TRANSCRIPT OF PROCEEDINGS

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

FOOD ADVISORY COMMITTEE MEETING ON PROBIOTICS

Pages 1 thru 274

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

FOOD ADVISORY COMMITTEE **MEETING ON PROBIOTICS**

Tuesday, September 26, 2000

8:30 a.m.

Hilton Towers-Gallery I and II
Ballston Metro Stop
Arlington, Virginia

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Thomas Montville, Ph.D.

Robert M. Russell, M.D.

Madeleine J. Sigman-Grant, Ph.D.

Roberto Villarreal, DrPH

EXPERTS, TEMPORARY VOTING MEMBERS

Stephen H. Benedict, Ph.D. (Acting Chair)

Mitchell L. Cohen, M.D.

Naomi Fukagawa, M.D.

Donna R. Richardson, J.D., R.N.

FDA

Robert Buchanan, Ph.D.

Cathy DeRoever

C O N T E N T S

P R O C E E D I N G S

Introductions and Administrative Matters

DR. BENEDICT: Good morning. Welcome to the FDA meeting to provide questions about probiotics. Before we start, let's just set a few ground rules. The idea here is that we entertain testimony and information, and that we question as stringently as we can everything to do with what we hear. The idea is to provide FDA with as much information for as many holes that we hear as we can. Pursuant to that, I would like to use the Ed Brandt style of questioning, which is as questions arise, please just make yourself known to myself and Ms. DeRoever, and we will actually entertain questions in the order in which people are recognized. The purpose for this is so the transcribers can get everyone's name down, and it is a very clear transcript that the FDA will then be able to study at a later time.

Before we go too much further, I think that those of us around the table should introduce ourselves so we all know who we are. My name is Steve Benedict. I am in the Department of Molecular Biosciences at the University of Kansas. My area of expertise is immunology and molecular biology.

MS. DEROEVER: Good morning. My name is Cathy DeRoever. I am the executive secretary for the Food

Advisory Committee. I work for FDA's Center for Food Safety and Applied Nutrition in the Office of Science. I would like to welcome all the members and all the guest speakers.

DR. HOTCHKISS: My name is Jo Hotchkiss. I am with the Department of Food Science at Cornell University. My major area of interest is food safety.

MS. RICHARDSON: I am Donna Richardson, and I am Assistant Professor of Medicine and Nursing at Howard University and with the firm of Joan Wilbon and Associates, and my background is in women's health and geriatrics.

DR. MONTVILLE: Tom Montville, Professor at the Department of Food Science, Rutgers, the State University of New Jersey -- I get a nickel every time I say New Jersey.

[Laughter]

I am a food microbiologist.

*DR. BUCHANAN: Good morning. I am Bob Buchanan. I am with FDA's Center for Food Safety and Applied Nutrition, Senior Science Advisor and the Director of the Office of Science.

DR. VILLARREAL: I am Roberto Villarreal, from San Antonio, University of Texas, and my interest is nutrition and diabetes.

DR. COHEN: I am Mitch Cohen. I am the Director of Division of Bacterial and Mycotic Diseases at CDC. My areas of interest are quite varied, food-borne disease,

antimicrobial resistance, molecular biology.

DR. RUSSELL: I am Robert Russell. I am Associate Director of USDA Human Nutrition Center, in Boston. I am a gastroenterologist and nutritionist.

DR. SIGMAN-GRANT: I am Madeleine Sigman-Grant, with the University of Nevada Cooperative Extension. I am a professor and maternal and child health nutrition specialist. My area of interest, obviously, is maternal and child health but I am also interested in consumer behavior and behavior change.

DR. BENEDICT: Thank you, all. Before we go any further, I think Ms DeRoever has some inevitable announcements and things to say.

MS. DEROEVER: Very briefly, for our members and guest speakers, the portfolios that we have provided have your expense vouchers in them. So, you want to keep that handy. You also have copies of, I believe, all the slides of the talks for today, except for one and that will be provided later. You have a copy of a letter from CalBio Yarine. This is a public comment. As in the past, it is provided for your information based on the request of the submitter. That is all I have at the moment.

DR. BENEDICT: Before we begin the actual meeting, Dr. Buchanan -- we would like to ask him to give us sort of an overview of the restructuring of the Food Advisory

ommittee of the FDA.

DR. BUCHANAN: Thank you, Steve. By this time all f you should have received a letter from Cathy explaining ome of the activities that have been taking place during he last year in terms of the -- oh, it came from Jo. Well, athy actually drafted it -- explaining some of the changes hat have been taking place or will take place in the Food dvisory Committee.

We are restructuring it to expand the activities nd to provide more in depth scientific information in ertain specific areas. We will have a structure that onsists of the parent committee, which is this body and hen there will be four standing subcommittees. These will e a subcommittee on dietary supplements that Dr. Chris ewis will be the executive secretary for; a standing ubcommittee on food biotechnology which Mr. Bob Lake will e in charge of; a standing subcommittee on additives and ingredients which Dr. Allen Willis from our Office of Premarket Approval will be leading; and there will be a standing subcommittee on contaminants and natural toxicants which Dr. Terri Troxel will be executive secretary of.

We have put out a call for nominations for this new body. We have received approximately 100 nominations to date, and we are expecting a few more to be coming in within the time frame that we requested them. At that point, they

will go under review, and we are hoping to have the membership finalized and announced by early December.

The membership of the parent committee will consist of approximately 15 people, a chair, two industry representatives, two consumer representatives, the four subcommittee chairs and six academic and/or public health scientists with the appropriate background. I might note here, if there is anyone among the parent committee that is dying to become a subcommittee chair, we would certainly entertain your interest with a great deal of fervor.

Just a little bit more about the membership of the subcommittees, these will be much more focused on specific technical matter experts, focusing on the four areas in question. The current thinking on how this will operate is that for very highly issue specific meetings of a very technical nature or of a more technical nature in one of those four areas issues will be sent to those appropriate subcommittees, whereas the parent committee will be dealing primarily with cross-cutting issues, things that involve more than one of these specific areas. So, for example, probiotics is a good example of a cross-cutting area because it hits in many of the different areas we just mentioned. It will also be there to deal with and respond to requests from the subcommittees on matters that are sent up that they feel should have more cross-cutting evaluation.

We are anticipating, as I indicated, that the memberships of the subcommittees will be announced in December, and we are anticipating that we will start the cycle of meetings in a new structure sometime in the early spring. At that point, we are anticipating that the parent committee will go back to a meeting schedule of approximately two meetings per year. It is also anticipated that the subcommittees will meet as often as necessary but, again, two per year is probably a realistic estimate.

We are assuming at this point, unless we hear from individual members of the parent committee -- you people -- that you want to stay on the parent committee. However, if you feel that you would better serve as a member of the subcommittee we will entertain that. Just please see either Cathy or me.

I think that is about it. If you have additional questions on the new committee or its new structure, please feel free to talk to either Cathy or me, or Linda Hayden, and we will sit down and try to explain it in more detail as needed and some of the intricacies of it.. Steve, thank you.

DR. BENEDICT: Thank you, Bob. Now we will enter the fascinating world of probiotics, and while he shuffles his notes I will just do a little song and dance because Bob Buchanan is going to give us our charge. We have a copy essentially of our charge in our folder, but such things are

always better delivered verbally and we will ask Dr. Buchanan to continue his oratory by giving the charge to the committee.

Focus of the Meeting

DR. BUCHANAN: And then I promise to be quiet.

DR. BENEDICT: No way!

DR. BUCHANAN: I know.

[Laughter]

I would ask you to pull out from your packet a two-page document that starts off with, up in the upper left-hand corner, Food Advisory Committee meeting and then it really doesn't have a title. It says "charge to the committee."

Certainly fermented foods have long been an integral part of the diets of various regions. In fact, one of the things that is unique as you travel around the world is that you get to sample the different fermented foods that are all unique and interesting and have the character of the country; in fact help make up the character. While these products have been an integral part of our diet, it hasn't been until the last half of the twentieth century that we really saw a scientific effort to study the organisms that are being ingested in fermented foods or as part of the normal contaminants of the diet, and their impact on the health of the consumer.

There has been a great deal of work, and it is increasingly focused on the health effects that can be associated with different microorganisms and, as in any type of science, typically after a latent period there is a transfer of that technology to people that will find it useful. So, as we enter the twenty-first century FDA is increasingly being called upon to look at the whole area of probiotics in terms of both its safety and its efficacy, two areas for which we have responsibility both in terms of foods themselves and for dietary supplements.

Now, as FDA is embracing this new challenge, this whole new area of foods and dietary supplements, we, as always, attempt to bring to our decision-making process the best science that we have available to us. We are at the stage now where we are trying to bring that science together and make it an integral component of a framework for which we will develop future programs for evaluating both the safety and efficacy of probiotics and related products.

This meeting today is part of our emphasis on bringing the best science we can to this new challenge we are facing, and what we have attempted to outline in the two-page document that we have provided is sort of the general approach that we would like you to deal with what we are going to be doing for the next day and a half.

I might note that this is a somewhat different

approach in terms of food advisory committee meetings than e usually do. Usually we have some very specific areas here we are asking some very specific questions and at the nd of the day we are looking for a yes/no answer or at east the best advice you can give us on a very specific uestion.

Today's meeting is much more of a fact-finding ype of a meeting, and the focus today will be on providing ou with some background information through the form of resentations and documents that we have provided. Then we re going to be asking you to sit and discuss the science hat underlies probiotics both in terms of safety and fficacy, and to give us your overall synthesized impression f the variables and parameters that should be considered by DA as we develop a framework for evaluating this new class f products.

To help you in that deliberation, we have provided you with a series of very general questions that are there at the bottom of the first page of the document and that follow on to the second page. You will note that they are basically divided into three areas. They are very general in nature. They are, one, related to the definition of , terms and whether we need to be more specific in the terms that we use. I know from my personal experience in dealing with advisory committees and scientific bodies, after three

or four days of deliberations we all find out that we are using different definitions and then we start all over. So, we would sort of like to get that out of the way early to get the communications started. The other two areas are broad questions in terms of factors or parameters associated with wither the assessment of safety or the assessment of health effects.

Now, our interest in you providing this advice is to help us get started in this process and, certainly, the advice we get and the commentary we get will be very important as part of our development of a framework of dealing with probiotics and related products. I might note also that we are not expecting everything to be done. This is the first of a series of steps that will lead to a framework, and it is highly likely that we will be coming back to you at least one more time as the framework is developing. So, in other words, there will be a test -- no, we are very much looking for your advice on this and we have, hopefully, set up a system now where you will be able to interact fully with the speakers. We will have a number of speakers make a series of presentations. Then we will also have them available to you for the entire deliberation.

Steve, with that, I think they don't need me to reread the questions. They are short. But I do ask you, as you go through your discussions, that periodically you just

go back to these questions because these are really the areas that we are looking for your help in. Thank you.

DR. BENEDICT: Thank you, Bob. So, we will begin directly with Dr. Douglas Archer who will give us what is entitled on the screen, probiotics, history of food use.

History of Food Use

DR. ARCHER: Actually, I will give a little more than that because I was asked to cover some very specific areas so I will go a little beyond just history of food use.

[Slide]

First of all, I guess importantly, I am here representing myself and no one else. I have been involved in the area of probiotics for a while and it is just an interest area of mine.

Some of the areas that I was asked to touch on were, first of all, the definition of probiotics, which I think is pretty critical; also, the history of food use and a little bit of safety and maybe some regulatory considerations. For those of you who don't know me, I was in Center of Food Safety and Applied Nutrition for twenty years. So, when I tried to put this talk together I kind of put my old regulatory hat on and tried to think like a regulator, which some might think is an oxymoron. Nevertheless, I did try and think if I was back at the FDA what kinds of things, if I was starting from ground zero,

hat would I want to know?

