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Probiotics: Time for a Dose of Realism

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Abstract

There is already a long research and retail history of probiotics, but progress in the scientific and medical validation of these products has been extremely slow. Even now, adequate information by which the consumer and health professional can judge the efficacy and safety of retailed probiotics is lacking. Probiotic products have not been subjected to large scale trials of efficacy that are used in the pharmaceutical industry. Without these trials and subsequent approval by fastidious regulatory agencies such as the FDA (USA), probiotics continue to languish in the self-care health market. Optimistically, a new generation of probiotics may be developed that have medical validity with respect to the prevention or treatment of atopic and inflammatory bowel diseases. These new products, however, will need to be targeted at the alleviation of specific medical conditions, and the mechanistic basis of their effectiveness will need to be provided.

Probiotics: a Primer

The origin of the "probiotic" concept rests with Elie Metchnikoff, director of the Pasteur Institute, Paris, in the early years of the 20th century. Awarded the Nobel Prize for the first scientific investigation of phagocytosis, Metchnikoff was a highly regarded scientist. Through his research concerning resistance to disease, he developed an interest in the ageing process and eventually published articles and books that recorded his views on the role of the large bowel as a source of toxic substances (for example amines, ammonia) that damaged the nervous and vascular systems when absorbed from the gut and circulated in the blood. Microbes inhabiting the large bowel produced these toxic substances, and were thus responsible for "autointoxication". The microbial products, it was explained, originated from the digestion of proteins by "putrefactive" bacteria. Metchnikoff proposed that humans would benefit by encouraging the correct balance of microbial types in the large bowel, especially from the reduction in putrefactive activity (Metchnikoff 1907, 1908). Milk fermented by lactic acid-producing bacteria had been noted to inhibit the multiplication of proteolytic (putrefactive) bacteria because of the low pH produced by the fermentation of lactose. Metchnikoff had also recorded, amongst his wide-ranging observations of factors affecting longevity, that Bulgarians consumed large amounts of

fermented milk as part of their diet and that they were known to reach advanced ages by the standards of the day. In a time of relatively unsophisticated knowledge of the large bowel ecosystem, it was a small leap of faith to assume that the consumption of fermented milk would "implant" lactic acid-producing bacteria in the human gut, and that these bacteria would proliferate and suppress the growth of putrefactive bacteria, just as had been observed with milk in the laboratory.

Milk fermented with the "Bulgarian bacillus" of Metchnikoff, the real identity of which bacteria will never be known because the culture was lost, subsequently enjoyed some vogue in Western Europe, and the healthy image of yoghurt has persisted and been actively promoted by the dairy and food industries. It is difficult to judge how much of the probiotic concept Metchnikoff believed, but certainly he was associated with a commercial product, something that his scientific peers strongly disapproved of. It is interesting to read the advertisement for "Le Ferment", that was produced in 1906 because of the similarity to the modern descriptions of probiotics:

"For a year, the *Le Ferment* company has organised a laboratory in which, using a lactic fermentation called "*Lactobacilline*", it prepares a curdled milk according to the method outlined by Professor Metchnikoff.

The fermentation is prepared using pure cultures of lactic bacteria, without any other kinds of microbes, such as those found in sour milk products such as kephir etc. We think that our clients will find our curdled milk has an agreeable taste, and irreplaceable from the point of view of hygiene.

***Lactobacilline* is also available as tablets for the use of people who don't like, or can't tolerate, curdled milk. This preparation offers the same therapeutic advantages as the curdled milk."**

Interest in the health benefits of consuming products containing lactic acid bacteria appears to have been lost with the advent of the First World War, but re-emerged in the United States of America during the 1930s. It was realised that the bacterial types commonly used to ferment milk did not survive transit of the digestive tract in numbers detectable by bacteriological culture so a faecal isolate (*Lactobacillus acidophilus*), presumably an intestinal inhabitant, was mixed into milk: the resulting "Acidophilus milk" remains a commercial product in the United States of America. Acidophilus milk, according to Rettger and colleagues (1935), "brought at least temporary relief to a large majority of these subjects" suffering from constipation, chronic diarrhoea, colitis, sprue, and eczema: a diverse range of maladies, still reflected in the literature pertaining to more recent probiotics.

