FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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VACCINES AND RELATED BIOLOGICAL PRODUCTS

ADVISORY COMMITTEE

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OPEN SESSION

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TELECONFERENCE MEETING

ON CBER LABORATORY SITE VISIT

LABORATORY OF ENTERIC AND SEXUALLY

TRANSMITTED DISEASES

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CONFERENCE ROOM C
BUILDING 29B
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

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MONDAY, NOVEMBER 18, 2002

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ADVISORY COMMITTEE: (Telephonically)

ROBERT S. DAUM, M.D.

Professor of Pediatrics
University of Chicago Children's
Hospital M/C 6054 Chairman

WALTER L. FAGGETT, M.D.
Medical Director
Medical Assistance Administration
D.C. Department of Health

BARBARA LOE FISHER
C-Founder and President
National Vaccine Information Center

JUDITH D. GOLDBERG, Sc.D.
Director
New York University School of Medicine
Division of Biostatistics

DIANE E. GRIFFIN, M.D., Ph.D.
Professor and Chair
Molecular Microbiology & Immunology
Johns Hopkins University School of Medicine

SAMUEL L. KATZ, M.D.
Wilburt C. Davidson Professor and Chairman Emeritus
Department of Pediatrics
Duke University Medical Center

DAVID M. MARKOVITZ, M.D.
Professor
Division of Infectious Diseases
Department of Internal Medicine
University of Michigan Medical Center

AUDREY F. MANLEY, M.D., M.P.H.
President Emeritus
Spelman College
Rear Admiral (Ret)
U.S. Public Health Service

GARY D. OVERTURF, M.D.
Professor of Pediatrics
Department of Pediatrics
University of New Mexico School of Medicine
Ambulatory Care Center

ADVISORY COMMITTEE: (Continued)

PETER PALESE, Ph.D.
Chairman and Professor
Department of Microbiology

Mt. Sinai School of Medicine of New York City

JULIE PARSONNET, M.D.
Associate Professor of Medicine and Health Research and Policy
Stanford University

DAVID S. STEPHENS, M.D.
Professor of Medicine
Division of Infectious Diseases Division
Department of Medicine
Emory University School of Medicine

RICHARD J. WHITLEY, M.D.
Professor
Pediatrics, Microbiology and Medicine
Department of Pediatrics and Microbiology
University of Alabama at Birmingham

CONSULTANT:

ESTUARDO AGUILAR-CORDOVA, Ph.D., MA.D.
Director
Advantagene,Inc.

EXECUTIVE SECRETARY:

JODY SACHS, D.P.M.
Scientific Advisors & Consultants Staff
Center for Biologics Evaluation and Research, FDA

COMMITTEE MANAGEMENT SPECIALIST:
DENISE ROYSTER
Scientific Advisors & Consultants Staff
Center for Biologics Evaluation and Research, FDA

DR. BILL FREAS
Director
Scientific Advisors & Consultants Staff
Center for Biologics Evaluation and Research

FDA PARTICIPANTS:

NEIL GOLDMAN, Ph.D.
Associate Director for Research
Office of the Director, CBER

KAREN MIDTHUN, M.D.
Director

Office of Vaccines Research and Review, CBER

RICHARD WALKER, Ph.D.
Division Director
Division of Bacterial, Parasitic & Allergenic Products
OVRR/CBER

DENNIS KOPECKO, Ph.D.
Laboratory Chief
Laboratory of Enteric and Sexually Transmitted Diseases
DBPAP/OVRR/CBER

SCOTT STIBITZ, Ph.D.
Principal Investigator
Laboratory of Enteric and Sexually Transmitted Diseases
DBPAP/OVRR/CBER (Telephonically)

PHILIP BOUCHER, Ph.D.
Staff Fellow
Laboratory of Enteric and Sexually Transmitted Diseases
DBPAP/OVRR/CBER

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1 **P R O C E E D I N G S**

2 (1:05 p.m.)

3 DR. DAUM: I'd like to begin by
4 calling the meeting to order. Jody, would you
5 like to go through another roll call first, or
6 are you satisfied with that?

7 DR. SACHS: No, I'm happy. There's
8 maybe two other people -- three other people --
9 we'll just notify everybody.

10 DR. DAUM: Everyone is on except Drs.
11 Griffin, Palese --

12 DR. PALESE: I'm here.

13 DR. DAUM: Hello, Dr. Palese.
14 Everyone is on except Drs. Griffin and Whitley at
15 this point. Dr. Diaz we know wasn't coming, and
16 Dr. Gellin is still a question mark.

17 ARBITRATOR HOCKENBERRY: Estuardo
18 Aguilar-Cordova is about to join us on the call.

19 DR. AGUILAR-CORDOVA: I'm here.

20 DR. SACHS: Great.

21 DR. DAUM: Okay. Thank you. You're
22 not on my voting list, D. Aguilar-Cordova, so I
23 made a mistake. I'm sorry.

24 So, with that attendance information,
25 Jody, why don't we turn the floor over to you for
26 announcements.

1 DR. SACHS: Okay. I just have a
2 little bit of a meeting statement to read. Good
3 afternoon. I'm Jody Sachs, the Executive
4 Secretary for today's meeting of the Vaccines and
5 Related Biological Products Advisory Committee.
6 I'd like to welcome all of you to the 92nd
7 meeting of the Advisory Committee. There is a
8 speaker phone for public participation located
9 here in Conference Room C of Building 29B, on the
10 NIH Campus.
11 This afternoon's session will consist
12 of a presentation and committee discussion that
13 are open to the public. We will then go into a
14 closed session until the meeting is adjourned, as
15 described in the Federal Register Notice of
16 October 30, 2002.
17 Should a committee member get dropped
18 from the teleconference, simply call back at the
19 888-324-0789 number and give the pass code as
20 VRBPAC. The operator is under instructions only
21 to connect the committee members to this line.
22 Again, you have the number so, if you get
23 disconnected, please call back. If you have any
24 problems while we're on the call, you can press
25 "*0" and the operator will help you.
26 We ask that you do not place us on

