and bring the mercury into the brain. And once it has breached that barrier, then, as we again earlier discussed, you get a process of de-alkylation or deethylation in this case, in which the inorganic mercury is released on the other side of the blood-brain barrier. So it's now behind the barrier, as inorganic mercury is unable to recross the blood-brain barrier back out to the rest of the body. And therefore, is more or less trapped, as inorganic

mercury, in the brain.

In addition, the de-alkylation step is very important in establishing the toxicologic activity of the mercury, because when the ethyl mercury has, when the mercury in ethyl mercury has ethyl group bound to it, that one ethyl group leaves only one remaining binding opportunity for the mercury. So it can only bind to one thing more than the ethyl group.

Once it becomes inorganic mercury, it has two binding opportunities. And it can bind simultaneously to, for example, to thiol or to sulphur groups as long as they are positioned close enough to each other. And when the inorganic mercury is simultaneously bound to two such SH or thiol groups, and even if one bond breaks, which happens rarely but does happen, the other bond keeps the mercury in place. So even if I lose one, I'm still not going anyplace, I still have a second one.

So when an inorganic mercury binds simultaneously to two thiols, it stays for an extraordinarily long time, much more longer than even if it was just one. And as it turns out, the kind of molecules that have two thiol groups in such a position to be bridged by a mercury, you know, inorganic mercury ion here, those molecules tend to be

involved in sulphur metabolism. And they tend to be the key regulatory proteins that are determining the amount of the antioxidant glutathione, as we'll discuss in more detail later on.

These properties of inorganic mercury, in general terms, mean that it's going to target sulphur metabolism, which is really the focus of my research now, and the focus of my comments here today. And it disrupts sulphur metabolism, not only in neurons, which of course provide the function of the brain that we're most familiar with, but it will disrupt sulphur metabolism in the other cell types, not only in the brain, but the liver and other tissues of the body, as well.

So the inorganic mercury disrupts sulphur metabolism in all cell types, I could say that broadly, and in the brain in particular, where it's trapped behind the blood-brain barrier, this is a particular problem.

Q Why does mercury and sulphur tend to go together?

A Well, it turns out the electrons that populate the mercury atom that are available for bonding are, I'll say a perfect match with the sulphur atom, in especially the so-called thiols, where the

sulphur has a hydrogen that can come off. And if the hydrogen comes off of the sulphur and the mercury comes on, the strength of that bond, because of the reciprocal nature of their electrons that they share in that bond is a strong one. And it's very difficult to break that bond.

And so this gives rise actually to the naming of these thiols that can start to form such strong bonds as mercaptans. The name "mercaptans," after mercury itself, because of the well-recognized likelihood that mercury in the body will be found bound to sulphur, bound to these thiols, otherwise named as mercaptans.

Q Now, when we see -- I know in the United Kingdom at least they call thimerosal thiomersal, T-H-I-O. Is the "thi" in thimerosal, is that related to the fact that it has a sulphur in it?

A That's right. The original you could say construction of this molecule by Lilly as a preservative recognized the fact that the molecule, thiol, which otherwise would be an H if the mercury wasn't there, that this thiol here was a perfect place to attach a mercury to. But then had the probability of being released, and releases the active ingredient here, the ethyl mercury.

And in fact, as we sit here and discuss the potential of that ethyl mercury as a causative factor in autism, we can recognize that the preservative value of thimerosal, as formulated and as included in the vaccine, that preservative role is fundamentally identical to the role that I am describing here. That is, the way that it acts as a preservative by interfering with sulphur metabolism in organisms such as bacteria, and as a result of interfering with their sulphur metabolism at the concentrations present in vaccine formulations, is liable to preserve or otherwise decrease the growth of bugs in those containers.

Q And it also has the same effect on fungus, doesn't it?

A That's right. It's the non-specific ability of mercury binding to thiols and sulphur compounds to disrupt sulphur metabolism that makes it an effective preservative against many different life forms. Because it's so critical for life forms to have normal sulphur metabolism.

Q And by preservative, we're preserving the integrity of the vaccine itself from the invasion of these bugs or fungus, is that right?

A That's right. The word "preservative" is a

relatively comforting word. I like my materials preserved, more or less.

But in this case, the preservative in the mercury has more or less an infinite lifespan. That is, the inorganic mercury especially can never be mutated to anything else other than inorganic mercury. Whereas a different preservative that we might think of, like sodium benzoate for example, might be able to be metabolized to other things, and might have a halflife in the body that's much shorter.

But the choice of mercury guarantees that wherever that mercury goes, it will have a toxic potential for the rest of its existence.

Q Is it fair to say that another word for a preservative in thimerosal as a preservative would be as a bactericide, or a fungicide?

A In a sense, yes. It has the ability to kill bacteria, although there are certain bacteria that apparently are resistant. Because in fact, different, lots of vaccines, for example, have been found to be contaminated despite the presence of the thimerosal.

So to a significant extent, it is a bactericide and a fungicide, but it's not infallible, even in those regards.

Q Are we ready to move to the next slide?

A I'm ready, except I did want to point out where we're going here. I specified these three cell types here, which we have heard and will develop further. But I want to make it clear that we'll next move on to it in a short time.

So the effects that are distinctive in neurons or neuronal cells, or microglial and astrocyte cells, so different cell types respond in their own way to the presence of the inorganic mercury. And so if we couldn't move ahead now, the next slide was really intended by me to be sort of a vocabulary builder here to make sure that all parties concerned recognize some of the terminology.

When I talk about thiols or talk about sulphur metabolism, it's necessary of course to use the biochemical terms. And here I'll just introduce three important thiols. And so the three here would be cysteine, which is the normal sulphur-containing amino acid that is, in fact, a thiol. It has an SH as a part of it. And it's also the limiting factor inside of cells for making glutathione, the anti-oxidant. So the concentration of cysteine and the availability of cysteine is critical for making the anti-oxidant glutathione, and its sort of cousin here would be homocysteine.

It turns out that it's an unusual amino acid; it's only formed in the body, typically, and the homo part of it here, refers to the fact that it has an extra carbon, compared to cysteine, and that gives it the name homocysteine. It's formed during the process of methylation, a cycle called the methionine cycle that I'll refer to. And so it's formed from the amino acid, and it can be a precursor for making cysteine as needed.

Q Now, you call these the three most important thiols. Why are they the most important?

A Well, they are important especially for the consideration here, because they are a part of the core sulphur metabolism that's involved in maintaining the anti-oxidant, or the reduced state of cells; maintaining a normal redox status of the cell.

And this is the area which I believe, and others, is the most critical problem in autism, and it's an area that mercury is active in. These compounds, each of them actually can bind to mercury directly. They are all thiols; they can bind mercury. Although, in fact, the effects of mercury are more than simply binding to these three molecules here.

Q Now, you used the word "reduce." Can you explain, you know, oxidation and reduction?

A Yes. I'm glad you asked me to do that, and actually I wish I had a better slide to present it. But let me try to illustrate here.

Each of these thiols, as a sulphur, let's say the tip of my finger would be the thiol. And when it's in its reduced state, the sulphur has a hydrogen atom attached to it. That's a reduced thiol.

But if we have two such reduced thiols, the hydrogens can be removed from both of them, and the two sulphurs join together. In this case you have a disulfide, which also is referred to as an oxidized form of the thiol, because the reducing equivalents, the hydrogens are off, and now they are oxidized as they are going together here.

So we could have a diathiol of two cysteines bound to each other, or two homocysteines bound to each other. Or most importantly, two glutathiones bound to each other that would be oxidized glutathione. And this would be reduced glutothiones with two hydrogens on either one.

Q Okay.

A And the glutathione I'm mentioning here, which one might consider the star player in this important drama here that we're a part of, the glutathione is actually a small peptide made of three

amino acids; and the cysteine is the important functional part, and it's the middle one. And on one other side there's a glutamate, and on the other side of the cysteine is a glycine. So there is three, this peptide has three amino acids to make it up.

And in fact, it is the major anti-oxidant in all of our cells. And by all, I mean not only neurons and astrocytes and microglia, but I mean cardiac muscle cells, I mean liver, kidney, and whatever. It has, through evolution, been chosen as the anti-oxidant that's going to keep us from oxidizing. We need to have enough glutathione in every cell in order to be able for that cell not to be damaged by oxidation. So it's our primary anti-oxidant. And when we run short of the reduced glutathione, then in fact that cell is in danger of not only dying, but certainly losing normal function and things like that.

And really, it took me a while to understand how important the glutathione synthesis is, to recognize its concentration inside of cells -- I make a note here that the concentration is 10 millimolar. Inside of cells as a typical value. Now, this is scientific terminology, and I recognize that. But we can compare that to the sodium ion, salt, which is an important part of the blood and all of our fluids.

This is actually just about the same amount of the glutathione as there is of sodium ions floating around. So it's a really impressive amount of this is produced and maintained by cells in order to stay alive in a oxygen environment.

Q Anything more about this one?

A I think I've covered all those aspects. And this again was meant as sort of background for the more detailed considerations of what the thimerosal does to cells.

Q Okay. Then we're ready for the next slide, please, 4. This is slide 4.

A So here I've tried to provide a little more detail about the brain, and about those three cell types that I alluded to before. And they are represented here as in the middle, a neuron, two astrocytes, and then one microglial cell.

And in the brain, these three cells work together. And they work together to maintain a satisfactory or a homeostatically normal redox environment. And the way they work together, I've tried to illustrate here, and I'll start the description here with the -- well, let me start with the astrocytes, if I could, up at the top here.

Q Let me ask you this.

A Okay.

Q Are astrocytes also called glial cells?

A Yes. Excuse me for not specifying, but yes, it's one kind of glial cells. A microglial would be another type of glial cell. That means non-neuronal generally speaking.

Q Yes.

A So if I start with the astrocytes here, the astrocytes take up the oxidized form of cysteine that I mentioned before, which comes from the liver. Just by way of background, it's cysteine, it's called here, the name for the oxidized cysteine, is provided by the liver in the bloodstream, and it crosses the blood-brain barrier readily. And in the brain it's taken up by the astrocytes, and converted into glutathione. And the astrocytes, because of their makeup, have the ability to make an excess quantity of reduced glutathione, GSH.

And so they have so much extra capacity that they export some of this reduced glutathione out of the astrocyte, into the environment around the neuron. And in that extra-cellular environment, that glutathione is converted into cysteine first by the removal of the glutamate, and then by the glycine, and now the cysteine content of the glutathione is taken

up by the local neuron cells by a particular transporter. And once inside of the neuron, this cysteine is now available to the neurons to make their own glutathione.

It's kind of an odd relationship between these two cells, why don't neurons simply take up the cysteine and do it themselves? Why do they need the astrocytes to make it into the glutathione first, and then break it down? Well, this is how nature carefully controls the access of cysteine to these neurons. The amount of cysteine available is dictated by the astrocytes.

And so they have like a working relationship. But astrocytes are sometimes considered to be nurse cells, taking care of neurons. And this is a very important way in which they do that: by providing a source of cysteine to neurons they influence the fate and the functionality of neurons that way.

I should mention that these transporters that take up the cysteine by astrocytes, as well as the ones that take up the cysteine in neurons, are transporters that can take cysteine and/or glutamates, the excitatory neurotransmitter glutamate. And in fact, this can be either together with the cysteine in

the same direction or in opposite directions; they can exchange for each other with the cysteine. And so this alerts us that there is a close relationship between the oxidative mechanisms of metabolism in the brain and the excitatory neurotransmitter, glutamate. We'll hear more about that from Dr. Kinsbourne.

But otherwise, this is how neurons get their glutathione. And this leaves us with the microglial cells, which serve as really vigilant sentinels for the redox status of the brain. Actually, they are positioned almost like in a matrix in the brain. Each one has their domain, their area around the microglial cell, and they monitor the redox status in their zone, in their area.

And when they detect something there that's not supposed to be there, perhaps a bacterial toxin, perhaps a metal ion, when it is in that area and they are impacted by that, the microglial response is to undertake an activation mechanism and to clean up the area. This is much the same as in the periphery, the so-called macrophage as part of our white blood cells. They go out and they scavenge things and they phagocytosis bacteria and so forth.

In a similar way, the microglial cells monitor materials, and the redox state in their

environment. And when they come in contact with or are affected by, in this case let's say inorganic mercury, they become activated. And the activated microglial actually change shape. They change morphology. They become phagocytotic, to take up materials, and they also put out oxygen species that are damaging, called reactive oxygen species, or ROS.

And I've illustrated here, what those could be, they can be hydrogen peroxide, that we're sort of familiar with. They can be another one in the middle here, super-oxide anion, or hydroxyl radical. And these oxygen species are meant to kill bacteria. This is how our innate immune system works, is that certain cells, by producing these nasty oxygen species, can damage bacteria that are nearby, and kill them. And by damaging them and then taking up the bacterial remnants, they can clean up their areas here.

But they are creating an oxidative environment. And so when the microglia are activated, it creates near the neurons, or in the neuronal area, a certain amount of oxidative stress, or an extra sort of load of oxygen species that the neurons have to deal with themselves, because they are in that same environment.

Q Now, you said that the microglia not only

detect and take in bacteria, but also metal ion.

A Well, I hesitate even again here, because the microglial interaction with metal ions, it's really one, I don't know whether they're like designed to take up the metal ions. But more precisely, they are actually affected by the metal ions. And they do respond to the metal ions. They don't have a choice.

For example, if the mercury ion is there, and it binds to sulphur groups and sulphur proteins in microglia, the microglia are affected by that metal ion. And they develop oxidative stress as a result of the interference with their metabolism.

So in a sense, they are sensing metal ions, but it's a slightly different way. They don't have receptors for mercury, whereas they do have receptors on the surface to detect bacterial components. So it's subtly different, but similar.

Q Okay. Anything more about the --

A Well, I think I've covered this. I just want to make sure the word neuroinflammation here is meant to describe the state where, as a result of this oxidative condition here and the effects of the mercury on each of these three tissues, cell types rather, that we can call that oxidative stress as a chemical term. But as a pathologic term, it's very

closely related, it is a part of inflammation, in this case neuroinflammation.

So the term neuroinflammation as it's used in different articles and so forth implies the presence of oxidative stress, and the sulphur metabolism changes. Please, the next slide.

Q The next slide.

A This is a simpler one. And the concept here is to say that sulphur metabolism, as I tried to allude to here, has an important role in maintaining cellular oxidative status, because of the glutathione synthesis. But at the same time, sulphur metabolism has other roles. And it has to balance these different roles.

One of the other important roles that I'll develop here is that of methylation, a process that's dependent on sulphur metabolism. And when thimerosal or inorganic mercury interferes with sulphur metabolism, it's going to affect both of these processes, because it's like having a limited amount of resources. You're either going to attend to the oxidative needs of the cell, or the methylation processes; and you have to make, the cell has to make decisions about that.

And so when thimerosal shifts the needs of

the cell toward oxidative needs, then in fact the methylation needs may suffer by default. And so to understand, it's a reciprocal relationship between these two, in that thimerosal interferes with the normal regulation.

Q And methylation means?

A I'm about to specify. I think the next line, if I have the order correct here.

Q Okay, you're right.

A I'd better check myself. I think that slide 6 gives us a chance to introduce methylation.

So methylation relies on the transfer of a methyl group, which is the CH-3, in chemical terms. It's a carbon atom, one carbon atom; and it can be transferred from a donor, which is usually the methyl donor, adenosylmethionine, here SAM. And that donation's molecule can give up its methyl group and physically attach it to another molecule.

So as the methyl group leaves and it gets attached to something else, the something else gets methylated. And that's the process known as methylation. It's like a methyl transfer reaction.

And the molecule that does the methyl donating, SAM, adenosylmethionine, is itself a sulphur amino acid. Because as it turns out, the chemistry

again of the sulphur here, when it has a methyl group attached to it, that bond is weak enough to be broken so the methyl group can be transferred. So sulphur atoms are chosen for methylation because of the ability to transfer a methyl group from this sulphur atom to something else.

And when the sulphur atom, excuse me, the methyl group is transferred from SAM, the remainder of the molecule is referred to as adenosylhomocysteine, or SAH, which is simply SAM without the methyl group. It's the leftover part.

And when we're talking about methylation, you can say it's just one of those biochemical reactions, another esoteric, something like that. But nature has found it useful, again during evolution, to develop many methylation reactions. And there are almost 200 different methylation reactions.

So when something affects methylation, it's going to affect 200 different processes, not just one. And examples are pretty important examples. Because perhaps the most important example and relevant to autism is the methylation of DNA, or genes.

When genes are methylated at certain locations where they are methylated, it leads to a process by which they become hidden, or silent, and

unavailable for expression. So methylation of DNA is the mechanism by which genes are turned on or allowed to be on if they're not methylated, or off if they are methylated. And so it's a pretty important process, and it's particularly important during development, because during development is when genes are turned on or turned off, and so forth.

This happens in conjunction with another set of proteins that are involved with the DNA, and that is the histone protein. Histones are proteins, I think of them as like a sphere that the DNA wraps around. And methylation of the DNA starts that process going, and methylation of the histones helps it along, as well.

So both DNA methylation and histone methylation are involved in gene silencing, which is also referred to as epigenetic regulation of genes.

Other things besides those two can be, other proteins can be methylated. Another important methylation target, the individual phospholipid molecules, the fats that make up the membranes of neurons and other cells. And that's where our work originated, was from studying phospholipid methylation. I'll talk more about that.