Dietary supplements for farm animals became a big business from the 1960s, and lactic acid-producing bacteria were included as health-promoting agents. Claims of improved weight gain, increased egg production, and decreased incidence of infections are amongst several that have been made in association with these products (Parker,

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1974). Fuller (1999) has reviewed the use of these products in animal husbandry and has concluded that “the claims for probiotics are many and varied but it is not always possible to provide good scientific evidence to support them” which is, unfortunately, a recurring refrain with respect to probiotics in general. The only probiotic product to have been approved by the Food and Drug Administration (FDA) of the United States of America is, nevertheless, one for use with poultry. PREEMPT is a mixture of 29 bacterial strains that is maintained in continuous culture, then freeze dried for administration to day-old chickens. Sprayed onto the birds, the bacterial inoculum is introduced into the gut when they preen their feathers. The mixture of bacteria colonises the gut of the birds and subsequently prevents the establishment of *Salmonella* species, commonly present as a contaminant of poultry feed (Anon, 1998). *Salmonella* colonisation of poultry can lead to contamination of egg yolks or meat with the cells of this pathogen, resulting in a serious public health problem.

At about the same time that probiotics became popular in the animal feeds industry, the dairy industry began to promote new yoghurts containing *Lactobacillus acidophilus* in addition to the usual starter cultures (*Streptococcus thermophilus* and *Lactobacillus salivarius* subsp. *bulgaricus*). In subsequent decades, other species of *Lactobacillus* have been included in some products (for example, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus johnsonii*) because they are considered to be intestinal bacteria with special properties. Increasingly, members of the genus *Bifidobacterium* (especially *B. lactis*) have been added to probiotic products, though the rationale is not historically clear. Perhaps interest in bifidobacteria is derived mainly from the observation of Tissier (1905) that these bacteria, which produce acetic and lactic acids as fermentation products, were commonly present in the faeces of infants. Indeed, they appeared to dominate the bacterial community present in the faeces of infants suckled at the breast. A more varied microflora was evident in the faeces of cow's milk formula-fed infants. Since paediatricians recognised, and still recognise, that infants receiving mothers milk have less intestinal upsets than formula-fed children, the inference that bifidobacteria were associated with health was made.

Probiotic products are not confined to the dairy industry. Capsules and tablets containing lactic acid bacteria are widely available in pharmacies, health food stores and via the Internet. Probiotics do not only contain lactic acid bacteria: products containing a yeast (*Saccharomyces boulardii* [*Saccharomyces cerevisiae*], *Escherichia coli* (the Nissle 1917 strain) are available.

The Gut Microflora: a Primer

The gastrointestinal tract of healthy mammals is inhabited by a large number of microbial cells that represent many species, particularly of bacteria. The microbial populations present in the gut form a community referred to as the “microflora”. The microflora of humans is confined in health to the terminal region of the small bowel (the ileum) and to the large bowel (colon). In the large bowel in particular,

total bacterial numbers are about 10^{11} cells per gram of digesta. The large bowel of humans, on average, contains about 200 grams of digesta; therefore each human carries 2×10^{13} bacterial cells within their colon even in health. The distribution of the gut microflora differs between animal species. While all species have an extensive bacterial community inhabiting the large bowel, ruminants also have an equivalent microflora in the proximal region of the digestive system known as the rumen-reticulum. Rats, mice and pigs also have numerous bacteria in anterior regions of the gut because the gastric region of these animals is partly lined by a non-secretory epithelium to which certain bacteria (lactobacilli) can attach. The proliferation of attached bacteria results in a layer of *Lactobacillus* cells on the epithelial surface and which serves to inoculate the digesta. Hence in these animals, relatively large numbers of lactobacilli are present throughout the small bowel as well as in the large bowel. In humans, however, the microflora is confined to the ileum and colon (Barrow *et al.*, 1980; Fuller and Brooker, 1974; Tannock, 1995; Tannock, 1997).

Much of our knowledge of the human gut microflora has been derived from the microbiological study of faeces (Savage, 1977). The microflora of faeces provides accurate information about the bacterial community of the distal colon, but may not be informative of the situation in the proximal colon. Bacterial metabolism is greater, and the pH of the digesta lower, in the proximal relative to the distal colon. This is because of the presence of substances newly delivered from the small bowel into the proximal colon that can be used as substrates of bacterial metabolism (Cummings and Macfarlane, 1991). There has been one recent report that the composition of the proximal colon differs from that of the faeces in that facultative anaerobes and lactobacilli are more numerous in the former than in the latter (Marteau *et al.*, 2002). However this report requires substantiation because earlier investigators did not report such differences, and because, in the recent study, an indwelling tube was used in the sampling procedure (Drasar and Hill, 1974). The presence of this tube may have altered the conditions within the proximal colon such that the composition of the microflora was altered.