1 hold because many clinical centers have
2 background music, and it can be very distracting
3 to those remaining on the teleconference line.
4 However, I strongly urge everyone to use "*6" or
5 a mute button -- it is the same as a mute button
6 -- since there are many lines connected, the
7 background sound will be decreased and the
8 quality of sound for everybody would improve if
9 you just use mute.
10 I just want to let everybody know who
11 is in the room wiht me, in front of me. Dr.
12 Karen Midthun is here. She's the Director of the
13 Office of Vaccines Research and Review; Dr. Neil
14 Goldman, Associate Director for Research, Office
15 of the Center Director in CBER is here. Dr.
16 Richard Walker, Director of Division of
17 Bacterial, Parasitic & Allergenic Products. And
18 we have two of the people in the Laboratory of
19 Enteric and Sexually Transmitted Disease Labs,
20 Dr. Dennis Kopecko, who is the Laboratory Chief,
21 and Dr. Philip Boucher. Also on the call is Dr.
22 Sam Katz, who is the Site Visit Team Chair.
23 I would now like to read a public
24 statement for the record. The following
25 announcement addresses conflict of interest
26 issues associated with today's meeting of the

1 Vaccine and Related Biological Products Advisory
2 Committee on November 18th, related to the review
3 and discussion of intramural research programs of
4 the Laboratory of Enteric and Sexually
5 Transmitted Diseases.
6 Based on the agenda made available, it
7 has been determined that the committee
8 discussions present no potential for conflict of
9 interest.
10 At this point, I'd like to turn the
11 meeting over to you, Dr. Daum.
12 DR. DAUM: Thank you very much. We're
13 now going to go to the open session portion of
14 this afternoon's meeting. The meeting, of
15 course, as Jody mentioned, is devoted entirely to
16 the findings of the CBER laboratory site visit of
17 the Laboratory of Enteric and Sexually
18 Transmitted Diseases.
19 So, we are first going to hear from
20 Dr. Walker regarding the overview of the Division
21 of Bacterial, Parasitic and Allergenic Products.
22 Dr. Walker.
23 DR. WALKER: Thank you. Good
24 afternoon. I'd like to take just a few minutes
25 to, as Dr. Daum said, give you a quick overview
26 of what goes on in the Division of Bacterial,

1 Parasitic and Allergenic Products.
2 Basically, the division is focused on
3 assuring safe and effective products for
4 immunological control of bacterial, parasitic and
5 allergenic agents affecting human health. It's
6 important to keep in mind that the scientists in
7 this division perform both research and review
8 functions. In some cases for our investigators,
9 this review function could be 30 to 50 percent of
10 their time.
11 Another point I'd like to make focuses
12 in on the review functions that these people do.
13 I'd like to emphasize that their review work is
14 a cradle-to-grave type operation because it
15 begins with pre-IND meetings with sponsors to
16 help provide guidance, and then through the IND
17 process, continuing meetings and review, license
18 actions, and then it's very important to keep in
19 mind that even post-licensure of a product that
20 the task of this division goes on because there's
21 review of biological deviation reports, there's
22 inspections, lot release issues, and so forth.
23 So, these people have a lot of work in the review
24 are as well as in the scientific area.
25 I'd like to make another point about -
26 - that focuses in on the scientific area that

1 these people in LESTD and other parts of the
2 division deal with is in there are quite variety
3 of products. When you consider the products that
4 are existing now or that are possible in the
5 foreseeable future, I think of respiratory
6 pathogens, from Streptococcus to Moraxella,
7 sexually transmitted diseases, pathogens that
8 might be encountered by penetrating inoculation,
9 like Malaria and Borrelia, and so forth; special
10 pathogens which has become a big issue in recent
11 years, like Bacillus anthracis, Clostridium,
12 Franciscella and Yersinia; diarrhea-causing
13 pathogens which will be discussed a bit today,
14 and so forth, allergenic products whether it's
15 latex or cockroach antigens and skin test
16 antigens. So there's quite a variety of things
17 that the people in this division have to cover.
18 And so the division, to do this, is
19 made up of about 100 people organized into eight
20 laboratories. There's the Laboratory of Methods
21 Development Quality Control, which seeks to
22 develop and standardize and evaluate quality
23 control methods for bacterial vaccines, and also
24 develop, evaluate and apply serological methods
25 to measure immune responses in vaccine trials.
26 And also this group more recently is working to

1 coordinate quality assurance activities within
2 the division and provide leadership and the
3 initiative to accredit CBER quality control
4 testing laboratories.
5 Another laboratory, the Laboratory of
6 Bacterial Polysaccharides is involved in
7 characterization of immune responses to
8 polysaccharide and conjugate vaccines, in
9 standardization of methods for relevant clinical
10 application. They also have been instrumental in
11 developing novel physical and chemical methods
12 for improved evaluation of licensed and
13 experimental vaccines involving polysaccharides.
14 A third laboratory is the Laboratory
15 of Biophysics, which use high-end instrumentation
16 to help characterize biopolymers such as
17 polysaccharides, DNA, proteins and macromolecular
18 assemblies such as vaccine/adjuvant complexes.
19 And the high-end equipment like applying NMR
20 technology to vaccine characterization.
21 Another laboratory in the division is
22 the Laboratory of Respiratory and Special
23 Pathogens, which deals with Bordetella pertussis,
24 as well as more recently with Bacillus anthracis
25 and Yersinia species. And the work that these
26 people are doing is to characterize the virulence

1 factors and studies on mechanism of actions of
2 these factors, and also the regulation of gene
3 expression of these factors.
4 Another laboratory is the Laboratory
5 of Bacterial Toxins, which deals with neurotoxins
6 as well as iron-regulated virulence factors in
7 Corynebacterium diphtheriae, and also in Bacillus
8 anthracis.
9 There are two other laboratories
10 before the laboratory that we're going to talk
11 about today. The Laboratory of Mycobacterial
12 Diseases and Cellular Immunology, which evaluates
13 protective innate and adaptive immune responses
14 to intracellular bacteria, and they are presently
15 involved in assessment of DNA vaccination
16 strategies against tuberculosis, as well as
17 identifying new antigens that might be useful in
18 anti-tuberculosis vaccines.
19 The seventh of the eight laboratories
20 that I want to talk about today is the Laboratory
21 of Immunobiochemistry, which deals with
22 allergenic products. They are concerned with
23 allergen structure and function, and
24 immunomodulation of the allergic responses, and
25 the study of chamokines and receptors in the
26 modulation of immune responses to allergenic

1 agents.

