FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

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ANTI-INFECTIVE DRUGS ADVISORY COMMITTEE

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Friday, July 28, 2000

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The Advisory Committee met at 8:30 a.m., in Conference Rooms G and H, Parklawn Building, 5600 Fishers Lane, Rockville, Maryland, Dr. Barth Reller, Chairman, presiding.

PRESENT:

L. BARTH RELLER, M.D., Chairman

GORDON L. ARCHER, M.D.

P. JOAN CHESNEY, M.D.

CELIA D.C. CHRISTIE-SAMUELS, M.D., M.P.H., F.A.A.P.

JUDITH O'FALLON, M.D.

KEITH A. RODVOLD, Pharm.D.

DAVID E. SOPER, M.D.

MURRAY WITTNER, M.D., Ph.D.

PRESENT (Continued):

THOMAS H. PEREZ, M.P.H., R.Ph.

CONSULTANTS AND GUESTS PRESENT:

JAMES W. BAYUK, M.D.

SCOTT DEITCHMAN, M.D.

ARTHUR FRIEDLANDER, M.D.

MARTIN HUGH-JONES, D.V.M.

JONATHAN MORENO

ERNEST TAKAFUJI, M.D.

DAVID WALKER, M.D.

MICHAEL M. WERTZ

FDA REPRESENTATIVES PRESENT:

GARY K. CHIKAMI, M.D.

SANDRA L. KWEDER, M.D.

DIANNE MURPHY, M.D.

PUBLIC SPEAKER:

ITZHAK BROOK, M.D.

C-O-N-T-E-N-T-S

P-R-O-C-E-E-D-I-N-G-S

(9:13 a.m.)

CHAIRMAN RELLER: Good morning. I'd like to welcome everyone to this meeting of the Anti-Infective Drugs Advisory Committee of the Food and Drug Administration.

I'm Barth Reller from Duke University Medical Center and will be chairing today's meeting.

At the outset of the meeting, we'd like to introduce those who are around the table and will be either guest speakers, consultants or members of the committee, and we'll begin at the end of the table with Dr. Diane Murphy.

DR. MURPHY: I'm Dr. Diane Murphy. I am the Director for the Office of Drug Evaluation-4, but presently on detail to the Office of Review Management. So I'm really being Mack Lumpkin this morning, and Dr. Kweder is being the Director of ODE-4.

DR. CHIKAMI: I'm Gary Chikami. I'm the Director of the Division of Anti-Infective Drug Products at the FDA.

DR. KWEDER: I'm Sandra Kweder. I'm serving as the Acting Director, Office of Drug Evaluation-4.

DR. MEYERHOFF: I'm Andrea Meyerhoff. I'm a medical officer in the Division of Special Pathogens.

DR. ARCHER: I'm Gordon Archer from Virginia Commonwealth University in Richmond, Virginia. I'm the Chair of the Division of Infectious Disease there.

DR. CHESNEY: I'm Joan Chesney from the University of Tennessee in Memphis, in pediatric infectious disease.

DR. O'FALLON: Judith O'Fallon, Mayo Clinic, statistician.

DR. SOPER: David Soper, Medical University of South Carolina in Charleston.

DR. CHRISTIE-SAMUELS: Celia Christie, University of the West Indies, Kingston, Jamaica, infectious diseases and epidemiology and child health.

DR. RODVOLD: Keith Rodvold, the University of Illinois College of Pharmacy and

Medicine.

DR. PEREZ: Tom Perez, Executive Secretary for this meeting.

DR. WITTNER: Murray Wittner. I'm with the Albert Einstein College of Medicine and Professor of Pathology, Parasitology, and Tropical Medicine.

CHAIRMAN RELLER: I would also like to introduce our guests who will be speaking. Dr. Arthur Friedlander, and maybe you could help me out by introducing yourselves as we go down the table here.

DR. FRIEDLANDER: I'm Art Friedlander from USAMRID, Fort Dietrich, Frederick, Maryland.

DR. WALKER: David Walker, Chairman of Pathology at the University of Texas, and Director of the Center for Tropical Diseases.

DR. HUGH-JONES: Martin Hugh-Jones, Department of Veterinary Epidemiology at LSU Veterinary School, and I'm the coordinator for the WHO Anthrax Working Group.

DR. TAKAFUJI: I'm Ernie Takafuji from the Office of the Assistant Secretary of Defense for Health Affairs.

DR. DEITCHMAN: I'm Scott Deitchman. I'm an occupational medicine physician and senior scientist with the National Institute for Occupational Safety and Health, which is a part of the Centers for Disease Control and Prevention.

DR. BAYUK: Dr. Jim Bayuk. I'm the Office of Environmental Health and Preventive Medicine at the Department of State, Office of Medical Services. Our office represents the health care responsibilities for approximately 25,000 men, women and children that are part of our U.S. embassies and consulates overseas.

MR. WERTZ: I'm Mike Wertz with Eagle Group International. I'm the project manager for the Anthrax Vaccine Immunization Program at the Department of State, Office of Medical Services.

CHAIRMAN RELLER: Thank you very much.

We're most pleased to have our guests and consultants, members to discuss the important issues before us today in the interest of the health of the nation.

I'd next like to turn the meeting over to Tom Perez, who will make the necessary statements

regarding this meeting.

DR. PEREZ: Good morning. The following announcement addresses the issue of conflict of interest with regard to this meeting and is made part of the record to preclude even the appearance of such at this meeting.

Based on the submitted agenda for the meeting and all financial interests reported to the committee participants by the committee participants, it has been determined that all interests in firms regulated by the Center for Drug Evaluation and Research present no potential for a conflict of interest or the appearance of a conflict at this meeting.

We would, however, like to disclose for the record that Keith Rodvold, Pharm.D., previously participated in meetings of Bayer's Moxyfloxacin Pharmacy Advisory Board and that he previously participated in Bayer's Speakers Bureau.

In addition, Dr. Rodvold was a co-investigator in a pharmacokinetic study of the lung penetration of vivofloxacin and cipro.

Lastly, Dr. Rodvold was an investigator in a study of the effect of cardiopulmonary bypass on cipro disposition.

With respect to FDA's invited guests, there are interests which we believe should be made public in order to allow the participants to objectively evaluate the guests' comments. Dr. Arthur Friedlander would like to disclose for the record that he has received speaker fees from Bayer for educational lectures that he has given to physicians.

In the event that the discussions involve any other product or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement, and their exclusion will be noted for the record.

With respect to all other participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose product they may wish to comment upon.

Thank you.

CHAIRMAN RELLER: Thank you, Tom.

I'd next like to take the opportunity to introduce Dr. Jonathan Moreno from the Center for Bioethics at the University of Virginia, who has joined us and is one of our guests for today's discussions, deliberations.

Now I should like to call on Dr. Diane Murphy whom you heard before is the Director of the Office of Drug Evaluation-4. Dr. Murphy will present opening comments and set the framework, the context into which this meeting is occurring.

Dr. Murphy.

DR. MURPHY: I wish to extend my sincere thanks to everyone who has made time in their schedule to be here today.

My task this morning is to delineate for the committee, in particular, but also for the discussants and the public, how this meeting is different because, as you will hear, this is not quite our usual situation. It is a unique situation.

So I'm going to -- my tasks are to go over how we got here. It's often important to understand what has happened, to understand the context of the

situation. So I'm going to spend a minute doing that and review then what are the components of this meeting which we think are important for people to understand or different.

Next slide, please.

In 1998, there have been a number of presidential directives, but we'll focus on this one for right now concerning bioterrorism that orders federal agencies to significantly expand and better coordinate their steps to protect against the consequences of biological and other unconventional attacks.

We, of course, are a federal agency and wish to facilitate the ability of our population to have access to therapies that they would need in such an event.

Next slide, please.

The particular mandates or directions under the presidential order that were addressed at HHS, and this is quite a condensation of those bullets, were that HHS is to basically be involved in improving the nation's surveillance network,

strengthening the medical response capacities, creating and maintaining a stockpile of pharmaceuticals for mass treatment, and expanding research into the disease agents and into improved treatment.

Next slide, please.

The research and development aspects do relate to FDA. As you will see, the agents that have been particularly targeted with an emphasis on anthrax, tularemia, and plague, and as you are aware this morning, the product which, along with input from national and academic and professional societies, and that has -- there has been numerous papers and publications in this area, and they are to develop the stockpile, the components of which that relate to FDA activities. This is a stockpile that will be deliverable within 24 hours, will cross interstate boundaries.

That left us at FDA with a number of options.

Next slide, please.

We have been working with a number of

other federal agencies in trying to streamline the IND process for use in the event of a mass exposure. That is an ongoing process so that products that would be under study and not yet approved for the indication could be used, if necessary, in such a situation.

That is not why we're really here this morning. We're not talking about that process. We're talking about really Item No. 2.

We also have been involved in identifying marketed products which do not have the indication for treatment in the event of a bioterrorist event, but which may be appropriate for labeling, and what that means is we have looked at the products in the stockpile and have looked at those which do not have the indication in their label, and have proceeded to look at the evidence, the body of evidence that is available to see if it would be appropriate to consider labeling these, the product, and if not, whether the activities or studies would be needed.

And then last is another area that we continue to be involved in, which is identifying marketed products which may need additional other

studies.

Next slide, please.

How is it different? This is an FDA initiated process. We basically reviewed the public data and the professional recommendations. We have determined, having done that, a need for submission of data, and we requested the sponsor submit the application, and we ask the investigators and sponsors to participate in this public discussion.

So that is different than the usual process.