In faeces, the predominant microbial types are bacterial species that are obligately anaerobic. About 40% of the total microscopic count of bacterial cells observed in faecal smears can be cultured in the laboratory using good anaerobic culture techniques (Tannock *et al.*, 2000). The remaining 60% of the gut inhabitants can be detected and enumerated by nucleic acid-based methodologies that target 16S ribosomal RNA gene sequences (a cornerstone of bacterial classification) in which is “written” the signatures of bacterial groups (Woese, 1987). Even so, a complete catalogue of the gut microflora of humans has not yet been achieved, but hundreds of bacterial species are likely to be residents. Indeed it is impossible to define the human gut microflora in other than general terms because it is now clear that each human harbours a microflora of unique composition (Zoetendal *et al.*, 1998). It is possible to generate a “fingerprint” of the faecal microflora of humans and on this basis one can easily differentiate between each

human (Stebbing *et al.*, 2002). It is likely, however, that since the major metabolic products of the gut bacteria (short chain fatty acids) differ little in ratio from one human to another, that the overall metabolism of the gut ecosystem is similar in all humans. If the taxonomy of the microflora differs from human to human yet the metabolic products are the same, there must be considerable redundancy associated with the composition of the human gut microflora.

Largely for historical reasons, as noted previously, lactobacilli and bifidobacteria are predominant in the use of microbes as probiotics (Goldin and Gorbach, 1992). An important observation relating to these bacteria is that neither group is a major component of the faecal microflora of adult humans. Lactobacilli are present at most at 10^8 per gram of faeces (0.1% of the total microflora), but usually at about 10^6 per gram (0.001%) when detectable (Tannock *et al.*, 2000). According to American observations, 25% of humans do not have lactobacilli at detectable levels in their faeces (single sample) (Finegold *et al.*, 1983). Even so, recent studies indicate that most of the lactobacilli detected in human faeces are transient in the gut because they are detected intermittently in faecal samples from subjects in temporal studies (Reuter, 2001; Tannock *et al.*, 2000). These lactobacilli belong to species that are commonly associated with foods such as cheese, fermented meats, and vegetables and have probably been introduced into the gut with the food. Some may be members of the oral microflora and originate in the saliva. Only two species have been observed to attain moderate populations that persist as long-term inhabitants of the human gut: *Lactobacillus ruminis* and *Lactobacillus salivarius* (Tannock *et al.*, 2000). Bifidobacteria are more numerous than lactobacilli and are harboured by most adult humans, but on average comprise only a few per cent of the total microflora (Franks *et al.*, 1998; Langendijk *et al.*, 1995; Sghir *et al.*, 1995). As noted earlier, bifidobacteria are the predominant bacteria in the gut of children during the first month of life (Harmsen *et al.*, 2000).

Much attention has been paid to the selection of bacterial cultures for inclusion in probiotic products on the basis of their ability to adhere to cultured eukaryotic cells in the laboratory (Morelli, 2000). The premise that persistence of bacteria in the gut requires that they adhere to mucus or epithelial surfaces is misplaced because the regions of the gut where the microflora exists are relatively static and there is time for multiplication of the bacteria in the digesta to such an extent that the constant loss of material (faeces) does not diminish bacterial numbers. The large bowel is a continuous culture vessel with a constant rate of digesta (the culture medium) flowing in from the small bowel, and an outflow of spent medium (the faeces). Mucosa-associated bacterial populations have only been shown to occur convincingly in the case of rodents and pigs in which cases lactobacilli adhere to the forestomach epithelium (rats, mice) or pars oesophagea (pigs), or where obligately anaerobic bacteria or microaerophilic spiral-shaped bacteria inhabit the mucus layer in the colon of rats and mice (Savage, 1977). The colonisation of the mucus is particularly noticeable between the rugae of the proximal colon where a thick accumulation of mucus occurs

(Tannock, 1987). Bacterial pathogens, of course, are well known for their ability to attach to epithelial cells in the small bowel so that they are not dislodged by the fast-flowing digesta in this region of the gut. Peristaltic flow of the digesta is an important protective mechanism of the small bowel where damage to the epithelium or competition for nutrients by bacteria is clearly undesirable. Reports of a mucus-associated microflora in the gut of healthy humans are controversial: the collection of biopsies uses methods that are less than optimal for bacteriological investigation because the extent of contamination of the tissue samples with intestinal "washings" (luminal contents) has never been measured. Additionally, numbers of bacteria associated with biopsies are very low when collected from healthy subjects, but increase in numbers when biopsies from patients with inflammatory bowel diseases have been examined (Schultsz *et al.*, 1999). Thus it would seem that a healthy mucosa should not be associated with an extensive microflora.