[Slide]

The first thing, the definition of probiotic and this seems to me to be the most important thing, the most important first step certainly. Not only is it a starting point for understanding what it is we are talking about but, certainly, it is a starting point for regulatory understanding of what these substances may be. Unfortunately, it is not that easy. It would be great if I ould put up a definition of probiotics that would cover the waterfront and everybody would be satisfied with it, but it sn't that easy as I will show you in a moment.

I am going to run through a series of definitions hat have been proposed and I will comment on each, but I ant to apologize ahead of time if anyone is here who wrote different definition than the ones I have; this is not ntended to be an all-inclusive list.

[Slide]

To start though, I will first ask a question, are ll lactose fermenting bacteria probiotics? Well, the short tnswer is no. Lactic acid bacteria are a very broad group of organisms including very well known ones like Lactobacillus. Certainly, they do not all fit into the rubric that many would call probiotic.

Some of the lactic acid bacteria may be

probiotics, but before moving them from the lactic acid bacteria category to probiotic there must be certain criteria, and I will come later to a list of some of the criteria that have been proposed that would make something fall into the category of probiotic. Worse yet in terms of defining these things, not all probiotics are identical. They vary among strains as to what they do.

[Slide]

Well, the simplest and oldest definition I guess is from Greek. Probiotic means "for life." I wish it was this easy. Certainly in the present regulatory environment you could construe this as a disease claim if you wanted to consider death as a disease.

[Slide]

First definition, "substances produced by one organism that stimulate the growth of another organism." Well, this is clearly not what we are talking about in the current context of what a probiotic is. It is a bit vague, as you will notice, and I think that is kind of an underlying theme in a lot of the definitions I will show you. They are a bit vague. The key point is that it stimulates growth via a produced substances, and the definition has certainly been expanded since this one was first written.

[Slide]

"Tissue extracts that stimulate microbial growth." Again, this is almost irrelevant in terms of what we are currently calling a probiotic.

[Slide]

"Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance." Well, now we are getting closer to what we currently are referring to as a probiotic.

So, some components of this I just want to talk about in terms of starting a regulatory understanding of what these things are. First, we are talking about organisms and substances -- two things. We are talking about a beneficial effect on the host, and that beneficial effect being contributed to the intestinal microbial balance with a positive effect on the host. Presumably then, we are talking about ingested substances in this definition and, again, we are getting closer to what we would considerthe modern definition to be.

[Slide]

"Viable bacteria, in single or mixed culture, that have a beneficial effect on the health of the host." Now we are talking about viable bacteria.-- not substances, just bacteria. Mixed or pure culture. Really with this definition to designated portal of entry, like in the last, but again the beneficial effect.

[Slide]

"A live microbial feed supplement which beneficially affects the host animal by improving the ıtestinal microbial balance." This is probably the most ɔmmonly used definition and commonly quoted one that you ill find in the literature.

So, let's take the components again. First, it is a live microbial feed supplement. Well, this refers to the ell established principle of competitive exclusion in nimals. It has a beneficial effect by improving the ntestinal microbial balance and, I can tell you for a fact aving sat in on certain hearing and meetings such as this, hat there are some FDA and other centers that make believe hat this definition alone makes these substances a drug.

[Slide]

This one, "a mono- or mixed culture of live ıicroorganisms which, when applied to man or animal, affects ɔeneficially the host by improving the properties of the ndigenous microflora." That is a mouthful. It means, gain, pure or mixed. "Applied to" leave the portal open so it can be ingested, or applied topically, or you name it. Beneficial effect again improved the properties of the indigenous microflora, whether they are bowel flora, skin flora, nasal flora, whatever. But, again, this definition seems a bit vague.

[Slide]

This one, one of the authors of which will be speaking to you later, Dr. Roger Clemens, is very specific. "A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity as well as improving nutritional and microbial balance in the intestinal tract." Well, microbial dietary adjuvant -- that seems to be very specific. It says exactly what it does. The beneficial effects here are more numerous, modulate mucosal and systemic immunity; improve nutritional balance in the GI tract and improve microbial balance in the GI tract. This is a somewhat different definition than the others, more specific as to what probiotics do, but not all inclusive actually as to what has been claimed probiotics can do, which goes well beyond this in terms of improved nutrition, in terms of lowering cholesterol which we will come to later in later talks.,

One that I tried to insert in here, which is another one I came across and I think is important and my zip just told me that if I tried to cram one more thing in, it would fail, so I didn't, but it is a definition proposed by Guarner and Schafernan in 1998. Their definition is rather simple, "live microorganisms that confer a health effect" -- and these are the words I want to, stress,. -- "when consumed in adequate amounts." I think that is a very

important concept that I will come back to later -- "when consumed in adequate amounts." There are many, many products on the market, and one of the big questions about some of these products is what is in it and how many, and is it realistic that what is in it and how many can effect a real change?

[Slide]

Is viability of the organisms an absolute requirement? Well, many papers say yes and other say no. For me, personally, it is kind of hard to reconcile a dead probiotic. It kind of seems contrary, nevertheless, it is out there in the literature.

[Slide]

So, a new category was proposed, again by Dr. Clemens and his colleagues, a probiotic-active substance, which is a cellular complex of lactic acid bacteria that has a capacity to interact with the host mucosa and may beneficially modulate the immune system independent of the lactic acid bacterium's viability. So, we have a new definition, probiotic-active substance, or a new term to deal with, and this can include dead things. We are talking now about cellular complexes, parts of the cell, dead cells, etc., and they work by modulating the immune system independent of the viability of the organism.

[Slide]

In terms of a definition, I had a hard time aying, okay, here are the definitions and then here are ome of the desirable traits of probiotics. I kind of lended them together a little bit because I think some of hat have been listed as desirable properties of probiotics ay well help define them. So, that is the reason I am utting it in this order.

[Slide]

Again, this is probably not a complete listing ut, again, I think they may help shape a definition ltimately. First of all, probiotics are supposed to be of uman origin. Well, as we will see a little bit later, that s not always so and with recombinant DNA technology owadays able to kind of tell us where things come from, not lways have these things come from human beings; they come from all over the place.

They must be resistant to acid and bile. Well, he simple thought here is that they have to get through the stomach and into the. intestine before they can do anything good. so, if they are killed in the stomach by gastric acid they are not going to be much good.

They should adhere to human intestinal cells. Well, the most common test that people have run these through is to see if they adhere to a cell line such as CAC02 but, nevertheless, the concept being they have to stay

round a while or they can't do much.

They need to colonize the human gut. Just as the nalogy was made before with probiotic supplement feeds for nimals, in order for them to do their thing they have to olonize, have a stabilized colony in the gastrointestinal ract in order to exclude the things they are trying to xclude.

[Slide]

Some others, production of antimicrobial substances. Well, here we are talking about things like acteriocins, organic acids and other things that might have a deleterious effect on pathogenic microorganisms.

Antagonism against cariogenic and pathogenic acteria, well, by the same token, either by crowding them out or competing successfully for nutrients and starving them out or by producing some substance that knocks off the bad guys. Remember that the mouth is one of the richest places to find lactic acid bacteria. They are there in huge numbers and we are constantly ingesting them from our own indigenous source.

They must be safe for human consumption. I think this goes without saying and I will talk a little bit about safety later.

They must have clinically validated health effects, and I think here is the crux of the controversy.

As you read the literature, there are literally thousands of papers about probiotics, some very well-designed studies and some not so well designed. So, what does it all mean? Are they clinically valid findings?

[Slide]

So, probiotic bacteria are more than just capable of fermenting lactose; they must do other things as well. They must possess some of the desirable properties that I have listed. They must be beneficial by some measure, and that measure I will leave up to you and to other speakers who are going to address you today. They must be taken in adequate amounts. I added that one. That is my own thought. I really think if they have any effect, they are certainly not going to have an effect if they are virtually a drop in a huge bucket. Foremost, they must be safe.

[Slide]

Now just a thought after seeing all the definitions, if they are to be recognized and presumably regulated somehow; they have to fit in some regulatory rubric whether we like it or not, an agreed on definition must be established. I think this is the first step. We must define what it is we are talking about. But how do we define them? Do we define them by functionality, by their effect? Do we define them by criteria based on their properties? How do we account for the differences among

robiotic strains themselves? One probiotic strain may make claim that it can do a certain thing; another probiotic train can't do that but it does something different. So, t is not going to be an easy task. In a regulatory sense, think the definition and the crafting of a definition is oing to be one of the hardest things to do and yet I think t is the requisite first step.

[Slide]

Kind of shifting gears a little bit into food use, do want to say a little bit about food use of lactic acid acteria, and here I am talking about all LAB not just robiotics. Now, as was stated before, use of lactic acid acteria to preserve foods is centuries old technology. Dairy foods certainly predominate but meats, vegetables, iruits, everything -- fruit juices, fermented beverages, all of these have been around for millennia.

[Slide]

Some of the applications of lactic acid bacteria are food such as kefir, yogurt, acidophilus and, I didn't put it but bifidus milk as well, buttermilk, fermented meat -- that means sausages and intact meats, fermented vegetables such as kimchi and wine and cider.

[Slide]

To take a couple of examples, kefir, indigenous food in Russia, Turkey, the Balkans, very, very old, the

ferment itself, the starter culture is dried into grains and that is how it is preserved from passage to passage. So, clearly, it is a mixed culture fermentation including yFeasts, bacteria -- not very well defined; not very well controlled. In a regulatory sense it would kind of raise some question markers right away. Most any kind of milk can be used to make this particular food.

[Slide]

Another one, kumiss, European, Russia, Central and Southwest Asia, usually mares milk is the milk of choice to ferment. In this fermentation the starter is usually either fermenting or decaying vegetable matter or even decaying animal matter. There must be some interesting side flavors generated in this kind of technology.

[Laughter]

[Slide]

Yogurt -- at least 4000 years old. It comes in a variety of terms and a variety of different languages.

[Slide]

The belief that yogurt consumption leads to good health has been with us for centuries. It has; it is just sort of a thing, you eat yogurt and you are healthier for it. Was there a basis for that? No. People just felt better when they ate it. It certainly is a good food nutritionally; good source of protein, calcium and lots of

other things but the belief goes beyond that. The belief is that other beneficial effects have resulted from the consumption of yogurt.

[Slide]

It wasn't really stated too clearly until Metchnikov said it in 1907 in his work, "The Prolongation of Life."

[Slide]

His proposal was that the consumption of large quantities of yogurt containing Lactobacillus would result in the replacement of toxin-producing bacteria in the intestine, and he referred to the concept as "longevity -- without aging."

Now, he came to this conclusion by studying the elderly in Bulgaria and noting that people tended to live long and they ate a lot of yogurt. That wasn't exactly what we would call an efficacious clinical trial nowadays but, nevertheless, this is what he did.

[Slide]

A more recent proposal, just to give you one example of the huge gap in what is going on in probiotics, "Biotherapeutic Agents: a Neglected Modality for the Treatment and Prevention of Selected Intestinal and Vaginal Infections," published in the Journal of the American Medical Association, with an accompanying editorial. The

conclusion of this article was that selected microorganisms, i.e., probiotics, may prevent and treat intestinal infections and may treat vaginal infections. For the FDA folks here, I have now said the "P" word and the "T" word which means I am talking about a drug and, for me, it is a hard concept to think that I would have to get a prescription in order to ingest yogurt but, nevertheless, clearly if we are starting to talk about foods that prevent and treat or mitigate diseases we are talking about a regulatory category that is very rigidly regulated.

