situ hybridization. That is easier said than done.

I hope you can see this, but we are looking at the same comparison here in which we fixed, with Carnoy's, and also added a buffer system, a hybridization buffer system, that is compatible with FISH and simply stained with a Gram stain.

But, once again, you get the picture. You can see, with this sample gram stain that, indeed, the bacteria are mainly caught up in this mucus web that covers the epithelium. So, again, fixed with Carnoy's in an effort to try to preserve the mucus layer, you get the view that, indeed, you see some interaction, perhaps, with the epithelial surface but, mainly, the because are in this mucus plug.

We think they are in the mucus plug because that is where the host wants them to be, and the host has learned, over a long period of time, how to keep bacteria away from the epithelial surface.

Interestingly, I think, perhaps our views on bacterial adhesion to the epithelium have been generated, in part, by the pictures that we have been able to take.

[Slide.]

One can find very beautiful pictures such as this scanning electron micrograph of, this happens to be streptococci adherent on the epithelium of the chicken

ileum. But, again, I just want you think about the protocol used to fix this prep. So these tissues are fixed with glutaraldehyde, which is extremely dehydrating. So if you have, indeed, a mucus layer that is filled with bacteria and you take your tissue sample and you plop it in a vial of glutaraldehyde, then, obviously, the mucins are going to dehydrate, disappear.

Then, where will those bacteria go? They will essentially just lay on the surface because of hydrophobic interactions and then you beam this with electrons, and you get this very nice picture of streptococci adhering on the surface of epithelial cells. And then you begin, you go home and you have dreams, or nightmares, whatever the case may be, about adherent bacteria and how the host has to deal with bacteria adhering on the epithelial surface.

I am not suggesting that, indeed, that phenomenon does not exist; but I think we have to be careful in how we, in effect, draw our pictures and try our best to preserve tissue in its natural state because all evidence that we have been able to generated and glean from the literature indicates that, indeed, the host is not interested in making a bed for bacteria on its surface.

[Slide.]

So at least we can conclude that bacteria are associated with the mucosal surface. So then, if that is

the case, the host compartment first encountered would be epithelium cells. So then, if one wants to ask questions about how the host responds to bacteria associated with the mucosal surface, then I think you must first ask questions about how epithelial cells, intestine epithelial cells, respond to normal bacteria.

What I would like to do now is just quickly show you two datasets that I think are interesting and illuminating in this regard.

[Slide.]

These datasets are utilizing the HT-29 cell line, and we fully recognize the limitations of cell-line studies. But, indeed, in such a complex system, our approach is to try to take a reductionist view first and try to survey some of the phenotypes, epithelial phenotypes, in response to normal "gut bugs," we call them, and then try to work back to a more complex system.

Of course, ideally, if one can afford it, you can do in parallel, both in vivo-type model approaches as well as in-vitro model approaches. But the next two datasets ask questions relating to how Lactobacillus plantarum, a commensal lactobacillus, modulates the expression of mucins by HT-29 cells and'in IL-8, in this case, one of a number of cytokines that are synthesized and secreted by intestinal epithelial cells.

[Slide.]

The first dataset was contributed by David Mack. He is at the University of Nebraska at Omaha. The paper was published in American Journal of Physiology in 1999. It is a very interesting study in which he is, in part, trying to understand how lactobacillus organisms may prevent the adherence of enteropathogenic E. coli.

He is doing that by co-culture studies so HT-29 cells that are co-cultured either with Lactobacillus plantarum 299v or with enteropathic E. coli for a short period of time, isolating RNA and looking at expression of mucin genes.

[Slide.]

On this left panel here, we are looking at the adherence of EPEC on the Y axis and two lactobacillus strains, very common lactobacillus strains, Lactobacillus plantarum 299v and Lactobacillus rhamnosis GG, and measuring EPEC adherence.

You can see, without the addition of lactobacillus strains, you get this degree of EPEC adherence to the cell surface. However, with the addition of Lactobacillus plantarum and Lactobacillus rhamnosis, essentially a complete ablation of EPEC adherence.

So, in the right panel, he is looking at MUC2 gene expression relative to 28S ribosomal RNA gene expression in

response, again, to no bacteria, an E. coli strain--not EPEC but a different E. coli strain--or Lactobacillus plantarum 299v. Here he sees a significant increase in mucin gene expression in response to Lactobacillus plantarum that correlates completely with the ability of Lactobacillus plantarum to prevent adhesion of EPEC to the cell surface.

[Slide.]

So those data are consistent with the idea that epithelial cells, specifically, perhaps, goblet cells, are sensitive to regulatory cues generated by normal gut bacteria and respond by increasing mucus production. That observation is also consistent with the idea that it is the host's intent to, indeed, to keep even these so-called good bacteria away from the epithelial surface.

I mentioned the fact that the host is equipped with second tiers of defense that enable it to respond also to bacteria if the physical barrier afforded by the mucus blanket becomes compromised. So we have also asked similar questions except we have looked at the response of epithelial cells to, again, Lactobacillus plantarum, looked at cytokine responses in response to Lactobacillus plantarum.

[Slide.]

So a similar assay, the same HT-29 cells. In this case, we are looking at both constitutive HT-29 cells and

HT-29 cells that were first activated with the proinflammatory cytokine, tumor-necrosis factor alpha. Then we added Lactobacillus plantarum 299v. So, comparing cells treated either without or with TNF alpha, a pro-inflammatory cytokine, and then isolating RNA for interleukin-8 expression, a potent neutrophil chemoattractant.

[Slide.]

One panel of data is shown here. First, IL-8 sxpression is very low or undetectable constitutively. So, in non-TNF-treated HT-29 cells, IL-8 expression is very low. If one treats cells with TNF-alpha, you see a significant upregulation of IL-8 expression in response to TNF.

I will also mention that IL-8 expression was not nodulated by Lactobacillus plantarum 299v in constitutive HT-29 cells. However, in TNF-treated HT-29 cells, you see a very significant increase. By the way, this represents three separate cell cultures and IL-8 expression, in response to Lactobacillus plantarum 299v.

A similar response was not observed in response to LPS and lipid tachoic acid, just two cell-wall components. Also we looked at the effect of viability, and this response was only noted in response to viable lactobacilli.

Again, just to summarize; upregulation of IL-8 in response to the proinflammatory cytokine TNF-alpha and a distinct potentiation of that response in the cells that

were also exposed to this nonpathogenic lactobacillus species.

[Slide.]

So we think those data are consistent with the idea that, indeed, if the mucus blanket is compromised, bacteria then are able to translocated through the epithelium, activating lamina-propria cells, for example, macropaghes, to secrete TNF-alpha. The TNF-alpha, then, would activate the epithelial cells to express yet undefined receptors that make those cells very responsive to even, again, normal gut bacteria.

That response would amplify. The inflammation would be amplified using cues from normal gut bacteria to rebuild this important barrier, protective barrier. So, if that is the case, we need to understand the extent to which the host is responsive to normal gut bacteria. We would like to understand something about the regulatory cues.

Viability was necessary. We have had mixed results with adhesion. We have blocked adhesion of mannoside. Lactobacillus adheres to epithelial cells in a mannose-dependent manner. 'We have blocked adhesion with mannoside. Sometimes we see the potentiation, sometimes we do not. So we are unable to conclude if adhesion is necessary. But we know that viability is necessary.

I think we are struck with the dramatic nature of

the host response and we would like to understand is this an unusual occurrence or is this a widespread occurrence. Just how sensitive is the epithelium?

[Slide.]

There is one study that I would like to draw your attention to that I think, when all is said and done in the field of host microbe interactions--and, by the way, a lot more needs to be done than said; so far, there has been a lot more been said than done. I think this paper is going to represent a seminal paper in the field.

I am speaking of the paper, perhaps that some of you are aware of. It was published in Science in 1996 and it was a very clever approach. Actually, it turns out, after this paper got a lot of attention, that a Japanese group had published very similar results in 1982 and they contributed a letter to Science to inform the public that, indeed, a similar observation had previously been communicated.

[Slide.]

But I would just like to magnify the picture to briefly summarize the findings because I think they represent kind of the tip of the ice berg as far as the dynamic nature of host-microbe interactions. This was a very clever study in which the investigators inoculated germ-free mice with two isogenic bacteroides strains,

Bacteroides theta iota omicron.

These were isogenic mutants, one of which the mutant strain was unable to use fucose as a substrate. So we are looking at two isogenic bacteroides strains, the wild type which utilizes fucose as a substrate and the mutant strain does not utilize fucose.

Then they stained the epithelium with a lectin that recognizes fucosylated glycoconjugates. In the panel on the left represents the mice, the germ-free mice, that were inoculated with the mutant bacteroides strain that is unable to utilize fucose as a substrate.

The panel on the right represents the mice that were inoculated with the wild type bacteroides strain that primarily utilizes fucose as a substrate. The observation then is that, indeed, somehow, this commensal bacterium was able to stimulate the host to upregulate its fucosylation program providing an appropriate substrate for that particular bacterium.