Homeostasis

A striking observation made in recent investigations of the human gut microflora is the stability of the bacterial community in terms of its composition (Zoetendal *et al.*, 1998). The genetic fingerprint of the community remains the same in samples collected during long term studies, even 18 months in duration (Tannock *et al.*, 2000). Homeostasis of microbial communities is a common feature of ecological studies in which a steady state is generated by the organisms themselves. Competition for nutrients and space, the inhibition of one group by the metabolic products of another group, and predation and parasitism all contribute to the regulation of populations in particular proportions, one to the other. Because all of the ecological niches are filled in a regulated microbial community, it is extremely difficult for "alien" microbes, accidentally or intentionally introduced into an ecosystem, to establish (Alexander, 1971). They have no way of earning their living in the ecosystem since all possible niches have been filled. Competitive exclusion of pathogens by the already resident gut microflora has attracted much attention. The non-specific resistance of mice to *Salmonella* infection is notable because about 10^9 *Salmonella* cells must be introduced into the gut to infect a conventional animal. In contrast, less than 10 *Salmonella* cells will infect a germfree mouse when inoculated by the same route (Tannock, 1984). A consortium of gut bacteria is required to achieve this degree of competitive exclusion, and obligately anaerobic bacteria that produce short chain fatty acids, especially butyric acid, are particularly important (Bonhoff *et al.*, 1964a, b; Meynell, 1963). Under the conditions of E_h and pH that exist in the gut, the short chain fatty acids are toxic to *Salmonella* cells. There are doubtless many mechanisms that operate in the gut ecosystem that are important in competitive exclusion. A collection of bacterial species that will suppress the growth of *Escherichia coli* in the gut of mice fed a refined diet, for example, will not have the same effect when a different diet is fed. When a crude diet is fed to the animals, a larger collection of bacteria is required to produce the inhibitory effect (Freter, 1988).

Competitive exclusion is a general ecological phenomenon and pathogens are not the only aliens to enter the gut ecosystem. It applies equally well to the introduction of food-associated bacteria and probiotic bacteria into the gut. These, too, are alien bacteria and they have only a transient existence in the ecosystem. As demonstrated in a study in which *Lactobacillus rhamnosus* DR20 was administered daily in milk for six months to human subjects, the probiotic strain was only detected while the probiotic continued to be consumed. Once consumption of the probiotic product ceased, so too did excretion of the bacteria in the faeces. Moreover, numbers of the probiotic strain were relatively low (10^5 - 10^6 per gram of faeces) and were detected only irregularly in samples collected from about 40% of the subjects who had a pre-existing, stable *Lactobacillus* population resident in their gut. The remainder of the subjects did not have stable *Lactobacillus* populations and the probiotic strain could be detected in all of their faecal samples during the period of probiotic use (Tannock *et al.*, 2000). Therefore the outcome of probiotic consumption for consumers is unpredictable: in some subjects the probiotic will be regularly present in the faeces, in others it will be irregular. The impact of probiotic consumption on humans will thus vary according to the pre-existing composition of the microflora and the degree of competitive exclusion that it generates. The expectation of Metchnikoff that lactic acid-producing bacteria could be implanted in the human gut was, in the light of modern knowledge, not realistic. Nor are the lactic acid-producing bacteria a suitable choice for modification of the large bowel microflora of humans: they are numerically minor members of an extremely complex bacterial community.

As knowledge of the microbial ecology of the human gut has increased, definitions of a probiotic have altered. First coined in an entirely different context by Lilley and Stillwell (1965) to describe substances secreted by one type of micro-organism that stimulated the growth of another (probiotic to contrast with antibiotic), "probiotic" was subsequently used to describe "organisms and substances which contribute to intestinal microbial balance" (Parker, 1974). Roy Fuller's definition (1989), "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance", has been widely used. "Living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition" has been suggested (Guarner and Schaafsma, 1998) as well as "Probiotics contain microbial cells which transit the gastrointestinal tract and which, in doing so, benefit the health of the consumer" (Tannock *et al.*, 2000). So too has "defined, live microorganisms administered in adequate amounts which confer a beneficial physiological effect on the host" (International Scientific Association for Probiotics and Prebiotics). The definitions of a "probiotic" have clearly become broader with the passage of time, to the extent where practically anything, dead or alive, can be included: "Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host" (Salminen *et al.*, 1999).