But I think the other conclusion in this paper was an important one, and that is that biotherapeutic agents hold the hope of decreasing our dependence on antibiotics, and I think that is something that we should keep in mind because clearly that would be a benefit.

[Slide]

In terms of live microbial food suppiements and health, to go back to 76 BC, Plinio advocated the use of fermented milks to treat GI infections. How he came upon this we can only guess. In fact, I kind of wondered when I read it that, in my way of thinking, probably all the milk was fermented very quickly in those days.

Later on, in 1907, Tissier recommended bifidobacteria for infants suffering from diarrhea. This is the same gentleman who, 1899, first characterized

Bifidobacterium as being a new kind of bacteria, and he found them to be the predominant flora in breast fed infants. So, from there he extrapolated to this theory that it would be good to treat infants suffering from diarrhea.

[Slide]

That proposal has raised its head even more recently. The controversy has been around for a while. For those of you who have ever come across it, U.S.D.A. Bulletin No. 319, called, "Fermented Milks," first written in 1916, rewritten in 1928 reviews the controversy surrounding the therapeutic use of fermented milks. Basically, back then the statement was made that well-designed clinical trials were lacking and a lot of evidence was anecdotal, which sounds a lot like the criticisms that we hear even presently.

[Slide]

In terms of what the organisms are that have been claimed to be probiotic or have probiotic properties -- lot os different Lactobacillus species, acidophilus, GG, the patented strain, plantarum, rhamnosus, brevis, bulgaricus, all of these at one time or another and certain subcultures of these have been said to have certain probiotic attributes.

[Slide]

And the same thing with Bifidobacterium. I would

point out Bifidobacterium animalis, as it name implies, is thought to not be a very human strain, nevertheless it does have some interesting probiotic properties.

[Slide]

Without stepping on Mary Ellen's talk too much but just to point out that a lot of the lactic acid bacteria have been around and consumed in huge quantities for a long time, and this gets us thinking, well gee, how can they be unsafe? We are so used to eating them. Cheese, for example,, projected consumption for the year 2000 is 30 lbs per person. That is quite a bit. You have to remember most cheese is, in fact, a living food. It has quite a few bacteria in it. Cheese consumption has increased by over 150 percent since 1970.

[Slide]

Human exposure to lactic acid bacteria in yogurt. Yogurt consumption through 1997 was 5.1 lbs per person. I found that surprising personally but there it was. This is an increase of over 500 percent in the U.S. since 1970. Maybe later speakers will address the point, I don't know if consumers are saying this is a healthy thing to do; I should eat yogurt for its healthful, beneficial properties, or what but, nevertheless, sales seem to be taking off.

Just a little side word, yogurt by definition does not have to contain any living bacteria. There is no

regulatory agency that says it must contain live bacteria. It can contain from zero to sky is the limit in terms of bacteria. The National Yogurt Association has set some standards for its members, and in order to use their live active culture seal the yogurt must contain at least 10^8 living microorganisms at the point of manufacturer, however, there is some die-off during the shelf-life of the product. It comes back to the issue I mentioned before, how many actually are we ingesting and is the number related somehow to the perceived benefit?

[Slide]

Again, thinking about safety as an issue, populations consuming lactic acid bacteria worldwide -- virtually everyone does. I mean, if you go to various contrives, certainly infants, children, adults, people of all health statuses -- and I know you will hear some clinical studies later that were done on some severely ill people and, yet, there are virtually no infections caused by the probiotic strains themselves.

[Slide]

The bottom line on lactic acid bacteria -- this is as much an editorial comment from me as it is anything, I believe from looking at all the literature I have reviewed, and it has been quite a bit, that the lactic acid bacteria are safe for human consumption.

[Slide]

A lot of people have written papers about the safety of lactic acid bacteria, and I would point out one, the last paper, by Gasser. There have been infections in human beings caused by lactic acid bacteria such as endocarditis, septicemias and certainly other organ infections as well, but I think the bottom line here is they are extremely rare and, in most cases, are felt to be of an indigenous source, usually associated with some kind of trauma. The bottom line of this investigator was -- and this is a quote -- is that "there is insufficient evidence to conclude they are unsafe." As a former regulator, that statement makes me a little uncomfortable. I wish they had worded it a little bit differently. It seems to be a negative statement and actually it is a positive statement, i.e., he thinks they are safe.

[Slide]

Advice on new probiotic strain use -- I think this is an interesting quote from Salminen, who has certainly published extensively in the area of probiotics, "it cannot be assumed that these novel probiotic organisms share the historical safety of traditional strains. New strains should be tested for safety and efficacy of their proposed use."

So, here is a little word of caution from someone

in the probiotic game, we can't just assume that they are all safe. I think getting a comfort level with the general area of lactic acid bacteria might be a healthy thing. I mean, we can put them into kind of a different concern level but, nevertheless, when someone proposes to use a probiotic strain there may be some other things that we might want to ask.

[Slide]

How would testing for efficacy be approached? Well, that would depend on the definition probiotics are assigned and whether it is recognized that they have therapeutic value. Again, this comes back to a very critical point, are these foods? Are these drugs? Are these dietary supplements? What are they and what can we say about them legally in the current legal confines?

I think it would be a shame, however -- and again, this is an editorial note of my own -- if progress was impeded by simply lumping them into an existing regulatory category such as a food additive and then. saying, hey, what is the technical effect? It doesn't fit any of our current technical effect definitions as they were originally assigned by the National Academy of Sciences.

[Slide]

Beth also asked me to touch on this area as well, good manufacturing practices, quality assurance and quality

control guides and requirements, what would they be for these substances? Well, I think it is clear that rigid adherence to good manufacturing practices must be required. These are living things that we are talking about, and that the industry should be a part of their development and refinement. The reason I add that is that there is an industry out there. It has a long history of doing things a certain way, and they seem to have come out in a positive way. People seem to be doing things, by and large, right. So, I think that the regulators do have a lot to learn from the industry in terms of how they have set up their GMPs; how they have set up their QA/QC procedures.

[Slide]

Quality assurance factors for probiotics should be standardized and developed with industry input. I think this is a given. Learn from industry; learn from its mistakes and learn from what it has done right. Obviously, there are going to be some unique quality factors that will apply to these living things. For example, how do we assure purity all the time? How do we assure consistency from batch to batch each and every time? And, how do we assure the genetic stability of these organisms? There are answers to all those questions, it is just a matter of sitting down and putting them on paper and learning from each other in the process.

Quality control measures will also be somewhat unique, and should also be thought of as safety controls, again remembering that these are living microorganisms that we are talking about, by and large. Dr. Clemens' definition of probiotic active substances aside for a second, we are probably talking about living subsets of lactic acid bacteria so, again, purity becomes a big issue. Make sure nothing bad gets in with the good.

[Slide]

Beth asked me to mention a few words about international considerations. In the area of probiotics the U.S. is behind the rest of the world in their acceptance in common use. Clearly, the European Union countries and Japan are light years ahead. In Japan there are vending machines as common as Coca Cola or Pepsi Cola machines that dispense probiotic formulas. In the European Union there are many products on the market, such as infant formulas containing probiotic cultures, that aren't quite here yet but some day may be, but they are generally accepted in European Union and Japan as being good things, being something positive and having some positive health benefit.

[Slide]

So, from a regulatory perspective, again, I think some of the steps that have to be considered are, first, to define a probiotic. What are we talking about? Again, I

don't think this is going to be an easy task. I think it is going to be very a difficult task to come to a definition that can be broad enough to capture all of what this concept of probiotics has become.

Decide of efficacy criteria. Well, are they biotherapeutic agents? I mean, let's just say are they or are they not? Again, going twenty years of Food and Drug Administration and seven years subsequently, I honestly wish that when they had written the Food, Drug and Cosmetic Act they had done food, drug and something in the middle, called the frog maybe, but we are caught now in a situation that is a Catch-22. If it is a food and has any therapeutic use, it is a drug. We do have dietary supplements that allow us some latitude in what we can say about them but then, again, we are constantly seeming to have to hammer round pegs into square holes and it is becoming more difficult as things get more complex.

Develop GMPs. QA and QC -- certainly, I think industry should have a voice at the table in how things have been done; what has been successful; what hasn't been successful but, clearly, this is something FDA needs to think about.

Standardize internationally -- I think we have a lot to learn from the European Union countries and Japan in terms of how they have developed their comfort level with

these substances and what the considerations were that they took into account. Part of the efficacy also, which I don't have on here, may well be determining how many are needed in order to have a beneficial effect, should there be one.

With that, I will end my talk.

DR. BENEDICT: Thank you, Dr. Archer. That is extremely informative. Before we go any further, I would like to recognize that we have been joined by Dr. Fukagawa, and I would like you to just do what we always do, wave and then introduce yourself and your expertise, just for the record and for everyone else.

DR. FUKAGAWA: I am from the University of Vermont, and I guess I can describe myself as a pediatric gerontologist.

[Laughter]

Questions and Answers

DR. BENEDICT: So, now we have a little time for questions. Before we do this, clearly, what we need to do is to begin to examine everything we have heard and the things we have already thought about in preparing for the meeting. We are going to have a lot of time tomorrow for a good, healthy discussion and we will have all of the speakers who can possibly be here available for additional questioning, and for discussion and for providing their expertise. So, in the next few minutes, I think it is a

good idea that we begin to formulate questions that even perhaps the subsequent speakers could address for us. And, one final thing that Dr. Brandt always says, if possible, when you are not in session let's not resolve issues among ourselves because what happens is then the discussion is blunted for the record and for the audience and for each other. So, perhaps if many of us find ourselves at lunch or at dinner, we can certainly discuss things, of course, but let's try not to discuss this because fresh ideas and fresh questions are the ones that stimulate other folks who aren't at dinner. So, I would like to ask you to bring all your questions to the table now during the day today or tomorrow for a more vigorous discussion. So with that, we can open the floor to questions for Dr. Archer on almost anything you have to think of.

DR. ARCHER: Just a comment, I will be here today but I will not be here tomorrow because I will be going up to the University of Vermont, actually, tomorrow.

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DR. BENEDICT: In which case, we had better pepper him now. Dr. Russell?

DR. RUSSELL: Yes, I have a couple of points that I wanted to get clear in my own mind. You mentioned that a desirable property of probiotic bacteria was colonization. Are we talking really about colonization or prolonged residence time in the GI tract, for example? I mean, are we

really expecting these organisms to colonize or just to have a longer effect in the GI tract but do they need to be fed on a regular basis, for example?

DR. ARCHER: That is an excellent question and by most measures, say, a single dose of a probiotic bacterium -- just simply following it in stool samples over a period of time, eventually it is going to disappear. And, I think from some of the clinical trials that probably Dr. Clemens will be commenting on, it does require more or less a constant dosing in order to achieve what you could call a stable population but I **am** not sure you could really define it as such. I don't think we understand the dynamics of these organisms or, for that matter, lots of organisms in the gastrointestinal tract well enough to say they are a stable population; they are **always** going to be there. My personal view is they are not. I think if you can increase the residence time they have a greater chance of doing something good.

DR. BENEDICT: Continue, please.

DR. RUSSELL: Thank you. Perhaps related to the first question a little bit is the concept of microbial balance that came up in a couple of definitions. Actually, **as** a gastroenterologist, I find that to be kind of a vague concept and I wondered is there a clearer definition of what people are thinking when they are talking about microbial

balance? I mean, I am not sure what is meant by that, I guess, exactly.

