

Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer

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In the UK all age incidence rates for large bowel cancer are second only to lung cancer in men, and breast cancer in women. Rates increase with age, from twelve cases per 100 000 in men aged 40–45 years, rising to 110 at age 60–65 years, and to 419 at age 80–85 years in England and Wales. Age-standardized rates are even higher in areas such as Australia, USA, New Zealand and parts of Europe such as North Italy, Denmark and Germany. Low rates are seen in areas such as parts of South America, rural Africa, China, India and Kuwait. In Japan, colon cancer incidence has at least doubled since 1960 and Fig. 1 shows that rates for colo-rectal cancer continuously recorded by the Miyagi Cancer Registry are fast approaching those recorded by the Birmingham registry in Britain (Muir *et al.* 1987).

Migrant studies have suggested that these international differences are largely due to environmental factors, although within any one population, genetic factors will affect individual susceptibility to the development of cancer. A mutation common to the same gene on chromosome 5 has been involved in both the rare genetically inherited disease familial adenomatous polyposis, which markedly increases individual risk of colo-rectal cancer, and the ‘sporadic’ form of colo-rectal cancer which results in somatic mutation

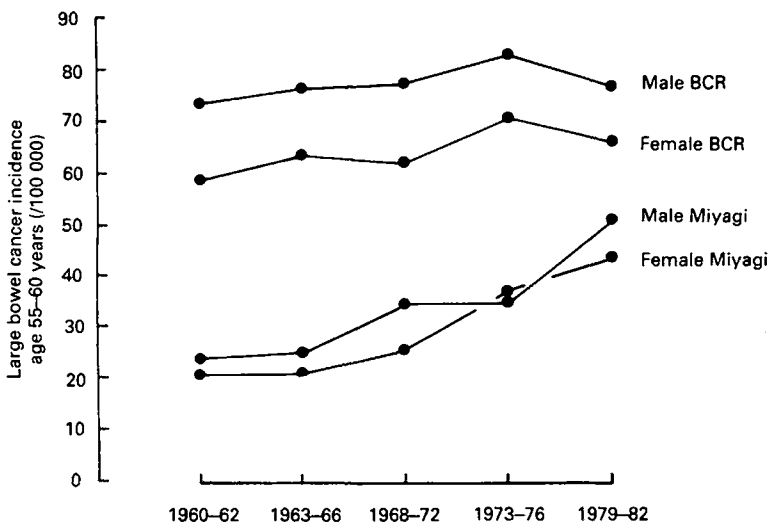


Fig. 1. Age-specific colon and rectal cancer incidence 1960–1982; England, Birmingham Cancer Registry (BCR); Japan, Miyagi Cancer Registry (Muir *et al.* 1987).

and is presumed to be subject to environmental influences (Solomon *et al.* 1987). Other allelic deletions are also implicated in sporadic cancer, for example, on chromosome 17 (Baker *et al.* 1989).

Large bowel cancer incidence rates also vary amongst different groups within any one population, rates for vegetarian Seventh Day Adventists, for example, being much lower than those of the general USA population (Phillips & Snowdon, 1983). Higginson & Oettle (1960) were amongst the first to document differences in colo-rectal cancer incidence amongst different groups in South Africa, and to attribute the low rates in the Bantu to the fact that 'in the Bantu a large amount of roughage is consumed and constipation in the Western sense is rare'. This suggestion, however, only received widespread attention following the classification of large bowel cancer with a number of other large bowel disorders common in Western societies whose aetiology was attributed to lack of dietary fibre by Trowell, Burkitt and co-workers. Burkitt (1969) suggested a mechanism for the protective action of dietary fibre in stating that 'with regard to bowel tumours . . . with the Western diet, the greatly delayed transit time (most of the delay occurring in the distal colon), together with the concentration associated with diminished stool bulk, might enhance the action of any carcinogen by the multiple of these factors'.

Since that time, the 'fibre hypothesis' has attracted much research into the definition, analysis, physiology and epidemiology of dietary fibre in relation to disease. The existing evidence regarding the possible protective effect of dietary fibre in colo-rectal cancer will first be reviewed, followed by a discussion of the mechanisms whereby fibre might be involved.

FIBRE SUPPLEMENTS AND CHEMICALLY INITIATED CARCINOGENESIS

In animal models of colo-rectal cancer, lesions are induced using the carcinogens 1,2-dimethylhydrazine (DMH), azoxymethane (AOM), and 3,2-dimethyl-4-aminobiphenyl (DMAB) which require microsomal activation. The direct-acting carcinogen methyl nitrosourea (MNU) may also be used.

The effect of purified sources of dietary fibre on chemically initiated colo-rectal cancer has been investigated in a large number of studies over the past decade. In these, the numbers of tumours induced by the administered carcinogen whilst the animals are fed on 'control' diets are compared with those found with fibre supplements. Although at first sight these types of experiments are easily standardized, comparisons between different studies are difficult due to differences in age, sex, strain and type of animal used, the chemical carcinogen used and its route of administration, the timing of carcinogen dosage (whether before or during fibre supplementation) and the level of dosage. These animal models are highly susceptible to nutritional factors such as the level of fat and energy in the control and experimental diets, and the type of fat used. Both increased levels of fat and energy are promoters of colon carcinogenesis, as are the $\omega 6$ polyunsaturated fatty acids (Ip, 1978). Further experimental variables are the level of fibre fed in the experimental diet and the level of fibre in the control diet, some studies having been carried out with chow as the control, and others a 'fibre-free' diet.