But also neurotransmitters are methylated in

order to terminate their activity. For example, dopamine, the neurotransmitter involved in attention awareness. It's a release of neurons, and then the enzymes that put methyl groups onto the dopamine. And when the dopamine is methylated, it no longer binds to its receptors, it terminates its activities. And any problem with methylation will therefore affect neurotransmission that way. And I could go on and on, and I'll try to restrict that. But in fact, methylation has a lot of different targets.

I mentioned methylation here, and the bottom point on this slide is we emphasize that whenever you have oxidative stress, you have reduced amount, or less methylation. It's like a reciprocal relationship. More oxidative stress, less methylation. That's the way the cells work.

Q Okay.

A So next slide?

Q Next slide, slide 7.

A And again, this takes us a little further, to our understanding of the relationship with glutathione here, we, humans, are aerobic organisms. And we take in oxygen bravely. We use it to make ATP and energy. But it's a risk. What we're doing is breathing in a risk of oxidation, and using it, which

works great for us as long as we have enough anti-oxidants to counter-balance the risks.

And I've tried to represent that here by saying usually there's a certain amount of oxygen radicals or damaging oxygen forms. And as long as we have enough glutathione, or buffer capacity to offset that, we're fine. And this is why we have so much glutathione in cells, as I mentioned earlier.

But under certain circumstances, which can be partly genetic and partly environmentally induced, under certain circumstances this balance shifts in favor of the oxidative conditions. And this would be the oxidative stress condition that I alluded to before, which can be -- you can conceptualize this -- as being either because you made too many oxygen radicals, so you have an over-production of them. For example, your mitochondria is not efficient in making ATP from oxygen; they have too many of these oxygen radicals. Or on the other hand, your defense mechanism, anti-oxidant glutathione levels might be too slim, or too limited, in which case the oxygen state is more on the oxidated side, rather than the reduced side.

So in any case, this balance can be due to genes that we carry. And if we do carry some genes,

whatever negative potential they have might be amplified by exposure to things that make the situation worse, that otherwise damage mitochondria, or otherwise limit the glutathione antioxidant synthesis.

So we can see the genes and the environmental factors, generally speaking, can give rise to oxidative stress, or contribute to neuroinflammation.

Q Let me ask you, you have different arrows on there. The arrow beside the word "oxygen radicals" pointing up, does that mean that that's increased?

A Yes. This would be under this condition on the right, compared to this condition. If you have a higher or increased level of oxygen radicals, or ROS, then they could be excessive with regard to the buffering capacity that you have.

Q And you have a down arrow beside redox buffer.

A Right. As compared to here, there's less buffer capacity; compared to here, there's more oxygen radicals. Either one could create the imbalance. And I think reasonably, probably they both contributed commonly to the imbalance.

Q And then another question. We've heard the

word occasionally the last two days, mitochondria. What are mitochondria?

A Okay. Mitochondria are a so-called sub-cellular organelle. That is, if the cell is a whole unit here, within the cell are these little factories, energy factories called mitochondria, where oxygen, molecular oxygen, O-2, is taken up by the mitochondria; converted into water, H2O. And in the process, the energy in the oxygen is converted into the energy molecule ATP. And it's a way in which we can use oxygen metabolically as an energy source, as long as we convert all of the O-2 into water. If we did that perfectly, we would have zero risk.

But inherently, that process releases some of the oxygen as hydrogen peroxide or super-oxide anion, the dangerous forms which then can otherwise attack other molecules, damaging the cells.

Q And do neurons and microglia and astrocytes, do they have mitochondria?

A Absolutely every cell in the body, and I know what I'm thinking of is red blood cell ghosts, but I think even they have mitochondria in them; they just don't have a nucleus. But every cell in the body has a number of mitochondria in them; and they need that, of course, as a source of energy for those

cells.

So just the bottom part of this, what if you have oxidative stress? If you have that, then in neurons in particular there are some consequences. And the most dire consequence would be on the right side here. That is to say, degeneration, which is another way of saying the cell could die. And certainly Alzheimer's Disease, Parkinson's Disease, other neurodegenerative disorders would be examples of neurodegeneration.

But at a lesser level, you would lose function. And inhibition of methylation is one way that function is lost. Because one example that's pertinent to our work on dopamine receptors is the fact that methylation activities are important for those dopamine receptors to provide for a synchronized firing of neural network areas of the brain together. And since that activity is dependent on methylation, then any oxidative stress that lowers methylation will give a functional consequence here. You'll lose the ability for this neurosynchronization, as well as other activities.

So the point I am trying to make is that short of cell death, which can happen from extreme oxidative stress, there is also a loss of the usual

abilities or the usual function of cells during oxidative stress.

Q Now, you mentioned Alzheimer's Disease and Parkinson's Disease. Are those associated in any way with neuroinflammation?

A In fact, both of those conditions are well known in the medical literature to be associated with oxidative stress, and with neuroinflammation in microglial activation. I have to be careful; I can't say as I remember the microglial part, I'll take that back. But I will say that they're associated with oxidative stress. And in particular with Parkinson's, recent evidence associated with pesticide exposure indicates that environmental exposure to xenotoxins is part of the pathologic circumstances for that condition.

Q Okay. Are we ready for the next slide?

A We could move on to slide 8, if we could. And we could probably do that a couple of times, because as we consider the sulphur metabolism, I mentioned before there's the glutathione, it's the really important molecule here. It's the main anti-oxidant.

So how do we get this glutathione? We get it from converting the amino acid cysteine to

glutathione. And again, the cysteine is limiting for that. And where do we get the cysteine from?

Well, one of the places we can get it from is from the homocysteine, through a pathway that goes through an intermediate -- again, this is terminology here -- cystathionine. I believe, I won't go there now, but later I'll have a slide with some vocabulary glossary terms, but the idea here is that the cysteine to make the glutathione can come from the homocysteine down here. And this pathway is called transsulphuration, as a homocysteine is converted to the cysteine. And it's the intracellular way to make glutathione from homocysteine.

Now, if you advance to --

Q Wait, hold on, Scott, if you would.

A Okay. Well, we can stay there, okay.

Q Just first of all, the thio in glutathione and the thio in cystathionine, are those indicative of sulphur groups?

A That's right. Really, with all of these compounds here, and I have to beg beware of those next ones that I introduce, are all sulphur-containing compounds. I'm showing you sulphur metabolism. And each of these, there's a sulphur in homocysteine; there's a sulphur in the cystathionine; there is

sulphur in cysteine. There is cysteine now attached to glutamate, so it's still here and still here.

And sulphur metabolism, I'm not going to launch into my evolution story here, but since the origins of life under the ocean, it's recognized that sulphur metabolism is critical for life, and for oxidative control. And so that's why we're looking at it.

Q Okay. Now we're ready.

A So where do we get that homocysteine from, that we can use as needed to make glutathione? We get that from the methionine methylation cycle is now added to the diagram here. And this cycle starts with the lower left, with the amino acid methionine, and essential amino acid thrusts we can get from the diet. It's activated by ATP to be the methyl donor that I referred to, SAM, before, adenosylmethionine, which gives up the methyl group to things like DNA. And then the leftover is the adenosylhomocysteine, and the adenosylhomocysteine is then converted to the homocysteine.

And this reaction, by the way, is reversible if those who can see it, the arrows go back and forth. And in fact, this reversibility is a key feature of this cycle.

Well, once a homocysteine is formed, nature has a decision to make: whether to send a homocysteine toward transsulphuration, to make more glutathione; or to reinvest it back in methylation. This is like a T junction here. And that decision that nature makes is guided by the redox state of the cell.

If the cell has the need for more glutathione, it's going to send more to the glutathione pathway, to desulphuration. If not, it sends the homocysteine back to methylation.

So we can see from these relationships how methylation is related to redox status of the cell.

The next slide introduces the enzyme, the key enzyme, the critical enzyme, methionine synthase. And this enzyme is obviously in a position to control the fate of the homocysteine. Because if the enzyme methionine synthase is not working or is turned off, the homocysteine instead of going down to making the methionine in methylation, the homocysteine accumulates and goes north to make the glutathione.

So regulating the methionine synthase activity is how nature controls the fate of the homocysteine. And this represents a switch mechanism. You can relate to this in any way you can. You can

give it water flowing, and shifting water from one direction to the other. In this case you might consider that oxidation is like a fire that has to be put out by the glutathione.

So as needed, you can divert more water towards that oxidative need, and less towards your methylation problem. And the methionine synthase does that.

Now, the last part that has appeared here as well is the part that has got into this story. Because through a coincidence, you could say, through our own molecular studies of receptors when we were studying cardiovascular systems, we discovered that there is a receptor for the neurotransmitter dopamine, specifically the D-4 dopamine receptor, that has its own methylation cycle.

This receptor, and I'll show it in just a second in the next slide, this receptor has a methionine sticking out from the receptor that has its own sulphur with a methyl group at the end of it; and it's able to activate that methionine to be a methyl donor methionine, a SAM, and then to give the methyl group to the membrane-phospholipids that are right next to the receptor, causing them to be methylated. And then to pick up a new methyl group using the

enzyme methionine synthase, and the new methyl group comes from the folate pathway.

And this enzyme, methionine synthase, is, number one, folate dependent. It's dependent on methyl folate for those methyl groups to keep the cycle going here. And number two, it's a B-12, or cobalamin-dependent enzyme. And vitamin B-12, again essential for humans, even for vegans to survive, is essential for methionine synthase.

So we discovered this activity again in 1998, which prompted our own interest in what's nature doing here. Why does it allow this one receptor, and only this one dopamine receptor, to carry out a methylation activity like this?

The next slide I hope --

Q Let me stop you. I want to ask, the picture that you have here, all of this is inside of a cell, is that right?

A That's correct. This is a segment, and even only a small segment, of cellular metabolism. Since this includes now a dopamine D-4 receptor, it's going to be cells that have that receptor, and not all cells do. And typically, neuronal cells have this dopamine receptor; in particular, the kind of intra-neurons called gabaurgic or inhibitory intraneurons, are rich

in the D-4 receptor.

So it's best to think of this as typically a neuronal cell, although some non-neuronal cells also have these D-4 receptors.

Q And then the fossil lipid methylation, that's the cell surface, is that right?

A The term phospholipid refers to the surface membranes, and actually makes the cell. The sort of bag-like structure is actually made of these phospholipids. And they're getting methylated.

And as I'll see in a second, I guess, the methylation of the membrane phospholipids changes the membrane.

Q And the ATP cycle down there, that's what the mitochondria produce?

A That's right. The, well, it can be produced in several ways. It can get ATP from glycolisis and non-mitochondrial sources. But the mitochondria is the main source of ATP.

Q And do the mitochondria also depend on this kind of regulatory pathway?

A Mitochondria depended upon the glutathione availability to protect them against the very oxygen-damaging species that they are producing. Mitochondria, however, to my knowledge, don't carry

out methylation directly in the mitochondria. That's more of an activity within, within the rest of the cytoplasm of the cell.

Q Okay. Now, the next.

SPECIAL MASTER VOWELL: Let me interrupt again, Mr. Williams. I remind you to use the slide numbers so we'll have an adequate reflection in the record.

MR. WILLIAMS: Okay, thank you.

SPECIAL MASTER VOWELL: The last testimony was on slide 8.

MR. WILLIAMS: It was actually the one --

SPECIAL MASTER VOWELL: I think we'll be able to pick it up, given the diagrams, but stay on the safe side.

MR. WILLIAMS: Thank you. So now we are on slide 8?

SPECIAL MASTER VOWELL: Nine.

MR. WILLIAMS: Slide 9.

BY MS. RENZI:

Q Excuse me, slide 9. I want to make the record clear.

Q Slide 9 is meant to provide a pictorial illustration of what I described in the previous one. And in the context of the membrane of the cell. So

these ocular-shaped molecules here, this is a slice of a cell membrane. The outside would be at the top, and the inside of the membrane at the bottom. So the cytoplasm would be down below here, and the neurotransmitter, dopamine, would be released from other cells, and would come into its receptor, which is represented here by these blue spirals, and define the binding site.

And when the dopamine finds its binding site, it causes a rotation of one of these receptor's helices or spirals, and the helix that moves is the one that has the methionine that's going to be the methyl donor. I've shown that in yellow here. So the dopamine makes that available for donating a methyl group, and the methyl group is transferred from the receptor to the fossil lipid, and the new one to replace it comes from the enzyme, methionine synthase, and the methylfolate co-factors that it requires.

So this process, when we were able to estimate how rapid it occurs, occurred in one second's time, about 20 to 50 times for each receptor molecule. Twenty to 50 times per second is a very robust activity. And it startled us to learn that. And when we did, we realized that the methylation of the membrane around this receptor, the methylation of it

would change the physical properties in a short period of time; would make that membrane, now methylated, makes it more fluid. It makes it biophysically a little different. And this seems to be the role that this methylation plays. And when dopamine does that, it's really changing the membrane of the neuron in this local area.

Moving ahead now to slide 10. What role does that methylation play in the brain? We don't know, step by step, exactly that. But we have proposed, I have proposed that it plays, the fluidity of the membranes that the dopamine causes plays an important role in attention. And I proposed that in the book that I wrote a number of years ago, in part because this D-4 dopamine receptor is the genetic risk factor for attention deficit hyperactivity disorder, or ADHD.

That is to say, if you have a particular form, a genetic form of that receptor, then your risk of ADHD is three- to five-fold higher than other people's. And this suggested that this receptor plays a unique role in attention and awareness. And I have proposed that this might involve the synchronization of information during attention.

And the study that's shown here, and the

data that's shown on this slide -- again, number 10 -- supports this suggestion or hypothesis. And this data shows that the synchronization during attention is stronger if you have the so-called seven repeat form of this D-4 receptor, and the strength of this synchronization is indicated by the more brilliant red color here, compared to other people with a different form of the receptor, with two or four repeats.

And as the title suggests, the D-4 receptor, polymorphisms or repeats, modulate the human, we call them gamma band responses. But this is gamma frequency synchronization between neural networks in the brain.

And if I can try to capture the idea here, during attention, let's say that I'm attending to, for example, my pointer here. If I focus my attention on that, everything else gets kind of blurry, and the attended information becomes sharp. And it turns out in your brain the gamma activity is associated in, the areas that are receiving this information are showing gamma activity.

And so it indicates this kind of information suggests to me, although it's not proven step by step, that the special methylation activity of the D-4 dopamine receptor is related to attention and

awareness, and to gamma synchronization of neural networks.

Q Now, do all people that have the seven repeat gene structure, do they develop ADHD?

A No. I indicated that there's a higher risk if you have this, three- to five-fold. There's more than 100 peer-reviewed papers now confirming this.

But in fact, it's not a guarantee. Interestingly, it's also associated with a personality trait of novelty seeking, which is a positive virtue, compared to a loss of attention, which is a disorder. And it's been suggested that this seven repeat that's associated with improved gamma activity is actually, places some people at risk of ADHD when they're exposed to environmental pollutants, or even -- the people who published this didn't specify -- but environmental factors that took a good gene, a gene with a positive evolutionary value, and now converted it into a risk factor.

And now it probably means that not everybody who has it has ADHD. But if you have an environmental exposure and you have that, chances are your, the chances of ADHD are greater.

Q Are we ready for the next slide?

A I am. That would be number 11. Here is the

same pathway that I illustrated before, but now I want to point out what happens during oxidative stress. The oxidative stress turns off this enzyme, methionine synthase. I'll in a second be explicit about how that happens, but when it does turn it off, we can see that the beneficial effect, which we are all happy to have occur, is that the homocysteine is now diverted to make more anti-oxidants, the perfect solution for the oxidated stress. You want more anti-oxidants.

However, the two methylation processes shown here, one involving let's say DNA and gene expression during development, the other that D-4 dopamine receptor I just talked about, they suffer the consequences of oxidative stress by having less methylation, or less methyl groups even available to support their activities. So the consequence you might expect to have, impaired attention, impaired gamma synchronization, as well as problems during development with inappropriate gene expression.

So how is it that the enzyme methionine synthase responds to this? Actually, the next slide I meant to expand on it. And if you'll excuse me, the slide 12 illustrates some additional methylation reaction, some of which I alluded to before, but I wanted to reemphasize that many different things

happen during the inhibition of methylation during oxidative stress. I'll leave that as such, and then ask that we move ahead to the next slide.

Which is a result from a paper published by a researcher, Dr. Jill James.

SPECIAL MASTER VOWELL: And we will be on slide 13.

THE WITNESS: And now we are on slide 13. Thank you for that.

And this slide and this data, gathered from a study of autistic children and neuraltypical control, 80 autistic, 73 neurotypical controls. And Dr. James measured in the plasma of these individuals the levels of those same materials that I just showed on the previous slide.

In fact, excuse me, the slide before that, slide number, would that be 9?

BY MR. WILLIAMS:

Q Yes.

A Excuse me. On slide 11, if we go back to slide 11, if we could just go backwards through this pattern here of showing impaired methylation and the diversion of more homocysteine here is expressed in those data. And when you don't have enough glutathione, when your glutathione is low, then this

pathway will be emphasized, and these pathways will be inhibited.

And what we should expect to see during oxidative stress is two little glutathione, associated with lower activity of the methylation pathways.

And now moving ahead two slides, again returning to slide 13 here with this data, we can see what she found. And what she found was the levels of glutathione in its reduced form in the plasma were reduced by 36 percent. So this means that these individuals, these autistic subjects had too little of the reduced glutathione that they needed to combat oxidative stress; whereas the methylation activity, reflected as the ratio of the methyl donor SAM to the SAH without the methyl group, that was reduced by 30 or 28 percent. That reduction means that methylation is decreased in the presence of oxidative stress, and suggests that these autistic subjects do suffer from oxidative stress and impaired methylation.

