In The Matter Of:

Paul Halderson, et al., v.
Star Blends, et al.

Lewis G. Sheffield, Ph.D.
March 14, 2014
Volume 1

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STATE OF WISCONSIN CIRCUIT COURT TREMPEALEAU COUNTY

- -

Paul Halderson and Lyn M. Halderson, N17388 County Road Galesville, Wisconsin 54630

and

Arctic View Farms, LLC 1919 Riley Rd. Sparta, Wisconsin 54656,

Plaintiffs,

vs.

Star Blends LLC 1919 Riley Rd. Sparta, Wisconsin 54656

and

ABC Insurance Company, a fictitious company

and

Northern States Power Company d/b/a Xcel Energy Services Inc. 1414 W. Hamlin Avenue Eau Claire, WI 54702,

Defendants.

- -

Case No. 12-CV-74
Code Nos: 30303 & 30201

VOLUME I

Deposition of LEWIS G. SHEFFIELD, PhD, taken pursuant to Notice of Taking Deposition, and taken before John T. Kirby, a Notary Public in and for the County of Dakota, State of Minnesota, on the 14th day of March, 2014, at 1 South Pinckney Street, Madison, Wisconsin, commencing at approximately 12:35, p.m.

APPEARANCES:

Natalia Blaskovich, Esquire, of the firm of REYNOLDS & KENLINE, LLP, 110 East Ninth Street, P.O. Box 239, Dubuque, Iowa 52004-0239, 563-556-8000, blaskovich @rkenline.com,

-and-

Scott Lawrence, Esquire, of the LAWRENCE LAW OFFICE, S.C., 403 South Fourth Avenue, P.O. Box 117, Saint Nazianz, Wisconsin 54232-0117, 920-773-2811, ATTORNEYS@LDLAWSTN.COM, appeared jointly representing the Plaintiffs.

Timothy R. Thornton, Esquire, of the firm of BRIGGS & MORGAN, 2400 IDS Center, Minneapolis, Minnesota 55402, 612-977-8400, tthornton@briggs.com, appeared representing Defendant NSP/Xcel Energy.

Catherine M. Rottier, Esquire, of the firm of BOARDMAN & CLARK, LLP, 1 South Pinckney Street, Suite 410, P.O. Box 927, Madison, Wisconsin 53701-0927, 608-257-9521, crottier@boardmanclark.com, appeared representing Defendant Star Blends.

ALSO PRESENT:

Theresa A. Peterson, DVM.

VIDEOGRAPHER:

Mark C. Haskins, HASKINS MEDIA SERVICES, 1071 Whitney Drive, Apple Valley, Minnesota 55124, 952-997-6455, mark@haskinsmediaservices.com.

EXHIBIT INDEX

EXAMINATIONS

* * * *

WHEREUPON, the following proceedings were duly had:

* * * *

LEWIS G. SHEFFIELD, PhD,
an expert witnesss in the above matter,
after having been first duly sworn,
testified under oath as follows:

CROSS EXAMINATION

BY MR. THORNTON:

Q It's Dr. Sheffield, right?
A Whatever. It is. It is.
Q You have a PhD?
A I do have a PhD, yes.
Q As you just heard, doctor, I represent Northern States Power Company in this lawsuit. Do you know anything

about this lawsuit?
A Very little. I knew it existed and that's about it.
Q All right. Have you talked to any of the lawyers except for the brief conversation that you and I had on the telephone?
A And introducing ourselves in the lobby. That was all.
Q What do you do for a living, doctor?
A I teach biology courses and occasionally chemistry at MATC, Madison Area Technical College, in Portage.
Q How far is Portage from here?
A I don't know. Takes me about 45 minutes or so to drive there. Mileage, I'm not sure.
Q And can you give me a brief overview of your educational background?
A Yes. I received a bachelor of science degree from Clemson University in animal science, stayed there for a master's degree, received a masters in 1980.
Q Also in animal science?
A Well, it was called animal and food industries, but essentially animal science, yes. 1983, I received a PhD in dairy science at the University of Missouri, studying mammary gland development. From '83 to '86, I was a post --doctoral researcher at Michigan State University with Dr. Shuford Welch, who is a breast cancer researcher.
Q Is there an overlap between mammary development and breast cancer?
A Yes.
Q And what did you do after 1986?
A I joined the faculty at the University of Wisconsin in

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the Department of Dairy Science.
Q Go ahead. I don't mean to interrupt you. Go ahead.
A I was just going to say, I was initially assistant professor, left there in 2010 as full professor.
Q And was there any area of specialty that you had at the University of Wisconsin?
A I worked predominantly on mammary gland development, which is what my training was in.
Q Did you have any special focus on immunology or genetics?
A Immunology. I did work a little bit in immunology, mostly on the stray voltage related work. I would not have considered myself an immunologist, per se, but I did do some work on that. Genetics, in terms of gene expression, I did some work on that, if that has some relationship to mammary gland development. So, I guess that depends a little bit on how you define genetics.
Q What did you do - can you give me a brief overview of what you did in the area of stray voltage?
A Yes. I don't recall the year, but sometime in the '90s, a researcher that was affiliated was in a different department, Ag. Engineering. Dr. Douglas Reinemann was doing some research on stray voltage, and the question arose: "Are there ways to measure physiological responses that might be relevant to immunology, or stress, in general?" And that began some work that we did in collaboration with him, measuring various aspects of immune function in dairy cattle that had been exposed to voltages.
Initially, we were measuring - I'm trying to think of the best way of wording it - levels of

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various proteins associated with immune function in the blood, such as immunoglobulins and interleukins, which are chemicals that regulate function of the immune system. And we measured activity of neutrophile cells, which are major phagocytic cells, and - -
Q Doctor, when you say some of the words like phagocytic, it would help Mr. Kirby if you spelled them, because you're probably the only one who can.
A Okay. Phagocytic. P-h-a-g-o-c-y-t-i-c.
THE REPORTER: Thank you.
Q Sorry I interrupted you.
A I apologize. If I use a term that you're not familiar with, please let me know and I can define it. Phagocytic simply means cell heat. And a group of cells called lymphocytes. Lymphocytes are important in a variety of aspects of immunology, and in the initial studies we measured the ability of the lymphocytes to respond to various stimulants. These are chemicals that are recognized by the lymphocytes as something they should respond to.
Q So, antigens, for example?
A Well, yes. Antigens, that's a good way of putting it. That's accurate.
Q So, when a bacteria or a virus or some invader that enters - -
A Yes. In fact, one of the things we measured in response to was a particular bacteria, staphylococcus aureas. Others were lectins from Pokeweed.
Q What is lectins?
A Lectin. It's a compound protein that binds to cell

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surface with ceptors on the surface of lymphocytes, and it often stimulates it to undergo cell division.
Later, we did another study in which we measured the level of messenger RNA of certain genes, some of which are associated with immune function. This is - perhaps I should explain what messenger RNA is, is each of the cells in the immune system has a set of genes. Coding for - a wide variety of things that they need to carry out their cell functions. The way they express these proteins, the interleukins, for instance, the anti-bodies are proteins, is, the, DNA is copied into the intermediate molecule called Messenger RNA, a processed called transformation. That is then used as a template to direct symphysis of the specific protein, a process called translation.
Early on, we had studied the production of the actual proteins. We then studied the production of specific messenger RNA's coding for those proteins.
Q And this work was done in cooperation in connection with Dr. Reinemann?
A Yes.
Q He did the engineering side of it and you did the biological side of it?
A Yes.
Q And did that culminate in a paper, a published paper?
A No, it did not.
Q What happened to that work?
A That, we never published that. We did not find a lot of great statistically significant findings. Some of the things were, I want to say inconsistent, erratic, and we

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did not pursue publication of that, or at least I did not. I do not know if Doug might have published any of that work or not.
Q What is CALS at the University of Wisconsin?
A College of Agriculture and Lab Science, that is the College of Agriculture.
Q Is there a unit called the ICCUC that meets with experimenters to make sure that their work is statistically correct and significant?
A That's not the purpose of that committee. That stands for the Institutional Animal Care and Use Committee. They monitor the use of experimental animals to make sure appropriate animal welfare regulations are followed.
Q Is there any entity associated with the University of Wisconsin that examines the statistical work of researchers?
A None that I'm aware of.
Q Was there any review of the statistical work that you and Dr. Reinemann did early on?
A The statistical analysis on this early work was done by Steven LeMire.
Q Who is Steven LeMire?
A He was associated with Dr. Lyman. I do not recall the details of that association, as to whether he was a graduate student or research associate or what the details were.
Q But was he a statistician or did he have expertise in statistics?
A He had expertise in statistics. I do not know exactly what his background was.

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Q And what's your basis for concluding that your early work didn't come up with anything that was statistically significant, and, in fact, was contradictory?
A Well, I didn't say - I didn't mean to imply contradictory.
Q Okay.
A If you look at the work, we have a table here that is Table 2.
Q What are you looking at now?
A I'm looking at this file here (indicating).
Q Okay. Can we mark that as an exhibit?
A Yes. You have a copy of that. I sent you a copy of this.
Q My people didn't give it to me, unfortunately.
A Oh, okay.
MR. LAWRENCE: Perhaps I can help. If that's the same data as the paper that was eventually published, I've got it along.
Q I've got the published paper. This is the early work. Why don't we mark it as 249.
A Okay. This is what I'm referring to.
Q Okay. Can the court reporter mark your copy as 249?
A Yes.
Q We'll get it back to you. That's what you missed, Scott, first 248 exhibits.
MR. LAWRENCE: Thank God.
A Okay. I'm referring here to Table 2.
Q Table 2. What page?
A 22.
Q Page 22. Okay.

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A Okay. Statistically, what these numbers mean, treatment is the effect of treatment, and treatment here is exposure to voltage. So, the smaller the number, the greater the degree of significance. Biologists generally want to see a number less than .05 to consider it statistically significant.
Q You're talking about P values?
A P values, that is what these numbers are.
Q Maybe for the record, why don't you just explain what P value is?
A P value is a messure of statistical significance. It ranges from zero to 1. And, although I'm not a statistician, so my interpretation here might not be exactly what a statistician would give, it is generally considered the probability of being wrong if you say there's a difference between two treatments. So, we want that number to be small.
Q .05.
A .05 is often used as the criteria.
Q And that's 95 percent certain?
A 95 percent certain that it's not due to random chance, or a 5 percent chance that it is due to random chance.
Q And - -
A And if we look down, most of these numbers are fairly large, with one major exception, and that is, immunoglobulin A. Serum IgA, and my lines are not numbered here, but it's very easy to find them.
Q And that's the .360 number? No.
A No, .015. So, Serium IgA, and the Treatment column, .015.