2 Then the eighth laboratory, which Dr.
3 Kopecko will be talking much more about this
4 afternoon, is the Laboratory of Enteric and
5 Sexually Transmitted Diseases, which of course is
6 dealing with enteric pathogens, but also in more
7 recent times is dealing with several bioterrorism
8 agents, and I'm sure Dr. Kopecko will mention
9 that a little bit more.

10 I'd like to conclude by just bringing
11 your attention to some of the realities facing
12 our Researcher/Reviewers in the division. Some
13 of the things they have to deal with are, as you
14 would expect in a large organization, are
15 bureaucratic hurdles, like personnel and other
16 things that sometimes make work challenging, even
17 though we've got very good people working with
18 us. Another issue these Research/Reviewers have
19 by working in the Federal Government, they have
20 the issue of funding levels are uncertain from
21 year to year, and dependent upon the
22 appropriation process.

23 But the most unique thing, I think,
24 about the FDA Researcher/Reviewers as opposed to
25 people in other government agencies like CDC or
26 NIH is that since these people are reviewers, the

1 timing of their workload is determined by the
2 sponsor submissions and not by CBER. And so they
3 have to carry out the research work and also meet
4 deadlines that are part of the review function,
5 and that is one of the challenges that our
6 investigators are meeting.
7 And, finally, when we had the actual
8 site visit, I asked the Site Visit Committee to
9 not only help us by reviewing the individuals and
10 reviewing the programs that they are directing,
11 but also comment on the current and future
12 directions of the research.
13 So, hopefully that gives you a very
14 brief overview of our Researcher/Reviewers and
15 sort of the nature of the work that goes on
16 within this division, and I'll be glad to answer
17 any questions that you might have.
18 DR. DAUM: Thank you very much, Dr.
19 Walker. The floor is open for committee question
20 or comment.
21 DR. KATZ: This is Sam Katz. I guess
22 I'll reserve mine until after we go through the
23 report.
24 DR. DAUM: That sounds fine, your
25 choice. Any others?
26 (No response.)

1 Then why don't we move on to the next
2 item, which is we're going to hear from Dr.
3 Kopecko. Welcome, Dr. Kopecko, and we will have
4 an overview of the Laboratory of Enteric and
5 Sexually Transmitted Diseases.
6 DR. KOPECKO: Thank you very much.
7 Good afternoon to everyone. It's a great
8 pleasure to have the opportunity to introduce the
9 lab to you. In ten minutes, I'll try to give you
10 a little bit of a flavor for who we are and what
11 we do both regulatory and research-wise, and
12 mention a few of our future directions.
13 If you have the Site Visit Notebook in
14 from of you, you can see from Section 2 that the
15 laboratory is comprised of three sections -- the
16 Pathogenesis Section, the Gene Regulation
17 Section, and Dr. Stibitz and Dr. Boucher will be
18 reviewing research in that area for the review
19 period. And the third is a new section, the
20 Immune Mechanisms Section, that Dr. Walker is
21 heading up, that's just begun, and there wasn't
22 any significant amount of research carried out
23 during the review period, but as we move more
24 into anthrax and Shigella vaccine development,
25 that Immune Mechanism Section carries great
26 importance.

1 The lab also is evolving from five
2 personnel to 14 personnel, so we're undergoing
3 some significant changes space-wise and
4 personnel-wise. We currently have 13 people
5 onboard, with one more hopefully to join us
6 within the next couple of months.
7 If you can see from Section 3, our lab
8 was started in 1994, replaced the former
9 Micoplasma Lab. It was initiated to handle a
10 large number of INDs and regulatory work in
11 enteric diseases, and also to cover sexually
12 transmitted diseases which, in fact, were thought
13 that there would be an onslaught of STD
14 applications, which has not actually come yet,
15 but there has been a tremendous increase of
16 enteric disease applications.
17 The lab, as Dr. Walker mentioned, has
18 a bifunction, as all labs here at CBER. We are
19 tasked with carrying out regulatory review in our
20 area of specialty, as well as to conduct research
21 which enhances our knowledge base, which allows
22 us to increase both the quality and speed of the
23 regulatory review of various products. So, our
24 research is aimed at our area of specialization.
25 Within LESTD, we conduct basic
26 research into the genetic and molecular bases of

1 disease pathogenesis and the host response to
2 infection. And the current events that have been
3 analyzed during the last review period involve
4 both prokaryotic and eukaryotic mechanisms
5 involved in bacterial invasion of mucosal
6 epithelial cells -- that is, induced endocytosis
7 and also induced exocytosis -- as well as the
8 global regulation of expression of bacterial
9 virulence components, which Drs. Stibitz and
10 Boucher will address.
11 Our regulatory responsibilities, you
12 can tell from the title, cover both enteric and
13 sexually transmitted diseases, but I wanted to
14 summarize some of the more common and some of the
15 less common types of agents that we cover.
16 This includes, obviously, cholera,
17 Typhoid Fever, Shigellosis, as you might expect,
18 but also enterotoxigenic E.coli and other
19 pathogenic E.coli such as Campylobacter, LOB-
20 attenuated bacterial vector based vaccine
21 development for multiantigen use. These include
22 both modified Salmonella strains, Shigella
23 strains and cholera strains. We also cover
24 Helicobacter pylori, the use of live enteric
25 bacteria such as attenuated Salmonella
26 typhinerium given IV to treat metastatic cancer,