So how is it that the enzyme, methionine synthase, is regulated? Let me provide that detail in the next slide, which is no. 14. It illustrates the molecular structure of the enzyme methionine synthase.

And this is a molecular model from an x-ray crystal structure. And the enzyme has five distinct

parts to it. I'm going to start with the pink part in the upper right. This is the part, or the domain as it's called. It's substrate homocysteine. And here the homocysteine is in yellow. And attached to the pink is the green domain, and here is the methylfolate bound onto that domain, so that is the folate domain. I'll get back to the intervening yellow-cap domain. But the B-12, the cobalamin, is bound to the red domain, and the final domain is this blue one, called the SAM-binding domain, because it binds a molecule of the methyl donor's SAM.

The way the enzyme works, in brief -- and it's easy to understand, even though this is like a molecular science -- if you just think of it as TinkerToys or something like that you can understand how it works. The B-12, in the middle of it is a cobalt atom. It's the heart of the B-12. And the cobalt atom, like the tip of my finger, sits there and waits for the green methylfolate domain to bring the methylfolate to it. And now the methyl group is transferred from the methylfolate to the cobalt.

So now I have methylcobalamin, or methyl B-12, in the red domain. Then this green domain backs away, rotates in space.

Now the pink domain comes in and brings the

homocysteine close enough to pick up that methyl group, and becomes methionine. So that completes a reaction cycle. The homocysteine has now been converted to methionine, and the methylfolate gets replaced with a fresh one, and that cycle can continue until interrupted by oxidation.

Q Now, a chemical term you're using, methionine synthase. What does synthase mean?

A Well, the word "synthase," I can clarify that. Synthesis means making something. In this case it's making methionine. It's methionine synthase. And it's making the methionine by adding a methyl group to the homocysteine, which makes it methionine.

So methionine synthase, the name of this enzyme as a whole describes its activity.

Now, as I indicated, the reaction continues of the formation of methionine; but the cobalamin, when it's waiting for the next methyl group, while it's bare, it turns out that it's the most easily oxidized substance in our whole body. It's the most easily oxidized material that we have.

And that means that if there's anything in its environment that could oxidize it, it will oxidize it. And so it's a censor of oxygen status. And if there is something around, it oxidizes the cobalt and

stops the reaction.

Well, while the reaction is stopped, the homocysteine accumulates and goes toward glutathione synthesis, so that we have more anti-oxidant. And now when the environment calms down, we can repair the oxidized cobalamin. And the oxidized cobalamin is repaired by the blue SAM domain, which comes in, brings a methyl group from SAM to put on there to make it into methyl B-12. And there's an auxiliary protein, methionine synthase reductase, that brings electrons just to help that reaction.

So this is how the enzyme is sent to do the oxidation. It's because the B-12 gets oxidized.

The last component to mention here is the yellow domain or region, which is called the cap region. It's called the cap because, in fact, it floats above the vulnerable cobalt, while it's exposed. And when you have a cap domain, it limits the oxidation. And as a result, we fix the stoppage of the enzyme.

And I won't present this data today, but what we've found in elder humans in their brains is that that cap domain can be removed with aging, so that more anti-oxidant can be made by trading greater vulnerability for the cobalt atom.

So in any case, it's a marvel of engineering at the molecular level, including an oxidative sensing ability which helps the cell to make more glutathione anti-oxidant.

So in any case, this is the description of a standard enzyme. The next slide no. 15 indicates that in cases of human neuronal cells that we've studied in the lab, and we've also done related studies on rat cortex, we find that in those cells, the ability to reactivate the enzyme using this blue SAM-binding domain is not working. It doesn't function.

In order to reactivate the enzyme in these human neuronal cells, you need to take out, physically let the oxidized cobalamin or B-12 float off and be replaced with a new B-12 that's already methyl B-12. And this, in net terms, shows us that the neuronal cells, human neuronal cells, need methyl B-12 to reactivate the enzyme.

And as the next slide no. 16 shows us, the methyl B-12, or methyl cobalamin as it's otherwise known, in order to have that methyl cobalamin available to reactivate the enzyme, you need to have enough glutathione to synthesize it. Because the synthesis of methyl B-12 is glutathione-dependent. This first step requires glutathione.

Now, putting this together, what you can see is that what nature has in mind in neurons, in human neuronal cells, is to keep the enzyme off unless there is enough glutathione to make methyl B-12 available. And if there's any deficit in glutathione, you won't have enough methyl B-12, and the enzyme will stay off, making more glutathione until you do have enough glutathione.

So it's like another solution to the problem of how to control the flow of homocysteine by keeping the enzyme in need of methyl B-12.

Q Now, the same need for glutathione that you say is in neurons, is that also true of microglia in the astrocytes?

A We don't know that. And I can't really comment with any authority about that. We've only been able to confirm this in human neuronal cells, and in whole brain preparations, which contain a mixture of neurons and microglia and astrocytes. So at this time, I can't say whether or not the properties of the whole brain reflect the neurons, or the microglia, or some complement.

So in any case, having made that point, I also want to extend the idea that human neuronal cells don't operate the same as other species. And to

illustrate that, the next slide no. 17 shows the levels of the SAM sulphuration intermediate cystathionine in human brain on the left, compared to the monkey brain, the rat brain, guinea pig, cat, cow, chicken, and duck.

And what we can see in this progression here is that as evolution or whatever is driving human development, that we have an extraordinarily higher amount of this cystathionine, which is the first step in making the glutathione from homocysteine, which is the first step in transsulphuration. But because it's accumulating, we can see that it's not getting any further than this first step. There appears to be a block in human brains after the cystathionine that limits its ability to go all the way to cysteine and glutathione.

And moreover, this is exclusive to the human brain. Because on the far right of this illustration, the levels of cystathionine in human liver, human kidney, and human muscle are 40-fold lower, indicating this is a brain-specific phenomenon.

So the point I wanted to make with this slide is that human brain -- and again, this includes a mixture of cells. We're not sure that this is all neurons. But if it was neurons, there would be an

even higher, if it was restricted to neurons, an even higher distinction. But otherwise, the human brain is different than other, I will say lower species here, even though it's a self-flattery to humans. The possibility is that our evolution and our abilities depend somehow on limiting the transsulphuration mechanism.

The next slide no. 18 illustrates that. Here I've tried to illustrate the human brain situation by introducing an arrow and a dotted line here, to indicate the limited transsulphuration activity, with the blockage here, would cause the accumulation of the cystathionine, as we just saw in that previous slide.

And if this is the case, if cystathionine is not fully allowed to go forward to make the anti-oxidant glutathione or cysteine, the consequence for the cell is that it needs to find extra amounts of cysteine from outside the cell. And it makes human neuronal cells all the more dependent upon the uptake of cysteine from outside the cell. Now, that's the cysteine that comes from the astrocytes that I introduced earlier. Now we can see in more detail, here is the glial cells or astrocytes releasing that glutathione that they have an excess amount of. And

the cysteine derived from that is now taken up by neuronal cells to a very specific transporter. A transporter labeled here EAAT-3, which stands for excitatory amino acid transporter 3, which transports the neurotransmitter glutamate -- that's why it's named excitatory amino acid transporter -- or cysteine. It can take glutamate or cysteine across the cell membrane.

Here we're thinking about its capacity for cysteine transport. And in neurons, when this is blocked at the level of transsulphuration, the EAAT-3 uptake of cysteine becomes absolutely critical for survival and normal function of neurons. And that extra importance is now, is attached to this transport, is taken advantage of because we have found that that transporter is regulated by growth factors, like brain-derived growth factors and signalling pathways that control that.

In any case, the bottom line here I'm trying to make is that human neuronal cells have extra vulnerability to oxidative stress because they don't have a robust transsulphuration pathway. And the remaining pathways really have to function normally; otherwise, a deficit of oxidative status will occur here.

So we can now move ahead. I'm going to do the next slide, just as in slide 19, has a list of, again, a sort of glossary of terms for those abbreviations for reference purposes here.

Q Right. I asked you to prepare this, even though we don't need to repeat them all here, just for reference if people are looking at this later.

A I have to apologize, but it's sort of necessary to use this terminology here.

So I'm moving now ahead to slide 20. I'm now going to review the results that we found for the effects of thimerosal at different dosage levels in most cases on the various processes, which I hope this background has provided identification of.

And this work, looking at the effects of thimerosal, is an offshoot of our earlier publication, Waly, et al, in molecular psychiatry, where we found and published there that the activity of the enzyme methionine synthase. And the activity of this dopamine methylation system that we were investigating was inhibited by thimerosal, by mercury, and also by lead and aluminum. And we were curious, having made that observation of the enzyme, as to what was causing that.

So we undertook a series of studies to probe

deeper into that observation, and find out at what point was the thimerosal doing what it does.

So in any case --

Q You've written inorganic mercury underneath thimerosal there.

A That's right. Because while the general terminology here is thimerosal role, we recognize that the culprit, if you will, or the active species here is likely to be inorganic mercury released from thimerosal.

So slide 21 brings us to some result here. What I'm showing you is that transporter, EAAT-3. We measured its activity by using radioactive cysteine and cultured human neuronal cells, and measured the uptake of cysteine. And even though I have not shown them here, we have otherwise confirmed through pharmacologic inhibitors that this transport is EAAT-3-mediated.

And here we see the inhibitory effects of thimerosal as a function of its concentration.

Q Now, the left-hand column there, with the numbers from 50 down to zero, what does that represent?

A Okay. On this graph, these are amounts expressed in chemical terms as nanomolars per

milligram protein of cysteine uptake. So this is the amount of cysteine being taken up during the three-minute interval in these cells, and then we've pre-incubated the cells in various concentrations of thimerosal for 60 minutes, one hour. And after those pre-incubations, we then went ahead to measure the cysteine uptake.

And as you can see, exquisitely low concentrations here caused a substantial two-thirds reduction in the uptake of cysteine. So this process of cysteine uptake is exquisitely sensitive to concentrations of thimerosal at or below the concentrations which occur in plasma, for example after vaccination, and concentrations which have been estimated to occur in human brain.

For example, earlier this morning we heard testimony suggesting that the concentration in the brain, based on Burbacher's study, might be of the order of 30 nanomolars. Thirty nanomolars would be somewhere here between 10 to the minus-8 and 10 to the minus-7. And these concentrations have at this point more or less completed the inhibition, again amounting to about two thirds of the uptake of cysteine here. So this is a very substantial, very potent effect.

The left side of this figure compares the

effect of 100 nanomolar thimerosal, 10 to the minus-7, which is the far-right bar, with equal concentrations of inorganic mercury, which is close to, but not significantly different from, the thimerosal. They're almost the same, each one slightly less than aluminum at that concentration, arsenic at that concentration, and lead at that same 100-nanomolar concentration. This would be the normal uptake here.

So what we can see from this comparison is that this, while it's not a unique activity of inorganic mercury or of thimerosal, even though they are the most effective at this concentration, it's an effect shared by other heavy metals which also have an affinity for thiols, and can do the same thing.

So if we were concerned about which of these materials might be important here, we would have to say that certainly the mercury and the thimerosal would qualify. But the other materials, should they be at these levels, would produce at least partially the same effect.

Q Let me ask you about the difference between the monkey brain and the human brain that we saw at an earlier slide.

If the cysteine uptake is being interfered with by thimerosal or by inorganic mercury, would you

expect, given that difference between human and monkey brain, the effect would be greater in humans?

A That's exactly correct. When the transsulphuration pathway is restricted, then this pathway becomes all the more important. So humans, based upon that comparison, would be more dependent on this pathway, and therefore more vulnerable to inhibition.

Q Even than the monkeys.

A Than monkeys, for example, or the other lower species even further down the chain there.

Q Okay.

A Slide no. 22, the next slide is again provided for convenience, as a comparison of the scientific nomenclature of concentrations which I use, such as molar concentrations, with other more toxicologic common expression, parts per billion here. And so we can see the conversion levels that can be applied here.

Again, the concentrations that we're using in finding effects of thimerosal are very low, either in the parts per billion or in the molar terminology.

Down at the bottom I've also included here the EPA's referenced dose, which is a dose per day that is considered by the EPA as safe, or without

effect. This dose of course is being given outside of the brain, to the body as a whole, and that corresponds to .1 micrograms per day per kilogram.

And the EPA also provides what it considers to be a safe reference level in the blood. That is, the blood levels of inorganic mercury that the EPA considers safe is this 5.8 micrograms per liter. Now, that's in the blood compartment. What we're talking about are neurons that are behind the barriers in the brain. So that concentration in the blood that's considered safe would be 30 nanomolar, whereas I suppose a lesser concentration would occur in the brain because of this restrictive compartmentalization. But nonetheless, our results show that concentrations of thimerosal, 30 nanomolar or less, if they occur in the brain, are going to inhibit this process.

Q And what did you say the level in Burbacher, in the infant monkeys, what was the level then?

A Well, the estimate, depending on the calculations used, gave I believe around ranges between approximately 15 to 30. It depends on the weights, the ages, so two months versus six months and so forth, of infants. They were estimating -- excuse me, that was human estimates in human equivalents.

But it was in the range of 15 to 30 nanomolar concentrations, which would correspond to this, but in the brain, not in the plasma.

Q But we also saw from the Burbacher paper there is a figure that shows the blood level of mercury going up, and then clearing fairly rapidly after each injection. And while the mercury is clearing out of the blood over a few days, it is still building up in the form of inorganic mercury in the brain.

A That's correct.

Q So is that relevant to the levels we're talking about here?

A Very much. We considered the organo mercurials; that is, the ethyl mercury. It's really a passport to the brain, the ethyl group. That means that even this level, which is the EPA's inorganic mercury in the plasma, if we attached an ethyl group to that and then let it have a passport to the brain, and equilibrate across the blood-brain barrier, then this concentration would reach the brain.

But in fact, the inorganic doesn't. It's only the organic form that are able to penetrate across the blood-brain barrier and achieve those brain concentrations. And now when they are hydrolyzed to

the inorganic form and are trapped behind the blood-brain barrier, then they gradually accumulate in concentration with, for example, every vaccination or other source of exposure, for years at a time.

So moving ahead, if I might, to slide 23. So having shown the inhibition of the uptake of cysteine, we would predict, if the cysteine uptake was blocked by thimerosal, and if cysteine was limiting for the synthesis of glutathione, then in the same cultured-cell model, which has limitations -- it's a cultured-cell system that we can use fruitfully for these studies, but it's not a brain as such -- we would predict that the blockade here should lead to a reduction in the glutathione levels. And indeed, the slide 24, the next slide, shows us the effects of these doses of thimerosal, again, a one-hour incubation at these concentrations, and this time the glutathione levels in the cell.

And we can see a graded reduction in the intracellular concentrations of glutathione, which reflect the blockage of the cysteine coming in, so you don't have enough to make glutathione. So naturally the cell has less of the antioxidant glutathione, and is therefore at risk intracellularly of the effects of oxidated stress. So these two things are very much in

correlation with each other.

Q And now again, explain the bottom numbers, those negative numbers along the bottom.

A This is the scientific terminology for concentrations of thimerosal here, in terms of molar concentration. The one nanomolar, or ten to the minus ninth, would be right here. I indicated before this 30-nanomolar level, that would be right here. So we can see that the concentrations that are estimated by some means to be present in the brain after a regimen of thimerosal exposure of monkeys, for example, in the Burbacher study, would produce significant inhibition, or significant reductions in this case of the glutathione that are attributable to the blockade of the transport that we saw in the previous data.

Q Now, when you said "right there," we --

SPECIAL MASTER VOWELL: You're using your pointer, and unfortunately we're not going to have your pointer when we go back and review your testimony next to your slides.

THE WITNESS: I'll try to --

SPECIAL MASTER VOWELL: So will you explicate what you just did, in terms of --

THE WITNESS: Excuse me, let me do that.

//

BY MR. WILLIAMS:

Q I'll ask the same question. You were pointing up and down between the numbers negative-7 and negative-8 there. And when you said "right here," would you explain that again?

A Excuse me for not recognizing the problem. Yes, I was indicating that if the data of Burbacher suggests that if there is approximately a 30-nanomolar concentration of inorganic mercury in the brains after a treatment with thimerosal, then that 30 nanomolar would lie somewhere between 10 to the minus-8 molar, which is 10 nanomolar, and 10 to the minus-7, which is 100. So the 30 would be approximately halfway between 10 to minus-8 and 10 to minus-7 molar concentration, which on this figure provides for approximately 75 percent of the full effect of thimerosal, a reduction amounting to two thirds of the normal level of glutathione, or reduction by two thirds.

Q So let me just try to summarize that. The level of organic mercury in the brain of those infant monkeys is equivalent to your 30 nanomolar, is that right?

A That's correct. Somewhere between minus-8 and minus-7 on this graph.

Q And on this graph, at that level of

thimerosal, how much of a reduction in glutathione did you have?

A Well, you can see that the Y intercepted at the left-hand side is about 750. And just looking at it myself here, I see that the area that would correspond to 30 nanomolar would be about 300, between 250 and 300. So a reduction approaching two-thirds reduction in the level of intracellular glutathione here. Substantial, an obviously significant reduction.

Q Your slide 25.

A Going further in the process here, if there is a reduction in the glutathione levels, as we just observed, then one might anticipate that thimerosal would also cause a reduction in the synthesis of the methyl B-12, or methylcobalamin, because its synthesis is dependent upon glutathione level. And the next illustration, slide 26, shows a bar graph in which a single concentration, a 100-nanomolar thimerosal, is used. And again, after a one-hour pretreatment of these cells, we see that the level of methyl B-12 is reduced to almost zero. This is a greater-than-90-percent reduction in methyl B-12.

