DR. SHERWIN: So, my question is if you knew the insulin content right away you could know the answer about the content the next morning when you did your transplant because an overnight currently is possible. I don't know the literature. Have there been studies done to actually look at insulin content acutely and try to get a sense of whether that has something to do with function of the islets later on?

DR. SHAPIRO: A flash analysis of insulin content doesn't tell you whether or not that islet has the capacity to regenerate and repair.

DR. SHERWIN: Of course. I realize that. Theoretically there is no question about that. You are absolutely right. That is why I said originally function. The problem is the functional assay is only in retrospect. Ideally, you would like to know what the product is before you put it in the patient. Maybe we can develop assays in an hour that work that are reasonable, and that would nice but right now we can do it at the time of isolation. Even though it isn't perfect because it clearly could be looking at insulin in a dead islet or that is about to die. Have people looked at this?

DR. HERING: Studies have been done to address the question whether the insulin content reflects the beta-cell mass but not the potency of a given transplant to revere

diabetes. The studies that I mentioned found that there is a tremendous variability and insulin content may, as you are well aware, reflect the degree of degranulation; may reflect a number of different things. It is not the perfect assay. I know that much, much better assays, for example insulin biosynthesis if you get into potency have been done in Brussels, and are very well documented, but this didn't predict graft outcome because so many other factors may be much more important. There is limited information but insulin content alone -- there is no evidence to support that this has predictive value.

DR. SALOMON: From the audience?

DR. OLACK: I can just say that in St. Louis, probably in the first 16 patients that were transplanted we measured total insulin content in those patients and found no correlation between insulin independence and the amount of insulin content that was transplanted into those patients.

DR. SHERWIN: If you don't have insulin in the preparation, then I assume it is not going to work. In other words, there is obviously no direct relationship but is it something that one should do to just be sure that there is at least some insulin? In other words, is it a criterion for a cut-off? I guess that is what I am asking.

DR. SALOMON: Sixteen patients? Did any of them

work?

DR. OLACK: Yes.

DR. SALOMON: Okay. So, what percent worked, and there was no correlation between insulin content and the percent that worked?

DR. OLACK: I guess our longest insulin-free patient was five years out with insulin independence. We had patients that ranged -- and Camillo was there at the time too -- from a few weeks out to 12 months. But our goal was to transplant like 100 units of insulin content, at the time, per preparation and sometimes we achieved it and sometimes we didn't, but we didn't set a criterion that that is what we needed to have. So, I can just say that we tried to find a correlation between the two and we couldn't. Even today, with all of our islet preparations, we still monitor in different ways insulin content but I haven't seen papers where someone said if you transplant this much insulin content you are going to have a successful transplant.

DR. SALOMON: The problem though is if you design your experiments that you are trying in every single one to do a certain amount and in a few you fall short, you can interpret that data as saying that insulin content is not a criterion to follow.

DR. OLACK: Right.

DR. SALOMON: Right.

DR. NOGUCHI: You would never transplant islets that didn't have insulin? Is that correct? I mean, that was the point being brought before. If we are talking about identity of something you stick into a person, it would just seem that it should have insulin in it at some point.

DR. OLACK: I am sure you would have islets that have some amount of insulin in them. If you had a preparation -- I can't imagine anybody that was close enough to be doing islet transplantation, getting the whole transplant preparation and having no insulin content. But the question is, is there a cut-off or is there not and I haven't seen that published.

DR. SHERWIN: I mean, it is such an easy measurement; it is child's play --

DR. OLACK: The measure is not hard but setting the criteria --

DR. SHERWIN: Oh, no, no, I realize that. I am just saying, you know, what kind of criteria are we going to establish and it seems to me that since it is such an easy measurement, even if it turns out to be useless, it is something that should be --

DR. SAUSVILLE: But I would distinguish between something that is utilized by the community and people who do this and data that would be collected along the way. To me, I have heard that an islet is something that sort of

looks like an islet, has a certain size and is dithiazone positive, and that is what we give back after two hours. Is that correct?

DR. SALOMON: Right.

DR. SAUSVILLE: So, unless some compelling evidence should emerge that that is not a good standard at this outset it becomes difficult to make things more onerous. On the other hand, I definitely agree with collecting more data along the way and then we will see if we can with the power of numbers -- we are not going to tell anything from an experience of 16.

DR. LAKEY: If I may comment, on our islet patients to date we have been collecting samples for insulin DNA, calculating the insulin/DNA ratio and the beta-cell content of the grafts. To date, we have not been able to demonstrate any correlation with any of those factors to the islet equivalence.

DR. SHERWIN: But you said that all your islets work and they function in retrospect. So if they all function, obviously, they are going to have islets and, surely, there would be no relationship. I would predict that. The key issue is let's say there is a minimum amount of insulin that is needed by content as being an acceptable product. I am sure all your islets exceed that. Therefore, there would be no relationship. But the issue is for other

people who may not be quite as good, is there going to be a minimum requirement? It seems to me that we don't have it now. So, I wouldn't stop you from doing anything but I think we should really try to come up with some standard about that because there will be people who will be putting in islets which have virtually no insulin -- not no insulin but very little, and they are doomed.

DR. BLUESTONE: It is not quite that straightforward. Right? I mean, what you are saying is that it works. Well, there is "works" and there is "works." If you ask how many became insulin independent after the transplant, the answer is they didn't all work. So, there are ways of doing some correlations. It is not like all of them became insulin independent within 24 hours and, therefore, you never need to look again. So, there may be a little more subtlety in the data that you can actually look at. Right?

DR. HERING: I think your question was to use insulin content as an identity assay, not as a potency assay. Here, of course, it can be done whenever you culture islets for one or two days because this simply takes a few hours or overnight and then you have to do an assay. In this particular setting it couldn't have been done because islets were transplanted right away. So, maybe one compromise would be a center which is interested in doing

islet transplantation could do non-clinical test runs and could document that they have a critical mass of insulin present in islet preps. Then, I guess, this should probably take care of the issue, and whoever is going to transplant islets after a culture period could use this assay as an identity assay.

DR. LEVITSKY: I will address this to everyone. I isolated islets at one time and I can't imagine an islet preparation that wouldn't have insulin in it that looked healthy otherwise. If they look like healthy, happy islets they are going to have insulin. I think it is something that can be done, but I wonder whether the dynamic assay isn't really more important, and to do that to islets that have just been through the isolation process would be cruel and inhuman. You would really have to wait for them --

[Laughter]

DR. SHERWIN: It is not that I don't favor functional assays, it is just that you would like to know -- clearly the amount of insulin in a pancreas -- you could digest the pancreas and the amount of insulin in it relates to the islet mass. My guess is that they don't always know how many islets they really have and, consequently, some minimum number, since some of the islets are dead anyhow -- it might be a useful thing and it is easy to do. It is not that it is a good thing to do, and it is not superior to

functional assay but functional assays currently won't have the information until retrospectively. You would like to know the information prospectively to eliminate those pancreases where you think the hopes are very low.

DR. LEVITSKY: I guess a dead islet is going to have insulin in it.

DR. SHERWIN: It will.

DR. LEVITSKY: So, that is not going to help you.

DR. SHERWIN: Well, it is really how much islet mass you have, yes, you are absolutely right.

DR. SAUSVILLE: This is where this calcium assay that was talked about -- of everything that is around, that can take about a minute or two. You look under a confocal microscope and immediately -- this is sort of Star Wars technology, but you've got a functioning islet. Although that is a very interesting thing from a research perspective to look at prospectively, I don't think anybody has experience with that to say that this should be a criterion now. But I would note that of everything that has been talked about that has the greatest hope for a short-term functional assay.

DR. SALOMON: What I would like to do is offer everybody a break at this point. We have done identity testing. We have answered specifically, and to review it, to make sure that we are providing a sense of the committee

to the FDA, my sense here is that we have to acknowledge the FDA's need to identify what is an islet as a product, which means, as much as you guys want to keep it vague and research based, you are going to have to agree as a group, not just the three of you but the whole field, on criteria. They may be criteria that have flexibility in them though.

Those criteria also need to be divided into criteria that are determined immediately and are a requirement for putting the islets into a patient, allowing you to move efficiently toward a transplant, and what I think I have heard there is that they are Gram stain negative and that they are dithiazone positive; that they have a sufficient islet mass, which I heard and which was not challenged, of about 5000 islet equivalents per kilogram; that there is reasonable volume distribution. That was Camillo's point. That would be figured into the algorithm of the IEC determination. And, that had to be fulfilled by two hours.

Within the next 48 hours, I think that the general concept is accepted by the group that there should be follow-up testing. I don't know that anyone is willing -- and I am not quoting anyone as saying this will be the rigid criteria but I think everyone accepts the concept that the next morning there will be criteria that should be fulfilled, and they would include insulin content, albeit it

is not totally clear; some sort of dynamic test of insulin release --

DR. RICORDI: You have forgotten the earlier marker. There is also the variability by exclusion --

DR. SALOMON: Yes, and I wasn't trying to be too clever but the next section is viability, when we come back from a break but, yes, fair point, Camillo.

The third part that I think we would all agree on is that there is really a research part of this that should be fostered by investment of the NIH, the JDF and other funding organizations where we would begin to look at things like gene arrays, rapid expression of apoptosis, looking at apoptotic genes as well as apoptotic markers such as fragmentation of existing caspace proteins, for example, which can be done by Western blots now; RNAs; protection assays, approaches like that; confocal microscopy for calcium current. All of those should be in the research criteria. Have I got sort of the general thing?

DR. BLUESTONE: Does this mean you are done with potency? I am confused about your numbering system right now.

DR. SALOMON: We have done viability, Jeff. That is all I am trying to get done -- appropriate identity testing.

DR. BLUESTONE: But you went well beyond identity

testing in what you were just talking about. So, do you want us to discuss the things you talked about even though they are later numbers, or do you want to wait until we get to later numbers?

DR. SALOMON: No, remember, I said that we included potency --

DR. BLUESTONE: So, you are adding potency now?

DR. SALOMON: Yes.

DR. BLUESTONE: So, one thing I brought up a while ago and Camillo brought up is, if you think about it, if we are doing things that are long term, shouldn't we be thinking about the ultimate potency assay which is an in vivo assay, not an in vitro assay? And, shouldn't we be thinking about using reconstitution of diabetic nude mice, not under the kidney capsule where it is ridiculous but maybe nude rats so you can actually inject them in the portal vein? Shouldn't we be thinking about some kind of true in vivo potency assay?

DR. SALOMON: I like that one a lot. Let's come back from the break and start there.

DR. CHAMPLIN: I hate to stretch things out but the issue in terms of standards for what you would demand to do the transplant is the first step -- the number of cells, their characteristics that you can do immediately and the fact that they are not infected. Everything else is

unvalidated. So, I would put everything else into the research pot. Maybe some of those things are going to be important but I don't think you can require them as a product definition today. Certainly they should be done and as, you know, they are validated scientifically they should be included and ultimately in vivo testing as well. But if we are looking for a description of the product I think we are done by that first group.

DR. SALOMON: Dr. Levitsky?

DR. LEVITSKY: The question I have is when I read the data from the transplant documents that we were sent, it seems as if until the new data from Edmonton, which sound like they are going to be spectacular when we know about them, the actual survival of most islet grafts was based entirely upon small amounts of C-peptide release that wouldn't be sufficient to sustain anything very much. And, those data are what the criteria for the number of islets that should be given are based on. Do we have real data based upon outcomes that would meet colleagues guidelines which tell you how many islets should be transplanted? I mean, I am just trying to find out here if any of these criteria have really reached --

DR. SALOMON: That is question three, viability number and size distribution --

DR. LEVITSKY: But you were mentioning that.

DR. SALOMON: My apology.

DR. LEVITSKY: Okay.