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Q Oh, got it.
A The other thing that you see, if you look at the figures here, on page 25, you see things like, Figure 2, we have an elevation, or we have a level, it drops and then it comes back up. We don't see flat lines or lines that are diverging. I'm not sure what that means. It's what we found. But the only thing in here that we found that was statistically significant was the IgA levels.
Q And that's under the treatment column, the .015?
A Yes. Yes. Correct.
Q And so you came to a conclusion that this research, to a reasonable degree of scientific certainty, didn't necessarily mean anything?
MR. LAWRENCE: I'm gothing to object to the form as leading. All right. Go ahead.
A I don't know if I would say it doesn't mean anything.
Q Could you draw any conclusions from these data to a reasonable degree of scientific certainty?
A To a reasonable degree of scientific certainty, this study, based on 12 treated and 12 controlled cows, showed a probability that IgA was lower in terms of statistics. That is not the same as biologic significance. Biological and statistical significance are different ideas.
Q Two different animals, right?
A Correct. Correct. So, statistically we saw a difference in Serum IgA.
Q Could you say to a reasonable degree of scientific certainty that there was biological significance in anything you - -

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A I've always - - I'll let you finish the question.
MR. LAWRENCE: Object to form. Go ahead.
THE REPORTER: Wait until he finishes his question.
Q I don't mind if you interrupt me and Mr. Lawrence, but it makes it hard on Mr. Kirby, and he's an old man, and we try and go easy on him.
A I'm sorry. I've never done this before, so if I do something wrong, let me know.
Q Just do your best not to interrupt Mr. Lawrence or I, and we'll do our best not to interrupt you, okay?
A Okay. Now, biological significance. There were two observations, IL1 and IL2, that were close to significant statistically. The one that was significant was IgA. Now, an important part in interpreting these data is to know what IgA actually is. Ig stands for immunoglobulin. That's effectively is an antibody. The major immunoglobulin that circulates in blood is the immunoglobulin G4. There are different forms of these immunoglobulins. Immunoglobulin A makes a very minor contribution to immunoglobulins in circulation. It's importance is in what is called mucosal immunity. The mucosal tissue is what lines many of the cavities of the body and surfaces. For example, the lining much of the intestine is a mucosal tissue.
Most of the IgA in the body is not found circulating, it's found associated with mucosal tissues.
Q In the digestive tract?
A And other surfaces. Digestive tract I used as an example, but there are many others, the lining of the

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lungs, the lining of the mammary gland, the lining of the genital urinary tract, and so forth.
Q The areas of the body that are most likely to come in contact with antigens?
A Yes. Those surfaces. So, where you would expect to find large amounts of IgA would be in lymphoid tissue, that is immune system tissue associated with surfaces, and secreted into the - sometimes into the secretions from these surfaces. So, a major question is whether the Serum IgA reflects the change in mucosal immunity or not. And I don't know the answer to that. It doesn't necessarily reflect a change in mucosal immunity. One could certainly imagine seeing no change in IgA or a change in Serum IgA that isn't reflective of mucosal immunity. It suggests a possibility. It doesn't establish it to a biological certainty.
Q So, if you were going to attempt to draw any conclusions to a reasonable degree of biological certainty, these data don't enable you to do that?
MR. LAWRENCE: Object to form. Leading. Go head.
A Very rarely do you see a single study in which you can say something to certainty. I'll start with that. I would suggest that - it suggests the possibility that further work might be worth doing, but it doesn't establish a change in mucosal immunity.
Q And ultimately you decided that these data were not significant enough or not certain enough to warrant publication, you and Dr. Reinemann?
MR. LAWRENCE: Objection to form. Leading.

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Go ahead.
A I do not know Dr. Reinemann's opinions. I only know mine. So I can't speak to Dr. Reinemann's opinions on this. I was not very excited about publishing it. I wouldn't object to publishing it, but I did not think it was a particularly exciting study from that standpoint.
Q In the scientific community, what does it mean to have a paper peer reviewed?
A The most scientific journals have an editorial board, and when a paper is submitted to those journals for possible publication, it is sent to reviewers who are, in the judgment of the editor at least, sufficiently expert in the subject matter of the paper to pass judgment on whether it meets that journal's criteria for publication.
It varies considerably with a journal as to what that might mean. Some of the things that are typically evaluated are novelty of work. Is it reporting something that hasn't been reported before? Appropriate methodology, whether the right measures were made, appropriate controls, whether, statistically, whether the experiment was big enough, for example, did you use enough animals?
And something that is a little harder, at least for me to get a grasp on, and that is, the significance of the finding; does it actually change the way we look at a particular field.
The reviewers evaluate these. They send a report as, in my experience, always anonymously, back to the editor who then communicates this to the author as to whether the paper is acceptable for publications, needs

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some modification, a very common thing, maybe generally acceptable, but they have some questions, or is not acceptable for publication in that it doesn't meet some of the criteria.
Q Does the validity or accuracy of the conclusions or findings have anything to do with the function of the reviewers?
A I'm not sure what you mean by validity or accuracy.
Q How about, let me restate it. Is there ever a situation where the reviewers say the experimentation, the data, simply doesn't support the conclusion?
A Yes. If, as an example, if you do an experiment, you observe a certain observation, and you make inferences far beyond what your data will actually support, yes, that comment can be made.
Q In any event, you decided 249 was not worthy of publication?
A That was my opinion.
Q By the way, what's a Type 1 error?
A I know the answer, I'm trying to think of how to explain it to you.
Q You're a teacher?
A Yes. Yes, but sometimes when I haven't explained something in a very long time, I have to think of it before I get into this.
Q If you can explain it so that I can understand it.
A I will use this experiment as an example. We had, for each of these measurements, we had two groups, control and treated. If I - I can make two decisions. The control and the treated are the same, they're equal, or I

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can decide that they're different. There is a truth, they either really are the same or they really are different. Now, if I say that they're the same, then they really are the same, there's no treatment effect. Then I haven't made a mistake. If I say they're different, and they're really different, then I haven't made a mistake. If I say they're the same, but they're really different, that is an error. And if I say that they're different, but they're really the same, that's also an error. But those two errors aren't the same. If I say they are different when, in fact, they're really the same, that's called a Type 1 error. And if I say they're the same when I really should have said they're different, that's called a Type 2 error.
Q So you're focusing on the pre-treatment conditions of the animals to be able to make a valid comparison between the control and the treatment group?
MR. LAWRENCE: Object to form. Leading.
A No. We're focusing on the differences after applying treatment.
Q But there can be pre-treatment differences between two groups of animals that are going to affect the end results after the treatment, is that correct?
A That's possible, yes.
Q And what did you do to ensure that, in your initial study, that the animals' pre-treatment really were the same?
A The initial study, the first thing that's done in any study is randomization. You randomly assign animals to a treatment group, so that you don't, for example, take the

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12 highest milk production cows and call them a control in 12 lowest ones, treatment. Where they're housed in the barn is random, for example. We don't house all of the treatment group together and all of the control group together, in case there's some local environmental effect.

So that's the first thing, and probably the most important in any experiment, is the randomization part.

The second, in this initial study, is a technique called analysis of co-variance. Analysis of co-variance makes a measurement at the start of the experiment. It doesn't have to be the same as what you're measuring later, but it can be; and statistically correct for any difference there between the two, the groups.

It's mostly the measure of actually reducing variability. And I'm not a statistician, so I'm hoping that makes sense. But that's the best I can do.

Q In your initial study, you were looking for a number of outcomes?
A Correct.
Q And in the study, the Part 3 of the Minnesota Science Advisory, that also looked for a number of outcomes?
A Correct.
Q And in the unpublished abstract that you did, that looked for scores of outcomes?
A That's correct.
Q Have you ever heard of statistically the Bonferioni Adjustment?

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MR. LAWRENCE: Do you mean Bonferioni?
A Bonferioni?
Q Bonferioni.
A Yes.
Q You didn't use those in these studies?
A I do not know that I used them for these studies or not.
Q Okay.
A I have heard of it though.
Q So you didn't do the statistics, is that correct, doctor?
A That's correct.
Q What was the next project that you did relating to bovine immune systems after 249?
A Okay. That was the study looking specifically at messenger RNA reference. When we started this, we thought it would be technically feasible to make very large numbers of measurements. We were able to get measurements on a reasonable number around the hundred or so.
Q Of different immune responses?
A Different messenger RNAs. Not all of them were related to immune responses. We included some that we were pretty sure wouldn't see an effect as a control for that. We also included some things that we shouldn't have even seen in the cells to make sure that we weren't detecting spurious signals. Does that answer - is that an answer to your question?
Q Yes. Well, let me just ask you this. Exhibit number 250. Do you have a copy?
A Yes, I have a copy.
Q Is that the next research that you did?

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A I believe he was referring to this (indicating).
Q No, no. Part 3.
A Oh. Okay. This is the same - that is this (indicating). This is just a different summary of this.
Q So, 250 is just a summary of 249?
A This is what was submitted to the Minnesota Public Utilities. This wasn't. (All indicating).
Q But 250 is just a different compilation - -
A 250 is a different compilation.
Q - - of 249?
A Of the same work. At least - -
Q Go ahead.
A To the best of my knowledge, that is true.
Q And at the time 250 was submitted to the Minnesota Science Advisory, you're indicated to be a professor of dairy science?
A That is correct.
Q And Dr. Reinemann was just an associate professor?
A That's what this says, and I don't recall, but that's what it says.
Q And Steve LeMire, he was the guy who was in charge of the statistics?
A He was in charge of the statistics. I don't know if he did other things as well, but that's correct.
Q What did Morten Dam Rasmussen, PhD, do?
A He was an associate of Dr. Reinemann's, and I'm not quite sure. Dr. Reinemann felt his name should be associated with it, I do not know why.
Q What about Milo Wiltbank?
A Milo Wiltbank did some of the assays. I believe the

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assays he did were the assays for the hormone cortisol. C-o-r-t-i-s-o-l.
Q Why don't you explain for the record what assays are? A-s-s-a-y-s?
A Correct. Measurements.
Q And there is a certain number of - at least in exhibit 250, there were a certain number of indicators or markers of the immune's function response that were identified for observation and testing. Why did you identify and test those?
A They are often used in immunology to assess immune function. They are accessible, meaning they were things we had the ability to actually assay. Those were the major reasons.
Q When we talk about immune response, are there two types of proteins and cells, those that stimulate an immune response and those that control an immune response?
A There are many proteins in the body that affect the immune response. Affect spelled with an A. Some of them stimulate certain activities, some of them inhibit certain activities. For example, there are pathways that stimulate antibody production, there are pathways that inhibit antibody production.