1 various UTI pathogens, proteus, E.coli,
2 intestinal strain replacement for probiotic
3 therapy, the use of bacterially derived enzymes
4 for treatment such as alosperigenase for acute
5 lymphocytic leukemia, bacterial phase for
6 therapeutic antimicrobial use, bovine or chicken
7 immunoglobulin concentrates from specifically
8 immunized animals for human use, and genetic
9 harbored vegetables, to mention some of the
10 products that we cover.
11 These encompass products that are
12 administered by oral, intranasal, parenteral,
13 intrarectoral, or transcutaneous routes, and they
14 also cover evaluation of new adjutants for orally
15 administered products.
16 Currently within the lab we cover
17 about 110 different IND products. We are
18 receiving about 12 to 14 new IND applications per
19 year, so you can see how that list is growing.
20 We probably drop about five to seven per year
21 that become inactive. We also are reviewing one
22 full biological license application on Cholera
23 currently, and we have several BLA supplements
24 under review.
25 So, that covers the general lab and
26 our regulatory responsibilities. I'd like to

1 take a couple of minutes and go back to Section 9
2 and just quickly summarize some of the research
3 carried out in Molecular Pathogenesis Section.
4 Our main focus was to look at the cell
5 biology of invasion of intestinal epithelial
6 cells by Campylobacter jejuni, which I'll get to
7 summarize in a minute, but we also carried out a
8 number of more minor projects which all turned
9 out to result in publications, a couple of which
10 I want to point out because I think they have
11 enhanced significance.
12 One involves Nanobacteria, which was
13 reported by a Alevi Pejander (phonetic) in
14 Finland, to be a new species of bacteria that
15 were very small, filterable through .1 micron-
16 filters, potentially important adventitious agent
17 biological products because they are found in
18 more than 80 percent of bovine and human serum,
19 thought to be the main cause for human kidney
20 stone formation, and more recently touted to be
21 the main cause of atherosclerotic plaque, still
22 being pushed by Dr. Pejander.
23 We followed up his results, which were
24 mostly phenomenological studies with the heaviest
25 piece of data being PCR 16Srod assembled DNA. We
26 were able to obtain growth of these same coccoid

1 like structures that had a calcified outer shell.
2 They looked like coccoid bacteria. However,
3 when we got into molecular evidence, we found by
4 PCR we obtained the same sequence that Pejander
5 obtained, but in going to gene bank, we found
6 that he had misinterpreted the data and, in fact,
7 this was the sequence from phylo bacterium, which
8 is a common water-borne contaminant that's found
9 in PCR reactions that are carried out for a high
10 number of cycles. Also, the growth of these
11 structures could not be inhibited in azides,
12 which all respiration-dependent organisms would
13 be inhibited by. So, the upshot of
14 these studies that we published in P&AS was that
15 there was no credible molecular data to support
16 the existence of Nanobacteria. We feel that
17 these structures are actually hydroxy-appetite
18 crystals that are triggered into initiation by
19 various macromolecules in blood and in saliva as
20 part of a natural process. And, in fact, as
21 Pejander reported, you can destroy these
22 macromolecules by heavy gamma-irradiation. So,
23 there is a way to knock them out, but we didn't
24 feel that there was any strong evidence to
25 support the existence of this new group of
26 organisms, removing it at least for the current

1 time as an important potential adventitious
2 agent.
3 The second project that I want to
4 mention quickly involved Shigella sonnei. This
5 was a project that we started some 20 years ago,
6 trying to make a modified hybrid vaccine in
7 salmonella that would protect against typhoid and
8 Shigella, and this involved moving the LPS genes
9 from Shigella sonnei in Salmonella typhi, Ty21a.
10 The difficulty is this block of genes is very
11 large, and more recently we were able to clone
12 downsize to about 12kb the essential genes. We
13 found that they are stably expressed in
14 Salmonella off of an illegal copied plasmid
15 versus the typical multi-copied plasmid that most
16 cloners use. We also found that there is an
17 adjacent insertion sequence, Is91, that causes
18 instability of this. Once that's deleted, it
19 stabilizes the clone region for production.
20 And we also found that this form one
21 polysaccharide is expressed in two forms in
22 Shigella and in E.coli, both as a lipoaliga
23 saccharide attached to core as normally, but also
24 as a capsule where it's lipid-bound and stuck in
25 the outer surface of the cell. And we were able
26 to transfer this into Salmonella and make a

1 candidate vaccine.

2 These studies were published recently
3 in Infection and Immunity, patented, and we had
4 one firm who's carrying out commercially the
5 development of this into a vaccine. We are
6 extending these studies to include Shigella
7 dysenteria and a couple of other Shigella
8 serotypes for future vaccine development,
9 together with development of a live oral vaccine
10 against anthrax.

11 Now, the last thing I want to mention
12 is our work on Campylobacter jejuni. We carried
13 out a number of in vitro tissue culture invasion
14 assays into various cell lines, using a variety
15 of different inhibitors. And to understand the
16 cell biology of entry, these studies have been
17 summarized in 2001 Trends in Microbiology
18 Summary, for the most part. We found that the
19 organism recognizes a receptor that's probably in
20 the junctional space, the receptor is not yet
21 characterized, and it's carried within cuveale
22 interactions of the bacteria with this receptor
23 trigger, we think, secretion of invasion
24 effectors -- this is work by Mike Comp
25 (phonetic), not ours -- these invasion effectors,
26 we found, trigger an up-regulation of PI3 kinase,

1 a release of intracellular calcium from
2 intracellular stores, activation of calmodulin
3 and protein CON-AC, which cause a structural
4 rearrangement -- and this is a microtubule
5 dependent up-take mechanisms as opposed to a
6 microfilament dependent that most other bacteria
7 use -- the cytoskeleton is reorganized, the
8 bacteria enter through a microtubular like
9 extension, they are carried in an endosomal
10 vacuole along microtubules via the molecular
11 motor dinaene to raise the lateral surface where
12 exocytosis occurs. So, that's the current state
13 of understanding for Campylobacter jejuni entry.
14 And if there aren't any questions, I'll turn
15 things over to Dr. Stibitz.
16 DR. DAUM: Dr. Kopecko's very
17 interesting presentation is open for questions
18 and discussion.
19 (No response.)
20 Well, I guess there aren't any
21 questions or discussion, Dr. Kopecko, which means
22 you were crystal clear. Thank you very much.
23 We will call on Dr. Stibitz for an
24 overview of research activities in his area.
25 DR. STIBITZ: Thank you. And thank
26 you for letting me phone in. I'm actually in

