BEFORE THE
UNITED STATES DEPARTMENT OF DEFENSE
ARMED FORCES EPIDEMIOLOGICAL BOARD

In The Matter Of:

ARMED FORCES
EPIDEMIOLOGICAL BOARD

U.S. Army Medical Research
Institute of Infectious
Diseases Auditorium
The Dalrymple Conference Room
Ft. Detrick, Maryland
Friday
July 8, 1994

The above entitled matter came on for hearing pursuant to notice, at 7:30 a.m.

BEFORE:

Dr. Lewis H. Kuller, M.D., President, and
Colonel Michael R. Peterson, U.S.A.F., B.S.C.,
Executive Secretary

APPEARANCES:

Dr. Kuller
Colonel Peterson
Colonel Takafuji
Captain Berg
Colonel Erdtmann
Colonel Parkinson
Commander Ungs
Colonel Leitch
Commander Clifford
Colonel Schuster
Commander Helmkamp
Commander Gray
Colonel Wright
Captain Robbins
Lieutenant Kelley
Dr. Weske
Major Klenke
Dr. Johnson-Winegar
Colonel Friedlander
Colonel Byrne
Colonel Hoover

APPEARANCES (Continued):

Colonel Taylor
Colonel Wade
Colonel Baze
Colonel Jennings
Dr. Brandt
Dr. Smith
Dr. Jahrling
Dr. Broome

I N D E X

OPENING STATEMENTS: PAGE

(None.)

Witness	Direct	Cross	Redirect	Recross

(None.)

EXHIBITS	IDENTIFIED	RECEIVED	DESCRIPTION

(None.)

CLOSING ARGUMENTS: PAGE

(None.)

P R O C E E D I N G S

(Time Noted: 7:40 a.m.)

DR. KULLER: Today, a very interesting meeting of the Board, very important meeting, so we will be getting started in a moment.

First, I would like to thank Colonel Takafuji and the staff here for, really, their hosting us and for the excellent accommodations for this meeting. Given this large number of people here and the heat of the discussion the facilities have been superb, and the help that we have had, so we really appreciate that. It really helps the Board and helps everybody. So, thank you, again, very much for this.

COLONEL PETERSON: I have just one quick announcement. Dr. Fletcher is going to be leaving sometime around noon today and he indicated if anybody is headed toward National Airport he would be more than willing to fill up their tank with gas for a ride to National Airport.

DR. FLETCHER: I will make it a tank and a half.

COLONEL PETERSON: Or, a tank and a half. If anybody has room in their car around that time and is headed that way, please see Dr. Fletcher.

That is all I have.

Okay, are we all in place now? Ernie, did you have any comments this morning? Ernie? Dr. Takafuji?

COLONEL TAKAFUJI: I would like to make a brief statement. I have been approached --

(Pause.)

I have been approached by several of the members of the Board pertaining to tours of the Institute, and the time today will not really permit that because of the need for us to really get into the many issues, but one of the things that I have been discussing with your President, as well as with Colonel Peterson is the possibility that for your next follow up meeting is to have the meeting here, which will then allow us to give you some time to spend time with not only the various aspects in terms of visiting the laboratories, but even with the scientists, themselves, as you have a chance to assimilate a lot of the information that is going to be presented to you.

So, one of the things that we might do for the next meeting is to have it here and then deliberately schedule some time for you in the laboratories.

The other thing that I would like for you to sort of keep in mind as we go through the day today is that many of you have been involved with vaccine production issues, or vaccine development issues. The Defense Department's perspective when it comes to Bio-Defense, though, is a little different in that the root of administration becomes a very critical issue.

You may have a very good vaccine, but in terms of its being protective against -- for example, the aerosol challenge raises a whole myriad of different issues that are a direct concern to us in the Defense Department.

So, you need to keep in mind that many of these agents can be delivered not only through skin, not only through the GI tract, but also through the lungs and, as you go through these assessments in terms of the threat agents, you sort of need to look at that from that perspective.

The issues that we are going to get into of a classified nature having to do with agents, we are not going to discuss issues having to do with capabilities of delivering those agents. We are going to be looking at just specifically those agents, themselves, and the theoretical threat that

they pose.

One final statement; the classified portions this morning is classified at the Secret level with no foreign attendees, and that is done deliberately to allow us in the military, in the United States Military, to be able to discuss some of these issues in a much more open forum than with any caveats put on that.

But, please understand that the research programs that we have and the development programs that we have are done in collaboration and conjunction with the efforts that are done by our allies. Thank you.

DR. KULLER: The mikes aren't on. They are on? Now they are on, okay. The -- can everybody hear now?

MALE VOICE: No.

DR. KULLER: No? Can you hear now? I don't think the mikes are working very well.

(Pause.)

Okay, they weren't a minute ago. Okay. Dr. Weske, you are the first -- you are first up. We are ready to roll.

(Whereupon, from 7:44 a.m. to 9:15 a.m., at the direction and request of Colonel Michael

Peterson, secret presentations were conducted off the record.)

DR. JOHNSON-WINEGAR: Good morning. Beginning from this point on, the meeting is no longer classified and you are free to take notes, and I believe that each of you has a paper copy in front of you of the briefing slides that I am going to be using.

My primary objective is to give you an overview of the Medical Biological Defense Research Program. As you can tell from the schedule, I will be followed by a number of speakers who will be dealing in much more detail with the specific medical counter-measures, primarily vaccines that we have developed in our program.

I have taken it upon myself as an additional goal to try to get us back on schedule so that I won't be going line through line of each of the briefing charts. As I said, you do have them to take away with you. I will be happy to answer questions.

First slide? (Visual aid provided.) Again, I just want to make the point from this slide that we certainly feel that a bio-threat to the U.S. has never been greater, and I would just like to

point out that prior to 1990 our program was perhaps little known and since that time there has been a great deal of interest generated at the highest levels, and I think from the two previous presentations this morning and the amount of questions that have been generated, it is obvious to this audience that the Bio-Defense Program certainly remains one of the very highest priority.

Next? (Visual aid provided.) I won't dwell on this slide. It just recaps some of the historical milestones and the specific questions of intentional use of biological agents.

I just want to re-emphasize that in 1969 the U.S. Offensive Bio Program was terminated. Believe it or not, there is still a tremendous amount of confusion, or perhaps even disbelief right here at Ft. Detrick, as one of the offensive centers for bio research, and still today when some of our people go to meetings, or make presentations they are accosted with, "Well, you work at Ft. Detrick and you must have an offensive program."

I am here to tell you that we do not have an offensive program. We are strictly defensive and all of the things that we will be pointing out today are medical counter-measures designed to provide a

defense for our forces.

One of the issues that does come up was the question that was raised earlier about how do we know that our vaccines are going to be effective, how do we know what is a deliverable dose, and all of those things impact on how we develop our program and how we measure the effectiveness of our medical counter-measures.

Next? (Visual aid provided.) Very briefly and very obviously, our mission is to sustain the effectiveness of U.S. forces. This is a fine point here, too. We really don't have responsibility for the civilian community. We are directed for U.S. Active Duty forces.

Next? (Visual aid provided.) How do we plan to do that? Our first and foremost priority is to prevent any casualties, and the things that we do in that line are to develop vaccines and toxoids and pre-treatment drugs.

The second thrust of our program is diagnosis, and for that we have two efforts. One, a far forward deployable type kit that can be used in field settings without requiring any sophisticated laboratory equipment, or training. A second, confirmation assay, based primarily on PCR. That

1 does, obviously, require a formal laboratory set-up.
2 The third prong of our approach is
3 treatment where we are developing antitoxins and
4 drugs and the reason that treatment is third is
5 primarily because of a logistical issue and also the
6 need to know what it is that you need to treat. We
7 don't have a detector and, so, oftentimes treatment
8 cannot be initiated until clinical signs are
9 obvious.
10 Next? (Visual aid provided.) Just a
11 little bit about the organization of the program.
12 The U.S. Army is designated as the Executive Agent
13 for Biological Defense for all of DOD. The slide is
14 a little bit out of date.
15 I have U.S. AMRDC, which is U.S. Army
16 Medical Research and Development Command. We are
17 now the U.S. Army Medical Research Development
18 Acquisition and Logistics Command, having assumed
19 two additional organizations in our function.
20 We are the primary lead for all medical
21 aspects of Bio-Defense. As was mentioned earlier,
22 the AMED Center and School is responsible for
23 developing requirements, and OTSG, up until the
24 formation of the Joint Program Office, has been the
25 procurer, the acquisition community, and that was

the thrust of Major Klenke's briefing.

It is, indeed, a joint program, a tri-Service program under the auspices of the ASBREM. That is the Armed Services Bio-Medical Research Evaluation and Management Committee. There are seven joint technology groups. We are Group Number 4 for Medical Biological Defense. We have tri-Service representation on that committee.

The things that outline our program are the Army Technology Base Master Plan and the Medical Science and Technology Master Plan. These are comprehensive documents. Suffice it to say that they are the cornerstone by which the real Army assesses its R&D Program and that the medical component is one piece of that program.

In the Advanced Development portion of our Program we base our requirements on a Concept-Based Requirements System. Again, we get input from all the Services. In the medical arena, our products generally don't have any problem being accepted by all services.

A vaccine is universally acceptable whether you are wearing a blue suit, a white suit, or a green suit, as opposed to pieces of equipment that may be developed that have to be specific for use on

a ship, or on a tank, or in a plane.

Who are the participants in the Program? We are very fortunate to be meeting here today at USAMRIID. That is the primary lab involved in our Bio-Defense Programs. We also have efforts at WRAIR. You have had some of your meetings there and I know that this group is familiar with that organization.

The Medical Research Institute of Chemical Defense where we are leveraging the technologies there because many of the biotoxins are similar to some of the traditional chemical agents. USAMMDA is our material development activity, responsible for products once they reach Phase I and phase II clinical studies.

The Navy, both at NMRI and the Naval Research Lab, are participants in our Program and recently we have expanded to include a small effort from the Air Force with their Armstrong Lab contingent that is based up at Edgewood, Maryland, working on some diagnostic devices.

We have an Extramural Program somewhere in the neighborhood of 30 contracts, primarily with universities and academia and some small industry. I do have to admit that that has been a bit of a

1 problem for us encouraging some of the academic
2 centers to get interested in the particular threats
3 that we have to address in the Bio-Defense Program.
4 I won't dwell on this slide. It is just
5 something for you to take away, because you will
6 hear us talk about the types of funds that we have
7 available, and I want to emphasize that ours is,
8 indeed, a comprehensive program that covers the
9 range from 6.1, which is very basic research,
10 isolating and characterizing the agents, all the
11 way through the various stages; development of
12 animal models, characterization of candidate
13 vaccines or other medical counter-measures all the
14 way through the traditional clinical trials as are
15 described in the F.D.A. regulations.
16 You will see at the bottom of the slide a
17 box marked "Contingency Fielding". That is between
18 our 6.3B and 6.4 Programs. What that means is when
19 we don't have human efficacy data we can't get our
20 vaccines licensed. They are held under I.N.D.,
21 Investigational New Drug status, and can be
22 administered under the provisions of the protocol
23 with written, informed consent. That issue came up
24 briefly earlier, and that does present an
25 administrative and logistical problem for us.

How do we manage the program? We have 22 Science and Technology Objectives with tasks underneath each of those. They are aligned with validated threat agents.

The next two slides briefly list for you what our broad Science and Technology objectives are, and you will see that in most cases we have used the generic term "Medical Counter Measure", as Colonel Takafuji described before. Quite often we think there may have to be a multi-pronged approach.

We may be looking at a vaccine, as well as an antiserum, an antitoxin, or drug therapy for some of these biological threats. The next slide is just a continuation of what our science and technology objectives are. That is not a prioritized list.

Just to give you a brief idea of the scope of the program, I wanted to show you that the funding for FY '94 in our basic research is slightly in excess of $17 million. Again, reminding you that is used to isolate and characterize the bio threat agents.

Next slide, please? (Visual aid provided.) Our 6.2 Program is our Exploratory Research where we are trying to develop animal models and some early preparation of candidate vaccines. About $15

1 million this year in that program.

2 Next slide? (Visual aid provided.) Our

3 6.3A is our real Laboratory Scale-Up of our pilot

4 candidates. Again, about $16 million this year in

5 that aspect of the program.

6 The last two program elements are our

7 Advanced Development portion of the Program. The

8 6.3B portion is where we assemble the pre-clinical

9 data, submit the I.N.D. and conduct Phase I and II,

10 Safety and Immunogenicity Studies.

11 You will see a significantly reduced amount

12 of money in this slide and also in the next slide

13 simply because we don't have that many products

14 right now that we are evaluating in the Program.

15 What is our vision and where are we going?

16 We continue to think that our Medical Biological

17 Defense Program is a superior program and that we

18 are doing everything that we can to deter, constrain

19 and defeat the use of biological weapons.

20 It is a premier program, both an in-house

21 and an extramural program, a tri-Service program,

22 and I think that we have focused our goals

23 sufficiently over the last couple of years that the

24 opportunity for pay-off by development of high

25 quality products is one that we can look forward to

in the near future.

How are we going to do that? We are going to continue to work to provide the very best material that we can by a comprehensive approach with both our in-house scientists and support and collaboration with extramural contractors.

This slide is probably not appropriate for this group, but I like to use it for non-scientific groups, and I thought I would throw it in anyway to show that we are aware of emerging technologies.

We are trying to take advantage of the various types of techniques and had some outstanding success lately in molecular modeling in some of the toxin work and potential drug intervention and therapies there. We are looking at all of the possibilities of vectored vaccines, synthetic vaccines and have a very strong molecular biology program.

What are some of the challenges? Again, development of the appropriate model systems. For some of the bio threat agents, USAMRIID and some of our other contractors are the only places that have the wherewithal to develop the animal models, partly because of the bio containment that is required for many of these threat agents and partly because of

the limited experience and expertise of the people in the program.

For many of the bio threats where we don't at this time have sufficient information on the genetic library that is going to be required for some of the molecular biology, the whole aspect of immunization methodology, delivery systems for vaccines, whether that be micro-encapsulation, polyvalent vaccines, viral vectored vaccines -- the possibilities are quite large there -- generation of immune responses to small molecules, some of the toxins on the threat list are low molecular weight materials and it is difficult to generate an immune response to them.

What are some of the challenges as far as the developmental side of the Program? I already mentioned the problem with the human efficacy studies. We have been working closely with the FDA on that as far as presenting to them all of the animal data that we have, trying to identify surrogate markers in human tests, whether that be an antibody level that we think equates to protection, or basically generating as much information as we can.

We have the whole issue of stockpiling,

both licensed products and those products that are held in an I.N.D. status. That was discussed briefly by Major Klenke, and what the requirement is and how we can meet that requirement has to be factored into both the amount of funds available and the changing threat.

The final slide talks about the acquisition part of the Program and that has been discussed briefly before on the possibility of a dedicated DOD vaccine production facility, or continuing to use the existing manufacturing firms to the best of their abilities to meet our Program needs.

I now have gone through the -- turn that off, please -- gone through these slides relatively quickly, but I think it was just very basic information, and I know that a lot of you are very anxious to see some data because that is what we are all interested in, after all, and the people that follow me will have some specific information on the medical counter measures, primarily the vaccines that we have, and we will be happy to discuss those with you in detail.

If you have any questions of a general nature about the Program, I would be happy to try to answer them now.

MALE VOICE: You are telling us that your funding in the last year, FY '94 -- we are now in the fourth quarter of FY '94.

DR. JOHNSON-WINEGAR: Right.

MALE VOICE: Are you optimistic about what you expect to get in '95? Are your funds reasonably adequate?

DR. JOHNSON-WINEGAR: Yes. We expect stable funding, approximately level over the POM. For those of you who don't know what the POM is, it is Program Objective Memorandum, which is our funding for the next six-year cycle, but we anticipate approximately level funding.

DR. KULLER: Thank you.

DR. JOHNSON-WINEGAR: Yes.

DR. KULLER: We are going to go on now, because when the next speaker has to leave, so, rather than take the break now, we will take the break after the next two speaks -- talks on Anthrax and Plague. Colonel Friedlander?

COLONEL FRIEDLANDER: I am going to give two presentations and, so, perhaps we can take questions after the first one and again after the second one if that is acceptable.

May I have the first slide, please?

(Visual aid provided.) The first slide, which you will see shortly, is a picture that most of you would -- I dare say perhaps none of you have seen.

This is inhalation anthrax. It is a disease that is very rare, but nevertheless, this is the disease that we are trying to prevent. Under natural circumstances, the most common form of anthrax is, of course, cutaneous anthrax. Inhalation anthrax is a very rare disease, but keep in mind, as I said, that the Army's objective is to develop a vaccine effective against inhalation anthrax because of the concern of the use of anthrax as a biological weapon by the aerosol route.

Up until the Sverdlovsk outbreak in 1979, there were something like 30 cases of inhalation anthrax reported in this century. There were 18 in the United States, with outbreaks in woolen mills in New Hampshire in the 1950's.

As a result of the release of spores from a military facility in Sverdlovsk, there were about 96 cases of inhalation anthrax, the largest epidemic of inhalation anthrax ever reported.

May I have the overhead, the first overhead? (Visual aid provided.) Our Program, as you heard, is geared to develop medical counter

measures against inhalation anthrax. These consist of prophylaxis, namely our concentrated effort on improving a vaccine and some effort on therapy.

I would like, first, to give -- to set the stage for the approach that has been taken in the development of the vaccine, to give a brief overview of the virulence factors and pathogenesis of anthrax, some of which I am sure is familiar to some of you.

May I have the next slide, please? (Visual aid provided.) This shows diagrammatically, if you look at -- if you look at the organism on the top left, schematically, this shows the fully virulent anthrax organism.

It consists in the middle of the chromosome, and on the left circle is a plasmid, which encodes for the genes for the capsule. This is a polydeglutamic acid capsule. It is antiphagocytic and it constitutes one of the prime virulence factors for anthrax.

There is another extrachromosomal element, another plasmid shown on the right. I am color blind, so I can't tell you the colors, but I was going to say, blue, or purple, but I am not sure what it is. In any case, it encodes for the toxin

1 proteins.

2 There are three toxin proteins which

3 constitute two protein exotoxins, as I will describe

4 shortly. These two protein exotoxins constitute the

5 two other virulence factors of anthrax.

6 Next slide, please. (Visual aid provided.)

7 This shows diagrammatically the anthrax toxin

8 components --

9 (Pause.)

10 This shows the anthrax toxin components.

11 There are three proteins, the edema factor, the

12 protective antigen and the lethal factor.

13 Individually, these proteins are inactive, they are

14 biologically inactive. In combination, protective

15 antigen plus edema factor produces edema in skin,

16 the characteristic that is seen in clinical

17 contagious anthrax.

18 The same protective antigen when combined

19 with a third protein called lethal factor is lethal

20 for some experimental animals. Work that has been

21 done over the last ten years, predominantly at this

22 institute, is demonstrated, in fact, that this edema

23 factor is an adenylate cyclase and raises cyclic A

24 and P. The lethal factor is lytic for macrophages

25 and appears to be a proteosome.

1 The important player in this scenario is
2 the protective antigen. It is necessary for both
3 toxin activities and it is the cell binding domain,
4 or component of the toxin.
5 It was called protective antigen
6 historically, before this information about its cell
7 binding activity was known, and it is continued to
8 be called protective antigen. It is the predominant
9 antigen responsible for immunity to anthrax and is -
10 - we call it P.A.
11 Next slide, please. (Visual aid provided.)
12 This shows just schematically the pathogenesis of
13 anthrax and we will concentrate primarily on -- this
14 is a misnomer here. This should be "inhalation",
15 not "pulmonary" anthrax.
16 The spore enters either the skin, the GI
17 tract, or the lung. It is taken up, then, by
18 macrophage. In the lung, in inhalation anthrax, it
19 is thought to be transported to the regional lymph
20 node where the spore germinates within the
21 macrophage to form the bacillus.
22 The bacillus, then, begins local production
23 of these toxins that I mentioned and that leads to
24 edema, hemorrhage and necrosis. In the skin, this
25 gives a characteristic necrotic lesion, but in

1 inhalation anthrax the business end of the disease
2 is in the regional lymph node and the media stagnum.
3 This results in the characteristic
4 hemorrhagic lymphadenitis and media stannitis and
5 the widening of the media stagnum on the chest x-ray
6 that you saw. From there, the organism spreads to
7 the blood with development of bacteremia and seeding
8 of the other organs, particularly the brain. Death
9 ensues from media stannitis and pulmonary edema and
10 often with hemorrhagic meningitis.
11 May I have the next overhead, please?
12 (Visual aid provided.) This is the current anthrax
13 vaccine that you heard about. It is produced by the
14 Michigan Department of Public Health. It was
15 licensed in 1972 and thousands of doses of the order
16 of, perhaps, 10,000 doses, now more, have been
17 given.
18 It is composed of a sterile, crude culture
19 supernatant from an attenuated non-encapsulated, but
20 toxin producing strain, which is absorbed through
21 lumen hydroxide. Formaldehyde is added and
22 benzethonium chloride, as well, as preservatives.
23 Next overhead, please. (Visual aid
24 provided.) This is the vaccination schedule. A
25 half an ml is given subcutaneously at zero, two,

four weeks followed by half ml doses at six, twelve and eighteen months with subsequent yearly boosters.

Under this regimen, more than 95% of the vaccinees will sero-convert after the first three doses.

(Pause.)

In terms of vaccine efficacy, there is human data and animal data. In terms of the human data, a controlled clinical trial was conducted with a self re-culture supernatant vaccine similar, but not identical to the current vaccine.

This was performed in a susceptible population in the New England -- the New Hampshire woolen mills in the 1950's where there was an outbreak of anthrax and there was contaminated goat hair that was being processed.

An assessment of that study revealed that vaccination compared to placebo provided about 90% protection against cutaneous anthrax with the lower 95% confidence limit of 65% effectiveness. Now, it is important to point out that the effectiveness of this vaccine against inhalation anthrax was impossible to assess because there were too few cases.

Next, please. Next overhead. (Visual aid

provided.) This is a summary of a lot of animal data over the years. There have been extensive tests done in guinea pigs which have established the effectiveness of the current licensed vaccine against both parenteral and aerosol challenge.

This is -- there is a -- the protection is not extraordinary because the guinea pig is very difficult to protect, but in the rhesus monkey, which was a model of human disease which is perhaps the best model of human disease and closely approximates what is described in human disease, the current license vaccine can protect rhesus monkeys against a lethal aerosol challenge up to two years after vaccination.

Now, what are the problems with this vaccine? First, the present vaccine uses a strain which is produced under Bio Safety Level III containment. The present strain is a spore forming organism and it requires a separate production facility.

The vaccine components are completely undefined in terms of quantitation, in terms of characterization of the protective antigen and in terms of anything else that is in there.

There is significant lot to lot variability

1 of the protective antigen content in the present
2 vaccine lots. It is only characterized in terms of
3 its ability to protect the guinea pigs. There is no
4 lot to lot standardization, and it is reactogenic.
5 Next overhead. (Visual aid provided.)
6 Depending upon how one assesses reactogenicity, the
7 numbers vary, obviously. In this study, with the
8 initial series of three doses, systemic
9 reactogenicity of significant myalgia headache
10 malaise was at 0.7% and what is called significant
11 local reaction, and that is defined below as
12 induration erythema greater than five centimeters in
13 diameter, with edema, local warmth and tenderness.
14 This does not include reactions of a lesser
15 degree, which are the most common reactions, and
16 that is about two and a half percent. With
17 boosters, it tends to rise somewhat, but then
18 actually tends to level off and decline. There are
19 occasional nodules that are present and can persist
20 for weeks to months, but no abscesses.
21 Next, please. (Visual aid provided.) What
22 are our plans in terms of an improved vaccine? The
23 course that we have embarked upon concerns the use
24 of a recombinant, protective antigen based vaccine.
25 This is our major thrust and it is based, as I

1 said, on the observation that P.A. alone can protect
2 against an aerosol challenge with anthrax.
3 By making the vaccine with highly purified
4 protective antigen from a recombinant, non-spore-
5 forming strain, we will avoid several of the
6 problems associated with the current vaccine that I
7 have discussed.
8 Namely, we will eliminate the need for Bio
9 Safety Level III containment, we will eliminate the
10 need for a dedicated facility because of using the
11 spore-forming organism and we will eliminate lot to
12 lot variability.
13 We have evaluated the potency of protective
14 antigen from other vectors besides bacillus. We
15 looked at baculovirus, as well as a bacillus vector
16 cloned protective antigen product free of the other
17 toxin components, and we have decided to proceed
18 with the bacillus product.
19 We had produced by recombinant means a non-
20 spore-forming anthrax strain which contains a
21 plasmid encoding only the P.A. gene and no other
22 toxin genes.
23 We are -- we have optimized the P.A.
24 production of this in a four and twenty liter
25 fermenter and are continuing to so do that and, as

1 well, develop rapid purification schemes for
2 producing the P.A. from this recombinant non-spore-
3 forming strain.
4 Next, please. (Visual aid provided.) The
5 other -- the other aspect of this -- of our plans in
6 terms of improved vaccine concerns the optimization
7 of the adjuvant for efficacy and safety.
8 Based upon the advances that are developing
9 in adjuvant technology in information and testing in
10 humans, particularly driven by HIV, we have decided
11 to compare the current adjuvant, all hydrogel and
12 newer adjuvants such as miopus, or lipide,
13 lipostomies, a salatin derivative, micro-fluidized
14 emulsion and ISCOMS (sic) and use them with
15 protective antigen for efficacy against aerosol
16 infection in rodents.
17 The difficulty, as you are well aware, is
18 that rodents are not monkeys -- are not man and the
19 only -- there were -- the only advantages that were
20 seen in the rodent studies were the lipostomies and
21 the monopus, or lipide.
22 We are in the process of testing this
23 recombinant produced protective antigen with,
24 initially all hydrogel, which has never been done
25 directly, comparing to MBPH and then, subsequently,

with the best candidates, predominantly MPL and lipostomies in the rhesus monkey model.

Now, a second generation approach, if you will, is the evaluation of microencapsulation of P.A. You, I am sure, have heard something about this and I am sure are familiar with it, but it offers, certainly, the advantage of altering the vaccination schedule to theoretically, at least, offer the opportunity of a single dose vaccination.

We have done some initial studies in rodents that suggest that at least the microencapsulation process does not alter the immunogenicity of protective antigen and we are going ahead in terms of evaluating this as a second generation effort.

Next overhead, please. (Visual aid provided.) Now, in terms of the other arm of medical counter measures, that concerns the therapy of inhalation anthrax.

Based upon some studies that we had an opportunity to perform and that I will discuss briefly, shortly, we came up with recommendations as follows; namely, that penicillin remains the mainstay of treatment if the organism is sensitive and that ciprofloxacin or doxycycline represent

alternatives if the organism is penicillin resistant.