Not being an immunologist, I am not quite sure how to answer that, but I'm not sure I would say that it's quite as simple as a protein is always either one or always the other.
Q And if you look at the abstract of 250, the last sentence says, "Correctively, these results suggest that exposure to 1 milliamp of current for two weeks has no significant

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effect on the immune function of dairy cattle." Was that the conclusion of this report?
A I did not write that, but that was the conclusion of this report.
Q Was this report peer reviewed?
A Not to my knowledge.
Q Is peer review the gold standard in your business?
A For publication awards, it certainly is. For - yes, I guess I could say that that's correct.
Q Now, the study reflected by 249 and 250, which is the same data, I guess you're telling me, was that in a stanchion barn or a free-stall barn?
A This was in a stanchion barn.
Q And how the current was delivered to the animals?
A It was a long time ago, but if I recall correctly, there were special stalls constructed with conductive mats on the floor, and I believe it was an AC. I'm not the engineer, so I may be remembering this wrong. But it was delivered through the floor of the stanchion, if I recall right. I didn't design the stalls, so I am relying on an old memory here to answer that.
Q And the animal was tethered in the stall so she couldn't escape the introduction of the electricity?
A I believe that is correct.
Q And going on the second page of exhibit number 250, the first full sentence says, "The consensus of the science advisors was that current in the earth can only interact with dairy cows through their associated electrical fields, magnetic fields and voltages, and that these parameters should be the focus of the analysis." Do you

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know what the author is attempting to say there?
A I am not entirely sure. Or - -
Q Let me - well, go ahead.
A I was just looking at it and seeing if I could think of a different way of saying the same thing. If we have a current in the earth, you need to create an electric field that the cow is exposed to, current flow and electric field and the magnetic field. So, either the electric field or the magnetic field interact with the cow or the current flows through the cow. I believe that's what you're trying to say, but I'm actually not sure that would be.
Q Would you agree with me that the early research on stray voltage primarily focused on behavioral responses?
A What I am familiar with, that's correct. There may be some things that I'm not familiar with, but that's what I am familiar with.
Q And the point of this research that you and Dr. Reinemann were involved in was to see if there were other responses?
A Yes.
Q And specifically, if there were immune responses?
A Correct.
Q Now, exhibit number 250 and 249, both of which tested the control group and the test group before the treatment, correct?
A I believe that's correct, yes.
Q So you had not only a comparison between the test group and the control group, but you had a comparison before and after the test was conducted?

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A That's correct.
Q And that's the best way to do it, isn't it?
A That is a powerful way of doing it. I don't know if I would say it's the best. There are many possible ways, but that certainly is a way of reducing the variability.
Q It is a more powerful way of reducing variability than to just compare the test group to the control group after the test is completed with no baseline comparison?
A Under most situations, that's correct. It would be - it is technically possible that that's not correct, but those situations would be pretty rare.
Q The second last sentence in the last full paragraph on page 2 says, "The absence of significant changes in these laboratory data in treatment cattle over time (each cow serving as her own control), as well as a lack of difference between treatment and control cows, indicate there was no alteration in circulating volume or acid-base balance, nor was there significant stress (as meausred by glucose concentration) or muscle injury inflicted by the treatment." Are you talking there - or is the author there talking about the testing you did or testing that had been done?
A I believe - let me read the whole paragraph for a moment to put it in context.
Q All right.
A I believe that is referring to this study cited Reinemann, et al, 1996, which is in the references of this.
Q Okay.
A That is not referring to this particular study.

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Q If you look on page 3 under Objectives, that was the objective of the study that's reflected by this paper?
A Excuse me?
Q Page 3, where it says Objectives, that was what this study hoped to accomplish, by this study that's reflected by exhibit 250?
A That, referring to the immune function?
Q Yes.
A Correct.
Q And do you agree with me that the stress that an animal is subjected to, is, in part, related to herd management?
A Yes.
Q And the way one group of cows might be treated, if it was different than the way another group of cows is treated, you might expect to see different stress responses?
A Could you repeat that?
Q Yes. If you had two herds, and their daily protocol, their daily management, was different, one would expect to see different stress responses in those two different herds?
A That's certainly possible.
Q And on the bottom of page 3, the last paragraph on the bottom of page 3 talks about how this herd was managed.
A Okay. Yes.
Q And it wouldn't be appropriate to draw necessarily a comparison between this management style and a completely different management style?
A (No response).
Q It looks like you're struggling with the question.
A I'm struggling a bit for several reasons here. If I

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understand the question correctly, you're asking, can we extend the results from this study, which was done in a stanchion barn, UW Madison herd, their particular genetics and so forth.
Q Milk two times a day?
A Milked twice a day, fed a certain type of ration.
Q May or may not have been administered BST, we don't know that.
A I do not recall when UW started using BST.
MR. LAWRENCE: I'll straighten that out. I don't mean to interrupt, but it's discussed in a lot of the page 3 also, if that helps.
A The extent to which these results could be extended to other herds. Basic biology is still constant. I mean, there are certain principles of biology that can be extended. Certainly it limits some types of responses. For example, if you are - and I believe there is research to support this, although it has been a long time since I looked at it, an animal that is housed in such a way that it can avoid a stress shows less of a stress response than one that's housed in such a way that it can't avoid the stress.
Q So, extrapolating that, an animal in a free stall barn that can avoid a stress is going to have less stress than an animal in a stanchion barn where the stress is administered?
MR. LAWRENCE: I'll object to the form as leading. Go ahead.
A There are some studies that would suggest that, if I remember the literature correctly. To state that that's

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always true I think is a bit of an overstatement. So.
Q It could be true?
A It could be true, but I wouldn't say it is true.
Q You wouldn't say it's always true?
A I wouldn't say it's always true.
Q All right. Then, on Page 4, the bottom of the last paragraph says, "The differences of the treatment cows were compared to the differences of the control cows using and independent t-test." What's a t-test?
A It is a statistical method of determining significance.
Q On Page 6, how is it determined when the blood samples were collected? The samples were collected - -
A Are you referring here to the duration of - I'm not sure what exactly you're referring to.
Q It says, "Samples were collected for one week before exposure and for the two weeks of exposure."
A Yes.
Q Who decided how and when to collect the blood samples and what to make the comparisons to? I mean, that sounds like more biology than it does engineering.
A It does. One of the factors - are you referring here to the duration of the collection, that it was for, say two weeks of treatment, or to the exact date it was collected on?
Q The latter.
A The latter. Okay. One of the things - let me refresh myself on how often we actually didn't collect these. One aspect of this is, these - some of these assays are quite difficult to conduct. It can't be conducted on stored samples. Some of the things like serum cortisol

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levels, if you put the sample in the freezer and do the analysis at anytime. But some of these tests require living tissue collected from the cow, and they take several days to actually conduct.
That timing allowed us the opportunity to collect the sample, process it and then go back and collect and process the next sample. We simply didn't have the personnel to, for example, take twice daily samples and process all of that for some of the assays that we were doing, like chemiluminescence in the lymphocite blastogenesis assay, in particular, are vary laborious assays.
Q On page 9, are those data the same data that were in 249? Because I noticed it in IgA serum, the mean difference is .017 rather than .015.
A I believe that it's based on the same row data set, but we did some slight differences in, I think, - I think that is reflected in a statistical difference that was made in how the details of how the statistics were analyzed that makes a slight difference in the exact number. But I - it does appear that these should have been the same data. There was only one data set with all of this.
What's in this first report may have been analyzed by a slightly different technique, and so the numbers may show very small differences, like the difference between .017 and .015.
Q But just from looking at, with my rudimentary understanding of P-values and statistics, there isn't anything in the far righthand column that's less than

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.05, is there? Well, I guess there is, staph. aureas.
A Staphylococcus aureas. I did not do those statistics. So that detailed independent t-test. Okay. Let me see what the difference is. Staphylococcus aureas. I am not sure what that refers to.
Q Okay.
A That is something that a statistician did that I do not know what that even refers to.
Q Take a look at page 13, Conclusion. The conclusion says, "Collectively, these results suggest that exposeure to 1 milliamp of 60 hertz electrical current for two weeks had no significant effect on immune function of dairy cattle." Was that the conclusion of this study?
A That would have been the collected conclusion of the authors in the study.
Q And you were one of the authors?
A I was one of the authors. There is, like I said, the one observation that was significant.
Q Now, 251 is your abstract?
A That's correct.
Q Is that the next research that was done on this subject matter?
A That I was involved in, that's correct.
Q And why was this abstract never published, Dr. Sheffield?
A I had - for basically the same reasons as the first. The study had not shown a lot of significant effects, and I doubted it would stand peer review.
Q Why did you doubt that it would stand peer review?
A By the time - when we started this study, the technology we were trying to use was in its infancy.