I think this observation just profoundly demonstrates the dynamic nature of host-microbe interactions. This paper'was published in 1996. The signal generated by this particular bacteroides strain has yet to be identified. So, again, a very significant challenge but a very interesting and dramatic observation.

[Slide.]

The model drawn by this group, Jeffrey Gordon's group at Washington University, is, I think, consistent with also the data from Mack and our own data illustrating that commensal bacteria are likely to generate a number of soluble signals, perhaps in a density-dependent fashion, that are modulating gene expression by epithelial cells.

Most likely, the collective response of epithelial cells is to mount both innate and acquired barriers that prevent adherence or interactions of normal bacteria with the epithelium. SO, if that were to be the case, then what we are 'talking about today is trying to impose upon that very dynamic system, or trying to modulate that very dynamic system by adding one particular organism.

I think, in most cases, we don't understand a whole lot about that organism. Perhaps, sometimes, we can't even define that organism. So I just leave you with the way we see some of the challenges associated with this objective.

Thank you.

DR. BENEDICT: Thank you.

So let's point out that we are not behind because of the speakers. We are behind because of the tardiness of the actual committee.

We will move, now, to Dr. Clemens again who, I am sure, in his normal fashion, will rip through his comments--

no; please don't. Please give us the full benefit of your intellect. Dr. Clemens will address you on the topic before you, Probiotics in Infancy, the State of Evidence.

Probiotics and Infancy, the State of Evidence

DR. CLEMENS: Thank you very much.

[Slide.]

You know who I am. You know where I have been. Do you know where I am going?

[Slide.]

It is really a kind of a pun, obviously, because we have discussed, throughout the day, really the beginnings and a bit of a history of probiotics. Now we are going to turn the corner just a little bit. We studied a little bit about mechanisms, how we assay, and what could be the possibility or the application for infancy. I hope to address some of the mechanisms or, perhaps, some of the exposure data and some of the opportunities at the same time.

So I have a few things to say about this. It is actually interesting that Johns Hopkins University has a great press service, and Dr. Jose Saavedra, better known as Pepe, has this release. It is interesting that, in his quoted release in Texas--notice I have highlighted areas which are consistent with the various definitions that have been proposed throughout the day.

Notice here, in Dr. Saavedra's comments, live bacteria help digest lactose, prevent and treat diarrhea, and improve carbohydrate digestion and control intestinal infections. The question would be what is the evidence for these kinds of positions, particularly in a pediatric population, for example in a pediatric population to digest lactose where, in fact, we have not identified any congenital lactase-deficient babies, particularly in North America. In fact, I believe, throughout the world, we have only identified 60 in the literature.

[Slide.]

Through the presentation objectives, to get you involved with probiotics at all, particularly in a pediatric population, we can address some of that. We also address promising clinical endpoints and, as in any clinical evaluation, what is the appropriate endpoint to be assessed. Keep in mind what might be of statistical significance and what might be of biological interest is not necessarily of clinical practicality or clinical significance.

[Slide.]

What are the modes of action? Some of the modes of action have been discussed today. I won't tell you necessarily how, but I think it is a point of discussion that we must address, particularly in a complex organism such as a baby that is developing.

Also, safety and tolerance, how do babies respond or how do young children respond, to the ingestion of these kinds of food. And what are the potential scientific and technical issues that are part of the whole area of probiotics.

As has been alluded to, of course, it started, really, at the turn of the century, if you will, more the history in this country. We look at Eli Metchnikov, who, in his last ten years of his research with Pasteur Institute, spent time on lactic-acid bacteria in the application of digestion.

Dr. Archer gave you the definitions presented by Parker and by Roy Fuller. The definition by Roy Fuller, of course, has been used predominantly in the literature. Some addition information indicated that it is a living organism. I think the viability seems to be dominant in the literature and digestion in certain numbers--this is the first definition that addressed dosage, to get certain benefits oeyond inherent nutrition.

Then we look at it as an adjuvant, and so forth, as indicated in the paper that I co-authored just a year ago.

Why probiotics in babies. Dr. Russell indicated, well, why? What is this microbial balance? What is our line of decision? What is our reference point? Let's look

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at "nature knows best." No; it is not a show but actually an opportunity to take a look at it.

We noticed that, in a paper presented today and it actually represents some really fine work by Erica Isolauri and Sepalis Almidden in Finland. There is clearly a difference in the microecology system of a child that either conventionally delivered or delivered by C-section. Also, various environments have quite an influence on how a baby is fed and the environment in which that baby is fed. Some of this fine work has been done by Rex's group and Rod Macki and others there at the University of Illinois. I give you a typical citation for that.

We also know that, again, some more work by Erica Isolauri and presented earlier today that the bacteria may, in fact, influence the development of the GI tract. In Rex' group, he summarized that really fine work and published by Gerald Tannock in 1999, the potential influence of GI-tract development might be.

Here, we are talking about the development of Peyer's patches, the development of IgA, and the development of a maturation process to exclude, perhaps, dietary antigens and, therefore, reduce the likelihood of different types of allergic responses.

Also, effective resistance to disease; Alan Walker, out of Boston, did really a fine paper in JPGN

earlier this year talking about cell signalling. Again, Rex Gaskins has some work on that as well. Also to assess normal nutrition. There is some evidence that says that through either digestibility or nutrient uptake, this may play an important role. This is a fine paper published earlier this year.

Let's look at a couple of promising endpoints. They have been belabored, perhaps, throughout the day. I won't address them in any particular sequence. But let's just take a look a little bit further what the evidence might be in terms of the pediatric populations.

Some of the mechanisms that we might be looking at have been discussed today. This is not a complete list. Obviously, there are some papers here the I have given you and others are readily available. We talked a little bit today about the acidification in the gut, the acidification through the production of short-chain fatty acids, the production of antimicrobials.

This work in actually the production, in the efficacious production of bacteriocins, for example, natural bacteriocins, was summarized in a paper which I helped write earlier this year put out by CRC Press, a book, a thirty-chapter book, in natural antimicrobials. I am sure you will all rush right out and get one. It is only-about 450 pages of reading.

Competition for nutrients; this is not only by the microbes by actually only microbes. It does not influence the competition for nutrients by the host. Competition for receptor sites, and particularly oligosaccharides. And, of course, immunomodulation. Again, Dr. Gaskins has a wonderful chapter on this topic.

[Slide.]

Nutrient interactions; it has been clear that some of the vitamins--for instance, look at vitamin B12 and vitamin K, for which we depend on microbes in our own gut as adults. The question might be how much does the flora impact on the nutritional status of an infant or young child.

There is some evidence that these vitamins are, in fact, synthesized by some of these organisms that may benefit the development of the GI tract. Also mineral absorption, particularly the work has focused on calcium, iron and zinc. A microenvironment for these bacteria shows that if the ionization of that environment under acidic conditions that, in fact, localized iron, calcium and zinc may be enhanced. The overall impact of the host, in this case, an infant or child, remains to be determined.

Protein digestibility. It is clear that many of these organisms possess proteases which there is evidence that peptides are formed. I think, as has been discussed

briefly today, peptides are formed through the breakdown of whey and casein and some of these peptides may have some physiological, if not tropic, effect on the development of the GI tract.

Carbohydrate metabolism; again, this refers to the lactase. Many of the organisms which have been discussing today are, in fact, beta-galactosidase-positive. Bile-salt hydrolysis; this refers, of course, to fatty-acid digestibility and other conjugates.

Nutrient composition; on competition, this refers to utilization of nutrients by these microbes, and so favoring the development of the friendly bacteria, if you will, and not so favoring the environment for the potential pathogens which are often iron-hungry, for example.

[Slide.]

Safety and tolerance; what if we were to add those microbes of infant formula? I think Madeleine--I have worked with Madeleine for many years and I just went brain-dead earlier this morning. I apologize for that. If you will just forgive me for that. What if we were to add these to infant formula or to foods that are directed to the pediatric population? Let's look at some of those questions.

It is clear that children and babies are not little adults. They are markedly different. Their GI

tracts are under development The immune system is underdeveloped and going through a rapid phase of development. Kids are going through rapid phases of development so we have to look at growth factors that might be associated or impacted by the introduction of these microbes.

At the same time, we have the very senior adult population, for example, which may have issues associated with achlorhydria and also relating to a condition of bacterial overgrowth.

What might be the population risk and what might be the population benefits associated with consumption of probiotics in these various groups? Is there a risk factor or are there potential benefits and what is the balance of those two areas, with a typical toxicological approach and a safety assessment?

In a pediatric population, Dr. Saavedra--and I will touch base with this briefly--Dr. Saavedra has a paper in press. It will be published in AJCN later this year, also a paper in JPGN later this year as well. It will talk about growth and development. The American Academy of Pediatrics, specifically looks at growth and development as a pre-criterion for adequate nutrition and support.

Also, the question, what is the impact of probiotics on the immature system of the newborn? Some of

this work is done by Ian Sanderson and Allen Walker published in '93 and in their subsequent papers as well. Obviously, I think it was Mary Ellen who commented that one of the areas that has been addressed, at least in Europe, is the immature immune system, also the immature gut system. Some of that work has been done in Europe.

