# INTERRELATIONSHIPS BETWEEN THE GASTROINTESTINAL MICROFLORA AND NON-NUTRIENT COMPONENTS OF THE DIET

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## CONTENTS



## INTRODUCTION

Man, in common with all creatures inhabiting the normal world, is host to a mass of microorganisms that colonize the epithelial surfaces and contents of the gastrointestinal tract. All substances entering the gut lumen are subject to the considerable metabolic capacity of the flora. The influence of the gut microflora on digestion, absorption and metabolism of nutrients has been well documented (Wostmann, 1981; Coates, 1987, and others). Its effects on additives, contaminants and other non-nutrient components of the diet have also been recognized for some time (Scheline, 1973; Goldman, 1978). Most gut microorganisms are strict anaerobes, hence their reactions tend to be mainly reductive and hydrolytic, in contrast to those of mammalian tissues which are generally oxidative and conjugative. Ingested foreign compounds may be converted directly by organisms in the gut into metabolites with greater or less toxicity to the host. Those that are absorbed are transported to the liver for detoxication, usually by oxidation and conjugation with glucuronate, sulphate or glutathione. The conjugates are re-excreted via the bile into the small intestine, where they may undergo deconjugation and reduction by bacterial enzymes to simpler compounds which are likely to be more readily absorbed. The overall effect of this enterohepatic circulation is to prolong the length of time the xenobiotic or its metabolites remain in the body of the host. This review deals only with constituents of foods, but it is evident that drugs given by mouth, and parenterally administered



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compounds or their metabolites that enter the enterohepatic circulation, are similarly affected by the activities of the gut microflora.

## THE HUMAN GASTROINTESTINAL MICROFLORA

The microbial composition of the normal human gastrointestinal flora has been described and discussed by Savage (1989). This indigenous microflora is usually established soon after birth and, as the term 'indigenous' implies, exists in symbiosis with the host. Interactions of its component species with each other, with the host and with the diet can have consequences that are beneficial, detrimental or of no importance to the host's wellbeing.

For obvious reasons, much of our knowledge of the human gut microflora has to be derived from studies on samples of faeces, which reflect the composition of the flora of the lumen of the colon, but give no information regarding the distribution or activities of organisms in the rest of the gastrointestinal tract. Investigations in various animal models have shown that different communities of the microbial population may be present in the luminal contents, or adhering to the epithelial surface or inhabiting the depths of the crypts of Lieberkühn. The truly indigenous organisms are in close association with the gut wall; many of those in the luminal contents may only be transient, and of little concern to the host.

A great deal has been learned of the activities of the gut microflora from comparisons between germ-free and conventional animals (Coates & Gustafsson, 1984), and this review inevitably draws on the results of such experiments. However, since the indigenous microflora tends to be characteristic for each animal species the validity of transposing data from animals to man is open to question. In an attempt to circumvent this uncertainty, rats born germ-free have been associated soon after weaning with a suspension of human faeces. Biochemical and microbiological tests have shown that the flora becomes established and remains stable for at least several weeks (Mallett *et al.* 1987). Preliminary results suggest that the human-flora-associated rat may provide a better model for man than its ordinary conventional counterpart.

## PLANT CONSTITUENTS

### *Glycosides*

The earliest workers to demonstrate an influence of the microflora on the toxicity of non-nutrient constituents of food were Laqueur and her colleagues, who investigated cycasin, a glycoside present in the nuts of *Cycas circinalis* (Laqueur *et al.* 1963; Spatz *et al.* 1967). Cycasin given orally caused liver tumours in conventional rats but was harmless when administered parenterally, indicating that it was converted into a tumorigenic compound on passage through the alimentary canal. Since cycasin was also ineffective when fed to germ-free rats it was concluded that gut microorganisms effect the conversion. Many components of the gut microflora possess $\beta$-glucosidase (EC 3.2.1.21) activity, hence the cycasin aglycone, methylazoxymethanol, was proposed as the oncogenic metabolite and was shown to be highly tumorigenic in germ-free rats. Furthermore, rats monoassociated with a strain of lactobacillus capable of hydrolysing cycasin also developed tumours, establishing with reasonable certainty that the toxic effects of orally ingested cycasin result from its conversion by bacterial $\beta$-glucosidases into its aglycone.