We have looked at a multitude of other anibiotics and many of them are active in vitro. May I have the last slide, please? Slide?

(Pause.)

(Visual aid provided.) Yes, thank you. This is a busy slide, but I will try to summarize the major points concerning this. This was a study that was performed in the rhesus monkey with an aerosol challenge with -- of anthrax spores, and it was addressed -- it was designed to address the issue as to whether or not prolonged antibiotic therapy could provide an effective treatment against an aerosol exposure to anthrax spores.

The animals were exposed on day zero and treatment was begun with three different antibiotic regimens for a period of 30 days. The control animals, shown in dark circles, died within about ten days. The treatment groups were ciprofloxacin, penicillin and doxycycline.

The penicillin is shown in the closed triangles, the ciprofloxacin in the open triangles and the doxycycline in the closed squares. I am color blind. This doesn't help me in the closed

squares.

Several major points to point out. During antibiotic treatment no animal died of anthrax. There was an animal that died, but this was an iatrogenic cause of death and the animal did not have viable anthrax. So, during treatment for 30 days the animals were completely protected.

When the antibiotic was stopped, in the penicillin group, the closed triangles, three animals died, on days 9, 12 and 20 after stopping the antibiotic. In the ciprofloxacin group, which was the triangles, one animal died on day six after stopping the drug. In the doxycycline group, one animal died -- I am sorry, I gave a square -- on day 28, after stopping the antibiotics.

So, antibiotics were effective while -- they were completely effective while the antibiotics were continued and did offer significant protection even when the antibiotics were discontinued, although there were animals that died.

It points out what has been described previously and what we confirm, namely that the anthrax spore can survive in the animal for an extended period of time.

The best efficacy was demonstrated by

combining antibiotics, doxycycline plus vaccination, and in that group none of the animals died when the antibiotics were discontinued, although the differences are not statistically significant. Slide off now.

COLONEL ERDTMANN: Real quick?

COLONEL FRIEDLANDER: Yes?

COLONEL ERDTMANN: Would you put that slide back on again?

(Visual aid provided.)

In the war, we were using a combination of vaccine and (inaudible).

COLONEL FRIEDLANDER: Yes.

COLONEL ERDTMANN: There is no reason to believe that if you didn't test that and just modeled it --

COLONEL FRIEDLANDER: Correct.

COLONEL ERDTMANN: There is no reason to believe there would be a difference, is there?

COLONEL FRIEDLANDER: None at all, in my view. I think it demonstrates the thesis, essentially, the antibiotic coverage. I don't think there is any differences; this is a monkey, this is not a human.

I don't think it shows essentially that

antibiotics plus the vaccination, in fact, provided the best protection, and what I don't have time to go into is that -- let me just mention that we challenged these animals several months later and the only animals that survived were those that had been vaccinated.

That is to say, the antibiotic therapy prevented the infection, or prevented development of an immune response. Yes?

FEMALE VOICE: When was the last time you did it?

COLONEL FRIEDLANDER: Day 1 and day 15. Okay. In summary, then -- slide off. (Visual aid removed.) The current vaccine, MDPH, Michigan Department of Public Health, appears to be effective in animal models of aerosol exposure, but suffers from problems associated with its production in that it requires BL-3 Level containment, it is a spore-former requiring a dedicated production facility and there is significant lot to lot variability.

The contents of this vaccine are totally undefined. It is also reactogenic. Our approach has been to use a recombinant strain which produces only the protective antigen and is a non-spore former. The production of purification schemes have

been devised to produce very highly purified protective antigen, free of other products.

It is anticipated that a vaccine made from this recombinant strain will allow easier and safer commercial production, will prove as effective, will enable precise quantitation of the vaccine components eliminating lot to lot variability and, hopefully, be less reactogenic. Thank you. I will take any further questions.

MALE VOICE: Have you tried aerosol immunization?

COLONEL FRIEDLANDER: No, we have not. That has been done before by the Soviets -- former Soviets -- and both in humans as well as in animals.

MALE VOICE: (Inaudible.)

COLONEL FRIEDLANDER: With -- well, it is hard to know what kind of reactogenicity you want to accept. That was done, actually, with live vaccine, a live attenuated vaccine. Yes, sir?

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: Right.

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: Was that patient here? No, this was actually a case report from New Hampshire.

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: That's right.

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: Yes.

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: Right, and the model and the monkey, essentially --

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: How was that patient affected?

DR. WOODWARD: (Inaudible.)

COLONEL FRIEDLANDER: I think there were two exposures, as I recall here. One, I don't think it was clear how it occurred, in another it was a clear exposure. Yes?

MALE VOICE: Oh, on a previous question, have you looked at the other routes of the administration, like nose drop, one, and two, any accelerated administration schedules?

COLONEL FRIEDLANDER: You are talking of the vaccine?

MALE VOICE: Yes.

COLONEL FRIEDLANDER: We have not looked at other routes of immunization and I am not sure how more accelerated -- we have not -- I mean, the

vaccine is given at zero and two weeks. These animals -- these animal -- these monkeys were protected after two doses of the vaccine.

MALE VOICE: Against aerosol challenge?

COLONEL FRIEDLANDER: Against aerosol challenge. Yes?

MALE VOICE: Since you have (inaudible).

COLONEL FRIEDLANDER: We have not tried --

MALE VOICE: (Inaudible.)

COLONEL FRIEDLANDER: With this -- we have a small effort with Plague that we are looking to that. We have not tried it with this vaccine. I think

that -- well, that is a separate issue, I think, that the issue of DNA vaccines, I think, is a fourth generation vaccine.

MALE VOICE: Did you collect any serum from Desert Storm to see if there was good protected antibodies?

COLONEL FRIEDLANDER: That's -- we haven't looked at that, myself, but, I mean, that has been looked at in terms of the fact that there was an effective -- there was an immune response in the vaccinees, and in the vaccinees we have been subsequently boosted, as well. Yes?

MALE VOICE: Is there a significant civilian need for this vaccine?

COLONEL FRIEDLANDER: No.

MALE VOICE: This is the only need?

COLONEL FRIEDLANDER: The vaccine -- significant is the operative word. The vaccine is used by some in the civilian community. Namely, workers in woolen mills where there is a continuing problem.

I mean, there are occasional cases of cutaneous anthrax that occur in states in occupational exposure, and there is no reason to think that there might not be another outbreak of inhalation anthrax in that situation.

It is not clear why that outbreak occurred in New Hampshire. I mean, anthrax had been -- lots and lots of cases of anthrax in the U.S. since the turn of the century. Essentially, one epidemic of inhalation anthrax.

Okay, I will take a deep breath and move on to Plague. Could I have the next slide? (Visual aid provided.) I like to begin this with chest x-rays. This is a disease I haven't seen. I am sure some of you have seen this disease. Well, maybe not this disease, but bubonic plague. Again, this is a

disease we are trying to prevent.

This is pneumonic plague. May I have the first overhead? (Visual aid provided.) Again, we are here to develop medical counter measures against pneumonic plague and, again, the emphasis has to be on pneumonic plague in the context of the use of plague as a biological weapon.

We are, of course, also interested in having an improved vaccine effective against endemic plague, mainly bubonic plague. The current program began about two years ago and is, again, focused on prophylaxis with the development of an improved vaccine and therapy.

The next overhead. (Visual aid provided.) I would like to spend a minute, or so to give you a brief overview of pathogenesis, again, to set the context for our approach to development of an improved vaccine.

Understanding of the virulence determinants of the organism and their role in pathogenesis; obviously, it is critical to the design of effective vaccines and the work that we are doing with plague therefore involves a significant effort in pathogenesis.

In the early studies in the 1950's, the

basic ideas about pathogenesis -- and earlier, I should
say -- sorry -- were worked out, but they were using phenotypically defined organisms, not genotypically defined organisms and, so, the results from the studies in that era are difficult to interpret with certainty.

But, basically the organism enters from the flea, or by aerosol having grown, we think, at ambient temperature. That is a significant issue with plague.
Under these conditions, it is felt that the majority of the organisms are initially phagocytosis sensitive. They can be phagocytose and they are killed by neutrophils.

However, some of the organisms are taken up by macrophages which are less efficient at killing, and some of those organisms proliferated in macrophage, now at 37 degrees, and a whole series of virulence genes are turned on by the elevated temperature and, perhaps, by the environment, or the macrophage, such that when those organisms come out of the macrophage they now come out with a fat capsule and antiphagocytic capsule called F-1, and a whole slew of other virulence factors. They are now

resistant to phagocytosis and, basically, the ball game is over because these organisms can replicate extra-saliably unimpeded.

There is more recent information that suggests that these phagocytosis resistant products are encoded on a medium size plasmid that I will describe below, and a lot of these virulence factors have not been demonstrated fully on plague organisms, so the complete story is as yet unknown.

These virulence factors -- next overhead, please (visual aid provided) -- are conveniently differentiated by where they are located, whether on the chromosome, or on three plasmids that plague possesses, and I will just mention them briefly.

There is iron uptake that is important. There is a so-called fibrilla antigen that is important. There are three plasmids. The smallest contains what is called the plasminogen activator, which is responsible for spread from a peripheral root to the blood stream, and there is this medium sized plasmid which is common to enterocolitica and pseudotuberculosis, the other urocynia pathogenic species.

Encoded on this plasmid is what is called the V antigen and several what are called YOPS.

1 Several of these have been implicated, as shown by
2 the asterisks, in virulence in pestis, but others
3 have not yet been shown, although we think they are.
4
5 The largest plasmid encodes this F-1
6 capsule. The F-1 capsule historically has been
7 implicated as being an important protective -- the
8 important protective immunogen in plague. The other
9 virulence factor that has been implicated in
10 immunogenicity is the V antigen, and we have
11 initially concentrated our efforts on those two
12 virulence factors as potential components of the
13 vaccine.
14 Next overhead. (Visual aid provided.)
15 Now, the current plague vaccine that I suspect many
16 of you have had, as opposed to -- well, many of you
17 probably had the anthrax vaccine, too, so you can
18 take your pick which one you don't like worst.
19 (Laughter.)
20 But, this strain is a 19th Century vaccine.
21 This vaccine is a 19th Century vaccine. This is a
22 whole cell. This is the -- the strain is a virulent
23 Indian isolate from Bombay. It is grown. This is
24 the way it is produced. It is grown for three days
25 at 37 degrees.

It is harvested, it is killed with formaldehyde and then preserved at 10 to the 9th organisms per ml with some phenol, and the final concentration of formaldehyde has to be lower than 0.19%

Next overhead, please. (Visual aid provided.) This is the vaccination schedule. The initial dose is one ml. That is 10 to the 9th kill bacteria, in your arm, like a type of an old typhoid vaccine.

It is given I.M. so that we don't know about it, and real deep. The second dose is two tenths of an ml at one to three months. The third dose is 0.2 ml's about three to six months later, and boosters of the same dose, 0.1 to 0.2, depending on how well you tolerated the first three, at six month intervals if exposure continues.

Next, please. (Visual aid provided.) Okay. I am sure some of you are well aware of this information, but I would like to go over what some of the evidence is for the protective efficacy of kill vaccines against plague, at least as I read it from literature.

The human data -- well, first of all, the effectiveness of vaccines and the prevention of

plague is to read the literature -- has been going -- I mean, the question of the effectiveness has been going on since the first development of vaccines by Hafkind (sic), and the live vaccines by Otten and Gerard, and it has been studied for many years, and particularly against pneumonic plague the efficacy has been -- remained controversial.

In March of 1942 commercial production of a kill plague vaccine was undertaken under the direction of K.F. Meyer. It was used in World War II, and no U.S. Armed Forces contracted plague. So, the statement is made, "despite potential exposure in the Mediterranean and the Orient, the final report after World War II says -- we are not sure whether -- there have never been extensive field trials, but we think it offers some protection."

Then we get to Vietnam, and in Vietnam there were thousands of Vietnamese civilians who were infected with plague and, please, those in the audience can fill in the gaps here since I wasn't there, but -- and I am sure -- I know several of you were.

There were supposedly only eight cases of plague diagnosed in ill Americans in a ten year period from 1961 to '71. There was also evidence

1 for subclinical plague infection in vaccinated
2 personnel in Vietnam. So, people who were
3 vaccinated and were working the fields in plague
4 epidemic areas came back with antibody rises. That
5 was some of the evidence.
6 There is also anecdotal serological
7 evidence of asymptomatic infection in vaccinated
8 workers, laboratory workers at WRAIR, after
9 accidental laboratory exposures. There were several
10 such episodes, again, in which there were presumed
11 exposures, presumed aerosol exposures, in fact, with
12 antibody rises. Not well documented, but
13 documented.
14 The next overhead. (Visual aid provided.)
15 There have been no double blind clinical studies
16 done, obviously. The data is in animal studies. In
17 previous animal studies it was demonstrated that
18 kill vaccines protect against parenteral, but not
19 aerosol challenge. However, if you hyper-
20 immunize the animals with the kill vaccine, or with
21 antigens given with very potent adjuvants, you
22 could, in fact, protect in some models against
23 pneumonic plague.
24 Next overhead. (Visual aid provided.) In
25 this, basically the major players in the field are

relegated to some final statements. This is K.F. Meyer, who I am sure many of you knew, and was a major researcher in plague.

This is essentially at the end of his career, and he said, "Additional experimental studies on non-human primates are required to appraise the efficacy of killed or live vaccines against aerosol infections."

Next overhead, please. (Visual aid provided.) This is a feeling, a summary of the feeling of the group at WRAIR. This is an annual -- this is a statement from an annual report in 1973 and it says, as you can read, "The cumulated experience supported by laboratory studies indicates that vaccination with kill plague vaccine affords a significant degree of protection against bubonic plague. The value of vaccines for protection of man against pneumonic plague is not well-defined. The efficacy of various vaccines protecting against experimental -- experimental animals against respiratory challenge, yersinia pestis should be evaluated."

Next overhead. (Visual aid provided.) And that was sort of what we were faced with when we began some of the studies here. Again, these have

just been done in the last few years.

In the studies that have been performed to date, the human vaccine does protect mice and guinea pigs against subcutaneous challenge, but could only prolong the time to death by one to two days after an aerosol challenge.

In a non-human primate model of pneumonic plague which we developed here, three doses of the human vaccine given in the recommended schedule had no effect on survival or mean time to death after an aerosol exposure. The only effect of the vaccine was that it appeared to delay the onset of bacteremia by a day.

Next, please. (Visual aid provided.) What are the problems with the current vaccine? Well, it uses -- it currently uses virulent strains to produce the vaccine, it is an undefined immunogen, the immunogenic content of the vaccine is undefined and, again, there is poor control of lot to lot variation.

There is a problem of reactogenicity and historically a problem with non-responders. This vaccine is probably not effective against pneumonic plague.

An issue that I will just touch on briefly

is the issue of -- I mentioned this capsule, F-1. There are organisms that do not have the capsule. They retain virulence as we determined here, and this vaccine which contains -- the current plague vaccine contains a lot of F-1 in it and may be even less protective against F-1 negative strains.

Next, please. (Visual aid provided.) Reactogenicity. Local effects at the site in about 10% of people, arm soreness in more than half the people, systemic effects in someplace like 4 to 10% and the reactogenicity tends to increase with repeated injection.

Next overhead, please. (Visual aid provided.) The issue of non-responders I don't think has totally been resolved yet. In the previous studies with the current vaccine with the current -- similar to the current vaccine -- 8% of 117 individuals failed to respond as measured by hemagglutination in addition to antibody.

In recent studies at USAMRIID less than 5% were non-responders when measured by the more sensitive eliza. The study also confirmed previous work which showed that when measured by this mouse protected index, about 65% of people developed what are thought to be evidence of relative immunity

after two doses of the vaccine, and this goes up with subsequent boosters.

Next, please. (Visual aid provided.) What are our plans to improve this vaccine? Well, we have taken the initial approach of trying to develop a multi-component recombinant protein based vaccine. Most of our effort to date has been on F-1. We know that F-1 is going to be in the vaccine. We know that F-1 has protected in the past against animals, and that is where we put our major effort in terms of cloning, expressing and purifying F-1 from a recombinant vector so that it will be safe to produce, and we are in the midst of studies comparing the protective efficacy of F-1 from this recombinant vector compared to F-1 from yersinia pestis, and the initial studies to date, that are very preliminary studies, suggest that they are equipped, which is what we had anticipated.

We also have an effort on V antigen which is following on the effort on F-1, and that is to clone, express and purify V from an E cholera recombinant together with collaborating efforts in the U.S. and the U.K., and tests for efficacy in rodent models.

Next, please. (Visual aid provided.)

1 There is this problem of additional virulence
2 factors and we intend to determine the importance of
3 these other virulence factors that I have alluded
4 to, and there are a multitude of them, and then to
5 examine -- to clone, express -- we have begun some
6 of this effort -- and test additional immunogens.
7 This is particularly important for the F-1 minus
8 strains which we have just recently found to retain
9 virulence.
10 Next, please. (Visual aid provided.) In
11 conjunction, then, following along the efforts that
12 are going on in the anthrax program with new
13 adjuvants and delivery systems, we plan to examine
14 initially F-1, the recombinant F-1, with a variety
15 of new adjuvants and delivery systems and
16 microcapsules, a cholera toxin based delivery
17 system, as well, to see whether or not, particularly
18 based upon the data in the older literature which
19 suggests that adjuvant -- that enhancing -- using an
20 -- a potent adjuvant will definitely enhance the
21 protective efficacy of a plague vaccine.
22 Finally, I would like to spend a few
23 minutes on our efforts of improving therapy against
24 plague, because we recognize for the immediate time
25 frame that antibiotics will remain the mainstay of

1 our medical counter measures against plague.
2 Next overhead. (Visual aid provided.) The
3 current therapy of plague is perhaps open to a
4 little controversy. There is a little bit of taste
5 in this as to who you are and what you like, but
6 basically streptomycin remains the treatment of
7 choice.
8 Gentamicin has been used and maybe
9 substituted, but there is less clinical experience
10 with this drug. Chloramphenicol can also be used
11 and certainly should be added when meningitis is
12 suspected, or when intravenous therapy is required.
13 Tetracycline can be used for mild cases of
14 bubonic plague. It has certainly been used for
15 pneumonic plague, as well, and is shown to be
16 effective.
17 Doxycycline should be acceptable as an
18 alternative, but based upon in vitro sensitivities
19 in some animal work, but there is no clinical
20 experience with this drug, other than here where we
21 give it to people who may have been exposed.
22 Fortunately, we haven't had a chance to see whether
23 or not it is effective.
24 Next, please. (Visual aid provided.)
25 These are our future plans, and that is to test the

1 newer antibiotics which have in vitro activity
2 against yersinia pestis in animal models of
3 pneumonic plague. This will initially be in rodents
4 and then move on to the primate with selected drugs.
5 These will include fluoro-quinalones,
6 ciprofloxacin and ofloxacin, ceftriaxone (sic),
7 gentamicin, natamycin and doxycycline. We expect to
8 complete these studies shortly. They -- the
9 pharmacokinetics have been performed and the studies
10 are to begin shortly.
11 Those will be our initial efforts,
12 particularly geared towards evaluating both
13 parenteral drugs, as well as oral drugs that are our
14 alternatives. Overhead off, please.
15 In summary, then, the current human vaccine
16 has several deficiencies. It is a whole cell
17 vaccine which is un-characterized as to the
18 protective immunogens and it is reactogenic.
19 Unequivocal data on its protective efficacy
20 in humans is not available. It likely confers some
21 protection against bubonic plague, but little
22 against pneumonic plague which is our concern.
23 Our current efforts are focused on
24 developing a multi-component recombinant protein
25 based vaccine consisting of the F-1 capsule and the

V antigen, which should protect against both F-1 positive and F-1 negative organisms. Additional immunogens will also be evaluated, as will improved adjuvants and delivery systems.

Additional studies are ongoing to assess the usefulness of the newer antibiotics in the treatment and in the prophylaxis of plague. Thank you, and I will take any questions now.

MALE VOICE: One of the arguments for the effectiveness of plague vaccine (inaudible) and not bubonic plague (inaudible). I am sorry?

MALE VOICE: (Inaudible).

MALE VOICE: Yes, that is correct. Right, both. The epidemiology is exactly the same. Now, the other thing, though, is that it has been seen bubonic plague that has been treated with streptomycin that is almost miraculous.

I visited the (inaudible) and there were these kids running up and down the ward whooping and hollering and I commented that obviously these kids got over their plague.

COLONEL FRIEDLANDER: Streptomycin?

MALE VOICE: Yes.

COLONEL FRIEDLANDER: It has been tested, again, in the primate.

MALE VOICE: But, do you have all the data you need?

COLONEL FRIEDLANDER: I think, given the evidence of its effectiveness, one can make an argument to test it to make sure that the primate model approaches the human model, but there is, I think, sufficient data with humans in the use of streptomycin to know that the drug is effective. It has been tested in the primate model, as well. Yes, sir?

DR. WOODWARD: Is there any interest at all in the paper and the TV to any of this?

COLONEL FRIEDLANDER: Well, I think there should be. I think there is a matter of resources. For those of you that don't know, this is the live attenuated vaccine strain of plague that was developed in Mada -- tested in Madagascar and is used currently in China, I believe.

We have been limited in terms -- to some extent, and we felt that this was the thing that was going to get it going the fastest.

DR. WOODWARD: Did it work?

COLONEL FRIEDLANDER: Yes, it worked, but some people, at least the U.S. volunteers in the hospital --

DR. WOODWARD: Like the smallpox.

COLONEL FRIEDLANDER: Right. You are right. I mean, I think that -- it is something, I think, that we should include -- we would like to include -- in our program. Yes, sir?

MALE VOICE: (Inaudible).

COLONEL FRIEDLANDER: That's right.

MALE VOICE: The concern was that the (inaudible) and that is why we discontinued working with the live virus.

COLONEL FRIEDLANDER: Yeah. It is not clear -- the studies that were done in the U.S. with the EV-7 -- EV-76 strain -- were done with subcutaneous inoculation vaccination, and the organism is more virulent when it is given by the subcutaneous route than by scarification, which is the way the Soviets give it and, in fact, it was never gone back in the U.S. -- when it was given by the subcutaneous route -- this is sort of analogous to smallpox, perhaps, but it is a vaccinia.

When it was given by the subcutaneous route, it put about half the people in the hospital. There were 12 volunteers, I think, that were studied, but it has never subsequently been done in the U.S. by scarification.

Those strains were not well-defined, of course. They were -- that was part of the problem. They were not genotypically well-defined strains and there was lots of variation EV strain to EV strain. So, I think there is a future for it. Yes?

MALE VOICE: Why do you think the vaccine which is immunogenic in humans protects primates in sort of a form that is abscessed, like, and it is hard to get at, doesn't work against the parcella, because it seems to me unless you know that, just making another vaccine that is more modern, but really not directed against the reason why the current vaccine doesn't work isn't going to be a successful approach.

COLONEL FRIEDLANDER: Well, I think that is a good point. I think the evidence from the literature suggests that even the cutter vaccine, if you jacked it up with a potent adjuvant, it will protect against pneumonic plague.

MALE VOICE: So, it is just a matter of immunogenicity?

COLONEL FRIEDLANDER: Yes. I think the immunogens are there. We don't know exactly which ones, but they are there.

DR. GWALTNEY: I would like to pursue that

1 question, too. I have been on the Board, the
2 Scientific Advisory Board at (inaudible)
3 Laboratories for several years, and where they had
4 some very sophisticated and excellent
5 electrobiology.
6 The question I raise is the same one, that
7 if the whole organism vaccine doesn't work, why do
8 you think that the set unit is going to be better,
9 and I don't know many examples I can think of where
10 that has been the case and, again, to get back to
11 the fact that with aerosol -- with protection
12 against respiratory diseases, we have had trouble
13 with parenteral vaccine with other examples, and I
14 wondered about pursuing immunization by the
15 respiratory tract in some of these other things,
16 rather than the components of the vaccine.
17 COLONEL FRIEDLANDER: In fact, we have done
18 an experiment by aeros -- attempting to vaccinate
19 using the cutter vaccine by the aerosol route, and
20 we are not able to protect in the rodent.
21 DR. GWALTNEY: Do you get antibody
22 response, local or systemic?
23 COLONEL FRIEDLANDER: We don't have the
24 data on the local antibody response. There was
25 systemic antibody results. But, the point about the

route of immunization and the adjuvants that are going to be necessary for protecting against the lung I think are very valid points, and they are ones that we are looking at.

This is a different disease than anthrax. This is big time pneumonia, and we have to protect the lung. Yes?

MALE VOICE: I think streptomycin (inaudible) is only available in this country under protocol now.

COLONEL FRIEDLANDER: Right.

MALE VOICE: And it could be important to tuberculosis, and that could be problematic in thinking about protection against TW threat as well as endemic disease. Probably some action with the FDA at this point to be able to use Canadian streptomycin would be important.

COLONEL FRIEDLANDER: Yes. Yes?

MALE VOICE: Since this vaccine has really no operational efficacy, what is the Board's recommendation for us in maintaining the stores of vaccines that we have, and know it all expires tomorrow, but when the new stuff becomes available should we continue to get this vaccine and give it to our troops?

COLONEL FRIEDLANDER: Well, I would also temper that. I mean, there is -- the -- to say that it is not effective I think is perhaps a little too strong. It is not effective in these animal models.

MALE VOICE: No, operationally effective.

COLONEL FRIEDLANDER: Well, if you talk to some of the people who were in Vietnam they will tell you some other stories that I can't disclose in mixed company. I guess that is not the right -- politically correct thing to say, but if you talk to Cavanaugh, he will tell you about some exposures to pneumonic plague, pretty good exposures, where people were vaccinated did not come down with plague.

Now, they are all anecdotal experience. I told you about the laboratory exposures that were aerosol exposures with antibody rises. It -- there may be some efficacy of this vaccine, and it certainly is probably efficacious against bubonic plague.

DR. KULLER: Anymore questions?

(No response.)

We will take about a ten minute break now and then we will continue at about -- according to the schedule. Off the record.

(Whereupon, a brief recess was taken.)

COLONEL BYRNE: My name is Russell Byrne. I am at USAMRIID in the Division of Bacteriology and the purpose of this presentation is to give the Board an update on a couple of diseases for which there is a long history of vaccine use and development at USAMRIID.

At the risk of being somewhat pedantic, I am going to give a brief review of the history, clinical features and epidemiology of the diseases, along with a little bit about treatment, a few comments about antibody counter measures -- this work was done a long time ago at USAMRIID -- a brief vaccine history and then a current status, what we are doing now and what we plan to do in the next year, or two.

I will start with Q Fever. Number 1. (Visual aid provided.) Q Fever is an acute febrile illness people get usually from contact with animals caused by coxiella burnetii.