And if we stop to reflect, we can see how the strategy of the neuronal cells pays off here.

That is to say, a reduction in glutothione, such as we just confirmed on the previous slide, here is turning off synthesis of the needed co-factor for methionine synthase. It's turning it off very efficiently. So that this assures that without methyl B-12 or methylcobalamin, that methionine synthase will stay off until the glutathione synthesis returns toward a normal level.

In fact, if this is not resolved, it will stay off indefinitely. That is to say, one can expect that methionine synthase and the activities it supports, including the D-4 dopamine receptor methylation pathway, will remain inhibited until normal oxidative status is regained. And if it's never regained, it's never allowed to reactivate.

So we can expect a persistent loss of whatever role it is that that D-4 receptor provides. And evidence is that it's necessary for neural synchronization during attention and awareness.

Q And because the inorganic mercury, according to the monkey studies, is trapped in the brain, is that going to create this persistent type of effect?

A It would, for as long as the trapped inorganic mercury remains in a position to block the uptake of cysteine, and otherwise maintain oxidated

stress in these neurons. Then this enzyme will remain inhibited, at least the co-factor synthesis in this slide no. 26 will remain inhibited, and the function it subserves will remain dysfunctional.

Moving on, the next slide allows us to say if you don't have the methyl B-12 availability, then one would predict that the enzyme, methionine synthase, as we've already alluded to, should be inhibited by concentrations that are relevant here for exposure to thimerosal.

And in the data in the next slide, which now brings us to slide no. 28, shows our measurements of methionine synthase activity, in the presence of either methyl B-12 measured with methyl B-12 as blue lines in this diagram, or hydroxy B-12 in red line. And the distinction between using those two co-factors is that the methyl B-12 is already methyl B-12, and doesn't require glutathione, whereas the hydroxy B-12 requires glutathione to be made into methyl B-12.

So we compared these two co-factor situations, and we compared them in the lower left-hand corner.

MR. WILLIAMS: Can you blow that up, Scott? The lower left-hand box?

THE WITNESS: The lower left-hand box in

this figure has thimerosal-dose-response relationship.

BY MR. WILLIAMS:

Q I ask these questions, and I don't know if I'm always going to get the right answer.

A Even though it says "aluminum" at the top, this is the thimerosal dose response curve here. And looking at this curve again, we can see potent effects of thimerosal if we look first at the hydroxy B12 assayed condition, we see that there's essentially a complete loss of activity of the enzyme with hydroxy B12 at concentrations as low as 10 to the minus-11.

Now, in our previous published study, Waly, et al, in 2004, we used hydroxy B12 in the assays in that paper. And as we reported in that paper, the thimerosal completely eliminates the methionine synthase activity. And so this is actually a replication, if you will, of that finding.

And we can see that when methyl B12 in blue is present, however, activity is still maintained at a higher level, even though it's going down as a function of thimerosal concentration. As long as the methyl B12 is provided, then you still have a significant amount of enzyme activity.

So from this comparison we can see that what thimerosal is doing is interfering primarily with the

conversion of the hydroxy B12 to the methyl B12 that's needed to sustain enzyme activity.

Now, the lower right-hand portion of this figure is again a measurement of the glutathione reduction that the thimerosal has caused, the far right-hand column is thimerosal. And again, you can see the reduction of approximately two thirds to three quarters of the glutathione concentration.

Now, the remaining figures, starting with the upper left, are different metal ions rather than the thimerosal. Again, at different concentrations for one hour, before measuring the enzyme activity here. The upper left-hand corner is lead, which is certainly associated with neurodevelopmental disorders, and is also recognized as an important environmental risk factor for ADHD.

To the right of the upper panel we have arsenic, an encountered environmental toxin. And the second from the top, in the middle panel on the left, is aluminum, which of course we recognize as a continuing adjuvant in vaccines, and shows important effects of aluminum, which aren't as potent, but are still very potent. Not as potent as thimerosal, but still very potent effect, sufficiently potent to inhibit here.

And then finally in the middle panel on the right is inorganic mercury. And the pattern for inorganic mercury most closely resembles that of thimerosal; it's only slightly less potent in its inhibition of the hydroxy B12 activity.

So the methyl, excuse me, the inorganic mercury and the thimerosal create a similar pattern of potent inhibitions here.

SPECIAL MASTER HASTINGS: Before you go on --

A Please.

SPECIAL MASTER HASTINGS: -- let me ask a question about this particular slide. This is a description, slide 28, of the experiments done in your lab that were reported in the Waly 2004 article?

A No. These are follow-up not-as-yet published results. The Waly article that you're referencing, we showed a similar result to the lower left-hand portion, but we used only a single concentration of thimerosal. And we showed a total loss of activity. The concentration we used was 10 to the minus-8, to my best recollection. And here in this more detailed follow-up study we used different concentrations, and we also used, by comparison, the methyl B12 as well as the hydroxy B12.

SPECIAL MASTER VOWELL: This is unpublished data.

A This is unpublished data from our lab.

BY MR. WILLIAMS:

SPECIAL MASTER HASTINGS: And let's go back, then, just to clarify in that regard, beginning with slide 21 through 28.

A That's right. Each of --

SPECIAL MASTER HASTINGS: All of these are from your latest unpublished experimentation.

A That's correct. The next one that I'll show next is from Waly, et al, but otherwise the preceding ones on the EAAT3, on the glutathione level, and the bar graph with the methyl B12, we have not yet submitted that for publication, because in fact the bar graph that I just showed, where I'm awaiting a particular graduate student's dose-dependent results on the methyl B12 concentration reduction. We wanted to show the dose dependence of that effect. And at this moment in time, that's data that's keeping us from submitting this for publication. So that's why I have only a bar graph instead of a dose-dependent graph.

SPECIAL MASTER VOWELL: Dr. Deth, I have another question while we're on slide 28. And that

has to do with the levels that are measured in the absence of whatever heavy metal you're testing.

There appears to be a significant variability. If we look at slide E, the upper limit is 100. If we look at slide C, it's 140. Why is that? I mean, it would seem to me if we're comparing effects, we would want to use the same scale.

THE WITNESS: I certainly could have used, maybe even should have used, the same scale. But I will say that these are not great variations, in my opinion. They range from let's say a Basal level of 70 to the highest and the lowest, and the highest that I see is, it looks like 128 to me, in panel C. It's less than a twofold variation.

Even though these are cultured cells, these experiments aren't done on the same day. They're not exactly twin studies in that regard. They may be done a week or two or three later, even if they're done in succession. And that amount of variation to me is not surprising, as experiment-to-experiment variability in the baseline activity.

I'd like them to be better, but the truth is the kind of variation that one encounters in using the cultured cells.

SPECIAL MASTER VOWELL: Thank you. Okay.

THE WITNESS: So these are indications that confirm the inhibition of methionine synthase activity is sensitive to thimerosal. If methionine synthase is sensitive to thimerosal, then the methylation processes that its supports should likewise be sensitive. And the next illustration just showed that schematically, slide 29.

So now we'll look at methylation events, not of the methionine synthase, but the actual methylation here. And then slide 30 has some of this. Now, this would represent published data from Waly, slide 30. Scott, thank you.

So in this data we examine the methylation of phospholipids again in the same cell system used in the preceding experiments. We see on the left the baseline activity. The lower line represents the activity of phospholipid methylation with nothing added to the cells. You see a level of approximately four.

When dopamine is added, we see one of those upper lines. In fact, the upper line that has the boxes as the symbols, and that's approximately 12 -- excuse me, approximately 13 -- indicating that dopamine has stimulated phospholipid methylation, as I described earlier.

And now, as thimerosal incubation are taking place, one hour at these different concentrations, we see a graded reduction in the baseline level. We can see that bottom line going down. And the dopamine-stimulated level likewise decreases, again potently affected by thimerosal here, reaching a maximum inhibition at about 10 to the minus-7 concentration.

Now, in the same figure is included the other stimulating agent, IGF1, or insulin-like growth factor 1. This is an example of a growth factor which acts similar to other brain growth factors, neuronal growth factor, brain-derived growth factor, and stimulates the signaling pathway that activates the cysteine uptake that I mentioned earlier.

And indeed, the IGF1 stimulation of methylation here is potently inhibited by thimerosal at the same concentrations that inhibit the dopamine-stimulated methylation.

The final line which I included in this published figure here was one in which we added divalent copper ions along with the IGF1. And we did that because of a paper showing that the signaling activity of this IGF1 was, in fact, copper-dependent. And we can see that when copper was added here, it offset some of the effects of the thimerosal. The

thimerosal is not as effective when this extra complement of copper was added in the experiment. And I can just mention here that what the copper is doing is affecting thiol status in such a way as to counter the effects of the thimerosal.

So in any case, this slide verifies the potent effects of thimerosal on phospholipid methylation, including dopamine-stimulated.

The next slide no. 31 shows a similar phospholipid methylation experiment carried out with lymphoblasts. Instead of using human neuronal cells, here we're using white blood cells in culture or lymphoblasts. And similarly, we're adding concentrations of thimerosal here.

And thimerosal again inhibits phospholipid methylation in these lymphoblasts. But the potency is somewhat less, perhaps tenfold or more less, than it is in the neuronal cells. The purpose of including this is to indicate that the higher sensitivity of thimerosal is associated with human neuronal cells, compared to even other human cells, in this case human lymphoblasts in cell cultures.

So the upshot of that would be to alert ourselves to the most vulnerable tissues, most vulnerable cell types would be the neurons in whom, as

I indicated, the transsulphuration is less efficient.

So we recently, even more recently than that previous data, have had the extraordinary opportunity to measure the levels of methionine synthase at the level of its messenger RNA in autopsy-based post-mortem samples of autistic subjects in age- and sex-matched control subjects.

The next slide just illustrates what I'm referring to here. Slide 32 shows us that the final protein, for example at the bottom, methionine synthase, its availability depends upon its gene in the DNA, which is transcribed to its MRNA, or messenger RNA, which then gives rise to the final protein enzymes.

And indeed, regulation of methionine synthase activity we can understand from this relationship, regulation of methionine synthase can be exerted at the protein level. For instance, the cofactor can be oxidized of B12, it can be exerted at the level of the messenger RNA, which can be, for example, determine how much messenger RNA is translated into protein. Or it can be at the gene level itself, how much original product from the gene is made into messenger RNA that is transcription.

So we can see that nature can regulate the

activity of methionine synthase in very short microseconds or millisecond waves, that's a level of the co-factor, or for days or hours at a time, depending upon which level of control is chosen.

We had the opportunity to use messenger RNA samples that were provided to us by the Autism Tissue Program, and maintained in part by Johns Hopkins Institute. And in fact, the samples that we were able to obtain and to analyze the messenger RNA from were the same samples in most part used by Vargas, et al, in their study, in which they concluded that there was neuroinflammation present in these post-mortem brain samples.

So essentially what we were able to do is, using those same samples, ask the question is what was the level of the methionine synthase messenger RNA in those same brain samples.

So the next slide --

BY MR. WILLIAMS:

Q Let me stop you just for a second.

A Excuse me.

Q To the extent that the DNA is affected here by the toxin, we're not talking about genetic damage, are we? We're talking just about shutting down the gene operation.

A I haven't shown you the results yet.
Q Okay.
A When we see those results, we could interpret them in light of the fact that regulation, rather than mutation, may be taking place. Okay?
Q Okay.
A I think we're ready to look. Actually, the next slide just illustrates how we did this experiment.
Q And its slide number?
A Excuse me now, it's slide no. 33. And we carried out a very basic, a very commonly employed laboratory procedure called PCR, or polymerase chain reaction, which is used to amplify the available messenger RNA. And by using comparison samples, one can estimate the relative amount or abundance of the messenger RNA for methionine synthase.
Usually the PCR reaction is carried out with one so-called primer set for the entire gene or messenger RNA. But we recognize that the methionine synthase has five of those different domains or regions to it. We devised a method, or very simply used a method where we had primer sets directed against each of those domains of the proteins that I introduced earlier: the pink one, the green one, the

yellow one, the red one, and the blue one.

And so we did what we can call primer, excuse me, domain-specific PCR. And in doing so, we found, although I won't include those results explicitly here, we found that that cap domain, the yellow one that is in the middle, is actually excised at the level of messenger RNA as a function of age. And in elderly individuals, it's missing.

But we then, using this strategy, analyzed, as I said, the autism samples versus controls. And now slide 34 has the first of that dataset. And shown here on the left, the open bars represent the abundance of messenger RNA for methionine synthase using primer sets directed against the cap domain. Or on the right side of the open and closed bars, the primer sets directed against the B12 binding cobalamin domain.

So these are two different types of messenger RNA that we're probing for here. And in either case, in both cases, we found a significantly lower amount of the messenger RNA for methionine synthase in the autism brain sample.

Again, if we think of these as samples in which neuroinflammation could be or was detected, we can then suggest the possibility that there is a

relationship between lower levels of the messenger RNA of methionine synthase, and the presence of inflammation.

Because indeed, if you have less messenger RNA level, then in fact you would have less of the enzyme, and more homocysteine would be diverted to make more glutathione, an appropriate response at that level of regulation to inflammation or oxidative stress.

The next slide no. is 34; 35 allows me just to capture the main thoughts from that result. And that result indicates that the brain levels of methionine synthase, MRNA, are indeed significantly lower in autistic subjects, and at that lower level of MRNA will lead to diversion of homocysteine to more transsulphuration and glutathione synthesis. And again, the Vargas study indicates the presence of neuroinflammation in these very same brain samples, suggesting that these two outcomes are related to each other. And we can propose as an end statement that reduced transcription of methionine synthase may be viewed as an adaptive response to the presence of oxidative stress and neuroinflammation.

Again, this alerts us to mechanisms that nature can employ at multiple levels to control the

flow of homocysteine towards transsulphuration or not.

Now, in this same dataset, the next slide no. 36, allowed us to do a paired comparison, because we had paired samples, age-matched samples and sex-matched samples. And we were struck by the pattern that we observed here.

On the right-hand side are the representative members of the pair from the neurotypical controls, and on the left, the autistic members of the pair. And we color-coded the samples here into age groups. That is to say, individuals that were between the ages of one to five are sort of a red color; correspondingly, six to 10, orange; 11 to 15, yellow; 16 to 20, green; 21 to 25, blue; and then finally 26 to 30, the samples that were in purple here.

And in the controls in particular, what you can see is an age-dependent pattern. You can see that the youngest individuals in this control group had the highest levels, and that progressively, as age increased, there was lower levels across the span of the ages that we had available to us.

And the number of samples is limited here, thankfully, because these are post-mortem samples. And the ones that we had available to us in this

limited number however do show this age-dependent pattern here.

On the other hand, the autistic samples, even at a young age, had a much lower level of methionine synthase activity. And in fact, the decrease associated with autism was dramatically lowered as a function of age.

So the implication here is that if you have oxidative stress and a reduction in methionine synthase as a compensatory or adaptive response to that, the impact is greatest when you're young.

So I think the slide 37 provides again a narrative summary. So in the limited samples that we had, again, the pattern of age dependence to the reductions in methionine synthase, the messenger RNA, was apparent. The age dependence was obvious in the controls, but not obvious in the autism samples. The MRNA levels in autism don't show an age-dependent pattern, we'll call it the normal age-dependent pattern.

And the conclusions from that would be that the inhibition of methionine synthase in this case by the neuroinflammation and oxidative stress, confirmed in the same samples by other investigators, is of greater significance at younger ages.

Now, the occurrence of autism is estimated to be one in 150 individuals, by the CDC. And so this tells us that exposure to thimerosal or other uniformly exposing agents in our society only affects a subpopulation of this society.

And this has led to the suggestion that the subpopulation with autism has certain genetic features. And the genetic features have in part been investigated. And the next slide no. 38 shows an illustration of the findings of Dr. Jill James, published in 2006.

And in her study, she particularly focused on normal polymorphisms; that is, normal variants of genes that are involved in the pathways that I reviewed here. Pathways involving methylation and transsulphuration.

The genes that she particularly investigated in her population were highlighted in pink in this illustration. For example, on the left, the enzyme MTHFR, methylene tetrahydrofolate reductase, a gene that has several, two distinct polymorphisms. And that enzyme normally makes the methylfolate that the enzyme methionine synthase depends upon.

Next to it we have the RFC, or the reduced folate carrier gene, RFC-1. As the name implies, this

gene product is the proteins, the carrier that brings folate into cells. Again, folate is required for methionine synthase activity.

Next in the middle we have transcobalamin II. And transcobalamin II is the enzyme, the transporter that brings B12 into cells. And in doing so, it limits the activity of methionine synthase.

On the right-hand side we have catacol methyltransferase, COMT, the enzyme that determines the duration of dopamine action.

And finally at the bottom, glutathione S transferase, particularly the M-1 form. And so Dr. James examined these proteins and their genes, because the polymorphism that they normally show might or might not be more prevalent in a particularly at-risk population.

The results that she found, that I'll present here because of how they relate to our work, in the next slide, which is designated as slide 39, the results that she found in this table is an association between the risk associated alleles; that is, the lower-activity alleles. And she highlighted them in bold print in this particular diagram.

And as we can see by the occurrence of bold print, these risk associated alleles in these

particular genes are associated with a higher frequency in the autism population. That is to say that they are at risk of problems with methylation, problems with methionine synthase, and problems with dopamine, if they have these alleles.

I actually didn't mention the last one on this list at the bottom. It's methionine synthase reductase. It's responsible for the activation of the enzyme. But she also found a significant association with methionine synthase reductase especially when compared in combination together. Each of these risk alleles brings an individual risk. But in the common pathway, such as we've described, they can be additive or even synergistic.