Next slide, please.

As you all are aware, this is a unique situation. Fortunately bioterrorist attacks do not occur on a regular basis, and we are dealing with what is the appropriateness of the IND process for a marketed product with extensive safety record and additional other studies, including significant animal study of inhalational -- typo there -- anthrax in a situation in which it is ethically unacceptable to conduct trials with the organism in humans.

That is the circumstances in which we are

addressing this morning.

Next slide, please.

What we will do today is to look at the body of evidence that is available, and Dr. Chikami and Dr. Meyerhoff will spend a lot more time on this with you, in addition to the speakers that we have asked to address this issue, but this slide is a very succinct summary in that we have a large body of clinical safety information. We have animal studies. We have PK/PD data in animals and humans, and in vitro microbiologic data that will be reviewed for you today.

Next slide, please.

Another thing that's different about today, we normally do not make a recommendation. We provide an assessment to the Advisory Committee. Clearly, we thought it rather disingenuous to come to the committee having outlined for you the process in which we have undertaken and not assume that we have a recommendation.

However, we felt this recommendation needs public input and discussion, and so that is the other

difference that you will see today.

We will be asking you, if you've seen the questions, you agree with the recommendation, but we will be making a recommendation of what our assessment is.

Next slide, please.

And my last opportunity this morning is to tell you that there have been a number of people at FDA who have put in a tremendous amount of work gathering information, reviewing it, and involve both Anti-Infectives and Special Pathogens Divisions' cooperation with a number of scientific individuals.

And I wish to personally recognize the effort and commitment they have put into this activity.

Thank you.

CHAIRMAN RELLER: Thank you, Dr. Murphy, for that important background information.

Later this morning we're going to have the opportunity to hear about the clinical manifestations of anthrax and its epidemiology presented by Dr. Martin Hugh-Jones; the pathology of inhalational

anthrax portrayed by Dr. David Walker; and a detailed discussion of the non-human primate model of inhalational anthrax by Dr. Friedlander.

Before that discussion of the epidemiology/pathophysiology of the disease for which the sponsor is coming forth with their presentation, we will now hear from Bayer their presentation of the relevant information regarding safety and other aspects of ciprofloxacin.

Andrew Verderame will present. He is the Associate Director of Regulatory Affairs for Bayer.

MR. VERDERAME: Thank you, Dr. Reller.

I'm Andy Verderame of Bayer Corporation.

I wish to thank the members of the Advisory Committee, the FDA, and the other invited guests today for their participation in a discussion of a new indication for our fluoroquinolone ciprofloxacin.

The agenda for my remarks is as presented. In the next 20 minutes or so, I'll spend some time reviewing the events that have brought us here today from the Bayer perspective, and because our submission

contains no human data for the proposed indication, I will present some indication that provides a strong rationale for the use of ciprofloxacin in the event of an anthrax release.

Now, in February of this year, Bayer submitted labeling supplements for all ciprofloxacin oral and IV formulations to the FDA for an indication of post inhalational exposure prophylaxis of anthrax. We have been told by the FDA that this is the first anti-infective drug application submitted to treat patients from the intentional use of a biological agent.

This slide presents our proposed labeling highlights. The indication is anthrax post inhalational exposure prophylaxis. The recommended dose for adults is 500 milligrams given twice a day as either the tablet or oral suspension. The IV dose is 400 milligrams twice a day.

The recommended pediatric dose is ten to 15 milligrams per kilogram given twice a day in either the oral or IV forms.

Treatment with ciprofloxacin should begin

as soon as possible after exposure. Once the susceptibility of the strain has been determined, the most appropriate antibiotic should be administered to complete a total of 60 days' treatment.

These recommendations are taken from those published in the Journal of the American Medical Association by the Working Group on Civilian Biodefense and are similar to those recommended by the Centers for Disease Control.

Ciprofloxacin tablets were approved in 1987, and subsequently the IV and oral suspension products became available in 1990 and 1997, respectively.

The otic and ophthalmic formulations have been out-licensed and are not currently marketed by Bayer.

Ciprofloxacin has been proved to treat a wide variety of indications as listed here. Important to note is that the approvals for many of these indications include the severe category.

Also, many of these indications were approved subsequent to the original NDA approvals of

the formulations, and as such, ciprofloxacin's safety and efficacy has been reviewed by FDA many times over the years.

The chain of events which ultimately has led us here today actually began about ten years ago. Bayer supplied over 30 million cipro 500 milligram tablets to the U.S. government prior to and during the Gulf War. It is our understanding that these tablets were provided to the air and ground troops to be used in the event of a biological attack.

We do not know if any tablets were actually used for this purpose.

After the war, Bayer was commended for meeting all production and delivery time lines necessitated by the emergency nature of the time.

Also during this period, the Department of Defense conducted the anthrax testing in Rhesus monkeys, which we included in our submission. My presentation will not include remarks on this topic as Colonel Friedlander, who performed the testing, is here today and will present his data to you shortly.

Fast forward now to 1998. The possible

threat of a biological attack in the United States has been raised by the media and others as an issue of public awareness. During this year, the third edition of the Medical Management of Biological Casualties Handbook was issued. Prophylaxis with ciprofloxacin 500 milligrams tablets is prescribed as a preferred treatment.

In 1999, two additional publications on this topic also came to Bayer's attention. The Centers for Disease Control's morbidity and mortality weekly report published recommended treatment guidelines for the post exposure prophylaxis of anthrax. Ciprofloxacin is listed as a treatment of choice.

Later in that year, the Working Group on Civilian Biodefense published their consensus statement in JAMA with the recommendations for the public health measures to be taken following an anthrax attack. Ciprofloxacin is again listed as a preferred agent.

It is from the consensus statements from this working group that the recommended doses and

durations of ciprofloxacin therapy are based.

I would also like to mention at this time that Dr. John Bartlett from Johns Hopkins University is here with us today. He was a member of this working group and is available during the morning session until about 11:00 a.m. to answer any questions concerning their recommendations.

Also during 1999, Bayer was asked by government agencies to provide information in the development of the emergency preparedness plans being generated in the event of a bioterrorist attack. These queries, coupled with the published recommendations for ciprofloxacin use for anthrax, prompted Bayer to evaluate our responsibilities and our options to further disseminate this information in the interest of public health through appropriate product labeling.

Now, the summary basis of approvals for penicillin, doxycycline, and all other agents with any product labeling regarding anthrax or Bacillus anthracis were reviewed, but unfortunately they provided no information on the data necessary for

those approvals.

So we gathered all information available to us and submitted a briefing document to the FDA to initiate and facilitate discussion with them. A teleconference was held in which FDA advised us that the information presented with a few other requests was sufficient for their review of a supplement and encouraged us to submit this information formally.

Now, because we have proposed that an indication be granted for pediatric patients as well as adults, the committee may be interested that occurring at this same time were discussions between Bayer and the division concerning the conduct of new ciprofloxacin clinical trials in children.

Discussions started in August 1998 and culminated in May 1999 with the issuance of a letter to Bayer requesting that pediatric patients be included in well controlled clinical trials.

Enrollment in two trials is currently underway, and I'll briefly discuss these trials in a few moments.

Finally, in November 1999, Bayer received

a call from a representative of the CDC's Bioterrorism Response Group. The representative asked if Bayer was aware of the recommendations for the use of ciprofloxacin in the event of an anthrax release and was pleased when informed that we had already contacted the FDA and were preparing a submission.

And as was mentioned earlier, this submission was formally made on February 29th of this year.

Because we cannot intentionally expose human subjects to the anthrax microorganism and because inhalational anthrax is an extremely rare disease, this submission contains no human data. We rely on the animal data to be presented by Dr. Friedlander and upon what we know about ciprofloxacin.

I would like to discuss now the additional points which lead us to believe that ciprofloxacin therapy would be safe and effective for this indication. I will review certain aspects of ciprofloxacin pharmacokinetics, especially in relation to the MIC of Bacillus anthracis. I will also present additional information concerning the efficacy and

safety of our products.

The MIC range for ciprofloxacin for all tested strains of Bacillus anthracis in our submission was 0.03 to 0.06 micrograms per mL. The MIC-90 was also 0.06.

The half-life is approximately four hours, and the protein binding is approximately 30 percent. The absolute bioavailability of the oral formulations is about 70 percent.

Plasma concentrations for adults and pediatrics observed that the dosages recommended by the Working Group and in our proposed labeling are shown here. All C-max and AUC values are fairly comparable, with the one C-max value somewhat striking from the pediatric IV study.

However, the infusions in this study were completed in just 30 minutes. One hour infusion, which is the recommended duration, provides results similar to that observed in adults. The minimum concentrations observed at the end of the 12 hour dosing interval for these studies is approximately 0.2 micrograms per mL, which is still three to fourfold

higher than the MIC of the infecting organism.

It has been suggested that for optimal antibiotic effect the ratios of C-max to MIC and AUC to MIC should be at least eight to ten and 125, respectively. The C-max to MIC ratio with ciprofloxacin 500 milligram oral dosing in adults is approximately 50, and the 12 hour AUC to MIC ratio is 228.

There are later speakers who will address the pathology and pathophysiology of anthrax infection. In short though, it is believe that inhaled Bacillus anthracis spores reach the pulmonary alveolar epithelium where they are phagocytosed by pulmonary macrophages. The spores are transported to the local lymphatic system where they are thought to germinate into vegetative Bacillus anthracis.