Also, Allen Walker has presented some of the issues we are here with, preterm babies, and, perhaps, we will have an opportunity to take a look at that. In terms of what is the nutritive value of these organisms; do they, in and of themselves, have an nutritive value? Do they have some other value beyond traditional nutrition?

And then, which has been discussed briefly throughout the day, actually the genetic stability of these organisms. We talked a little bit about, and it has been talked about, in terms of if you ferment these bugs up, are they going to changing at such a rate that you are going to change the functional characteristic as well as their genetic potential.

Gerald Tannock does an excellent overview on that in his publication. To a comment made by Dr. Russell today, and it has been discussed by the other speakers, the antibiotic resistance of organisms--there is an excellent review on this topic. Gerald Tannock, in his book, does, in fact, talk about plasmins and potential antibiotic

resistance. Also, in that publication, the group goes on to say that there are not any organisms that have blanket resistance to any of the antibiotics that we know of today.

While they have natural resistance to some of the antibiotics, none of the organisms which are under study for probiotic applications have a blanket resistance to any of the antibiotics which are used today in clinical practice.

[Slide.]

You have seen a list of these. If you look at all the probiotic studies that have been conducted and reported in all the literature, and I have over a thousand of them, these are the organisms which have received the greatest attention and to a point that is made throughout the day, starting from Doug Archer's comments, many of the organisms, with few exceptions, have they gone so far as to identify the strain, have actually had the molecular tools to identify specifically the strain.

To the credit of Dr. Saavedra and others, the fact that the B. lactis, now known as Bb12 from Chris Hanson, has, in fact, looked at genetic makeup and characterized that to the fullest.

You see, generically, Strep thermophilous very much in the literature. Very seldom, however, have you seen the variation presented, the strain presented. And then Saccharomyces boulardii. A comment earlier today--I believe

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Dr. Russell made a comment, "What about HIV of AIDS patients?" if I recall, correctly,

There have been three studies with this population group, and perhaps we will have a chance to address that a little bit later.

[Slide.]

This is a page on your printout that doesn't show because it was in yellow, so the numbers are gone. I did a survey with my colleagues. I did all the literature that was printed, that I was aware of, between 1961 and 1998. There are, of course, papers which I may not be aware of but Norraine, Wayne and myself reviewed every single reprint that we could find.

You can see, in this case, we found, up through 1998, when this was submitted to the publisher, we had about 8,000 subjects in clinical research, about 3100, almost 3200, infants and children in clinical research.

I know of several studies that are about to be published later this year. A study that just was completed in Finland, really an outstanding study by Erica Isolauri and others. So almost 3200 subjects of infants and children, that will clearly tip over 4,000 infants and children when those papers are published later this year and the first of next year.

So our total exposure of the clinical study has

been on adults, infants and children. The numbers are very, very rapidly approaching 9,000 subjects. Yes; they are varying lengths. They are varying credibility. And they have taken two approaches, both of a prophylactic basis and that of a therapeutic trial.

[Slide.]

As Dr. Sanders had indicated, many of those of you who are addressing clinical trials, these studies have been of varying lengths, different ages. We have had some studies that have been three or four days with severe diarrhea. For example, we have had some studies as long as three or four years of varying ages. Particularly in a pediatric population, we look at kids who were given these probiotics when they are pre-term babies, all the way up through birth, obviously and then through twenty years or so of age.

Sample sizes are quite variable from the sizes of four, for our case histories, to sample sizes of several hundred, like the study in Finland that just was completed. Culture and strain; they have not been readily identified in many of the studies. The studies that have been printed in the 1990s are much better at this. We didn't have the tools really prior to that time, or were not exercised by the principle investigators.

Often, in the publication, we don't know what the

feeding dosage is. But, in the more recent studies, that information is readily available. Also, the daily exposure is readily available in the newer studies.

Study endpoints. Some of the study endpoints may not be clinically relevant but sometimes there is serendipity and we get some endpoints that might be of biological interest that are of clinical relevance. We will take a look at a few of those.

Interestingly enough, however, if you look at all the studies--obviously, I have reviewed over 150 studies; now that number is approaching 200. And I believe Dr. Sanders and others have pointed out that no adverse effects have ever been reported, none whatsoever. This is true even in HIV-positive patients or subjects.

In studies with Saccharomyces boulardii and other studies with Lactobacillus GG, for example, no adverse events have been reported. In Dr. Saavedra's own study, he had three patients in the study in 1994, and neither one of those patients presented any secondary symptoms to potential infection by the organism of choice. Of course, the study results are quite variable throughout the literature.

In brief, there is always a question, what about the virulence, Rog. Reported today by Gasser, Donohue and others, there have been some compromised subjects and they will sometimes present a history. These subjects have often

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been compromised already and you will isolate these organisms.

Some two really outstanding studies, one in Canada and one, a review of work that was done in Paris, show between 0, in one study, and 0.2 percent in others, those patients which presented septicemia, lactobacillus that were actually isolated in the blood culture.

[Slide.]

They did not say that it was an increased risk, but these organisms were isolated. There is no information that any of the probiotic strains that have been discussed and researched in clinical perspective have presented any toxins or poisonous substances as assessed through normal toxicological methodologies.

In fact, none of the bacteria have presented anything among high-risk populations. Those patients which come from parents that have a history of allergies and they, themselves, may present--say, peanut allergies, milk allergies, dust-mite allergies and so forth--none of these subjects has presented any symptoms, in terms of allergic symptoms relative to the presentation of these bacteria.

In fact, the work by Erica Isolauri and others are just looking the way, in fact. In the most recent study, they are looking at eczema as a potential outcome, as a potential benefit of consuming certain probiotic strains.

[Slide. 1

The other aspects of adverse events that we reported in the literature in pediatric studies; here we have looked at well over 100 pediatric studies in all ages in pediatrics. No adverse events have ever been reported. Even the most severely compromised, and I referred to the Gallardi, Guandini, for example. That was a multicenter study throughout Europe where kids who had C. difficile, rotovirus infection, chronic diarrhea. Again, in those studies, none of the kids presented any adverse events.

Adult studies; typically, if you see something, usually the subject has an underlying disease. We are not aware of any pathogenesis in normalized healthy adults, certainly not in pregnant women. None has been reported, anyway. We certainly have seen a plethora of exposure data and nothing has been deemed to be pathogenic.

And no case has been linked, significantly linked, or case linked to the consumption of fermented foods or those with lactic-acid bacteria even though that one study with rhamnosis, I think Mary Ellen alluded to that, reported in 1999, there was also a history of additional hepatic abscesses reported in the literature but none linked, per se, to the consumption of foods with lactic-acid bacteria.

[Slide.]

With that as a brief background, an exhausting

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background, I would like to address two studies that were conducted at Johns Hopkins University under the direction of Dr. Saavedra. One of the first studies deals with high-risk patients dealing with diarrhea management and prevention of diarrhea, and the second one deals with day-care centers.

You might ask why these particular subjects. First of all, rotoviral diarrhea accounts for the majority of diarrhea in pediatric subjects. Over 500 kids in the United States die from rotoviral diarrhea disease and to the cost of the health-care system of $2 billion a year for health care.

In day-care centers, you know, if you have had children, you know that when you take Johnny and Sally to the day-care center, what happens. Usually, in a very short period of time, Johnny and Sally present diarrhea as the ingested microflora. So the question might be, is there an opportunity here to decrease the incidence of diarrhea in these kids.

[Slide.]

It is clear, if you look at the infant-formula regulations in collaboration with the FDA and the infant-formula industry, you see here these criteria are rather self-evident. In a publication by the FDA in 1997, written by Chris Lewis who is still with the FDA, it is quite clear, and everyone who has dealt with infants agrees that infant

formula never will match that of human milk and there should not be any attempt to, that is in composition.

But the question might be can you make some modifications to these kinds of products for functional endpoints. Let's look at some of those functional endpoints.

[Slide. 1

In Dr. Saavedra's first paper, and let me summarize this very briefly, in this particular case, we had kids five to twenty-four months of age. I think Dr. Sigman-Grant will appreciate this. This met all the criticisms of previous work. In fact, this is a randomized double-blind placebo-controlled trial with children from five to twenty-four months of age, 55 kids, evenly distributed between a control and study group.

This was in a high-risk population at Mt. Washington facilities outside of Baltimore. These were in a chronic-care facility.

In this case, some of the patients were exposed to up to seventeen months of product, getting nearly 4500 patient days. In this case, they were exposed to B. bifidum, now known as B. lactis, and thermophilous TH4 organisms on a daily basis throughout their stay at the hospital.

[Slide. 1

The incidence of diarrhea was markedly decreased, significantly decreased, in the supplemented group of roughly 7 percent versus 31 percent. Also, in fact, the rotovirus setting was markedly decreased, which was significant, particularly from a public-health perspective.

[Slide.]

So, in this particular study, it was important to note that even in high-risk kids, they did not present any intolerance problems and, in fact, their product intake was normally consistent with both the control and the subject and study groups, and also they actually improved their nutritional status.