And the next slide now, no. 40, illustrates combinations of these alleles further increase the odds or ratio of autism. That is to say, when you have three, four, or more of these common alleles, then your risk is accordingly higher than if you have only one or two.

So this genetic data especially from Dr. James's study, which focus on the methylation and redox cycles, starts a process of identifying the at-risk population from their genetic features.

Q And they are at risk because of an

interference with the redox?

A The presence of these polymorphisms indicates that the enzymes, let's say in the case of MTHFR for example, that they function at a lower rate than if that polymorphism was not present.

Alone, in the normal circumstances, in a circumstance or an environment where there was no extraordinary challenge by stressful factors on the system, those polymorphisms are not a commitment to the outcome of autism or any other disorder. In fact, their occurrence at high frequency in the population suggests that they may have a favorable role to play under most environmental conditions.

However, in the presence of adverse environmental conditions, such as perhaps the introduction of heavy metal toxicity, then these otherwise latent polymorphisms, or risk factors, can be activated to in fact be real consequential risk factors. So it's really reflecting the fact that our evolution in one environment may not be ideal for a more hostile alternative environment.

So the final slide that I've prepared here, and it's part of the review article that I published in The Journal of Neurotoxicology in January of this year, is an attempt to summarize the relationship and

the interaction between genetic risk factors identified here in red, and environmental exposure, a broad term. Environmental exposure includes things other than in genetic nature that are, that are the organisms that we are exposed to during our lives. In this case, in this proceeding, it's meant to include thimerosal and the inorganic mercury that it releases in the brain.

And such exposures, for reasons that I outlined, impair sulphur metabolism, especially when their focused target is sulphur compounds. And individuals that possess these risk factors in combination are at high risk.

The risk arises because of the importance of sulphur metabolism for oxidative stress, and in responding to oxidative stress. I've illustrated that the enzyme methionine synthase is a particularly important factor, and that the polymorphism affecting methronine synthase, directly or indirectly, introduce a high level of risk.

The consequence of inhibition of methionine synthase are manifested throughout methylation reactions. There is 100 to 150, 200 such reactions. They will all be affected. Some of the most important include DNA methylation, as I specified earlier, whose

consequence will be altered gene expression during development.

So the idea of this mechanism accounting for developmental disorder is rather direct.

Q Now, I have one other question I wanted to ask you. It was a question that was put to Dr. Aposhian this morning by the defense. And that is, you studied oxidative stress and have been working on oxidative stress.

If chelation is shown or found to be effective in helping autistic children recover some of their function, but it doesn't pull inorganic mercury out of the brain, is there some explanation related to your work that could explain that phenomena?

A The oxidative stress that's actually the last slide illustrated, and otherwise we've talked about, in the case of autistic individuals, it is a whole-body oxidative stress. The fact that you can draw a plasma sample and find a 40-percent reduction in circulating blood of glutothione indicates very strongly that it's a systemic, not a problem restricted to the brain.

And as such, the mercury effects that I mentioned and spoke about as a general feature of sulphur metabolism are affecting peripheral tissues,

like liver, important metabolic organ that it is.

And so the chelation of peripheral mercury can have useful effects by restoring normal metabolism and normal redox state peripherally, helping peripheral cells to work better. And as a result, the beneficial peripheral metabolism can affect brain. A most explicit example would be, for example, would be reducing the amount of inflammatory cytokines in the blood that otherwise could contribute to neuroinflammation, or the availability of the cysteine that's ultimately the source of sulphur compounds for the brain, and for neuronal inflammation.

So there are benefits from correcting heavy metal exposure and toxicity peripherally that can have benefits for neurological function, even though the chelating agents don't penetrate the brain and directly remove the mercury from the brain, in my opinion.

Q So do you have an opinion, then, as to whether thimerosal exposure and inorganic deposition in the brain at the levels we've seen in the monkey studies, can those levels of thimerosal and inorganic mercury cause interference to the brain that can lead to autism symptoms?

A Based upon both my understanding and reading

of the literature, the results of others that I've incorporated into my own presentation, as well as the direct result that we have obtained when we looked -- and the important thing is that we have looked -- and when we looked, we found basically at every turn the effects of thimerosal, which suggests that it has the molecular capability to cause autism, and to account for the major symptoms of autism, which include impaired attention, awareness, sociability, and neuronal synchronization in the gamma range.

All these things together lead me to the unavoidable conclusion that it's involved as a causative factor in autism.

MR. WILLIAMS: Thank you very much. That's all I have.

SPECIAL MASTER VOWELL: It would appear to be an appropriate time to take our mid-afternoon break. So let's plan on 15 minutes, or do you need a little longer?

MR. MATANOSKI: May I have a little bit longer than that?

SPECIAL MASTER VOWELL: How much time would you like?

MR. MATANOSKI: May I have until five after?

SPECIAL MASTER VOWELL: Certainly.

MR. MATANOSKI: Thank you.

SPECIAL MASTER VOWELL: So we will reconvene at five after 4:00.

MR. MATANOSKI: Thank you.

(Whereupon, a short recess was taken.)

SPECIAL MASTER VOWELL: Please be seated. We're back on the record in the Autism Omnibus Proceeding Theory II in the King and Mead cases.

You may proceed, Mr. Matanoski.

MR. MATANOSKI: Thank you.

CROSS-EXAMINATION

BY MR. MATANOSKI:

Q I am Vince Matanoski, and I'm representing the United States. Good afternoon, Doctor.

Doctor, could you tell me the strongest piece of evidence you have to support your hypothesis?

A I would say the strongest piece, especially in the context in which I presented today, is probably the post-mortem samples showing, in the real brains of real people with autism, a down-regulation and alteration in the enzyme methionine synthase, that serves the role, as I described it, relating to dopamine receptors, as well as other important methylation events.

Q Do I understand this to be the information

that you were giving us towards the end of your talk this afternoon, that had to do with work in your own lab?

A That's correct, as I presented it. I took your question to, I think you had the term as you. So I'm not saying our work is the most important. I'm saying that among the work that I presented today of ours, that's the one piece that I think is most important in describing, in relating these findings to autism.

Q I'm sorry, anything else?

A That was how I took your question. Were you asking about our work? Or were you asking about work in general?

Q I'm asking what you believe, in forming your hypothesis, is the strongest piece of evidence to support that hypothesis.

A I must defer to the work of Dr. Jill James, whose studies involving both measurements of sulphur metabolites, but also the genetic polymorphisms, which I presented, I think are clearly the strongest evidence in favor of that hypothesis.

Q Would those be the two studies that you referenced in your report? In your report you referenced, and your report being Petitioner's Exhibit

No. 23, I believe you referenced those as 9 and 10?

A That's correct.

Q Okay. And I was hopefully going to be able to tell everyone, so that we'd have it for the record, I believe No. 9 was PNL No. 49, and No. 10 was PNL No. 5, I believe.

(Discussion held off the record.)

BY MR. MATANOSKI:

Q Doctor, we'll turn to your CV. The memberships that you list there, in terms of these various memberships, are these memberships -- how does one join these different organizations? How did you join them?

A Well, the first several are, they invite you as honor societies; Rho Chi happens to be in the pharmaceutical area that I'm in.

Q I'm sorry, that was pharmaceutical?

A Rho Chi is the honor society for pharmaceutical areas.

Q Okay.

A The AAAS is just science, and you just subscribe basically to Science Magazine, and you are a member of the AAAS.

Q So if you buy a subscription to their magazine, you become a member?

A That's my understanding. The Society for Neuroscience, you have to be, I'm trying to remember because I've been a member for many years. You have to have approval of other members who sponsor your membership, and evidence of your publications.

Q Are you still a member of the Society for Neuroscience?

A Yes. You want to check my dues? The date, or something like that?

(Laughter.)

Q And once you're invited, you pay dues to stay in, is that --

A That's correct.

Q And the American Association of Colleges of Pharmacy?

A That's an education one.

Q How did you enter that?

A It's, as a faculty member in a school of pharmacy.

Q You enter by, is it dues?

A You decide to join.

Q So it's by your own decision to join.

A Yes. It's not a distinction. I hope you're, I hope I didn't misrepresent these as points of distinction or something like that.

Q No, I'm just asking. You haven't; I don't believe you have. I'm just asking on each of these how one becomes a member of that organization.

And the American Society of Pharmacology and Experimental Therapeutics? Is that by your voluntary --

A You likely have to I think be sponsored. Again, I've been a member of that for 30-some-odd years. I probably don't recall the exact criteria. But I know you have to nowadays be sponsored by someone who is a member.

Q So it's a membership that's voluntary; it's not an honorary membership.

A None of these are honorary memberships. They are just indications of my affiliation at some point with these organizations.

Q So the last one is also not an honorary membership.

A No.

Q Okay. Also in your curriculum vitae, you mention some grants. And then you have a section after you talk about the grants where you say "grants pending."

Now, by "pending," did you mean that those grants had been approved, and you were just awaiting

funding for them?

A No. I think the common use of the term "pending" in these circumstances is that application has not been acted upon at the time at which this document was prepared.

Q Okay. So these are just grant applications, then.

A That's right.

Q They don't necessarily reflect any approved research by the organization.

A That's right. That's what the word "pending" means.

Q And the timeframes that you have there, some of them extend back to 2005. Does that mean that --

A It probably means that this is an out-of-date CV.

Q I see, I see. Well, then, maybe you could tell me, on the one that you submitted, the grant you applied for from the Nancy Lurie Marks Foundation, was that approved?

A No, that was not approved.

Q And the one that you submitted to NIH, was that approved?

A The one that I submitted to NIH. That one sticks in my mind, because that one was not approved

with the -- instead of the review of my project, there was instead a cutting and pasting of a statement from the FDA indicating that thimerosal does not contribute to autism. And therefore, that particular grant should not be funded.

So in fact, it was not funded because there was a sentiment on the part of the primary reviewer that it was inappropriate to study thimerosal, because it doesn't cause autism.

Q I see. So the conclusion was, so this grant wasn't --

A That's correct.

Q Wasn't approved.

A That's correct.

Q And the conclusion in not approving it was essentially the money shouldn't be spent there, because there has not been any verification that thimerosal causes --

A Because the FDA website posted a statement indicating -- and it was literally cut and pasted -- so it was clear that that was the factor, a factor.

Q If you know, when NIH receives a grant, do they ask for other individuals outside of NIH to review the grant to determine whether or not it should be approved?

A They constitute a study section. Each grant is assigned to a study section, which may have inherently the appropriate expertise to review and evaluate that application. If not, then the chair of that study section can opt to bring in additional reviewers. It's not a necessary part of the process, but it can occur.

Q And the study section, so do I understand that it is not necessarily NIH employees that review your grant application, or a grant application?

A No, in no case is it really NIH employees. But there are fixed study sections whose membership includes people from broad aspects of academia and non-academia.

Q So the study section isn't necessarily the government, in other words; it's a spectrum of academia that has an interest in that area, an expertise in that area.

A That's true.

Q And that was true in the case of the grant application you made concerning methionine synthase, methyl B12 synthesis in autism?

A I'm sure it was true then, yes.

Q You mentioned you had several book chapters on autism pending?

A That's correct.

Q By "pending," do you mean that they are awaiting publication?

A That's correct.

Q Could you describe those book chapters?

A One, under the editorship of Dr. Gene Blatt, Boston University, a chapter which he asked me to contribute relating to D4 dopamine receptor methylation mechanism that I described and its relationship to autism.

Second, I'm a co-author of a chapter of a book focused mainly on nutritional aspects of childhood diseases in the chapter on autism, co-authored with Dr. Patricia Cain.

Q Are these expected out any time soon?

A Books can take most of a year. I wouldn't expect them any sooner than the end of this year.

Q In the D4 -- have you written a chapter?

A Excuse me?

Q Have you written a chapter that you're giving to James Blatt?

A No, Gene Blatt. Yes, I gave him that chapter several months ago.

Q In that book chapter on D4 dopamine, did you conclude that there is sufficient evidence to conclude

that thimerosal-containing vaccines cause autism?

A This was not a chapter about thimerosal, although I included a paragraph about environmental factors including, but not limited to, thimerosal that can contribute to autism.

Q And did you say that it's established that these environmental factors, such as vaccines, cause autism?

A I don't believe I did, because that wouldn't reflect the general establishment.

Q That wouldn't reflect the -- this is your book chapter, though. This is your writing.

A The term that you used, could you repeat your question? Then I could --

Q Yes. I wasn't understanding, because your answer doesn't reflect the establishment.

A I asked what your question was. I wanted to clarify my answer, if I could have your question.

Q Why would you write something different in that chapter than what you're testifying to here? That ought to be an easier way to ask it.

A The chapter was about, was not about thimerosal. And in fact, I wanted to not make thimerosal the focus of the chapter. I wanted, as I was requested, to make the D4 dopamine receptor the

focus of the chapter.

Q I'm sorry, I wasn't clear enough. I did say the chapter, and I should have said the paragraph that dealt with environmental factors. As I understand --

A And your question was?

Q I understand your answer to my previous question, that you didn't put in there that you believe there is sufficient evidence to conclude that thimerosal causes autism. And then your answer to that, to subsequent questions, had something to do with the establishment. And I was trying to understand what you meant by that.

A I was answering your answer to me. I'm not sure we have a transcript here, but you asked, you said something to me about established -- what did you ask me? I was responding to what you said.

Q Maybe we should start again.

A I think we should. So if you'll ask your question, I will respond.

Q Well, I had one.

A If you ask it again, I'll respond again.

Q What does your paragraph concerning environmental factors say in the book chapter that you submitted?

A I included a paragraph about environmental

factors, including, but not limited to, thimerosal as possible causative factors interacting with the D4 receptor causing oxidative stress, and contributing to the causation of autism.

Q So there you used the term "possible."

A I used the term "possible," that's right. Referencing, of course, the range of factors. Because the probability of each of them, and the probability of exposure of large numbers of children across the country to individual agents is not as predictable for each of these possible agents. So the term "possible" certainly applies to a group of agents with differing possibilities of exposure and contribution.

Q So your conclusion there is that it's only possible; you didn't say it's established.

A I don't have it in front of me, and at this point in this inquiry, thinking that I would have to refer to that which I don't have in front of me. If you would like precise language, I'd have to have access to that.

Q Your recollection is --

A My recollection is?

Q -- on this, that you said "possible," but not that you said that it was established.

A The precision that you're looking for I

don't have available to me, because I don't have the chapter available to me.

Q So you don't know.

A I don't know? I don't have the chapter available to me. If it means I don't know or it means something different.

Q Well, let me ask you this about that particular point. Would you be more cautious in what you say in a book chapter than what you say in the courtroom about what you're willing to conclude?

A There's no reason to be differentially cautious. The theme of a chapter might be different than the theme of a courtroom proceeding, in which case different questions, different facts, different specifications would apply.

Q So what you write for the scientific community in general would reflect your true beliefs about a subject.

A I would hope so.

Q Now, funding for your research in general, what's your research budget, say on an annualized basis, for this year? How much money do you have for research in your lab?

A Not much, but I can be specific. My lab, for the past year -- I'll use as reference my past

year -- I've had a post-doctoral fellow, Dr. Musafa Waly. I've had two doctoral graduate students and a number of Master's students and undergraduates.

The post-doctoral fellow pay at the rate of $40,000 annually, plus fringe benefits of 25 percent, would bring it to $50,000, and the graduate students and supplies and things like that probably add $30,000 to $40,000.

So I suppose at a minimum, somewhere at $80,000 to $90,000 for that year. Now that post-doc, because I don't have the money to pay him going forward, he'll be terminated June 13.

Q So the past year you figure your research budget was about $90,000.

A That's a reasonable estimate.

Q What are your sources of funding for your research budget?

A Over the past five years, they have been largely organizations that have an interest in autism, typically parent-supported organizations, including Cure Autism Now, which later merged with Autism Speaks. The Safe Minds, the National Autism Association, and the Autism Research Institute. These are the primary sources.

Q For your last year, since we are talking

about $90,000, do you know how much of that would come from the various organizations? Do you know how much, let's say, came from Safe Minds?

A This past year I had a 50/50 shared grant with the National Autism Association and Safe Minds. My recollection is it amounted to $43,000. So Safe Minds would have been half of that.

Q I'm sorry, so half of $43,000 was the contribution from Safe Minds, and then the other half was from another one?

A National Autism Association, NAA.

Q And the balance of that would be made up from Autism Research Institute and Cure Autism Now?

A I had two grants during that time period, from Autism Research Institute. The Cure Autism Now, I remember, I don't know whether this was this past year. They funded me for a two-year period. I'd have to be more precise about whether it overlapped with the past calendar year.

During the past calendar year I had two separate grants from Autism Research Institute, one to investigate the importance of methyl B12 in methionine synthase activity in the brain and the neuronal cells. And also another one to investigate the methods for measuring homocysteine thiolactone.

Q So do you know roughly in the last year, since we're using that as our example, how much came from Autism Research Institute? How much of that $90,000 would represent money from Autism Research Institute?

A Let's see. The thiolactone study I referenced was for $35,000. Although that is still ongoing, so only a portion of that would be attributable to the previous 12-month interval. The other one would -- so I would apportion $10,000 into that, if you like. Thirty-five thousand for another one, maybe $40,000, something like that.

I didn't come prepared with the numbers.

Q I was just asking for your rough estimate. Doctor, doesn't at least Safe Minds at least have an explicit research agenda to find a credible, as they call it, credible findings to support the mercury autism hypothesis is true?

