DEPARTMENT OF HEALTH AND HUMAN SERVICES

NATIONAL VACCINE PROGRAM OFFICE PRESENTS:

WORKSHOP ON ALUMINUM IN VACCINES

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I N D E X

I N D E X (Continued)

P R O C E E D I N G S

WELCOME AND INTRODUCTION

MARTIN MYERS

DR. MYERS: Good morning. I am Martin Myers and I am the acting director of the National Vaccine Program Office and we are sponsoring this meeting on aluminum in vaccines, along with our advisory committee, the National Vaccine Advisory Committee.

We are hoping that Dr. George Peter, who is the chair of the NVAC, will be here to chair the second session this morning but he called last night. There were no planes out of Boston or Providence yesterday evening so he may not make it.

Someone just told me that they like coming to these meetings that the NVPO sponsors because they tend to be on topics they do not know anything about and my answer to that is that is, of course, why we do these and, therefore, Marty Myers' education, as well as education for a lot of other of you.

Last summer we started a series of what we hope will be a series of symposia on the attitudes to vaccines. We talked about thimerosal last summer. We are talking about aluminum today and we plan to talk sequentially about each of the additives within vaccine.

Perhaps the most important thing that I took away from the last meeting was that those of us who

deal with vaccines have really very little applicable background with metals and with toxicological research. Of course, that is the reason that the meeting is occurring today in San Juan is because of the metals -- metal ions in biology and medicine meeting that occurred here earlier this week and an opportunity for a number of us to attend that meeting and I am delighted to see that we have a number of individuals here from that meeting who have come to join us.

Dr. Jose Centeno, who was the host of the metal ions meeting that was here earlier this week, is going to join us in this meeting and, indeed, he is going to lead a session tomorrow morning for us.

I have to say that it is a bit intimidating to have a meeting immediately following his magnificent affair that he put on earlier this week.

We are an eclectic diverse group. Dr. Vogel was asking me a moment or two ago about who all was here. We have vaccinologists. We have rheumatologists. We have metal ion specialists. We have people who are interested specifically in aluminum. Others who are interested specifically in adjuvants. We represent academia, government, more than one government, the WHO, industry and interested individuals.

One of the things I took away from the metal ions meeting earlier this week is that infectious disease people and vaccinologists are really used to coax (?) principles for establishing causality. Get an organism, put it in an animal, reproduce the disease and so on. But the dominant difficulty in hazard assessment based -- is that it is based upon whatever data is available and it may not be complete data. And the difficulty of establishing causality of risk is very difficult. In fact, one of the speakers earlier this week used the term "pervasive uncertainty," which is a term that I think describes issues relating to things such as mercury and aluminum and trying to assess the potential hazard and risk.

So our objectives for the next two days are to explore and consider the complexities of the use and need for adjuvants and vaccines; to consider the potential benefits and potential hazards of the use of salts, of aluminum of adjuvants; and then we will discuss tomorrow morning the newly described entity of macrophagic myofascitis.

Just a couple of important issues for everybody in the -- in attending a meeting like this. The bathrooms are right around the corner here. If you have not discovered the ocean and the ocean breezes, I invite you to do that. Our breaks -- by

the new regulations in government, we are able to have breaks were we are able to sponsor the coffee and light snacks but you are on your own for your meals.

When you ask questions or when you make a comment, if you would please identify yourself by both name and affiliation, it is not that the moderators may not know you, it is that we are transcribing the meeting and the transcriber will be -- would greatly appreciate knowing who each person is as they speak.

From a format perspective, we are set up as a series of plenary presentations. We have asked each of the speakers to leave a few moments at the end of their presentation to allow for questions. Those questions should be oriented specifically to the presentation by the presenter because each session will have a discussion period at the end where we will invite all of the speakers to come forward and we will have a discussion that involves everyone.

So with no further ado, we will begin with our first session this morning, which is the use of adjuvants in vaccines. Dr. Fred Vogel is going to be our moderator for that session. Dr. Vogel is the program platform leader at Aventis Pasteur, more

importantly, of course, as most of you know, he has been a leader in adjuvant research for some time.

So I will turn the microphone over to you, Fred.

SESSION I: USE OF ADJUVANTS IN VACCINES

MODERATOR: FRED VOGEL

DR. VOGEL: Thank you, Dr. Myers. I am very happy to be here and I would like to also extend my welcome to this Session I: The Use of Adjuvants in Vaccines.

We will start with Dr. Robert Hunter. Dr. Hunter comes to us from the University of Texas in Houston where he has been since 1997 and before that Emery University since 1980.

His interests now are the properties of immunogens that control the type and rate of immune responses and his current research is in immunopathogenesis and vaccines for TB. Dr. Hunter's presentation today will be an overview of adjuvants in vaccines, present and future.

Dr. Hunter?

OVERVIEW OF ADJUVANTS IN VACCINES (PRESENT & FUTURE)

ROBERT HUNTER

DR. HUNTER: Thank you very much. I need to find all the paraphernalia. I have a laser pointer and a forward button.

(Slide.)

I got interested in adjuvants in vaccines really in the first week of my research career as a sophomore medical student. A professor gave us the problem. He says, "Immunity to tuberculosis is related to cell mediated immunity. We can elicit cell mediated immunity with PPD, skin tests, and it appears to have all the antigens that one would need. But if you try to immunize with that to induce a cell mediated immunity, you cannot get it. And if you keep pushing hard enough, you, in fact, desensitize the animal so that they are incapable of making delayed sensitivity to the infection."

In fact, this was an often repeated experiment. It was first done by Robert Caulk (?) in trying to treat people with tuberculin for TB with disastrous results.

So the question there was the tuberculin protein in that mixture of things has the antigens that are necessary for the protection against tuberculosis. That can be argued for the sake of argument but by itself it cannot elicit a protective response.

It has something to do with the milieu of the organisms, the waxes, the lipids, the vital principles, whatever, that are essential to get the appropriate response against the antigen. So just

getting the antigen itself is not enough and that question fascinated me. We have been working on it for most of my career.

But to understand the adjuvants I think it is important to understand vaccines. To understand vaccines requires an understanding of infectious disease. To understand infectious disease, it helps to know something about the history of this.

(Slide.)

This is a diagram of the spread of the black death through Europe in the Middle Ages, which we all know was a terrible thing and decimated the planet.

Actually a man named William McNeill in the late '60s wrote this book Plagues and People, which he said, "This is part of a pattern which has gone with the development of civilization; that as people came together in large groups we acquire infections from animals, those spread rapidly through the new immune population." It then took a period of many generations to develop a natural immunity, which those would become childhood infections. And that this has really been a major controlling factor, the development of civilization.

(Slide.)

One of the examples: In the time Columbus discovered America there were more people per square mile in Central America than there were in

Europe because corn is a better food crop than rice. When the settlers arrived 100 years later 90 to 95 percent were dead and the major players of that were small pox and measles but malaria and yellow fever and all the rest of it were part of it.

When the Pilgrims arrived in Massachusetts in 1620, I believe, they found corn fields had been abandoned only three years later because of small pox among the Indians. So that is a very clear example of the effect of infections in a population that has not developed immunity to them.

(Slide.)

Closer to home, yellow fever in Memphis Tennessee in 1878: 45,000 people when the epidemic came; 25,000 fled; 18,000 caught the disease; 5,000 died. The City of Memphis lost its charter, went bankrupt, was managed and the old river city never recovered from this in the 1870's.

(Slide.)

A little closer to home: Philadelphia, 1918, influenza. Influenza is estimated to have killed 20 to 30 million people and some people think that is a gross under estimate in 1918, which is far more than the first World War. 730,000 Americans died with influenza that year and Philadelphia was the hardest hit city in the Western World.

(Slide.)

And we have pictures like this of the health workers.

(Slide.)

And the ones carrying people off with a disease to which they had little immunity.

(Slide.)

Well, when this book came out, people said, "Well, we have modern science. This will never affect us anymore. If something comes up we will find a way to deal with it."

Well, this is -- Barbara Coltrane (?) wrote for the Washington Post on 4/30 of this year that AIDS has now been designated by the Clinton Administration as a national security threat.

It is a first time a disease has ever been so designated and the reason for that is the dramatic declines in life expectancy are the strongest risk factor for revolutionary wars, ethnic wars, genocide, disruptive regimen, transitions in developing states, and that their figures are that 20 to 25 percent of the population of parts of Southern Africa, Southeast Asia and the former Soviet Union are likely to die from AIDS in the next 20 years.

There was another article in the Houston paper two weeks ago saying that nations in the Caribbean, very close to where we are, are second

only to Southern Africa in the incidence of a disease.

So I think we can say that the days of when mass societal problems with uncontrolled infectious disease are not behind us. This is something we need to think about very seriously.

(Slide.)

Switching over to adjuvants then. The conventional view of adjuvants is that the primary mechanisms are the formation of depots of antigen in tissue and stimulation of macrophages. Two, toxicity increases with potency.

If you want a better adjuvant you can expect to get some more toxicities with it. We kind of avoid it in a search for new vaccines. We find some way around this to find a magic bullet of some way or other. And that scientifically it has not been a terribly interesting idea. We are talking about depots and mineral salts and genomics and specificity and the various things.

(Slide.)

I would like to show you that this is not the best way to look at it. This is a picture of an adjuvant. This is an oil and water emulsion that is about 80 percent water and 20 percent squalene that is floating on the surface of water. It is a

very stable water and oil emulsion that contains egg albumen.

(Slide.)

This is an older picture of Freund's (?) incomplete adjuvant, which is a similar emulsion but made with mineral oil. This is showing what adjuvants do.

This is in guinea pigs injected with an injection of egg albumen to make a little antibody titer which rapidly disappears, injected in that adjuvant. They make a titer which is fully three logs higher and persists out here for 350 days plus. So something has gone from a very weak and very transient response to very strong and very prolonged response.

Adjuvants can have very major effects on the production of immune responses.

(Slide.)

We then take that same kind of preparation and boost the animals at intervals with soluble egg albumin. If you boost very early it does not do very much.

You have to wait a while but then each time you boost, they are showing boosting at different time periods, starting earliest to latest, there is a short transient rise but it comes right back down about to the level that you were before until you get

to the latest one where it looks like it is maybe staying up longer.

So the soluble antigen -- even though you have -- the adjuvant has produced this very prolonged response, additional injections give you a transient boost but will come right back down to the level that was there before.

(Slide.)

So we look at what are the mechanisms of adjuvants and aluminum adjuvants are clearly a major portion of -- and act similarly in many ways, different in others. They form a repository of depot of antigen in tissue produced in prolonged exposure to antigen.

There is a pretty good correlation that if an antigen does not persist in tissue, it will not make prolonged response. There may be a memory response eventually but the response itself will be limited.

It proves particulate antigens that facilitate targeting to antigen presenting cells. It is very clear that simply aggregating with various things or making a particulate will produce a very different response than the soluble protein.

And they activate complement, other mediators to stimulate macrophages, and induce retention and activation of lymphocytes in lymphoid

cells. They stimulate components of the immune system and these are sort of the major mechanisms that have been published over the years.

(Slide.)

This is a slide showing the effect of formulation on adjuvant activity where there is exactly the same components. This is an adjuvant where the antigen is given in saline or the antigen is given in an oil and water emulsion.

Tiny oil droplets with the antigen inside of that and we see that the antigen in the oil to make it a particulate gets a response after the first injection. Where the other one makes a response really only after the booster injection.

This is something one has to keep in mind when reading the literature, is how many injections are people talking about. They say mine makes just as good CTL's or antibody responses as another one but it took four, five or eight injections and then how long did it last. So these are important paramters, particularly for vaccines, that we cannot give multiple injections.

One of the priorities the World Health Organization published a few years ago was to make multiple shot vaccines into single shot vaccines so that they could reach people more readily with them.

(Slide.)

Now the retention of antigen can involve the body's own mechanisms in addition to mechanical things we do with adjuvants. This is an autoradiography with a lymph node of a guinea pig that has an antigen bovine serum albumen labeled with radioiodine in germinal centers.

The germinal centers have specialized cells whose function it is to take up and retain antigens and there is a reasonable correlation that the duration of an antibody response roughly correlates with the duration of antigen in germinal centers if there is not some external depot some place.

The ones that go in here for a short time, antigens like salmonella flagella, the animal will make antibody response his entire life from a single injection. It is retained for a very long time. Other ones are not. And the adjuvants will upon multiple injections induce responses that promote this localization.

So when one gets prolonged responses after multiple injections it is likely that they are stimulating the body's own mechanisms to retain antigen in sites for antibody in germinal centers.

(Slide.)

What are the effect of -- those are animal studies. What are the effect of such studies on humans? It is pretty hard to find controlled studies

in the literature on adjuvant versus nonadjuvant with human vaccines.

This is one that was published in the -- reprinted in the 1960's from a study in the 1940's comparing alum toxoid, one, two or three doses. Alum precipitated with one or two doses.

The bottom line is that with one dose alone only four percent -- eight percent respond at four months and four percent at three years. When you get up to two doses, up to 96 and 86 percent. So it makes a very large difference in the human studies on the proportion of children who have antibody responses and the duration.

(Slide.)

The uses of aluminum adjuvants are they are very good for enhancing primary responses to protein antigens, diphtheria toxoid, pertussis and polio. Pertussis, they are not -- that I have seen -- as necessary as adjuvants but there is papers they reduce the toxicity of pertussis.

They tend to stimulate Th2 lymphocytes, which is IgG1 and IgE antibody, which may be protective against some things but clearly not against others. They are not good generally for inducing cell mediated immunity, the Th1 lymphocytes. Things like influenza and typhoid fever. And they are seldom found to be good for peptide antigens. So

they can be very good for some things but have a limited range of applicability.

(Slide.)

If you look now at what are the properties of antibodies, what are the things that adjuvants and the antigens together can influence, and there is really four things.

The specificity, what it will bind with; quantity; avidity, how tightly it binds; isotype, which is really what kind of heavy chain you have and what subsequent reactions, immune or inflammatory, can be induced by that antibody. The evidence is that all these things can be influenced through relatively selective ways by adjuvants.

(Slide.)

Most of our work was on these compounds: Copolymer adjuvants, which are simple polymers of polyoxy ethylene, polyoxy propylene, which is the same thing in the methyl group, and polyoxy ethylene by varying the lengths of these chains.

One can produce a broad spectrum of polymers or nonionics or factones because this was hydrophile, hydrophone, hydrophile to cover virtually the entire functional range of nonionics or factones that have been widely used in food and drugs and cosmetics.

(Slide.)

This diagram, the black ones show the hydrophone, the white the hydrophile drawn sort of scale to show you the length of them. So this is a series that were tested. There are some interesting ones here.

L121 and 122, which are absolutely identical hydrophones. A little tiny bit more sticking out here of the hydrophilic end. The L180 series, the same thing, the same hydrophone, a little bit longer, hydrophile on each end, and one is much longer than either one.

(Slide.)

These were made up in many different experiments but this is a particularly informative representative one. I mean oil in water, that is the squalene in water, about two percent oil, with TNP egg albumen, measuring antibody titers at 28 days after a single injection.

And we find that the titers go from almost nothing, 200 to L122 to 300 and some thousand. This one -- adding a small amount of hydrophile from 10 to 20 percent, your titers go from 11,000 to 200, and in general the titers get larger with larger hydrophones and better with the smaller hydrophile.

(Slide.)

If we look at the isotype of antibody to these and we find there is a relationship between the

ratio of IgG1 and 2B mouse isotypes to the molecule weight of the hydrophone when the proportion of hydrophile is constant. And since IgG2b is the one that is more likely to be protective in many instances than 1, this could be an important kind of consideration.

(Slide.)

A key experiment in looking at the mechanisms of this was how do -- these polymers are essentially adhesive agents. Surface activity and physical chemistry is defined in terms of adhesion and surface tensions with each other.

The experiment here is to take copolymers, put them on the surface of plastic in concentrations from .001 to 100 micrograms per ml, and then measure the amount that is there by two methods. One is the comasy (?) blue, just measure the total amount, and then we add the protein to this and see how much protein sticks.

So we are measuring how much protein will stick to defined layers of the copolymer, the adjuvant.

As the amount increases, the protein goes down a little bit, 20 percent roughly, when you get to about between .1 and one microgram per centimeter squared where the amount of -- the red line is the

amount of -- using an ELISA for the ability of that protein that is bound to bound antibody.

What we see is the amount of protein itself is going down as the polymer -- and right about here is where you get a single monolayer. So when you get above a monolayer the total amount of protein goes down. Its ability to bind antibody goes up. So what we are doing is binding antibody in a way that is binding sites are more accessible than they would be otherwise.

(Slide.)

This is a diagram of these molecules drawn to scale with a hydrophile and hydrophone at a water interface. The ones that are effective adjuvants are underlined. Their characteristics are the hydrophobic chain is long enough to make a complete loop and they have a small hydrophile and these will bind proteins at this oil-water interface.

If they fail to bind proteins, either because their hydrophilic part is too large, makes too much of a hydrophilic surface, or because they are unable to fold and end up still needing a hydropic surface, then they are not effective adjuvants, the ones that are able to bind this combination of hydrophilic and hydrophobic interactions.

(Slide.)

And our model for them is this where they have a hydrophobic surface and the surface can be the polymer itself because these are things that are right at the border of solubility and so it can be a particle of the polymer itself or an oil droplet or any other hydrophobic surface. They will fold to put the hydrophilic on the surface.

They will bind antigens. Bind antigen is a way that retains the native conformation much better than binding to a plastic hydrophobic surface. They will also bind complement and activate that via the alternate pathway.

And this is -- complement binding is important in getting antigen to localize in germinal centers and in activating numerous parts of the immune reactions. It almost certainly binds other contact activated factors.

So here is presenting antigen in a -- instead of individual molecules coming to the immune system individually, you have a condensed two dimensional matrix of antigens that retain native confirmation in a milieu of activated host mediators. We believe that that is the mechanism by which these works.

(Slide.)

Then one varies that and gets variation within that adhesive mixture that can drive responses in different ways.

This is a scanning electron micrograph of one of these particles. This is about a micron diameter squalene droplet. In this protein you see this fuzzy stuff is stuck to the surface. If it will bind to the surface like that it would be a good adjuvant. If it would not bind it basically would not be in the kind of models we were using.

(Slide.)

Now in addition to that, which is a binding conformational and some host activation, one could add other things and the things we have studied most was detoxified lipopolysaccharide LPS. From these we produce a synergy.

This is toxified Ra-LPS by itself, the polymer by itself, the two together, get a striking synergy between them, both were increased titers, some change in isotype and also get into a deeper change in the specificity of the antibody to be made by these various combinations.

(Slide.)

This you cannot read from back there but it is a very informative experiment and it is sort of patterns of what is important. We are working on

malaria vaccines with the group at the CDC in a mouse peolei (?) model.

We took whole killed peolei organisms or a membrane fraction of them, and injected the mice with 16 different adjuvants, which is shown across here, boosted them once, challenged them, and this is measuring the parasitemia and the ones that are above this line are not protected. Here is the controls over here. The ones below the line are protected so these are basically protected pretty well over here. The ones in this end are not.

We had lots of theories about what we were going to do with these different adjuvants but the only thing that held up really was the adjuvant vehicle. These were water in oil vehicles by themselves or with LPS and there is Freund's complete adjuvant in here some place. They did not protect even though they made very high titers. The ones that -- all the oil in water or no oil, which is Sabin and Pertussis, the polymer alone and polymer plus LPS, were protecting rather well.

So it is the adjuvant vehicle that is determining that we are getting protection.

(Slide.)

Look a little farther at this and this -- also the important part of this is the pattern and the colors. This is measuring antibody of the IgG1,

which is white; 2a, red; 2b and Ig3 are the other colors, and this is measuring by two methods, by immunofluorescence, which measures antigens on the surface of viable or intact parasites or ELISA, which is where the parasites are ground up and stuck to a plate, and you get different responses, and the critical difference is in this area here there is nothing but white except for the one here which is IgG1 antibody.

These animals were not protected. The correlate with protection on these is the red bar, IgG2a antibody measured by immuno-florescence, which are epitopes on the surface.

So what we see here is that given them in Freund's complete adjuvants or other water in oil emulsions they are getting very high titers of antibody and by ELISA higher than the ones that are protected but they are getting a different isotype for the particular epitopes on the surface and, therefore, are not protected.

So this is a good instance where we know we have a protective antigen that protects very well but unless you get it in the right formulation, the right -- unless you induce Ig2a antibodies specific for conformational epitopes on the surface you get no protection. If you do get an antibody you get very good protection that is quite long lasting.

(Slide.)

This shows this a little bit more. This is saline formulation of the antigens with -- by itself and with LPS, water in oil, measuring antibody ELISA and IFA. This one here you get very high titers by ELISA and very low by IFA. These are not protected. The ones over here are protected.

(Slide.)

What is critical for protection on this one turns out to be the response that the animals make upon challenge, not what they have beforehand, and this you get by just reducing the dose of the antigen. We have almost no detectable titers before they are challenged but they make an Ig2a response of the appropriate type after challenge and they are protected.

(Slide.)

How does this work? Part of it we think we know. Part we know we do not know. One of it is the antigen is internal to an emulsion. Then the evidence is that one tends to get antibody against internal epitopes. It works very good with peptides and the -- so we think that this antigen is presented to cells in the immune system once it has been through macrophages and has been degraded so it sees the parts of what is on the inside.

If -- whereas in the oil in water emulsions or otherwise where the antigen is presented on the surface it then maintains its confirmation so that it is presented to the cells or the immune cells in this native confirmation.

Now in T cells things have to be broken down and presented as peptides. B cells, the antibody would be completely the opposite. This can be specific for sequence segments but some of the most important antibodies are dependent upon the confirmation and the confirmation may be two unrelated molecules happen to be stuck together but they just physically come apart or if you somehow denature the protein you lose the protective antibody. And the ability of vaccines to maintain that confirmation in this malaria model at least is the critical component of protection.

DR. VOGEL: About five minutes, Bob.

DR. HUNTER: Pardon?

DR. VOGEL: Five minutes.

DR. HUNTER: Five minutes. Thank you.

(Slide.)

So what are the mechanisms of these adjuvants that we know now? They are hydrophobic adhesive agents. They vector antigens to appropriate environmental areas. They recruit and activate antigen presenting cells. They bind complement and

other host mediators. They deliver B epitopes preserved and altered or with preserved or altered structure.

They can deliver epitopes either to Class I or Class II pathways depending on how it is done, sometimes both. They facilitate antigen directly in tissue in germinal centers or by depot formation.

(Slide.)

This is a picture of a titanium dioxide marker. Particles just under the dome of a pyres patch of a mouse illustrates that one can use the adjuvants to vector antigens to particular areas of tissue and this happens to be one in mucosal immune studies.

(Slide.)

The parameters that we now know are influenced by adjuvants: Antibody, specificity titer duration, memory, class, isotype avidity, which all the parameters of antibody can be influenced by adjuvants. Cell mediated immunity, the generation of CD4 or CD8 cells, generation of mucosal immunity.

Even the incidence of genetic nonresponders. Some genetic nonresponders is maybe due to specific epitopes but other ones are things that can be overcome by adjuvants and there are clear examples of this in the literature.

(Slide.)

This is a study published -- this is my last slide -- about three years ago by Science magazine looking for the most urgently needed vaccines in the world, HIV, malaria, tuberculosis.

I have here a copy of the Scientist newspaper that came out two weeks ago and the headline here is "New era in vaccine development." The first paragraph starts out, the first sentence says, "When all else fails, try something new."

What it is saying in here now are the new genomics. We are going to have a tremendous boost in development of vaccines because we can make more antigens and that is true. We can make vastly more antigen before either proteins or DNA but to me this is a story I have heard before. The first time we can make peptide, the first time we can make recombinants, the first time we can put things in viruses.

What has happened is people can make it but until we come to grips with what is the appropriate immune response and how do we get that antigen to induce the appropriate immune response, I do not think we are going to get -- unless we get very lucky -- to the real potential that we have in vaccines.

Because as we saw in that malaria model, and there are other ones, there are places where we know we have the right antigen. But unless you get the

right response to it at the right time at the right duration, you do not get protection and the antigen itself is not sufficient to do that.

Thank you.

DR. VOGEL: Thank you very much, Dr. Hunter.

(Applause.)

DR. VOGEL: This paper is open for discussion. Okay. If there are -- Carl? Carl, please identify yourself.

DR. ALVING: Carl Alving, Walter Reed Army Institute of Research in actually Silver Spring now, Silver Spring, Maryland.

One of the major elements of adjuvants that has actually had a tremendous influence and, in fact, may be one of the motivating factors for this meeting is the question of toxicity. When you were looking at all your nonionic glycopolymers, did you find any relationship between the structure of the polymer or other adjuvants and the toxic effects if there were any?

DR. HUNTER: Some of the polymers are quite toxic and those are not the ones that are best adjuvants. It seems to me that there are fundamental issues here that frequently get confused. We have a long way to go on the basic science of how do we direct immune responses. And for this field, which I think is critical for -- the infections we are

looking at now, AIDS, malaria and TB, are bugs that are very capable of invading immune responses.

We need to know a lot more on the basic science. To get that hung up because we cannot yet handle all the toxicity issues, seems to me, is a fundamental error. On the other hand, I think that most of the toxicities are beyond my area of expertise quite obviously.

But in the case of this malaria vaccine, the most effective formulations were the least toxic. Freund's adjuvant and LPS and all those things made it worse. It was the one that -- the simplest one with the power by itself or even the antigen by itself given multiple shots will protect better than if you had these adjuvants in it.

And the total height of response was not nearly as critical as getting memory for the appropriate response. And that could take a very low dose of very nontoxic materials in that particular model.

DR. VOGEL: Are there other questions?

If not, we will move on to the next speaker. Our next speaker is Dr. Norman Baylor, acting deputy director of the Office of Vaccine Research and Review and associate director for Regulatory Policy at the Center for Biological Evaluation of Research at FDA.

Dr. Baylor's talk today will be "Aluminum salts in vaccines: A U.S. perspective."

ALUMINUM SALTS IN VACCINES - U.S. PERSPECTIVE

NORMAN BAYLOR

DR. BAYLOR: Good morning.

(Slide.)

What I am going to try to do in my talk is focus on aluminum and sort of give you a historical perspective of how we got where we are and a couple of my slides will be redundant to Dr. Hunter's.

(Slide.)

The first thing I want to do is sort of differentiate the different types of aluminum adjuvants. I mean, you hear often people will say that a vaccine is -- the adjunct for a vaccine is alum. Well, alum is not the only aluminum adjuvant.

And, in fact, one of the aluminum adjuvants is aluminum hydroxide and aluminum hydroxide is a crystalline. It is an aluminum oxyhydroxide and it has an isoelectric point of 11. It is positively charged at physiological pH and depending on the antigen that you are using with the adjuvant it will dictate which adjuvant you will use depending on the charge of the antigen.

(Slide.)

And then we have aluminum phosphate. This is eumorphic. It is aluminum hydroxy phosphate. It

has a PI of about 5-7. It is related to the phosphate content and the phosphate aluminum ratio is in the range of .3 to .9 and this particular adjuvant is negatively charged.

(Slide.)

And then there is alum. And alum is actually potassium aluminum sulfate and in alum precipitated vaccines the adjuvant is an aluminum hydroxide that contains some sulfate anions as well as anions that are used in the buffer, often phosphate, and this isoelectric point depends on the precipitation process and it is usually in the range of 6-7 and the phosphate aluminum ratio is usually in the range of .3 to .6. This adjuvant is, therefore, negatively charged at physiological pH.

(Slide.)

Now aluminum phosphate, as far as the biodegradability of the aluminum adjuvant, aluminum phosphate is more readily or more rapidly absorbed to interstitial fluid or citrate buffer in aluminum hydroxide. On the other hand, it has been reported by Gupta, et al,. that aluminum is detectable at the injection site in mice and guinea pigs for as long as a year.

(Slide.)

As far as the adverse reactions that have been reported with aluminum, they are generally local

reactions, mal reactions, sterile abscesses, erythema, subcutaneous nodules, granulomatous inflammation, a contact type of sensitivity, and aluminum containing adjuvants may increase the levels of antigen specific and total IgE antibodies.

(Slide.)

Now I want to switch gears a little bit and go over some of the historical data to demonstrate the effectiveness or lack of effectiveness of aluminum salts as adjuvants.

And Dr. Hunter showed this slide here and basically what we are demonstrating is that we are comparing the fluid -- a diphtheria toxin, fluid versus alum precipitate, and we notice that with the -- you do see -- if you just look at the fluid versus the alum precipitate after one dose, here four months after the first injection, you do see a higher percentage of children responding and showing detectable antitoxin fluid. At first dose, eight percent; here after first dose, 56 percent.

(Slide.)

In another study from the Lancet in 1952 looking at diphtheria toxoid fluid versus alum precipitate, these children were either six to ten days, older than seven months, or greater than or equal to six weeks.

And if you look at the injections given, plain toxoid given three injections at 25 LF versus plain toxoid -- well, I am sorry. Looking at the adjuvant precipitate, two doses, 25 LF, and you see the number of infants with detectable antitoxin. Nine out of 15 with the plain; 23 out of 23 with the adjuvant precipitate. And then greater than seven months six out of six number of infants detectable antitoxin with plain, 43 out of 43 with the alum.

And so you do see an increase -- you do see somewhat of an increase in the number of infants with detectable antitoxin with the alum precipitate.

(Slide.)

And then if you look in another study, DPT trial of Barr, Glenney and Butler in '55, looking at the geometric mean antitoxin in the aluminum hydroxide vaccine, you had 61 children at one, six and fourteen weeks versus plain vaccine at six months, twelve months, three months post booster. You really do not see any effect here at all.

And then tetanus, not really significant differences. So here is an example where with the aluminum adjuvant no effect -- no great difference is seen between plain versus the aluminum hydroxide adjuvant.

(Slide.)

And then another trial in '56 looking at DPT one month after the last of three primary half well injections. Plain versus aluminum phosphate at two different concentrations. And looking at the mean antitoxin titers, you see somewhat of an increase when you go up to five micrograms of D using the aluminum phosphate, 1.024 versus the .28, and then for tetanus similar results.