They didn't show any disease scores here, but if you will look at the paper, itself, these kids went from a negative-3 Z-score up to a negative 1 or negative 2 Z-score. Keep in mind that these kids were particularly nutritionally compromised at the beginning of the study.

So, in fact, they increased their Z-scores. There was a marked improvement.

[Slide.]

Secondly, and in general from this particular study, even with high-risk subjects with chronic illness, they showed that this product was well received and is well tolerated. We believe, because I was part of that clinical study, that the chemical endpoints, in terms of diarrhea

management, were met. And if you see the microfloral balance--Dr. Russell has asked, what is the microflora balance.

In this case, you are looking at a balance that might be adopted with babies right after that first few hours or first few days of birth. It was presented earlier today that that balance really is dominated by a lactobacillus and also by bifidobacteria. That might be the balance which we are trying to achieve, or one might try to achieve through probiotics.

[Slide.]

In the day-care study, this study is yet to be published. It was presented at NAS, began just two years ago. In this particular case, the infants were introduced to the product at entry, at seven months of age. A large number of subjects in this particular case increased by five-fold the number of exposure days. And now we are up to 68 subject years to exposure versus 12 in the last study and it is the same organisms that were used in the previous study.

[Slide.]

This will be the first study in which actual growth data are presented. Many of the studies that were out there that said that normal growth was experienced will show is the data. Now, in God we trust, and everybody else

must have data.

In fact, very few, if none, of the previous investigators presented data. Dr. Saavedra has presented data. This is just a sample of the data. These are using the Year 2000 CDC charts on growth. I took the subject raw data and plotted them. You can see that if you look at the general pattern for all these infants, they follow the traditional growth curve just established a few months ago.

Actually, you see that one subject which was below the chart, below the third percentile, markedly improved and tracked a reasonable percentile for that child.

[Slide.]

We also looked at intake for this particular case. All the probiotics were assessed throughout the study both in the product, itself-- this was assessed quarterly--and also all the product was assessed for microbial growth for these organisms when the formula was taken home and returned and all those samples were analyzed to look at exactly what was taken in.

Here is a plot indicting that the subjects were consuming anywhere from 10^8 to 10^5 organisms per kilogram of body weight. No impact whatsoever in terms of a negative outcome but, perhaps, a positive impact in terms of clinical outcomes.

[Slide.]

Here is one of the positive outcomes. In fact, we had a decrease in bm's with kids that were supplemented. This was quite significant based on the sample size versus that of the placebo, and what is the significance of decreased bm's. They know this was not due to constipation, as some of you might rush into.

[Slide.]

But we did examine the diaper rash, the incidence of diaper rash and the frequency of diaper rash and it showed, in this particular case base, that there was a decreased incidence of diaper rash with the decreased bm's.

You might expect that to be rather obvious, but it had not, in fact, been documented and this was an observation from the first study and was now clinically demonstrated in the second study.

[Slide.]

The bottom line with this particular study, the products that were formulated, at this point in time, and monitored throughout the study, there was decreased prevalence of diaper rash and, perhaps, more desirable stool pattern. This stool pattern mimicked that of a breast-fed child.

[Slide.]

It is clear, for the first time, we had the evidence that the kids grew normally. We had the direct

data. There were not any differences in consumption and there weren't any tolerances, there weren't any aversion to the product with these microbes. In fact, there were not any differences in clinical manifestations, both in GI health-care visits, the use of antibiotics or any type of therapy by a health-care center and due to illness, which you might expect in a day-care center.

[Slide.]

There are other clinical trials in progress. There are many of them. Let me just give you a nutshell. Give me two more minutes. Immune-modulation; there is a great deal of work--Dr. Gaskins has truly outstanding work in immune-modulation. There is some really excellent work looking at upper-respiratory infection in otitis, particularly in the pediatric population.

There is really a large study going on in Europe looking at allergy reduction using probiotics, particularly eczema, as an outcome. There are some studies underway to examine nitrogen balance. Even though we have demonstrated that babies grew normally, what is the impact of nitrogen balance on babies, particularly newborns. So that work has not been done.

What is the impact of mineral balance. We had indicated that calcium, zinc and iron may be enhanced. Any absorption of those minerals may be enhanced, but what is

the impact on balance. That information has yet to be done in the pediatric population. Different gas production and flatulence has yet to be reviewed.

There is a very large study underway right now with the pediatric population in inflammatory bowel disease, particularly looking at Crohn's relapse. That is a multicenter study being conducted here in the United States. And some really fine work with probiotics in H. pylori and ulcerative colitis.

[Slide.]

Lastly, two graphics here. Clearly, it has been indicated in the presentations so far and, really, reiterated in Todd Klaenhammer's work at North Carolina State University, historically, the species and strains have not been well-identified. I think, in the new studies, in fact, this is turning around and that, using modern techniques, you will see that they are being better identified and be able to be traced.

There are multiple probiotic expectations. What /is the active principal mechanism yet to be identified. Is it cell-wall debris? Is it enzymes? Is it fermentation products or other components that are involved with the cell? We don't know all the mechanisms but many mechanisms have been identified and any others remain to be elucidated.

Clearly, as Todd points out, historically, we have

had inadequate designs. They are, in fact, expensive. I can attest to that, having funded a couple of them. Poor statistics in many of them, but that is changing. More and more studies are being conducted. I know Dr. Vanderhoof, who I think is in the audience somewhere--John is involved with several of the studies and some very credible work is now being done.

So these issues, while they are expensive, the statistics are much better and principles are being identified. But, technically speaking, from a manufacturing perspective, if you were to produce this in a product, in a food product, particularly, you might say, can we isolate that so it doesn't get into other foods. If you are producing a bar or a beverage, you certainly do not want to increase the SPC.

So that needs to be contained. That is a manufacturing issue. Also, as has been pointed out today--Dr. Russell and others have pointed it out--what is the dose. How do you assure that the dose is right and that it doesn't overdose and what is an overdose. We can talk about that later.

The homogeneity; is it going to be the same throughout the product? Physical stability; how does it impact the stability of the organism versus the stability of the product. That remains to be determined. Does that

matrix have any impact on the genetic stability of the organism. As part of that genetic stability, is the functional stability still intact.

As was pointed out by Dr. Gaskins a few moments ago, we need to be able to assess the functional characteristics, too, and fermentation versus in vivo functionality may, in fact, be different.

[Slide.]

Lastly, we need to identify differential microbiologies more than a dilution effect, and as been pointed out by Dr. Sanders and others, in fact, by isolation of microbes, we often will put antibiotics to select different bacteria. Are we going to, in fact, be able to do that when we look at probiotics?

Also, we are looking at orders of magnitude and difference here. If you look at some of these organisms, from 1 to 10 per 500 grams, and now we are going to look at, say 10^{10} in 500 grams, so there is quite a bit of difference, which leads us to the next issue.

If you look at the Federal Register, as published in 1996, a maximal load in a powdered infant formula, for example, was suggested at 10^{4}. But here, in fact, for an efficacious dose, it may well be in the order of 10^{8} to 10^{10}. So, again, how do you differentiate the probiotics at a load that is several orders of magnitude larger than the APC as

stipulated by the regulations.

[Slide. 1

Evidence of safety; historically, we have not seen any outbreaks in the pediatric population, even in the most vulnerable population. I think what we have seen, historically in the literature, is, in fact, the more compromised the pediatric population, the results are more definitive. We have not seen that to be the case in typically healthy kids, although we have not seen any outbreaks.

As indicated by Dr. Archer, B. dentium was identified as one of those bifidobacteria that should not be considered to be safe. But it is not found in the food supply and, to a point by Dr. Russell, we have seen several studies by AIDS patients and SKIDs patient, but none of those patients have presented any--have we seen any microbial outbreaks and we do not have any data on those patients with chemotherapy.

[Slide.]

Two slides; we see that products may be beneficial for kids. If you look at outcomes, clinical outcomes, there may be a rationale that if you can decrease the incidence of certain kinds of disease to match so that we have the flora of a breast-fed child.

[Slide.]

Here I am, cycling down Haleakela which is in Maui with my family.

With that I close. I do have fun.

DR. BENEDICT: Thank you.

Let us ask Dr. Gaskins to join Dr. Clemens in the vicinity of a microphone. We can begin questioning. We are not that far behind, folks.

Questions and Answers

DR. BENEDICT: Dr. Sigman-Grant?

DR. SIGMAN-GRANT: This is for Roger. The day-care study, was the only difference--or how well maintained were the outcomes based on the only difference being the type of formula received? Was there nothing else different between these groups?

DR. CLEMENS: The only difference in that particular--there were three formulas administered in that particular study. The two formulas were supplemented at different levels, as indicated by placebo control. That was the only difference between the two products, and the consumption of formula between those three groups was not any different.

DR. SIGMAN-GRANT: Was the formula consumed at the day-care center or was the formula sent home with the child?

DR. CLEMENS: All of the above. It was administered through its normal routine, both at the day-

care center, if they were on formula at the time, as well as it was consumed at home.

