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The meeting took place in Versailles Rooms I and II, Holiday Inn, Wisconsin Avenue, Bethesda, MD, at 8:00 a.m., Harry B. Greenberg, M.D., Chair, presiding.

Present:

Harry B. Greenberg, Chair
Nancy Cherry, Executive Secretary
Adaora Adimora, M.D., Member
Robert S. Daum, M.D., Member
Kathryn M. Edwards, M.D., Member
Mary K. Estes, Ph.D., Member
Walter L. Faggett, M.D., Member
Barbara Loe Fisher, Member
Diane E. Griffin, M.D., Ph.D., Member
Alice S. Huang, Ph.D., Member
Kwang Sik Kim, M.D., Member
Dixie E. Snider, Jr., M.D., M.P.H., Member
David S. Stephens, M.D., Member
Robert Breiman, M.D., Invited Participant
George Carlone, Ph.D., Invited Participant
L. Patricia Ferrieri, M.D., Invited Participant
Emil Gotschlich, M.D., Invited Participant
David Karzon, M.D., Invited Participant
Bradley Perkins, M.D., Invited Participant
Dr. Carl Frasch, FDA Representative
Dr. John Donnelly, Industry Presenter
Dr. Peter Fusco, Industry Presenter
Dr. Robert Ryall, Industry Presenter
Dr. Dace Medor, Public Comment

OPEN

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8:00 a.m.

DR. GREENBERG: Can we take our seats. If people take their seats, we can get started. We have a full agenda today and lots of people that need to get to the airport so I would like to get started.

One announcement that I have is for those of you on the panel who need cabs, some of you have found out that the red phone in the lobby is next to useless. If you could speak to Denise at half time, she can make a reservation for a cab if you need it. Any other announcements?

MS. CHERRY: The only thing I know is that I've heard that Dr. Eickhoff will not be joining us at all at this meeting.

DR. GREENBERG: Okay. That's a loss. Okay. I don't have any other announcements so we'll proceed to the first topic. This is Session 7, Use of Immunologic Surrogates for Demonstration of Protective Efficacy of Meningococcal Conjugate Vaccines.

Now, as you know, the short year or two that I've been on this panel, the question of surrogates and their use as correlates of protection is one that continues to surface and befuddle us. I am hoping that in the world of meningococcus it will look a

little clearer. Carl Frasch will introduce the topic.

I would like before we get started again remind the speakers if they can be as brief as possible, clear, and leave some time for questions within their allotted time.

DR. FRASCH: Okay. Regarding brevity I will try to be. As you know, the subject of today's presentation is the Use of Immunologic Surrogates for Demonstration of Protective Efficacy of Meningococcal Conjugate Vaccines.

The reason we are here today to discuss this is because with the development of the hemophilus meningococcal conjugate vaccine, for example, interest in other kinds of vaccines have increased greatly.

We have pneumococcal, meningococcal, group B streptococcal. Therefore, the interest is high. The FDA fully anticipates getting applications for a meningococcal conjugate vaccine of some kind probably within -- maybe within the next two years.

Therefore, in order not to give any misinformation to companies that would be interested in approaching CBER, we would like to bring this information before the advisory committee today.

Therefore, the purpose of today's meeting is to discuss the ability to use immunologic correlates

to demonstrate the effectiveness of meningococcal conjugate vaccines for the purpose of licensure.

A number of aspects relating to protection need to be considered: (1) meningococcal epidemiology; (2) nature of the antibody response; (3) how antibodies are correlated with protection.

Now, these aspects relating to protection will be covered in the following presentations. First, Dr. Bradley Perkins will present information on the epidemiology of group C meningococcal disease in the United States. And then relate that to what we know about epidemiology in some other countries.

Dr. Emil Gotschlich based upon his pioneering work with Goldschneider and Company on building some of the very first correlates of protection against meningococcal disease. We will discuss correlation of bactericidal antibody for protection against meningococcal disease.

Next, to show that we have really been working on invitro assays, both functional and binding, for a number of years Dr. George Carlone has come up from CDC to discuss standardized and validated immunoassays to major group C polysaccharide antibodies using bactericidal and ELISA methods.

Now, to show the advisory committee that

there is indeed interest within the vaccine manufacturers with group C or ACYW135 conjugate vaccines, we have invited presentations from manufacturers and we have received presentations from three of them. The first one is Pasteur Merieux Connaught. Dr. Robert Ryall will present for them. Second is the Chiron Corporation. Dr. John Donnelly will present for them. Then North American Vaccine and Dr. Peter Fusco will present for North American Vaccine.

However, this is not the entire list of companies that are working on meningococcal conjugate vaccines. We will hear more about that in a little while.

Therefore, with this brief introduction, I would like to introduce our first speaker, Dr. Bradley Perkins who will present the epidemiology of group C meningococcal disease.

DR. PERKINS: The first slide, please. Thank you, Carl. It's a pleasure to get to address this subject today. In the time allotted I'm just going to have an opportunity to give you a thin veneer about the epidemiology of meningococcal disease.

I caveated my assigned title slightly to indicate that it's really not possible to talk about

the epidemiology of serogroup C meningococcal disease in a vacuum without considering the other groups. I'm just going to hit a couple of items on the epidemiology in other countries that I think are particularly relevant to our discussions today.

This graph shows surveillance data in the United States for meningococcal disease from 1920 through 1998. Prior to the end of World War II there were routine large epidemics of meningococcal disease in the United States caused by serogroup A meningococcide. Since that time rates of disease have been relatively stable at an incidence of one to two per 100,000.

I've listed here some of the highlights in progress in meningococcal disease starting with the isolation of the organism in 1887, proceeding to the introduction of serum therapy, antibiotics, the discovery of the protective capability of the polysaccharide on down to the first conjugate that was developed and published in 1992.

During this time we have seen a steady decline in the case fatality ratio. You can see here the impact of serum therapy and antibiotics and presumably the progressive delivery of better medical care. We have leveled off over the last decade and a

half at a fairly consistent case fatality rate of about 10 percent. The total number of cases that occur in the United States is variable year to year, but in general ranges from 2,500 to 3,000 cases resulting in 250 to 300 deaths.

Since the introduction and success of hemophilus influenza B conjugates, meningococcal disease has become a much more prominent public health target for improved control. These are data showing the epidemiology of bacterial meningitis in the United States in the post Hib conjugate era.

You can see that neisseria meningitides has become the leading cause of bacterial meningitis among older children and young adults with, of course, strep pneumo the leader overall in a broader range of ages.

Although the rates of meningococcal disease have been relatively steady over the last couple of decades, there have been some important changes within the context of that incidence.

Let me flash to Europe at this point as I'm going to do throughout the talk and give you a little bit of a European contrast. These are data from a surveillance project that has been ongoing for a couple of years and show the first quarter of 1997 in Europe.

In contrast to the United States where the incidence is about 1 per 100,000, there are a number of European countries that have rates that are substantially greater than that. I think a number of you attended the meeting last week in Manchester in the U.K. where they highlighted the current epidemiology of meningococcal disease in the U.K. with overall population based rates of about 5 per 100,000, five fold the current U.S. rates.

Let me just comment briefly on that and say that those numbers may be augmented based on their use of PCR to confirm and classify cases of meningococcal disease in a way that we currently do not in the United States. What this shows is that since 1996 a number of their confirmed cases shown here in light blue have been confirmed by PCR only. We think this is good technology and we are working with them to evaluate this application in the U.K. and look at the possible use of this technology in the United States.

This slide shows a comparison of age specific attack rates of meningococcal disease in the United States and Europe and basically highlights the similarity between Europe and the United States with the general pattern of age specific attack rates with the highest rates being in children less than one year

of age gradually descending and then a slight bump here among older children and young adults, and then some increase in the elderly population.

I think everyone here is probably aware of the mechanism or the methods used for serologic classification of neisseria meningitides. Basically they are characterized based on immunologic characteristics of their capsular polysaccharide into serogroups and they are class II or III OMPs and class I OMPs for serotype and subtype designation so the nomenclature is serogroup, serotype, and subtype separated by colons.

The distribution of serogroup in the United States has changed remarkably through this last decade. In the period 1990 through 1992 you'll see that serogroup C and B roughly accounted for about half of the total cases of invasive disease in the United States at 40 and 43 percent respectively.

During that time period serogroup Y accounted for only 9 percent. By the end of this decade the rate and the proportion of serogroup Y disease in the United States had increased dramatically accounting for a full third of invasive cases of meningococcal disease in the United States.

We've done a number of things to try to

investigate the explanations for this. Thus far it's unclear other than the acknowledgement that there is cyclical variation in serogroup predominance which has been noted over the last 20 or 30 years.

A comparison between Europe and the United States reveals two facts worth noting. The first is that Y still represents a very small proportion of disease in Europe. The proportion of serogroup B disease is, in fact, about twice as large as the current proportion of serogroup B disease in the United States.

This slide shows the serogroup's specific incidence of meningococcal disease by age group. These are collapsed data from 1990 through 1998 in a very sensitive surveillance system we have in what the CDC refers to as our emerging infection program sites which are a number of sites scattered around the United States that do population based surveillance for meningococcal disease as well as other disease.

The points that I want to point out here are the predominance of serogroup B meningococcal disease in the less than one year population. Basically the equivalence of B and C in the one-year-olds and two to four-year-olds with some edging out of serogroup C in the older children and young adults which has

relevance to the issue of college students which I'll address briefly momentarily.

Finally, in the elderly population a predominance of serogroup Y disease that we've recognized over the last half of this decade.

One other point just to mention is that in the infant population contemporary epidemiology the rates of serogroup C and serogroup Y disease are basically equivalent.

It's important I think not to get swayed too much by those age specific attack rates in thinking about the numbers of cases that occur in groups. Because we are collapsing multiple years in those older age groups, the numbers of cases that occur actually out here on this end of the curb are quite substantial.

This shows the serogroup's specific contribution by age group to the cases of meningococcal disease that occur in the United States. You can see that the burden of serogroup Y disease out here in the elderly population is fairly pronounced, whereas C shown in yellow is fairly consistent across the board. Again, the disproportionate burden of serogroup B disease in the infant population.

This is a similar slide shown for deaths.

You can see that the actual number of deaths of meningococcal disease is actually concentrated out here in this population.

Another phenomenon that we have noticed through the decade is the increased occurrence of small outbreaks of meningococcal disease. These are outbreaks that include or result in the occurrence of anywhere from two to about 20 cases and have prompted an increased use of the vaccine for mass vaccination campaigns usually at a county level or at an organization based level such as universities or other close populations.

You can see that the geographic distribution of these outbreaks is fairly general across the United States and generally reflects the population distribution in this country.

As a result of those outbreaks, we have developed guidelines along with the ACIP for use by state and local health departments in deciding when to use vaccine to conduct mass vaccination campaigns. In brief, outbreaks are defined for organizations as two or more cases in three or fewer months reaching an attack rate of 10 per 100,000. For communities we have used three cases in less than three months with an attack rate of 10 per 100,000.

