Manipulation of the human gut microflora

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Manipulation of the human gut microflora is being attempted as a means of introducing into the digestive tract new micro-organisms which are beneficial to the host or which are able to bring about advantageous changes to the equilibrium populations and metabolic activities of the indigenous flora. In theory, this can be achieved by ingestion of nutrients or growth substances specifically favouring certain species, or by administration of exogenous selected micro-organisms. These micro-organisms, through their own complement of enzymes, may be able to compensate for some deficient intestinal enzyme activities of the host. However, the administration of the exogenous flora is primarily aimed at improving the digestive flora acquired under abnormal conditions, such as (a) inadequate transfer of micro-organisms from the mother to the infant in a highly hygienic environment. (b) consumption of sterile or nearly sterile food, (c) frequent antibiotic treatment (Fuller, 1991). Improvement in the resistance of the host to intestinal pathogens, whether by a 'barrier' effect of the flora or by stimulation of the immune system, and alteration of the profile of the colonic flora and its enzymic capacity are among the most widely investigated putative effects of exogenous micro-organisms. A few studies, which have dealt with their potential extra-gastrointestinal actions will not be reviewed here (see Marteau *et al.* 1993).

Micro-organisms that are likely to be used to achieve these goals have been called probiotics, i.e. 'live microbial feed supplements which beneficially affect the host animal (or human) by improving its microbiological balance' (Fuller, 1991). However, some of the putative effects of probiotics are beyond the limits of this definition.

An essential determinant in the choice of a probiotic is its ability to survive in the environment where it is intended to act. Unfortunately, most studies on probiotic actions have ignored this basic requirement. Moreover, a micro-organism cannot affect its environment unless its population reaches a certain minimum level which has not been exactly determined, but is probably about 10^7–10^8 CFU/g in the large intestine (Ducluzeau & Raibaud, 1989).

The probiotic preparations currently available are still mainly the lactic acid bacteria and bifidobacteria, which have been used empirically for as long as fermented milk has been consumed (Table 1).

INTESTINAL COLONIZATION BY EXOGENOUS BACTERIA

Several conditions, some related to the properties of the inoculum, are needed to facilitate prolonged colonization of the intestine by an exogenous bacterium. Being host specific (Conway, 1988) and belonging to a host dominant bacterial genus (Ducluzeau & Raibaud, 1989) seem to be prerequisites. As suggested by the study of infectious diarrhoeas, the size of the inoculum is probably important. The viability in the upper digestive tract of exogenous bacteria depends on the buffering capacity of the vehicle,

Table 1. *Composition of probiotics*

Origin . . .	Human	Animal	Plant
Streptococcus thermophilus		X	
Lactobacillus bulgaricus		X	
Lactobacillus acidophilus	X	X	
Lactobacillus casei (GG)	X		
Bifidobacterium longum	X		
Bifidobacterium breve	X		
Bifidobacterium animalis		X	
Streptococcus faecium	X		
Saccharomyces boulardii			X
Saccharomyces cerevisiae			X

the capacity of the micro-organism to resist acid (Conway *et al*. 1987; Pochart *et al*. 1989) and possibly other upper digestive secretions. This resistance is mainly a function of its cell wall stability.

Adhesion to the intestinal mucosa is probably a major determinant of colonization by the exogenous strains (Conway, 1988). Compared with the great deal of knowledge concerning pathogens, little is known about the adhesion capacity and mechanisms of organisms used as probiotics. It has been shown that *Lactobacillus* spp. may be cultured from human jejunal and colonic mucosa (Conway, 1988) and that this genus of bacteria adhere, to an extent that varies among strains, to human adult ileal cells (Conway *et al*. 1987) and to human fetal intestinal cells *in vitro*, but not to *Streptococcus thermophilus* (Kleeman & Klaenhammer, 1982).

The mechanisms of adhesion of *Lactobacillus* spp. to the squamous stomach epithelium of rodents have been studied (Conway, 1988). The role of membrane polysaccharide structures as receptors for adhesion has been questioned, and lipoteichoic acids and bacterial proteins have been proposed as possible alternatives. Surface proteins are probably involved in ileal adhesion of lactobacilli. In the large intestine, bacteria adhered to the mucosa by a chemotaxic mechanism, attracting them towards the mucus (Ducluzeau & Raibaud, 1989).