For these reasons, it is relevant to examine the tissue penetration of ciprofloxacin in the bronchial epithelial lining fluid, alveolar macrophages, and peripheral lymph fluid. As shown here, ciprofloxacin concentrations do remain above the Bacillus anthracis MIC for the full 12-hour dosing

period.

Now that I have discussed ciprofloxacin from a pharmacokinetics perspective, I'd like to speak with you about ciprofloxacin from an efficacy and safety standpoint. It is the most widely used and studied fluoroquinolone, and Bayer has conducted over 850 ciprofloxacin trials.

There have been over 140,000 adults and 3,400 children treated in these protocols. The safety and efficacy of ciprofloxacin therapy has been well established through these clinical trials and the post marketing experience.

Now, as highlighted, there are no human anthrax data in our submission. To support the anticipated efficacy in this pulmonary indication, we have conducted a review of the U.S. ciprofloxacin trials conducted in lower respiratory tract infections which can serve as a reasonable predictor of efficacy against anthrax.

This analysis includes a review of 34 controlled studies, many of which were conducted in severe diseases. These trials employed well known and

established comparators and confirmed the efficacy of ciprofloxacin therapy in lower respiratory tract infection.

Clinical success, defined as cure plus improvement, was demonstrated for 86 percent of ciprofloxacin treated patients in this pool. Patients treated with comparator drugs had an 85 percent clinical success rate.

In addition, we have also reviewed the clinical trial safety database from all patients enrolled in ciprofloxacin protocols. We have found that there were over 1,000 patients, including 104 children, who have received ciprofloxacin for 60 days or longer. The most frequent indications for these patients are listed here.

This slide reviews for you the safety database which includes adverse event rates from ciprofloxacin in controlled patients from comparative trials, those who received cipro from 30 to 59 days, and those who received ciprofloxacin for 60 or more days.

The data show that for the comparative

trials that regardless of severity or drug relationship, 31 percent of patients reported an adverse event versus 33 percent of the patients receiving comparator drugs.

The most common adverse events reported for ciprofloxacin therapy were nausea and diarrhea. Of the over 1,000 patients who received ciprofloxacin for over 60 days, we note the 29 percent reported an adverse event and that the frequency of events was similar to those who received shorter durations of ciprofloxacin therapy.

To shift now to ciprofloxacin use in pediatrics, Bayer has data available on over 3,400 patients who have received therapy for a variety of indications, the most common of which is cystic fibrosis. For the 104 patients who received ciprofloxacin for over 60 days, there were no reported serious adverse events.

In comparative pediatric studies conducted, again, primarily in cystic fibrosis patients, the incidence of nausea, vomiting, and rash is somewhat higher for ciprofloxacin treated patients.

However, these patients reported fewer arthralgia and joint disorder complaints than those in the comparator group.

An overall review of the global clinical trials database for pediatrics is presented here. Data from all ciprofloxacin patients from clinical trials, those who received 30 to 59 days of therapy, and those who received 60 or more days of ciprofloxacin treatment are shown. The adverse event rate is quite similar for all groups regardless of treatment duration.

Now, as mentioned earlier, Bayer is presently conducting two clinical trials in pediatric patients. We designed these studies in partnership with the FDA.

The first study is a randomized, double blind comparative trial in patients with complicated urinary tract infections.

The second trial is a long term, post dosing observational study in children treated with ciprofloxacin for any indication.

Both trials are currently in the

enrollment phase.

Even though Bayer has never promoted ciprofloxacin to pediatricians, we know that it is used to some degree off label in this community, despite the well known quinolone class labeling warnings. Data show that about 14,000 ciprofloxacin prescriptions are written annually in the U.S. for patients under the age of ten. This represents about 0.1 percent of all U.S. ciprofloxacin scrips.

An additional 28,000 prescriptions are written for patients between the ages of ten and 14 years old, and 140,000 scrips or about one percent of the U.S. total use are for patients between the ages of 15 and 17.

All told, we estimate that approximately four and a half million courses of ciprofloxacin therapy have been administered to pediatric patients worldwide since approval.

And I'll now discuss briefly the post marketing safety experience for ciprofloxacin. Ciprofloxacin has been available for prescription use for 13 years. My next overhead will present raw

numbers for adverse events reported to Bayer from anywhere in the world, regardless of relationship to ciprofloxacin therapy or any concomitant medications.

These numbers should be viewed in the context of the over 250 million prescriptions dispensed in over five billion individual doses taken worldwide. About half of this exposure has occurred in the United States.

The ten events reported most frequently for all formulations over 13 years are reported here. As you can see, rash is the most commonly reported event, more than twice that of the next event, tendon disorder. For any of these listed events, the frequency of reporting is less than five per one million treatment courses.

Now, as one reviews this data when the age of the patient is known, you can see that the distribution of adverse events is generally similar regardless of the patient's age. Remember that the estimated denominator for the under age 18 group is four and a half million treated patients.

From this database we can say that there

does not appear to be a signal for excess joint related adverse events in pediatric patients treated with ciprofloxacin.

Finally, a review of the most frequently reported serious events is shown here. The frequency of serious reports is low for all events, and these events are consistent with the currently approved ciprofloxacin product labeling.

So now to summarize, ciprofloxacin has been available for 13 years, and over 250 million treatment courses have been completed by patients throughout the world.

The pharmacokinetic data shared today supports expected efficacy in the indication of anthrax post inhalation exposure prophylaxis.

Our extensive clinical trials and post marketing experience have shown that ciprofloxacin therapy is safe and effective, including treatment durations up to and exceeding 60 days.

Bayer Corporation, at the encouragement of government agencies, has submitted this labeling application to respond to a public health need. And

given the seriousness of the indication and the extraordinary hazards that an anthrax release would entail, the risk-benefit ratio supports ciprofloxacin therapy for this indication.

This concludes my prepared remarks. I and my Bayer colleagues would be happy to answer any questions on ciprofloxacin.

CHAIRMAN RELLER: Are there any questions on the material that Andrew Verderame presented? David.

DR. SOPER: You have a pretty extensive experience now with pediatric exposure. What about pregnant women? Clearly there probably has been opportunity over the years for pregnant women to have been administered cipro. Do you have similar sorts of outcome data on them?

MR. VERDERAME: I would address that to Dr. Felix Monteagudo, who is our Vice President for Drug Safety.

DR. MONTEAGUDO: Felix Monteagudo. I'm in the Drug Safety Group from Bayer.

We really do not have any well controlled

studies looking at the use of ciprofloxacin in pregnancy. However, we have had cases where women have taken ciprofloxacin during the pregnancy at various stages of pregnancy, and looking through the data, we have no suggestion of any teratogenic potential arising out of that.

Now, looking at that, clearly, in terms of the indication with anthrax, one would have to look at the benefit-risk ratio that would ensue out of that and possibly the consideration of the consensus document that came out of the JAMA article last year.

CHAIRMAN RELLER: Dr. Archer.

DR. ARCHER: What about data on QT prolongation, particularly in children, and is there any indication that if it were even a small risk that prolonged exposure for like 60 days would increase that risk?

DR. MONTEAGUDO: We have, as you heard from Mr. Verderame's presentation, we have about 250 million prescriptions for this product over about 13 years, and if we were to look at the database that we have, we have only had four cases of Torsade de Point

with ciprofloxacin over this entire period of time, and this would lead us to believe this very small incidence in the background of such wide exposure would lead us to believe that this is not a signal for our particular product.

With the specifics regard that you say about children and prolonged duration of therapy, we would not have any additional data to that regard.

CHAIRMAN RELLER: Dr. Chesney.

DR. CHESNEY: Two questions. The first one: have you looked at the cipro MICs for organisms that are resistant to other drugs and are they the same?

The second question is: has there ever been a cipro resistant strain identified?

And the third one: how long does it take for the spore to become the vegetative state in vitro?

MR. VERDERAME: If this is okay with you, there are other speakers who are going to cover all of those topics. We at Bayer are ciprofloxacin experts. We're not necessarily anthrax experts, and I'd rather that be experts answer those questions.

CHAIRMAN RELLER: Thanks.

Yes, Dr. Deitchman.

DR. DEITCHMAN: I'm cursed with a name that everyone stumbles over.

MR. VERDERAME: Me, too.

(Laughter.)

DR. DEITCHMAN: I appreciate your showing the information from the adverse effects database. Recognizing that we don't know much about under reporting in this data, I'm not sure how fair it is to estimate rates, but in reading some of the articles, such as the one by Segev, it is my impression that that database encompasses patients that received a variety of dosages, including some as low as 250 milligrams BID.

Since the indication that we're looking at here would be 500 milligrams BID for most adults, what does the data look like if you break it out by the higher dose experience?

MR. VERDERAME: It's all very similar.

DR. DEITCHMAN: And secondly, how quickly do you achieve MICs following the initial dose? And

should there be consideration of a larger loading dose for the initial dosing?

MR. VERDERAME: I'll address that to Dr. John Lettierie.

DR. LETTIERIE: John Lettierie from Clinical Pharmacology Group at Bayer.

Cipro is very rapidly absorbed. So you reach a concentration of 0.06 within a half hour or so, 15 minutes to a half hour.

DR. BROOK: Itzhak Brook from AFRRI.

I have a question about the use of ciprofloxacin in endemic anthrax, which is very prevalent in Thailand, Turkey, Africa, and Russia. Do you have any information about the use of those, of ciprofloxacin, in those countries of course not for inhalation mostly, but for cutaneous or gastrointestinal?

CHAIRMAN RELLER: I should like to ask that we come back to this question later when we've heard the presentations about anthrax, its epidemiology and pathophysiology.