DR. ARCHER: I would be hesitant to answer that question, particularly to a gastroenterologist. I think the concept as people have proposed it is, let's say, lessen the number of clostridia, lessen the number of Eubacterium versus increasing the number of Bifidobacterium. I think that is what they are talking about but, again, I think you are absolutely right, it is a vague concept and possibly that is something that needs to be looked at a little more closely in terms of the definition. Probably some of the other speakers may address that.

DR. RUSSELL: I have one last question. You mentioned that there are very, very few infections reported with probiotics. I was wondering if any infections have ever been reported in AIDS patients.

DR. ARCHER: I don't think so, or at least I am not aware of any in AIDS patients. From the literature I have seen, those that have occurred, the septicemias and particularly endocarditis, most have been traced back to some kind of a dental source, whether it is a severe dental infection of some kind of dental trauma. I am not aware -- this doesn't mean there haven't been but I am not aware of any in AIDS patients.

I think when the discussion comes on clinical

trials a little bit later on, Dr. Clemens will be talking about a population that was severely at risk and yet suffered no adverse effects as a result. I might as well say this now,. there is one publication in the literature, rather recent, a letter that did reflect a possible dietary source of a human infection. I think that is the first one that, using genetic fingerprinting, has suggested that something that is in the food supply actually did wind up in a human infection. A little bit vague on how that happened, how the opportunist which I think this clearly was, an opportunist gained the upper hand is sort of a mystery.

DR. RUSSELL: Thank you.

DR. BENEDICT: Dr. Fukagawa?

DR. FUKAGAWA: Is it expected that any of these probiotics might influence then the oral flora in the oral cavity? Since one of the big issues in medicine and dentistry now is the effect of peridental disease and the flora there on the evolution or pathogenesis of common medical disorders, such as atherosclerosis and things, would you think that an imbalance of the intake may influence?

DR. ARCHER: That is an excellent question, it is also one that I don't think has really been addressed in the literature with regard to probiotics, what it would actually do to the oral flora. One of the criteria or the desirable properties that I listed was that they not be cariogenic.

Whether they would be good bacteria that overpopulate and crowd out cariogenic bacteria, I think that is probably an open issue. I am not aware of a whole lot that has been done with that.

If you go back to the list of organisms that I put up under the bifidobacteria species, there is one that was omitted, and that was Bifidobacterium dentium, I believe, which is a known cariogenic bacterium.

DR. BENEDICT: Yes, Dr. Hotchkiss?

DR. HOTCHKISS: A question, Doug, and I think you would agree, that the standard to which probiotics, in terms of efficacy, must be dependent, at least in part or large part, on what category of product they are put in -- a drug, a supplement, a food or whatever, and I presume that claims, label claims and other kinds of claims will certainly in some way be related to efficacy, as I looked through some of the literature on probiotics I have come away a little mixed on what the status of methods of determining efficacy are. What is your opinion about where we stand in terms of being able to measure or quantify efficacy in a rather complex system of biological-biological interaction as opposed to a drug-biological interaction? Where do we stand?

DR. ARCHER: I think that the best way to answer that is if you view the totality of the literature one of the things that is very striking is that it has only been

recently that people have sat down and really designed good, solid clinical trials to prove a point. They have decided on their endpoint. They want to see if there is an effect, a lessening, a mitigation in, for example, rotoviral diarrhea in children. One endpoint to measure is a good clinical trial and get the result. When you look at the totality of the literature, I think it is intriguing to look at the number of things that these organisms can do in vitro that are slightly different than the run of the mill that they can do. I mean, they really do some interesting things. From there, there has been an awful lot of speculation as to what they might do in humans but very few clinical trials have set out to actually prove those points at this point in time. I think we are sort of at a threshold and, frankly, I think if I were a company right now and I were thinking of marketing something in the line of a probiotic, I would probably be taking a little bit of a low profile to wait and see what the regulatory dust does before I invested millions of dollars in a clinical trial.

So, I think it is again almost a Catch-22. People are a little hesitant to go ahead and invest mega-bucks if, all of a sudden, they are going to face a set of regulatory criteria that are impossible to meet or if they are going to be thrown over into the drug category. I think that is a basic thing that possibly FDA sort of needs to reconcile

internally before you are going to see a lot of progress.

DR. BENEDICT: Dr. Buchanan?

DR. BUCHANAN: Doug, just a quick question, and it revolves around definitions. I like the list of characteristics that you put up there, understanding that that was derived primarily from the historic research in probiotics with lactic acid bacteria, but if I was going through that list the organism that would jump to mind to me would be Escherichia coli. It has all of those characteristics or at least certain strains. How far do these characteristics go beyond just lactic acid bacteria?

DR. ARCHER: Well actually, Bob, that is a good example. Escherichia coli has been proposed as a probiotic, not very recently but it has been proposed in terms of establishing "an intestinal balance." So, that is not outlandish and what you are saying is exactly true. I think the difference would be when you get into the context of do they do something beneficial and, again, the breadth of the definition becomes important here. I mean, they can have those criteria and be E. coli 0157. That is not exactly what we are after, is it? What we are after is a good thing, a good endpoint. So, I think again that somehow freezing a beneficial health effect -- it has to be a little tighter than that, but somehow that concept has to be woven into the definition.

DR. BENEDICT: Dr. Montville?

DR. MONTVILLE: I would just like to bring up the consideration of not only the definition of what they do but what they are because, having worked with lactics, it is a somewhat muddled group. You know, I worked with Lactobacillus bavaricus and three years later people said, no, that is really a saki. The species names are really based on descriptive biochemical characteristics that are sometimes hard to call. So, I just think we have to grapple with that and when someone makes a claim, at least that they better define the organism they are making the claim for.

DR. BENEDICT: Thank you. That actually leads into a question I have been pondering for a while and that is, I don't know anything about the lactics but I know that a number of gram-positives undergo significant gene transfer through transformation, and I would like over the course of a couple of days for someone to just answer for me just what is the probability of this in these organisms, and since it is transformation in raw DNA is there ever a case of pathogenicity islands being absorbed? Perhaps that doesn't happen but I think somewhere along the way we have to address gene transfer and perhaps shifting of what appears to be species. Dr. Russell, did you have a question?

DR. RUSSELL: Yes, it had to do with food use as the sort of second part of the talk. I understand that some

of these organisms as customarily used in this country are in yogurt or perhaps milk, beverages. These organisms, I assume, can be freeze-dried and be taken as a pill and are marketed that way as well? Is that correct?

DR. ARCHER: Yes, there are products on the market that are freeze-dried products.

DR. RUSSELL: And they are stable and active? No quality control?

DR. ARCHER: Well, you have asked a group of questions there. Are they stable and active? Well, they can be resuscitated. That is one step. Are they active in the GI tract? People think so.

DR. RUSSELL: I see. I was wondering whether there are any food products that have been developed using freeze-dried organisms in a dry bar, for example, or a dry product.

DR. ARCHER: Well, Nestle test marketed a product called LC-1 and that is available. It is a European product but it is a freeze-dried product that can be added to beverages, cereal or whatever.

DR. RUSSELL: I guess I was thinking of a prepackaged sort of thing like a candy bar.

DR. ARCHER: 1 am not aware of it but Mary Ellen Sanders is going to be talking in a lot more depth about current foods.

accessible from humans. You can't just bleed them and get that kind of gamma-delta cell. So, I am just asking is there a way to do this, and if there isn't should we not spend some time thinking about it? The answer to your question is yes, we should but I am really not aware of any other models that have been applied and, again, maybe one of the other speakers might have a much better idea than I do.

DR. BENEDICT: I suspect we will get further into it. Dr. Buchanan, you have a comment?

DR. BUCHANAN: Just a question, Doug. Your presentation focused largely on bacterial probiotics. Based on a lot of the work that has been done on the fermentation type of environment has been focused on protozoa as a factor that determines what is the microbiological balance. Is there any work in probiotics that you ran across in protozoan or higher species?

DR. ARCHER: Not protozoa but yeasts.

DR. BUCHANAN: Yeasts?

DR. ARCHER: I am not aware of any studies that have been focused on a probiotic effect that would involve a protozoan.

DR. BENEDICT: Additional questions from the table? Seeing none, this is a real good start. Thank you very much for a very informative presentation. We will take our break six minutes early but we will, nonetheless, resume

at ten o'clock. It is now 9:39 and at 10:00 we will start promptly. Thank you.

[Brief recess]

DR. BENEDICT: Dr. Mary Ellen Sanders will address the topic of food in the marketplace.

Foods in the Marketplace

DR. SANDERS: Thank you very much. It is a pleasure to be here to work with this group in an area that clearly is becoming much more important and into the limelight in terms of food as well as dietary supplements.

[Slide]

I am going to go ahead and jump right into my presentation. Everyone here has seen the list of questions that this group has proposed that they hope to answer, and the focus of my presentation is going to be on foods and later presentations will focus on dietary supplements.

[Slide]

Specifically, I am going to be looking at the probiotic organisms, products, labeling, safety and effectiveness considerations, standards for levels and, finally, a comment or two about the future. Of course, we don't have enough time to develop any of these topics in depth but I am hoping that with the question and answer period and our discussion tomorrow this will at least serve as a backbone for those types of discussions.

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Some of the information on probiotic microbes was already discussed in Dr. Archer's talk, but I have maybe a little different information so I will go ahead and go over it quickly. Microbial probiotic species, as he mentioned, really do cover a range of bacteria. He mentioned a variety of Lactobacillus species, including the acidophilus group, casei, reuteri, plantarum and rhamnosus; the bifidobacteria, including a variety of species there.

I would also like to include the yogurt bacteria, Streptococcus thermophilous and Lactobacillus bulgaricus that are used primarily as starter cultures in the preparation of yogurt, but have also been shown in several publications to have impact on the immune system as well as on improving lactose digestion.

Another organism that has been studied fairly extensively is a yeast known as Saccharomyces boulardii. This organism has been studied relative to antibiotic-associated diarrhea, and the approach there has not been inclusion of this organism in food but use as a biotherapeutic agent.

Enterococci have also been used and are currently being used in many probiotic products. In this country it is not uncommon to find Enterococcus on the label of dietary supplement probiotics, and we will bring up some questions

when I discuss safety.

Finally, E. coli, as was mentioned earlier, has been discussed and actually currently is being used as a probiotic organism in supplement type products in Europe. So, this is quite a diverse group of organisms and certainly extends beyond what our traditional lactic acid bacteria groupings would include.

[Slide]

I am going to just really quickly go through this and the next slide, just to point out that there is a variety of companies worldwide that have a vested interest in the probiotic area, and many of these companies have been involved in defining and studying very specific strains of probiotic bacteria, and many of these are the subject of many different studies that are published, and the research goes on with additional companies and additional strains that have been defined and characterized at least to some extent.

[Slide]

Now if we look at probiotic organisms and their habitats, I want to point out that even though many of these organisms are associated historically with fermented dairy foods, not all of them. Certainly, bifidobacteria have as their primary habitat the GI tract of man and animals.. They have primarily an intestinal source.

There are some organisms that may, in fact, both be associated with the intestine and also associated with fermented dairy foods, and that group might include these organisms. Yogurt starter cultures, in fact, do not survive intestinal transit and are not, for example, bioresistant as was listed on one of the slides as an important characteristic of probiotics but, as I mentioned, in fact, may have probiotic properties.

So, it is important to realize that we are not just talking about microorganisms that are associated with traditional fermented foods.