DR. SIGMAN-GRANT: So the treated and nontreated, experimental and control, everything else about their environment was exactly the same?

DR. CLEMENS: Exactly the same. It was as if in a real-life, real-use, type of environment. Yes.

DR. BENEDICT: Dr. Russell?

DR. RUSSELL: Dr. Gaskins, thank you very much for the talk. I was wondering, when you heat an organism, a probiotic, to immune-modulate this intricate system you have described, have there been studies to know how sustainable that modulation is over time?

In other words, does the system demodulate after while even if you keep feeding--I don't know the right words here, but even if you keep feeding the same probiotic? Over time, does it go back to where it was before or does it adapt?

DR. GASKINS: Actually, I am not aware of such temporal studies. I am not aware.

DR. RUSSELL: How long have the studies been done where you look and you can demonstrate that immune-modulatory effect? Is it a month or two?

DR. GASKINS: I think it varies widely but, typically, the outcomes are measured only a few times. So,

for example, the T-cell assays and T-cell proliferation and so forth. I am not aware of studies in which they have traced, at the same time, persistence of the dose microbe and an immune response.

DR. BENEDICT: You would probably happily speculate about the existence of memory T- and B-cells, though, wouldn't you?

DR. GASKINS: So, again, greater than 50 percent of secretory IgA is directed against normal bacterial antigens, or antigens from normal gut bacteria. So, clearly, that system is primed against those microbes. I am also not aware of studies that have looked at secretory IgA responses to dose probiotic bacteria.

DR. RUSSELL: It seems to me that, just as a follow up, if you are feeding one organism the amounts that we have been talking about, compared to the amounts that are in the colon, for example, that it seems like a drop in the bucket and that it might demodulate over time.

But, as you said, even though you might get an acute effect because something new has come into the system, but I wonder how sustainable that would be.

DR. GASKINS: I am not sure on sustainability, but certainly organisms differ in their relative antigenicity. So we have tried to survey that in the paper, comparing germ-free responses or responses of germ-free

animals to different bacteria. You do find that relative conventionalization parameters differ according to the bacteria dosed.

I am not sure how easy it would be to categorize bacteria based on their relative antigenicity because the measures are so vastly different. But such a systematic approach could be taken and, perhaps, should be taken.

DR. BENEDICT: Dr. Russell, again.

DR. RUSSELL: Again, I suppose the reason I am honing in on this is that I am interested in inflammatory bowel disease. I am wondering how--here you have a system where you want to decrease the immune response, perhaps, to a protein being made by some innate bacteria. I think that is one of the theories of how a probiotic might work, is that it is somehow decreasing the activity of the numbers of some "harmful" bacteria that is making some antigenic product that you are trying to deemphasize.

So I am wondering, isn't that an important property to try to learn how to characterize the antigenicity of the probiotic?

DR.' GASKINS: I think the first--so, for example, if the host response to normal bacteria by increasing mucous production, then that would also be consistent with the anecdotal evidence from IBD studies in that, in effect, what one is doing is increasing barrier function to prevent

translocation of bacteria to which the host has already mounted an immune response.

So, in other words, memory cells are present. But if you can effectively prevent those bacterial cells from translocating, then you can effectively prevent acquired immune responses to those bacteria. That is also consistent with the bouts of disease activity and relapse, and so forth.

In other words, I think the relative barrier function is key as to explaining--it appears most clear that, indeed, at least some fraction of IBD or the immune responses are directed against normal bacteria and so you are aware of all of the studies with the knockout inbred mouse models, the knockout models, that spontaneously develop colitis. None of those models develop IBD or colitis in the germ-free state.

But, first, of course, the host had to be exposed to normal bacteria to mount the response. And then, after that, barrier function becomes very important. So it could be that this adjuvant type effect that we were talking about--and I think the observations that Mack has communicated are consistent with that; in other words, potentially explaining the mechanism of this so-called adjuvant effect.

So, in that sense, the goodness of that response

is the relative activation of immunity. So you kind of get a bystander type effect.

DR. BENEDICT: Dr. Cohen?

DR. COHEN: I am also interested in this issue of sort of temporal, the question, because a lot of the things that you are talking about seem to be related to short-term exposure. But are there models of other antigenic exposures that are chronic exposure to the gastrointestinal tract that allow you to evaluate either histologic changes, biochemical changes, the issue of functioning as an adjuvant.

The concept of long-term exposure with one of these agents raises a variety of questions about those kinds of impacts and are there models that you could extrapolate from?

DR. GASKINS: Of course, with a defined antigen, then one can measure memory. That is how it is traditionally done. The problem is, of course, there are very few defined antigens that correspond to common probiotic strains. So if memory cells are generated in response to antigens associated with probiotic organisms, then you could very simply determine the persistence of that response.

I think that varies according to the nature of the antigen.

DR. COHEN: So, in some instances, if I interpret

what you are saying, it is not predictable that an antigenic response will either continue to be upmodulated or will be downmodulated over time. It would depend on a variety of other factors.

DR. GASKINS: So an overt immune response against an antigen associated with a bacteria I think, if persistent, will relate to the nature of the antigen. I mean, typically they are long-lived. In other words, the memory cell is present. The adjuvant-type effect, I think, is short-term, dependent on the signals that are contributed by those bacteria.

So, to generate this type of bystander activation, I think you need the organism present to achieve that. But once you have generated a memory cell against an antigen then, of course, that memory cell is going to be present for some period of time. That seems to vary, at least the work I am aware of, according to the nature of the antigen.

DR. BENEDICT: Dr. Buchanan?

DR. BUCHANAN: This is a question for Dr. Clemens. It has to do about the sustainability of the responses we are seeing. I would like to focus here on the attributes associated with the prevention of disease due to pathogens. If we look at national disease statistics, they are amazingly consistent among the developed countries.

This includes North America, Western Europe and

Japan. We have two of the three areas, these products are consumed quite extensively. Is there any indication, at all, in terms of a long-term sustained effect that these products have had, have they either decreased the incidence of disease or decreased the severity of disease when it happens?

My impression is that it doesn't jump to the fore in my remembering disease statistics. But do you have any more additional information on that?

DR. CLEMENS: That is a good question, Dr. Buchanan. There have been a number of studies, small studies, particularly those with diarrhea1 studies, both in this country and across in Europe and other parts of the world that are suggestive that, in fact, you can decrease the incidence of diarrhea1 disease regardless of the cause and, in many cases, you can decrease the severity.

Now, in terms of a long-term effect, to what Dr. Gaskins was saying, that has not been assessed. During the incidence by the continued consumption of this, during that period, such as the day-care center in Bangkok and Thailand or in Shanghai, it has been demonstrated that, in fact, it decreased the severity and it decreased the incidence.

But still there are kids that, obviously, present diarrhea1 disease, for example. Will that go away? If you stop taking the organism, does the incidence go back up or

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does the severity go back up? I think the data would suggest that it probably does.

If you look at those kids that were given oral rehydration solution, particularly in that multicenter study, had rotoviral infection, Salmonella infections, E. coli and C. difficile, they all improved. The question is, will they have the same incidence.

During the study period, those five years, the incidence of diarrhea1 disease was markedly decreased and, certainly, by hospital stay. You decrease hospital stay by a day to a day and a half. If you multiply that by the thousands of kids who present, the answer is it may have a temporary effect or a transient effect.

But we do not now if, in fact, it has a lasting affect at this time.

DR. BENEDICT: I am not sure I even want to ask this question, but either of you could address it. I am aondering about model systems for infant formula. Given what is at least my understanding, that the antibodies that arise, that allow us to do blood typing, are essentially against enteric organisms, the opposite ones being tolerized away.

So the question that I have is do we know how long it takes these antibodies to appear and if, in fact, we know how long it takes, can we test the infant formula for the

affect on this naturally occurring immune phenomenon and see if we shift the response one way or the other?

DR. CLEMENS: Could the work be done? Yes. Has the work been done? No.

DR. BENEDICT: Thank you.

DR. CLEMENS: Clearly, a lot of these outcomes, and I think of the work that Dr. Gaskins has done, and others, in terms of immunological responses, it is usually speculative based on some animal models, both in mice and pigs in particular.

The IgA model, for instance, was examined in a study in Shanghai reported by Fernande Hashke a year or so ago, if I recall correctly, in a German publication. That was salivary IgA. The question is is salivary IgA the appropriate assessment for IgA production from the systemic perspective and that would concern the question of methodology.

DR. BENEDICT: Dr. Sigman-Grant?

DR. SIGMAN-GRANT: Again, this is for Dr. Clemens. Given the state of the art, as you have it now, the evidence about the immune-modulation with URI and otitis media, do you think that a claim that addition of these probiotics to infant foods is warranted, in particular, for those two conditions?

DR. CLEMENS: The preponderance of the evidence is

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really associated with GI function and diarrhea1 management, if not diarrhea1 prevention. The evidence, in terms of gut signalling or cell signalling with mucosal cells is preliminary at this point in time, in my opinion.

