1   Let's see. Is that number one? Could you

2   go back one just to make sure I didn't hold it down 12

3   times? Yeah, okay.

4   So what I'd like to do initially is talk

5   about this basic premise about tumorigenicity as it

6   relates to cell substrates. I think that's an area

7   of concern when I read over the documentation. The

8   potential risk statement that Dr. Lewis has provided:

9   what are the determinants of experimental tumor

10   formation that are being used to characterize these

11   tissue culture cells used for vaccine production?

12   And then some general observations

13   thinking about experimental tumorigenicity as the data

14   that we understand from that relate to potential risk,

15   and then, as I mentioned, tell you something about E1A

16   induced sensitivity to apoptotic injury and E1A

17   induced rejection of cells expressing these proteins,

18   and then just a very little bit at the end about E1A

19   in humans.

20   So the focus here is on the E1A as the

21   immortalizing, enabling oncogene in the cells that are

22   being created, and I guess theoretically its potential

23   risk as a contaminating bit of DNA that would go

24   across in the vaccine.

25   So the potential risk statement cut down

1 . a bit says something like tumorigenic cells are more

2 risky than non-tumorigenic cells as vaccine

3 substrates, and that seems to be a focus of -- an

4 appropriate focus of concern, and taking that on

5 directly, I think, is an interesting thing to do, and

6 this comes, as Dr. Lewis mentioned, from this Armed

7 Forces Experimental Board in 1954 where there was a

8 preference for normal cells over human tumor cells,

9 not cells like 293 or PER.C6.

10 And so I think the question at least as I

11 think of it is the concern about the use of human

12 tumor cells, like HeLa or something of that sort, the

13 same thing as the concern about using human cells that

14 form tumors. Those things may seem to be similar, but

15 I think they're different, and that is form tumors in

16 nude mice.

17 So what I'm going to try to convince you

18 is that I think experimental tumorigenicity as we

19 measure it is not the same thing as primary tumor

20 development because I think this is an important

21 distinction. We're talking about the tumorigenicity

22 of E1 expressing cells or E1 immortalized cells, and

23 I don't think that's the same thing as tumor formation

24 from a single immortalized or mutated cell _in vivo_

25 that goes on to successfully form a tumor in us or in

1   a mouse.

2   And it's important, I think, not to

3   confused these things.

4   So the variables that can be affected,

5   that can affect experimental tumor formation, that is,

6   the ability to form tumor in an animal like a nude

7   mouse with a set of tissue culture cells, the first

8   thing is the host that you select. That makes a big

9   difference.

10   And this shows you something about some

11   experiments we've done where you can see a huge host

12   difference in the apparent tumorigenicity of the

13   cells.

14   Let me crank this up.

15   So these are three sets of types of mice.

16   These are normal C57 Black 6 adult mice. These are

17   C57 Black 6 mice that are nude and, therefore, lack of

18   functional thymus and lack of functional T cells that

19   have an intact natural killer cell response, a

20   different kind of host defense.

21   And these are CD3-epsilon transgenic mice,

22   and it's not interesting to know what they are, except

23   for the fact that they lack both T cell and NK cell

24   responses.

25   And then what's been done here is to take

1 a mouse sarcoma that is a relatively non-immunogenic

2 mouse sarcoma, and this is collaborative study done

3 with Jack Routes, and these cells were either tested

4 in the three types of mice, either in their E1A

5 negative parental state or in the state that they

6 would be after being transfected with the E1A gene

7 alone, no E1B, not the whole E1 region, just the E1A

8 gene.

9 And the question was: did E1A expression

10 affect the tumorigenicity, and if so, did that relate

11 to the type of animal that you use to test tumor

12 development?

13 And the answer is obvious. All of the red

14 lines are the control cells not expressing E1A, and

15 all of the green lines are the efficiency of tumor

16 formation using the parameter that Dr. Lewis

17 mentioned, the TPD-50, the efficiency of tumor

18 formation by the cells in the different animals.

19 And the lower the TPD-50, the more

20 efficient tumor formation occurred. So you can ignore

21 all of the red lines because they're essentially all

22 the same, and that is the non-E1A expressing sarcoma

23 cells were very efficient at forming tumors in all

24 three types of animals. The E1A expressing cells were

25 essentially unable to form tumors in normal mice,

1   formed tumors sort of in a medium fashion reasonably

2   well in nude mice, those that lacked a T cell

3   response, and formed tumors with equal efficiency as

4   the E1A negative cells in the animals that lacked both

5   types of cellular immune defenses.

6   And so the type of host you pick

7   determines a lot about the type of answer you get.

8   You can say that this cell is, quotes, non-

9   tumorigenic, whereas this cell is highly tumorigenic,

10   but it really just depends on the animal chosen. So

11   I think that's clearly an important parameter to

12   consider.

13   The next thing that's important is the

14   threshold effect. Dr. Lewis mentioned numbers of

15   cells used to challenge animals, and so the cell dose

16   you pick determines the result you get as well. So,

17   again, you could create a non-tumorigenic cell by just

18   picking too low a dose.

19   And here is an experiment from a study

20   that Dr. Lewis did with these Adeno. 12 transformed

21   mouse cells. These were Balb/c cells, and they were

22   inoculated into adult immunocompetent animals at

23   various doses over time. This is the number of cells

24   injected. This is the tumor incidence of these

25   animals.

1   And what you can see is there are

2 essentially no tumors up to 100,000 cells. So if you

3 happen to get unlucky and pick 100,000 or ten to the

4 fifth cells as your number, you'd call these non-

5 tumorigenic cells.

6   On the other hand, if you picked 100

7 million or 10 million cells as your dose, you'd say

8 they're highly tumorigenic. So dose matters. Host

9 matters, dose matters.

10   The next thing is the rout of inoculation.

11 This is something that's not as well quantitated, but

12 there's no question in my mind that the route that is

13 used for a given challenge makes a difference, and in

14 our experience intraperitoneal inoculation results in

15 an apparently more efficient tumor positive result

16 than subcutaneous inoculation, and there are other

17 kinds of things you could do, like inoculate into the

18 immunologically privileged sites.

19   Another result is to think about the

20 observation period. How long do you wait before you

21 score tumor development versus non-tumor development

22 when you're doing an experimental tumor formation

23 assay?

24   This in our laboratory is a notorious

25 experiment because we did a collaboration with another

1   investigator. He had published a paper saying that

2   human breast cancer cells expressing the E1A gene were

3   non-tumorigenic and E1A had, in fact, converted these

4   cells into non-tumorigenic cells. It had reversed

5   their tumorigenicity.

6   Well, the thing was he looked at these

7   cells only after about 14 to 18 days. We repeated the

8   experiment, and so what's shown here is the percentage

9   of animals that are tumor free over time after a

10   challenge, with the breast cancer cell itself. These

11   are all nude mice. So they lack T cell responses,

12   have some NK cell defenses. So this is the E1A

13   negative breast cancer cell. This is a ductile

14   epithelial cell carcinoma.

15   There was the E1A expressing cells, and

16   sure enough, if you look at about two to two and a

17   half weeks, you get zero tumor formation or 100

18   percent tumor free animals, whereas all the animals

19   that had been inoculated -- that's not true -- some of

20   the animals that had been inoculated with the E1A

21   negative cells developed tumors, and that tumor

22   incidence continued to increase over time or the tumor

23   free number decreased.

24   But if you waited a bit longer, then the

25   E1A positive cells begin to make tumors in up to 20

1   percent of the animals over time. So if you get

2   unlucky and pick a very short observation period, you

3   might come up with the wrong conclusion and call a

4   cell nontumorigenic.

5   So the host matters. The cell dose

6   matters, and the route of inoculation matters, and how

7   long you wait to call the endpoint matters.

8   Well, then the last thing, I don't have

9   any data to show on this, but there are several other

10 things when you look at the literature that can make

11 a difference in the methodology chosen to determine

12 whether cells make tumors or don't.

13   One thing that I find interesting because

14 almost all of these experiments are done by sticking

15 a needle into a mouse or a hamster or a rat, injecting

16 some cells into that needle track, and then asking

17 whether they form tumors or not. That's not normal.

18 That's not what happens after a UV irradiation or

19 exposure to a carcinogen. This causes some kind of

20 trauma at which site the cells now have a chance to

21 grow.

22   And so I think there may be a wound

23 effect, and we were talking earlier about some of the

24 observations that have been made in Rous sarcoma virus

25 where tumors form at the site of wounds.

1   That is an artifact that can affect tumor

2   formation, I think. Foreign bodies can affect tumor

3   development as well. For example, you can attach

4   cells to plastic plates and put them into animals and

5   get tumors. These cells wouldn't form tumors at all

6   through any route of inoculation like 3T3 cells if you

7   didn't attach them onto these little disks, and so the

8   foreign body can make a difference in your tumor

9   assay.

10   So you have to be careful about how you

11   interpret those, and the other thing that can happen

12   is if you just provide fibroblast as a mixed culture

13   in with your putative tumor cells, you can get tumor

14   forming efficiency that is much greater. So there is

15   some feeding effect or something that goes on when you

16   can do certain kinds of mixed culture inoculations.

17   So my point is that tumorigenicity is not

18   all the same. It depends on how you rig the system,

19   how you set it up. All of these experiments can be

20   used quite effectively to ask questions about one cell

21   versus the other, but I don't think they are anything

22   like at least in my mind what goes on when you get a

23   spontaneous single transformation event and ask that

24   cell to grow into a tumor, especially when you think

25   about cell dose.

1   There are very few spontaneous

2   transformation events that result in the immediate

3   generation of 100 million cells or ten million cells.

4   So what I'd like you to think about as a

5   possibility is the fact that tumor formation is not

6   the result of a single activity at least as we look at

7   it in experimental tumorigenicity, but it's really an

8   orchestra of events, and if all of these things aren't

9   working right, you don't get a very good outcome or in

10   our case you don't get a tumor.

11   You need to have in most cases that we

12   know about continuous oncogene expression or

13   continuous in our case E1A expression. That probably

14   isn't always true, but almost always true.

15   That gene has to successfully cause cell

16   cycle disregulation, and it probably has to hit to

17   Achilles heel in the cell cycle that relates to what

18   some people call the p53 Rb pathway where something

19   has to occur to cause that to go out of whack.

20   Otherwise you don't get immortalization.

21   The cells gave to survive this, and it's

22   important to think about the fact that when you try to

23   over express E1A in normal mammalian cells it usually

24   kills them. So it is a proapoptotic event itself. So

25   it's not easy if you're trying to transfer a lot of

1 . E1A and some contaminating DNA to get those cells to

2 survive because they would tend to die as a result of

3 E1A expression itself.

4   Then you have to have this magical

5 threshold cell number. I don't think single E1A

6 positive cells probably ever form tumors. I was

7 taught early in medicine never to say "never" or

8 "ever," but I think it's probably true that it's

9 probably true that it's very hard to get a tumor with

10 a single E1A positive cell. You probably need

11 millions at least.

12   There probably would have to also be local

13 tissue factors. Steve Frisch from California has

14 shown that if you plate E1A positive cells on collagen

15 matrices as opposed to plating them on plastic, they

16 tend to do poorly. So you could argue that maybe the

17 reason that E1A positive cells grow poorly in nude

18 mice compared to normal mice is that they don't like

19 being on biometrices and that tends to be inhibitory

20 to their growth. Most of us grow them on plastic

21 dishes and they look fine.

22   And then they also have to escape from

23 this cellular immune response that I described in this

24 three-mouse experiment. If they don't escape from

25 that, then the cells are likely to be killed, even if

1   they make it through all of these other hurdles.

2   So I think it's very unlikely that this

3   combination of events would occur in a well

4   orchestrated fashion with transmitted DNA without a

5   lot of help.

6   I'm sorry. Would you go back one?

7   This is just a summary of a lot of work

8   done on trying to characterize how E1A sensitizes

9   cells to a variety of proapoptotic injuries. We've

10   mostly been interested in immunological injuries since

11   they relate more to whether a cell will form a tumor

12   or won't form a tumor in vivo, but a number of other

13   injuries do the same thing, and that tells us

14   something about the mechanism, I think.

15   So if you look at a variety of types of

16   things, chemotherapeutic drugs, hydrogen peroxide,

17   potassium ionophores, irradiation; this is a protein

18   synthesis inhibitor; where the things we're more

19   interested in like natural killer cells, cytolytic T

20   cells, TNF alpha, or the TNF receptor apoptosis

21   inducing ligand trail, all of these things can

22   selectively induce apoptosis in an E1A expressing

23   cell.

24   So there were a lot of things going

25   against E1A positive cells in trying to survive in

1   vivo or trying to survive when exposed to a variety of

2   types of injuries.

3   This may say something about the pathway

4   through which E1A sensitizes, but what it does say is

5   that an E1A positive cell doesn't have an easy time of

6   it trying to survive in vivo, especially when you

7   consider these kinds of defenses.

8   It's also important that these events,

9   this sensitization of cells to immunological mediated

10   proapoptotic outcomes are not necessarily dependent on

11   normal cell genetics. So p53 minus cells are cells

12   that lack expression of the Rb gene, are also

13   sensitized by E1A just like normal cells are.