[Slide]

Let's run through some of the products. As was mentioned earlier also, the probiotic market in Europe as well as in Asia is much more developed than in the United States. This is a slide that just shows one grouping of probiotic beverages that are sold in Europe. Many of these products we do not see in this country, with the exception of the Actimel product. We now see that being marketed in Colorado by Dannon. Probiotic beverages in Japan include a variety of drinkable yogurts, tetra-pack type products that include probiotic bacteria. This particular slide is showing products that are produced by the company Yakult.

[Slide]

I am going to discuss a little bit the Yakult

beverage. Again, this is not a product that is available currently in the United States but it is a product that is noteworthy for a variety of reasons. It was developed back in 1935 by a Japanese scientists, and it is a flavored milk-based product containing a Lactobacillus casei strain Shirota which carries the scientist's name. Dr. Shirota is the one who did the research on this strain. This product is marketed in 65 billion cfu/serving which is comprised of 10^9/mL which, when we look at the levels that are currently present in dairy products in the U.S., this is a much higher level than we would normally see in this country.

The company claims that about 10 percent of the Japanese population consumes this product daily. That, to me, seems to be a very huge penetration. That is a large number of people that are consuming this product. They estimate about 24 million, and I have seen numbers as high as 28 million sold daily worldwide in a variety of countries. This particular product does also have what is called FOSHU status, which is essentially a Japanese definition of functional foods.

[Slide]

Now the way this product is labeled, its FOSHU status was obtained in May of '98. The claims that they are allowed to make based on the review of the Japanese Ministry of Health are shown here. The Yakult strain can reach the

intestine alive. It helps increase beneficial bacteria in the intestine. It suppresses growing harmful bacteria in the intestine. It improves the environment in the intestine and it maintains the intestine in good health. All of those are allowable statements on this product but, to my knowledge, they don't use any of those on the labeling of the product.

[Slide]

Just a quick comment on how this product is distributed, as was mentioned, it is available in vending machines. It is available in supermarkets. And I know as it is marketed in Europe, the Yakult product in Europe is marketed as a seven-pack, with the obvious implication that you get enough to take one a day for an entire week. It is also marketed or distributed using door-to-door approaches with what they call their Yakult ladies. These women actually go door-to-door with Yakult and serve as a point to point reference with the consumer to provide information about these products and how they may help with general health. So, they have a very diverse approach to getting this product to the market.

[Slide]

Now, the Actimel product might be discussed a little bit more in the dietary supplement discussion, but I wanted to point out that in Europe it is my understanding

that this product is marketed as a food, not as a dietary supplement, although in the U.S. it is labeled as a dietary supplement. It contains Lactobacillus casei at 10^{10}/serving and also contains yogurt cultures. Again, this is a very high level of microbes in this product.

[Slide]

Infant formula is another food product that does contain probiotic bacteria. This is a product that contains S. thermophilous and a Bifidobacterium strain. This formulation is based on some research that was conducted in 1994 on the reduction of rotovirus shedding as well as infant diarrhea. This particular product is available in Europe and Asia but not in the U.S. From what I understand, other brands of formula with probiotics are also available in Europe.

[Slide]

This product is a food product offered in Japan. It perhaps isn't technically a probiotic product because it is a pasteurized product. But I wanted to show this as an example because this particular product is marketed toward an antihypertensive claim or clinical effect, and what was found to be the active ingredient in this product, which is called Ameal-S, produced by the Calpis Corp., is a series of tripeptides that are fermentation end products from the proteolytic activity on casein. It is a FOSHU product also,

and there is evidence from spontaneously hypertensive rat studies and one human study a reduction in both diastolic and systolic blood pressure of hypertensive people. This product, as I mentioned, has FOSHU status. The claim that they are able to make according to the Japanese Ministry of Health is that this food contains lacto-tripeptides VPP and IPP, and is suitable for people with mild hypertension. So, that is an interesting product and we don't have anything similar in the U.S.

[Slide]

This product line is produced in Finland by a company called Valio Dairy. It contains Lactobacillus rhamnosus GG. This particular strain is a very highly researched strain. This company has put a lot of effort into developing clinical evaluations of this strain. It is marketed in a whole array of different types of products including yogurts, unfermented milks, fermented drinkable yogurts, as well as juices.

[Slide]

This Gefilus product, which is similar in a way to the Yakult type product where it is designed in single servings, is designed to be consumed once a day. The other point I would like to make is that the advertising campaign for this product has this ring around the products and that is what they call their ring of protection, which is the

implication that this product will provide some sort of ring of protection for the consumer against the bad things that life has to offer.

[Slide]

There is a Swedish product that contains a Lactobacillus rhamnosus strain that indicates that it is effective for the treatment of diarrhea, constipation and other GI tract product. It is buttermilk as well as yogurt.

Just recently I received information that the Valio Dairy, again with the Lactobacillus GG strain, is now producing a cheese product. This is produced in Finland. It has somewhere around 10^8 Lactobacillus GG/gram of cheese. They indicate that four to six slices of this cheese provides an adequate daily dose. So, this is the first example that I am aware of, of a ripened cheese that is on the market that contains probiotic bacteria.

[Slide]

So, really there is a much broader diversity of products that are available in Europe. What I would like to do now is quickly run over what we find in the United States. Perhaps one of the first probiotic targeted products in this country was when the concept of sweet acidophilus milk came out, back in the mid '70s. This particular product is unfermented milk where Lactobacillus acidophilus and some products now also contain a bifidus

culture, bifidus bacterium, are added to fluid milk. Generally speaking, the target is to deliver about 2 X 10^6 or about two million bacteria per mL. So, you are in a position to get about 4 X 10^8 if you drink 200 mL of milk or what would be close to a serving of milk.

[Slide]

Kefir products are also available. This is just one example. However, these products, although they are oftentimes listed under probiotic beverages, in fact, the defined content of these is minimal. In other words, they contain undefined mixed cultures of lactobacilli. So, we really don't have any sense with any of these kefir type products of how these organisms specifically might interact with people in a true probiotic manner.

[Slide]

Here is a product that we found on the shelves at the Wild Oats Natural Grocery, which is in Colorado. It is in Nevada. I don't know whether it is an East Coast chain or not, but this is a natural grocery store, general groceries but also quite a huge display of dietary supplements, vitamins and those types of things as well. They produce a non-dairy drink, soy-based. This is the vanilla flavor, which contains soy milk and it provides calcium and vitamin D, but also Lactobacillus acidophilus and they say "L-bifidus," and we know, of course, that these

organisms are not Lactobacillus bifidus any longer but that labeling hasn't quite caught up with the taxonomy of twenty years ago.

[Slide]

Of course, as everyone knows here, the yogurt products in this country are perhaps the most dominant in terms' of their positioning in carrying probiotic bacteria. Unfortunately, many of those products, and they are produced by most of the major yogurt manufacturers in this country, do very little to promote or label their probiotic content, and they don't label it based on strains; sometimes they don't label it even on species; but they also don't label it based on count. So, the consumer doesn't really have any idea of what levels of bacteria are being produced in those products.

[Slide]

Stonyfield Farms a couple of years ago came out with a commitment to include six different species of probiotic bacteria in all of their lines, including their regular yogurt. This is a new product of theirs, Yosqueeze, which also contains these bacteria. Again, we have no idea what levels are being offered in these products but they are labeled as containing these six bacteria.

[Slide]

I point this particular product out, Dairy DeLite.

I am not sure what kind of distribution it has in the U.S., but I point it out because the name of this yogurt is probiotic yogurt, and it is produced by Noga Dairy. They do indicate on the label that it contains helpful bacteria, vitamins and minerals. If you look at their promotional literature, they are much more aggressive in what they say about this product. They say it promotes healthy digestive tract; minimizes the effects of poor diet, stress and aging on your inner body's bacteria; strengthens the body's natural defense against harmful bacteria; and then they also state probiotic bacteria and probiotic dairy foods replenish the body's intestinal tract, maintaining the positive ratio of good to harmful bacteria. And, I would challenge anyone here to give me a definition of what a positive ratio of good to harmful bacteria is in the intestinal tract because, as far as I can tell, there is not a good definition of what the good or the "friendly" bacteria. Other than the clear pathogenic organisms, I don't think we have a good sense of what those ratios really are but, regardless of that, this maintains it.

[Slide]

Cottage cheese -- some cottage cheese products also contain probiotic products. The Horizon line does contain both Lactobacillus acidophilus and Bifidobacterium.

[Slide]

I want to take just a moment and talk a little bit about some of the health statements that are used in the United States for probiotic foods. I haven't really identified the product because I didn't want to get into a finger-pointing exercise here, but I just want to provide some understanding of where manufacturers in the U.S. are in terms of making statements about probiotics in their foods.

"The cultures may help keep your digestive system healthy and balanced, and may even help you digest foods you cannot now eat comfortably," which is probably an indirect relationship to helping alleviate lactose-intolerance symptoms.

"Yogurt is made from the finest ingredients and cultures with L. acidophilus and B. bifidus o assist in lactose digestion and to maintain a healthy and balanced intestinal system." Again, what does that mean?

"Helps you efficiently digest the foods you eat, and the milk you drink."

"Bifidus has been with you since birth, and it's important to maintain the amount your body requires."

"This culture helps to keep your system operating the way nature intended." So, those are some statements that are on products. So, that was a very quick overview of what the food products are in the U.S. and in Europe.

[Slide]

Now I would like to move to discussing some issues relative to efficacy and/or standards. Of course, this topic in itself could be the subject of an entire workshop so we are only going to do a very cursory approach to this, but I would like to just point out a few things.

[Slide]

First of all, if we look at what the probiotic foods primarily are targeting, it is GI tract health and enhanced immune function. Those seem to be the areas that are of most interest or at least most used by people marketing these products. GI health though, of course is a huge area and it might comprise improved lactose digestion, a balanced or healthy GI tract flora. Again we are not sure what that is but it may, in fact, be one of the target statements. Decreased incidence or duration of diarrhea1 diseases and, in fact, this particular bullet point reflects the area where there are probably more appropriately controlled clinical studies that demonstrate this effect than any of the other areas, probably with the exception of. lactose intolerance. Certain types of inflammatory diseases, inflammatory bowel disease, Crohn's disease, ulcerative colitis, pouchitis -- those areas are all currently being studied relative to the impact that probiotic bacteria may have on recurrence or progression of those diseases, and it may even include colon cancer. There

is a variety of indirect studies that suggest that there may be an influence of probiotic bacteria on colon cancer.

So this area of GI tract health, in fact, can be quite a broad area. Enhanced immune function at this point is focused on improved levels of immune markers. I am not aware of any human study that actually shows a causative effect of probiotic bacteria on immune function that has then led to a decreased level, for example, of infections or some type of disease. So, primarily the studies are on immune markers, many times on healthy subjects.

There is also an increased research area on the effect of probiotic bacteria on decreasing allergy symptoms, and I think this is a very, very fascinating area. I am sure if we watch over the next couple of years, there will be some interesting work that comes out on this. Other health statements that may come out would be cholesterol lowering, antihypertensive effect, vaginal/urinary tract health and stomach health. Those are all ones that could possibly be targeted for probiotic foods.

[Slide]

Now, the concept of significant scientific agreement, I know, is a very specific understanding relative to the FDA, but I just want to comment that there are many positive placebo-controlled studies for probiotics but, as was mentioned earlier today, many of these are not -- well,

it is hard to get quantitative but they can suffer from small study size; small numbers of subjects. Short study duration -- that is one of the criticisms that I have heard of both the immune studies as well as the cholesterol studies, that you follow people for eight weeks, twelve weeks and how meaningful is that really? Sometimes I think there is questionable statistical analysis of the data.