The overall findings of experimental studies were collated by the Federation of American Societies for Experimental Biology (FASEB) in 1987 (Pilch, 1987). Table 1 shows a summary of these for wheat bran, cellulose and pectin. In general, bran appears to have a consistently protective effect against chemical carcinogenesis. Of seventeen

Table 1. *Effect of fibre on protection against chemical carcinogens (Pilch, 1987)*

Type of fibre	No. of studies	Fibre protective	Fibre no effect	Fibre enhancing
Wheat bran	17	13	3	1
Cellulose	9	6	3	0
Pectin	7	1	3	3

studies, bran decreased the number of tumours in thirteen studies, and increased the number of tumours compared with control levels in only one study. Cellulose also appears protective in six of nine studies, with no significant difference in three. In three of seven studies, pectin apparently enhanced carcinogenesis, possibly due to the variety of experimental variables in these studies. Other brans from rice, soya beans, maize and oats have only been studied in a few experiments using variable protocols. No consensus was possible for the gums, guar gum and carrageenan (Pilch, 1987).

EPIDEMIOLOGY OF DIETARY FIBRE INTAKE AND COLO-RECTAL CANCER

Much of the evidence relating risk of large bowel cancer to lack of dietary fibre was based on the epidemiological observations of Burkitt and co-workers in Africa. Since that time, however, extensive epidemiological studies have been undertaken.

Correlation studies. A number of investigators have made international comparisons between colo-rectal cancer incidence or mortality rates with estimates of national average consumption of dietary fibre. McKeown-Eyssen & Bright-See (1985) for example, correlated mortality rates for colon cancer in thirty-eight countries with estimated dietary fibre intakes calculated from Food and Agriculture Organization food balance sheets. Estimated dietary fibre intakes were higher in countries at low risk for colon cancer, with correlation coefficients relating total dietary fibre and cereal dietary fibre consumption to colon cancer death rates -0.66 and -0.72 . However, the strongest association between colo-rectal cancer and large bowel cancer epidemiologically is with meat (Amstrong & Doll, 1975), and animal protein intake and cereal consumption are inversely related world-wide (Perisse *et al.* 1969). Hence, these relationships are substantially reduced on partial correlation analysis controlling for meat ($r -0.36$) and fat ($r -0.18$; McKeown-Eyssen & Bright-See, 1985).

Population studies do not, therefore, suggest that there is a clear association between fibre intake and large bowel cancer. Other anomalies have also been reported. Age-standardized colo-rectal cancer rates of the Maoris in New Zealand are approximately half those of New Zealand whites, and yet intakes of dietary fibre are virtually the same (Smith *et al.* 1985). In South Africa, different racial groups are also at very different risks of large bowel cancer, yet recent reports suggest that fibre intakes in these groups are also very similar (Walker *et al.* 1986). The marked increase in large bowel cancer in Japan over the past 30 years (Fig. 1) has not been associated with changes in intakes of dietary fibre (Kuratsune *et al.* 1986). However, other items of the Japanese diet have changed substantially, and since 1950 there has been at least a threefold increase in fat consumption, a ninefold increase in meat consumption, and rice consumption has fallen by one-third (Minowa *et al.* 1983).

Table 2. Case-control studies of colo-rectal cancer

Study	Area	No of cases	No. of controls	Fibre	Vegetables	Cereals	Starch
Higginson	USA, Kansas	340	1020	NR	0	NR	NR
Wynder <i>et al.</i>	Japan	107	307	NR	0	dec risk	NR
Bjelke	Norway	278	1394	NR	dec risk	NR	NR
Haenzel <i>et al.</i>	Hawaiian Japanese	179	357	NR	pos risk	pos risk	NR
Modan <i>et al.</i>	Israel	198	396	dec risk	String beans	Rice	NR
Phillips	USA, SDA	41	123	0	dec risk	0	NR
Dales <i>et al.</i>	USA, blacks, San Francisco	99	280	(dec risk)	NR	NR	NR
Graham <i>et al.</i>	USA, whites, New York	982	1764	NR	dec risk	NR	NR
Moskovitz <i>et al.</i>	USA, Connecticut	15	23	0	NR	NR	NR
Esser <i>et al.</i>	Germany	49	60	0	NR	NR	NR
Hunter <i>et al.</i>	USA, veterans	25	25	0	NR	NR	NR
Calder <i>et al.</i>	Kenyan Africans	12	100	0	0	0	NR
Jain <i>et al.</i>	Canada	542	1077	0	0	0	NR
Miller <i>et al.</i>							
Martinez <i>et al.</i>	Puerto Rico	461	461	pos risk	NR	pos risk	NR
Manousos <i>et al.</i>	Greece	100	100	NR	dec risk	0	NR
Pickle <i>et al.</i>	USA, Nebraska	86	176	NR	0	0	NR
Bristol <i>et al.</i>	UK	50	50	0	NR	NR	NR
Berta <i>et al.</i>	France	94	94	0	NR	NR	NR
Potter & McMichael	Australia	419	732	pos risk(F)	NR	NR	NR
Lopez <i>et al.</i>	Brazil	?20	?20	0(M)	(dec risk)	0	NR
Macquart-Moulin <i>et al.</i>	France	399	399	0	dec risk	pos risk	0
Kune <i>et al.</i>	Australia	715	727	dec risk	dec risk	dec risk	NR
Lyon <i>et al.</i>	USA, Mormons	246	484	dec risk	NR	NR	NR
Tuyms <i>et al.</i>	Belgium	453	2851	dec risk	dec risk	dec risk	dec risk
Graham <i>et al.</i>	USA, New York	428	618	dec risk	0	NR	NR
Young & Wolf	USA, Wisconsin	353	618	NR	dec risk	NR	NR
Slattery <i>et al.</i>	USA, Mormons	229	384	dec risk	NR	NR	NR
Nakaji <i>et al.</i>	Japan	45	45	dec risk	NR	NR	NR
Lee <i>et al.</i>	Singapore Chinese	203	425	dec risk	dec risk	0	NR
O'Sullivan <i>et al.</i>	Ireland, polyyps and cancer	NR	NR	dec risk	dec risk	NR	NR