It was originally described in Australia by Derrick in 1937. The organism was characterized as rickettsial by McFarland Burnet, hence the name, and it is isolated from ticks by Gordon Diggs and Harold Cox in 1938.

Next. (Visual aid provided.) It is a --

MALE VOICE: Turn the mike so that everyone can hear.

COLONEL BYRNE: Turn the mikes what?

MALE VOICE: We can't pick you up real well back here.

COLONEL BYRNE: Talk into the mikes?

MALE VOICE: Yeah, talk into it.

COLONEL BYRNE: Alright. It is a pleomorphic coccobacillus, it is an obligate intracellular parasite for growth and reproduction although it survives in a spore-like form for a long time under very hostile conditions at times, it is grown in embryonated eggs or cell culture, it survives within the host phagolysosome, normally a very hostile environment with an acidic ph and a lot of digestive enzymes, it is resistant to heat and desiccation.

There are two phases. I mention this because it has some implications with respect to the vaccine. Phase I is smooth, virulent, infectious; Phase II occurs after serial passage in the laboratory. It is a result of a mutation, presumptively with a loss of enzymes for sensitis of lipopolysaccharide.

Number 3. (Visual aid provided.) There are rare reports of outbreaks of this disease in the United States. The distribution is worldwide. American soldiers could pick it up almost anywhere we deploy to. The only place that I know of where it has not been found where it has been looked for is New Zealand.

Primary reservoirs for human disease are livestock and farm animals, mainly goats, sheep and cattle, particularly parturient animals; lambing sheep, or cats having kittens and so on. There are a lot of organisms in the placenta and these are aerosolized quite easily.

It is highly infectious by aerosol. A single organism may cause disease and that was well-established in studies almost -- formed almost 50 years ago.

Number 4. (Visual aid provided.) After entry into the organism there is little or no reaction at the site of entry, as compared to tularemia. There is phagocytosis by macrophages without killing of the organism.

There is multiplication within the macrophage, initially, without harm to the cell, followed ultimately by cell lysis. There is

subsequent dissemination of multiple organs, particularly in the fetal system.

There is a brisk antibody response, but this is largely ineffective and infection is ultimately controlled by a cell-mediated immunity on the part of the host.

Number 5. (Visual aid provided.) The incubation period is 10 to 40 days. It is inversely correlated with inoculum size. When you give less than ten organisms you get a longer incubation period. This is work that was done long ago here.

The acute disease. The reason I list all these things mainly is to show that there is nothing specific about it. It is easy to miss this disease if you are not looking for it, because it is self-limited most of the time.

The fever, chills, malaise and headache are almost universal, like with other rickettsial diseases. Cough and chest pain are less common. Sore throat, nausea, vomiting and diarrhea are not common at all, but are reported.

The pneumonia occurs in a variable percentage of cases and frequently occurs without other symptoms referable to the respiratory track. It can look like acute hepatitis. It can be

1 asymptomatic, and this was also worked on by
2 Tigertt-Benenson in 1956
3 Individuals who were infected with a single
4 organism, two out of four developed disease and two
5 out of the four who sero-converted did not develop
6 any symptoms of acute Q Fever.
7 Number 6. (Visual aid provided.) Chronic
8 disease results in a small percentage of cases,
9 probably endocarditis is seen in less than 1%,
10 granulomatous hepatitis is probably just as
11 infrequent. I listed this -- this is a
12 personal communication from Barry Marmion who has
13 done a lot of work on the vaccine and the disease in
14 Australia, and it is his opinion in Australia that
15 20% of the acute cases are followed by something
16 that very closely resembles chronic fatigue
17 syndrome. That has not been published yet. This is
18 an observation that he is working on right now.
19 Number 7. (Visual aid provided.) The
20 diagnosis -- it is dangerous to try and culture this
21 organism, so it is not done very much. The
22 diagnosis is primarily by serology and you see
23 antibodies to the rough -- a very little organism
24 first, particularly Phase I. You see those in two
25 to three weeks.

The IGM will last six months, a year. Some of the cases described in Nova Scotia, the IGM has persisted for five years after the initial diagnosis, followed by development of IGG antibody in several weeks to several months, and the IGG antibody, one of the antigens is usually present for at least five years and I suspect probably life long, at least in the ELISA we perform here at USAMRIID.

Number 8. (Visual aid provided.) Treatment of Q Fever. The tetracyclines remain the mainstay of therapy. In chronic disease, tetracyclines are kind of the anchor for them. Attempts have been made to add other antibodies, for famp and trimeth from sulfamethoxazole have been used, and most recently D.D.A. Rowe in France has added hydroxychloroquine to alkalinize the Phagolysosome in an effort to treat Q Fever Endocarditis medically, which is very interesting. Quinalones have also been used with variable success.

Number 9. (Visual aid provided.) Prevention of infection with Q Fever in humans has been studied. Tetracyclines were used. Oxytetracycline, 20 grams is given over five to six

days and it was a 3 gram loading dose followed by 750 milligrams four times a day, starting -- when it is given 24 hours after exposure, when you administered for five or six days, the only thing that happens is the incubation period is prolonged.

If it is administered late in the incubation period the clinical disease is prevented, and if started within 24 hours after the onset of fever the fever subsides within 24 to 48 hours. So, the illness is shortened if it is -- if they are administered early.

Number 10. (Visual aid provided.) The history of Q Fever vaccines started shortly after the organism was isolated and grown in chick embryo yolk sacs. The Smadel Vaccine introduced in 1948, I believe, was a very effective vaccine.

Note the dose there of the antigen, 120 micrograms. At that time it was measured in competent fixation units and it was described as one to eight, and it is estimated that it was 120 micrograms. It was probably a mixture of Phase I and Phase II organisms, and the Smadel Vaccine was protective against aerosol challenge in human volunteers.

Number 11. (Visual aid provided.) There

1 were problems with the early vaccines, and this has
2 to do with the vaccine that we are working on now,
3 and the earlier vaccines were occasionally
4 associated with severe reactions.
5 Particularly, the things of most concern
6 were the sterile abscesses with sinus track
7 formation requiring surgical excision. If somebody
8 gets a big, swollen deltoid, that doesn't deter very
9 many investigators, at least in this room, but the
10 abscesses were a problem that it was an attempt made
11 to avoid it.
12 The reactions were subsequently associated
13 with either naturally induced immunity from
14 vaccines, and a skin test with a dilute Q Fever
15 vaccine antigen was used to screen people, and it
16 was found that in duration of five millimeters or
17 greater, a week after the administration of the skin
18 test it was associated with the development of
19 abscesses and, so, this -- the number of severe
20 local reactions, persistent reactions, was markedly
21 reduced after this was -- this procedure was
22 instituted.
23 Severe reactions were noted in the majority
24 of patients. These were prisoners in Montana, I
25 believe, who were inadvertently vaccinated who had

positive skin tests in 1963.

Number 12. (Visual aid provided.) There is a Q vaccine that is used. We use a whole cell vaccine here now. There is a vaccine licensed in Australia I will refer to as Q-Vax, manufactured by CSL Limited. The dose is 30 micrograms, and that is effective. 20 micrograms has been administered inadvertently, and that appears to be just as effective as the 30 microgram dose, and contrast that to the 120 microgram dose of the Smadel Vaccine.

The pre-vaccination screening with the 20 nanogram skin test is read a week after administration, and you vaccinate those that are negative. A few cases of acute Q Fever have been identified in vaccine recipients, and they were all felt to have occurred within the first two weeks of vaccination during the incubation period of Q Fever.

The immunity appears to be 100% There were no cases after two weeks following vaccination in any of the vaccinees, and it appears to last at least five years. It is very safe.

A single abscess among more than 5,000 vaccinees has been reported. This was in a master butcher who had been working in the industry for 30

years and had questioningly positive serology in a skin test that may not have been applied correctly, so it is thought that he was already immune.

The reason Dr. Marmion made his contact with us is because the cost of vaccinating somebody for Q Fever could be reduced almost by 50% simply by eliminating the skin test. So, that is why there is interest in it.

Next slide, number 13. (Visual aid provided.) Following a chloroform methanol extraction the residue that is remaining -- and this comprises about 78% of the weight of the organism -- this was found to be less reactive in mice.

It looked like a lot of the toxicity was removed when this procedure was performed, with a retention of the immunogenicity of the vaccine. Now, a lot of fatty acid is removed, and Jim Williams hypothesized that we are removing something that he called the immunomagetory complex. Be that as it may, the organism -- the CMR Vaccine does appear to be safer in laboratory animals.

Mike Ascher in 1983 reported diminished granulomatous reactions in guinea pigs with this vaccine when compared to whole cell vaccines. Protective immunity is induced in laboratory

1 animals.

2 Jeff Chulay looked at the skin tests

3 compared to whole cell and found that it was less

4 reactive than the whole cell skin test in --

5 particularly in individuals who had been vaccinated,

6 not so much in those who were immune by reason of

7 natural infection.

8 Dave Waag last year found that the

9 protective immunity was comparable to whole cell

10 vaccine in laboratory animals. There was a little -

11 - quite a bit of variability, actually, in the

12 ration of the CMR Vaccine to the whole cell vaccine,

13 but if all the results were pooled for mice and

14 guinea pigs via aerosol, interparenteral challenge,

15 the ratio is about one to one, so they are roughly

16 comparable.

17 Number 14, please? (Visual aid provided.)

18 What has been done recently -- oh, I neglected to

19 mention the safety on the last slide. Leave that

20 slide up. (Visual aid provided.) Lou Fries studied

21 the safety and immunogenicity of different doses of

22 the vaccine and humans were not screened for

23 immunity with the skin test. There were a lot more

24 reactions in the 120, 240 microgram groups than in

25 the 30 and 60 microgram groups. This was done at

1 Johns Hopkins.

2 Expanded safety trials were formed by Jenny

3 Losonsky at the University of Maryland. These were

4 double blind placebo controlled studies of 100

5 microgram CMR in non-immune individuals. These were

6 people that were screened with a skin test.

7 There were 50 vaccine recipients and 10

8 placebo recipients, and the safety profile looked

9 very good at that dose and, as a matter of fact, the

10 systemic reactions in the placebo recipients, such

11 as headache and fever, were the same as in the

12 vaccine recipients.

13 What we have got planned this year, we are

14 going to try and project an effective human dose for

15 this vaccine in non-human primate studies, and

16 Louise Pitt is going to start those studies later

17 this year in cynomolgus monkeys.

18 We are going to compare the 30 micrograms

19 of Q-Vax to 30 micrograms and 100 micrograms of CMR

20 Vaccine. They are going to be vaccinated and

21 challenged with aerosolized coxiella burnetii six

22 months later, and this is also going to be a placebo

23 controlled.

24 We are also developing a protocol to study

25 the safety of the CMR Vaccine in escalating doses in

individuals with positive skin tests. The whole purpose of the CMR vaccine development program is to get a vaccine that we can put into anybody, positive skin test, or not.

We are going to look at .3 micrograms of the CMR Vaccine. There are going to be groups of seven individuals. After that, if the -- if the safety profile is satisfactory, we are going to go to 3 micrograms, 10 micrograms and 30 micrograms -- 100 micrograms if we need to, and these are all Phase I safety trials.

That is -- that brings you up to date on what our plans are for the Q Fever vaccine. If the safety trials are acceptable, then we are going to have to look for a place to do a vaccine efficacy trial.

The Australians are still interested. They are not going to run a placebo controlled trial, because they already have a licensed effective product, but we will be looking for that. That will probably take some time to develop, because we don't have enough of the disease in this country, or we haven't identified enough disease in this country to study it.

The Tularemia, the first slide. To start

1 off with, I would like to thank Henry Eigelsbach who
2 provided me with the source material for a lot of
3 this. He gave me the re-prints and a re-print of a
4 review that he put together for the product license
5 for application, and also his personal recollections
6 were very valuable in my learning a little bit about
7 the history of this vaccine and the development.
8 Also, to Phil Pippen for sharing the data
9 that he developed here at USAMRIID in the Med
10 Division on the safety of the Tularemia Vaccine.
11 Tularemia is an acute febrile zoonotic illness like
12 a lot of BW agents, caused by a gram negative
13 coccobacillus Francisella tularensis.
14 A plague-like disease of rodents was
15 described by McCoy and Chapin in 1911. The organism
16 is isolated a year later and two years later after
17 the first bacteriologically confirmed case was
18 reported, Edward Francis pretty much spent his
19 career defining this disease in 1925 and published
20 the definitive description of it.
21 Number 2. (Visual aid provided.)
22 Tularemia is a fastidious strict aerobe. The
23 virulent organisms are encapsulated, but it is easy
24 to remove the capsule, even a saline wash will take
25 it off.

Now, there are two major groups of pathogens; the Old World and New World, or the via var tularensis and palaearctica, or Type A and Type B. The Type A tends to be the more virulent. That is what we see in North Carolina -- North America here. It is virulent for rabbits and mice. Type B is seen in Europe, Asia and also in North America, but with less frequency and is much less virulent.

Next slide. (Visual aid provided.) Tularemia is acquired by bites of ticks or deer flies and occasionally an animal bite by an animal infected with Francisella tularensis. The disease is seen in hunters handling animal carcasses.

If you eat an infected animal, or drink water that is infected -- it does grow in water, it grows in mud -- you can acquire the disease, and it is also easily acquired by aerosol. The infectious dose is one to ten organisms by the aerosol or intradermal route.

Number 4. (Visual aid provided.) After phagocytosis, there is initial multiplication within macrophages, followed by spread to regional lymph nodes and dissemination to the reticular and theo system.

There is involvement of the lungs

1 secondarily, in spite of an antibody response, cell-
2 mediated immunity, as with coxiella burnetii, as
3 required for control of the infection.
4 An important aspect is, immunity from
5 natural infection is not 100% Don Burke in his
6 review of the experience here in 1977 commented on
7 the fact that Ed Francis had the disease four times
8 during his career, and they are well-described
9 laboratory cases here in people who were infected
10 with the SCHU S4 strain who acquired infection
11 subsequently. So, natural infection is not -- does
12 not produce 100% immunity.
13 Next slide. (Visual aid provided.) The
14 incubation period is a few days to three weeks. The
15 mean is about 4.5 days. Three days after aerosol
16 exposure, symptoms are relatively non-specific;
17 fever, chills, headache, backache, malaise and
18 asthenia, as with other coxiella-like organisms.
19 In contrast to coxiella burnetii, there is
20 a marked reaction at the portal of entry. There is
21 erythema, edema and quite frequently an ulcer. It
22 may resemble an eschar, and that may be persistent.
23 In one case I saw, a man had been sick for
24 a month and the eschar was still there on his thigh.
25 There are six clinical types, but the vast majority

are the ulceroglandular.

Number 6. (Visual aid provided.) Again, culturing the organism is hazardous. There are dozens of cases in the 1950's here at Fort Detrick from people working on the organism. The diagnosis is usually done by serology.

The agglutinins appear within 10 to 14 days, and maximally in three to four weeks. The micro-agglutination test is done here in the Applied Research Division.

Next one is Number 7. (Visual aid provided.) Treatment is with streptomycin. Gentamicin is also effective. Tetracycline and chloramphenicol work if you give the drugs a little bit longer. The untreated mortality is 5% unless there is pulmonary involvement, and then the mortality is estimated to be 20-30% Treated mortality is 1 to 3%

Next slide. (Visual aid provided.) Antibiotic prophylaxis, the bactericidal antibiotics are more effective. Five days of streptomycin following a challenge is effective in protecting against infection, whereas five days of chloramphenicol failed, or only worked in two out of the five recipients.

1 Aerosol challenge; tetracycline initiated
2 within 24 hours after a challenge. If you give 15
3 days of a gram a day the disease occurs in 20% of
4 the individuals afterwards. If you give a gram a
5 day for 28 days, or if you increase the dose to two
6 grams a day, you get complete protection.
7 As I mentioned, the bactericidal
8 antibiotics are considered to be more effective,
9 although the bacteriostatic antibiotics are
10 effective if the dose and duration are adequate.
11 Number 9. (Visual aid provided.) Vaccine
12 history starts pretty much with Dr. Foshay here in
13 this country. I don't know how to pronounce that
14 other name. I could guess at it. I put that on
15 there. This was mentioned in Henry Eigelsbach's
16 review. This was a Russian who worked on a kill
17 vaccine back in 1931, so the Russians were
18 interested in this vaccine for a long time, too.
19 The kill vaccine is probably ineffective in
20 preventing a clinical disease, although it does
21 modify the course of the infection. People get
22 better quicker, the temperatures aren't as high and
23 the need for antibiotic therapy is diminished.
24 Number 10. (Visual aid provided.) Since
25 the kill vaccines on review were not felt to be

1 tremendously effective, a live vaccine was
2 speculated as a -- postulated as a solution both
3 here at Ft. Detrick and in Russia.
4 The vaccine was acquired during United
5 States/USSR Medical Exchange Mission in 1956 and
6 members of that Exchange Mission included Dr. Karl
7 Meyer, along with Sabin, Debow, Smadel and Enders
8 (sic), and they knew of the work in the tularemia
9 vaccine and requested a sample of it from Professor
10 Olsuflev, and they got an ampule of the vaccine from
11 him, and that is how we -- well, that is not
12 directly how we acquired it.
13 It had instructions for use, a lot number
14 and expiration date, and a lot of it had been given
15 in that country. Dr. Eigelsbach received his sample
16 from Cora Downs at the University of Kansas, who
17 received her sample from Karl Meyer.
18 Number 11. (Visual aid provided.) The
19 Soviet vaccine initially was very safe. It was very
20 benign, you know, but it didn't protect against
21 challenge with the Schu S4 strain which was the
22 virulent test organism used at that time here, and
23 Dr. Eigelsbach increased the virulence by serial
24 mouse passage.
25 There are two colony types, one blue and

one gray, and the blue colony type is more virulent and more immunogenic, and that was selected as the live vaccine strain. The decision was made to go ahead with the Soviet vaccine, Dr. Eigelsbach told me, because the conversation with Colonel Tigertt.

They felt there was a lot of concern at that time about reversion to the wild type and they felt that if this vaccine reverted to the wild type they would get something that was less virulent, as opposed to the polio vaccine which reverts to something that causes disease.

This is a little out of place, but it was initially produced at Ft. Detrick here, after which it was made by the national drug company, Swiftwater of Pennsylvania, which became the Salk Institute, subsequently.

It was interesting to me that within three years of the time we got this vaccine from the Russians that we were using it here. This was right in the middle of the cold war and the Berlin Wall was going to go up, Hungary had been invaded, and it still amazes me that we had so much faith in this vaccine.

Next slide, Number 12. (Visual aid provided.) This vaccine, after the virulence was

increased by Dr. Eigelsbach, was protective against aerosol and intradermal challenge of up to 20,000 organisms.

The efficacy ranged from around 40% to up to 95% It was not 100% It was a lot better against the lower challenge doses than against the higher challenge doses. It did not appear that if you immunized people by aerosol that the immunity against challenge by aerosol was improved significantly, so we continued to administer it intradermally, as before.

When somebody is vaccinated, the correlates of protection are, number one, induration at the site and, number two, development of antibody, any antibody above the background, non-specific binding.

Immunity is very long-lasting. Here at USAMRIID we don't give boosters routinely. The individual's antibody is checked and if there is any antibody present it is not re-administered.

Number 13. (Visual aid provided.) The experience here at USAMRIID was summarized in 1977 by Don Burke. I was surprised to find out he had a life before Retrovirology. That was interesting to me. Well, it is true, he compared the vaccine

experience from 1950 to 1959 with the Foshay vaccine, compared to the following decade with the live vaccine strain.

The incidence of typhoidal tularemia decreased with the live vaccine strain from 5.7 to 0.27 cases per thousand, the at risk employee years. These were all civilians here, by the way. The incidence of ulceroglandular tularemia remains stable because it doesn't -- it doesn't appear that this vaccine is 100% effective under any circumstances.

Number 14. (Visual aid provided.) The vaccine as it is currently utilized is lyophized. It is administered by scarification. It is a lot like -- it's a lot like smallpox vaccine, .1 ml of the vaccine solution. The solution contains 2.4 times ten to the eighth organisms per ml.

It is scarified with a bifurcated needle and there is normally a small area of induration, perhaps with a tiny ulcer, and this is considered a normal primary take, like smallpox.

Complications include local pain, erythema, induration in excess of what is considered to be the primary take. Also, they can vesiculate, it can get secondarily infected, the ulcer can enlarge.

1 Ipsilateral axillary adenopathy has been described,
2 kind of like with the typhoid vaccine we used to
3 use.
4 Fever and headache is also described, and
5 at one time it looked like liver function tests were
6 elevated in response to that vaccine, but that does
7 not appear to be the case when compared to placebo
8 controls.
9 Number 15. (Visual aid provided.) The
10 experience here at USAMIID, the total vaccinees
11 numbered more than 5,000 over the 31 years, in that
12 time span -- this was the NDBR 101 -- in addition to
13 that prepared at Ft. Detrick.
14 The experience with this law was reviewed
15 by Phil Pittman; 838 individuals, local reactions
16 exceeding primary take were seen in 6.1%, systemic
17 reacts as a one and a half percent, and both local
18 and systemic reactions in one half percent. He
19 concluded the vaccine was very safe from that
20 experience.
21 Last slide, Number 16. (Visual aid
22 provided.) One lot was compared to placebo here at
23 USAMIID by Phil Pittman and he noted that
24 tenderness, pruritus and diarrhea were more common
25 in the live vaccine strain group by statistical

analysis -- excuse me -- Lou Fries examined the other two lots at Johns Hopkins and found similar findings.

The micro-agglutination ties the human immune response to the geometric mean (inaudible) were not statistically different from the NDBR 101 product, and a product licensure application for the lots is being prepared. Bud Cole is responsible for that at USAMDA, and he is working closely with other people, including a contractor.

That concludes the presentation. Thank you. Are there any comments or questions?

(Pause.)

Yes?

DR. JORDAN: I have a general comment. It seems to me that as you develop these vaccines that if you start with the most effective adjuvant (inaudible), you might take a lesson from (inaudible).

You have a situation where you want to give (inaudible) protection against potentially (inaudible). You might consider that possibility, which means you have got to do some more work (inaudible).

GENERAL RUSSELL: Was a license issued for

the first national drug product? Was that a licensed vaccine? (Inaudible.)

COLONEL BYRNE: By the way, there is a hand-out for those who want them out on the desk.

DR. ENGLEY: Let me be a picky, picky microbiologist. You listed the organism as being a fastidious highly aerobic organism. Actually, we published it here in 1949, the fact that it only takes (inaudible). Now, that is not very fastidious. I think on ten to the tenth micro-organism (inaudible).

COLONEL BYRNE: I appreciate that.

DR. ASCHER: In the next month, or so vaccine policy on Q Fever will be listed where to date the development has been in the area of biological defense, but most of us would agree that the real threat is the natural exposure in certain environments.

So, it will be interesting to see how that plays out as we decide how monies that are compartmentalized which can or can't be used for one purpose for a product like this, which is a very well developed product, a very effective program, with a very well recognized threat in nature, but not as much as a biological warfare threat.

So, every combination is present in our matrix of things to think about.

COLONEL BYRNE: I think soldiers pick it up anywhere they go. We saw at least four cases in the Persian Gulf.

MALE VOICE: Thank you.

MALE VOICE: Sure.

DR. KULLER: Colonel Hoover will talk about brucellosis.

COLONEL HOOVER: Thank you. Could I have the first slide, please? (Visual aid provided.) In contrast to the previous speakers, the Brucella Program is relatively new. We began this program about two years ago and we very soon realized that fielding an immunologic defense against brucella would pose some interesting scientific challenges.

What I would like to do today is not only give you the goals of the program and the strategies we have devised to reach those goals, but also to provide you with some conceptual framework that we base the program on.

Next slide, please. (Visual aid provided.) We operate under Science and Technology Objective 4-B, which is to develop medical counter measures against the biological threat of brucella species.

1 Specifically, by FY '99 we are charged with
2 transitioning to development a vaccine that will
3 protect any percent of immunized personnel against
4 an aerosol challenge of any species of brucella and
5 will induce minimum reactogenicity in soldiers when
6 immunized; by Fy '96 to demonstrate the feasibility
7 of producing a vaccine against brucellosis using one
8 species as a model approach; and during the FY's
9 from '94 to '99 to investigate new biotechnology
10 methods for counter measures against all three
11 species of the genus brucella, and that is the three
12 classic pathogens.
13 Next slide, please. (Visual aid provided.)
14 These are the brucella species for those who don't
15 remember. Those in yellow are the organisms that
16 cause disease in man and they are listed in
17 approximate descending order of virulence.
18 B. melitensis which classically affects
19 sheep and goats is the most virulent. B. canis
20 which is a naturally rough strain infects dogs, but
21 causes disease in humans pretty much
22 indistinguishable from that caused by brucella
23 abortus. The last two members, B. ovis and B.
24 neotomae are not known to cause disease in people.
25 Typically, brucella is a zoonosis and is not a

1 primary pathogen of man.

2 Next slide, please. (Visual aid provided.)

3 Man, therefore, acquires in nature the disease by

4 exposure to animal products. It is an occupational

5 disease of abattoir workers and herdsmen.

6 Worldwide, a very important mode of

7 acquisition is the ingestion of unpasteurized dairy

8 products. That is particularly goat cheese in

9 Mexico, South America and throughout the

10 Mediterranean Basin, but, really, the disease is

11 found wherever large animals are located.

12 Interestingly, recent reports suggested

13 that camping in a contaminated environment, that is,

14 the Kuwaitee Desert, was associated with acquisition

15 of disease even in individuals who did not give a

16 good ingestion history, or history of exposure to

17 animals.

18 Laboratory workers are at very high risk,

19 and outbreaks of a number of individuals in

20 laboratories have occurred simply when a plate was

21 opened and streaked on the open bench top without

22 use of bio containment.

23 These natural routes of infection indicate

24 that infection, then, can occur by either ingestion,

25 direct inoculation, or by inhalation. One would

1 expect in a bio warfare scenario that soldiers would
2 be exposed to all three routes of infection.
3 Next slide, please. (Visual aid provided.)
4 The disease is actually a fairly non-specific
5 febrile illness. 95% of individuals have fever, and
6 that is the most characteristic sign. From that,
7 myalgias, weakness and chills are described.
8 Lassitude and neuro-psychiatric symptoms
9 received a great deal of attention in earlier
10 reports, but have received less attention recently.
11 Actually, only a minority developed localized
12 disease.
13 Bone and joint disease occurs in about 30%
14 This is usually non-destructive, although a large
15 joint, particularly hip and vertebral disease, can
16 be destructive.
17 About 20% of patients have respiratory
18 complaints, mostly cough. In spite of that, chest
19 x-ray is usually normal and this is true no matter
20 what the route of the infection. Occasionally,
21 there is GU disease, hepatitis, or central nervous
22 system disease. Fatalities are rare, usually
23 from endocarditis and treatment of this endocarditis
24 from brucella requires valve replacement, as well as
25 antibiotics. Central nervous system infection also

occasionally has resulted in fatality.

