DIETARY FIBRE IN THE PREVENTION OF COLORECTAL CANCER: LESSONS FROM STUDIES IN ANIMAL MODELS

Graeme P. Young

Summary

While there is good evidence that dietary fibre protects against large bowel cancer, the types of fibre and mechanisms responsible are not clear. It has been suggested that characteristics of fibre, both dependent on and independent of microbial fermentation, are important. Studies in animal models show that fibres which are rapidly and completely fermented, e.g. guar gum and pectin, are not usually protective and do not influence the distal colonic environment, where tumours are most common, to a great degree. Even though such fibres can increase faecal bulk, they are not protective. Slowly fermentable and nonfermentable fibres are protective, however. The physical presence of unfermented fibre in distal colon during the initiation phase of carcinogenesis protects against cancer. The presence of fermentation products in distal colon, especially butyrate, also appears to have a protective effect. Such can only be achieved by feeding slowly fermentable fibres. While it is not yet certain at which phase in tumorigenesis this effect of butyrate occurs, it seems likely to be a direct effect of butyrate on neoplastic cells, rather than interaction with epithelia during initiation. Butyrate is known to interact with nuclear histones and so it has the potential to directly influence the genetic events of tumorigenesis - this might provide a good model for studying gene-environmental interactions in bowel cancer.

I. INTRODUCTION

The primary cause of large bowel cancer is unknown, although critical genetic changes (Winawer and Sherlock 1983; Willson 1989) play a role as well as environmental influences (Wynder et al. 1969; Burkitt 1973). Diet appears to be a major environmental influence and, given that the genetic make-up of cells throughout the large bowel is identical, it may be that environmental factors such as diet account for the regional distribution of cancers (Schottenfeld and Has 1978; Freeman et al. 1978). Many but not all epidemiological studies suggest that diets high in fibre can protect against the development of large bowel cancer (Kune et al. 1987; Cummings and Bingham 1987; Wargovich et al. 1988; Vogel and McPherson 1989). While there are various views on the mechanism responsible for this protective effect, the actual mechanisms responsible remain uncertain. Furthermore, in experimental studies using specific fibres in carefully defined diets, the effects of fibre have been variable (see below). The confusion surrounding both the protective value and the mechanism of action of dietary fibre is probably due to a number of factors: dietary "fibre" is physically and chemically heterogeneous; the degree to which fibre is fermented varies with its type and physical state; and the balance of fermentation products vary with each fibre (Cummings 1981; Mortensen et al. 1988; Fleming et al. 1989).

II. PROTECTIVE MECHANISMS OF DIETARY FIBRE

Fibre has various effects on intestinal physiology and the large bowel luminal environment. It increases transit rate (Jacobs and Lupton, 1986) and bulk and so dilutes constituents, modifies intestinal flora and so alters bile salt and carcinogen metabolism (Gregoire et al. 1989), adsorbs carcinogens and mutagens (see Jacobs 1986), decreases faecal bile salt excretion (Jacobs 1986), lowers colonic pH, and increases colonic and faecal short chain fatty (SCFA) concentrations (Fleming et al. 1989). The relative importance of these mechanisms is uncertain. Furthermore, because dietary fibres are so diverse in their physicochemical characteristics, it seems probable that different fibres will

University of Melbourne Dept. of Medicine, The Royal Melbourne Hospital, VIC 3050.

act by different mechanisms.

One property which varies between fibres is fermentability. Some fibres are fermented by anaerobic bacteria producing energy, H_2, CO_2, CH_4, lactate and SCFAs (principally acetate, proprionate, and butyrate) (Cummings et al. 1987). This fermentation process provides nutrients for the epithelium, principally as butyrate (Roediger 1982), modifies the luminal environment, e.g. changing pH, and results in a breakdown of the fibre itself. It other words, fermentation might both generate and destroy important protective features of fibre.

