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MODIFICATION OF GUT FLORA METABOLISM BY PROBIOTICS AND OLIGOSACCHARIDES

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SUMMARY

Gut bacterial metabolic activities play an important role in influencing the toxic effects of many ingested chemicals by activating them to more toxic, genotoxic or carcinogenic derivatives. Modification of the activity of these enzymes by ingestion of lactic acid bacteria or of oligosaccharides which stimulate the growth of lactic acid bacteria in the colon could have beneficial effects for the host.

INTRODUCTION

The metabolic activities of the gut microflora, particularly towards ingested xenobiotics, can have wide-ranging implications for the health of the host, resulting in both detrimental and beneficial effects (Table 1; *Rowland* et al., 1985; *Rowland* and *Walker*, 1983).

Metabolic reactions occurring in the gut can have consequences both locally, on the gut mucosa, or systemically. For example, amines and phenols generated by gut bacteria from amino acids, can have effects on the central nervous system, the vascular system and potentially on tumorigenesis in various organs of the body (*Bakke*, 1969; *Boultwell* and *Bosch*, 1969; *Drasar* and *Hill*, 1974).

The region of the gut which harbours the greatest number of bacteria is the colon, indeed other areas of the human gastrointestinal tract are very sparsely populated (*Drasar*, 1988). This does not mean, however , that only poorly absorbed ingested chemicals encounter the colonic flora. Substances, and their metabolites, may partition across the intestinal wall from the blood or may reach the colon after excretion in the bile (see below). Thus there is ample opportunity for a wide variety of materials in diet to encounter, and be metabolised by, the colonic microflora.

Table 1: Some health implications of gut flora metabolism

1. Production of toxic, carcinogenic or mutagenic metabolites from substances derived from diet or produced endogenously
2. Detoxification of dietary toxicants
3. Enterohepatic circulation of drugs, food additives and steroids

Table 2: Some bacterial enzymes that generate toxic, genotoxic, or carcinogenic products

Enzyme	Substrates
ß-Glycosidase	Plant glycosides • Rutin • Franguloside
Nitroreductase	Nitro compounds • Dinitrotoluene
Azoreductase	Azo compounds • Benzidine-based dyes
ß-Glucuronidase	Biliary glucuronides • Benzo(a)pyrene • IQ • Benzidine
IQ "hydratase-dehydrogenase"	IQ, MeIQ
Nitrate/nitrite reductases	Nitrate, nitrite
Bile acid dehydroxylase	Cholic and chenodeoxycholic acids
Amino acid deaminase	Tyrosine and other amino acids

BACTERIAL XENOBIOTIC METABOLISING ENZYMES

A list of the major bacterial enzymatic reactions leading to alterations in the toxicity of substrates is given in Table 2. Some of the reactions are considered in more detail below.

ß-Glycosidases

Plants produce a wide variety of secondary metabolites including azoxy, anthraquinone, diterpenoid and flavonoid structures which are usually stored as glycosides (*Brown*, 1988). Their presence, often in large quantities, in edible fruits and vegetables and in beverages, such as tea and wine, results in significant human intake (*Hertog* et al., 1993). Glycosides are poorly absorbed in the small intestine and pass into the colon, where the action of bacterial ß-glycosidases cleaves the sugar moiety releasing aglycones, which exhibit a wide range of biological activities. For example, methylazoxymethanol (MAM), the hydrolysis product of cycasin, is carcinogenic (*Laqueur* and *Spatz*, 1968) and many of the flavonoid, anthraquinone and diterpenoid aglycones exhibit mutagenic activity in *in vitro* assays, although apart from MAM, their carcinogenic effects are debatable (*Brown*, 1988).

Assessment of the toxicological significance of glycoside hydrolysis by intestinal microflora is further complicated by reports of anti-carcinogenic and anti-mutagenic effects of flavonoid aglycones against a wide variety of carcinogens including benzo(a)pyrene (*Wattenberg* and *Leong*, 1970), 4-dimethylaminoazobenzene (*Nagase* et al., 1964) and the cooked food mutagens IQ, MeIQ and MeIQx (*Alldrick* et al., 1986).