NR, not reported; 0, no difference; (), no test of significance; (F), female; (M), male; dec, decreased; pos, positive.

Table 3. *Case-control studies of dietary fibre (thirty studies 1969-89)*

	No. of studies	Reduced risk	Enhanced risk	No effect
Fibre	22	11	2	9
Vegetables	19	12	1	6
Cereals	13	3	3	7
Starch	2	1	0	1

Case-control studies. In epidemiology, case-control studies offer major advantages over population studies because individual exposure to a dietary variable can be related to individual outcome. The most common type of study is the case-control, whereby individuals with a particular cancer are studied after diagnosis and the results compared with those for individuals who are healthy.

The results of thirty case-control studies carried out in Europe, Australasia, North and South America, and Africa, over the past 20 years are shown in Table 2. Values for 'fibre' were derived either from various indices of 'fibre-rich' foods, crude fibre, or dietary fibre using the British food tables. Several of these studies were very small scale and tended to yield non-significant results.

Table 3 summarizes these studies and shows that, in studies where it has been measured, fibre has tended to be associated with a reduction in risk. Eleven studies, for example, of twenty-two have shown that cases reported less 'fibre' than controls, and in only two had they reported eating more. There were no significant differences in nine.

Much of this apparent protective effect of fibre is accounted for by the fact that in twelve of nineteen studies, cases reported eating smaller amounts of vegetables than controls. In the thirteen studies in which cereal consumption was reported, risk was reduced by cereal consumption in three, but increased in three. However, in the largest study of 818 cases in Belgium, starch, fibre, and vegetable consumption were all protective factors, with relative risks for colo-rectal cancer being reduced to 0.71, 0.37, 0.82 and 0.67 respectively in the highest levels of cooked vegetables, raw vegetables, starch and fibre consumption. Peas and beans were associated with increased risk (Tuyns *et al.* 1987, 1988).

The consistently protective effect of vegetables may reflect the fact that vegetables are a major source of fibre in Western diets. Vegetables also contain numerous flavours and colours, such as flavanoids, tannins, isothiocyanates, indoles and phenols which have been found to reduce the incidence of some chemically-induced cancers (Wattenberg, 1990).

However, a major drawback of case-control studies is that the most common symptoms in large bowel disease are pain and altered bowel function (Cummings, 1981a), which patients may attempt to ameliorate by a change in diet. An attempt to avoid this potential bias is made in case-control studies of diet by asking for details of past dietary habits, rather than present ones. This procedure is impossible to validate with certainty, but circumstantial evidence suggests that recall of past diet is strongly influenced by present dietary habits (Bingham, 1987). The apparently protective effects of fibre and of vegetables, documented in case-control studies may, therefore, be reflecting the effect of cancer rather than the cause.

Prospective studies. There have been three prospective studies of diet and cancer in which several thousand individuals have been assessed for their consumption of fibre, vegetables or cereals, and then followed up for several years. In the largest, of 265 000 Japanese, individuals who later developed colo-rectal cancer reported eating less vegetables and cereals than those who did not (Hirayama, 1981). The 141 individuals who developed colon cancer, of a study of 35 000 Californian Seventh Day Adventists, also reported eating less crude fibre and some vegetables than the rest of the population (Stampfer *et al.* 1987). No significant findings for crude fibre or fibre-related foods have so far been found in a prospective study of 89 534 US nurses (Morgan *et al.* 1988).

More definitive results will be available in several years' time from European collaborative prospective studies of diet and cancer. In these studies, to be carried out in nine European and Scandinavian countries, the food intake of in excess of 500 000 individuals will be measured using properly validated methods suitable for the assessment of food intake in individuals (Riboli, 1989). As importantly, accurate methods for the assessment of dietary fibre consumption can now be included, as non-starch polysaccharides (NSP).

DEFINITION AND ANALYSIS OF DIETARY FIBRE (NSP)

As mentioned previously, various methods for assessing fibre consumption have been used by epidemiologists, and it is only recently that accurate values for fibre in foods, as NSP, have become available (Englyst *et al.* 1988, 1989). NSP include all the carbohydrate fractions and types of dietary fibre: soluble and insoluble, pectins, gums, hemicelluloses, together with storage polysaccharides such as inulin, guar, gums and exudates (e.g. sterculia, karaya), cellulose, β -glucans and non-cellulosic polysaccharides. During analysis NSP are hydrolysed into their component sugars and uronic acids, after previous removal of the other main polysaccharide in food, starch. The component sugars and uronic acids are then measured directly by gas-liquid chromatography or a colorimetric procedure to give the NSP or dietary fibre content (Englyst & Hudson, 1987; Englyst *et al.* 1987a).