14   So if you wanted to postulate the worst

15   scenario, you'd say, well, maybe E1A would be

16   transmitted, and it would hit a cell that already had

17   a mutation and that would give a chance for a bad

18   outcome.

19   The fact is the most common mutations in

20   human cells that lead to neoplasia are in this

21   pathway, E1A sensitized cells expressing mutations in

22   that pathway to immunological injury. So they're not

23   going to be protected by that second mutation.

24   This just shows you something about the

25   range of experiments that have been done with human

1   cells. This is just an experiment with natural killer

2   cells to show you that human cells are very much like

3   other cells.   These are mouse 3T3.   This is a

4   continuous rat line that was immortalized with ras.

5   This is BHK21, a spontaneously transformed hamster

6   cell line. THE BHK21 is highly tumorigenic, and its

7   tumorigenicity is eliminated by expressing E1A.

8   These other lines, all when expressing E1A

9   become more sensitive to natural killer lymphocyte

10   induced lysis, and this is just a human fibrosarcoma

11   cell line, stably expressing E1A, and it does the same

12   thing.

13   If you generalize this to other types of

14   things that we know about, a variety of types cells

15   from   different   tissue   origins   from   humans   are

16   sensitized to various apoptotic injuries. Epithelial

17   cells, notable among those 293. We've also looked at

18   this breast cancer cell I told you about; fibroblastic

19   cells. This is a fibrosarcoma line.

20   Hematopoietic cells, K562 has been shown

21   by Stiewe   in a   publication   in   2000   to   become

22   sensitized   to   reactive   oxygen   intermediates   when

23   expressing E1A, but not when not expressing E1A, and

24   ACLS2, which is an interesting cell line because it

25   doesn't express either p53 or Rb, when it expresses

1   E1A becomes much more sensitive to natural killer cell

2   injury, and I've already told you about some of the

3   injuries. These are the ones that have been tested

4   against human cells expressing E1A.

5   So this is like the atomic chart or the

6   intermediary metabolism pathway. It's one of these

7   things that we never remember, and I just show it for

8   the purpose of saying that E1A probably hits multiple

9   targets in the apoptosis cascade. This is an

10   incomplete representation of what goes on during

11   apoptosis, but there are preliminary data. This isn't

12   well worked out, but there are data that E1A causes

13   over expression of p53, which can drive apoptosis.

14   E1A positive cells -- this was all done in 293

15   cells -- express some kind of what's called an

16   oncogene associated factor that is probably like a

17   mitochondrial-like factor that can be involved in

18   apoptotic activity.

19   E1A can probably facilitate the conversion

20   of Procas Base 8 to Cas Base 8 (phonetic). In certain

21   cell types, it can increase cell surface depth

22   receptor expression, and we've done several studies

23   now, and I've just submitted a publication about E1A

24   repression of the cellular NF kappa B (phonetic)

25   defense against apoptosis.

1   So probably the bottom line is that there

2 are multiple sites in cells that are targets for E1A

3 rendering cells sensitive to a variety of apoptotic

4 injuries, especially immunological ones.

5   So the last point is E1A in humans, and I

6 guess to try to put the idea of contaminating E1A in

7 perspective, we should think about what goes on

8 elsewise (phonetic), and that is there are studies.

9 This is a study by Jimmy Hogg from Canada, from his

10 laboratory, that shows that E1A actually may persist

11 in normal human tissues after infection.

12   In these studies what they did is they

13 took a lot of people that had lung disease, and they

14 asked whether those that progressed to emphysema or

15 chronic bronchitis had something unusual about them,

16 and I think the truth be told, he had somebody who had

17 done a post doc in Alex's laboratory or somebody's who

18 had learned how to look for E1A, and they looked for

19 E1A in these lung tissues, and what they found was a

20 lot of E1A sequences in both normal people and those

21 that progressed to emphysema based on PCR technology.

22   So it's not easy to do, but when people

23 have looked, they have found E1A, at least in this one

24 laboratory, have found E1A in normal tissues, and that

25 may speak to the persistence of adenoviral infection.

1   It may say that there is integration that goes on that

2   we aren't very good at detecting, but it's probably

3   not true that E1A is never around and these viral

4   oncogenes are never expressed in humans anyway, even

5   vaccine recipients.

6   And then there are two clinical trials

7   that are going on now and fairly extensive studies in

8   which E1A is actually being used therapeutically.

9   It's being put into people that have cancer to ask

10   whether it can help reject tumor cells. One of those

11   is in a company that has the name of the virus that's

12   called ONYX. It's really an old Arnie Levine that has

13   a mutation that allows it to selectively kill p53

14   minus cells, which many human tumors are.

15   So this virus infection is given to people

16   who   have   p53   minus   tumors,   and   that   virus

17   preferentially replicates in those tumors and destroys

18   the tumor tissue preferentially to normal tissue.

19   It's not that simply, but that's the idea.

20   There is also an M.D. Anderson study in

21   which E1A is being used in gene therapy to inject in

22   liposome vectors into people that have, for example,

23   metastatic   ovarian   or   peritoneal   metastasis   with

24   ovarian carcinomas, and it's also being considered for

25   breast carcinomas.

1   And here the idea is that E1A represses

2 expression of a cell surface receptor that's necessary

3 for growth factor stimulation of the tumor cells. So

4 not only do we probably have E1A already in our

5 tissues. If we were to look carefully and certainly

6 can support persistent infection with adenoviruses,

7 but E1A is being used therapeutically in humans, and

8 so far now admittedly these aren't long-term

9 vaccinations of babies, but so far there's no evidence

10 from the safety testing that this has caused an

11 adverse outcome in these patients.

12   So my conclusions are that experimental

13 tumor formation in nude mice does not predict

14 tumorigenicity of an isolated oncogene, in this case

15 E1A. Even if the E1A oncogene were transmitted in a

16 contaminating DNA, it's very unlikely that it would

17 become stably expressed in recipient cells because of

18 transfection inefficiency. Dr. van der Eb just told

19 you how hard it is to transfect human cells with

20 sheared adenovirus DNA or certainly with E1A itself

21 it's actually even harder.

22   Direct apoptosis that occurs during

23 attempted establishment of these cells would probably

24 kill many of the cells in which the DNA would try to

25 get itself inserted.

1   Even if E1A did become stably expressed in

2 recipient cells, it's very unlikely that such cells

3 would provide a tumorigenic risk because there

4 wouldn't be a threshold cell dose. You wouldn't have

5 the millions of cells up front that you'd need to get

6 over the hurdle of getting tumor formation initiated.

7   Now, the viability of the E1A positive

8 cells on biometrics, Steve Frisch's data, suggesting

9 that these cells do relatively poorly when cultured on

10 things like we might have in our soft tissues, and the

11 susceptibility of these cells even if they did get

12 established to immune mediated apoptosis, I think,

13 would rule against their survival.

14   And then also we have to think about the

15 fact that expression of E1A in humans might be normal,

16 and when therapeutically used in humans, it can

17 actually be directly apoptotic and can reduce

18 expression of critical growth factor receptors.

19   So I think E1A expression as a

20 contaminating force from the vaccine would probably

21 not likely be a major problem.

22   I'd be glad to answer questions.

23   ACTING CHAIRMAN DAUM: Thank you very

24 much, Dr. Cook, for an enlightening presentation.

25   We'll take a few questions. Dr. Griffin,

1   then Dr. Stephens, please, and then Dr. Faggett.

2   DR. GRIFFIN: It's my understanding that

3   the cells we're talking about express both E1A and

4   E1B.

5   DR. COOK: Right.

6   DR. GRIFFIN: And that E1B is a counter

7   force, an anti-apoptotic factor. So how does that fit

8   in with all of your conclusions that you're making

9   about the safety of E1A when you're really talking

10   about giving both?

11   DR. COOK: Right. I specifically didn't

12   talk about E1B.   There's no evidence that E1B

13   expressed alone does anything in terms of its ability

14   to immortalize cells.

15   They're looking at both. The only reason

16   that E1B is interesting in the contest of E1A is it

17   allows E1A -- it prevents E1A from destroying cells.

18   So without E1B what you get is a tremendously

19   inefficient immortalization event, very few colonies

20   formed. So E1B really has to be around to prevent the

21   apoptotic response and to bind p53, the two proteins

22   that E1B makes.

23   So I don't think that E1B is a factor.

24   All I can tell you is when it gets down to what I

25   think is the key event at least from our perspective

is that if you look at the immunological injuries and ask whether they still happen, from a killer cell point of view, a natural killer cell, a cytolytic lymphocyte or an activated macrophage still kills cells expressing E1B perfectly well, even though it does it in an E1A specific manner.

The only thing that E1B can do in human cells when co-expressed with E1A is to repress the TNF induced apoptotic response. That's a unique thing that Linda Gooding showed years ago.

So I don't think E1B really adds a whole lot to the mix, other than allowing the cells to be immortalized in the first place.

Then the other thing that I would have to think about is how do you think about transmitting this DNA when these are two separate genes under the control of two different promoters, and now what you have to do is get the orchestra to work even harder. You've got to put the two genes together again to get them expressed in the same cell if you're going to try to transmit this as contaminating DNA.

So now the odds even go up higher.

ACTING CHAIRMAN DAUM: Thank you very much.

Dr. Stephens and then Dr. Faggett.

1   DR. STEPHENS: I was struck by one of your

2   papers talking about this family of oncogenes, and you

3   compare the HPV oncogene, which has very different

4   properties than EA-1, and I just wondered if you could

5   comment   on   the   structural   relationships   and

6   possibility of mutation of EA-1 to produced an HPV-

7   like oncogene.

8   DR. COOK: Well, E1A and E7 have some

9   sequence similarities. You can actually do with an

10   analogous gene SV40, you can do mix and match

11   experiments where you can cut out a bit one, stick

12   into the other and get a competent gene.

13   So they have conserved sequences. I think

14   the reason for this is that all of these genes need to

15   do the same thing when they get into the cell, and

16   that is to regulate the cell cycle, and they do that

17   by binding Rb and by binding p300 and Kreb binding

18   proteins.

19   So there are reasons that they have co-

20   evolved these. They aren't the same virus. As far as

21   anybody know, they didn't evolve together, and they

22   aren't just recombinations.

23   The data you're referring to are that E1A

24   does all of the things I'm talking about, and so far

25   as we can tell, when E7 is expressed in cells with the

1   exception of one or two reports, it doesn't sensitize

2   cells to some of these other kinds of injuries like

3   TRAIL or to killer cell injury, and so E7 is a

4   different beast, even though it shares some of these

5   same cell cycle regulatory bits.

6   The ability to convert E1A into E7, other

7   than making a chimeric molecule, which can work --

8   Jack Routes has done that. He's made an E1A E7

9   chimera. What happens is the E1A phenotype is

10   dominant, and so E7 sort of lacks what E1A has. It

11   doesn't look like it has any evil force that's trying

12   to outdo E1A.

13   There are no mutations so far that we have

14   been able to make in E1A that's eliminated these

15   activities, although if you eliminate enough of the

16   genome, if you take enough of E1A out, you can reduce

17   its ability to sensitize things, but it's not just a

18   simple mutation. It's a deletion.

19   So I don't know that we could, other than

20   chimera formation between E1A and E7 just making

21   single based changes or some kind of frame shift or

22   something in E1A create an E7-like gene product.

23   ACTING CHAIRMAN DAUM: Thank you.

24   Dr. Faggett, please.

25   DR. FAGGETT: Thank you very much.

1   It was a very clear presentation of a very

2   complex topic.

3   You did mention that you were comfortable

4   in terms of safety issues with the A-1. What was the

5   level of sensitivity and types of tests used to bring

6   you to that level of comfort in terms of safety?

7   DR. COOK: Well, what I'm saying is that

8   I think the -- I'm talking about odds. I suppose we

9   could talk to statisticians, which I'm not, but the

10   odds of getting multiple unlikely events that occur at

11   a   rate   of   maybe   one   in   a   million   to   occur

12   simultaneously to get the event that you want to see

13   happen, which is the transfer of an E1A oncogene into

14   a vaccine recipient at the level of DNA contamination

15   you're talking about, and have that gene by itself now

16   be   able   to   immortalize   the   cell,   have   that   cell

17   survive itself, and then have it survive an immune

18   response. I think the odds could be -- probably can't

19   be   calculated   because   you   don't   know   all   of   the

20   numbers, but I think they're very low.

21   DR. FAGGETT: It's   a   question   of   ten

22   nanograms   of   DNA   fragments   estimated   to   be   the

23   smallness to get some effect. Is that a level?

24   DR. COOK: Ten nanograms per vaccine dose

25   is what I think I heard earlier.

1   DR. FAGGETT: Okay. So how do you --

2   DR. COOK: I've have to try to do some

3   math. I think it would be mental gymnastics, but it

4   probably could be done.

5   DR. FAGGETT: Thank you.

6   ACTING CHAIRMAN DAUM: We will return to

7   this very subject in our discussions later.