(Slide.)

And then for pertussis vaccine, plain versus alum precipitate, you -- absorbed, I am sorry -- cases of pertussis inoculated versus control, plain versus absorbed. No real significant differences in the plain versus absorbed with the pertussis.

(Slide.)

Now there were studies -- I quote these other two studies from the Canadian Journal of Public Health. Fraser and Halpern in 1935 demonstrated that one dose of alum toxoid was not as effective as three doses of plain toxoid.

Another study in 1936 by Schuhardt and Cook also demonstrated that one dose of alum toxoid was found to be inferior to two doses of plain so demonstrating that it is not necessarily across the board that if you have the aluminum adjuvant that you are going to increase the response when you are comparing it to the plain.

(Slide.)

Now, of course, the different results may be accounted for by the differences in antigen at dose. They also may be accounted for by the stability of the aluminum adjuvant complexes and, of course, the levels of circulating maternal antitoxin come into play depending on how early these children were immunized.

In one of the previous slides you saw that one group of children were immunized at six weeks.

(Slide.)

There was a consensus of early reports if you just take all the data from the 30's, 40's, 50's and early 60's. Basically the consensus was that aluminum precipitated toxoid dose for dose is distinctly more effective than plain toxoid but that is for the primary immunization of children. For the secondary or booster immunization there is little difference between plain and alum toxoid.

(Slide.)

It is interesting that there was concern about aluminum even in the early days debating the usefulness and whether there was some -- whether there was concern about the hazards of using aluminum in vaccines.

And in a 1957 British Ministry of Health, the recommendation was to use aluminum-free vaccines.

However, in 1964, the American Academy of Pediatrics Committee on the Control of Infectious Disease advised the use of alum precipitated DPT or absorbed with aluminum hydroxide. Whereas, in Canada, for decades they had used many vaccines free of aluminum.

(Slide.)

Now in the United States in the Code of Federal Regulations under 610.15, our constituent materials, including preservatives and adjuvants, the amount of aluminum in the recommended individual dose of a biology product shall not exceed .85 milligrams of elemental aluminum if determined by assay. And this is equivalent to about 15 milligrams of potassium aluminum sulfate. This is alum per dose of toxoid and so this is a requirement as per the regulation, the FDA regulations in the U.S.

(Slide.)

Now this can -- this amount, there -- of course, with the regulations there is always an escape clause and this can increase if you can demonstrate that it is needed, number one, that you need a higher level and that you can demonstrate that it is safe.

(Slide.)

Now I will go over some aluminum containing vaccines and some of these slides are going to be

very busy so I will hone you into where to focus. Okay. I warned you.

(Slide.)

This is the aluminum content of licensed vaccines and what we have done here is just put vaccine, trade name, manufacturer. The important thing here is to look at the aluminum per dose and the total aluminum for the series. And for the acellular pertussis vaccines the aluminum per dose ranges from as small an amount as less than 170 micrograms per dose to upwards of over 500 micrograms per dose.

And then if you look at -- focus on the total aluminum for the series, and this series includes five doses, you are talking about 3.1 micrograms. Let me just start here: .9 micrograms up to 3.1 micrograms for the whole series with the -- for the five doses with acellular pertussis.

Another example might be the hepatitis B ranging anywhere -- between 225 to 250 micrograms of alum aluminum per dose and then for the total series approximately between .68 to .75 milligrams for the total series.

You will also notice that there are many vaccines without aluminum -- the inactivated polio does not have aluminum, OPV, the measles, mumps and

rubella, the varicella vaccine and the rhodavirus vaccine.

(Slide.)

Now if you look at the -- break this out by age, looking at a child at age of one, the vaccine -- receiving acellular pertussis vaccine, Hib conjugate vaccine and hepatitis, and here are the number of doses in those series. The aluminum per series in milligrams. A minimum of -- for the acellular -- .51 mgs. A maximum of 1.88. And this just depends on which vaccine you receive. The Hib conjugate can be anywhere from a minimum of zero to an exposure of .45 mgs and then the total aluminum from 1.2 to a maximum of 3.1 if you take the whole series of receiving acellular pertussis, Hib and hepatitis B.

And then a child at age five receiving acellular pertussis, the complete series of five doses, and Hib conjugate vaccine, a complete series of three doses. A minimum exposure of .85 mgs to a maximum of 3.13.

Again total aluminum, minimum of 1.5 and this is for the complete series of both -- obtaining both vaccines at the age of five, 1.5 to a maximum exposure of 4.6. And then at 60 there are -- that -- this would vary also. The total aluminum from 10.3 to 18.7.

And the adult vaccines such as Td, Hepatitis A, Lyme, Anthrax and Rabies. These vaccines all -- except rabies -- well, even including rabies -- a minimum of zero to 1.6.

But again, I mean, a six year old individual is not going to receive all of these vaccines and may not receive any of these vaccines so this will vary.

(Slide.)

I can skip this slide.

(Slide.)

And again here is a demonstration that some of the other adult vaccines do not -- they are not absorbed to aluminum such as typhoid, plague, cholera, small pox, what have you.

(Slide.)

So, in summary, looking at the historical data, there have been few clinical trials in which a given a batch of vaccine with or without adjuvant has been tested in a comparable population so that just has not been done.

Plain toxoids and polio vaccine absorption onto aluminum phosphate or alum precipitation usually gives superior antigenic activity especially in the primary series. Immunization against tetanus, the aluminum phosphate toxoid appears to be better than the fluid toxoid. However, aluminum adjuvants do not

improve the protective activity of pertussis vaccines.

With whole cell pertussis -- DPT, different trials using plain versus absorbed vaccines, as you saw in the data I presented, gave different results and the aluminum containing vaccines appear to give more local reactions than plain vaccines and that is especially true in children.

Now, I guess, bringing this all together, one might argue that why do we have aluminum in the vaccines after the primary series and there are a lot of -- you can think of the practicality of making a number of formulations, especially for the manufacturers, where you would make a formulation for the primary -- speaking specifically of the pediatric vaccines -- making a vaccine formulation for the primary series and then having a separate formulation without aluminum for the booster doses and for the adult vaccines.

I just throw that out there. I mean, that is a difficult challenge. Also, to -- there have been a -- there are a number -- let me back up. As we know, as all of you know, the only adjuvant used and licensed products, vaccines, in the U.S. is the aluminum salts.

There are a number of adjuvants that are under -- that are being used in study under

investigational or the IND process but none of those have come to fruition yet. And thinking about going back to -- if one of those new adjuvants pans out, the logistics of going back and applying to -- are you trying to use some of those to the older vaccines would also be very difficult because we are talking about basically a new product and it would require new clinical trials so it would be years coming on line.

So I will stop there and take some questions.

(Applause.)

DR. VOGEL: Thank you very much, Dr. Baylor. Lizzie?

DR. LEININGER: Hi, Lizzie Leininger, SmithKline Beecham. Norman, when you talk about pertussis not requiring adjuvant or here alum, that is true for whole cell pertussis. Can you comment on acellular pertussis antigens? Is that true also?

DR. BAYLOR: When you say require -- I hope I did not say -- I did not use that term "required." If I did I --

DR. LEININGER: We are not --

DR. BAYLOR: -- I retract that statement. Not required. But I do not think it is -- I mean, in the older studies for the whole cell pertussis it did not appear as though you needed to have an adjuvant.

That is probably not true for the acellular pertussis because you do not have -- it is a purified preparation and so you do not have the contribution of the whole cell to provide some of that adjuvant effect.

DR. LEININGER: So taking your challenge one step further then boosters with acellular pertussis may need aluminum salts, adjuvants, in those booster vaccines?

DR. BAYLOR: But I would say the operative word is "may" because remember we do not have pertussis by itself so you are going to get that adjuvant effect with the other two antigens, the diphtheria and the tetanus.

DR. VOGEL: Dr. Clements?

DR. CLEMENTS: Thank you. John Clements, WHO. You mentioned in 1954 that the United Kingdom backed away from aluminum in adjuvants. Could you give us the background to that because that was obviously strange to what the U.S. did subsequently?

DR. BAYLOR: That was in 1957 and that was -- I could provide you later with a reference on that but I just cannot remember it off the top of my head. But I thought that was interesting at that time that -- you know, I really do not know where we were. I really did not find out where the U.S. was in the '50s as far as a recommendation that the products are

-- would be advised to use a product with aluminum precipitate.

DR. HALSEY: (Not at microphone.) (Inaudible).

DR. VOGEL: Microphone, please.

DR. BAYLOR: I cannot hear you.

DR. HALSEY: Neal Halsey from Johns Hopkins University.

In your list of adverse events that you attributed to the aluminum adjuvants, you included a couple of things that I guess I was not aware were shown to be causally associated, and that is the abscesses and the hypersensitivity. Could you elaborate on the evidence for the causal association with the abscesses? I know there is an association but is it due to the adjuvant? I mean, there is an association with several of those vaccines. DTP being the most obvious, the whole cell product.

DR. BAYLOR: That was in the old literature. There was a study in New Guinea where they looked at aluminum, some of the aluminum salts, and they noticed -- they observed the sterile abscess.

DR. HALSEY: Well, the sterile abscess has been associated with DTP in a variety of products due to several different reasons but I -- but it is convincing evidence that it is the aluminum that was responsible. That is what I am trying to get at.

DR. BAYLOR: I cannot say that. I mean, no. And I am not trying -- I am -- I retract that statement also.

DR. HALSEY: And the hypersensitivity is the same way. Is there hypersensitivity other than the local inflammatory response?

DR. BAYLOR: And that is all I am referring to there. It is just the local. But as far as a causation specifically to the aluminum, no, that paper did not suggest that.

DR. GELLIN: Bruce Gellin.

Norman, this is for clarification and not retraction purposes.

DR. BAYLOR: Okay. I will see.

(Laughter.)

DR. GELLIN: But your final comments about new adjuvants and how they are obviously going to require, you know, a whole set of new clinical trials, would it not be the same if one were to take aluminum out of existing vaccines? Wouldn't they be seen as new products without such a component?

DR. BAYLOR: Yes.

DR. GELLIN: And require similar data for clinical trials?

DR. BAYLOR: Similar. Maybe not -- of course, you know, it is all case by case, but definitely starting with the new adjuvant. We

started with a new product and so you are going to have to start from scratch.

DR. GHERARDI: Romain Gherardi from INSERM, France. In France, only three types of vaccines contain aluminum. All hepatitis B vaccines, all hepatitis A virus vaccines and most tetanus toxoid vaccines. I understood that in the U.S. maybe more than these three types of vaccines contain aluminum. Untrue or not?

DR. BAYLOR: I would have to go back to my slide and count them. Some of the acellular pertussis vaccines are hepatitis B vaccine, some of the Hibs, also some of the adult vaccines. Lyme vaccine contains alum.

DR. GHERARDI: So many more than in France.

DR. BAYLOR: Yes. See here is the list here. Hep-A, Lyme, Anthrax, some of the rabies vaccines, and then we have our DT absorbed, Hib vaccine. So, yes, there are more than in France.

DR. GHERARDI: I have another question.

In France it is very difficult to know what is the adjuvant which is used in the vaccines because usually it says only aluminum hydroxide. And it is not clear to me whether it means that it is alum or it is really aluminum hydroxide. Both are used or when aluminum hydroxide is said to be in it, this means that it is alum --

DR. BAYLOR: Well, not necessarily. I mean, we have the same problem here. I mean, if you look in the older package inserts for the products, sometimes they just say "alum."

And so we went back and I think we got them all. There may be a few still out there. And specifically asked the manufacturers to put if it is aluminum hydroxide, if it is aluminum phosphate, if it is alum, specifically state that because there are differences as I have demonstrated here.

And, also, something I did not mention about the combination vaccines, some manufacturers have demonstrated that if you are trying to combine two vaccines and you have one in aluminum hydroxide and one in aluminum phosphate, you are going to have problems, manufacturing problems.

DR. GERBER: Michael Gerber, National Institutes of Health.

Norman, the standard of 0.85 milligrams of aluminum per dose set forth in the Code of Federal Regulations, can you tell us where that came from and how that was determined?

DR. BAYLOR: Unfortunately, I could not. I mean, we have been trying to figure that out. We have been trying to figure that out as far as going back in the historical records and determining how they came up with that and going back to the preamble

to the regulation. We just have been unsuccessful with that but we are still trying to figure that out.

DR. MYERS: Norman, would it be possible to get copies of these to circulate to the people who are in attendance, these particular slides?

DR. BAYLOR: Sure. Dr. Myers just asked whether it would be possible to get copies of the slides for those who would want them and I said, "Yes."

DR. VOGEL: Go ahead.

DR. KEITH: And, I guess, one last comment concerning the --

DR. VOGEL: Identify yourself.

DR. KEITH: This is Sam Keith from ATSDR.

As far as aluminum hypersensitivity, in '93 we published a paper concerning nodule formations following vaccinations and if the nodule lasted more than about six weeks a general aluminum hypersensitivity resulted, indicating that it perhaps is hypersensitivity to aluminum itself is opposed to the hypersensitivity to the antigen.

Also, if one goes into the PDR and finds that the vaccines with alum adjuvant are specifically pointed out as aluminum potassium sulfate.

DR. BAYLOR: Okay.

DR. GARCON-JOHNSON: I had the same comment. If you look at any vaccine in France --

DR. BAYLOR: Can you come to this one because that one is pretty bad?

DR. VOGEL: And identify yourself and institution.

DR. GARCON-JOHNSON: Nathalie Johnson, SmithKline Beecham.

I had the same comment about the vaccines containing aluminum. If you look at any insert of vaccinia they do not just put aluminum salt, it is specified if it is hydroxide or phosphate or alum precipitate so you know what you are using.

DR. BAYLOR: So you are saying in France it is identified?

DR. GARCON-JOHNSON: Yes.

DR. BAYLOR: Okay. And it is the same in the U.S. We require that.

DR. VOGEL: Okay. Thank you very much, Norman.

The next speaker today is Dr. John Clements. John Clements is a medical officer with the expanded program on immunization for the last 14 years at WHO. Prior to that he was the head of disease control and the Minster -- in the Ministry of Health in New Zealand. Dr. Clements' talk today will be "Adjuvants in Vaccines - A Global Perspective."

ADJUVANTS IN VACCINES - A GLOBAL PERSPECTIVE

JOHN CLEMENTS

DR. CLEMENTS: Good morning, everybody.

(Slide.)

I want to first thank the National Vaccine Program and Marty for inviting me to come here. I must say I have been looking forward to it, especially as this is the first time my wife has traveled with me on business. We now have an empty nest at home and so I am delighted that she is with me.

But I had my hopes dashed about the success of this week because of things that happened. I am unable to say "I love you" to her any longer in case she thinks that I am going to send e-mails to all her friends and replicate on her hard disk.

(Laughter.)

I hope the rest of this meeting will go well.

Here on the screen you see my clients. I thought that was a nice way to start off.

(Slide.)

I am going to talk to you about WHO's perspective about adjuvants and I knew before I came and it has been confirmed that it is very difficult to be the third speaker following the two gentlemen, who have been already, not to overlap somewhat so I apologize in advance for any minor overlaps and I

will be prepared to skip quickly over slides which duplicate.

(Slide.)

So what do I want to speak about this morning? I thought I would ask three questions. What aluminum adjuvant vaccines have been widely used? And I would like to just draw your attention to that second word there and count the number of "I's" in it. What impact have they had globally and what conclusions can we draw from this?

(Slide.)

I am afraid I do not have any wonderful maps about the plague going through Europe that we have just seen but it is important, I think, just to look at the historical perspective of how vaccines were developed. Since Genna and Pasteur did their wonderful work in the early history of vaccines, then we had a phase going through to the 1930's where the classical vaccines were developed, and right in the middle of that was diphtheria-pertussis-tetanus as you can see.

(Slide.)

And then a little bit later, the second generation -- I am calling them the second generation, that is my term and nobody else's -- of vaccines were produced because viral technology allowed this to happen right up to the '70s.

(Slide.)

How were those vaccines used? Well, in the initial programs in -- vaccine programs nationally, small pox was the principle vaccine which was used and gradually certain countries introduced BCG, the toxoids, IPV and measles vaccine up to the 1960's and 1970's. But the use of them was very much confined to industrialized countries and even there to within the better off or the better educated.

(Slide.)

It was clear in 1974 that with only five percent of the world's children in industrialized countries having access to vaccines that this was unacceptable. The World Health Program -- the World Health Organization formed the expanded program on immunization and brought in six classical vaccines and they called it expanded because it built on basically the success of the small pox program up to that date.

They expanded it with the six classical vaccines that you see there. BCG, diphtheria, tetanus, pertussis, oral polio vaccine and measles vaccine. Then later we have added three other vaccines, hepatitis B and Hib, and yellow fever in endemic countries. So right at this point I would say those are our classical vaccines.

(Slide.)

I want to draw your attention to some of the wide spectrum of adjuvants that are currently in use and I put at the top of the list the aluminum calcium salts because from our perspective they are the key adjuvants. You will see BCG itself is an adjuvant and a whole range of other items there. And really as far as I can see and as far as the books seem to say, the properties that these have in common are simply that they are adjuvants. They vary enormously.

We are particularly interested in Quil-A and immune stimulating complexes, that third one down, because it has looked for a time as if we would get a new measles vaccine using ISCOMS.

(Slide.)

And I think from our point of view and for the discussion for the rest of the two days I want to draw attention to the first bullet there, the formation of the antigen at the site of the inoculation, which is slowly released. This is our principle activity that we are looking at in terms of DTP.

And, as I understand it, the absorbed vaccine is absorbed on to a lattice work formed by the aluminum salts and those salts change their property around the freezing point, around zero, and the lattice work breaks down. So from our point of

view in the field these vaccines are -- must crucially be held above zero. Otherwise they lose their potency.

From the practical point of view, as well, it is important to understand that there is probably going to be a granuloma formed, which attracts plasma cells, and these present the immune -- the antigen to the immune competent cells.

(Slide.)

Now WHO has been aware of adjuvants for a very long time as, indeed, the FDA has. And this culminated in the most recent report specifically targeted at adjuvants which was in 1976. Report number 595. And if any of you need to look in the library and get the details of some of that, that would be the gold standard that WHO has produced up to this point.

(Slide.)

Now in practical terms where are these adjuvants in the immunization program? Well, we have just seen the United States schedule and the global schedule that we work on is really not very different. The first and important group of vaccines which have the aluminum in them is the DTP and the family there, the tetanus toxoid, DT with a large D and dT with a small "d" and also the hepatitis B vaccine.

Now we heard there ar other vaccines as well that have the aluminum adjuvant in them but these are the two groups of vaccines that we are interested in globally.

(Slide.)

Why are we so pleased with them in the vaccines that we are using? Well, with minor qualifications that we have already touched on, in part, they are safe. They are effective. They do produce a priming. They do seem, in general, with some exceptions to be successful to boost. They do attract eosinophil. And as far as we can tell, at this point, we have no evidence that they cause immune complex disorders so they do have a lot of very positive properties.

(Slide.)

Looking at the vaccines themselves and how these vaccines have become adjuvated and how they have been used globally, it was clear that the diphtheria vaccine in the 1940's was suffering from a reputation of fairly high reactogenecity. It was -- mostly it seemed to be a type 4 hypersensitivity reaction.

And this resulted in a search for a better vaccine which was less reactogenic and the way that was done was to reduce the antigen content somewhat,

to purify the toxoid and to use it as an aluminum -- to build in an aluminum adjuvant.

So now generally the vaccine used globally is with an absorbed aluminum hydroxide or aluminum phosphate and, of course, as you all know, mostly it is given with other antigens.

It still does have the tendency and the worry in the program for us that it is reactogenic and this has led to the recommendation that we use Td with a small "d". That is a smaller dose in children from seven years of age up through adulthood.

And we do have reports from several countries in any one year that complain that the DTP is very reactogenic and this is generally those vaccines where there is a relatively higher level of diphtheria content in the DTP.

But because most of the diphtheria vaccine given in the world now is either with tetanus toxoid or as DTP or even as a quadrivalent, feedback about what the reactogenecity of the diphtheria content is now very difficult to ascertain.

(Slide.)

Diphtheria has been a major disease through the history of mankind. Just as we heard about the passage of measles and small pox to the Americas, diphtheria was doing a lot of damage early on in the history of Europe. Even up into the 20th Century

immediately after the first -- the second World War, there were major epidemics still occurring and another major epidemic occurred in the former U.S.S.R. in the 1990's.

(Slide.)

If you look at this graph of the number of cases that are reported to WHO -- and these are not complete, of course. These are incomplete numbers reported -- you can see in red how the number went down up to about 1992 and then started to go up again but that increase was due solely to the blue, which is the European region and the U.S.S.R. cases.

This graph does not go back far enough but it was estimated that around a million cases of diphtheria were occurring in 1943 in Europe alone so this disease has caused havoc throughout the world throughout history.

That is just to show you the age distribution of cases in the U.S.S.R. outbreak. Young adults predominately.

(Slide.)

And the reason is very difficult to identify why a country which had been using DTP for literally generations ended up with an outbreak and low and decreasing immunization coverage certainly contributed. There were large movements of populations which contributed to spreading the

organization. And, lastly, a lack of immunity to diphtheria in adults and that is something that worries us as to its long-term implications.

(Slide.)

In terms of how the vaccine is used globally, we only know how DTP is used and the -- it mirrors very much the use of BCG, which is in red there. The DTP is behind. That is DTP3. So up to until 1985 when I appeared on the program there you can see it was not very good but after I arrived it improved a lot.

(Slide.)

The countries in red are the ones that are still not doing very well. They have low DTP3 coverage and they are still a problem regarding elimination of tetanus.

(Slide.)

If you look at -- a number of countries in Africa have falling DTP levels and again this is something which is of greatest -- highest concern to us in the program.

If you look, for instance, at the two countries I have just indicated, Ethiopia and Nigeria, although Ethiopia only has a six point drop and Nigeria has a 24 point drop, the high population levels of infants in these countries indicate enormous numbers of children unprotected still.

(Slide.)

Diphtheria is not just a problem in industrialized countries but in developing countries, as I am sure you know. It is not so much a forschal (?) or a tonsilar disease as one of the skin, and recent population changes -- political changes that have been about in many, many developing countries have, in fact, brought with it epidemiological changes and we now have outbreaks of forschal diphtheria in countries like these that are shown on the screen there.

(Slide.)

Turning to tetanus quickly. We have heard that it can be a liquid nonabsorbed or an absorbed vaccine. It can include phosphate or a hydroxide. And the most important impact that it makes for our program is to try and reduce neonatal tetanus and the principle way it does that is by protection of mothers before they give birth, either before the antenatal period or receiving two doses within their pregnancy.

We have problems to a small extent with reactions to the vaccine, and in sensitivity to the questions and discussion that we have just had, it is difficult to identify that this is necessarily directly to do with the absorbed vaccine but nonetheless we are concerned that a small proportion

of mothers do get sterile abscesses following tetanus toxoid and it does seem to be proportional to the number of doses they get.

A very small proportion of cases get brachial neuritis afterwards so it is something approaching one case per million doses administered. And individuals do go down with Guillain-Barre syndrome afterwards -- after tetanus toxoid but it is far from certain that it is cause and effect.

(Slide.)

Coverage with the vaccine of pregnant women throughout the world is very mixed and, indeed, even in countries where it is -- should be -- where it is required because there are a lot of cases of neonatal tetanus we do not seem to be able to get up much above 50 percent and that is, I think, the weakest vaccine that is administered through EPI and reflects a different target group. It is mothers that we are targeting and not infants.

(Slide.)

In terms of cases -- well, we get cases reported to us throughout -- from all the countries in the world and the number of cases in 1980 dropped from 31 to 15,000 but that is the number reported and the number that really occur is clearly higher than that.

(Slide.)

The type of mother and infant that are at risk are these. I took this picture in one of the slums in Bangladesh. And this woman is at high risk from her next pregnancy of getting tetanus and neonatal tetanus for the baby.

And we estimate that the figures are very much higher. Something around 200,000 neonatal deaths are continuing to occur a year and, just as tragic, 30,000 maternal deaths from tetanus.

(Slide.)

The whole cell and the acellular pertussis vaccine both have aluminum adjuvants. We think most of the reactions that are recorded are caused by the whole cell and not by the acellular. There is significant difference in the reaction rate there.

And again aluminum phosphates or aluminum phosphate sulfate are the adjuvants that are involved in that but it is an interesting to note that the pertussis toxoid itself acts as an adjuvant for the diphtheria and the tetanus components of DTP.

(Slide.)

The impact again of this looks very impressive. This is cases reported to us by WHO regions since 1974 and you can see a very impressive decline in the incidence of pertussis but again this is reported cases and many of you will know the difficulty in diagnosis and reporting of pertussis.

Although there is a clear trend in reduction, the actual incidence cannot be accepted as what you see there.

It is estimated that between 20 and 40 million cases of pertussis still occur every year with between 200 and 300,000 fatalities annually and nearly all those are in developing countries.

(Slide.)

Quickly, with hepatitis B and Hib, the areas in red demonstrate the high prevalence areas for hepatitis B in the world. Indeed, 30 percent of the world's population have serological markers of infection against hepatitis B so it is a phenomenonally common disorder.

(Slide.)

I am sorry about the title there but this is the number of countries that have adopted infant immunization throughout the world using hepatitis B. So you can see large areas here where we would like to see infant immunization and although there are some trial areas within Delhi this area here is not implementing immunization at this point.

(Slide.)

There is no doubt at all that this vaccine has a tremendously positive impact. Just using -- looking at studies from these countries alone and looking here, the percentage of chronically infected

before the immunization program. If we take Alaska, 16 percent of infants were becoming chronically infected. Whereas, after the immunization program, zero. And you can see all the way down here the tremendous impact, 12 to 2.9 percent.

The number of children that are subsequently getting infected with hepatitis B after the introduction of a successful immunization program is very much reduced.

(Slide.)

I do not have a graph to show you the fantastic impact that Hib immunization has had but I think many of you know from the Americas better than I do the very -- the tremendous success it has had, and this graph shows the areas that are now using Hib in their immunization programs.

Of course, we hope that this will spread to the rest of the world and, indeed, the Global Alliance for Vaccines and Immunization, GAVI, which has recently been formed, is specifically targeting introduction of new vaccines to countries, particularly developing countries. So stay tuned on that one.

(Slide.)

The last issue that I want to raise with you is the fragility of the DTP market and although we are providing just about enough DTP for the needs of

the world at the moment you will see that the yellow there is locally produced vaccine and the other two colors represent that which is produced probably mostly by industrialized countries, although not exclusively, and either donated -- purchased through UNICEF or purchased directly by countries.

However, if one of -- even one of the major manufacturers that is making DTP at the moment were to pull out for any reason because this is a marginal vaccine for them, they do not make their profit through DTP, it would put the whole of the supply system in jeopardy. It is very important to realize that so many -- that so much of the DTP is produced locally.

(Slide.)

And a similar story for the hepatitis B locally produced in green. And particularly in the Western Pacific for China and other countries, the majority of it is locally produced.

(Slide.)

So why are we here discussing adjuvants at all? Well, we have heard that it is a new era and there are many vaccines coming along that are going to need adjuvants. There is no question that new vaccines equals the need for new adjuvants.

And WHO is involved in that and are looking at the development of a tetanus toxoid vaccine which

can be given as one shot and deliver three slow release boosts subsequently to get the same effect of having three doses. That is not completed yet but research is well advanced in that era.

It is clear that the adjuvants that have been used in the past for the classical vaccines are unlikely to be suitable without modification for the future vaccines.

Secondly, just as thimerosal emerged its -- can I call it -- its ugly head last year and we were all thrown into a situation of siege momentarily until we got the facts out to the public, the public is very much interested in what is in vaccines and what their children are getting, and I believe this is something that we need to discuss in the next two days.

The public is very much concerned with mercury and it is not so surprising that thimerosal with its mercury generated so much interest. Aluminum is not perceived, I believe, by the public a dangerous metal and, therefore, we are in a much more comfortable wicket in terms of defending its presence in vaccines.

But nonetheless we have to be very much aware that the communities are watching what we do and how we handle the issues of the safety of the world's vaccines. I know there are many of you in

the room here that I have worked with who are concerned alongside with me but WHO takes that very seriously and is looking to the outcome of this meeting with great interest.

I think the public does have a right to know what is going on. I think the days of hidden administration are over and I do not think we should have any problem in disclosing what is in vaccines and what the risks are. The days for WHO and, I believe, all administrations is over where tight lips and closed doors are the response to the press. We must share what we know. And if we do not say what we know then it will be made up and we need to get our point across about vaccine safety from a strong point of view with good communications.

(Slide.)

So, in wrapping up, Mr. Chairman, my conclusions would be that these vaccines that have had aluminum adjuvants in them have had an excellent track record of safety and efficacy for over 70 years. They have had a dramatic positive effect on the control of major infant, child and adult diseases. DPT vaccine supply is potentially fragile; that nonaluminium based adjuvants could not easily replace aluminum adjuvants for the reasons that our last speaker has eloquently outlined; and that new generation vaccines will probably need new generation

adjuvants with all the requirements of safety, which we have just heard about as well.

(Slide.)

So I hope we will be able to take those points further in discussion and I thank you for your attention.

(Applause.)

DR. VOGEL: Thank you very much, Dr. Clements.

This paper is open for discussion. Dr. Myers?

DR. MYERS: John, could you say something about the calcium adjuvant? I just noticed that you had -- that there were -- which vaccines and were they utilized?

DR. CLEMENTS: Not off the top of my head, no. If I can pull back the table.

(Slide.)

I can only tell you that we are aware of calcium phosphate in DTP. I would have to go back to the books to find out which countries are manufacturing it. There may be people in the room who can see better than I. I think some of the European manufacturers.

DR. VOGEL: Dr. Armand?

DR. ARMAND: Yes. Calcium phosphate was utilized in the past by Institut Pasteur for their

DTP. When we merged our activities with their activities, this vaccine has been dropped. I have no special information regarding the comparison in terms of safety between aluminum phosphate and calcium phosphate.