A Quite frankly, I don't know explicitly what their, what their website is. I guess we're about to find out what it says.

Q And they funded that particular study of yours. Is that the one you were referencing, or was that a different one?

A That's an earlier one.

Q So they funded other work that you'd been doing, as well.

A Right, before this calendar year.

Q In all, how many studies did they fund for you?

A Two. The one that we see here on the screen, and the more recent one that I mentioned was co-funded with the National Autism Association.

Q Thank you. How many publications do you have that directly deal with oxidative stress?

A Well, since we only recognized the role of oxidative stress subsequent to the Waly paper that I mentioned earlier today, which was published in 2004, it only really, it wasn't immediately after that paper that we uncovered, I guess, the role of oxidative stress in regulating methionine synthase.

And so we've researched on that in the interval of I would say 2005 now until current times, and have only published a review paper during that interval that is directly about oxidative stress.

Q A review paper? That's the --

A A review paper.

Q -- only publication that you have on oxidative stress?

A Yes, that's what I said.

Q So there's no publication that you have that's direct research that you've done into oxidative stress.

A Not at this time.

Q How many years did you work in cardiovascular diseases?

A My PhD work began in that area, and it began roughly 1972, and then through 1996. So you're talking about 24, 25 years.

Q And how many years have you researched mercury?

A Well, I don't actually -- we have done research on mercury. But again, as an offshoot of our interests in this D4 dopamine receptor, and the mercury aspect only came in I suppose 2003, I believe. Wait a minute, let's see. I believe 2002 or 2003 would be my estimate of when we first did our first studies with thimerosal and mercury.

Q So five or six --

A Leading to the publication that was Waly, et al, which was 2004.

Q So for five or six years, you have had some research interest in mercury.

A Four or five.

Q Four or five years. And is the 2004

publication, Waly, et al, that you referred to, is that your only publication on mercury?

A Let's see. Actually, in my monograph there was a figure involving using effects of thimerosal that preceded actually the Waly paper. Then I believe that is the only, those two would represent the only two.

Q There's one before the 2004 Waly, et al paper?

A The book that I wrote the monograph I wrote, I believe had a figure in it. Obviously the book was not about thimerosal or mercury. But my recollection is the chapter on autism in that book included a figure, and that was something. The book was sent to the publisher in 2002 or something like that.

Q Is that book a peer-reviewed book?

A Not really, no.

Q It was written by you, it wasn't submitted to an editor?

A It's not a peer-reviewed, it's, it would be a monograph that I wrote.

Q So there was no scrutinizing what you had worked to determine whether it reflects --

A It wasn't the nature of that publication that it would be scrutinized.

Q What was the nature of that publication?

A Let me put it into context. We had made what seemed like an unusual finding, where a neurotransmitter receptor, the neurotransmitter being dopamine, which was linked to dopamine being linked to schizophrenia being linked to ADHD, other disorders seemed like finding a new action of that neurotransmitter and its receptor, the D4 receptor, might be worthy of pause and worthy of analysis in terms of what role it might play.

So I took a sabbatical year, and used that sabbatical year to do that; to look into the literature not only about methylation and lipid events, but neural network and theories of attention and cognition and neurosynchronization, as well as look into the biochemical foundations for various neurological disorders. Asking myself, but at the same time using the opportunity to express what I found in a monograph. And so that resulted in the publication of that book.

Q Did you take any parts of what you were writing, and attempt to put them into a paper and have a published, peer-reviewed paper?

A Well, the thimerosal figure that I mentioned before appears in the Waly article, so I suppose that

would be an example of that. But it wasn't the purpose of the book to somehow use it as a stepping stone for publishing the same information in the form of papers. I thought the book was sort of an end point in itself, and it wasn't on my mind at least to somehow create papers.

Really, the book represented an opportunity to synthesize bodies of information and integrate them. And it's really provided me with a very useful framework from which to go forward to do research. But that research is not necessarily already in the book.

Q Do you receive, did you receive payment for your work, this monograph? Did you get paid for this?

A I think it's like two percent of the, I'm not sure whether it's two percent of the total. But I think it amounted to a total of $500 over a period of five years, or something like that. Maybe even less.

The other day I think I sold five copies of it. We're not talking about a bestseller here. And so I think, I don't even think they bothered to put a check in, as a matter of fact, for the possible royalties. It might have been Starbucks kind of money.

Q So you didn't have to pay to get this

published, did you?

A No. Not quite.

Q Okay. But it really hasn't generated a lot of sales.

A Let's see. Have you checked the price of this book?

Q No.

A No, okay. One of the unfortunate things I learned, besides the science that I gathered from my book, was to be a little more careful in choosing -- not that I had that much choice -- your publisher and the arrangements. Because the book, the last time I checked, was $180 for a 200-page, rather small, modest book, or something like that. It was designed to not sell, from a financial standpoint.

(Laughter.)

A And I was disappointed, because I think the information in that book, as relative to the testimony I gave today, is actually very worthwhile. And it was a heartfelt effort on my part to write it. So it was worthwhile doing it, but it certainly was never really one for monetary gain; and in fact, it never resulted in monetary gain.

Q Publishers don't price their books not to sell, though, do they? I mean, they go out of

business if they try to publish a book that's not going to sell.

A We're way off base in terms of why I'm here. But otherwise, publishers sell books to libraries at whatever price libraries will pay, and it's not necessarily the consumer market that they have first on their minds. They may be just looking to have something available for institutions to buy for their libraries.

Q Doctor, I've looked at some of your presentations at Defeat Autism Now conferences, and actually some other works that you've put out that are on the public sphere. And I think every time I've seen you reference articles written by Mark Geier as support for your hypothesis. Is that right? Do you usually cite Mark Geier as support for your hypothesis?

A Am I aware of their work? Is that what you're asking?

Q No. I'm wondering with your presentations, for example at Defeat Autism Now conferences, you cite Mark Geier.

A I am aware of the Geiers, I am aware of their work, and on occasion I have cited their work.

Q I didn't see it as one of the references in

your opinion here, and I was wondering why that was.

A The citations that I gave here support the work that I included in my remarks.

Q In your 2007 review paper, did you cite Dr. Geier there?

A To which paper are you referring to?

Q I think you were talking about a review paper that you just, that you had come out.

A In 2008? I thought you said 2002.

Q Oh, 2008. I'm sorry, 2008.

A I may have. Some of their work is very interesting, and they are very active clinicians and investigators in the autism area.

Q So you've relied on their work?

A I rely on their findings within the context to which it's presented.

Q So then I take it since you didn't cite it here, it doesn't support you, you don't find it to have value in the context of the hypothesis you're giving us today?

A I wouldn't necessarily draw that conclusion.

Q Oxidative stress, would it be fair to say that that describes a very general mechanism of injury?

A Oxidative stress status, or redox status, is

fundamental to cellular function for many different cells. As such, it represents a very general mechanism that could express itself as, and does express itself as different diseases in a general way. The question is a bit vague, and I'm not sure if that's an adequate answer.

Q I think that is. In other words, it plays a role, or is thought to play a role in a wide variety of diseases, correct?

A That's correct.

Q And isn't it thought to be caused by a wide variety of events?

A That's a reasonable statement.

Q For example, exposure to infectious agent? Is that right? Would that cause oxidative stress, or could it cause oxidative stress?

A It could. We recognize that part of the innate immune system, the resistance is mounted against an infectious organism. Includes an important role for oxidative events.

Q Let's say I went out for a jog. Will that create an oxidative stress state in my body?

A While I'm not expert in exercise physiology, I wouldn't expect that it would. So there are limitations to what I know about oxidative stress

during exercise. I wouldn't define the experience of exercise as oxidative stress, although it's such as dynamic and moment-to-moment.

So the response system that I suppose that mitochondrial activity being heightened during exercise might change, even increase the generation of oxygen species. So there might be a dynamic change in the redox systems. But it's certainly beyond my expert knowledge to say more than that.

Q So, okay. So you don't know anything more than what you've stated just now, in terms of --

A About exercise and oxidative stress?

Q Yes.

A That's probably a reasonable statement.

Q Now, your mechanism of thimerosal triggering oxidative stress then would potentially implicate a wide variety of diseases as a possible outcome. Is that right?

A Potentially, it could.

Q I believe you were talking a little earlier about Parkinson's disease?

A Parkinson's disease is another oxidative stress-related neurodegenerative disease.

Q Alzheimer's?

A Too.

Q Do you believe that either of those diseases are caused by exposure to thimerosal?

A I don't. I would recognize that those are diseases of advanced age. And our work that I alluded to in the background is consistent with the idea that the ability to tolerate oxidative stressors decreases with age, because aging itself is considered an advancing oxidative state. And with thimerosal exposure that we're concerned with here being restricted to younger individuals, it's unlikely that it contributed to the lives of those old enough to suffer these degenerative diseases of old age.

Q So younger individuals are better able to tolerate oxidative stress.

A That's consistent with what I know. However, let me qualify that. By tolerating, it means surviving it. And when you survive an insult, it doesn't mean that you don't carry the scars, or otherwise the consequences of that even temporary episode that you survived.

And so one might consider certain conditions to reflect the influence of oxidative stress, but not to the catastrophic end that autism can represent.

Q And you have hypothesized that thimerosal, through this mechanism of oxidative stress, can lead

to obesity?

A I've noted at a recent conference that a paper was published, perhaps six months ago now, by researchers in Italy. And I found that paper of interest. It popped up on a search mechanism that I use to follow methionine synthase-related literature.

Because what these researchers found was that some of the same genes that I referred to from Dr. Jill James's study, explicitly methionine synthase itself, methionine synthase reductase, and I believe MTHFR, methylfolate synthesizing enzyme, that the polymorphisms of those enzymes, according to their study carried out in Italy, were highly associated with obesity. And that is, in combination, they found up to a 16-fold increase in obesity risk, odds of ratio for obesity, with the same genes that I have, or I have been paying attention to, and Dr. James has associated with autism.

And at least it caused me to entertain the hypothesis, which I publicly passed along, the hypothesis that, in fact, other conditions that we perhaps are experiencing epidemic outcomes of, might also be related to shared mechanisms.

Q Under your hypothesis or mechanism of thimerosal causing autism, what's going to happen to

neurons?

A The consequences would, at the level of function, be a loss of methylation related, or reduction methylated related activities. Most important of those for our laboratory interests are the D4 dopamine receptor function would be compromised. And whatever role that does play, and there is reason to think that the D4 receptor has a unique role to play in the ability of neuronetworks to synchronize their firing activity to a particular frequency, gamma frequency, during attention, that I would suppose that a consequence would be impaired gamma frequency synchronization during attention.

And as I alluded to, there's many other methylation reactions, each one of which, although not necessarily to the same extent, but each one of which would likely be reduced in its efficiency as a result of inhibition of a thioneine synthase consequent to oxidative stress.

Q Are the neurons going to die? Or are they going to be spared? Are they going to look any differently than they look before the oxidative stress that you hypothesized results in autism?

A At doses which are within the range of typical exposures that we've discussed here, I would

not expect death of neurons. At higher concentrations, as demonstrated in a number of studies, thimerosal and certainly mercury can cause neurons to die and cells to die.

But I think my expectation of the concentration ranges that we're talking about here, by that I specifically mean 100 nanomolar or less, those concentration ranges, rather than causing the cells and neurons to die, or even to show overt anatomic differences, might rather instead of that cause a loss of function or impaired function.

Q In your studies that you've done in your lab, published in 2004, and then I believe you spoke about some other studies being done currently in your lab, what happened to the neurons there?

A The neurons did not die. In fact, there was a discernible change in the cell shape while they were exposed to thimerosal. They rounded up and lost their processes; that is, they became more spherical. And this was reversible.

And so recovery under these concentrations, and the concentrations that we used, as I showed, these were actually 10 to the minus-7; again, not necessarily extremely high concentrations, but lower concentrations.

And it was clear to us that the cells had changed over an influence, but they had not been killed. This is actually a point of encouragement, I hope, and I take for the possibility that recovery from damage or oxidative stress conditions, neuroinflammation might be reversible. And there is encouragement along those lines in the treatment of autistic children, including the administration of methyl B12 and folinic acid supplements, which not only improve their metabolic characteristics, but also, as reported recently, improve their neurologic function.

And so this is consistent with the idea that the underlying cellular components are still there, and in a significant number. But alas, not all children can be improved significantly, if not fully recovered. So it's an important distinction to say that we're not killing, not proposing the death of neurons as an explicit part of autism.

Q And you said when you looked at these cells, when they were under these conditions you had put them under, they actually looked different than they did prior to your treatments.

A That's correct. These are SY5Y cells.

Q So the treatment actually changed the

physical appearance of the cells.

A They were more round.

Q And is that because of the --

A Let me make it clear that these are again cells in cell-culture petri dishes that have a typical morphology of having some processes or extensions, pointy features. And it was those features that were not maintained as well in the presence of concentrations of thimerosal of 10 to the minus-7, in that range.

Q In your report, you stated that thimerosal is toxic to human cortical neurons and neuronal cells grown in culture. Thimerosal caused 50 percent of the cells to die after 48 hours. Concentrations between five and 100 nanomolars.

So in those instances, the --

A I would like for you to reference which page?

Q Page 3. So in those instances thimerosal caused neuronal death?

A As reported in those references I gave you? You're not talking about my lab work now, I don't believe.

Q No, no, I'm not.

A Thank you.

Q So it caused neuronal death.
A Uh-huh.
SPECIAL MASTER VOWELL: Is that a yes?
THE WITNESS: That's what the report says, and that was reference no. 5? Oh, is that a -- I hope that wasn't a typo. Is that where we're headed here? Because if that --
BY MR. MATANOSKI:
Q You don't need to anticipate where I'm heading, Doctor.
A I think I --
Q I hope you'll hear my question, so you'll know exactly what I'm asking you.
A Okay, good. Because I am noticing that's what it says, and that was reference 5 here. But on my personal reflection, I'm thinking oh, that's an impressively low concentration for causing cell death.
Q Doctor, doesn't the body have numerous compensatory processes for coping with oxidative stress?
A Yes.
Q Doesn't your hypothesis -- I'm sorry. Does your hypothesis apply only to regressive autism?
A No.
Q Doctor, do you believe there's an epidemic

of autism directly tied to the use of thimerosal in vaccines?

A I do.

Q Do you believe that the incidence of autism -- never mind, strike that.

Do you believe that there is also a rise in the, in obesity in children linked to thimerosal?

A I don't believe that with anywhere near the same certainty that the relationship to autism is both believed and supported by my work and others. Because in fact, there hasn't been a parallel investigation of that. I raised it as a hypothesis.

Because the exposure of the public as a whole, our population as a whole, to an agent that induces oxidative stress and impaired methylation, might -- and hypothetically here, might -- result in more than one consequence.

If we generalize what I presented, in regard to autism, it's those who have a certain number of polymorphism or risk genes, and are exposed to thimerosal; have a high risk of having a neurological condition, in which impaired methylation of methionine synthase activity plays a role. We might imagine that people with another set of risk genes, perhaps involving instead of neurological functions, perhaps

metabolic functions more closely aligned to, with the metabolism VLDL formation, might manifest a different set of symptoms.

And if one hypothesis is true, then the other should be looked for. And that's why I was struck by the finding that there was an association of obesity with some of the same genes.

Q And you've publicly spoken to imply that the two epidemics, that is in your view two epidemics, autism and obesity could be linked to thimerosal.

A The way I presented it was an interesting finding, which in fact it is. And I presented that, because I think the public, who at some level our information serves, should be aware of the possibilities that different disorders, for whom an explanation is frequently not directly available, might have something in common with another disorder.

Q In 2003 you authored the paper for a Defeat Autism Now conference. And in that, you essentially said that it would be interesting to see, since thimerosal had been virtually removed from vaccines in the U.S., to observe whether the incidence of autism decreases in the next three to five years.

Do you recall making that statement?

A I can easily, it sounds correct. It sounds

certainly as a thought that I have held, so I can acknowledge that.

Q And are you familiar with the information that was recently published about the incidence of autism in California?

A I am.

Q And that information showed an increase, a continued increase in the rate of autism, is that right?

A That's correct. It's certainly a troubling finding.

Q I agree. And your observation in 2003 where you, you didn't say which way you expected it to go; you say it would be interesting to observe whether the incidence of autism -- I'm sorry, you said it would be interesting to see whether it decreases in the next three to five years.

By that, did you mean your expectation was that with the removal of thimerosal, it would decrease?

A I think those words are clear on their own merit, that what I meant is exactly what they say. I don't, I didn't want to, and I don't want to take them in any one direction or another any further than what they say.

Q Well, casting your mind back to 2003, I just want to make sure I'm --

A Is there a particular day you had in mind?

Q At the time you wrote this statement, was your expectation that with the virtual elimination of thimerosal in vaccines, the incidence of autism would decrease?

A I was hopeful it would.

Q Was that your expectation?

A I was hopeful that it would.

Q Based on your hypothesis, was that your expectation?

A Because of my hypothesis, I was hopeful that it would.

Q And it did not.

A The data in California does not show a decrease.

Q What percentage of individuals are genetically predisposed to react to thimerosal-containing vaccine?

A I don't have an absolute answer. That's obviously a question for which the data is not available to answer it, not only by myself or anyone else. If I said it, perhaps one in 150, it would be just on the basis of the fact that rate might indicate

we're experiencing that percentage of sensitive individuals.

Q You mentioned polymorphism in the population.

A Uh-huh.

Q That you were interested in. Do those, are those polymorphisms shared by more than one percent of the population?

A Yes.

Q Are they shared by more than five percent?