The role of microorganisms in the metabolism of cyanogenic glycosides is less clear. Enzymes capable of hydrolysing the sugar residues from amygdalin and prunasin have been found in the kidneys, small intestine and intestinal contents of germ-free rats, and in

specimens of intestinal tissue from human subjects (Newmark *et al.* 1981). The LD50 of amygdalin after oral or parenteral administration is much lower in conventional than in germ-free mice (Williams, 1970), but the relative importance of the mammalian and microbial enzymes to the hydrolysis of cyanogenic glycosides is not known.

Flavonoids are polyphenolic glycosides that occur widely in edible plants, including citrus fruits, berries, root vegetables, cereals, pulses, tea and coffee. They are hydrolysed by bacteria in the saliva and the intestine to the respective aglycones of which quercetin, kaempferol and myricetin are the most common. Although antimicrobial properties have been attributed to the aglycones, there are no reports that they influence the gut microflora. The metabolic fate of the aglycones is a matter of concern since they exhibit mutagenicity in the Ames test, and there is conflicting evidence, largely negative, of oncogenicity in laboratory animals (Aeschbacher, 1982). In contrast, other experiments indicate an anticancer effect of flavonoids. For instance, several aglycones have been shown to inhibit proliferation of stimulated HeLa cells (Nishino *et al.* 1983) and administration of flavone acetic acid delayed tumour growth in mice implanted with adenocarcinoma (Corbett *et al.* 1986). The potential deleterious or prophylactic effects of ingestion of flavonoids seem unlikely to be of much significance to the consumer in view of the evidence from studies in vitro and in vivo (reviewed by Brown, 1988) of microbial C-ring fission of aglycones which, combined with rapid excretion, reduces the risk of exposure to their activities. Several components of the human intestinal flora capable of hydrolysing flavonoid glycosides or cleaving the aglycones have been isolated and identified (Bokkenheuser & Winter, 1989).

Stevioside is an intensely sweet glycoside found in the leaves of a South American plant, *Stevia rebaudiana*. As might be expected, incubation of stevioside with rat caecal bacteria results in formation of the aglycone, steviol (Wingard *et al.* 1980). After administration of an oral dose of the tritiated glycoside to rats it was metabolized and excreted within 72 h. Steviol was the main component in the faeces, and the presence of conjugates of steviol and other metabolites in the bile suggests that the aglycone enters the enterohepatic cycle (Nakayama *et al.* 1986). Pure steviol has been shown to be a bacterial mutagen (Pezzuto *et al.* 1985), although in long term toxicity studies rats fed on extracts of leaves did not develop tumours. Nevertheless the potential mutagenicity of its aglycone metabolite has prevented its approval for use as a sweetener in many countries including the United Kingdom.

*Lectins*

Lectins are proteinaceous substances occurring throughout the plant kingdom in seeds, bulbs, bark and leaves, where they act as defensive molecules against predators. The antinutritional properties of raw legume seeds, e.g. soya (*Glycine max*), kidney (navy) (*Phaseolus vulgaris*) and jack (*Canavalia ensiformis*) beans have long been known. The beans contain a trypsin inhibitor, and consequent impairment of protein digestion is partly responsible for the poor performance of animals fed on the raw beans, but it cannot account for the full severity of the effects (Liener, 1969). From early experiments with chicks, quail and rats it became apparent that the toxicity depends on the presence of the gut microflora. The growth of chicks on a diet of raw soya meal is severely depressed, but that of corresponding germ-free birds is almost normal (Coates *et al.* 1970). Diets containing high proportions of kidney or jack bean meals were lethal to conventional quail but relatively harmless to their germ-free counterparts (Jayne-Williams & Hewitt, 1972). The toxic effects of kidney beans were much less severe, and the digestibility of protein was more efficient, in germ-free compared with conventional rats (Rattray *et al.* 1974). In subsequent fractionations, different lectins were identified as the toxic principles of the beans. In particular, the phytohaemagglutinin (PHA) of kidney beans has been the subject