ß-Glucuronidase

Many xenobiotics and endogenously-produced compounds are metabolised in the liver and conjugated to glucuronic acid before being excreted into the small intestine in bile (*Smith*, 1966). In the colon, hydrolysis of the glucuronide linkage by bacterial ß-glucuronidase can release the parent compound, or its hepatic metabolite. Reabsorption of the compound can result in its enterohepatic circulation with concomitant potentiation of its pharmacological or toxicological effects. Many carcinogens including polycyclic aromatic hydrocarbons, heterocyclic amines (e.g. IQ) can be conjugated and secreted into the intestine where ß-glucuronidase action may release the parent carcinogen in the colon. For example, benzo(a)pyrene (BP), a contaminant of the human diet, undergoes activation and conjugation in the liver. Gut bacteria have been shown to release reactive metabolites from biliary conjugates which covalently to DNA and are genotoxic (*Renwick* and *Drasar*, 1976; *Chipman* et al., 1983).

Nitrate reductase

Nitrate, ingested with the diet and drinking water, is readily converted by gut bacteria to its more reactive and toxic reduction product, nitrite which can react with nitrogenous compounds in the body to produce N-nitroso compounds many of which are highly carcinogenic (*Rowland*, 1988). Using germ-free rats, we have demonstrated the importance of the gut microflora for this nitrosation reaction (*Massey* et al., 1988) and recently have shown that the reaction can occur in man by measuring N-nitroso compounds in faeces (*Rowland* et al., 1991).

Azoreductase

A number of dyes used in food, cosmetics and for textiles and leather are based on azo compounds which are reduced in the gut by the intestinal flora to produce, ultimately, amines. In some cases the reduction products are toxic. For example, workers exposed to Direct Black 38, a dye used in the leather and textile industry, have an elevated risk of bladder cancer which has been attributed to the reduction of the dye by the gut microflora to benzidine, a known human bladder carcinogen (*Powell*, 1979; *Cerniglia* et al., 1982).

Nitroreductase

Heterocyclic and aromatic nitro compounds are important chemical intermediates (*Hartter*, 1984), are used as antibiotics and radiosensitising drugs and are ubiquitous environmental pollutants resulting from combustion of fossil fuels. Many of these compounds possess toxic, mutagenic and carcinogenic activity and so may contribute to the environmental cancer risk in man (*Rosenkranz* and *Mermelstein*, 1982; *Busby* et al., 1985). Reduction of the nitro group is usually required for the pharmaceutical and toxicological activity of these compounds to be expressed (*Lindmark* and *Muller*, 1976; *Reddy* et al., 1976). Although reduction of the nitro group can be effected by both mammalian and bacterial reductases nitroreduction by the gut microflora appears to play a more important role than hepatic enzymes particularly in the cases of nitrobenzenes (*Reddy* et al., 1976), dintrotoluenes (*Doolittle* et al., 1983; *Mirsalis* et al., 1982) and nitrated polycyclic hydrocarbons, such as 6-nitrochrysene (*Cerniglia* et al., 1982; *Rickert*, 1988).

Bacterial metabolism of 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ)

IQ is one of several heterocyclic amine compounds which are produced in small amounts when meat and fish are grilled or fried (*Felton* et al., 1986).

Table 3: Possible mechanisms by which probiotics exert beneficial effects via gut flora metabolism

1. Probiotic displaces or dilutes indigenous gut flora organisms with high enzymic activities, thus suppressing, overall, reactions which result in generation of toxic or carcinogenic metabolites.
2. Probiotic generates conditions in the gut which alters the rate of bacterial activation of ingested chemicals e.g. lowering of pH (affects ammonia production, bile acid metabolism).
3. Probiotic stimulates reactions which lead to the production of potentially beneficial products in the gut e.g. anti-carcinogenic flavonoids.

These compounds are normally mutagenic in *Salmonella typhimurium* only after activation by hepatic cytochrome P450-dependent mixed function oxidases (*Yamazoe* et al., 1983; *Alldrick* et al., 1986). IQ is also carcinogenic in rodent bioassays and induces tumours at various sites including the large intestine, suggesting that it may play a role in the aetiology of colon cancer in man (*Ohgaki* et al., 1986).

Incubation of IQ with a suspension of human faeces yields the 7 keto derivative, 2-amino-3,6-dihydro-3-methyl-7*H*-imidazo[4,5-*f*]quinoline-7-one (7-OHIQ) (*Bashir* et al., 1987) and the reaction has been shown not to occur in gut contents obtained from germ-free rats (*Rumney* et al., 1993). Unlike IQ, the bacterial metabolite is a direct-acting mutagen in *Salmonella typhimurium* (*Carman* et al., 1988, *Rumney* et al., 1993). Thus there is strong evidence for the bacterial formation in the human gut of a directly genotoxic derivative of a dietary carcinogen.