(a) Mechanisms by which dietary factors influence tumorigenesis

It appears likely that dietary factors can have a direct or indirect effect on tumorigenesis. *Direct* effects might be as the initiating carcinogen, or as a tumour promoter or suppressor acting directly on the cells. The mechanisms responsible for *indirect* effects are uncertain, but a popular view at present is that they occur via an effect on the luminal environment. Conditions such as pH, concentrations of calcium or SCFAs, bacterial flora and metabolic activity, bile acids and nitrogenous products, are modulated by changes in the diet. Each has been implicated (by inference or postulate) as being important. There are various examples of these indirect mechanisms. Fat is thought by some to promote tumorigenesis by increasing bile salt excretion; bacterial metabolism creates toxic derivatives such as secondary bile acids (Gregoire et al. 1989; Friedman et al. 1989). Bacteria also activate carcinogens (see Freeman 1986); the feeding of an inhibitor of bacterial β-glycosidases, or suppression of anaerobic bacteria by antibiotics, change cancer risk. Nitrogenous products resulting from bacterial action might also be important, fibre modifies such products (Cummings and Bingham 1987). Calcium, on the other hand, has been postulated as an important protective factor (Gregoire et al. 1989). It binds bile salts, modifies pH and so might protect against enhancement of tumorigenesis by dietary fats.

(b) Overview of the inter-relationships between luminal events and tumorigenesis

Dietary factors (not being carcinogens themselves) might act either early or late in tumorigenesis:

a) *During the initiation phase of carcinoma when a dietary component might "sensitise" or "protect" mucosa to the carcinogen.* It is generally supposed that this is achieved via an effect on proliferation since proliferative activity of epithelium is considered to increase its susceptibility to malignant transformation (Craven and DeRubertis 1988). A convenient model can be constructed (Fig. 1) which is based on the assumption that the effect of a dietary factor is dependent on its influence on proliferation of epithelium exposed to the initiating agent (Reddy et al. 1981; Craven and DeRubertis 1988; Gregoire et al. 1989; Friedman et al. 1989).

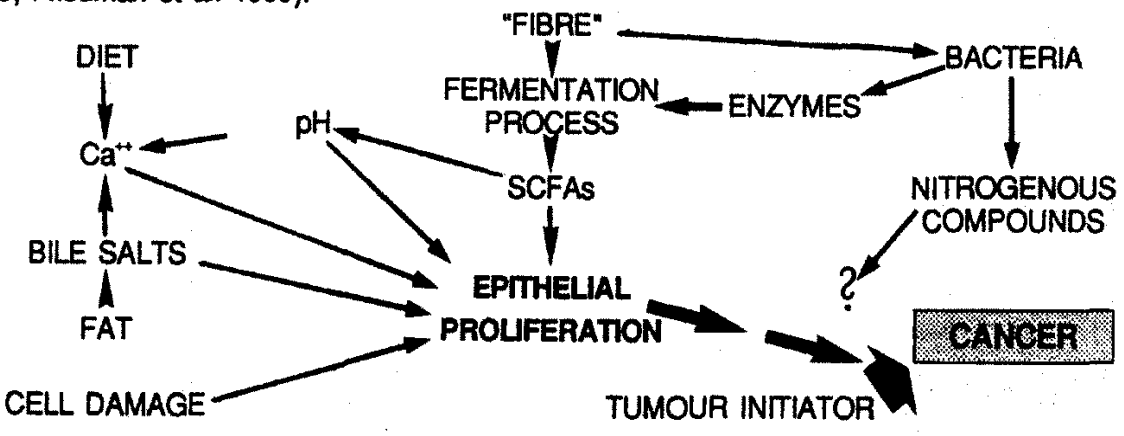

Fig. 1: Inter-relationships between luminal events, epithelial proliferation and cancer.