DR. SALOMON: Obviously it is all the same thing but we are trying to arbitrarily divide it up into a series of questions.

DR. SIEGEL: I know you want to get to the break but let me just help provide a little bit of an intellectual framework, a regulatory intellectual framework so that you can understand why we are asking these questions. These are experimental products so we are not going to expect any testing criteria to be validated, which is to say nothing at this point do we expect to be validated to provide an effective product because we have no data that there is such a thing as an effective product.

So, why are we asking for testing? Well, in a sense there are three reasons we are asking for testing, and these have been underlying a lot of the comments but I want you to kind of think of these a little more discretely so you can understand where we are coming from and what we need.

One is safety of patients. So, if you tell us that when the Gram stain is positive it is unsafe; if the volume is too large it is going to cause portal hypertension or whatever, we may put a specification that you cannot do an experiment with dead cells in people; that is not safe.

Or, with contaminated cells, or with whatever specification.

Second is rationale. It is closely related to the issue of efficacy. This gets to Dr. Zoon's comment about validation in animal models. Why do we ask for rationale? Well, it gets to a very complex issue which has to do with the relationship of rationale to risk, which is much harder to think about than benefit to risk. To make this more concrete, for example, when we were talking about whether something should come from a donor with pancreatitis, we are going to assume that there is a risk associated with entry into any clinical trials and it may differ with different ones. For example, some of these protocols may put a patient on immunocompromising therapy that they otherwise wouldn't be on. So, if you are going to come in and say I am going to put somebody on immunocompromising therapy that I think carries an infectious risk, or I think carries a risk of malignancy, or I don't know but it may carry that risk, then there is an issue of rationale. It may well be that if you have a hypothesis that cells with low trypan blue exclusion work as well as cells with high viability and, therefore, you want to study that, or cells from pancreatitic donors do as well, we may want to see the science behind that. It may come from an animal model; it may come from an <u>in vitro</u> model; a secretion model, but something to provide some evidence that that aspect of the

product is sufficient to make it appropriate to expose patients to the intrinsic risk of being on the protocol.

So, that is two areas. One is product testing to make sure it is safe. Another is product testing to have something that has a reasonable probability of yielding useful data and some likelihood of providing benefit or at least being helpful in the development of the product that may compensate the risks.

The third issue is the one I outlined before that you have all taken into account, and I think is a very critical one, which is the issue of what data need to be collected. In this one, unlike the other two which are likely to be very small numbers of things -- those first two areas of rationale and safety, just might be, as we have been discussing, a small handful of tests, three, four, five or whatever they are that we are going to put a specification on. The other issues are the questions of collecting the data so that at the end of the stream, whether it is a year from now or ten years from now when we have something that works, we also have the data to be able to say what it is that predicts that it works. Because what will happen is when it works it will go out of the hands of the five or fifty laboratories that are expert in controlling how to do it and into the hands of a far broader population. If at that point in time we do not know what

are the tests that need to be done to ensure quality, then experience teaches that you can magically lose efficacy -- it works in these people's hands and it doesn't work in those people's hands and nobody knows why.

So, those are the three things we really need to know in terms of knowing what testing needs to be done, and the three reasons why we want product testing.

DR. SALOMON: And a last word from Carole?

DR. MILLER: Can you clarify for me whether or not, from a regulatory standpoint, we are talking about the fact that this is a product and you are assuming that, after you make these minimum regulatory criteria for what makes a product, all further pancreatic islet cell transplants will be done on clinical trials or not? Because I think it does make a difference for what we say and how we say to use it. you know, I am from the drug side more than from the cellular therapy side so some of these things we are trying to do when you are talking about the regulation while you are doing the experiments is you are writing the package insert before you have the pharmacokinetics. So, that is why I think I am having trouble trying to figure out how anybody can answer these questions. If you are saying, okay, this makes the minimum product -- you know, sterility and so many islet equivalent -- does that make it so that anybody who wants to collect a pancreas can do it? Or, does

it have to be done in the setting of a clinical trial? Do we know that answer?

DR. SIEGEL: Yes. This is an experimental product and it can be used only in the setting of a clinical trial.

DR. MILLER: So, would the fact that these were all registries, these patients have all signed informed consent for a clinical trials that have been done and will be done in the future. Is that true? Registries usually collect data on stuff that is not part of a clinical trial, and this registry that is being funded is collecting data and so the data doesn't mean it has to be part of a clinical trial. Like a bone marrow transplant registry collects all the transplants that are done in the United States on a voluntary basis, or is it the fact that like the National Bone Marrow Donor Program where you can't get a product without being part of a clinical trial?

DR. SIEGEL: Well, we may be using the clinical trial in different ways. I am using the word clinical trial in a rather broad sense which includes protocol-defined and controlled collection of data even in what you may consider a registry experience. I think we would probably all agree that this field is at a point in time where certain steps forward would best be made through multi-center controlled, well-designed clinical trials. We are supportive of that. But in saying that this product should only be used in a

setting of a clinical trial, I am not saying that it can only be used in the setting of that sort of clinical trial. Is that what you are asking?

DR. MILLER: My question is these guidelines you are making assume, if we say we want this minimum but we want to collect all this data, that there is some control over this because you, as the FDA or as the regulatory body, can only approve trials that are going through your mechanism and getting that. Is that what we have? Or, no matter what is said here at this meeting, if somebody wants to collect pancreatic islet cells in their hospital and infuse them off an IRB-confirmed clinical trial without going through a protocol for the FDA, that can still happen? Correct? Can it or can it not still happen? That is my question.

DR. SIEGEL: It should be done under IND.

DR. MILLER: They all have to be done under IND. So, there is a control. That is really the question about determining a minimum. You are then making a minimum for the clinical trials, not a minimum for the product to be used outside of clinical trials. Correct?

DR. SIEGEL: If I understand you, I think that is correct. No, we are not setting regulations here, which means we can decide in this group that cells should always have a viability of 50 percent or 70 percent or whatever

number, and if somebody comes to us and says I have a clinical trial in which I want to use cells of a lower viability, and the reason is because it is a very low risk trial and I have a better measure that is not dye exclusion that is really telling me that I have a highly functional cell even though dye exclusion is low -- you know, this is guidance and guidance simply is that and has substantial flexibility.

DR. MILLER: Thank you.

DR. SALOMON: We are going to have a break and we will be back to start again at four o'clock.

[Brief recess]

DR. SALOMON: We can get started. When they presented all these questions to me in a pre-meeting telephone conference last week, I said you don't really think we are going to get done with all of them? But we are going to try. Okay? So, when we finished, just before we ended Jeff Bluestone brought up something that I also agree very much with, and that is the idea of a biological assay, and animal transplant assay as another criterion to be considered for defining the quality of the product. That really is crossing the line at this point into something that would be experimental but it certainly is a test that all of us do in our laboratories all the time. There is no assay to develop here, right?

So, let's start with that, although I don't want to spend hours on it, but it is a very, very good point that Jeff has made. So, what kind of comments do you have on that? Bernhard, how about if I put you on the spot because I know you do this routinely?

DR. HERING: We have discussed that here between the centers and our approach right now is to transplant islets -- this is still not validated but this is what we want to study, to transplant islets into diabetic nude or SCID animals, mice, and transplant two animals at least, one that receives 2000 islet equivalents and one that receives 1000 islet equivalents. The mouse that receives 2000 should become normal glycemic basically with every single prep, and the one that receives 1000 islet equivalents and becomes normal glycemic obviously received a better prep. Then, we would study the time to cure and the proportion of cured animals, So, this is what a number of us are doing, and this is what we would like to discuss as a potential approach.

DR. SALOMON: I would like to add that when we get to talking about shipping islets I think these bioassays may be the only way to really do this correctly. In other words, if you take islets at the purification center and say that 2000 islet equivalents in that particular prep cured diabetes under the circumstance and you then ship that islet

prep to me, I should be able to demonstrate that it takes 2000 or 3000 or 4000 to do the same thing. I would submit that that is a very sensitive and also very valuable way of looking at things. Jeff, did you have a comment you wanted to make?

DR. BLUESTONE: Well, I just wanted to comment that what Bernhard is talking about is putting islets under the kidney capsule. Correct? Which I think for islets that are cultured overnight is quite doable, but I think for islets that are going to be used immediately and not cultured overnight it is problematic because of the exocrine tissue. So, I think that we need to be thinking simultaneously about different kind of assay, and that is why I proposed rats or maybe something where you could go actually into the portal vein and perhaps have a model that is more akin to what the human experience is going to be, only because of this issue of non-culturing. I don't know what the Edmonton group thinks but I don't think they would think that would be a very good assay for their islets because it would probably kill all the islets when you go under the kidney capsule. Right?

DR. SHAPIRO: Well, we do that. We don't do it routinely but we do put them under the kidney capsule. Also, it would be technically possible to put them in the portal vein of the mouse. You would have to do it under a

microscope.

DR. SALOMON: I mean, people put fragments of pancreas under the kidney capsule, adult pancreas, and that works. So, why are you saying it would kill them?

DR. BLUESTONE: We have done it and it is much more difficult when you don't purify your islets to get it to work. Now, if you purify them over ficoll it is better, and if you culture them it is best. And, I have talked to Bernhard about it and others and --

DR. RICORDI: You are talking about fragments of fetal pancreas?

DR. SALOMON: We do that but I wasn't bringing that up. I was talking about adult pancreas.

DR HERING: I think Jeff's point is how are you going to approach this if you have an unpurified islet prep. Let's say your aliquot that contains 200 islet equivalents is such a tremendous volume that you cannot possibly place it beneath a kidney capsule. So what are you going to do? That is, I guess, your question.

DR. BLUESTONE: That is part of my question. With the unpurified islets you have several problems. You have the problem of volume. Right? You have the problem of a lot of other stuff you are putting in and so you pack it in there and you get a lot of death of the exocrine tissue which seems to kill the beta tissue.

DR. AUCHINCLOSS: If I could just make another point about the non-quantitative aspect of this assay, which I also think is a nice assay to have around, is that we have taken aliquots of the same preparation and put them into a variety of mice all at the same time. Some of them get cured; some of them don't. And, I don't know whether some of them are more diabetic than others or whether technically it is better in some cases. It is not a purely quantitative assay.

DR. RICORDI: Remembering that some islets still need to get to the patient at the end of all this testing.

DR. SALOMON: Yes, that is the problem we need to discuss. You are too stingy with the islets!

So, if I can summarize, I think there is general agreement that there is a place for such bioassays. Jeff has made an interesting point that I didn't catch the first time through, I am sorry. That is, you are suggesting that putting them under the kidney capsule is really not the same as modeling what your clinical plan is, putting them in the portal circulation. I don't think we are going to solve anything on that today but it is an interesting thing to think about.

DR. RICORDI: I think it is a very important point and I don't know if anyone wants to comment on nude rats instead of nude mice. My feeling is that there are much

more problems actually with nude rats than with nude mice is a biological system to assess synergenic tissue transplant. Otherwise it would be a perfect model, and maybe even for mice we should reconsider doing intraportal transplant in nude mice because the aliquot that you would need for an intraportal transplant would be less than what you need in the renal subcapsule. So, the total volume may be inferior.

DR. BLUESTONE: That was the other point. If you picked up on Bernhard, he was talking about using 1000 --

DR. SALOMON: Two thousand.

DR. BLUESTONE: Two thousand islet equivalents in a 25 g animal. Calculate that back to humans; there is no relationship at all. Whether you could come up with an assay that had a closer relationship would be very important.