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Q A ray analyzer?
A Yes.
Q Did you have a lot of trouble with that?
A Yes. As we progressed through this, some of the things that we would liked to have done were technically not feasible, at least not at the time with the technology that we had available.
Q The ray analyzer was a new - -
A It was a very new technology and it really had not been applied to cattle at the time.
Q And the notes, underlying notes, seems to suggest that your lab assistant had some difficulty using that technology?
A That's correct.
Q And you heard of the expression, garbage in, garbage out?
A Yes.
Q And there's some aspect of garbage in, garbage out in the data that were generated, is that correct?
MR. LAWRENCE: Object to form. Leading.
A I don't know if I would say in the data that were generated. The - well, I'll just say that.
Q Well, is there some reason to believe that the underlying data that were suspect because of the new technology and the unfamiliarity of the people who were applying that new technology?
A I think the data included here are as reliable as we could have made them.
Q I understand that. I know that - -
A You're asking me about technical abilities. Certainly, today there are much better ways of doing it than what we

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did.
Q I'm not suggesting - -
A By today's standards, the results would be very noisy.
Q By the way, was there any observation in the work that you and Dr. Reinemann did before exhibit 251 of a drop-off in milk production or an adverse effects on animal health associated with the administration of electrical currents to the animals?
A We did not notice any change in milk production.
Q And is that true - - go ahead.
A And, well, you asked also about animal health. The numbers would have been pretty small to have detected any health effects at all.
Q And is that true with the animals in the experiment of 251, no drop-off in milk production?
A I do not recall any.
Q Now, in the abstract, the last sentence says, "These results suggest that electrical effects on disease processes are likely to be modest, probably more long-term and likely to be very difficult to detect in small samples." Was that the conclusion of this study?
A That's what I would have concluded, yes. Perhaps I should define modest, meaning, we basically found, out of a hundred genes, only a couple of things were actually different that we could detect at all, and when you're doing the hundred statistical test, you expect a certain number of false resuts.
Q False positives?
A Yes.
Q So, some of the results that you see could just as likely

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have been attributed to false positives as they could be to the effects of the administration of - -
A I don't know if I - -
MR. LAWRENCE: Object to form. Go ahead.
A I don't know if I would say just as likely, but it could be.
Q And you also said there could be a Type 1 error in the data that you generated?
A I probably said that in the caveats here somewhere. That sounds like something that would be in here.
Q And again, this was in a stanchion barn?
A Correct.
Q And unlike exposure in a real life situation, you essentially attached electrodes to the legs of the animals so they were constantly administered electric current?
A Correct.
Q They couldn't avoid it?
A Not without physically detaching the electrodes by rubbing against the stanchion.
Q Did that happen?
A Well, we did check those. Each time the cows were milked, that got checked. You will occasionally - we would on occasion see the electrodes detached. They were immediately repaired. But, in general, they did stay in place.
Q And in the last paragraph on the second page, first sentence, it says, "In a previous study." Is that referring to the study that you and Dr. Reinemann did? It says, "In a previous study, we observed that

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electrical exposure of dairy cattle had minimal effect on most immune function measures, including chemiluminescence, lympho - how do you pronounce it?
A Lymphocyte blastogenesis, is how it's pronounced.
Q So that was - -
A That refers to the previous study that we've just discussed, yes.
Q All right. And you're talking about interleukin 1 approached significance of less than .01, but I thought statistical significance was less than .05.
A Where do you see this? Next sentence. "Increase in serum interleukin 1 approached significance at P of less than .01."
A No. .10.
Q Excuse me. .10.
A That is greater than .05. That's why we say "approached" rather than "reached."
Q So that could be attributable to chance?
A Anything can be attributable to chance. It's more likely to be chance than if it were a smaller number. That's what that means.
Q In your business, .05 is what's regarded - -
A Most - - excuse me. You're correct. Most biologists consider .05 to be, for lack of a better word, the gold standard.
Q And it says underneath the animals, so the CALSIACUC, that makes sure you're not abusing the animals?
A That's correct. I served on that committee, so, if you want, I could discuss for you what they do. But that's a short version and accurate enough.

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Q Bottom line is, you're not electrocuting the animals?
A That's correct. If we had tried to give them a voltage that the committee felt was truly dangerous, for example, we're going to use 110 volt 20 amps, which is quite serious stress.
Q Probably wouldn't get the assignment.
A You probably wouldn't get the approval to do that. But that is what they assess, yes.
Q Do they look at what they do with the rhesus monkeys and the mice and the rats?
A Well, the college of agriculture's committee isn't the one that does that. But there is a committee that does. Any vertebrae animal research goes through such a committee at the University of Wisconsin.
Q Hopes of keeping the PETA people happen, huh?
A I wouldn't comment on that.
MR. LAWRENCE: If you ask if that's true in Harry Harlow's days, it was.
A These laws are more recent than Dr. Harlow's work. His work would not have been subjected to that.
MR. LAWRENCE: Thank you.
Q Then you say underneath Animals, "Blood samples was collected - probably should be were collected - via the tail vein immediately prior to applying the current and at a the end of a three week exposure period." So you took two blood samples, one at the beginning and one at the end?
A That's correct.
Q How come your data doesn't reveal anywhere what the blood samples showed at the beginning of the test?

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A I do not know.
Q Your comparison is between the control group and the test group?
A That is the comparison we did.
Q No cow to cow - within cow comparison?
A No, as I recall, we did not do that.
Q And then, if you look at, it's not numbered, but the next page, under Results and Discussion. Are you with me?
A I'm with you, yes.
Q You said, "Most measures were not affected, suggesting that those that were could be Type 1 errors, due to a large number of hypotheses tested." What do you mean by that, doctor?
A You mentioned earlier - well, I guess you explained it. If you measure one thing and you have a Type 1 error of 5 percent, there's a 5 percent chance that if you measured the conclusion, that there's a difference, there's a 5 percent chance of being wrong. Follow me so far?
Q I think so.
A If I'm measuring one thing, let's say milk production, and I should conclude milk production was changed at a P value or Type 1 error P value of .05, there's a 5 percent chance to be wrong. If I measure two things, there's a 5 percent change of each one, of either one. So, the chance that at least one of them is a Type 1 error goes up. The more things you measure, the greater the chance that at least one of them will show a difference even though it wasn't really there.
Q Kind of like, if you flip a coin a hundred times, every time you flip it, there's 50-50 chance it will be tails.

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But the chances that if you'll be able to flip it a hundred times, it'll come up tails becomes less and less?
A I don't know if that's a good analogy or not. But.
Q But the point is, one of the reasons the Bonferioni, if I'm pronouncing that correctly, the adjustment is, is taking into consideration that possibility?
A Yes. Now, that is a major issue in statistics anytime you're making mini comparisons. And the Bonferioni approach - again, I'm not a statistician, but I think I'm getting this close to right. The critical question is, what should the Type 1 error rate be based on? Each individual comparison for the whole experiment. And there's great debate, at least in my understanding, among statisticians about how to correctly do those corrections. The Bonferioni is one approach. Some statisticians criticize it by saying it over-corrects. But that is the idea of the Bonferioni approach, is to correct that.
Q But what you're saying in your reference to Type 1 errors, when you were studying dozens of outcomes and only three or four showed it's a statistically significant difference, that could be due to chance?
A It could always be due to chance. It's more likely to be due to chance when you've got a large number of comparisons and a small number of significant results.
Q That's why many scientific studies just look for one outcome?
A I don't know. Many scientific studies, at least in large animals, look for multiple outcomes. So, I guess I don't know if I would have an opinion on that one way or the

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other.
Q Now, on the last page of your report, you say, "In conclusion, these studies suggest that electrical impacts on immune function are of relatively small impact compared with infection and inflammation." What are you saying there, doctor?
A All right. Good question.
Q All my questions are good.
A This is important. Let's suppose we took a cow and, whether intentionally or unintentionally, gave her something that the immune system recognizes as poor, this happens with vaccination, for example, if you give her a vaccine or intentionally give an infection.
Q That's what a vaccine is, is introducing a foreign - -
A Yes.
Q - - entity into the animal.
A The immune system responds very strongly to these. When that happens, you see major changes in the immune functions, much larger than what we saw here. And that's what I was referring to there. The magnitude of the changes that we did see are generally small compared to what you would expect to see if the cow were truly ill. For example, if you gave the cow a strong vaccine, you would expect to see bigger changes than this in at least some of the immune function measures. That's what that's referring to.
Q So, if what you were observing was biologically significant as opposed to statistically significant, you would expect to see a much greater reaction.
MR. LAWRENCE: Object to form. Leading.

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Go head.
A Not necessarily. And here is the reason - well, a reason for this, and a weakness of this study, by the way. So I'm kind of being critical of myself, but I think you should do that. This study measures base line responses, what the base line is. These cows were, as far as we knew, healthy. They weren't being exposed to any known pathogens other than the things that's normally in their environment.
An important thing to remember about the immune system, you don't really want the immune system to be active all the time, because it's very damaging. Inflammation is very damaging, but it's also very beneficial because it gets rid of infections.
What we didn't look at in this study was how strongly and rapidly the immune system responds to a challenge. So, what we looked at was, you got a base line here, and that base line didn't change.
A second important question that we didn't assess was, if you give a challenge, a vaccine or a disease, would the immune system respond strongly or would in one group the response be less than the other group? So, what's not assessed here is that ability of the immune system to respond to a challenge. But in terms of base line, we didn't see, except for, I believe it was IgA, we did see a drop in the IgA message. But other than that, the base lines were the same.
Q So, is there sufficient or insufficient data here to be able to draw any conclusions to a reasonable degree of scientific certainty about the animal's immune systems

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ability to respond to an insult?
A This says very little, if anything, about ability to respond to an insult. It's just a base line study.
Q And then you go on to say, "Any effects observed appear to affect only a small set of immune response regulators, compared to most disease processes, which affect a wider spectrum of regulators." Are you saying there that, when you introduce a real disease, there's a much more robust response in the animals than the response you saw to the administration of current?
A I think that's what I was trying to say, yes.
Q And that you conclude by saying, "As a result, the impacts of electrical exposure on animal health and disease is likely to be difficult to detect reliably, particularly without examining a large population." We need to study a whole lot more animals before we can come to any conclusions. Is that what that means?
A Okay. That sentence is referring to disease processes. So. One of the questions that comes up is, even if you see something such as, say a change in IgA levels, if you see that, will that indicate that this animal is more susceptible or less susceptible, depending on what you see, to a disease. To actually study a disease itself generally takes a very large number of animals, much larger than what was involved here.
For example, if you look at mastitis, small studies don't have a lot of what is called statistical power, and that's what I was trying to get at here, although it probably didn't do a very good job of explaining. Remember this Type 1 error, if you say

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there's a difference, you might be wrong. That's the Type 1 error. The Type 2 error is also important, that is, if you say there is no difference, you could still be wrong about that also. That's called a Type 2 error. That's the biggest reason why we use the largest number that's practical in experiments.
To have a low Type 1 error depends on several factors. One is, it depends on how big an effect you're looking for. If I want to see something with a 10 percent change, that's going to be the larger Type 1 error than if I'm looking for 10 volt change.
Another thing that influences Type 1 error is what you said calls significant, what your P value is. Most biologists use .05.
Q Even .05, there's a 5 percent chance of being wrong?
A If you say there's a difference. It's a little more complicated than that. There's a 5 percent chance that you would see that big a difference by random chance. It's not quite the same as you'd be wrong.
But a big factor that influences Type 1 error, that you have a lot of control over, is not just how big an effect you're looking for, but also how many animals you use. The bigger your experiment, the more reliably you can say that there is no difference when you make that conclusion. And that's what takes large numbers of animals - that's what was trying to get at here.
If you wanted to study disease, if I take a dozen animals and look at an instance of a particular disease, I'm probably not going to find any difference,

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simply because 12 animals for most diseases is not merely enough. So, for a study of actual disease instance, and whether something affects that, does take very large numbers of animals.
Q So, is it fair to say, doctor, that, based upon Dr. Reinemann and your joint studies, and the abstract that you did, the data simply isn't sufficient to draw any conclusions to a reasonable degree of scientific certainty about disease effects on animals associated with electricity?
MR. LAWRENCE: Object to form. Leading.
A We did not measure a disease itself. That's important to know. We measured some things that may be correlated to sensitivity to disease. We found most of those measures were unchanged, and a few were changed, few enough that I cannot reliably conclude that it's not due to random change.
Q Now, last topic. You were a full professor at the University of Wisconsin, tenured, specialized in the area of mammary gland development?
A Correct.
Q You are now teaching at a junior college in a suburban or a small town in Wisconsin. How come?
A I reached a point in my life where I simply disliked the various stresses associated with doing research and wanted to do something that was more pure teaching.
Q Got tired of publish or perish?
A And getting grants and various other stresses. My life, I don't know that the job is any easier, but I find it more enjoyable.