It is difficult to modulate just one of these factors without directly or indirectly affecting others. The effect of any substance will almost certainly be influenced by the sum total of effects of other elements. A number of studies demonstrate the complexity of

changes resulting from a single change in the diet, e.g. changing the type of fibre affects stool bulk, pH, and concentrations of SCFAs, enzymes and Ca^{++}. Whether such a model accounts for all effects of diet is questionable, however.

b) *During the postinitiation phase as a tumour "promoter" or "suppressor" when the agent acts on committed preneoplastic cells or frankly neoplastic cells* (Reddy et al. 1981; Friedman et al. 1989). In this way, environmental factors might influence tumorigenesis independently of an effect on epithelial proliferation. There are at least two examples of this. Certain diglycerides can act as tumour promoters *in vitro*, yet they do not affect normal epithelium (Friedman et al. 1989). Butyrate is a tumour "suppressor" *in vitro*, and possibly *in vivo*, yet it has opposite effects on normal epithelium (see below).

III. FIBRE FERMENTABILITY AND BOWEL CANCER

Dietary fibres can be broadly classified (Kay et al. 1978) into three groups:
a) Highly fermentable, e.g. guar gum, oat bran, pectin.
b) Slowly fermentable, e.g. most wheat brans
c) Poorly or non-fermentable, e.g. most celluloses, lignin.

Fermentability in this context relates to the degree to which a fibre is broken down and not detected in the faeces. This is usually paralleled by production of SCFAs from the fibre, but the degree of SCFA production does not necessarily correlate with fermentability, e.g. pectin is totally digested in rat large bowel, but has little impact on pH in SCFA concentrations (Thomsen et al. 1984).

Many dietary fibres are not pure. Wheat bran consists of cellulose, hemicellulose, lignins and small amounts of other fibres (Kay et al. 1978) and so is generally found to be slowly fermentable, although this also depends on its physical nature. This complicates the study of dietary fibres since different sources of the same fibre will possibly have different characteristics. Thus, some researchers evaluate purified fibres for their protective effect, but this, in turn, has the disadvantage of being an artificial situation relative to what is consumed in the typical diet.

TABLE I: Summary of number of published reports on effects of dietary fibres on tumorigenesis in rat models of large bowel cancer (see Jacobs 1986; Cameron et al 1990).

Fibre type	Effect on tumorigenesis			Total
	Protective	Equivocal	Enhanced	number
Poorly Fermentable				
Cellulose	8*	3	0	11
Lignin	2	0	0	2
Slowly Fermentable				
Wheat bran	7	9	0	16
Highly Fermentable				
Guar	0	2	1	3
Pectin	0	2	3	5
Oat bran	0	0	1	1

* Number of publications.

Various human studies, particularly case-control studies, have attempted to relate type of dietary fibre to cancer (e.g. Zaridze 1983; Kune et al. 1987). The conclusions reached have often been conflicting and of uncertain relevance (Wargovich et al. 1988). In attempts to be more specific, and to evaluate specific fibres in a more definitive fashion, the protective effect of various fibres have been studied in animal models of bowel cancer. The vast majority of these studies have used the rat and the carcinogen 1,2-dimethylhydrazine (DMH) or its metabolic derivative azoxymethane. Table I summarises

the results of these studies, the material being drawn from two reviews (Jacobs 1986; Cameron et al. 1990) and certain other recent publications. Fibre in the form of cruciferous vegetables has not been studied in the animal models for practical reasons. While the summary presented in Table I does not take into account all of the subtle and not-so-subtle differences between the studies, it is apparent that slowly fermentable and poorly fermentable fibres are generally protective, while highly fermentable fibres are not, and may indeed promote tumorigenesis.

(a) Protective effects of fermentation products

Despite this body of evidence (Table I) that suggests that fermentability *per se* is not protective, it has been proposed that both lowered pH (Thornton 1981) and increased butyrate (see below), both consequences of fermentation, are protective.

The evidence that a low pH is protective is largely circumstantial. Walker and co-workers (Walker et al. 1986), when studying four South African populations, showed that faecal pH was highest in those with a high incidence of cancer and lowest in those with a low incidence. pH directly influences the growth of cells in culture and it modifies the degradation of bile salts (Cummings and Bingham 1987). However, there is evidence that pH is not important. Cameron and co-workers (Cameron 1990) have not shown a relationship between luminal pH and cancer incidence in careful studies of a rat model of bowel cancer.