DR. SALOMON: All those points are well taken. So, let's move on to question three, which is viability, number and size distribution of islet preparations. I would like to focus first on 3(b), which is the initial lot specification viability for 70 percent for islets, is that appropriate? So, again, trying to be a little bit specific, we are at this point where I am hoping the discussion we have already had will enhance the efficiency of the discussion planned for the rest of this session. How about specifically starting with what is the best test to measure

viability? Then, the second question is what percentage of viability -- is 70 percent of viable islets a good cut-off, or do you want to discuss that? So, those are the two questions on the table now.

DR. RICORDI: I think an acceptable guideline, or the initial one was 50 percent but if you consider that this is based on fluorescent dyes and exclusion dyes it doesn't reflect necessarily also apoptosis; 70 percent may be an acceptable limit as determined by exclusion dyes.

DR. SALOMON: Bernhard, James, anyone else?

DR. HERING: I think there is consensus so we hardly see viabilities that would not meet the criteria. Most of the islet preps are in the range of 80, 90, 95 percent anyway.

DR. SALOMON: You measure by trypan exclusion. We mentioned earlier other alternatives such as apoptosis or someone mentioned a mitochondrial dye. There are some new mitochondrial dyes for molecular probes which can be used quite efficiently. Do you guys have any suggestions that there should be work on what kind of measure you use for viability?

DR. HERING: In the past we compared exclusion assays and FDA PI microfluorometric assays, MDT mitochondrial assays that basically gave pretty similar information. So, we didn't identify one that was more

suitable than another one.

DR. BLUESTONE: Could I ask a question? I understand that in the best centers, and we happen to have the best centers here, you always get 80-90 percent. So, you really don't care if it is 50 percent or 70 percent. But if you had to rely for your success on another collaborator and what their viability should be, if someone called you up and said I have some islets for you; they are about 50 percent viable, would you want to transplant them?

DR. HERING: I would not transplant, no.

DR. BLUESTONE: So, if the answer is no, then it seems to me that when one is setting viability, number one should be to pick a number where you would be comfortable transplanting because, in fact, the FDA is not trying to influence you guys, but for the community, trying to come up with a baseline number that we would all feel comfortable meaning that it was a good islet prep. It sounds to me, of everything we have talked about already viability is the single criterion right now that we are all somewhat in agreement with. So, we should pick a number where you would transplant the islets.

DR. RICORDI: Well, let me ask you a question. If you would have 300,000 islets that are 85 percent viable, would you prefer that preparation compared to a million islets that are 65 percent viable? So, I think it is very

difficult to set an absolute criterion for exclusion but 70 percent I think is a number we would feel comfortable with because we would expect to have a slightly higher variability that still would allow you to include the preparation that is 70 percent viable.

DR. BLUESTONE: Well, I am going to assume that was the question. So my answer to that question would be that I actually think that if you are lucky enough to get a lot of lousy islets that that doesn't make it okay. I feel pretty strongly that the islets you get should be really good and you shouldn't be able to overcome bad preparations by high numbers. So, I was hearing until ten seconds ago that you were saying 50 percent. Now, if you are arguing that 70 percent is what it should be -- because in Bernhard's earlier statement it was 50 percent up on the thing -- if you are arguing that it should be 70 percent, then I am much more comfortable. I thought I was hearing 50 percent.

DR. HERING: We reached consensus two minutes ago.

[Laughter]

DR. SALOMON: I would like to say this is a record.

DR. RICORDI: You know, also when you define lousy preparation you may have an outstanding preparation or processing and a lousy donor or a marginal donor but still

have --

DR. BLUESTONE: Don't take it personally.

DR. RICORDI: No, it is not personally but I am saying there are so many variables that you don't know about. Is it better to obtain 350,000 islets from a donor whose native islet mass is 500,000 islets, or is it better to have 600,000 islets from a donor whose native islet mass is 1.2 million? You get into potency or like donor factors. I think at this stage the less dogmatic we are with these criteria -- I would go with 50 percent but I can agree with 70 percent if it is something that will introduce more confidence about the level of purification. I don't think it would be a problem one way or the other because most preparations will fall above the 70 percent.

DR. SALOMON: Dr. Harmon?

DR. HARMON: When you have gotten these crummy batches of islets, do you just throw them out or have you ever gone ahead and done the studies in the nude mouse or any of the other assays to see whether or not they function just as well, or if they function the way you think they are, which is crummy?

DR. RICORDI: Well, I can tell you, and it is all anecdotal evidence at this time but, for example, for the first successful long-term islet allograft of the Pittsburgh series we spent one hour debating, with the patient in the

operating room, whether that lousy preparation was good enough to be infused in that patient because it was scarcely purified with mantel islets around it, and a little rim acinar, and that has so far been the best islet transplant ever with five years insulin independence. There are many other variables but I am saying it is very difficult right now to determine by eye-balling a lousy islet preparation. I mean, you can do it but I don't think there is enough data to say for sure that islets will not work unless you see that they are severely damaged or non-viable or that you don't have islets.

DR. SHAPIRO: It would be unusual to have a fantastic islet isolation and have them all dead. That would be really unusual unless you have added cyanide to the media afterwards.

DR. LAKEY: I guess we have spent too much money isolating islets jut to throw them out. So, a lot of experiments are done on preparations that don't fulfill our clinical criteria for clinical islet transplantation, and we have looked at the function and categorized islets that meet minimum criteria in terms of numbers and compared that to groups of islets that didn't meet certain criteria and certainly the function of those islets was significantly reduced as compared to the islets yielding more islets.

DR. BLUESTONE: So, I am hearing that there is

consensus then. Right? So, 70 percent -- the better the prep, the better the viability. Right? And, there is a general sense that the better the viability, then the better the prep. Right?

DR. HERING: No. I think the only thing that you can say, Jeff, is that 70 percent is acceptable as a cut-off but you cannot talk about potency. It doesn't give you any information regarding potency.

DR. BLUESTONE: This is just the first level or cut. It is not beyond that but it is the first level of cut.

DR. SALOMON: Okay. The next question to finish this group is to discuss the recommendations about the assessments on the size, distribution and the amount of so-called maximum dose to go into a portal vein.

DR. HERING: I don't think we know the maximum volume that can be safely transplanted in the portal vein. The approach that we took is the following, we continuously monitor portal pressure and we stop once we would approach 30 cm water. So, this is how we deal with the problem. There is no tissue volume that we consider too much. So, we monitor the pressure.

DR. SHAPIRO: We always keep our tissue volume under 10 cc. We have never seen any significant change in portal pressure.

DR. SALOMON: So, that is a very different approach than the one Dr. Hering just mentioned because he might give 15 cc in that situation.

DR. RICORDI: You may want to consider to keep it below 10 cc because if you count your minimal number of islets and viability and potency based on the total prep that is in 15 cc and then you arrive in the operating room and the portal pressure increases after 8, then you lose half of your preparation and you don't meet your product release criteria. So, you have to be pretty confident that the volume you infuse you actually do infuse it, if that is how you base your viability and potency calculation.

DR. SIEGEL: I just have a couple of questions. Are you also then measuring portal pressure as you infuse in addition to volume?

DR. RICORDI: Yes.

DR. SIEGEL: In talking about the volume and portal hypertension, I guess in the review of the IQR this morning we saw that there have been some cases of clinical significant hypertension. Are the data reasonably good? I realize they are not controlled studies but are the data reasonably good that volume is the most important determinant, or might the number of very large particles be a determinant, or the amount of non-islet cell particles be a determinant? Do we really know what the risk factors are

for portal hypertension?

DR. HERING: I think we looked at the Minnesota transplants, some 125 transplants and there was no correlation between the tissue volume that was infused into the portal circulation and the increase in portal pressure. I think there are other factors that are important -- what type of washing is done and there may be soluble factors that also result in an increase in portal pressure. Those cases were reported in the early '80 or late '70s and I guess I can say ever since Dr. Ricordi developed this new approach this has not been documented and maybe this is related to the fact that more washing is now a part of the process, and maybe it is not simply the amount of tissue that is infused and that is why I think monitoring pressure as you infuse is important.

DR. SHAPIRO: Another big factor is that these islets are all purified now and in the previous studies where portal hypertension and DIC occurred were unpurified transplants using autografts, again, with very minimal washing.

DR. RICORDI: You are talking about an experience in the old days when this event occurred and they were infusing 40, 45 cc of digested tissue or pancreas homogenate. It was a very different kind of cellular composition or situation to what you have today.

DR. SALOMON: Before we go from that, just to summarize this and move on, there are a number of people on the committee that are experts in hematopoietic stem cell transplantation and that is a field that has many parallels, as we have discussed among ourselves here. Do any of you have any comments about how you, in the earlier stages of hematopoietic stem cell transplantation, determined the total number? I mean, you didn't keep injecting stem cells until the portal circulation went up. Any comments on that?

DR. CHAMPLIN: There was initially with bone marrow cells a rough correlation between the cell dose and recovery, and one ended up giving about 10^8 whole bone marrow cells per kilogram. Then, with blood stem cells there has been a better correlation with the number of CD34 positive cells and the engraftment time. One now has worked out pretty much that 5 million CD34 positive cells per kilogram maxes out the engraftment and giving more than that number doesn't help you that much. So, that is presently the standard of care. If you can get that dose, that is what you would give.

DR. SIEGEL: We are discussing here the upper end. I am not aware of any --

DR. CHAMPLIN: There isn't data on the lower end of what the minimum number is that can engraft, and the lower dose, you know, the greater the risk of graft failure

or slow recovery. But, in principle, one pluripotent stem cell might, with enough time, reconstitute hematopoiesis.

DR. RICORDI: But you would still transplant a bone marrow preparation even if the viability is less than 70 percent.

DR. CHAMPLIN: Fortunately, with living donors viability isn't usually a problem and we get the cells right away and they are given promptly without losing viability.

DR. SALOMON: I was just curious how these guys would respond to this. In a sense, the challenge is somewhat similar and the questions, of course, would be if we go back at some point it might benefit the field to think a little bit along those lines. In other words, is there a CD34 cell equivalent? You could measure it on the number of islet cells but we ought to be measuring on the number of beta cells being given, for example. The question of how fast you get reconstitution of the bone marrow, which has been a measure for them on what is clinically practical, might also be another parameter that we could consider as a measure of the functionality or the quality or the number of an islet infusion.

DR. SAUSVILLE: But a critical distinction is that we expect that the stem cells that go into bone marrow reconstitution will actually divide and propagate themselves. Nothing I have heard, and I don't know whether

the science is such that we know that there is a beta stem cell equivalent, or actually you are having function by just the mass of cells that were injected. You know, that is a preclinical model issue that I think could be quite useful.

DR. CHAMPLIN: Have you done any late biopsies to show survival of the allogeneic cells over time?

DR. RICORDI: Yes, there are biopsies all the way. They are not protocol biopsies but there is a histological sample of islets obtained from livers in biopsies. It is rather a chance because if you do a needle biopsy in the liver and you have the luck to actually hit one islet, it cost me a dinar on one occasion as a bet --

[Laughter]

-- but it is possible and there is now evidence of intrahepatic islets all the way to over five years post-transplant and maybe even longer in an autograft, but an allograft for several years.

DR. LEVITSKY: Are they budding and look like new islet formations? Has that been seen, the way you see in islets that have been stressed?

DR. BLUESTONE: Are they bigger than the ones you put in?

DR. RICORDI: I didn't see bigger islets, like a mass of beta cells that are bigger than the islets. They tend to interdigitate more with hepatic parenchyma and you

may have direct opposition with some hepatocytes. Dr. Ryler did a whole study on one of the early cluster patients on islet morphology when this patient, unfortunately, died from reoccurrence of the original malignancy that determined the surgical cluster resection before the islet transplant. So, they had the opportunity to study the whole liver five years after islet transplant that remained completely off insulin for the duration of the follow up.