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Q So now you're teaching basic biology rather than highly specialized mammary gland development?
A That's correct. Yes. I teach - this semester I'm teaching not only Physiology I, mostly to nursing students, Anatomy Physiology II, the microbiology.
Q Your choice?
A Yes.
Q That's all I have.
MR. LAWRENCE: I have a bunch, doctor. We've been going about an hour and a half. If you'd like a break, we can take one, if not, we can go for a while longer.
MR. THORNTON: I have to leave at 3:30 to catch an airplane.
A Actually, I'm fine.

(At this time a recess was taken - 2:00 to 2:09).

RE-DIRECT EXAMINATION

BY MR. LAWRENCE:

Q Mr. Sheffield, let's go back to exhibit 249 and 250 for a moment, please, and I would like to look at Table 2 on those two documents with you for a moment. It's on page 9 in 250, and I'm not sure what page it's on in 249.
A Table 2, you said?
Q Yes. Page 9.
MR. THORNTON: I think Table 2 is on page 22 of 249.

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Q Okay. Then get the same table out from exhibit 249. I think that was the - it is page 9, originally paginated on the published paper. Let's talk about the - well, let's talk about the various columns. I'd like to cover this with you in some detail.
First of all, and just as background, could you please describe what your major functions were in both designing and carrying out these studies that resulted in the data that's set forth in Table 2 on exhibit 249 and 250?
A I was responsible for coming up with the list of things we would measure, so I made the assessment as to what to measure, what I reasonably felt we could measure, and my laboratory did the actual measurements.
Q So those would be your two principle functions with respect to this work?
A Yes.
Q And that would probably apply to the second studies where messenger RNA assays were used?
A Yes.
Q Generally speaking, do you think these measurements were done in an appropriate and accurate manner?
A I think so.
Q From your discussion with Mr. Thornton this morning (sic), I take it you had no responsibility for the statistical analysis that resulted in Table 2, is that correct?
A No. You are correct.
Q Thank you.
A I was not responsible for statistics.

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Q Thank you.
A Sorry.
Q No, the problem was my question, not your answer. I apologize. We get double negatives in this business too often.
And could you briefly describe for me your academic training in immunology in terms of course work you've taken and the research you've done as it relates to that subject? And I'm looking for the short version of that, a short version.
A Well, like any graduate student whose specialty was physiology, I had a reasonable amount of immunology in courses. I was included in a lot of other courses I took in microbiology and physiology. I have used immunological techniques as research tools for some time. Some of these assays were new to me, but in terms of actually doing them I was familiar with what the assays were, but I had not actually performed them before doing this study.
Q The assays involved on Table 2, page 9 of exhibit 250, in particular, or other ones in the other study?
A Well, we'll just talk about these for now. But, yes, these I - some of these would have been new assays to me.
Q And who actually did the physical work of making the assays? A number of people? Can you describe who they were or what - -
A The end is, there's technicians. I believe all of these were done - this was a very long time period. By full-time technicians as opposed to graduate students, although it might be possible that a graduate student

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carried out some of the assays. I do not know about the cortisol assay. That was not one that I was responsible for. I believe Dr. Wiltbank had done that. But most of them would have been done by full-time technicians of working under my supervision.
Q For example, were the assays involving the cytokines, c-y-t-o-k-i-n-e-s, with interleukin 1 and interleukin 2, those would have been done by full-time technicians, is that correct?
A That's correct.
Q That was the machinery or the equipment and the apparatus and whatever else was needed to do those assays, is that something that all had been in the lab for some time?
A We had had access to. Some of the equipment is very expensive and so shared by several labs, so some of it may have been physically located somewhere else. These things they would have used before.
Q Okay. That was my next question. Thank you. By "they" you meant the technicians?
A The technicians, yes.
Q Thank you. The units for each of the various variables are indicated in parentheses, and it looks like you made, you or the statistician, made a logarithmic transfer on each of the numbers, is that correct, or transformation - -
A That's what the other one would refer to, ye.
Q That's a referral to natural logarithm?
A That's a logarithm, yes.
Q So you take the absolute number and before the statistical analysis is performed, the natural logarithm

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of that number is the number that's actually used to analyze, correct?
A That's my understanding of what was done, yes.
Q All right. Was that done, the use of the natural logarithm done at your direction?
A I do not recall how that procedure was arrived at. I believe, if my memory serves me correctly, that Steve, the individual who was doing that work, was concerned about the statistical problem called heterostevasticity (ph).
THE REPORTER: Called what?
A A-l - - Let me use a different word. The heterogeneous, h-e-t-e-r-o-g-e-n-e-o-u-s. Unequal. Let's use this word. Unequal variances. V-a-r-i-a-n-c-e-s.
Q And making a natural log transformation, is a standard, unique in those circumstances?
A Is one of several commonly used techniques. I believe Steve - -
MR. THORNTON: Try not to interrupt.
A Sorry.
MR. THORNTON: Or we're just going to have a terrible transcript. Because this is hard enough as it is.
Q Why don't we do that one more time, just for the record, doctor, to make sure John got it correctly.
Is it true that making a natural logarithmic transformation in the circumstances you described is standard statistical technique?
A That's true.
Q And in your opinion, was it appropriate?

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A I did not - I did not go through the data in extreme detail to check that, but it seemed reasonable.
Q With respect to the first two main response variables, concanavalin A and phytochemagglutanin, the units appear to be DPM, is that correct?
A That is correct.
Q What does that mean?
A Disintegrations per minute. These - should I explain the assays?
Q Please.
A These assays are based on taking lymphocytes from blood, culture them in the presence of a stimulant, and measuring their DNA symphysis. The DNA symphysis is measured by adding a radioactive isotope of the phymidine p-h-y-m-i-d-i-n-e, and measuring how much of the phymidine is incorporated into the cells. And for this, we measured the amount of radioactivity in the cells that the units for that were disintegrations, how many radio-active phase per minute occurred.
Q And then, I'm sorry, this is all done out of the body?
A Correct, yes.
Q The term of that is in vitro or in vivo, one or the other.
MR. THRONTON: In vivo.
Q In vivo.
A In vitro is literally in glass. So that's in test tube. In vivo is in the whole body.
Q Thank you. I always get them mixed up. So these were done in vitro, is that correct?
A That's correct.

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Q In fact, I think everything here was - that would apply to, is that true?
A Not quite. Many things it does.
Q Okay. The third main response variable, chemiliminescense, PMA. First of all, what does PMA mean?
A Phorbol miristate acetate. I know I'm going to have to spell this. P-h-o-r-b-o-l, m-i-r-i-s-t-a-t-e, I believe. Acetate, a-c-e-t-a-t-e.
Q And then the number apparently has the acronym, RLU, is that correct?
A That's correct. That stands for relative luminescence, l-u-m-i-n-e-s-c-e-n-c-e, units.
Q Describe the assay in some detail, if you would, including what relative luminescence units means.
A Yes. Here we take lymphocytes from the blood, and we add to them a stimulant. There's several that we could have used. Phorbol miristate acetate, or PMA, is the one that we used here. This stimulates certain cells, mostly from blood, a cell type called a neutrophil, which is a component in the immune system that engulfs some digest type bacteria.
We also add a detector, I believe, luminol, l-u-m-i-n-o-l, was added. And the active neutrophils produced oxygen radicals, this is part of the pathway that they use to kill bacteria. This interacts with the luminol and gives off light, hence the name, chemiluminescence.
The instrument that we use to detect the light is called a luminometer, it's effectively a light

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detector. And the relative luminescence units is simply how many protons of light we detected for the output of the instrument. It's called relative luminescence units because it really has no specific number, like disintegrations per minute does with radioactivity. It's used in association with it.
Q So, every unit would be a whole bunch of protons, is that correct?
A Probably. I don't know the details of that.
Q All right. Is there a particular reason or reasons that you chose these three at the top of Table 2 as the main response variables? And please describe that system.
A I don't recall that discussion at all about how that was going to be presented in the table.
Q Well, picking out these various variables was your primary responsibility, is that correct?
A Picking out the whole list was my primary responsibility. But I don't recall discussing calling any of them primary and secondary. I don't know why that distinction is made there.
Q Well, who was the lead author of the Part 3 table, if it wasn't - -
A The composition of it, as I recall, was by Dr. Reinemann.
Q So he would have done the drafting?
A He would have done the drafting of this paper, I believe.
Q And I would assume - -
A I did not. So, I am assuming that Dr. Reinemann did.
Q Was it then circulated for comments to all the co-authors?
A I believe so.

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Q Going then down the list, the next variable, the next response variable, which is the lead one, top one, under secondary response variables, is S. aureus, or staphylacoccus aureas, is that correct?
A That's correct.
Q But again, if they were measuring DPM, which would be the same procedures as before, is that correct?
A That's correct.
MR. THORNTON: Mr. Lawrence, you should probably make clear that you're dealing with the table on 249, excuse me, 250 or 249, you started talking about both and now a different one.
Q You're absolutely correct. We are looking at the table on 250 at the moment, correct?
A That is the one I'm looking at.
Q Thank you. Thank you, Mr. Thornton.
Going down the list in 250, the next response variable is pokeweed. You may have explained this to Mr. Thornton a bit. Can you tell us what that's all about, briefly?
A That is an agent causing in pokeweed that stimulates certain lymphocytes to proliferate.
Q So again, lymphocyte proliferation that's being determined here?
A That is correct.
Q And that would be true of the staph. aureas?
A That's correct.
Q And then the next one is IgG in the serum, correct? That's correct.
Q And the units are milligrams per milliliter, correct?