1 southern Illinois, in my ancestral home, and I'm
2 willing to bet I'm the only person who literally
3 dialed-up to the conference call.
4 The section of LESTD that I'm
5 concerned with is interested primarily in the
6 study of one particular aspect of bacterial
7 pathogenesis, and that is the regulation of
8 virulence factor expression. The model system
9 that we use is that of Bordetella pertussis,
10 which has a very well-developed regulon governed
11 by the BvgAS two-component system. Now, BvgAS,
12 like other two-component systems, contains an
13 environmental sensor protein which is localized
14 in the inner membrane, BvgS in this case, and
15 this protein communicates with the cytoplasmic
16 response regulator protein, which is BvgA in this
17 case. These two proteins communicate by the
18 kinase activity of the BvgS sensor protein, and
19 when BvgA is phosphorylated, it binds to the
20 promoter regions of virulence.
21 Now, in pertussis, these include two
22 which I'll mention, the fha which encodes the
23 filamentous hemagglutinin, an important adhesion,
24 and ptx which encodes the pertussis toxin
25 responsible for some of the hallmark symptoms of
26 whooping cough.

1 Now, we know from in vitro
2 transcription studies that BvgA phosphate is both
3 necessary and sufficient to activate
4 transcription of virulence seen. However, there
5 are interesting and significant differences in
6 how this occurs, and a classic experiment which
7 demonstrates this is that if one grows pertussis
8 under conditions where the virulence regulon is
9 turned off and then shifts it to permissive
10 conditions, one sees that fha transcription is
11 initiated within minutes while that of ptx takes
12 several hours. And it has been suggested to
13 represent a temporal pattern of gene expression
14 upon infection.
15 Now, this difference in one of the
16 aspects of virulence gene regulation that we're
17 most interested in, and our ultimate goal is the
18 complete understanding at a molecular level of
19 the interactions of BvgA with promoter DNA and
20 with RNA polymerase in the process of
21 transcriptional activation. And to accomplish
22 this, we've taken a combined biochemical and
23 genetic approach.
24 Now, until now, our efforts have
25 concentrated on the fha promoter. This is one of
26 the simplest, and actually the simplest, BvgA

1 regulated promoter described. And it was our
2 hope that we could best learn the details of BvgA
3 action in this system and then broaden our sights
4 to include other promoters. And, in fact, that's
5 exactly the juncture that we now find ourselves
6 at, and it has been quite satisfying.
7 Now, Dr. Philip Boucher, who will be
8 speaking in a few moments, has been the driving
9 force behind the biochemical analysis, and he'll
10 be telling you more about that, but I just wanted
11 to mention that that work has recently been
12 accepted for publication in Molecular Cell.
13 I have been primarily involved in the
14 genetic analysis, and I'd just like to go over
15 that briefly. Past work includes both forward
16 and reverse genetics. In terms of forward
17 genetics -- in other words, screening for mutant
18 phenotypes and then determining the site and
19 nature of mutations involved -- we have isolated
20 mutants which are affected in the differential
21 regulation -- in other words, ones which can
22 express fha but not ptx, and a reverse of that,
23 those which are hyperactivated for ptx expression
24 and down for fha expression.
25 In terms of reverse genetics -- in
26 other words, introducing specific mutations like

1 bacterialistic mutagenesis and then determining
2 their phenotypic effect -- we have confirmed to
3 role of the amino acid D54, we have made short C-
4 terminal deletions of BvgA which had an
5 interesting phenotype in that they were lethal to
6 the cell and were suppressed by mutations in the
7 alpha subunit of RNA polymerase and, more
8 recently, we've made a number of 16 substitution
9 mutants at different positions, and many of these
10 had interesting phenotypes as well either in
11 terms of differential regulation or, in one case,
12 we believe the mutation which affects
13 oligomerization of BvgA.
14 Now, my future genetic analysis of
15 BvgA involved a full-scale genetic assault on
16 BvgA and rests on some genetic screens which
17 we've developed, which will allow us to identify
18 mutations that specifically affect the ability of
19 BvgA to interact with RNA polymerase or to
20 interact with itself in the process of
21 dimerization or oligomerization. And I won't go
22 into those, but input into these will be BvgA in
23 which we've done either intense in vivo
24 mutagenesis or, in fact, saturation mutagenesis
25 by alynase scanning. And what we hope to
26 identify are specific messages in BvgA which are

1 involved in interacting with RNA polymerase or
2 with itself in the process of oligomerization and
3 dimerization.
4 Now, recently two other people have
5 joined the laboratory, and I'll just briefly
6 describe their projects. Dr. Wendy Veal Carr is
7 an ERDA Fellow who came to us from Bill
8 Schaeffer's lab at Emory University, and Wendy is
9 embarking on a project which, if successful, will
10 allow us to zoom out from our detailed
11 mechanistic analyses of BvgA to an organismal or
12 host pathogen interaction level. And she's
13 adapting RIVET, which stands for recombinase-
14 based in vivo expression technology. This system
15 was developed by Andy Kanuli, who is not at
16 Tufts, and it was to examine in vivo gene
17 expression oligliocollar. The system uses a DNA
18 recombinase as a reporter gene, and the readout
19 is the degree of recombination of a construct in
20 the chromosome which, upon resolution, leads to a
21 block with antibiotic resistance. And that's
22 after appropriate strain construction one is able
23 to infect an animal -- in our case, a mouse --
24 and then recover the bacteria at different time
25 points and, by affecting the degree of
26 recombination, affect the level of expression