All plant foods except oils and sugars contain NSP, with contents ranging from 1 g/kg in cornflour to 690 and 360 g/kg in soya-bean bran and wheat bran. Values for some commonly eaten foods are shown in Table 4. Of these, whole-grain wheat cereals, such as Weetabix, and wholemeal bread are the most concentrated sources, although some leafy vegetables, such as cabbage, and root vegetables and legumes contain appreciable amounts. The NSP content of white rice is very low (4 g/kg).

INTAKES OF NSP

To date, only six populations have been studied for their NSP consumption in Scandinavia, Britain and Japan (Table 5). In the UK diet, approximately 10% of total NSP is supplied by uronic acids, 20% by cellulose and 68% by the remaining pentose and hexose sugars.

NSP intake has been related to colon cancer incidence in two of these populations, in Britain and Scandinavia, both of which consume high amounts of meat (150–215 g/d) and fat (100–150 g/d). In these populations, protective though weak associations between large bowel cancer occurrence and NSP intake were found. In Britain, the correlation

Table 4. *Non-starch polysaccharide (NSP) content of common foods (from Englyst et al. 1988, 1989)*

Food	NSP (g/kg)				
	Total	Ratio insoluble: soluble	Cellulose	Pentose + hexose	Uronic acids
Apples	15	15	5	6	4
Baked beans	35	7	8	21	6
Cabbage	27	13	9	11	7
Carrots	25	8	8	9	8
Lettuce	12	10	5	3	4
Weetabix	97	21	15	80	2
Oats	70	6	6	62	2
Wholemeal bread	52	33	8	42	2
Brown rice	19	All insoluble	6	9	1
White bread	15	7	2	14	0
Cornflakes	9	13	3	5	1
White rice	4	All insoluble	2	3	0

Table 5. *Non-starch polysaccharide (NSP) intakes of six populations*

	NSP intake (g/d)				
	Total	(SD)	Cellulose	Pentose + hexose	Uronic acids
Rural Finland	18.4	7.8	4.2	12.7	1.9
Rural Denmark	18.0	6.4	3.7	12.1	2.2
Helsinki	14.5	5.4	3.4	9.2	2.0
Copenhagen	13.2	4.0	3.2	8.1	1.9
UK	12.4	—	2.6	7.9	1.8
Japan	10.9	—	3.0	4.7	3.1

between regional NSP intake and age-standardized colon cancer mortality rates was -0.74 ($P < 0.05$), see Fig. 2. There was no significant association between NSP intake and colo-rectal cancer mortality and the strongest association was with vegetable consumption ($r 0.94$) (Bingham *et al.* 1985).

In the four regions of Scandinavia, Helsinki, Parrikala in rural Finland, Copenhagen and Them in rural Denmark, there was a significant negative association between colo-rectal cancer incidence and NSP intake (Fig. 3), but in contrast to the findings in Britain, this negative association was largely accounted for by cereal consumption, rather than vegetables. In rural Finland, the low cancer area, consumption of cereals, including rye bread made from high extraction flour, was nearly double that of Copenhagen, where cancer rates were highest (IARC Large Bowel Cancer Group, 1982).

Also shown in Table 5 are NSP intakes measured in composite diets derived from the Japanese National Nutrition Survey for 1979 (Kuratsune *et al.* 1986). Despite their

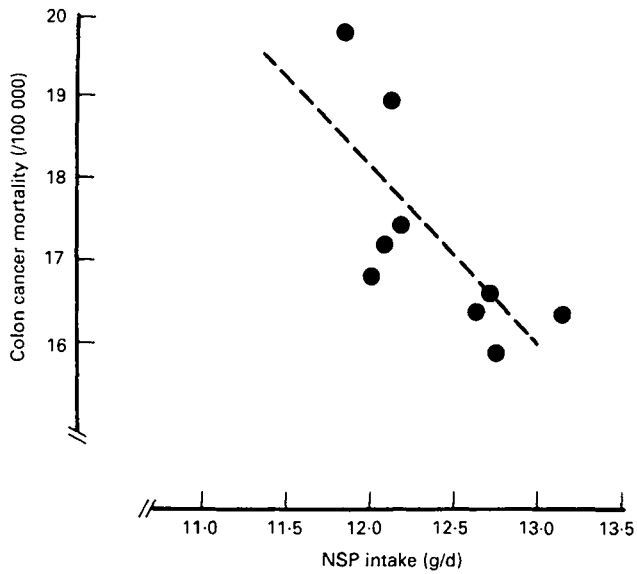


Fig. 2. Age-standardized colon cancer mortality and non-starch polysaccharide (NSP) intake in the nine standard regions of the UK, 1969–1975 (Bingham *et al.* 1985).