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A That is correct, yes.
Q And again, the logarithmic transformation made on the absolute number, correct?
A Correct.
Q And then the statistics are round?
A Correct.
MR. THORNTON: Doctor, if you could - -
A I'm sorry, I'm just - I'm just wondering that if it should be micrograms per milliliter rather than milligrams.
MR. THORNTON: Doctor, take your hand down.
Q If you would.
A Excuse me.
MR. THORNTON: It was okay when you were looking at me, because the court reporter is between us. But now you're facing the other way.
Q Okay. With that understanding, we will continue.
What's the difference between IgG in the serum and the next variable IgG in vitro?
A In serum, we collect the blood sample from the cow and measure the IgG in serum of that cow at that time. The in vitro, we collected cells, placed them in culture and measured their ability to produce IgG in culture.
Q Then the next variable is IgA in serum, correct?
A Correct.
Q And the reported units are the same again, milligrams per milliliter, correct?
A Correct.
Q And then you explained to Mr. Thornton what IgA is, so I won't ask you that again.

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We then have IL1 of first in serum and then in vitro, with the units being picograms per milliliter, correct?
A Correct.
Q And hypo - that prefix indicates 10 to the minus 12, is that right?
A Correct.
Q So, we're a couple order - well, as compared to a milligram per milliliter, we're a couple orders of magnitude down, is that correct?
A Much more than that.
Q Okay. Well, comes to the minus 6 down, correct?
A Yes.
Q All right.
A There is considerably more IgG than there is interleukin 1.
Q And what is interleukin 1?
A Interleukin 1 is - interleukin means between leukocytes. So, it is the factor, protein factor, produced by certain leukocytes in the body that regulate other leukocytes.
Q Would the chemical messenger be another way of expressing it?
A That would be another way of expressing it, yes.
Q And what is the significance of serum interleukin 1 levels to the status of immune function in a cow at a particular time?
A Elevated interleukin 1 levels is often associated with inflammatory processes and disease processes.
Q Are there things other than inflammation that cause elevated interleukin 1?

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A Possibly. I am not familiar enough with the work on that to know for certain.
Q All right. If there are no inflammatory processes going on in a cow, would you expect to find any interleukin 1 in the blood?
A You would expect to find small amounts.
Q Is there a particular reference that you would - that refers to a discussion of these various subjects related to interleukin 1?
A Not off the top of my head, but they do exist.
Q Well, for example, there's a standard text, perhaps even a couple of them on veterinary immunology, one of them is by Tizard, T-i-z-a-r-d, is that correct?
A I am not familiar with that particular work, but it could be. There are standard texts available. That could be one of them.
Q Okay. You can't think of the name of one as you sit here?
A Not off the top of my head.
Q Are there any standard immunology texts, not necessarily directed just at animals, but at humans, that you rely on in your - that you have relied on in your immunological studies in the past?
A There are. I would have to go back to my records and look them up to give you an exact reference, but there are such references available.
Q Have you ever done work in the nature of, for example, of doing vaccine trials for drug companies and that sort of thing?
A No, I have not.

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Q Would that type of work typically be done by veterinary immunologists?
A I would think so.
Q That's not something you have ever been involved in?
A I have not.
Q All right. Why did you look at both interleukin 1 in serum and in vitro in this particular study?
A The interleukin 1 in serum gives us a base line of where the animals are at. In vitro, as I recall how these studies were done, we're measuring a stimulation, so we're measuring the ability of the lymphocytes in the blood to elevate interleukin 1 in response to a challenge.
Q And the challenge in this case was, hopefully, the electric shock that was going on at levels or something else?
A No, no. The challenge in this was - I hope I am remembering this correctly. Method section for this. The challenges that were used for this was propylene nitrogen. What was done was, the cows were treated either as control or voltage. We took the lymphocytes from the blood of both control and treated cows, and we stimulated them with propylene nitrogen. This will elevate their production of the interleukin. And we measured how much elevation we saw. So we're going from very, very little, essentially none, if I recall correctly, without the stimulation, to detectable levels. So we're measureing whether the voltage changed, whether or not they could produce interleukin 1 and 2 in response to the propylene nitrogen.

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Q Okay. Is that described in the text of the paper somewhere?
A It is described in - -
MR. THORNTON: 249.
A 249. I don't know if all of it is described in detail in this one or not, but it is described.
Q We can look at 250. Well, let's do the math here a little bit as to the interleukin 1 in serum. The next column over has two numbers, one on top and one below, correct?
A That's correct.
Q And those numbers represent the mean change of controls on top and the mean change of treatment on the bottom, correct?
A That's what it looks like, yes.
Q And the treatments would be those cows getting the shock from what's described in the paper, exhibit 250, correct?
A That's correct.
Q So, if we are looking at the concentration in picrograms per milliliter of the controls, the mean change when exposed to the pokeweed was - the natural logarithm of that number is minus 0.085, correct?
A That's correct. Yes.
Q And that indicates a very small change, correct?
A I don't have a variance associated with that, so I can't really say that. But it looks to me to be a small change.
Q All right. Well, if we're looking at the absolute value of the change, we'd have to invert the natural logarithm, in other words, raise E - the number E to that power to

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see what the factor is, correct?
A That's correct.
Q And E to the zero power is 1, indicating no change, correct?
A Correct.
Q And the number we have associated with the interleukin 1 mean change controls is very close to - not equal to zero, but very close, correct?
A That's what it looks to me like, yes.
Q Then the mean change of treatments is 0.450, correct?
A That's what this shows, yes.
Q Okay. If you wanted to get the absolute number, you would raise the number E to that power, correct?
A That would give you the actual levels, or it would give you the geometric means of that number, yes.
Q Well, when you say - well, let's go through this a bit more. I want to make sure I've got this right. When you say the mean change of the treatment, how is that number 0.450 calculated from the data? Could you describe the math?
A I did not do that calculation, and I am not entirely sure exactly how this table was calculated. I would interpret that, just based on what is here, as before treatment and after treatment. What I don't know is which time point after treatment would have been used for this table.
Q Well, assuming one before treatment measured and one after, whenever they were taken before and after, what's the math by which you arrive at the 0.450?
A You would take the mean after treatment, the way I would do it. I would interpret this as taking the mean after,

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and subtract from that the mean before.
Q And then taking the natural logarithm of the resulting number?
A No. The natural logarithm would be taken before the - before the means were taken.
Q Okay. So you determined the mean, you take the natural logarithm of that number after and subtract - -
A You take the natural logarithm would be raw data, and take the mean of that natural logarithm.
Q And then take the difference of those numbers?
A That's how I interpret what was done here.
Q Okay. Very good. Let me then show you exhibit - I think I handed you exhibit 253, which I will represent to you is that same Part III paper, but it was printed off the electronic data that was produced by the University in response to subpoena back in late 2007, from the data that was compiled that were labeled as yours, as the copy service hired by the University indicated, and it appears there's a whole bunch of data attached to that copy of the Part III paper.
A Okay.
Q Are the documents attached, do they look familiar to you?
A They don't really look familiar, but that's because I haven't looked at this in a very long time.
MR. THORNTON: You're talking about, Mr. Lawrence, Appendix 3?
Q Yes. I'm talking about - well, actually Appendix - yeah, it would start with - -
MR. THORNTON: Sheffield 304.
Q 304, maybe even 303.

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MR. THORNTON: Thank you.
Q And on through the end of that document. Assuming that data came from the disk produced by the University in response to subpoena back in late 2007, do you have any argument with the conclusion of, that that's data from this study, Part III?
A I see no reason, from what I'm seeing here, to say it otherwise.
Q Okay. Then back to Table II on page 9 of 250, you have a pair of variables for IL 2, or interleukin 2 in serum, and then the following one in vitro, correct?
A That's correct.
Q By the way, where did you draw the cells from the cows to do the in vitro measurements? What part of the cow did it come from?
A They came from the - I believe they came from the tail, that's where we usually collect blood samples from.
Q But the cells would come from there also?
A Yes.
Q All right. And why did you choose to study interleukin 2?
A Interleukin 2 is a - one of the interleukins that is often changed in response to inflammation and infection. It's also, at the time we did this, if I recall correctly, interleukins were not very easy to study in cattle, as opposed to humans, of immunological assays. To measure them very easily wasn't available, so we were having to rely on rather tedious bio-assays for doing these. So we did not have the ability to measure a lot

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of different things. These were two that we felt we could measure.
Q Well, were the assays and the measurement techniques utilized in this study any different than would be done on human blood or human cells to determine interleukin 1 or interleukin 2 levels?
A They were assays that could be done on human blood, and in the past were done on human blood. But today they have been supplanted by other methods.
Q Was that true back in '99 or 2000 when this work was done?
A I don't recall for certain, but I believe that the immunological assays would have been available at that time for humans, but not for cattle.
Q And then, the last response variable is cortisol, correct?
A That's correct.
Q And what is cortisol?
A Cortisol is a glucocorticoid produced by the adrenal gland. It's often seen elevated in stress situations.
Q Will all of these variables necessarily show change for any challenge of any type to the immune system?
A Not necessarily.
Q Are there many, many other response variables associated with immune function of cattle that could be studied as part of - of studies such as this?
A Yes.
Q And I take it you've studied quite a few more and did the follow-up study later, is that correct?
A We studied some more. Fewer than I would have liked to

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have studied, but some.
Q You indicated about a hundred total, but not all associated with immune function?
A Right.
Q Then we get, in the third column on Table 2, exhibit 250, the column with the mean difference or treatment minus control, is that correct?
A That's what it says.
Q And the arithmetic there is simply to subtract one number from the other that's contained in the column to the left, is that correct?
A That's what it appears to have been done, yes.
Q And in that column, under IgG serum, we see the number 0.017, correct?
A That's shown here, yes.
Q And you spoke to Mr. Thornton about that earlier this afternoon, correct?
A I recall discussing IgGs. I don't recall if I recall talking about that specific number, but, yes.
Q And I think you indicated that the comparable number in exhibit 249 was 0.015, is that correct?
A What I said there was based on a misunderstanding that I had at the time. I recall this discussion now. I was comparing apples to oranges there.
Q Okay.
A Let me go back and correct.
Q Please. That's what I was getting to.
A Let's go back, because I was getting a little confused here. In exhibit 250, the number here is a difference in means, it's not a P factor. Table 2 in exhibit 249 is