We've been monitoring the occurrence of these outbreaks in the United States fairly carefully. What you see here is an epi curve, if you will, of those outbreaks from July 1994 through July 1997 by serogroup, B in red, C in yellow, and Y in blue. What you can see if there is a predominance of the C outbreaks. We've had a number of B outbreaks. Since our mid time point here in the frame of reference, we've had four serogroup Y outbreaks. One has resulted in a vaccination campaign. All of those outbreaks occurred in nursing home settings – interestingly enough.

One other phenomenon that I think is important to mention is that during the 1990 through 1998 time period there has also been a gradual increase in disease rate among older children and young adults. You can see this here. The data are broken '90 to '92, '93 to '95, and '96 to '98. You can see that there has been rather consistent rates or a decline in these age groups but substantial increases proportionately in these age groups which has caused us some concern and prompted a great deal of interest in what was happening among college students in the United States. Primarily because outbreaks had occurred in that setting and a number of

people recognized the parallel or the possible parallels between college students and military recruits which have been routinely vaccinated with meningococcal disease since the '70s.

This concern prompted the American College Health Association, which is a group that represents about 60 percent of all colleges and universities that have health clinics associated with them, to issue the following statement that suggested that students consider vaccination to reduce their risk for potentially meningococcal disease and that college health providers take a more proactive role in providing information and access to the meningococcal vaccine.

This and our concern about the increased rates in these age groups prompted us to initiate a couple of studies in collaboration with the Counsel of State and Territorial Epidemiologists and the American College Health Association. Over the last year we have been doing supplemental surveillance for meningococcal cases in persons 17 to 30 years of age where we complete a supplemental information sheet about their college attendance status.

From 9/98 and 7/99 we identified 83 cases among college students. This is the serogroup. For

the 66 known isolates that occurred among those 83 patients, 50 percent of them were due to serogroup C meningococcide and roughly a quarter attributable to serogroup B and serogroup Y.

This is just a gloss view of some of the results of this effort. What we found is that compared to the normal population based rate for 18 to 23-year-olds, which in our active surveillance system is 1.3 per 100,000, college undergraduates were actually at less risk from meningococcal disease with an overall rate of 0.7.

As you march through freshman students and dormitory residents, the rates gradually creep up with the highest rate 5.2, a five-fold increase risk occurring among freshmen living in dormitories. These data and a complimentary case control study that was done also during this period of time will be the focus of discussions with ACIP on the 20th or 21st of October as they help to guide us about the possible need of guidelines or recommendations for vaccination of college students, particularly freshmen or freshmen living in dormitories.

I did not touch on the serogroup B vaccine development track but I will just mention as I close out the talk that most research has been devoted to

the use of these outer membrane proteins as potential immunogens for vaccines because the serogroup B capsular polysaccharide is not immunogenic in man.

As we see it, the current obstacles to improve control and prevention of meningococcal disease in the United States surround these five items. The current limitations in the performance of the currently available polysaccharide vaccine, most notably its inability to provide long-lasting durable immunity in young children.

We do not have a serogroup B vaccine at this point. The protection provided by the outer membrane protein vaccines appears to be strain specific and there is a high degree of strain variability both in the United States and Europe so that problem remains unsolved.

The outbreak control, although we think it's important we'll have a minimal effect on disease burden, secondary cases now with good chemo prophylaxis in the United States are rare. Other risk factors that we've identified such as maternal smoking or exposure to passive smoke, which contribute to a substantial proportion of meningococcal disease cases in the United States, are not easily translated into public health prevention modalities.

So we see as our current priorities the further development, licensure, and use of conjugate vaccines including some combination of those polysaccharides that have already shown to be effective.

We need a B vaccine that is appropriate for infants and toddlers as well. With these vaccines we would suggest that they be implemented into routine vaccination programs with catch-up at school entry among adolescence and young adults that we are already in the midst of trying to proactively address some of the issues about combinations of these vaccines; meningococcus with strep pneumo, Hib, or other combinations, schedules, and cost. I think I will stop there.

DR. GREENBERG: Thank you, Dr. Perkins. We have time for some questions from the panel. Kathy.

DR. EDWARDS: I was intrigued by the large percentage of B disease in the under one year of age. I really hadn't appreciated that. I thought it was more a third like the later ages. I wonder if you could comment just a bit more about the B vaccines. Is it totally inconceivable that a capsular vaccine for B could be generated or is there too much concern about cross reactivity with neuro tissue and those

issues?

DR. PERKINS: There are a number of people here that might be able to comment more authoritatively than myself on that issue. It is an active area of research currently within academia and industry looking at the concept of a modified or conjugated group B polysaccharide vaccine. It's not the leading approach and there is very little human data regarding the immunogenicity of vaccines using that approach. There is no clinical protection data at this point.

DR. GREENBERG: Dr. Breiman.

DR. BREIMAN: Brad, you mentioned the 10 percent case fatality rate. Could you comment on the severity of nonfatal sequelae and just the nonfatal presentations and how serious that could be. Is there any variability in that by serogroup?

DR. PERKINS: We've looked at that very closely recently both in the United States and in South America. About 15 percent of survivors of meningococcal disease will be left with some permanent sequelae ranging from relatively mild hearing loss or deficits based on IQ testing right up through amputation of limbs or severe mental retardation. We think that number is about 15 percent of survivors.

DR. BREIMAN: I was just wondering if there are any data on serogroup. I mean, is there any difference in the severity?

DR. PERKINS: The serogroup C right now in this country appears to be associated with fatalities more tightly than the other serogroups but that's not an incredibly tight association. In a recent multivariate analysis we were able to show that. As far as the sequelae profile, we don't see any variability in sequelae based on that.

DR. GREENBERG: Ms. Fisher.

MS. FISHER: Has there been any attempt to look at the common denominators of those who come down with meningococcal disease? Are they usually persons who are immune compromised or in poor health generally? You have a lot in the elderly group.

DR. PERKINS: We've done a number of case control studies in the United States both in outbreak settings as well as endemic disease settings. The proportion of meningococcal cases that are accounted for by persons with known immune defects is relatively small. Probably does not exceed five to 10 percent.

In that group I would include people with a known compliment disorder either functional or anatomic splenectomy are the leading causes. Some

people with some particular kinds with soft and blood cancers are at increased risk but the proportion that they contribute to the total is very low.

DR. GREENBERG: Dr. Stephens.

DR. STEPHENS: Two comments or two questions. The rates in Europe obviously are considerably higher than they are in this country. Some of that may be more effective diagnosis by PCR but not all of it. Can you comment on why you think those rates are consistently higher?

Second, there has always been this issue of why serogroup A disease disappeared from this country and hasn't returned in any significant way. Can you comment on why you think that happened?

DR. PERKINS: Yes. David Stephens, of course, a leading meningococcal world expert, challenging me with some tough questions. I think part of the explanation for the higher rates in Europe is hyperendemic serogroup B disease. Of course, as you know, a number of those countries have experienced relatively clonal epidemics of serogroup B disease. Some of the rates throughout that region remain elevated.

In Norway, for example, they had a serogroup B epidemic where rates reached about 15 per 100,000

population and have slowly declined. That process actually occurred over a 20-year period. Epidemics of serogroup B can be quite prolonged and hover in the hyperendemic range. I think that contributes a lot to the rates that we see in Europe.

The serogroup A issue, I don't think anybody knows. What I can say is based on some of George Carlone's work is that people in the United States do have high levels of antibody against the serogroup A polysaccharide suggesting that there is some cross protective antigen that is probably circulating in this population.

DR. GREENBERG: Dr. Daum.

DR. DAUM: I've always been intrigued clinically that at least simplistically speaking you can classify people who get meningococcal invasive disease as the overwhelming meningococcemia syndrome or a more indolent kind of meningitis picture.

I'm wondering with the very nice epidemiology that you showed whether there is any differences in age specific attack rates, occurrence of sequelae or mortality rates to be gleaned from your data regarding the different clinical syndromes?

DR. PERKINS: Yes. We've tried to look at that very carefully, especially with the application

of good molecular subtyping that we now have for all these isolates. We haven't really found anything conclusive linking molecular subtype or serogroup to any of those outcomes. What I can tell you is that case fatality by age does vary a little bit. Older children and young adults have higher case fatality rates than the young children. Basically among older children and young adults case fatality rates year to year can reach 20 percent.

We also see increased fatality rates when we look at our series of outbreaks. That series of cases actually has a case fatality rate up around 23 or 25 percent. Some of that may be reporting bias so we haven't hit that hard yet and we are still trying to look at that. That is what prompts the high degree of concern in this population because we frequently have previously completely healthy people that are in their so-called prime of life that get sick and die within 24 to 48 hours. Of course, that generates a huge amount of attention in the community and press.

DR. GREENBERG: Dr. Karzon and then Dr. Faggett.

DR. KARZON: First I would like to congratulate you and CDC and the state health departments for putting together a very excellent

picture of what faces us. But also you have presented a lot of dilemmas for the immunologists who try to divert these diseases.

Do you have information about immune responses? Is there such a thing as natural immunity? What does it look like? How is it acquired? Can you get B infection? Can you account for the particular distributions that you find immunologically?

DR. PERKINS: There's a substantial amount of knowledge about naturally acquired immunity, especially in early life. I don't know if Emil -- how much of that are you going to address, Emil?

DR. GOTSCHLICH: Well, perhaps the question will be better asked after.

DR. PERKINS: Okay. Yeah. I will let the world authority respond to that in the next presentation. There is a substantial amount of interesting information that actually is quite challenging to link to a lot of the epidemiology data that I showed. Maybe ask Emil after his talk.

DR. GREENBERG: Dr. Faggett.

DR. FAGGETT: Brad, I want to second Dr. Karzon's comment that it is a very eloquent study and presentation. In your slides you did document increase in the Y serogroup in the United States.

When you showed the one on outbreaks, it would appear there was less increase in Y and C was predominate. Can you explain that? Is that an accurate observation first?

DR. PERKINS: Yes, that's correct. There are a couple of things that are tough about Y. We know that Y is more frequently associated with respiratory disease than the other serogroups. Our current surveillance definitions don't lend themselves well to capturing serogroup Y in the absence of a positive blood culture. We are concerned that we may be missing some predominately respiratory outbreak of serogroup Y.

Y accounts for the minority of outbreaks but this is the first time that we have seen Y outbreaks in the United States among a civilian population. To have all those outbreaks occurring in the nursing home setting is quite an unusual and remarkable finding. The one situation where we ended up vaccinating for control of a serogroup Y outbreak in a nursing home, the median age of the population we vaccinated was actually 90. We don't know very much about the performance of this vaccine either against serogroup Y or among 90-year-olds.

DR. FAGGETT: Which is another immune

response, too.

DR. PERKINS: Yes. We did not look at that but it would have been interesting to know.

DR. GREENBERG: Other questions? If not, thank you very much, Dr. Perkins. We will move on to Dr. Gotschlich who will talk about immunity.