DR. MYERS: So the only utilized adjuvant now then would be salts of aluminum?

DR. ARMAND: Yes. I think to the best of my recollection, Institut Pasteur was the only manufacturer having utilized calcium phosphate.

DR. VOGEL: Do we have other questions? Okay. If not, thank you very much. We will now take a break and rejoin here at 10:35.

(Whereupon, at 10:13 p.m., a break was taken.)

DR. VOGEL: Okay. We would like to get started again.

Our next speaker is Dr. Carl Alving. He has been on active duty with the U.S. Army since 1970, stationed at Walter Reed Army Institute of Research, where he is the Chief of the Department of Membrane Chemistry. His special interests include liposomes as vaccines carriers, emulsion technology and the biological effects of complement. Dr. Alving's talk today will be on adjuvant immunology.

Carl?

ADJUVANT IMMUNOLOGY

CARL ALVING

DR. ALVING: Well, the purpose of this talk is really to discuss what are adjuvants and how do they work, and I feel somewhat in the position and in the dilemma of Elizabeth Taylor's seventh husband. You know, I know what I am supposed to do but I do not know how to do it any better with the eminent people who have preceded me.

(Laughter.)

(Slide.)

But the question is what are adjuvants? Well, my simplified view of adjuvant is anything that has a beneficial effect on the immune response and there have been hundreds, perhaps thousands, of adjuvants that have been described and I think perhaps it is just as well to ask what do we expect adjuvants to do.

This is the same thing that you would ask of a vaccine. What do you expect the vaccine to do? And I am going to go through a large number of adjuvants and a large number of mechanisms. I am going to discuss a variety of different mechanisms of how adjuvants work today.

But what do we expect adjuvants to do? Well, I have put it into five categories. The first is you want -- ideally you might want to bring the

antigen -- you want to help bring the antigen into close contact with the immune system.

Number two might be to influence the type of immunity, whether it is humoral immunity or antibodies or mucosal immunity.

The third is to influence the quality of the immune response. For example, affinity of the isotypes or the specificity as was discussed by Bob Hunter.

And fourth is to influence the quantity of the immune response, namely the magnitude and the duration and so forth.

And, finally, we are always worried about the stimulation of appropriate immunity. For example, except for cancer vaccines and certain other exotic vaccine applications, we normally may not want to stimulate autoimmunity. We want the vaccines to be safe.

Now there are numerous different classifications of adjuvants that have been put forward. I happen to like this one by Bob Edelman at the University of Maryland who classified as adjuvants, carriers and vehicles as being separate. The aluminum salts would be among the adjuvants as would be saponin, muramyl diad tripeptide, monophosphoryl lipid A, bordetella pertussis and cytokines, and so forth.

Now he puts the carriers, the bacterial toxoids of fatty acids, the living vectors and so forth as being carriers but I would say they ought to be called adjuvants as well just in the generic type of definition that I am talking about.

And then he calls vehicles with the mineral oil emulsions, Freund's adjuvant, vegetable oil emulsions, peanut oil, and squalene, nonionic blocked copolymer surfactant, the squalene or squalene, liposomes and biodegradable polymer microspheres.

And then finally it is most appropriate to talk about adjuvant formulations which are mixtures of the above. Now very -- there has been very little talk in this meeting so far and perhaps in the rest of the meeting on incomplete Freund's adjuvant. Incomplete Freund's adjuvant has been widely used. Most people do not realize it has been given to more than a million people worldwide.

(Slide.)

Now the incomplete Freund's adjuvant -- maybe that could be focused a little bit. The incomplete Freund's adjuvant consists of -- it is a water and oil, a Drachy (?) oil, which is a light paraphrenic mineral oil emulsion that is stabilized with LSLA (sic) as the emulsifying agent.

Well, when the idea of having adjuvant formulations -- mixtures of the above -- we had the

idea of putting liposomes actually emulsified into incomplete Freund's adjuvant and here is the incomplete Freund's. And then we thought that the liposomes would compete with the LSLA and, sure enough, when you get too much liposomes moving in with the LSLA you get a separation of the oil and water so you get an unstable emulsion that occurs.

However, at a proper combination of liposomes and incomplete Freund's adjuvant it is possible to get a mixture of the two and get a stable emulsion so that you could have liposomes containing antigen encapsulated within them with an antigen such as lipid A or some other sort of thing actually intact sitting inside an oil and water emulsion, and then you would get -- presumably you would get slow release that would occur.

(Slide.)

I do not expect you to read any of this at all but most people -- as I mentioned, the incomplete Freund's adjuvant -- let's go to the question now of the safety. The reason that incomplete Freund's adjuvant is not widely used is because it is perceived as not being a very safe formulation.

However, there has been a wonderful study that was done in which Jonas Salk in 1951 through 1953 used an incomplete Freund's adjuvant, influenza formulation, to immunize 18,000 soldiers in the U.S.

Army and it was found to be unexcelled as a stimulant of antibody reactions for the influenza reaction.

Then there was a nine year six -- nine to ten year, 16 to 18 year, and a greater than 30 year follow-up of this cohort. And simply to summarize for you there were found some -- initially there were found some cyst-like reactions that were observed. However, they -- according to Salk he could remove those by purifying the LSLA later. However, they did occur in a certain percentage of the individuals early on, a few, as much as three or four percent. Actually one to four percent.

However, most dramatically in the greater than 35 year follow-up there was no increased adverse effects whatsoever found in the stimulation in this cohort. Particularly there were no increase in autoimmune diseases when this was looked at very carefully and, in fact, there was a significant decrease of reduced mortality due to tumors of the digestive system in this cohort.

(Slide.)

In any case, because of the perceived dangers of incomplete Freund's adjuvant, people have gone from water and oil emulsions more towards oil and water emulsions.

An excellent example of it is called MF59 manufactured by Chiron. This formulation actually is in a licensed influenza vaccine in Italy. It was given during the current flu season to perhaps more than 300,000 individuals and it appears to be a highly effective and very, very safe adjuvant. It is an oil in water. It contains squalene oil and water emulsion so that is one of the licensed adjuvants.

Another licensed adjuvant, just while I am thinking of it, that I might point out is the Swiss Airman (sic) Vaccine Institute has actually licensed a hepatitis A vaccine that contains liposomes as the basis for its formulation.

(Slide.)

The saponin derivative -- the Quil-A derivatives actually were mentioned earlier. These are derived from saponin. Saponin, as you may know, binds to cholesterol. It punches a hole in red cells and, in fact, this may be one of the toxic mechanisms of saponin.

However, the QS-21 when it is put in an oil and water emulsion together with monophosphoryl lipid A by SmithKline Beecham in their malaria vaccine, the combination has been found to be a very highly effective combination.

(Slide.)

Now how does these adjuvants work? Well, Bob Hunter -- and actually how does the Quil-A work? It binds to cholesterol. This may be a mechanism of what it does. What are some of the other mechanisms?

(Slide.)

This is a slide that Bob showed earlier. Another mechanism -- and I think Bob is a pioneer in this area -- is the effect of complement activation. The complement activation and perhaps the binding to cholesterol of the previous adjuvant are mechanisms which promote -- which could be viewed as promoting interactions with the antigen presenting cells.

Now when we get to the -- the immunological mechanisms of how do the adjuvants work, the first thing that happens is that the antigen is brought into contact with the antigen presenting cell, then it can go into -- it goes through the T helper cells, it can go into two types of pathways.

Either through B cells, stimulation of B cells or through stimulation of cytotoxic T cells. B cells would lead to antibody formation. The cytotoxic T cells would lead to the direct killing of the tumors.

Well, there are ways to influence this by the use of cytokines. The cytokines that can stimulate what are called either the Th1 or the Th2 response. The Th1 response is useful for inducing

cellular immunity such as cytotoxic T lymphocytes. Maybe generated by cytokines such as interferon gamma, interleukin-2, interleukin-12 or TNF alpha.

The Th2 lymphocytes classically are generated by interleukin-4, interleukin-5, interleukin-6, interleukin-10, interleukin-13, and there may be other indirect types of ways of generating these materials such as TGF beta, which induces IL-10, which induces Th2 lymphocytes.

And then the other thing you might want to do is you might want to have more of your antigen presenting cells so there are cytokines that can do that. GM-CSF can promote the recruitment of dendritic cells to the site of injection.

(Slide.)

And this is a paper that came out of Jay Brezhovsky's laboratory just as an illustration of how this can be used. Here this was from the Journal of Immunology in 1997 and what he showed was that, yes, the incomplete Freund's adjuvant with different cytokines that he found that he could use both the recruitment of dendritic cells with GM-CSF when combined with IL-12 that he could get a generation. He could direct the immune response towards the generation of cytotoxic T lymphocytes.

(Slide.)

And then with the -- I was pleased to see in

Roiit's Experimental Immunology actually that there was an article -- that there was a story -- a little thing about liposomes where they call it the do it all in one omnipotent liposome particle.

This is not -- I did not do this but this is -- Roiit did this. And he is talking about being able to add things in the gastrointestinal tract that have resistance, have multiple antigens, put various adjuvants such as monophosphoryl lipid A or MDP in the liposomes, target lymphocytes with IL-2 or IL-4 or interferon gamma or interleukin-12 or to target the material to a particular site such as the cholera toxin or have C3B from the complement system or to have antidendritic cell antibodies.

(Slide.)

Now if you want to generate cytotoxic T lymphocytes, which is classically what you might want to do for intracellular viruses or for tumor cells, you have to actually get the antigen in the cytoplasm so the mechanism is that the cytoplasmic antigen then goes into a proteosome, which has a variety of proteases. These are broken down into peptides through the so-called TAP complex into the endoplasmic reticulum where it combines with the MHC Class I molecules and goes into the Golgi.

In going into the Golgi then we get the MHC Class I going and being presented at the surface of

the macrophage where it generates cytokines that generate the various T helper cells.

The antibodies may be produced through the MHC Class II pathway where they are broken down in the emecitic (?) compartment and then they are presented in combination with MHC Class II histocompatibility antigens.

(Slide.)

This is simply a slide showing that it is possible using different -- a variety of kinds of adjuvants to actually generate things that will go into the cytoplasm and will generate a cytotoxic T lymphocyte.

This is work from my laboratory where these are just two separate cells. Here we have unencapsulated protein that is stained red and we have a stain which can actually identify where the Golgi is located. This is a vital stain. These are living cells and this is the combination of the two.

The unencapsulated antigen by itself, just a soluble antigen, this is conalbumin that was being used, did not go into the Golgi apparatus. It went here. However, when it was put into the liposomes it then enters into the Golgi apparatus and as a result of that you should expect to see the phenomenon that is shown here, mainly that the liposomal antigen flows into the cytoplasm.

(Slide.)

This is the only way that it can get into the Golgi apparatus. It goes into the cytoplasm and goes through the classical pathway for inducing.

Now this is not just liposomes that do this. Any particle appears to do this. You can do this with an albumin particle. You can do it with polystyrene beads, microcapsules, microspheres. You could do the same thing. They flow into the cytoplasm. This is now an established phenomenon, the mechanisms of which are not totally understood at the moment but we have actually visualized this by electron microscopy.

(Slide.)

And then you can get cytotoxic T lymphocytes. This was actually under a grant that we did with the CDC where we actually had the hypothesis that it might be possible to induce cytotoxic T lymphocytes more effectively against Ebola virus by intravenous immunization than intramuscular immunization. In fact, that did turn out to be correct with liposomes containing lipid A.

(Slide.)

And then we get -- and in this challenge model in mice actually we have gotten currently 100 percent survival of the mice that have been immunized in that way.

But just to show that not all things are very simple, when we now have gone to the monkeys looking at the ability of this system to protect monkeys, we found that we get huge neutralizing antibodies against the Ebola virus in the monkeys but the -- it does not appear to protect them.

It gives a little bit of protection. It prolongs their life span a little bit. There may be an antigen dose. We are still investigating that at the present time.

(Slide.)

What about some other adjuvants? Here is an example: Cholera toxin is a classical adjuvant. It is classically used as an adjuvant for the mucosal immunity and it is sold by a variety of different suppliers. I just point this out here.

Again our chairman, Fred Vogel, actually has done a great service to the field in working with Mike Powell and now I have gotten involved in this in producing a compendium of vaccine adjuvants and excipient. This compendium of vaccine adjuvants excipient was, in fact, on a very expensive volume that was published. It cost more than $100. It is now free. It is on line on the NIH's web site and the address for it is given right here.

Now the -- sorry. It is niaid.nih.gov/aidsvaccine/adjuvants.htm and it is on a PDF file.

(Slide.)

And, in fact, I would encourage anyone to add to that if you have adjuvants that you would like to have added to that.

Now the cholera toxin worked by binding to a glycolipid ganglioside GM1. When it binds to ganglioside GM1 it completely loses all of its adjuvant activity.

(Slide.)

This is a very, very interesting experiment that was done. Here we are trying to induce an immune response against cholera toxin and when we have the cholera toxin alone we are getting mainly IgG1 predominance in the immune response. Namely this will be a Th2 type of reactivity. However, when it binds to the GM1 on liposomes, liposomes containing lipid-A and GM1, it is converted to a Th1 type of response, predominantly IgG2A. So that it is possible with adjuvants to direct the immune response in different directions that may be desired.

(Slide.)

Mucosal immunity. What about this? Mucosal immunity -- the mucosal services are very important. They occupy about 90 percent of a basketball court.

I do not know if anybody actually spread them out to measure but I think that is a calculated value.

But IgA represents greater than 60 percent of all antibody isotypes in humans according to McGee, et al., in this publication that was shown here.

(Slide.)

Mucosal immunity represents another way in which adjuvants can work and that is by direct access to the immune system. Here we are directly applying the antigen directly to the immune system and the most commonly thought mechanism for going into the immune system through the gut, for example, is through -- entry through what is known as M cells that are phagocytic cells that basically lack lysosomal apparatus but process the antigen so that they come into the underlying tissues and can gain access to the immune system.

(Slide.)

However, I am not going to talk about that anymore at this point but I am going to talk about how can we gain access to the mucosal system by other mechanisms. We have recently discovered a wonderful mechanism actually that should be of great interest, I would think, to WHO and that is by direct application on the surface of the skin.

It happens that when the skin -- directly under the cutaneous layer of the skin there is a huge -- there are a huge number of Langerhans cells that are quintessential antigen presenting cells under the surface of the skin. When you hydrate the skin what happens is that the -- through the little cracks and things like that that are in the skin this becomes permeable down to the level of the Langerhans cells.

(Slide.)

So that we have actually discovered that it is possible by putting cholera toxin mixed with an antigen simply on the surface of the skin with a bandaid on top of it, it is possible to get an immune response. So, for example, here we have cholera toxin on the surface of the skin. We get a tremendous anticholera toxin immune response.

When we mix cholera toxin together, let's say, with diphtheria toxoid or serum albumin or tetanus toxoid, we also get an immune response against the other antigen so that with no injection, a needle free immunization procedure, direct application to the site of the immune system, namely the Langerhans cells directly under the cutaneous layer without any kind of permeability enhancers or anything other than moisture that is put, it is possible to get an immune response.

(Slide.)

This actually gives a classical IgG response with the boosting, such as Bob was talking about earlier, and the titers can be equivalent to those obtained after parental or oral immunization with a classical boosting. Published in the Journal of Immunology in 1998, this particular one.

(Slide.)

And then this protects against challenge with an intranasal challenge. So, for example, here we use heat labile enterotoxin as the antigen and then we immunized on the surface of the skin and then challenged with heat labile enterotoxin intranasally giving a lethal challenge and we got protection by immunization on the skin, presumably due to the mucosal immunity that occurred as a result.

So this mechanism just looks like an injection procedure but, in fact, it is an injection procedure with the simple thing that is missing is a needle. There is no needle placed no here. It was simply put on a bandaid with an occlusive bandaid left on for a few hours. Actually you could probably do it for as little as about 15 minutes and get the same response.

(Slide.)

Now this is the last slide and I put it to show my co-inventors, Greg Glenn and the individuals that he is working with now in the IOMAI Corporation,

which is located under a cooperative research and development agreement in my department at Walter Reed at the present time, and I will just read the names, Tonya Schartonkersten, Corey Mallet, Larry Hale, Russell Vassell and Debbie Wharender (?).

But the real reason for showing this slide in addition is to show this is -- these are the Langerhans cells actually that are beautifully stained. This is with a histocompatibility antibody.

An antibody against histocompatibility antigens looking at the virtual confluence that you can see -- you can get them actually almost confluent under certain circumstances when you stimulate them enough under the surface of the skin.

So, in summary, it is possible to think of adjuvants in a variety of different ways and you have to think about what do you want to achieve, whether you want to achieve antibodies or CTL's. Do you want to focus the reaction better? Do you want to make it less reactogenic?

I would like to make a plea in addition for incomplete Freund's adjuvant. I think the incomplete Freund's adjuvant actually is not as toxic as it has been said to be in the past. It is an extremely potent immune response. It could be used for influenza. It could be used for other antigens. It

was given to 900,000 people in the U.K. and there were a number of granulomatous reactions, some of which required surgical excision, but I believe that can be taken care of by purifying the LSLA. According to Jonas Salk that is a possible thing to do.

Thank you very much.

(Applause.)

DR. VOGEL: Thank you very much, Carl.

This paper is open for questions.

DR. GHERARDI: I want a precision about the site of antigen presentation after immunization. Do you agree that presentation must be performed within the draining lymph node from the site of biopsy and it cannot be presented by dendritic cells directly in situ into the skin or not?

DR. ALVING: Well, that is, I think, a matter of semantics to some extent. Clearly the dendritic cells migrate all over the body. They leave the site where they are. The question is where, in fact, are they processing the antigen? The presentation to the lymphocytes, of course, has to be where the lymphocytes are located. Namely in the lymphatic system.

So that it -- but it is well known that the dendritic cells do not -- a huge percentage of the dendritic cells do not remain simply in a depot at

the site in which the antigen is explored -- is found.

Now, you makes it perfect sense that the skin is such a huge organ and it is being assaulted so constantly by outside organisms that it probably constantly has to deal with organisms and things where it has to induce an immune response and it does not necessarily do that directly at the surface of the skin.

DR. GHERARDI: Okay. So the granuloma at the depot formation, for instance, vaccines is not the site of antigen presentation. It is the site from which cells take the antigen and go to the draining lymph node. Is that correct?

DR. ALVING: I think that is probably mainly correct. See, what --

(Laughter.)

DR. ALVING: I mean, because there is a -- there are some instances where there could be lymphocytes directly in the location of the -- of where the responses -- you know, where the immune response -- but let me give you an example.

When we first did our first liposome vaccine, the FDA required -- they said they had never heard of anybody injecting liposomes intramuscularly previously and what might happen if you injected liposomes intramuscularly. And we said, goodness, we

never thought of that. You know, what does it matter if we get a good immune response.

But, in fact, we were first to actually do a study looking at the -- what happened to the liposome so we made fluorescent liposomes and then we injected them and we found that the fluorescent liposomes remained at the site of injection for weeks, maybe months. For a long period of time you could demonstrate that they were there.

However, gradually they were escaping into the lymphatic system. Now that escape into the lymphatic system could have been through two mechanisms. The macrophage may have been coming up and feeding at the injection tract and then returning -- and then going into the local lymphatic circulation or the antigen may have been released for a period of time into the draining lymphatic system.

My believe is that the major mechanism of things like -- that are said to have a depot, like Freund's and aluminum adjuvants and so forth, is to serve as a place where cells can come up and feed and then go away and go into the draining lymphatic circulation.

But the granuloma that is formed promotes that because it generates all kinds of cytokines, chemotactic materials and things that would stimulate

the macrophage to bring more cells into the local area.

DR. GHERARDI: A final question if you allow me. Skin and mucosa are filled with dendritic cells but it is not the case for muscle, for instance.

DR. ALVING: For where?

DR. GHERARDI: For muscles. In muscle tissue you get resident macrophage but they do not correspond to what is called dendritic cells. So as far as I know, only the dendritic cell can elicit memory T cells from naive T cells. How can muscle inoculation and immunization -- muscle immunization achieve immunization?

DR. ALVING: Well, actually this -- I am glad you asked that actually because this was actually the subject of why we requested funding from the CDC to study intravenous versus intramuscular injection in order to achieve the generation of cytotoxic T lymphocytes against Ebola virus.

One thing you must remember is that mice are really quite different than humans and as you say humans you are injecting intramuscularly. Mice generally you do not inject intramuscularly. You inject intraperitoneally. It gets more into the circulating lymphatic system.

So this is the genesis of our feeling that maybe it would be better for inducing cytotoxic T

lymphocytes to inject directly into the intravenous system. Now whether we are able to achieve that or not in a human vaccine with an intravenous injection is another issue but I think the -- certainly, as you say, you can expect to get carrying of antigen away from the injection site into the lymphatic circulation. It can be through binding the particles. It can be binding the cells. It can be through a variety of mechanisms.

DR. VOGEL: Francois?

DR. VERDIER: Yes. Francois Verdier, Aventis Pasteur.

You mentioned during your presentation the use of MF59 as a safe adjuvant.

DR. ALVING: Yes.

DR. VERDIER: And also the use of squalene as a potential component of this adjuvant.

DR. ALVING: Yes.

DR. VERDIER: But there are also rumors and even one scientific paper describing the potential link between squalene and the Gulf War Syndrome. Squalene could have been used as an adjuvant in the British and the U.S. Army during the Gulf War. Could you comment about this potential toxicity of squalene? Is it just a rumor and not scientifically based?

DR. ALVING: Well, I am not sure I am glad you asked that but I would be certainly happy to --

(Laughter.)

DR. ALVING: -- happy to respond to it.

Number one, squalene has never been used in the Gulf War. Never. The U.S. Army has actually examined all of the lots of the anthrax vaccine that were immunized and an assay was set up by Stanford Research Institute in Menlo Park, California for squalene and they looked at the amount of squalene that is in a fingerprint and that sent the test off scale for squalene.

Then they extracted a door knob, which is a fairly unusual thing to do, and that sent the assay off scale too because squalene is so common in the skin oil. Using that test there was no detectable squalene whatsoever in any of the vials and all of the vials of the anthrax vaccine are currently being tested for that.

Now what you are referring to is the hypothesis that antibodies to squalene, in fact, are responsible for the Gulf War Syndrome. And, in fact, there has been one paper that was published. I do not really have the time to get into the pros and cons to that. We believe that paper was highly technically flawed. They had no negative controls. It had no positive controls. It had no controls

whatsoever and, in fact, it was claiming that it had developed a new assay for antibodies to squalene and that these antibodies to squalene were found only in sick Gulf War veterans and not in normal Gulf War veterans or in normal people.

All I can say about that it is that it is not 100 percent clear that the assay was suitable for detecting antibodies to squalene, number one. And, number two, it is not at all clear that there was a proper selection of sampling of individuals, normal versus Gulf War and so forth.

And it was not 100 percent clear whether this may not be something that could occur as an epi phenomenon in people who are sick. For example, if you get various connective tissue disorders or rheumatic disorders of various sorts it is certainly possible that these antibodies may occur if they do occur, may occur in the normal population.

But one thing I will tell you is that I have actually been studying this and I have found that it is possible to manufacture antibodies to squalene and I, in fact, have made monoclonal antibodies to squalene myself through a new immunization procedure that can actually differentiate squalene from squalene, the hydrogenated form of squalene.

So that is does -- it is possible that antibodies to squalene could have effects in certain

types of disorders. The relationship to the Gulf War is not at all clear at the present time and certainly there was no squalene whatsoever in any vaccines that were administered by the U.S. Army.

DR. VERDIER: Thank you.

DR. VOGEL: Bob?

DR. HUNTER: Robert Hunter.

I have a few comments about the Freund's in the adjuvants. First, some of the very nasty local reactions that were gotten in the '40s and '50s were very clearly shown to be use of very crude materials making them, which cleaned up after this period.

Secondly, the question about whether it is the local or the, you know, lymph node. There have been a number of studies in animals where people resect ejection site and you resect the injection site within a relatively short time after injection and the response you get is close to what you would have gotten leaving it on. So it is very clear that you do get antibody formation in the granuloma going in the major site and things leave the site of injection quite quickly and stimulate things elsewhere in the body.

Finally, if you look at the dose things, if you are using a Freund's in a complete adjuvant, it is usually given at a much higher dose as needed. One of the problems is you cannot get a syringe to

inject 50 microliters very effectively. If you can get a microsyringe and do that you are going to get good responses with it so you are usually given a half a ml or a quarter ml, or something much more than it is. So I agree that these adjuvants are something that can be very effectively looked at but one can reduce the dose a great deal to what was there before and change the formulations to get things that are not going to produce those side effects.

DR. ALVING: I agree.

DR. VOGEL: Okay. I think we need to go on.

Our next speaker is Dr. Bruce Fowler and we will switch gears a little bit here and talk about binary metal mixtures. Dr. Fowler is a professor at the University of Maryland School of Medicine and Graduate School where he is the director of the program in toxicology.

DR. FOWLER: I think I am --

DR. VOGEL: Oh, sorry.

(Laughter.)

DR. VOGEL: It would have been good though. It would have been a really good introduction.

I meant to say that our next speaker will be Dr. Harm HogenEsch. He has been a professor of immunopathology at Purdue University since 1993, received a D.V.M. from the University of Utrecht in

1984 and his Ph.D. from the University of Illinois in 1989. He is a diplomat of the American College of Veterinary Pathologists.

His talk will still be on aluminum and the adjuvant -- this is the adjuvant properties of aluminum.

Dr. Hogen?

ADJUVANT PROPERTIES OF ALUMINUM

HARM HOGENESCH

DR. HOGENESCH: I would know very little about metals so I am in a better position to give a talk on the immunological aspects of aluminum adjuvant and that will be the focus of my talk.

(Slide.)

A little bit of an historical perspective. As many people before me already have done and also I want to thank Dr. Alving for setting the stage for my presentation.

The idea that aluminum could be used as an adjuvant is based on a study by Glenny that he published in 1926 where he injected guinea pigs with diphtheria toxoid precipitate with potassium aluminum sulfate or alum and found that the guinea pigs that received the aluminum precipitate of diphtheria toxoid had a better immune response than the guinea pigs that received soluble diphtheria toxoid.

Since then aluminum has been used -- widely used as previous people have said -- in human vaccines and also in many -- about 50 percent of veterinary vaccines. Before that there are different types of aluminum based adjuvants, aluminum hydroxide, aluminum phosphate, and alum, which again is potassium aluminum sulfate. And, in general, aluminum adjuvants have an excellent safety record.

(Slide.)

However, they do have a number of limitations. Aluminum adjuvants are relatively weak adjuvants as compared to say something like complete Freund's adjuvant and they are not as effective as certain candidate vaccine antigens such as certain peptide antigens, for example.

In addition, aluminum based adjuvants only induce a type 2 immune response, which can lead to IgE responses and set an individual up for allergic reactions to vaccine components.

And the opposite side of that is that there are also poor inducers of Type 1 immune responses and cytotoxic T cell responses so the ideal adjuvants for those pathogens in which antibody based responses are not protective such as certain viruses and HIV may be one example of those.

(Slide.)

But a number of mechanisms have been proposed for how aluminum adjuvants work and the most quoted theory is the depot effects and it plays a role and better absorption is also important of the antigens to the aluminum particles.

The other mechanism is immune stimulation indicating that the immune system is triggered for enhanced immune response by the aluminum salts and, as I mentioned, aluminum based adjuvants tend to give a Type 2 immune response and not a Type 1 immune response. I will go over these three topics in the next couple of slides.

(Slide.)

Glenny again, five years after he first published a paper on the adjuvant effect of aluminum salts, injected guinea pigs with soluble diphtheria toxoid or alum precipitate toxoid. Let me use a pointer here. And then three days after the injection he removed the injection site material and injected back into naive guinea pigs and found that the guinea pigs that received the injection site material from the guinea pigs that had been immunized with a soluble diphtheria toxoid were not immune. Whereas, the guinea pigs that received the injection site material from the alum precipitated diphtheria toxoid were, indeed, immune. Suggesting or indicating that there are still diphtheria toxoid

present at the injection site of these guinea pigs here and so that the alum, of course, is able to keep the diphtheria toxoid at the site of injection for at least three days.

This study was followed up by Harrison. It was published in the American Journal of Public Health in 1935 where he injected guinea pigs again with an alum precipitated diphtheria toxoid and then extended the interval to up to seven weeks and he found that seven weeks after the injection he could still -- he could remove the injection site and inject it into naive guinea pigs and still get an immune response, indicating that there was -- even after seven weeks there is still enough diphtheria toxoid at the site of inoculation or injection to induce an immune response.

(Slide.)

I apologize for this slide. You cannot really read it well. I will take you through it. Holtz, in 1950, published a monograph in which he discussed several experiments on the aluminum adjuvant effects in relation to diphtheria toxoid and he challenged the depot effects.

What he did is he sort of turned the experiment that Glenny did around and he said, "Okay. If I take out the injection site after various time periods, do I still get an immune response?" And

this graph -- this line here is the -- are the guinea pigs that received a diphtheria toxoid and the injection site was left intact. So days guinea pigs can induce proper immune response.

If he excised the injection site after four days, this line here, he did not get an immune response. If he excised the injection site after seven or ten or fourteen days, there was no significant effect on the immune response, indicating that, sure, there is still antigen present at the injection site after 14 days or three weeks but it is not relevant anymore to the induction of the immune response.

(Slide.)

Now an interesting twist to this depot effect is -- was given by experiments in recent -- that were recently published in Vaccine by Ulmer and his colleagues, who are at Merck, and they looked at the effect of aluminum adjuvants on the immune response to DNA vaccines. DNA vaccines have been termed the third vaccine revolution and are very promising but they tend to give a relatively weak antibody response.

Ulmer and his colleagues evaluated several compounds, Saponins, cytokines and also different aluminum salts to see whether they could enhance the antibody response to DNA vaccines and they found that

of all these compounds that they evaluated only aluminum phosphate enhanced the antibody response to a significant level.