A Question of Safety

Feeding relatively large numbers of alien bacteria to humans raises concerns because, although the body is daily exposed to a myriad of microbial types including those of the resident microflora, the intentional introduction of cultures of bacterial cells into the gut cannot be considered normal. Fortunately, lactobacilli and bifidobacteria are relatively avirulent bacteria. Lactobacilli can cause bacteraemia, rarely with localisation on heart valves or liver abscess, usually associated with underlying conditions such as cancer, *diabetes mellitus*, or recent surgery (Husni *et al.*, 1997; Rautio *et al.*, 1999; Woo *et al.*, 2002). Often, the bacteraemia is polymicrobial: the lactobacilli being but one of a mixture of bacteria circulating in the blood of extremely ill patients. The recent trend to administer lactobacilli as probiotics to neonates and patients suffering from inflammatory bowel diseases requires careful monitoring, however, considering the bacteraemia due to *Lactobacillus rhamnosus* recently reported in a woman with ulcerative colitis (Farina *et al.*, 2001), and the mortality associated with colonisation of neonatal, immunodeficient mice with *Lactobacillus rhamnosus* and *Lactobacillus reuteri* strains (Wagner *et al.*, 1997). As pointed out by Mary Ellen Sanders (Montreal international symposium on probiotics and health, 2002), the expression "Lactobacilli are GRAS (Generally Regarded as Safe)", often heard in relation to probiotic constituents, is an incorrect phrase. Some microbes are GRAS for 'general food use', hence "lactobacilli are GRAS for use as fermentation agents in foods" is a permissible statement. Regardless of GRAS status, the safety standards remain the same for all microbes: there must be reasonable certainty of no harm occurring under the intended conditions of use.

The Quality of Human Probiotic Studies

A relatively large number of papers relating to probiotics have appeared in the scientific literature during the past twenty years (Naidu *et al.*, 1999). Much of this research has been conducted with in vitro "models" or using experimental animals. There has been little attempt to validate the results of in vitro observations by proceeding to studies with humans, and the bacterial preparations that have been used were not the commercial products that the consumer would purchase and consume (Naidu *et al.*, 1999; Morelli, 2000). An almost arbitrary dosage of 10^9 probiotic bacteria per day appears to have been concluded as optimal, presumably based on the appearance of the probiotic organism in the faeces of the majority of human subjects when this daily dose is consumed. However, until recently, medical or scientific end-points by which to judge the efficacy of probiotic action have been neglected. As pointed out by Gregor Reid (1999), the probiotic concept is not new and it would be difficult and costly for any company to obtain patent exclusivity. Probiotics in the United States of America are marketed as dietary supplements and are not regulated as over-the-counter products or foods. They are governed by the Dietary Supplement Health and Education Act (DSHEA, 21 USCA § 301). The producers of probiotics are not forthcoming in their health claims on product labels, and the products have

not passed rigorous tests of efficacy required in the development of pharmaceutical drugs. The evaluation of a new pharmaceutical drug generally requires four phases of human study. Phase one concerns the demonstration of the short-term safety of the product; phase two is aimed at demonstrating that the drug produces a specific, desired effect in relation to dosage (there should be a dose-dependent effect), and the study must be of sufficient statistical power (calculated number of subjects to show a significant clinical outcome). Phase three requires that the new drug is at least as efficacious as the current treatment (the “gold standard”) for the particular medical condition, a placebo group is included, and the subjects are randomly placed into treatment groups and the subjects and clinicians are blinded as to the nature of the substance administered to the groups. The code identifying the groups and their respective treatments is not broken until the study has been completed. In phase four, the drug is marketed and large numbers of patients are monitored. In all of these phases, the documentation for each subject is thorough and must follow procedures described by the governmental agency. These files are scrutinised carefully before approval to market the drug is given. The benefits of this process are that the producer can make medically valid claims, the health professional can be confident that the product is efficacious, and the consumer can have confidence in the safety of the product and that health benefits will accrue. Reid (1999) has reviewed human studies with probiotics conducted since the 1960s, has pointed out the variability in outcomes between different trials, and concluded that “there is a relatively large volume of literature which supports the use of probiotics to prevent and treat intestinal and urogenital infections and other ailments. However, the basis for the claims is often weakened by the lack of proven reliability of the preparations, and an inability to prove conclusively that the contents are safe and efficacious for probiotic therapy to truly be accepted in general medical practice, it must undergo rigorous clinical trials”.

Mary Ellen Sanders (1993; 2000) has reviewed publications about probiotics in relation to the following health targets: lactose digestion, diarrhoea, serum cholesterol concentration, cancer prevention, stimulation of the immune system, constipation, and vaginitis. She concluded that “responsible promotion of the health-promoting bacterial inoculants requires more evidence that some strains of lactic cultures under some conditions have been shown to have some effect. The conditions necessary for causing the effect, the level of the effect, and the importance of the effect to the overall health of the human must be known. For most applications, this information is not available”.