Are there any other questions regarding

the safety, pharmacodynamics, pharmacokinetics of ciprofloxacin?

Yes.

DR. ARCHER: Just a question about drug accumulation with cipro, once again, over long periods of time, like 60 days. Is there any evidence that it actually accumulates in lung tissue, for instance, which might be an advantage if you're trying to prevent spore germination with time?

MR. VERDERAME: Again, Dr. John Lettierie.

DR. LETTIERIE: I'm not aware of any data on lung accumulation specifically. It does not accumulate in plasma to any significant degree.

DR. ARCHER: How about other tissues, liver?

DR. LETTIERIE: No, I'm not aware. There's no prolonged -- there's no accumulation.

DR. ARCHER: No accumulation.

CHAIRMAN RELLER: Yes, Dr. Deitchman.

DR. DEITCHMAN: I have a practical question. I'm sort of concerned as some of my CDC colleagues who manage the stockpile. What's been the

experience, if any, with the use of diluents if you needed to crush tablets to prepare a suspension for oral use? This might be a practical issue in a community prophylaxis situation.

CHAIRMAN RELLER: I'd ask Dr. Posner to answer that, please.

DR. POSNER: I actually don't have the answer to that question, but what I could say is that ciprofloxacin, I believe, is one of the few antibiotics that we do have an oral suspension available. So it actually is available in an approved marketed oral, non-tablet form. But I don't know the answer specifically to the question about crushing tablets and how it can be diluted.

MR. VERDERAME: And, Dr. Lettierie, can you add something?

DR. LETTIERIE: There is at least one published report of giving crushed tablets to children, and they did attain adequate plasma concentration.

CHAIRMAN RELLER: I'd like to remind everyone that after lunch there will be an open public

hearing session, and at this time I should like to thank Mr. Verderame for his succinct, but complete presentation.

MR. VERDERAME: Thank you.

CHAIRMAN RELLER: We now will move into the discussion of anthrax, its epidemiology, pathophysiology, and clinical manifestations.

Dr. Martin Hugh-Jones will be our first invited speaker on these topics.

DR. HUGH-JONES: Thank you, Mr. Chairman.

It's a privilege to be here. Good morning, ladies and gentlemen.

The first half of my talk will be the sort of basic background of what anthrax is, and then the second half will be aspects of the Sverdlovsk epidemic, as we discovered when David Walker and I were there and in retrospect.

Next slide, please.

This is, as I tell my graduate students, Bacillus anthracis is a Swiss army knife as far as an organism goes. It's very straightforward. It's agile on its feet, and what it does it does very well.

It's been known for a long, long time, clinically described first in the 1830s. It's a Gram-positive organism. You can see from this it has a nice capsule around it.

The vaccines were first developed in the 1870s by Greenfield in London and Toussaint in Paris. Pasteur then had a public demonstration Toussaint's vaccine, not his own, I may add, in '82, and it was very successful, and there are a whole series of livestock and human vaccines.

In general, the livestock vaccines are live vaccines and the human ones tend to be dead, except the Russians, in fact, use a live vaccine as they do for brucellosis, as well.

Next please.

It's derived from the Bacillus soil organism. It's Gram positive, and it survives by killing. It has no reason to hang around in the body. Its whole purpose is to kill and do it as quickly as possible, and then form spores, which are capsulated and have a very good survival for decades of years sometimes.

I've got a very large collection in some of our material that's come out of desk drawers, say, in Maputo where it was first isolated from a partridge in '42, and it's still growing very nicely. Thank you.

(Laughter.)

DR. HUGH-JONES: It's a junior relative of the B. cereus/thuringensis group, and recent molecular biology puts it into a group here of a subset, as you might say, which are pathogenic, which cause disease, but it's just a junior member of a larger series, and its pathogenicity depends on its two plasmids, pX01 and pX02.

Next slide.

We've been involved with a study with Northern Arizona University in Los Alamos in the molecular strain definition, and what we've been using is variable number tandem repeats. We have presently -- we have been using eight, but we've now, in fact, expanded it to 36, and these are just sequences which repeat, and without getting too complicated into this and so all of you can understand the level that I

understand it, it's a bar code. Just think of it as a bar code.

And we can read these. At first it would take us six months. Now it takes is less than six hours to do that work, and it does a very nice job, and it works extremely well.

Next, please.

A few years ago all anthrax was anthrax. You couldn't tell them apart. There were some which grew better on this culture than that, killed faster than this, but really there was not much difference.

But thanks to being able to take it apart, and the team I'm a member of, we're way in advance of everybody else, I may add, we've been able to work out some very interesting things in it, and I'm not going to bore you with, as my daughter says, once I get started on anthrax, I don't stop, and I know I've only got 30 minutes, and so I'll be very brief.

(Laughter.)

DR. HUGH-JONES: But basically the B strains at the bottom are probably the very first pathogens out of this group and probably existed from

Cape Town to the Horn in wildlife, and then with domestication of livestock, it managed to get into these cattle and sheep, and through changing its habits, to go around the world.

What basically it does, it takes advantage of a dormant infection so that an animal can be taken on a caravan or a ship journey and gets to somewhere else before it dies 12 months later.

Also, the A strains appear to be more flexible in the environment in which they live. The B strains need high pH, high calcium levels. The A levels are much more flexible, and there are different forms in different parts of the world, but I'm not here to tell you about that.

But it is all around the world and a fascinating bug. The way it works is pX01 produces three toxic factors. There's the protective antigen, called that because this is the basis of the human vaccine. If you use this as an antigen, it protects. Therefore, it's known as a protective antigen.

This binds to cell surface receptors, and there are about 200, 300 per cell. So it has no

difficulty catching at that end.

And then furin comes along and pops the top off rather like you pop the top off a beer can, revealing an adhesion surface at the top.

These then form heptamers, i.e., seven PA groups together clustered, and those open surfaces compete for edema factor or lethal factor that the adhesion surface is where it stick on.

This structure is then drawn into the body of the cell by endocytosis, and then the heptamer structure acts as a portal into the cell itself where the edema toxin or the lethal toxin is produced.

The edema toxin does just that. It produces edema, leakage. You get a lot of fluid like in the lungs. The lungs fill up with fluid. Your kidneys; even in your brain to a certain extent. It allows a substrate for further multiplication.

The lethal factor, through a complicated process not totally understood yet, an oxidation causes the release of large amounts of cysteine and the induction of shock, and when we were in Nekatminsberg (phonetic) talking to the Director of

Hospital No. 20 --

PARTICIPANT: Forty.

DR. HUGH-JONES: Forty -- no, 40 was -- well, whichever, the hospital which dealt with the initial triage, and this woman had been there for many years, and these people came in from the local community with high temperatures, substernal pain, anxiety, and she'd be taking their temperatures, taking their pulse, talking to them, and she said, "They would die in mid-sentence." And that is absolutely characteristic of this disease.

One moment you're alive. The next you are dead, and this is as true of animals as it is of human beings.

Next, please.

As I said, for us we regard this as a veterinary disease. If we've failed to control it, it then gets into the human population, and then we see a whole lot of other things.

But very quickly, basically what we see is a farmer calls us up and says an animal that was fine yesterday is dead today. Why? Sometimes you can go

out and find an animal, and I have friends who have gone onto a farm and literally as they came in the farm they'd see animals fall over dead in front of them.

I've seen pictures of hippopotami dead on their feet. They died so fast they didn't fall over.

Next, please.

The acute form, yes, you find animals sick. You've got to realize that livestock normally get it from eating. We don't see the pneumonic form.

Dogs and pigs tend to be more resistant, but in this form, they die normally within about 36 hours. Horses take a big longer.

Next, please.

What I call hypoacute -- I'm afraid I had to make a work up because it's not chronic -- but you get internal lesions in dogs and pigs, and they rare die of it, to be quite frank, but they can get very sick.

Human beings at this point, you see cutaneous lesions. A lesion once seen you can never forget it. It's usually around a cut or insect bit,

frequently on the hands. It's inflamed. There will be small vesicles forming, and characteristically there is no pain involved in it whatsoever, and I'll show you some pictures of what it looks like.

Next, please.

This is a case of a man in London -- no, Liverpool, and this is absolutely characteristic. It's about the size of a quarter, and there are little vesicles around the side. Once the ulcer is form, the better the ulcer is black, which is why the French call it "charbon." It's otherwise known as Siberian ulcer, and in fact, if you ever see anybody with a circular scar on the inside of their wrist, you'll know that they've had anthrax. It's absolutely pathognomonic.

Next, please.

This was a gentleman in England whose son worked in a bone meal plant, and he lent his father, who was a postman, his scarf, and he developed lesions all around his neck. It was touch and go whether he would live, but he did, in fact.

But without treatment, the skin lesion

carries a ten percent fatality risk. With treatment, as long as you get it early, it resolves almost within minutes.

I've had a friend who was treated, and it took 120 minutes for the signs to disappear, although it took much longer for the lesion to resolve.

It responds extremely well to early treatment. Late, you cannot save people; you cannot save animals.

Next, please.

Workers in plants processing woolen hair. This is a slide that Phil Brackmann gave me from his work in New Jersey.

Next, please.

And most frequently on the hands, arms, not seen much elsewhere. People carrying stuff on their shoulders, you'll get lesions up there.

Also, it's in relation to insect bites. If, say, a horsefly has been feeding on an animal that's moribund, they can transfer it on their mouth parts or you get somebody who's been butchering an animal that died, and they then scratch their face,

and you may sometimes get a lesion around the eye. It looks terrible, but with treatment, it resolves extremely nicely.