The evidence is rather intriguing that has been presented so far because we have been looking at the breast-fed child. If that is your model system, I think it is a good model, frankly.

DR. SIGMAN-GRANT: It has worked.

DR. CLEMENS: You agree with that, I suspect.

DR. SIGMAN-GRANT: Yes.

DR. CLEMENS: Clearly, if you look at the breast-fed child versus the child that is infant-formula-fed, that URI and otitis are clearly much higher in the formula-fed child. And then the question becomes why. You can speculate for a number of reasons, I am sure.

One of them might be that, in fact, the GI makeup is different. The maturation process of the GI is tract different and, therefore, one could speculate that, based on cell signalling and the presence of a variety of things such as probiotics, such as a lot of other things, may, in fact, modulate response, mucosal response, in URI and otitis.

It is clear that, if you look at prebiotics, these compounds which were readily identified in one of the presentations today, that breast milk contains over 130

different oligosaccharides at micromolar amounts. What role do they play? We don't know. It is speculation at this point in time versus what is available on the American market today. It is quite limited, as you know.

But it is interesting, that, in fact, parents and physicians are very much interested in URI and otitis, more so than they are in diarrhea. I don't know why. But I think, right now, the evidence that URI and otitis is interesting, yet certainly not definitive and certainly warrants further investigation.

DR. SIGMAN-GRANT: Thank you.

DR. BENEDICT: This question is mostly for Dr. Gaskins, but Dr. Clemens could also respond. Thank you for a very nice presentation. When you were talking about the common mucosal immune system and mentioning migration of effects, does this mean, or at least could you please comment on, the participation of tonsil and saliva and potential models for measuring effects without being too invasive.

Could we use tonsil T- and B-cells? Could we use saliva? How close will they get to modeling what is going on in various, or any, portions of the gut system?

DR. GASKINS: Do you mean to determine if you have T- or B-cells responsive against the probiotic or against normal intestinal bacteria?

DR. BENEDICT: just probably any and all. I would like to get a feel for what the tonsils, the various sets of tonsils, reflect and for what comes out in the saliva relative to the gut, normal or probiotic or disease, anything.

DR. GASKINS: Certainly, B-cells migrate to tonsils, so I would guess yes. I am not aware of data, but it sounds like a reasonable approach. All of the epithelial tissues are comprised of B-cells, for example, that are differentiated in Peyer's patches.

DR. BENEDICT: Do you know of any information on saliva for antibody--

DR. GASKINS: Secretory IgA?

DR. BENEDICT: Yes.

DR. GASKINS: The levels are very high. I am unable to give you a number.

DR. BENEDICT: No; certainly. But I mean if you look in saliva as Dr. Clemens suggested a moment ago, could we expect to look for probiotic reactive antibodies there?

DR. GASKINS: Yes.

DR. BENEDICT: Would you speculate this came from the gut or came from exposure in the buccal cavity to some of the various lymphoid systems?

DR. GASKINS: I would like to see the data and speculate from that, but B-cell differentiation is appearing

in Peyer's patches. So I would have to guess from the gut.

DR. BENEDICT: Is it not also occurring in the tonsils, though? There are follicular areas in the tonsil.

DR. GASKINS: For IgA differentiation?

DR. BENEDICT: This, I don't know.

DR. GASKINS: I don't think so. Certainly, B-cell development in tonsils, but I don't think--1gA development seems to be unique to Peyer's patches due to the cytokine, combinatory cytokine concentrations found there; for example, high concentrations of IL-5 and TFG beta.

DR. BENEDICT: Thank you.

Dr. Clemens, do you have anything else to add to that?

DR. CLEMENS: In that particular study, you may have read it, by Dr. Hashke and others, there weren't any differences in the IgA that they assessed, but IgA, perhaps, could be very specific to the antigen and that was not addressed.

DR. BENEDICT: Anyone else? Dr. Russell?

DR. RUSSELL: This is for Dr. Clemens. In your slide of "nature knows best," you spoke, or listed, about assisting in normal nutrition. Were you referring, there, to the increase, or possible increase, of mineral absorption brought about, that you have mentioned, by probiotics?

DR. CLEMENS: Yes; there is actually a paper that

was published in Nutrition Research last year and others have suggested that, perhaps, in a microenvironment, that there may be some assistance in normal nutrition through either calcium, iron and zinc absorption or in the hydrolysis of carbohydrates and protein.

That is to what I was referring. But the significance of that contribution remains to be determined. It is interesting to speculate, and perhaps it is dangerous to speculate, that isn't it interesting if you look at, say, the mineral content of breast milk, for example, and iron, for example, is a classic, or zinc or that of calcium, it is much lower than you find in infant formula today.

The question is, it is a bioavailability issue. Is part of the bioavailability based on the buffering capacity. I think Dr. Benedict used the word "buffering capacity" this morning. If you look at the buffering capacity of a breast-fed, the milieu of the breast-fed, child in the stomach, for example, versus that of a formula-fed, it is markedly different.

The protein level and the nature of that protein in breast milk is markedly different from that which is typically used in infant formula. I can't help but think, and I don't have the evidence, that it may have some impact in terms of the potential availability of nutrients.

DR. RUSSELL: Some of these probiotics, I think we

heard this morning, compete with other bacteria for iron, for example. But, evidently, they don't compete for iron crossing the epithelial cells. Is that the--

DR. CLEMENS: I believe that is true. There isn't any evidence in any of these long-term-fed kids that they present any signs, symptoms, of anemia or zinc deficiency. As a matter of fact, if you look at the length data, if you assume that as a possible indicator of calcium deposition in normal skeletal growth, in fact, all these kids are normal in terms of length for age.

DR. RUSSELL: As a follow up, with regard to the vitamin synthesis by some probiotic organisms, vitamin synthesis can take place with so many organisms in the normal GI tract, but you mentioned specifically vitamin K. That has been looked at in the adult, that vitamin K synthesized by intestinal microorganisms really contributes very, very little, if anything, to vitamin K nutriture.

We used to think it contributed a lot. Is that different in the infant that, in fact, we know that the organisms do contribute a lot to vitamin K nutriture?

DR. CLEMENS: It is a good question, Dr. Russell. Actually, we don't have direct evidence. If you look at the vitamin status of these kids as well as the mineral status of the kids, it doesn't appear to be one way or the other, as a matter of fact.

You can do some calculations based on in vitro modeling, based on production of the various B vitamins, for example, folic acid as well as some of the fat-soluble vitamins such as Vitamin K, and then you look at that amount that could be produced, say, in a 24-hour period versus that which is a requirement, it appears to be very, very small relative to their actual requirements.

DR. RUSSELL: Thank you.

DR. BENEDICT: We have allotted some time, as always, for public comment, public participation. Thank you both for joining us. I hope you will be here with us tomorrow. Thank you both for illuminating presentations.

Public Participation

Our first speaker is Dr. Gregor Reid, who may or may not be prepared physically. Are you ready to go there?

DR. REID: Yes.

DR. BENEDICT: Oh; the analog method.

DR. REID: Yes. Slides.

DR. BENEDICT: Dr. Reid, if you would just introduce yourself. We have allotted about ten minutes. Thank you.

DR. REID: I am Gregor Reid.

[Slide.]

I am a Professor of Microbiology and Immunology at the University of Western Ontario, Associate Scientific

Director of the Larson Health Research Institute. I own the intellectual property to several lactobacilli strains including GR1 and RC14, which I will talk a little bit about today.

I thank you for giving me the time. I felt like a supervisor in a graduate-student exam desperate to say something but couldn't. So I guess I now get my ten minutes.

[Slide.]

The first question I am going to ask is why are we here? I think we need to be reminded of some of these numbers. The Burden of Disease World Health Organization '99 figures, leading cause of disease; cardiovascular, 31 percent, infectious diseases, number two of which diarrhea1 is 2.2 million people died because of that.

I would have to say that countries such as Malaysia, India, China where large numbers of populations are present, and Africa, would be responsible for most of these, but that doesn't mean that, as a society, North Americans shouldn't be trying to help. I think, through probiotics, we can.

Diarrhea accounts for 2.5 percent of total health-care costs in Brazil and antibiotics have no effect on 85 to 95 percent of pediatric cases. The work that I am referencing today--I have included a whole bunch of

references because I like to back it up with hard stats--the urogenital tract, which has been my primary interest and, therefore, I make a very strong case for expanding the term "probiotics."

We have been working on probiotics for the urogenital tract for eighteen years and I think it is important. The estimated bacterial vaginosis cases in the U.S. is 10 million a year. This is associated with preterm labor, increased risk of sexually transmitted diseases. Antibiotic therapy for BV does not prevent onset of labor.

There are over 11.4 million cases in the U.S., again, of urinary-tract infections in 1997, 2 million cases in the hospital get UTI and this is an annual cost of $2 billion per year to the U.S.

Vaginitis is a little bit more difficult to get all the numbers, but 15 million has been estimated for yeast vaginitis. So this is a huge problem.

[Slide.]