8   Dr. Diaz.

9   DR. DIAZ: You mentioned that there was a

10   lot of E1A just naturally occurring in human cells,

11   different types of human tissue. Could you expound on

12   that a little bit in terms of what's known? Is it

13   expressed?   Does it perform similar immunologic

14   regulatory functions in humans?

15   And, secondly, is there any known E1B-like

16   genes in human tissue also?

17   DR. COOK: Yeah, it's important to put it

18   into perspective so that I don't overstate this. So

19   there is a laboratory in Canada in which they have

20   found evidence based on PCR analysis of E1A sequences

21   in lung tissue. It was in a lot of different lung

22   specimens that they sampled.

23   As far as I know, there aren't other

24   laboratories that have done the same thing.

25   There's a laboratory in Australia where

1   they've found E1A-like activity in mammalian cells,

2   including human cells, but haven't convinced anybody

3   that it's E1A yet.

4   So there's a different in finding E1A, as

5   Hogg's lab did, and finding E1A-like activity, as many

6   other people have. So there probably are E1A-like

7   functions in normal cells, and that's probably why E1A

8   decided to ape this during evolution anyway, because

9   it helps regulate cell cycle.

10   A far as E1B expression in normal human

11 cells outside of initial infection or persistent

12 infection, I'm not aware of any data that E1B

13 independently has been detected in human tissues

14 unrelated to infection.

15   ACTING CHAIRMAN DAUM: There are four more

16 hands. We'll take these four questions, and then

17 we're going to move on. Dr. Minor, then Dr. Ketner,

18 Dr. Aguilar-Cordova.

19   DR. MINOR: I'm interested in your

20 threshold description. If you had a PD-50 of 1,000

21 and you give it to ten mice, five of them will go

22 down.

23   If you had 100 cells, for example, went

24 into 100 mice, are you saying that none of them will

25 go down?

1   DR. COOK: I think that's true, actually,

2   yeah.

3   DR. MINOR: Has anybody ever done to see--

4   DR. COOK: Andy, you've given a lot of --

5   you know the answer.

6   DR. LEWIS: Yes, we did that very

7   experiment with the Adeno. 12 because we found that

8   the TPD-50 for the Adeno. 12 transformed Balb/c mice

9   embryo cells was a million cells, and so we asked the

10 question: what would happen if you put 100,000 cells

11 into 100 mice?

12   And we did that experiment, and we got no

13 tumors. So it looks like that threshold is real.

14   We did several other experiments to

15 address that question the same way, like cloning and

16 doing this, that, and the other, and the TPD-50 on

17 sub-clones of the population were again a million

18 cells.

19   So nothing we could do would alter that

20 threshold. I can't say that there's thing that

21 couldn't be done to alter the threshold, but we were

22 unable to do it considering that as a criticism for

23 the proposal we were making.

24   ACTING CHAIRMAN DAUM: Can I ask a follow-

25 up and this be -- save one of the four questions?

1   Is this threshold phenomenon in your

2   belief a probability event where if you just give

3   enough cells, one will do it, or is this a quorum

4   sensing kind of thing where if you give enough cells

5   they will start doing something?

6   DR. LEWIS: Yes. Well, I think what we

7   ruled out by the experiments we did was the

8   possibility that one cell out of a million cells was

9   doing the job. All the data that we have suggested it

10   took the collective action of million cells to

11   overcome whatever it was that was prohibiting the

12   cells to form a tumor mass.

13   Now, exactly what it's overcoming I don't

14   know. I think the question was as to whether this was

15   -- and these were adult Balb/c mice, by the way. They

16   were not newborns or immunoincompetent animals. These

17   were adult animals.

18   So the question I think we raised but were

19   never able to answer was the possibility that it was

20   something about the immune system that it took a

21   million cells to overcome. In other words, that was

22   the level of competence at which that animal could

23   function immunologically with all of his anti-tumor

24   defenses intact.

25   And if you challenged him with enough, he

1   could no longer respond. Jim may have some other

2   ideas about that, but that's as far as we were able to

3   take the logic. We were never able to test that.

4   ACTING CHAIRMAN DAUM: It may be fun to

5   return to that this afternoon as an issue. I'd like

6   to get the focus back to your presentation.

7   Dr. Ketner is next, and then Dr. Aguilar-

8   Cordova.

9   DR. KETNER: Yeah, this is just a comment

10   on the persistence of E1A sequences in human lung

11   samples. It turns out it was shown a long time ago,

12   20 years or so, that human peripheral blood contains,

13   after an adenovirus infection has come and gone,

14   contains residual, intact viral genomes. They're

15   probably replicating slowly in peripheral blood cells,

16   and so those E1A sequences that were found, these are

17   detectable by relatively insensitive techniques like

18   Southern blotting.

19   So those E1A sequences that were detected

20   by PCR may well just have been intact replicating

21   genomes in the blood in those tissues. So I think it

22   overstates it to say that there are cells in which

23   there are, you know, E1A persistent sequences.

24   ACTING CHAIRMAN DAUM: Thank you for a

25   clarifying comment.

1   Dr. Aguilar-Cordova.

2   DR. AGUILAR-CORDOVA: Yeah, I was

3   wondering on the cell thing you were mentioning that

4   had E1A-like activities if 'these cells could

5   complement the E1A minus vectors, and what other known

6   genes, such as E7 or other viral genes, may have that

7   function of complementing and then producing the

8   replication competent activity.

9   DR. COOK: The clearest experiments have

10   been done with mouse embryonal carcinoma cells, the

11   cell line F9, and I think some of the break-away

12   studies from Australia may be similar, but in F9 cells

13   what happens is these are interesting cells because

14   they can be differentiated _in vitro_ with retinoic acid

15   into terminally differentiated cells. Without

16   retinoic acid, they're undifferentiated embryonal

17   carcinoma cells.

18   The undifferentiated cells will complement

19   a virus called DL312, which has no E1A and grows very

20   inefficiently in normal cells. When these cells are

21   differentiated, that complementation goes away.

22   So this is the basis upon which it has

23   been concluded there is some E1A-like activity in

24   normal cells. This is a mouse embryonal carcinoma

25   cell.

The question about whether other human persistent viruses or like HPV where E7 is expressed in an epithelial carcinoma or something like that, I don't know of any evidence that E7 can be expressed in the cell and complement a defective adenovirus. I don't think -- at least as far as I know, that's not the case, but I don't know the data.

ACTING CHAIRMAN DAUM: Thank you very much, Dr. Cook.

We're going to move on now to our final morning, so to speak, presentation. We'll hear from Dr. Peden at the FDA regarding quantitative assessment of the risks of residual DNA, and after which we'll take a break for lunch.

DR. PEDEN: Thank you.

ACTING CHAIRMAN DAUM: While setting up, I'd like to congratulate Dr. Peden for providing us with a handout with his slides on it. It makes it much easier to follow the talk.

DR. PEDEN: Thank you.

I was going to say good morning, but it is, in fact, good afternoon.

This presentation will discuss issues of whether residual cell substrate DNA in vaccine manufactured in neoplastic cells poses a risk to

1 . vaccine recipients.

2 At the outset, I should like to say that

3 while these views expressed are largely my own, I

4 would like to acknowledge the sage and helpful

5 discussions of many colleagues, particularly Andy

6 Lewis, Phil Krause, and Becky Sheets.

7 I would discuss what the perceived risks

8 are, the proposed mechanisms whereby there could be a

9 risk. I will review the relevant in vivo data that do

10 exist and present some calculations and assumptions

11 upon which they are based.

12 The overall conclusion I want to leave you

13 with is that the small amounts of residual cell

14 substrate DNA, and by this I mean ten nanograms, poses

15 an acceptably small risk to vaccinees, and this value

16 of ten micrograms did not come from my whim.

17 Oops, can I go back one more?

18 It has been discussed for many years, and

19 at the WHO expert commission in biological

20 standardization in 1998, the residual DNA from

21 continuous cell lines, ten nanograms or less, was

22 proposed as a reasonable level, raising it from 100

23 picograms, .1 nanograms or less, that was reached in

24 1986.

25 So what types of risks are there? There's

the perceived risks associated with residual cell

substrate DNA, has generally considered to be

oncogenic risk, but there is another risk which I'm

going to discuss, too, the infectivity risk.

So the proposed mechanisms whereby an

oncogenic risk by cell substrate DNA comes for several

ways. One is with the introduction of a dominant

activated oncogene. For example, an activated rat

oncogene or in the issues we're discussing today, the

dominant activated oncogenes could be E1A or E1B.

Insertional mutagenesis is another

mechanism whereby DNA could be oncogenic. While this

mechanism has been found in certain cancers of

animals, such as the disruption of cellular tumor

suppressor gene or activation of a cellular dominant

oncogene, by promotion of insertion mechanisms,

calculations have been done by many people, and the

risk associated with a single integration event by

this type of -- to disrupt the cellular tumor

suppressor gene or dominant oncogene is considered by

people like Reinhart Court (phonetic) to be one in ten

to the minus 18 or one in ten to the 18.

So this is a very low probability event,

and it comes about -- this has been seen in animals

were activation has occurred from an integrating,

replicating system, such as a retrovirus, and a single

insertion event from residual DNA is unlikely, very

unlikely to pose this.

Another mechanism that's been pioneered by

Walter Doerfler is that DNA methylation pattern

changes following integration. While this occurs in

system trends, the lack of a phenotypic consequence of

this makes this very difficult to study.

The other risk of integrating -- of cell

substrate DNA is the one I talked about, cell

substrate DNA they incurred in infectious genome. The

DNA virus, which is polyoma virus, Herpes virus, and

papilloma virus exist as integrated into cells.

Well, in addition, of course, retroviruses

have a provirus state, such as HIV-1 and HTLV and

human retroviruses. So by infectious DNA what we mean

is that if this DNA is introduced into the appropriate

system, then infectious particles are produced, and an

infection can be established.

So one other thing that influences the

nature of the inoculum. For example, we concern

linear versus circular DNA as not being studied

greatly. The single stranded versus double stranded,

where the state of the DNA is free versus the

chromatin associated. In most lysed cells, of course,

1   the DNA will not be free, but be chromatin associated.

2   This has not been assessed in any study except one,

3   that chromatin was injected into animals.

4   The size distribution of the DNA is

5   obviously very important. If the DNA is sheared to a

6   size that's below the size of a normal gene, such as

7   normal genes excluding neutrons are about 3,000 base

8   pairs.   Then clearly this DNA will not be able to

9   produce a product.

10   Now, the route of inoculation can be

11   important as you heard from Dr. Cook. Most of the

12   studies, various animal studies were done with

13   intramuscular, intradermal, subcutaneous, intranasal

14   and oral route, and also by intraperitoneal route.

15   However, an application in Phase 1 would be considered

16   unfavorably, I would imagine, with this route.

17   Assumptions. Before we can go into the

18   calculations, we need to make certain assumptions

19   here, and I hope you can read that, but what I wanted

20   to do is to go through these assumptions. I'll read

21   them slowly, and I hope you have the handout.

22   For a given DNA, the level of response of

23   the cell to that DNA is proportional to the amount of

24   that DNA. That makes somewhat sense. The activity of

25   the gene integrated in the chromosomal DNA was part of

1   a plasmid or phage vector is equivalent or lower.

2   Now, we have to make this assumption

3   because we have no other way of doing this. However,

4   it's likely that the DNA integrated into chromosomal

5   DNA being controlled by its own promoter, requiring

6   certain perhaps tissue specific transcription factors,

7   may not, in fact, be active at all, whereas we put a

8   gene on a plasmid or phage vector and we've designed

9   it such that it expresses very well.

10   So if anything, this is going to over

11   estimate the risk rather than underestimating the

12   risk, but that's the only thing we can do. So we're

13   making this assumption.

14   And the amount of uptake of a given gene

15   by a cell and the expression of this gene in the cell

16   is related to the concentration of the gene in the

17   DNA.

18   Now, because a single copy is represented

19   at approximately one-millionth of the haploid

20   mammalian genome, the amount of DNA corresponding to

21   a single copy gene is a million-fold less abundant for

22   equivalent amounts of cellular DNA compared with the

23   plasma DNA with the same gene.

24   Now, as I say, the genome is of nine --

25   the haploid genome is three billion base pairs, where

1   as I say, the normal gene is maybe 1,000 base pairs.

2   So there's a million-fold difference in the size of

3   the genomes.

4   So, for example, that is, for the uptake

5   and expression of a gene in mammalian genomic DNA

6   equivalent to one microgram of plasma DNA, one million

7   micrograms or one gram of mammalian DNA is to elicit

8   an   equivalent   biological   effect,   giving   the

9   assumptions on the previous slide.

10   Conversely, the single oncogene is present

11   at one microgram of mammalian DNA, the equivalent

12   amount of the same oncogene if cloned in the plasma is

13   one times ten to the minus six micrograms or one times

14   ten to the minus three nanograms or picogram.

15   So there are the assumptions on which

16   we've made some calculations.