EFFECT OF PROBIOTICS AND OLIGOSACCHARIDES ON XENOBIOTIC METABOLISM BY THE GUT MICROFLORA

One of the most important ways in which a probiotic organism may exert a beneficial effect on its host is to modify the reactions (described above and in Table 2) leading to the generation of potentially toxic products in the gut. Such a beneficial effect could be achieved in theory in a number of ways (Table 3).

The colonic flora of man and other mammals is a highly complex ecosystem comprising over 400 different species. Some limited information on the ability of some of these species to catalyse metabolic reactions *in vitro* has been reported and these studies indicate a wide range of metabolic capacity within and between the various groups (*Cole* et al., 1985; *Saito* and *Rowland*, 1992). In general, however, species of *Bifidobacterium* and *Lactobacillus*, which are commonly used as probiotics, have low activities of xenobiotic metabolising enzymes such as azoreductase, nitroreductase, nitrate reductase and ß-glucuronidase, by comparison to other major anaerobes in the gut such as bacteroides, eubacteria and clostridia (Table 4). Conversely, they have high levels of glucosidase activity which, as described above, may result in the generation in the gut of flavonoid aglycones with genotoxic and anti-carcinogenic properties.

The number of organisms that has been investigated in this way is limited,

Table 4: Enzyme activities of intestinal organisms *in vitro*

Species	Enzyme activities (µmol/h/10^{10}cells)			
	Azo	GN	GS	NR
Bifidobacterium				
longum	ND	0.001	0.20	NE
adolescentis	ND	ND	0.65	NE
breve	ND	ND	3.67	NE
infantis	ND	ND	2.73	NE
bifidum	ND	ND	0.11	NE
Bacteroides				
fragilis	0.01	0.007	0.514	NE
vulgatus	0.03	0.012	0.064	NE
thetaiotaomicron	0.02	ND	0.815	NE
uniformis	0.09	0.006	1.860	NE
sp IY37	NE	0.680	5.380	0.01
Clostridium				
perfringens	0.343	0.02	0.84	NE
paraputrificum	7.530	0.03	8.20	NE
innocuum	0.230	0.04	0.55	NE
Eubacterium				
aerofaciens	0.348	0.012	0.166	NE
lentum	ND	ND	0.004	NE
Streptococcus sp.	NE	0.04	0.03	0.0004
Lactobacillus				
salivarius	NE	ND	0.10	ND

After *Cole* et al. (1985), and *Saito* & *Rowland* (unpublished observations, 1991).

NE = Not estimated
ND = Not detected
Enzymes Key : Azo = azoreductase; N = ß-glucuronidase; GS = ß-glucosidase; NR = nitroreductase.

hence the abilities of the many component species of the microflora to metabolise nutrients and foreign compounds are not known in any detail. A further complication is that although a species may express an enzyme activity when cultured *in vitro*, the same species may behave very differently when it colonises the gut of an animal. This phenomenon was demonstrated by comparing enzymatic activity of microflora strains *in vitro* with the activity *in vivo* by using gnotobiotic animals monoassociated with specific gut microorganisms (*Cole* et al., 1985).

Despite these caveats, the evidence suggests that increasing the numbers of lactic acid bacteria in the gut could modify, beneficially, the levels of xenobiotic metabolising enzymes.

Two main strategies have been employed in attempts to increase the level of potentially beneficial organisms in the gut. The direct approach is to supply live preparations of probiotics which can be added to the diet. The indirect approach is to use non-digestible, carbohydrate food supplements (oligosac-

charides) which support and stimulate the growth of lactic acid bacteria in the colon.

Effects of probiotic consumption on gut bacterial metabolism

The effect of administration of probiotic organisms on bacterial enzymes of toxicological importance has been addressed in a number of papers.

In a rat study, supplementation of a high meat diet (72% beef) with *L. acidophilus* (to provide 10^9-10^{10} organisms/day) significantly decreased by 40-50% the activity of faecal ß-glucuronidase and nitroreductase. It is noteworthy that the modulating effect of the lactobacillus strain was dependent on the type of diet fed - no significant effect on enzyme activities were seen when the rats were fed grain-based diet (*Goldin* and *Gorbach*, 1977).