Next slide, please. (Visual aid provided.) Diagnosis, although a number of ELISA's and other diagnostic approaches have been worked on and are being very actively pursued both within the military and without, the standard still remains a tube agglutination test and culture of both either marrow or blood, or infected tissue, but marrow culture appears to be better than blood.

In the range of positive cultures in infected individuals, ranges from about 10% to 100% partly depends on the skill of the laboratory and the length of time that the cultures are held. A culture usually has to be held for up to two months before you can discard it as truly negative.

Next slide, please. (Visual aid provided.) Treatment in general is pretty straight forward. Doxycycline and rifampin is the current recommended regimen. There are some alternative drugs that are available, as well.

Interestingly, the Soviets reported a couple of years ago the development of a multi drug-resistant strain to at least streptomycin, tetracycline and a few others. So, in a bio warfare scenario we would have to contend with the potential

1 for a drug resistant organism.

2 Next slide, please. (Visual aid provided.)

3 Pathogenesis of brucella is probably similar to a

4 number of the other organisms we have heard about.

5 It is a facultative intracellular parasite in a

6 macrophages. It enters phagocytic cells at the

7 portal of entry.

8 The best evidence for this is M cell

9 penetration in the small intestine. It is then

10 carried to the regional lymphoid tissue and then to

11 the rest of the reticular endothelial system.

12 Control of replication is primarily mediated by T

13 cells and in murine models, primarily, macrophages

14 can be shown to be activated for non-specific

15 microbicidal activity.

16 Anti-gamma, interferon, anti-TNF reduced

17 resistance to infection, but do not result in a

18 fatality, and both CD4 and CD8 positive T cells may

19 play a role in infection. The cellular immunology

20 of brucella infection has really not been well

21 elucidated with modern techniques.

22 Next slide, please. (Visual aid provided.)

23 Although we are interested in an aerosol route of

24 infection, primarily, the standard murine model of

25 brucella that is available in the literature is one

that uses interparenteral or intravenous inoculation.

By this route, a chronic infection of the liver ensues, then the spleen is also rapidly infected. This gradually declines over a period of 12 to 16 weeks and the mice eventually become sterile. Mice are not killed and do not look ill when infected with brucella, so there is no lethal model.

Guinea pigs have also been used and in very high doses one can induce lethality, but, in general, the models are not lethal. Therefore, successful interventions, that is, vaccines or other treatments, are measure by a reduction in the number of spleen colony forming units at the peak, and that is usually about two weeks after inoculation.

Unfortunately, if you go and look out at six to eight weeks, usually a vaccinated animal will have a very similar number of organisms, as compared to an unvaccinated animal. So, there is a transient reduction in the peak spleen colony-forming units.

Next slide, please. (Visual aid provided.) There are several large animal vaccines that are available. Again, this is primarily a zoonosis, a big problem in animal husbandry. These vaccines are

1 designed to protect against abortion and secondarily
2 they protect against genitourinary disease.
3 There are some killed whole cell H-38. B.
4 melitensis based vaccine was in use, but has been
5 largely superseded by either strain 19, which is an
6 attenuated brucella abortus strain, or REV 1, which
7 is an attenuated B. melitensis strain.
8 An interesting strain that is currently
9 under development is a strain called RB-51 developed
10 by Gerhardt Shurig and others at Virginia Polytech.
11 This is a rifampin resistant organism and it is
12 rough.
13 It is very poorly infectious in animal
14 models, but those give heterologous cross
15 protection. This unfortunately would not be very
16 useful for us because it is rifampin resistant and
17 rifampin is one of the major antibiotics that we use
18 to treat brucella.
19 In fact, it is likely that it is not just
20 the roughness of this organism, but probably its
21 rifampin resistance that is also related to the
22 attenuation. Both strain 19 and REV 1 have either
23 been tried in people and have caused symptomatic
24 bacteremia and, as a matter of fact, veterinarians
25 who administer these agents occasionally develop

1 symptomatic disease.

2 Next slide, please. (Visual aid provided.)

3 There have been a couple of human vaccine attempts.

4 The French developed a preparation. In the 60's

5 there was a phenol insoluble derivative of

6 delipidated bacteria. It has caused local reactions

7 up to ten centimeters of erythema and induration in

8 about 85% of recipients. It was immunogenic, as

9 measured by antibody responses and by skin tests to

10 brucellin.

11 Its efficacy is unknown formally, although

12 anecdotally the developer has indicated that

13 individuals, laboratory workers vaccinated with this

14 strain, or with this preparation, had reduced

15 numbers of infections. This has not been looked at

16 in a clinical trial.

17 Next slide, please. (Visual aid provided.)

18 Soviets also developed a live vaccine which was

19 based on a selected brucella abortus strain 19.

20 This was administered not really subcu, but by

21 scarification. It costs about two to four

22 centimeters of erythema and 47% of the vaccinees

23 which lasted five to six days, or more.

24 A very small number had systemic

25 complaints, but there was no systemic true illness

1 observed by physicians over a period of six weeks.
2 Of 161 individuals who were followed with serial
3 blood cultures, only one developed a positive blood
4 culture in this group.
5 This was actually an immunologic reactions,
6 and that is not well-defined, actually, from the
7 literature and were maintained at about 60% for two
8 to three years with this vaccine.
9 Now, this was administered to a couple
10 hundred thousand people in an open basis and the
11 infection rate was a half a percent in those
12 receiving the vaccine versus 12.3% in unvaccinated
13 individuals, and the comment was made that during
14 about six to nine months following vaccination the
15 disease in those who did become infected was milder.
16 I think the interesting aspect of this
17 vaccine is that it suggests that a live attenuated -
18 - to some extent, attenuated -- vaccine may actually
19 have some promise in terms of reducing clinical
20 disease.
21 Next slide, please. (Visual aid provided.)
22 Now, with that background, we would like to refocus
23 the vaccine goals. We would like to protect against
24 an aerosol challenge with minimal side effects.
25 An immunization schedule should use a small

1 number of doses at close intervals and there are
2 really -- I think we have an ultimate goal and an
3 interim goal. The ultimate goal is to produce a
4 long-lasting sterile immunity against a high dose
5 challenge. This may require a live vaccine, or
6 alternatively a live vector vaccine with expression
7 of, quote, unquote, "relevant antigens" in a vector
8 such as salmonella.
9 As an interim, however, we may be able to
10 increase the infectious dose 50 -- or, reduce
11 symptoms with a subcellular vaccine. All the data
12 from mouse studies indicate that the -- the -- in
13 the first place, LPS is an immuno-dominant antigen
14 and all the protection that can be transferred by
15 serum is based on anti-LPS antibody.
16 Next slide, please. (Visual aid provided.)
17 We will -- our primary concern is to develop models
18 that were appropriate for this development of these
19 vaccines. We need them to screen potentially
20 attenuating mutants to characterize the efficacy of
21 antibody search for target antigens and establish
22 correlates of protection.
23 This would be important since we will not
24 be able to do a challenge study. We will need a
25 suitable animal model that should mimic the expected

respiratory challenge route, and we will need to characterize the immune response both to natural infection in that model and also characterize the immune response to candidate vaccines so that we can have some correlates of protection that might be useful in either primate, or in immunized human situations, and we also, of course, need to provide a mechanism to screen candidate vaccines for toxicity and efficacy.

Next slide, please. (Visual aid provided.) I thought I would just mention one administrative issue. We don't have a BL-3 facility at WRAIR, so we have established an MOU with the AFIP in the division of microbiology, and the program at WRAIR is primarily in the Department of Bacterial Diseases.

We also have established collaborations with three contractors taking advantage of the expertise in the veterinary community, and these individuals should be performing various -- particularly screening aspects of the program.

Next slide, please. (Visual aid provided.) The study was divided into several tasks and these are the -- these are the studies that we actually have ongoing at the present. I will show you a

1 little bit of data at the end to show you what
2 progress we made.
3 First, we are using human immune sero to
4 screen brucella lysates and DNA libraries to
5 identify immuno-dominant proteins that might be
6 vaccine candidates. We are making deletional
7 mutants in brucella both as functional tools to
8 understand the path of the intercellular
9 pathogenesis and also to develop vaccine candidates.
10
11 Screening for brucella antigens stimulate
12 proliferation of lymphocytes from infected mice and
13 were -- and we are getting ready to immunize mice
14 with these antigens using vaccinia virus as a live
15 vector. This is actually being done by the
16 contractors, not by us. We are also developing
17 potentially attenuating auxotrophic mutants and I
18 will show you some data on that a little later.
19 Next slide, please. (Visual aid provided.)
20 Interestingly, we are trying to identify bacterial
21 genes that are selectively activated during
22 residence and host cells. If we can identify these,
23 we may be able to knock them out and use the -- use
24 the deleted mutant as an attenuated organism, or
25 express these genes in a live vector.

Looking at mechanisms of entry of brucella into human monocytes while we are using this as a screening mechanism for attenuated vaccines, or antibody, now we are examining the role of antibody and compliment in both direct killing opsonization and intracellular killing of smooth and rough strains.

Next slide, please. (Visual aid provided.) We are establishing murine models, as I mentioned. We are examining the effect of the antibiotic treatment on protective immunity and we are investigating the effects of immuno-modulating agents on the course of infection and host defenses.

This would be an immuno-prophylaxis, or immunotherapeutic approach which is a relatively -- we expect to be a minor component of the program.

Next slide, please. (Visual aid provided.) We have purified LPS from brucella immunized mice with it alone, or with adjuvants, and I will show you some data, and we are characterizing host immune responses during the course of immunization, and we are examining host defense mechanisms during infection with either the live attenuated strains, or challenge infection.

Next slide, please. (Visual aid provided.)

1 We have done some work with highly purified B.
2 melitensis LPS. As it turns out, the brucella LPS
3 is a lot tougher to purify than bacterial LPS. We
4 found that this highly purified LPS is immunogenic
5 by its subcutaneous route in mice. It gives us
6 about 4,000 O.D. units after a boost and is also
7 immunogenic in rabbits. That is not new. That is
8 well-described in the literature.
9 Interesting, N. meningitidis outer membrane
10 protein, which enhances responses of
11 polysaccharides, also enhances IgG antibody
12 secreting cells, as determined by an LA spot (sic)
13 technique and also peripheral serum antibody
14 response.
15 We have been interested in looking at
16 intranasal immunization. We are clearly concerned
17 about a mucosal route of infection and we thought it
18 would be interesting to see whether intranasal
19 immunization, either with LPS alone, or with the LPS
20 outer membrane and meningitidis outer membrane
21 protein conjugate would be effective.
22 We found that LPS alone induced IgM and IgA
23 antibody-secreting cell response, but little
24 antibody in the lung lavage fluid, but these
25 responses were markedly boosted by the outer

1 membrane protein. I will show you that on the next
2 slide.
3 Next slide, please. (Visual aid provided.)
4 Ooh, I am sorry, I don't think this shows up well.
5 This is their control mouse. These are mice
6 immunized with two different doses of LPS and these
7 are mice immunized with LPS with a high dose and low
8 dose of LPS with ten micrograms of N. meningitidis
9 outer membrane protein.
10 This is a one intranasal -- one dose
11 intranasally. The mice are sacrificed at ten days
12 and lungs are lavaged and antibody is detected by
13 ELISA against purified LPS. So, you can see we get
14 a very nice boost with N. meningitidis outer
15 membrane protein as an adjuvant.
16 We think -- this is one of the areas we are
17 clearly planning to pursue as a potential route of
18 enhancing mucosal immunity.
19 Next slide, please. (Visual aid provided.)
20 We have also developed a mutant -- deletional
21 mutant --in the purine pathway. This is a 278-base
22 pair deletion in the purE gene, which is very early
23 in the purine synthesis pathway. The B. melitensis
24 with this defect infects, but does not replicate in
25 human monocytes, and I will just show you a slide

1 next on that.

2 Next slide, please. (Visual aid provided.)

3 This is a slide of human monocytes one week old,

4 infected with either the wild type 16M strain of B.

5 melitensis, or with the purE mutant, and here is

6 another purE mutant down here where you don't have

7 all the time points.

8 You can see that the wild -- and the

9 macrophages are viced and colony-forming units per

10 culture are determined by serial dilution and

11 plating at various time periods. You can see that

12 the 16M replicates over a period of a couple days in

13 monocytes, whereas the purE mutant declines.

14 There is a couple of interesting points

15 here. One is that it is not killed right away. It

16 does -- in this study it persisted for five days,

17 and that was repeated on a couple of occasions. So,

18 it may be that a mutant of this type will persist

19 long enough to generate an appropriate immune

20 response, but hopefully will not be virulent enough

21 to cause disease.

22 Next slide, please. (Visual aid provided.)

23 I would just like to point out that Dr.

24 Bhattacharjee, Van de Verg and Warren were principal

25 players in the development in looking at the

antibody responses in intranasal challenge. Dr. Huo-Shu Houng and Warren were the principal -- the principal developers of the purE mutant.

Next slide, please. (Visual aid provided.) Where are we going to go from here? Well, we need to establish our standard murine model, which is the IP infection route.

We need to develop a murine intranasal challenge model to mimic the expected route and then we will -- we will take the preliminary data we have so far and we will test protective efficacy of subcu and intranasal immunization in these -- particularly in the intranasal challenge model -- and then we will determine attenuation and protective efficacy of the purine mutant.

We are also collaborating with individuals at the USDA to test the purE mutant in goats, because they are interested in this approach as a vaccine against brucellosis in animals. Thank you.

DR. ENGLEY: There is evidence there is a relationship between the antigens of tularensis and brucella.

COLONEL HOOVER: Right.

DR. ENGLEY: Have you checked to see how much antibody results from immunization of one or

the other through crossing and also skin testing for hypersensitivity?

COLONEL HOOVER: As a diagnostic -- as a diagnostic measure?

DR. ENGLEY: Well, I was speaking about some of your personnel who might get tularemia, then get brucella vaccine.

COLONEL HOOVER: Well, it is true there is some -- it is true there is some cross reactivity and this appears to be relatively minor -- serologic cross reactivity -- but it can be a problem, actually, in diagnostic tests.

You have to exclude the possibility of tularemia in individuals who have a positive brucella (inaudible).

DR. ENGLEY: But, you don't routinely check to see how much antibody you get against tularensis when you give it to someone?

COLONEL HOOVER: We have not done that. I mean, we have not administered this to people at this point. We are only in mice and we haven't looked for that. That is an interesting idea, though. It would be worth doing.

DR. ENGLEY: Because that would bring up Dr. Jordan's question about putting vaccines

1 together.

2 COLONEL HOOVER: Um-hum.

3 DR. KULLER: How much work is there going

4 on in this area in other places? In other words,

5 this has some public health significance, in

6 general. Is there a lot of work going on in vaccine

7 development outside of this program, or is this

8 unique?

9 COLONEL HOOVER: There is very little in

10 terms of human development. There is very little.

11 The Canadians actually do have a very small brucella

12 research effort with -- but, again, they are mostly

13 at this point looking at testing in animal models.

14 But, most of the work that is being done is

15 being done under the auspices of agricultural

16 departments, because this is a big zoonotic problem,

17 that the RB-51 vaccination is an offshoot of that.

18 There are somewhat different goals with

19 the -- and problems -- with the animal vaccines

20 compared to human vaccines. The big problem in

21 animal vaccines is that the standard agglutination

22 tests measures anti-LPS antibody and, so, the

23 current vaccines are about 80% effective in

24 preventing disease in animals.

25 But, the agricultural folks would like to

have a vaccine that does not induce anti-LPS antibody and that is the push for RB-51, because then they can use their screening test to decide what animals are infected and which have just been vaccinated.

We would anticipate, actually, that if the -- that if we can develop good attenuated mutants we might be able to combine those, and we are working on that, as well, with -- either put them into rough strains or knock out genes in the LPS biosynthetic pathway and use a double deletion mutant which might have efficacy both in human -- for human use and for animal husbandries.

DR. KULLER: So, is there some attempt to see whether the vaccine program that you are developing would have some impact on the -- in the agricultural community? I mean, is that the --

COLONEL HOOVER: We have -- we have three of our collaborate -- all three of our collaborators, in fact, are from the University of -- are contractors. One is from D.P.I., Dr. Shurick (sic), who developed the RB-51, Dr. Splitter (sic) at Wisconsin and Dr. Rupe (sic) at L.S.U.

They were all in veterinary schools and, so -- and the USDA is very interested in their -- as

a matter of fact, I think they inoculated the goats today to test to see whether purine mutant will be attenuated in their animals.

So, yes, there is a lot of interest, but -- and, interestingly, the budget for brucellosis vaccine research in this country has been going down because the Brucellosis Eradication Program has been pretty successful, particularly for abortus.

DR. KULLER: Thank you.

DR. BENENSON: Under therapy, you did not give tetracyclines to children, but you gave it to pregnant women. Isn't that contradictory?

COLONEL HOOVER: Well, I think the problem is the -- and, you know, the alternative, trimethoprim sulpha is ciprofloxacin rifampin. You can't use rifampin alone, but trimethoprim sulpha and ciprofloxacin probably would not be ideal drugs in pregnant --

DR. BENENSON: I think four weeks (inaudible).

COLONEL HOOVER: I don't think there are -- I think there are problems, but I don't think there are great alternatives in pregnant women, frankly.

DR. ASCHER: There are about half a dozen cases a year in California from soft Mexican cheese,

and the last one had a lab infection and (inaudible).

COLONEL HOOVER: It really -- it is in a comment in 1977 that said this was the most infectious threat to laboratory personnel, and that appears to be true.

DR. KULLER: Colonel Taylor is going to talk about cholera.

COLONEL TAYLOR: May I have the first slide, please?

(Pause.)

There are three main reasons that we are interested in developing cholera vaccine from an infectious disease standpoint. One was the impact of E tech diarrhea that we had seen in travelers over now many years, and also in the military.

The data coming back from the Navy Forward Laboratory from the Persian Gulf War definitely indicated that E tech was one of our most important causes of illness out there.

Also important is the introduction of epidemic 01 cholera into Latin America. This happened beginning in January of 1991, and since that time there have been over a million cases with nearly 10,000 deaths throughout Latin America, going

1 from Mexico all the way down to Chile. This is
2 introduction after a hundred year absence of cholera
3 and, so, definitely is one of the important emerging
4 infections in Latin America.
5 Finally, the emergence of vibrio cholera
6 0139. This is a new 901 sero-type that has emerged
7 as an important cause of epidemics in India, in
8 Bangladesh, as well as other regions outside of that
9 area. There have been outbreaks now in, I think,
10 half a dozen, or so countries in that area. Right
11 now, from our standpoint in Thailand where we have a
12 laboratory, we see 0139 in about 40% of all the
13 cholera cases now. So, these are the
14 important reasons why we are studying cholera
15 vaccines and also inter-toxigenic E. coli, and we
16 really got into the business of studying cholera
17 vaccine because of the cross protection that was
18 seen with the whole cell B subunit cholera vaccine
19 in Bangladesh.
20 Now, just a reminder, vibrio cholera has
21 two biotypes and two serotypes, as well as the El
22 Tor and classical biotype and the Inaba and Ogawa
23 serotypes and, so, there are four combinations there
24 that you could have.
25 0139 has a lot of similarities with the El

Tor biotype, but there also seems to be some exogenous DNA that makes it a 901, as well. So -- and it also has other properties, such as encapsulization which is -- makes it similar to the 901's.

Then, in terms of pathogenesis, what is important to remember is that the toxin that causes secretory diarrhea is cholera toxin with vibrio cholerae, and there is a highly related toxin, heat labile toxin, of enterotoxigenic E. coli which causes secretory diarrhea with that organism.

So, that is the underlying similarity which allows for the cross protection between cholera vaccines which produce an anti-toxic immunity in E. coli.

These toxins have this A and B structured subunit type, and the B subunit is a large sort of a circular shaped pentameric structure which causes attachment. It is very immunogenic, and the A subunit is the active unit which actually activates the secretory process through an enzymatic reaction.

Now, these are the vaccines that are available for cholera. Of course, the killed whole cell vaccine is the one that is licensed in the United States. This requires two doses a week

apart, with boosters, and is quite reactogenic and is poorly immunogenic and only gives about a 50% protection, which is short lived.

So, that is the vaccine that is on the books that we are trying to improve on. The killed whole cell plus B subunit vaccine is an oral two or three dose vaccine. We know that it is very safe.

It has been used extensively in -- extensively evaluated, I would say, in a trial in Bangladesh that began in the mid-80's. It was shown to give short term protection over the first year of around 75 -- 70% protection, and over the years about 50% protection and, of course, this is an endemic population there. This vaccine is available at least in the Scandinavian countries.

Another vaccine under evaluation now is the live attenuated vibrio cholera strain, CVD 103 mercury resistant. This is a single oral dose. It has been extensively tested for safety. It is very safe and gave a 70 to 100% protection in the challenge model at the University of Maryland.

The vaccine is licensed in some countries in Europe where it is made by the Swiss Serum and Vaccine Institute and is right now under field efficacy evaluation in Indonesia.

1 The strain that we have been evaluating is
2 another live attenuated strain which is called Peru-
3 14. This is, again, a single oral dose vaccine
4 which is safe and produces high level -- perhaps not
5 100% -- protection in the challenge model and it is
6 undergoing continued Phase I and Phase II testing in
7 a product type preparation.
8 Finally, another vaccine which we have
9 looked into is this polysaccharide, conjugated to
10 CTB, the cholera toxin B subunit. So, this is a --
11 the de-lipidized cell wall polysaccharide from
12 vibrio cholera conjugated to CTB.
13 So, this is a conjugate type vaccine. It
14 was developed by John Robins at the NIH in his
15 group. It is a single dose IM vaccine, produces
16 some mild local reactions, but like other
17 polysaccharide conjugate vaccines it is quite safe
18 and well-tolerated. We don't know whether this
19 vaccine is going to be protective, but it is
20 certainly immunogenic, producing a nice immune
21 response, and it is undergoing further evaluation
22 for safety and immunogenicity.
23 Now, the four vaccines that we have been
24 working with most closely at Walter Reed and here at
25 USAMRIID are these vaccines; the inactivated whole

1 cell plus B subunit vaccines, and these are made by
2 SBL Vaccines which is a -- the newly formed company
3 that came out of the Swedish bacteriological
4 laboratory.
5 This is a vaccine that's indication is
6 for -- the cholera vaccine part of it is for 01
7 cholera and it contains a mixture of El Tor and
8 classical strains, plus a milligram of the B
9 subunit. Again, it is an oral, two-dose vaccine,
10 and we will talk more about it. It is testing now.
11
12 They have also been able to take E. coli
13 cells, preserve the colonization factors from these
14 -- from the inter-toxigenic strains, and we are now
15 evaluating that as a vaccine purely against inter-
16 toxigenic E. coli, and we are doing the safety
17 studies now.
18 Then, in addition to the Peru-14 strain,
19 which is this attenuated 01 cholera El Tor strain
20 that we are looking at, we are also evaluating an
21 0139 vaccine. Since the 01 El Tor strain is very
22 similar genetically, we were able to use the same
23 sort of genetic manipulations to make an attenuated
24 0139 vaccine.
25 These have been evaluated at doses of

around ten to the eighth organisms and I will show you more about the safety and immunogenicity and protective data later. Again, these are oral, single-dose vaccines.

Now, we started out with the whole cell B subunit vaccine as a result of the Persian Gulf War and, so, we have the most experience with this vaccine, and since 1991 we have done an extensive safety and immunogenicity testing here in the United States and also in Latin America.

We have done several studies. They are in the middle here. Is there a pointer?

(Pause.)

-- where we have tried to evaluate these vaccines for -- as vaccines for inter-toxigenic E. coli. This is a study we did in collaboration with Dr. DuPont's group down in the University of Texas to look at students going to Mexico, and we also vaccinated the crew of the U.S.S. Saratoga to see whether we could diminish the amount of diarrhea that they had on port call.

Unfortunately, both these studies did not give us a definite answer, and I think that now that we have an E tech version of that vaccine we will go on and test that one in further studies, rather than

continue our work with the cholera vaccine as an E tech vaccine.

Right now, we have just completed our pilot studies for field efficacy studies in Peru and began in January a study in Peruvian military recruits. Approximately a thousand in the group were anticipated, and we ended up with about 700 in each group, and also beginning a little bit earlier than that, last fall, last October, we began a large field efficacy study in South Lima with about 10,000 people in each cell.

So, we are well along the way to developing the protective efficacy data of this vaccine. Now, I should back up just a minute and say, "Why are we testing this vaccine that has already been tested and evaluated in Bangladesh so thoroughly?"

The reason is several reasons. One is that this vaccine is slightly different in its formulation. It uses a recombinant B subunit rather than a purified B subunit that was used in the original one. So, this is -- this recombinant technology has allowed the vaccine to be made in a relatively inexpensive way and making it a viable product for both, to develop in a developing world.

The other aspects are the idea of whether

or not this vaccine will work in Latin America. There are several things that, at least theoretically, work against this vaccine. You know, these were observations made in the Bangladesh trial, and one was that the vaccine did not work as well against El Tor biotype strains of vibrio cholera compared to classical and, of course, all of the disease that we are seeing in Latin America is with El Tor 01's and, so, that might make it less effective, or not effective at all.

That population in Latin America obviously is naive immunologically, or, at least it was when cholera was introduced back in 1991 and, so, the vaccine may not work as well in that population. Of course, that is very germane to us, who would be vaccinating non-immune North Americans.

There were a number of other factors which suggested the vaccine really needed to be evaluated completely over again in the Latin American sort of setting, so that is why we went through all of this and why we are doing the field efficacy studies over again.