DR. SALOMON: The fact remains that there is no formal data to exclude the possibility that there isn't proliferation of islet endocrine cells after transplantation, and there is some data suggesting, for example, a constant turnover in the islets by measuring apoptosis and showing that 1-3 percent of the cells in intact pancreatic tissue and in the islets, that at that rate you have to have some proliferation of beta cells or you would have basically lost your islets within several months. So, there is some evidence that is kind of encouraging, and it is an area that is worth considering.

So, in terms of time, I think we have addressed this viability, number and size distribution of islet preparations. There has been consensus that a 70 percent viability would be good. There is no consensus on how one would measure the upper limit of islets going in. Two of you have suggested that it be 10 cc or less and Bernhard

argued, I believe reasonably, that if you measure the consequences of that injection one might be more efficient. And, I don't think there is data to satisfy a specific answer there.

We talked about how to measure viability. What I understand from you guys is that you have measured several currently available tests for viability and that we can always pretend like there may be a new generation of viability assays around the corner, but that is not really on the table now.

DR. SIEGEL: I thought I heard some interest from some members earlier in measurements that may detect impending or early signs of apoptosis as being areas where, although we might not be ready to set any standards, we ought to be collecting data as to whether those are going to predict the quality and the success rate.

DR. SALOMON: Yes, I think that is absolutely so. We have covered that before. I mean, I think that is true.

Question four, purity and composition of islet preparations. I think this is really an important and an interesting question. Historical data in the Islet Transplant Registry reveals that functional transplants of islets have ranged in purity from less than 5 percent to greater than 95 percent. What we are talking about here is islets and other pancreatic tissue. There was a report, I

guess it was from David Sutherland who suggested that less processed, so-called impure islet preparations might actually provide acinar tissue signals that might be important. But I understand that in general the field does not go along with that, and that you would be happier with higher levels of purity, basically fairly well-defined islet cells without a lot of rim tissue. So, do you guys want to pick that one up?

DR. RICORDI: Well, if you assume that you stay with the volume of less than 10 cc this would still provide a vastly predominant non-islet infusion so you will have plenty of ductal cells and acinar to provide. So, again, the consideration is more with respect to safety for the patients but none of us right now is striving to have the kind of super-pure islet with no extra endocrine tissue. So, no, I wouldn't even pose a lower or upper limit of purity at this time, but just collect the data and make sure that we don't have more than whatever volume we want.

DR. SALOMON: I would guess again that the FDA isn't going to be happy with that, that they are going to want some limit, albeit it is up to you to be pragmatic about it.

DR. RICORDI: Well, something between 1 and 99 percent --

[Laughter]

DR. SIEGEL: Well, what does 10 cc translate to? If that were pure islet equivalence, how many would that be?

DR. RICORDI: Well, one million islets equivalent pure theoretical volume would be 1.767145 ml. So, you assume it may take as much as 3, 4, or maybe even 5 ml if you have a million pure islets, let's say 95 percent or more. So, if you have 500,000 islets in an average good islet preparation and you have 10 cc, you are already in the range of something like 20, 25 percent purity.

DR. SHERWIN: Does that mean that most of the tissue is exocrine and ductal?

DR. RICORDI: Yes, you could have 70 percent non-islets. That is still much more than having 98 percent non-islets as in the original pancreas.

DR. HERING; So you want to call is a pancreas transplant or an acinar transplantation.

DR. SHERWIN: Well, it sounds like it is a copped up pancreas transplant, yes.

DR. HERING: But there is some evidence to suggest that embedded or mantel islets are high quality islets because cell matrix disruptions have not progressed to the point that the islet is over-digested. And, if you have embedded of mantel islets, then you have to make compromises as far as purification is concerned because you cannot purify to the very same point using density gradients

compared to completely cleaved islets. So, if you want to transplant this preparation and if you may want to succeed with a single-donor pancreas, then you have to accept that the total tissue volume is higher and it may approach 10 cc or 15 cc.

DR. SHERWIN: And the exocrine tissue dies?

DR. HERING: Yes, when patients were biopsied weeks or month after islet transplant only islets, not acinar tissue, was demonstrated in the liver.

DR. CHAMPLIN: Does the acinar tissue induce DIC?

DR. HERING: Patients are heparinized if they receive a significant amount of tissue. So, in islet autotransplantation patients are always fully heparinized. So, they receive 70 units per kilogram prior to islet transplant and DIC has not been noted in a single patient since the mid or early '80s who received intraportal islet transplantation and heparinization.

DR. SALOMON: So, if you had a procedure five year ago, before the more recent and apparently more encouraging data was around, that wasn't working and I was getting purities of 5 percent to 25 percent -- I am not trying to be over-obvious here, then at some point you must have said, huh, maybe the acinar tissue that is 75 percent of what I am transplanting is having a negative effect on the success of my procedure.

DR. RICORDI: Actually, I think that the thinking was different. If you start with a composition that is 2 percent islet, 98 percent non-islets, with a volume of 80 or 100 ml of tissue or more in a big pancreas, that clearly imposes risk if you inject it intraportally. So, initially it was thought that pure islets have less antigenicity than unpurified preparations and this has been a question of debate but right now, bringing down that volume from 100 ml to less than 10, maintaining the islet component, would provide you a relatively rich -- it is a little like when in bone marrow transplantation you talk about CD34 stem cell transplant from peripheral blood mobilized stem cells after leukopheresis, you don't have like 100 percent or 90 percent pure CD34 or hematopoietic stem cells. You just have an enriched preparation, if I understand correctly.

DR. SALOMON: The question is, is it working?

DR. RICORDI: Yes, because the alternative -- you know, you have a different setting for engraftment, if you have like a radiation conditioning or litter conditioning you have to reconstitute the patient, meaning that in mouse or in rodents you carry out reconstitute animals with a few stem cells. You can even reconstitute them with an organ transplant with passenger stem cells within that organ. So, I think you are in a different setting of issues and not dealing with engraftment of a very delicate cell population

that is highly susceptible to cytokine mediated beta cell damage and other factors that you may not have equally relevantly in stem cell transplantation.

DR. SHAPIRO: Purified cell preparations transplanted in Edmonton range between 50 and 90 percent. The majority are around about 75 percent, and the packed cell volume is around 3.5 cc. That may not be the optimal way of doing things ultimately since we require more than one donor.

DR. SHERWIN: If I caught that, you said it rather quickly, are you saying that there is more endocrine tissue in your preparation?

DR. SHAPIRO: Yes, the islet component consists of between 50 and 90 percent, the average about 75 percent.

DR. SHERWIN: Right, and the implication is that if you concentrate the endocrine tissue to a higher level the results might be better?

DR. SHAPIRO: No, it may be the opposite. We don't know. We have to carry out experiments and find out.

DR. MILLER: Do you have any idea of the difficult immunogenicity or the difficult components of the graft, whether or not the acinar cells or the islet cells have a different immune stimulation effect? That is the only reason why it maybe matter how pure it is, if there is a different immune function related to it.

DR. HERING: Studies have been done in small animal models and some studies suggested that acinar tissue may contribute immunogenicity but others show just the opposite. In the clinical setting there is no evidence to suggest this, and you see that rejection of a pancreas transplant can be prevented. There is no evidence that I know of that would clearly indicate that this would increase immunogenicity of an islet transplant. It is not only the immunogenicity, it is also the susceptibility to immune-mediated mechanisms and islets are highly susceptible. I think transplanting an islet prep that is 10 percent, 20 percent, or 50 percent pure -- I think we cannot really exclude any form of transplant. There is no data to support this.

DR. SALOMON: The question I had, trying to come to grips with what the FDA is grappling with in the sense of what criteria can we set for quality islets and what is important, I am still a little hung up here in that 75 percent of your tissue is destined in a relatively short period of time to apoptose and/or necrose and die, all of which is terribly inflammatory cytokine inducing, and we accept the fact that the islets being transplanted are struggling and very susceptible to injury by cytokines -- again, as I said, I am not trying to be obvious, you guys know exactly what I am talking about -- wouldn't it be a

good idea to have more pure populations?

DR. HERING: Yes, in that direction there are some conflicting results. For example, a more purified islet preparation induces more nitric generation of the intrahepatic infusion than a less purified islet preparation. Then, the second consideration is that the clusters are dispersed throughout the hepatic parenchyma so it is not like if you do a kidney capsule transplant that the more unpurified would have -- the purified islets may remain on their own without an effect of surrounding dying acinar. If we see at five years beautiful islets and we know that we had like 70 percent or 60 percent non-islet tissue at the time of transplant we cannot prove that those islets are just because everything else died. You may have some proliferation from early elements.

DR. CARA: As you try to purify the prep is there loss of viability? In other words, is there an inverse relationship between how pure a preparation you get and how viable the islet cells ultimately are?

DR. RICORDI: I don't want to monopolize the answers but in general there is not. There have been some conditions, like in theory, if you damage the islets you could have a situation where you can purify them better because they are lighter or less dense than non-exocrine. In general, our consideration is generally that the cut-off

for the level of purification that you want in the final preparation depends on how many islets you want to recover from the prepurification digest. Generally, as you cut towards more a purified fraction, you assume a loss of islets that are lost because of the migration together with the exocrine. So, if you assume of a core of islet purification decrease, then you would have an increase in the islet number recovered from prepurification. So, the reason we like to set up the cut-off on safety in terms of volume is that we try to sacrifice some of the purity to increase the actual number of islets that you can retrieve from the pancreas. Edmonton and other groups are using continuous gradients where you can very easily determine how much purity you want and choose at what level you want to assume an islet loss.

DR. CARA: Just a quick follow-up question, so would it be worthwhile looking at purity versus, say, survival of islet cell transplants?

DR. RICORDI: It could be a nice research project to look at the most purified fraction from the 100 percent pure islets versus the ones that remain in the bottom that are, like, 10 percent pure -- if there is a difference in potency or survival.

DR. SHAPIRO: Again, we are going to get some of these answers back from the multi-center trial since in

every case all this data will be here, collected prospectively, including the beta cell mass, the exact purity, etc.

DR. SAUSVILLE: But of concern remains if we are going to define today -- getting back to this thing of what the trial is going to use, again, we saw a nice picture this morning from the NIDDK representative where there were, you know, orange sort of globules floating around and I didn't see any acinar or other things. So, I am a little confused about how we then exactly define what will be the substrate for the research. In other words, what will you reject and not use? Would you ever reject anything based on this criterion?

DR. SHAPIRO: Speaking for ourselves in Edmonton, if the islet transplant mass is adequate, in our case if it is more than 4000 islet equivalents per kilogram, we will dial in the purity appropriately to enhance the mass. So, we might be able to carry out a transplant with 3.5 cc of tissue, but then the total islet equivalence might be just under 4000. We might increase that, say, with another cc or two of tissue to bring ourselves up to 4000 threshold for transplant.

DR. SAUSVILLE: But of concern in any protocol that is written, and again this enzyme preparation, liberase I think it was called, I mean, one could imagine that you

will have very different outcomes depending on how active your liberase is in making the islets. Right? So, would this better then be cast in terms of for a certain specific activity of liberase, collagenase or whatever you should expect? Something; some number?

DR. SALOMON: Well, I think these guys are being honest. This is not an answer that they can give you. We have to respect that. Has anybody ever done a transplant where you purposely transplanted no islets and demonstrated that it didn't work? In other words, put all this acinar tissue in there?

DR. SIEGEL: Want to see an informed consent for that procedure!

DR. SALOMON: There is data on endocrine progenitors arising from the tubular ductal tissue. Okay?

DR. SHAPIRO: I have done such an experiment in a dog where the isolation is totally hopeless; you can scarcely see an islet with a microscope. I put that preparation in the spleen, probably put it as an impure preparation in the spleen. That dog is always normoglycemic the next day. It is quite amazing. So, presumably there are fragments of cells, etc. that are working that we can't identify.