Butyrate, which is an important energy source for colonocytes (30) being metabolised in preference to glucose and other substrates, is of particular interest as this SCFA brings about a concentration-dependent slowing of the rate of cancer cell proliferation (10,11). At concentrations equivalent to those encountered in the colon, butyrate increases by 100% the time taken for LIM1215 cells grown in monolayer culture, to double in number (Whitehead et al. 1986). Furthermore, it promotes expression of a differentiated phenotype *in vitro*. Alkaline phosphatase and dipeptidylpeptidase-IV, both markers of colonocyte differentiation, are increased during culture of LIM1215 cells in the presence of butyrate (Whitehead et al. 1987). Many workers believe that these effects on the differentiated phenotype are at least as important as effects on proliferation. Neither acetate or propionate shared the effects of butyrate. As different amounts of butyrate are produced during fermentation of different types of fibre (McBurney and Thompson 1987), this might account, at least in part, for the conflicting results with respect to the protective effect of "fibre" on large bowel cancer.

In addition, butyrate is unique amongst the SCFAs in that it acts in a number of cell types to stabilise DNA via effects on histone deacetylase (Candido et al. 1978). Post-translational modification of histones by acetylation is one mechanism for modification of gene expression. Thus butyrate has the potential to interact with genetic material. Apart from restricted studies of a few putative colonic carcinogens in the diet, there have been no attempts to examine the influence of dietary factors on genetic events *in vivo*.

(b) Sites of butyrate production in the large bowel

Before testing the real protective capacity of butyrate, however, there has been a need to identify the types of fibre which result in production of substantial luminal concentrations of butyrate and which sustain concentrations of butyrate along the length of the large bowel. This is particularly important as colorectal cancer is more common in the distal rather than the proximal large bowel (Schottenfeld and Has 1978) and fermentation is normally most active in the proximal large bowel (Cummings et al. 1987). Thus we performed a study in which we manipulated SCFA concentrations in the rat colon by providing different substrates for bacterial fermentation in the form of four different fibre-containing diets: a low fibre diet containing 2% residual fibre (basic diet), a diet containing 10% guar gum, one with 10% oat bran, and one with 10% wheat bran (McIntyre et al. 1990a). A number of luminal environmental variables having possible modulatory effects on colorectal tumorigenesis were measured with the specific aim of identifying a diet which elevated concentrations of butyrate in the distal large bowel.

Short chain fatty acids showed a falling gradient along the large bowel with the low

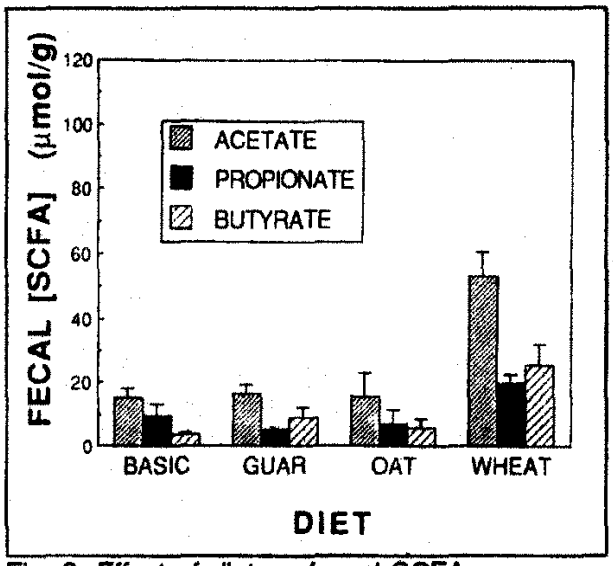

Fig. 2: Effect of diet on faecal SCFAs.

fibre, guar and oat bran diets. None of these diets lowered distal colonic pH. Wheat bran, however, maintained total short chain fatty acid levels in fresh faeces at three times the levels seen with the other diets; faecal butyrate concentrations were maintained at high caecal values in distal large bowel (Fig. 1), and faecal pH was maintained at the low levels seen in caecal contents. Thus, dietary fibres have differing effects on different regions of the luminal environment depending on their fermentability.