The other major difference between our studies and the original studies is that we are using a shorter, abbreviated, you know, regimen for

1 immunization. In the original trial, they used
2 three doses spread over -- that were six weeks
3 apart, so it took 12 weeks to immunize someone.
4 We are using two doses, two weeks apart
5 with a booster given after the first year to try and
6 give relatively quick immunity at the beginning and
7 then boostering them to provide longer term
8 immunity, because the study in Bangladesh showed
9 that there was a definite waning of immunity after
10 the first year. So, a number of differences which
11 could be important, I think, in terms of the wider
12 acceptability and dissemination of this vaccine.
13 Now, we have some encouraging news about
14 this Peruvian military study. We broke the code
15 after the recruits finished their recruit period and
16 after the cholera season finished just this last
17 month, and we had 16 evaluable cases of vibrio
18 cholera in people that had received two doses and
19 that occurred two weeks or more after the
20 immunization series, and found that only two of
21 those cases occurred in the vaccinees and 14 of them
22 occurred in the placebo recipients.
23 So, that is about an 85% efficacy and shows
24 us that this product definitely can work in this
25 setting of Latin America where at least giving short

1 term protection in adults there against the El Tor
2 disease.
3 I might also mention that all of these
4 people who had cholera had the -- were O blood type,
5 which is a blood type that has been associated more
6 with the -- with a severe cholera illness and less
7 efficacy in other studies.
8 So, I think that harbors well for our next
9 study and in addition to the military studies which
10 are in -- which are now in full -- we are about a
11 year over this study and, so, we will continue it
12 for another year to look at two years of efficacy
13 data. Another study probably will be starting this
14 year in South Peru in the town of Arequipa, and this
15 is going to be a Pawho, W-H-O, sponsored trial.
16 Now, the one problem that occurs with the
17 CVD 103 strain, which is an excellent vaccine, but
18 in terms of its safety and immunogenicity, but it
19 does give slightly different rates of protection
20 against the classical versus the El Tor strains.
21 CVD 103 is a classical strain and gives higher
22 protection rates against the classical strains than
23 it does against the El Tor strains.
24 Now, these are some of our data on Peru-14
25 which is an El Tor strain and, although the numbers

are quite small here -- and these are studies that are done in, you know, closed ward type settings -- but, this is the -- among vaccinees who were given the vaccine a month -- or, two months, I am sorry -- before we had one breakthrough, and this was a relatively mild disease and we had five of five controls come down with cholera. So, that is about an 80% protective efficacy. Now, with the Bengal strain, the results actually are quite similar in that we had one breakthrough out of seven vaccinees, and that was quite a mild illness, so it changed the severity of illness, and we had five of six controls develop diarrhea, and that was quite severe, about 3.6 liters of purge over a week's period of time from these people.

The other thing that the vaccine did in terms of modulating this one breakthrough case that we had was that it changed the incubation period from about a day to around four days. So, it prolonged it. So

that -- this is work that we have just completed here at USAMRIID on the closed ward here.

So, this vaccine -- both of these vaccines -- look like they are going to be good candidates for future trials, and the next step that we would

1 like to do is try and make a bivalent vaccine by
2 combining these two into one oral formulation.
3 Now, the vaccines that give the best
4 antitoxin immunity are not the vaccines that give
5 you the best vibriocidal antibodies. These live
6 attenuated strains, they have unquestionably given
7 much higher vibriocidal tider than the whole cell B
8 subunit vaccine. So, they produce terrific
9 bactericidal antibodies in the serum which are quite
10 protective. The whole cell B subunit vaccine,
11 because of that slug of a milligram of B subunit,
12 produces a much better -- a much higher antitoxin
13 response, and this really is not projected well here
14 by this (indicating), but, really to look at the
15 affect of preventing cholera toxin disease by
16 aerosolization, the whole cell B subunit vaccine
17 would be the preferred vaccine.
18 So, this is just some experimentation that
19 was done in animals by the Department of Enteric
20 Infections at WRAIR, Larry Hale and his group, in
21 collaboration with the Department of Pathology who
22 did some of the pathology.
23 So, they are taking that whole cell B
24 subunit vaccine and trying to determine what is a
25 mouse dose here, and giving a regimen on day 0, day

14 and day 28, and then coming back on day 56 and challenging intranasally with the cholera toxin.

These are the results of two experiments, so these black lines (indicating) are the vaccinees, the mice that received the vaccine, three doses, and the open circles and squares there (indicating) are the respective control group for theirs. So -- and this is the percent survival of mice.

So, there was about a 70% survival in the vaccinated mice, compared to about 35% in the un-vaccinated group. So, there is about a 50% protective ratio there, 50% protective efficacy.

So, the vaccine does offer some hope as a control of inhalation cholera toxin and I think that we can work with this experimental model, this sort of mouse/lung challenge to see how we can improve the immunogenicity of this vaccine and try and work out some of the parameters.

If you look at survival here, the IgG antitoxin titers were definitely higher here in the mice that survived than the ones that didn't. So, there is a correlation both with IgG and with IgA in surviving with this.

Okay, that is all I have to say. Thank you.

MALE VOICE: Is there any cross protection between (inaudible) and Peru (inaudible)?

COLONEL HOOVER: I don't think so. We have not done the cross challenge specifically, so we don't have that information. The vibriocidals do not appear to be cross protective.

MALE VOICE: They have a common B substance (inaudible).

COLONEL HOOVER: Right. Well, the big question, I think, is will the whole cell B subunit vaccine protect, you know, because of that antitoxin immunity. So, I think that is a very important experiment to do, and we have not done it.

MALE VOICE: There is two lines of evidence that there is no cross protection. One is that in cross neutralize -- cross protection studies in (inaudible) done in Bangladesh, there was no protection, so immunization (inaudible) 100% protected immunity (inaudible), classical protects against classical model, 0139 protects against 0139, no cross protection.

The second is epidemiological, and that adults that have been living in areas where it is highly endemic have been getting much higher percentage of the 0139 disease, and we have actually

seen more disease in adults than children, and it has been lethal, and that is what has made the 0139 so frightening, that you could live all your life and survive childhood in a cholera endemic area and then come down with a new strain of disease.

It does indicate that antitoxin immunity in the field situation is probably not nearly as important as we thought, or you wouldn't be seeing this, and that other types of immunity against other factors are probably much more important in humans.

COLONEL HOOVER: Yes, sir?

MALE VOICE: Is the mechanism of mice dying in the inhalation of cholera toxin experiments the same as what you would see if they were exposed regularly?

COLONEL HOOVER: Well, they get pulmonary edema, so I think that what they are seeing is that mice that are vaccinated develop milder pulmonary edema for shorter periods of time, and 70% survive, whereas those that are unprotect -- unvaccinated -- get rather severe pulmonary edema and die at a much higher rate.

DR. GWALTNEY: What happens in non-human primates is that this (inaudible) feasible as a biological warfare agent.

COLONEL HOOVER: I have no idea.

DR. GWALTNEY: Have non-human primates been challenged with aerosol, cholera toxin aerosol?

COLONEL HOOVER: Not to my knowledge.

(Pause.)

MALE VOICE: It looks like most of the work that is being done, both internationally and nationally in-house where the Army is looking at it from the endemic threat, the last question related to what are we doing in terms of the potential biological warfare threat of cholera as an agent? It doesn't sound like much is going on.

COLONEL HOOVER: Well, all of these animal experiments are really what we are doing on that. We are looking at the whole cell B subunit vaccine, which is the vaccine that we have tested that produces the best anti-toxic immunity for its ability to prevent inhalation disease in mice.

So, that is -- I mean, that is the model we have been using.

MALE VOICE: The date that we found -- that Larry has found -- is that if you had adjuvants you could increase the level of (inaudible) and significantly increase the protection against aerosolized cholera toxin so that B subunit when

added to some type of adjuvants (inaudible) type response, such as Vitamin D, or other types of adjuvants that we are working with, might enhance this inhalation approach.

But, I think that one thing we don't know is how much of a threat inhalation of cholera toxin is and, so, therefore our desire to work on it extensively is tempered by our -- whatever the threat might be.

DR. KULLER: Thank you. We will meet again at 1:30 -- 1330, excuse me -- to talk about botulinum.

(Whereupon, at 12:15 p.m. a luncheon recess was taken.)

A F T E R N O O N S E S S I O N

(Time noted: 1:35 p.m.)

DR. KULLER: Quiet, please. We are going to talk about botulism now, Lieutenant Colonel Wade.

COLONEL WADE: Okay, good morning. Could I

have the first view graph, please? (Visual aid provided.) Okay, I would like to talk to you this afternoon about our work in botulinum toxin, and this talk will cover both the existing vaccine which was not developed here, but clearly is in the field, or in the ability for use right now, and we need to cover its potential capabilities, as well as where our research focus is going in the future, both in terms of an improved vaccine, which is where I will spend most of my time, and then slightly, as an aside, some of our other efforts that we are working on in the arena of botulinum toxin.

Our goal here is to try to transition and improve vaccine by about fiscal year '97 that is effective against an aerosol challenge of botulinum toxin. With regards to aerosol challenge, unlike some of the toxins that you will hear about this afternoon, botulinum has the same pathophysiologic effect in terms of its role on neuronal function by predominantly any route.

So, we have nothing unique in terms of its aerosol delivery, but clearly if we want to talk about toxins as the non-living free-form sort of toxic chemicals that are considered bio, you would have to consider botulinum the gold standard.

This is one of the more toxic, if not the most toxic substance known to man. I always like to use large scale analogies, and one teaspoon of botulinum toxin effectively disseminated could take out Los Angeles. I will leave you to judge the pros and cons of that.

MALE VOICE: Yeah.

(Laughter.)

COLONEL WADE: Our approach, then, right now is going to be focused on -- never mind, no Simpson jokes.

(Laughter.)

We'll focus on the development of a genetically engineered vaccine candidate and we will also talk about some of our drug developmental efforts.

Could I have the next slide, please? (Visual aid provided.) To start off talking -- well, we have an existence right now and this is the pentavalent and botulinum toxoid. I think many of you became very familiar with this during operations Desert Shield and Storm.

This is an IND vaccine. It has been used widely for about 20 years. Again, like many of these older products produced by Michigan State

1 Department of Public Health, it has demonstrated
2 safety and efficacy, both in humans and in primate
3 studies.
4 We have a considerable amount of data with
5 regards to the vaccine in man, relevant to its
6 safety. In fact, here at USAMRIID, the data
7 collected through the end of '90 -- mid-'93 are
8 roughly 3,600. 4,000 doses have been given.
9 Next slide, please. (Visual aid provided.)
10 This is a pentavalent toxoid of clostridium
11 botulinum. It includes type -- toxin types A
12 through E. The five serotypes are grown separately
13 in either static culture or fermenter systems. The
14 toxin is recovered by precipitation and partial
15 purification, and this partial is an important
16 point.
17 Very much as you heard this morning with
18 the anthrax product, this is not a good, clean, pure
19 vaccine and that becomes more important as we talk
20 about the potential for product improvement.
21 It is inactivated with Formalin to produce
22 a toxoid. Then, these five monovalent toxoids are
23 re-blended in proportion to the mouse dosage that
24 would give you the appropriate protection. Alum is
25 used as an adjuvant, and then it is bottled as a

1 pentavalent product.

2 Next slide, please. (Visual aid provided.)

3 As far as use of the vaccine, the primary

4 immunization schedule consists of three deep

5 subcutaneous injections. Those of us who have had

6 this know that it is not without some possible side

7 effects. It is painful, but tolerable. I kind of

8 rank it somewhere halfway between the flu shot and

9 the typhoid shot.

10 It is a half ml for each injection, given

11 at 0, 2 and 12 weeks. Following this, the first

12 booster is given at 12 months thereafter and then

13 subsequent boosters are given every one to two years

14 based on serum levels of antitoxin.

15 The bottom bullet here is somewhat

16 misleading. We say that approximately 80% are

17 seropositive two weeks after that third dose. The

18 reason I say this is somewhat misleading has to do

19 with the sensitivity of our assay for sero-

20 conversion in man.

21 Clearly, evidence would indicate that if

22 you do show a positive titer you are protected, but

23 considering the threshold of what we can measure a

24 positive titer, there is a very good likelihood that

25 these folks that don't fall within this 80% of sero-

converters may very well, themselves, have sufficient protection, also.

Next slide. (Visual aid provided.) As I said, up to 10% of the recipients will experience a mild discomfort. Again, tenderness and swelling at the site of inoculation for up to three days. This frequency increases with subsequent inoculations, as you might expect. However, severe -- though we do see local reactions, severe reactions are rare, and systemic reactions are not very common, either.

As you can see, only a small percentage of recipients have a standard fever, malaise, headache, myalgia and, obviously, no long term sequelae demonstrated with a long history of its use.

Next slide. (Visual aid provided.) As I have said, this product does have some perceived shortcomings. It is there, it is available for contingency use as an IND product, but, again, we have this difficulty of use as an IND product and the problems associated with determining informed consent, particularly in a battle field scenario.

As I said, it is Formalin treated. We have a problem there in terms of making sure the Formalin is removed down to a certain level, and this certainly probably contributes to some degree to an

amount of the reactogenicity we see.

At present, we are only looking at five serotypes of the seven, so we are leaving ourselves with a hole, if you would, on three other serotypes of botulinum.

It is costly to produce. This is produced from a spore-forming organism, so where we talked this morning on anthrax vaccine, the need for BL-3 containment, we also need that here.

When you look at that purification and separate lot development, there is clearly a better way to do this in terms of a cost per unit dose, and perhaps even encouraging other manufacturers in addition to the MSBP to get into the game with that.

Therefore, we think we need to be looking at a vaccine that, one, is homogeneous in composition, easy to produce, covering all seven serotypes and giving us effective long-term protective immunity.

Next slide, please. (Visual aid provided.) Shortly after Desert Storm also is more indication of what I said about that 80% sero-conversion. We have some very good data in non-human primates demonstrating that using this licensed product on a short-term scheduling at 0 and 14 days rather than

having a third dose, of these animals all survived aerosol challenge, and a point is made here that, in a hurry, if we had to get people to the field quickly, though this is not in concert with the IND, people always -- the question always asked is how soon after what dose is the individual protected.

I think, from this data, we could comfortably say that there is a very good likelihood that if you only get two doses on board, you probably have a good protection from a challenge, maybe even in the absence of an obvious sero-conversion that we can measure by the assay system that we have available now.

Again, I say our focus right now is looking at a genetically engineered vaccine which we will be looking at in terms of protection against all seven serotypes from botulinum.

Next slide, please. (Visual aid provided.)

DR. CHIN: Excuse me, when were those organisms challenged?

COLONEL WADE: I am going to have to defer a few of these questions.

MALE VOICE: Three weeks after the second shot.

COLONEL WADE: Three weeks after the -- thank you. I am two months on this job and some of the stuff that happened before my arrival, I might have to pick on a few people. So, we will see how we do on that.

What we are doing on our approach is looking at a portion of the toxin, rather than taking the entire toxin and toxoiding it. This focus has been on the C-fragment of the heavy chain.

We have gone through, now, in using this chemical synthesis, nucleotide oligomers have constructed and sequenced the gene and have come up with a synthetic gene code for about a 50 kl portion from the C terminus.

At this point in time we have done BoNT A and B and, and we have actually progressed further along here than would be implicated. Bont E is completed in collaboration with the folks at Porton Down in the United Kingdom.

We are working on serotype F, and the beauty of this product is, one, it is non-toxic. In and of itself it does not produce any toxicity, which would be expected, because when you look at botulinum as a heavy chain and a light chain, the

1 light chain is what is the toxic component when it
2 becomes intracellular in the neuron. We do not have
3 that here. It is a non-toxic product. It is well-
4 characterized. It is clean.
5 Now, both for the Bont A and the B
6 serotype, when it is expressed in an E. coli vector,
7 mice are protected from one million lethal doses,
8 and this is an astronomical increase in efficacy
9 over what we see with the pentavalent product that
10 is currently out there.
11 Next slide, please? (Visual aid provided.)
12 Where we are now, as I say, we have got all seven
13 serotypes working. Our goal in this in terms of
14 proof of principal new product is to take one to two
15 serotypes, most likely A plus B, as far along as we
16 can to demonstrate that we have a good potential
17 product, and then go back and catch up with the
18 other five to six serotypes.
19 Right now, this expressed in E. coli is
20 protective in mice, very efficacious, very good
21 yield. The product is not really well-soluble.
22 This is somewhat of a drawback.
23 We have also, however, expressed the
24 product in a baculovirus system. This is soluble,
25 about a 40-fold yield above and beyond the best

1 expression we can see, just using the clostridium
2 organism and it, too, is efficacious in protecting
3 mice from just super lethal doses of the native
4 toxin.
5 We recently have worked -- started working
6 in the area of a pichia pastoris yeast strain. We
7 are hoping that this is where we will end up, simply
8 because if we look at the analogous use of tetanus
9 toxin fragment in the same system, some calculations
10 have been done that perhaps one 100 liter fermenter
11 run, if we see the sort of yield on product that we
12 are anticipating, it would produce, what? Was it a
13 million doses? Correct me if I am wrong. John?
14 100,000,000 potential doses of this product to use
15 as a vaccine.
16 We are very excited about this work and we
17 think this is a good logical extension from where we
18 are with the product that is currently available for
19 contingency use, if we needed to.
20 Next slide, please. (Visual aid provided.)
21 Another item that we are working on has to do with
22 therapy. One of the difficulties with botulinum
23 toxin is you have a very succinct window of
24 opportunity. Once the toxin is internalized
25 into the neuron, most likely immune products are not

1 going to be beneficial to you. It is out of touch
2 and out of reach at that point.
3 Clearly, the first course of action that
4 would be most beneficial to us is a highly
5 efficacive vaccine. I think we are going to be in
6 that realm with what I showed you with the C-
7 fragment product.
8 In the absence of that, clearly we need to
9 have a way of providing therapy to someone who is
10 stricken with this, and we do have somewhat of a
11 target of a window of opportunity in which this can
12 be done.
13 We currently have a stock of another IND
14 product which was a botulinum immunoglobulin, and it
15 has been shown in animal studies that if that is
16 given in a window anywhere between, say, 8 and 24
17 hours post-exposure, that you are capable of
18 rescuing these animals from the results of toxin
19 exposure.
20 That, too, was an old product, and shortly
21 after Desert Shield and in the throws of Desert
22 Storm this laboratory scaled up to come up with an
23 improvement on that as a therapeutic. What this has
24 led to is a product which is an equine antibody, but
25 it is despeciated.

1 We are only looking at the FAB-2 fragment
2 of this to try to cut down on the cross reactivity
3 between humans that may be sensitized to an equine
4 origin product. We currently have six horses for
5 each of the seven serotypes that are being
6 hyperimmunized individually.
7 Those animals are then being plasmapheresed
8 and then the product will result as an add mixture,
9 again, of those individual serotypes of
10 immunoglobulin back to give the right
11 proportionation of A, B, C, D, et cetera, up to H,
12 to give us one product for therapeutic in that
13 window where therapeutics may be possible.
14 The difficulty at the present time, other
15 than vaccination in this sort of immunotherapy, our
16 only other alternative is early supportive care when
17 you reach a point of cessation of neuronal function.
18 That is where we are.
19 We would clearly like to also have in our
20 armamentarium a pharmacologic intervention. I am
21 not going to talk about a lot of the number of
22 studies that we are working on right now, but just
23 within the last two years there has been a virtual
24 explosion in what we understand about neuronal
25 mechanism. In fact, botulinum toxin has been a key

tool for the neuroscientist in trying to sort that story out.

We know that botulinum acts by preventing normal release of osteocholine into a synapse, which is a vesicle release fusion process. Over the last several years there has been a tremendous growth in the characterization of a number of key and integral vesicle proteins that are involved in here and, low and behold, the botulinum light chain, which is the culprit when it gets into the neuron, has been shown to have an enzymatic activity that is involved with these peptides.

So, with this, also as a tool in our armamentarium, we are going into an aggressive campaign to do molecular modeling of that structural relationship of the light chain.

It's possible enzymatic activity highly traverses and gets to that target of toxicity in the cell, and then how, possibly, if we somehow have missed our window of opportunity by not having a vaccinated individual, not being able to provide an immunoglobulin as a therapeutic, to now perhaps come up with a pharmacologic intervention that will be successful in resuscitating and maintaining the patient.

Next slide. (Visual aid provided.) Again, within the scope of our research efforts, then, a lot of work looking at this mechanism of action down at the subcellular level, investigating various pharmacologic agents, perhaps as pre-treatment, most likely as therapeutics.

Again, looking definitively at aerosol challenge with botulinum, though its effects should not differ, I think there is always a need to go back and ascertain that that is where we are in that assessment.

As I said, the C-fragment work coming on is, I think, a very promising second generation candidate product, and then a number of other ongoing efforts looking at what even may be a third generation to really target the vaccine to get to the neuron where it really needs to be.

Next slide, please. (Visual aid provided.) This, again, is just an abbreviated time line of the projects going on. As I say, we have the pentavalent toxoid as an IND product.

Therefore, it is now -- and I missed part of Major Klenke's presentation this morning, so I am not sure if he gave you actual stockpiles of doses, but I know that information is available through

him.

We feel that there is a likelihood of a transition decision to go forward to produce a recombinant product, perhaps in fiscal year '96, and then, as I say, we have some follow on efforts. Depending on the success of this, we may not even really need to pursue to their obvious fruition.

Again, we are working right now on a contract with our horses to get the electro-free immunoglobulin product. We are also doing some work looking at other approaches in terms of either some monoclonal antibodies against botulinum and, as I said, with the chemotherapy as a treatment, much of this being done in collaboration with the neuroscience group down at the Institute of Chemical Defense.

Other efforts that have been ongoing within our division deal, again, with some of the standardization within the world community as far as what is an international unit dose of botulinum, what are the assays, and improving that sensitivity so that we can detect serum titers and levels down at meaningful concentration.

That is very brief and abbreviated. Colonel Takafuji asked me to try to get us a little

back on track because the speaker after I, also has to try to catch an airplane. I think there is a pattern developing here today, but I am open for any questions you might have. Yes, sir?

MALE VOICE: Is it part of -- if you have a casualty and you are treating them with an immunoglobulin, is there any interference if you try to then immunize the individual at the same time with (inaudible.)

COLONEL WADE: With -- I honestly don't have a real good answer to that question. I -- Colonel Franz, you are shaking your head this way. What's that?

MALE VOICE: (Inaudible.)

COLONEL WADE: I would mix -- yeah. I can't see that you would, and I am not really sure that I would see a pressing need.

If you had someone that -- you know -- and again, the difficulty on this is you have a window of opportunity there of about 8 to 24 hours post-exposure, which is going to be prior to symptoms.

Once they start expressing, a patient begins to express symptoms, you have already internalized toxin into the neuron and you can't -- you cannot do much with this, so I am not sure that

you would be gaining a lot by simultaneous vaccination and treatment.

MALE VOICE: (Inaudible.)

COLONEL WADE: Yeah, that is true, because it is -- he has got an immediate problem, which boosting immunity is a later issue. Yes, sir?

DR. ASCHER: Just a comment on how these programs sometimes relate to the outside world.

The globulin that was prepared became the basis for the treatment of botulism and was later then made into a different product through the FDA Product Program. This institute sort of gave that program a leg up.

COLONEL WADE: Um-hum.

DR. ASCHER: That is very important. Not any money, but a leg up.

(Laughter.)

COLONEL WADE: Yes, ma'am?

DR. BROOME: I understood you to say the antitoxin is still horse product?

COLONEL WADE: It is a horse product, but it is -- the current one is a horse product. The one that we are working on is a horse product, also, but it is being despeciated.

In other words, when you take the antibody

chain, we are only going at the FAB-2 fragment which gets away from that portion of it that is uniquely recognized as horse by man, and this should be a less immunoreactive product.

MALE VOICE: The current one is also despeciated.

COLONEL WADE: The current one is also despeciated. Okay. I am sorry. Thank you.

MALE VOICE: The one that is produced by the Army.

COLONEL WADE: Right.

FEMALE VOICE: But, the one that Dr. Ascher is referring to is a human antitoxin.

COLONEL WADE: Any questions? Yes, sir?

MALE VOICE: I am excited about the recombinant technology that you and others will prescribe, and it seems to me that the use of that kind of technology for making these new vaccines will be to more greatly simplify the manufacturing facilities that you need.

COLONEL WADE: Absolutely, and that really is one of the key points, is when you look at the -- the scale up as far as the yield that those products -- as I said, when you can get one fermenter run that is going to give you all the doses you would

ever need, whereas -- I think that we were discussing it earlier.

If you tried to achieve the same thing right now with what we know the yield of serotype H is, you would have to do 30,000 fermenter runs to get the same amount of product. So, the magnitude of scale there very quickly comes around in terms of cost savings. Plus, it is a much better product, the efficacy and immunogenicity of this, and it is non-toxic. There is just a whole lot of things that speak in its favor. We are very excited about it.

If we have no further questions, we will move on, then, to the two-by-two slides, change gear slightly, and I am going to talk to you about our work in Ricin.

Rather than a bacterial toxin, Ricin is a plant toxin. These are derived from the bean of the ricinus communis plant, which is produced worldwide for the production of castor oil. This is the castor bean. In fact, when I moved into my office about two months ago, I was rummaging around. I have a huge desk. It used to belong to the Institute Commander and no one had the heart to throw it out. I reached into this bag and I thought

I was set with a supply of coffee that would last me months, except the beans were a strange color.

(Laughter.)

Colonel Franz keeps some strange mementos that he has never moved. I have got 20 pounds of castor beans up underneath my desk. Very pretty, but it is a medium like low eight -- well -- protein that -- well, I think we are all familiar with ricin as it is used as a biochemical tool, and has been for a number of years and is an inhibitor of protein synthesis.

I think one of the things that makes this a tremendous concern to us is that, "Hey, if I have got 20 pounds of beans under my desk, what does the rest of the world have?"

It is very much readily available. It is grown as a commercial product and in the process after you remove the castor oil, which is what you are trying to achieve, you have got a lot of bean hull left over, and without a lot of sophistication you can go ahead and get a relatively crude toxin out of that.

Also, most importantly, it is highly toxic by aerosol exposure, and I have to really emphasize this point because unlike some of the things you

have heard today where route of exposure is more or less an immaterial process with regards to the path of physiology of the agent, here it truly makes a difference.

When we think about systemic exposure of ricin I think we have all had recollection of the poor fellow that -- the Bulgarian that caught the little pellet out of the umbrella in his thigh with ricin in it, and as a protein synthesis inhibitor I think he wasted away after several weeks of a sort of a malaise, progressing into an illness that was very difficult to diagnose.

That is not what we see with aerosol exposure to ricin. Upon aerosol inhalation the lion share, if not the majority of the damage, seems to reside in the lung. You have a fulminating pulmonary edema, a lot of hemorrhage.

If you look at the slides of these lungs, this is not a happy animal, or we would certainly expect not a happy human. Because they die from the pulmonary edema that ensues, it is hard to sort out specifically how much of this is direct lung affect, how much of it is specific protein synthesis inhibition in pulmonary cells and then how much of it actually ultimately reaches systemic circulation

to have more of a systemic effect. But, clearly this is the organ of exposure that we are concerned with.

Again, right at the moment we have no product in terms of either a pre-treatment, prophylactic or therapeutic, and clearly this is an area that is much needed.

Next slide, please. I have got this one, that's right. As I said, with aerosol exposure the lung is the primary target organ. We see a fulminating pulmonary edema that takes some while to develop. There is about an eight hour latency period during which the animals are asymptomatic and then progressively they worsen.

Our goals here, then, have been, again, to understand this mechanism of action. Is it different, particularly at the biochemical level in the lung than if you had systemic administration. We clearly know that the physical signs and symptoms in the clinical progression of illness is different.