A One cannot generalize, because a polymorphism of more than one percent might be five percent for one example, or 50 percent for another.

Q So these polymorphisms you are talking about could cover broad areas of the population.

A They do cover broad areas of the population, because they're normal. They are not mutations in the sense we might think of an aberrant feature of the DNA, but they are, in many cases, risk-inducing, especially under changes in the environment. Under circumstances which their otherwise potentially useful role is, in fact, reversed to be a risk-inducing role.

Q So these, again, these polymorphisms are normal, and shared by a large percentage of the population. Is that right?

A Again, the percentage varies. It can be as high as 50 percent. Or otherwise it's, if you're talking in general about polymorphism, polymorphisms are shared by all of us.

Q I was talking about the polymorphisms that you were identifying as potential risk factors.

A Right.

Q Do these fit these general -- are they shared generally amongst the population?

A Yes.

Q In the manner you just described?

A Yes.

Q Do you believe, under your hypothesis, that it's thimerosal, ethyl mercury, or inorganic mercury that is responsible for oxidative stress?

A They each take their turn, don't they? There was no thimerosal administration, then in fact the risk would be rather low. And so that thimerosal has its role to play, as the original molecule and the ethyl mercury had its role to play as the facilitator of trans-blood-brain barrier movements.

But ultimately, the final and longest-residing form of toxicity can be assigned to the inorganic mercury within the brain.

Q So is it the inorganic mercury in the brain

that is the mercury of interest, in terms of your hypothesis?

A Yes, as far as the neurological manifestations of the disease, that's correct.

Q With your hypothesized mechanism, will the same effect be seen after exposure to methyl mercury?

A Are you talking about the same dose of methyl mercury? The same rate of administration? The same route of administration?

Q It's a very general question.

A It can't be a general answer, then. Because the thimerosal as administered as a bolus dose, it is relatively quickly absorbed within a matter of hours. It is in fact available faster than a tuna sandwich delivers methyl mercury, over a period of a week or two. So these things can make a very big difference in terms of what the same amount of these different materials will do. Because a proportion of elimination, the proportion that crosses the blood-brain barrier are driven by the concentrations. And the concentrations achieved by a bolus dose are much higher than by the dribbling in of small amounts, for whom the excretory pathway, detoxification pathways maintain a very low concentration.

So this makes it difficult to answer your

question.

Q How much -- I just need to know whether you believe that the amount of thimerosal was important. Now that we've established that that is important to you, as well as other factors, start with how much thimerosal would one need. I'm sorry, how much inorganic mercury would one need to have the effect that you hypothesize results in autism?

A How much inorganic mercury would be needed where? Behind the blood-brain barrier in the brain?

Q Wherever it's important for your hypothesis. Is it in the brain?

A It's important --

Q It doesn't matter if it's elsewhere for your hypothesis?

A Uh-huh.

Q I'm sorry, you'll have to say yes or no.

A You're changing it. I'm not sure, you're asking me several questions.

Q No, I'm trying to get to where you can answer the question.

A Let's both be on the same wavelength. I think you're asking me how much you need in the brain.

Q Let me step back. Where does the inorganic mercury need to be in your hypothesis for it to have

an effect that you see autism?

A For the neurological symptoms of autism, which my work reflects most closely on, it is in the brain. And so therefore, it's the concentration in the brain that's most relevant.

Q How much inorganic mercury would be necessary in the brain to see the neurologic effects that you, in your hypothesis, that you say are consistent with autism?

A Okay. I obviously went out of my way to emphasize the concentration-dependent effects on the various contributors to disturbed sulphur metabolism, which let's call my hypothesis here. And as we reflect, I saw concentration-dependent effects at very low concentrations, concentrations that are sub-nanomolar, and in fact concentrations that are in that range of nanomolar and above would likely cause graded levels of interference with sulphur metabolism.

Now, if you'd care to get into more detail, which if we want to be more sophisticated, would you like to?

Q I just want to know, sir, I'm just looking for a number. How much?

A I'm not going to share a simplistic view here. Because a certain concentration at moment zero,

let's say, will result in adaptive responses such as I outlined. And it's possible that you will get little effects from a one-nanomolar concentration, because the cell can handle that. It can adapt to that without any consequential loss of function.

But there will be a threshold concentration. I think that's really what your question is getting at. Is there a threshold concentration at which loss of function occurs because cells can no longer compensate for the presence of toxic substances like inorganic mercury.

Q What is that threshold concentration?

A Again, I don't know what that threshold concentration is in the intact brain. But the studies which show concentrations after vaccination of monkeys, I guess it would be the administration of equivalent concentrations that produce 30 nanomolar, estimates of human autism, excuse me, human brain levels are in that same range. I'm drawn to that range as saying well, I guess at those concentrations, if autism symptoms do occur, then that might be -- and I can only say might, I'm trying to help your interest in finding an estimable number. I'm not dealing with facts here.

But I would guess that in the range of 10 to

100 nanomolar, in that range, would be sufficient to cause a loss of function. But I have to qualify that by saying it's really just my efforts to focus on a number in response to your question. It's not based on an experimental measurement.

Q So there is no experimental measurement that you know of that would give us that threshold concentration.

A There is none at hand. And if we're talking about which concentrations would cause autism, you can imagine that the subjects for such a study would be prohibited, and such a study would be prohibited. And so we're at a difficult situation of extrapolation here from other experiments that we can do.

Q Are you willing to extrapolate from your in vitro studies as to what the threshold dose would be?

A No, I'm not. We should also recognize that the free concentration -- when I carried out our studies, we have a concentration in the bathing medium for cells. And that represents, at the time we added the free concentration, whereas a concentration in brain tissue and the extrapolation from the amounts in microgram or milligram quantities, or parts per billion, probably represent bound forms, not free forms.

I can, with great confidence, say that there are probably very marginally small free concentrations of inorganic mercury in, say, the Burbacher primate studies, or in humans as we estimate that. It's bound. It's bound to some sulphydryl-containing enzymes. So our concentrations in free form are sort of a different experimental system.

Q In free form, there is more of it available to react with the cell, than in the human body?

A The amount available is, for concentration -- well, the amount available, if we're now trying to convert concentration into amounts, is that the nature of your question?

Q My question started with, from your cell studies, do you feel comfortable calculating a threshold concentration for which, under your hypothesis, you would see this neurologic reaction?

A No. Our studies, as I presented them, indicate that when certain concentrations, as free concentrations, are presented to human neuronal cells, they inhibit these processes at the stated level. And so that it's different, it's a different system than saying that the amount or the concentration in an intact animal's brain, which is not a free concentration, but rather a net amount that is

normalized to the weight of the tissue and so forth.

So it's really difficult for me to extrapolate one to the other. I'd like to do that; it's important to do that. And we all need to do that. But we have to sort of temper that by the recognition that those are two different circumstances. And the truth is, we don't know the free concentration in the brain, and it's likely to be very low in the case of the brain studies.

Q In other words, in the brain there would be less freely available to be presented to the cells.

A It's going to be bound to an extremely high percentage, especially at a given time. You can imagine presenting even within the brain a free concentration, and then over time a greater and greater proportion of that will be in a bound form, as it finds its targets and binds so strongly that it doesn't come off of those targets. It's going to be bound.

Q Can you extrapolate from any other research work that you know of, besides your own, to tell us what the threshold concentration might be under your hypothesis, through which you would see this neurologic event?

A No.

Q So I take it then you have no opinion as to whether one vaccination with, say, hepatitis B vaccine would be enough to create this neurologic effect.

A The extrapolation, I think I didn't indicate I have no opinion. I think your question was do I have any additional knowledge. I assume, therefore, the facts that we at most discussed prior to that final question are not erased. There are reasons to think that individual doses create individual concentrations in the brain that can summate over time, especially with inorganic mercury.

Q For your hypothesis, do you need to have an efflux disorder for the effect you hypothesized?

A The efflux disorder, which is a reasonable way to describe the impairment in glutathione-based, especially in glutathione-based detoxification, although there are other efflux pathways. But the term "efflux" is really one way to think about the reduced clearance of mercury and its various forms, when you don't have enough glutathione.

For example, if you're autistic and you have 40 percent less glutathione, we could think of that as a biochemical cause of an efflux disorder.

Of course, at some dosage of mercury or ethyl mercury, whether you have a normal efflux

capacity -- i.e., normal glutathione -- or not, you'll suffer consequences and have an overwhelming effect of that high concentration. It's really a titration issue.

We can even view the tolerance for mercury exposures, but in truth, other heavy metals as well, as a titration issue, as saying how much can you clear per day. And are we all equal in our ability to clear that amount. And if some among us are not, then those individuals will tolerate less.

So given a standard rate of administration shared by a heterogenous population, we can and should anticipate that some individuals will be less able to clear and efflux that mercury, even at the levels that vaccination provides, albeit seemingly modest levels. There may be individuals for whom even that modest level is not excreted, and therefore causes a problem.

Q Is it important to your theory that this be shown in an individual? Or does your hypothesis stand independent of an efflux disorder?

A It's certainly important to that individual.

Q Is your hypothesis independent of the existence of an efflux disorder? Or do you also rely on that in forming your opinion?

A Especially when one considers the role of

glutathione in efflux, and the role of glutathione in controlling methylation, it's hard to separate those two. And I have a hard time, under those conditions, imagining somebody who had, let's say, low glutathione peripherally, and therefore has an efflux problem, but has normal glutathione centrally. So it's really, to me, likely that you would find an efflux problem when you have a redox problem.

And so I think these two are sort of inextricably part of the central role of glutathione.

Q In the work in your lab that you described in the 2004 paper that was published, and the unpublished work that you described today, the cells that you were using were not cells from a human brain, were they?

A No. The SY5Y cells, described as neuroblastoma cells -- the "oma" indicates that in fact they were originally isolated from a tumor of neural origin, but not necessarily brain origin. In fact, this is a tumor which originally was of peripheral origin.

Nonetheless, they are human. Nonetheless, they are neuronal. And in fact, the cells that we chose here to use are by far the most common cultured cell, neuronal cultured cell model used in, by medical

science. And there's upwards of 3,000 papers published using these cells.

And it's been our experience that the things that we learn from these cultured human neuronal cells, albeit non-brain-derived, have a high predictive value for brain functions in animals, as well as in humans. And the fact that we found, as we did, abnormal levels of methionine synthase, especially among young subjects, in human brains, our motivation for looking for that in the first place came from cultured cell studies.

So it's a very good example of what you get from using these cultured human cells. You get ideas that you can then go ahead and test as best you can in the more satisfactory systems and materials, as they are available.

Q So Doctor, from your answer I take it these are cancer cells?

A Tumor cells.

Q While they're neuronal, they're not from either the brain or even the central nervous system, is that right?

A I think that's what I specified.

Q In your 2004 study that was published, didn't you find that -- you used both thimerosal and

inorganic mercury, amongst other agents, in testing, in the testing that you did, is that right?

A That's correct.

Q And didn't you find that -- and as I understand it, inorganic mercury now is the focus of your attention, which you believe we should be looking at in terms of what might be the mercury species in the brain that's of interest, at least under your hypothesis, is that right?

A That's correct.

Q In that 2004 paper, you found that inorganic mercury didn't lower glutathione as great as the thimerosal did, isn't that right?

A We didn't measure glutathione in that paper.

Q The ability --

A It took us a while. That paper showed an inhibition of methionine synthase, and inhibition of phospholipid methylation. But it was really, as a result of our trying to find out more about why that occurred, that led us to this other series of more deeper investigations, including glutathione measurements.

Q Let me put it in perhaps a more simplistic way, because that's where my level of understanding is.

The thimerosal and the inorganic mercury ability to go across and attack cells was not the same in your experiment, is that right?

A It never would be. Assuming that the ethyl group was on top of the mercury, it should facilitate its transfer across the cell membrane in the case of cultured cells, roughly analogous to the blood-brain barrier.

Q So the inorganic mercury, the cell was actually better protected against inorganic mercury in your experiment.

A It critically depends upon where the target is. The target need not be intracellular, but might well be considered to be intracellular. If it was intracellular, and if time was a factor, then the rate at which the ethyl mercury would enter the cell would be faster facilitated.

However, if inside of the cell, the target preferred, inorganic mercury, or was more affected by that than the ethyl, then you'd have sort of a confounding issue about both the target and the transports to the inside of the cell where the target is located. And not to mention there might be more than one target.

So all these factors in the end give you

what you get. When you do the experiment, you get the result.

Q I turn your attention a moment to the article, the Charleston article that's listed as PML-32.

A Uh-huh.

Q This article, you've referenced it, and you say in your reference ethyl mercury. Is this article about ethyl mercury?

A The Charleston article no, that was about the methyl, you know, that was an earlier paper. It was a microglial paper I guess. Is that the one where Charleston was writing that? Okay, methyl.

Q Okay. So when you cited this as an article about ethyl mercury, that was just --

A I guess, could you reference on my report which particular page you're --

Q I believe, I'm not sure which reference it was. I can find that. I just wanted to know whether you believe that that article was about ethyl or methyl mercury, because you cited it as ethyl.

A Okay. I just wanted to verify that citation that you're alluding to. It's possible it could be an error, or not. Okay.

Q Actually, Doctor, it's not important about

whether you made an error in citing it or not. I just wanted to know, because I've seen this cited by you a number of times in other presentations, and it always says "ethyl mercury." And I wanted to know whether that was what you were believing this article was about.

I'm sorry. To speed us up, you believe the article is about methyl mercury.

A I don't need to be speeded up, I need to find the reference here, and I'm looking for it here. So monkeys with thimerosal --

MR. WILLIAMS: Page 4.

THE WITNESS: Uh-huh. It's page 4, and it's reference 20?

MR. WILLIAMS: Reference 20.

THE WITNESS: Okay.

BY MR. MATANOSKI:

Q Doctor, actually, all I want to know is when you were discussing it, whether you thought this study was about ethyl or methyl. And you've answered that you do understand that this study is about methyl.

A Actually, I'm sort of shocked. I'm looking at the reference which says "ethyl" in the title. Is there an M missing in that title?

Q Yes, but in discussing this, in discussing

it, it's not --

A It's probably not going to make a difference when we're done figuring this out, because ethyl and methyl will result in the same trans-blood-brain barrier potential, and a similar release of inorganic mercury. But it will be important to make sure we have either the M or -- so it is, if I'm looking at the title here, it should be methyl, not ethyl.

Q Okay. And as you just said, it's not important because, in terms of the differences as far as you're concerned, because both methyl and ethyl will eventually become inorganic, which, as you're saying now for your hypothesis, that's the target species of mercury that's important.

A Yes, the long-term source of toxicity is the inorganic mercury.

Q And we know that methyl mercury is available from a variety of sources that are not vaccine, obviously actually isn't available through the vaccine agent.

A True.

Q So there are a number of environmental sources of methyl mercury which will eventually end up as inorganic mercury in your brain.

A True. Especially with advancing years, as

it accumulates. Probably not common in younger children.

Q Do you know how, in the Charleston article we were just talking about, do you know how many micrograms of mercury per kilogram per day these squirrel monkeys were receiving?

A No.

Q I asked you earlier what your strongest evidence was. And you said the two James articles?

A My strongest evidence?

Q For your hypothesis.

A That's right, the strongest. Not mine, but the --

Q I'm sorry.

A -- strongest evidence for a role of impaired methylation and oxidative stress I believe comes from those two papers in particular.

Q And the first one that you cited in your report was cited as no. 9. And it's PML No. 49.

Did Dr. James ever directly measure the activity or level in the methionine synthase in this study?

A No.

Q Did she ever measure thimerosal, ethyl mercury, or inorganic mercury in this study? I'm

sorry, we'll need an audible response.

A No. I'm just trying to understand why you might expect that she would. The study was not aimed at doing that. I mean, it had nothing to do with mercury administration, it had nothing to do with specifically measuring methionine synthase. It did what it did, it measured what it planned to.

But if you want me to answer, I'll just say no.

Q And did Dr. James provide any information on caloric intake and diet for the patients in these studies?

A Not to my recollection.

Q Can GSH levels change depending on diet?

A They can.

Q And are you aware that in this study, the authors state that -- we'll bring this up for you in a moment. Our attempts to interpret these preliminary metabolic findings are clearly speculative, and a better understanding of the abnormal one-carbon metabolism in these children will require additional research efforts.

A Uh-huh.

Q Let's turn for a moment to the other James study. Can you pull that up?

Are you aware what Dr. James stated in that study?

(Discussion held off the record.)

Q "Clearly these new findings should be considered preliminary until confirmed in larger population-based studies." Have such studies been conducted and published?

A Following up on her studies in larger populations? I don't believe anybody has, to my knowledge.

Q You were asked a question about chelation, and you talked about oxidative stress to the body, of areas of the body besides the brain. Does oxidative stress in these other areas of the body affect neuroinflammation in the brain?

A There are metabolic relationships between the rest of the body and the brain. I particularly focus on the liver, important metabolic organ that it is. And ultimately the sulphur material in our diet processed through the liver, put into the bloodstream, and eventually transferring out of the bloodstream and across the blood-brain barrier represents the source of sulphur resources to the brain.

Q Will decreasing oxidative stress in these other areas of the body affect neuroinflammation in

the brain?

A Not -- well, let's make sure what the limits of direct and indirect are. Indirectly, yes. By making resources available, antioxidant resources available, not utilized preferentially in the periphery. They have a better chance of being available for use in the brain.

And there's a lot of resources to consider here. For example, when one wants to consider what keeps glutathione reduced. I had made this sort of visual analogy between oxidized and reduced glutathione. The enzyme that does that requires NADPH. NADPH arises from glucose metabolism. And the reduced NADPH ultimately becomes available, as well as even the glucose that becomes available to the brain, you know. It depends in part on peripheral metabolisms, as well.