These studies have been extended to humans who were given milk supplemented with 10^9 viable lactobacilli per day (*Goldin* and *Gorbach*, 1984). Prior to lactobacillus feeding, faecal ß-glucuronidase activity ranged between 1.74 and 2.14 units/mg faecal protein. After ingestion of the lactobacilli for 30 days, the activity of ß-glucuronidase declined in all 21 subjects (mean value of 1.12 units/mg protein). Enzyme activities returned to the baseline value 10 days after lactobacillus consumption ceased. In the case of nitroreductase and azoreductase, decreases in enzyme activity during lactobacillus exposure of over 75% were reported and control values were not restored until 30 days after lactobacillus supplementation ceased. The fact that the changes in enzyme activity were not sustained when the ingestion of lactobacilli ceased indicates that the intestinal tract was not permanently colonised.

In an analogous experiment to those of *Goldin* and *Gorbach*, *Cole* and colleagues (1989) investigated the effect of *L. acidophilus* administration on bacterial metabolic activities in germ-free rats colonised with a human faecal microflora. A significant reduction in ß-glucosidase and ß-glucuronidase activities was observed when lactobacilli were given for 3 days with the effects persisting for 7 days after dosing ceased.

Effect of probiotics on *in vivo* metabolism of xenobiotics

The changes in enzyme activities seen in the animals and humans treated with lactobacilli would, in theory, be expected to result in changes in rates of metabolism of their substrates *in vivo*, although only if the enzymes catalysed the rate limiting step in their metabolism. It is important, therefore, to test that this is in fact the case and that the changes in enzyme activities observed do result in corresponding changes *in vivo* metabolism of potentially toxic chemicals. *Goldin* and *Gorbach* (1984a) have investigated this by feeding to rats aromatic nitro compounds (nitrofluorene, nitronaphthalene), an azo dye and a glucuronide (2-naphthylamine-N-D-glucuronide) and monitoring the production of the reaction products, i.e. free amines, in faeces. It should be noted that the rats were fed a high meat (72%, w/w) diet to increase enzyme activities and so maximise the effect of the lactobacilli on the enzymes (see above).

The reduction in activity of nitroreductase, azoreductase and ß-glucuronidase in the rats given oral lactobacilli supplements, was matched by a decrease, about 50% by comparison to controls, in the excretion of free amine products. These results show that administration to laboratory animals of lactobacilli can decrease the production of toxic and carcinogenic amines (e.g. 2-naphthylamine, a human bladder carcinogen) from ingested substrates.

The effect of ingestion by human subjects of *Lactobacillus casei* on urinary excretion of potentially toxic amino acid metabolites was studied by *Tohyama* and colleagues (1981). The urinary concentration of indican (from tryptophan) and p-cresol (from tyrosine) was significantly decreased by feeding 10^{10} organisms/day for 5 weeks with mean reductions of 29 and 43% respectively.

Effect of probiotics on tumorigenesis

The influence of *B. longum* on induction of colon and mammary tumours by the cooked food carcinogen IQ has recently been investigated by *Reddy* and co-workers (1993). Rats were fed, for 58 weeks, a high fat diet containing 125 ppm IQ with or without a dietary supplement (0.5%) of freeze-dried *B. longum*. In male rats, significant decreases were reported in both the incidence of colon tumours (43% incidence in rats fed IQ alone, no tumours in those fed IQ plus *B. longum*) and the number of tumours per animal. In female rats, the number of mammary tumours per animal, but not tumour incidence, was significantly decreased. One of the postulated mechanisms for the anticarcinogenic effect was inhibition by the bifidobacteria of the deconjugation of biliary conjugates of IQ in the colon, which would have the effect of reducing the release of free IQ and/or its genotoxic metabolites in the colon (see section on ß-glucuronidase above)

Effect of milk (fermented and non-fermented) and yoghurt on metabolism by gut microflora

Ingestion by elderly (>65 yr.) volunteers of non-fermented milk containing *L. acidophilus* was associated with minor and inconsistent changes in activity of ß-glucuronidase and ß-glucosidase in faeces (*Ayebo* et al., 1980). The lack of effects were possibly due to the low level of lactobacilli (about 2 x 10^6 organisms per ml) in the milk which resulted in only minor increases in the faecal lactobacillus count.