So, I think what we really are forced to do is to look not specifically at one study and put a lot of weight on it but look at the group, the body of literature in this area.

Another question I have is how meaningful many of these published clinical studies are to generally healthy consumers. You know, if we have a study that shows a clinical effect on decreasing inflammatory flare-ups with IBD patients or rotovirus diarrhea in infants -- you know, those types of studies which, in fact, are very nicely conducted studies, how do we then ultimately translate that type of clinical data into a recommendation for probiotics for generally healthy consumers? And, I don't know the answer to that.

Then, I think to elevate the body of research in this area -- unfortunately, many of the studies that were conducted maybe prior to 1980 don't indicate what strain they are using. Sometimes they don't even indicate what

Levels are being fed. They might, for example, just feed commercial sweet acidophilus milk and you don't even know what levels of organisms are there or what organisms are even there. So, those studies suffer from those types of difficulties and it is important I think, as we move forward, that those issues are clearly defined.

What type of evidence is important? This, of course, has everything to do with the discussion on efficacy with probiotics. Are we going to require to make some sort of a health statement? Controlled human studies? Are animal studies adequate? Are in vitro studies adequate? Do we need to know what the mechanism is? And, I am not clear on where the FDA stands on that type of issue.

Finally, an area that I have real difficulty with in this whole area of nutrition research is what the meaning of the biomarkers that are oftentimes used in this research is.

[Slide]

I have a cartoon to show my frustration. This is a kid. "I dropped a dime here. Help me look for it," he says to his friend. His friend goes over there and he says, "Why are you looking over there?" And, he said, "it's cooler here in the shade."

You know, that sort of struck me because I think to some extent that is what we do with nutrition research

aith biomarkers. It is much easier to do biomarker research than it is to do controlled human studies. It is cheaper; it is easier. Many times you get more clear-cut results but ultimately are we looking where we need to look? In my opinion, until the biomarkers are validated to, in fact, have some meaningful physiological relevance to humans the focus on the biomarkers is really questionable.

I think a good example of that is the area of adherence. That came up earlier. There are reams of studies that have been published on the ability in vitro of probiotic strains to adhere to cells in tissue culture, but the bottom line is that those results seems to have nothing to do with how these organisms adhere in vivo. In fact, the Lion's share of studies show that when these organisms are consumed, within two weeks you can no longer recover them from the stools and, regardless of their high level of adhering ability in vitro, they do not seem to show adherence in vivo. So, these tests have not been validated to be meaningful.

[Slide]

So, this whole area of biomarkers I think is an important one to discuss. Biomarkers must be validated by correlation of the biomarker with physiological and clinical effects. Many used are not. Specifically, the culture adherence assays, cholesterol assimilation in test tubes,

the immune markers. People make a big deal out of the fact that these organisms produce bacteriocins but no one has taken a bacteriocin-producing strain and compared it clinically to a bacteriocin non-producing strain, preferably the isogene derivative of it, to see if there is any difference i probiotic effects, and it would be an easy experiment to do.

[Slide]

If we talk a minute about levels of probiotics, what is our current status? And, I alluded to this through some of the product slides but most dairy products containing probiotics, in the U.S., deliver about 10^8 cfu/serving and for the most part -- in fact, I don't know of any that are labeled with probiotic count in the U.S.

The exception to that is a dietary supplement/food, depending what country you are in, but the Actimel product does deliver 10^{10}/serving. That is available in Colorado. So, to me, I think that Dannon has come to the plate and has made the commitment to deliver what they consider to be efficacious levels of the probiotic organism, and they are putting the reputation of their company on the line by labeling that product with the levels of organisms that are contained within it.

Many capsule or pill products claim to deliver about 10^9 or 10^{10} per dose but, of course, many studies that

have been published that have done surveys of these type of products show, in fact, that what they contain is very different from what they are labeled as containing.

Clinical studies target approximately 10^8 on the low side, 10^{11} on the high side per day. There have been dose studies which have suggested that 10^9 or greater, 10^9 is probably a minimum for many of the clinical studies but, of course, you can't make generalizations because one particular strain may, in fact, be more capable of surviving the acid in the stomach; more capable of surviving the bile in the intestine; a variety of physiological differences may, in fact, relate to what levels of that organism actually reach the target site. So, it is very difficult to generalize about in general probiotics should be delivered X level. It is really going to be very strain specific and few dose studies, unfortunately, are published.

[Slide]

Now, if we look at standards for levels, what considerations do we need to use? Standards establishing minimal levels are going to require specific evaluation of the dose studies for each strain.

Required levels may be dependent on the levels resulting in the clinical effect; what your target consumers are -- are they the same group as in research? Are you targeting infants who may need a different daily dose than

an adult or a geriatric population?

Here is what I consider to be a very important point, what is the active principle or mechanism of the effect? That is one of the reasons why I showed that Calpis product slide. That particular "probiotic" effect is not mediated through viable cells; it is mediated through a fermentation endpoint. There is some evidence that suggests that immune-enhancing properties are mediated through cell wall components -- again, not viable cells.

The ability to alleviate lactose mal-digestion, in fact, very likely is due to the delivery of lactase to the small intestine and, again, may not require viable cells.

So, I think in order to really have a rational discussion of standards we need to know what the mechanism of effect is. In fact, standards for one product may not be the same as standards for another.

Also, physiological traits of the strain may vary or may change this. If you have a strain that is able to survive in the product in the GI tract, possibly colonize, maybe even truly adhere, you might be able to deliver much lower doses of that strain than you would for another. Finally, there may be synergy for certain clinical effects between multiple strains that are used. So, I think it is going to be very difficult to make huge generalizations about this area.

[Slide]

Now safety -- this is a very big area which I think needs to be carefully considered. I think it is safe to say that generally the lactobacilli and bifidobacteria are considered to have a very low pathogenic potential, and that opinion has been published by many people of diverse laboratories across the world. So, I don't think that is a hugely controversial comment. We know there are normal comensuls. We know they are present in high number in foods, and we have a huge history of safe use with many lactobacilli and even bifidobacteria.

However, having said that, we know that dozens of documented infections resulting from lactobacilli and bifidobacteria have been reported in the literature. These people almost always have an underlying illness, however. Generally it is thought that the source of the microbe causing the infection is indigenous to the patient and not from foods that the patient is consuming.

I think it is interesting that one report out of Europe indicated that the lactobacilli isolated from clinical infections are most commonly the species of rhamnosus, casei, paracasei and plantarum. So, maybe the "potential" for infection by these different lactobacilli may be somewhat species dependent.

I will also comment that this report in Europe

indicated that L. rhamnosus, although they considered it essentially equivalent to other lactobacilli for the most part, they said its more frequent association with infection deserves -- this organism, therefore, warrants further surveillance. So, they sort of set this particular species aside and said, yes, it is safe and certainly we know it is present in a variety of products that are sold in Europe and also dietary supplements in the U.S. now, but they think it is worth watching.

[Slide]

I do want to point out two publications. Again, you have to keep in context that this is two publications out of a huge history of safe use of these organisms, but these publications are useful in that they are the only two that I am aware of that have made a suggestion that the infection that they are reporting is due to a food source or a supplement source of the microbe.

Rautio et al., in '99 indicated that L. rhamnosus caused a liver abscess. A 74-year old hypertensive diabetic woman consumed about half a liter of a Lactobacillus rhamnosus GG-containing drink each day. The strain that was isolated from her live abscess was indistinguishable both in phenotypic characteristics as well as pulse-field gel electrophoresis chromosomal patterns from the strain that was in the product.

Now, the people publishing this also made the point, and I think it is a valid one, that in fact the same genotype can be isolated from people and, in this particular case they made the point that it was isolated from an infant that had never been exposed to this product. So, they ask a reasonable question, which is was this strain associated with the infection derived from the GG drink or derived from an indigenous Lactobacillus rhamnosus and, of course, we don't know the answer to that.

This other case is an endocarditis, again caused by a rhamnosus strain, a 67-year old male with a history of mitral valve prolapse and tooth removal -- again, that was pointed out earlier. This particular gentleman chewed dried mixed strain probiotic capsules. He would open up the capsule, throw the powder in his mouth and chew it. It contained a mixture of organisms including rhamnosus, enterococcus faecalis and acidophilus. Again, the strain isolated from the infection was indistinguishable and, in this case, they only used only phenotypic characteristics, not genotypic ones, but it was indistinguishable from the strain in the probiotic supplement.

Those both are noteworthy comments about infections but, of course, they have to be evaluated within the context of the total field.

[Slide]

Let's talk a moment about enterococci. Let me skip to the third bullet point, enterococci are currently sold in many dietary supplement products in the U.S. and worldwide. One of the reasons -- and, I being very cynical by saying this, but one of the reasons why I think these organisms are so regularly included in dietary supplement dried, room temperature products is that they are stable. They are shelf stable. They are much easier to keep viable than lactobacilli or bifidobacteria are. Therefore, if you want to put on your product that it contains 10^9, if 10^9 of that is enterococcus and you have 10^4 lactobacilli, who is going to stop you from saying that it contains enterococcus and lactobacilli? There is just no stopping that.

But, being a non-cynic, I will say that some clinical studies certainly have been done, primarily in the area of alleviation of diarrhea1 symptoms as well as cholesterol lowering, on enterococci. So, there are health benefits associated with that. These organisms are, of course, normal comensuls to humans. Enterococcus faecalis and faecium are readily isolated from the stools or the intestinal tract. They are present in foods as contaminants. They certainly can be food spoilage organisms, or as intentional additives in starter cultures, primarily more in the Mediterranean countries.

[Slide]

Now, recent review indicated that in the past enterococci were viewed as harmless comensuls with low pathogenic potential, but today, in the context of increasing multiple levels of antibiotic resistance, and increasing association of enterococci with nosocomial infections -- they are the number one cause of nosocomial infections today; they didn't used to be -- they may be considered opportunistic pathogens. I know they are a primary pathogen also for urinary tract infections.

So, essentially, even though there might be species specific safety concerns -- faecalis is considered a higher risk or is more commonly associated with infection than faecium is -- I think there are some serious questions that should be asked about the incorporation of enterococci into these types of products, but we have to conclude that the link between foods and Enterococcus infection is currently now known. There is no published study that shows that enterococci consumed in a product were, in fact, isolated from an infection.

[Slide]

So, if we look at the context for safety issues, on this side I sort of have the pro lactic acid bacteria, pro probiotics, and the lactobacilli, bifidobacteria and enterococci are normal comensuls found on the body and also in food and the environment. A tremendous volume of

probiotic bacteria are consumed safely worldwide. There have been many human studies that have been conducted with no adverse incidence reported, including a study that I am specifically mentioning because I don't know who could have gotten approval for this study, but in Finland they administered high levels -- I don't remember the exact number -- of Lactobacillus rhamnosus GG to neonatal premature infants, and they had no adverse incidence reports in there. So, I mean, to me that is -- wow!

Also, I would like to point out that a study in Finland found no similarity -- and this was using only phenotypic analysis -- between clinical isolates and food strains. So, they went back over the blood cultures that had been isolated. They looked at thousands of these, looked at carbohydrate fermentation patterns and other phenotypic characteristics and said that there is really no similarity between the clinical isolates of lactobacilli and the ones that are currently used in food. Of course, that is not a conclusive response, or that doesn't conclusively indicate that that can't occur but it is supportive of that.