And these two, they don't operate in isolation from each other. And so if you have oxidative stress in the periphery of your body, you will have consequences in the brain. Not even to mention the cytokines and inflammatory remediator substances produced by activated lymphocytes in the periphery finding their way to the brain, otherwise causing inflammation.

Q So it will affect neuroinflammation in the brain if you reduce or affect the levels of oxidative stress in other areas of the body.

A Peripherally, that's right. Another area, if you care to pursue it, would be the intestines, the gut, it we consider that part of the periphery. The ability of the intestine to extract, transport in a normal manner, nutrients necessary for the brain. It might depend on whether the gut is inflamed.

MR. MATANOSKI: Now, if I could ask counsel, opposing counsel if they could put up the slide presentation. Can we switch back then over to the presentation? Now if you can move it forward to slide no. 7.

BY MR. MATANOSKI:

Q I'm going to ask you a series of questions, Doctor, hopefully moving through this very rapidly. I've seen some of these slides before in presentations you've given in other, in other --

A I gathered that.

Q Yes. But I'm not sure which ones in all I've seen elsewhere. And I wanted to take you through some of these slides and ask you what is different about this slide, if anything, from what you prepared before and what you have here today?

Now, let's start with 7. Can you tell me on that slide if you, if it's one that you've used before, and if you altered it in some way for the presentation today? And what that alteration, if any, was, or alterations, if any, were.

A Sure. The basics of the slide are the same, and you recognize that, as well. The genetic risk factors used to be on the right side, and I moved it over to the left, and I added on the upper right, mitochondrial dysfunction and neuroinflammation in recognition of the fact that this terminology, neuroinflammation, was going to be central to the proceedings here.

I wanted to make it clear that neuroinflammation was in fact associated with an increase in oxygen radical numbers, and also recognizing that because of the Poling decision and related events, that the role of mitochondria as a source of those oxygen radicals was worth adding to this slide.

Q So prior to this litigation, when you were discussing this theory and explaining it, you never talked, or at least you never used neuroinflammation and mitochondrial dysfunction in explaining your hypothesis.

A On this slide? On this slide.

Q Right.

A On this slide, right? That would be incorrect.

Q The part, the part of your hypothesis that you use in this slide to explain, you had never previously discussed mitochondrial dysfunction or neuroinflammation.

A I actually can't really say that. Even though we're nitpicking here, I frequently include neuroinflammation and mitochondria as a source of oxygen radicals in my talks.

And so you're asking me now what the verbal accompaniment was to this slide; did I ever talk about where the oxygen radicals came from? It wouldn't be unlikely that I'd mention that they come from mitochondria.

And here what I'm really doing is just sort of bringing that to a visual form, rather than thinking it. Again, I don't think that's a big point.

Q So you didn't feel that it was important enough to include on your visual depiction of the process you were describing.

A Theres always a balance of how much information to put on a slide. One has to be careful

not to clutter it. And for the most naive reasons one can imagine, I decided to add that here. I think --

Q Not only add it, but discuss it here, correct?

A Well, if it was there, I felt an obligation to discuss it.

Q Can we move to slide 18? Have you used this slide before?

A Maybe. Let's see. Not in the precise form.

Q Was it altered in some way for this presentation today?

A I added glutamate to the EAAT-3 to indicate that glutamate can be alternatively transported. Whenever I give this talk, I say that it's excitatory amino acid transporter 3, and the excitatory amino acid is glutamate. But having explicitly presented that here, it makes it easier for the viewer to grasp what I'm communicating or saying.

Q Any other alterations to this slide?

A Well, I added the hydroxocobalamin over there in green. And let's see, did I say, did I change glial cells to healthy, and in parentheses astrocytes? I have a suspicion that you know better than I about that.

Q I'm just trying to find out what you --

A Again, I don't remember at what point I changed that. It may be that I didn't use that in prior presentation, but I can't remember. I make presentations regularly.

Q So from your recollection right now, you altered this slide to add glutamate, and perhaps glial cells.

A The hydroxocobalamin, I now I added that.

Q And the hydroxocobalamin.

A And I'm not sure about that last part, the healthy glial cells. It was meant to illustrate yes, that when they're healthy they're putting out glutathione. Whereas if they weren't healthy, otherwise they were oxidatively stressed themselves, they wouldn't be putting out the glutathione.

Q Will you turn to slide 20? Take a look at this. Have you used this slide before?

A No.

Q This is, I see this is essentially --

A Custom for this occasion.

Q Well, it looks like it's a repeat of the slide that you had before.

A If you're going to ask me about whether it's the same one, it's obviously not the same one because it's got thimerosal on it, and the others didn't.

Don't.

Q Are you sure this isn't just taking the slide that you had in 18 and changing it? If you look at the slide in 18, and then you look at the --

A They're all based on, they're all based on some original slide. And I'm sure 18 is not equal to 20. What are you -- I'm not sure. Are we on the same wavelength about what your question is?

Q I'm sorry, because you don't have them side by side the way it's set up here. Do you have the presentation in front of you?

A Okay, let me do that here. So what is the question? Is this the same, is 20 the same as 18?

Q Eighteen you believe you used before, and made some alterations to it for this hearing.

A Uh-huh. And here --

Q And if you look at it side by side with 20, it looks like it's largely the same slide, with a few changes to it. You're working off of the same slide that you originally used previously, and making additional changes to it here.

I think, I'm sorry. If you could just --

A I wish we were helping autism by doing this, but I don't think we are. I can't understand at all what's --

Q Take a look at the two side by side.

A Yes?

Q Are you saying you started from scratch on slide 20?

A I didn't say I started from scratch. No, these are all a template. It takes a certain effort to do that, so I'll use the same, the players are the same, they're always there. It's a question of whether there's more or less of one of them. I'm trying to depict that by making either arrows or something like that different between them? Perhaps I've erred in some manner here, but this is the way that slides was added.

Q No, I just -- and this one, working from the template that you've used previously, glutamate and perhaps thimerosal, is that it?

A Of course it is. That's what the purpose of this slide is, to show that thimerosal in slide 20 is illustrated as inhibiting EAAT-3, because the data coming up in slide 21 shows that.

Q So when you previously -- moving back to 18, then. When you previously gave this, glutamate and these other things, additions didn't appear on this slide. They weren't important for your explanation at that time.

A I added glutamate because I got tired of, I did a better job. Because usually I'd talk about the fact that glutamate can also be transported there. But instead of having to remember to do that, if it is on the slides, no one can then otherwise ignore the fact that this transporter, named for glutamate, and otherwise can alternatively transport glutamate. Which is an important function that it plays.

Q Doctor, I'm going to move on, then, to --

SPECIAL MASTER VOWELL: Mr. Matanoski, how much longer do you think this will take?

MR. MATANOSKI: I'm very close to the end.

SPECIAL MASTER VOWELL: Mr. Williams, do you anticipate redirect?

MR. WILLIAMS: About two minutes' worth.

SPECIAL MASTER VOWELL: Okay. We are just approaching what we said was our 6:00 p.m. stop time, and I wanted to make sure that we weren't -- it complicates getting people out of the building.

MR. MATANOSKI: Yes, ma'am.

SPECIAL MASTER VOWELL: Okay. Please proceed.

BY MR. MATANOSKI:

Q Slide 21, is this published data?

A No.

Q And slide 24, is this published data?

A As I indicated during my testimony, these experiments are not published yet. They are experiments that were undertaken to follow up on the Waly, et al published results, showing that methionine synthase was inhibited. And we wanted to know why was it inhibited. Why could it be zero activity after thimerosal, for example, but also after other interventions. Because inhibitors don't normally go to zero. They may have partial effects.

And so we were certainly trying to understand in more detail what was going on. And when we did, we found that the zero activity was because the methyl B12 was not available, and was required by the enzyme. So we undertook these series of additional, but not-yet-published, studies to flesh out the details of why methionine synthase was being inhibited.

Q When did you complete the work in this study?

A Which one are you referring to here?

Q Twenty-one.

A Twenty-one. I'm thinking that that was November, November of 2007.

Q This isn't discussed in your expert report,

then.

A Not explicitly, no. Actually, I think, did I reference the general idea that cysteine availability was limiting? And I may have also, and I'm trying to refresh my memory, mentioned that cystine uptake was important, as well, but not this exact result, because we hadn't obtained it at that time.

Q And slide 24, that's new. And when did you finish that work?

A Slide 24?

Q Slide 24. That's new, isn't it?

A Yes. Actually, that was obtained somewhat earlier, I believe, perhaps even over the summer, or even slightly before that.

Q And slide 26, is that new? Unpublished material?

A That was last April, I believe. April, perhaps March, the time period of I would say March of 2007.

Q Slide 28. That's not published. When was that available? Because that's not published, correct?

A That's correct. And --

Q When was that available?

A When was it performed? Performed, I guess available to me?

Q Yes.

A Again, I estimate this is probably February of 2007.

Q And slide 30, same question. Is that new?

A No, that's published.

Q I'm sorry, I'm sorry, you're right. Slide 31? Was that published?

A We've had that one for a while, so that then is probably some time in 2006, maybe September of 2006.

Q And you said that's not published?

A Correct.

Q Slide 34. When was that available? Is that unpublished?

A It is unpublished, and that was, let's see. I think it was early summer of last year. So, let's see. I guess it would be over the summer of 2007, when the experiments were done. The analysis took us a little longer to do.

Q Now, I think the rest of this is that same study, the rest of these slides, 35, 36, 37 all are descriptions taken from that study that you had the data in summer --

A Well, 36 was just available to me actually only a matter of a few weeks ago.

Q Okay. And I'm sorry, 36 did you say was only available to you a matter of a few weeks ago?

A Uh-huh.

Q The other two slides, that information was available before? Is that --

A Correct.

Q And that would have been summer of 2007 on those?

A Correct. Correct.

Q And slide 41, this represents your -- as I took it, the way the presentation went, this represents the hypothesis that you laid out today. Is this --

A This is published. I didn't put the reference here. This is, with the exception of the addition of the word "neuroinflammation," it was published in that review article on neurotoxicology.

Q So this is the, with the exception of the addition here of "neuroinflammation," this is essentially -- well, put the neuroinflammation aside. This represents the pictorial representation of what you described to us as your hypothesis.

A Yes, that's a reasonable description.

Q And this was put in a publication that came out in 2008, you said?

A Correct.

Q And the only change is that you've added neuroinflammation.

A Yes, I believe that's the only change. Yes.

Q Does the representation here, even with neuroinflammation, does that represent the same understanding or the same idea that you were trying to express when you made the publication in 2008?

Has your, has your hypothesis changed because you're added neuroinflammation.

A No. The word "neuroinflammation" was just added for occasion here so that we could make it clear that neuroinflammation and oxidative stress are closely related principles.

Q So the hypothesis is the same one that you published, in other words.

A That's correct. Yes.

Q And in that publication you discussed the role of neuroinflammation as far as your overall hypothesis, is that right?

A I included neuroinflammation, a discussion of that.

Q Let me bring that up. This is your 2008

paper, as you pointed out. I think I might have the, that was one of the recently submitted articles by the PSC. I'm trying to find the Petitioner's Master List Number for that, and I don't seem to have it here. I apologize.

MR. WILLIAMS: 563.

MR. MATANOSKI: Thank you, 563.

BY MR. MATANOSKI:

Q In that, you said elevated levels of inflammatory cytokines and evidence of microglial activation -- oh, there's a typo there, you use it twice -- was observed in post-mortem brain sections, including the presence of neuronal inflammation.

I don't want to read to you, Doctor. This lays out your hypothesis, as far as the neuroinflammation goes. This is how it fits in overall with your theory?

A This is certainly a component of it.

Q This, though, is meant to express the role of neuroinflammation as you've discussed it here today?

A In this paper, it served the purpose that it served now, today? I'm not, I don't expect it as a conflict between this. I don't expect were going to find one. If we are, then I'd like to know about it.

But otherwise, hopefully I'm consistent in saying that neuroinflammation includes oxidative stress, and that certain studies have revealed neuroinflammation in the brains of autistic subjects or biomarkers indicating oxidative stress. And that these are terms that interdigitate with each other.

Q So is it fair to say that the hypothesis that you've described in this paper is the same one that you gave here today?

A In general terms, yes.

Q And in that you stated that -- move to the quote about the starting point.

You describe this hypothesis that it "may serve as a useful starting point that can be critically tested, accordingly revised, and even discarded?"

A Yes.

Q So the hypothesis you presented to the Court today still awaits critical testing?

A There are many ways to test it, and we are in the middle of continuing to do that, yes.

MR. MATANOSKI: I have no further questions.

SPECIAL MASTER VOWELL: Go ahead, Mr. Williams.

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REDIRECT EXAMINATION

BY MR. WILLIAMS:

Q I just have one quick topic. There was a question about your source of funding, and Mr. Matanoski pulled a web page out of Safe Minds to imply that there was motivation of that organization to prove the thimerosal-autism connection.

There was also a question to you about why the NIH had turned down your application for funding. And I just want to show you a quote from the former head of the NIH, and then ask you about that for a second.

We can put it up on the screen. This is from the website of CBS News from yesterday. And let's just look at what she said. Could you read that, please, Doctor?

A Starting at the top?

Q Yes, just start at the top.

A "Dr. Bernadine Healy is the former head of the National Institutes of Health, and the most well-known medical voice yet to break with her colleagues on the vaccine autism question.

"In an exclusive interview with CBS News, Healy said the question is still open. 'I think the public health officials have been too quick to dismiss

the hypothesis as irrational,' Healy says. But public health officials have been saying they know they've been implying to the public there's enough evidence, and they know it's not causal, Atkinson said.

"'I think you can say that,' Healy said." You can't say that. I mean, this is out of context, and I really have a hard time.

Q There's more coming that will help put it in context. Okay.

SPECIAL MASTER VOWELL: Mr. Williams?

MR. WILLIAMS: Yes?

SPECIAL MASTER VOWELL: Are you going to get to a question here? Because having witnesses read documents that you may introduce is not helpful to me. I don't know about my colleagues.

MR. WILLIAMS: Okay. I thought I was doing exactly what Mr. Matanoski did, which was reading off a website and asking him what he thought of it.

SPECIAL MASTER VOWELL: Well, that's why I asked if you were going to get to a question.

MR. WILLIAMS: All right.

BY MR. WILLIAMS:

Q I'll read this statement from Dr. Healy, and then ask you whether you agree with it.

She goes on to say that public health

officials have intentionally avoided researching whether subsets of children are susceptible to vaccine side effects, afraid the answer will scare the public. And then she says there is completely expressed concern that they don't want to pursue a hypothesis, because that hypothesis could be damaging to the public health community-at-large by scaring people.

Now, my question to you is, do you have any reason to think that the NIH turned down your grant for these reasons?

A I do. I expressed before the fact that reviewers, primary reviewers in the very first paragraphs of their supposed review instead took a government statement indicating that thimerosal does not cause autism; and on the basis of that, indicated it was therefore not appropriate to study thimerosal. This is, as I'm saying, unfortunately not restricted to government agencies. I've had the same response from reviewers, you know, in the private sector, foundations.

And so it's unfortunate. And I understand the importance of preserving public confidence in vaccines. I think the most, the best way to gain that confidence is by scientific validated studies of their safety and their components' safety. But nonetheless,

it's frustrating to not be able to pursue important questions because of lack of funding.

MR. WILLIAMS: Thank you. That's all I have.

SPECIAL MASTER VOWELL: Mr. Matanoski?

MR. MATANOSKI: Briefly.

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RECROSS-EXAMINATION

BY MR. MATANOSKI:

Q Doctor, just to be clear, your particular study was denied by your colleagues in academia, is that correct?

A I don't remember. Because it's blinded, I'm not allowed to know who the reviewer was who took that action. I can assume it was the panel; I don't believe it was exclusively from academia.

Q It was not governmental officials involved in that decision.

A Again, the people on these panels are not government officials, though they may have government positions. They could be scientists at government locations. They could be in the private sector they are probably likely to be academicians. But that is, I really don't know. I don't know who that person was

who chose to do that.

Q And you just stated that you've had other grants turned down by private foundations.

A This is a general issue. The general issue is will agencies, will reviewers allow studies of this issue, or not. Will they fund studies of this issue or not? And let's be honest. The truth is there has been restricted funding. And while the parent supporting groups have taken on the necessity of funding those, their resources are limited; and as a result, there probably isn't an influence on the findings in terms of how broad the question is explored.

Q So is it your personal belief that it didn't have anything to do with the merits of the grant that you put in?

A I can't be confident it had nothing. But just the actual cutting and pasting of an official position has no real role, in my opinion, in the evaluation of the scientific validity of the research proposal.

MR. MATANOSKI: I have no further questions.

SPECIAL MASTER VOWELL: I take it we are concluded for today, and we can excuse Dr. Deth.

(Witness excused.)

SPECIAL MASTER VOWELL: All right. We will reconvene at 9:00 a.m. tomorrow morning.

(Whereupon, at 6:12 p.m., the hearing in the above-entitled matter was recessed, to reconvene at 9:00 a.m. the following day, Wednesday, May 14, 2008.)

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REPORTER'S CERTIFICATE

DOCKET NO.: 03-584V; 03-215V

CASE TITLE: King and Mead v. U.S.

HEARING DATE: May 13, 2008

LOCATION: Washington, D.C.

I hereby certify that the proceedings and evidence are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the United States Court of Federal Claims.

Date: May 13, 2008

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