17   So now I want to go through some of the

18   data that has been presented in the literature about

19   oncogenicity, and this is the work done by Hsien-jen

20   Kung, and colleagues, in 1983, where they're using a

21   cloned src gene, two micrograms induced tumors in

22   seven out of ten chickens inoculated subcutaneously in

23   the wing web.

24   If you inoculated a cloned Rous sarcoma

25   genome, the entire genome, this two micrograms induced

1   tumors in six and six. Presumably this comes about

2   because the Rous sarcoma virus DNA initiated an

3   infection.

4   So two micrograms was found to induce

5   tumors in about 70 percent of the animals. A study in

6   1990 by Halpern and colleagues got essentially the

7   same or similar results using in this case 20

8   micrograms of DNA by the same route, and inoculated

9   intravenously. Also it gave tumors.

10   So we can conclude that two micrograms of

11   DNA, which is equivalent to about two and a half times

12   ten to the 11 molecules of cloned _v-src_ oncogene is

13   oncogenic in chickens.

14   The study that was done by Burns and

15   colleagues inducted the oncogenicity of _ras_, of the

16   activated _ras_ gene in mice. Activated _ras_ from the T-

17   24 gene, ten micrograms inoculated by scarification in

18   the mouse skin.

19   Lymph anginose sarcomas developed in 33

20   out of 34 animals within 12 months, usually within 12

21   weeks. However, the normal _ras_ gene failed to induce

22   tumors. So at least in this route, ten micrograms,

23   which is equivalent of about one times ten to the 12

24   molecules of an activated _ras_ gene is oncogenic in

25   adult mice.

1   If we looked at the oncogenicity of

2   polyoma virus DNA, polyoma virus DNA, of course, is

3   infectious in mice, but in hamsters it is not

4   infectious. It causes tumors by interperitoneal,

5   subcutaneous routes, using either super clonal DNA or

6   linear DNA. As you can see, it's tumorigenic.

7   This is the viral DNA isolated from the

8   virion. If you clone the polyoma virus DNA and inject

9   in newborn hamsters, it is also tumorigenic.

10   Now, the minimum amount of DNA they found

11   activity was .2 micrograms of the linear DNA, and gave

12   it to 22 percent of the animals. So if we use that

13   figure, it can conclude that .2 micrograms, which is

14   about two times ten to the ten molecules of polyoma

15   virus DNA is oncogenic in newborn hamsters.

16   So then we're going to move on to what

17   data are available on the infectivity. Now, I'm going

18   to talk about several studies on retroviruses, and in

19   the first ones I'm going to talk about is work done by

20   three groups over the years, using Simian

21   immunodeficiency virus through various routes and

22   establishing infection in various animals, and the

23   number of genomes, for infection.

24   In the bovine leukemia virus using ID

25   route with either a facilitated Achiheinig (phonetic)

1   lipid facilitator DOTAP in the Caprine (phonetic)

2   arthritis encephalitis virus in goats, again, and

3   feline leukemia virus and feline immunodeficiency

4   virus.

5   Now, the only reason for presenting this

6   slide is, although you probably can't see it, is that

7   the number of genomes for infection with these

8   disparate systems and different routes of inoculation

9   is remarkably similar.

10   The highest number of genomes required is

11   about two times ten to the 13, and the lowest is one

12   times ten to the 12 in this system. So it's somewhat

13   remarkable. Even though a dose response study was not

14   generally done, with the possible exception of the

15   Purcell group, it is quite remarkable the number of

16   genomes or molecules of these proviral clones.

17   And yet to establish an infection, we're

18   very similar within about a 20-fold range. The dose

19   response study was done by Portis and colleagues with

20   a murine retrovirus using 19, 3.8, .38, and .038

21   micrograms intraperitoneally and found that 3.8

22   micrograms intraperitoneally was the last of the level

23   to establish an infection.

24   Interesting, they also found that super

25   core DNA was unable to establish an infection, whereas

1 linear DNA established an infection very well.

2 That result disagrees somewhat from other

3 people's, but probably reflects the system as well as

4 the clones.

5 So the summary then of the infectivity

6 with cloned viral genomes, viral DNA coming from the

7 retroviruses, 15 to 500 micrograms intramuscular

8 injection, and so that if you calculate the number of

9 genomes, it's about one times ten to the 12 to 2.3

10 times ten to the 13 genomes required for infection.

11 Polyoma virus, in contrast, from five time

12 ten to the minus five micrograms or one microgram

13 variously, and the minimum estimate then is 1.3 times

14 ten to the seven genomes. So if we can conclude from

15 that the infectivity of different retroviral DNAs is

16 quite similar, quite surprisingly perhaps is similar,

17 and depending on the route of inoculation, 15

18 micrograms can be infectious for retroviruses, but the

19 infectivity of polyoma virus DNA is higher than

20 approximately 50 picograms or .05 nanograms is

21 sufficient to establish an infection.

22 So it may be informative to compare the

23 oncogenicity and infectivity where it's possible. So

24 for polyoma virus DNA, that's the only one where we

25 can, in fact, compare directly the oncogenicity.

1   Point, zero, two micrograms is the minimum amount of

2   DNA that was tested that was oncogenic, and that's

3   about 3.6 times ten to the ten genomes infectivity,

4   however. The infectious dose 50 is 1.3 times ten to

5   the minus four micrograms. So this is about 2.3 times

6   ten to seven genomes.

7   So there's about three orders of magnitude

8   difference with the infectivity of polyoma versus the

9   oncogenicity, and SV40 is about an oncogenicity of a

10 microgram. Retrovirus, the infectivity, again, is 15

11 to 30 micrograms. It's about one to two times ten to

12 the 12 genomes. V-src was two micrograms at about

13 two, two and a half times ten to the 11 molecules.

14 Inactivated ras, ten micrograms, is nine times ten to

15 the 11 molecules.

16   So we went in the literature to find out

17 what evidence, what data are available for intranasal

18 inoculation. Unfortunately there is almost none in

19 terms of quantitative estimates. So the only one we

20 could find was looking at this study by Timer and

21 colleagues in Japan several years ago by using DNA

22 vaccine administered intranasally.

23   So this was a clone gene of the

24 hemagglutinin gene from influenza. It was inoculated

25 into mice via the nasal route. Different amounts of

1   DNA were administered in PBS, and after four weeks, a

2   second intranasal inoculation of 1.5 micrograms was

3   given as a good.

4   So down here we can zero micrograms, 0.2

5   micrograms, .4 micrograms, and .6 micrograms, and they

6   measured two responses, hemagglutinin inhibition assay

7   and mucosal IgA to the vaccine, and after the primary

8   inoculation, nothing was found except for the six

9   micrograms. There was a weak response.

10   However, after the second inoculation,

11   there was nothing. No priming inoculation was given

12   demonstrating that this 1.5 microgram secondary -- the

13   boost was not immunogenic. However, for all the

14   levels of DNA, there was a substantial boost.

15   So what this means to us is that even 0.2

16   micrograms of DNA, in other words, 20 nanograms,

17   administered intranasally elicits immune response

18   since the secondary inoculation boosts this response.

19   Therefore, we're concluding that 20

20   nanograms of DNA can be biologically active when

21   administered intranasally.

22   So the other route that we're considering

23   is the oral route. Now, there aren't that many

24   studies of this either, but Malcolm Martin and Mark

25   Israel's group fed mice polyoma virus DNA between one

1   or 0.5 micrograms, and zero out of 25 became infected

2   with one microgram and zero out of 30 became infected

3   with 0.5 micrograms.

4   However, if you stick a gastric tube

5   through, you can get some infectivity, but how this is

6   doing this is through damage of the gastric tube in

7   mice is not clear, but the important point is though

8   that it's unlikely that small amounts of DNA will

9   survive passage through the stomach intact.

10   Now, the next study that looked at oral

11   routes is a study by Duffler (phonetic) and

12   colleagues, and they fed a phage DNA -- it's a 7.25 kb

13   phage DNA -- large quantity DNA and followed its fate

14   to see whether anything did escape through the

15   stomach, and they did find DNA in feces, blood, small

16   intestine, and large intestine in the leukocyte

17   population.

18   But the size, as you notice, is 100 base

19   pairs to 1,700 base pairs, whereas the original genome

20   was 7,000 base pairs.   So it's clearly getting

21   degraded during passage through the stomach.

22   They did find some in the blood, 194 base

23   pairs to 976 base pairs, and perhaps interestingly,

24   they also found about a 700 base pair fragments in

25   blood, and they also found it integrated in 0.1

1   . percent of the cells.

2   However, these cells did not survive, and

3   these cells came from the peyer's patches and from the

4   spleen.

5   So conclusions that small amounts of DNA

6   can, in fact, pass through the stomach, but no full

7   length DNA was detected, and DNA can be found

8   integrated into the mouse chromosome in a few percent

9   of the cells. So you have to feed large quantities of

10   DNA.

11   So now we can go on to try to get some

12   estimate of risks. We'll first consider the

13   oncogenicity.

14   The cloned activated oncogene, for a

15   single dominant activated oncogene per cell, one

16   microgram of DNA has 152,000 oncogenes, and this

17   figure comes from the DNA in 152,000 cells that's one

18   microgram or one microgram carries 152,000 cell

19   equivalents. So that's where that figure comes from.

20   We know from our previous studies of the

21   literature and for polyoma virus DNA about 3.6 times

22   ten to the seven genomes or molecules are required for

23   oncogenicity, and the activated ras DNA, 9.1 times ten

24   to the 11 molecules are required for oncogenicity.

25   Therefore, the number of tumors expected

from one microgram of residual DNA with a single dominate oncogene per cell is between 152,000 divided by this. It gives you a value of 4.2 times ten to the minus six for polyoma, and a value of 1.7 times ten to the minus seven for activated ras, and if you take that the other way, therefore, for ten nanograms of DNA, not one microgram, the probability of an oncogenic event is between one and two times ten the five or 200,000 for polyoma virus, and one in ten -- six times six million for the inactivated ras. So that's where the calculation comes from.

For infectivity, we've looked at polio virus DNA, and viral genomes required for infectivity is ten to the seven. So it's ten million.

The probability of an infectious event using ten nanograms of mammalian DNA with a single copy of polyoma virus DNA is one in 7,000 events, one in 7,000 events. This is rather high.

This also points to the fact that it is very important to be able to determine that an adventitious agent, such as polyoma, not just polyoma; other adventitious agents are not present in the cell substrate since infectivity is a very easy -- much easier than oncogenicity.

For the retrovirus cloned proviral DNA,

1   the number of viral genomes we've determined is about

2   four times ten to the 11, as a minimum, and the

3   probability of an infectious event for ten nanograms

4   of mammalian DNA with a single provirus is about one

5   in three times ten to the eight, one in 300 million.

6   So it's much more difficult for this. OF

7   course, these values all change if there are more than

8   one copy of the oncogenic agent or of the retroviral

9   provirus.

10 So we can draw some conclusions from this.

11 Infectious risk of DNA can be more important than

12 oncogenic risk, and as I said, therefore, it's very

13 important to determine what level of adventitious

14 agents are present.

15 For the IM, intramuscular and subcutaneous

16 routes, ten nanograms of DNA provides an estimated

17 risk for the polyoma virus DNA, one in about 200,000

18 for an oncogenic event and about one in 7,000 for an

19 infectious event.

20 For the more likely events of cellular DNA

21 now, cellular activated ras, there's one in six times

22 ten to the sixth for an oncogenic event, and for

23 proviral DNA for an infectious event, there's one in

24 300 million, three times ten to the eight.

25 For the IN route, the intranasal route,

ten nanograms of DNA as I've shown you has an estimated safety margin of one in ten to the sixth for what I'm quoting an expression event. This is because you can detect the activity of that DNA. However, that is not an unoncogenic event. That's just what we can detect by immunological means. So that's the best one we have.

And for an oral route, one micrograms of polyoma DNA administered orally is not infectious. So, therefore, ten nanograms by arithmetic calculation with one copy of polyoma DNA, the safety margin is at least one in ten to the eighth.

However, it's not all bad for the risk. There are some mitigating factors for DNA. As you probably all know, the uptake, expression, integration are inefficient processes. Integration requires cell division, and so therefore, not all cells in the body, of course, are dividing. In intramuscular routes, the myocytes, and muscle cells have a very low division rate, and integration is required for maintenance of DNA in the absence of the replicating system.

The degradation of DNA vaccine manufacturer procedure usually requires degradation of the DNA, and of course, DNA is degraded _in vivo_. It doesn't exist for very long _in vivo_.

1   So if the DNA is degraded below the size

2 of a gene, then that increases its safety.

3   There's a host immune response to

4 transfected cells, as Dr. Cook just mentioned. So

5 even if the DNA does get in and express these cells in

6 an immunocompetent individual, it will likely be

7 removed.

8   What also Dr. Cook talked about eloquently

9 is the multi-step nature of carcinogenesis. We now

10 know that it's not just a single event. So even if an

11 activated ras did get into that cell, that cell would

12 not be established as a tumor right there. There are

13 many more steps that have to proceed before

14 carcinogenesis can occur.

15   And, of course, we all know that

16 transmission of human cells in culture is much more

17 difficult than transmission of rodent cells, and Dr.