of extensive studies to elucidate the mechanism of the toxic action of lectins. Lectins are extremely resistant to proteolysis and can therefore survive passage through the alimentary tract. Some, including PHA, bind strongly to the brush border of the small intestinal epithelium, where they act as a metabolic signal which induces hyperplastic overgrowth of the small intestine (Pusztai *et al.* 1990*a*). The increased growth is preceded by an accumulation of polyamines, particularly spermidine (Bardocz *et al.* 1990), and accords with the reported increase in the fractional rate of protein synthesis in the mucosa after intubation of pure PHA (Pusztai *et al.* 1990*b*). These events may contribute to the antinutritional effects of lectins, since they lead to wastage of energy and body proteins. However, the full toxic syndrome is not realized unless the lectin enters the cell, and endocytosis only occurs if microorganisms are present. In the germ-free rat PHA binds to the brush border and causes overgrowth of the small intestine, but there is little or no endocytosis (Pusztai *et al.* in preparation).

The microbial ecology of the small intestine is considerably modified by ingestion of PHA, which induces an overgrowth of coliform organisms. A dramatic increase in non-haemolytic *Escherichia coli* was observed in rats given diets containing a variety of kidney bean with a high lectin content, but no such overgrowth occurred in animals given another cultivar with low lectin activity (Wilson *et al.* 1980). The proliferation of *E. coli* was preceded by disruption of the microvilli, and the authors suggest that consequent malabsorption of digesta together with the presence of cell debris and cellular exudates could provide a good substrate for bacterial growth. The properties of lectins and their interactions with the mucosal epithelium and the indigenous microbial population of the gut are discussed in detail by Pusztai *et al.* (1992).

Lectins bind to specific sugars of the epithelial membrane glycoconjugates. The fimbriae of bacteria which govern their attachment to the gut epithelium are also lectins (Sharon, 1987), raising the possibility that food lectins with sugar specificities similar to those of bacterial adhesins may prove effective blockers of microbial attachment to the mucosa. It would be of obvious advantage to the human and animal host if appropriate food lectins could be found to compete for the sites of attachment with harmful bacteria such as salmonella spp., thereby reducing their proliferation in the gut. This process has been termed 'chemical probiosis' (Pusztai *et al.* 1990*c*).

## NITRATES

Nitrate is present in substantial quantities in foods, either as an intentional additive for preservation of cured meats or to a much greater extent as a component of vegetables, notably celery, beetroot and lettuce, that accumulate the ion from nitrogenous fertilizers. It is also present in drinking water which in some areas contains undesirably high concentrations. Its potential toxicity depends on its reduction to nitrite which in sufficient quantity can induce methaemoglobinaemia, and which reacts with secondary and tertiary amines in the acid medium of the stomach to form carcinogenic *N*-nitrosamines (Bartsch & Montesano, 1984).

Microbial nitrate reducing activity has been detected in the gastrointestinal contents of man and laboratory animals. In man the most active site is the mouth, where oral bacteria reduce nitrate recirculated in the saliva (Spiegelhalder *et al.* 1976; Walters & Smith, 1981). The so-called 'blue baby' syndrome occurs in areas where the nitrate content of the drinking water is high because the stomach microflora of infants is rich in nitrate-reducing organisms. The condition is aggravated by the fact that the methaemoglobin reducing system is less efficient in babies than in adults (Green & Tannenbaum, 1982).

The existence of a mammalian nitrate reducing system has been established by experiments in germ-free rats, which developed methaemoglobinaemia after administration of nitrate for several days in the drinking water. Mucosal scrapings from the small intestine of germ-free rats exhibited considerable nitrate reducing activity which was heat labile, so presumably enzymic. Much less was detected in similar preparations from the stomach (Ward *et al.* 1986).