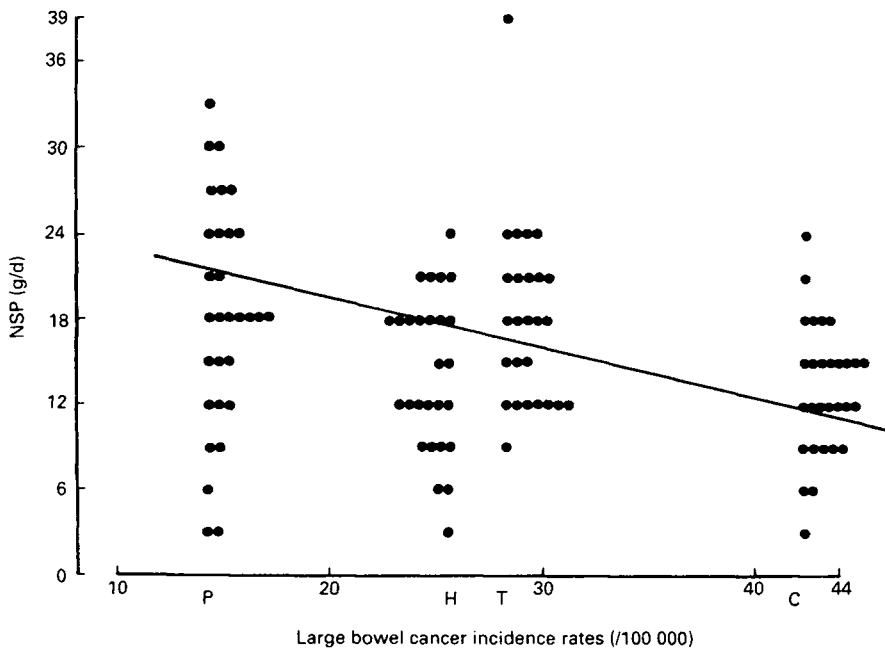


Fig. 3. Age-standardized colon and rectal cancer incidence *v.* non-starch polysaccharide (NSP) intake in four Scandinavian populations ($r = -0.78$) (IARC Large Bowel Cancer Group, 1982). P, Parrikala (Finland); H, Helsinki (Finland); T, Them (Denmark); C, Copenhagen (Denmark).

comparatively high cereal consumption, the Japanese consume no more NSP than that consumed by European populations: 11 v. 12 g/d, for example, in Britain. The reason for the low NSP content of the Japanese diet is the low NSP content of rice, see Table 4.

In all populations so far studied, remarkably little dietary fibre is found. Table 5 shows that the greatest amount of NSP found in average diets is 18 g/d. Intakes of NSP based on food consumption level estimates in Finland were also 18 g/d (Varo *et al.* 1984), as were the intakes of Finnish adolescents (Virtanen & Varo, 1988). This is substantially less than the other major polysaccharide in food, starch.

Starch. Starch is chemically distinct from NSP but contaminates most analytical methods for dietary fibre (James & Theander, 1980). Resistant starch is a name originally introduced by Englyst for retrograded amylose that is difficult to remove from food samples by normal enzymic hydrolysis without previous treatment with alkali or dimethyl sulphoxide. The amount of resistant starch in a particular food sample is highly dependent on the way in which the sample is treated before analysis; freeze-drying, for example, introduces large amounts (Englyst *et al.* 1982).

Other forms of starch may also reach the large intestine and may, or may not, contaminate analytical methods for the determination of dietary fibre. Whole or partly milled cereal grains and ungelatinized potato- and banana-starch granules have been classified as 'partially resistant starch', although starch granules, once cooked and gelatinized, are readily digested in the small gut. A further type of partially resistant starch is retrograded amylopectin which can be made to revert on heating to its more digestible form (Englyst & Cummings, 1987a).

The amount of starch reaching the large intestine has been assessed in careful studies using volunteer patients with an ileostomy, a fistula constructed so that the ileum opens onto the abdominal wall, usually after surgical removal of the colon and rectum. In these patients, known amounts of different starches were fed and the ileostomy fluid obtained from the small bowel analysed for its content of undigested starch (Englyst & Cummings, 1985, 1986, 1987b). These studies have shown that substantial amounts of starch may reach the large intestine, but the amount that does so depends on cooking, processing and even the ripeness of food. Of the starch in bananas 90%, in the form of starch granules, is not digested in the small gut, so that when only 'just ripe' bananas, with green tips, are eaten as much starch as an average daily intake of NSP may reach the large intestine (Englyst & Cummings, 1986). Of the starch in white-flour products, corn-flakes, and freshly cooked potatoes 3–5% is not digested, but this increases to 12% when potatoes are cooked and then cooled before eating (Englyst & Cummings, 1985, 1987b).

Large quantities of starch may, therefore, reach the large intestine. For this reason it has been argued that starch should be included in the definition of 'dietary fibre', despite the fact that one of the original and leading pioneers of the dietary fibre hypothesis, H. R. Trowell, has stated that 'starch was never named . . . as a constituent of dietary fibre' (Trowell, 1985). As yet little work has been done on the effect of starch on bowel habit, although *in vitro* and *in vivo* studies suggest that it behaves differently to NSP on becoming available for fermentation by the large bowel flora (see p. 165).

MECHANISMS IN COLO-RECTAL CANCER

The physiological importance of starch, particularly in cooked and cooled foods, has only recently been realized and there is consequently little epidemiological information

available on amounts consumed. Nevertheless, the available information suggests that NSP and possibly starch interact with meat and other aspects of diet, particularly meat and fat, in affecting risk from large bowel cancer. In considering possible mechanisms whereby this interaction in dietary habits could be important in cancer aetiology, the colonic flora and the control of its activity by diet play a key role.