What they did is they immunized mice intramuscularly with 10 micrograms of influenza hemagglutinin with a plasmid for the influenza hemagglutinin gene and then 450 micrograms of aluminum adjuvant. After eight weeks they collected serum and looked at the antibody response to hemagglutinin.

And this graph here shows the -- this bar here is for mice immunized with the influenza hemagglutinin plasmid only. This bar here is for mice that were immunized with the influenza hemagglutinin DNA with aluminum phosphate adjuvant and you can see that this enhanced the immune response. This is a log scale so there is about a tenfold increase of the antibody titer.

If they used aluminum hydroxyphosphate or aluminum hydroxide the immune response was not enhanced or actually suppressed.

(Slide.)

Why was that? Well, they again followed it up. They mixed -- they looked at the binding of the DNA plasmid to the aluminum and they mixed the DNA plasmid with different adjuvants and after 50 minutes

collected the supernatants and evaluated the supernatants for the presence of plasmid.

The plasmid comes in two forms. An open circle form and a super coiled form. And what you can see here is that when you incubate the plasmid with the buffer only -- of course, they still find the DNA plasmid as your positive control. If you incubate the plasmid with aluminum phosphate you also still find the plasmid in the supernatant.

However, if you incubate the plasmid with aluminum hydroxide or aluminum hydroxy phosphate, there is -- virtually all the DNA plasmid is gone from the supernatant indicating it has bound to the aluminum salts and apparently the binding of the aluminum salts interferes then with the induction of an immune response, which follows the expression in the muscle and in the induction of an immune response.

(Slide.)

They followed up with yet another experiment where they immunized mice intramuscularly with myoblasts or muscle cells that were transfected, stably transfected, with influenza, a nuclear protein and then with or without 450 micrograms of aluminum phosphate, and nine weeks later looked at the antibody response again.

And this is -- this bar here shows the immune response after injection of the myoblasts only. This is with the aluminum phosphate only so you do not get an immune response here.

You get an immune response here and then you inject -- with the aluminum phosphate you do get an increased immune response. However, interestingly enough it did not make a difference whether they injected the aluminum phosphate three days before or three days after the injection of the plasmid.

So this -- I think really is sort of the demise, I guess, of the depot theory. I think this shows that aluminum phosphate at least does not act by -- as a depot but is in direct -- directly stimulates the immune system.

And they further examined the effect of aluminum phosphate on the expression of the antigen in muscle cells and did not find an effect there but they speculated that, indeed, aluminum phosphate has a direct immunostimulating effect and that is why it enhances this antibody response.

(Slide.)

Now this is based on a question. If aluminum salts do not act as a -- if the main mechanism is not in the depot effect, is it important then to have the antigens absorbed onto the aluminum particles. In fact, these DNA experiments where you

do not even have here protein antigens suggest that it is not the case.

We did an experiment where we used the information generated by Dr. Stan Hem's lab at Purdue on the interaction between different proteins and aluminum adjuvants. For example, lysozyme and fibrinogen have approximately the same absorption -- I am sorry. Aluminum phosphate has about the same absorption capacity for lysozyme and fibrinogen but lysozyme has a much lower absorption coefficient than fibrinogen meaning that fibrinogen binds much more strongly to aluminum phosphate adjuvant than lysozyme.

And, in fact, if you precoat your aluminum particles with fibrinogen, lysozyme cannot absorb any more so by using this we could inject the aluminum particles with a lysozyme in the same -- at the same localization in animals and make sure that there was no absorption of lysozyme to the aluminum particles, and I will show you the results in the next slide.

(Slide.)

So we did this. We injected mice with aluminum phosphate only, with lysozyme only, with lysozyme and aluminum phosphate, in which case the lysozyme was absorbed to the aluminum phosphate, or with aluminum phosphate that was blocked by previous

binding of fibrinogen and then in combination with the lysozyme.

And the immune response was evaluated after three weeks by ELISA methods and so here is the titer, and you can see that aluminum phosphate markedly enhanced the immune response over the hen egg -- the lysozyme only but there was no difference between the fibrinogen blocked aluminum phosphate and the absorbed -- and the case where the lysozyme was absorbed to the aluminum phosphate, indicating that at least in this case absorption was not critical to the adjuvant effects of lysozyme.

(Slide.)

I want to talk now a little bit more about the Type 2 immune responses and several of the speakers have already alluded to this. So it has been known for a very long time that the immune response consists of two components. A cell mediated immune response and a humoral immune response.

And it was about 15 years ago that Mossman (?) and Kaufman at DNX showed that you could explain these type of immune responses in terms of the cytokines that were produced. So interferon gamma is the prototypical cytokine that is produced in a Type 1 immune response and it drives the development of cytotoxic T cells and the activation of macrophages. Whereas, IL-4/5/13 drive the production of antibodies

and are the prototypic cytokines or typical cytokines for Type 2 immune response.

This is one of the few concepts in immunology that has held up. It has a half life of more than ten years, I think. Immunology tends to change very, very quickly.

(Slide.)

This is a graph that just illustrates the fact that aluminum adjuvants induce a Type 2 immune response and it is from an article in the Journal of Immunology last year from Paul Lehman's group in Cleveland at Case Western where they immunized mice, Balb-C mice and B-10 mice, so two different genetic backgrounds, intraperitoneally or subcutaneously with hemic (sic) lysozyme, with or without adjuvants, and they used complete Freund's adjuvant, incomplete Freund's adjuvant, aluminum, and then he had soluble without any adjuvants.

And you can see here -- I hope you can see it in the back -- is that the aluminum, which is -- are the triangles here -- does induce an IgG1 response in both strains of mice and about -- at the same level as incomplete Freund's adjuvant and complete Freund's adjuvant but only complete Freund's adjuvant induces an IgG2A response and I meant to mention that.

(Slide.)

Here in this graph IgG2A is a characteristic. It is a characteristic for the Type 1 immune response and IgG1 is characteristic for the Type 2 immune response. Of course, interferon gamma is a switch factor. It switches B cells from the production of IGM to IgG2A, where as IL-4 switches B cells from the production of IgM to IgG1. So you can use the IgG2A to IgG1 ratio as a measure of how much Type 2 immune response you induce.

(Slide.)

So aluminum induces primarily an IgG1 response, indicating it induces a Type 2 immune response.

(Slide.)

Now the regulation of these responses is complex and it is still not completely understood but what we know is that naive T -- CD4 positive T cells and T helper cells are activated by dendritic cells and the activated CD4 positive T cells can produce a variety of cytokines but then somehow they decide to differentiate in either T helper 1 cells that produce interferon gamma or T helper 2 cells that produce IL-4/5/13 and some other cytokines as well.

We have some -- we have a very good understanding of the factors that drive the T helper 1 response and IL-12, interleukin-12, seems to be the primary factor that drives the T helper 1 response.

We do not quite understand the factors that drive the T helper 2 response although there is more and more information coming out.

(Slide.)

It was already mentioned in the previous talk and in the questions in the follow-up that tissues -- that dendritic cells occur in an immature form in the nonlymphoid tissues, in the skin, mucosal organs, and to a lesser extent in some of the more internal organs, the heart and the kidney. There may be some in skeletal muscle but, indeed, there are very few in skeletal muscle.

The immature dendritic cells, when they are activated and exposed to antigen, they take up the antigen and migrate to the lymph node and during that process they mature into cells that are now capable of activating naive CD4 positive T cells.

However, they do not just -- their soul function is not just to take antigen to the lymph node but also to convey information about the type of insults that occurred in the nonlymphoid tissues and that helps them to induce a proper immune response in the CD4 positive T cells.

So if you have an infection -- for example, bacterial infection with LPS produced, LPS is a potent inducer of dendritic cell maturation and it results in dendritic cells that are -- that can

produce a lot of IL-12. So the mature dendritic cells use IL-12 to instruct the naive CD4 positive T cells to differentiate into T helper 1 cells and produce interferon gamma.

There is some evidence now that there are certain factors that inhibit IL-12 production by the mature dendritic cells and some of these are prostaglandin E2, complement factor 5A and certain chemokines.

And so this local production of factors in the nonlymphoid tissues at the injection site might induce the -- or will induce the immature dendritic cells to mature into dendritic cells that are not capable of producing IL-12 and maybe produce other factors which have not been identified, and that induces then the CD4 positive T cells to differentiate into T helper 2 cells.

Aluminum adjuvants induced response may directly affect differentiation of dendritic cells or may induce the production of some of these factors and that is still not known. There is very little research that has been done in this area.

Although we do know that aluminum adjuvants can activate the complement cascade and so potentially they can produce C5a, complement factor 5a, locally at the injection site, which then may inhibit the production of IL-12 and then result in a

dendritic cell that activates the T cells to produce T helper 2 cells.

It seems very important in order to understand how aluminum acts as an adjuvant to look at the local injection site and see what is going on at the local injection site soon after injection and we have done a very preliminary experiment on this and I should really stress that this is a preliminary experiment.

(Slide.)

Only three mice were used here. Where we injected the mice in the left leg with aluminum and in the right leg with a control, saline, and then looked at chemokine production in the -- at the injection site 24 hours after injection.

And this is a ribonuclease protection assay which allows you to screen for and quantitate the expression of mRNAse for a range of chemokines or cytokines. In this case chemokines. There are some controls here on the left side but I would like you to focus on these lanes here, the right six lanes.

These are individual mice. So this -- let me just -- for example, this is one mouse here but this is the injected leg, injected with the saline, and this is injected with the aluminum. This is from another mouse, saline and aluminum. Another mouse, saline and aluminum.

And so what you can see is that the aluminum increased expression of MIP-1 beta, MIP-2, IP-10 and MCP-1, monocyte chemoattractant protein-1, and this -- probably the strong expression here is interesting because recently MCP-1 has been implicated as being one of the chemokines that is at least necessary for the induction of Type 2 immune responses and maybe drives Type 2 immune responses.

Again these are very preliminary data that we need to repeat and work on to make it more complete.

(Slide.)

Now, do I have some time still?

DR. VOGEL: Yes, you have some time.

DR. HOGENESCH: Okay. Is it possible then to change the immune response using aluminum to another type of response and then use the Type 1 immune response? Some people have used IL-12 mixed with aluminum adjuvants and found that, indeed, you can induce a Type 1 immune response if you absorb IL-12 on to the aluminum particles.

Another study showed that CPG DNA, which is basically bacterial DNA which has unmethylated CPG nucleotides, that those nucleotides can -- oligonucleotides can induce a Type 1 immune response when mixed by themselves but also when mixed with aluminum adjuvants.

That is shown here. These are mice that were injected with -- immunized with the hepatitis B surface antigen alone or mixed with the oligos and then with and without aluminum hydroxide. You can see here the black aspect is the IG2A response and the open bars are the IG1 responses. The oligos that induce -- that enhance the immune response, they have adjuvant effects by themselves. This bar here. Induced mostly IgG2A response and some IG1 responses.

If you inject aluminum by itself, this bar here, it induces mostly IG1 response and a little bit of an IG2A response.

If you mix this CPG DNA with the aluminum adjuvant then you get this response here with digital encephalograph with a separate -- on a separate scale. And you can see that there is a marked enhancement of the immune response to the aluminum by mixing it with the CPG DNA but you continue to have a strong IgG2A response. So it is possible to change the Type 2 expression of cytokines by -- aluminum adjuvants by mixing it with other compounds such as these oligonucleotides.

(Slide.)

Now I want to spend the last few minutes to talk about an experiment that we are currently conducting that has particular relevance, I think, to this conference, and that is to very -- try to find

out how aluminum and antigen are transported to the draining lymph node, what are the kinetics of this and particularly also what is the role of cells and dendritic cells.

It has been known for a while from early studies that showed that after injection of aluminum you can find aluminum in lymph nodes but how it got there and how much of the aluminum got there was not investigated and the tools were simply not available at that time.

(Slide.)

The experiment that we are conducting involves sheep so what we want to do is we want to look in the efferent lymph, the lymph that drains from the injection site, and see how much aluminum is in there and where it is in the lymph. And what we -- we use sheep because it is possible to cannulate lymph vessels in these animals.

Now even in sheep, large animals, it is difficult to cannulate the efferent lymphatics, the lymphatics that drain directly from the skin and you get a very small yield of fluid. So what we do is we remove the prefemoral lymph node, which is located approximately here, and then after about eight weeks the efferent lymphatics -- and then we can cannulate the efferent lymph vessels, which is a larger, and a single lymph vessel, to collect efferent lymph.

So we can -- after -- we can cannulate the efferent, the efferent lymph vessel and inject the aluminum and protein, and look for the presence of protein and aluminum in the efferent lymph.

Now in order to find aluminum we have labeled aluminum with an isotope and a stable isotope in 26 aluminum, which is different from the normal 27 aluminum by just one neutron. And we mix it with a carbon-14 labeled ovalbumin (?).

We then analyze the presence of aluminum 26 and carbon 14 by accelerator mass spectrometry. Purdue has a facility for that. It is one of the few institutions in the world that actually has this capability. It is actually an accelerator that was built in the '50s that has been converted into a mass spectrometer.

And I should also point out that Dr. Hem will talk about it this afternoon, we have done studies with Richard Flarend (?), doing similar studies in rabbits, and this is just basically a follow-up on these kinds of studies.

(Slide.)

Now we are in the middle of doing these experiments and so there is not a whole lot of data that I can share with you at this time. But I wanted to show you these data here where we look at a lymph fluid and in cells, and we are looking here for

aluminum 26, and you can -- what you can se here is that we have analyzed two sheep so far, is that there is, indeed, aluminum in the lymph fluid, aluminum 26 in the lymph fluid, one day after the injection. And there are some that continues to be present, although it peaks at one day but there continues to be some presence even two, three, four, five days after the injection.

Interestingly, there is also -- this different scale. Obviously -- there is also aluminum in the cells. We have not determined at this time yet whether those are dendritic cells or macrophages or other cells but you can see that there is again an early peak of aluminum present in the cells and that decreases then fairly rapidly, more rapidly actually than in the fluid, and at four or five days very little aluminum is found in the cells.

(Slide.)

I would like to acknowledge Adam North, a technician, in my lab for his help with the ribonuclease protection assay and also with some of the sheep experiments. My collaborator, Stan Hem, at Purdue. This is graduate students, Ayishi and Seema Mudholker (?). The accelerator mass spectrometry is performed at Purdue with David Elmore and George Jackson at the Prime (?) Lab. Steve Adams is a surgeon that helped with the sheep cannulation. And

this study was, in part, supported -- is, in part, supported by the Showalter Trust.

Thank you.

(Applause.)

DR. VOGEL: Thank you very much, Dr. HogenEsch. That is a very interesting paper and is open to discussion.

DR. GHERARDI: I have a question about the possible implication of an immune reaction directed towards Th2 due to aluminum. At first babies have Th2 directed reaction in their lymph nodes. Afterwards Th1 and Th2 recuperate (?) presumably because of viral infections in infancy.

Do you believe that injection of aluminum compounds very early in childhood can retain the recuperation (?) between Th2 and Th1? Do you think that this could imply that kids vaccinated with aluminum compound may be high IgE producers when getting adult --

DR. HOGENESCH: Yes. Well, I think there is certainly some -- there is certainly evidence that aluminum adjuvants increase the total IgE levels in the serum. There are some studies in mice which have been conducted to see whether if you induce a Type 2 immune response -- say, for example, by infecting the mice with Helman's (?), which induce a Type 2 immune

response -- that it will not affect the immune response to other -- say protein antigens.

And the data are somewhat conflicting but there are some evidence that suggest that, yes, if you induce a Type 2 immune response by immunizing or by infecting the mice with Helman's that you set up or change the balance in the immune system and the mice then respond with a Type 2 immune response instead of a Type 1 immune response to other antigens.

So there is a potential that certainly individuals -- and there are a lot of factors that play obviously in allergies but that certain individuals by exposing them to aluminum adjuvants could have a little more reactivity -- allergic reactivity to allergens.

I do not think that this is a major contributor. Aluminum adjuvants have been around for a very long time. We have seen in the last 20 or 30 years what some people have called an epidemic of allergic diseases, and I suspect that other factors are more important than aluminum adjuvants.

DR. VOGEL: Thanks. Let me just make a comment as well on that.

DR. HOGENESCH: Yes.

DR. VOGEL: One thing that we do in adjuvant work a lot is work on mice and there is an awful lot

of work done on mice but when we try to make the jump between mice to other primates, particularly, you know, primates and humans, sometimes we do not always get this nice separation between Th1 and Th2 responses.

And, in fact, there was a study done by Kingston Mills looking at infants that were injected with acellular pertussis vaccines and they looked to see if they -- what kind of cytokines they made. And from the mice you would predict that they would not -- you would not see any Ig -- any gamma interferon at all. But, in fact, the infants make gamma interferon.

So it may be one of the -- you know, maybe when we base most of our data on this very nice system in the mouse of, you know, Th1, throw a little IL-12 in there, it switches over to Th1 responses, we may not really see quite that same separation when we get to human.

So it is a -- I do not know really -- it is very difficult for me to comment on whether or not, you know, IgE would be --

DR. HOGENESCH: Right.

DR. VOGEL: Carl?

DR. ALVING: Carl Alving, Walter Reed.

Just from a logical standpoint, I would find it difficult to imagine how it is that an aluminum

absorbed antigen could become an intracellular antigen that could be processed by an antigen presenting cell for a Th1 response.

So from a logical standpoint you would not expect to get a Th1 type response from an aluminum based vaccine just by itself. If you had CPG on there perhaps the CPG may be acting independently and the aluminum would then be serving as a depot for that.

Do you agree with all that?

DR. HOGENESCH: Well, it is interesting. The CPG requires to be internalized in order to have its effect and the experiment that I showed here was done with aluminum hydroxide so you would anticipate that it would bind the CPG oligonucleotides very tightly.

So I am not quite sure how that -- how exactly how it works but one of the possibilities is that if aluminum adjuvants, aluminum particles are taken up, that the change of pH in the antigen presenting cells releases both the antigens and the CPG oligos, and that then triggers the immune response.

DR. ALVING: Would you expect the CPG to be bound to aluminum phosphate?

DR. HOGENESCH: Not aluminum phosphate but the experiments with the CPG actually was done with aluminum hydroxide.

DR. ALVING: I see.

DR. HOGENESCH: I did not point it out, I guess.

DR. VOGEL: Okay. Are there other questions? Nathalie?

DR. GARCON-JOHNSON: Just to complete on the CPG story, we know that CPG does not bind to phosphate, which is logical. But the experiment you were talking about, we know also that the amount of DNA that was used was such that the aluminum hydroxide was saturated and there was a vast excess of CPG that was free in the system.

DR. HOGENESCH: Okay.

DR. VOGEL: Okay. Moving along. Our next speaker is Dr. Bruce Fowler, who is a professor at the University of Maryland, School of Medicine and Graduate School, where he is director of the Program in Toxicology and a fellow of ATS.

Dr. Fowler's talk will be on "Binary Metal Mixtures."

BINARY METAL MIXTURES

BRUCE FOWLER

DR. FOWLER: Okay. Thank you.

Well, I am very pleased to be here. I think

the talk I am going to give you is going to be very different from the ones you have just heard. I am going to try to -- I will start out with some general considerations, which may be of use, useful data, to illustrate them and then conclude with the issue of risk assessment, which is, I think, ultimately where a lot of this has got to go.

(Slide.)

There are many ways to introduce talks on toxic metals. I happen to like this one. It is original to Carlos Gustafalender (?) from the Delta (?) workshop some years ago but I think it is still quite valid. It proceeds from an axiom in pharmacology, which says that a drug is any substance which when injected into an animal produces a publication.

(Laughter.)

And what you can see is that we have more people injecting lead than anything else into their animals and we have mercury and we have cadmium and arsenic. The so-called big four.

The other thing it shows you, though, I think, which is useful, is that for a number of the other elements, the toxicological database for these things is relatively small. What is more -- and this is the topic that I am going to try to address for you -- the issue of mixtures, chemical mixtures is an

area of -- a very thorny area in toxicology because the reality is that all of us are exposed to not just one thing at a time but to mixtures of things. Some of these things are in our -- well, they are in our air, food and water. Also, dental amalgams in our mouths, for example.

So there are a number of sources of these things. The question then becomes how can we make an informed judgment about relative risk?

(Slide.)

Now I am going to talk about interactions and these are some general terms. These are definitions according to Fowler about different kinds of interactions. First you have the possibility that there is not one, that the two things simply do not interact at all. The most common, however, for many toxic agents, metals in particular, is that of additivity. That is you can think of this as stacking blocks, chemical insults from one agent acting independently from those of another. You could have a synergistic interaction, that is to say that the response that I am getting in terms of a deleterious response is much more severe than I would predict from having either one of these agents acting by itself.

And you can also have a case where you can have an antagonistic interaction. Mercury and

selenium will be one that I will show you in the course of this but this ha been around for quite some time.

(Slide.)

Then we have the issue of populations at risk. We can define this in a number of ways and sometimes it is not what you think. We have general principles from pharmacology having to do with dose and time and things like that, but we also have the issue -- the fact that we are individuals and that we vary as a function of age and gender, and males and females are not the same. Believe it. And I am going to give you an example of that. Okay.

This holds up down at the molecular level. It turns out we also have cellular protective mechanisms which have evolved over time so that the administered dose of a particular substance under a given set of circumstances is important but it is also important as to what the organism, let's say humans, do with it once it is inside.

We have a number of protective mechanisms. Metallothionine is one that I will show you. We also have this stress protein response or the heat shock response which you may be familiar with. And then this is the heart of the matter: Multiple chemical exposures and the fact that exposure to one substance can alter the system so that perhaps, let's say, the

stress protein response is not the same as it might have been if that other substance were not there.

(Slide.)

And, finally, in terms of just generalities, I am going to bring up the issue of biomarkers and, simply stated, you have your idea of toxicity and I have mine. And it is one thing to say that, well, you know, we have this dead twitching organism laying there that was exposed to a substance, and most of us would agree that there was a linkage between the two things.

However, that is not usually the case. Usually the case is that, well, we have got exposure to this or we have got exposure to that or we have got exposure in this case to a mixture of things, and how can I discriminate between the -- what is the pharmacological bullet? Let's put it that way.

Toxicology has come a long way in the last 20 years with regard to these -- biochemical tests is really what they are mostly. They give us a way of looking at interactions under a sublethal context. That is to say I can find a biochemical response that I can measure noninvasively or relatively noninvasively. And then if I add another substance, in this case metals into that paradigm, I can see what it does to this without killing the organism.

These responses as end points are for this reason enormously valuable to us as tools, is what I will call them, for detecting ongoing effects prior to the onset of clinical disease.

(Slide.)

Now this is an example of one. This is from the EPA criteria document on lead and I am sure some of the folks here from CDC may well recognize this. The fact is that over the last 30 years -- this is the heme biosynthetic pathway which is essential for life and is highly consortant across species, so rats do it, mice do it, people do it, basically plants do it too. Anything that requires heme, as in hemoglobin, will need it. It is also used for a number of other things.

But it has been known for some time that lead, in this case Pb, interrupts this pathway in a number of places and that if you are dealing with a human or you are dealing with a rat, and you can get a urine sample you can measure the precursor. ALA, aminolevulinic acid, for example, is a result of inhibition of ALA dehydratase in blood. It will be appear in the urine so you can say, well, not only was there exposure but there was enough of that stuff that got in that it caused a biochemical effect.

(Slide.)

Now it turns out a number of other metals do this as well, including mercury. This is from some studies that Jim Woods and I did some years ago with methyl mercury hydroxide in rats but actually it had been reported in workers exposed to mercury some years before that even.

The important thing is that we are looking at the excretion of a couple of metabolites in the pathway, synthesis of heme, uroporphyrin and coproporphyrin. In this case this is -- it shows a nice dose response relationship. That is another handy thing in pharmacology. We have a porphrynurea that is dominated by coproporphyrin with lesser amounts of uroporphyrin.

(Slide.)

Now this is useful because you can go back in the case of experimental animals and look at the enzymes in the pathway that are involved in this. Ferrochelatase is the terminal enzyme in the pathway. It is the enzyme that inserts the iron into the porphyrin ring to make heme. ALA synthetase is the rate limiting enzyme in the pathway which is induced under a variety of conditions where there is a depletion of heme.

Now in this case -- can you see that all right? I think you just wrinkled ALA dehydratase.

(Slide.)

Okay. This is associated morphologically -- and this prophyrinurea, I should tell you, is coming from the kidneys -- with a variety of changes in the kidney proximal tubules. These are mitochondria here, for those of you who are familiar with ultrastructure, that are swollen.

Now the important thing about this is that this is a change in the organism, a biodetectable, statistically analyzable biochemical change that can occur prior to the onset of -- let's call it overt clinical symptoms. In this case in rats.

(Slide.)

And this is not an uncommon phenomenon. In other words, if you knew what to look for, you can pick up changes early on in the course of an exposure and be able to say, yes, there is something going on here. There is enough of this stuff getting in to do something. Or on the other hand to say, no, by the most sensitive technique we have there is no evidence whatsoever that this is producing an effect. So it is a powerful tool.

(Slide.)

Now you can carry this a step further and it has been carried, thanks to the advent of high performance lipid chromatography, it turns out that uroporphyrin are systematically decarboxylate from

the eight to the seven to the six to the five and finally you end up with coproporphyrin.

(Slide.)

And with the advent of HPLC you can measure these various porphryins in the urine. So this is something that has come along in the last, oh, 10 or 15 years.

(Slide.)

Now we are getting to the binary mixtures. This happens to be from a series of experiments, in hamsters in this case, that looked indium arsenide as the binary compound. And it looked at response of the heme pathway in terms of porphyrin in the urine for two different doses of arsenic. We tried to bracket what we thought the internal dose would be with indium.

Now the reason somebody might want to study indium arsenide is faster than you can say computer or cell telephone or satellite or anything else. Indium arsenide and gallium arsenide, which are the two compounds I am going to talk about the most, are semiconductor materials and if you have one of those cute little clock radios with red numbers on it you have gallium arsenide. That is a light emitting diode.

This is now a growing area of concern with something called e-waste. That is to say what do you

do with 100 million computers that are full of things like this that people turn over every two years. It is also of interest from the point of view of replacement dental materials. The next time you go in and your dentist wants to put something called indalloy in your mouth in place of a mercury amalgam, that is what it is.

Now again the tox database on this is very limited. However, the useful thing about this and I think you can see this perhaps right here. We looked at 10 and 30 days post injection. If we just look at the low dose of arsenic here at the copro and at the -- this is the 5-carboxyl, the pentacarboxyl porphyrin. You get -- this is presented as percent of control. You can see that when the two things are together, we basically get an additive effect of the indium and the arsenic. Now this is what I meant by a additive kind of interaction.

The value of this is that it is something that can be measured. It can be analyzed statistically. You can say yes or no. Or if you want to say maybe, you can say I am going to accept a certain level of risk with regard to this particular parameter but you have that choice.

(Slide.)

Now, as I mentioned at the beginning, we -- a lot of what happens to us with regard to chemicals

depends on what we do with it and how good our cellular protective mechanisms are. I am sure in immunology we recognize that individuals vary over the spectrum in terms of their responsiveness, in terms of their susceptibility to infection.

This is again -- this is a chemical concept paper from again Carlos Gustafalender (?) from the Delta (?) workshops. But it says that the N rate of any chemical -- we have a capacity, we being an organism -- to adapt or protect ourselves but that if I raise the dose up high enough I am going to get breakthrough to a target. In other words, I will get toxicity.

The little dotted line going up here, this says leakage to a highly susceptible target, is sort of the Murphy's Law of biochemical defense systems. It says that no matter how good that defense system is, it is not 100 percent, and that if there is even a small amount that gets through it can go to some place where we really do not want it. For example, an oncogene activation.

(Slide.)

Now I am sure there are a number of you in the room who are familiar with stress proteins but this is just simply a 2D gel map, and for those of you are not, these little -- each one of those little

black spots in there is a gene product. Okay. And they are labeled with S35 methionine.

The 2D gel separates proteins on the basis of isoelectric points in this dimension and the basis of size in this dimension. So it spreads it out and it gives you a very good snapshot of what the genetic machinery of a cell is doing or a group of cells in tissue in response to a chemical.

(Slide.)

And this is a little -- well, this is a control up here. This is again our friend indium arsenide and I put arrows on the gene products that are induced. This is the low dose of arsenic, the high dose of arsenic, indium, and the combination.

Now what I hope you can see and you may not be able to see it from the back is that there are a whole lot of arrows down here, relatively few arrows in here, and relatively few arrows there.

Now the reason that is important --

(Slide.)

-- is -- well, there is two things. I need to back this up a little bit. -- is that the stress protein response is increasingly regarded as one of the very important protective mechanisms which all organisms have in dealing with toxic substances, reactive oxygen species, metals. And the problem with it is analyzing the data. I nearly sent one of

my early post-docs around the bend with counting all these little spots looking for differences.

Now, thankfully, thanks to Star Wars, we have computerized image analysis programs now.

(Slide.)

And you can use this in this case to look at and compare things. So I am comparing now gallium arsenide at two different time points, 10 days and 30 days, within indium arsenide at two different time points.

(Slide.)

And you get a data set that looks like this. Can you drop that down just a second? Okay.

What I want to show you is that the data here that the computer gives us are relative changes in gene expression for a given size of stress protein or gene product and the way to read this is we are looking from the top of the gel down to the bottom. So the higher proteins are up here at the top, the lower ones down at the bottom. And everything is ratioed to the control so up here at 90 to 100 for indium arsenide it is the same as the control. For gallium arsenide it is 2.1 times greater.

You can also see that there are some of these that are smaller. In other words, it goes to .1 so it measures both up and down regulation of gene expression. The important thing here is that you

will see more numbers that are up with the gallium arsenide than with the indium arsenide.

The reason is that the indium component, the moiety of this particular material is a very effective inhibitor of protein synthesis.

(Slide.)