DR. GOTSCHLICH: Good morning. I have been asked to review the evidence that systemic meningococcal infection is dependent on the immunity and systemic meningococcal infection is dependent upon the presence of circulating antibodies to the particular meningococcus. I have been asked to focus particularly on the serum bactericidal reaction.

Before I begin I would also like to tell you that I take it as a given that it will not be possible to do a placebo controlled field trial of group C meningococcal conjugate vaccines. The reason for that is I think that the logistics of such a trial in terms of the number of children involved is simply formidable and it is confounded by the ethical problem of having available a vaccine which is at least partially effective which you could not withhold from the control group.

Thus, I think we are faced with the fact that we must, as Carl has mentioned at the beginning

of this session, arrive at a set of human immunogenicity standards that we will apply to these vaccines, that we will demand from these vaccines.

The question first of all is what is wrong with the current group C meningococcal vaccine? There are two principal shortcomings. The group C vaccine is poorly immunogenic in young children and the immune response that it engenders is rather evanescent.

Therefore, I believe that the central issue is to figure out what are the immunological characteristics of the conjugate vaccines which will address these specific deficiencies of the current group C polysaccharide vaccine.

What should these standards be? I don't think this is an easy decision. If we set the standards too high, we will find it will be very difficult to produce vaccines with a mandated level of immunogenicity and we will both delay the introduction of the vaccines and make them quite expensive or probably raise their cost.

On the other hand, if we set the standards too low, we will find out in Phase IV follow-up of these vaccines that there will be a large number of vaccine failures and that will result in a loss of confidence not only of that particular vaccine but

also of the establishment that promoted this vaccine.

Between those two choices, I must say I would prefer to make an error on the high side. Now, what can we marshall as evidence to allow us to arrive at such standards? Before I would like to face that, I would like to show you a slide that summarizes immunological tests that have been used to test antibodies to meningococcide.

Included on the slide is the first introduction of set tests. I have also included on that slide whether the test tests antigens of the whole microbe or whether it depends upon the presence of purified antibodies.

The reason that I've done this is to transport you back to 1966 and 1968 which is the time when Goldschneider, Artenstein and myself did some of the experiments which I will relate to you later that are the foundation for the evidence that antibodies are the determinant of human immunity to meningococcus.

If you look at this slide, what you find out is that ultimately the only test that you can apply to a large number of factors in 1966 is the bactericidal reaction. It is reasonably sensitive. It has no particular bias for any particular antigen. It can be

done in a relatively large number of samples.

Immunofluorescence was also available at that time but with the format that was used in those days having to be read by microscopy it was a very demanding technique and it was used by Goldschneider to confirm data obtained by the bactericidal test.

Finally, the high molecular weight group specific polysaccharides became available in the fall of 1967. At that time hemagglutination with sensitized red cells was widely employed. The gist of this slide is to show you that the matter of using the bactericidal test was a default choice.

What is the evidence that meningococcal immunity is antibody dependent? There are two principal aligns that I would like to tell you about. I guess I'll do the next slide.

This slide demonstrates the incidence of the disease versus age and the incidence of bactericidal antibodies versus age. The first two curves here demonstrate the age specific incidence for meningococcal meningitis in the United States for the year 1965 and 1966 respectively. Each point represents the number of cases between that point and the previous point.

As you can see, there is clearly -- actually

already shown by Brad today -- there is clearly a higher incidence in this particular age group. In order to obtain more specific information on the age distribution of cases within that group, a series of meningitis cases that was carefully studied at Children's Hospital in Los Angeles in the period of 1944 to 1953 was used. That is plotted in this line here. What this line shows you is that meningococcal meningitis is essentially an unknown disease in the first month of life.

At the time when the maternal antibodies in the child decline, the disease becomes manifest and increases to its maximum level in the period between six months to two years. After that time the incidence decreases again.

These lines here demonstrate the bactericidal activity found in sera of children or adults of that particular age. The data between zero years and 12 years is based on a set of 282 sera obtained from children. It indicates that at the time of birth the child is endowed with essentially the same level of antibodies that his mother was. These antibodies are lost over the first six months of life and that they are then regained slowly over a period of time by natural immunity.

Parenthetically natural immunity is believed to be essentially the immune response that is acquired by contact with either meningococcide of a lesser virulence or of organisms that contain capsule polysaccharides that strongly cross react. Quite a few of such organisms have been identified by the work of John Robbins.

This is the bactericidal activity against three prototypic organisms, respectively group A, group B, and group C. This particular pattern of age related incidence of disease and the inverse relationship of antibodies was also the one that was shown in 1933 by Fothergill and Wright for hemophilus B influenza disease.

Let me briefly review now the bactericidal reaction that was employed to get this data. It was a lipotiter format. The total volume was 200 microliters and it contained one quarter the test serum, either neat or diluted, one quarter complement, one quarter organisms, 500 CFU, and one quarter PBS. The reaction was incubated for 30 minutes and then one tenth of this mixture was plated to look for survivors.

In the instance where the only interest was to establish whether bactericidal antibodies were

present or absent and the intent was not to quantitate them by dilution and, of course, if the sera had been properly stored the complement was omitted.

The second major piece of evidence for the role of serum antibodies in immunity to meningococcal disease came from a prospective study done at that time. Nearly 15,000 recruits at Fort Dix had their serum collected during the first week of basic infantry training. The serum was stored at -70 degrees. At that time group C meningococcal disease was a very serious problem in that particular post.

By the end of the eight weeks of basic training of these recruits that were bled, there were 60 cases of meningitis. In 54 instances it was possible to obtain both the initial serum that was obtained at the time that the individual came to the post and the group C organism with which that particular individual was infected.

That particular strain of group C meningococcus was then also tested against 10 sera obtained from individuals from the same training platoon and again obtained at the time that they entered the post. The short of it is that only three out of 54 of the individuals who did get disease had bactericidal activity, whereas their platoon mates 82

percent of them had bactericidal activity to the particular strain in question.

I would say that in the interest of time I will not show you the additional data that this was absolutely dependent upon the absence of antibodies and no other factor. I will not show you the additional data that if yo followed these individuals prospectively within the group, you actually find out that an individual who lacks bactericidal activity and does encounter group C meningococcus has a 40 percent chance of getting the disease.

Finally, the success of meningococcal polysaccharide vaccines, the efficacy of them is clearly additional evidence that the immunity to this disease is antibody mediated. Just parenthetically this is the rule for all encapsulated organisms.

Okay. Now I want to reiterate the use of the bactericidal reaction to measure these antibodies. I want to reiterate that the choice was essentially imposed by the technology of the time. I would say, and I would particularly like to hear Dr. Carlone's remarks, if investigators today were faced with a similar epidemiological question and a similar opportunity, I think it is very hard to believe that we would choose the bactericidal reaction with all its

various faults and warts above all the others as the primary test. There is nothing magical about the bactericidal antibodies. This was recognized in the original paper from which I would like to quote. I'll show you the following quote.

"It is important to emphasize that the results of the experiment are not interpreted to indicate that the serum bactericidal activity per se is the protective factor in natural immunity against meningococcal disease. The serum bactericidal test was used only as a sensitive indicator of specific antibodies to meningococcide. Such antibodies may have other functions in addition to bactericidal. For instance, opsonisation or other nonbactericidal antibodies may play a role.

I have provided this background in order to counter the excessive adherence to the notion that bactericidal antibodies are the gold standard. We should remain open to using the much more readily standardized and quantitative tests that are now available to help us make the decision that we must make in terms of devising these immunogenicity standards.

So now let's shift to the issue of what I would recommend, what my first recommendation would be

in terms of immunogenicity standards. They would obviously imply a correlation between the immune responses seen in children and the efficacy of the vaccines.

The next slide lists the particular trials that I wish to discuss with you today. In the military in 1970 and 1971 two independent trials were carried out and they very clearly proved that the group C vaccine had a 90 percent effectiveness for a particular age group and for the eight-week period for which the recruits were followed.

However, it is pretty clear from a great deal of ancillary data, (1) that there has been no recurrence of the meningococcal problem in the military since that time; (2) from the equal efficacy of these vaccines in other military and similar type of institutional settings; (3) and also from the studies of Zangwill, et al., which demonstrate that the immune response of adults to the group C polysaccharide lasts for years. There is very little doubt that the immune response of an adult to a single dose of group C polysaccharide gives high level of immunity for several years.

When we come to children, there are three trials that I wish to briefly discuss and the dates at

which they were done. The first trial I wish to briefly discuss is the mass vaccination that was carried out in Quebec. They obviously had one of these major outbreaks that Dr. Perkins was talking about and it resulted ultimately in the immunization of 1.6 million people which was approximately 84 percent of the population. The target population that was immunized was the population of six months to 20 years. The follow-up period was approximately a year.

I would like you to note that the control group was the not-vaccinated group which is obviously a control that is not as good as placebo controlled. Nevertheless, with this shortcoming aside, the efficacy of the vaccine was very high and it was high in all the groups that it could be seen. Incidentally, this figure which is in your original paper is incorrect. If you recalculate it, it's 80 percent.

However, the data does not allow you to stratify the efficacy in the age group between six months to five years. It simply says that age groups had an 80 percent efficacy.

The next trial I would like to show you is one carried out by the CDC and it was typically one of the county-wide outbreaks. Over a series of three

vaccinations it resulted essentially in the vaccination of 36,400 people in this particular county.

In this instance it was a case-controlled study. The population that was vaccinated was the two to 29-year-old. As you can see, in this case-controlled study the effectiveness of the vaccine in the two to 29-year-old was 85 percent. In the two to five-year-old was essentially the same. Again, not sufficient data was available to stratify the efficacy. In other words, to determine at what particular age group is the vaccine effective and at what age group might it not be.

For that we have to turn to a study that was done in 1974 in Brazil where 67,000 children were vaccinated with group C vaccine and an equal number of children received tetanus toxoid as a placebo. In this instance you can see that the children in this particular age group had -- what I'm showing here is the incidence of disease in the vaccinated group and the incidence of disease in the placebo group.

As you can see, there was no protection in the six to 11-month-old children, nor was there any protection in the 12 to 23-month-old children. However, there was partial protection in the children

who were protected at the age between two and three years of age. Note that this was an 18-month follow-up.

What do we know about the immune responses of these children? This cohort of vaccinated children was examined at that time and was examined in my laboratory and is, therefore, comparable to the data that I will show you later that was also obtained in Connecticut. It was determined by radioimmunoassay. What it shows you is the immune responses of the various age groups and in bold is the immune response of the age group that did show some protection as compared to the children that are slightly younger which showed no protection.

I must say I have no idea how you would distinguish between these two means with these two standard deviations. There is, therefore, no particular way to look at the immune response in a raw way to distinguish between the children that had no protection and the children that did have partial protection.

What could the explanations for this be? We do not know at this point in time but there are two obvious ones. Either these antibodies are qualitatively different, and we will get to that issue

a bit later, or they are longer lasting in terms of the immune response because, after all, this is what you see at one month following immunization but it must persist for the 18 months in order to protect these children.