1 that that particular gene -- in our case, we'll
2 expand an fha and ptx -- has experienced.
3 And we plan to use this to determine
4 if the differential regulation seen in vitro with
5 fha and ptx develop in a manner accepted in vivo
6 and, secondly, to determine the effect of
7 mutation -- for example, which knock out
8 important adhesions such as fha -- on the
9 expression of pertussis toxin.
10 And then, finally, Dr. Brian James has
11 just very recently joined us, actually, since the
12 site visit, and he joins us from Dr. Robert
13 Bender's lab at University of Michigan. And
14 Brian is responsible for getting our anthrax
15 project up and running, and in the initial phase
16 he's developing new genetic tools specifically
17 for powerful molelic exchange vectors and an
18 enhanced efficiency of DNA transfer, and then we
19 plan to use those tools to study genetic
20 regulation of virulence in anthrax. And I'll
21 stop there.
22 DR. DAUM: Thank you very much, Dr.
23 Stibitz. Dr. Stibitz' interesting presentation
24 is now open for committee discussion and comment.
25 (No response.)
26 I was wondering if you could possibly

1 say a couple of sentences about the big-picture
2 role of fha and the pathogenesis of pertussis --
3 why is this of particular importance? Of the
4 vaccine antigens that we talk about and bandy
5 around their importance of the acellular
6 vaccines, why this particular antigen important
7 to the pathogenesis?
8 DR. STIBITZ: Well, I guess the most
9 sort of convincing evidence of that is that the
10 mutants of pertussis which lack expression of fha
11 are decreased -- they have decreased ability to
12 adhere to host cells, and in some models have
13 decreased virulence.
14 DR. KATZ: If you ask John Robbins, he
15 says it doesn't do anything.
16 DR. STIBITZ: Fair enough. I think it
17 is safe to say that the animal models that we use
18 may not be the best animal models for assessing
19 the effect of fha, but at least, you know, one
20 aspect of -- I think whether or not fha is
21 absolutely important in infection in a mouse
22 model, we can still assess the degree of in vivo
23 regulation using the system that we're going to
24 use.
25 DR. KATZ: I guess what I would like
26 you to comment on -- I was one of the visitors,

1 so I heard this, but I don't know if the
2 committee members did -- what is the relevance of
3 Bordetella research to a laboratory of enteric
4 and sexually transmitted diseases?
5 DR. STIBITZ: Yes, I get this question
6 every time.
7 DR. DAUM: I imagine you do.
8 DR. STIBITZ: There are two answers to
9 that. One is sort of the historical story of how
10 I came to be where I am, and I don't think that's
11 particularly relevant. I think what's more
12 relevant is the fact that the type of approach
13 that we take, until recently, we were probably
14 the only group within the division taking a very
15 genetic approach. That set of knowledge has
16 turned out to be very useful for the type of
17 products which we particularly find in the
18 Enterics Lab. For example, rationally attenuated
19 live vaccine which use the exchange methods that
20 we use often in pertussis and actually developed
21 for use in pertussis, and then also issues
22 generally relating to mutation rate, population
23 genetics, and so forth, that impact upon genetic
24 stability, and these also impact upon live
25 vaccine preparation, as well as issues that
26 relate to products we see, such as the

1 replacement flouro-type of approach that Dennis
2 mentioned, as well as bacteria therapy, which is
3 also something that's coming to the fore again.
4 So, I think pertussis itself is not
5 relevant. I think the system that we study, the
6 regulatory system, is widely relevant to
7 bacterial pathogenesis as a whole, including
8 enterics, and then our specific method technology
9 base help us greatly in the reviews that we do.
10 DR. DAUM: Thank you.
11 MS. FISHER: Dr. Daum?
12 DR. DAUM: Yes.
13 MS. FISHER: Barbara Fisher. May I
14 ask a question?
15 DR. DAUM: Please.
16 MS. FISHER: Is any of your work going
17 to lead us to genetic screening techniques for
18 those who would be at high risk of potentially
19 reacting, say, to pertussis vaccine?
20 DR. STIBITZ: I don't believe so. In
21 other words, that would be primarily a host
22 characteristic, and our techniques are not
23 designed to assess that aspect of the
24 host/parasite relationship.
25 DR. DAUM: Thank you, Dr. Stibitz.
26 Are there other committee questions or comments?

1 DR. PALESE: Yes. Peter Palese,
2 please. Can I ask a question about the anthrax?
3 DR. DAUM: Certainly.
4 DR. PALESE: A new person who is
5 coming, we work on real characteristics of the
6 anthraces. Now, are there facilities available
7 at your institution, or how are you going to test
8 for A-virulence factors?
9 DR. STIBITZ: Well, initially, we're
10 trying to develop new genetic tools, and that may
11 take some time. When we do get to the point
12 where we're working directly with anthrax, much
13 of our work can be done with the A-virulent
14 strain. At this point, we're seeing a need for
15 doing actual work with fully virulent anthrax,
16 although, if that happens, we do have a petri
17 facility. In fact, we're actually going one
18 better and we're doing much of the initial work
19 in Bacillicereous, which is a very close relative
20 of anthrax, but much, much less virulent. Does
21 that answer your question?
22 DR. PALESE: Yes. Thank you very
23 much.
24 DR. AGUILAR-CORDOVA: This is Estuardo
25 Aguilar. I wonder, Dr. Stibitz, if you could
26 comment -- I was also on the review team -- but

1 if you could comment on the independence of Dr.
2 Boucher within the work that you are doing and
3 the collaboration that involved.
4 DR. STIBITZ: The word you use is the
5 word I would use to describe it. Basically, Phil
6 brings a set of knowledge and tools to the
7 problems that I don't possess, and he's developed
8 all the biochemistry. He's done that essentially
9 on his own. I work in some of the mutant
10 construct, and so on and so forth, but Dr.
11 Boucher has done all that biochemistry from the
12 ground up, and it's a project that he initiated
13 and it's really a collaboration. I did the
14 genetics and he does the biochemistry. If you
15 have more specific questions, I could elaborate,
16 but the degree of independence is very high.
17 DR. DAUM: Dr. Palese, a followup?
18 (No response.)
19 All right. Thank you. Okay. Other
20 committee questions, comments?
21 DR. SACHS: I just have a very quick
22 comment. This is Jody Sachs. I just wanted to
23 remind everybody that a Transcriber is recording
24 this telecon, and it's easier for the Transcriber
25 if everybody repeats their name before speaking
26 when they comment. Thank you.