Highly fermented fibres (e.g. guar, oat bran), which are largely fermented in the proximal large bowel, have little effect on distal bowel environment, while a more slowly fermented fibre source such as wheat bran, which has been reported to be only 30% digested by caecal bacteria (Kay and Strasberg 1978), can influence the environment along the length of the large bowel. Perhaps the site of fermentation is much more important than fermentability itself - this could account for the failure of highly fermentable fibres to protect against bowel cancer.

(c) Site of fermentation relative to tumour production

To pursue the importance of the site of fermentation, we examined the antitumour effects of the three fibre-containing diets referred to above in the rat-DMH model (McIntyre et al. 1990b). The incidence of tumours was examined in relation to the effect of each fibre on various components of the distal colonic luminal environment, as reflected in faeces.

To induce tumours, we treated four-week old Sprague-Dawley rats with DMH by i.p. injection, 30mg/kg/week for 10 weeks. The distribution and histological nature of tumours in this model are similar to findings in humans, and the time-course of events (hyperproliferation, dysplasia progressing through polypoid lesions to frank cancer) (Heitman et al. 1983) has major parallels with the dysplasia-cancer sequence proposed in humans. Also, alterations in crypt cell kinetics are similar (Willson 1989). To assess tumour production, the LB is removed, opened lengthwise, rinsed and examined under a dissecting microscope. Tumours are counted and site recorded; from the product of two transverse dimensions, a tumour size index (TSI) is computed. The TSI for a rat is the sum of individual TSIs; log-TSIs are distributed normally.

TABLE II: Influence of dietary fibre (10%) on distal colonic luminal environment, faecal weight and tumorigenesis in rats treated with 1,2-dimethylhydrazine.

"Fibre"	Fermentability	pH	Acetate	Propionate	Butyrate	24h-Weight	Tumour #	p*
None	Control	6.9±0.1	17±2	8±2	3±1	1.8±0.1	3.53±0.60	NS
Guar	High	6.6±0.1	20±3	11±3	7±1	4.3±0.3	4.79±0.90	0.05
Oat	High	6.5±0.2	21±4	12±3	6±2	1.9±0.1	4.58±0.98	0.04
Wheat	Medium	6.0±0.1	41±5	16±2	15±3	4.0±0.2	2.92±0.44	-

* P values refer to comparison of numbers between wheat-bran and the specified diet.

Table II summarises *faecal* parameters and tumour numbers and shows that tumour incidence was affected by dietary fibre. Changes in activities of the bacterial enzymes, β-glucuronidase and β-glucosidase, also occurred with changes in fibre, indicating how complex alterations in the luminal environment can be, in response to a change in a single ingredient. The highest faecal butyrate levels occurred with wheat bran as expected ($p<0.01$); this fibre also gave the lowest tumour incidence. Multiple regression analysis of the effect of all faecal parameters on tumour incidence as measured by TSI, showed that

only butyrate had a significant effect (P=0.043), while the effects of pH, other SCFAs, stool weight, and bacterial glycosidases, were not. Neither pH nor stool weight, both of which were affected by diet, correlated significantly with tumour development, agreeing with the findings of others (Cameron et al. 1990).

This constitutes the most direct evidence that the ability of a fibre to produce butyrate in distal large bowel points to a protective effect of the fibre.

IV. PHYSICAL EFFECTS OF FIBRE

Despite this body of evidence implicating butyrate there is also evidence suggesting that the mere physical presence of fibre is beneficial.

It has been shown that a purified nonfermentable cellulose leads to increased crypt cellularity, but reduced cell proliferation in rat large bowel - in other words it reduces cell turnover rate (Cameron et al. 1989). Such cellulose also reduces tumorigenesis in the DMH model. Table III shows the results of a similar study to that reported in Table II, in which rats received either a low fibre diet, a 20% cellulose diet, a 20% wheat bran diet, or a diet containing 20% kaolin as inert bulk. What is apparent from these studies is that the physical presence of fibre in the distal colon is as protective as the presence of butyrate-producing fibre, although inert bulk is not in itself adequate.