Is there an antibody apocalypse? Every day, in the U.S., we give 190 million doses of antibiotics. We estimate that there are 133 million doses prescribed to outpatients each year. In Ontario, Canada, and maybe it is typical in the U.S., 20 percent of all oral antibiotics are given out for urinary-tract infections.

Is there an antibiotic resistance problem? In

Japan, multi-drug resistance in Staphylococci is now at 60 percent. There have, in fact, been strains of an isolated that combine the MR assay and VRE properties, potentially leading to a bug that we cannot cure.

The fluoroquinolones were seen as the big savior. There is now over 30 percent resistance against E. coli in Spain, 90 percent in Bolivia to trimethoprim sulfamethoxazole, and 99 percent resistance to tetracycline in Trinidad.

[Slide.]

so antibiotics are no longer the gold standard. YOU maybe can't see this, but the amazing thing to me is that we give out antibiotics, and really we kind of don't think about it.

I went to the Compendium of Pharmaceutical Agents. These are the side effects of trimethoprim sulfamethoxazole, one of the most common antibiotics. The first thing is fatalities. That is not very good. And then there is Stevens-Johnson syndrome, and hepatic necrosis and anemia and, well, a lactating woman can get renal impairment and actually causing renal failure in asymptomatic meningitis and depression and shortness of breath.

Fluoroquinolones, some of them, have killed people but there are also some serious fatal reactions. There is hypertension. There is vaginitis, but don't worry about it;

we can cure that. There is kidney failure, joint pain, anemia, Stevens-Johnson syndrome.

And then there is amoxicillin which gives superinfection with fungi. So here is a case where we could actually claim on the antibiotic label, "Causes yeast infections," and yet we are having trouble saying probiotics don't? so maybe if we can't say yeast infections, maybe we could say, "Probiotics stop the itching," and get around the FDA's silly terminology.

[Slide.]

The percent for probiotics--1 have said 16 million. I think Mary Ellen is correct. It is 24 million, but there are lots of examples and I am not going to repeat what has been said today. Brazilians consume 120,000 tons of fermented milk per year. The French; it is 100,000 kilograms per year. This is a $400 million market for those of you who like numbers.

Consumption of fermented milk is highest in Finland, 36.4 kilograms per person per year. Sweden, Germany, UK; lots of people take it. These products are going to come on the North American market and I would argue, again, that there are no side effects.

Interestingly, one of the future areas, and that is why I kind of went back to cardiovascular, over 125 million Europeans have high cholesterol. If probiotics

has a role there, it could be kind of exciting.

[Slide.]

I am not going to go over the ones like Naidu, et cetera, because they have been mentioned, but we have done studies with three strains, rhamnosis GR1, B54 and RC4 or fermenturn strains with over 100 patients with no adverse effects, and certainly no yeast infections.

I am going to describe to you wound infections in a minute which is a very exciting new area that we have discovered that probiotics can be applied to, once again expanding your area.

[Slide.]

Wound infections in the U.S.; you spend $200 billion a year on managing wound infections. So if probiotics can have an effect, that is exciting. Then there are other papers on newborns and in children, newborns particularly. Reducing the rate of necrotizing enterocolitis in premature babies is potentially very exciting.

I was at an antibiotic conference in Atlanta where all they talked about was antibiotics. In fact, the first thing they give a newborn is gentamicin-ampicillin. I suggested probiotics and he looked at me as I have had three heads. Clearly, we need to educate some of the physicians that this is a potential option.

[Slide.]

This is a wound-infection study that we did. This is Staph aureus. When you put it under the skin of a rat for four days, you get horrendous sepsis. This strain, GR1, didn't make any impact on this at all. It was blood and awful. It was pretty terrible.

When you put under bovine serum albumin, you still get this horrendous sepsis, wound infection. But when you put under RC14, we get absolutely no infection, which is kind of remarkable and we have since isolated a biosurfactant from that strain which also gave no infection and then, subsequently, isolated a collagen-binding protein which also gave us no infection.

So, again, I think the potential applications of some of these strains, given that diabetic patients, basically, get their legs amputated and other limbs amputated when they have severe wound infections, let's not hold it to the gut.

[Slide.]

Evidence for probiotic colonization; the probiotic bugs colonize the gut. We have seen and given good examples of Gerald Tannock's work. I think there are, now, enough studies with these strains and we have talked about it today, so I am not going to repeat that.

[Slide.]

We have concentrated on the vagina. Do probiotic organisms colonize the vagina? I think the answer is yes. We have shown clearly that GR1 does for at least seven weeks. We did not follow it longer. We have also shown that RC14 does.

Not only that, we have shown that RC14 produces the P29 protein on the vaginal epithelial cell. Thirty-three patients who received this therapy for a year did not experience a yeast infection nor did fifty-five patients who received therapy for one month. This is equivalent to thirty-nine patient years without yeast.

The expected yeast prevalence in recurrent UTI patients is two to four per year, or 264 expected, which is remarkable.

There is another strain which Sean Hillyer is working on, CTV05, which FDA and HPB are probably aware of. It has been shown to colonize and persist in the vagina and it looks like it has got a chance of reducing the risk of BV.

[Slide. 1

I have said for quite a long time we need to have a scientific basis for probiotics. These are some of their characteristics. I would have to agree that adhesion, itself, is not necessarily important but we have shown that in vitro adhesion actually can correlate with in vivo

colonization levels. I can happily show you the data.

I think it is important, though, if adhesion is followed by exclusion of pathogens, and we have, again, shown that persistence to multiply production of antimicrobial products like hydrogen peroxide. But the problem with hydrogen peroxide is that spermicide kills it, the bugs that produce it. So you can't just simply go with that.

It should be antagonistic to the growth of pathogens, able to resist vaginal microbicides. There is no point in putting in a lacto when someone is using an anoxinol 9 that will probably get rid of it. It should be safe. And it should form a balanced flora. To the best of our knowledge, a balanced flora in the vagina is one that is dominated by lactobacilli so we are a little bit clearer on that than on people in the gut.

So then I raise the question, should we have a claim that states something like, "Well, lactobacilli strain," in our case, these two but it could be any one, "reduces the ability of pathogens to adhere and grow." We have to think about that. There are arguments for and against.

A claim should not, in my opinion, be done by association with work on other strains. If you go to the website of people who are producing products with

probiotics, it is classically filled with reference to the Literature. The literature has nothing to do with the strains that they have in their product.

[Slide.]

I think this is the last slide. Studies should be able to show that the use of specific probiotic strains make an impact in the incidence of infection. So, for example, I will give you, again, our work in UTI. The recurrence rate for women not receiving antibiotics is 2.6 per patient per year. This is Walter Stamm, a very well-known figure in Seattle.

However, the risk of recurrence rises up to 5.5-fold in women with a history of UTI. The recurrence rate in UTI women who also get trimethoprim sulfamethoxazole--this is an antibiotic that is given every day for up to five years--imagine. They still get 2.3 per patient breakthroughs.

When we gave lactobacilli in a combination of vaginal therapy once a week, the infection rate, we then found, after a year, was 1.6 per patient per year. Lactobacilli, three strains, all proven by molecular typing to colonize the vagina and by Nugent score, which is very well regarded, restore the flora to normal.

Also, there is a correlation between lactobacilli presence, fewer recurrences of UTI; therefore, surely, there

at

is proof of persistence of vaginal lactobacilli after probiotic therapy and therefore should we not allow--I know there is not going to be a decision made on this today; I am just challenging you, I guess--the use of lactobacilli strains, et cetera, is safe and can help restore the vaginal flora and reduce the ability of pathogens to adhere, grow and infect--I know there is that awful word "infect--"thereby helping to maintain the health of the host."

Thank you again for your time.

DR. BENEDICT: Thank you.

Next we have Dr. John Vanderhoof who is in the house who also uses the analog method of presentation.

DR. VANDERHOOF: Thank you very much. I am also going to go low-tech here today.

[Slide.]

This is the "who I am and why am I here" slide. I am John Vanderhoof. I am a pediatric gastroenterologist. I run the Pediatric GI Section at the University of Nebraska and Creighton University since 1976. About four years ago, I received a call from some people at ConAgra, which is a large food company in Omaha, and they said, "Dr. Vanderhoof, your name was given to us by somebody in New York as somebody who might be able to tell us about probiotics. Do you know anything about that?"

And I said, "Yeah; they don't work."

Subsequently, they said, "Would you review some stuff for us?" And I reviewed a bunch of material for them and told them that, in my opinion, they ought to try to get hold of something called Lactobacillus GG. I didn't know very much about it, but, from reading the literature, it seemed to do something.

That was my introduction to probiotics. It was also my introduction to ConAgra and that has ended up resulting in a number of clinical and basic studies and a fruitful collaboration with these people. They have even given me a title. They funded the work that David Mack did that was in my laboratory that was presented by Dr. Gaskins and some of the studies that I want to show you today.

When I initially met with them, they asked me several questions. These are some of them, and they are some of them I want to pose to you. What are the potential uses of probiotics? What kind of basic and clinical investigations should be done prior to marketing probiotics? How should clinical data be used to direct probiotic use? What is needed to make health-related claims about probiotics? At what point do the claims about probiotics become meaningless and misleading to the public?