Now the reason that is important is because of this: These are -- those were from kidneys, kidneys of animals who were exposed in vivo. These are silver strained urine samples from those same animals so the control is up here and basically all this black stuff are proteins coming out in the urine. Okay. Proteinuria.

What you can see is that in the indium treated animals in comparison with the gallium treated animals there is a lot more protein being dumped. In other words, that the inhibition by indium of the expression of those stress proteins in the target tissue resulted in a greater toxicity. In other words, the protective mechanism at the level of these cells was compromised by one side of the compound.

(Slide.)

Now these are in vitro. Basically what I am doing here is comparing males and females. Okay. So this is from hamster. We have also done this for humans. And we are looking again at changes in gene

expression so the controls are up here, which you cannot see, but if you look back and forth across these you can see that there are differences between the males and the females.

What that is, is down here, but the important point is that these are cells exposed in vitro to these chemicals and if we go to the combinations we get a different set of patterns.

Now the reason this is important is that these cells -- in the case of the humans, in particular, were grown up from liquid nitrogen cultures that had been stored. They had not been treated in vivo and they had not been in a human body in a long time.

The point is that there seems to be cellular programming with regard to changes in gene expression in response to a given stress. The idea is simply put that male cells and female cells respond differently. There are some general -- there are some similarities in certain areas but there are also some differences.

(Slide.)

Now where am I going with this? I am going with this in the general direction of risk assessment and how we make an informed judgment about -- or betterly -- more better -- a better judgment about exposures and the relationship between exposure to

something and what we might have to worry about in terms of risk.

This is a diagram that has also to do with what cells do with toxic metals in vivo or in situ or in vitro. This is original to RJP Williams from Oxford and it has to do with metals in solution and metals out of solution. The idea is that the metals are -- most metals are very reactive. They do not sit around as ions for very long. They complex with something.

If they complex with a monomeric substance such as glutathione, for example, then the equilibrium of the steady state between metals in solution and out of solution is not affected. If they, on the other hand, become bound with metal binding proteins such as metallothionine in the form of a cluster, then a you raise the exposure you begin to take them out of solution.

The best kind of buffer is, what Dr. Williams refers to this as, is a precipitate. That is to say under a given set of conditions, temperature, pH, whatever, the metal and its components fall out of solution. Okay.

(Slide.)

Now from the point of view of protecting the cells inside from toxic substances, these are important mechanisms and they can greatly shift the

dose response curve or the predicted dose response curve one way or another.

The best studied of these is metallothionine. This is a small protein, about 6,800 daltons, highly conserved across species, binds up to 7 gram atoms per mole, that is to say each protein chain will bind up to 7 metal atoms and two clusters. The sulphur -- it is a cysteine rich protein with four sulphurs to one metal atom.

The metals that are abound include cadmium, zinc, mercury, bismuth, silver but not aluminum. But it has a dissociation constant of -- on the order of 10^{-16} molar for cadmium. This is a very great intracellular chelator and it is inducible.

Where this has presented problems in the area for things like cadmium, for example, is that people who have tried to remove cadmium from the body by chelation have not gotten anywhere. This is just simply too good so it hangs on to it.

The way it hangs on to it --

(Slide.)

-- and this is from some work from Ian Armitage -- is it forms these two clusters and cadmium 113 was used as the way they figured this out. But basically each one of those metal atoms has four sulphurs on it. It is a dynamic molecule but the fact is that it is a very, very good chelator and

from the point of view of protection, for example, we have done experiments where you can induce this protein with zinc, which is relatively nontoxic, and then challenged the animals with cadmium and virtually attenuated the toxicity so it is important in that regard. But remember that mercury also binds it.

(Slide.)

This particular protein, there are polymorphic forms as that one slide indicated. There is Type 3 metallothionine in brain. As you may know, it sounds like you have solved the problem of thimerosal but I will just mention this in passing, even alkyl mercurial such as methyl mercury and ethyl mercury are demethylated to release inorganic mercury. That inorganic mercury is going to wind up predominantly bound to metallothionine. Okay. There is a place for it to go.

(Slide.)

The other way that metals can be complexed is in precipitate. This is a nucleus in a kidney proximal tubule cell from a rat that was drinking water containing both mercuric chloride and selenium for a prolonged period of time and you will note that there is a kind of unusual structure in here.

(Slide.)

They are actually crystalloid but if you do x-ray analysis of them you can find that there is mercury and selenium complexed in those structures inside of the nucleus of those cells in a two to one ratio.

The important point here is that we can think of -- we need to think broadly in terms of interactions between essential elements and toxic elements.

How much time do I have?

DR. VOGEL: Five minutes.

DR. FOWLER: Okay. I can wrap this.

(Slide.)

So these mechanisms and the concomitant exposure to other metals, whether they are an essential metal such as selenium, can also greatly influence the results, the outcome.

(Slide.)

Now the problem of assessing risk. Risk assessment, and I am sure you are familiar with the differences of opinion that have existed between several federal agencies with regard to mercury, stem in part from the assumptions, the underlying assumptions and the uncertainty factors that have been applied.

Those uncertainty factors tend for some reason -- factors of 10 or 100 seem to be very

popular, not 8.5 or 6.2, but five or ten. The magnitude of those uncertainty factors decreases with increased scientific understanding of what is really going on. Okay. In other words, the more precise the data,the better the data that go into those assessments, the more sagacious they become.

(Slide.)

There is also the fact that again these risk assessments frequently do not take into effect multiple chemical exposures, which can greatly alter the outcome. So if you have a high zinc diet, you eat your Wheaties every morning an you have a lot of metallothionine around, your risk based on exposure to something else, cadmium, perhaps mercury, may be very different from someone who is let's say alcohol, who is zinc deficient, who has a very small pool to receive some of these toxic ions.

(Slide.)

Now let's deal with the perception of risk. Does that look risky? Okay. We have some questions we can ask here legitimately? How big is that shark? When did he last have lunch? What are these crazy fools doing in here anyway? (Slide.)

Now does that look risky? Actually it turns out he was curious and just wanted his picture taken.

These guys know the drill. The point here is that in the absence of scientific data we get

stuck in the problem -- and I think Dr. Clements said it earlier -- of people basically arguing over what they do not know. And what we can say out of this is that interactions between chemicals such as toxic metals do occur.

(Slide.)

Overall, additivity, if there is an interaction, is the most common form of that and risk assessment should be conducted based on a variety of parameters. Again, not to beat a dead horse, the quality of those risk assessments very much depend on the data.

And, as I showed you in the very first slide, what we have -- we have more data, we have better data for certain elements rather than others, and we have relatively few data for interactions between substances.

And with that, I shall stop. Thank you.

(Applause.)

DR. VOGEL: Thank you very much, Dr. Fowler. This paper is open for discussion.

DR. FOWLER: It is also time for lunch.

DISCUSSION: SESSION I PAPERS

DR. VOGEL: Due to the time I think I would just like to open the discussion up generally for Dr. Fowler and for the other participants as well if there are any other questions at all.

Dr. Tchounwou?

DR. TCHOUNWOU: Yes. Paul Tchounwou, Jackson State University.

I have a question with regard to the binding of metals to metallothionine. On the list that you have shown up here I did not see arsenic. I do not know if you have done some work with it because we are doing some molecular study with arsenic and we see a lot of significant induction of metallothionine when we expose human cell lines to sodium -- to arsenic. So I am wondering if --

DR. FOWLER: Okay. The answer, I believe, is that a number of other folks have found that, too, that there is induction of metallothionine but not binding. What I think might be happening is that the arsenical will produce oxidative stress inside of the cells and I think that may be the inductive mechanism. The arsenical, as you know, tend to undergo a methylation process and to be excreted in the urine as monomethyl or dimethyl arsenic acid chiefly. That seems to be the way -- you know, 90 -- you can account for about 90 percent of it in those terms.

So I think -- I mean, what you are saying has been -- you know, is certainly affirmed but the -- whether or not there is binding of arsenical to this, I think, is -- that has not been clearly

demonstrated. In other words, you can have induction of metallothionine but it does not mean the arsenic is there. It may be something else.

Is that clear?

DR. TCHOUNWOU: Yes.

DR. FOWLER: Okay.

DR. TCHOUNWOU: (Not at microphone.) And, also, on the list of chemical interaction and potentiation of activity and the antagonisms -- do not forget the potentiation --

DR. FOWLER: Well, I used synergy in place of potentiation but basically the idea is that you can get an enhanced effect so that, I think, is maybe a little semantic. Okay.

DR. VOGEL: Are there other questions for Dr. Fowler or for any of the speakers?

Dr. Myers?

DR. MYERS: Maybe I will take the prerogative of tossing out a general question for the -- all the discussants this morning. And that was one of the things that struck me was that we did not see comparative -- very many comparative human trials of potential vaccine antigens with and without the presence of adjuvants.

I wondered generically how then the decision is made as to whether to include an adjuvant in a product that is presented to the FDA is, in fact,

from a manufacturer's perspective, for example, a decision made mostly on animal data? Are human trials done? If so, is that data available for us to understand the differences in immune response between aluminum containing candidate products?

DR. VOGEL: Dr. Alving?

DR. ALVING: Carl Alving.

I would say it is partly determined on intellectual property rights. I mean, there are thousands of adjuvants that have been developed. The ones that are actually being developed individually may not necessarily be the best ones. The best may be combinations and so this is one of the problems.

The question of whether you can test for adjuvant activity or compare different adjuvants in experimental animals is an important issue to raise because it is our impression from numerous studies, both in animals and in humans with different types of adjuvants that the animal studies frequently, in fact usually are not very predictive of the relative efficacy of one adjuvant compared to another.

So a lot of this is empirical. If you look at a mouse, for example, they may be -- mice, generally, are extremely reactive to a variety of different adjuvants. Those same adjuvants when you put them into humans, there may be no reactivity. There is nothing. And so what is really missing is

comparative adjuvant studies in humans. I think that is an important thing.

Now I have attempted to -- my group at Walter Reed has attempted to address that issue with respect to one vaccine formulation and that was prostate cancer. An immunotherapeutic vaccine where prostate specific antigen was put in liposomes with a variety of different adjuvants. We went through sequentially six Phase I trials actually.

And the results were quite amazing that you could -- by going -- doing just five patients at a time you could actually differentiate the relative efficacy of one particular formulation compared to another one.

Now we have not published that yet because we have now moved into Phase II trials and those trials are still ongoing but nonetheless this is a huge deficiency, I think, in the -- in knowledge and I do not think that animal studies alone are going to be the answer.

DR. VOGEL: Go ahead.

DR. CHEN: Bob Chen, CDC.

I guess we have heard about a number of potential future adjuvants that are promising but they look like they will be some ways off. In the meantime, I guess trying to understand the potential risks that are associated with the current

vaccination programs -- I guess, one way to think about it is that when we add the different alum adjuvant vaccines, initially starting with the DTP vaccines and more recently with the Hib and the hepatitis B in an increasing number of immunization programs, what is that risk?

Now, of course, that is somewhat difficult to study in children. And in Norman's presentation he showed in adults they may get a number of different vaccines. They will frequently -- they may not get all of them at one time but there are certain populations perhaps in the military where they would get several of them. Presumably the tetanus. They would get the hepatitis A and hepatitis B and others.

And I guess the question perhaps to some folks in the audience, are there any lessons from that -- from presumably recruits that are receiving several of those vaccines all in short -- relatively short period of time, over a couple of years?

I guess we did hear about some of that data and maybe we will hear more about that but I just wanted to kind of probe that a big more.

DR. VOGEL: Anyone like to respond to that?

DR. GRABENSTEIN: John Grabenstein, United States Army.

I think Bob is asking a question -- where is he? Where did he go? Okay.

Alving may want to pitch in to what -- I do not know.

We know of no acute toxicities in -- I was starting to calculate the number -- the amount of aluminum that we would give at basic training, which is not all that much, and be relative to starting an adult on a full series of tetanus diphtheria, for example, which is unusual in the U.S. but anyway -- I will work out a number for you by the end of the day.

We know of no special toxicities that we have recognized beyond what is recognized in the literature for acute toxicities from a dose or two, the injection site reactions and that sort of thing.

And over time we have not recognized anything different from what we have seen in the normal adult population so it is an absence of data and an inference of safety rather than explicit studies per se.

DR. VOGEL: I think one thing that might be contributing to this in a way maybe indirectly is the desire now to move from individual vaccines to combination products. We kind of started thinking about this when we were thinking about mercury but also with aluminum that it takes the same amount of mercury or thimerosal to preserve a combination

vaccine as it does to preserve individual vaccines. It also takes the same amount of aluminum as in aluminum gel type adjuvants to adjuvant combinations as well. So it would be an indirect effect of combination vaccines to be able to lower the dose of aluminum in -- if you look at the immunization schedule.

DR. MYERS: Let me ask the hard question. Is an adjuvant needed in any of the currently licensed vaccines? Is it absolutely something that is necessary?

DR. VOGEL: Well, I think you have to go back to what you really do with adjuvants and some of the work that was brought up before. One of the adverse reactions that are seen with vaccines is having too much antigen around. That is why we have small "d" and big "T" for, you know, adults or adolescents.

If the immunologic adjuvant can be used to reduce the dose of antigen to get the same response then that would be a good effect of the adjuvant. So it is not just always like a gas pedal to drive the response higher and faster. It can also be to direct the immune response.

So there may be vaccines that will work fine by themselves on aluminum adjuvants or on -- or with no adjuvant at all but there may be vaccines that we

cannot build at all now unless we are able to direct the immune response with an adjuvant in the appropriate direction.

We talked about ability to deliver mucosal vaccines to use transdermal immunizations to drive responses specifically towards cell mediated immunity and not necessarily antibody. So there may be reasons for adjuvants other than simply boosting the response but more their ability to direct immune responses. I think that is the real advantage for adjuvants in future vaccines.

Dr. Alving?

DR. ALVING: I think there are -- well, there are clearly some vaccines where an adjuvant is going to be needed like malaria, I think, and HIV and other things where that may be important.

With response to John Grabenstein, I do not have any particular response to the question that he asked but I would like to say that I believe that John is involved with some kind of a vaccine publication on line. Is that -- I was surfing around a while ago and saw some wonderful vaccines -- a current vaccine page that might be of use for just general information.

Is that right, John?

DR. GRABENSTEIN: (Not at microphone.) I compiled an internet web site called www.immunofacts.com. (Inaudible).

DR. PLESS: I am Robert Pless, CDC. It is more of a challenge to the speakers this afternoon because in response to Bob Chen's question about the number -- the increasing exposure to aluminum given the increasing number of doses being given and Norman's slide showing the exposures through the series in children and adults, it is sort of a deja vu from the thimerosal workshop where we were shown the exposures to mercury with increasing numbers of doses.

So, hopefully -- I mean, if aluminum does not behave like mercury in terms of its cumulative neurotoxic effects, hopefully this afternoon's speakers could enlighten us as to whether it really makes a difference in terms of toxicity if one has a depot of aluminum that hangs around, whether it really does make a difference whether there is an increase in quantity or not in relation to the adverse effects.

So maybe if they have not incorporated those into their slides they could spend the lunch hour doing that.

(Laughter.)

DR. MYERS: I think we probably ought to break for lunch at this point and we are a few moments late.

Someone asked me earlier this morning, they said I am getting -- I have gotten a little softer in my older middle age and letting people off at 4:30 in Puerto Rico, what did I think they would do, go for a swim or something.

What I would suggest having been here for the last several days is that we probably ought to reconvene at 1:45 and give everybody time enough to get lunch. It takes a bit of time to get through the dining area here and maybe we will run over a few minutes at the end of the day.

So reconvene at 1:45.

(Whereupon, at 12:30 p.m., a luncheon recess was taken.)

* * * * *

AFTERNOON SESSION

DR. MYERS: I think we will go ahead and get started even though we do not have everybody back from lunch yet but we have a lot to cover this afternoon so I think we better get started on time.

Dr. Georges Peter, as I mentioned this morning, his plane did not get off last night from Providence. It did not get off again this morning so he is not going to be able to join us and I asked Dr. Stanley Music to step in as the moderator of the second session this afternoon, which is "Aluminum Pharmacotoxicology."

Dr. Music is -- has a diverse background. He was the environmental epidemiologist, which is something I did not know, in North Carolina so he fits right in with metals. He was the state epidemiologist in Wyoming. For 28 years he was at CDC in part of the small pox program. And now he is with Merck Research Laboratories on the WorldWide Safety and Epidemiology Program.

So thank you very much, Dr. Music.

SESSION II: ALUMINUM PHARMACOTOXICOLOGY

MODERATOR: STANLEY MUSIC

DR. MUSIC: Thank you. Can everybody hear me?

Our first speaker this afternoon is a fellow Brooklynite and another Stan. Stan Hem is a

professor --

(Technical difficulties.)

DR. MUSIC: -- adjuvants where I came in contact with him in my recent Merck move.

His work in this area was recognized by --

(Technical difficulties.)

DR. MUSIC: -- on time and I will remind you when we have five minutes left.

ABSORPTION AND ELIMINATION OF ALUMINUM-CONTAINING ADJUVANTS

STANLEY HEM

DR. HEM: Okay. Thank you very much. It is a pleasure to be here and I appreciate the invitation. I am a chemist in background so I am really learning a lot from getting involved with vaccine adjuvants but I think some of the chemistry is an important part of this story that we are all thinking about.

What I would like to do is talk about -- a little bit about the properties of aluminum hydroxide and aluminum phosphate adjuvant, then talk a little bit about *in vitro* dissolution experiments, and simulated interstitial fluid, and then finish with some *in vivo* experiments that show that the aluminum adjuvants are dissolved by the citrate in the interstitial fluid and that they leave the body.

So let's begin, and the speaker this morning mentioned that aluminum phosphate adjuvants are really chemically amorphus aluminum hydroxy phosphate. Aluminum hydroxy phosphate is not a stoichiometric compound and so you get all kinds of combinations and different ratios of phosphate and hydroxyl making aluminum hydroxy phosphate.

I think most of the in situ precipitations, people refer to them as alum adjuvants, that kind of bothers me as a chemist because alum is the chemical of potassium aluminum sulfate, which is a very water soluble compound. So if you had alum you would have -- it would be a solution.

So it -- and alum is the starting the material, it is the source of your aluminum, and you are precipitating it with your antigen. So I prefer alum precipitated adjuvant to refer to the adjuvant that is produced from alum. And if you have a phosphate buffer in any way involved in that then you are making aluminum hydroxy phosphate very much like these aluminum phosphate adjuvants.

So basically the properties that I will describe for aluminum phosphate adjuvant really are attributed also to the -- at least every one that I have looked at where people use alum and precipitate an antigen in the presence of alum and have a phosphate buffer somewhere in the story.

(Slide.)

I cannot give you an x-ray pattern because aluminum phosphate adjuvant is amorphus. Here is the infrared spectra and the infrared spectra shows a nice band here that is the phosphate band so we know that it has got phosphate in it.

This band here is the hydroxyl stretching band. That can come from the water, the hydroxyls in water, as well as any structural hydroxyls. When we heat this up to drive off the water we are left with a very small but very sharp band which tells us that it is a hydroxy phosphate. Hydroxyl is a part of the structure and so it is a phosphate compound and a hydroxyl compound and so hydroxy phosphate is a good name for it.

(Slide.)

This is the morphology. This bar is 50 nanometers and so these are very, very small particles. The primary particles are small plates. So they are basically individual primary particles, small plates, plated like morphology, very thin, and aggregated together and when people measure the particle size they say that the particle size is two microns or five microns. They are really measuring the size of the aggregate.

The primary particles are very, very small and when you start looking at the absorptive capacity

you just do not understand how you could have such a high absorptive capacity for these materials because they are made up of these very, very small primary particles.

(Slide.)

Now interstitial fluid contains some alpha hydroxy carboxylic acid. It contains citric acid, lactic acid and malic acid. Alpha hydroxy carboxylic acids are chelating solubilizing agents for aluminum. In fact, many soil chemistry tests are -- when they do the soil chemistry one of the steps is to dissolve the aluminum compounds out of the soil with a citrate solution. So it is well-known in mineralogists that these alpha hydroxy carboxylic acids are able to dissolve in soluble aluminum compounds.

(Slide.)

Here is an in vitro experiment that we did. We took aluminum phosphate adjuvant and we used the normal amount per dose, which is 850 micrograms, so we are not showing the whole scale here, the total amount was 850 micrograms, and what we are doing is we are doing an in vitro dissolution experiment. We are adding that to a citrate solution at the concentration of citrate and interstitial fluid.

We are doing this at room temperature and we are just mixing it and stirring it for 12 hours and taking samples periodically. You can see that in 12

hours we have about 450 micrograms out of the total of 850 has dissolved. So these aluminum phosphate adjuvants dissolve in citrate solution similar to the citrate at the same concentration that citrate is in human interstitial fluid.

(Slide.)

This is the isoelectric point experiment for aluminum phosphate adjuvant. This is the zeta potential versus the pH. And, as you can see, it is positively charged below pH, about five, and negatively charged above pH-5. And so it would be a good absorber by electrostatic forces for positively charged antigens and I think it has its main use with those kind of antigens.

We will look at this -- this is the aluminum hydroxide adjuvant but we will come to that in a minute. That isoelectric point depends upon the degree of substitution of phosphate-4-hydroxyl. So this point could move around in different samples depending upon the recipe and the phosphate and hydroxyl ratio.

(Slide.)

And this illustrates that. Here we have precipitated aluminum hydroxide without any phosphate and the isoelectric point of AOLH3 (?) is around ten, and then we precipitated the same amount of aluminum but with increasing a little bit of -- little

quantities of phosphate added to the recipe, and notice what is happening to the isoelectric point, 0.0 charge. It comes down -- it becomes asentotic along about pH4.

So aluminum hydroxy phosphate could have any isoelectric point between 10 and 4 depending upon the degree of substitution of phosphate. I guess everybody might be familiar with the commercial aduphos (?) and rehydrophos, and they have isoelectric points around 4.5 to 5.5. So they are in this ball park but it is possible to adjust the recipe and make aluminum phosphate adjuvant with higher or even lower isoelectric points.

(Slide.)

The other adjuvant that we have to think about is aluminum hydroxide adjuvant and the speaker this morning was also good to get you thinking about the aluminum hydroxide adjuvant also being misnamed. It is really aluminum oxyhydroxide and it corresponds to a mineral in nature that is known as bomite (?).

(Slide.)

And here is the x-ray pattern for aluminum hydroxide adjuvant. I was really surprised that we got an x-ray pattern because I know that aluminum hydroxide adjuvant was used to absorb proteins and so I expected it to be amorphus. Generally amorphus materials have high surface areas and have high

absorptive capacities. And son of a gun, here is aluminum hydroxide adjuvant with a very strong x-ray pattern and this x-ray pattern matches the x-ray pattern of the mineral bomite and is aluminum oxyhydroxide.

(Slide.)

The sharpness of these x-ray -- of these peaks tell us how highly crystalline it is. There is degrees of crystallinity. We could go from something being very poorly ordered and we would get very broad x-ray bands to something that was very highly ordered, and we would get very sharp x-ray bands.

So we use the width of these bands, we call it the width that have height, to characterize how highly organized the aluminum hydroxide adjuvant is. And so when we have a small width at half height, it means the peaks are sharp, it is highly crystalline, highly organized. When the width at half height is larger it means the peaks are broader. It is less crystalline and less highly organized. And that will make a difference in the solubility you will see in just a second.

(Slide.)

Here is the infrared spectra. This band at 1072 and this shoulder at 3098 are characteristic of bomite, aluminum oxyhydroxide.

(Slide.)

And here is why it is a good absorber. How a crystalline material can have a high surface area. This -- again this bar is 50 nanometers and aluminum oxyhydroxide or what we use in vaccines, aluminum hydroxide adjuvant, has a fibrous morphology. All needles. Each one of these is an aluminum oxyhydroxide fiber and there is millions of them and this is why you can have a crystalline material but have a terrifically high surface area.

This also explains an earlier speaker this morning who talked about the problems when you freeze the adjuvants. So you can just imagine when you freeze this, all these fibers are going to stick together, and when you thaw it they are not going to pop apart. So you are going to lose all your surface area when you freeze these adjuvants so do not -- the speaker this morning was right in advising you not to do that.

This also tells why it is hard to get a surface area number because the normal way to get surface area is to dry the material to a powder and then measure the nitrogen or some gas absorption. So again if you dry this to a powder all these needles are going to stick together and you will get a very low surface area.

We determined the surface area by absorbing phosphate. We exhaustively absorbed phosphate from

the footprint of a phosphate eye and we assumed they were lying flat on the surface. We then calculated the surface area and it came out to be 525 meters squared per gram. That is a terrifically high surface area. The swelling clays (sic) are around 600 meters squared per gram. The fume silicas are around 800 meters squared per gram. So 525 is a terrifically high surface area and I think that is an important part of the wide use of aluminum hydroxide adjuvant.

(Slide.)

This is a dissolution experiment in an in vitro experiment with a citrate buffer -- with a citrate solution. When we use the concentration of citrate that is in interstitial fluid we could not measure any detectable amount of aluminum in solution in a normal time period.

So what we are looking at here is 100 times the concentration of citrate in interstitial fluid and at 37 degrees. That aluminum phosphate was at the concentration in interstitial fluid and at room temperature but we had to speed things up to get this student out in a normal -- to get her thesis done.

So here is two different aluminum hydroxide adjuvants. The total amount we put in was 850. We have broken the graph here and so we are in about 120-140 hours, you are getting dissolution but it is

much slower. It is coming up to about 80 of the 850 micrograms.

The one that is dissolving faster is the one with the broader x-ray pattern. The one that is dissolving slower is the one with the sharper x-ray pattern. So you are going to learn a lot from the x-ray pattern about how these things are going to behave in the body.

In contrast, this is aluminum phosphate adjuvant. So in about 12 hours the aluminum phosphate adjuvant at this 100-fold concentration completely dissolves. The aluminum phosphate adjuvant dissolves much more rapidly than the aluminum hydroxide adjuvant does in this in vitro dissolution experiment.

(Slide.)

And the aluminum hydroxide adjuvant -- here is its isoelectric point. It is up around 11. So that at -- the normal pH is where we formulate that, it will be positively charged. And so the industry is in a nice position. It has got one adjuvant that will electrostatically attract negative antigens and it has got another adjuvant that will electrostatically attract positive adjuvants. So we are in a good position to have both of them available.

(Slide.)

Interstitial fluid. It has got a lot of interesting things in it. I do not think everybody knows everything that is in it but it has got a good amount of phosphate. It has got a substantial amount of albumen and fibrinogen and it has got six m. equivalents per liter of citrate. So that was the concentration that we were using in those in vitro dissolution experiments.

(Slide.)

We are really lucky at Purdue to have a physics department that has an accelerator and rather than bury it when the funding stopped, they converted it into a mass spectrometer.

I would like Richard Flarend to stand up. He was the graduate student. He was a graduate student when he did the work that I am going to be showing. You might want to speak with him. He is now an assistant professor of physics at Penn State, the Altoona campus. So this work is his and part of his thesis was also to take the antiperspirant, aluminum chlorohydrate, and incorporate aluminum 26 into it and apply it to humans and see how much aluminum was absorbed from under arm antiperspirant use.

(Slide.)

The normal person -- we generally get about 10 milligrams a day of aluminum. The plasma

concentration -- there is a mistake here. This is five micrograms of aluminum per liter. It is the plasma concentration so there is a typo there that I apologize for.

In terms of tissue concentration it ranges from one to 100 milligrams per kilogram of tissue. And in comparison then the maximum dose of aluminum that is allowed in a human vaccine is .85 milligrams. That will give you a little bit of perspective. We are not talking about very much aluminum compared to what we are exposed to in our daily life in our daily contacts.

The neat thing about accelerated mass spectroscopy is it can measure incredibly small amounts of aluminum 26 and this is not a typo. 10^{-17} grams. That is the amount that it can detect and quantify. That is not a typo. That is real.

The physics people tell me that Purdue has a football stadium that holds 70,000 people. They tell me that if you fill the football stadium half filled with sand and every grain of sand represented an aluminum 27 atom and you put on aluminum 26 grain of sand in they could tell it. They could detect it and measure it.

So this is an incredibly sensitive way. It is completely safe because they are measuring it by its mass. They are not measuring it by its

radiation. So when we work at these numbers of 10^{-17} there is no radiation. Your geiger counter does not click. There is no measurable radiation so it is completely safe.

We had no trouble with either the human -- the Humans Committee at Purdue or the Animal Committee at Purdue. If you eat one banana you get an exposure of .12 m. rem per year. The aluminum 26 study that we are going to show you with the bunnies was just a little bit more than that. So it is a nice technique. No worry about safety. Easy to -- able to do in humans without any concern for any injury or problems.

(Slide.)

This just supports what I just said. Here is some data on the exposures that we may have and the natural background is around 300. The average x-ray is around 20. And the amount of aluminum in one of these studies is less than one. So I just want to impress upon you it is safe. These kinds of experiments are safe.

(Slide.)

They have got to do a little chemistry to work up this sample. They have got to take the blood or plasma or urine or take the tissue and they actually end up making aluminum oxide out of it. So they treat it with acid and digest it and finally

heat it at 1,000 degrees and they make aluminum 26 oxide and that is what they -- that is what goes into the accelerator.

(Slide.)

So what we did is we precipitated aluminum hydroxide and aluminum phosphate adjuvants in our lab in the presence of a little bit of aluminum 26 chloride. Aluminum 26 does not occur in nature. It is made in accelerators.

We got this from Oak Ridge and so we took just a pinch of the normal aluminum 27 out of the recipe and put an equivalent pinch of aluminum 26 chloride into the recipe. We precipitated them. We tested them to see if they had the properties that they -- that we expect to have that I have just shown you when they did and then we went to the bunnies.

(Slide.)

And we dosed New Zealand white rabbits with .2 mls of each adjuvant and that contained .85 milligrams of aluminum. We decided to use the human dose in the bunnies even though we know that there is less interstitial fluid but we thought we would give the worst case situation.

So we have got the human dose in the bunnies. We collected blood and urine for 28 days and there were two bunnies with each adjuvant.

(Slide.)