The colon is host to a large and diverse commensal flora of anaerobic bacteria that until recently has attracted little attention in medical microbiology. However, with >400 species (Finegold *et al.* 1983) the colonic flora can be considered as an important organ in its own right, with a flexibility and potential for metabolic transformations at least as great as that of the liver. Enzymes are readily induced by changes in the metabolic environment and a wide variety of substances can be hydrolysed, reduced, degraded, or synthesized. All of them may be important in the generation of carcinogens or in degrading or re-activating carcinogens secreted in bile.

The importance of diet is due to the fact that the major control on the metabolic environment of the flora is via residues entering the large gut from the small bowel. These in turn are determined by dietary intakes, particularly protein and carbohydrate.

Carbohydrate entering the large bowel stimulates anaerobic fermentation, leading to the production of short-chain fatty acids (SCFA), acetate, propionate and butyrate, gas, and an increase in microbial cell mass. The SCFA produced are absorbed by the intestinal mucosa, where they stimulate sodium absorption and bicarbonate secretion (Cummings, 1981*b*). Carbohydrates stimulating fermentation include in the caecum the laxative lactulose and, in lactase-deficient individuals, lactose. Normally, however, sugars are absorbed in the small gut and it is the polysaccharides that are quantitatively the most important.

Fermentation has a number of important consequences in large bowel physiology and possible implications for protection against large bowel cancer. First, the increase in bacterial cell mass results in an increase in faecal weight (Stephen & Cummings, 1980). Cereal fibre, from bran and some resistant starch, has an additional effect because it is only partly fermented and the residual polysaccharides absorb water, also contributing to faecal bulking (McBurney *et al.* 1985). With the increase in faecal weight, transit time is reduced and the contents of the large bowel lumen are diluted, which would reduce the time putative carcinogens are in contact with the large bowel mucosa, an effect originally suggested by Burkitt (1969) to explain the way in which dietary fibre would be protective against colon cancer.

These protective mechanisms depend on the assumption that the initiating factors against which NSP and starch are thought to protect are present in the faecal stream, and there is accumulating evidence that faeces do contain at least mutagens, and possibly carcinogens.

Faecal mutagenicity. Faecal mutagenicity may be assessed with the Ames test, TA 98 detecting frameshift mutations, and TA 100 base pair substitutions (Maron & Ames, 1983). Both strains may be tested following stimulation with the microsomal fraction S9 to stimulate *in vivo* metabolic activation. Not all individuals produce mutagenic samples, the proportion who do so in any one population depending to a large extent on the solvent used to prepare sterile extracts from faeces (Schiffman, 1986).

Using organic solvents, eighteen of fifty individuals in Helsinki exhibited high faecal mutagenicity. Fifteen of these individuals then took part in an intervention study, whereby fibre intake from whole-grain bread was increased by 11 g/d for 4 weeks. Stool

bulk increased by 35% and faecal mutagenicity decreased by 2.5–4.5-fold (Reddy *et al.* 1987).

The standard Ames test is open to difficulties when used for faecal extract testing, but Venitt (1988; Venitt *et al.* 1986), using methods designed to overcome both toxic and nutritive factors in faecal extracts, also found a reduction in faecal mutagenicity with bran, albeit in only one subject in a preliminary study. Kuhnlein *et al.* (1983), using aqueous faecal extracts, has also shown reductions in faecal mutagenicity with cellulose and with pectin in rats.

The fact that different results are obtained from different extracts in Ames testing suggests that faeces contain a variety of mutagens (Schiffman, 1987). Polycyclic hydrocarbons, phenols, and N-nitrosocompounds are possible candidates, but most attention has been focused recently on the fecapentaenes and heterocyclic amines.

Fecapentaenes. Fecapentaenes are compounds with a central pentaene structure, and a chain length of 12 or 14. They are produced by the large bowel bacterial flora, possibly by *Bacteroides*, the most common species in the human colon (Finegold *et al.* 1983). The precursor(s) is unknown. Fecapentaenes can now be isolated from faeces, thus avoiding the necessity for mutagenicity testing, and a synthetic fecapentaene 12 (FP 12) has now been synthesized (Schiffman, 1987).

No relationship between dietary fibre intake and fecapentaene excretion was detected in a questionnaire study, although high excreters were less likely to be consuming supplements of vitamins C and E (Schiffman, 1987). Little work has otherwise been done on the interaction between dietary fibre and fecapentaenes, and two recent studies suggest that its importance in colon cancer may be limited. Although FP 12 has been shown to exhibit a high degree of genotoxicity, for example, causing DNA single-strand breaks and sister chromatid exchanges, it has not been shown to be a carcinogen in rodent assays (Ward *et al.* 1988). Second, patients with colo-rectal cancer have been shown to excrete substantially less fecapentaenes than patients without colo-rectal cancer (Schiffman *et al.* 1988).