So the next slide shows you these same data. These two groups are the ones that I have focused on. This is the youngest group. This is the group that had essentially the same antibody responses as this group but was not protected. This is all the children.

I merely show you this scattergram simply because it allows us to now calculate the proportion of children that do have antibodies above one or two micrograms of antibody per ml. Again, these are the two groups I focused on. This is the group in which we saw no protection. This is the group in which we see at least partial protection.

So to arrive at the issue of immunogenicity standards, I would certainly say that this is a marginal response and one that clearly in quantitative terms we would have to ask the conjugate vaccines to exceed.

Let us look at the same time now at data that was obtained in Connecticut. What we have here

is a cohort of children, a rather large one, that was followed over a five-year period. These children received vaccine at the age of 24 months and this is the immune response one month later. It is somewhat higher than that seen in the children of about equal age in Brazil.

This then shows a decline of the antibodies over a period of time as has been noted before the antibodies are evanescent. This is what you would see if you do not immunize these children. This is the same antibody response of the children that are not immunized which showed a gradual increase in naturally acquired antibodies.

These children at the age of 66 months were revaccinated and showed this immune response. I think taking together what I have said before that there is absolutely no question that a child between the ages of four and five that is vaccinated with the group C vaccine will have protection for at least a period of one year.

Taking that together with this immunological data, and with the immunological data that has been presented by many others for this age group, and most recently by Maslanka and her associates in children in Montana using the ELISA test that has been

standardized at the CDC, I would say that this is where I would aim at in terms of quantitative immunogenicity for the conjugate vaccines.

Finally, I would just like to make a comment about the fact that I'm really looking forward to hearing the presentations on the conjugate vaccines that we are going to hear. I think all of us, particularly the manufacturers of conjugate vaccines, were a bit surprised that the quantitative immune response to the conjugate vaccines was not what we have seen with the hemophilus B. They are really quite substantially lower.

What is interesting is that the serum bactericidal activity that is engendered by the conjugate vaccines is quite high. In fact, it is entirely disproportionately high in relationship to the quantitative immune response. While I think this is clearly encouraging, I think it must be established what this difference is due to.

This difference could be do to isotopic differences in the immune response. It could be due to differences in favoring a high affinity immune response. I think we must establish why the D sera was really rather low. Total immunoglobulin levels have such high bactericidal activity. I believe that

when we do figure out what the mechanistic basis for that is, we will be much better prepared to write truly intelligent standards for immunogenicity for the conjugate vaccines. Thank you.

DR. GREENBERG: Thank you, Dr. Gotschlich. We have time for some questions. I would like to start out. You showed very nice old data from Fort Dix which very clearly -- I guess that was your data -- associated the presence of bactericidal antibody at a specific titer with protection in an actual setting. Are those sera available to be reanalyzed using any of the newer assays to see whether that very clear distinction can be obtained?

DR. GOTSCHLICH: No. In fact, they were essentially exhausted by 1968. I mean, we did everything conceivable on them.

DR. GREENBERG: Dr. Kim.

DR. KIM: In your slide which shows an inverse relationship between bactericidal and any activity and age distribution, you have shown high bactericidal activity at the time of birth against prototypes of strains A, B, and C. Can you elaborate on the nature of epitopes of bactericidal activity against group B?

DR. GOTSCHLICH: Okay. Against group B.

Well, let's first talk about group C and group A. There is very little doubt that if you take a gammaglobulin preparation and you absorb it with polysaccharides, you markedly reduce the bactericidal activity of that gammaglobulin preparation.

The second thing, and now I shall be responsive to your question, is it is not true that the group B meningococcal polysaccharide is nonimmunogenic. What is true is that you cannot change in a human being by immunization the natural titer of group B antibodies. When you inject the group B polysaccharide a very short, very evanescent immune response but it is extremely short. Literally it is just weeks.

But there are antibodies I believe, and there is evidence and some of the evidence was done years and years ago with Carl Frasch, that these antibodies are bactericidal.

DR. GREENBERG: Dr. Daum.

DR. DAUM: I have two questions, both of which are probably going to need to see the relevant slides again because they may have just gone by too quickly. The first one is in the Brazilian antibody data that you showed. I thought I understood that you said in children younger than two years of age there

was no protection based on the data.

DR. GOTSCHLICH: That's correct.

DR. DAUM: And in children two to three years of age it looked like there was from the data you showed. In looking at the antibody responses that strattled that line, 12 to 23-month-old children -- if we could put the slide on for a second, it would help a lot.

DR. GOTSCHLICH: I would be glad to.

DR. DAUM: I didn't see much change in the antibody response across those two.

DR. GOTSCHLICH: That is correct. You are absolutely correct. There was no way that the total quantitative antibody response could be distinguished. Okay. This was basically to tell you that there was at least a 50/55 percent efficacy. The next slide is these two particular lines and then the ones that I was focusing on and the ones that you are focusing on.

DR. DAUM: That's what I'm asking about.

DR. GOTSCHLICH: Right. You're right. There ain't no difference.

DR. DAUM: What would you like us to infer from that?

DR. GOTSCHLICH: What I would like you to infer from that is that merely a quantitative number

is not enough. We do not know what would have happened to the antibody levels of these children six months following this particular vaccination. This is four weeks following vaccination. We don't know what the antibody levels would have been six months later.

In other words, we do not know whether the kinetics of the decline is different with age. Nor alternatively do we know -- and that speaks very much to the data where the conjugate vaccines were unexpectedly high bactericidal activity has been seen. Nor do we know whether the quality of antibodies raised at different ages in terms of affinity or isotopic specificity is different. What I was trying to do --

DR. DAUM: That having been said, we can't come away with a protective correlate from that.

DR. GOTSCHLICH: That is correct. I came away telling you that I believe that this is definitely too low. What I finally came away with is that I think all the data clearly show that children four to five years of age that are vaccinated are protected. Let us look at what their immune response is and let us use that as a quantitative guide.

DR. DAUM: There's my follow-up question right there on that slide. That's the only other

thing I didn't really understand about what you said. As I understand these data, 66-month-old children receive one dose of an unconjugated polysaccharide vaccine.

DR. GOTSCHLICH: That is correct.

DR. DAUM: And then got a second dose of an unconjugated polysaccharide vaccine at 67 months.

DR. GOTSCHLICH: No.

DR. DAUM: No. Good.

DR. GOTSCHLICH: These children were vaccinated at the age of 24 months and then simply followed. At the age of 66 months this is the antibody level that they have. At that time they received an immunization and one month later this is what they look like.

DR. DAUM: This just reflects my lack of immunology knowledge. I thought that we thought about these kinds of vaccines as T-cell independent. That is to say, there was no booster response. Is that just a difference in response by age do you think? Or do you think that is an anamnestic response or can't you tell?

DR. GOTSCHLICH: Had one taken any other group of children that had never seen a group C vaccine, one would have gotten quantitatively the same

results. In other words, you are quite right. This is not an anamnestic response. This is simply the most convenient population where I could lay my hands on to make a nice slide.

DR. GOTSCHLICH: Alice, please.

DR. HUANG: The question that I have really follows on that which concerns reinfection and the extent of immunity after one injection. The fact that your Fort Dix population showed that there was consistent high-levels of bactericidal activity, is that just continued natural infection?

DR. GOTSCHLICH: Do that again.

DR. HUANG: I guess I'm wondering whether there are many cases of reinfection. That is No. 1. Also, how much protection does one have over time after a natural infection.

DR. GOTSCHLICH: Okay. You know, you come to the days here where it's preantibiotic days. Yeah, it is very likely that reinfection was extremely rare. There is considerable natural immunity that results from an infection. It does not have to be -- in fact, it usually is not -- it does not have to be a septicemic infection. It can be the carrier state. The carrier state is highly immunizing and is, in fact, what protects the majority of us.

I mean, you know, if you took this room and really challenged them with meningococcide, less than 10 percent of us would be able to get the disease.

DR. GREENBERG: Dr. Ferrieri.

DR. FERRIERI: Emil, in reviewing literature, have you encountered any data to suggest that the immunogenicity of a given vaccine, serogroup C, for example, differs according to racial ethnic groups or is there any evidence to suggest the response may be governed by one's genetic makeup?

DR. GOTSCHLICH: Undoubtedly our immune responses are governed by our genetic makeup. I don't think there are any very clean data showing that there are significantly important genetic bases of immune responsiveness or not to these particular polysaccharide vaccines.

DR. GREENBERG: Bob.

DR. BREIMAN: I have a question also about carriage. I'm assuming that the reason that we see outbreaks in institutional settings like prisons and college dormitories and military training centers has something to do with spread of meningococcide via people who are carrying the organism?

DR. GOTSCHLICH: That is correct.

DR. BREIMAN: And I'm wondering what -- sort

of reversing the question that you answered that Alice asked a moment ago, what would be the expected impact of these antibody levels on carriage and the ability to transmit?

DR. GOTSCHLICH: Okay. So to just back off one moment to amplify a little bit. You know, the living that meningococcus is trying to make is to live in people's throats. To cause disease is a terrible accident for the meningococcus. That clone is lost whichever way you slice it. That is what its lifestyle is. To answer your second question, studies have been done repeatedly and they have shown that immunization with the polysaccharide vaccine, both group A and group C, do provide for a relatively sort period of time immunity to the acquisition of the carrier state. Let me really say what that means.

That means if you take a set of recruits and put them into a situation, vaccinate them, and follow them very carefully as to how they acquire group C meningococcide, you will in most instances see a significant diminishment of the acquisition. You will not under any circumstances by a vaccination have a loss of the carrier state.

DR. GREENBERG: We have first Dr. Estes and then Ms. Fisher.

DR. ESTES: I have a technical question. Could you clarify for me what antibodies to what are you measuring in the RIA test versus the ELISA?

DR. GOTSCHLICH: Okay. The RIA test was done against the purified capsulate polysaccharide. The ELISA tests that are being done by Dr. Carlone, etcetera, are, again, true depurified polysaccharide. The bactericidal reaction, of course, is whatever the organism faces you with.

DR. GREENBERG: Ms. Fisher.

MS. FISHER: To what extent will the use of this vaccine in all children have an impact on future generations of the ability of mothers to transfer protective antibodies to their babies for the first two years of life?

DR. GOTSCHLICH: I think the best way to answer that question is to point out that we have not had essentially any group A meningococcide in the United States since 1945. Yet, all of us have protective levels of group A meningococcal polysaccharide antibodies which we acquired by contact with other organisms.

MS. FISHER: The same for group C?

DR. GOTSCHLICH: Yes.

MS. FISHER: So you are saying there would

be no impact? There should be no impact by the use of the vaccine?

DR. GOTSCHLICH: That's what I would predict.

DR. GREENBERG: I'd like to ask a question. You started off your discussion with saying that it was given that nobody could test the efficacy of a group C vaccine. I'm not familiar with this issue and I think I need to understand that given a little bit more.