Besides the intrinsic properties of the exogenous organisms, exogenous and host factors could play a major role in the gut. Animal studies have shown the influence of diet and environmental stress (Tannock, 1983); also, the possibility in some cases of immunological modulation has been considered. However, the barrier effect of the indigenous flora is the main factor in the elimination of exogenous micro-organisms. The mechanisms proposed, which are poorly understood at present, include space occupation, competition for substrate, secretion of bacteriocins or other antagonistic proteins, production of antagonistic substrates such as volatile fatty acids and H_2S and adverse physico-chemical (pH, redox potential) conditions (Ducluzeau & Raibaud, 1989). *In vivo*, these factors usually have a bacteriostatic action on the exogenous micro-organisms, which are cleared at the same rate as an intestinal transit marker.

These numerous obstacles to colonization of the gut by non-pathogenic micro-organisms may be responsible for the usual failure of attempts to implant exogenous bacteria. A few studies (Gilliland *et al*. 1978; Ayebo *et al*. 1980) have shown faecal excretion, at a high level, of living *Lactobacillus acidophilus*, in fermented or non-

fermented milk, but whether the recovered faecal strain was identical to the one ingested usually was not checked.

Attempts to use antibiotics to weaken resistance to colonization within the gut (Barza *et al*. 1987) failed to promote *Lactobacillus* spp. implantation (Lidbeck *et al*. 1988). Interestingly, in a genotypic study with *Bifidobacterium* spp. by Bouhnik *et al*. (1992*a*), no colonization of the distal intestine by the exogenous strain was demonstrated. However, during antibiotic treatment, indigenous strains appeared and remained in the dominant flora at the same population level several weeks after antibiotic treatment had ceased.

These essentially negative results contrast with the successful treatment of relapsing pseudomembranous colitis with human fresh stool or bacterial mixture enemas, which are supposed to restore the barrier against *Clostridium difficile* (Bowden *et al*. 1981; Tvede & Rask-Madsen, 1989). However, a similar attempt to implant an exogenous *Bifidobacterium* spp. by enema after antibiotic treatment was unsuccessful (Y. Bouhnik, N. Bisetti & J. C. Rambaud, unpublished results). Similarly, the transient reduction of colonic bacterial mass by oral administration of a polyethylene glycol solution failed to facilitate colonization by the *Bifidobacterium* spp. previously used in the antibiotic experiments (Bouhnik *et al*. 1991).

Most of the obstacles for implantation of an exogenous flora in adults theoretically do not exist in the newborn, in whom indigenous flora is absent or limited and in whom the immune mechanisms are not fully developed. It has been shown that very early administration of a strain of human *Escherichia coli* (the dominant flora at this age), which is without plasmids, is non-toxigenic and sensitive to all the usual antibiotics, it was able in most cases to establish itself for a prolonged time in the dominant flora and to exert a barrier effect on antibiotic-resistant strains of *E. coli* (Duval-Iflah *et al*. 1982). Unfortunately, few further attempts have been made to go beyond this study of a barrier effect within a species to ascertain its potential as a barrier against challenge from other species, and its possible value in establishing a beneficial flora. Recently, a strain of *Bifidobacterium bifidum* was implanted in a small proportion of inoculated neonates; no effect of the implanted bacterium was found on the faecal level of enterobacteria or streptococci (Hudault *et al*. 1993).

SURVIVAL OF INGESTED MICRO-ORGANISMS

Even in the absence of intestinal colonization, exogenous micro-organisms can exert significant probiotic effects, provided they are in continuous transit in the gut at a sufficient concentration (Fuller, 1991). Currently, all probiotics used in humans act according to the latter mechanism. In contrast to colonization, it is clear that host specificity does not influence the activity of exogenous micro-organisms, as shown by some yogurt bacteria and yeast effects.

Lactobacillus bulgaricus and *S. thermophilus* in pure culture have a poor intrinsic resistance to acid, and are destroyed within a few minutes at pH 1 and in about 1 h at pH 3 (Conway *et al*. 1987). However, yogurt has a potent buffering capacity and, after 430 g yogurt were ingested by volunteers, the duodenal concentration of viable *L. bulgaricus* was over 10^5 CFU/ml, i.e. a survival rate of at least 1% (Pochart *et al*. 1989). In ileostomists, the concentration of viable *L. bulgaricus* in the ileostomy fluid was 10^5–10^6 CFU/ml after consumption of yogurt containing 10^6 CFU/ml *L. bulgaricus* (Lindwall &

Fonden, 1984). The excretion of yogurt bacteria in faeces cannot be accurately quantified with confidence by the usual techniques.