Heterocyclic amines. A number of heterocyclic amines are formed in meat and fish cooked at relatively low temperatures. After microsomal activation these were found to be highly mutagenic in the Ames test and carcinogenic in several organs, particularly the liver (Sugimura, 1985). The quinoline derivatives, such as IQ, MeIQx, and MeIQ, result probably from Maillard reactions between a hexose such as glucose and an amino acid with linkage of the resulting Strecker aldehyde with creatinine (Sjodin *et al.* 1985). MeIQ and IQ are damaging to mouse colon cells *in vivo* (Bird & Bruce, 1984) and IQ induces large intestinal tumours in mice (Ohgaki *et al.* 1986). MeIQx can be extracted from faeces using blue cotton which can adsorb organic complexes having three or greater numbers of fused aromatic rings. Hayatsu *et al.* (1986) showed an increase in faecal mutagenicity in volunteers subsequent to a meal of 150 g fried beefburgers, but could not identify the major mutagenic component as MeIQx. This finding would have offered a direct link with the epidemiological association between meat consumption and colon cancer. No studies as yet have investigated the effect of fibre on heterocyclic amine metabolism. Fermentation does, however, have marked effects on two other substances present in the large bowel, ammonia, and bile acids.

Ammonia. The main product of bacterial metabolism of nitrogenous residues is ammonia, which enhances cell proliferation, alters DNA synthesis, and has been implicated in large bowel carcinogenesis (Visek, 1978). When carbohydrate availability

is limited, ammonia concentrations increase *in vitro* (Cummings *et al.* 1979), but in the presence of active fermentation ammonia is used for bacterial protein synthesis and faecal ammonia concentration falls (Macfarlane *et al.* 1986). Diets high in meat generally also contain little starch and NSP and can, therefore, be expected to increase ammonia concentration partly by an increase in available nitrogen and because fermentation is limited. It was also suggested that high-protein diets have an added effect via increased diffusion of urea into the colon when blood urea levels rise in response to high-protein diets (Wolpert *et al.* 1971). However, in humans, colonic perfusion experiments showed that the large bowel mucosa is virtually impermeable to urea, and studies in which plasma urea was labelled with ^{15}N showed that endogenous urea makes only a small contribution to total faecal N content, about 8% (Wrong *et al.* 1985). Levels of faecal ammonia have not yet been measured on an epidemiological basis.

pH and bile acids. Fermentation may also affect risk of colon cancer via its effect on pH and bile acids. Bile acids are thought to promote colo-rectal tumours, although the actual mechanism involved is uncertain. Proliferation, via increased ornithine decarboxylase (*EC* 4.1.1.17) activity or protein kinase C has been suggested (Takano *et al.* 1981; De Rubertis *et al.* 1984; De Rubertis & Craven, 1987; Fitzer *et al.* 1987; Sakata, 1987). Bile acids alone, however, are unlikely to affect colon cancer risk because there is no difference in faecal bile acid output either between cases and healthy matched controls, or between individuals living in a high-risk area compared with a low one (Setchell *et al.* 1987).

Cereal fibre, from bran, dilutes faecal bile acid content, although total output is increased (Cummings *et al.* 1976). It has also been proposed that a key step in the development of bowel cancer is the conversion of primary to secondary bile acids (Thornton, 1981). The SCFA produced during fermentation are thought to lower pH, and as a result this conversion is inhibited (Thornton, 1981). However, other considerations are important. In the perfused colon, there is little damaging effect of deoxycholic acid at pH 5.9, whereas damage can be extensive at pH 7.9 (Rafter *et al.* 1986). This effect has been attributed to the insolubility of bile acids at low pH, so that bile acid concentration in faecal water may be more indicative of risk (Bruce, 1987).

Gas production. The gases carbon dioxide, hydrogen, and in some individuals methane, are other end-products of fermentation. H_2 diffuses into the bloodstream, and excretion in breath has been extensively used as a marker of oral caecal transit time in humans. Attempts have also been made to quantify the amount of carbohydrate reaching the large gut by comparing breath H_2 production with a known amount of fermentable carbohydrate, such as lactulose (Bond & Levitt, 1975). However, this is unlikely to be an accurate procedure, since there are other alternatives for disposal of H_2 in fermentation, depending partly on the bacterial flora, including methanogenic bacteria (Christl *et al.* 1989) which themselves are in competition with sulphate-reducing bacteria for H_2 (Gibson *et al.* 1988a). The end-product of the activities of sulphate-reducing bacteria, hydrogen sulphide, is highly toxic and may be important in the aetiology of large gut disease (Florin *et al.* 1989). Only 15% of rural black South Africans at low risk of colon cancer carry sulphate-reducing bacteria, compared with 70% of samples from British individuals (Gibson *et al.* 1988b). Correspondingly 84% of black Africans are methane producers compared with 52% of whites (Segal *et al.* 1988). Methanogenesis appears, therefore, not to be risk factor for colon cancer, contrary to previous observations (Haines *et al.* 1977).

SCFA and butyrate production. In addition to their probable effect on pH, SCFA are involved in the nutrition of the large bowel mucosa, and in the control of cellular proliferation and differentiation.

Approximately 60, 20 and 20% molar ratios of acetate, propionate and butyrate are formed during fermentation in the human large gut (Cummings, 1981*b*). Molar ratios can, however, be varied *in vitro*, according to the substrate. In batch cultures of human faecal slurries, 29% of butyrate can be produced from starch, compared with 8, 3 and 2% from an arabinogalactan, a xylan, and pectin (Englyst *et al.* 1987*b*). This effect of starch fermentation in increasing butyrate production has also been shown *in vivo*. Eleven individuals were fed on the glucosidase inhibitor acarbose in order to induce starch malabsorption in the small gut. Molar ratios of butyrate in faeces increased from an average of 21% without starch malabsorption to 30% with malabsorption, a 50% increase. There was no change in the molar ratio of acetate (41%), and a fall in propionate production, from 27 to 21% (Scheppach *et al.* 1988).