*N*-nitrosation occurs non-enzymically at low pH, so conditions in the stomach are conducive to nitrosamine formation from nitrite swallowed in saliva. Increased incidence of stomach cancers in some areas has been attributed to high concentrations of nitrate in the drinking water (Hill *et al.* 1973; Tannenbaum *et al.* 1979). Later epidemiological studies report the reverse effect (Beresford, 1985; Forman *et al.* 1985), but it has been suggested that other influences may have confounded these findings (Pocock, 1985). The gastric achlorhydria experienced by patients with pernicious anaemia or partial gastrectomy results in a higher stomach pH which does not favour *N*-nitrosation. They nevertheless have an increased risk of stomach neoplasms, probably because the increased pH permits colonization of the contents by nitrate reducing microorganisms (Bartholomew *et al.* 1980). An epidemiological finding associating a high carbohydrate diet with increased incidence of stomach cancer (Modan *et al.* 1977) could be similarly explained, since an excess of carbohydrate in the mouth and stomach might support increased numbers of nitrate reducing bacteria.

Nitrosamines are also formed in germ-free rats, but to a lesser extent than in their conventional counterparts (Ward *et al.* 1986). This might imply a direct microbial involvement in the reaction, since studies in vitro have shown that rat intestinal bacteria can catalyse the nitrosation reaction (Klubes *et al.* 1972). Alternatively or as well, it may be a reflection of the significantly lower gastrointestinal pH in the conventional rat (Ward & Coates, 1987*a*), which would enhance the formation of *N*-nitroso compounds.

Most nitrosamines are metabolized in the tissues but *N*-nitrosoproline (NPRO) is a stable nitrosated amine and its excretion in urine can be used as an indicator of *N*-nitrosamine formation (Ohshima *et al.* 1982). Inclusion of high amounts of fat in the diet greatly decreased the amounts of NPRO excreted, and, by implication, of *N*-nitrosamines formed, by conventional but not by germ-free rats (Ward & Coates, 1987*b*; Ward *et al.* 1990*a*). As the presence of fat affected neither the absorption of NPRO nor the intragastric nitrosation reaction it seemed likely that the decrease was due to inhibition of nitrate reducing ability. The effect was absent in germ-free rats indicating that the microbial rather than the mammalian reductase was implicated. This hypothesis was strengthened by the fact that nitrate reductase, which was absent from the stomach contents of germ-free rats, was much reduced in the stomachs of conventional rats given the high fat diets.

The inhibitory action was shared to a greater or lesser degree by all the fats tested, which included butterfat, maize, coconut, olive and safflower oils. The strength of the action could not be related to the fatty acid composition or the degree of their unsaturation, but butterfat was consistently significantly more inhibitory than any of the vegetable oils. In a small trial with human subjects high fat diets depressed excretion of NPRO but butterfat was not more effective than vegetable oils (Ward *et al.* 1988). In a subsequent comparison between butterfat and maize oil given to conventional rats and rats associated with a human flora all groups receiving the fat supplemented diets excreted less NPRO than corresponding groups on the low fat diet. As expected, butterfat caused the greatest reduction in the conventional rats but, in accord with the findings in human subjects, it was no more effective than maize oil in the human-flora-associated rats (Ward *et al.* 1990*a*). The mechanism of the inhibitory effect of fats on nitrosamine formation has not been elucidated, but it appears to be due to suppression of microbial reduction of nitrate. The

stomach is the main site of nitrate reduction and nitrosation in the rat, but in man the stomach is virtually sterile and the oral cavity is a more important site of nitrate reductase activity. It may be that the responsible organisms in the rat, or the conditions under which they act, are more susceptible than those of man to interference by butterfat. Whatever the explanation, these findings emphasize the uncertainty of extrapolating data from laboratory animals too closely to man.