DR. SALOMON: That was my point. Thanks. That is very interesting and important, isn't it, in terms of

thinking about it. I think we could argue that in this particular case -- and, again, I am trying to summarize so we can move on, there probably is no purity standard that we can set and maybe even go so far as saying there is no purity standard we should be setting at this point for the trials. Whether or not this is a reasonable research issue, I think is obvious to everybody, including these guys here but probably just for the record I am stating it.

DR. SIEGEL: Just one question on that. Dr. Ricordi, you mentioned that you have a de facto purity standard, that you have a numerical standard at the 10 ml volume and I am just wondering, Dr. Hering and Dr. Shapiro, you don't put that volume limit on it. How commonly would you be going over that 10 ml volume in order to get the yield that you need?

DR. HERING: In islet autotransplantation we more or less almost routinely go over this limit, but in islet allotransplantation this has rarely occurred.

DR. SALOMON: Is everyone okay with my summary of this? The last point was other cell types in the islet preparation but I think, given the answers, that really makes little sense. Any more discussion?

DR. SHERWIN: Is there a way of monitoring the amount of ductal material that is in the graft as a learning experience?

DR. RICORDI: There are actually also publications analyzing the cellular composition of human islet preparations that go into patients and will be part of the documentation with the morphology studies that will be done on each preparation. That will document not just the beta cell content but also the known islets, other cell populations and the relative purity of each component.

DR. SHERWIN: So, you can specifically look at ductal material that would quantify that?

DR. SHAPIRO: All of our preparations that we test at Edmonton are stained with CK19 exactly to measure the ductal cell element, yes.

DR. SALOMON: Anything else? Then let's go on. Although I promised not to, I did already do potency out of order. We will go to six, which is demonstration of control and islet processing. Again, I think we have definitely danced around these issues throughout the afternoon but, in the interest of making sure that our discussion does focus on anything specifically that we want to communicate to the FDA on that, let's discuss these two sub points.

So, we all agree that the investigators who are going to be doing this need to demonstrate that high quality islet preparations are consistently made prior to initiating a clinical research study in the human patient. I think it is particularly important for you guys to give us some sort

of feedback on that since you know what is going to happen when the first two or three trials come out showing that you can now get 80 percent 1-year graft survival with islet transplants. I am hoping it is even better than that, of course. Then everyone who is interested in this area is going to want to quickly set up their islet purification program and start treating all their diabetic patients. I think Dr. Goldstein's point this morning was we want to cure this disease. So, at some point here there are going to be some real issues that I think the FDA is concerned about, particularly being sensitized recently over what we have seen in the gene therapy area. So, can we spend the next couple of minutes talking about exactly what should be the criteria for experts before they say I have an islet preparation lab; I am doing my first patient.

DR. BLUESTONE: Well, I would make the point that it is really simple. If I heard correctly a half hour ago, you start out with 70 percent viability, with the criteria of being within less than 10 ml or whatever. That is about the best you can actually do at this point. Every other assay is interesting but if it is not going to make or break any transplant how is it going to make or break for somebody else? It has to be fairly simple stuff. It has to be sterile. It has to be viable. Beyond that, I don't know what other criteria we are prepared to do right now.

DR. HERING: Islet number.

DR. BLUESTONE: And the number?

DR. HERING: Yes.

DR. BLUESTONE: Obviously, you have to have the minimum 5000 but beyond that I don't know what else you can do.

DR. SALOMON: Well, I think that we have to be a little more specific than that. I mean, I don't disagree with you, Jeff. Obviously, one criterion is that I can reproducibly provide this data, but what if I told you on Wednesday last week I had an islet prep that fulfilled those criteria?

DR. BLUESTONE: Obviously there are two stages. One is the criteria and the other is how reproducible it is. Okay? We can discuss how many times in a row and --

DR. SALOMON: That is what I want to discuss now. I want to discuss what criteria we want to provide, again in general terms, for the FDA. I come to them, I say I want an IND. You say can you make islets?

DR. BLUESTONE: So, if you don't have a proven track record to come up with the highest legitimate viability level you can with the number of islets and the sterility -- because what I am hearing is that everything falls from that; that if you have an approach to purifying islets to give you that kind of viability everything else

sort of comes along for the ride. Then you guys can make a choice of whether you have to do that ten times in a row, or five times in a row, or what. But I would start with the criteria that we have, and maybe it should be even a little bit higher than the 70 percent that the established centers have, but there should be some criteria based on viability and number and volume. Beyond that it is a question of how many times you have to do it, over what period of time.

DR. SALOMON: So, let's start with what Jeff said. Where do you guys want to go with that?

DR. RICORDI: You have to consider that for centers to make this proof of concept, for new centers you would have to imply that they will then have access to quality grade pancreas that cannot be then used for transplantation. So, I think that the actual trial runs could be easily done with research grade pancreases because we already got into trouble with part of the UNOS subcommittee on kidney/pancreas allocation and it took us over one year to get permission to use 12 pancreas a year for clinical islet transplant of clinical quality grade.

If you are asking organ procurement of UNOS to now allocate clinical grade pancreas to every center nationwide to prove that they can consistently achieve, like, five out of five isolations we would probably have a mutiny in the transplant community.

DR. SALOMON: If I procure the pancreata I want them -- to heck with you!

DR. RICORDI: You mean you would take them away from a potentially life-saving procedure?

DR. SALOMON: I am telling you I am getting 85 percent new grafts with my islets. That is as good as you are getting with your whole organ pancreas.

DR. BLUESTONE: No, what Camillo is saying is that every time you use a pancreas as a practice you have taken it away from the transplant, and if you take the best pancreases to practice, is that ethical? And, if you don't, are you going to get the purity I just said? And, Camillo says no.

DR. RICORDI: Actually, I am just saying that you have to be less strict. I would never ask that this center has to document in five consecutive isolations to get this kind of result, but to document that they at least have two or three isolations in which they can show that they have 70 percent viable cells and at least a certain number per pancreas. Those are more reasonable, assuming that they are utilizing a very valuable source of tissue that can come from different sources of different quality.

DR. SALOMON: The first thing we agree on is that you have to demonstrate on multiple occasions -- is it 5, 10, something like that? You have done at least 5 in a row

consecutively or 10. I mean, let's give some guidelines. And, that you get 70 percent viability, and I understand the other point; I didn't mean it that way -- you don't use clinical grade pancreata for these first 10. Is that the right idea? At least 8 out of 10 should be over 70 percent?

DR. SHAPIRO: Also, it is unrealistic to expect to be ready to do a clinical islet transplant having done 5 or 10 isolations. I mean, you have probably done 1500 or 1600. You have to put that in context too.

DR. SALOMON: How high is the bar going to get set then? I am ready to go here.

DR. RICORDI: Actually, I would be extremely low with the entry. I mean, if someone has been trained in the procedure -- these are not magic like some kind of protocols, if you have been trained in a center with experience and you apply the SOP, and go through your training for processing, and can document that you can produce this kind of islet isolation two or three times, the only risk is that they will not be able to meet this requirement in an isolation and then, you know, you can reassess.

DR. SALOMON: So, another point comes out that if you are going to apply for an IND to do islet transplantation in a new unit, you should demonstrate some objective measure of the training of the director of that

unit, the same way I have to demonstrate that I have UNOS approval to be the director of the kidney and the pancreas transplant programs. Right? That makes sense to me. So, some criteria are going to have to be made in agreement with the islet purification experts, and that probably should be done in collaboration with UNOS committees, much the same way we now agree that someone can be a director of a program for transplantation.

DR. SIEGEL: I want to ask a little more about that comment though that if you can succeed two or three times and move ahead, then the only risk is that they won't be able to do that well and you can stop them. Generally in manufacture, if you can't reach a quality standard consistently, if you don't have consistent control of the process, then even when you get a product that meets standards there are concerns. For example, just to put it in simplistic terms with pancreas, if I came to you and said I just did 12 pancreases and, by George, they were Gram stain negative 6 of the times and they were only Gram stain positive 6 of the times, you would probably be worried about it if I said I put those 6 into people because they were Gram stain negative. Right? That is the way it is in my laboratory. By the way, I don't do pancreases, I do tissue culture.

DR. RICORDI: Actually, if you would be doing Gram

stain or culture from the transplant solution of each organ, then you would never transplant an organ because the majority are all contaminated.

DR. SHAPIRO: That is exactly right but those are ideal organs still for whole pancreas transplantation. They are all contaminated; it doesn't necessarily mean they can't be used. The entire process is a huge washing machine that dilutes and washes out all the bacterial load.

DR. SALOMON: How about your thoughts about the actual place where it is done? Do you believe that it can be done anywhere as long as it is a nice little room somewhere? Do you believe that it should be under all hepaflow GMP sort of conditions? Should you have separate rooms for tissue coming in and preparation, and a third room for where the actual islet isolation is done?

DR. RICORDI: I think provided that the best series of islet transplants, including the Edmonton trial, have been performed not in cGMP hepafilter, or the Pittsburgh series, or autografts, I think that we can set up some general guidelines about the processing to be done, within like class 100 hoods or within facilities -- you know, we can discuss some minimal requirement for tissue processing but the product release criteria is what will determine it. If you start setting up a processing facility and you find out that you have a contaminated product one

out of three times you may want to release it the way you are designing the facility because it is not going to give you the kind of quality that will allow you to do any clinical trial.

DR. SALOMON: Can we get some comments from the group that have done stem cell processing? What kind of a facility do you guys believe is necessary for doing clinical stem cell processing and cell separation?

DR. MILLER: In general it would depend upon the degree of manipulation. So, as the manipulation increased the amount of regulatory oversight -- you know, the GMP facilities are coming into play more. The issue that comes up with this is when we are talking about what stage of development is this. You said that according to regulatory you don't need to have GMP facilities until you are in stage three.

DR. WEBER: Let me clarify it. You do need GMPs. You don't have to be under full GMPs until you are at phase three. So, it is a progressive scale. You are going to have to increase your level of GMP compliance as you progress.

DR. MILLER: So, what stage would these be in right now? Are they Phase I, Phase II, phase III trials?

DR. WEBER: I would say Phase I, as far as I know.

DR. SIEGEL: I can go into that question but this

isn't one of the questions we asked, and I am wondering if we shouldn't focus on the question at hand which was the demonstrate of control and processing.

I am a little unsatisfied with where we left that issue of contamination because I think my point wasn't clear. I guess the point I was trying to make is that if some labs can get sterility 95 percent of the time, then one that is getting it 50 percent of the time by Gram stain, you are worried about a process problem, even if it is negative that time. The same thing is true for viability. If your lab always gets 80 percent, I am going to worry if another lab gets 80 percent like 10 percent of the time. Even when they get 80 percent there is a suggestion that they have a process control problem that may even impact negatively on those products. So, I guess what I was getting at is, on the one hand I am hearing you really need to do thousands of these to know how to do them right and, on the other hand, I am hearing, well, if you do two or three and they meet the specifications you should be going ahead. I am not exactly sure what the right answer is here. How do we know that somebody is ready and qualified in terms of the control of their process to begin human experimentation?

DR. RICORDI: Now, for example, this question of training or of the regulatory issue of qualification of investigators or centers performing these kind of procedures

may develop. I know that now there will be a cell transplant subcommittee of the American Society of Transplant Surgeons that will be set up to discuss some of these issues. But I completely agree with your point, I don't think that if someone starts processing islets and sees that he is having contamination five out of ten times, or he cannot meet these criteria most of the time that he would feel comfortable to process towards clinical trials. And, you have another safety net which is that he would not be able to transplant any cell because it doesn't meet the minimal product release criteria. But, at the same time, it would be very difficult to determine this consecutive series of positive isolations because you could do it if you assumed that you have a similar starting product. So, you could demonstrate, for example, the effectiveness of your islet procedure if you say, well, we will do it with a non-human primate pancreas, and we will do five out of five with a controlled donor condition in order to have perfect pancreas, and you have to show this before moving to human. But when you start seeing the human experience, it depends so much on the quality of the pancreas that you accept also for distribution for research or which source are you going to use exclusively for clinical grade pancreases; or, you keep working to try to expand the number of pancreases and the conditions that you are getting from these pancreases.