We also have to realize that there are recent reports of Lactobacillus rhamnosus infection that is possibly linked to product consumption. The recent increase of enterococci associated with nosocomial infections I think cannot be ignored and needs to be evaluated relative to the

use of these particular organisms. Finally, modern approaches to strain and species use differ from historical use. The point I want to make here is that in the past, when we talk of history of safe use of these organisms in foods, primary what you are looking at is organisms that are natural to the dairy environment or to green plant material. They were natural contaminant to the food products and, in fact, they were not organisms that were specifically isolated from human sources.

I am sorry, am I out of time?

DR. BENEDICT: You are pretty close.

DR. SANDERS: Okay, I am sorry. I didn't bring my watch with me.

We also have the technology today to concentrate these organisms to very dense populations where historically we relied on the ability of these organisms to grow in the product. Of course, I think these provide a slightly different spin on what we are doing today. We are using different strains and even different genera than were used traditionally in products.

[Slide]

This is close to my last slide. What kind of anticipated uses for probiotics are coming? I think that the use in animal agriculture for these organisms will really increase. There is going to be a real advantage I

think for substituting for antibiotics as growth promoters with probiotics, as well as decreasing the pathogen carrier state in food animals.

I think we are going to see more medical use. My particular focus has been on foods, but I think that areas being researched, such as oral vaccines, intravaginal installations, prevention of GI tract disease are going to be true medical biotherapeutic applications for probiotics, which are really going to fall into the drug category and not foods. Optimized strains and blends will be developed, I believe. We are going to see applications to foods beyond dairy in the U.S. Finally, I think we are going to increase our understanding, hopefully, of clinical effects and mechanisms.

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Finally, I would just like to end with this because I think that this is a powerful message that is being conveyed by the Nestle people. If you sign on to www.diallc1.com this is what you pull up on the web page, and they are giving a very direct message to the consumer -- "eat more bacteria." I think that companies are going to try to get this message out more and that is why I think the activity of this group is so important because we have to come to some sort of an agreement on how to approach these products in this country. Thank you.

DR. BENEDICT: Thank you. Before you step down, I was remiss in the beginning to ask you to just tell us your affiliation.

DR. SANDERS: Oh, I am sorry. I primarily spend my time consulting. My company is Dairy and Food Culture Technologies and I am based out of the Denver metro area. I do have an affiliation with CalPoly State University at St. Louis Obispo as a research.professor, where I collaborate with other professors in the dairy products technology center on probiotic-focused research, primarily in the in vitro analysis of these strains.

DR. BENEDICT: Thank you very much. We will hold our questions. I hope you will be available --

DR. SANDERS: Yes, I will be here today and tomorrow.

DR. BENEDICT: So, now we will hear from Dr. Roger Clemens. Please, also state your affiliation for the record. In addition to that, we are a few minutes over. I would like for us just to ignore that. The presentations are so interesting and useful that I think if we stretch a little bit into the time we are allowed for lunch, it is probably not going to hurt. So please, Dr. Clemens, don't feel exceptionally rushed. We can hold a lot of our questions for tomorrow if we need to. Will you be here tomorrow as well?

DR. CLEMENS: Yes.

DR. BENEDICT: Good. I am getting grumblings from the nutritionists about ignoring lunch. If you went around this table of over-achievers you would probably find 95 percent of the people ignore lunch, and it is probably pretty embarrassing. So, there is the five percent.

Dietary Supplements in the Marketplace

DR. CLEMENS: Good morning.

[Slide]

I am Roger Clemens. My affiliation -- I have had 21 years experience in the food industry, specifically as the scientific adviser to Nestle. I was intimately involved with probiotic research. I am now independent, free-lancing, and a professor of food science and nutrition at CalPoly Pomona in the southern California area.

Today we are going to talk about straight science. For the next few minutes we are going to talk about dietary supplements, and Dr. Sanders just did a superb job in reviewing what we have in the foods, and many of the areas which we know a great deal about is thanks to the research conducted by Mary Ellen.

[Slide]

I have a couple of presentation objectives, along with a little humor -- I trust that is allowed. We need to identify the current supplements and what we anticipate.

Mary Ellen did a great job of anticipating what the future might look at, but let's take a look at what we currently have on the U.S. market. I am going to talk about what is there, also some levels of organisms that are there, which organisms are there, and what is coming up in the near future.

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Also, we will talk about manufacturing Q/A principles. I appreciate comments by Doug and by Mary Ellen. We need to look at these areas and I proposed some comments to the panel this morning regarding what the current practice is and where practices might want to go.

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Also, we are going to look at labeling criteria, what should be the standard for label declaration.

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What is the business for probiotics in the United States? Specifically, let's start with the nutrition business. This graphic comes from the Nutrition Business Journal, published earlier this year. In this particular case, it breaks out the entire nutrition business in the United States as found in 1999. You see the distribution there, and obviously this category of functional foods, which is yet to be defined, is grabbing great parts of nutrition business. But, of interest here for today's

presentation is dietary supplements. In nutrition business, this represents 35 percent of what people send on their dollar nutrition.

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Well, what does that mean in total dollars? That is 44.9 billion dollars in nutrition business. That is billion with a "b"; 44.5 billion dollars is spent on nutrition. We go, apply this then to probiotic supplements and we see from the very traditional approach that 70 million dollars are spent every year for probiotic dietary supplements. This does not include the Internet. We have a final report published by the FDA in October of this last year, a survey of dietary supplement usage and an excellent assessment of where the various supplements were purchased, including the Internet, and they clearly indicated that the vast majority is swinging to purchase of supplements through the Internet.

Specifically, thanks to the work by the FDA, we see that ten to twenty percent, depending on which market you are looking at, represents probiotics within this market, the total dietary supplement market. We also see through Mary Ellen's excellent comment that the interest appears to lie primarily in these two areas, both immune function or immune system, as well as the digestive system. Somebody out there, the general consumer, is looking for

something very positive, some benefit by consuming probiotics relative to immune enhancement or immune modulation as well as digestive assistance.

A very fine article that Mary Ellen authored earlier this year talks about the probiotic potential. If you are looking for something that is very concise and direct, I urge you to grab a copy of Dairy Foods, January, 2000. Many of the graphics that I will show you actually come from Mary Ellen's work.

But it is interesting, you see here, in the upper right-hand corner, that the kefirs and various black current drinks with biocultures in the American food system are increasing, indeed.

[Slide]

Mary Ellen did a good job also of identifying key manufacturers. I went on the web site to access all these manufacturers and it is really interesting what they say or shouldn't be saying, and what they cite, and I will talk to you briefly about what they can or can't deliver. The other ones were mostly domestic. Here we have domestic applications. It is interesting that many of the international companies are selling their products in the United States as foods and sometimes as supplements. This list may be found also in Mary Ellen's presentation today, and also in the publication that she had back in January.

[Slide]

Notice that many of the companies have been involved with pharmaceutical agents and have a history of pharmaceutical applications and development, and now we see more food companies getting involved with produced probiotic-containing products.

This is the company. Here is the web site. This is the information I pulled off the **web** in September, just a week or so ago. This particular company is promoting its unique strain. This one is Lactobacillus reuteri and they are promoting the product that will help biological systems. In this particular case they are addressing biological systems in terms of immune function.

[Slide]

They have a really unique way of educating the consumer. Everyone has taken a different approach to educate the consumer. In this particular one for Lactobacillus reuteri they have a video on how they believe Lactobacillus reuteri will interact with the GI tract and provide some benefit. Here, they are also promoting that it produces substances -- to go back to the definitions that Doug presented earlier this morning. In this case, they are identifying the bacteriocin reuterin, and that is a key factor in the production of the effectiveness of Lactobacillus reuteri. Also they talk about the reducing

stomach disorders, and also enhancing that, in fact, it adheres or modifies or modulates the product of gut mucins, and we can talk about that later when we discuss mechanisms.

[Slide]

Chris Hansen is well established in Milwaukee of this country and also in Denmark. They now have their own coined phrase of probiotech. It is real interesting in fact that while they are promoting this, if you go back on the web, some of the clinical studies used their organism and, in fact, probiotic was not in vogue at that time.

This goes back to the work though that Dr. Saavedra did at Johns Hopkins. Now they have embraced those organisms, in this particular case the BB12, in this family of organisms called probiotech, and the list of organisms is shown on the right-hand side. In this case, you see that Bifidobacterium is now called lactis. And, I appreciate Mary Ellen's comment that we really need to address strain characterization, and I appreciate Tom's comment that it is more than genus species; it is more than just chemical characteristics; we really need to understand the genetics of these particular organisms. As you see, the changes in taxonomy have been quite demonstrable, particularly in the last ten years.

[Slide]

Culturelle, produced by ConAgra -- Lactobacillus

product. It is a very delicious product, by the way.

[Slide]

Here is Natren. Natren is a small company, based in the Los Angeles area. They have taken a rather unique approach to show probiotics. They believe that their products are among the highest quality. I haven't defined what that is yet. Quality, potency, service -- operation down in West Lake Village.

[Slide]

It is interesting, in the survey that the FDA conducted, most of those companies were based in California. It is also interesting that they have created their own triangle here, the inverted pyramid. At the top of the pyramid they show probiotics as really essential for everything that is done.

[Slide]

Then, Nestle in the United States, as May Ellen indicated, has this on their web site -- "eat more bacteria" and a number of publications to support their position.

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They have actually taken a very unique approach. Instead of being a capsule of any kind or a tablet form, it is actually in a powder as a supplement, and it is monitored this way and it provides 2 X 10^{10} organisms per dose.

[Slide]

Another company that actually is in the business of producing probiotic organisms is Nutraceutix. It is based in Redmon, Washington in the northern part of the States. They say they are the premiere research center for human resource and human and animal health. In fact, I have had the opportunity to visit that fine institute and evaluate the research. I have had the opportunity to visit a fair number of production centers. I can comment on the various production techniques, or the lack thereof; the adherence to pharmaceutical GMPs and GLPs as well.

[Slide]

Well, what are those GLP and GMP standards? Those of you who have been in the pharmaceutical industry for quite some time certainly understand this. If we are going to set standards, we need to understand what is the primary culture and how is it managed? What are the characteristics that show that that culture doesn't drift?

We need to understand the fermentation media. What is essential to make the bugs grow, under what conditions? And, what is the load to get that culture going? What are the ingredients to use for that culture media? One of the characteristics is that media contains non-fat milk. For some of those people who are on non-fat milk are, in fact milk protein allergic and the addition of the milk protein to the supplements could cause them to

Light up and none of wants to see that happen. But that is not declared on the label.

What type of techniques are used to isolate their organism or concentrate their organism? There are a variety of techniques that are out there. The primary techniques chat are used are ultra-filtration or centrifugation. I have seen that used in many of the production facilities.

Interestingly enough, some of the facilities don't take this approach at all. In fact, what they do, they take the entire milieu of the culture and they dry that matrix. So, whatever the culture media happen to include, those compounds are included, which are not declared in the labeling. Interestingly enough, many of the companies take the approach that Doug has mentioned. In fact, these organisms often will produce enzymes, or produce short-chain fatty acids. They will often produce bacteriocins or other compounds. So, those compounds, they believe, are included when they freeze-dry the entire matrix and therefore their product may be better. Hence, yes, they do freeze-dry. But, to Mary Ellen's comment, they have varying viabilities, and they have varying instabilities, and they have various properties, and a lot of them don't survive. They may be pelletized and you grind them up into powders, and so forth. So, there are a lot of technical issues that have to be addressed in this area as well that go beyond just freeze-

drying.