We need to further understand this aerosol threat. We are very much interested in immuno-prophylaxis, and because we have the potential that even with protection from death, or -- we may not be able to fully ameliorate the lung damage.

We are also looking at possible means of intervention as far as pharmacologic treatment for any residual problems in the lung that we may not be able to solve with a vaccine product.

Again, looking at the scope of the research program active immunity, I think, right now is where we are going as our number one target, and when you look at the fact that the ricin appears to, once inhaled, bind quite readily, there is a latency period towards onset of signs, but I think really we need an individual that is protected before exposure rather than trying to rely exclusively on some sort of a treatment afterwards.

To that regard, I am not sure passive immunity is much of an answer, though we are looking into that potential and, again, part of this may depend on how much of the toxin actually enters systemic circulation rather than having the profound effect that it does locally in the lung.

Again, as I said, however, we think we may need some sort of ancillary support. This hasn't been fully born out yet and, as such, we are looking at, again, molecular modeling and structure activity functions of the ricin A chain to try to determine whether or not there might be a rational drug design

process and screening that we can go to to help us in that area, again, looking at these various pathogenic mechanisms that are occurring.

Today I want to focus predominantly on a product that we have here that is being pushed towards the clinic, and that is a ricin toxoid. Here at USAMRIID our laboratory made a toxoid several years back that, in fact, was capable of protecting mice and has protected monkeys against the lethal affects of an aerosol ricin challenge.

Again, we have said it is more difficult to protect against aerosol challenge compared to systemic, but we have achieved that with this product. This
is -- this slide kind of goes through some debate and maturation depending on the data and how it is examined and whether or not we have an optimal immunization schedule.

But, it appears that the toxoid that we are going to be talking about may very well be able to protect against a majority of the lung damage. It is hard to say that anything is complete, but I think we are a good ways toward the solution of that problem.

But, again, this lung lesion may require a

little bit of separate intervention, perhaps in the way of a pharmacologic method. Again, right now the toxoid we are going to describe is the most viable option for a first generation product.

Looking at the program, our objective, then, was to try to take this toxoid and get it out to an IND for contingency use. Right now we don't have anything. We need a product that we can call upon if we need to call upon it.

This is a formalin-based toxoid. Is this old technology? Yes, but I think you have seen through some of the products that are already fielded that old technology may give us what we love to call the 70% solution.

I think a lot of times we look for the perfect vaccine and it may be off on the horizon and we have got some second generation efforts, but when you lack a first line defense, right now I think you go with something that works, and this works very, very well.

In our strategy we did -- in fact, as a first for this laboratory -- scale up and produce the toxin using good manufacturing practices. There was an initial toxoid pilot lot of small scale followed by a two gram production lot.

1 This is significant that we got up to that
2 two gram capability, because from the data that I am
3 going to show you, if this goes through as we
4 suspect it will and we can support an IND submission
5 on it, that right now represents a potential of
6 perhaps 150,000 doses that are there for use should
7 the need arise.
8 Again, we have actually started the pulling
9 together the data package for a transition decision
10 to get it into Phase I in the clinic late this
11 summer, early fall, looking towards what is
12 necessary for IND submission.
13 To show you a little bit about the product,
14 the original pilot lot that was toxoided, number
15 one, it is safe. It is three log orders less toxic
16 than the parent toxin, which is really down in the -
17 - pretty much the background noise. You would have
18 to push the dosage tremendously to get any adverse
19 effect.
20 To just emphasize that, what would be a
21 hundred immunizing doses is non-lethal to the mouse.
22 It is very immunogenic, it is protective and this
23 is important in the mouse.
24 We have a very good surrogate marker. We
25 seem to get a fairly good correlation between a

serum anti-toxin titers and protection against the aerosol challenge, and this will be important as we go to trying to get potential approval of product through the FDA.

As I alluded to scale-up to the GMP product, we have 150,000 potential doses, and this was bottled some with and some without alum. I am going to show you some data in a second here regarding the efficacy of this compound with and without adjuvant. This may not be the most ideal, but at the present time we have it bottled in both fashions.

We have done GLP on contract, pre-chemical safety and toxicity. The product is coming through with a clean bill of health as far as those sorts of tests are concerned. With regard to validation, we are fairly confident that our data on efficacy will stand the test, particularly in mice.

All these studies are complete. We have additional data in the rat. Originally, we looked at doing non-human primate studies, and I think at this point when we consider the sensitivity and the cost involving that, we are waiting on this to see whether or not they are necessary.

These are not historically required for

approval of a biologic product and particularly in that we have a good surrogate marker in terms of anti-toxin titer, we feel that as we go into the Phase I safety testing in man, if we get the demonstrable titers that we really believe we are going to, we have a direct cognate, then, that equates to protection which may obviate doing these studies, and perhaps it might be better to wait for FDA to say, "Yeah, verily we need that," rather than go out. I think we can get things moved ahead without that.

This shows you some of the data. We have got two slides here. This, again, is showing the efficacy of the GMP product against an aerosol challenge. The mice were immunized with 5 micrograms per mouse immunization schedule here at 0, 2 and 4 weeks, and this on the next will show challenges of ricin at 6 weeks, which is obviously 2 weeks after the last dose, at two different levels.

This one is showing the range of 12 and a half to 13 micrograms of ricin per liter of air. What was the time exposure on those?

MALE VOICE: Ten minutes.

COLONEL WADE: Ten minutes, okay. What we can see here is that with the ricin toxoid alone, we

have 18 out of 20 survivors. With alum, we see 20 of 20 survivors. Control, 0, 20 survivors. So, we have a very efficacious product.

This is a very high challenge dose, though, and we were concerned a little bit about whether or not alum was giving us a substantial improvement over the toxoid alone.

Did we need the adjuvant, or particularly that adjuvant, and there was another group that was actually being performed simultaneously, same dosage regimen of the toxoid; however, looking at a probably more realistic challenge in terms of what man might see on a battlefield of ricin concentration.

Here, we get to the point that we have 19 out of 20 with the toxoid alone surviving, and there is really no statistical difference between the two of these.

Additional supporting data with that first pilot batch of ricin, studies were also done in the rats. These were really looking more at measuring some of the parameters of lung injury, edema, composition of the edemal fluid through lavage, and what have you. But, clearly what we see is that with this toxoid we do see an efficacy that is

substantial, and with an adjuvant it is improved.

What I would like to point out is, don't be misled by these appearing to be low numbers, because this study was not really designed to answer the survival question, but to characterize the lung injury, and it is unclear retrospectively whether in the rat the optimal immunization regimen was used.

What is important, though, is that with a multitude of parameters measured as far as indication of lung injury, the lung injury was markedly attenuated in those immunized rats and, as we saw in the mouse data study, toxoid with adjuvant was superior to toxoid alone.

The plans that we have ongoing right now, we are continuing on in the rat model to thoroughly characterize the lung injury parameters using the GMP toxoid, not the original pilot lot.

We are looking, then, again, at the acquisition of immunity, the duration of immunity, protection, post-immunization with regards to aerosol challenge and, again, where we see a perhaps residual bit of lung damage, whether or not various anti-inflammatory drugs, or logical first choice treatments to ameliorate the pulmonary edema and the lung injury would be efficacious.

Along this line, this laboratory, Major Estep's group in aerobiology is the first to really do an extensive characterization of ricin aerosol toxicity and, really, when you look at the nature of these species, this is one of these things that makes USAMRIID unique, is the ability to do the challenge, and that is critical as you are going through product development. We have bracketed a range here with a number of sources of the toxin in a number of species, looking at various modulations in the exposure system.

As I told you and what is driving the potential for looking at pharmacologic intervention is we do see a latency period, and it would be interesting to note what you might want to do if you knew you had been exposed to ricin, perhaps in the absence of immunization, but maybe even as an adjunct with immunization.

Is there a -- is this latent period an opportunistic treatment window, as well, and we have studies ongoing in that, again, characterizing this just massive cellular gammish of damage that occurs in the lung with exposure to the ricin as an aerosol.

Again, we are going forward with further

studies in the rat with the GMP toxin. Another question that comes up that harkens over to some of the chemical defense work with such things as phosgene that caused pulmonary edema, what happens if you have exercise stress on top of someone who may be in the process of incubating lung damage?

We are going to be looking at this in immunized animals to ascertain -- and, clearly, our troops while they are being exposed to this sort of a threat are going to be called upon to exert themselves, because it is a combat situation, as well.

Again, looking at anti-inflammatory drugs, looking at this question about ricin distribution with aerosol exposure, how much of it really is reaching the systemic circulation? Is that a problem, is there any long term sequelae? Right now, survival looks real good and perhaps even, if necessary, to look at the pathophysiology more definitively, going into a larger animal model than a sheep -- than a rat -- that is more amenable to that.

Again, looking at our time lines, this one snuck by us by about a quarter and a half, but we really think as far as a transition decision, we

have met with the product developer. We are looking here at third/fourth quarter of '94 and moving this into Phase I testing to demonstrate safety in man and then to go forward with that as far as IND submission with animal efficacy data.

Our efforts in the chemotherapeutic realm, we hope to be able to have come up with a recommendation with regards to potential pharmacologic intervention and therapy in the fiscal year '96 time frame, and then really have a good definitive characterization of the full time course of pathophysiology with and without the various products that we are working on shortly thereafter.

That concludes that presentation which was advertised as "Ricin and Other Toxins". In support of that, Colonel Base will talk about S.E.B. and that it is one of the last big other toxins, and we are doing a number of studies, particularly in the basic science realm, on some of the other items that you saw up on the charts earlier this morning.

I think one of the challenges before us right now is that there are thousands of toxins in the world. We have chosen to focus our product and programmatic thrust on those that we think are currently the greatest threat, particularly as we

get into the second generation products which are using the biotechnology tools that are recently coming available. We will stand poised, I believe, to address anything else that may come up in that emerging list.

Clearly, one of the concerns is the same technology that may allow us to solve these problems and could be used in an offensive sort of capacity to take an obscure toxin and bring it to the four, and I think what we are learning on such things as botulinum, ricin, S.E.B., as you will see here in a bit, will put us in a good position for that and, as such, we are keeping a close watch on a number of the people, both in this country and abroad that are using these toxins as tools in cellular biochemistry and just making sure that we follow that progress and that data.

General Russell?

GENERAL RUSSELL: Have you got studies with ricin right now to relate to the isotyping and (inaudible) with specificity to the protective immunity?

COLONEL WADE: Do we have isotyping on the protective immunity on the ricin animals at this point? Do you care to address that? Did you hear

General Russell's question?

MALE VOICE: Sir, we have looked at the -- on the active immunized animals, the principal serum component is IgG. There -- and the epitope using monochlorum antibodies, we have looked for the location of protective epitopes on both subunits of the ricin.

COLONEL WADE: Yes, sir?

DR. WOODWARD: I was very interested when you had mentioned pulmonary edema and bacillitis, the toxic form of bacillitis, which is not dissimilar to toxic bacillitis. Now, you indicated you are going to look at the blood vessels in pulmonary disease, and so they are mostly (inaudible).

COLONEL WADE: Yeah, clearly these animals we see an increase in just -- in bronchoalveolar lavage, for instance. Protein concentrations go up considerably, be it serum protein, you know, or immunoglobulin response.

There is obviously a vascular leakage. It would be interesting to see just exactly which cells may be most involved and what is driving that sort of

a -- it is probably a hydrostatic non-cardiogenic

sort of an issue, but --

DR. WOODWARD: (Inaudible.)

COLONEL WADE: We have some preliminary dose results with one non-steroidal drug that seemed to suggest it had an affect. You may be right. The only thing I draw back on is my personal experience working with phosgene where often dexamestero (sic) makes them worse. So, you are never really sure.

DR. WOODWARD: (Inaudible.)

COLONEL WADE: Which is why we are going to look at it, sir, because it clearly -- I think a key informational product that oftentimes comes out of these studies is not only what can we do to protect the soldier, but what shouldn't we do because it may cause him more harm, and we need to look at what the clinician intuitively is going to go for when presented with someone with an endemagenic situation. Any further questions?

(No response.)

If not, I think in the interest of getting us back on schedule, I will turn it over to Colonel Baze.

COLONEL BAZE: Good afternoon. I am -- is there a question back there? No?

Okay, I am Colonel Baze from the Walter

Reed Army Institute of Research, and I would like to bring you up to date on the current status with staphylococcal enterotoxin B, that is, S.E.B.

Colonel Wade has already said many things that I was going to say as they applied to ricin and the other toxin he mentioned, botulinum, but we will apply some of them to the S.E.B. program, as well.

I would like to say at the beginning, I can't in 15 minutes cover all the work that is being done with S.E.B., and I was asked to focus on the vaccine development project that was underway -- or, is underway.

I would also mention that this is a multi-institute program involving Walter Reed, as well as USAMRIID and, of late, several years ago the Institute of Chemical Defense.

Could I have the first view graph, please? (Visual aid provided.) Okay, let me back off here a minute and remind everyone that was here this morning that S.E.B. is a recognized biological warfare threat. The tasking of the Army's scientist is similar to what we have heard earlier. It is to develop a prophylactic compound against aerosol challenge with the S.E.B. toxin.

The question was raised earlier this

morning about how these things apply to the battlefield. I would say with S.E.B. there has been modeling that has been done to evaluate the threat on the battlefield, and based on those findings we still -- S.E.B. is definitely a threat -- potential threat -- on the battlefield.

(Pause.)

Okay, so, let me reiterate that I will focus on the vaccine development program. Next view graph, please. (Visual aid provided.) As far as the briefing, I am going to divide it into three parts.

I am going to give you some background on the program that is some 25 plus years in progress, I will give you a research update, particularly research within the last five years and, as a preview, give you -- at that time give you the results from some exciting findings we have had as far as S.E.B. vaccine development. Based on those recent -- the research findings of late, I will predict or project what we plan to do for the future.

Next view graph, please. (Visual aid provided.) This is to remind you that S.E.B. is an extracellular protein produced by the ubiquitous

bacterium staphylococcal aureus. It is easily produced, it is stable and it is one -- you are probably aware of at least six other enterotoxins produced by the staph aureus organism.

You are probably most familiar with it as a cause of food poisoning in civilian populations when ingested. It is usually -- will usually lead to vomiting, diarrhea, acute gastrointestinal symptoms; however, it is usually not fatal.

In a military setting with military personnel, we can expect oral ingestion of the toxin to cause -- to cause -- similar to the vomiting and diarrhea that I explained in the civilian population; however, as I mentioned at the beginning, the aerosol route is the perceived threat route as far as the military is concerned, and soldiers exposed by the aerosol route to include -- do you have trouble with this?

MALE VOICE: It was getting a lot weaker on a white background.

COLONEL BAZE: Okay, I don't know if we can see the corner there very well. But, anyway, aerosol exposed military personnel could be expected to develop vomiting and diarrhea that could progress to shock and death.

(Pause.)

Thank you. The bottom line would be that S.E.B. --

(Pause.)

The bottom line would be that S.E.B. can cause -- now, I am getting mixed results -- but, can cause incapacitation and/or death, lethality via respiratory challenge.

The other bottom line to get from the background that I am giving you here is that we have no vaccine against S.E.B. and, even at that, there is no specific treatment, either, other than symptomatic.

Okay, could we have the first slide, please? (Visual aid provided.) There was a publication recently on an extramural contract for the Army that showed the -- based on the crystal structure of S.E.B., I wanted to show you what the molecule looked like.

This is 239 amino acids. This is a carboxyl N, the N -- amino N. This is a disulfide loop over here, various alpha helixes and B. depleted sheet type configuration, to show you what we are talking about. This was generated out of Dr. Sax's lab.

This has given the molecular biologist and the genetic manipulation people areas to concentrate on to develop genetically engineered prophylactic measures against this compound, a rensis molecule.

Okay, next slide, please. (Visual aid provided.) You may or may not know it, but I am a pathologist, and everyone has been talking about lesions today, but nobody showed any, so I decided --

(Laughter.)

When I took my pathology oath -- you have to show lesions when you give a talk, so -- so let me show you a few here to bring you to the real world, as it were. As I mentioned, in a lethal exposure, aside from the initial vomiting and diarrhea, then we go through a progression of depression leading to shock and death.

Along in that cycle the most severe complication, at least pathologically, is pulmonary edema which we just mentioned awhile ago with the ricin.

So, this is a section from an animal that was lethally exposed. This is a large vessel, large airway, a bronchial -- and you can -- if you are a pathologist you can criticize me, but if you are not

you can take my word that this is significant perivascular and peribronchiolar pulmonary edema.

I am usually safe on that, because usually nobody is out here that will challenge me and, so, they have to take what I say. As well, there is edema in the parenchyma that is not -- I don't think you can see it from back there.

Next slide, please. (Visual aid provided.) Just to show you a few other highlights. This is another section of lung, a large vessel, and you may notice all the large mononuclear cells within this vessel.

I didn't mention in talking about S.E.B. awhile ago that as far as the mechanistic, or the pathogenesis of the lesion that I will mention in a minute is stimulation of T cells as a T call antigen.

These are lymphoblastic cells within the parenchyma that we can suppose are certainly involved in the pathogenesis elaboration of cytokines that are contributing to the pulmonary edema.

Next slide, please. (Visual aid provided.) This is a final section here showing lung parenchyma proper. These are the -- the clear

spaces here are alveoli, they are highly cellular.

The fibrinous material in here is edema and/or fibrin and you may notice, or maybe you can appreciate there is an increase in cellularity here. So, an acute inflammatory response is going on within this lung, in addition to the obvious pulmonary edema. Okay, can we go back to the view graphs, please? (Visual aid provided.) As far as research update, to date, only pre-clinical studies have been performed with S.E.B. These studies have incorporated rodent models, in vitro models and non-human primate model.

I would tell you that the non-human primate model is the recognized model for S.E.B. and the non-human primate most closely mimics the intoxication as it occurs in man.

In the past, the -- in these pre-clinical studies, there has been focus on pathogenesis of the intoxication, as well as the prophylactic measures that I will be mentioning here a little bit more in a second.

As far as the prophylactic measures that have been done in these pre-clinical studies, the toxin has been manipulated primarily with formalin toxoiding, and these results over these many years

1 that I have mentioned before were that the toxoid
2 either provided inadequate protection and/or there
3 were hypersensitivity reactions, some sort of
4 reactivity, negative reactivity, to the toxoid that
5 made it undesirable and made them -- made
6 investigators continually look to find new and
7 improved products.
8 Anyway, more recently -- well, I can't see
9 that. More recently, the toxin has been toxoided
10 using essentially the same basic mechanism as in the
11 past; however, with modernization of techniques and
12 molecular biology and biochemical techniques, the
13 toxoiding has made a toxoid that is slightly
14 different from the toxoid the Army had used in the
15 past.
16 More specifically -- I won't go into the
17 details of this -- but, more specifically, this
18 newly formalinized toxoid is non-mitogenic for
19 lymphocytes. Very important point which goes to the
20 superantigenicity of the S.E.B. toxin, itself.
21 So, using this newly formalinized toxoid,
22 the toxoid has been enhanced with various carriers,
23 or adjuvants. The last two bullets here are going
24 to
25 it -- going to this point. The toxoid has been

microencapsulated. This is with a poly lactide, poly glycolide polymer, if you will, similar to resorbable suture material, little microspheres.

The toxoid has been incorporated in this. These vary in size from one to ten microns. It has also been formulated with a compound with proteosomes. Proteosomes were mentioned earlier this morning. These are outer meningococcal outer membrane proteins. That is with and without alum, and it is these two latter toxoid formulations that I want to expand on and discuss a little bit more.

Next view graph, please? (Visual aid provided.) First of all, the microencapsulated S.E.B. toxoid. There have been two large studies within the last five years using this toxoid.

The rhesus monkey aerosol challenge model was used, and this was really the bottom line on this study that I have written here, these three bullets (indicating). The protection correlated with serum and bronchial wash, anti-S.E.B. antibodies. In particular, serum and bronchial wash IgG and bronchial wash IgA.

The last bullet, 77% protection was achieved following a lethal challenge. Although the toxoid was given to the animals by various

1 immunization routes, to include intramuscular, oral
2 and intertracheal. The IT stands for intertracheal.
3
4 The only animals -- this 77% protection was
5 achieved with the animals that had been given a
6 primary intramuscular immunization followed by a
7 secondary intertracheal.
8 At the time that these studies were done,
9 at least the first of the two studies, the
10 intertracheal immunization was considered to be
11 similar to an aerosol immunization. So, if you are
12 thinking, "Well, that is not practical," we realize
13 that, but we are talking about animals here that at
14 the time the technology wasn't available. We didn't
15 have the expertise to do the aerosol immunization.
16 So, anyway, to date these have been some of
17 the most impressive results that have been achieved
18 with any of the toxoids, as far as the S.E.B. is
19 concerned.
20 Okay, next view graph. (Visual aid
21 provided.) Let's jump now to the proteosome
22 formulated S.E.B. toxoid. Again, we are using the
23 definitive model. As far as we are concerned, the
24 rhesus monkey aerosol challenge model.
25 Again, the proteosome formulated toxoid

1 induced high anti-S.E.B. antibodies in the serum and
2 bronchial wash. I say high. I can't compare them
3 directly with the microencapsulated study that we
4 just mentioned, because they were done by -- under a
5 different set of circumstances by different people
6 and there were different -- somewhat different
7 laboratories.
8 But, I think it is safe to say that the
9 antibodies induced by the proteosome formulation
10 were several fold higher than I have described for
11 the microencapsulated.
12 In this case, significantly 100% protection
13 was achieved. This is with an N of 20 against
14 lethal challenge. Also, significantly, the animals
15 in this case -- there were at least three -- in this
16 case, two immunization routes that were evaluated
17 that we, based on our information from the
18 microencapsulated data, chose to attempt.
19 In this case, the animals that received
20 three intramuscular injections, or a priming
21 intramuscular followed by two intertracheal, these
22 animals were all protected.
23 Next view graph, please. (Visual aid
24 provided.) Let me summarize this.
25 Microencapsulated S.E.B. toxoid showed us an

1 efficacy, or protective -- protective efficacy of
2 77% In comparison, the proteosome S.E.B. toxoid was
3 100%
4 Again, I won't go into the details, but
5 this was a significant number of animals, and these
6 observations were in comparison to the years of past
7 and were highly significant as far as saying that we
8 have got something going on here.
9 In addition, we did some clinical
10 pathologic studies in association with these two
11 vaccine protocols, and at least with our preliminary
12 observations both of these vaccines appear to be
13 safe. At least they were in the two models we
14 tested, the mouse and the rhesus monkey.
15 Next slide -- or, view graph. (Visual aid
16 provided.) Okay, let me finish up by talking about
17 the future. In the future we would focus on the
18 proteosome S.E.B. toxoid. Having said that, we
19 won't ignore the microencapsulated S.E.B. toxoid
20 and, as well, there is some evidence that the newly
21 forminalized toxoid alone may be efficacious.
22 So, we have some -- we have effort in that,
23 as well, okay? We are not eliminating that
24 possibility. I could give you some history on the
25 proteosome. When we did that, we decided to take

1 our best shot at what we thought would be -- give it
2 the most opportunity for success.
3 If we succeeded, then we could back off
4 from there. One of the ways that we could back off
5 from there was to try the toxoid alone. So, that
6 won't be eliminated.
7 By within fiscal year '95 we would hope --
8 we had planned out already the three clinical
9 studies that we feel like we need to do to complete
10 the pre-clinical proof as far as the proteosome
11 S.E.B. toxoid, as well as hold -- I have written
12 here, "pre-IND meetings during that time."
13 Next fiscal year we would hope to submit an
14 IND and begin and/or conduct parts or all of Phase I
15 or Phase II clinical trials with the ultimate goal
16 being gaining FDA approval sometime in the future.
17 Of course, all these steps that I have outlined here
18 are obviously based on success at the step above
19 that, or the previous step.
20 So, in conclusion let me say that I think
21 there is reason to be optimistic in the S.E.B.
22 vaccine program. The two vaccines, in particular
23 the proteosome S.E.B. formulation, have given
24 outstanding results, results unattainable -- or, un-
25 attained within the last 25 years as far as this

vaccine is -- as far as this program is concerned, and we will pursue them as vigorously as we can. Thank you. Any questions? Yes, sir?

MALE VOICE: (Inaudible).

COLONEL BAZE: Right. We -- that is -- we have that programmed into these. When I said "complete the pre-clinical studies," we have that programmed in.

The microencapsulated and the proteosome are not -- like I said -- are not exactly comparable, because they were done under different circumstances and different people were involved.

The proteosome results I gave you, these animals were challenged approximately 30 days after the last immunization. The microsphere -- microencapsulated animals were challenged what, Bob, 80 days?

COLONEL HUNT: 87 (inaudible).

COLONEL BAZE: There were some logistics problems. This was done in collaboration with Southern Research Institute as an extramural contract, and also with USAMRIID. So, there was -- in the shipping of the animals and what have you, there was a delay as far as the challenge time.

So -- but the longevity, the protocols are

already in place. They have been approved and we were waiting for support from USAMRIID, as well as from the Red 4 in this area to help us get those done, but we have incorporated that into this time line of fiscal year '95.

COLONEL TAKAFUJI: How far out are you going to go?

COLONEL BAZE: We are going to go to a year. We are going to do it in a step-wise fashion; approximately three months, six months, assuming we have success on those, and we will go out to a year. I think that is the guidelines we have been given, as a year, approximately, to -- as far as longevity. Someone had their hand up? Yes, ma'am?

DR. BROOME: Given that the normal, if you will, clinical syndrome, though so much more severe with toxic shock syndrome toxin, I wondered if you have ever looked in aerosol challenge with TSS T-1?

COLONEL BAZE: I haven't. I don't know if anyone else here has. Some of the people here from USAMRIID might be able to comment on that, but I think the answer is no.

But -- do you think that is right, Colonel Franz, or do you know?

COLONEL FRANZ: I didn't hear the question.

I am sorry.

MALE VOICE: The answer is no.

(Laughter.)

COLONEL BAZE: Yes?

COLONEL ERDTMANN: Several of the (inaudible) presented with a pulmonary clinical expression, and we learned earlier today that that is also true of some of the bacterial infections that could be biologically delivered as BW weapons.

In the threat of war, is there anything going on to be rapidly assessed and diagnosed? What is happening with soldiers that might be coming in in a large group (inaudible).

COLONEL BAZE: Do you mean as far as, maybe --

COLONEL ERDTMANN: Diagnostic -- yeah, rapid diagnostic --

COLONEL BAZE: -- as far as diagnostic? Yes, there is. I am not necessarily the one to answer that question, but there is an element within the biological program assessing diagnostic -- diagnostics.

Maybe --Dr. Winegar, can you answer that question, or can you address that? He is talking about diagnostics.