18 Alex van der Eb discussed some of these issues earlier

19 on.

20   So what are we doing at CBER to try to

21 help to address this? Well, Dr. Hughes mentioned that

22 we have now initiated a study. We have finally got

23 some funding from the FDA, and in a collaborative

24 study with CBER and the National Cancer Institute and

25 the Center for Drugs, we're going to study in a

1   systematic way to develop numbers that have been

2   alluding everybody for 40 years.

3   These issues come up every five to ten

4   years, and still nothing is being done.

5   So we're trying to develop sensitive

6   animal models to detect oncogenic activity of DNA.

7   The models that have been heretofore used have not

8   been systematically developed and are not very

9   sensitive. So what we're going to try to do is test

10   newborn NIH Swiss and C57 Black 6 mice, the athymic

11   nude mice, and K6/ODC mice, which is a constitutively

12   promotive mouse line, and we're going to use them

13   making the activated ras genes.

14   And our colleagues, and we're very

15   grateful to have John Coffin and Don Blair and Steve

16   Hughes from NCI and Frank Sistair (phonetic) from CDER

17   to help and advise us on these studies.

18   The other assay we're trying to do with

19   CDER is to get some quantitative estimate of DNA

20   infectivity, and we want to develop quantitative in

21   vitro assays first, of course, to affect the

22   infectivity of proviral DNA in retroviruses. It is

23   not known what level of proviral -- levels of provirus

24   that is in mammalian DNA are infectious.

25   Howard Tammin (phonetic) has shown that in

1   times.

2   If you could go into a system where some

3   of those things are already present, if you would --

4   I don't know -- give us a better readout.

5   DR. PEDEN: Yes, you're right. I didn't

6   mention it, but, in fact, the idea of a first hit and

7   then you give to a vaccinee and then subsequently you

8   get additional hits is a worrying issue.

9   We don't know how stable those initial

10   hits are. If you give it to a cell, where that cell

11   eventually gets lost, you don't retain that so-called

12   activated state in the cell. We don't know that.

13   As for the models, the ODC mouse is a

14   carotin 6 promotor that drives olefin de-Kalb oxylase

15   (phonetic), and that's been used in carcinogenesis

16   activities because it's constitutively what they call

17   promoted. So if you add an initiator such as ras,

18   it's a very sensitive model on the skin, of course,

19   because --

20   DR. GRIFFIN: So it does provide a more

21   sensitive --

22   DR. PEDEN: So it does provide that. We

23   discussed with Steve Hughes and John Coffin and Don

24   Blair about which mice to use. We're thinking about

25   using the heterozygous p53 mouse, and perhaps Steve or

1   the others could comment on that, but we decided in

2   the first run through to do these mice. I think the

3   homozygous p53 knockout was generally felt, I think,

4   by Steve just to have too much background.

5   And so we're trying to balance it. I'm

6   glad you do point out the importance of doing these

7   quantitative studies. I don't want to lessen what's

8   done in the past, but they were never intended for

9   that role, and of course, the infectivity in monkeys

10   is an incredibly expensive thing. So there isn't any

11   information on that.

12   So I don't know whether Steve wants to

13   comment on the particular animal models or Don or

14   John.

15   DR. BLAIR: No, I think we chose, you

16   know, sort of a reasonable spread of different strains

17   of mice which might show up some strain differences.

18   The nudes as an immunosuppressed, perhaps more

19   sensitive mechanism, and as you say, the problem with

20   some of the p53 knockouts and things is as you

21   increase the sensitivity, you increase the background,

22   and you may obscure things. So we went with something

23   where we felt the background wasn't going to be

24   insurmountable and we should see positive.

25   DR. HUGHES: I also would add that I don't

1   think that the studies that have been outlined so far

2   will necessarily be the only things that will ever be

3   done, and I think that based on what we would like to

4   do is to have what we hope is a reasonable preliminary

5   survey and see, in fact, the degree to which we get

6   response with relatively lower amounts of DNA.

7   And depending on the outcome of these

8   experiments, I think we would be better prepared to go

9   back and actually plan and propose a second round in

10   which, depending on what the outcome is, something

11   like a p53 heterozygous animal might be employed.

12   But because they're actually, as you point

13   out and Dr. Peden has pointed out, there's relatively

14   little really quantitative assessment, we thought we'd

15   make what we intend to be the first attempt to do

16   that, and then based on what we learn from that, try

17   and go ahead in a reasonable fashion and maybe expand

18   this to other model systems.

19   ACTING CHAIRMAN DAUM: This is, again, an

20   issue that I think we should encourage everybody to

21   raise in this afternoon's session when we're talking

22   about the big picture issues with respect to this

23   approach.

24   I'd like to focus now on Dr. Peden's

25   presentation though, and Dr. Aguilar-Cordova is next

1   and then Dr. Faggett and Dr. Moulton.

2   DR. AGUILAR-CORDOVA: Yes. Given the

3   numbers that you presented and there may be various

4   oncogenes in any one cell or activated genes or

5   whichever, in your interpretation would it make a

6   significant difference whether it was a spontaneous

7   tumorigenic cell line versus a designer cell line in

8   which one of the many events might be known?

9   DR. PEDEN: So now are you saying the

10   oncogenes from a spontaneously transformed cell is

11   studied? I mean, these are cloned oncogenes injected.

12   DR. AGUILAR-CORDOVA: This is like the

13   worst case scenario. What I'm saying is based on your

14   statistical analysis that you presented --

15   DR. PEDEN: Well, I wouldn't grace it with

16   statistical analysis, but --

17   (Laughter.)

18   DR. AGUILAR-CORDOVA: Or statistical

19   deductions. I guess the general question would be

20   would, say, in the presentation we heard earlier from

21   Dr. Lewis, do you think that based on that would A549

22   residual DNA pose any significant different risk than

23   293?

24   DR. PEDEN: I think there's not universal

25   agreement, I think, among us about that, but I think

1 the general feeling is yes. The main reason, you

2 don't know what the oncogenes were that brought about

3 the A549 phenotype. So that's one thing.

4   And also the other issue about A549 is you

5 don't know what's in there. Is there an oncogenic

6 agent that brought about that?

7   So I think in general terms there is a big

8 difference between a cell that you know hits passage

9 history and you transform it by another mechanism. I

10 think that's why we are calling these designer cell

11 substrates and we call them at low risk, such as A549.

12   So there's two reasons for that. The both

13 I've just said to you.

14   I think for the study what we want to do

15 is, in fact, determine ultimately the most sensible

16 animal model where we can look at genomic DNA, DNA

17 from A549, from PER.C6, to see where that can induce

18 an oncogenic event. So that's really where we're

19 going at, although I didn't, in fact, say that. It's

20 to see whether you can, in fact, detect an oncogenic

21 event using DNA from such things as A549 cells.

22   That's where we want to go, but we need to

23 develop these animal models, sensitive animal models

24 for that reason.

25   ACTING CHAIRMAN DAUM: Thank you very

1   much.

2   Dr. Faggett, and then Dr. Moulton, and

3   then we will adjourn for lunch.

4   DR. FAGGETT: Now, what prompted the

5   change from the WHO requirements for residual cell

6   substrate DNA levels from 0.1 nanograms in 1986 to the

7   ten nanogram level in 1999? Was there some --

8   DR. PEDEN: I think as we get more and

9   more information, we do these experiments. See, more

10 information came, more experiments were done. You may

11 think they were deficient, and we may agree with you,

12 but we have more knowledge and more data and also more

13 clinical experience with some of these cells, such as

14 Vero cells.

15   So I think it's a combination of all

16 things, but I wasn't at that meeting. So maybe Phil

17 and Andrew were at the meeting. I don't know what

18 specifically lowered except for these numbers that I

19 just presented to you.

20   But, Phil, do you want to say anything?

21   DR. KRAUSE: Yeah, maybe I could just

22 comment. I think an important thing to remember is

23 that these meetings that have gone on in the past, in

24 general, have been talking about the older kind of

25 cell substrates, and here we're talking about newer

1   kinds of cell substrates.

2   And so I don't think that the idea of

3   using an A549 cell, for instance, was specifically

4   considered as one of the things that was going into

5   raising that limit from 100 picograms to ten nanograms

6   at that meeting, but instead the idea was to consider

7   the kinds of cells with which there was much more

8   substantial experience in vaccine and other biological

9   production.

10   ACTING CHAIRMAN DAUM: Thank you very

11   much.

12   Dr. Moulton.

13   DR. MOULTON: Yeah.   This is a great

14   start, I think, towards the full risk analysis.   I

15   think a full risk analysis would have probability

16   distributions associated with every one of the

17   assumptions there. There's a lot of assumptions we're

18   making. For example, the potential mitigating factors

19   are going to reduce the risk.   There's other

20   assumptions that could increase it, and a couple of

21   people have already mentioned the problem of the low

22   dose extrapolation, which could affect things by a

23   couple of orders of magnitude.

24   And I was wondering if there's a plan even

25   before we get the result of these future experiments

1   to try and put some probability distributions on

2   there, every one of those assessments, and also a more

3   general question of how does this risk analysis fit

4   into the general risk management program at FDA.

5   It's one thing to estimate or calculate a

6   risk, estimate that point estimate, and if you're

7   lucky get some kind of tolerance interval around it.

8   It's another to actually manage the risk once it's

9   been estimated.

10   DR. PEDEN: Well, the way we manage the

11   DNA risk is to require that the levels are low. I

12   mean that's the simple way to do that.

13   As for the probability, many people have

14   tried to make estimates, probability estimates at all

15   of these steps, and i can give you lots of references

16   where that was done. I don't find that that's

17   satisfactory nor useful because there isn't -- I mean,

18   the probability of integration event, the probability

19   of out-take of DNA, that's all those events you're

20   talking about, and that's the evidence.

21   The consideration of those is all done

22   from _in vitro_ studies. They've been transfecting DNA

23   in culture and measuring events, and so to me that's

24   not very satisfactory to do that, to extrapolate from

25   what goes on _in vitro_ to what goes on _in vivo_.

1   That's why I think we need the <u>in vivo</u>

2   data to try to get real numbers as opposed to these

3   estimates.

4   ACTING CHAIRMAN DAUM: This is a topic

5   that I think must be also returned to this afternoon.

6   So perhaps, Dr. Moulton, you will remind us that we

7   need to address this and bring it up again.

8   I'd like to thank Dr. Peden at this point

9   very much for another fine presentation that helped us

10   think.   Dare I say you provided us a great deal of

11   thought for food.

12   We will now break for lunch.   It's 12:45

13   here in the Eastern time zone, and we will reconvene

14   at 1:45 exactly.

15   Thank you.

16   (Whereupon, at 12:45 p.m., the meeting was

17   recessed for lunch, to reconvene at 1:45 p.m., the

18   same day.)

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

2   (1:49 a.m.)

3   ACTING CHAIRMAN DAUM: Okay. We are in

4   afternoon session.

5   Welcome back from lunch, and we'll begin

6   with the adventitious agent issue this afternoon. We

7   have three or four presentations on that, and we'll

8   begin with Dr. Philip Krause from FDA who will

9   introduce us to the topic.

10   DR. KRAUSE: Vaccines are among the most

11   effective public health interventions ever devised.

12   Vaccines, however, are very dependent on public

13   confidence in their safety, and that's really for

14   several reasons, but one of the most important is that

15   vaccines are generally given to healthy people, many

16   of whom may never be exposed to disease which one is

17   trying to prevent, and another reason, of course, is

18   the need in many cases for many vaccines to generate

19   high enough immunization rates to get some level of

20   herd immunity, without which one doesn't get maximal

21   vaccine benefit.

22   And of course, since many vaccines are

23   given to healthy children, these issues are even more

24   important.

25   An   important   component   of   public

1   confidence in vaccine safety, of course, is their

2   freedom from adventitious agents.   So what is an

3   adventitious agent?

4   Well, for the purposes of this discussion

5   at least we're defining an adventitious agent as an

6   infectious agent that is extraneous to the product,

7   and so a vaccine strain obviously is not an

8   adventitious agent, but something that's carried in

9   from the outside or carried in with the cell

10 substrate, is potentially an adventitious agent, and

11 an obvious goal then is to insure that final products

12 don't contain adventitious agents.

13   Now, there are two additional points I

14 want to make, and just because I say final products

15 here doesn't mean that intermediate products should

16 contain adventitious agents. In fact, all through the

17 production process it's important to keep adventitious

18 agents out of vaccine manufacture, and the other thing

19 which we've spent some time talking about already is

20 trying to define in some quantitative way the

21 terminology of "should not contain."

22   So any negative result obviously needs

23 some quantitation associated with the sensitivity of

24 the testing, which allows one to exclude that

25 something is there.

1   Now, in general the OVRR approach to

2 adventitious agent issues has included identifying

3 potential issues, including theoretical ones,

4 discussing these issues in public, and Dr. Lewis

5 described the two previous Advisory Committee meetings

6 at which this came up, as well as the public

7 international meeting on cell substrates, and of

8 course, this meeting is another component of that,

9 making decisions based on the best available science

10 and, in addition to that, insuring that any potential

11 issues are known and understood by research subjects

12 and investigators.