Thank you. Next.

Pulmonary form in human beings, normally occupational. It's not been seen in this country for many years. The onset is sort of like a bout of flu. Then so things calm down for a bit, and then somewhere around the second or third or fourth day usually, you'll develop cyanosis, dyspnea, rapid heart beat, and you get all the lesions which Dr. Friedlander and Dr. Walker will describe to you. So I won't get into that.

But treated at the early influenza stage, feeling like -- yeah, they respond beautifully. Once it's got to the systemic infection stage, there is nothing you can do for them. They die.

Next, please.

In the old days, this is one of Phil's photographs. This young man feeding wool into the hopper for work, and there would be tremendous amounts of spores in the air, high risk. This is why it was

known as Bradford disease, from Bradford in England, which were wool mills.

Next.

Gastroenteric. It's seen -- at the moment, it's largely seen in Central Asia and in Africa. It comes from eating an animal that's died of or with anthrax, nausea, malaise, abdominal pain, bloody vomiting, diarrhea. Carries about a 30 to 40 percent case fatality risk.

Next, please.

This is an animal that I photographed being butchered outside a village in Somalia. In Africa, people may eat meat only once in six months, once a year. So they'll risk it.

The better the cook, the more likely you are to die of gastroenteric anthrax. The worse the cook, in other words, the more the meat is cooked, the safer it is for everybody, and we worked it out, Peter Turnbull and I. It's basically about one in 67 anthrax livestock cooked which results in gastroenteric cases. It's at that level of risk.

And for them, if you've got malnutrition,

it's worth taking the risk.

You'll also find cutaneous lesions in those people who are butchering the affected animal.

Next please.

This is the most recent map we have for the disease. The areas in red are where it is hyperendemic. Brown is where it is endemic. Yellow is sporadic. There are a few countries, areas where it is free, like in Malaysia, Taiwan, Norway, Finland, the Guyanas, Egypt apparently.

But in Europe it is, in fact, disappearing, and I think in a few more years we can start coloring in Europe as probably free.

In the United States it's endemic in Southwest Texas and in the Dakotas. In Canada, Alberta, Saskatchewan, and the southern part of the Northwest Territories, they get cases regularly up there each year, but the rest of the United States it's very infrequent to say the least.

I could take you to places where it might occur, but it has, in fact, disappeared beautifully in North America, and we'll just be left with these few

areas where there are problems.

Next.

Okay. Sverdlovsk. In 1979, there was a -- from what we can make out, they were grinding some weapon fill, and the outgoing team found that the filters were clogged, and they left a note for the incoming team that they'd taken the filters out, and they'd better replace them with clean ones before they started up.

Again, they didn't read the note, and they started grinding this weapon fill.

The work we did on some tissue from these people indicated that minimum of at least five strains were involved. In fact, we've now been informed that six strains were involved in the Russian weapon mixture.

The number of people who died is probably in the region of 90. The reason we say that is that some early cases were missed. The military cases were definitely not revealed. The Russians said they had 64 cases because that was the number of pensions they were willing to pay, and there was a certain amount of

musical chairs involved. If somebody was confirmed not to have had anthrax, they were taken off, and somebody came in and got a pension.

But we reckon somewhere around 90 people died. How many people became ill is any number you care to think of between 120 and 400. They initially reported as due to contaminated meat, which they insisted on for many years.

The local team diagnosed the first case as anthrax on the 10th of April by Faina Abramova and David will tell you about the story with that.

The local team did a very good job, but they missed the original cases. I've forgotten. The first man to be diagnosed was what, number 12 or number 20-something, Markhov. He wasn't the first one. That's for sure.

And the level -- there was a constant wind from the northwest at the time of the release, and calculations that I've been involved in, based on exact time of exposure of the people concerned, indicated some half a kilo of spores were released.

The exposure from people I measured who

were living inside the plume and working outside or working outside and -- sorry -- living outside and working inside the plume or having to cross the plume in some way or another indicated that release was between 6:15 to 7:45. So it was quite a prolonged period of exposure. It was not one puff that went by and you had to be standing outside and take a deep breath. It was out for quite a while, and this is why there was so much.

And it killed animals where you see the letters of the alphabet, and the furthest out was some sheep who certainly would have died from the aerosol 53 kilometers out. The furthest human case was 4.3 kilometers.

Next, please.

Going over the records, which we did have, a very interesting thing comes out of this. Now, there are all sorts of problems with the database, but just we'll take what we've got, is that normally you would have a normal Gaussian distribution for onsets, but it starts collapsing on the 15th of April, and then we just get sporadic cases.

Next, please.

The deaths stopped on the 16th, after which there were sporadic cases.

If we overlap the two -- next, please -- we get this pattern, and I went back over my notes, and I checked with Alex Shelakov, who was with us, and we both were told that, yes, people were taking oral antibiotics.

The normal routine was when the medical team when to the household where a case was, is the family was prescribed oral antibiotics, and Olga Yampolskaya, who was with us, who had been on the original Moscow team that came down on or around the 11th or 12th of April, she said, yes, they were on oral antibiotics, but there was a community-wide prescription on the 15th of April.

I then checked with my colleague in Moscow, Benjamin Cherkosskij, who is a long time colleague of General Burgasov and had been involved with him in the vaccine trials in the Ukraine in the '70s when they vaccinated three million people. He said, yes, at the time they had a laid down procedure

for five days of antibiotics, which confirmed what Olga had said, and the drugs were ampicillin, tetramycin (phonetic).

I'm afraid I prepared a special slide for this, but it seems that academia has better computers than FDA, and they can't read my version of Power Point, but I'll make sure you get a copy of exactly what these drugs were and their protocols, but it was once or twice a day. They were penicillin derivatives or tetramycin.

Next, please.

Something else you've got to keep in mind is that we had these addresses of these people. The compound wall was 900 meters from the source, and it was three stories high, and there was a constant wind for quite a number of hours, and you can see that the residences where people were living who got sick and died are not evenly distributed.

Next, please.

If you take where people were working, similarly that bit about 25 to 27 meters -- 2,700 meters is where the ceramics factory was, which is

where the majority of people died.

Next.

And if you put the two together, it comes out that we have a periodicity of 900 meters. In other words, we have a leewave (phonetic) formed by the wall of the compound. A leewave is just one that does literally this. If you're a glider, you'll know exactly what I mean.

And so as it hit, if you were in part of the town where it hit, that's where you were at risk. Otherwise you weren't.

We had confirmation on this one in talking to the locals where they said, "Yes, nobody died in our street, but they did in the street over." So it's different.

Next, please.

I'm involved in a very large study, as I said, in looking at the molecular biology of this bargain, the molecular epidemiology. It is a large collection, and we recently took receipt of the Italian national archive and, along with other Italian isolates we have, it comes to about 53. They're

essentially from Rome and Sardinia south.

And in preparing the DNA for this, we now routinely make archival preparations, and in the first time my graduate students responsible for this noticed that we had three that were gammaphage resistant.

Normally, in the screening of this organism in culture, you look for organisms which are penicillin sensitive and are gammaphage sensitive. Now, these were penicillin sensitive, but they were gammaphage resistant, something we hadn't seen before. It's in the literature. We were expecting it to happen, and so she said, "Well, let's try out a whole range of antibiotics," which is something we hadn't done before. We had ordered it, but we had never done whole range.

And lo and behold, the Sample A, 850 up at the top there, was resistant to ciprofloxacin. This was a goat that died in '96 in Sicily, and that's essentially all we know, but as friends of mine say, if you give antibiotics to sheep and goats, you've doubled their value. So they tend not to be treated all that much.

Sheep and goats are, in general, I'm sorry to say as a veterinarian, are under treated, under cared for by their owners as opposed to cattle who sometimes get far too much treatment.

Kudu '93 at the bottom is our standard strain out of the Kruger National Park. It is the commonest strain in that park, and so that we put up against it.

And so what I'm trying to point out to you is this. This is the first time we tried out any other drugs than penicillin, and in the first attempt we stumbled on this ciprofloxacin resistant isolate. We have no idea of how common it is at all. I had no idea how commonly ciprofloxacin is given to livestock. In my experience not at all, but people from Bayer can tell you what their veterinary sales of this drug are.

Thank you.

CHAIRMAN RELLER: Are there questions from the panel for Dr. Hugh-Jones? Yes, Dr. Chesney.

DR. CHESNEY: We were wondering what the numbers represented. Are those number of strains or MICs in the table, the last table?

DR. HUGH-JONES: The last table? No, the numbers was the distance from the edge of the paper disk to where the lawn started growing again.

CHAIRMAN RELLER: Dr. Hugh-Jones, has the strain that you found resistant by disk testing --

DR. HUGH-JONES: Yes.

CHAIRMAN RELLER: -- has that been confirmed at a reference laboratory by dilutional MIC, agar dilution, other measures?

DR. HUGH-JONES: We only discovered it a few weeks ago. It hasn't been passed on for MIC testing.

CHAIRMAN RELLER: I'd like to encourage that. I mean, there are a lot of pitfalls with the disk testing. In concert, I know that recently in the National Committee for Clinical Laboratory Standards has published the susceptibility guidelines for veterinary medicine that are cross-linked with those for human.

But very important in that as a general comment is in newly recognized phenomena of potential public veterinary health interest and importance of

reference laboratories of confirming the mechanism and degree of resistance. So that would be very helpful, I think.

DR. HUGH-JONES: I would agree with you. We hadn't expected it, and suddenly it was there, and now we've got to think about, okay, if we set up MIC testing, how much and who pays.