DR. JOHNSON-WINEGAR: Yeah, I heard the question. We have a number of specific assays in place, some of which are derived from traditional ELISA type assays, and are now incorporated into membrane closed group type assays, and we are trying to incorporate a broad spectrum of all of the biological threat agents. So, if the specific question was for SEV, yes, and BoNT toxin, yes. I mean, this is --

COLONEL BAZE: I think that is what you are asking, right?

DR. JOHNSON-WINEGAR: How many threat agents do we have in diagnostic test form?

COLONEL ERDTMANN: Well, the concern is that the clinical expression is very similar with a lot of the agents --

DR. JOHNSON-WINEGAR: Right.

COLONEL ERDTMANN: -- that you compare it to and, so, the question the Commission is going to ask is, "What is causing these hundred people to come in with these pulmonary symptoms?"

We have got to get a quick answer on it. Are we working on getting the capability to answer the question quickly?

COLONEL WADE: Yes?

COLONEL TAKAFUJI: Let me answer the question. That is a very serious concern, and we are addressing that as part of our program.

As you well know, with all of these toxins the actions are so fast, the binding occurs so rapidly, especially with things such as neuro-toxins, that this doesn't give me much of a window of opportunity.

Plus that the amount of toxins that you are referring to at any one time is such an amount that just detection, itself (inaudible), but there are some innovative ways, when you think about it, that you can go about it.

For example, if you look at how these toxins would enter the body, they enter through (inaudible) of the nose, for example. So, it may very well be that the most appropriate way to diagnose it is not actually by drawing a serum sample for actually doing an (inaudible). So, those types of things are in the (inaudible).

Toxins are going to be our biggest challenge from a diagnostic perspective.

COLONEL BAZE: Yes, sir?

DR. ASCHER: Mike Ascher. Some of the work that was done in this Institute when I was here a

long time ago was really trying to hit this issue right on the head.

Looking at physiologic changes of various (inaudible) and S.E.B. was kind of fun, because it had this profound lymphocyte effect and you see a very, very profound lymphopenia in some humans, basically no lymphocytes, and it scares the hell out of you when you see it. That is how it got connected and was -- finally toxic shock was (inaudible), and that all fit together.

But, I think the general result of that program has been not completely satisfactory, that the attempt to put a combination of a panel together of things, at least in the physiologic sense, that would show elevated iron decrease, zinc and all of these things, it really never came out completely clear. It was a nice try.

COLONEL BAZE: Yes, sir?

DR. ALLEN: Have any of these toxins that might be disseminated through the aerosol route, what happens if the military personnel are wearing the standard physical gas masks, or other types of protective gear? Is there a means of perhaps prophylaxing through that kind of physical route before it gets into the system?

COLONEL BAZE: John, can you -- can you comment on that?

COLONEL WADE: We know considering particle size and cut-off, the protective mask is one that protects against anything that you have seen here today as far as inhaled aerosol. The difficulty is, if you look at the size of the community that is working on detection, you have to know to put the mask on.

You know, take today outside, raise the temperature 20 degrees, drop the humidity and that is what we saw in Saudi Arabia. You didn't want to have the mask on long.

I mean, alarms went off and you did it, but you were very happy to hear the all clear. Therein lies the challenge, because most likely with an aerosol it is going to be odorless, colorless and perhaps tasteless, and you won't know that it happens. So, the physical protection, yes, it would work if you have it on.

(Pause.)

DR. KULLER: I think we can take about a ten minute break now and then we will continue on with smallpox. Off the record.

(Whereupon, a brief recess was taken.)

COLONEL TAKAFUJI: Good afternoon. There has probably been no issue as emotionally charged, or politically charged in terms of immunization policies in the area of bio-Defense as the smallpox issue, and this is an issue for the members -- the newer members of the Board.

This has been an issue of concern for many, many years and it actually came to a head way back in the 60's and the early 70's when the World Health Organization initiated a campaign to stop smallpox and eventually in 1977, in fact, the last transmitted -- endemically transmitted related case was heard in Somalia.

The policy in the country had been to stop routine smallpox immunizations, but, of course, because of the military concerns about smallpox, the policy had continued for some time. Later on in the course of events, what had happened was that the issue of the stockpile of smallpox, of course, became smaller and smaller as time went on.

The transfer of the stock -- the stockpile of the vaccine was transferred to the Centers for Disease Control, Health and Human Services, and in 1988 we finally ended up, in fact, temporarily stopping the routine administration of smallpox

vaccine to our recruits for a variety of reasons.

But, much of it centered on the fact that we were now also running out of vaccine immunoglobulin, and it was considered to be unethical to be able to continue a smallpox vaccination policy in the atmosphere when, if we had an adverse event occur, we would not have adequate supplies of V.I.G. That, in essence, summarizes the sequence of events that transpired regarding smallpox.

What we find ourselves with now, as a result of the continuing threat that still faces us, as you heard this morning, is an issue about how do we continue to address protecting the forces in view of the development of now a new vaccine that we have been tasked by the former Defense Health Council, chaired by at that time Dr. Bud Mayer, to develop.

Dr. Russell, when he was Commander of Medical Research and Development Command, was intimately involved in this decision pertaining to what we would be able to do in terms of delivering a new product that would be a safer vaccine to the Defense Department.

I will be providing to the Board a copy of all of the letters and memoranda that took place

pertaining to this issue of smallpox so that -- I believe, Dr. Ascher, you will be the one that will be working on that, so I will provide you a copy of the whole packet so you have a complete history of everything that has transpired in the task groups that resulted thereof of us.

I really want to get into the science and get your questions and input on this issue, so I really don't want to spend much time covering the politics. This afternoon I will be joined by Colonel Jerry Jennings, who is the Chief of Virology here at USAMRIID, who has inherited the task of making sure that this program happens in terms of the new vaccine, and also Dr. Will Brandt from USAMDA who has been particularly involved with making sure that we work all these issues with FDA and get our supplies of V.I.G. up to par.

He will also have some comments to make pertaining to our current status with the V.I.G. Jerry?

COLONEL JENNINGS: Thank you, sir. Good afternoon, ladies and gentlemen. May I have the first slide, please? (Visual aid provided.)

The efforts that I will be discussing this afternoon represent the group effort from the

1 Virology Division, which includes current efforts by
2 Dr. Dave McLean who, as we speak, is at Ft. Sam
3 Houston conducting a study to initiate efforts by
4 the late Dr. Dalrymple and who has honored this
5 conference room his name.
6 Current stocks of the Wyeth Vaccine are
7 diminishing in supply and particularly in potency,
8 as you heard this morning. Due to that fact and due
9 to the old manufacturing process, we were tasked
10 with developing a safe efficacious and replenishable
11 smallpox vaccine.
12 That new vaccine is called TSI GSD-241, for
13 lack of a shorter name. It was produced in cell
14 culture from the New York Board of Health strain.
15 It was developed for subcutaneous inoculation to
16 minimize the possibility of spread to others which
17 can occur following -- which can occur from the
18 cutaneous vesicles which result from scarification.
19 The initial biological and safety
20 characterization of the new vaccine included a pock
21 formation in chicken eggs, a virulence testing in
22 mice and lesion formation following intradermal
23 inoculation in rabbits.
24 All of those tests indicated that the new
25 vaccine was comparable to the original parent

1 strain, and also that the test indicated that it was
2 within the classical guidelines developed for
3 smallpox vaccines.
4 A primate study was conducted to compare
5 the new vaccine to Wyeth strain. Those primates
6 that were inoculated by the intradermal route
7 developed somewhat milder lesions than those with
8 the Wyeth Vaccine. Primates that received
9 subcutaneous or intramuscular inoculations did not
10 develop any cutaneous lesions. Those primates
11 which received internasal inoculation failed to
12 develop an adequate neutralizing antibody response.
13 However, all three of the other routes, ID, SQ, IM,
14 did develop adequate neutralizing antibody response.
15 Based on that group of data, a dose
16 escalation trial was conducted in human volunteers.
17 As a point of reference, the Wyeth Vaccine, when it
18 is given by scarification, delivers approximately
19 ten to the fifth, or five PFU's to the person
20 inoculated.
21 None of these individuals developed any
22 complications. However, several of the individuals
23 developed pox lesions at the site of inoculation.
24 In addition, those -- there was a correlation that
25 the neutralizing antibody response appeared to be

1 dependent upon the formation of a pox lesion.
2 However, those -- the numbers were rather
3 small, as you can see from the sample groups. This
4 is an example of the lesion in one of the
5 volunteers. This is on day 5 -- day 0 being day of
6 inoculation -- so day 5, day 9 and, lastly, day 14.
7
8 Because we wanted to minimize the formation
9 of these pox lesions, we conducted a second safety
10 trial to determine the safety of the vaccine if
11 given intramuscularly or intradermally. Again, none
12 of these individuals developed any complications.
13 Those individuals that received the
14 inoculation intradermally developed erythema
15 induration at the site; however, only one individual
16 developed a pox lesion. None of these individuals
17 developed an adequate immune response, with the
18 exception of the one individual who had a pox
19 lesion.
20 As I mentioned at the beginning, we
21 currently have a study in progress at Ft. Sam
22 Houston with Colonel Shannon to look at the
23 immunogenicity of the new vaccine compared to the
24 Wyeth Vaccine. There are three groups of
25 individuals, as you can see there.

As far as what vaccine and what route they received, the first group is divided into two groups. Half of the intradermal group receives an alcohol wipe in an attempt to decide if that will minimize the possibility of a pox lesion at the site of inoculation.

As Dr. Johnson-Winegar alluded to this morning, one of the problems in working with smallpox is that we are unable to directly test the efficacy against the variola virus. Therefore, we are

testing -- or, we are examining -- surrogate markers, antibody response and T cell response in great detail in these individuals.

That is being accomplished by Dr. Alan Smalljohn here at USAMRIID and, in addition, through an extramural contract with Dr. Frank Innis at the University of Massachusetts.

To date, we have enrolled 30 individuals in this study. None of them have had any serious complications. Of the 12 individuals who have developed -- who have received inoculation intradermally, six of them have developed pox lesions; three of them in group 1-A that received the alcohol wipe and three in the group 1-B.

1 The conclusions to date is that we have
2 produced a safe and replenishable vaccine, and we
3 hope that the current study and future studies will
4 help us to establish the optimal route and dose of
5 the vaccination.
6 I can take questions now, or if Dr. Brandt
7 wants to go ahead and speak and then maybe we will
8 just take it as a group. So, I will introduce Dr.
9 Brandt from USAMDA.
10 DR. BRANDT: I will just give you the
11 status of the vaccinia immunoglobulin, the currently
12 available material both from the military and from
13 the Centers for Disease Control that had expired
14 back in June of 1990, and the FDA agreed to a two-
15 year extension at that time if we gave them potency
16 data every four months, which we did.
17 We got contracts going in 1992 to start
18 immunizing and plasmapheresing military volunteers,
19 retirees and discharged military. We didn't get
20 enough material produced in time. We had to go in
21 for another two-year extension in June of 1992.
22 That expired last month in June of 1994 and the FDA
23 gave us another one-year extension.
24 As the potency data continued to roll in
25 every four months, there has been no change. The

1 FDA performed HPLC studies on this ten-year-old
2 product and it still looks like it is fresh
3 material.
4 They only allow the one-year extension
5 because they are fairly sure that they can arrange
6 for the release of a 6,000 bottle lot that is being
7 held out at Highland right now.
8 The issue is hepatitis C. Some of the
9 early units went in before the hepatitis C testing
10 was established, and the FDA's own PCR testing --
11 they have gone through it twice now -- is absolutely
12 negative. They are still a little concerned
13 about the fact that some untested units had gone
14 into that first batch, and they are currently
15 resolving that issue of whether we would do heat and
16 activation curves to show that hepatitis C is
17 destroyed at the treatment temperature of this
18 V.I.G. It is three days at 45 degrees centigrade.
19 That is the only immune globulin product that is
20 treated that way.
21 We don't know, but we are waiting to hear
22 if they will release it, or if they want us to
23 completely re-bottle it, put in a detergent bar, or
24 an inactivation step, or -- I don't know.
25 But, at any rate, we took care of

1 repackaging both the military and the CDC supplies
2 down at Atlanta, so that V.I.G. continues to be
3 available for those clinical centers that are doing
4 vaccine studies with vaccinia vectored vaccines.
5 The supply is getting low. I think CDC
6 only has about 150 bottles left. The military has,
7 oh, about 300. We have about 50 bottles, ten each,
8 out at the Plasma and Pheresis Centers in case of an
9 adverse reaction.
10 Since 1992, January, we have had 1,865
11 donors that have been vaccinated and one serious
12 adverse reaction that required 50 mls of V.I.G. So,
13 right now the availability of fresh V.I.G. is still
14 in question, but we hope to have it resolved within
15 the next several months. Yeah?
16 DR. STEVENS: I am a little puzzled about
17 the hepatitis C concern. Is this an IV
18 immunoglobulin,
19 or --
20 DR. BRANDT: No, it is intramuscular and
21 that is -- so far, there are two people that feel --
22 within FDA -- that feel that they should release it
23 to us, but they are still agonizing over their
24 decision.
25 DR. STEVENS: I didn't think there was an

issue about hepatitis C with IM immunoglobulin.

DR. BRANDT: We wouldn't think so, but nonetheless they are conservative and --

DR. STEVENS: In fact, there has been a recent outbreak of hepatitis C with IV immunoglobulin in material that was tested by -- donors all tested by a second or third -- second generation (inaudible), so there is concern of the reverse issue. That had me puzzled.

DR. BRANDT: Yeah, up to November of 1991, of course, they allowed hepatitis C positive material to go in, because they figured that the hepatitis C antibodies were beneficial, but then they backed off from that position and we had to institute then the hepatitis C testing which delayed us somewhat. Any others?

MALE VOICE: This is in reference to the vaccinia vaccine. Do you have any idea whether this vaccine would alter other vaccines that utilize vaccinia vectors, either (inaudible) potency of those vaccines?

DR. BRANDT: It remains to be seen. What I discussed was our BDRP program as far as the vaccinia goes. Under our infectious disease research program, we are in the process of

1 developing a vaccinia vector hunt on vaccine, and
2 then there is numerous other groups out there who
3 are looking at vaccinia vector vaccines.
4 I think I can say that, other than the
5 military, we would probably be the only ones that
6 are really concerned with the effect of those
7 vaccines on smallpox immunity. A lot of the
8 vaccinia vector ones are more interested in how the
9 immune response is to the gene that has been
10 incorporated into the vaccinia virus.
11 GENERAL RUSSELL: What kind of a yield do
12 you get from these subcultures and how do you arrive
13 at the doses that were used in these studies?
14 DR. BRANDT: The -- if I am not mistaken,
15 there may be some here who remember it better. I
16 believe that new vaccine is bottled at ten to the
17 eight PFU's. I believe that is correct, and the
18 initial dose was just a dose escalation.
19 Subsequent studies were essentially because
20 we wanted to compare it to the Wyeth Vaccine, which
21 was given at ten to the fifth and, hence, that is
22 the dose that we had used.
23 GENERAL RUSSELL: Do you have any studies
24 of deep IM with ten to the seventh and ten to the
25 eighth that you might expect antibody response?

DR. BRANDT: To my knowledge, I am not aware.

COLONEL TAKAFUJI: Going back to that question that was asked about the vaccinia, and that is a concern that we have had, because when we look at this program we are not just looking in terms of meeting a requirement in terms of protecting the force against smallpox, we are looking at it in terms of protecting the force against orthopox viruses and we are looking at it from the standpoint of also the booster.

So, that may be necessary down the road and, as you already are very much aware of, there is a lot of that from looking at not only things having to do with vaccinia as carrier vaccines, but other types of vaccinia related carriers that could have an impact in terms of our total program.

So, there is a concern that we have. We just don't have the data that would substantiate either where there is going to be some competitive problems, or what, at this point in time, but it is a concern that we have.

The other concern that I think I want to emphasize that Colonel Jennings made was that it appears that what we still need to really elicit the

best etiological response is some type of pustular formation.

Originally, when we were tasked to develop a vaccine, it was with the idea that the vaccine that would be produced would be, number one, cleaner, number two, safer in terms of less reactogenicity. We have done that. We have a vaccine now that is cleaner, that is safer.

That is basically the same virus, but it is a cleaner and safer product, but what we are finding out is that in order to elicit the appropriate etiological response, as evidenced by new titers and so forth, it appears that we still need that pustular formation, which still creates a potential problem for us from the standpoint of the risk of an autoinoculation, as well as risk of transmission to other people in the community.

GENERAL RUSSELL: You can't say that, Ernie. You have only got data with a low dose, and a low dose --

COLONEL TAKAFUJI: Well, you have an open pustule.

GENERAL RUSSELL: (Inaudible.)

COLONEL TAKAFUJI: But, you have a pustule.

GENERAL RUSSELL: But, with other more attenuated pox viruses, including the generic pox you give at high dose, with no evidence of skin (inaudible), but you do get a response to that virus. (Inaudible), so you can't say that you need a pustule formation until you do the --

COLONEL TAKAFUJI: What I am saying -- yeah.

GENERAL RUSSELL: -- ten to the eighth.

COLONEL TAKAFUJI: I understand, but what I am saying is that based on what we have so far, it appears that there may be --

GENERAL RUSSELL: You have done the wrong experiment.

(Laughter.)

COLONEL TAKAFUJI: You know, the thing is that if I am -- if my statements happen to be right and if, indeed, we do need to still do some type of scarification methodology, we haven't really solved the problem about potential risk.

Now, if you notice, those slides that I had Colonel Jennings show you, specifically, those are small pustules, so in terms of risk, the risk is small, but the problem that we have to deal with is where we are going to be exposing recruits, for

example, of people who we would be immunizing, nonetheless, to an un-immunized population where there may be some very serious risk factors that may predispose them to contracting infection.

There is also another issue that had come up as a result of this program, and that is a health care provider issue, because as we look at alternate routes in terms of how we would deliver the vaccine, there is a danger that there may be some splash resulting from the administration of a vaccine intradermally if you were to use a method other than the bifurcated needle and, therefore, splash through the eyes, which could create a vaccinia eye infection.

(Pause.)

MALE VOICE: Colonel Jennings said that when the virus is given subcutaneously at various doses, if you go up to ten to the eighth, ten to the eight CFU's or ten to the 7.8, or 7.8 logs, he described the immune response as inadequate.

We don't really know that it is inadequate. What we know is that it is several fold less than the antibody response you get when giving Wyeth by scarification. The same is true for L-vac and for MVA attenuated viruses given in large dose, subcu,

historically.

They are not necessarily inadequate when you test against smallpox. They are -- they pale by comparison serologically, and we think in a T cell response compared to a scarification response.

We don't know what is sufficient. We know what is -- we know that what we are getting is less than what is typically gotten with scarification.

COLONEL TAKAFUJI: I asked Dr. Benenson, who has, of course, been involved quite a bit with the smallpox issue through his lifetime to look into some of the aspects pertaining to the jet injector gun and the use of jet injector methodologies in terms of how smallpox could be administered using an intradermal muzzle.

Do you have any comments that you would like to make to the group, sir?

DR. BENENSON: Well, first of all, I would like to go to the issue of (inaudible). We carried out a four University study and we were very cautious still. We didn't dare go above ten to the five. We gave subcutaneous great care. There was dose examination on the needle and we used ten to the three, ten to the four, ten to the five.

The surprising result, although it is a

poignant result, was that there was no dose response that occurred. It was just flat. About one-third developed antibody. There were more that developed antibodies by hemagglutination inhibition than by neutralization.

Now, we have decided that we would determine how effective the immunity was by their resistance to a challenge with a standard (inaudible) vaccine. We did that six months afterwards and the disappointing thing was, first of all, about a third developed a primary type lesion to the challenge, the first cutaneous challenge.

The other thing that has had me intrigued since that time, you could not look at the demonstrable antibodies and say this person is immune. Now, it -- the neutralizing antibody didn't mean that they resist infection, the hemagglutination antibodies didn't. That leaves the cell immunity, the thing we didn't look at and maybe would be the measure, but the important thing is that after getting the vaccine (inaudible) we did not get immunity (inaudible).

Now, that was not done (inaudible). That was way back in 1956 (inaudible). We gave with care subcutaneous vaccine, and I think that they were

probably working around the ten to the seventh and eighth and (inaudible).

Yeah, only one of them showed any cutaneous lesions. We then challenged (inaudible) and one quarter of them developed (inaudible). So, that much for subcutaneous vaccination. I think it is unreliable. You don't know what you have got.

Now, the other issue was one of angular injection. First of all, the angular injection was put on for two reasons. We did that because, for one thing, even though a regular injection worked very nicely in the three of us, three males who were working on establishing the characteristics of the orifice, when I called in my secretary (inaudible), gave her a shot and there was no (inaudible).

Her skin was much more tender and less course than the males, so the thought is that we had the issue of the resistance of the skin (inaudible). In one way, to minimize that as a problem, we had the injection go angular, which would give us the longer area of the skin, but added to that was the advantage that we would have more virus situated at the skin level, and assuming that that was subcutaneous (inaudible).

So, that is where we were. CDC then took

over and (inaudible) and with the jet injection a one to fifty dilution of the ten to the eighth vaccine, ten to the eighth (inaudible) vaccine was as effective, or more effective in producing immunity, period, based on antibodies. No, visible takes. I think they used visible takes pox as the criteria.

(inaudible) out with a conclusion that I think you did, Ernie, and that is that in order to know that you have immunity against that (inaudible). Now, one of the issues that you had mentioned to me was a concern -- and we considered this very seriously -- when you prime your injector you have to fill the system. You do that by squirting a couple of shots into the air.

(Laughter.)

That is perfectly all right if you are dealing with (inaudible), but we didn't. We were concerned about what would happen if live virus got into (inaudible) and the solution is very, very simple. When you do that, you have a (inaudible) or jar of cotton (inaudible) and shoot into that, so you don't generate an open aerosol.

I think that (inaudible) of the -- I guess that is as far as I will go (inaudible).

MALE VOICE: Did you guys change needles?

DR. BENENSON: Did we change?

MALE VOICE: No, did the guys at USAMRIID change needles?

MALE VOICE: I wasn't there when they actually did, but I believe so. (Inaudible.)

MALE VOICE: Yes, at the time they drew it into the syringe with one needle, replaced the needle and inoculated with another. So, what went in subcu, or IM was a clean needle.

DR. ASCHER: Another way to look at this is, we heard about tularemia, which is also a scarification, and the way I have always thought about both of these is that you are giving whatever dose in that situation into a place where it is part of the target pathology, and the organism will replicate to a certain level. The immune response kicks in and you have basically a corrected, automatic immune response based on the equilibrium between that dose and the immune system.

I think General Russell is right, though, that if you really got a pure product which wouldn't do that, you could get the same affect, but it is a lot easier, to me, to use the low dose and let the equilibrium find its own balance. You can see it,

because you know who has got immunity.

DR. BENENSON: (Inaudible.)

MALE VOICE: It is just the safety issue.

MALE VOICE: Oh, yeah, I know, it is the safety.

MALE VOICE: I don't remember seeing it in the literature, but maybe -- in the study you have chick embryo vaccine (inaudible) and they went down to Puerto Rico and gave a bunch of shots with it and developed a lot of abscess, so that the (inaudible).

Now, that is not consistent. It hasn't happened anywhere else and I don't understand it.

MALE VOICE: (Inaudible.)

MALE VOICE: (Inaudible.)

DR. BENENSON: The whole point was, and we have been criticized for it, our (inaudible) intradermal (inaudible) very low bacterial count, but it is a live bacteria and (inaudible) of his argument was we have no bacteria. (inaudible.)

MALE VOICE: We could --

COLONEL TAKAFUJI: We have answers we didn't anticipate. In fact, some -- many of these issues will be coming up as we move ahead, but clearly there are some issues having to do with the methodology in terms of how we immunize, be it by

needle, or be it by gun, and no matter how you do it, I mean, you know, you are going to drag something through a hole that you put in someone's arm.

Whether it is leaking backwards, or whether you are dragging it forward, I mean, the point is that in a certain number of people you are going to have a pustule formation, so we have got to address that issue, or look at, perhaps, an alternative way of immunizing altogether.

So, that is going to be a concern that we have. We have basically met the charge in terms of coming up with a cleaner vaccine. The question is now how do we do it, and do it in the most safest way possible. That is an issue.

The second issue is having to do with the immunization and what is adequate protection, and tied in with that is the issue of, "Well, how often do you boost," because I am sure all of you remember that when we started this smallpox vaccination program many years ago, it was like every year and then went to every three years, and it was extended to every five years and, you know, what is the appropriate time interval to boost individuals? That is going to have to be addressed somewhere in

there, too.

DR. BENENSON: The CDC has put out (inaudible) amendment to the --

COURT REPORTER: Excuse me, sir. Excuse me. Excuse me, sir. Could you take that microphone, please? Otherwise, a lot of the people I don't think are hearing all that you are saying there. The other one, please. Thank you.

DR. BENENSON: CDC has put out the guidelines for the -- oh, you are not ready?

(Pause.)

I will try to talk loud enough so it won't matter.

COURT REPORTER: That's fine, that's fine.

DR. BENENSON: Alright, the CDC has put out guidelines on how to handle this particular issue and they have indicated -- I think you should be saying this, not me -- that the priority is that those people who are actually handling the vaccinal material should be vaccinated and CDC will provide the vaccine for it.

There are others who are peripherally exposed in which one may well consider that they should be covered, too, if they are handling -- are potentially exposed. But, the one group, they are

very positive and say that they should be vaccinated.

I don't remember whether it was every three years, or five years they should be re-vaccinated, but I -- you know, I can tell you that in the next edition of the "Controlled Communicable Diseases in Man" that is going to be specified.

DR. BROOME: (Inaudible.) You are aware because of the increased use in laboratories using the vaccinia as a vector for various procedures so that the recommendations were updated to deal both with laboratory workers and with health care workers who were caring for investigational vaccine, or other exposures.

MALE VOICE: (Inaudible.)

COURT REPORTER: Excuse me, sir. Could you use the microphone?

DR. BENENSON: If this mode of immunization in which we are using the vaccinia as a carrier depends on multiplication of the virus and therefore the other antigens, how can you boost if you have created immunity against vaccinia to start with?

This is something that I -- you -- maybe some of you are working in this and can explain it to me. I know I have read the publications that say

that, yes, they were able a year later to elicit immunity against another disease, but somehow or another, basically, there is an illogical step in here. Can anyone explain that?

MALE VOICE: (Inaudible).

DR. ASCHER: Could you rephrase the question? If you would then ask the question, would someone given a recombinant vaccinia vector carrying another protein, would a person with a primary response to that give a different immune response to the carried material than someone with a secondary response?