13   So today we're going to be talking about

14 two potential adventitious agents or types of

15 potential adventitious agents. One is transmissible

16 spongiform encephalopathy agents, and the other is

17 viruses.

18   Directly following this brief

19 introduction, Dr. Sue Priola will talk in some more

20 detail about TSE agents, and then after that, I will

21 provide some general principles of adventitious agent

22 testing, as well as a more detailed discussion of

23 virus as potential adventitious agents.

24   So to introduce Dr. Priola's talk, I'm

25 just going to go over a few things related to our

consideration of transmissible spongiform encephalopathy agents and cell substrates. And the kinds of issues that can come up that might increase the risk that a cell substrate could harbor such an agent.

And I guess one possibility is that because some transmissible spongiform encephalopathy diseases, such as Creutzfeldt-Jakob disease and Gerstmann-Straussler syndrome are at least in part genetically determined. The question arises of whether a cell substrate which contains mutations that are associated with these types of diseases might then harbor a greater risk of either having TSE agents in it or, if exposed to TSE agents causing infection or being infected with them and thereby transmitting them to a vaccine recipient.

The second question, which is a general one which comes up and which this committee has addressed before to some degree at least, is the consequence of potential exposure to serum from countries where bovine spongiform encephalopathy or the risk of BSE exists.

And this is an issue which has come up in the context, in particular, of cells which have been in laboratories in the early to mid-1980s at a time

before this risk was as widely appreciated as it is now.

And then, of course, there are other factors that could theoretically increase the risk of TSE infection of cell substrates. One of these is PrP expression levels. It's been shown at least in some systems that cells that express higher levels of the PRNP gene that encodes PrP, that the risk of those cells being able to propagate TSE agents is higher, and then also there's the question of whether cells of neuronal or retinal origin might also have greater risk of at least propagating TSE agents if exposed or of containing them in the first place.

Now, there are a couple of neoplastic cell specific notions that are also TSE related. One of them is that because neoplastic cells often have some associated genomic instability, whether, in fact, that genomic instability might itself induce mutations in the PRNP gene, which might then have some consequence on the ability of those cells to harbor TSE agents.

And then also, it appears that at least in some cells the ability of a cell to undergo apoptosis helps to prevent infection with TSE agents in them, and because neoplastic cells have in most cases at least some apoptosis pathways abrogated, the question

1   then is whether a neoplastic cell might a priori be a

2   little bit more susceptible to infection with TSE

3   agents.

4   So at this time, we're following the

5   approach to TSE issues that's outlined on this slide,

6   and that is where possible to determine the family and

7   medical history of the cell donor with respect to TSE

8   risk factors; to ask that the PRNP gene be sequenced;

9   to ask that Western blots be performed to look for the

10   presence of protease resistant PRP; and to insist

11   that they be negative, of course; and to determine if

12   exposure to fetal bovine serum from countries with BSE

13   or with the risk of BSE could have occurred, and then

14   if some possible exposure to questionable fetal bovine

15   serum has occurred, to perform a risk assessment based

16   on the dilution factor from the time of that exposure,

17   based on the assumption then that the cells cannot

18   support replication of a BSE agent.

19   There are several evolving ideas which we

20   don't think can be applied right now, but which may be

21   applicable in the future. One of them is if possible

22   exposure to fetal bovine serum of unknown origin has

23   occurred, to document that the cells cannot support

24   replication of the bovine spongiform encephalopathy

25   agent.

1   And this time I don't believe that there

2   are any cells in tissue culture that have been

3   demonstrated to replicate the BSE agent.

4   To evaluate the level of PrP expression,

5   which although it is generally accepted, I think that

6   cells that express higher levels of PrP might have a

7   greater risk of propagating TSE agents if exposed.

8   The question then also comes down to defining what

9   exactly would constitute a safe level and

10   understanding in a way that can be applied

11   quantitatively what kind of controls would be

12   necessary to use such an assay in a regulatory

13   setting.

14   To evaluate for the presence of TSE agents

15   by animal inoculation, and as many of you know, the

16   monkey, the primate models take a very long time to

17   carry out, but there are promising transgenic mouse

18   models which could potentially be used after they are

19   appropriately validated to get more rapid answers to

20   these kinds of questions in an animal model, and of

21   course, the general principle that once new assays for

22   detection of TSE agents become available, to introduce

23   them for cell substrate testing as soon as is

24   feasible.

25   So with that, I would like to stop here.

1   I'm happy to take any questions, but if there aren't

2   any, then we'll go on to Dr. Priola's talk on TSE

3   agents as an issue in the use of neoplastic cell

4   substrates, and I think she'll concentrate, in

5   particular, on issues associated with TSE agents in

6   cell culture.

7   ACTING CHAIRMAN DAUM: I think we'll go

8   right on if that's okay because we'll have a change to

9   ask questions.

10   DR. KRAUSE: You'll get another crack at

11   me.

12   ACTING CHAIRMAN DAUM: That's right.

13   Thank you.

14   Dr. Priola here? There she is.

15   DR. PRIOLA: I would just like to thank --

16   can everybody hear me? -- to thank Dr. Krause for

17   giving such a terrific introduction. He left me with

18   a few things to talk about, but he hit really on every

19   major point that is going to be an issue in what I go

20   over.

21   What I want to do is basically give a very

22   brief introduction, basic introduction to TSE

23   diseases, and then describe using experimental

24   examples what we know about the difficulties

25   associated with passaging TSE infectivity into tissue

1   culture.

2   So do you have the presentation up? There

3   you go.

4   I think as we all know that TSE diseases

5   are long term transmissible, degenerative diseases.

6   Have you frozen up there? It's coming on.

7   It's the good at explanation point. Oh, no, there it

8   goes.

9   (Laughter.)

10   DR. PRIOLA: I want to wait until this

11   comes up. Okay. There we go. Thank you.

12   All right. So the TSEs are slow, fadable,

13   and transmissible brain diseases that affect a variety

14   of   mammals,   including,   of   course,   humans.

15   Creutzfeldt-Jakob disease in humans is the prototypic,

16   one of the prototypic TSEs, scrapie in sheep, and of

17   course, BSE in cattle.

18   One of the things about the TSE diseases

19   is there's this phenomenon called the species barrier

20   to infection, and it's this that has caused all of the

21   concern when it was realized that SE from cattle

22   apparently has crossed over and caused a new form of

23   CJD in people in the U.K.

24   And usually the species barrier is quite

25   strong in these diseases, and so it was unexpected

1 that cattle BSE would necessarily pass into people.

2 And, of course, it's this concern that now

3 that we have BSE in cattle populations that

4 contamination of bovine products used in tissue

5 culture could then passage that TSE infectivity into

6 tissue cultures using those products.

7 Experimentally, the primary systems are

8 TSE infections of mice and hamsters, and most of what

9 I'm going to talk about today deals with that.

10 It's important to remember that within the

11 TSE there are different strains of TSE agents. So

12 there has to be some caution in interpreting

13 experiments using one strain of agent and one species

14 of animal and extrapolating those data to other

15 species and other strains. I'd like you to keep that

16 in mind.

17 Infection, of course, is believed to be

18 primarily from ingestion or inoculation. It's an

19 extremely long disease course. It can take from

20 months to decades to appear. It is always fatal.

21 There are no preclinical diagnostic tests available.

22 There's no effective post or preclinical treatments

23 available.

24 If that weren't bad enough, it turns out

25 that we're still not entirely clear what the exact

composition of the infectious agent is, but the fact that it's unusually hard to kill and that there's no viral or bacterial association with TSE infectivity -- good Lord. Can I go back? Good luck. Okay -- led to the hypothesis that, in fact, it was an infectious self-replicating protein that was responsible for these diseases.

In the early '80s, a protein was found that was very closely associated with TSE infectivity in Stan Prusiner's lab, and that protein was called prion protein, or PrP, and I think as most of us are aware here, PrP is, in fact, a normal host cellular protein. It's something that's expressed in all mammals, and it's expressed almost ubiquitously. It's very difficult to find a tissue or cell that does not express PRP.

In its normal form it's sensitive to digestion with cellular proteases, and for that reason I'm going to call it PrP-sen for protease sensitive. As I said, it's almost ubiquitously expressed, and it's soluble.

During TSE disease, the normal form of PrP, which is shown here -- it's a cell surface glycoprotein -- is converted to an abnormal form that is now partially resistant to proteinase K, and the

1   partial proteinase resistance comes about because when

2   you expose this molecule to proteases, what happens is

3   instead of digesting everything away as it would if it

4   were just PrP-sen, it basically can only clip away

5   part of the end terminus, and you see this very

6   characteristic side shift down like this, and that is

7   what we define as PrP-res or protease resistant PrP.

8   This form is now insoluble. It's heavily

9   aggregated, and it is completely TSE specific. It's

10   the marker that everybody looks for. If you find PrP-

11   res, by definition you have a TSE disease.

12   It's found primarily at high levels in the

13   CNS   in   some   animal   models   it's   found   in   the

14   lymphoreticular system as well.

15   It   has   a   different   confirmational

16   structure   than   normal   PrP.   The   normal   PrP   is

17   primarily optihelical. When it gets converted to the

18   abnormal form, it becomes beta sheeted structure, and

19   it's believed that it's this difference, it's this

20   change in confirmation that accounts for the different

21   properties of these molecules.

22   Now,   while   there's   still   controversy

23   within the field about the role of whether or not PrP-

24   res is the infectious agent, the role of PrP in TSE

25   diseases is absolutely undeniable. You need normal

1   PrP for infection. Mice that don't express this gene

2   do not get sick following exposure to TSE agents.

3   Mutations in this molecule can strongly

4   influence disease susceptibility and species barriers

5   to infection.   PrP-res, the abnormal form is

6   associated with toxic events in the brain and is, of

7   course, always associated with infectivity.

8   So in terms of human TSE diseases and the

9   dangers involved, not dangers, the possibility

10   involved with deriving neoplastic tissue culture from

11   human cells, there are three groups of human TSE

12   diseases.

13   There is the sporadic form known as

14   Creutzfeldt-Jakob disease or CJD. There's no known

15   exposure to TSE infectivity. This form of the disease

16   is not associated with mutations in PrP. It accounts

17   for the vast majority of TSE cases. It's extremely

18   rare. It happens annually worldwide at an incidence

19   of about one case per million people.

20   There's also familial forms of the human

21   TSEs.   These include familial CJD, Gerstmann-

22   Straussler-Scheinker syndrome. These forms are also

23   not associated with any known exposure to infectivity.

24   They are, however, associated with mutations in the

25   PrP molecule. They are even more rare than this radic

1 (phonetic) disease with about one to ten cases per 100

2 million people. There are very few families in the

3 world that have been identified that carry these

4 mutations.

5   Finally, I have lumped together here under

6 the term "infectious TSE" infections which are a

7 result of exposure, a known exposure or presumed

8 exposure to infectivity, and these include iatrogenic

9 CJD cases that arise following exposure to

10 contaminated medical instruments, and of course,

11 variant CJD in Great Britain.

12   Is there a problem? You can't see the

13 slides? I'm not speaking clearly enough for you to

14 see the slides? Sorry. I'm glad you told me.

15   All right. So where in the heck was I?

16 Okay. So in terms of it being an issue in cell

17 culture, the problems are can you infect tissue

18 culture cells easily with TSE agents.

19   You're going to have to wait a minute.

20 I've got to reorganize myself.

21   And as alluded to by Phil, there are three

22 basic issues. First of all, development of new cell

23 lines from CNS tissue of patients who are potentially

24 contaminated or who are potentially infected with

25 Creutzfeldt Jakob disease or a derivation of cell

1   lines from an individual carrying familial PrP

2   mutations, and I'll address both of these issues.

3   There's also what has become a concern

4   particularly in the last couple of years whether or

5   not tissue culture cells exposed to bovine derived

6   products could become TSE infected, and I'll address

7   this and the susceptibility factors that might be

8   involved.

9   And   finally,   given   these   two

10   possibilities, what is our ability to detect TSE

11   infectivity in these cells?

12   All right. So the first thing I want to

13   address is is it, in fact, possible to derive

14   persistently infected TSE infected cultures from

15   immortalized cell lines derived from human cells, and

16   these experiments were all done many years ago, 20 to

17   30, almost 40 years ago now.

18   And   in   these   experiments   essentially

19   tissue was taken from the brain or spinal cord of CJD

20   or Kuru patients who had died of CJD or -- this thing

21   is extremely sensitive. I've got it. It's okay --

22   had died from CJD or Kuru, were maintained in tissue

23   culture for over 300 or up to 300 days, and then the

24   presence of infectivity assayed for by bioassay in

25   primates, and they could always detect infectivity.

1   Similar experiments were done in mouse and

2   sheep models of scrapie. The point I want to make

3   here is that even though you could maintain

4   infectivity if you just kept the cells alive, the

5   minute you start passing them, you lose infectivity.