Naidu, Bidlack and Clemens (1999) carried out an extensive review of the probiotic literature and noted that 143 trials involving humans had been carried out between 1961 and 1998. They concluded that “most probiotic LAB (lactic acid bacteria) are used in dairy (*sic*) with a ‘Metchnikoffian philosophy’ and a telemarketing trend – “so what, if the expected and claimed effects fail to appear, at least the consumer is satisfied and has had a tasteful meal. Future research on probiotic LAB should break this dogma and focus on the selection of Target-Specific Probiotics for

prophylactic and therapeutic health benefits”.

Ouwehand, Salminen and Isolauri (2002), on the other hand, are representative of scientists who have a strong commitment to the promotion and use of probiotics. They reviewed studies concerning lactose intolerance, gastroenteritis, inflammatory bowel disease, colorectal cancer, and constipation, and concluded that “properties like relief of lactose intolerance symptoms and shortening of rotavirus diarrhoea are now widely accepted for selected probiotics. Some areas, such as the treatment and prevention of atopy hold great promise. However many proposed health effects still need additional investigation. In particular the potential benefits for the healthy consumer, the main market for probiotic products, requires more attention”.

Even when reliable trials of commercial probiotic products are conducted, it is likely that only trials where a promising outcome has been obtained will be published in the scientific literature. Companies who fund studies of their products would be unlikely to want a null result to be published. Even if companies were not concerned about a lack of efficacy under certain conditions, it would be difficult to have a manuscript accepted by a peer-reviewed journal if there were only negative results to be reported. There is a danger, therefore, that the probiotic literature is somewhat biased.

Overall, one must conclude that probiotic research has been weakened by the empirical nature of the research in which companies search for ways in which probiotic products impact on the biology of humans. Efficacy of products has not been proven following the procedures required for pharmaceutical drugs. Rather than target a disease and formulate a preventative or curative treatment, producers have developed products for the self-care health market estimated to be worth billions of dollars in North America alone (Sloan, 2001). These products are purchased and consumed by humans in general good health, but who subscribe to the “wellness philosophy” in which dietary supplementation with vitamins, anti-oxidants and herbal extracts is seen as desirable, and magazine articles provide “examples of possible functionality” of probiotics as “improved gastrointestinal function, enhanced immune system, lower risk of colon cancer”. The health professional and the consumer has no guarantee that any of this is true and such statements are dismissed by many scientists who laughingly refer to probiotics as “myths”, “foo-foo dust” or “conbiotics” (Berg, 1998).

Probiotics and the Immune System

The human gut is home to a complex bacterial community that exhibits a multiplicity of antigenic substances. The gut microflora is believed to be involved in the “programming” of the immune system in the newborn child, although definitive descriptions of the events that occur, and the mechanisms by which they occur, are lacking (McCracken and Gaskins, 1999). The overall result, however, is one of immune tolerance to the presence of the millions of bacterial cells that inhabit the human intestine (Duchmann *et al.*, 1995). The intestinal mucosa is rich in immunological cells, and continual monitoring of the intestinal ecosystem

is carried out to ensure early detection of invasion of the intestinal wall by bacteria. Although the consumption of probiotic lactobacilli and bifidobacteria does not have a major impact on the composition of the gut microflora, their presence is probably detected by the gut-associated immune system because they are “new” and “foreign”. There are reports in the literature concerning observed alterations in immune parameters when subjects are fed probiotic bacteria, but while the results between test and control groups are statistically significant (though only at a low level of significance), the biological significance of the results is unknown (Gill *et al.*, 2001). This is because the studies did not have a medical end-point but monitored only the immune system of healthy individuals. These effects on the immune system, in any case, may be transient because continued consumption of the probiotic bacteria would likely lead to tolerance, just as occurs in the case of the gut residents. Nevertheless, the initial exposure of the gut-associated immune system to probiotic bacteria could have a useful adjuvant effect in the case of infections of the small bowel. An adjuvant is a substance that enhances an immune response to an antigen. The feeding of probiotic bacteria to children with rotavirus infection may explain the slightly shortened symptomatic period and reduced shedding of the virus in the faeces. Saavedra (2000) has summarised studies in which lactic acid-producing bacteria have been administered to children with the aim of preventing or minimising diarrhoeal diseases. He concluded that the effect of probiotic products appears to be most significant against rotavirus infections, and suggests that an immunological mechanism is responsible for the beneficial effects. It is likely that this is due to the adjuvant effect of the lactic acid-producing bacteria.