The body content of nitrate is not solely dependent on dietary intake. The existence of an endogenous non-microbial system for nitrate synthesis was established when germ-free rats were shown to excrete more nitrate than could be accounted for by amounts taken in air, food and water (Green *et al.* 1981). Dietary protein appears to be an important source of nitrogen for nitrate synthesis, since rats given diets containing 200 g/kg casein excreted two or three times as much nitrate as their counterparts receiving only 50 g casein/kg (Ward *et al.* 1989). Nitrate excretion by germ-free rats is generally higher than that of conventional controls (Witter *et al.* 1982; Ward *et al.* 1986, 1989). This implies that microorganisms are not involved in the synthesis but instead suggests that some of the synthesized nitrate or its precursors are metabolized by the gut microflora. A number of anaerobic bacteria are known to be capable of incorporating nitrate nitrogen into microbial amino acids (Payne 1973). Although microorganisms seem not be directly involved in nitrate synthesis they may have an indirect influence as a result of toxin production. In febrile conditions nitrate synthesis is increased through the activity of immunostimulated macrophages (Stuer & Marletta, 1985, 1987; Miwa *et al.* 1987). Injection of *E. coli* endotoxin causes a similar increase in nitrate synthesis both in germ-free and conventional rats (Nielsch *et al.* 1991).

Much concern has been expressed about the potentially harmful effects of exogenous nitrate ingested in vegetables and tap water, but it is clear from the studies quoted above that endogenously synthesized nitrate makes an important contribution to the total body pool. It is also apparent that the composition of the diet influences the amount of nitrate formed as well as the subsequent conversion of exogenous and recycled endogenous nitrate into nitrosamines. The main activity of the gut microflora appears to favour nitrosamine formation by providing a source of nitrate reductase, but it also has a reverse effect by utilizing some of the available nitrate.

## MUTAGENS

The cooking and processing of meat and fish at high temperatures gives rise to compounds, all heterocyclic amines, with mutagenic and carcinogenic activities (Sugimura & Sato, 1983). Several of these have been shown to exert strong mutagenicity in the Ames test with *Salmonella typhimurium* TA98 and carcinogenicity in laboratory rodents (Sugimura, 1985). In particular, IQ (2-amino-3-methylimidazo(4,5-f)quinoline) and MeIQ, its methyl derivative, are extremely potent. These substances are indirect mutagens, i.e. they require an activation system to convert them into the product directly responsible for the mutagenicity. Liver enzymes of the cytochrome P450 series effectively perform the activation. The livers of conventional rats are heavier, and the activities of several hepatic enzymes are greater, than those of their germ-free counterparts (Reddy *et al.* 1973), which raises the possibility that the presence of the gut microflora may influence the activation of food mutagens.

To explore this possibility, liver fractions from germ-free and conventional rats were examined for their ability to activate aflatoxin $B_1$, MeIQ and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) in bacterial mutation assays with *S. typhimurium*. No

difference in the activation of aflatoxin was observed, but significantly greater activation of MeIQ and PhIP occurred when germ-free liver fractions were used. However, there was no difference between germ-free and conventional liver fractions in the specific activity of cytochrome P450 activity. No differences were found in the specific activities of several other hepatic enzymes except for glucuronyl transferase, which was significantly lower in fractions from germ-free rats (Ward *et al.* 1990*b*). The greater capacity of the germ-free livers to activate these two compounds suggests that the presence of a gut microflora indirectly protects the host against their mutagenic effects, but there is as yet no explanation of the mechanism of this protection.

The influence of the gut microflora on the metabolism of heterocyclic amines was investigated by Rafter & Gustafsson (1986) using $^{14}$C-labelled Trp-P-1 (3-amino-1,4 dimethyl-5H-pyrido (4,3-b)indole), a tryptophan pyrolysis product present in various foods. A single oral dose of the compound given to groups of germ-free and conventional intact and bile-fistulated rats was rapidly excreted in urine, faeces and bile, with a similar pattern of metabolites in all the groups. Although the gut microflora did not appear to affect the metabolism of Trp-P-1 the radioactive residues in the faeces of conventional rats were strongly bound to the faecal mass, whereas they were readily extractable in water or methanol from the faeces of germ-free rats. The metabolism of 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline), another heterocyclic amine found in cooked meat products, was also largely unaffected by the presence of the intestinal microflora. Faecal excretion was much slower in germ-free rats, due no doubt to the longer transit time characteristic of germ-free animals, but the metabolites were similar in urine and faeces of rats in both environments. Some of the rats in this study were treated with $\beta$-naphthoflavone (BNF) which induces the cytochrome P450 proteins in animal tissues. The induction of intestinal enzymes was 6 × greater in conventional compared with germ-free rats. Induction caused increased faecal excretion in both environments and, as in the previous study, a greater proportion of the residues in the conventional rats was bound to the faecal mass (Knize *et al.* 1989).