One of the reasons you gave was that, of course, you now have an effective vaccine that would have to be given and it would be unethical. But I thought that the effective vaccine was not effective in one group, and that is children under the age of 2. Could you or could you not test the efficacy of a vaccine in that population since you don't have an effective vaccine?

DR. GOTSCHLICH: I believe that the logistics in terms of the incidence of the disease plus that ethical compounding factor would make it essentially impossible. However, I am not an epidemiologist so if somebody else would like to differ with that, that's fine.

DR. GREENBERG: I think this is a very

important point because it really goes to the heart of how critical it is for us to make a determination of immunologic correlates and I just didn't want to let it pass as a given without having somebody talk to the data on which that given is based.

DR. GOTSCHLICH: Okay. The only thing that I could add to that is that there simply was no stomach for testing the group Y or the group W135 vaccine at the time that they were introduced. They were accepted by virtue of the fact that they gave the same immune responses as A and C where efficacy data was obtained.

DR. GREENBERG: Carl, is this question going to be addressed by anybody, this given that nobody could never do an efficacy trial?

DR. FRASCH: I think Brad Perkins from his epidemiology standpoint could comment.

MS. CHERRY: Carl, could you get closer to the mike?

DR. PERKINS: Yes. I was not asked to directly address this but I think it's a critical question. It is something that we've looked at fairly carefully at CDC, and that is the possibility of doing a controlled trial. It would require a population much larger than the populations that have been used

to get Phase III data on both Hib and pneumococcus.

The most readily accessible large populations that we could conceivably do such a study are the emerging infection program sites where we do have very good surveillance. And with a population of something in the range of 30 million people which is the collective population that is included in those six to eight sites.

It could be over a three year period possible to do a controlled trial. The logistics and the cost of doing that trial I think are prohibitive. We have made an internal decision at CDC being that is not something we would advocate.

What we will advocate very strongly for our Phase IV case controlled studies, I think, with some nested immunogenicity studies both in the U.K. and in the United States as these vaccines are introduced.

DR. GREENBERG: Can I ask just one follow-up? That is since the vaccine is said to decrease the acquisition of carrier state, is this a marker of efficacy?

DR. PERKINS: I think that is a very important aspect of the potential of vaccine to provide herd immunity. In situation like Africa where one of your main objectives may be to prevent

epidemics in addition to prevention of endemic disease, that is a critical question.

In the United States I think that is a high bar to clear in terms of just clinical protection. I think it is very likely you would have clinical protection whereas you may not have protection against carriage. We have also looked at sample size calculations to evaluate the impact on the serogroup, specific impact of carriage in this country and think that it's probably not possible to do those studies in the United States either.

DR. GREENBERG: Kathy.

DR. EDWARDS: I have two questions. First of all, is it clear that capsular switches occur with meninge? I mean, if you're a C can you become a B and whether that will have any impact if there is widespread immunization?

Secondly, could you comment again about the immunity to type B. Is the immunity that is naturally seen specific for capsule or is it the outer membrane proteins that are conferring the bactericidal activity or is it known?

DR. GOTSCHLICH: The first question is yes, it is possible to do that switch in a laboratory. Yes, that switch has, in fact, been seen in nature and

it was such a lovely switch that it made it into national academy. However, it is quite rare.

The second thing that I would add to that is that in the military situation there has been plenty of opportunity for group B meningococcus to reenter the military population in terms of a problem. It has not done so. Keep in mind that group B was the main military problem in the early late '50s and early '60s.

There is no particular reason why if you prevent group C or whatever other meningitis, group Y or group A, there is no particular reason that now there is an empty nest that must be filled by group B.

Lastly, the immunity to group B meningococcide is controversial. My own view is that the capsular antibodies that are present have been underestimated in their importance.

DR. GREENBERG: We have three questions. First, Dr. Karzon.

DR. KARZON: The inverted bell-shaped curve which you showed is fascinating to me. It is very similar to a monotypic bell-shaped curve of, say, measles. A single disease, the antibody is high at birth, it rapidly falls by six or so months, then gradually goes up to a very high number.

It would suggest, as you have, that the antigens involved and the epitopes involved in that phenomena is a summation perhaps of all related organisms in meningococcal and perhaps other families.

It seems to me then that the antigenic recognition signs, the epitopes in this material could be sorted out and find the contributions to the individual ones, the cross-reacting ones, and their specificity. There are a lot of clues that you can get from this phenomenonology that you and others have shown.

DR. GOTSCHLICH: I would certainly say that even in the original papers that we discussed today, there was an effort made to determine what the antigenic specificity was within the frame work of that day. It was clear that a good proportion of the protective antibodies as determined by this bactericidal assay were to the polysaccharide but that they were also antibodies that could cause the bactericidal reaction that were directed at further antigens.

DR. KARZON: One could conceivably generate an antigenic profile which would cover any desired organisms that are targets at that time.

DR. GREENBERG: Dr. Stephens and then Dr.

Daum.

DR. STEPHENS: Emil, can you comment on the issue that is certainly in the literature regarding blocking antibodies and the potential issue of using the total ELISA in that kind of context?

DR. GOTSCHLICH: Blocking antibodies were described in a particular setting. Let me just briefly remind the audience what they are. It was found that sera that were obtained from army recruits that did not have bactericidal activity to a particular organism would show bactericidal activity after the IgA antibodies were absorbed. This is a fact that does occur. It is not, I believe, a terribly common fact.

The second issue is I'm not advocating adherence to the quantitative total immunoglobulin level as a standard. What I am advocating is that we use all the information that is available to us and do not fixate on the bactericidal reaction as being the only thing that measures effective immunity.

DR. GREENBERG: Could you just elaborate a little bit more on what you are advocating as opposed to what you are not advocating? I got it that we should not be fixated on bactericidal. I also understand that the specific level quantitative is

measured by radioimmuno assay or bioassay may not be perfect. Tell me a little bit more about what other information will be available to us.

DR. GOTSCHLICH: I think actually, if I may be -- I do not wish to avoid your question but I think it would be best if we asked Dr. Carlone.

DR. GREENBERG: Fine. Good. Dr. Daum.

DR. DAUM: Something that went by a little too quickly I would like to ask you to reframe for me. With the H. flu vaccines certainly the unconjugated capsular polysaccharide did not interfere with asymptomatic carriage, although the conjugate vaccines in some settings do.

I believe that you mentioned that unconjugated meningococcal polysaccharide vaccines did, I think you used the words, protect against acquisition of carriage. I would like to ask you to repeat what you said because, I'm sorry, it went by too quick. Also the comment on the mechanism by which that might occur.

DR. GOTSCHLICH: The experiments that were done were done initially in 1968 within this Fort Dix setting that I have already described to you. Within that setting in two of the --

MS. CHERRY: Sir, would you use the mike?

DR. GOTSCHLICH: I'm sorry. Within two of the training companies in which this experiment was done, the effect was approximately a 50 percent reduction in the acquisition of the carrier state. In an additional training company the effect was even somewhat larger. They were all statistically significant. They are not like what you would expect. They are not 100 percent effective. They are clear partial protection against the carrier state.

Similarly, all the other experiments where this phenomenon has been seen have been in that same general range whether they be with group C in the United States military recruits when this has been repeated, or with group A in Finnish military recruits where it has been repeated. It is not an absolute prevention of the acquisition of the carrier state. It is a partial one.

What might be the mechanism of this? The immune response to the polysaccharides does produce all classes of immunoglobulins. It is perfectly possible that IgA will occur in the secretions. What has been generally overlooked is the rule of IgG in mucosal secretions. There is a hell of a lot of IgG. In most instances there is more IgG than IgA and we have generally overlooked the biological significance.

DR. GREENBERG: Other panel questions?

DR. ESTES: I have one more.

DR. GREENBERG: Oh, I'm sorry. Both of you? Dr. Estes.

DR. ESTES: So you have told us about percent micrograms of total immunoglobulin. Have people measured total IgG and does that have a correlative protection?

DR. GOTSCHLICH: The isotopic antibody responses will be reviewed by Dr. Carlone.

DR. GREENBERG: Dr. Stephens.

DR. STEPHENS: A question also for Brad dealing with this issue of carriage. To my knowledge, I'm not aware of any data that is out there that has looked carefully at carriage with the conjugates. Can you help me with that? Is there data on carriage?

The other issue has to do with certainly strains that may not be expressing capsule and, therefore, will not be serogroupable but have the potential to express capsule can be found in a carriage state. Can you comment on that particular issue?

DR. PERKINS: Yes. There are very little data on the use of conjugate vaccines and the impact they might have on carriage. They have been

collecting some in one of the studies in Niamay Niger. They are collecting it, although I don't think we are going to have the sample size. It is recognized in the study that we are not going to have the sample size to make definitive conclusions.

We think that the only place -- I mean, in a closed military population it may be possible to look at some of the aspects of the conjugate impact on carriage but I think in the Africa setting carriage among older children in school may be high enough for serogroup A to actually do that study and we are planning to do that with one of the conjugate vaccines in Africa.

Now, extrapolating the results of serogroup A impact on carriage to the other serogroups may be difficult but I think it's unlikely we are going to find a setting that we will be able to look at the other serogroups directly.

What I do think we can do is some of the similar type of observational studies that were done with hemophilus influenza B and those were as the vaccine was introduced into the routine program, the impact on disease occurrence was out of proportion to what would be expected on vaccine efficacy alone suggesting that you were getting substantial herd

immunity.

I think those kinds of observational studies are possible and that indirect way is probably the way we will get most quantitative information about the impact on carriage in the United States.

DR. GREENBERG: Any other questions? Dr. Snider.

DR. SNIDER: Emil, no one has mentioned, or maybe I was asleep, the issue of the susceptibility of people with complement deficiencies C3, C5 through 9, to this disease. My question is what does that tell us about the kinds of antibodies that we ought to be looking for as correlates of immunity?

DR. GOTSCHLICH: You raise a very interesting issue which we should adumbrate a little bit more. That is that any instances where you see repeated either gonococcal septicemia or meningococcal septicemia it is almost invariable that you will find a late complement deficiency.

This does give you pause. There is no question that invitro you can get a very nice opsonic effect. There is no question that in vivo the polymorphonucleukocites of people with meningococcal meningitis or gonorrhea are loaded with organisms which they are busy killing.

Yet, there is this peculiar hint that maybe the serum bactericidal reaction is particularly important in niseral disease. I don't think we can give it much more credence than that.

DR. GREENBERG: Anymore questions?

DR. ESTES: Can I have a follow-up to that? If you look at the antibodies in people who are lacking complement in the tests that you've been talking about, are they behaving in the same or a different way?

DR. GOTSCHLICH: Generally those people will have bactericidal antibodies if you repair the particular specific complement defect. On top of that, those people do benefit from polysaccharide vaccination in terms of the recurrence of the disease. You can't prevent recurrence of the disease among these people with polysaccharide vaccination.

DR. ESTES: But in the ELISA or RIA assays were you measuring total immunoglobulin, people with complement defects?