And the physics people wondered when we should take the first sample. I said, "Well, you know, let's wait a day or two after we inject it." I am picturing this crystalline material dissolving slowly in interstitial fluid, going to the lymph, getting to the blood. They said, "Let's do an hour." I said, "That is crazy. You are going to waste -- this is an expensive assay. You are going to waste money."

But they prevailed and I am glad they did because here is the aluminum hydroxide blood level data and the one hour blood sample -- the one hour blood sample showed aluminum 26 in it. So the adjuvants is beginning to dissolve and aluminum 26 is appearing in the blood within an hour. I wish we had done a shorter time than the hour. So the body has a very powerful mechanism for processing and eliminating these adjuvants.

It kind of reached a nice steady blood level over the -- this is 28 days out here and we need now to look at the urine data. Each of the data points is one of the bunnies and the triangles is the average of the two bunnies. So in a nice normal kind of cumulative urinary excretion behavior.

(Slide.)

Now we will go to the aluminum phosphate adjuvant. Remember that is amorphous. Remember in

our in vitro experiment with citrate solution it was a lot more soluble so the dotted line now is the aluminum phosphate adjuvant and so here is the blood level and we are getting -- the area under the curve here is about three times higher than the area under the aluminum hydroxide adjuvant.

So both adjuvants are dissolving in the interstitial fluid, ending up in the blood, but the rate of dissolution is different and it is nicely understood when you go back to the crystallinity, the crystalline material is not as soluble as the amorphus material.

(Slide.)

And here is the urine data and this is also why I like to be a chemist and do not like to be a biologist because these data points are the -- these data points are for the urine for the aluminum hydroxide adjuvant. We had one bunny that just did not -- that just did not excrete the aluminum very rapidly.

The blood levels of the two bunnies were very similar but one of these bunnies just somehow held on to the aluminum and so what we have got here is the mean of the two bunnies but those two bunnies varied a lot. The bunnies that got the aluminum phosphate were very consistent so this is -- this

confirms my belief that I should stick to beakers and test tubes and not do animal stuff.

(Slide.)

This is probably what you are interested in seeing. This is the pharmacokinetics data and the aluminum hydroxide adjuvant with the bunny one and two, the percent of aluminum that appeared in the urine in 28 days was 13 for one and 22 for the other, for an average of 17. For the aluminum phosphate adjuvant, one bunny was 47 percent of the aluminum that appeared in the urine in 28 days, the other bunny was 55 for an average of 51. So it was about three times more soluble, three times more dissolved -- faster dissolving. The blood level curves were about three times different.

The urine curves -- remember it has got to go into the blood and then it will be distributed to tissues and then from the tissues it will go out in the urine so it is going to take a little bit longer before we start seeing aluminum 26 in the urine because it has got to distribute and be taken out of the tissue.

(Slide.)

So here is the two bunnies with the urine, 5 and 6.2, for an average of 5.6, and 10 and 32. This was the bunny that I was not happy about but an

average of 22 so that might be a little bit higher if we had more bunnies.

(Slide.)

The tissue distribution, after the 28 days we sacrificed the bunnies and examined the different organs, and in every case the aluminum phosphate -- here is the aluminum phosphate two bunnies. In every case the amount of aluminum 26 in the tissues was higher from the aluminum phosphate than the aluminum hydroxide but it is three times higher, which is exactly the same proportion that the blood levels were higher.

So you have more aluminum in the blood. You are going to get more in the tissue just by mass -- just by mass balance. And the distribution here is the same as you normally see aluminum in these different organs. So the aluminum 26 was not going to a special place and the aluminum from the adjuvant was not going to a special place in the body.

And that is it. So I hope you got a little sense that the body has a way -- I was really pleased with this study because I wanted to do it because I did not think anybody knew what happened to these particles in the body.

I had seen these papers where people excised the site and tried to look for aluminum there but I did not think that was very dependable and when the

physics people started working with this accelerated mass spectrometer I really got excited. And I do not think there is any doubt that the body has a way to eliminate the adjuvants, the citrate, the alpha hydroxy, carboxylic acids in interstitial fluid, chelate, dissolve them, they go through the lymph, into the blood, to the tissues, and out, and out in the urine.

Thank you.

(Applause.)

DR. MUSIC: Thank you.

DR. GHERARDI: I have two questions. You explained the removal of aluminum by the composition of interstitial fluid but we know that shortly after injection most of the aluminum is inside the cells, into cells.

DR. HEM: How do we know that?

DR. GHERARDI: Yes.

DR. HEM: Who knows that?

DR. GHERARDI: I do.

DR. HEM: From what data?

DR. GHERARDI: From data you have -- from the IM injection in rats. After a few days you have no aluminum outside cells.

DR. HEM: I have not seen your data. We are trying to do an experiment right now with aluminum 26. Dr. HogenEsch described it. So I think we will

seen have aluminum 26 data that will answer the question does the cells take up these particles or not. So I am not aware that anybody knows that.

DR. GHERARDI: Okay.

DR. HEM: But we will assume it is true.

DR. GHERARDI: I will show tomorrow some pictures.

DR. HEM: Good.

DR. GHERARDI: And the second question -- so this is an important point. Second, I would like you to tell us about --

(Technical difficulties.)

DR. GHERARDI: -- such discrepancies from rabbit one to rabbit two?

DR. HEM: Bad bunny.

(Laughter.)

DR. GHERARDI: Do you think that bad humans exist too?

(Laughter.)

DR. TODD: Charles Todd, CDC.

Stan, we almost had a half life --

DR. MUSIC: I wish we had gone longer. We did this with Purdue money. If somebody will give us some money we will do it longer.

DR. TODD: Do you have any idea in people what the half life is in people or whether it would differ in children and adults?

DR. MUSIC: We have the tool to do that. So the reason I was excited when you invited me to speak here was to tell the world that those experiments now can be done but they have not been done.

DR. HUNTER: Robert Hunter, University of Texas.

Was their baseline level normal?

DR. HEM: Yes.

DR. HUNTER: How high is it above baseline?

DR. HEM: The baseline in bunnies of aluminum is 30 nanograms per ml and the increase was to 32. The average increase was for both -- to combine the four bunnies. The average aluminum plasma level went from 30 nanograms per ml to 32.

DR. HUNTER: The second question is the --

(Technical difficulties.)

DR. HEM: I do not like to work with animals so we did not collect feces.

(Laughter.)

DR. HEM: I will assure you that we did not. Urine was bad enough for a chemist.

DR. HUNTER: Once you get it ready for aluminum assay there is no difference.

DR. HEM: There is no difference. Okay. Richard did the work up so we did not look at the feces so I cannot answer that.

DR. GARCON-JOHNSON: I have two questions.

(Technical difficulties.)

DR. HEM: Yes, it was equivalent to the commercial -- with isoelectric point of around 4.5 to 5. So it had a lot of phosphate on it. If you had an aluminum hydroxy phosphate with less phosphate substitution I think it would be more soluble.

DR. GARCON-JOHNSON: It is nice when you just say it like that to have a nice balance at the end. Did you manage to -- never mind.

DR. HEM: I did not think of excising the site of injection and having Richard run through the AMS. So I wish I had done it and now that I see the interest I really wish I had done it but we euthanized the animals and we took all those organs that we described but we did not mark the site and we did not take the site so I wish we had done that.

DR. GARCON-JOHNSON: Okay. So you did not repeat the experiment to see how much was left.

DR. HEM: We will certainly be happy to do it if we do it again.

DR. GARCON-JOHNSON: You can do it on the goat, I guess, or whatever.

DR. HEM: Hmm?

DR. GARCON-JOHNSON: The goat you are using, you could do it on this one.

DR. HEM: The sheep.

DR. GARCON-JOHNSON: The sheep, whatever. The beast.

(Laughter.)

DR. HEM: We hope that sheep stays around for a while. We have got big plans for that sheep.

Thank you.

DR. MUSIC: Thank you very much.

Our next speaker -- we are going to change the program a little bit -- will be John Wheeler. John Wheeler is a toxicologist in the Division of Toxicology at ATSDR, the Agency for Toxic Substances and Disease Registry, which is not CDC but a sister agency of CDC. He works in the Office of the Assistant Director for Science on a variety of toxicity issues concerning hazardous waste sites.

HEALTH GUIDANCE VALUES

JOHN WHEELER

DR. WHEELER: I want to thank the organizing committee for having ATSDR speak to you today on some of the things we are doing.

(Slide.)

We are not doing or we -- this is new to us, anything to do with vaccinations except for maybe the thimerosal incident. So we have a kind of different perspective but I hope that perspective that we bring is something that you find useful.

Our experience has been mostly in the environmental field. ATSDR is funded by Super Fund and we deal with toxic waste sites so our experience with aluminum has been at toxic waste sites.

(Slide.)

What I wanted to talk about were reference values that ATSDR sets. Now there is a million definitions for reference values. Reference values can be references for instrumentation or they can be reference values for allowable daily intakes of there is many different definitions of reference values.

What I am talking about in respect to ATSDR are health guidance values that are used for screening environmental contaminants to determine if further investigation is warranted. So we derive these values and take them out into the field to examine what is going on in the field and screen samples with these values.

(Slide.)

The ones that are important in the field of environmental valuation that we use a lot, and there are some more additional ones than these on this slide, is ATSDR derives what they call "minimal risk levels" or MRLs.

The EPA has something that is somewhat analogous known as reference doses or if it is an inhalation exposure they are reference

concentrations. That is the amount that you can be exposed to for a lifetime without any appreciable risk to health.

Health Canada has something that is similar to that called "tolerable intakes and concentrations."

And our Division of Health Assessment and Consultation takes the MRLs and does exposure data on them and creates what they call an EMEG. So now you have an environmental -- it is an environmental media evaluation guide so they become media specific for soil or for air or for drinking water or whatever they are looking at.

(Slide.)

The way we got into this was essentially just a congressional mandate that we were to prepare toxicological profiles. I think most of you have seen toxicological profiles for priority hazardous substances and certain significant human exposure levels.

Now we are still struggling with what exactly significant human exposure levels are but minimal risk levels is our first effort to try to get to this. They were to be of acute, subacute and chronic health effects also.

(Slide.)

So we came up with a minimal risk level which is defined as an estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specific duration of exposure.

(Slide.)

The purpose of them is to serve as screening values so that when our health assessors go out into the environment they can get large amounts of data, of environmental data, of soil samples, of water samples, of air samples. They can screen this data rapidly and determine what they do not need to worry about.

Now an MRL is not a threshold of toxicity. An MRL sits way below a known threshold of toxicity so there is a grey area in between. If you are at an MRL or just slightly above that does not mean you are at a toxic value. But if you are below, we believe that there is -- you do not have an appreciable risk.

(Slide.)

MRLs cover oral exposures, inhalation exposures, and dermal exposures, and they do it for the three durations that were required by CIRCLA, which we have defined as acute, intermediate and chronic. Acute is any exposure less than 14 days. Intermediate is from 15 days to a year. And chronic we consider an exposure over a year.

If you look at the EPA values, the RFDs and the RFCs, those are chronic values. Those are chronic lifetime values. So that considers a 70 year exposure.

(Slide.)

The first thing we do to determine an MRL is to take a look at the literature and we do that primarily through our process of developing the toxicological profiles when we pull all the toxicity information we can find together about a given substance. This is an LSE table of -- for aluminum from oral exposure. These are all the studies that we have identified -- actually this is a subset of studies. We take the -- all the studies that we find and examine them for whether or not we would think they are well done studies, whether controls were properly used, whether there is problems with the studies. And the ones that we think that are well done we put into the LSE table and we group them according to the endpoint that they study.

As you can see here there is immunological effects, neurological effects, reproductive effects, and developmental effects. This is just part of one of the tables.

(Slide.)

From that we can identify NOAELs and LOAELs. NOAELs are no observed adverse effect levels and LOAELs are least observed adverse effect levels.

(Slide.)

We take that level which -- let me see if I can back up here.

(Slide.)

These that are in the open would be considered NOAELs. Those that are half shaded are LOAELs. We look for the highest NOAEL that we can find in a dataset or the lowest LOAEL. We take that number and divide it by an uncertainty factor and we call that the MRL.

(Slide.)

Unfortunately, that looks like a very simple deterministic approach that you can do quite rapidly but the uncertainty factors become quite a tangled web. You can quickly extrapolate down with some essential metals until the level is below what would be a recommended daily intake.

(Slide.)

So let me talk about some of the uncertainty factors. There is an interspecies variability uncertainty factor, which if we are -- if we have animal data and we are extrapolating the human data, we would use an uncertainty factor for that. That is

traditionally 10. However, in some instances we have used something less than ten.

Say that we have monkey data with an enzyme that we see being induced that is very similar to a human enzyme, we may use a factor less than 10.

Interspecies variability would be for within human variation. This is what we typically call a sensitive population and we are looking at effects to children or effects to elderly. We are looking for different genotypic expressions. Anything that we see in there and that factor is typically a ten.

We also -- if we cannot identify a NOAEL -- so we have a -- all we have is data that has some adverse effect associated with it. We use an uncertainty factor to extrapolate from a LOAEL to a NOAEL.

EPA will use another uncertainty factor for database deficiencies which is another factor of 10. These are all multiplied times each other so you can see that they get quite high quickly.

ATSDR traditionally does not use this uncertainty factor. We think if there is a database uncertainties that are that great, we do not derive an MRL.

And the EPA will also use an extrapolation across exposure duration. They will take a subacute study and make a chronic RFD from it.

The EPA has realized that there are overlaps between these different uncertainty factors, that there is a interdependence, they are not all independent variables, and so they have put a limit on their uncertainty factors of 3,000. You could see you could get to 100,000 here if you wanted to multiply ten times ten times ten times ten but they stop at 3,000. The largest we can have since we only use the first three uncertainty factors is 1,000.

(Slide.)

Dealing with uncertainty factors is certainly one of the most difficult issues. There are some things that have come to light in recent years or in the last ten years or so that we have been trying to use to reduce some of the uncertainty around these traditional factors of ten that are used.

One thing that we use is the human equivalency concentrations that are published with the RFC guidance. Those human equivalency concentrations are a database of information on extrapolating from animals to humans on inhalation studies. It will have both particulates and gas determinations so that you can make a dosimetric adjustment from an animal to human.

ATSDR also recently put in a computational toxicology facility and we have brought staff on board to do some computational toxicity testing. We think that with the pharmacokinetics based PBPK type of efforts that we can reduce some of the uncertainty. If you look at the WHO documents from '93 and '98, they suggest that the uncertainty extrapolating from animals to humans can be broken down into both pharmacokinetics and pharmacodynamic parameters, and both of those weigh about the same. So we think with good pharmacokinetics data that we can reduce some of the uncertainty there and we are working on that.

(Slide.)

And something that has been around for quite a long time but has not really come into this field until recently is providing benchmark dose modeling.

(Slide.)

With benchmark dose modeling we take a dose response curve. Let me see if I can use this.

If you look at this dose response curve, these are actual experimental doses. If you take a model and fit a model through that curve, this is probably a Wivel model. It is one that we found that is very successful at getting the low end of the curve. The Wivel model occasionally falls apart at the top end of the curve.

It will fall apart a little bit up here but since we are worried about the bottom end of the curve, it is a good model to use. You can take -- generate this curve and then you can generate the 95 percent confidence intervals around this curve. And by setting what is known as a benchmark response, a certain response that you would see in the population for the endpoint that you are looking at, you can extrapolate to the 95 percent confidence limit -- extrapolate down and find a benchmark dose from that level.

This has several advantages. One is that it uses all of the data to generate this curve. If you are doing the traditional NOAEL/LOAEL approach like I was talking about a minute ago, in this example you would have simply taken this point right here and called it the NOAEL and began your uncertainty divisions from that point.

But with the benchmark dose you can extrapolate in between points and get points that are not determined by the dosing that was done in the study. So you are using all the data and you are able to generate points in between.

The 95 percent confidence intervals also give you somewhat of a feel of how confident you are in the data and whether you have a bad dataset -- this -- line will move on up so your benchmark dose

will move down. So your benchmark dose becomes lower as the dataset becomes worse.

The benchmark dose has been studied and compared to the NOAEL/LOAEL approach and it is found that most of the time the benchmark dose approximates the NOAEL. So we accept it as a NOAEL and no longer have to use the uncertainty factor of a LOAEL.

(Slide.)

Sam is going to talk about, in a minute, the MRL for aluminum that we have derived and I hope that this has kind of set the stage of where we are coming from with the MRL and our health guidance values.

Here are some resources for where you can get information on different health guidance values. TERA, which is out of Cincinnati, has a good web site that you can -- that compares health guidance values across several different agencies. The EPA/IRIS database has all their RFDs and RFCs in it. And you can go to our web site and get all of our MRLs off of there.

Thank you.

(Applause.)

DR. MUSIC: This paper is open for questions.

DR. GHERARDI: There is a problem of the dose that has to be used to assess toxicokinetics for aluminum is an important problem. We use usually

small animals, rabbits of 300 grams or rabbits a bit more, and we frequently use a full dose vaccine and subsequently try to assess the kinetics. What dose will you recommend for such small animals to -- what --

DR. WHEELER: Well, Sam has got those numbers and I do not want to step on his talk but all these numbers are in milligrams per kilogram per day. So you have adjusted on body weight. You could certainly do that for surface area or something else that you found more appropriate.

DR. GHERARDI: That means that we should use a very small dose of aluminum adjuvant if we want to reproduce the human situation.

DR. WHEELER: That is correct.

DR. TCHOUNWOU: I have a question with regard to the benchmark dose.

DR. WHEELER: Okay.

DR. TCHOUNWOU: I know the reference dose, for example, EPA usually recommend that it should be based on the critical effects and in the development of the benchmark dose what effects do we base that on?

DR. WHEELER: For aluminum?

DR. TCHOUNWOU: Yes.

DR. WHEELER: We have not done that for aluminum because we have not identified that but for

many other substances -- we have only done benchmark doses on about six substances and several of those have been volatile organics and I believe two of those have been neurological effects and one has been a developmental effect. When we go through our initial procedure of that table that I showed you, the LSE table, you can identify which target organs are the most sensitive.

And if we have a good database, and we have a fairly good database with aluminum, you can then use those studies. If those studies are quantile data or if they are continuous data that you can change to quantile data, then a benchmark dose would be appropriate. But looking at endpoint is an important part of the whole assessment.

DR. TCHOUNWOU: I know for nonsystemic -- let's say carcinogenic effect, usually you do not have any such reference dose because of the effect but do you think for chemicals like arsenic, for example, where it has been recently recommended for the treatment of a certain type of leukemia, is it possible to develop a reference dose for such chemicals?

DR. WHEELER: A chemical such as what?

DR. TCHOUNWOU: Arsenic. Because on one side it is used in the treatment of certain cancer and on the other side it is --

DR. WHEELER: I do not know. That is a loaded question. I do not know if I could answer that.

DR. TCHOUNWOU: Okay.

DR. WHEELER: You know, this has been a debate among us as we derive MRLs even if the substance is cancer, a cancer causing agent. And the reason that we do that is most of the time a clean up around the site will be driven by the lowest number and quite often that is a cancer number. But in reality we have a lot of people that get exposed to acute duration exposures to carcinogens and they are not worried about the cancer at that time. They are worried about what are the acute effects that I am going to see from my immediate exposure and so we find that the MRLs provide a useful tool when we do that. And so we have MRLs for cancer causing compounds.

DR. MUSIC: I would like to thank you for making that pretty clear. I spent a couple of years as an environmental epidemiologist and the transition from infectious diseases to environmental epidemiology is not an easy one but you made MRLs and those reference doses very clear. Thank you very much.

DR. MYERS: I think that is true for most of us, too, Stan.

DR. MUSIC: Our next speaker is going to be Sam Keith. He is an environmental health scientist with ATSDR in the Division of Toxicology. He is involved in the development of toxicological profiles for substances such as radionucleids, uranium and aluminum or aluminum. The title of his talk as noted in your book is not correct. It will be "Toxicokinetics."

TOXICOKINETICS

SAM KEITH

DR. KEITH: Good afternoon.

(Slide.)

We at ATSDR, among the other products we develop, are toxicological profiles. We have profiled a number of -- several hundred substances over the years and most of what we look at are what we consider to be the three primary routes of exposure. Inhalation, oral and dermal.

A few years ago the powers that be had some insight that other routes of exposure may also be of interest and so we have started including other routes as information was available.

I just did not happen to realize that I would be one that would have a couple of profiles that were relevant from the, you know, transdermal injection route. One being uranium and the other aluminum.

And with uranium it is a military situation using depleted uranium penetrators that are shafts of dense uranium that is shot at tanks. And when discussing this with some toxicologists it was generally agreed that once the penetrator had penetrated the skin and exited the other side of the body it was likely that there would be some adverse health effects.

(Slide.)

With aluminum, with it being injected by a syringe, the situation is a bit more subtle. Be that as it may, aluminum, as uranium, is very prominent.

Aluminum is the third most abundant element behind oxygen and silicon, which means it just happens to be in every media that humans enjoy in taking into their body. It is in the air we breathe. It is in the water we drink. It is in the food we eat. And typically the intake for a day for an adult human is around 12 to 14 milligrams, which is a pretty substantial amount, but the uptake tends to be, you know, quite low.

(Slide.)

But how about the foods? One of my favorites, being from Atlanta, is cornbread. And if you notice, it is one of the higher ones up there. So I guess my intake may be higher than some who are

on a salmon diet but those of you who want to kind of balance things. Salmon and hush puppies work well.

(Laughter.)

(Slide.)

Aluminum is interesting in that it is always present in the ion stage as a trivalent ion. Aluminum was once thought to be, you know, very averse to any changes. You build aluminum buildings and they last forever but as acid rain happened it occurred that when pH hits around five and below aluminum dissolves. Aluminum compounds dissolve.

In the stomach acid aluminum dissociates from whatever ligand that it is associated with and hydrates to the hexahydrate. And it can recomplex with anything that is there with the original complexing ions or with carboxylic acids, lactate, citrates, whatever. But once it hits the intestines and the pH increases there is a great precipitation as sequentially three of the water molecules will deproteinate forming very insoluble aluminum hydroxide, which perhaps has an uptake factor of .01 percent.

So with low absorption why does this occur? Looking at the literature, it is not apparent that there is any active diffusion. Perhaps there is. Perhaps there is some. It has been suggested that transcellular and pericellular mechanisms are

involved to allow it to passively cross the intestinal wall. Some suggest that citrate somehow may mediate that and enhancing the absorption but it is not really clear what is happening.

(Slide.)

So solubility, human data, rat data show solubility and uptake. Citrate, lactate, nitrate are pretty high. And some of the others, oxides, hydroxides are pretty low, which is -- I guess that is pretty good for those who are heavy antacid users because their intake can be as high five grams of aluminum a day.

Once aluminum arrives inside the body what happens to it? Take an adjuvant, for example. How does it release itself from the site? Heimlich recently performed a study which made mock antigens and absorbed on to aluminum hydroxide adjuvant. He also took interstitial and serum proteins and absorbed them on to the adjuvants.

Then he took the adjuvated complex and the raw solution, mixed them, and he found that the aluminum -- the antigen quickly released from the adjuvant and the adjuvant bound to the interstitial or serum protein. And over half of that occurred in 15 minutes, indicating there is a way to release aluminum from the injection site.

Once it is released from that site there seems to be quite a competition between it and magnesium, calcium, phosphorus. And the first and last -- the magnesium and calcium is kind of interesting because they are divalent and aluminum is trivalent.

Once in the blood most of it seems to be bound to transferrin, some to citrate and other things. And once it is inside the blood several references cite different transfer rates.

The one at the bottom by Priest is an actual human study. It is an IV study using radioactive aluminum 26 citrate and it was found that over half of the aluminum transferred from blood to body tissues within 15 minutes in over 99 percent in two days, indicating there is a rapid transfer to other tissues.

So starting at the beginning there is a potential rapid release from the injection site of an adjuvant. Once systemic, there is a rapid transfer to bodily tissues.

(Slide.)

Rick Flarend has information out. So does Walker. Rabbits and rats, and they both tend to show the same thing after a reasonably short period of time. Bone seems to be the greatest depot followed by kidney and brain and muscle toward the end. One

might question the relationship with Alzheimer's, I guess, at this point.

(Slide.)

But from the Priest study, the -- after the injection inside a human, this was done first in one and then in several others, and they seem to support each other. Large excretion within 24 hours, initial half time of less than one day, 85 percent through 13 days.

But here is a critical one right here: 96 percent had been excreted through 1,178 days. And what does that mean? It means that there in the body is a depot that once it grabs on to the aluminum retains it and does not want to let it go. That depot is likely bone. But it also tells us that perhaps aluminum never reaches a steady state in the body but accumulates over a number of years. That seems to be what we find as the human body tends to accumulate aluminum in the lung from almost nothing at birth to perhaps 20 or 30 milligrams at a ripe old age.

(Slide.)

So the aluminum body burden when one is trying to figure out how it is retained, it is interesting that by measuring urine, feces and whole body monitoring, a curve was generated for human retention of a single dose following the format of

whatever dose it was, .354 times the dose times time in days to the -3.2 power. Pretty steady except for day one and day one under estimated it so in the graphs following that was accommodated.

Also, for steady state intake and uptake through the gut or whatever manner, a build up can be resolved essentially by integrating that function.

(Slide.)

Now how does this relate to humans? What we decided to do at our last meeting was really to take a look at infants and the vaccine dosing schedule, and what else they are exposed to. Typically one would expect the infant to contain a small amount of aluminum, perhaps a milligram at birth. Perhaps less.

But during the first six months what we are looking at is an intake formula or breast milk of 670 to 900 milliliters a day from day zero on to six months increase. With breast milk having a concentration of 40 to 50 micrograms of aluminum per liter. You know, a reasonably wide range, this 380 is in Croatian women, it is not really clear. It has not been resolved why that occurs, whether it is anomaly or whether it is something associated with their diet.

Cow's milk is a little bit higher and formula is even higher. Formula tends to be higher

perhaps due to the added ingredients that definitely contain aluminum as well as the process method in the equipment that does contain aluminum and a transfer in that process.

Then we assume for the second six months a published value of .7 milligrams of aluminum intake per day. And we were using an uptake factor of around .7 to .8 which for the hydroxide is .01 up to a maximum of around one percent so we considered that was probably a pretty reasonable value for available aluminum.

(Slide.)

And on this chart with the breast milk at the bottom because breast milk was -- has the lower concentration, using the previous formula for retention and incorporating into that a progressive intake of breast milk over time and a progressive growth of the child, it followed -- and this is logarithmic scale -- followed this and then here is the point at 180 days which we transferred to the .7 milligrams per day.

Now in nature what one would expect is some sort of transition unlike the uranium penetrator that penetrates the body. That occurs instantaneously. But when looking at formula, the higher level increases and the second part of this curve actually

is -- it is the same curve but on a logarithmic scale they tend to join up at the higher times.

(Slide.)

What does this mean? Well, as far as toxicity, the mechanism of action really has not been totally elucidated. Perhaps there is an interference with the second messenger system. An interference with calmodulin allowing calcium uptake in cells higher than it should be.

We do know that when aluminum binds to the larger proteins it tends to, as the protein is larger, it tends to bind more irreversibly, and it can inhibit the formation of neuronal microtubule.

Neurologically, from the studies we have reviewed, neurological seems to be the most sensitive health endpoint that we are considering for aluminum dealing with memory problems, fatigue, depression, behavior. A number of these in pot room workers -- aluminum workers that were dealing with aluminum fumes, those who were associated with aluminum vapors for many years, neurocognitive tests, psychomotor tests have indicated that some of these workers perhaps have a slower response to the various questions, delayed response.

It was recognized fairly early, dialysis dementia, that individuals with renal impairment put on dialysis developed a relatively nonresponsive

neurological dementia state. And it was identified that the very small concentrations of aluminum that was in the drinking water used to make the dialysis solution actually fed aluminum into the body and since aluminum bounds to transferrin and since it cannot be filtered in the dialysis equipment, there is allowed an increase -- the hospital was dosing the patients with aluminum tap water and there is no way to get it out and the result was the dialysis dementia.

That has been resolved because now there are standards for making sure that the aluminum concentration is extremely low.

We found some respiratory effects primarily in early days of programs in which pulmonary fibrosis was observed, an increase in the number of alveolar macrophages, also a decrease in the mobility of those macrophages.

But what we were seeing over and over again was symptoms that were indicative of dust overload from diverse inorganic dust. So it was not apparent that the aluminum was always toxic in that case. In other cases it appears that the aluminum was playing a toxic role.

(Slide.)

We recognize aluminum as a dermal irritant. We also immunologically recognize when there is a

vaccination if the nodule remains for more than about six weeks the body tends to achieve a hypersensitivity that can be identified in an aluminum chloride patch test.

Lesions in the tracheal or bronchial lymph nodes also can be immunologic in nature.

Then we have musculoskeletal. Many studies have found developmental problems associated with the skeleton, not so much as the muscles but the skeleton, osteomalacia. Pathological fractures where aluminum replaces or it competes with the phosphorus, either in not allowing the phosphorus to be taken into the body or competing with it at the osteon formation site.

Bone pain, also, which is a study from the U.K. A town had aluminum sulfate dumped in the water and there was joint pain but it was not clear whether it was related to the aluminum or whether it was associated with other high levels of metals with lead and copper.

(Slide.)

After looking at the full range of studies we had available to us, ATSDR developed a minimal risk level for aluminum based on the oral route of exposure, intermediate duration, ingestion with spontaneous motor activity interference in mice that were observed for periods of time.

And both horizontal and vertical movements produced a no adverse effect level of 62 milligrams per kilogram per day of aluminum. Uncertainty factors, three for interspecies and ten for human variability produced two milligrams aluminum per kilogram per day MRL.

And we are in the process in this effort of looking at the data and assessing whether an injection MRL is resolvable.

(Slide.)

So at the MRL level, two milligrams per kilogram per day, considering that the fetus starts from an average 50 percentile female weight of 3.2 kilograms at birth to around 10 kilograms at a year, the MRL curve follows this path, which is significantly higher than the intake due to either breast milk or formula. That is refreshing.