The intrinsic *in vitro* capacity for survival of *L. acidophilus* in acid conditions is clearly higher than that of *L. bulgaricus*, although differences among strains do exist. In the stomach, the *in vivo* survival of *L. acidophilus* is pH-related and is increased by the buffering capacity of milk (Conway *et al.* 1987), and *L. acidophilus* ingested in fermented milk has been found to survive well in the proximal (Robins-Browne & Levine, 1981) and distal small bowel (Marteau *et al.* 1992). The ileal recovery of the oral inoculum, measured by the intubation technique, is about 1·5% (Marteau *et al.* 1992). Studies on the capacity of *L. acidophilus* to survive through the whole human gut have yielded variable results, probably due to differences in strains, inocula and level of indigenous faecal lactobacilli. The ingested dose seems to be an important factor in recovery of viable exogenous lactobacilli in faeces (Gilliland *et al.* 1978). Based on the findings of Gilliland *et al.* (1978), the maximal survival rate is 2–5%.

Lactobacillus casei strain GG has been isolated by screening human stools for strains that were resistant to acid and bile (Silva *et al.* 1987). However, its survival rate through the digestive tract is low, since the lowest dose rate at which strain GG passes into the faeces was $1·5 \times 10^{10}$ CFU/d in the majority of volunteers studied (Marteau *et al.* 1993). After oral ingestion, *Lactobacillus* strain GG is found in stools, sometimes at very low concentrations, following antibiotic treatment (Siitonen *et al.* 1990).

In vitro sensitivity of bifidobacteria to acid differs among strains. Some of those used in fermented dairy products are highly resistant, so that they survive passage through the human stomach (Berrada *et al.* 1991). In two studies using an intestinal perfusion technique, 23·5 (Pochart *et al.* 1992) and 37·5% (Marteau *et al.* 1992) of two strains ingested in fermented milk were recovered in the distal ileum. Owing to the ingested load and ileal fluid flow-rate, the concentration of the two *Bifidobacterium* strains in ileal fluid reached 2×10^6 CFU/ml in both studies. A significant increase in the faecal concentration of bifidobacteria was observed after ingestion of viable bifidobacteria, but whether the strain in faeces was the ingested one was not checked, and the possibility that a 'bifidus growth factor' affected the indigenous flora could not be ruled out (Pochart *et al.* 1990). By using milk fermented with a selected *Bifidobacterium* sp. variant that could be distinguished from the indigenous strains, it was shown that the exogenous strain reached a mean level in stools of $6·3 \times 10^8$ CFU/g for as long as the product was being consumed (Fig. 1). Faecal recovery of the oral load was 30% (Bouhnik *et al.* 1992*b*). This high value makes bifidobacteria a potentially privileged vector of probiotic actions.

Enterococcus faecium, which showed partial resistance to acid *in vitro*, was found at low concentrations in the duodenum after ingestion of three tablets containing 10^7–18^8 organisms. Faecal counts of *E. faecium* also increased significantly during the period of ingestion (Friis-Moller & Hey, 1983).

Very little is known about the fate of ingested yeasts in the human gut. During administration of $3·2 \times 10^{10}$ CFU *Saccharomyces boulardii* for 14 d, steady-state and faecal elimination of living *S. boulardii* was 0·36%, and the faecal concentration was 1×10^7 CFU/g (Bléhaut *et al.* 1989).

In summary, several micro-organisms can pass live through the human gastrointestinal tract. The concentrations of exogenous living micro-organisms (or bacterial enzyme activities) at the different levels of the gut are more significant variables than the overall
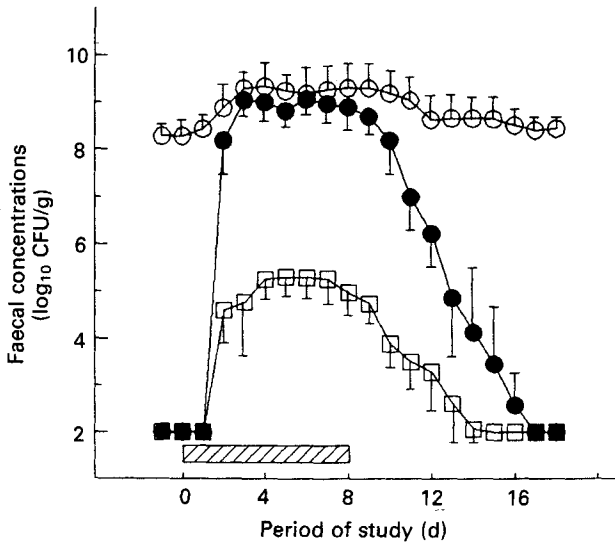
Fig. 1. Faecal concentrations of total bifidobacteria (○), the exogenous strain of *Bifidobacterium* sp. (●) and spores of *Bacillus sterothermophilus* (SBS; □) obtained in eight healthy volunteers after ingestion of 125 g three times daily of fermented milk containing 1.6×10^9 CFU selected exogenous *Bifidobacterium* sp./g and 3.2×10^5 CFU SBS/g for 8 d. Values are means with their standard errors represented by vertical bars. (From Bouhnik *et al.* 1992*b*.)