DR. GOTSCHLICH: They will tend to be -- well, okay. Let me give you my impression rather than claim this as fact. They will probably have a normal immune response before they had the disease. If you took that particular serum and enhanced it with the

particular missing complement component, they would have a perfectly good bactericidal response.

DR. GREENBERG: If there are no other questions, I would like to -- are there anymore? No? Thank you, Dr. Gotschlich. We'll move on to our last -- actually we have a break scheduled and we are a little ahead of time. I would like everybody to take a 15 minute break. I would like you all to be back here at 10 of 10:00.

MS. CHERRY: But before you do, it has been suggested that we should ask any of you that are taking planes to call your airlines and check on cancellations. Apparently the hurricane is doing strange things to airline schedules all across the country. It would be wise that you do that now.

(Whereupon, at 9:36 a.m. a recess until 9:55 a.m.)

DR. GREENBERG: If people could take their seats, I would like to begin. I would like to make just a brief request of all the speakers and panel members. As many of you know, hurricane Floyd is making his way up the east coast. Evidently airlines are beginning to decompensate.

All things being equal, I would like to move this meeting along as crisply as possible since many

of us would not want to spend the night in the airport. This is a very important subject and we have to cover the issues but I would ask all the speakers and the questioners to formulate your presentations and your questions precisely and I'm not going to let people dawdle. With that in mind, we now have Dr. Carlone from the CDC.

DR. CARLONE: Would you cue me at 25 minutes so I can go right to my conclusions if I'm getting close?

DR. GREENBERG: There will be a hook.

DR. CARLONE: Okay. I'm going to talk a little bit about the laboratory issues that we are discussing today so this will be a very laboid type presentation. I'm going to talk about the standardized ELISA and the bactericidal assays for the measurement of meningococcal serogroup C.

Let me give you the overview first and then we will go back and talk about these things. First of all, the standardized assays can be used to predict immunogenicity just as Dr. Gotschlich said. What we are going to talk about today is the ELISA which is a finding assay. I'm not going to mention the RIA. We are going to talk about the bactericidal assay which measures functional assay. I'll refer to this

occasionally or most of the time as SBA.

Also, I think we are going to try to get at that immunogenicity can be used as a surrogate for vaccine efficacy, also what Dr. Gotschlich talked about today. We are going to spend most of the time on the laboratory type issues and the status of the current assays and what are they.

Well, first off, the ELISA which we will spend some time with. It is the method of choice. When I say method of choice, I mean it is the method that most people like to use for ease of measurement.

And we do have a standardized ELISA and I'll be drawing your attention to that because standardized ELISA means that it binds both low and high avidity antibodies. We will be spending some time on the details about this.

I will be talking about another modification of the ELISA called the high avidity ELISA. We will also be talking about the SBA which, as Dr. Gotschlich has told you, bridges to previous studies. What we need to do is we need to talk a little bit about the correlation of the ELISA with the SBA.

We see that the ELISA bridges to previous studies. It is a correlative protection. We need to make sure that the ELISA in this case is a valid

correlate of a correlate, if you will.

The other issue that we are going to talk about today, and I'll show you some slides, has to do with the complement source. This may answer some of the issues that were described or talked about earlier today. We will talk about some human versus the rabbit complement source.

Hopefully, the use of immunogenicity data will reduce or eliminate the need of efficacy trials, we hope, and permit vaccine licensure and public health recommendations. Hopefully, we will get to that last statement.

Let's just talk about the ELIZA and the SBA requirements. First off, the assays that we've chosen, both the SBA, have to correlate with protection. That was provided earlier by Dr. Gotschlich. The ELISA has to correlate with the SBA. Obviously if we don't have a correlative protection, which we do the SBA, we have no choice but to use clinical efficacy as the endpoint.

These assays also have to be very specific, reproducible, and robust. The robust part we'll talk about a little bit later. The assays have to be able to tolerate some changes in them.

Also, the very ability from a statistical

standpoint needs to be within, if you will, "acceptable limits." Brian Plikaytis has spent a lot of time on that from CDC making sure that we stay within these acceptable limits.

The last part is these assays also have to be generalizable. I think this is very important and I will come back to this issue as we go through with the graphs and table later. What I mean by generalizable is you can't have four or five of these so-called standard assays to measure various aspects of what we are looking at.

You can't have two or three ELISA assays to measure vaccines and different formulations. The schedules have to all be generalizable and that's what I will try to show today, that the ELISA and the SBA are, for the most part, generalizable.

When we talk about standardization and validation, we've done a number of things. First of all, we have standardized these protocols. Now, originally the protocols were standardized where everyone used exactly the same method and the exact same protocol.

What we found over the years is that they drifted away from using the standard protocol and we have gone to more performance based. That's where the

aspect of robustness with these standardized assays come in. You can make slight deviations and still have these assays perform optimally.

We also have looked at reagent standardization which is also another very, very important aspect obviously of any standardized test. We currently have reference sera that are produced or used for both the SBA and the ELISA. We have solid phase antigens. We have quality control, etcetera. These reagents are distributed both by the CDC and the NIBSC, National Institute for Biological Standards and Control at the U.K. for everybody to get those reagents if they choose.

Also, another aspect of the standardization, which Brian Plikaytis of the CDC has spent a lot of time on, is the data analysis standardization. You clearly need to use in these assays appropriate algorithms. You can't spend a lot of time pondering over the right antigens and tests and protocols and then put your data in a black box so to speak and accept the numbers.

We have spent a lot of time in trying to standardize these with this as well. All of these tests have been validated through the use of multiple laboratory settings. The standardized ELISA, let's

get to that.

MS. CHERRY: Would you use the mike?

DR. CARLONE: Is this not working now?

MS. CHERRY: I think the other microphone is fine.

DR. CARLONE: Is this okay?

DR. GREENBERG: Yes.

DR. CARLONE: Okay. We'll talk a little bit about the standardized ELISA that is being described in this talk for serogroup C. We developed that in 1994 in a paper by Gheesling, et al., in JCM. It was validated in a multi-laboratory study. In that study we had regulatory agencies that participated, manufacturers, Government agencies, and folks from academia. There were a total of 11 labs that participated in this validation study of the group C ELISA.

We found, as I mentioned earlier, that it did meet some of the criteria of the standardized assay. It was robust. It could tolerate slight differences in the protocol. The variability again was in acceptable limits as determined by Brian Plikaytis.

Also, these assays were used in many of the serogroup C immunogenicity studies. Just a snapshot

of some of those studies, here are some of the studies that are listed from 1994 to recently where this ELISA was used. Actually, the SBA in most of these.

If you'll take a look at that, one of the things you can see is we have used them in a wide variety of settings. They have been used on a U.S. population, an African population. We've looked at adults, toddlers, and infants. It has also been used on the polysaccharide vaccine and the conjugate vaccine. We have used these assays on a wide range of populations.

Now, let's get to some of the limitations of this assay. First off, the protective level -- the precise protective level is unknown. As Dr. Gotschlich said, it's been estimated to be approximately one to two micrograms per ml. This is very similar to what we see with hemophilus influenza.

Also the reference in the quality control sera that we used are from adults that have been previously vaccinated with the polysaccharide vaccine. This does not appear to be a problem at this point. Also, I don't think we have many alternatives. We clearly can't get reference here from adults.

I think it would be inappropriate to get reference here from an individual who has been

vaccinated with a particular vaccine formulation. We also can't get the sera obviously from toddlers that we would like to use it on so we have to use these reference sera for these standardized assays from adults.

Now, also this aspect of it. The antibody avidity which is important. The word came up a few times earlier today and we are going to spend a bit of time on that.

The standardized ELISA binds both low as well as high avidity antibodies. It's important to know why that is important because the low avidity antibodies can have no or low functional activity. This could affect the correlation.

We also had the high avidity ELISA and I'll talk about that. Only it selectively binds or favors binding of the high avidity antibody. This is by the addition of a reagent. This has been published in Granoff, Maslanka, Carlone, et al., all of this about the antibody avidity.

What we have found in this publication is that the high avidity antibody does correlate well with the SBA.

We'll talk a little bit on the next slide and I'll try to show you what this is. This is from

the Granoff paper. This is published.

We are looking here at the geometric antibody concentrations as assessed by the different assays. These are toddlers vaccinated with the Chiron vaccine. The way I have this slide set up is, first off, we have three panels. We have the standardized ELISA which measures low and high avidity antibodies, the high ELISA, and we'll look at the bactericidal test and how it relates.

Over here we have the geometric mean of the pre post-dose one, post-dose two sera. Then we have the P values that compare it in this direction. As we look across with the two vaccines that are being compared, the polysaccharide and the conjugate vaccine, we see in the presera that these antibody levels are very close to one another and they are not statistically different.

As you look at post-dose one and post-dose two you get a slightly different picture. You see here that the polysaccharide level is higher than the conjugate level but it is not statistically different. You look at post-dose two in these toddlers and you see that they are almost the same with a P value of .81.

Now, if we look at that in terms of just

looking or selectively looking or preferentially looking at the high avidity ELISA with the two vaccines, let's take a look and see what picture we get.

When we look at the prevaccination sera you can see again that they are very close and there is no statistical difference. However, when you look at the post-dose one and post-dose two, what you see is a decrease in the post-dose one and post-dose two with the polysaccharide vaccine. You can see that the conjugate vaccine stays pretty much the same here. This is higher most likely because you have much better binding of these very high avid antibodies.

What you see also is the P values now between here and here are statistically different, whereas they weren't here when we used the low of the standardized ELISA.

Now, let's look at how that compares to the functional assay. We have the bactericidal test for the polysaccharide in the conjugate. Here too you see that the levels are very similar and are not statistically different.

As you look at the post-dose one you see a relatively low level here and a lower level here with the conjugate vaccine. There is a statistically

significant difference. Look at this difference over here. What you see is a very low level of the bactericidal test and a very high level of bactericidal with the conjugate vaccine.

This pattern clearly more closely mimics this with the high avidity ELISA than it does with the low avidity or standardized ELISA over here. This kind of gets the stage as a snapshot of how that works.

Now, let's talk about the geometric mean concentrations of the IgG high avidity as determined in infants after conjugate vaccination. This was from Borrows, presented by Ray Borrows at the meningococcal workshop in 1999 in Geneva. The way I have this set up is I have the IgG levels over here and the bleeds over here. We have a prebleed post-dose one, post-dose two, and three, prebleed, and post bleed.

We are looking at it with two of those same tests. We are looking at the standardized IgG test, which I've labeled as No. 1, the high avidity IgG test which is No. 2. What you see here is the median values which is the large bar and the confidence intervals over here.

As you go across and just kind of look at the picture, what you see is using the high avidity

and the low avidity test as you go across, you don't see much difference in the pre. This is on a log scale by the way.

You do see a considerable difference over here on the post-dose one and not much on the post-dose two, post-dose three. Maybe a little difference on the prebleed and then the post booster, prebooster. Then the post booster you see that you get a nice level here.

So it's the post-dose one in this case here where you see some of the differences. You have the standardized ELISA that gives you a higher absolute value. Some of those values as based on preferentially measuring it with the high avidity ELISA you get a lower level.