I couldn't answer any of these questions. And I still can't answer them, but I have an opinion on them. I think it is the same opinion that the members of my GI

section, and also the people at ConAgra might share. So that is what I want to tell you about today.

[Slide.]

Lactobacillus GG is the organism that we have worked with the most. We have worked with a few others. This was developed by Gorbach and Goldin at Tufts or, if you talk to Barry Goldin, it was discovered by Goldin and Gorbach, and, hence, the name GG, a human organism. It is probably the most clinically studied probiotic that there is.

[Slide.]

I think we have adequately demonstrated efficacy in the following conditions. Viral diarrhea, antibiotic-associated diarrhea, relapsing C. difficile--maybe we have a little bit more work to do there--and traveler's diarrhea. By demonstrating efficacy, what I mean is we did double-blind placebo-controlled studies.

If you don't do double-blind placebo-controlled studies in humans to establish efficacy about probiotics, I think you are wasting your time and I think these studies need to be published and they need to be published in refereed medical journals where everybody can read them and where they undergo the scrutiny of the review process.

Until that happens, I don't think we ought to be making claims about things.

[Slide.]

A lot has been said about colonization and a lot of the things that I see about probiotics are based on, "Well, this one colonizes better than that one and so forth and so forth." So what? I don't know that we know that this is an important thing. It seems to be, but it, in and of itseif, I don't think it tells us that--at least tells me, as a physician--that that probiotic is going to be useful.

Perhaps the most well-established indication for the use, at least of Lactobacillus GG, is in viral diarrhea. This was a study by Erica Isolauri in Finland where she showed that GG in either a milk or a powder reduced the diarrhea1 days of viral diarrhea in children relative to a placebo.

One thing I think that is always important, as honest as all physicians are, it is nice to have something done by groups working totally independently. And that is why I think another feature of this is you ought to have at least two studies that show the same thing.

[Slide.]

Here was a multicenter study in Europe done by the European Society for Pediatric Gastroenterology and Nutrition that basically showed the same thing. If you looked at the viral diarrhea, specifically rotovirus-

positive diarrheas, although the effect wasn't dramatic, the drop in diarrhea1 days was still there.

Interestingly enough, it doesn't seem to work in bacterial diarrheas. There have been about four studies now that have all shown the same thing with Lactobacillus GG. It, interestingly, works in viral diarrheas. It does not work in bacterial diarrheas.

[Slide.]

What about prevention of diarrhea? Here is a study that was done in Peru in toddlers, some of them breastfed and some of them not breastfed. In the non-breastfed group, Lactobacillus GG significantly, although modestly, reduced the incidence of diarrhea.

A similar study in a large number of children in a day-care center was done by us in association with an investigator in Brazil and basically showed the same thing. So I think we have at least two studies here but only one of them is published. I think that they both need to be.

[Slide.]

Here is another example. Children get diarrhea on antibiotics and children, as you know, and as we just heard, get antibiotics for almost everything, unfortunately, in this country. We took 200 children who got antibiotics and gave half of them GG and half of them placebo. The percentage of the children that got loose stools on the

placebo group was almost 50 percent, and it dropped by about three-quarters with Lactobacillus GG.

We published this in the Journal of Pediatrics. Fortunately, at about the same month in the journal, Pediatrics, was a paper from Finland showing an identical percentage reduction in diarrhea on antibiotics. So, again, two published studies in refereed journals, double-blind, placebo-controlled studies, demonstrating clinical efficacy in that particular area.

[Slide.]

Preliminary data suggesting that, after the first relapse of Clostridium difficile diarrhea in adults--this being done at Cornell University Medical Center by Mark Pochapin--demonstrated a significant reduction in relapse rates. The chronic recurrent relapsers, it didn't seem to work, but further data suggest that more prolonged administration of Lactobacillus GG greater than the two weeks that we did it in this first study does, in fact, work.

This study is under way. It is not published. This is abstracted and presented in a meeting but since it is not published in a refereed journal, I think it doesn't really count toward what I think needs to be done in order to say what these probiotics really do in that case.

Lactobacillus GG and traveler's diarrhea. Next

week, I go to Brazil. I can promise you I will have my Lactobacillus GG with me. Protection rate from people going from Long Island to Mexico, published by Dr. Hilton back in 1996, of about 47 percent. Again, the corresponding double-blind, out of Finland, Fins going to Turkey, showing a comparable reduction in the risk of traveler's diarrhea.

So I think, in these areas, we have been able to clinically demonstrate by the use of double-blind, placebo-controlled, studies that this agent is effective.

[Slide.]

Here is another example of something that you are commonly seeing; it enhances your immunity. "You ought to take this because it enhances your immunity." Everybody puts this on the box. But we did this with Lactobacillus GG. We gave a bunch of adults typhoid vaccine. This was done by Larry June at Creighton University.

They were either placed before they were vaccinated on GG or placebo and he drew antibody levels on. them. These were IgG antibodies to typhoid. You can see that the GG group got higher antibody titers. Does this mean that they are less likely to get an infection? I don't think it does. Maybe it does, but we didn't study that.

I don't think we could make any claims about that. I think when people read this on the package, they say, "I am not going to get a cold if I take this stuff." In fact,

this is all we did.

[Slide.]

Here is another study. Severe lung infections in cystic-fibrosis patients in Naples, Italy done by a very respectable investigator, Dr. Guarino and colleagues, showing about a 50 percent reduction in lung infections. Now, that gets my attention. That, to me, suggest that it does have some protective effect.

It is so astounding that it needs to be repeated and we are in the process of trying to repeat it at an American medical school at this time. But if you had two of these in the literature suggesting that that happened, that would tell you, yes, people should maybe take that. Maybe it won't make them sick. Maybe it is worth doing

I think that is something that you can substantiate.

[Slide.]

What about safety? I think lactobacillus has got about as much data on safety as you can have. With clinical trials involving over 5,000 patients, no untoward effects have been recorded. There has also been a major survey done in Finland where the stuff is consumed in large quantities because that is where ConAgra gets it and it is sold in large quantities in Finland and there have been no reported cases of lactobacillus infection until recently.

[Slide. 1

And then one case pops up.

I think this is a diabetic lady who developed a liver abscess and was treated and got well, and the strain was indistinguishable from Lactobacillus GG. What does this case point out? There are tons of cases of lactobacillus bacterial infections in the literature, and you have to remember that anything that gets into the bowel can get into the blood stream, and anything that gets into the blood stream and infect you.

It doesn't matter what probiotic you take, no bacteria is totally innocuous and they all can cause an infection. As these things become more commonly used, you will see more and more of these. Does it mean they are dangerous? No. Quite likely, this lady would have gotten infected with another strain of lactobacillus that the GG replaced.

But you are going to see these and I think that we are going to have to find a way to deal with these kinds of reports as they come up.

[Slide.]

So, in answer to the questions that I posed, I would like to give you my opinion. These are my opinions only. I think most of the people at ConAgra would probably agree with this since they funded a lot of this research.

But, nonetheless, first of all, I think we can say that probiotics are most useful in intestinal disorders, but they may have other benefits.

We don't know that yet. The studies haven't really been done. The second thing is that double-blind, placebo-controlled, studies published in peer-review medical journals, in my opinion, should be directing the use of probiotics and not little deflections of laboratory values in one way or another or studies that were done in rats or mice.

This is the endpoint that we ought to be using as physicians to determine whether or not these things should be used, just like we would an antibiotic or a cancer drug or anything else.

The study should be species- and strain-specific. These are very different. When comparative studies have been done with probiotics, there have been big differences on how well they work to do one thing versus another versus another. I think you should know what strain you are getting. You can't apply what was done with one probiotic to another. The claims, based on these kinds of double-blind, placebo-controlled, studies I think are probably--

DR. BENEDICT: Is this your last slide?

DR. VANDERHOOF: This is it. Finally, I think that we should say that claims based upon demonstration of

colonization or changes in laboratory values, by themselves, are meaningless and I think misleading to the public.

Thank you very much.

DR. BENEDICT: Thank you.

We have come to the end of Day 1. What we will do tomorrow, as it says on your schedules, is we will have a discussion and we will ask the invited speakers to join us at the table. We will be provided with a more focused charge but we also will deal with the questions that we were provided with today.

What we would like to encourage you to do is please don't check your intellect as you leave the room but, in fact, think about how all of the things we have learned today can be applied to what FDA needs to do over the next period of years.

We want to try to think tomorrow about how all this information can be made useful to FDA. I encourage you to think creatively; to think about anything you can think about and then we will have our discussions tomorrow. I think all the speakers were very illuminating presentations.

Ms. DeRoever, do you have anything else to add? Apparently not. So we stand adjourned until 8:30 sharp.

[Whereupon, at 5:05 p.m., the meeting was recessed, to be resumed at 8 o'clock a.m., Wednesday, September 27, 2000.]

C E R T I F I C A T E

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.

Alice Toigo

ALICE TOIGO