DR. SALOMON: But I still have not heard anything that we can summarize.

DR. BLUESTONE: Maybe I can try something quantitative.

DR. SALOMON: Okay.

DR. BLUESTONE: So, it is a two-step quantitative event. Right? The one step is the learning curve, in which case you are not going to use the best pancreas that you can get and you are going to learn how to them. And, you should come up with a number. So, let's say I said that you have to do a minimum of ten pancreases --

DR. SALOMON: Let's stop there. We will come back to you but ten? Twenty? Give us a number. Or, if you don't give us a number tell us why you can't give us a number.

DR. HERING: Well, I think 10 or 20 consecutive is fine, and I would think you at least 90 percent of the preps should be sterile. At least 80 percent of the preps should have a viability greater than 70 percent. Now, I am not sure how many organs should have islet yields beyond -- I don't know -- 500,000. This may be a difficult goal to accomplish, but sterility and viability, I would say the majority should be within the specification range.

DR. BLUESTONE: Yes, and the only difference between what Bernhard said and what I was going to say is

that I am not sure I would add all of them together to get the number. I would do it two-stage. You know, you should always have a chance to have batting practice and get the system up and running. So, whatever that number is -- let's say 10, then at that point the next 10 should be your 80 percent and whatever. That way, you are not averaging the first 5 times when you are trying to figure out how to do it and things are working out. So, you should do it a two-stage thing. What the numbers are you can discuss, but there should be a stage in which you don't necessarily work with the best pancreases; you work it out; and then a stage where it counts.

DR. SALOMON: That is a good idea. You could say the last 10 consecutive or the last 20 consecutive when you got down to the details. That would allow you to sort of build up because you could start with a really bad pancreata at that point. Okay.

DR. SHERWIN: Is there a level of subjectivity in the assay of, let's say, viability or any of these issues? And, how do you control for that?

DR. HERING: Well, we have discussed whether clinical site training -- you see, we are discussing a multi-center trial. Now we are faced with the issue of whether we should have clinical site training and monitoring and to make sure that every site is doing the very same

thing. This is something you can discuss if you do multiple center trials, but not for the community at large. We will do it within the trial, and we will document that we do what we are supposed to do.

DR. SHERWIN: The reason I asked -- I mean, I don't know how you go about doing it; I am ignorant, but there may be ways of blinding the assessment using other people who don't really have -- you know, it just seems to me that if it is a subjective assessment, and I don't know, then it would seem to me some sort of independent assessment would make sense because there may be a lot of pressure if subjectivity enters into it.

DR. SALOMON: I think that is an interesting point. The question then would be should you, in addition to demonstrating that you consistently meet the 70 percent viability range and reasonable numbers of islets per processed pancreas, which we have been a little vague about, that we also show that at 24 hours in the last 10 quality pancreatic processing that you did at least all of the islet preparations had some glucose-stimulated insulin release that was greater than or equal to 2.5, which is the usual lower limit of a functional prep.

DR. HERING: You are asking whether we should send samples to a reference lab to document.

DR. SHERWIN: Yes. Something like that, yes.

DR. SAUSVILLE: But the nature of the viability tests that we heard would make it difficult to transport. I mean, fluorescence or trypan, etc. -- that is something you would want on site.

DR. SHERWIN: All I am saying is an independent person. It could be someone else in the institution.

DR. SALOMON: Well, there is no reason why you can't take pictures of these. Albeit there is nothing we can say that you don't cheat, we all know that, the bottom line here is that I don't think -- unless you send out inspection teams to all these places, which I know is well within -- well, comment on that, Phil.

DR. SIEGEL: I will comment. Traditionally, FDA has largely inspected clinical trials that are definitive efficacy trials at the point of licensure. However, recent events in the area of gene therapy have led us, both internally and externally, to ask the question should we use more of those resources to do on-site investigations of early developmental clinical trials to ensure that protocols are followed, that human subject rights and their welfare are protected, and that data of good quality is generated. We are, over the course of the next year, diverting some our standing resources, because it is a fixed pool in the short term, into that area and from that we will, among other things, have a better assessment at least in gene therapy

and possibly somewhat more broadly than that as to the value of routine inspections. At the present time most of our inspections in early experimental therapy are on a for-cause basis.

However, there is also some significant possibility, given what is being discussed out there, that in the not too long term picture there may be additional resources as I think Congress, and public groups and agency groups as well are certainly recognizing some of the potential merits of that sort of oversight. So, that is the long answer.

The short answer is in the immediate future or at least at the present time we have not been conducting substantial numbers of inspections in this and related fields, but that is under discussion and review and there may be some significant change.

DR. SALOMON: Did I understand then that you don't want to talk about facilities? That that is an area that you would like to stop the discussion, at that point? Because I had sort of gone to talk about the minimum facility requirement.

DR. SIEGEL: Phil, is that an area you would like to pursue?

DR. NOGUCHI: Well, it is related to question number six, and I think we are pleased to hear that the

advice that is coming back is that there is a certain learning curve and that there is a certain amount of stringency that the field wants to see before others, besides those who have pioneered the techniques, really get into the field. So, I think we have gotten quite a bit of information here and I don't think we need to go to the actual construction of facilities or anything like that, but it is the idea that, yes, you need to learn; that it is not a given. It is just like surgery; you don't operate the first chance you get.

DR. SIEGEL: Since I did abruptly change the topic but I did want to get to that other part of the question -- but the question did come up when do you need to produce during GMPs, and I know many of you are involved in manufacturing different types of cellular products where that question arises. What our regulations call for is that there always needs to be GMPs, but they say GMPs as appropriate for the stage of development of the product. That doesn't necessarily mean the phase of clinical trials. Usually what that means, usually the areas that will phase in later in GMPs are the validation aspects of it. So, we would expect from the very start that you have appropriate procedures to maintain sterility but we would not necessarily expect that you have challenged those procedures with different spore-forming organisms and done particle

counts and tests to prove, if you are operating in a hood, that the levels that if you were in commercial manufacture we might require to validate when you are, you know, bench to bedside, early basic research -- those can be somewhat stultifying.

So, it is hard, therefore, to give the exact answer of what is necessary when, but we require quality manufacturing and good manufacturing practices from the start for products going into humans. However, there is a lot more flexibility in how to achieve that and how to meet that standard than our regulations will impose at the time of licensure.

DR. AUCHINCLOSS: In this area of discussion, I thought I heard the comment made that UNOS should get involved in the certification and, boy, do I think we are way ahead of ourselves there. I think this is a small field of people where, yes, you want to see experience but I wouldn't get UNOS in as a regulatory body for determining who can run an islet isolation facility.

DR. SALOMON: I actually disagree with that. I brought that up. I will take credit for that one. I think that if we are talking about setting up criteria there should be some criteria for what is training that is adequate to do these sorts of studies in human beings. Now, if you want to say, okay, maybe the first couple early Phase

I studies -- I didn't mean to be that specific but I certainly think somewhere in the run-up here to a clinical trial, which is what I think the NIH, the JDF and the FDA want, there has to be some way of demonstrating that these guys have training. Certainly in bone marrow transplantation purification most of these people are certified clinicians and they won't get JCO approval for their bone stem cell processing without that sort of thing.

MR. BENEDI: To that point, I am not a huge fan of UNOS but they do have a system that has worked for many years and there is a procurement process in this country, organ procurement organizations that go into hospitals and do the consent to donor family members. When you go into that process, as you said earlier, if I procure an organ it is not really mine; it is the system's, and there are a lot of hospitals right now that are in trouble because they have gone around that system. So, I think it is a very sensitive issue when we are talking about donated organs to be used for whatever when there are, you know, 30,000 people waiting for organs in that specific category.

DR. SALOMON: Good point.

DR. BLUESTONE: I think the concern about UNOS is only the potential for intrinsic conflict of interest that UNOS has, which is that there are already a lot of politics about whether these organs are going to be available for

islet transplantation and to set that group as the monitoring group to decide whether or not an islet facility is capable to do transplants is putting a little bit of an undue set of politics into a system. So, I would much rather see a set of recommendations that support having training, support having some criteria without necessarily designating the group as UNOS at the git-go.

DR. SALOMON: So, in trying to summarize then this last point, which I think has pretty much been summarized in the last couple of minutes but, essentially, we recognize that there is a training curve and that should be factored into any such decision; that there should be a point, however, at which there is a commitment that the center says we have reached our training curve and demonstrates data that a minimum, probably 10 at least, maybe be better 20, purifications are meeting the kinds of criteria that, perhaps because they are not using clinical grade pancreata, are a little bit relaxed. But none of them should be infected or, at worst, maybe 10 percent of the first 10, 1/10. There should be some criteria demonstrated by these centers that there is training and experience that goes beyond the fact that they just read up in a book and did 10. There are some issues about facility and those need to be addressed but I think we are trying to be pretty flexible about those early in the process. Am I missing something?

There are ideas about at least demonstrating some minimum secondary viability/potency issues right now, not just showing in the preparations that they are -- whatever, dithiazone positive and viable but there should also be data showing a minimum, say, 1.5-fold glucose-stimulated insulin release the next morning, and I have stopped short of the idea that there should be a bioassay but I think a lot of places would probably be able to do that as well.

DR. BLUESTONE: And, I think Bob's idea is a good one. I don't know how we would say it but something like objective analysis, or objective results --

DR. SALOMON: Yes.

DR. BLUESTONE: -- whether it has to be blinded or someone else at the institution, but it should be objective

DR. SALOMON: Yes, I agree with that too. They should provide objective documentation and, again, there might be another scientist who warrants that they are not involved in this at all but perhaps in a neighbor institution, and the idea of a site visit team was raised and described by both Jay and Dr. Noguchi.

DR. SHERWIN: Yes, I was less enthusiastic about a major site visit --

[Laughter]

-- but I do think that you should have someone at the institution or someone local to just assess the

situation and have an independent sort of assessment.

DR. CHAMPION: It sounds to me like this might not be consistent with other precedent in terms of those kinds of requirements. I think normally one submits data supporting your manufacturing process that is reviewed and then acted upon, to my knowledge at least, without routinely having confirmation from other parties or inspections, in the current time frame, for things that aren't involving high-level manufacturing gene therapy or whatever.

DR. SIEGEL: That is right. We don't usually require that the sponsor of an IND or the manufacturing has some independent body certifying their data. Sometimes that is done.

I should clarify that although there are many, many areas in which there are professional standards and professional certifying bodies, and where those exist we often adopt those standards and require that those certificational standards be followed, and this sounds like an area in which there may well be room for that to develop.

I should be clear on this issue of FDA inspections. It is not in the cards -- unless Congress should see fit to have a massive increase in the FDA budget -- it is not in the cards I think that we would be a body that you could expect or hope would -- I don't know if you would hope for this anyhow, but that you could hope would be

there at every site on a regular basis to make sure that the data are right. What we might be seeing is, you know, some sort of spot checking and auditing on a somewhat higher level than we are currently able to do.

DR. SALOMON: For the seventh question I am again going to take the chair's prerogative to ignore and try to follow tomorrow. I think we have kind of touched on it and I think it is reasonable and can be discussed when we get to the clinical trials. Are you okay with that? All right.