(Slide.)

But how does that relate to vaccines? Well, using the CDC vaccination schedule there is a range of times that hepatitis B and DPT can be given. Hepatitis B, the first dose is right about at birth before the child leaves the hospital.

And these are -- these can be in a range of times but what is interesting to do to represent perhaps the worst case is injection at specific points in time simultaneously. Looking at hepatitis

B, the typical formulation is .25 milligrams of aluminum. For DPT .25 to .85.

(Slide.)

So in looking at all of these, looking at the high dose, here is a curve for infants following this path indicating that the body burden for aluminum from injection from vaccinations is higher than from dietary intake. And for most parts of the curve, less than the MRL curve.

There is an overlap here at the very beginning, an overlap here. And when taken out and expanded, this -- these two curves merge around one or two days, and this one around less than one day because there is a quick release of the aluminum.

Yet how would the lower end of the doses find that curve? Overlap at a center period of time, no overlap here or here, indicating that by a years period of time the body burden of the infant may be equivalent to the dietary intake.

Now since these are on a logarithmic scale, if one was to add the diet to the vaccine for total added body burden, it should not vary very much from that line there. If it was on a linear scale it would be obvious that there was very little addition.

(Slide.)

And that is how we stand here on aluminum adjuvants, aluminum toxicity and thank you for the

opportunity to come down here during almost hurricane weather. Have you been outside? The last time I came in here I was sailing from Aruba up here and fortunately it was in a large boat and it was all like 30 to 40 foot seas and I am glad it was not quite that bad of weather when we were arriving although I think some of the individuals from the Northeast were delayed quite a bit.

But thank you for your attention. I appreciate the opportunity to be here. I find that some of the previous presenters, Dr. HogenEsch with his rapid effects of aluminum, Dr. Fowler with the stress protein response, which may be similar to the in vivo study initially, and Dr. Hem with his distribution, have really helped us a long way with looking at aluminum toxicity in an area that may have lost a little bit of its spectacular nature of years ago. But perhaps with new tools we can revive aluminum toxicity studies, toxicokinetic studies and pharmacology in a way that we have never been able to do before with perhaps aluminum 26 and ICP mass spec.

Yes?

DR. CHEN: Bob Chen, CDC.

Sam, in your review of the literature, there are studies looking at kind of different ages in let's say animal models, newborn mice versus kind of

older mice? To what extent is kind of developmental age an issue in these exposures?

DR. KEITH: Well, developmentally the weanlings are definitely more sensitive. If you have an adult whose skeletal structure is already developed and then there is an exposure to aluminum there should not be any effect other than over time there is not going to be any great adjustment other than if pathological fractures could result in the long-term.

DR. VERDIER: Francois Verdier, Aventis Pasteur.

Do you think that the positive -- the few positive patch tests with aluminum are sufficient to give the conclusion that aluminum can trigger a positivity reaction?

DR. KEITH: I think it is a start. We know that there is a response and when nodules -- after a vaccination if a nodule remains for several weeks, after that the person tends to be hypersensitive to aluminum. How that occurs is not totally clear in my mind and yet there appears to be some derived sensitivity to aluminum and aluminum compounds and so there is an indication that if aluminum is retained in the site and there is a response over an appreciable period of time there is some effect, some lasting effect, on the body. It may be that an

aluminum chloride patch test is not sufficient but it is indicative, I think.

DR. VERDIER: Do you know if there are aluminum product -- things like nickel because with nickel you have reaction with different source of nickel?

DR. KEITH: Well, my --

DR. VERDIER: Do we have such data with aluminum?

DR. KEITH: Well, I guess we do. There are some studies over the last few years about Alzheimer's in which Alzheimer's -- neurofibrilar tangles from Alzheimer's patients brains on autopsy were taken and stained and fixed and aluminum was found. And so now that told us, you know, throw away your aluminum pots and pans, especially if you are cooking spaghetti sauce. It has a low pH and dissolves it. What does that mean about aluminum pans too? I do not see any here. That is good. But what it also meant is in recent studies it has been found that -- well, when aluminum -- when glass bottoms are formed, glass bottles for reagents, the glass is formed around aluminum ingots and just as rubbing my hand across a table transferred atoms in both directions, aluminum is transferred inside the glass bottles. Reagents fill up those glass bottles and solubilize the aluminum. You do not even know

that you have aluminum in your reagent and so using ICP mass spec some of these tangles recently evaluated by a couple of researchers, aluminum was not found. And then after performing normal staining and fixing, aluminum was found. The implication was that perhaps the staining effects and process themselves could have contaminated the samples in some way.

So it is an equivocal situation here not totally resolved but, hopefully, with ICP mass spec techniques and more interest in aluminum, we can resolve some of these pressing issues.

DR. MUSIC: Dr. Halsey?

DR. HALSEY: Yes. Two questions. Neal Halsey from Johns Hopkins.

The MRLs that you are showing us are based upon the oral slow, same amount each day, and then you are calculating out the body burden, and you are showing intermittent dose. You expressed an interest in or a suggestion that you might have to develop these MRLs for injectable aluminum.

I am curious why you have not been able to do that or if the data are insufficient from the dialysis, the dialysis patients, where there was neurotoxicity? Why you were not able to use those data because you should be able to estimate the aluminum exposure in those situations? And I do not

know that would be any closer to what the intermittent exposure from the vaccines would be.

DR. KEITH: Well, to answer that question it is a developmental process right now because when the profile was developed we did not envision vaccinations being a prominent role and so we are currently looking into those matters and we already held the first series of meetings to derive this MRL and it is passed this first hurdle but it is still developmental in nature.

But to get back to the point of the MRL, this MRL was developed for an intermediate duration exposure period. In going back and taking a look at the available data, there is an indication that perhaps on an acute basis, a one time basis or over a period of a week or two, this -- the MRL is actually -- would be increased.

Looking at all the ASTDR MRLs that have been derived, all of the ratio between acute and intermediate fall in the range of three to 250 depending on the substance. So based on anecdotal information we just would suspect that this curve, if we adjusted it for acute intake, would be perhaps a factor of three higher.

DR. HALSEY: The next part of my question is taking into account any variability by age and is there any evidence of a difference in neurotoxicity

by age as there is with mercury and some of the others?

DR. KEITH: In the derivation we have included an uncertainty factor of 10 for human variability so we are -- that initial value integrated review of children, infants, elderly, you know, various population groups that may be more sensitive than those who are not renally impaired, for example.

DR. HALSEY: But are there data that there is an increased susceptibility of infants as compared to older individuals? Do you have any data that would point in that direction?

DR. KEITH: Well, as far as osteogenesis, I guess there is but, you know, in people of my age, you know, we have very little of that. But, you know, during developmental years, of course, aluminum can play a role in childhood toxicology that it may not in adult toxicology and yet in our various -- in our review process we did not find developmental effects occurring close to the neurological effect level so we were looking at primary neurological and it appears that the neurological is the more appropriate endpoint for computing a health guidance value for aluminum than developmental toxicity.

DR. GERBER: Michael Gerber, NIH.

You mentioned the substantial information about deposition of aluminum in the central nervous system of rodents and I was going to ask you about what we know as far as the deposition of aluminum in the central nervous system of humans. You mentioned in part about Alzheimer's. I wonder if there is anything else beyond the Alzheimer's information that you mentioned.

DR. KEITH: Well, it is interesting. I went to a paper yesterday afternoon in which they were looking at the bonding links and electrostatic charges surrounding various molecules and how various monovalent, divalent, trivalent cations might fit into those complexes, and I asked about aluminum, and he scratched his head and he said, "You know, that is really interesting." He said, "We just had not looked at it." And I said, "Well, why?" He said, "There just does not seem to be an interest in it but we have the capabilities. We could have run all this at the same time."

And there are so many interesting new tools that if aluminum can gain a new place in the research arena it can piggy back upon some of the other metallic studies that are being conducted.

For ICP mass spec I do not suppose it takes any more time to get an aluminum result than it does to get results for copper, cadmium, nickel, zinc,

whatever, although I think there are a couple of elements that there is some interferences that I saw a poster right here indicating hexapol. An ICP hexapol mass spectroscopy system that can adjust for some of the interferences that were seen with isotopes that had -- that when bound with the argon transporter gave the same mass as some of the iron isotopes.

DR. BAYLOR: Norman Baylor, FDA.

You presented in one of your slides a toxicity summary. Do you have any numbers on the levels of aluminum that it would take to reach some of those? Like, for instance, neurological, memory, fatigue, depression, what kind of levels are we talking about when we -- exposure -- are we talking about?

DR. KEITH: Well, in the mouse neurological effects became apparent in the range of 120 milligrams per kilogram per day. No effect was observed at the 62 milligram per kilogram per day level.

DR. FLAREND: Richard Flarend, Penn State, Altoona.

Can you get that slide back that is on there right now?

DR. KEITH: Did I mess up? Did I mess up on your work? I apologize if I did.

DR. FLAREND: No, just the slide that was up there at just time -- there you go.

DR. KEITH: Oh, okay.

DR. FLAREND: Okay. The red line representing the vaccine or adjuvant contribution --

DR. KEITH: Yes.

DR. FLAREND: -- to the body burden. It looks like you have pretty much put in a bolus dose and made all of the injection available to the body at the time of injection but according to the, you know, previous study that Stan Hem had talked about that we did, that injection is really spread out. It does not dissolve right -- I mean, it starts dissolving right away but it really takes several weeks to dissolve and so that would have been averaged out quite a bit.

Do you have a calculation that takes that into account and where that would put the red line relative to your MRL?

DR. KEITH: You know, I would like to get you and Heimlich together and see if this can be resolved because his study -- his was <u>in vitro</u> but it indicated a quick transfer -- a quick dropping of the antigen and a binding of the aluminum to interstitial proteins. It seemed to be very quick at least in solution. Most of it happened within 15 minutes.

I am not sure exactly what is happening here or what is driving that. All I can say is that it appears that there is a good mechanism for aluminum releasing itself from the site after the immunological response is initiated.

I guess in one of the previous studies it was identified that by clipping out the tissue it was found that after -- what was it? -- maybe three or four days, the aluminum that was still depoted at the injection site may not have been really useful for the vaccination purposes.

What I wanted to show here, between this one and this one was the drop in the red line indicating that perhaps going on the low side of the aluminum dose in the adjuvant perhaps is maybe an acceptable way of injection. In some of my readings it seems like once the minimum amount of aluminum hydroxide is there, if more is available, the titer increase is higher. So we are looking at both bound and unbound aluminum hydroxide to the antigen as far as releasing from the site and it is not clear, I guess, how much aluminum hydroxide you really have to have in order to achieve an acceptable titer.

But FDA has its limitations.

DR. MUSIC: We have time for one more question.

DR. BRAUN: Okay. This is a quick one. Miles Braun from FDA. Excuse me if I miss this but this MRL level -- is this something that is, you know, published and disseminated?

DR. KEITH: This book right here -- these are called Toxicological Profiles. This is Toxicological Profile for Aluminum. Now look it up in the dictionary. This is not a profile. It is more like a tome. It is a great door stop if anybody has problems with that.

The one on uranium and ionizing radiation in mercury and lead, the new ones, and dioxin especially, it is weighty. But these profiles started out in the 50 to 70 page region and they are now up upwards of 300, 400, 500 pages, and the reason is because through the years we are finding out our health assessors and the public really need more answers to more questions.

We have recently added a child health section to it. We -- like I mentioned earlier, they added an "other routes of exposure" section. Various things we have tried to do to enhance the usability and yet it has increased in size.

DR. BRAUN: Is that the answer is no?

DR. MYERS: I think most of them that have been requested is from the interagency group. Is that right?

DR. BRAUN: Oh, it is in the profile. So those --

DR. KEITH: As far as they were -- oh, yes.

DR. MYERS: More requests for profile came from the interagency group.

DR. KEITH: Yes, a lot from interagency group. We distributed a couple thousand of these. Just like for uranium and the military bombing range here. You know, we have had tremendous interest in the uranium profile both overseas, the European Commission, the European Union, the Royal Society in the U.K., Armed Forces Radiobiology Research Institute, Army, so there is quite a bit of interest.

Thanks.

DR. MYERS: Should we take a break at this point?

DR. MUSIC: I think that is a good idea. So my watch shows 3:18. If we can be back here at 3:40. Thank you.

(Whereupon, a break was taken.)

DR. MYERS: Our moderator is trying to reconvene us, trying hard. While everybody is going back to their seats, there have been a number of requests for copies of slides. All the speakers are, I know, putting together manuscripts for us for our proceedings but people specifically asked if they could provide copies of the slides and if you -- so,

speakers, if you have it no disk or have some easily accessible way, Lena or Theresa would be glad to make copies for us to put out tomorrow.

Thank you.

DR. MUSIC: Thank you. If we could lean out that door and close it so that the people out in the hall know that we are serious.

(Laughter.)

DR. MUSIC: Our first speaker this afternoon is Peggy Rennels, Professor of Pediatrics in the Center for Vaccine Development at the University of Maryland, School of Medicine, my medical alma mater.

She has a special interest in the development of pediatric vaccines it says here but that is clearly an under statement. She is a member of the American Academy of Pediatrics Committee on Infectious Diseases, better known as the Red Book committee, and is also a member of CDC's Advisory Committee for Immunization Practices, the ACIP. She is also the only voting member of both bodies.

Peggy?

EXTENSIVE SWELLING REACTIONS AFTER BOOSTER DOSES OF DTaP VACCINES

MARGARET RENNELS

DR. RENNELS: Thank you. Good afternoon.

(Slide.)

On behalf of my colleagues, the NIH

Supported Vaccine Evaluation Units, I am going to present to you an evaluation we did of extensive swelling reactions after booster doses of acellular pertussis, tetanus, diphtheria vaccines in young children. And my colleagues are listed here. This was published in the electronic pages of Pediatrics this past January.

(Slide.)

As way of background, it had become appreciated that rates of local reactions increased with subsequent doses of these diphtheria, tetanus subunit or acellular pertussis vaccines. And, in fact, there had been two reports of entire thigh swelling after the fourth or toddler booster dose of two different vaccines manufactured by the same company.

(Slide.)

And here is a picture of one of these children. For the biochemists who do not like biology, this is the abnormal leg.

(Laughter.)

This is a child who had extensive leg swelling after a fourth DTaP.

(Slide.)

This led to my doing a retrospective evaluation of severe swelling after the fourth and the fifth booster doses of multiple different DTaP

vaccines that were evaluated in the multicenter NIH sponsored trial. This was a unique trial in that it was a head to head comparison of 13 different DTaP vaccines evaluated in the same way with all of the serology being in the same laboratories.

(Slide.)

So this would afford an opportunity to determine the rates of severe swelling after these two booster doses. The fifth booster dose, for those of you who are not clinicians, is given just before school or at four to five years of age.

(Slide.)

We also wanted to ascertain whether the severe reactions occurred with different products and this was really the only database that could be used for that.

We also wanted to look at associated reactions and then explore the relationship between the rates of these swelling reactions and antigen contents, including diphtheria, which is why I was invited here.

We also compared the pre and post-dose levels of antibodies to the common components, which were pertussis toxoid, diphtheria toxoid, and tetanus toxoid in children who did and did not have entire limb swelling.

(Slide.)

Methods: Toddlers who had been given a primary series, that is at two, four and six months of age, one of 13 different DTaP's or one or two different whole cell DTP's received a fourth dose of the same vaccine. A fifth dose of the same vaccine was then given to children who were still available, which unfortunately was a small cohort by the time we tracked them down. Different vaccine was given at dose of four or five if the original one was no longer manufactured and available.

(Slide.)

Reactions: We asked the parents to measure in millimeter the greatest diameter of redness and swelling and report it on a diary card. Now, unfortunately, because entire limb swelling reactions were not anticipated, they were not prospectively looked for.

The comment section instead of each reaction form was afterwards reviewed for spontaneous reports of entire limb swelling and I did those and some of that required some interpretation but most of them were quite straight forward.

One quote was "thigh swolled (sic) up so big we could not believe it."

(Slide.)

Serology blood was obtained before and one month after vaccination and antibody assays, among

others, but specifically for this evaluation were pertussis, antibodies to pertussis toxin by ELISA, to tetanus toxin by ELISA, and to diphtheria toxin by variceal neutralization.

(Slide.)

And here are the rates we found: After dose four, of children getting the same DTaP, 20 out of 1,105, or two percent, had entire thigh swelling reported by the parents. The actual rate was probably higher because we did not specifically solicit it.

One out of 16 or 6.3 percent of kids getting this same whole cell DTaP for all four doses had entire thigh swelling. And, interestingly, none of the children who got the first three doses with whole cell pertussis DT and then boosted with DTaP had entire thigh swelling, and that difference is statistically significant. I think that is real.

Post-dose five, none of the 121 children who got five doses of the same DTaP had entire upper arm swelling reported. The fourth dose was given into the upper arm. However, four of 146 or 2.7 of those who got a mixed DTaP series because the first ones they got were no longer available did have entire upper arm swelling. And I think these differences are just because of small numbers. The difference is not statistically significant.

(Slide.)

Parents reported these reactions within the first 24 hours.

(Slide.)

And associated reactions: The children who had the entire thigh swelling versus those who did not in green, there was no more fever in those having these reactions but there was more irritability, pain and erythema.

But what I think is very interesting is that 40 percent of children were reported to have no pain whatsoever in spite of massive thigh reaction -- swelling reactions and 40 percent had no erythema.

(Slide.)

And those who were reported to have pain, most of it was mild. Three out of 20 children or 15 percent were reported to have severe pain. Meaning they cried when the leg was moved.

So, indeed, these reactions look worse than they are most of the time.

(Slide.)

All of the reactions resolved usually by four days and there were no permanent sequelae. There was no ulceration, no bow formation, no necrosis.

(Slide.)

We found no correlation between the rates of entire thigh swelling in either pre or post-vaccination serum levels of antibody to any of the toxoid in the vaccine. So it did not look to be an arthus reaction as had been reported in the past with diphtheria reactions.

(Slide.)

And one of the interesting and maybe most important observations was that entire thigh swelling was reported after dose four with nine of the 12 different DTaP vaccines studied and the ones where no swelling was reported, it has been detected in subsequent studies. So this is a phenomenon of all the DTaP vaccines.

(Slide.)

The different rates of the vaccines, post dose four, entire thigh swelling are shown here and because there was a suggestion of a difference in rates among the different products, we looked then at the concentration of antigen contents in the different products and looked at the rates of swelling.

The numbers were small and I would encourage you not to over read these rates. We do not know that the rates are different among vaccines.

(Slide.)

Now the rates of swelling greater than 50 millimeters was also looked at post-dose five, because remember none of the children who got five doses of the same DTaP vaccine had entire arm swelling. So instead we looked at greater than 50 millimeters and here are the rates of different vaccines here. The ones in white are U.S. licensed DTaP vaccines.

(Slide.)

Now the involved DTaP vaccines contained anywhere between one to five pertussis antigens. So if it is the pertussis component of the vaccine, it has to be the pertussis toxoid.

(Slide.)

Now the other common components of the vaccines were diphtheria and tetanus and aluminum. Shown on these graphs, which I doubt you can see in the back -- sorry -- are the percentage of children who had entire limb swelling after the fourth dose plotted against the quantity of the different common components. This is a regression line. Each of these diamonds represents one vaccine.

What you can see is after the fourth dose DTaP there is a suggestion of a trend for increasing rates of swelling with increasing rates of each of the contents -- quantities of antigens with a significant one being for diphtheria, a p of .02 for

the relationship between the rate of swelling and the quantity of diphtheria.

And this made great sense to me because we know we, in fact, had to decrease the quantity of diphtheria in vaccines given to adults because of excessive reactions and I thought, great, end of story.

(Slide.)

Unfortunately, we went further and I looked then at the greater than 50 millimeter swelling and these lesser degrees of swelling were not consistent.

(Slide.)

What we saw was that post dose four, greater than 50 millimeter, again you see a trend for an association with pertussis toxin toxoid, p of .06, but with none of the others. And here is aluminum, p of .66.

(Slide.)

Greater than 50 millimeter swelling after the fifth dose, shown here. This time the significant association is with a quantity of aluminum and that should be milligrams per dose.

(Slide.)

And just review, in slides that maybe you can see better, the relationship with a quantity of

aluminum, again should be milligram. This is entire thigh swelling after the first dose. The p is .72.

(Slide.)

Greater than 50 millimeters after the fourth dose. Aluminum association, p of .66.

(Slide.)

And after the fifth dose, greater than 50 millimeters, the p is .02.

(Slide.)

So, in summary, the severe swelling reactions were seen post booster doses of many DTaP vaccines. They are associated with other local reactions but fortunately severe pain is uncommon and it was amazing how unconcerned the parents were in those children who did not have a lot of pain. It is self-limited.

(Slide.)

The etiology is probably multifactorial because of the inconsistent statistical associations. I think those associations were probably due to small numbers and were statistical artifact. And that probably aluminum is one of the factors but not the only factor.

(Slide.)

Now a question that I should have said that these immunizations are given deep IM with a one-and-a-quarter inch needle that -- although I cannot

guarantee that some of it did not get given subcutaneously, there was not a concentration of reactions at any one of the sites suggesting one of the nurses was giving it sub-Q. And I mention that because there has been association with severe swelling reactions with aluminum absorbed vaccines when they are given subcutaneously.

Any questions?

(Applause.)

DR. RENNELS: Thanks.

DR. MYERS: Peggy, after the fifth dose the babies are bigger.

DR. RENNELS: Correct.

DR. MYERS: And the legs are fatter. So are you as confident on that dose about the sub-Q versus IM?

DR. RENNELS: Well, I think so because they are fatter but you give it into the deltoid and so I think it probably got there.

DR. ALVING: Do you have any idea of the mechanism of the chemical or biological or biochemical mechanism of why a sub-Q immunization would cause a reaction and an intramuscular would not?

DR. RENNELS: I do not know but perhaps there is somebody here in the audience who can

address that. In fact, the next speaker, I think, is going to.

DR. BRAUN: Miles Braun, FDA.

Was there any attempt to do multivariat analysis or were all those graphs univariat?

DR. RENNELS: They were univariat.

DR. GHERARDI: What was the imaging aspect of the thigh?

DR. RENNELS: The imaging?

DR. GHERARDI: Yes.

DR. RENNELS: We did not do any imaging. There is one report of imaging done of entire thigh swelling from that -- that the previous report, and it just showed diffuse swelling.

DR. GHERARDI: Do you know the condition? Is that well known by veterinary doctors of the painful resistant nodules in cats that are immunized with aluminum containing vaccines? This is a most important feature of veterinary pathology and even some aluminum ascites sarcomas have been described in cats.

DR. RENNELS: Well, certainly nodules, you know, do occur after some of these vaccinations. In these particular children that was not noted.

DR. HENDRICKX: Bernadette Hendrickx, SmithKline Beecham. I just want to bring some piece of information. We developed in the company a

special form for swollen limbs and we just received the first results where we have the results of more than 2,000 booster doses of DTaP combined vaccines, amongst which more than 1,600 with the hexavalent vaccine, DTP/IPV/Hib.

DR. RENNELS: Yes.

DR. HENDRICKX: And the results, although solicited, are completely in line with the results of your publications. We have 3.7 percent of swollen limbs, although it is solicited. And what is very interesting is that as you mentioned in your publication the grade 3 pain is very low. It is even lower than in your publication. It is six percent.

DR. RENNELS: That is great.

DR. HENDRICKX: So -- there is also no difference between the hexavalent and other smaller combined DTPa vaccines.

DR. RENNELS: Let me clarify. Was that doses four or five combined?

DR. HENDRICKX: Four. Only four.

DR. RENNELS: Dose four. Do you have the data on dose five?

DR. HENDRICKX: I have some slides with me and I have the form also that we used.

DR. RENNELS: Yes. Really my reason for looking into this and publishing it is not that I think it is a show stopper. DTaP vaccines cause much

fewer systemic reactions but I thought practitioners and parents needed to be aware of it, otherwise these kids may all get admitted to the hospital on i.v. therapy or thigh cellulitis.

DR. HALSEY: Peggy, Neal Halsey.

DR. RENNELS: Neal, yes.

DR. HALSEY: You did show in one of the tables that there were trends for some differences by manufacturer and you urged caution. But did you -- you did not tell us whether the aluminum adjuvant varies for these different DTA products. I have not looked at that. Is there a difference between aluminum hydroxide, aluminum phosphate and alum?

DR. RENNELS: I think -- and do not hold me to this -- but I think all but one was aluminum hydroxide but I would have to go back and clarify that.

DR. CHEN: Bob Chen, CDC.

Peggy, I am trying to reconcile kind of two bits of information that seems to be somewhat discrepant in my mind. In your study you showed that the rates with whole cell are, in fact, higher than --

DR. RENNELS: Yes, based on one trial.

DR. CHEN: Okay. All right. Sure. Okay.

With that caveat then, for the long time pediatricians in the audience, yourself included, and

presumably -- I do not know if you have spoken with Jim Churry (?) and his study, et cetera, is this just something -- a phenomenon that seems different with the acellular compared to whole cell or --

DR. RENNELS: It is hard to get a handle on that. Okay. I think -- in going back -- certainly it occurs with whole cell. We know that.

And, in fact, there was a Connaught whole cell product that was used a number of years ago in Canada that was associated with a lot of extensive swelling reactions. It was a Connaught whole cell DTP.

But I am not able to find much other than that in the literature. Now maybe it just was not paid attention to but certainly practitioners of my vintage I ask about it, they do not recall it being a particular problem.

DR. CHEN: And the reason I mention Jim Churry, in his large trial, and I do not recall him mentioning anything like that.

DR. RENNELS: But again, you know, if these children are not having pain, it may not get brought to the attention of the investigator or the pediatrician.

DR. CHEN: Might not but you would not think that so few of them --

DR. RENNELS: I agree.

DR. CHEN: Yes.

DR. RENNELS: I agree. Okay. Thanks for your attention.

DR. MUSIC: Thank you. The last paper before I open this up for discussion, general discussion, will be by Phil Pittman.

He earned his B.S. at Jackson State University and his M.D. and M.P.H. at Harvard University. He has a fellowship at NIH from -- or had a fellowship from 1984 through 1987 and at the moment is with USAMRIID at Fort Dietrich, where his current position is Senior Medical Scientist. And he is Chief of the Division of Medicine, Emeritus.

Dr. Pittman?

ALUMINUM ASSOCIATED ADVERSE EVENTS:
ROUTE OF ADMINISTRATION AND GENDER

PHILLIP PITTMAN

DR. PITTMAN: Thank you very much. I am waiting for this to load.

DR. MUSIC: The title of Dr. Pittman's paper is "Aluminum Associated Adverse Events: Route of Administration and Gender."

DR. PITTMAN: Great.

(Slide.)

I will present this talk in the following manner: First, we will present some background data

on the adverse events that we have noticed in the special immunizations program at USAMRIID.

We will then proceed to discussing data from the dose reduction route change pilot study that we conducted there. And compare the safety profile of the IM versus subcutaneous routes of administering the anthrax vaccine. We will then look at gender differences in adverse events and describe briefly the antibody response of these two routes.

And, lastly, we will go through a brief description of a planned pivotal study that we have with the CDC and the NIH.

(Slide.)

For those of you who do not know this, why in the heck to discuss the anthrax vaccine at an aluminum vaccine meeting. Well, the anthrax protective antigen, which is the protective component of the vaccine is absorbed to aluminum hydroxide at the rate of 2.4 milligrams per 0.5 CC dose.

The licensed administration schedule is rather hefty. It requires 0.5 milliliter doses given sub-Q, not IM but sub-Q, at weeks zero, two, four and at months six, 12 and 18. And annual booster doses are required as long as the subject is in an at risk situation. In our case at USAMRIID the at risk situation is working in a biological containment laboratory.

(Slide.)

I hope this is not a prelude to the rest of the slides but in any event what I have listed here is the frequency of injection site reactions by gender that we have noticed in the special immunizations clinic at USAMRIID. We have induration and erythema that occur at a rate -- of course, here we have male and female. The total number of individuals -- of individual doses is over 10,000, 10,722.

When we break it down by gender we have about 9,000 males and about 2,000 females.

And the rate of induration is two percent for males versus over six percent for females. A significant difference. For erythema the rate is about the same. For tenderness, again the rate is higher in females. And as well as warmth -- rash at the injection site is also more common but not significantly so in females. But other symptoms such as itching at the injection site is -- do occur more commonly among females as do lymph node tenderness.

(Slide.)

This is a look at the -- at whether or not the second dose in that series is necessary. As you recall, the immunization schedule requires a dose at zero, two and four weeks, and then Q6 monthly

starting at month six. And in this slide we look at -- unlike laboratory animals, which are nice to work with, with humans who have free will, they tend to come in to get their shots when they want to but we can use that to our advantage and in this case we looked at people who came in for their second dose at -- on time, that is at week two, and those who came in later at week three and those who came in at week four, and this is our antibody response.

There is an increased -- there is a trend towards increasing antibody concentration as the time between the first two doses increase. In this particular study we looked at the IgG antibody response two weeks after the second dose at each interval.

(Slide.)

On the next slide -- and so we are looking at it at a constant time. From week two we looked at the antibody response at a constant time from dose one and in this situation -- i.e. at week seven. In this particular situation we also saw an increase in the antibody response as the time between the first two doses increased.

(Slide.)

So we asked the following question: (1) What is the antibody response to a single dose of AVA? And then what is the -- is the two week dose

necessary? Can local reaction be prevented by administering the aluminum hydroxide absorbed vaccine IM rather than sub-Q? And is the gender effect real or are women more effective complainers than are males?

(Laughter.)

Well, that put me in the dog house for a couple of nights.

(Laughter.)

If real, can the gender difference in adverse events be prevented by IM administration of AVA? A slightly different question than this one. And what is the effect of doing -- of giving the vaccine IM versus sub-Q, i.e. is there more or less of an antibody response?

(Slide.)