TABLE III: Influence of dietary fibre (20%) or bulk on distal colonic luminal environment, faecal weight and tumorigenesis in rats treated with 1,2-dimethylhydrazine.

"Fibre"	Fermentability	pH	Acetate	Propionate	Butyrate	24h-Weight	Tumour #	p
None	Control	6.8±0.2	18±2	11±3	5±1	0.7±0.1	2.55±0.28	0.01
Kaolin	Inert	7.6±0.1	13±2	4±1	2±1	3.8±0.2	1.86±0.34	NS
Cellulose	Low	7.4±0.1	8±1	3±1	1±1	4.8±0.6	1.60±0.40	NS
Wheat	Medium	5.9±0.2	49±7	22±2	29±11	3.4±0.2	1.38±0.33	-

* P values refer to comparison of numbers between wheat-bran and the specified diet.

Whether cellulose acts because it alters patterns of faecal bile salt excretion or because it adsorbs and so inactivates carcinogens (be they secondary bile salts or otherwise) is not clear. It seems likely, however, that it is not merely due to high stool bulk - guar gum produces the biggest and most liquid stools, yet does not protect against cancer (see Table I).

V. STAGE OF TUMORIGENESIS

The genesis of LB cancer involves a multistep process (Willson 1989) pathophysiologically manifest as: 1) the preneoplastic stage (hyperproliferative epithelium); 2) the precancerous stage (adenomas of varying size, villosity and degree of dysplasia); and 3) the cancerous stages (ACPS stages A to D). There is now increasing evidence for multiple genetic events. Deletions on chromosome 5, DNA hypomethylation and expression of the *ras* oncogene are considered important in the early stages, while deletions on chromosomes 17 and 18 occur commonly later in tumorigenesis (Vogelstein et al. 1988). The factors controlling progression from one stage to another are not clear (Willson 1989), although environmental factors such as those in the diet are thought likely to interact with genetic events.

The DMH model provides the opportunity to determine the most critical timepoint at which dietary factors are required, but little use has been made of this to date. Cameron *et al* (1989) have shown that cellulose is effective at suppressing tumorigenesis when given only during the initiation period (i.e. concomitant with DMH) consistent with it interacting directly with the carcinogen. In contrast, some wheat brans when given only during initiation seem to promote tumorigenesis (Jacobs and Lupton 1986); this observation still needs to be reconciled with frequent findings that wheat bran suppresses tumorigenesis when given throughout the animals' life-time (Tables I and II).

The *in vitro* studies with butyrate referred to above clearly show that it suppresses

proliferation of cancer cells, but it is not known if it influences adenoma cells (pre-invasive neoplastic cells), or preneoplastic hyperplastic epithelium. We have recently performed some in vitro studies using normal colonocytes isolated from patients undergoing colectomy, with a view to testing the effect of butyrate on phenotypic markers of differentiation (Gibson et al. 1990). The results were compared with those of two cancer cell lines, LIM1215 and LIM1863 (from Dr R Whitehead at the Ludwig Institute). This confirmed that butyrate caused expression of a more differentiated phenotype in the cancer cells but, surprisingly, there was a reduction in expression of differentiation markers (glycoprotein synthesis, alkaline phosphatase, and dipeptidylpeptidase-IV) in normal cells. To further substantiate the apparently opposite effects of butyrate on cancer and normal cells, Sakata et al have shown that butyrate stimulates proliferation in normal epithelium (Sakata and Engelhardt 1983).

VI. CONCLUSIONS

These studies in animal models clearly demonstrate that dietary fibre can protect against large bowel cancer provided that the fibre is not completely fermented in the caecal region. The mechanisms by which fibre suppresses tumorigenesis is still not clear, but the evidence favours a multiplicity of actions. There is increasing evidence that the physical presence of fibre during tumour initiation is important. Fermentative production of butyrate also appears likely to be important, although its presence during initiation might not be necessary. How these aspects of fibre physiology interact with the genetic events of tumorigenesis is unknown, but butyrate has the potential to directly modulate gene expression.

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