Again, as post-dose two and post-dose three in this case, there is really not a difference whether or not you measure the standardized IgG or the high avidity IgG ELISA.

So let's talk now about the bactericidal assay. After this I'll get back and try to put all these things together so you can see what the interplay is.

The standardized bactericidal assay that was published in the WHO Technical Report Series, 1997.

It was the recommendations from meningococcal polysaccharide vaccines. It did include an SBA protocol at that time.

In 1998, or actually a little before, we decided that we would look back at this assay because, as Dr. Goldschneider said, this was set up with the technology at the time. We went back because we knew we could miniaturize and so forth. We asked the question do we still get the same results and performance when we use our modifications or compare this to what was published in 1976.

We looked at the critical parameters and we evaluated a number of aspects. In particular, which I'll talk about in a few minutes, is the complement source. What we found in this assay is that we did have a variability of plus or minus one dilution within a lab and plus or minus two dilutions between a lab.

For all intents and purposes there were really little differences other than volumes and a few other very minor aspects of the original paper that was published that we came up with on this 1998 paper evaluation that said that this is more or less an optimized or validated test.

What has been done with this test is we've

demonstrated functional activity with various vaccines in different age groups and with different vaccine regimens. This gets back to my point about generalized ability. You don't want to have a test that is only specific for or you have to have multiple tasks to demonstrate these in different groups. We've looked at it in a number of different situations and it seems to be good.

Also as Dr. Gotschlich said, but I'll end this part on that, that it appears to provide a valid bridge to previous sera epidemiologic studies that he described back in 1969 and '70.

Now, there are some limitations with the SBA that we have to consider as well. Dr. Gotschlich told you about, for example, the protective levels in the Goldschneider, et al., study in 1969. The protective SBA titer was determined to be greater than or equal to a titer of one to four.

Also as he mentioned, this was done on natural infection on adults and that they used an endogenous human complement source to generate their data. It's a particular note to understand that in the standardized assay that we're using, we now use rabbit complement.

The decision was made after this study and

during the standardization of the WHO publication, that I told you, the switch from a human complement source to a rabbit complement source.

Basically one of the reasons for this is the rabbit complement source is much easier to get and it's much more standardizable. The human complement source you can get. It's clearly just for difficult and you have to have a lot of quality controls to qualify the complement source. I think for the most part people have used rabbit complement.

Now, we also have standard reference sera and quality control sera as with the ELISA that are also from adults immunized with the polysaccharide so that may be a limitation of the study.

Also, as mentioned by Dr. Goldschneider the bioassays or, in this case, the SBA, does tend to be more variable than the ELISA so people, I believe, tend to want to do these kinds of tests, the ELISA, as compared to the bioassays. Although the question came up what would my lab prefer to do. Because we've done so many over the years, this is not the consensus but in our lab we would much prefer to do the SBA than we would the ELISA, although we do both.

Also the SBA titers are higher with the rabbit complement than the human complement. This has

been an issue of a debate and concern. I think, as Dr. Goldschneider said, or at least implied, that what is important is maybe the absolute titer may really be less important than the demonstration of functional activity. I hope I interpreted what he said correctly. This is an important point to keep in mind as I go through the series of slides, that the rabbit complement titer does, in fact, give a higher titer than the human complement.

This is a little busy slide but I wanted to show you both here and kind of give you a snapshot of the way the points are around the line. This is toddlers vaccinated with two doses of the Chiron vaccine, combined data. Over here we have the bactericidal titer. What we have down here is the ELISA IgG levels. This is for group C and you can see some of the statistics.

What I want you to see is this basically. If you notice down here on the standardized ELISA where you measure both high and low avidity antibodies, you see that there is a string of -- a series of measurements down here that go as high as 10 micrograms per ml but you don't have any functional antibody activity. That is obviously concerning.

However, if you draw a regression line

through there, the R is not all that bad. Now, if we look at using a high avidity ELISA again which preferentially measures high avidity antibody binding in the ELISA, again you can look at kind of the shape of the curve. What you tend to see is we lose a lot of these sera that had these high levels shifted in this way. We lose some of these because of the lower limit of detection as well.

I think over all what we see is maybe a better correlation or higher correlation when you preferentially measure the high avidity antibodies over the standard assays which measure both high and low avidity antibodies.

I picked out just a few slides to try to illustrate some of this point too. This is just adults vaccinated with a single dose of the Chiron AC vaccine, bactericidal over here, and the ELISA levels down here for group C. You can see what the statistics are. What we have are the prelevels, prevaccination here, post conjugate vaccinations here, and a linear regression.

What I wanted to show you here is that in adults that generally produce high avidity antibodies, even with the standardized ELISA, you can see that you still get a pretty good R value here. Again, this is

the standardized ELISA and you're getting with adults a pretty good response.

This happens to be from the Anderson paper that was done in 1994. One of the first papers, at least, that we were involved in applying some of the ELISA technology to.

I want to shift gears just a little bit and take a look at some more data generated by Ray Borrows with the Wyeth Lederle vaccine and, again, in infancy, the SBA geometric means for infants. What we did is we determined them with two different complement sources, the same standardized assay, but we just changed the complement sources. We only have data for the post-dose one, post-dose three, and the post booster.

What Ray Borrows showed was again in the same way. We have No. 1 and No. 2 which is the human complement. No. 1 is the geometric mean and the 95 percent confidence intervals. Again, just looking at this what you see is you still get the clear rise regardless of whether you use the rabbit complement or the human complement.

Clearly, and this is what's important, is that the human complement gives you a much lower titer, approximately four dilutions lower in many

cases. Even thought there is overlap here, the point I'm trying to make with this slide is the difference in the complement you do see a lowering of the titers when you use the human complement as compared to the rabbit complement up here.

Now, again, the toddler is vaccinated with two doses of the Chiron vaccine. I wanted to kind of plot these in different ways. Again, here you have the bactericidal titer with the human complement on the Y axis and the bactericidal with the rabbit complement on a log scale.

What I've drawn here is a line of identity. We would hope they would all fall on that line. That would be nice but it doesn't. What you can see is a systematic shift to the right where again the rabbit complement is much higher. Here is the linear regression over here.

Even though the R value is pretty good here, you can clearly see that there's a difference in the absolute titers between the human and the rabbit. This was from Maslanka, Granoff, et al., that was published in 1996.

This slide again is toddlers vaccinated with two doses of the Chiron vaccine. It's a similar slide to what you saw before but here we have the

standardized ELISA versus the SBA with human complement. We have the high avidity ELISA with the SBA here with using human complement. Again, bactericidal titers on the Y, ELISA levels on the X.

Again because of the standardized ELISA you see these lines over here. It looks very similar to what you saw using the rabbit complement. The R value is not quite as good. That's what you get with N=83. Again, you get sort of a similar picture of a nice titer cluster.

When you preferentially measure the high avidity ELISA and using the human complement, the picture here, if you will, the cluster looks very similar, although, again remember the absolute titers with the human complement are shifted down.

What this slide shows is somewhat of a summation. It's a correlation using -- in this case I added the standardized IgG. That's measuring G, M, and A. The standardized ELISA again measuring high and low avidity for IgG and the high avidity ELISA as well as the SBA titers using the rabbit complement on infants that were vaccinated with these schedules. Again, this is the Ray Borrows data.

What I wanted to show you is just to look at these correlations. It's not the correlations that

degenerate. It's the absolute values with the rabbit and the human. Here we have the three different assays, the new one being just total IgG immunoglobulin, standard IgG, high avidity. You can see what the correlations are here in the confidence intervals with the rabbit complement.

We then take a look at the same thing with the human complement. Again, very similar and overlapping. Again, I think what's telling is you look at the SBA, as I showed you before, between the rabbit and the human complement and you get a good correlation between the two. However, the titers are significantly shifted down towards giving the rabbit complement much higher titers.

In concluding remarks, I would say that the standardized assays can be used to evaluate immunogenicity. I think we've heard that all morning. As Dr. Goldschneider said, the SBA provides a bridge to previous sera epidemiologic studies and that the SBA does detect differences in the immune response between different vaccines and regimens in infants, toddlers, and adults. Again, the important part that I think is the generalizability part.

Also, as I've shown you, the rabbit complement clearly gives higher titers than the human

complement. One possibility is that maybe we need to increase the higher protective threshold level that we have imposed instead of a one to four. Four rabbit we may need to move that up a little bit to make up for the differences. What that titer is is unclear at this time.

Also, again, as I mentioned, Dr. Goldschneider said -- Gotschlich said, excuse me, the SBA measures functional antibody, whereas again the ELISA can measure both functional and nonfunctional antibody. I think these are important distinctions as I've tried to show you. We would really like to look at functional antibody and correlate them and have a correlative of a correlative, if you will, which is ELISA to give people ease of measurement.

The standardized ELISA does correlate well with SBA when measuring particularly high avidity antibodies in some cases. Clearly the high avidity ELISA can, I would say, improve or give a better correlation with the SBA when you are preferentially measuring antibodies that are of high avidity.

I think to close, the last statement I said is I do believe that immunogenicity data can be used as a surrogate to evaluate and license vaccines and to help in the development of public health

recommendations. That's it.

DR. GREENBERG: Thank you, Dr. Carlone. We have some time for some questions. Diane.

DR. GRIFFIN: What do you know about isotopes and the antibody in these assays? It seems to me from having heard all of this for the first time basically that complement is extraordinarily important. We know that the bactericidal activity, as far as I understand it, is dependent on complement. We know that only certain isotopes of human IgG can bind an activate complement. It seems like you might be able to get to a better functional correlate if you knew something about the isotopes.

DR. CARLONE: Well, we do know something about the isotopes but clearly I can't stand here and tell you all these studies the isotopes have been looked at. But, in fact, most of these we find for IgG it's IgG I and IgG II. In fact, both of those work reasonably well with the SBA for functional bactericidal activity.

I can only tell you without the data in hand that I don't believe that spending the time looking at the isotope differences would help clarify any of these issues.

DR. GRIFFIN: And how about your high

avidity antibody? What isotope if being measured there?

DR. CARLONE: Well, it think it depends upon the vaccine. If you have the conjugate vaccine, you are basically getting mostly IgG I in that. What we're looking at is the difference in the population, what the high avidity to low avidity population is. But we don't know what the percent of -- we don't know what the isotopes are within that population.

DR. GRIFFIN: And what's the reagent that you're using to determine the difference between high avidity and standard? Is it UREA?

DR. CARLONE: No, it's a chaotropic agent. It's sodium isothiocyanate. This was described in a publication by Granoff, et al. This is a classical --

DR. GRIFFIN: Unfortunately I haven't read that.

DR. CARLONE: Yes. It's a classical method to use a chaotropic agent to prevent binding of the antibody.

DR. GRIFFIN: And that's used in the initial at the time you add the antibody in the ELISA or it's used as part of the wash after you have bound antibody?