CHAIRMAN RELLER: Right. I'm sure the CDC would be delighted to work with this, Dr. Tenover and colleagues.

Other questions from the panel for Dr. Hugh-Jones?

And, again, all of the consultants, speakers will be available later at the time of the public discussions for further -- yes, Dr. Friedlander.

DR. FRIEDLANDER: Yes. Just a comment about this. I noticed that several of the strains were resistant to vancomycin.

DR. HUGH-JONES: And variably so, and I don't understand it.

DR. FRIEDLANDER: I mean that would be, at

least in our experience, and I believe in the literature as well, almost all the strains as sensitive to vancomycin.

DR. HUGH-JONES: Yeah.

DR. FRIEDLANDER: So further reason to look at these, I think, carefully.

DR. HUGH-JONES: Yes.

DR. FRIEDLANDER: It may be something different about these strains. Have they been tested for virulence in animals? Do you know?

DR. HUGH-JONES: These are all from clinical field cases.

DR. FRIEDLANDER: I understand that, but have they been reconfirmed in terms of their virulence for mouse?

DR. HUGH-JONES: I have only a limited amount of research money, Colonel Friedlander. I'm not funded by the DOD for such experiments, which are not inexpensive, I may add.

COL. TAKAFUJI: Colonel Takafuji.

Could you make some comments about the mode of resistance, chromosomal mediated, plasmid

mediated resistance and the implications thereof here?

DR. HUGH-JONES: Well, the little I know about the ciprofloxacin resistance in anthracis is frequency is about five times ten to the minus nine. It's chromosomally based. It has nothing to do with the plasmids.

There are further genes involved if you then challenge the organism against higher and higher doses of ciprofloxacin. These have been defined for modest increases of ciprofloxacin, but not for the highest levels.

There's been very little work done really in the genetics of it.

CHAIRMAN RELLER: Dr. Archer.

DR. ARCHER: Do we know that if spores exposed to an antibiotic for a period of time can develop resistance before they germinate into vegetative cells?

DR. HUGH-JONES: No reason why they should. The spores are just dormant. They're susceptible to disinfectants. That's how you get rid of them.

CHAIRMAN RELLER: Dr. Friedlander.

DR. FRIEDLANDER: If I might address that, I don't think there's any evidence that anything works on the spore per se, but the antibiotics do work very quickly in the early germination of the organism. I mean, if you look at the organism, you basically can prevent the development to the Bacillus. It works very quickly, as soon as uptake starts probably.

DR. HUGH-JONES: I mean, at field decontamination you can get a hell of a long way just with a hose and water, believe it or not, but once it sporulates, it's much more resistant.

DR. ARCHER: So with a relatively low inoculum of persistent spores, for instance, there's no reason to think that chromosomally mutant vegetative cells would develop during the course of prophylaxis if they're not resistant to begin with.

DR. FRIEDLANDER: Right. There should not be a significant multiplication going on.

DR. HUGH-JONES: The evidence that we've seen so far is that when you expose it to antibiotics, you're just finding those one or two on a plate which

are resistant already. They had it before they started.

CHAIRMAN RELLER: Yes, Dr. Christie.

DR. CHRISTIE-SAMUELS: In the outbreak, do you have any more recent information as to why there were no children involved?

DR. HUGH-JONES: The only reason I can think is that the exposure dose was really rather small, and what we were seeing was people with industrial, occupational damage with ongoing like welder's lung, poor clearances.

The youngest person that died was 26, but she every morning went and took a shower at the ceramics factory, and that was a hit point. Where her day care center was was not. So I think she got it by walking into it.

There was a young teenager who was reported ill, treated, and recovered, but that's all we know. There were a couple of teenagers, but none died, and it was a puzzlement with us as to why we had so few, well, virtually nobody under the age of 40.

There was a question from the commandant

over here.

PARTICIPANT: That was my question.

DR. HUGH-JONES: Oh.

CHAIRMAN RELLER: Thank you again very much.

And now Dr. David Walker will present the human pathology of inhalational disease with Bacillus anthracis.

DR. WALKER: The purpose of my presentation is to show you what the inhalational anthrax does to humans, and the first part here is merely an excerpt from Alibek's book in which he gives a second hand version of what the exposure was, which Martin Hugh-Jones has just told you about.

And so I'm going to move directly to presenting to you the quantitative pathology of inhalational anthrax.

The most important person here is Dr. Abramova, who was a senior pathologist in Sverdlovsk. She made the first diagnosis that was made. She is a pathologist, and she recognized the hemorrhagic meningitis as being likely due to anthrax, assimilated

anthrax in the first autopsy that she did, and she confirmed that by making smears of the brain, seeing Gram positive bacilli, and cultivating the amount with cultures available the next day.

And they went on to do autopsies on all of the patients that came through during that period of time.

A resident in pathology, her protegee at that time, Dr. Grinberg, participated in all of those autopsies. He subsequently came to my department, brought the material to the United States, and we have studied it further, and what I will be showing you are the results of those, some of the results of some of those studies.

The pathologist at the University of Texas Medical Branch in Galveston, who is responsible for working with Dr. Grinberg, who produced together the data here, is Dr. Jerome Smith.

I already mentioned to you Olga Yampolskaya was a protegee; an anthrax expert who was sent from Moscow, Dr. Nakiferov. She worked with patients in the intensive care unit. She went down to

the morgue and saw the results of the autopsies. She went back and served as a member of our team and translated with me as I worked with the Russians in reviewing the material there.

She also came to the United States during the period that Dr. Grinberg was in my department, and communications help to make the progress in that report occur during that time.

This is Dr. Yampolskaya, Dr. Grinberg, and Dr. Abramova. And there we are at work, and the amazing thing is that although records were -- can we lower the lights a bit here at least in the front of the room? -- the amazing thing is although the KGB came in and took all records, everything that was written, the primary material was maintained, and so the picture here shows us in the morgue actually during my visit there.

And you can see that she actually has saved the organs, and they saved all of the slides, and although written words were destroyed, the primary material was still available.

And this shows some of the brains with

hemorrhagic meningitis and the hematogenously disseminated lesions in the gastrointestinal tract, which I'll describe to you and show to you.

And this is an example of what she saw in the first case, and there were many cases that had hemorrhagic meningitis, and this is one of them. So we see the skull cap opened up, and she recognized that. It has a name. It's called the cardinal's cap because of the red color, and it's really a subarachnoid hemorrhage of hematogenous dissemination to the brain, and this is an important part of the pathology in about half of the cases.

Anybody that knows how to work this better than I am, I'll take some lessons.

The key pathology is after the spread of the spores to the thoracic lymph nodes, they germinate, proliferate, secret the toxins, and you get a lot of local damage right there in that area.

So here we see the lungs with the trachea opened up and the bronchi, and all of this very dark material are lymph nodes that have got hemorrhagic necrosis extending out into the mediastinum so that

there's actually hemorrhagic mediastinitis, and this is characteristic of the inhalational form of anthrax.

Next.

And it was seen in all of the cases.

Next.

Just to reiterate, here we have opened posteriorally the esophagus, and you can see in the mediastinum the severe hemorrhagic mediastinitis.

Next.

I was very impressed that they had saved this, and this is sitting there in the museum of the medical school with the huge hemorrhagic lymph nodes, and you'll notice that the lungs themselves in this particular case don't show very much pathology at all, but there is severe enlargement, hemorrhage, and necrosis of the tracheal-bronchial lymph nodes.

Next.

I realize that I'm overdoing this, but I'm doing this on purpose just to show you this is consistent, and it's present in every case, and it's really the thing that's most important to prevent.

I think most of the things that lead to

patients' death occur in the chest, and this is what the major lesion is. Again, trachea opened up, massive hemorrhagic necrosis of the lymph nodes.

Next slide.

Now, the histology shows a lymph node here with hemorrhage in it and spreading out around into the mediastinal fat.

Next.

Histologically we can see virtual replacement of the lymph node here with hemorrhage, and hemorrhage also extending into the mediastinal fat.

Next.

So hemorrhage is a very important component of what's killing the patient. A lot of sophisticated work, and we're going to hear a lot of correlations from Dr. Friedlander who is really the expert on this subject of how the organism does this, but at the level that I'm looking at it with you, a lot of it is truly mechanical.

Yes, the organisms are there. You can see the Gram stain here. This is a lymph node, the

marginal science in which you can see the Gram positive bacilli that have spread and grown in that location and certainly must be producing the toxin.

Next slide.

And the hemorrhage here has been looked at and classified by Dr. Jerome Smith into two categories: a high pressure hemorrhage, which really distorts the surrounding tissue and compresses the structures.

Next slide.

And in these areas of mediastinum where the hemorrhage is occurring, one also finds the Gram positive bacilli.

Next slide.

So the effects clearly are coming from some damage to blood vessels.

The other effect is the effect of edema, edema toxin, a combination of protective antigen plus legal factor, very, very apparent.

And here we see gelatinous edema. This is the rib cage opened up, and we see the lungs here, but the mediastinum is massively swollen by edema, and

that's a very gelatinous edema that -- next slide -- shows histologically to be very fibrin rich. So there's a lot of fluid in the interstices between exudates of fibrinogen that had polymerized to form fibrin. So it's a very gelatinous material.

And this also forms the space occupying lesion in the thoracic cavity.

Yes, the next slide.

So in the original publication based upon looking at the slides and the material with the Russians, their slides, their microscopes, and under the conditions that we had, we had 42 cases, and the 42 cases that we felt were anthrax, and this was published in proceedings of the National Academy of Science article, and we showed that many of them were confirmed by culture or confirmed by identification of the organisms histologically.