In contrast to the findings of no microbial influence on the metabolism of Trp-P-1 or DiMeIQx in rats, experiments in vitro have shown IQ to be converted by human intestinal bacteria into its 7-keto derivative, 7-OHIQ (Bashir *et al.* 1987), which is a direct acting mutagen. The derivative was also found in the faeces of human subjects after ingestion of a meal of fried meat (Carman *et al.* 1988). Comparisons in vitro showed that caecal contents of mice and rats converted IQ to 7-OHIQ more rapidly than human faecal samples. Similarly, rats associated with a human flora effected the conversion at a lower rate than rats with their indigenous microflora. Human-flora-associated rats were used to investigate the effects of diet on the conversion of IQ, which was increased when the diet included a high supplement of beef dripping, but not when similarly supplemented with olive oil. Addition of several different types of fibre at the rate of 100 g/kg also increased the conversion to 7-OHIQ. In further studies with human diets, those containing high proportions of fat or fibre again supported high rates of conversion of IQ to its directly mutagenic derivative (Rumney *et al.* in preparation).

These findings, particularly as they were made in rats associated with a human flora, suggest that in spite of the undoubtedly beneficial effects of dietary fibre in human diets, immoderate consumption should be avoided. Fermentable fibres have been shown to cause proliferation of the mucosal epithelial cells of the colon in conventional but not in germ-free rats (Goodlad *et al.* 1989, 1990), a condition that has been linked to carcinogenesis in animals. The implication is that products of microbial fermentation of fibre are responsible, and it may be that such products provide a suitable medium for the conversion of potential mutagens into their active metabolites.

## ARTIFICIAL SWEETENERS

Interactions between the gut microflora and the artificial sweeteners saccharin and cyclamate, as well as stevioside (discussed above), have been noted. The effects of saccharin are only observed at very high levels of intake, and they constitute changes in metabolic activity rather than in composition of the flora (see review by Renwick, 1988). Inhibition of $\beta$-glucuronidase activity and of proteolysis, accompanied by accumulation of carbohydrates and proteins, leads to enlargement of the caecum in laboratory rats. Although these effects may confuse the interpretation of toxicological studies they are of little or no significance to the consumer within the acceptable daily intake.

Cyclamates (sodium or calcium cyclohexanesulphamate) are metabolized by the gut microflora to cyclohexylamine which is considerably more toxic than the parent compound, although early claims that it is carcinogenic have not been upheld by international organizations (Bopp *et al.* 1986). The appearance of cyclohexylamine in the faeces of man and several species of animals given cyclamates orally, and its suppression following administration of a variety of antibiotics, has been well documented (Collings, 1971; Bickel *et al.* 1974 and others). Several strains of bacteria are capable of converting cyclamates into cyclohexylamine (Renwick, 1988), but induction of the process varies widely among individuals. Chronic administration of cyclamate appears to be necessary to induce the reaction, but the metabolizing activity both in rats and in human volunteers can vary from being undetectable to converting more than half of an administered dose (Renwick, 1983). Diurnal variation in individual subjects may also vary widely. This variability is surprising in view of the number of strains of intestinal organisms capable of effecting the reaction. It may be that a particular combination of organisms or concentration of substrate is necessary, or that further metabolism of cyclohexylamine occurs at different rates in different individuals and at different times.

## METALS

Although toxic metallic compounds are known contaminants of foods the interrelationships with the gut microflora have only been investigated in the case of mercury and arsenic.

### *Mercury compounds*

Mercuric salts are ingested in very low amounts from food and beverages, but are poorly absorbed. In contrast methyl mercury (MeHg) is very efficiently absorbed and exerts highly toxic effects, particularly on the central nervous system. Its main source in human food is fish, which accumulate considerable quantities of the compound particularly if caught from mercury-polluted waters.