DR. CARLONE: Actually, you can do it both

ways. You may get slight differences if you do it both ways but, in essence, you get the same outcome. The lower avidity antibodies, whether you put it in before or after it binds, the lower avidity antibodies are removed.

DR. GRIFFIN: Lastly, it seems to me that the high avidity antibodies must be a subset of those that are being measured in your standard assay.

DR. CARLONE: Yes.

DR. GRIFFIN: But your data, particularly at baseline, showed higher numbers for the high avidity levels than for the standard for the same sera.

DR. CARLONE: I would be a little careful about the numbers because I think Dan Granoff, who was the major contributor to this paper, made an important point about not using the mass values in there. He called them ELISA units. What may happen is there may be a little difference in terms of what a mass value and an ELISA unit is. When you remove the low avidity antibodies, it's likely that these higher avidity antibodies can fit better. I can conceive in my mind where you may get a higher absolute number with a lot of really high avidity antibodies.

DR. GRIFFIN: Even at a time when you have very small amounts of antibodies so you would think

competition --

DR. CARLONE: I think overall the binding. Dan.

DR. GRANOFF: So you can't compare them directly.

DR. GREENBERG: Please identify yourself.

DR. GRANOFF: Dan Granoff. Sorry. The standard ELISA is calibrating micrograms per ml and the modified ELISA is in units per ml which is an arbitrary unit. You can't compare the absolute values. They are only relative values.

DR. CARLONE: Relative.

DR. GRIFFIN: But when you plotted them you plotted them --

DR. CARLONE: Well, I had ELISA units at the bottom and I had micrograms per ml at the top. I was trying to give you a sense of the pattern of how they cluster better as opposed to the absolute value.

DR. GREENBERG: Dr. Ferrieri and then Dr. Gotschlich.

DR. FERRIERI: I had a couple of technical questions, Dr. Carlone. Was your human complement source by luck or choice, A gammaglobulin anemic or not? Secondly, were these baby bunny complement sources rather than older rabbit complement sources?

Then lastly, I guess I don't have a good grasp of the mechanism for the difference as a function of the species of complement source.

DR. CARLONE: First off, we did not use A gammaglobulin anemic sera. What we did is we, if you will, qualified the complement source such that we could use it in the assay and give the controls low levels.

We used baby rabbit complement for a number of reasons. One is because it's available commercially and they pull hundreds of baby rabbits for one lot.

Your third question is what is the mechanism that would allow us to see this difference or enhancement with the rabbit serum. There have been a couple of publications out that speculate about, or at least identify the fact that there is an enhancement. The exact mechanism of that is unknown. However, if you saw the infant slides that I showed you, the confidence intervals are pretty wide and there is an overlap between the two. Clearly there's a tendency for the human to be lower than the rabbit.

DR. GREENBERG: Dr. Gotschlich.

DR. GOTSCHLICH: I presume in your --

DR. GREENBERG: Use your microphone.

DR. GOTSCHLICH: I'm sorry. In your standard versus your high avidity ELISA test where you expressed it in micrograms per ml, did you --

DR. GREENBERG: You're still not talking into the microphone.

DR. GOTSCHLICH: I presume you used the same standard serum to standardize it against.

DR. CARLONE: Yes.

DR. GOTSCHLICH: Okay. If the standardized serum contains a proportion of low avidity antibodies which is higher than what might be seen in a new response to the conjugate, one would then see a response that seems higher than one would expect from the data from the standard test. Is that correct?

DR. CARLONE: Yes.

DR. GOTSCHLICH: Okay. There is one little problem there. Let's just extrapolate this to the ridiculous. Suppose you have a standard serum that has all low avidity antibody? Then the immune responses that you will see will be totally fictitiously high.

DR. CARLONE: Well, we also --

DR. GOTSCHLICH: What are you going to do about that?

DR. CARLONE: Well, we also looked at the

bactericidal test. This is an area where you wanted to look at both of them to see. That's why I showed you that graph or the table where you want to match one to the other. I think looking at both the binding antibody as well as the functional activity gives you a lot of information. I think that goes back to what you were saying that maybe there's not just one assay that you need. Maybe there's a combination that can give you information that you don't get with one alone.

DR. GREENBERG: Dr. Edwards.

DR. EDWARDS: Just being very practical, George, would you say that if you look at the old data that conferred protection with titers greater than 1 to 4, then since there's a variation in the human and the rabbit, in your assays you would say anything greater than 1 to 8, 1 to 16, 1 to 32? What sort of magic number are you envisioning with the rabbit complement assay?

DR. CARLONE: Well, first off, there is no magic number. Secondly, we are working on that right now, as some of you know in the room, with Dan Granoff, Ray Borrows, and others. We are working towards that to try to understand what that is.

Clearly, as Dr. Gotschlich said, you don't

want to raise the bar too high, but yet maybe it's potentially a little too low right now based on what we see with the rabbit and the complement.

I can only skirt around your question by saying right now that there is no magic cutoff but we are looking at that very closely to see if we can give some recommendations and if there is going to be a difference, what that difference is. We want it to be based on obviously good science and good numbers.

DR. GREENBERG: Can you by any chance go back to the first slide that you put up that had the Chiron data. If was two graphs, one on top of the other, comparing regular ELIZA and high avidity ELIZA to bactericidal? Because I must be missing something here. I assume that the bactericidal titers in the top and the bottom -- no, that's the second one that actually was put up. There is one before that.

But even in that one shouldn't there be equal numbers of bactericidal titers of what looks to me to be about 5 in both graphs? In other words, the bactericidal titer is the same in top and bottom for all dots. Isn't it? The only thing that's changing here is the ELISA assay for any individual.

DR. CARLONE: Right.

DR. GREENBERG: But it looks like you

have --

DR. CARLONE: By shifting those some of the dots then turn because you're shifting one of the axes. By shifting some of the dots are going to go on top of each other. Is that what you mean?

DR. GREENBERG: So that's the reason it looks like there are a lot fewer dots on the bottom?

DR. CARLONE: I don't have a bubble plot.

DR. GREENBERG: Okay. I thought you were monkeying around.

DR. CARLONE: No.

DR. GREENBERG: Okay. So that doesn't look possible.

DR. GRIFFIN: That doesn't look possible.

DR. GREENBERG: I don't believe you.

DR. CARLONE: I will revisit that.

DR. GREENBERG: That was the one that had me concerned when I saw it. It just didn't seem like it could be.

DR. CARLONE: I will revisit that but --

DR. GREENBERG: Is it your graph?

DR. GRANOFF: Well, no. It's not mine. I contributed with Dr. Granoff. George is right. I mean, at the lower limit there are many, many points. I mean, you should put an end there. There could be

30 points or something there at one dot. I think when you look at it, it will show that. George, if you could put the table up.

DR. CARLONE: There's a typo.

DR. GRANOFF: There's a typo, yes.

DR. CARLONE: I went over that because I got the essence.

DR. GRANOFF: It really shows -- okay.

DR. GREENBERG: Okay. Dr. Kim.

DR. KIM: I guess another practical issue regarding serum bactericidal assay is that I know you indicated you pulled a lot of bunny serum as a source of complement. What is known about the variation of a bunny serum as a source of complement for bactericidal activity?

DR. CARLONE: All I can tell you is our personal experience. We published some of this a couple of years ago. Over approximately a period of four to five years we had about eight to 10 lots of complement. When we looked at our quality control sera that we ran along, the variability was very low. That's the only experience that we have with lot to lot consistency.

Dixie?

DR. SNIDER: Yes, George, for you or Emil.

What I've heard so far is that we might be looking for antibodies with high avidity and doing a functional assay. What else are you thinking of in terms of -- there was an implication that there might be even more than two correlates we would look for as we evaluated vaccines. Are there other things you have in mind? Are there other developments you haven't talked about?

DR. CARLONE: Well, yes, there is. I brought a slide but I probably don't want to dig it out right now unless someone really wants to see it. I can tell you what that is. We've been looking very carefully at opsonophagocytosis as another functional correlate of protection. What I can tell you is it's not fully validated yet.

We've gone from doing a killing assay to a flow cytometic very automated assay. We presented this at a meeting in Denmark a while back. The bottom line is we get very good correlation from what we've done so far by using opsonophagocytosis on this. Again, I just want to stress it's not fully validated and it's still under development.

DR. STEPHENS: A couple of questions regarding the immunogenicity issues of the C capsule. I think we'll hear later about acetylation as a component. Have you looked at different strains in

terms of acetylation versus nonacetylation in these assays?

DR. CARLONE: Again, we published this a while back where we looked -- it's sort of a side bar, if you will. I think that Peter Fusco is going -- is Peter here? I believe he's going to actually spend more time on that. The answer is yes, there are differences in acetylation.

DR. STEPHENS: Significant differences in serum bactericidal titer given?

DR. CARLONE: I don't want to use the word significant. I would rather let Peter talk about that. In our hands we found that there can be differences.

DR. STEPHENS: And a more general comment regarding have you looked also at serogroup A conjugate data?

DR. CARLONE: Yes.

DR. STEPHENS: And similar kinds of observations?

DR. CARLONE: I think serogroup A is a different story. We had a workshop in Geneva a number of months ago that clearly we brought the experts together and showed that group C is clearly more stable in all of these aspects than group A. I mean

that only in a qualitative sense that we still need to do a bit of work with the group A to validate a few issues. The group C, I would say, is more stable. I don't have any data to show you on group A.

DR. STEPHENS: A couple of technical questions regarding the assay. Rabbit complement alone doesn't kill. Is that correct?

DR. CARLONE: That's correct.

DR. STEPHENS: The serum killing titer, the percent that you're using, is 50?

DR. CARLONE: Twenty-five.

DR. STEPHENS: Twenty-five percent serum concentration. What percent kill are you using as your cutoff?

DR. CARLONE: Fifty percent.

DR. STEPHENS: Fifty percent.

DR. CARLONE: But we do a full titration curve so that you could cut that wherever you chose to.

DR. GREENBERG: Dr. Daum.

DR. DAUM: I guess I begin by apologizing for the naivete of this question but why doesn't low avidity antibody have functional activity? Isn't it what protects most of us in this room from being infected with meningococcus right now?

DR. CARLONE: Well, I would answer that by saying that I can't answer the question specifically. I can't quote why or give you a reference. I mean, in general I look at it this way. There is a threshold level of binding that is required on the organism in order to activate complement and kill.

What we know is that when you look at putting the polysaccharide on the plate, you have a different confirmation. We know that you can bind lower avidity antibodies to that. All we're doing is making a correlation of the amount that binds that we consider to be low avidity and where when you go up in that does it start to kill. We are really just making a correlation between the two.

That does not directly answer your question but that's an explanation of what we're trying to do. We are trying to optimize the killing and the binding to be similar to one another and correlate better.

DR. GREENBERG: We have time for a few more questions and then I'm going to call a halt.

Dr. Kim.

DR. KIM: Looking into some numbers of that you and Dr. Gotschlich indicated about bactericidal activity of 1 to 4 might be protective. Using the same serum have you done any other assays like the