Next slide.

Subsequently one of the cases that we've taken now, Case No. 24. Case No. 24 was a lady who received eight days of antibiotics. So we didn't find any organisms, and she really had recovered from her

disease, and she died of complications.

Her original disease very well could have been anthrax, but we couldn't prove it, and so that case has been removed. So now there are only 41 autopsied cases.

So what are the characteristics of these patients? One of them was a man of unknown identity who was found dead. It was a forensic case. We don't know how old he was. We don't know who he was, and so he's -- n is only 40 for those that we know the age.

This is an older age population, ranged from 25 to 71, with the mean around 46.

The gender was predominantly male. A lot of these males were working in a particular factory, a ceramics factory on night shift, and that was a work unit that probably was predominantly male and probably -- it may explain in part at least the gender predominance.

Twenty-two of the patients were known to have received antibiotic therapy after admission to the hospital for a mean of a little over 16 days, but you can see there's quite a bit of variation there.

The incubation period could be calculated for 30 patients and was a mean of 16 days. So that also is a long time. If you take the overall interval from exposure to death, it's got a very long range of six to 69 days. It's on the average about 20 days from the exposure time to death, but a wide range indicating that some of these organisms must remain as spores and germinate late, but the latest one was the germination period plus the incubation period, plus the disease period only added up to 69 days in this series.

The duration of illness is less than four days on the average, 3.85. Patients came to the hospital, and they didn't survive long. The average survival was just under a day.

Post mortem interval really did not correlate with any of the factors and probably indicates that autolysis is not an important factor in the analysis of the material.

Two observations that I haven't read about and need to examine the literature yet again that was impressive to us was the fact that there was a

vascular damage, vasculitis and capillaritis, and these were identified in a high proportion of patients and believe they must be present in all of the patients.

And we believe that this is why the hemorrhages occur, that there's damage to blood vessels. It's not a very sophisticated idea, but I think that this is a very important component of the pathology of systemic anthrax.

Next slide, please.

And this is an example of that vasculitis. Here we see a blood vessel with inflammation in the wall, fibrin rich edema adjacent to it.

Next slide, please.

You see a very good example of necrosis of the blood vessel wall certainly weakened because of all the cells that are making up the media of this small vessel or necrotic.

Next.

And here we see an example of an aneurism, a blood vessel here with a weakening of the wall and an out-pouching.

Next slide.

Dr. Smith and Grinberg analyzed these materials with some semi-quantitative data in which they tried to quantify the number of organisms as they observed them histologically. If you'll notice that two-plus is somewhere between one and ten organisms per 25 objective field, to sort of see what a mid-range is.

Next slide.

They also did a semi-quantitative tissue concentration of inflammatory cells, and there you can see that in the range of one would be up to ten cells per 25X objective field. So you're going to get some pretty high numbers before you really get into intense inflammation.

Next.

Criteria for quantification of other parameters, and as is always true of pathology, it has the jeopardy of being subjective and not reproducible, but I believe that these actually are valid.

If you will notice that two, which they find as moderate and being present and significant;

three marked prominent; and then down at one is really clearly present. So there's no question that it is there, but they don't believe that it was significant.

So they have put into their analysis, our analysis, a judgment factor where between one and two one shifts from it being there to it being there and being important.

Next.

So as we look in the mediastinum and the peribronchial soft tissue in inhalational anthrax where most of the action really is, we find that there are, you know, 1.4 bacillus burden. So we're finding only about four organisms per 25X field, and then we found organisms in 54 percent of the cases.

You can see that the fibrin rich edema is more than two. So we're getting into a range of lesion in this location, and it's present in almost all of the tissues that were examined. So it is clearly a very important lesion in that location, as are the hemorrhages, both the permeating hemorrhages, the low pressure, and the high pressure hemorrhage.

You will notice that although you can find

neutrophils among the nuclear cells, that the quantities are very low, and that those are -- there's not much inflammation occurring in response to this rapidly progressive infection.

An important lesion is lymphangitis. All of this process of increasing fluid exudation, transudation, and blockage of the lymphatics by all of the hemorrhage definitely is contributing to the accumulation of fluid in the thorax.

Next.

So there is pneumonia, and I would like to try to clarify what I mean by pneumonia. When a pathologist says pneumonia, he really means just inflammatory consolidation of the lung. That does not necessarily mean that Bacillus anthracis germinated there, produced its toxin there, and caused the primary disease to be pneumonia, and indeed, I believe most of the lesions are caused by the bacilli, but they're probably caused by bacilli that are spreading back to the lung through the blood stream because we find most of the organisms in the blood.

Nevertheless, you can see some examples

where the damage in the lung is localized.

Next.

Another example, and you can see it's hemorrhagic, and hemorrhage is a theme I've probably already emphasized enough, and it also occurs in the lungs. So a lot of those consolidations are, indeed, hemorrhages.

Next.

Some of the hemorrhage is tracking back along the bronchi from the mediastinum. So this is high pressure hemorrhage going back along the bronchi.

Next.

And here we see an example of that high pressure hemorrhage that's displacing lung tissue, and that would give a consolidation, and you probably would prefer to think of that as hemorrhage than pneumonia.

Next.

Here's a low pressure hemorrhage in which it's not distorted, but it is filling up the alveolar spaces with erythrocytes.

Next.

Another area you get a true exudate. This is that fibrin rich edema occurring in the lung. Again, the pathology of anthrax can occur in the lung, as well as in other organs.

Next.

And here is an example of what most people would truly think of as a bronchial pneumonia with exudate in the center, edema, and hemorrhage around the outside.

Next slide.

There were seven of these patients who were arc welders, and there's evidence in those patients of pneumoconiosis. So you can see some scarring and deposition of hemosiderin in the lung associated with their profession.

We hypothesize that this scarring may have led to decreased clearance of organisms from the lung and caused some, in some cases, the possibility of the organism germinating in the lung because of its not being cleared efficiently, as efficiently to the mediastinal lymph nodes.

Next.

To illustrate to you the lymphatic dilatation, this is a hugely dilated lymphatic vessel.

Next slide.

Here's in the lung. We can see a pulmonary vessel here, and around the vessel these hugely dilated lymphatics. So this is -- there could be some fluid coming from the lung, but there may also be fluid that's backing up because of the damage in the mediastinum.

Next slide.

Organisms can be found in these lymphatics with concentration being the greatest the closer one is to the mediastinum, decreasing as one goes into the lungs, but sometimes in association with an inflammatory lesion that one could call a lymphangitis.

Next.

So the quantitative microscopic findings in the lungs are that we actually found bacilli in half of the lungs, but more than half of the bacilli were intravascular, indicating they're being spread to the lungs through the blood stream, and the patient

has got systemic bacteremia.

Only about, you know, a sixth of them are intra-alveolar, and presumably those are spilling over from the blood vessels into the alveoli most of the time.

The exudates and neutrophils I think I've said enough about already. It's not an impressive inflammatory reaction, but there is some.

Next slide.

There's also inflammation in the interstitium of a similar nature, and again, not very much of a cellular response.

Next.

The hemorrhages, I think, are really very important, and a lot of things that we've been calling pneumonia really are due to the hemorrhage and due to congestion, the hemorrhage coming after the congestion, and due to damage to the capillaries, blood vessels, and with a lot of the increase of the mass due to lymphatic vessel obstruction, with dilatation being prominent.

Next.

So pneumonia in the fatal cases of inhalational anthrax really probably is mostly due to hematogenous anthrax pneumonia, some due to retrograde lymphangetic pneumonia, possibly some pneumonia of anthrax organisms themselves, although I certainly cannot sort out an organism that's germinated in the lung in situ versus when it's spread to the lung in a germinated state.

Respiratory insufficiency is clearly a very important event, and it's greatly due to atelectasis, and I must not have emphasized it in the slide when we went over it, but the average volume of pleural effusions in these patients is over 1,700 milliliters. So that's like around 900 cc's on each side, and that's replacing lung tissue, compressing the lung.

You also have got the ascites in some patients elevating the diaphragm; got the expansion of the mediastinum because of the hemorrhage and because of the gelatinous edema, and so there's really a great deal of atelectasis, and I believe this is the primary or a major factor in patients' respiratory

insufficiency.

There's really only one case we found of aspiration pneumonia, pulmonary edema per se or nosocomial pneumonia did not appear to be major factors that we identified.

Next slide.

Hematogenous spread to the lung or to the brain we show so grossly causes this hemorrhage in the subarachnoid space. Here's the brain, and there's the meninges and the subarachnoid space full of blood.

Next.

Here we can see it's a vasculitis, the blood vessel here, with inflammation in the wall and hemorrhage coming no doubt from hemorrhage from a damaged blood vessel.

Next.

Bacilli very, very frequently found in the brain, in the subarachnoid space of the brain. Lots of Gram positive Bacillus anthracis.

Next slide.

In a few cases there was hemorrhage in the brain parenchyma itself. We can see a blood vessel

here with a ring hemorrhage. Again, the same phenomenon of vascular damages that I have emphasized enough.

Next.

So the quantitative microscopic findings in the meninges were that we found organisms in 79 percent of the 29 brains that were taken at autopsy, and there was a significant amount of low pressure hemorrhage and fibrin, but the cellular response was modest.

Next.

Hematogenous spread occurred also to the gastrointestinal tract. It's very well described, been in the Russian literature for well over five decades, and we see these high pressure hemorrhages in the submucosa. This is a small intestine that's been opened up, and we're looking at the luminal surface, and we see these localized areas of hemorrhage.