Incubation of MeHg in vitro with suspensions of human faeces or gut contents from laboratory mice results in its demethylation to elemental mercury (Rowland *et al.* 1983). Demethylation occurred much more rapidly in gut contents from adult mice than in those from mice being suckled, and when mice were retained on a milk diet beyond the normal weaning time the rate of demethylation remained slow. A similar phenomenon was observed in human subjects. Faecal suspensions from infants being suckled demethylated MeHg much more slowly than those from older children, and faeces from children aged 10 months still maintained on a milk diet also showed a lower rate of demethylation than those from children of similar age consuming solid diets. A major change in the composition of the gut microflora occurs at weaning and these findings suggest that organisms capable of demethylation are more prevalent in the gut microflora of adults than of sucklings. They

also imply that unweaned infants are more susceptible to the toxic effects of MeHg than adults.

*Arsenic*

Inorganic forms of arsenic occur in drinking water and wine whereas organic arsenicals are present in fish and shellfish. Although incubation of sodium arsenate with rat caecal contents in vitro resulted in its reduction and subsequent methylation (Rowland & Davies, 1981), there is no evidence to suggest that microbial activity is important in the metabolism of arsenic in vivo. Comparisons in germ-free and conventional rats showed no difference in the extent of methylation of orally administered arsenate (Vahter & Gustafsson, 1980). According to Rowland & Davies (1982) the rate of the reaction in vivo is extremely rapid whether the arsenate is given by mouth or intravenous injection, which makes any involvement of the gut microflora highly unlikely.

## IMPLICATIONS AND PERSPECTIVES

The existence of a massive microbial population in the gastrointestinal tract, and its metabolic potential, have long been recognized. Nevertheless, the presence of this microbial burden has largely been taken for granted, probably because its influence on the nutrition of a healthy simple-stomached host eating an adequate diet is minimal. As evidence accumulates of interactions, for better or worse, between the gut microflora and non-nutrient components of the diet the need to recognize and understand the microbial contribution becomes imperative. Interest has been particularly aroused since a variety of food additives, contaminants and natural non-nutrient constituents of foods have been implicated in the aetiology of human cancers. Frequently the oncogenic agent is a microbial metabolite rather than the parent compound, e.g. the aglycone of cycasin and the derivatives of the promutagen imidazoquinolines. Conversely, microbial activity can be beneficial, as evidenced by the demethylation of MeHG, and the (postulated) anticancer effect of the flavonoid aglycones.

Once the role of the gut microflora is recognized, even if its mechanism is not fully understood, the logical procedure would seem to be an attempt to modify its composition or metabolism to the advantage of the host. Although in general dietary changes do not have a profound effect on the indigenous flora they may nevertheless induce some metabolic modifications. The inhibition of *N*-nitrosamine formation by high supplements of fat in the diet is obviously mediated through a change in microbial activity since it occurs in rats and human subjects with a gut microflora but not in germ-free rats. The major change in composition of the microflora that occurs when infants are weaned from a milk diet to solid food is concurrent with an increase in bacterial demethylation of toxic MeHg.

Another possible advantage from manipulation of an interaction between gut organisms and food constituents is evident from research on food lectins. Their use as competitors with certain pathogens has great potential in human and animal nutrition. Inclusion of such compounds in feeds for farm animals, especially poultry, could reduce the risk of salmonella infection in the livestock or birds and consequently ensure safer meat products for human consumption. If chemical probiosis could take the place of antibiotic supplements for growth promotion the danger of emergence of antibiotic-resistant strains of pathogens attendant upon the present widespread use of antibiotics in animal feeds would be largely eliminated.

In view of the wide-reaching nutritional and toxicological implications of the interrelationships between dietary components, both nutrient and non-nutrient, and the

gut microflora there is a compelling need for a multidisciplinary approach to obtain a clearer insight into the complex of interactions between host, gut microorganisms and dietary chemicals.

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