So, we are almost done until we go on to the xeno-session but I don't want to leave this, if you will bear with me for another couple of minutes. I would just like to ask if Dr. Goldstein and Dr. Harmon and then our three or four guys over here who are doing islets -- the overall purpose here has been from the very beginning to discuss the area but not, you know, in niggling over all these little details lose the concept of what is best for moving this field forwards; what is best for our patients with diabetes. So, just to make sure that everybody is on the right track, I would like to start with Bob, if he has any comments he will share with us.

DR. GOLDSTEIN: Nothing that I have heard sounds like an obstacle, and everything that I have heard sounds like something that says let's go forward together in some intelligent manner, to be defined with data, which is

progress. We would see that as progress.

DR. HERMAN: I would agree with everything Bob said. I certainly see an awful lot of research that has to be done and should be supported by anyone who has funds to support it. We look forward to helping out along those lines.

DR. SALOMON: Gentlemen, you have been on the hot seat for the afternoon. I hope you will all not hold it against us too long.

DR. HERING: Well, I think this has been a very helpful discussion. I certainly learned a lot and I think the objective is to move the field forward and not to protect those people who don't do the job. But, at the very same time, we don't want to prevent innovation because we don't know what the answer is going to be tomorrow. So, I think we have to find a compromise.

DR. SHAPIRO: Yes, I was surprised how open-minded a committee like this, with a firm regulatory view, has been in terms of this process, and I think it is very important that all the facilitation that has occurred so far continues.

DR. RICORDI: I actually want to say that my reasoning for decreasing the requirement for training is not that I want to see people out there with no training performing a dangerous procedure in patients, but it is that

I strongly believe that we are at a stage where a new center and new young investigators entering the process shouldn't be inhibited from setting up their own facility and trying to improve on these procedures. It will be critical what the flexibility is because if you impose upon any center to access 20 clinical grade human pancreas and demonstrate 80 percent achievement of the standards, you will have no new centers in islet transplantation. I just want to make sure that advisors or experts on the panel are here to perpetuate the job, to make sure that they are the only centers that will be able to continue accessing the grants and stuff --

[Laughter]

-- but that there are other institutions, like Yale and others, that are thinking about entering the process may have equal access to these opportunities.

DR. SALOMON: Okay. This has been a public hearing. Is there anyone from the public audience who feels that we missed something in our summaries, that we were biased or created an issue for any of you?

[No response]

Clearly, this is an FDA meeting. Does the FDA have any last comments or major questions? Have we addressed the issues that were set for today?

DR. SIEGEL: I just want to say thank you very much. I personally, and I think my colleagues as well, have

found the discussions extremely informative and helpful.

DR. SALOMON: Then, as chair, I would like to also formally thank Gail Dapolito and Rosanna Harvey and Bill Freas and the other groups at the FDA. They are absolutely necessary to the conduct of such a meeting. I don't know about you guys but they have to call me two or three times to make sure I made my hotel and plane reservations, and I am always embarrassed when do that and promise I will do better but, of course, I don't. But, anyway, without them I wouldn't be here and I think probably some others of you wouldn't be. So, I really always thank them very much. Their process in this is really critical.

What happens now is that we go into a session where everyone is okay to stay, but we take a five-minute break and we come back to review the xenotransplantation subcommittee report.

DR. DAPOLITO: Right, we will release everyone except the standing committee and the xeno members who are here, although we hope people will stay as members of the audience to listen to the discussion of the xenotransplantation report.

DR. SALOMON: I see John Coffin there, if you will join us at the table. John is a standing member of the xeno advisory committee. Edith, please join us.

I want to make sure that I take a minute to thank

all of those members who were here today that are not a member of the regular standing committee, and to take time out of your busy schedules and provide this kind of expert help to the FDA and to the committee is greatly appreciated. So, I want to, for the committee and for the FDA also, thank all of you for coming, and you all know who you are. Thank you. And, I am looking forward to seeing you all tomorrow, of course.

Topic II - Report of the January 13, 2000 Meeting of the Biological Response Modifiers Advisory Subcommittee on Xenotransplantation

Are we ready? Dr. Auchincloss?

DR. AUCHINCLOSS: First of all I want to thank John Coffin who filled in as chairman for a portion of the meeting. So, the summary of this involves my having read a transcript of a meeting that I was not present at.

Let me try and take you through this. I believe that members of the committee have received a copy of the chairman's summary report which was compiled after attending the meeting, reading the transcript actually several times, making a draft of the report, circulating it to committee members for comments and then trying to incorporate those comments into something that I hope represents close to a consensus.

By way of background, the central topic of the day

really was blood donation deferral. By way of background, in the past the xenotransplantation subcommittee had addressed that issue by suggesting that xenotransplant recipients and their close or intimate contacts should be indefinitely deferred from blood donation, and that this policy should be implemented primarily by the education of the xenotransplant recipients by the xenotransplant team. That seemed like a reasonable approach because the number of patients involved was very small, and it was a highly educated group as a result of the nature of the procedure they were going through.

Subsequent to that discussion and the institution of that policy or that recommendation for policy, the FDA, again with the subcommittee's agreement, expanded the definition of xenotransplantation from recipients of xenotransplants themselves to recipients of human cells or tissues that have come in contact ex vivo with live cells or tissues or organs that were of non-human origin.

With the inclusion of that group in the definition of xenotransplantation, it became apparent that the group of xenotransplant recipients was larger than we had originally imagined. We don't know exactly how large it is, but we used numbers between the range of 500 and 1000 recipients of xenotransplants and many of them, according to this definition that they are xenotransplant recipients were not

even aware that they had received xenotransplants and, therefore, the original policy of blood donor deferral could not apply to those people because they had never received any kind of education.

So, the issue was now how to deal with that. The committee considered that issue in great detail during the course of the morning and basically came back with -- let me summarize the conclusions and then people can comment on whether I have summarize this correctly: that the committee still believed that recipients of xenotransplants as defined in that definition should be deferred from being blood donors; and that that deferral process should still primarily occur as a result of education of the xenotransplant recipients; and that to a degree that should extend to intimate contacts of xenotransplant recipients, but the committee was somewhat divided about exactly how far that deferral should extend to intimate or close contacts.

Now, the crucial additional item that the committee added was that it was appropriate for the FDA to consider on a case by case basis examples of ex vivo contact with cells or tissues from non-human sources and exempt recipients of a xenotransplant product, and examples were the cell line with which contact had occurred -- the cell line had been so well characterized that the blood donor deferral would not be required.

So, the committee in the morning said it is okay for the FDA to exempt certain recipients of xenotransplant products under some circumstances but we didn't, at that point, define what the circumstances were. So, then we came back to discuss topic number two, in which an FDA gave us an example, and the example is epicell, of a product that involves this kind of ex vivo contact with cells of an animal source. Epicell-3 is a cultured epidermal autograft and it basically involves culturing autologous epidermal cells from a burn victim in most cases and the recipient of the final product, but they are cultured in vivo with irradiated 3-T-3 cells, 3-T-3 cells being a mouse cell line derived from a mouse more than 30 years ago.

So, now the question was in this example was this the kind of ex vivo contact where the cell line had been so well characterized that it was, in fact, possible for the FDA to exempt recipients of this xenotransplant product from blood donation deferral? Here, I think the committee reached several conclusions.

The first conclusion that I think we were in agreement on was that although extensive testing of the cell line had been done in the past, given the kinds of infectious disease concerns that related to xenotransplantation that have come up in the past several years, still further kinds of testing would be useful in

this kind of situation, including in particular co-culture assays designed to seek evidence of transfer of endogenous retroviruses and, in addition that there might be state-of-the-art improved assays to characterize this cell line.

So, that meant that there was a group of people who had received epicell-3 in the past, 500 or 1000 however many it is, in which the testing had been less than we would like it to be given our current state of knowledge, and that in the future some people will be able to receive epicell-3 which is tested with the best possible assays as discussed by the committee.

Now the question was how to handle blood donation deferral either by those who had donated in the past or those who will donate in the future. The committee was split on the recipients of future epicell products, feeling that to defer them -- the vote was not exactly 50-50 but a mixed vote -- a mixed vote felt that, well, it is awfully easy to tell people not to be blood donors and the numbers of people is not very large, so why don't you go ahead and defer future -- recipients of the new epicell product, meaning the one that is tested in the best possible way? That recommendation was not unanimous. In fact, actually technically the majority of the committee felt that those people did not need to be deferred, but it was a split vote.

What the committee was agreed on was that that

future deferral did not need to extend to intimate or close contacts of the xenotransplant product recipient and, secondly, what the committee, I believe, was unanimous on was that previous blood donors who had received the old epicell product, those blood products did not need to be withdrawn from the existing pool.

So, those I think were the principal conclusions and, again, we can spend some time talking about them. The FDA then asked us to go beyond the individual product of epicell-3 and talk about other characterizations or generalizations that we might be able to make about cell line characterization that might be useful to them in the future. Specifically, the committee considered species of source animal, non-primate mammals, non-mammalian animals including invertebrates, cell lines versus fresh tissues, use of barriers and/or encapsulation, trans-unit low-dose exposures, and the state of immunosuppression of the recipient.

In general terms, the committee agreed that all of those factors play a role in the degree of risk associated with xenotransplantation, but that the degree of risk was not changed in such a dramatic way as to enable us at this time to use any of these criteria to exclude people absolutely from the requirements for follow up and testing those listed in the FDA guidelines for xenotransplantation.

I think there is an exception to that in that the species of source animal -- the subcommittee agreed with the FDA recommendation to exclude non-human primates as source animals and I believe that it was agreed that source animal testing for cell lines was not necessary.

There was a third part of the meeting, which was more for information's sake, and I will describe the two presentations that occurred there. One was a presentation from the people at Novartis who basically presented the data that had been published in Science of 160 patients who had come in contact with pig tissues in one form or another, looking for any evidence of PERV infection in those recipients. The bottom line conclusions was no evidence of infection was encountered, and the committee in general thought it was a good study and was glad to see that, but also felt that ongoing testing of future xenotransplant recipients was appropriate.

Then the committee heard a presentation by Dr. David Onions of some unpublished experiments, preliminary experiments in which he injected large quantities of PERV virus into guinea pigs and was able to demonstrate that infection of guinea pig cells did occur without any evidence of viremia and without any evidence of any particular ill consequence as a result of that. I believe that there was some discussion about that finding, in general accepted with

the sense that this was useful now as a small animal model in which to examine, at least in this species combination, the potential effects of in vivo infection with the PERV virus, recognizing that what happens in one species may not be predictive of what will happen in another.

So, that is my summary of my summary of the meeting, and let me turn it first to John Coffin and see if he wants to modify what I said there or amplify.

DR. COFFIN: I have very little to add -- I think that was an excellent summary of the part of the meeting that you weren't at -- except to note that the presentation by Dr. Onions was of a highly preliminary study which was actually originally designed for a different purpose. So, there will be many, many questions that one would have about the import of this, but it seemed clear from the study that there was truly infection of guinea pig cells in the course of this experiment.

DR. SALOMON: Is there any discussion? I certainly would say that I was there up until the time Dr. Auchincloss left and I also felt that this was a very good summary, and i think that covered it really well.

DR. BLOOM: I think both your presentation and your summary reflect very well what the committee discussion was about.

DR. SIEGEL: I would also add as feedback that, as

you know, there were representatives there from our sister agencies, the NIH and CDC, and we received considerable feedback that it was an extremely fruitful, helpful, welcome, excellent discussion, and a lot of useful advice received by the FDA but also by the other public health agencies at that meeting.

DR. SALOMON: Can I see a show of hands for accepting the report as provided by Dr. Auchincloss and Dr. Coffin?

[Show of hands]

Nine. And, can we see "nays," not accepting the report?

[No show of hands]

Zero. And, anyone abstaining?

DR. DAPOLITO: Dr. Miller is not here.