The influence of probiotic bacteria on the immune system is topical because of the observed increase in atopic diseases (allergies and asthma) in children in affluent countries over recent decades, which cannot be genetic in origin (Murray and Woodcock, 2002). Over the same period, many aspects of lifestyle have changed in these countries and theories have been put forward to explain the altered incidence of allergies. The “Hygiene Hypothesis” proposes that the prevalence of allergic diseases is inversely related to infection and other microbial exposure in early childhood, yet a specific “infectious protective factor” has not so far been identified. Attention has turned to the gut microflora and the possibility that colonisation of the gut with specific bacteria may be more important than the impact of sporadic infections. The environmental conditions under which babies are born and nurtured may affect which microbes they are exposed to, and subsequently influence the composition of their gut microflora. Exposure, or the lack thereof, to particular bacterial species that are capable of colonising the gut might affect the development of the immune system of children (Bjorksten *et al.*, 1999; Farooqi and Hopkin, 1998). Studies involving the administration of probiotic products to children in an attempt to minimise or prevent allergic diseases have been reported (Kalliomaki *et al.*, 2001). Murray and Woodcock (2002) have reviewed these studies, most of which have originated in Finland. They

concluded that these are preliminary studies that offer a potentially exciting new treatment for allergic diseases but point out, however, that “these are small studies, in a population of exclusively breast-fed children with mild allergic disease”. The authors raise a number of important questions, including: what is the defect in the intestinal microflora? Will the results of the Finnish studies transfer to other parts of the World? Will probiotics be effective in treating more severe disease? Will they prevent allergic asthma? What is the mechanism of action?. They conclude their review “We badly need large controlled studies to explore these concepts. However we must proceed cautiously, with initial safety studies in infants with established allergic disease, before moving to primary prevention studies in at-risk children”. The large controlled studies will need to evaluate the effect of exposing hundreds of infants to probiotic administration in comparison to exposure to placebo. The results must be subjected to rigorous statistical evaluation and the children will need to be followed for several years. This will entail a large investment of time and money on the part of the investigators and the participants, but it is the only way in which the efficacy of probiotics in the prevention of atopic diseases can be evaluated.

Loss of Tolerance

Crohn's disease and ulcerative colitis are serious inflammatory diseases of the gastrointestinal tract (inflammatory bowel diseases, IBD). Both diseases are mainly diagnosed in humans less than 30 years of age and most cases follow a chronic relapsing course with exacerbations and remissions (Chadwick and Chen, 1999). Results from experiments with germfree rodents that have a dysfunctional immune system (gene-knockout or transgenic animals), in which intestinal inflammation is absent, have indicated that bacteria are indispensable contributors to the chronic intestinal inflammation characteristic of these diseases. Once associated with a gut microflora, these animals develop intestinal inflammation (Rath *et al.*, 1999; Sellon *et al.*, 1998;).

In the case of humans, a variety of observations also point to the involvement of the gut microflora (D'Haens *et al.*, 1998; Hugot *et al.*, 2001; Schultz *et al.*, 1999; Van Heel *et al.*, 2001). It has been tempting to think that a gut microflora of “abnormal” composition might be present in the gut of IBD patients but, so far, this has not been confirmed scientifically (Schultz and Rath, 2002). A small number of controlled human trials involving the administration of probiotic products to IBD patients have, however, been carried out with the rationale that probiotics might alter the composition of the gut microflora and modulate the immune response. Alternatively, the probiotics might affect the regulation of the immune system without altering the composition of the microflora. Schultz and Rath (2002) have reviewed empirical studies with probiotics and human patients and concluded that “oral administration of probiotics was able to prevent or at least significantly attenuate disease. However, treatment of established colitis seemed difficult”. As Shanahan (2001) has stated “Enthusiasm for probiotics in IBD is strong on rationale but

still weak on rigorous evidence for clinical efficacy. There are also worrisome gaps in knowledge of the normal flora. In addition, the notion that a single probiotic will be equally suited to all patients is simplistic, given the apparent heterogeneity of IBD". Again, the need for double blind, placebo-controlled crossover trials that will include large numbers of patients is evident.