In a study by *Marteau* and co-workers (1990), volunteers consumed a fermented milk product containing higher numbers of lactic acid bacteria : *L. acidophilus* (10^7/g), *Bifidobacterium bifidum* (10^8/g) and *Streptococcus lactis* and *S. cremoris* (both at 10^8/g). Although azoreductase and ß-glucuronidase activities did not change in response to consumption of the fermented milk, nitroreductase activity was decreased by 38% and remained depressed for at least 3 weeks. In contrast, ß-glucosidase increased after ingestion of the fermented milk - a change attributable to the high activity of this enzyme in *B. bifidum*.

In the various studies, described above, performed using either lactobacilli or milk products containing lactobacilli, the most consistent finding is a decrease in nitroreductase activity in faeces. The inconsistency of the other changes in metabolism may be due to differences in the types of probiotic fed or their concentrations in the product.

Effect of oligosaccharides on gut bacterial numbers and metabolism

A number of oligosaccharides (short chain-length sugars, usually less than 20 monomers in the chain) have been isolated which are not substrates for mammalian hydrolytic enzymes and so pass undegraded into the colon where they may be fermented by colonic bacteria. These oligosaccharides are usually only fermented by a limited range of microorganisms and so in theory can selectively stimulate the growth of chosen organisms. They are sometimes co-administered with a specific organism (usually a *Bifidobacterium* species) in

order encourage its multiplication in the gut and hence potentiate the beneficial, "probiotic" effects of the organism. Although not strictly probiotics, these indigestible sugars have been included in this review since they possess many of the properties of probiotics.

The main oligosaccharides that have been studied for beneficial effects on the consumer are :

a) Transgalactosylated oligosaccharides (TOS; *Tanaka* et al., 1983). TOS, a mixture of tri-, tetra-, penta- and hexa-saccharides of galactose and glucose, was utilised by all *Bifidobacterium* species tested and by some lactobacilli, bacteroides, streptococci and enterobacteria (*Tanaka* et al., 1983).
b) Soybean oligosaccharides extract (SOE; *Hayakawa* et al., 1990). SOE comprises a mixture of sucrose (44%), stachyose (23%), raffinose (7%) and monosaccharides and is obtained from defatted soybean whey. The extract can be further refined (SOR) to increase the stachyose and raffinose content to 71 and 20% respectively. The efficiency of utilisation of SOR by intestinal bacteria *in vitro* was greatest with *Bifidobacterium* species although other genera including *Lactobacillus* and *Bacteroides* were capable of fermenting the sugar.
c) Fructo-oligosaccharides (*Hidaka* et al., 1986; *Rumessen* et al., 1990). A commercial preparation of oligosaccharides ("Neosugar"), a mixture of tri-, tetra- and penta-saccharides of glucose and fructose, is utilised by most bifidobacteria, bacteroides and some streptococci, lactobacilli and enterobacteria, but not *E. coli* (*Hidaka* et al., 1986). Another oligofructose, derived from inulin which is found in garlic, chicory, artichoke and onion, behaves in a similar manner towards gut bacteria, being fermented mainly by bifidobacteria (*Wang* and *Gibson*, 1993).

The effect of ingestion of TOS (3 or 10g/d), *B. breve*, or both, on faecal bacterial counts and faecal ammonia has been investigated in human volunteers (*Tanaka* et al., 1983). In general, significant effects were seen only during periods when both TOS and *B. breve* were ingested simultaneously. For example, during these periods, the viable counts of bacteroides and enterobacteria in faeces declined and faecal ammonia concentration decreased markedly in 4 out of the 5 volunteers. Ingestion of TOS alone even at 10g/d , gave inconsistent effects on these parameters although the study may have been limited by the small number of participants. A study in human-flora-associated (HFA) rats has extended the above human study to encompass bacterial metabolic activities as well as bacteriological analyses (*Rowland* and *Tanaka*, 1993).

The HFA rats were fed, for 4 weeks, a purified diet with or without TOS (5% w/w). Caecal concentrations of bifidobacteria and lactobacilli were significantly increased in TOS-fed rats, whilst enterobacteria were decreased and bacteroides were unaltered. Bacterial ß-glucuronidase and nitrate reductase activities, pH and conversion of the dietary carcinogen IQ to its directly genotoxic 7-hydroxy derivative, were significantly reduced in caecal contents of TOS-fed rats. Bacterial ß-glucosidase activity was increased presumably as a consequence of the elevated numbers of bifidobacteria which have high levels of this enzyme (Table 4; *Saito* et al., 1992). The results of this study indicate that TOS-induced changes in microflora may be potentially beneficial due to decreased bacterial activities associated with generation of toxic, genotoxic and carcinogenic products in the gut.