Then, the question might come up, well, how do you preserve the viability if that is essential for the action of this particular organism? Some of the manufacturers use various preservatives which are GRAS. They use MSG, BHT and some other approaches, even natural vanilla to preserve the viability of the organisms. Also, they say they contain proprietary cryoprotectants. What are these? Some may use a beta-keratin. Some might use a vitamin C or other unknown substances to protect the organisms so that, in fact, they don't go away, and viability is maintained not only through distribution but also through the use by the consumer.

[Slide]

We typically look here at the general morphology and physiology of organisms but I think we have to really go beyond that. As indicated by Mary Ellen and by other speakers today, and certainly by Doug, we have to understand not only about carbohydrate fermentation profiles, but we need to understand what is in the cell walls. What components allow them to be active? Therefore, do they provide the functional characteristics of that particular strain? Therefore, it is essential that we understand the DNA composition; that we actually have a DNA fingerprint, if you will -- whatever approach you might take, to analyze and substantiate that that particular product, not only that

particular organism remains stable, that the DNA characteristics do not change through the use of that particular organism.

I visit a number of companies, and many of them are reputable companies and actually have extensive databases where they have followed their product for a considerable amount of time and demonstrated, in fact, that in their particular strains they maintain consistency here. But that isn't the case in all companies, of course.

To a comment that Mary Ellen made, it is important that we know the potency. How much do we give those people? The limiting factor is not how many bugs you can put in there. My experience has been that the limiting factor really is cost. You get upwards of 10^{11} 10^{12} and 10^{13} and that particular supplement becomes cost prohibitive to put on the market. You price yourself right out of the market. And, we can talk about cost a little bit later.

Contaminants is an issue, and I appreciate Mary Ellen's comment about E. faecium and E. faecalis. Actually, she is absolutely right. We often will see those organisms in plentiful supply in various supplements because they are stable. When you don't have to declare how much of an organism or really all the details of an organism in a particular dietary supplement, you can load them up with either one of these organisms and, yes, you will grow

something out but are you growing out what you want to be doing? Of course, that all relates to the stability of that particular product.

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Packaging distribution must be considered not only for a dietary supplement, but it must be considered as well for the food supply. Moisture levels are really critical. If you get moisture levels above five or so percent in these supplements, the viability of the organism goes away. And, it is more than just assessing moisture levels in a finished product, usually a powder of some form, but also the water activity. If you get water activity above about 0.25, again the viability goes away and you do not have a viable product -- no pun intended.

Also, a concern might be moisture barriers -- we have some wonderful polymers in packaging today. They are wonderful moisture barriers but, in fact, they may not be sufficient to maintain the viability of these organisms. Secondly, we do not have any data, to my knowledge -- and help me out -- that looks into the migration of some of the monomers that are part of these barriers so that, in fact, those monomers may migrate and, therefore, affect the viability of the organism. Hence, we might look at the use of glass.

Capsules -- are these two-piece capsules if they

are in a capsule form, or are they in a single-piece capsule? We don't know which is the best. Some of them actually take a probiotic and put it in an oil base in a one-piece unit and, based on a vacuum assessment, they don't Leak and can maintain viability, usually some type of a soy bean oil.

And, at what temperature is the distribution and at what temperature are they sold? Often you will see that the products will go to a normal health food store -- where are they located? Typically, you will find them on the shelf with all the other supplements, and if you assess those particular products, the viability of those products is often quite low. We are going to see in many of these stores an increased refrigeration section where probiotics may be maintained.

Testing laboratories -- which laboratory should be doing an evaluation of these organisms? Which laboratory is already set up to do that? In fact, the various companies that produce these organisms and dietary supplements, do they have the adequate facilities to assess? There are a number of qualified laboratories in the country, but not all the companies are assessing and accessing these particular laboratories. Obviously, we have to have confirmed and established procedures, and the traditional barn approach is not necessarily viable at this time.

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Let's look at the health food store with that as a background, and this will not be a surprise to any of you. Mary Ellen did a really fine job of looking at some of these issues, frankly. Are they going to declare the dose? Some do; some don't. What is the culture viability? They really don't talk too much about that. We see the banner on yogurts -- "live and active cultures," but you don't see that in too many dietary supplements. What is the stability of that culture and do they give an expiration data of that particular dietary supplement, like we have on a medication? And, how is the culture identified? Sometimes they just say probiotics or they give a genus species which might be nice, but the reality is we need to be looking at strains. They often include the DNA disclaimer and, unfortunately, many of them use references and many of the references really are much too old to support where they are going with their particular product.

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This is a piece that Mary Ellen demonstrated in her publication, and I have some work to do to substantiate a little bit further, and here we see that in this particular case Mary Ellen and her team examined the stability of four different strains over a period of six weeks. You see here four degrees, typical refrigeration in

a yogurt matrix, and we see that different strains do, in fact, have different stabilities and this is not unexpected.

I did initial studies and I went back and evaluated a lot of the strains that are on the U.S. market, and it is clear that they will make a declaration of dose; it is often not there. They will make a declaration of a strain; it is often not there. As a matter of fact, many times the bug that they declare is not even there.

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Interestingly enough, back in July of 1989, the National Nutritional Food Association made a proposal and actually adopted some standards for labeling. The question is do all the manufacturers adhere to this? The answer is no. I believe that this only represents about four percent of the dietary supplement market. So, it has not had an impact on the dietary supplements, particularly those in probiotics.

Here, they called for those producing probiotics that they have a viability cell count, and that is cfu's per particular unit. They have a particular minimum to allow natural die-off. Also, they recommended they have an expiration day like we see on foods and also medications. They called for species identification and more than just genus and species. It is clear that we have to look at strains today. it is clear that we have to examine what the

contaminants are and not actually declare one thing and actually say another. We need to have tools to assess genetic stability.

We need to examine storage requirements. Often you will look at supplements and storage requirements are not indicated on the label. That has to be specified and should be specified on the label. Also, all ingredients that are used, however they manufacture them or grow them out should be declared so those who might have allergies or be sensitive to that can avoid the product.

Then, in 1995, as part of the congressional record, these kinds of standards that were read into the congressional record but, again to my knowledge, these have not been adopted by the particular industry.

That gives you an overview of what is happening in the dietary supplement world. There are a lot of dietary supplements out there. It represents a fair amount of the market, 70 million dollars-plus; probably double that with the Internet. We realize there are a lot of standards that have been proposed but have actually been adhered to by a very small percentage of the dietary supplement market.

As I close, this is interesting. The Europeans are, in fact, ahead of us and I attended a meeting in Toulouse, France and this is what we see --

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Really I have a Superman piece attached to this out I attended a pediatric meeting and showed dietary supplements, and you see that in Europe, that probiotics are ready to fight intestinal disorders. Thank you very much.

DR. BENEDICT: Thank you, Dr. Clemens. Thank you also for putting us right exactly on schedule.

DR. CLEMENS: I tried hard.

DR. BENEDICT: I am sure the panel is relieved that lunch is still in the offing.

DR. CLEMENS: I am not pitching baseball today either!

DR. BENEDICT: Well, I didn't want to ask that question. So, now let's open the floor to questions to both of the speakers. Perhaps Dr. Sanders could come forward and be near the microphone as well.

Questions and Answers

DR. MONTVILLE: Does the EU have standards or have they grappled with any of these questions of potency, identity, validation?

DR. CLEMENS: The answer is no. If you go back to EU and their CODEX standards, it is interesting and perhaps I will address it a little bit later -- Doug brought up in conversation that they have addressed L plus lactic acid bacteria in infant formula, but I am not aware of any standards in terms of a dietary supplement or a product

other than L-plus.

DR. BENEDICT: Dr. Sigman-Grant?

DR. SIGMAN-GRANT: Yes, I have a question about the infant formulas. Are they marketed for the general pediatric population or as a product to use if the child is suffering from a GI disorder?

DR. CLEMENS: The products marketed in Europe are for the general pediatric population. They are marketed to the older child, the kids that are six months of age and older right now.

DR. SIGMAN-GRANT: So, as a supplement or in place of breast milk?

DR. CLEMENS: As a regular infant formula.

DR. BENEDICT: Dr. Russell? Dr. Sanders, you talked quite a bit about Yakult in Japan, and I was wondering if there was anything to learn from the Japanese regulators with regard to the FOSHU claims. For example, Yakult increasing beneficial bacteria or suppressing harmful bacteria. Have they defined that or made any effort to make that much more specific? I am trying to get at some specificity about health GI tract and what that means, or flora and what that means.

DR. SANDERS: Within the context of understanding GI tract flora, as I mentioned, I think it is going to be difficult to be able to state with confidence that certain

levels of the different normal comensul bacteria in the intestinal tract are "healthy" and others may not be. So, you know, I don't know that we know that we want 90 percent Bacteroides and 10 percent lactobacilli and 50 percent -- well, I guess you couldn't do that, but the different levels of bifidobacteria versus lactobacilli. There is not that level in my understanding of a sense of what that means.

But I think in your reference to the Japanese and what we can learn from them, they clearly buy into the fact that lactobacilli and especially bifidobacteria are beneficial, that they are better bacteria than some of the others that we have, including Clostridia and possibly enterococci.

DR. RUSSELL: And that is based on what?

DR. SANDERS: Well, I would suppose some of it is probably based on what they consider to be certain noxious end products of metabolism, for example, that Clostridia may produce or certain potential carcinogenic type of enzymes that are higher levels than some bacteria. They have probably looked at it from that point of view. But I think when they make a claim like that on the Yakult product, when it says improves healthy bacteria or healthy intestinal flora, probably what they are referring to is that if you consume this product your levels of lactobacilli and/or bifidobacteria increase, and my guess -- this is supposition

on my part, my guess is they view that as improved intestinal flora.

Now, whether or not you can argue that point with a group of GI tract microbiologists and say, okay, is that a defensible position scientifically? You would probably have to debate on it.

DR. BENEDICT: Yes, Dr. Russell, continue.

DR. RUSSELL: I have another question, Dr. Sanders. It has to do with the issue of lactose digestion. Quite a bit is made I think in a lot of the advertisements about these products improving lactose digestion. I understand in yogurt products, if they are warmed up to a certain degree and so forth, the bacteria will have lactase which will digest the lactose in that product. But have there been any studies at all where one has been able to convert a lactose mal-digester into a lactose digester by feeding a probiotic? I am not talking about the digestion within the product that was fed --

DR. SANDERS: Right, right.

DR. RUSSELL: -- but can the person then drink milk at the next meal?

DR. SANDERS: There is actually no evidence that -- well, let me back up. The amount of digestion that takes place in the product in terms of the conversion of the lactose in milk during the yogurt making process into lactic

acid is minimal. I mean, you might go from 5 percent lactose down to 4.5 percent lactose, but oftentimes current formulation processes supplement with non-fat dry milk solids anyway and, therefore, your lactose is boosted back up. so, the actual lactose content of yogurt in the U.S. is probably not significantly different than the lactose content in milk even considering the fact that you did have this fermentation occur.

So, you can't say any effects that are seen -- and oftentimes the studies are done with controlled lactose levels anyway, but the effects seen with the mediation of probiotic bacteria or, I should say, the lactic bacteria is not from reducing the lactose in the product. It is really coming from some sort of in situ activity once the yogurt of milk is consumed.

So, to answer your question, it is all a balance game. You can put in too much lactose and overload the system. Most of the studies are done, for example, in consuming and 8 oz glass of milk or an 8 oz cup of yogurt. So, it is a moderate consumption level. With that type of consumption level, the systems and/or the biomarker of decreased hydrogen excretion -- breath to breath hydrogen -- are reduced. So, the systems and breath hydrogens are both dropped. Does that answer your question?

DR. RUSSELL: Well, I am wondering if you can