1 DR. DAUM: Thank you.
2 DR. AGUILAR-CORDOVA: This is Estuardo
3 Aguilar one more time.
4 DR. DAUM: Please.
5 DR. AGUILAR-CORDOVA: Just another
6 quick question for either Dr. Stibitz or Dr.
7 Kopecko. Both of you have mentioned an increased
8 number of people. Is there also an increase in
9 space that's being considered?
10 DR. STIBITZ: Do you want to field
11 that one, Dennis?
12 DR. KOPECKO: Yes, I will. This is
13 Dennis Kopecko. Yes, we do have an increase in
14 space. We still would like to get more space
15 because even with renovations that are ongoing,
16 we are still going to be fairly cramped. But
17 within the limitations of the division and the
18 Center, we can't ask for anymore, really,
19 currently. But, yes, there is some additional
20 space, and certainly some additional funding for
21 per capita research.
22 DR. DAUM: Thank you.
23 DR. KATZ: Refreshing refrain. This
24 is Dr. Katz. You said you were going from five
25 to 14 people, which is what you said in your
26 introductory remarks. Those additional nine

1 people, at what level are they scientifically?
2 Are these doctoral, post-doctoral, what level
3 people are these?
4 DR. KOPECKO: Good question. Let's
5 see here. If we go back to Section 2, to the
6 organizational chart, you'll see that in the
7 Molecular Pathogenesis Section, there are two
8 Post-Doctoral level individuals joining. Dr.
9 DeQi Xu, who just recently joined within the past
10 week, who is going to be working on Shigella
11 vaccines, and a second Post-Doctoral Fellow to
12 work on Campylobacter pathogenesis. Dr. Stibitz
13 mentioned two additional individuals, Dr. Wendy
14 Veal Carr and Dr. Brian James, who joined at the
15 Post-Doctoral level. Dr. Manuel Osorio is a
16 Staff Fellow Ph.D. Cellular Immunologist, who
17 joined us back in July, in the Immune Mechanisms
18 Section, and then we have an additional beginning
19 research technician, and that sort of fills out
20 the field. So, mostly professional level, on one
21 technical level.
22 DR. KATZ: Thank you.
23 DR. DAUM: Other comments, questions?
24 DR. FAGGETT: This is Dr. Faggett.
25 One question relative to constant research versus
26 regulatory. What is the breakout in terms of how

1 much time -- sounds like the new Fellows will be
2 doing primarily the research -- but in terms of
3 how much time just on a regulatory?
4 DR. KOPECKO: This is Dr. Kopecko.
5 That's a very good question. In order to carry
6 out competitive research, you obviously have to
7 have some people in the lab that spend the
8 majority of time there. And what Scott and I
9 have sort of evolved into is a situation where he
10 and I take the majority workload for regulatory,
11 so we're splitting up around 40 to 45 IND
12 applications each, and then the remaining 20 to
13 30 are parceled out with other members of the
14 laboratory, that way trying to keep most of the
15 other individuals in the lab most of the time,
16 but they are all involved to some extent with
17 regulatory, other than a couple of the Post-
18 Doctoral Fellows who are not FDA personnel, but
19 they are actually individual contractors.
20 DR. KATZ: This is Dr. Katz. I think
21 when we made our review, if I remember correctly,
22 Dr. Kopecko, you told me 50 percent of your time
23 is spent on regulatory, is that correct?
24 DR. KOPECKO: That's correct.
25 DR. KATZ: So, I think that gives you
26 a little idea, Walter.

1 DR. FAGGETT: Thanks, Sam.
2 DR. DAUM: Other input?
3 (No response.)
4 Good. thank you, everybody, for your
5 comments. And let's move on to hear from Dr.
6 Boucher, who will give an overview of his
7 research activity. Dr. Boucher, welcome.
8 DR. BOUCHER: Thank you. I thought I
9 would very briefly summarize the work I presented
10 to the Site Visit Team a couple of months ago,
11 then discuss a current project, and then finish
12 off with what is planned for the future.
13 My interest in this lab since my
14 arrival here in 1994 has been on a biochemical
15 approach to an understanding of how a bacterial
16 response regulator effects control of expression
17 of virulence factors. This work has evolved from
18 early investigations describing how the
19 phosphorylated BvgA regulator of Bordetella
20 pertussis binds to simple promoters to more
21 recent experiments that have global implications
22 for molecular genetics in bacteria.
23 Initial studies employing DNA binding
24 and both in vivo and in vitro transcription
25 assays formed the basis of our understanding as
26 to how BvgA activated transcription at the fha

1 and ptx promoters that have already been
2 discussed by Scott. For example, it was
3 discovered that activation of both promoters
4 involves multiple dimers organized in such a way
5 as to suggest an interaction with the C-terminal
6 domain of the alpha subunit, one of the
7 polymerase subunits.
8 Again, as Scott mentioned, the most
9 recent work presented a couple of months ago will
10 be coming out in the January issue of Molecular
11 Cell, so I'll be going through it very briefly
12 here. Using a technique termed affinity cleavage
13 of DNA, we have developed a structural model of
14 how both the BvgA regulator and RNA polymerase
15 interact at a virulence factor promoter,
16 specifically the fha promoter.
17 The technique is based on the ability
18 of a chemical nuclease to cleave DNA primarily at
19 sites dictated by the binding specificity of the
20 DNA-binding protein to which it is bound.
21 Analysis of cleavage patterns generated by such
22 modified proteins bound to promoter DNA allows
23 the development of structural models capable of
24 addressing certain critical questions such as how
25 many molecules of a given regulator bind a
26 particular promoter and how are the different

1 proteins in a transcription initiation complex
2 oriented relative to each other.
3 Using this technology, we described a
4 novel architecture in which multiple dimers of
5 BvgA bind along one fact of fha promoter DNA
6 while a part of the polymerase complex, the alpha
7 C-terminal domain, binds to the opposing face
8 along the same linear stretch of DNA.
9 In addition, modeling studies revealed
10 that the orientation of BvgA monomers within the
11 dimers is the same as that recently demonstrated
12 by x-ray crystallographic methods for the C-
13 terminal domain of the related response regulator
14 called NarL bound to its DNA-binding site.
15 Since these experiments were
16 conducted, we have extended our analysis of
17 transcription initiation complexes to other
18 virulence factor promoters of Bordetella
19 pertussis. Previous studies using relatively low
20 resolution analyses made certain predictions
21 whose validity, in light of our current
22 understanding, were now being brought into the
23 question. In the pertussis toxin promoter, for
24 example, stoichiometry of BvgA binding and novel
25 orientations for how BvgA maneuvers itself onto a
26 very unusual binding site had previously been

1 suggested.