Ingestion of Neosugar (8g/d for 14d)

by elderly patients led to a slight increase in total bacterial count in faeces and about a 10-fold increase in bifidobacteria (*Hidaka* et al., 1986). However, the increase in bifidobacteria was seen only in those individuals who originally had low faecal counts ($<10^8$/g) of the organisms. Similar inter-individual differences in response were seen in gut bacterial metabolism when Neosugar was administered for 2 months at a dose rate of 8g/d. An increase in bifidobacteria was accompanied by a rise in short-chain fatty acid (SCFA) concentration in faeces and a decrease in p-cresol and indole. In the same person, however, faecal ammonia was increased nearly two-fold. Again, changes in these metabolic profiles were not seen in a person whose bifidobacteria count was initially high. (*Hidaka* et al., 1986). These studies need to be interpreted with extreme caution since only two individuals were studied.

Further experiments were performed in rats fed a purified diet containing tyrosine and tryptophan. Incorporation of Neosugar into the diet at 0.4-10% appeared to reduce p-cresol concentration in faeces especially at the highest dose of the oligosaccharide (*Hidaka* et al., 1986). As might be expected, high dietary concentrations of Neosugar (10-20%) in the diet of rats also markedly increased SCFA concentration in faeces (*Tokunaga* et al., 1986). The total daily excretion of neutral steroids was also increased although an increase in bile acid excretion was seen only at the higher (20%) dose, which is outside the normal human intake level. The changes in faecal neutral steroid excretion in rats were not reflected in serum cholesterol concentrations suggesting that Neosugar stimulates cholesterol synthesis rather than just increasing faecal excretion (*Tokunaga* et al., 1986).

A study of soy bean oligosaccharides (SOE) (10 g/day) in human volunteers demonstrated that the viable count of bifidobacteria in faeces increased slightly but significantly during the period of ingestion of oligosaccharide preparation (*Hayakawa* et al., 1990). Intake of *B. breve* with SOE appeared to have little additional effect on bifidobacteria numbers in faeces. Faecal pH and amino acid degradation products in faeces (indole, p-cresol and phenol) were also determined, but no consistent significant differences were observed between the various dietary periods.

The refined soybean oligosaccharide SOR has been studied in an *in vitro* continuous flow culture system which models the human colonic microflora (*Bearne* et al., 1990; *Saito* et al., 1992). Numbers of bifidobacteria were determined and various bacterial enzyme activities assayed before and after SOR was incorporated into the growth medium at a concentration of 0.1% (w/v). After addition of SOR, there was a 5-fold increase in concentration of bifidobacteria in the culture, which mirrors the changes seen in human volunteers (*Hayakawa* et al., 1990). The activity of some bacterial enzymes was also altered during the period of SOE supplementation. Azoreductase activity decreased significantly ($p<0.05$) by 40% and ß-glucuronidase and ß-glucosidase activities also decreased (by 38 and 32%, respectively), although the results were not significant. In a subsequent study, HFA rats fed 3% refined soy-bean oligosaccharides in the diet showed decreased excretion of N-nitroso compounds in faeces suggesting that soy bean oligosaccharides may decrease exposure of the colon to these potentially carcinogenic substances.

CONCLUSIONS

There is good evidence that certain strains of lactobacilli can modify intestinal bacterial metabolism of foreign compounds. In some cases, the biological and toxicological significance of the changes seen has been established and indicates that ingestion of such probiotic organisms may have beneficial effects in humans. Other probiotic organisms such as bifidobacteria have been studied less extensively with regard to their influence on intestinal bacterial metabolism. Their effects on the bacterial flora of the gut have been investigated, but such studies, although valuable in establishing that a treatment can modify the flora, give little information on the biological consequences of for the host animal. Further metabolic and toxicological studies are urgently needed therefore in this area. The use of indigestible oligosaccharides to stimulate growth of lactic acid bacteria in the intestine holds great promise and presumably these food supplements will continue to be refined in terms of the specificity of their effects. Again, metabolic and toxicological studies will be necessary for assessing their beneficial consequences for the consumer.

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