DR. SALOMON: Because we needed ten, right?

DR. AUCHINCLOSS: Is that right?

DR. DAPOLITO: Well, we don't need it but we have ten votes. We will have to get a vote from her.

DR. SALOMON: Okay. I believe we are close to done. There was a question about the Blood Products Advisory Committee, BPAC, but the person who was going to that is not here. Edith, do you want to tell us about the BPAC results?

DR. BLOOM: I was actually not able to attend. I

was at a Council of Europe Working Party on xenotransplantation meeting, at which I was fortunate to be able to summarize some of the findings of that committee meeting that Dr. Auchincloss just spoke about, and it is a privilege actually to be able to convey that kind of discussion internationally. But Ruth Solomon, from the Office of Blood, is here. Perhaps she could address some of that.

DR. SOLOMON: The topic was presented at the BPAC this past Friday, and the committee was asked originally two questions but then that became three questions. The first question asked about including information about xenotransplantation in the educational material that is given to the donor before they actually donate, the blood donor. And, there was a split vote. The question asked does the committee agree that donors should be required to read this material before donation? A vote of five said yes, seven said no, with zero abstentions.

Then the committee was asked do they agree with a question to be asked on the donor questionnaire, and the question was framed exactly how it would appear on the questionnaire. So, as stated, the committee voted two yes, ten no, and no abstentions.

However, a third question was asked to the committee, do you agree that any question should be asked on

the questionnaire about xeno because there was a discussion about modifying the nature of the question and coming up with the so-called ideal question, which was very difficult. So, when asked do you agree with asking any question to the blood donor, the vote again was split. There were five yes, four no and three abstained.

There was also some discussion during the open public hearing. The blood industry, some members, voiced the opinion that they did not feel that intimate contacts should be deferred, but that was not a question asked to the committee.

DR. SALOMON: I was waiting for that last one to come up but I could almost see that one coming. Well, I would start off by saying I don't buy that; that is not right. I mean, that is certainly in any way the spirit of what we had suggested at the xeno subcommittee meeting. Do you want to pick up on that, John and Hugh?

DR. AUCHINCLOSS: Well, the question specifically has to do with the questions that are presented to the potential blood donors.

DR. SALOMON: Start with the material. Remember, I thought we said that they would have to have some sort of material explaining xenotransplantation that the donor would read.

DR. AUCHINCLOSS: Yes, I guess I can't tell you

that I specifically recall the conversation about material, but let me tell you what I think the sense of our committee was. We considered three questions that might be added to the blood donor questionnaire, and the very strong feeling of the subcommittee was that it would mean absolutely nothing to the population at large. They wouldn't know what a xenotransplant was; they wouldn't know whether they had had one, or whether their intimate contact had had one; and it was simply going to be confusing, not productive in deferring those that you wished to defer and potentially harmful in making it still more cumbersome and confusing to go and donate blood. Specifically, our committee voting 16-0 in favor of not including any questions on xenotransplantation on the blood donor questionnaire. I assume that that would apply to material as well.

DR. SALOMON: I thought that the way we dealt with that was that we bowed to the concerns of the blood product industry that they didn't want to do anything to reduce blood donation, which we thought was perfectly appropriate for them to defend, but we thought that there should be -- in fact, they assured us that there was some material in there, material that they share with donors for xenotransplantation. And now they are saying they are not going to share data? I mean, maybe this is just a mis-impression.

DR. AUCHINCLOSS: We are talking first about the questionnaire, where I know my memory is correct because we have it recorded here. John is going to make a comment about material, reading material.

DR. COFFIN: There is something on the questionnaire about transplantation generally, if I remember correctly, that defers for a period of a few years -- I forget what it is.

DR. SOLOMON: Actually, the FDA does not specifically ask such a question be asked the donor, but the AABB, the American Association of Blood Banks, has a uniform donor questionnaire which the FDA has reviewed, and on the AABB donor questionnaire they ask, within the past 12 months have you received blood or a blood product, or a tissue, or organ or cellular product? But it is restricted to within the past 12 months.

DR. SALOMON: So, the last thing that they said was that intimate contacts should not be excluded. Again, if I am wrong, correct me but my understanding was we specifically thought that intimate contacts of a xenotransplant -- in fact, I thought we went to kind of great lengths to insert the term "intimate" rather than "close."

DR. AUCHINCLOSS: We inserted the term "intimate" instead of "close" trying to make it clear that we were

talking about a very narrow group of people, but the votes were consistently split on the issue of whether or not intimate or close contacts should be deferred, roughly 50-50.

DR. SOLOMON: Again, I am just giving you some comments made during the open public hearing. That is not necessarily the FDA position, and we did not put a question in front of the committee as to should intimate contacts be deferred. That was not on the table. The BPAC was to answer questions regarding the implementation of the suggestions of the January 13th xeno advisory committee.

DR. SALOMON: My only point now is discussion. I am not trying to make a conclusion for the committee, but my opinion is that what I thought is correct. In other words, what you are saying is that they are rejecting any question, which is okay by itself; any discussion of what a xenotransplant is, which means a lot to me; and no proscription even for intimate contacts, which then says that if we do a xenotransplant and pass an endogenous retrovirus it is coming right through the blood pool, which was the whole point of this meeting, I thought --

DR. AUCHINCLOSS: No, no --

DR. SALOMON: -- how we were going to prevent a movement from the patient to the blood pool.

DR. AUCHINCLOSS: No, that recipients of

xenotransplants should be educated about the fact that they had had a xenotransplant and should be deferred -- that wasn't the issue. The question was whether you, me and the next person who goes in and wants to donate blood should be asked, hey, did you have a xenotransplant, the person who hadn't presumably had one. And, there, the committee felt that is just noise to those people; that is not information.

DR. SALOMON: That is a good point, Hugh. I accept that as a good point, but the point I am making is that what you are saying then is that there is going to be nothing in the blood procurement process. All there is, is going to be a statement that I was trained because I got a xenotransplant, which is something back maybe three years ago, whatever, and if I had had a transplantation within a certain period of time or if I am on immunosuppressive drugs.

DR. SIEGEL: Let me clarify a few things about process. First of all, the BPAC, like the BRMAC, and the xeno subcommittee are advisory committees. So, she is not saying anything as FDA policy. We have a lot of advice and now we need to make to policy.

But also, Ruth, correct me, I think you may have mis-characterized the vote of the committee when you suggested they said that no question is appropriate, and I thought I hears you say that five to four and three

abstentions. They were in favor of a question, they just didn't like the one that was proposed.

DR. SOLOMON: The vote was split on both including in the educational material and asking any question. It was a split vote.

DR. SIEGEL: Another thing just as a matter of process, somebody said, well, we heard industry say one thing at our meeting and then apparently -- the BPAC is not industry; it is a federal advisory committee just like you people.

DR. SALOMON: John?

DR. COFFIN: I came away from the xenotransplant meeting with the firm conclusion that the committee strongly believed that xenotransplant recipients should be deferred but was firmly confused as to what the mechanism for that would be.

DR. SALOMON: And I don't have a problem with that. I think that characterizes -- I think the way I came away from the last meeting was that we were trying to be very, very sensitive that, based on the fact that no one had proved yet that we were transmitting any sort of infection, that we didn't suddenly saddle a very sensitive area, blood donation, blood product donation and processing, with a bunch of ridiculous ideas based on a fear that we haven't yet proven. And, I am very comfortable with being cautious

in that regard. Except what I am hearing now -- again, I think I have made my point -- is that it sounds like, faced with some ambivalence on our part, the response was then, you know, nothing.

DR. AUCHINCLOSS: We always try to be very, very sensitive.

DR. SALOMON: And you are not even from California!

DR. CHAMPLIN: Having some experience, you know, getting consents for transfusions, you know, you have a list of 44 questions or so and adding a 45th, when you have already asked them if they have had sex with somebody who was in jail last week as one of your previous questions, and it isn't going to be offensive, I think it is a burden to give them another book to read on xenotransplants for somebody who is a potential blood donor. So, I think some sort of simple question that could just be added to the questionnaire would be an appropriate middle ground here.

MR. BENEDI: I am not sure, as recipients do we really want a handful -- as a percentage of xenotransplant recipients to give blood without knowing what the long-term consequences are.

DR. CHAMPLIN: We don't want them to give blood.

MR. BENEDI: Exactly.

DR. CHAMPLIN: Everybody agrees --

MR. BENEDI: But you don't want to ask the question.

MR. CHAMPLIN: No, I am saying we should ask the question and we should add it to the other 44 questions that we ask as the way to address that, in addition to educating everybody who gets a xenotransplant that they have had one and that they shouldn't give blood. But, certainly, there are going to be people that don't hear that instruction or forget, so there should be something in the system to catch those people.

DR. SALOMON: Thank you. Carole?

DR. MILLER: You were saying that many people who get a xenotransplant with the expanded definition don't know they have had one. Did the committee make any recommendations about how to educate the rest of the people who are truly getting the full explanation, and are they going to ask that the package inserts or the documentation for, like epicell, get strengthened. Is there a consensus on how we are going to do that?

DR. AUCHINCLOSS: I took the position of the committee to be that in the future recipients of epicells should be told that they were receiving a xenotransplant and all that that implied. But I also took it as the position of the committee -- and I don't think we were precise on this; I think we were precise that you did not need to

withdraw blood products donated in the past by old recipients of epicell, but I don't think the committee was necessarily clear about whether there should be some effort to get back to previous recipients of epicell and tell them, hey, you had a xenotransplant. Do you think that is a fair characterization of what we said, what we didn't say and what we might have said?

DR. SIEGEL: I think so. I think on this issue of retrospective looks there was also some discussion about, well, what if somebody who was like one of twenty close contacts of one of these 500 people -- and I am mentioning these numbers for a reason -- then donated blood that was then pooled with blood from a few thousand other people to make a pool of plasma? The reason I mention that is because there was some thinking that, well, if it is 500 people it doesn't matter but, in fact, 500 people have thousands of contacts and if you are making a plasma product from thousands of donors the likelihood that one of those thousands of people will be one of those thousands of the donors is non-trivial. So, the question came up, as sometimes happens in blood donations, someone comes back after they have donated and after there is now, say, all this albumin out in the market, or whatever, and says, oh, I just remembered that I had this procedure or that test or that whatever it is, that behavioral experience that I

forgot to tell you about, and there was some discussion about what might trigger then product withdrawals. If I recollect correctly, that was also discussed in terms of looking back and there was a sense from the committee that one needn't go out and pull things off of the shelf because somebody came back and remembered that he had received a skin transplant.

DR. AUCHINCLOSS: Specifically, you made precisely that statement to the committee and nobody objected, and so I took that to mean that, indeed, withdrawal of blood products on the basis of old donation was not required. But we did at various times talk about the possibility of going back to epicell recipients in various look-backs, and my recollection of the conversation is that various people said, boy, that would be impossible and other people said, no, that should be quite easy, and no, it would be impossible, and we never really ended up with any formal recommendation for you.

DR. SALOMON: Any more discussion on this point?

[No response]

Just to fulfill my official capacity here, Carole, I have to ask you to join us in a vote on the report that Dr. Auchincloss and Dr. Coffin have given on the xenotransplantation committee.

DR. MILLER: I approve.

DR. SALOMON: Okay. So, I would like to note into the record that we have the approval of Dr. Miller on this, which makes it ten to zero and no abstentions.

Then, I move that we are adjourned, and see everybody here. So, the committee is at eight o'clock, however, those who are not on the committee, who aren't sitting here any more, are starting at ten o'clock. Thank you, all.

[Whereupon, at 6:13 p.m., the proceedings were recessed, to reconvene at 8:00 a.m., Wednesday, March 22, 2000.]

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CERTIFICATE

I, **ALICE TOIGO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

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