6   Okay. So after one or two passes in the

7   human system, you lose infectivity, and you can't

8   recover it upon further passage. You can, however,

9   derive immortalized cell lines infected with TSE

10   agents and other systems because this approach did

11   work in both the mouse and sheep system.

12   So even though it didn't work for human,

13   theoretically it's been shown to work in at least two

14   other system. So the possibility exists.

15   What about familial TSE diseases? There

16   are a wide variety, up to 20 mutations in the PrP gene

17   that have been associated with familial TSE disease,

18   and they're spread throughout the molecule, and we

19   don't know exactly how they lead to disease, but the

20   idea based on the protein only theory of the TSEs is

21   that these mutations lead to altered biochemical

22   properties of the normal PrP molecule.   It then

23   spontaneously converts over to the abnormal form,

24   accumulates, and causes disease.

25   So what is the evidence that this actually

1   happens? Because, of course, this is the concern if

2   you should immortalized either cell line derived from

3   an individual carrying a familial mutation. Does this

4   actually happen?

5   And in my laboratory over the last few

6   years, we've been working with PrP mutants that

7   involve insertion of extra copies of an octapeptide

8   repeated motif, and when you look at these mutations,

9   so what we've done is basically taken a PrP molecule

10   and just added more and more numbers of these repeats

11   and asked about the properties.

12   I hope you can see this. What you find is

13   that if you look at two of the cardinal properties of

14   PrP-res, the abnormal form, protease K resistance and

15   aggregation, you can see the red line here. As you

16   increase the number of repeats, you do, indeed,

17   increase the relative protease resistance of PrP.

18   To put it into perspective, the green line

19   represents the abnormal form PrP-res. At the level of

20   PK where all of the protein is destroyed, PrP-res

21   hasn't even been touched. So this molecule is not

22   PrP-res by this definition.

23   Similarly, when you look at the amount of

24   it aggregated, yes, as you increase, as you have these

25   mutations, the molecule begins to aggregate more. It

1   still does not do so to the same extent as PrP-res.

2   So while the biochemical properties of

3   these molecules change and they begin toe acquire some

4   properties reminiscent or res, they are not PrP-res,

5   and believe me, we've tried. We cannot get them to

6   spontaneously form PrP-res.

7   All right. So this is all in tissue

8   culture. What about _in vivo_? If you take these cells

9   and stick them into animals, you also don't get TSE

10   disease. Okay? So just sticking in the cells.

11   If you make transgenic mice over

12   expressing these mutations, so try to mimic what

13   happens in people, what do you get?

14   And what I've done here is really very

15   briefly summarized experiments done by several labs

16   where they basically inserted human associated

17   familial TSE mutations in to the PrP gene, overexpress

18   them in mice, and then assayed them for disease, and

19   a couple of points I want to make here.

20   Number one, you need overexpression to get

21   any sort of neurological disease. You don't need the

22   mutation necessarily because without the mutation and

23   over expression, you still get neurological disease.

24   Okay. So overexpression itself is sufficient to cause

25   symptoms in the animal.

1   For these top two models when they looked

2   at these mice, they did show signs of neurological

3   dysfunction, no TSE disease, and they certainly didn't

4   passage infectivity. There's this one GSS associated

5   mutation where this was overexpressed in animals, had

6   the neurological disease, and there may have been some

7   transmission into limited -- in a limited way into

8   other mice.

9   So this is the one instance where there

10   may be some evidence that this mutation could induce

11   disease.   When you look, however, at this same

12   mutation in a context where it is not overexpressed,

13   which is much more analogous to the human system, all

14   right, so this is an instance where mouse was made

15   where the mutation was put into the PrP gene and that

16   gene put right back into the correct spot in the

17   chromosome at the correct copy number. You don't get

18   neurological disease. You don't get TSE disease, and

19   you don't get infectivity, and the laboratory that did

20   this the last I heard had looked at 800 and some mice.

21   So they're looking very carefully.

22   So there's no really strong, convincing

23   evidence that mutations, familial TSE mutations will

24   generate spontaneous TSE disease in tissue culture or

25   in transgenic mouse models.

1   Now, what about infection of tissue

2   culture cells, particularly in regard to exposure to

3   bovine derived tissue culture products?

4   What are the susceptibility factors? How

5   much infectivity do you need? How is easy is it to

6   get an acute versus persistent infection?

7   And what I've tried to do on this next

8   slide is summarize all of those points in a little

9   model, and what I've shown here is each of these black

10   boxes represents what I think is a major block to

11   persistent TSE infection of tissue culture cells.

12   First of all, you need extremely high --

13   you need high multiplicity of infection. So routinely

14   what we used to do is brain material, high titered

15   infectious brain material. This automatically calls

16   into question whether or not the very low amounts of

17   TSE infectivity that have been hypothesized to be

18   carried around in blood would be sufficient to

19   overcome this barrier where you need a whole lot of

20   agent to get a little bit in.

21   The second thing, even if some of this

22   agent gets in that has to be considered is, first of

23   all, does the cell express PrP-sen theoretically any

24   cell that expresses normal PrP is susceptible. So if

25   it expresses PrP-sen, it's got the right sequence. So

1   say this is bovine, BSE, PrP-res, and this is human

2   PrP-sen.   Right away you've got a problem.   The

3   sequences of these genes do not match, and that will

4   be an issue in terms of the abnormal form reacting

5   with the normal form and causing propagation of the

6   abnormal form.

7   Okay. So let's say all of this is okay.

8   This matches that. There's enough PrP there. The

9   sequence is the same. It's folded appropriately.

10   This process occurs.   You've passed the second

11   barrier.

12   Then comes the third one, and this is a

13   big one, and that is that as you start to passage

14   these cells and dilute out whatever cells might have

15   become infected, you lose infectivity.

16   Even if you get an infected monolayer, in

17   many cases there are as few as one percent of these

18   cells infected. All right? And the consequence of

19   that is that if you look at an infected cell layer

20   like this for PrP-res, by current techniques you're

21   probably not going to find it. Okay?

22   So while the presence of PrP-res is a very

23   good indicator that the cell is infected, the absence

24   does not mean it is uninfected, and that's what I want

25   to show here. This is an experiment I did years ago

where we tried to infect cell lines that express
relatively low levels of PrP-sen. We assayed at
various times after exposure to an infectious brain
homogenate.

We looked both for PrP-res, which we could
not find, and we looked for infectivity in mouse
bioassay, and we easily found infectivity, and at
every pass and at equivalent levels.

Okay. When we did this experiment in
cells that had been maintained in tissue culture a bit
longer, so that these were relatively new cells; these
had been maintained a couple of months longer; all of
a sudden we couldn't infect them, and this gets back
to the point that Dr. Hughes made earlier this morning
that cells change over time in culture, and in this
instance, it changed in a good way for you guys and a
bad way for me because, you know, I want infected
cells.

The other point I want to make, and Dr.
Krause referred to this a couple of times in his talk,
is this issue that if you overexpress PrP-sen, and
this again is probably not something that should be a
concern with the cell lines that are being used here,
if you artificially overexpress PrP-sen, now you get
a tissue culture line that is more susceptible to

infection, and the idea is with more PrP-sen, you can accumulate more PrP-res, which you can then detect more easily, and you can infect more easily.

And our experience has been this is simply not true. It's completely unpredictable, and this is a piece of data that hasn't been published yet that just shows in neuronal and non-neuronal cells exposed to four different strains in this instance of mouse scrapie. So eight combinations. In only one case is there very clear evidence of formation of the abnormal form, and that's this one right here. All the others are negative.

And I could not have predicted. I mean I could have thrown a dart at this thing and predicted it just as easily as the result I got. So even overexpression, while it could be considered a susceptibility factor, you can't use it to predict susceptibility.

And just to summarize, all the different cell types that have been looked for susceptibility to TSE infection, there have been both neuronal and non-neuronal cells described that are susceptible to mouse and this bottom one here, the kidney epithelial, to sheep scrapie if they expressed the sheep PrP gene. All of these cells, including a couple of cell lines

1   that were referred to earlier this morning by Dr.

2   Lewis, they're using vaccine production. These guys

3   have not been shown to maintain human TSE infectivity.

4   So there are a lot of nonsusceptible cell

5   lines, and you can see a lot of these are human

6   derived.

7   Okay. So finally, to briefly mention what

8   our ability to detect TSE infectivity in the absence

9   of detectable PrP, in the absence of bioassay, there

10   are now three approved and marketed tests for checking

11   for PrP-res in infected animals, and these are being

12   used extensively in Europe at slaughterhouse levels to

13   look at cattle, potentially BSE infected cattle.

14   These tests are all maximized for high

15   titer tissue, such as brain or spinal cord, and

16   they're only sensitive to PrP-res levels that you

17   detect shortly before or at clinical signs. So this

18   is where you have a lot of infectivity present in the

19   animal.

20   This is not like tissue culture cells

21   where you have very little infectivity present. Okay?

22   There is another test that's coming

23   through in the approval process. This is the Adelphia

24   test, which may be a bit more sensitive than these

25   three. There are a couple of future tests that have

1 not really been -- in particular, the capillary

2 immunoelectrophoresis have not been rigorously tested,

3 but have been proposed to be able to detect extremely

4 low levels of PrP-res in tissues such as blood.

5   So the difficulties with all of these

6 tests, the postmortem test, they're based only on res

7 detection. They're maximized to high titer tissue,

8 and we haven't actually tested them against our tissue

9 culture cells.

10   Their sensitivity, again, is not terribly

11 -- not as good essentially as bioassay in either the

12 same species or in appropriate transgenic mice. So

13 bioassay remains the gold standard test for detection

14 of TSE infection, and as Dr. Krause referred to, the

15 time required for this assay often makes it

16 impractical, but it's very important.

17   So given the data that we currently have

18 in tissue culture, it's, you know, unlikely that human

19 neoplastic cell substrates will be TSE infected or

20 that exposure to potentially BSE contaminated bovine

21 tissue culture products could lead to persistent TSE

22 infection, but of course, as we're all well aware, you

23 cannot guarantee zero risk.

24   And in the absence of that, what can you

25 do to at least assess it as carefully as possible, the

1   presence of TSE infectivity in these cultures? And

2   this is the answer I came up with, and after finishing

3   it, I realized it's basically what we do in the lab to

4   assess whether our tissue cultures contain infection.

5   There are now commercially available tests

6   that can be used to do this, and as, again, Dr. Krause

7   mentioned, you assay for PrP-sen, its expression

8   level, determine its sequence. If it's a familial

9   mutation, throw the cell line out.

10   Assay for PrP-res, and this would best be

11   done in multiple cell sub-clones from the passage, as

12   well as periodically at different cell culture passes

13   because of the potential instability of the cell.

14   Bioassay for infectivity if all of this is

15   negative. If any of these are positive, you know,

16   again, throw out the cell line. If these are all

17   negative or if this is negative, bioassay for TSE

18   infectivity would have to be done, I should think.

19   And in terms of looking for human TSE

20   infectivity or BSE, perhaps the best bet is to use

21   human PrP or bovine PrP expressing transgenic mice.

22   Keep those animals for up to two years, watch for

23   clinical signs, and at the end of the day, look in the

24   brains for pathology and/or PrP-res, and to do this,

25   you know, relatively routinely if you're worried.

1   So I'll stop there pretty much on time and

2 take any questions.

3   ACTING CHAIRMAN DAUM: Thank you very

4 much, Dr. Priola.

5   And we'll have this presentation open for

6 discussion. We'll begin with Dr. Decker.

7   DR. DECKER: Just a simple clarifying

8 question to see if I've absorbed your implicit

9 definitions correctly. When you and Dr. Krause used

10 the term "infected," you're referring to a cell line

11 that actively is producing the abnormal prion protein,

12 and if we're just talking about pouring in some

13 preformed abnormal prion protein, you're going to use

14 the word "contaminated" or something like that?

15   DR. PRIOLA: Yeah, that's correct. When

16 I use "infected," I mean persistently infected.

17   DR. DECKER: So we've got a cell there

18 that's actually --

19   DR. PRIOLA: Producing infectivity.

20   DR. DECKER: -- producing.

21   DR. PRIOLA: Yeah. It's making PrP-res,

22 it's accumulating PrP-res, and it's making new

23 infectivity.

24   DR. DECKER: Rather than simply pouring

25 contaminated --

1   DR. PRIOLA: Exactly.

2   DR. DECKER: -- bovine serum in there which

3   eventually gets diluted out and doesn't reproduce.

4   DR. PRIOLA: Right, which is what's

5   suggested by those immortalization experiments, yeah.

6   ACTING CHAIRMAN DAUM: Thank you.

7   Dr. Coffin, please.

8   DR. COFFIN: It's technically feasible,

9   although probably not very pleasant, to take cell

10   lines that one imagines one might use for vaccine

11   production purposes and actually knock out both copies

12   of PrP. From what I understand, such cells would be

13   expected to be perfectly viable and you wouldn't

14   notice the difference. The whole animals are

15   perfectly viable without --

16   DR. PRIOLA: Yes.

17   DR. COFFIN: -- PrP. Do you think that

18   would be an advisable thing to do?