Your prediction is that a secondary responder previously immune to vaccinia would have a blunted response to the thing that is being carried, and that is very reasonable.

DR. BENENSON: Well, if the specific --

MALE VOICE: Yeah, right.

DR. BENENSON: If the specific booster response to the same epitope -- I accept the fact that a very small amount of it is enough to give you the booster effect, but our friend in Albany used different antigens and hypothetically the vaccinia virus was not multiplying and, yet, he claimed that he got antibodies through these other factors.

I think it is very good if it works, but I am puzzled as to why it would.

COLONEL TAKAFUJI: Well, the presentations that you heard on the smallpox, clearly they are multi-faceted, and I would like to be able to in the future bring some of these issues to the Board in a much more formal manner, because, as you can see, we are faced with a myriad of different issues.

But, I am not just concerned about the development from a research laboratory perspective, but I am very concerned about how we are going to administer a policy that would basically utilize the new vaccine that we are doing.

I would like to have the freedom to be able to come to the FEB and ask for your guidance in that regard, and whether it would be your committee, Dr. Ascher, or another committee, it clearly is something that we must come to some consensus on.

The worst thing of all, as I see it from my perspective, is to spend a lot of research dollars which are already at a premium to develop a vaccine that never gets adopted by the military.

So, I would like to see us develop a product that makes good sense and is very practical at meeting the needs of the military. Thank you

very much.

(Pause.)

DR. KULLER: Dr. Smith?

DR. SMITH: Are any of these working? Can you hear me?

(Pause.)

May I have the first slide, please? Good afternoon. (Visual aid provided.) Okay, in my summary this afternoon I will review our current efforts to design and develop improved vaccines for the equine encephalomyelitis viruses.

These are alphaviruses which are mosquito-borne in nature and cause epizootics associated with high mortality in equines, and an incapacitating or encephalitic disease in humans.

However, they are also highly infectious and stable in aerosols and are easy to produce in large quantities in relatively cheap systems. As such, they are classical BW agents and, hence, our concern here.

I will speak to the outline shown here and try to summarize the problems with the existing vaccines, problems which are specific to alphavirus immunization and our current vaccine development efforts, which include live vaccines derived from

infectious clones, which is a collaborative effort with investigators at the University of North Carolina, microencapsulated vaccines, which are -- which is a collaborative effort with investigators at the University of Alabama and recombinant baculovirus expression products.

The alphaviruses of direct concern to the BDRP are the three equine encephalomyelitis viruses, Venezuelan, Eastern and Western viruses. Actually, Venezuelan exists as a complex of serologically related viruses and that complicates vaccine development somewhat, as I will discuss later.

The specific objectives -- directives -- to the alphavirus program are summarized here. The objectives are to develop vaccines which will protect over 80% of soldiers from an aerosol infection with minimal reactogenicity and transition to advanced development of vaccine for Venezuelan by 1996 and analogous product for Eastern and Western by 1998.

Previous efforts within the Research and Development Command have resulted in four alphavirus vaccines which are currently used under IND status. None are licensed vaccines. A live attenuated vaccine for Venezuelan, TC-83, and formalin-

inactivated vaccines for Venezuelan, Eastern and Western viruses.

However, extensive experience with these vaccines in humans under the auspices of the Special Immunization Program at USAMRIID has demonstrated that they have multiple and unacceptable deficiencies with respect to immunogenicity and/or reactogenicity.

Furthermore, use of these vaccines has revealed other problems related to alphavirus vaccination, including vaccine interference, non-responder populations, insufficient protection against heterologous strains and questionable protection against aerosol infections.

TC-83 is a product attenuated by serial passage in cell culture. This product is used under IND status to protect at-risk laboratory personnel and has also been used as a veterinary vaccine. However, TC-83 is known to consist of heterogeneous virus populations which vary markedly in their virulence for rodents.

It is also reactogenic in 18 to 20% of human recipients, some of whom manifest a syndrome not unlike the natural disease and shed virus in the nasopharynx which is virulent for hamsters. It is

unclear whether these more virulent isolates result from true genetic reversion, selection of more virulent subpopulations, or perhaps both.

TC-83 produces a spectrum of adverse reactions which occur with a bimodal distribution with peaks at one to two days and seven to ten days after vaccination. This chart plots severe and total reactions by day of onset.

The severe reactions include high fever, headache, photophobia and disorientation. The more mild reactions typically include lower fever, myalgia and headache. Due to its reactogenicity and the severity of some of these reactions, it seems unlikely that TC-83 would be used immediately prior to troop deployment.

Surprisingly, for a vaccine with such high reactogenicity rates, TC-83 also fails to elicit adequate levels of neutralizing antibody in at least 20% of recipients. TC-83 has been shown to be transplacentally transmitted in mice. It also reverts rapidly to virulence upon passage in hamsters or infant mice.

TC-83 causes fever and viremia in some vaccinated horses and was reported to be transmitted to mosquito vectors following vaccination of horses

in Texas during the 1971 epizootic.

Based on a few cases of laboratory acquired disease here and elsewhere, it also appears that successful seroconversions in humans to TC-83 does not provide adequate protection against some enzootic subtypes of Venezuelan.

I would like to now turn to the formalin inactivated vaccines which the Command has developed for Venezuelan, Eastern and also Western. These vaccines were produced in the 1960's nad 70's and made from media fractions of infected chick embryo fibroblast cultures.

In general, these vaccines produced in cell culture have proven to be safe and are well-tolerated. However, they are also poorly immunogenic, requiring multiple injections and periodic boosters, and they are also relatively expensive to produce and there have been questions raised about their ability to provide protection against aerosol acquired infections.

The formalin inactivated product for Venezuelan, C84, was produced from TC-83 to provide an added level of safety in light of previous difficulties with incomplete inactivation of virulent Venezuelan strains.

It provides efficient protection in hamsters against subcutaneous challenge, but does not protect hamsters against an equivalent challenge delivered by the aerosol route.

It is unknown whether these results can be extrapolated to primates, although in the absence of data to the contrary, it has seemed prudent to assume so and, thus, C84 is currently used only to boost TC-83 vaccinees and not as a primary vaccine.

If we now look at our experience with the inactivated Eastern and Western vaccines, the first point is that these are the only vaccines currently available. There have been no live attenuated vaccines developed for Eastern and Western.

The second point is that they are poorly immunogenic. Multiple inoculations are necessary and seroconversion rates are low. After the primary series, only 58% of Eastern vaccinees develop one to forty neutralizing titers, and the rates for Western are even less, about 50%

In addition, antibody titers decline rapidly. Less than half will maintain a minimal titer for 12 months. We don't know whether individuals whose titer declines become susceptible, but we must assume that some are and, thus, the

laboratory staff are frequently tested and boosted to maintain titers.

It would, of course, be considerably more difficult to maintain titers in large numbers of troops in the field, so I think it is clear that the classical approaches to vaccine development have failed to provide vaccines which meet the requirements of the BDRP and that the existing vaccines are inadequate by a number of criteria.

They have been useful in a serologically and medically monitored laboratory population, but they would be of limited use in a deployment situation, so in the late 1980's a fresh look at these problems was made with the objective of newer technologies that would overcome the inherent limitations of the more classical approaches.

A number of strategies were considered and both live attenuated and non-replicating vaccine strategies are being pursued. The approach that we have taken to develop live attenuated vaccines has taken advantage of the fact that the alphavirus RNA genome is infectious as naked RNA.

It is therefore possible to make a DNA copy of the viral genome by genetic engineering techniques, introduce attenuating mutations by site

directed mutagenesis and derive vaccine candidates which are completely defined at all 11,000 nucleotides.

The basic premise of this rational approach is that optimal characteristics of attenuation, immunogenicity and low reversion rates can be achieved by engineering the genetic loci controlling these characteristics.

The overall strategy is outlined here. Can that be made smaller, or -- well, I think you can see it. The first step is to make DNA clones from viral RNA covering the entire Venezuelan genome and ligate them together to form a full length clone downstream from a T7 RNA polymerase promoter. This is basically a transcription plasmid capable of making viral RNA.

The next step is to identify specific nucleotide changes which will attenuate the virus. This was accomplished by sequence comparison of attenuated and virulent strains and also by targeted mutagenesis of highly conserved areas of the viral genome thought to be critical for virus replication.

The identified mutations are then incorporated into the full length DNA clone by site-directed mutagenesis, either individually for

evaluation of the mutation, or in various permutations to produce candidate vaccines.

The modified clones are then transcribed to produce RNA, which is transfected into certified cells in culture to recover the modified viruses for animal testing. All of this has been done for the Venezuelan virus.

The individual attenuating mutations which we have identified and studied are shown here along with our assessments. Essentially, all of the attenuating mutations discovered are in the E1 or E2 glycoprotein genes. The mutations above the line were defined by sequence analysis of attenuated mutants and those below the line were discovered by targeted mutagenesis studies.

A realistic vaccine candidate would, of course, require multiple independently attenuating mutations to maintain genetic stability and prevent reversion. The various permutations of the individual mutations which we have assessed are shown here.

All of these have been tested in mice and hamster models for attenuation and also for their efficacy against both parenteral and aerosol challenges.

A typical assessment study sheet is shown here which is pretty tough to read, but really there is only two important points that I want to show you here. One is that we have made several constructs here that are completely attenuated following subcutaneous inoculation, and also that there are credible neutralizing titers which are generated and that these vaccine candidates are capable of completely protecting these mice against not only aerosol -- not only subcutaneous, but also aerosol challenge.

Based on the results of the preliminary rodent assessment, we have chosen the most attractive three candidates which are now being tested in primates. So, overall we are very optimistic about this program and expect to transition a safe and effective vaccine for Venezuelan virus on schedule.

One of the advantages of this rational approach and that we have -- that we have a completely defined vaccine and that there is a direct extrapolation to Eastern and Western viruses. In fact, we have already developed a full length clone for Eastern for this purpose.

However, in this context and within the

broader context of the BDRP objectives which include three alphaviruses, there may be a potential problem with live alphavirus vaccines. That is to say, a generic problem, and that is the phenomenon of vaccine interference.

In recent clinical trials carried out at USAMRIID, we have made the observations that are shown here. In non-immune recipients we see about an 83% seroconversion with TC-83 VEE vaccine and about a 98% conversion with our new Chikungunya vaccine.

However, in TC-83 immune individuals, Chikungunya vaccination only seroconverts 46% In Chikungunya immunes, the seroconversion rate for TC-83 drops from 83% to 40%

This was not actually entirely unexpected, as alphaviruses are known to cross react serologically and this leads to some heterologous immunity. However, we assumed that this would not be a problem with simultaneous or polyvalent vaccination. That, however, may have been wrong.

In this study, individuals were given the Chikungunya vaccine in one arm and either TC-83 or a placebo in the other. What you can see from this study is that when the two vaccines are given

simultaneously, there is still a significant reduction in seroconversion to the Chikungunya vaccine that is shown here.

With the Chikungunya vaccine alone, we have 100% response. That is, no non-responders. In the individuals that got both Chik and TC-83, we have 9 out of 25 responders to Chikungunya. The response to TC-83 is about the same.

So, quite possibly, this could be overcome by varying relative doses, but that has not yet been studied. So, I think interference is a problem that we will need to address.

To maximize our options, we have also pursued non-replicating vaccines. The approach that we have selected to develop improved non-replicating vaccines has used microencapsulation technology which incorporates an activated and concentrated virus into five to ten micron microspheres of poly lactide-co-glycolide, as shown here.

This material is the same as used to make some resorbable sutures, and it has a long history of safety in humans. Other potential advantages are listed here.

In addition to the safety, other investigators have described an adjuvant effect,

mucosal antibody responses following oral immunization protection from mucosal challenges in a timed release capability.

We would also expect that such non-replicating vaccines would be less susceptible to interference and that the technology would lend itself to the production of polyvalent vaccines.

Those points which we have confirmed with microencapsulated Venezuelan vaccines are preceded by check marks on the slide and the other issues are now being addressed. So, for example, if -- in this study mice were immunized with 50 micrograms of Venezuelan protein, actually, purified virus, either in PBS or microspheres boosted at day 50.

Their neutralization titer was monitored and what we see is that following the boost we get a 20-fold increase in neutralization titer with a microencapsulated product.

The response to these microencapsulated vaccines is also protective. This is following a subcutaneous inoculation -- a subcutaneous challenge. This is a single inoculation of the vaccine, and with 25 or 50 micrograms we get 90 or 100% protection. This is a parenteral challenge.

We are also seeing protection against

aerosol. In this experiment, mice were immunized with 50 micrograms of either free or microencapsulated vaccine and then boosted either subcutaneously, or orally, or intratracheally and what we are seeing is a very good protection by basically all these routes against an aerosol challenge. So, we think that microencapsulation technology also offers attractive options for alphavirus vaccines.

I think I am running a little over, so I am going to go right on by our baculovirus studies and just say that we have -- we have developed both live attenuated and microencapsulated vaccine candidates for Venezuelan virus and are pursuing similar strategies for Eastern and Western viruses. Venezuelan is the lead agent. We are about 18 months behind Venezuelan with Eastern and Western efforts.

Which strategy is eventually chosen will largely depend not only on safety and induction of homologous immunity, but how each approach solves the issues that are shown here, some of which may be specific to alphavirus immunization and thereby maximize our options with respect to vaccination policy. Thank you.

DR. KULLER: Questions?

DR. SMITH: Question? Yes, yes, ma'am.

DR. STEVENS: You raised the issue of reversion to (inaudible).

DR. SMITH: Yes. We see -- we have looked actually at candidates that have single mutations, double mutations and three and four mutations, also.

The single mutations do revert, some of them more frequently than others. Interestingly, some of them revert at the same site and others revert at different sites, so we have second site reversions, as well as primary site reversions and -- but, we have rarely seen reversion with double mutants and have never seen reversion with triple mutants.

In fact, when we started the studies we assumed that the problem was going to be preventing reversion. As it turned out, we over-attenuated these candidates and, so, we are backing off now on the mutations. Yes, sir?

DR. ASCHER: A general comment about Western. In California, for a number of years a number of our people have been predicting that Western was going to come back with a vengeance, Dr. Reeves, most notably, and he was right on last

summer.

I don't know if a lot of you know it, but we had a couple of hundred Chikun seroconversions and positive mosquitos all the way from the Sacramento River to the top of the State, and that was the bad news.

The good news is, with active searching we could not find a single human case. Obviously, the virus that was in that group is going to be of interest in the mosquitos in such a big study, but it always concerned us that this, of all your herbivorous vaccines, is the one that is our shortest supply.

Every time we called up and said, "What happens if we started getting humans," and this is an orphan problem that other people have talked about, and fortunately we didn't have hundreds of human cases, but we certainly could have.

MALE VOICE: Horse cases?

DR. ASCHER: We had one, an un-immunized horse. So, it appeared to still have that potential, and it does knock the hell out of emus, and I didn't make that up.

(Laughter.)

MALE VOICE: So does Eastern.

DR. ASCHER: A commercial virus for an emu vaccine.

MALE VOICE: Yes.

MALE VOICE: $20,000.00 in emu runs.

MALE VOICE: That's right.

DR. SMITH: The veterinary vaccines are very similar to our formalin-activated products, and actually about 20% of the horses in Florida that are sick and dying with Eastern have clear histories of immunization.

GENERAL RUSSELL: Is there any evidence of these attenuated strains (inaudible)?

DR. SMITH: The fair answer is that we haven't looked, okay, and I don't -- I don't know why. I would bet that they would not be, because the mutations are in the structural genes, but I -- but, it is just a guess.

GENERAL RUSSELL: That subcutaneous vaccination (inaudible).

DR. SMITH: Yep, it was an unpleasant surprise to us, also.

GENERAL RUSSELL: All four (inaudible).

MALE VOICE: (Inaudible.)

DR. SMITH: Yeah, there was -- some of the very --

GENERAL RUSSELL: Jack interferes with himself.

(Laughter.)

DR. SMITH: Some of the very early polio virus, they looked as if there was some interference with some of the early polio trivalent vaccines and --

MALE VOICE: (Inaudible.)

DR. SMITH: Yeah. I think we could maybe fix it just with dose.

DR. KULLER: Dr. Jahrling?

DR. JAHRLING: I believe this is the last formal presentation of the afternoon and I see people kind of shuffling their feet, so I will try to keep this quite brief.

In fact, the title listed in the program is "Viral Hemorrhagic Fevers," but, in fact, I will restrict my comments today only to filoviruses. That is, Marburg and Ebola.

As you heard at the classified briefing this morning, these viruses are now on the threat list. In response to that threat, we have reactivated our research program here under the BDRP to develop effective counter measures for filovirus infections.

Filoviruses, as you know, have been associated with explosive outbreaks of hemorrhagic fever, first recognized in 25 laboratory workers handling Marburg virus infected monkeys in cell cultures in Yugoslavia in 1967.

Later, Ebola virus emerged as the agent responsible for over 500 cases in two major outbreaks in 1976 with case fatality rates of 86% in Zaire and 53% in Sudan.

Recently, another Ebola related virus, now known as Reston, emerged from monkeys that were imported into the United States from the Philippines in 1989. Reston virus, while uniformly fatal for monkeys, appears to be infectious for humans, but seems not to cause serious disease.

Epidemiologic data for all filoviruses suggest that aerosol and/or fomite transmission occurs commonly, and the published data suggests -- using minimal numbers of animals -- suggests that these viruses are infectious by the aerosol route and cause lethal infections in primates.

The aerosol infectivity of filoviruses, coupled with their high lethality, are factors in determining that these agents pose a significant biological warfare threat.

Can I have the first slide, please? (Visual aid provided.) To address the threat we are seeking to develop a bivalent vaccine, or perhaps multivalent, if necessary, to protect against aerosol exposures to Marburg and Ebola viruses.

Toward this end, we are developing animal models for the human diseases and using them to test candidate vaccines and other symptomatic or viral specific regimens, including passive antibody and antiviral drugs.

We know that ribavirin is not effective against the filoviruses. Dr. Huggins in Virology is investigating another class of compounds known as the S-adenosly methionine hydrolase, or SAH inhibitors, which hold real promise for Marburg and Ebola.

We are also aware that cytokines may well play a central role in the pathogenesis of these infections, and we believe that cytokine antagonists may find application in the symptomatic treatment of viral hemorrhagic fevers.

(Pause.)

In restarting this filovirus program, we obtained authentic reference viral stocks from a number of sources, all of which have been at least

partially, or completely cloned and sequenced, and we did the basic virology which will then establish the basis for comparison with more contemporary tools of modern virology, which include antigen-capture ELISA's for real time evaluation of viremia and virus content of other samples, PCR for rapid diagnosis and evaluation of viral latency.

Remarkable advances have been made in the past few years in the areas of immunohistochemistry and in pseudo-hybridization, immune electromycroscopy. All of these find application in evaluating filovirus infections.

This investment in virologic and immunologic tools is, of course, essential to model building. It will enhance our ability to identify these agents and to recognize newly emerging viruses as something -- as just that, something new, as occurred with Reston-Ebola in '89. Also, epidemiologic investigations may lead to identification of potentially useful test beds in evaluating candidate vaccines and treatment strategies.

The requirement for maximum biologic, or BL-4 containment imposes significant constraints on the size and the feasibility of the experiments we

can attempt. This is a file photo of a Class III hood line, and in the next slide is a picture of someone working in a spacesuit.

This combination of space suits and cabinets gives USAMRIID a unique capability and flexibility to deal with a number of BL-4 agents simultaneously, and in that regard we are truly a national resource.

Yet, even with these superb facilities, logistics are difficult and for these reasons published animal model studies to date have used minimal numbers of animals with limited virologic, immunologic and pathophysiologic data.

Our experience with filovirus infections in guinea pigs and primates frequently differs from that reported by the CDC and European BL-4-capable laboratories. Our first priority, after our viral seeds and immunologic assays are characterized, will be to develop reproducible animal models for Marburg and Ebola.

We will pay special attention to the influences of dose and route on pathogenesis, especially aerosol. We will quantitate as many parameters as possible, correlating the clinical pathologic profiles with virologic, pathologic and

1 immunologic events in animals and compare it with
2 what we think we know about these diseases in
3 humans. Ultimately, we will use the animal models
4 to test the vaccines and drugs.
5 We have invested in African green monkeys
6 versus cynomolgus and rhesus macaques. We know the
7 strain 13 guinea pigs are much more susceptible to
8 filoviruses than strain 2's. Our experience with
9 outbred Hartley guinea pigs is that they are very
10 variable in their susceptibility, which may explain
11 a number of the inconsistencies in the published
12 reports.
13 We have adapted some filovirus strains to
14 uniform lethality by serial passage in guinea pigs,
15 and this may be an alternative to relying on the
16 inbred and costly strain 13's.
17 For some studies, especially those
18 involving cell mediated immunity, inbred mice are
19 clearly more appropriate, and we are testing a
20 number of mouse strains for susceptibility to
21 reference filovirus strains.
22 For some studies, we might find it
23 preferable to work with filovirus strains with
24 reduced biohazard for man, such as Ebola Reston,
25 which, as I mentioned, is almost uniformly lethal

for cynomolgus macaques, but not for humans.

This is a partial list of the parameters that we routinely measure in animal models. In addition to what is listed here, we are getting platelet function and coagulopathy assays on line. We will attempt to relate recovery from acute disease with protective immunologic responses, both cellular and humoral.

We are aware of reports in the literature of immunologic enhancement following challenge with heterologous and sometimes even homologous filovirus strains. If true and confirmable, immunologic enhancement could force us to rethink our entire vaccination and passive immunization strategies.

Passively administered antibodies have been used on several occasions to treat laboratory workers exposed to filoviruses. The results have been equivocal and often contradictory. Conflicting reports probably result from virus strain fluctuations and poor characterization of neutralizing antibodies.

We have raised neutralizing antibodies to Ebola strains in guinea pigs and rhesus monkeys and, in fact, it was through this method that we classified Reston-Ebola as a new virus, based on its

neutralization profile.

In guinea pigs, cross neutralization predicts cross protection against Ebola virus challenge, in my hands, so selection of a protective antibody might be relatively straight forward in that model; however, we found quite recently that Ebola Reston does not protect monkeys against a challenge with Ebola Zaire, even when the neutralizing antibody to the challenge virus is already on board before challenge. This might be the dread immunologic enhancement that we have read about.

The need to define the conditions under which filovirus neut antibodies are protective, versus deleterious is a high priority. These principals may be established with polyclonal antisera. More likely, a precise understanding will come from studies involving monoclonals.

Certainly, if passive immunotherapy is to be seriously considered today, we will have to invest in monoclonal antibodies and eventually production of chimeric, or humanized antibodies for reasons of both safety, as well as enhanced efficacy.

Finally, there are many theoretical

obstacles to the development of the filovirus vaccine. One of these, as I mentioned before, is the belief that these viruses are, as a class, poorly immunogenic, especially with regard to neutralizing antibodies.

I am not sure that this is really true. Filoviruses are no less immunogenic than Old World arenaviruses, such as Lassa virus, using specialized in vitro neutralization assays.

As I mentioned, the three Ebola's can be serologically distinguished by cross neutralization, as can at least two different Marburg isolates. Whether or not this signals a multivalent vaccine will be required will only come from cross protection studies.

Immunologic enhancement, I think, is a bigger concern, especially now that we have the indication Ebola Reston sets up monkeys for rapid death following challenge with Ebola Zaire. This shakes our faith in neutralizing antibodies to predict protection.

The Russians have reported that a Marburg glycoprotein clearly contains a region clearly analogous by sequence analysis to the reputed immunosuppressive protein known as p15e, associated

with a number of retroviruses.

German investigators working at the CDC with Tony Sanchez and others have extended this observation to three serotypes of Ebola. The role, if any, of this protein in the pathogenesis and immune responses to filoviruses remains to be determined, but it is, of course, a concern.

Another potential problem in filovirus vaccine development is that virulence for primates may not be predictive for humans. Reston-Ebola is a good example of that since, as I mentioned, the virus kills almost all macaques, but produced only inapparent infections in animal caretakers exposed by both parenteral and aerosol routes.

Finally, the natural history of filoviruses is essentially unknown. Thus, there is no target population that would benefit from participating in a filovirus vaccine trial, but we are a long way from having to deal with that problem, because we need to make the vaccine first.

I think before we totally discard it out of hand, we need to revisit the old concept of conventional inactivated whole virus vaccines before going the high tech route.

As you heard from Dr. Smith's presentation,

1 the use of adjuvants, microencapsulation techniques
2 and inactivation strategies, other than formalin,
3 might be sufficient to validate this approach.
4 Availability of uniformly responding animal
5 models will certainly facilitate these studies. I
6 have listed here attenuated strains which might have
7 use in model building and might give some insight
8 into what is important to the way of protective
9 immunity; however, live attenuated filovirus
10 vaccines will probably never be approved for this
11 class of agents because of safety concerns,
12 including potential reversion frequency.
13 Kevin Anderson, Virology Division, is
14 working on baculovirus-expressed protective
15 antigens, as well as poxvirus-vectored filovirus
16 antigens to augment those already engineered by Tony
17 Sanchez at CDC. We will be testing those
18 constructs, both Kevin's and Tony's, soon in guinea
19 pigs, followed by primates using homologous
20 challenge strains. This reiterates our thrust in
21 the primate models.
22 I included it to remind me to mention that
23 effective protection against aerosol challenge is an
24 absolute requirement since these are -- these
25 filoviruses are BDRP agents. In that regard, all

candidate vaccines will have to be evaluated in animal models that are well-characterized by aerosol.

There are several examples that we heard about today of vaccines that protect against parenteral challenge, yet fail to elicit a protective mucosal immune response. Therefore, all critical virus-host and vaccine combinations will be tested by the aerosol route.

This is not just one more cell in the test matrix. Aerosol challenge with a BL-4 agent is a major logistical increment, but we will have to make that investment in the animal models that we have developed so far, particularly cynomolgus monkeys, African greens and guinea pigs challenged with adapted strains.

I had intended to conclude this talk with a brief sampling of data from a recent experiment using Ebola-Reston in macaques to give you some confidence and flavor of our ability to deliver on the multi-faceted program that I have described, but because of the hour, I think I will spare you those details and simply assert that we do have reasonable capability to model Ebola and Marburg infections in primates and guinea pigs.

Our next experiments will be enhanced by the use of radio telemetry for continuous monitoring of body temperatures, blood pressure, electrocardiograms and the like.

I want to leave you with the impression that in the nine months that our filovirus program has been reactivated we have made significant progress on a number of these fronts and we are cautiously optimistic that this progress will translate soon into effective counter measures against these rather nasty agents. Thank you.

DR. KULLER: Questions?

(No response.)

Thank you very much.

(Pause.)

We will have about a five minute pause now, and then I hope that the Board members and the preventive medicine consultants could stay with us for a short meeting. Off the record.

(Whereupon, the above proceedings were concluded.)

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