Next.

Here we see histologically the mucosa here. It's lifted up by hemorrhage in the submucosa. This would be the submucosa, and a great big hematoma

-- next slide -- will demonstrate how this comes about.

We see blood vessels in which the Gram positive bacilli are present, and so it's hematagomous spread to the intestine that results in these numerous lesions in the submucosa.

Next slide.

So what are the mechanisms of death that we believe we have identified in inhalational anthrax? Atelectasis, as I've already discussed is a primary mechanism of death we felt in 39 of the cases and a major contributory mechanism of death in 46 percent of cases.

The hemorrhagic meningeal encephalitis is very important and is a primary mechanism of death in 34 percent of cases.

The pneumonia is -- and you've already gotten what I mean by pneumonia. It's all of those damages in the lung added up together, with the lung damage being the most important thing only in two out of the 41 cases.

Clearly there's more to the

pathophysiology of systemic anthrax than one can see looking through a microscope, and the pathophysiology that Dr. Friedlander will tell you about will speak to that,b ut undoubtedly septic shock, which we attributed as being a contributing factor in 51 percent of the cases, could easily be more important than that, although these changes are pretty impressive, and I think that they really can kill you, that much pleural fluid accumulation and that severe hemorrhagic meningeal encephalitis.

Next slide.

This is my last slide, and these patients were treated and admitted to the hospital, and some of them were treated, and some of them, they didn't treat them. I guess they didn't have in mind what the diagnosis was. They're rather non-specific symptoms, to begin with.

And so among the patients that were not treated, the organism was cultivated at autopsy from 83 percent, where those that received any treatment at all is received from only 23 percent.

The treatment that I have been told about

is penicillin, cephalosporins, and forinphenocol (phonetic), and so I'm not sure of the doses, and certainly some of these patients didn't receive very much in the way of treatment in terms of duration.

In fact, those that were treated for less than 24 hours, we did continue to find organisms. Histologically we detected them in all of the patients that were not treated, and in 55 percent of those that were treated.

Histological detection of an organism, of course, doesn't prove that it's still alive. It could be that the dead organism is still lying there and has just not been removed yet.

Thank you. I'll be happy to answer any questions if I can.

CHAIRMAN RELLER: Are there questions from the panel for Dr. Walker?

Dr. Archer.

DR. ARCHER: Was there any evidence that antibiotic treatment had any effect on death? Of those who got antibiotics, was there a lower death rate than those who didn't?

DR. WALKER: Yes, some patients survived. I'm told that there were five survivors. There is the suggestion that there may have been prevention of death by some of the prophylaxis. Martin Hugh-Jones would be better to answer that, but it seems to be not the right curve of cases, and there's some missing towards the end that might have been exposed and got their disease prevented.

The five cases that survived, I have not had a chance to examine the records to know exactly ho long they had been ill and what they got treated with.

I think the image of anthrax as a virtually untreatable disease is probably close to true. I mean there are the experiments where they have taken animals and treated them with antibiotics past a certain critical phase, sterilized them of the organisms, but the animal still died of the effects of the toxin.

CHAIRMAN RELLER: Dr. Chesney.

DR. CHESNEY: Is the vasculitis present in all sizes of vessels? Is it in the larger vessels as well as the --

DR. WALKER: We didn't identify it in the larger vessels. We saw it mainly in medium sized and small vessels. I don't --

DR. CHESNEY: And is it your impression that the hemorrhage is coming from leakage from the medium and small sized vessels?

DR. WALKER: Yes.

CHAIRMAN RELLER: David, in Koch's day this was called splenic fever. What did the spleens look like in these patients?

DR. WALKER: We got a whole section in the article on that, on the spleen. I didn't think you guys would be interested in the spleen.

(Laughter.)

DR. WALKER: The spleen is enlarged, and it's got bacilli in it. No fatal lesions in the spleen though.

CHAIRMAN RELLER: Thanks.

Dr. Chikami.

DR. CHIKAMI: You describe in the pathology this high pressure hemorrhage within the mediastinum. Was there any evidence that this high

pressure hemorrhage led to compression of other structures within the mediastinum and, say, compromise or led to something akin to cardiac tamponade or some effect on the cardiac function in these patients?

DR. WALKER: No.

CHAIRMAN RELLER: Thanks very much, Dr. Walker.

Oh, sorry. Dr. Friedlander.

DR. FRIEDLANDER: I just wanted to make a comment. First of all, it was a delight to see these pictures. I haven't seen them before.

And to make two points, if I might. One, to reemphasize this point that I think there's been some misconception, and I think people have tried to rectify it, that this is not primarily a pneumonia. This is a mediastinitis. This is a disease of the lymph node, and even these pulmonary findings appear to be mainly hemorrhagic findings in the lung.

The second relates to the question of survivors, and I was interested to hear that because as I read the articles -- and I think this is an important point, and I certainly don't have the

answers for -- in the articles from Sverdlovsk, it said that there were nine survivors of inhalational disease.

As best I can read that data -- I wasn't there to visit either -- there is absolutely no evidence whatsoever given, either histologic, serologic, or microbiologic that these patients had anthrax.

So it becomes very difficult, I think, to posit, in fact, that there were survivors.

On the other hand, I think there is some data to suggest that in animals at least, in primates, that even when animals are bacteremic and even when they have mediastinitis, that some animals will survive.

So I don't think that it is -- at some point in any infection, there's a point of no return, but this concept, I think, is somewhat overstated that once bacteremia occurs or once mediastinitis occurs, it is absolutely fatal. I don't believe that myself, although I don't know any data in humans. There is some data in primates.

CHAIRMAN RELLER: Thank you.

And it's time for Dr. Friedlander to present the primate data.

DR. FRIEDLANDER: Thanks very much.

I appreciate Dr. Chikami and Meyerhoff asking me to present some of our previously reported work on post exposure prophylaxis in the non-human primate model of inhalational anthrax with antibiotics.

I'd like to begin with a few introductory remarks about the pathogenesis. I think we know a lot more; we've learned a lot more actually from Dr. Walker's presentation. We don't know a great deal about the disease in animals or in humans. What we know a great deal more about is the toxin and how it works in vitro, and that that relevance is to an in vivo situation remains still primarily conjectural.

Then I'll discuss some of the pathology in the non-human primate and contrast it to some extent with or compare it to that in the human, and finally present a review of the studies that we did during the Gulf War addressing the question of how to treat in

the post exposure scenario.

Did that work? No, the lights. Okay.

You've heard this before, and I'll just reiterate it if I can find this thing. Okay. The spore is the infectious form that we're concerned about. Very rarely the bacillus can be infectious, but it's the spore particularly for inhalational disease.

It enters, as you heard, the skin, the GI tract or the lung. It is thought to germinate in the macrophage. This is the central player so far as we know either locally, if it's the skin or the GI tract, or is transported to a regional lymph node in the case of inhalational disease.

There there's the local production of toxins leading to edema and necrosis, the characteristic lesions that were pointed out just recently, and then spread from the node with bacteremia and toxemia.

This is shown -- this is an old slide from Dutz, who was a pathologist who studied this disease intensively or had a lot of experience with the

disease, no nearly as much as in the Sverdlovsk now, and as I said, it's an old slide, but I like it because it points out this central player here.

Particularly for inhalational disease, this is the disease. It is not a pneumonia. It is a disease of the regional tracheal-bronchial, hiler (phonetic), mediastinal lymph node which spreads the mediastinum causing mediastinitis.

Now, the organism germinates -- you're going to do that for me. Okay.

This is a slide from Eli Metchnikoff. This disease as you heard is associated with the very origins of infectious disease and immunology. It was a big bacillus. It was easy to see under the microscope, and it was an important agricultural disease of domesticated animals.

The organism germinates. This is the macrophage from the liver of a rat. It germinates in -- the spore germinates in the macrophage.

Once the bacillus forms, it makes two toxins, edema toxin and lethal toxin that you've heard about. Both of them have anti-phagocytic effects.

One of the prime manifestations of this disease in the cutaneous form and also in the inhalational form is a relative paucity of inflammatory cells. Malignant pustule is not a pustule, in fact. There are very few inflammatory cells.

So these two toxins probably have other effects as well, but we know in vitro they have dramatic effects on macrophages and neutrophils.

It also make a capsule, a polyglutamic acid capsule. Once that capsule is made and these organisms escape, they never see a phagocyte again. That capsule, as with many others, prevents phagocytosis. This is an extracellular infection once it is released from the macrophage.

Now, the characteristic finding, as we said, was when it's released was this spread to the lymph node, the damage to the lymph node, the hemorrhage, the necrosis, and then spread to the surrounding mediastinum.

From the mediastinum it spreads through the lymph, and you've seen dramatic pictures of lymphatic dilatation. It spreads from the lymph to

the systemic circulation and to all the organs, particularly to the brain.

As we've heard and has been described before, about half of the cases have meningitis, and most often it's hemorrhagic.

Oh, sorry.

This is just a more modern version of Metchnikoff's slide. This happens to be an example of fluorescence microscopy from a group at the Pasteur Institute. These are mouse macrophages. You can barely see the outline of the cell, but it shows the spore essentially co-localized, both the F-actin into lysosomal markers.

So that the spores are ingested. They germinate. There's fusion, and eventually some of the spores germinate to the bacillus, destroy the cell, and the organism is now free to replicate.

Next slide, please.

Now, the clinical and pathologic findings of this disease were well described in the latter part of the 19th Century with the development of the industrial revolution. Basically a new disease