So we planned a pilot study to look at that point and this was a prospective randomized study of healthy males and nonpregnant females. Both military and civilian volunteers were involved between the ages of 18 and 65 years.

(Slide.)

We looked at a total of six study groups and the control group, which is the standard licensed vaccine schedule, and this was the only group that received the six, 12 and 18 month doses of the vaccine. But the other groups received the vaccine -

- the study groups, either as a single dose given sub-Q or IM or as two doses given two weeks apart sub-Q or IM, or again as two weeks -- as two doses given four weeks apart at either sub-Q or IM.

(Slide.)

This slide shows the randomization process. The number -- the n in each group ranged between 22 and 28 and the mean age is listed here and that ranged between 32 and 35. There were no differences in either numbers or the mean age among the various groups.

(Slide.)

We looked at -- when we look at the adverse event by the IM or sub-Q route, in this case the number of doses given IM, 118, and this is the percent of individuals -- the number and the percent of individuals with an adverse event present.

This is sub-Q. The number of doses and the number and percentage of individuals with a given adverse event and the p value. There -- as one can see, as far as systemic events go, there are no significant effects -- a difference between the IM and subcutaneous routes of administering the vaccine.

(Slide.)

However, when we looked at the local or the injection site we do see a difference and these are listed here. We looked at tenderness, erythema,

induration, warmth and subcutaneous nodules. Even for tenderness there is a difference between the IM and subcutaneous route in this particular study as well as for erythema and for induration. And, also, for subcutaneous nodules.

This was quite remarkable. We saw absolutely no subcutaneous nodules when this vaccine was given IM versus when it is given sub-Q, in which case 38 percent of individuals experience subcutaneous nodules. The next slide, we will stratify further on these looking at gender differences.

(Slide.)

Because there were so few reactions in the IM route we will concentrate on the subcutaneous route because of time limitations. In doing so, when we look at subcutaneous nodules, again we looked at males and females, and the p value. For subcutaneous nodules, females had two-and-a-half times the rate of development of subcutaneous nodules as did males.

For erythema, again about three times.

And for induration, over ten times the rate of just redness at the injection site without induration.

So, I have to say that after this one would be pleased to inform people that women do, in fact,

not complain more than men, that the difference is actually real.

(Slide.)

Just briefly, I will go through the immune response. In this case, specifically the antibody response. And in this case we used a validated ELISA to look at the geometric mean, NTPA IgG concentration and the proportion of individuals with a detectable NTPA IgG antibody at peak. And peak in this case was about -- and the concentration used was 25 micrograms per milliliter or greater.

(Slide.)

Once again we see the schedule, a single dose sub-Q, the geometric mean and body concentration, and the percent of individuals with detectable IgG NTPA antibody. We can see that a single dose gives a very low antibody concentration and, of course, we have from 30 to 60 percent having detectable antibody after a single dose of AVA, the anthrax vaccine.

If you look at two doses given two weeks apart, the antibody concentration is higher than a single dose and every -- and the response rate by IgG concentration is 96 to 100.

When we increase the distance between the first two doses from two weeks to four weeks we see that there is a two to threefold increase in the NTPA

antibody and again the rate is 96 to 100 percent by IgG concentration and that these rates, both -- this is the control, zero, two and four. You can see there is not a difference between the geometric mean antibody concentration of these groups and the response rate again are the same as well.

So the second dose is not needed.

(Slide.)

I should say, too, if you simply look at titer, there is one person in each group -- in each of these groups that did not quite reach the 25 microgram per milliliter level but they did have a protective titer. So they did have some antibody. It is just that it did not reach the 25 milligram per milliliter cut off for the study.

(Slide.)

Another not so good -- these look better up here by the way.

(Laughter.)

Once again we looked at weeks through week 24 and the log IgG concentration. These two represent the single dose. The single dose given IM. Single dose given sub-Q and the standard zero, two, four dosage. It is not clear why there is a -- why this little second bleep occurs in this group but it occurs in both single dose, whether given IM or sub-Q.

Of course, this difference is significant so a single dose is not equivalent to the three dose schedule.

(Slide.)

This slide shows two doses given two weeks apart, IM and sub-q, and this is again the control. The axis are the same. But there is a statistically significant difference in the peak titer.

(Slide.)

This slide represents the zero-four groups given IM and sub-Q. And, again, the peak occurs at week six and there is not a statistically significant difference in the peak although the titer is higher for the zero-four sub-Q, as well as the zero-four IM, than is the zero-two-four but that difference is not statistically significant.

I should say also that the decline in the antibody is not different between week six and week 24. We have extended this now out to a year and there is no difference in the rate of decline. In fact, it goes back down to zero.

(Slide.)

So next we have planned a large study. This is a congressional mandated CDC/DOD/NIH cooperative study, which will be a perspective randomized double blinded placebo control multi-center study in which the endpoints will be safety, local and systemic,

gender differences and antibody responses. These things are written in the congressional.

(Slide.)

This is the study outline and I will go through this very quickly. What we are pondering over here is -- and the reason that I show this is what -- because we need the study to be double blinded, we need to give a placebo at week two. So the question is should that placebo be normal saline or should it be alum, aluminum hydroxide?

We will, of course, have aluminum hydroxide controls for both the IM and subcutaneous route for all doses.

But the question is in the study groups -- should the two week dose be aluminum hydroxide or should it be normal saline? And this schedule here kind of goes through it more clearly. There will be 260 volunteers in each group except for the placebo groups. Of course, the first dose given at week one will be vaccine for all five study groups. It will be aluminum hydroxide for the placebo groups all the way through.

The second dose is -- we are debating on which will be better and we hope to get some input from individuals at the conference as to whether if, in fact, that dose two was saline or aluminum hydroxide. If aluminum hydroxide, would it have some

effect on the immune response, whether positive, negative or neutral. So that will be very useful to get your input.

(Slide.)

So, in conclusion, what we can say is that without significantly affecting -- without a significant reduction in the GMC at peak there is a significant reduction in the local adverse events to the anthrax vaccine when the vaccine is administered by the IM route. Certain events such as subcutaneous nodules disappear completely when the vaccine is given IM. As well as a marked reduction in erythema and induration.

Since the IM route of administration is preferred for all other aluminum hydroxide containing vaccines, this may be a preferable alternative vaccination route for AVA, and it is the purpose for doing the pilot study and it is the reason for doing the larger pivotal study that is planned to begin with the CDC next year.

Thank you.

(Applause.)

DR. MUSIC: Dr. Pittman's paper is open for discussion. I have a question.

This vaccine used to be made at the Michigan Public Health Laboratory. It has now become a

private company if I recall, although it still requires public subsidy to keep it alive.

But the original work that was done by Phil Brockman on this vaccine, I think had lower reaction rates than you are showing, and did it have the same adjuvant or was it a different adjuvant?

DR. PITTMAN: Actually that was a different vaccine. This was a precursor to the current vaccine. The manufacturing process was different. You are referring to the Brockman pivotal study done in the '50s. Right. That was a precursor vaccine. Different manufacturing processes. One was anaerobic and the other aerobic, for example.

The current vaccine was noted even back then -- vaccine candidate -- to have about four times the amount of protective antigen than did the older vaccine. So there were a number of differences between those two vaccines.

DR. MUSIC: Okay. Thank you.

DR. PITTMAN: The adjuvant in his vaccine was an alum precipitated vaccine. Alum precipitated. Whereas this is aluminum hydroxide absorbed.

DR. MUSIC: Okay. We could begin in the back with Dr. Eickhoff.

DR. EICKHOFF: Ted Eickhoff, University of Colorado.

As you just alluded to, that is quite a dose of aluminum hydroxide in that vaccine. Could you relate it? Is that aluminum hydroxide as aluminum hydroxide or two-and-a-half milligrams of elemental aluminum as the hydroxide? And depending on your answer, could you relate that to what Norm Baylor told us this morning about maximum levels approved by the FDA?

The second part of the question is are there any data supporting the use of that high a dose of aluminum hydroxide?

DR. PITTMAN: That is a manufacturing question and I am lucky to say that I am not involved in the manufacturing process. But according to the insert in the literature it is aluminum hydroxide and I do not know the answer to the second question.

DR. EICKHOFF: If Norm Baylor is still in the audience perhaps he can clarify that.

DR. PITTMAN: I see a hand in the back there.

DR. EICKHOFF: Or John Grabenstein.

DR. GRABENSTEIN: John Grabenstein, U.S. Army.

The same -- the anthrax vaccine that the DOD is using meets all of the FDA standards that every other vaccine licensed in America does. So the 2.4 milligram quantity Phil mentioned is aluminum

hydroxide salt and it is the 0.83 or 0.85 elemental aluminum. The same standard as all the other vaccines.

DR. EICKHOFF: Thank you.

DR. BAYLOR: In the regulations it is elemental aluminum so that is a difference in the amount of elemental aluminum versus the amount of aluminum hydroxide. So it should, as was stated, it should not be any more than that.

DR. GHERARDI: You mentioned a pretty high level of systemic symptoms, including malaise and myalgias, about four or five percent. What was the duration of these symptoms? It was a long survey or a short survey?

DR. PITTMAN: We queried individuals five times over a one month period and all of the symptoms occurred within the first three days. We looked at them 30 minutes after vaccination on day one, two and three, one week later and at day 30. All of the symptoms resolved by day seven so that by day 30 we do not have any new or lingering symptoms except an occasional subcutaneous nodule but all of the systemic ones resolved.

DR. GHERARDI: Was this vaccine the same one as that was used for Gulf veterans or not?

DR. PITTMAN: Yes.

DR. GHERARDI: The same one.

DR. PITTMAN: The same. So we just added more experience.

DR. GERBER: Gerber, NIH.

In thinking about the gender differences and the local reactions when the vaccine was supposedly given sub-Q, because women have more subcutaneous tissue and less muscle mass in general than males, is it possible that a lot of those injections in the males were, in fact, IM?

DR. PITTMAN: We do not -- certainly none of them in the study were IM. We do not think that that is the case with the larger vaccination program. But the -- we use a half inch needle so we do not think that we are going IM for the -- for either males or females. But I think that you are probably on the right track, that body mass probably plays a difference. Especially the amount of fat.

Perhaps 0.5 might be a hefty -- more of a hefty dose for a female compared to a 100 kilogram soldier -- male soldier that is. So there could be some differences there.

DR. HALSEY: Neal Halsey.

You asked for advice about your placebo in your forthcoming trial. You are actually in a very unique situation to answer an important question. Since in your situation you do not have the usual problem that most of us do in clinical trials in that

you can get an abundance of recruits. I would encourage you to use both. You will have to increase your sample size but you could just in a random manner have the placebo recipients receive either a saline or an aluminum hydroxide adjuvant as the placebo and then you will be able to compare and see what is attributable to the aluminum hydroxide alone.

DR. PITTMAN: Actually that is a great suggestion and that is something that was brought up at the last meeting. I will take that back to the organizing group that we have some support for it.

Actually I had that idea in the group discussion so I think that you probably are correct.

DR. MUSIC: Dr. Chen?

DR. CHEN: A comment and a question. Some of you in the audience may be familiar with a study that Lisa Jackson at Group Health Cooperative of Seattle and us did recently which we were able to look at the gender issue in a different population of college aged students. While we initially were not intending -- we did not intend to do an analysis by gender in that study, what we did was we gave influenza vaccine, which does not contain aluminum adjuvant so it is an interesting comparison here, and we were comparing two different injectors and then regular syringe and needle administration. When we saw Phil's data and analyzed our data, to our

surprise there was this similar kind of male/female difference in that study in those results. So it is not -- at least we can say that the aluminum adjuvant is not an issue in this gender difference.

Phil, I have kind of turned this around in my mind multiple times before and this is perhaps as much a question for you as well as for Norman. It has never been clear to me given the current political situation with anthrax vaccine in the U.S., why is it that we need to wait until the pivotal randomized trial result before we move to routine IM administration of the AVA?

What is preventing us from doing that routinely? I mean, the p value is already less than 0.001. The GMTs were, you know, marginally different between that and the routine dose. Wouldn't we benefit a lot in terms of decreased public -- kind of, you know -- the recruits in terms of their complaints, et cetera, if we just shifted to IM based on the data that you have and perhaps just even submit that to FDA for their approval?

DR. PITTMAN: Good question. I think I will let FDA go first.

(Laughter.)

DR. BAYLOR: I really cannot --

(Laughter.)

DR. BAYLOR: -- answer that question, Bob. I mean, really I do not think this is the place to answer that question. It is just not appropriate at this time to answer that question. I will leave it there.

(Laughter.)

DR. MUSIC: I think you understand his situation but actually I think Bob Chen has a good point but I think the FDA has to respond to your initiation so if you were to make the proposal they could either accept it or reject it on its merits, and if they found it acceptable then we could proceed from that point.

DR. BAYLOR: They could ask for more information.

DR. MUSIC: Yes.

DR. PITTMAN: Clearly in the various forms, the vast majority of complaints are local reactions, and those could be prevented by using the IM route.

And without a doubt in my mind at least that would be the proper thing to do in order to decrease the morbidity associated with the use of the vaccine. I mean, we could save several thousands of people sore arms.

DR. GRABENSTEIN: Mr. Chairman, I would be willing to give you a semi-official Department of Defense answer to Bob's question and that is that the

Department of Defense has been criticized before for stepping away from the package insert and "experimenting" on guinea pigs, and we are very leery of going beyond the science -- going and assuming that the pilot study is the definitive study.

In fact, FDA has said to change the label we need to do the definitive study and so we are working on compromises to enable and empower clinicians to go to IM routes after you have reacted to an initial dose but we are in a conundrum of what the meaning of the FDA endorsed or FDA approved labeling means.

DR. YORK: Laura York from Wyeth Lederle. I just wanted to add to the male/female gender differences in that -- I am sorry I cannot remember who did the studies but I think they have been looking at intradermal administration of hepatitis B vaccine in people who do not respond and, in fact, the gender differences have been seen where females respond better to the vaccine. You will get response then. So I think there are definite gender differences we have to be considering.

DR. PITTMAN: Thank you. We have also noted gender differences in immune response with other vaccines as well. So that completely agrees with what you just said.

DR. BAYLOR: I just wanted to make a final comment on that. Of course the FDA is working

closely with the Department of Defense to work this out. I mean, at the current time of course we cannot support for any group off label use of a vaccine.

So it would require the submission of data and the evaluation of the data prior to making a change to the package insert. But it is not something that -- you know, it is -- we see the preliminary data but it is not -- we are going to have to review all of the data in a properly controlled study before we can make a final decision to actually change the package insert.

I mean, we -- your point, Bob, of the public outcry, if you will, we do not want to do something abrupt. We want to make sure that what is done is done no differently than the regulatory process for any vaccine.

DR. MYERS: I can understand Dr. Chen's frustration but I think he has to recognize that there are three actually options for the FDA. One is to accept. One is to reject and the second one is to ask for more information if the information is insufficient. So I think we probably ought to leave that topic.

(Laughter.)

DR. PITTMAN: Yes.

DR. MYERS: I would like to ask Phil just one question and that had to do with the total arm

swelling phenomenon with this vaccine just to link it with Dr. Rennels' presentation. Did you see in this preliminary study any total arm swelling?

DR. PITTMAN: We did not in the preliminary study but in the special immunizations clinic that occurs at a rate of about one in 1,500.

And let me just add that in further analysis of the bigger special immunizations clinic, which has been administering the vaccine since 1970, so we have 30 years of experience with this, and I have been doing it for 10 years myself, that even after one has had a significant reaction that does not predict a similar reaction to the next dose.

So, also, there are pretreatment methods that one can also use if one thinks that a person may have a significant reaction.

DISCUSSION: SESSION II PAPERS

DR. MUSIC: I was not an original part of the planning of this meeting so I am relatively unconstrained in what I can say about it. I was recruited as a last minute replacement for a moderator who could not be here but I really want to thank everybody who did organize it because it has been a very instructive day taking us from the very general to the very specific, and has set the stage for a lot of knowledge that did not exist in as widespread a fashion as it now is for some

intelligent questions, which I hope we will now get.

So thank you all for organizing such a great conference and I look forward now to some serious questions that will shed some light where there yet remains some dark.

DR. MYERS: Did you want to have the speakers come forward or do you want to do it like we did before?

DR. MUSIC: We have room for the speakers and I think that is a very good idea actually.

DR. MYERS: Maybe we can turn the lights up a little bit. That will give everybody a seventh inning stretch. I am not quite sure if somebody from AV could turn off our screen here and turn up our overhead lights. I would appreciate that.

While everybody is organizing, maybe I can go back to this morning's question. I asked this morning sort of a combined question that the preparation of a vaccine for submission to the FDA is a somewhat empiric formulation. That is a statement, I guess, based on sort of the answers that I got this morning.

And so I asked the question about do we need an adjuvant for any of the presently licensed antigens and a number of people asked me to re-ask the question because Fred Vogel gave an excellent answer in saying that it would reduce the number of

doses. It could reduce the number of antigens. But a number of people said, "Do we actually need an aluminum containing adjuvant for the presently licensed antigens?"

I make that point, of course, because some of the antigens in the future will probably undoubtedly be an adjuvant so maybe I could toss that question out again if that would be appropriate, Mr. Moderator.

DR. MUSIC: I think that is excellent because I do not think you did get an answer.

You got an answer but you did not get a definitive answer.

Does anybody want to respond? And have all -- I do not think all of the presenters are up here. Can we get everybody who did present this morning up here as well?

DR. MYERS: You want the whole group?

DR. MUSIC: Yes, I think so.

DR. MYERS: Well, Norman, should we just let you start on that question? Do we need an adjuvant?

DR. BAYLOR: I think it is more appropriate for the manufacturer to address that question since we are -- the FDA is not generally in the business of formulating vaccines. But I think it is going to depend on the antigen and I think it is obvious from the old data that some of the vaccines, like the

diphtheria toxoid, had much better levels of -- gave much better immune responses when those vaccines were adjuvanted. So again it is going to depend on the antigen.

DR. GARSON-JOHNSON: Nathalie Garson-Johnson, SmithKline Beecham. First, I would like to correct the statement you make. Formulation of vaccine is not exactly black magic. I mean, we do try to do something about it. I think for the current existing vaccines which are based on aluminum salt we should go back to the history of the aluminum.

One of the reasons why aluminum was added to the vaccine and there was essentially diphtheria and tetanus was because they were very reactogenic as a standard antigen. And aluminum was added to it because it was decreasing the reactogenecity. The main reason why there was endotoxin present in those vaccines that was decreasing it because of the absorption effect.

The next step was -- I mean, it was observed then that you could benefit from the carrier in decreasing the antigen dose. By decreasing the antigen dose you were also decreasing the antitoxin label and that was -- I mean, a Catch-22 situation where aluminum appeared to be a very efficient way to have the vaccine less reactogenic with the same

efficacy. So that -- and nobody answered that question really, the use of aluminum in vaccines.

But I think there is another thing, too, which is fairly important, is that one added benefit to aluminum, although you can question the amount, which is present in the vaccine, but nonetheless aluminum does stabilize the antigens and usually when you prepare a vaccine you would like the vaccine to be stable enough so that you can prepare it, release it, distribute it, and use it and that takes about two to three years so you do need aluminum for some vaccines in order to stabilize your antigen, and you do not have the variability and the efficacy of the vaccine over time.

So I think it is probably a big step to say that you have to eliminate aluminum. Maybe you can work on reducing it although you will have to make sure that reducing that aluminum content will not have any effect on the persistence of the immune response, which is another level of the vaccine. And only long studies will allow you to get the response for that. So it is an interesting question but I think we should think twice before jumping from all of it to none of it.

DR. MUSIC: I think that is a very reasoned and logical answer. If you change it, it is going to be something very different and we have what we have

and we are going to have to modify it slowly on the basis of data.

DR. ARMAND: Just to complement what was previously said, first, do not believe that all the vaccines are adjuvanted. Many vaccines are not. Polio is not adjuvanted. The flu vaccine is not adjuvanted. Some vaccines are not adjuvanted. The rabies is not adjuvanted. It is just when preclinical data are there to justify the use of adjuvant that we put it in with the antigen.

And as it was said, it was for reducing the dose of antigen. It is for stabilizing the vaccine. It is true for hepatitis A, for instance. Hepatitis A, there are just nano amounts -- nanogram amount of hepatitis A antigen in the vaccine and it is thanks to the adjuvant that we are able to fix it and to avoid the loss in the glass (sic) that we put some aluminum oxide or aluminum phosphate.

But we put adjuvant mainly because preclinical data justify the use of these products.

DR. KEITH: Could I make one comment?

DR. MUSIC: Please.

DR. KEITH: There is some literature out there about the use of aluminum adjuvated vaccines in the first dose but not in the boosters. That could possibly indicate that its presence in the first vaccination is extremely important to increase to

enhancing the titer but its presence in subsequent boosters may not have been fully assessed at this point and perhaps in the Department of Defense study maybe perhaps there would be an opportunity to test a group with the anthrax vaccine with nonaluminum adjuvated boosters to assess that in a human population.

DR. GARSON-JOHNSON: I can give you a very down to earth answer to this one. As Dr. Armand said, usually in vaccines you have a minute amount of antigen and if you are giving a liquid form you would like it to remain that way and not have everything stick to your syringe or to your vial before you give it. So that is the first thing.

The second very down to earth answer, and that was given this morning already, is that you can imagine what a nightmare it would be to have a different form for the priming and the booster. Usually you prefer to have the same vaccine, which is delivered -- the same formulation for the first and the second injection for obvious reasons. I mean, the -- it is not really making the vaccine, which is the most cost -- the highest cost of the vaccine. It is the release of the lot -- preparation of the various -- registering of the different form of the vaccines and you have to make sure -- I mean, can you imagine if somebody comes and wants to have -- forget

if it is as a second or the third or the fourth administration. I mean, how are you going to deal with that?

So usually it is much more simpler to have exactly the same formulation for all the injection rather than starting with or without alum.

DR. MUSIC: And in addition to all of those practical considerations if you had to have the existing refrigerator capacity in many countries, multiplied with yet another set of duplicative dose formulations, minus adjuvant, you would have to buy more refrigerators and you would quickly run into a whole logistical nightmare.

DR. ALVING: Carl Alving.

DR. MUSIC: Go ahead.

DR. CLEMENTS: Thank you. Well, one of the things that WHO is criticized for probably most of all is being slow to do anything and I think in this context it is one of its greatest assets.

(Laughter.)

DR. CLEMENTS: Having established some guidelines I think the attitude that we have certainly in the regulatory area is that you have to have a very good reason to start changing it again so having got as far as this with what we feel is working in the world market for these vaccines, while we are open to change, it would certainly need a very

strong and convincing argument to get us to change and at the moment I am not hearing it.

In fact -- whereas, I think some of you feel you have a big constituency in front of you in terms of if you are producers, you are producing many millions of doses. If you are part of the U.S. vaccine program you are thinking of millions of kids.

The constituency for the World Health Organization is in excess of 100 million children a year with three doses or more of DTP, which is 300 million doses.

And the last comment that you made is very apropos. When you do any change at all, however small, you multiply it by 300 million times and then you begin to understand that you have to be very sure of a change before you want to introduce it.

DR. MUSIC: Carl?

DR. ALVING: Carl Alving, Walter Reed.

I think it was pointed out before my talk that not only am I interested in adjuvants but I am also interested in complement and the biological effects of complement. I would like to make a proposal that, in fact, some of the adverse events that are being seen with the aluminum based adjuvants are perhaps based on the very thing that may make them effective in the first place. Namely one being complement activation.

Some of the symptoms that have been described a slight degree -- a tendency towards somnolence and other myalgias and so forth are classical symptoms of complement activation. And there are ways to inhibit complement activation.

I do not know whether this could actually be done but I would like to propose that perhaps when you are injecting subcutaneously maybe you are getting more complement activation than when you are injecting intramuscularly just because of the F- or if the complement activation is occurring there, maybe the target cells, the effector cells that are stimulated by complement activation are more numerous in the subcutaneous area. I would not be surprised at that.

But the fact that you see -- that sometimes there are some symptoms that are seen that are parenteral symptoms suggest the possibility that this could be tested by looking for split products of complement actually perhaps circulating in the blood, C5A or C3A or something like that.

I wonder if anybody who is knowledge, maybe Bob or others, who know about complement activation would be able to comment. I am just throwing this out as a proposal. I think it might be a -- at least bring some light as to what might be the cause of this and there may be other symptoms also. Other

things that could be affected by complement which has such a broad spectrum of events that occurs after complement activation.

DR. MUSIC: Bob Hunter?

DR. HUNTER: Carl is always my friend.

Rheumatologists measure complement routinely as a measure of arthritic rheumatologic diseases. What we are talking about here is primarily very localized reactions and it is conceivable if one had very sensitive methods you could pick up something with that but my suspicion is it can only be on the more severe reactions and even then it is going to be a borderline reactivity compared with what you see people with rheumatoid arthritis and lupus and the sorts of systemic diseases where we measure these things.

DR. ALVING: This could be looked at in experimental animals, though, couldn't it? Sub-q versus IM?

DR. HUNTER: One could do that, yes.

DR. ALVING: It is not in the nature of a question so much as it is a proposal.

DR. GELLIN: Bruce Gellin.

This is a question that you will recognize comes from someone who is naive in the aluminum subject since I still refer to aluminum foil as tinfoil. I think that it is not tin.

The question is really directed to the toxicologists. In helping us put the exposure to aluminum into perspective, we have heard the different sorts of aluminum. But it strikes me that particularly as we have begun to look at injection safety, the small percentage of injections that are immunizations, given all the injections, I think the number is five percent or something even smaller than that -- are there other sources of aluminum in injectables that are not vaccines?

DR. VERDIER: (Not at microphone.)

DR. GELLIN: Then the other question based on your description about glass is not always what you think about, and the thought that maybe aluminum might be leached from glass containing compounds. Would that be possible -- would it be possible that aluminum would be present in vials of injectables that are leached in the glass while they sit on the shelf prior to injection or did I totally misinterpret what you were saying about glass as a potential source of contamination being in a laboratory or otherwise.

DR. KEITH: I do not think you misinterpreted. I think the answer is unless we measure we do not know. One assumes there is no aluminum. Pick up a box of anything and look at the ingredients and there is no aluminum there. Does

that mean it is not there or does it mean it is a contaminant in one of the ingredients but was not recognized as being present or it is present in such quantities that it does not require labeling? I do not know the answer to that. I suspect that there are many reagents, many dietary products that contain aluminum where aluminum is not an ingredient listed on the label and yet it may be present in small or large concentrations.

So, yes, availability of aluminum is -- and since aluminum is the third most prominent element on this earth, it sometimes may be difficult even in analytical situations to preclude contamination of analysis results because of the presence of aluminum. Somebody mentioned that. In ceiling tiles,. What if something falls down into your dish. You have aluminum there. Where did it come from?

DR. FOWLER: I would just like to add to that. If any of you have worked in an analytical laboratory and you buy reagent grade chemicals, and you read the labels, typically they will say things like less than five percent lead, less than three percent aluminum. I mean, if they look for it. It is not that hard even in supposedly relatively pure reagents to get contamination from the supplier of these reagents.

In certain cases they will actually -- the semiconductor industry is a good example of this because they have to have ultra-pure things and they actually have a different category --

DR. MUSIC: They cannot hear you in the back.

DR. FOWLER: Okay. Can you hear that? I will try this one.

All right. The point here is that they have something called semiconductor grade, which is one cut above reagent grade. So you need to be careful particularly for the manufacturers when you buy something that says reagent grade. It may have more things in it than they think. Beware of that less than designation.

DR. GHERARDI: Stanley Hem told us that the kinetics of aluminum phosphate was different from that of aluminum hydroxide this morning and that aluminum phosphate was much more quickly than aluminum hydroxide. What are the comparative benefits of these two adjuvants as adjuvants under immunological point of view? Are they equivalent or not?

DR. MUSIC: That is a very interesting question. Do I have anybody who wants to take a first cut at that?

DR. _____: (Not at microphone.) You

are buying different antigens, right, that is what he said. You are buying different antigens depending on their charge. Their isoelectric point is different.

DR. MUSIC: Yes, their isoelectric point is different. They are different but the question is immunologically as an adjuvant, as a helper to immunization, are they different and how different are they?

DR. HOGENESCH: I am not sure that those studies have been done to compare aluminum phosphate with aluminum hydroxide as an adjuvant but one of the concerns is the absorption so if we would conclude that absorption is not critical then we can do those studies. If absorption is critical then you are sort of comparing apples and oranges when you are -- if you use aluminum hydroxide with lysozyme versus aluminum phosphate with lysozyme because one does absorb and the other does not absorb. So it depends on how critical absorption is in order to -- for aluminum to have its adjuvant effects.

DR. MUSIC: I would guess that we probably do not have the data to answer the question and it would require some pretty complex and very rigid studies to get the answer because you are essentially changing the formulation of a licensed product.

DR. HUNTER: I do not know much specifically about different types of aluminum but I have compared

lots of other adjuvants. I think that one -- it would be extremely difficult to get a general answer. You could say for this toxoid this was the better one. If you try and do another antigen you are going to start over again. And to get a real -- principles that would go across all of them, I think, is not realistic.

DR. MUSIC: I see Dr. Grabenstein coming to a microphone.

DR. GRABENSTEIN: It took me some time to process it but I thought of an example of a medication containing aluminum that a moderate number of people would have gotten and that is aluminum -- there is a -- you know, many people with hay fever get immunotherapy with allergen extracts. A subset of them get alum precipitated allergen extracts. A small fraction. It has gone, I think, into less favor than previously but assembling a cohort of them might give you some information if you needed it.

DR. MUSIC: I do not see any clamoring for microphones or questions. Do any of the panelists have anything that they would like to volunteer at the moment?

Marty?

DR. MYERS: Well, I would like to thank all of our speakers and our panelists and our moderators

for a great session today. I think everybody has done a great job. Thank you all.

We will stand adjourned now until tomorrow morning. We have a continental breakfast out here at 8:00 and we will reconvene promptly at 8:30.

(Whereupon, at 5:00 p.m., the proceedings were adjourned.)

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