percentage of survival. However, from the few findings available, it seems that the main factor governing upper and lower small intestine and faecal concentrations of an exogenous strain is its resistance to upper digestive tract secretions. That the percentage of flowing ingested bacteria remains almost constant from the ileum to the faeces is consistent with there being a bacteriostatic action within the colon.

EFFECTS OF PROBIOTICS IN THE INTESTINE

Alteration of bacterial flora and its metabolic activities

Some probiotics are antagonistic *in vitro* to pathogens or indigenous bacteria, but very few pertinent findings are available on their *in vivo* action in humans. Administration of lactobacilli has been seen to induce a drop in faecal counts of *E. coli* in some studies (Ayebo *et al.* 1980; Lidbeck *et al.* 1987), but not in all (Gilliland *et al.* 1978).

Changes in the metabolic activities of the indigenous flora during various regimens of probiotic administration are more interesting, as they reflect the variation of whole flora. Significant variations in faecal output of enzymes which could play a role in colonic carcinogenesis (Goldin & Gorbach, 1976) have been reported during consumption of milk fermented with various lactic acid bacteria (Table 2). In some of these studies a probiotic effect of the vehicle was ruled out. Enzyme levels returned to their basal output within 3 weeks of stopping the administration of exogenous bacteria.

Bifidobacterium longum reduced lactulose-induced H_2 breath excretion, but no modification *in vitro* of lactulose-induced production of H_2 or CH_4 by the left and right colonic flora was observed during ingestion of *L. acidophilus* and *Bifidobacterium* spp. (Marteau *et al.* 1993).

Table 2. *Effects of lactic acid bacteria ingestion on faecal bacterial enzyme activities in humans*

Ingested bacteria	Daily quantity	Subjects	Azoreductase (EC 1.6.6.7)	Nitro-reductase	Glucu-ronidase	Reference
Lactobacillus acidophilus†	10^9	12	ND	ND	↓*	Ayebo *et al.* (1980)
L. acidophilus†	10^9	21	↓*	↓*	↓*	Goldin & Gorbach (1984)
L. acidophilus†	10^9	7	↓*	↓*	↓*	Pedrosa *et al.* (1990)
L. bulgaricus + *Streptococcus thermophilus*	5×10^8	9	–	–	–	Pedrosa *et al.* (1990)
L. acidophilus + *Bifidobacterium* sp. + *Streptococcus* spp. +	10^9–10^{10}	9	–	↓*	–	Marteau *et al.* (1990*b*)
Bifidobacterium sp.	10^{10}	6	–	↓*	↓*	Bouhnik (unpublished)

ND, not determined; –, no effect; ↓, decrease in activity.

† No effect with milk or killed bacteria.

* The decrease in activity was significant ($P<0.05$).

Lumen enzyme effects of probiotics

By using the H_2 breath test and intestinal intubation, it has been well established that lactose malabsorbers digest lactose more efficiently from fresh yogurt than from pasteurized yogurt or milk (Marteau *et al.* 1990*a*). This is partly due to the presence of a highly active bacterial lactase activity in *L. bulgaricus* and *St. thermophilus*. It has been shown that this lactase activity is not completely denatured during its passage through the stomach, and can be detected in the human intestine (Kolars *et al.* 1984; Pochart *et al.* 1989; Marteau *et al.* 1990*b*). This microbial lactase is able to digest lactose in the lumen and, thus, compensate for the mucosal deficiency. Similarly, Harms *et al.* (1987) demonstrated that in children with sucrase (EC 3.2.1.48)–isomaltase (EC 3.2.1.10) deficiency, sucrose digestion can be improved by administering *Saccharomyces cerevisiae*, a yeast which possesses a sucrase 1000 times more active than the sucrase of normal small intestinal mucosa.

Oral administration of *E. faecium* was found in one study to modify the colonic metabolism of bile salts (Salvioli *et al.* 1982). Recently, it has been shown that a milk fermented with *Bifidobacterium* spp., *Lactobacillus* spp. and *Streptococcus* spp. could deconjugate bile salts in the small bowel. This potentially harmful effect should be further studied (P. Marteau, P. Pochart, Y. Bouhnik & J. C. Rambaud, unpublished results).