19   DR. PRIOLA: Well, if you took away the

20   PrP, then I think you'd have a cell line that could be

21   very strongly argued would be completely resistant to

22   TSE infection, and I have never made knockout cells

23   myself, except from knockout mice, for example, PrP

24   knockout mice, but if it could be done, and it was not

25   technically too difficult, then, yeah, I think it

1   would be -- it would take away an element. You know,

2   what little risk there is, it would take that away

3   because PrP knockout mice are resistant completely to

4   infection.

5   ACTING CHAIRMAN DAUM: There was one more

6   hand here. Was it Dr. Myers?

7   DR. MYERS: When you're growing, in

8   effect, cell in culture and it loses the infectivity,

9   talking to people about this, the implication for me

10 is that if you're growing the cell fast enough, the

11 cell outgrows the PrP-res. Is that not an appropriate

12 way of looking at it?

13   DR. PRIOLA: Well --

14   DR. MYERS: Because an awful lot of your

15 infected cell lines are very slow growing things, and

16 you mentioned this business about the TSE from the

17 infected brains and so on. Is that a misconception?

18   DR. PRIOLA: You know, I don't think it's

19 a hard and fast rule. The neural 2A cells, which are

20 mouse neural blastoma cells, we have troubles with

21 losing infectivity in these cells if we don't use very

22 specific types of medium.

23   There's another persistently infected cell

24 line called an SMB cell that is perfectly stable. It

25 is slow growing. These fibroblast cells I showed you

1   where it appears we got infectivity in there based on

2   the res signal, we have to split those if we're not

3   careful every two to three days. They are not slow

4   growing, and they are very reproducibly infectable

5   based on PrP-res with this one strain. So I don't

6   think it's a hard and fast rule.

7   ACTING CHAIRMAN DAUM: Thank you.

8   Dr. Katz.

9   DR. KATZ: I have a naive mechanical

10 question. When you talk about passages and failure or

11 success at pass, you're talking about passing

12 suspensions of cells, not just tissue culture fluid;

13 is that correct?

14   DR. PRIOLA: That's correct. These are

15 passage of TAP cells, yeah.

16   DR. KATZ: Thank you.

17   ACTING CHAIRMAN DAUM: Dr. Aguilar-Cordova

18 and then Dr. Stephens.

19   DR. AGUILAR-CORDOVA: I was wondering.

20 When you talk about some people being contaminated

21 with hospital instruments, would that be expected to

22 be a large inoculum, and then from your experiments in

23 vitro, it seems like you really need a huge inoculum

24 to get anything going. So could that be a big

25 differential between _in vivo_ and _in vitro_?

1   DR. PRIOLA: Yeah. I mean, _in vitro_ you

2   definitely need much more infectivity to get a

3   productive infection of tissue culture cells. As to

4   how much infectivity is actually on a contaminated

5   surgical instrument that passes this, I don't know,

6   but I do know that when it happens, the people who

7   have -- what essentially happens is an operation is

8   done on a subclinical CJD patient, and those

9   instruments are used in other patients before it's

10   realized, and those other patients do get CJD.

11   So it's pretty effective, and with

12   presumably, you know -- well, I don't know the dose.

13   I don't know the dose, but it may be very low.

14   ACTING CHAIRMAN DAUM: Dr. Stephens, and

15   then Dr. Coffin.

16   DR. STEPHENS: Just a follow-up on the PrP

17   knockout question. What does PrP do in normal cells?

18   DR. PRIOLA: That's a really good

19   question. It's not precisely known, but there was a

20   recent really nice paper in science that suggested

21   that if you cross-link it in differentiated neurons,

22   it activates the Finnkinees (phonetic) pathway.

23   DR. STEPHENS: And as a follow-up, was

24   there any evidence, is there any evidence of an

25   inoculum effect in new variant CJD in terms of BSE

1   · exposure?

2   DR. PRIOLA: You mean inoculum effect, how

3   much you need?

4   DR. STEPHENS: Yeah, how much? I mean, is

5   there a relationship in any of the epidemiological

6   studies to beef exposure?

7   DR. PRIOLA: Yeah.   Not being an

8   epidemiologist, I don't know the precise answer, but

9   I do know that the current thinking right now is it's

10   inefficient because -- it's inefficient. You're

11   crossing a species barrier. You're going orally, both

12   of which are bad things. So you presumably need more,

13   a pretty good bolus.

14   DR. STEPHENS: I appreciate that, but is

15   there data that supports that?

16   DR. PRIOLA: Not that I'm clear on.

17   ACTING CHAIRMAN DAUM: Michael Decker

18   might have data about that, an answer for that.

19   DR. DECKER: I don't know if there's the

20   type of hard data we'd like, but an illustrative

21   anecdote is the town in England where the butcher shop

22   that served the local community was using centuries

23   old techniques that heavily contaminated the resulting

24   meat with neural tissue, and there was a very high

25   attack rate, a cluster of human variant CJD in that

1   town.

2   So   they   found   both   an   unusual

3   preponderance of disease and an unusual practice that

4   would give them a high load.

5   DR. PRIOLA: Yeah, I'm not sure what

6   the -- I know there was a cluster of five patients,

7   but I'm not clear what the total number of people were

8   that were exposed, and so what the exact attack rate

9   was.   Do you?

10   DR. DECKER: No, but five out of the 90 in

11 England is a pretty high proportion.

12   DR. PRIOLA: Oh, for sure. Oh, yeah.

13   DR. DECKER: The proportion of that town's

14 population to the total English population.

15   DR. PRIOLA: Definitely, yeah, yeah.

16   ACTING CHAIRMAN DAUM: Dr. Coffin and then

17 I'd like to move on.

18   DR. COFFIN: So what do we know about

19 infectivity in serum of affected animals and their

20 offspring?

21   DR. PRIOLA: In terms of cattle?

22   DR. COFFIN: Yeah.

23   DR. PRIOLA: In cattle?

24   DR. COFFIN: In cattle, yeah.

25   DR. PRIOLA: There are only two reports

1   · that I know of that have suggested there might be very

2   low levels of infectivity in blood of cattle, one of

3   which is in an experiment where they experimentally

4   inoculated cattle and assayed tissue over the entire

5   course of disease. There was a point at which they

6   detected infectivity in bone marrow cells from a pool

7   of three samples.

8   And there was a recent report last year

9   where it's a bit more indirect, where sheep infected

10   with BSE -- a bunch of sheep were infected with BSE,

11   and at various time points blood was transfused from

12   those sheep into naive sheep, and one of those sheep

13   got sick, the implication being that infectivity got

14   out into the blood.

15   DR. COFFIN: But have there been serious

16   attempts made to look into --

17   DR. PRIOLA: There have.

18   DR. COFFIN: -- serum products and other

19   blood products?

20   DR. PRIOLA: Commercial serum products?

21   Not that I --

22   DR. COFFIN: Or in serum that's obtained,

23   you know, large amounts of serum from large numbers of

24   significant --

25   DR. PRIOLA: Yeah, and in terms of

1   hamsters, yes, there was. Bob Rohwer did a really

2   heroic effort to do that, and pure concentrated

3   hundreds of mLs of hamster blood and was able to

4   detect using lots of animals that there's less than 1

5   LD-50 out of all that material.

6   DR. COFFIN: So it seems --

7   DR. PRIOLA: It's extremely low.

8   DR. COFFIN: -- at the moment, based on

9   our current thinking, it seems improbable --

10   DR. PRIOLA: Extremely.

11   DR. COFFIN: -- that there were these

12   significant risks from serum, to begin with.

13   DR. PRIOLA: Based on our current

14   knowledge.

15   DR. COFFIN: Based on our current

16   knowledge.

17   DR. PRIOLA: Absolutely.

18   ACTING CHAIRMAN DAUM: A burning final

19   comment.

20   DR. MINOR: I mean, while the bone marrow

21   result is out there, okay, I think it was regarded as

22   slightly questionable, and I think also that the

23   efforts that people have made to find infectivity in

24   cow blood as opposed to bone marrow have uniformly

25   been negative, and that's even going cow to cow, as I

1   understand it.

2   DR. PRIOLA: Yeah.

3   DR. MINOR: And I think there's also an

4   issue of the species that you're looking at will give

5   you a different level of infectivity in the blood

6   perhaps.

7   DR. PRIOLA: Sure, absolutely.

8   ACTING CHAIRMAN DAUM: Thank you for that

9   clarification.

10   I'd like to move on to Dr. Krause, who

11   promised we would have our opportunity to have at him

12   again, and here it is. Dr. Krause will talk about

13   adventitious   agent   testing   of   neoplastic   cell

14   substrates.

15   DR. KRAUSE: I'm just going to dive right

16   in and view this as a continuation of where I left

17   off. This slide shows a number of different episodes

18   throughout the history of the world and the U.S. of

19   contamination of biological products with adventitious

20   agents, and I don't want to go into this in very much

21   detail, but I do want to focus on the contamination of

22   polio and adenovirus vaccines in the late 1950s and

23   early 1960s with SV40 as an example of something we

24   really want to avoid repeating.

25   And as many of you know, millions of

1 people received SV40 contaminated polio and

2 adventitious vaccines into the late 1950s and early

3 1960s, and these vaccines were produced in primary

4 Rhesus monkey kidney cells.

5   SV40 was identified as a virus; it was

6 discovered after this, and the basis for discovering

7 SV40 was the fact that supernatants of primary Rhesus

8 monkey kidney cells cause tumors in laboratory animals

9 and also were shown to cause cytopathic effect in

10 primary Circa Pithacus (phonetic) monkey kidney cells,

11 or different species of monkey, which then led to the

12 discovery by Dr. Hillaman (phonetic) at Merck of SV40.

13   The vaccine seeds were treated with anti-

14 SV40 neutralizing antibodies in the early 1960s to rid

15 them of SV40. Epidemiological studies suggest luckily

16 that there was no adverse sequelae to the vaccinated

17 children, and however, recently SV40 DNA has been

18 detected in some human malignancies by PCR, and this

19 highlights a significant problem that one has with

20 adventitious agents, which is that sometimes the

21 concerns and the potential effect might not become

22 apparent for a substantial period of time, until a

23 substantial period of time after the contamination

24 event has occurred.

25   So a couple of lessons learned here are

1   obviously the value of insuring that the products are

2   free of adventitious agents, and also the particular

3   importance of insuring the freedom of products from

4   oncogenic agents or potentially oncogenic agents,

5   especially for vaccines that are given to children.

6   So I've listed here a few basic principles

7   of adventitious agent testing. We've already talked

8   about how this kind of testing should consider

9   quantitative issues and how important it is to

10 understand the sensitivity of one's assays and what

11 one precisely is tested for and what the meaning of a

12 negative result actually is.

13   It's important to consider issues that are

14 specific to the material in question, and I would

15 argue that also where possible one should use tests

16 that have the potential to detect unsuspected or even

17 undiscovered agents.

18   Now, to talk about adventitious agents, I

19 think it's useful to think a little bit about how

20 viral vaccines are produced. The center of viral

21 vaccine production is the cell substrate, and of

22 course, for live viral vaccines or inactivated viral

23 vaccines, some cell substrate is required to grow the

24 virus.

25   You add to that a vaccine seed and various

1 other raw materials, which can include serum, medium,

2 stabilizers or things like that, which then together

3 in the production process after usually some kind of

4 purification or inactivation lead to the final

5 product.

6 This entire thing takes place in a

7 facility which hopefully is designed in such a way as

8 to minimize the opportunity for contamination at any

9 of these steps, although there is the risk, of course,

10 that personnel who perform these steps might

11 contaminate a vaccine.

12 But a basic principle of producing these

13 vaccines is that all of the materials that go into the

14 vaccine, the vaccine seed, the raw materials and the

15 cell substrate, need to be shown to be free of

16 adventitious agents as they enter the process.

17 Now, purification steps could potentially

18 reduce adventitious agent burden if some were able to

19 get into the production process, and there is a model

20 for thinking about that that has been used in

21 evaluating therapeutic products at CBER in which some

22 cell substrates might contain viral particles, and the

23 ability of a purification scheme then to remove those

24 particles is then determined, and that's called

25 investigation of viral clearance.

1   And a general principle there as well then

2 is that the purification scheme which is used needs to

3 be able to remove no only the amount of material that

4 you think could be present in the cell substrate, but

5 also some additional amount which represents a safety

6 factor.

7   It's also worth noting that for live

8 vaccines a purification process could actually

9 potentially concentrate adventitious agents.

10   Okay. So as we look at adventitious

11 testing then, I've talked about the importance of

12 quantitation, but I just wanted to go through four

13 individual points here because in policy making not

14 only do we need to understand how sensitive an assay

15 is, but it really does need to estimate the pretest

16 probability of a problem because that will influence

17 the kinds of assays that one thinks needs to be done.

18   One needs to consider the number of doses

19 or dose equivalents that can be tested. One needs to

20 understand the sensitivity of the assays, and a very

21 important component of that then is in performing

22 assays to include the appropriate controls that allow

23 one to define how sensitive any given assay was, and

24 the need to consider safety margins in deciding what

25 kind of assays need to be performed.