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just the P value. So they're completely unrelated - well, they're not completely unrelated, but they are not comparable numbers. You would not expect them to be the same. I apologize, I - I was looking at the two tables and I was thinking this was a P value and it's not.
Q Can I take a look at 249, because I don't have a copy of that one. I'll get a copy of that one after today's deposition.
A I have an extra copy for you.
Q If you could. I appreciate that. While she is copying 249, let's talk a little bit more about 250. In 250, the P-value is, as calculated by Mr. LeMire on behalf of the researchers, is in the far right column directly across from the label IgA serum, correct?
A That seems to be correct, yes.
Q And that P value is 0.796 as reflected in Table 2, exhibit 250, correct?
A Oh, yes, IgA. IgA, yes.
Q So, the response of IgA was nowhere even near statistical significance, correct?
A That's based on this test. That's what that would say to you.
Q Okay. Go ahead. I'm sorry.
A However, the statistic done in exhibit 250 is more extensive than what's done here and it did show the difference.
Q Exhibit 250 is the one in front of you - -
A Oh, okay. I'm getting my exhibits mixed up.
MR. THORNTON: When you say here, - -

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A This, exhibit 250, as I recall, this is just a simple t-test, and there are other statistical ways of assessing this.
Q The simple t-test as reflected on Table 2 of page 9 of exhibit 250 is the one that the researchers collectively decided to include in the published paper again, is that correct?
A That is what was in the - this part. I do not recall the time course of when the various ways of analyzing this was done. This may have been done before the statistics of the other paper were done. Probably was. But I don't know that for certain.
Q Well, exhibit 250, which is, the front sheet entitled, "Dairy Cow Response to Electrical Environment, Final Report, Part III, Immune Function Response to Low-Level Electrical Current Exposure, submitted to the Minnesota Public Utilities Commission. That was the final paper that came out of that initial study, correct?
A That was the final submission to the Minnesota Public Utilities, that's correct.
Q By the way, did you have anything to do with the activities leading up to the University or Professor Reinemann's obtaining the contract, if you will, from the Minnesota Public Utilities Commission of terms - the request for proposal or anything like that?
A No, I was not involved in any of that work. My involvement came after the involvement with that.
Q And, generally speaking, what was your understanding of what the study was supposed to have done, in general, broad terms?

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A My understanding was that the initial study was to initially look at animal behavior responses and stress responses in response to voltage. I was not entirely clear from the very beginning as to the exact nature of the very initial proposal.
MR. THORNTON: Can I just ask one question.
Q Oh, sure. Go ahead.
MR. THORNTON: When you say initial proposal, are you talking about the entire Minnesota Science Advisors' study or are you talking about Part III?
A I'm talking about the entire one.
Q And there were papers labeled Part 1 and Part 2 in this series also, correct?
A That's my understanding, yes.
Q But they did not address items that were specifically aimed at assessing immunological function, is that a fair - -
A That's correct.
Q Okay. Let's look at exhibit 249, the comparable table, if you will. I realize it's not exactly the same format.
A Page 22.
Q Thank you. And I think you told Mr. Thornton this morning (sic) that exhibit 249 was something on the order of a preliminary draft of exhibit 250. Do I have that straight or not?
A I don't think so.
Q Okay.
A This is written as if it were to be submitted to a journal for publication. I do not recall the order that these were prepared in. It may be that 249 was prepared subsequent to 250. I don't know.

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Q Okay. And in 249, page 22, under - well, the columns to the right have a P value over the top of both of them it appears, is that correct?
A That's correct.
Q So, what do those numbers mean? Can you tell us starting with the chemiluminescence as an example, the top one?
A Yes. An alternative way of looking at the statistics here, that I would, with my non-professional understanding of statistics, say, is better than what was done in this table. But that is perhaps debatable.
MR. THORNTON: This table you pointed to was exhibit 250?
A This table is 250. So, in the table in 249, there were two things that were going on here. If you look at the figures that follow, you will see that there are two lines shown here, say on page 24 for chemiluminescence. One line, which has solid filled in circles, is the control group, the other line that has an open circle is the group exposed to current. So, there are two things that you can look at. You can look at whether this had changed over time and whether there's a treatment difference. So the treatment, in effect, is averaging all of these together and say, is the overall effect of treatment different?
The other thing is, an important question is, perhaps the overall effect isn't different, but you've got two lines that are not parallel, the two lines - the control group isn't changing and the treatment

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group is going down. And that's what that treatment by time interaction, the third column, is measuring. And for chemiluminescence, for example, we see a P value of .679, suggesting that there's no difference in the average chemiluminescence. But the treatment by time is whether those two lines are parallel to each other or converging with coming together. That's what that column will represent.
Q Would these P value calculations also be done following a natural logarithmic transformation?
A I believe that is correct.
Q Of the two columns on page 22 of exhibit 249, does the treatment column, those numbers, should they correspond to any of the columns in exhibit 250?
A Not directly, no.
Q Why not?
A The methodology was different in how they were assessed. This (indicating) was done using the technique known as analysis of co-variance.
MR. THORNTON: You've got to say which number you're talking about when you say "this."
A 249 was done using a technique called analysis of co-variance.
Q And 250 was not?
A 250 was done using a t-test, which is not directly comparable.
Q All right. Let's go back to 250 for a moment, Table 2. The paper itself describes three different groups of 8 cows of 4 treatments and 4 controls for each group, correct?

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A That's correct.
Q And they were done with different cows, different times, is that right?
A That's correct.
Q In your judgment, if you have a judgment on this subject, what would be appropriate statistical analysis of data collected in that manner be?
A Given that we were measuring things at multiple times, at the time this was done the method used in 249 would probably be considered the most appropriate.
Q Why?
A It takes into account the trends over time, which the method in 250 I don't believe does as complete the job.
Q Okay. Fair enough. And again, you don't claim to be a professional statistician, but you've had much contact with the subject?
A I have had contact with the subject. It is not my profession.
Q All right. Is there a concept in statistics that attempts to analyze multiple replications of the same experiment on different subjects?
A I'm not quite sure what the question is.
Q All right.
A So let's try to clarify that.
Q Fine. Is there such a thing in statistics as a two-way cross analysis?
A The terminology that you're using there, that I think you are referring to, is a crossover design, where - you could be referring to a couple of different things.
Q Okay.

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A This type of design, where you have the same experiment repeated three separate times, is called a blocked design, and there are statistical methods of dealing with that. I do not recall, it may be mentioned in here, if that was accounted for in the analysis that was done or not, but there are statistical ways of doing that.
The other thing that I wondered if you were referring to was taking one cow as a treatment, but then later making their control and switching the group around.
Q I wasn't referring to that.
A You were not. Okay. So, yes, I do not know if the blocking effect, the fact that it was done on three separate times was accounted for or not, but there are fairly standard ways of dealing with effect.
Q You would generally not do the statistical mathematics the same with a blocked design as you described, 8 cows per block for treatment or control, as you would in all 12 cows control and 12 cows treatment had the work done at the same time, is that a fair statement?
A The second situation that you mentioned is a little bit simpler analysis. The analysis was actually very similar, but you would normally account for the fact that it was done on three separate occasions by including a time called block in the statistical model.
Q The details of that would be more appropriate - -
A That would be done by a statistician, yes.
Q Do you know whether, in exhibit 250, Table 2, the P value for the detailed independent test, was that done simply aggregating all of the data together and treating it as

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12 controls and 12 treatments without accounting for the block design?
A I do not know.
Q Okay. Going on then to the second set of experiments involved in messenger RNA, I have a set of data that I would like to discuss with you. That will become 253.
THE REPORTER: 254.
Q 254. Excuse me. And I'll represent to you, Dr. Sheffield, that that data came out of the materials again that were provided by the University of Wisconsin subpoena seven years ago, and they were among your materials, I've got a photocopy of the disk they came off of, if it will help.
MR. THORNTON: I don't think that's correct. I think the last four lines were calculated by somebody else.
Q I don't believe so, but we can find out.
MR. THORNTON: At least the data he produced in response to a subpoena had the upper data out of the last four lines.
A Not everything that I provided to you is, not everything that was in the original subpoena, I have kept copies of. What I sent to you was a - what I currently had. There are things that are still - I do not know where they are, I assume they are still at the University of Wisconsin, Legal Services, but I don't physically possess them now. So, it is possible that this table was part of what I had, but it no longer is part of what I have in this form. Does that make sense?
MR. THORNTON: The only thing I'll tell you

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is that the top two blocks of numbers were in the material that you gave me, the last four lines, the control mean, the test mean, the fold T over C and the P value line were not.
Q You may be right about that. In any event, do you recall - and I'll stick to the block, for the moment, maybe forever.
MR. THORNTON: You can get to whatever you want.
Q I will.
MR. THORNTON: I think there's an issue about who did the last four lines.
Q You may be right. Regardless. Do you recall the second experiment where the messenger RNA techniques were used, which you described earlier, resulting in a set of data that looks like these top two blocks across the page - -
A Yes.
Q - - for the variants?
A Yes.
Q And this goes on and on for four pages of - -
A Right.
Q And those would be almost a hundred variables that you talked about?
A I think there's actually a little more than a hundred, but in that vicinity.
Q What is the - can you describe - well, let's talk about the interleukins in particular for a moment, find them here. I think they are on page 2.
A These are mostly in alphabetical order.
Q Right. And about halfway across, from left to right,

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page 2, we have IL1a and IL1b, is that correct?
A That's correct.
Q I think those are usually referred to as interleukin 1 alpha and interleukin 1 beta, is that correct?
A That's correct.
Q And this set of experiments distinguish between the two sub types with interleukin 1, correct?
A Correct.
Q What's the difference between the two? Can you describe what it is and the significance biologically, briefly?
A It's been a long time since I have looked into interleukins, but they are very similar. They are what we refer to, if I recall correctly, as molegus genes, that is, they originated as a gene duplication. So they are slightly different protein sequences. In terms of the biological activity, I believe they are very similar. That's why, in the bio-assay that we did, previously we could not detect the difference between - we were detecting total interleukin 1 and you can't detect the difference between alpha and beta forms.
Q Then, for example, in the interleukin 1a or alpha column, as an example, what do the numbers mean?
A These are best known as - how to explain this? Relative means, they are intensity of light multiplied by the area that that light covers. That's the best way of thinking it. They really don't have any standard mix of measures associated with it, like disintegrations per minute, or micrograms per milliliter.
Q Does that intensity of the light correspond to something about interleukin 1 alpha or beta?