Parietal effects of bacteria in transit

Oral administration of *S. boulardii* markedly increased lactase (*EC* 3.2.1.108), sucrase and maltase (*EC* 3.2.1.20) activities in jejunal biopsies, whereas histological morphology and protein content of the mucosa remained unchanged (Buts *et al.* 1986). These results were interpreted as an effect of the yeast on either the synthesis or catabolism of intestinal disaccharidases. Contrasting with this study, it was subsequently reported that ingestion of fresh yogurt containing viable *L. bulgaricus* and *St. thermophilus* did not modify significantly lactase activity in the duodenal mucosa of lactose malabsorbers (Lerebours *et al.* 1989). However, the site studied was perhaps not the best choice, since no spontaneous lactase activity exists in the duodenum of such subjects.

The putative effects of probiotics on the local or systemic immune system in humans is an attractive field of research, but present knowledge relies mainly on animal studies. In humans, ingestion of high doses of *L. bulgaricus* and *St. thermophilus* raise the levels of serum γ-interferon and circulating NK cells (De Simone *et al.* 1991).

Applications in medicine

Apart from remedying lactase and sucrase–isomaltase intolerance mentioned previously, probiotics have been largely used in the prevention or treatment of infectious diarrhoeas and antibiotic-related digestive disturbances. In the past papers reporting uncontrolled studies have raised doubt about the value of using probiotics. However, more recent well-controlled studies have provided a few unequivocal positive results (Marteau *et al.* 1993).

Several studies have demonstrated a protective effect of inoculation with bacteria or *S. boulardii* during antibiotic treatments (Table 3). In the management of intestinal infections, two goals can be defined for 'transiting micro-organism therapy', amelioration of symptoms, and elimination of the pathogen. The first goal has been far more frequently attained than the second. Several controlled randomized trials have demonstrated a beneficial effect of yogurt, *E. faecium* SF68 (Bellomo *et al.* 1980), *L. casei* strain GG, or *S. boulardii* for infants recovering from gastroenteritis; however, this is not a general property of all transiting micro-organisms.

Several studies in which transiting micro-organisms were used to prevent traveller's diarrhoea gave negative results. In a trial in subjects travelling to Turkey, *L. casei* strain GG had no significant effect on the overall diarrhoea rate, but a retrospective analysis of the results showed a reduction of the incidence of diarrhoea in a subgroup of travellers to one of the two destinations studied (Oksanen *et al.* 1990). A double-blind controlled study did not reveal any curative effect of *E. faecium* EF68 for cholera or *E. coli* diarrhoea (Mitra & Rabbaani, 1990), and treatment with lactobacilli was found to be inefficient in another study as a cure for *E. coli* diarrhoea (Silva *et al.* 1987). Open trials of several therapies using living micro-organisms to treat intestinal disease caused by *C. difficile* have suggested that lactobacilli or *S. boulardii* have interesting effects (Marteau *et al.* 1993); however, controlled studies are not yet available to confirm these results.

In conclusion, it is clear that much more solid scientific work needs to be undertaken before the medical profession can fully recommend probiotics, despite authority of Metchnikoff (1908). We might adapt the well-known legal maxim 'Justice must not only be done, it must be *seen* to be done' to our present enquiry, thus 'A theory must not only

Table 3. *Randomized controlled studies using transiting micro-organisms to protect from antibiotic-associated diarrhoea* (From Marteau *et al.* 1993)

Antibiotic	Probiotic	Incidence of diarrhoea†	
		Probiotic	Placebo
Ampicillin	Lactinex®	8·3*	21·0
Neomycin	*Lactobacillus acidophilus*	20·0*	42·0
Erythromycin	*Lactobacillus* GG	‡	
Erythromycin	*Bifidobacterium longum*	‡	
Antituberculous	*Enterococcus faecium*	5·0	18·0
Miscellaneous	*Saccharomyces boulardii*	‡	
Miscellaneous	*S. boulardii*	4·5*	17·5
Miscellaneous	*S. boulardii*	9·5*	21·8

Values were significantly lower than those for the placebo group: *$P<0·05$.
† Percentage of subjects suffering diarrhoea.
‡ Values were significantly lower than those for the placebo group; values not given.

be right, it must be *seen* to be right'. A rational, rather than an empirical choice of the putative probiotics is obviously advisable. The use of genetically engineered micro-organisms that can colonize the gut or pass through it at a high concentration and which also possess useful biological activity, are the most appropriate candidates for achieving this goal.

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