Evaluation of Probiotics by the Consumer – by Label

For many consumers in the USA, the only information about a specific probiotic product that they obtain will be from the label on the package or container that they see in the pharmacies that stock "natural health" remedies, stores belonging to large health food and nutrition chains, large food markets, and small health food stores. Overall, the consumer can learn from labels on probiotic containers and packages that these products contain, at least at the time of manufacture, large numbers of living bacteria that, in most cases, belong to two bacterial genera (*Lactobacillus* and *Bifidobacterium*). Consumption of the products, in the manufacturer's opinion, will contribute to a healthy gut but official evaluation of their scientific and medical validity is lacking. Daily dosages are given as broad recommendations (1-2 capsules) and more intensive regimes are sometimes suggested (3 capsules per day). The individual consumer, however, has no means of knowing what exact dosage to take, or what outcome they can observe or measure in order to know if the dosage is appropriate to them. Advice from a health professional is sometimes recommended, but one wonders how the doctor can calculate a desirable dosage any better than the consumer given the lack of information provided.

The labels reveal a degree of laxity in the regulation of the sale of probiotic products in the USA because of the use of invalid bacterial nomenclature on the labels (eg "Lactobacillus sporogenes" is not a recognised bacterial species), outdated bacterial nomenclature (eg "Streptococcus lactis" instead of *Lactococcus lactis*), and the incorrect statement on one label that *Lactobacillus acidophilus* produces antibodies (a feature of animals not bacteria). Further investigation should be made of the use of terms such as "super strain" which is a relative term and which needs to be supported by analysis of experiments in which different probiotic bacterial strains have been compared for efficacy. While some product labels contain the phrase "This statement has not been evaluated by FDA. This product is not intended to diagnose, treat, cure or prevent any disease", others do not. It might be appropriate that all probiotic product labels, except where FDA approval has been obtained, carried this phrase in order to provide a fair commercial environment and to make clear to the consumer that approved trials to show the efficacy of the product haven not been conducted.

Evaluation of Probiotics by the Consumer: by Website

The unregulated state of the Internet, designed for instant and unfettered communication, provides open slather for probiotic advertising and sales. Claims stated on company web sites are extravagant in many cases, and are

unsupported by cited literature referring to the specific retail product. From reading the information contained in these web sites, the consumer would, overall, receive the impression that probiotic products are very important in the maintenance of health. The lack of evaluation of the specific probiotics in human trials would not be apparent to the consumer. The liberal use of the word "helps" in relation to all of the medical conditions mentioned on the web sites, however, might alert the consumer to the very general nature of the discussions of particular diseases and the possible benefits of probiotic administration. An impression remains that the manufacturers' are not too sure whether their product really is helpful. Where references to the scientific literature are provided, the articles cited deal with experiments with lactobacilli and other microbes in vitro or in rodent models. Few human studies are quoted, and there is a paucity of references concerning the application of actual retail products in medical situations. Thus the health benefits associated with the retailed probiotic products are generic. Statements by manufacturers attempting to substantiate the benefits of their probiotic product based on the whole body of literature that relates to many different bacterial strains, and based on studies of highly variable quality, does not provide confidence in the efficacy of these commercial products. The consumer unfortunately does not have any way of evaluating the labels or web sites in a scientific manner. There is no guidance offered by the regulatory authorities because the products are sold without requirement for FDA validation. Therefore, the consumer must presently take it on trust that the label and internet claims about probiotics are true. The only way to find out is for the consumer to purchase the product and try it, but they will be no further ahead because they have no way of measuring any effect (placebo or otherwise), except that on their pocket book. This is hardly a satisfactory situation because it is clearly open to abuse and fraudulent claims.

Conclusions

According to Sanders (2000), the retail market for probiotic dietary supplements in the United States of America is between ten and twenty million dollars, yet the scientific and medical validation of these products remains elusive. The probiotics industry appears to currently exist at two levels: generic products that have not been tested for efficacy but which rely on support from information contained in general probiotic articles published in the scientific literature, and on anecdotal information disseminated by word of mouth and in magazines. At a second level are the probiotic products that have been developed, notably by Japanese and European food companies, with high profile marketing support. These products, generally dairy-based, have received considerable research funding during their development. These companies have aimed to produce products that are supported by scientific publications that relate specifically to the probiotic bacteria contained in their products. Unfortunately, even these probiotic products have not been subjected to rigorous trials of efficacy using large numbers of human subjects, and none have been submitted for evaluation to the FDA. Without such scrutiny,

probiotics are destined to languish in the self-care health market that, although presumably lucrative for the manufacturer, offers little to the consumer in terms of documented efficacy or safety. Optimistically, a third level of probiotic product can be imagined: products that will be designed and derived for the support of patients suffering from specific diseases (such as IBD) and for which a mechanistic explanation of efficacy can be provided. These products will have been demonstrated in large clinical trials to be efficacious, safe to administer even to immune dysfunctional patients, and available by medical prescription. Clearly, it is time for a dose of realism for the probiotics industry.

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