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A Not directly.
Q How about indirectly?
A There are a number of factors that affect it. Obviously, the amount of interleukin 1 alpha, for example, messenger RNA affects it. So, within interleukin 1 alpha, you can make comparisons. So, if you see a larger number, you would interpret that as having more interleukin 1 alpha messenger RNA. What you can't do is go across genes and say that it means a bigger number for interleukin 1 alpha than interleukin 2 means that there's more interleukin 1 alpha than interleukin 2. You can't make that comparison.
Q So we can't get to, for example, picograms per milliliters?
A No.
Q But you can compare the quantity of interleukin 1 alpha to itself in two different times or two different groups of cows, fair to say?
A Fair enough.
Q And were these numbers then the basis for the analysis of changes in interleukin 1 alpha and interleukin 1 beta and the various other paramaters here?
A Yes.
Q And they were the basis upon which the statistical analysis was performed which are reflected in the draft paper, draft abstract that counsel discussed with you this morning (sic)?
A Yes.
Q I believe that was in 251.
I would like to then go through with you

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what these various variables are. I know we've got about a hundred of them, close to it. And maybe with respect to each one, if you can tell us why you chose to study it, if you can recall?

(Discussion held off the record).

Q Dr. Sheffield, I would like to take the variables on exhibit 254 and have you explain to us what each one is a little bit, and whether serum or vitro or something else, and a little bit about why you chose to study each one, if you can recall. I realize it's a long time ago, and there's a lot of variables here. You may not recall.
A I will do the best I can - -
Q Thank you.
A - - on this. First of all, some of these - many of these, our initial hope was to actually study far more than these. Technically, we were not able to do that. These were chosen, in part, because they are the ones that we had reliable ways of studying in cattle. That was an important thing, because the technology to do things in cattle often lagged behind what it is in medicine for many reasons. But, okay. Almost need a magnifying glass.
Q I actually brought one along, believe it or not, somewhere. I'll get it for you.
A ACK2 is a fairly general gene for acetate kinase. It has important roles in a lot of different cell metabolisms. So it's not something that would be restricted to the immune system. But adenylate cyclase is an enzyme. By

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the way, all of these are messenger RNA.
MR. THORNTON: You're going to have to spell some of these, doctor.
A Let me finish my thought here. All of these are messenger RNAs. So the messenger RNA is a cell. I've tried to indicate whether the protein is something that is going to be in the cell or outside of the cell. But adenylate cyclase, a-d-e-n-y-l-a-t-e, c-y-c-l-a-s-e. This is a cell protein that's very important in hormone C. It is found in many cells, probably most cells in the body, there may be some that don't have it. So you would find it in the immune system, but a lot of other places.
It's one of these signal pathways that some hormones used to give their signals across the membrane into a cell.
ATP synthase is a very general gene. It does exactly what the name suggests, it is responsible for producing ATP, so it's something all cells would have, and expressed at a fairly - maybe not completely constant level, but it's responsible for using ATP, which is the main energy source of cells.
CFos, F-o-s, is what is called a transcription factor. This is a protein that binds to the DNA in the nucleus of the cell to promote messenger RNA production of certain genes. It's widely distributed, and it is often seen elevated during stress events in the cell. But there are quite a few things that will elevate CFos.
Those same comments hold for the next column, CJun. J-u-n. CaATPase, the Ca refers to

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calcium. This is an enzyme that grades ATP and transports calcium across cell membranes. Cells often use calcium as a message. Normally, calcium in the cytosol cell is very, very low. And the calcium ATPase is involved in pumping calcium out of the cell to keep its concentration in the cytosol very low.

The next column stands for casein, c-a-s-e-i-n, kinase, k-i-n-a-s-e. There are two of those, and the same comments will apply to both of these columns.

At first glance, this is a bit of a misnomer. Casein kinase you might think of as the enzyme that transfers phosphate to casein in the mammary gland. And we do call that that enzyme casein kinase, but this is a different casein kinase. It's an old terminology. Kinase, by the way, is an enzyme that transfers a phosphate from ATP to a protein or to something. Doesn't have to be a protein, but in this case it is a protein.

Many years ago, these were named sometimes based on what they transferred phosphate to. This one, it was found that casein would receive the phosphate very easily, so it was called that, even though physiologically, it does far more than that. These are often involved in hormone signaling mechanisms inside the cell.

The next are a series of proteins. CD 14, 23, 8, 3. These are cell surface antigens found in different types of lymphocytes. They are involved in self cell recognition and interactions of the cell with their environment. These are frequently found in

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lymphocytes. Different lymphocytes would express different proteins.

Cdk1 is cyclin, c-y-c-l-i-n, dependent kinase. The cyclins are a group of proteins that regulate proliferation of cells. And the cyclin dependent kinase is a part of this family.

The next column is cleavage poly adenosine. A-d-e-n-o-s-i-n-e. Or cleavage poly A. Messenger RNAs in most messenger RNAs, carryoffs. After they're initially transcribed, that is, the gene is used to synthesize the messenger RNA. The messenger RNA has to undergo several processing steps. One of those processing steps is called poly adenolation.

A large number of about 200 avenine residues, a-v-e-n-i-n-e, is added in somatically at the end of the messenger RNA. It's true in most avenine messenger RNAs, but not quite all.

Q But maybe?

A But maybe, yes.

MR. THORNTON: You got a duce.

A The lower is not absolutely required to make a protein, but it does seem to have a role in stabilizing that RNA. Messenger RNAs not only need to go up, you need to be able to downgrade them to get rid of that is no longer needed. One of the first things that frequently happens is degrading the unneeded messenger RNA that poly avenine is bleeded off. That's what poly A does. This again is going to be a very widely distributed gene.

CREB1 and 2, we often pronounce those CREB. That stands for cyclic A&P response element. And in B1

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and B2, which is different forms of this. Earlier I mentioned adenylate cyclase. This produces a compound called cyclic A&P. Cyclic A&P is what's called a second messenger. One of its effects is to activate a series of enzymes culminating in various responses. One of those responses is to increase the expression of certain genes. Those genes are those that have what's called a cyclically impede response element in the DNA sequence. The CREB is something that, it is a protein that actually binds to the DNA to promote expression of those genes.

I hope that makes sense.

Q That's fine. Is there any particular reason you chose CREB 1 and 2 to start with?

A They are cyclically a major regulator of cell functions.

The next column is copper, zinc, super oxide. Dismutase. D-i-s-m-u-t-a-s-e. This one is extremely important in nonspecific types of immunity and in antioxidant responses. During metabolism, the body produces large numbers of oxygen radicals. These are very damaging, and we have a whole series of enzymes that detoxify these oxygen radicals, using one of the enzymes involved in this.

The next one, I do not recall exactly what that is.

Q Fair enough. Looks like the initials are F-A-S, I think.

A No, there's another one before that.

Q Oh, I'm sorry.

A And I'm not quite sure about that. FAS is another regulator of self-signaling.

Q What do the - -

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A I do not recall off the top of my head what it stands for. I know it stands for something, but I just don't recall what.

Q Fair enough.

A The next column is FASLigand. The FAS, you can think of like a hormone receptor, the ligand is what binds it. There are several things that could refer to in the next column.

Q And you're not sure of the - -

A I'm not entirely sure which that refers to.

Q Okay. Fair enough. The last two on the page?

A Glutathione, g-l-u-t-a-t-h-i-o-m-e, peroxidase, p-e-r-o-x-i-d-a-s-e. This is another of the enzymes involved in oxygen radical metabolism.

The final column stands for glucose transport IV. Glucose does not cross cell membranes very well. So, we have to have specific cell membrane proteins to carry it across. This is one of the more common of the glucose transporters that would be present in cells.

Q Let's stay on Page 1 for just a moment, Dr. Sheffield, because it is approaching 3:30. Let me ask you a couple more questions about this data, and we'll be done because of Mr. Thornton's schedule being concluded for today.

MR. THORNTON: Sorry about that.

Q Okay. But for each of these variables, there are a series of a block of 10 numbers below, and then a space, and then a block of another 10 numbers below, is that correct?

A That's correct.

Q And my understanding is, the top block of 10 represents the control, the 10 control animals in this study, and the second group represents the treatment animals?
A That's what it looks to me like, yes.
Q And the application of the electricity to the treatment animals is as described in your draft abstract, correct?
A That's correct.
Q And that would hold - that characterization of the data would hold true throughout all four?
A As far as I know, it would, yes.
Q And in this case, this study was not done in separate blocks, but all 10 and 10 were studied at the same time?
A That is correct.
Q So, it's the same set of 10 animals or same sets of 20 animals, if you will, throughout?
A Correct.
Q And in those circumstances, a simple two tail t-test would be one appropriate - -
A That would be a reasonable thing to do.
Q It would be a reasonable statistical methodology, you wouldn't have to worry about the blocking effect, is that correct?
A There is no blocking in that, so you wouldn't worry about it.
Q Fair enough. Unfortunately, why don't we go off the record. Let's go off the record for a moment.

(Discussion held off the record - 3:28 to 3:30).

MR. LAWRENCE: Doctor, while we were off

the record, it was agreed that Friday is good for your schedule, and we've agreed to continue this May 9 at 9:00 a.m.

MR. THORNTON: Sure.

MR. LAWRENCE: And I know counsel mentioned a subpoena earlier by mail. Is that sufficient for you, if I write you, send somebody else to serve you with a subpoena.

MR. THORNTON: I can give you one right now, if you like.

A I will be here May 9, 9:00 a.m.

MR. THORNTON: Okay.

MR. LAWRENCE: Very good. Thank you.

(3:31 o'clock a.m.)

* * * *

READING AND SIGNING CERTIFICATE

I, LEWIS G. SHEFFIELD, PhD, do hereby certify that I have read the foregoing transcript of my deposition, recorded by John T. Kirby, of 3-14-14, and believe the same to be true and correct, (or except as follows, noting the page and line number of the change or addition and the reason why):

WRITING IN TRANSCRIPT WILL NOT BE ACCEPTED

_____ _____
DATE SIGNATURE

STATE OF MINNESOTA)
) ss.
COUNTY OF DAKOTA)

Be it known that I took the deposition of LEWIS G. SHEFFIELD, PhD, Volume I, on the 14th day of March, 2014, at Madison, Wisconsin;

That I was then and there a notary public in and for the County of Dakota, State of Minnesota, and that by virtue thereof, I was duly authorized to administer an oath;

That the witness before testifying was by me first duly sworn to testify to the truth and nothing but the truth relative to said cause;

That the testimony of said witness was recorded in computerized Stenotype and thereafter transcribed by myself, and that the testimony is a true record of the testimony given by the witness to the best of my ability;

That I am not related to any of the parties hereto nor interested in the outcome of the matter;

That the reading and the signing has been executed as evidenced by the preceding page.

WITNESS MY HAND AND SEAL THIS 16TH DAY OF MARCH, 2014.

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