2 Using our affinity cleavage assay, we
3 recently determined that the previous studies had
4 underestimated the number of BvgA dimers bound to
5 the toxin promoter and that the unusual BvgA
6 binding site within this promoter bound 2 dimers
7 of BvgA in a very usual and unconstrained manner.

8 At another promoter, the bipA
9 promoter, we and others have shown that
10 activation at this promoter requires a
11 concentration of BvgA that is intermediate
12 between that required to activate the fha
13 promoter and that required to activate the toxin
14 promoter. Indeed, at the relatively high in
15 vitro concentrations needed for toxin activation,
16 the bipA promoter appears to be repressed. Our
17 in vitro affinity cleavage assay has aided us in
18 distinguishing sites that are likely involved in
19 activation from sites that are likely involved in
20 repression. Studies of other critical virulence
21 factor promoters are currently ongoing.

22 Conclusions from the work just
23 described also suggested other interactions
24 between BvgA and the RNA polymerase. For
25 example, our data suggests that BvgA may interact
26 with one or both of the following: The C-

1 terminal domain of the sigma subunit of RNA
2 polymerase and the N-terminal domain for the
3 alpha subunit of RNA polymerase.
4 Experiments utilizing both an in vitro
5 biochemical approach and an in vivo 2-hybrid
6 protein-protein interaction assay are underway to
7 address these possibilities. As there are only
8 two copies of the alpha subunit per polymerase
9 complex, it is also important to understand
10 whether both copies are required for full BvgA-
11 dependent promoter activity. In vitro
12 transcription experiments are also underway to
13 address this question.
14 And, lastly, another project is in the
15 proof of concept phase of investigation. How
16 exactly phosphorylation of BvgA engenders an
17 active complex is not fully understood. Based on
18 structural data of unphosphorylated forms of
19 homologous proteins, it has been suggested that
20 phosphorylation results in the unmasking of the
21 DNA Binding Domain.
22 Perhaps due to the instability of the
23 phosphorylated form of response regulators, it
24 has thus far not been possible to crystalize this
25 form. In an attempt to circumvent these
26 limitations, we are opting for the use of a

1 physical method that would structurally compare
2 the two forms of BvgA. This technology termed
3 FRET, for florescence resonance energy transfer,
4 is based on the ability of an activated
5 flourophore to transfer its resonance energy to a
6 neighboring flourophore.
7 As the probability of this transfer is
8 dependent on the distance separating the two
9 flourophores, molecular distances can be
10 calculated if energy transfer is monitored for a
11 series of flourophore pairs distributed within a
12 single biomolecule. If measurements are made for
13 each pair in both the phosphorylated and
14 unphosphorylated forms of BvgA, a model can be
15 derived that describes the motion of one domain -
16 - for example, the C-terminal DNA binding domain
17 -- relative to another -- for example, the N-
18 terminal masking domain. The construction of
19 preliminary BvgA mutants that will eventually
20 harbor flourophore pairs is currently underway.
21 That about concludes my brief summary
22 of my lab activities, and I can entertain any
23 questions.
24 DR. DAUM: Dr. Boucher's presentation
25 is open for committee comment and questions.
26 (No response.)

1 I would like to ask the same question
2 I asked of Dr. Stibitz, if you wouldn't mind, and
3 that is, can you tell us a little bit about how
4 fha, which is good after some impressive
5 regulation, factors into pertussis status
6 physiology, and how important it is to the
7 conduct of the organism or the ability of the
8 organism to go about its business?
9 DR. BOUCHER: Certainly. First of
10 all, it is clear that fha, the expression of fha
11 in Bordetella plays an integral role in its
12 pathogenesis. Without the fha, it really has no
13 clear way of adhering to epithelial cells.
14 However, with the work that I am conducting, fha
15 really here is looked upon more as a readout of
16 the activity of the two-component systems that
17 we're studying, and less really as an antigen for
18 any possible vaccine.
19 DR. DAUM: I understand that, but it's
20 still an interesting issue for me, so I have to
21 ask it anyway.
22 Other committee comments? Questions?
23 (No response.)
24 Input?
25 (No response.)
26 Dr. Boucher, thank you.

1 DR. BOUCHER: Thank you.
2 Jody, I think it's that bewitching
3 time for the open public hearing. Do we know if
4 there's anybody interested in addressing the
5 committee?
6 DR. SACHS: No, but I'll just read a
7 statement.
8 As part of the FDA Advisory Committee
9 meeting, we are required to hold an open public
10 hearing for those members of the public who are
11 not on the agenda, who would like to make a
12 statement concerning matters pending before the
13 committee.
14 I have not received any requests at
15 this time. If there is anyone who would like to
16 speak -- address the committee at this time, this
17 is the opportunity. And I look around the room
18 where I am right now, and there's no one from the
19 public wishing to speak. So, I will close the
20 open public hearing and ask you, Dr. Daum, to
21 continue the meeting.
22 DR. DAUM: That sounds fine. So,
23 we're going to move into closed session at this
24 point, and I guess that's going to ask those
25 individuals who need to take their leave at this
26 point, to please do so.

1 (Whereupon, at 2:00 p.m., the open
2 portion of the meeting of the Advisory Committee
3 was concluded.)
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