SCFA are absorbed from the large gut, less butyrate being found in the human portal vein (Cummings *et al.* 1987). In most species, butyrate is used preferentially to acetate and propionate as a substrate for energy metabolism in the large bowel epithelium (Cummings, 1981*b*). In the isolated human colonocyte, butyrate accounts for about 75% of oxygen consumption, and glucose is able to replace butyrate to a lesser extent in the proximal colon compared with the distal colon. This, together with less ketone-body formation, suggests that butyrate is a major source of energy for the distal colonic mucosa (Roediger, 1980). Acetate was not investigated in these studies, due to an absence of effect in the rat colonocyte.

In the conventional but not germ-free rat, wheat bran and ispaghula significantly enhance epithelial cell proliferation compared with animals fed on fibre-free diets (Goodlad *et al.* 1989). SCFA, however, directly enhance proliferation rates in both germ-free and conventional rats (Sakata, 1987). Intracolonic infusions of SCFA have also been shown to promote healing in anastomoses of the surgically divided colon of rats (Rolandelli *et al.* 1986). This beneficial effect of SCFA on proliferation and healing is perhaps to be expected, if they are to be considered as nutritional factors for colonic epithelial cells. Excessive cell proliferation is a putative aetiological factor in carcinogenesis, but SCFA at physiological levels are unlikely to promote epithelial proliferation beyond the normal range (Sakata, 1987).

In cultured cell lines, butyrate is a well recognized anti-proliferative agent. At the 1–2 mM level, it acts directly as an inhibitor of DNA synthesis and cell growth, mainly via inhibition of histone deacetylase. Other SCFA are much less active in this respect (Kruh, 1982). The effect is reversible, with a 24 h lag in G₁ phase before normal growth rates are resumed after removal of butyrate from culture media (Smith, 1986). Acetylation may be a general mechanism for relaxing DNA, allowing access for repair enzymes; ultra violet radiation damage repair is enhanced and survival increased in human adenocarcinoma cell lines exposed to butyrate for example (Smith, 1986).

Butyrate is also a known differentiation-inducing agent, reducing cloning efficiency and increasing the expression of membrane glycoproteins such as alkaline phosphatase (EC 3.1.3.1) and carcino-embryonic antigen in some human colo-rectal cancer cell lines (Chung *et al.* 1985; Whitehead *et al.* 1986). Other SCFA have little effect compared with butyrate (Whitehead *et al.* 1986). The effects of butyrate, however, vary amongst different cell lines; in one line derived from familial adenomatous polyposis, butyrate

seemed to select for increased malignancy. This effect, however, was not seen in another line derived from a patient with non-inherited, sporadic colon cancer (Berry & Paraskeva, 1988). Very large doses (100–200 mmol/l) of butyrate added to drinking water have been reported to increase chemical carcinogenesis in rats treated with DMH (Freeman, 1986). The effects of butyrate on microsomal activation of DMH, and on a direct-acting carcinogen in the large bowel have not been determined.

Butyrate may, therefore, be involved in protection against carcinogenesis in the colon and rectum, the only organs in the body exposed to elevated levels. Two small studies have shown lowered faecal butyrate levels of 8 v. 12% total SCFA in patients with colo-rectal cancer compared with healthy controls (Vernia *et al.* 1988; Bonnen *et al.* 1989).

SUMMARY AND CONCLUSIONS

The cause of human colo-rectal cancer is unknown, although international and racial comparisons suggest that diet may be important. Within populations, risk of cancer is also affected by genetic factors which remain to be elucidated. Dietary fibre and NSP consumption is not always high in populations at low risk of colo-rectal cancer, but rates are fast increasing with westernization (and meat and fat consumption) in Japan.

The suggestion that dietary fibre is protective in colo-rectal cancer is based on the fact that cereal fibre from bran increases faecal weight, dilutes large intestinal contents, and speeds up transit time. In animal models, bran reduces the number of tumours induced by chemical carcinogens, and cellulose may have a similar effect. The faeces of some individuals contain mutagens, some of which have been identified as fecapentaenes and heterocyclic amines. Bran reduces faecal mutagenicity, although the mutagen concerned is unknown.

Most dietary fibre is fermented in the large gut by anaerobic bacteria and little remains in faecal matter. Recent observations have shown that substantial amounts of starch survive digestion in the small bowel and are available also for fermentation in the large gut. The metabolic consequences of fermentation may be important in carcinogenesis via altered N metabolism, SCFA production, and pH reduction. Methane is also produced in some individuals, but, contrary to previous findings, is not a risk factor for large bowel cancer. Starch appears to be beneficial as a substrate for fermentation because yields of the SCFA butyrate are increased both *in vitro* and *in vivo*. Butyrate is an energy substrate for the colonic mucosa and an anti-proliferative and differentiating agent in cell culture lines. Possible mechanisms whereby starch and NSP may protect against colo-rectal cancer, therefore, exist.

The majority of individual case-control epidemiological studies suggest that fibre-containing foods are protective in colo-rectal cancer, although this effect is largely due to vegetable, rather than cereal, consumption. Case-control studies of diet and large bowel cancer may, however, reflect the effect rather than the cause of the disease, so that confirmation of the possible protective effects of starch and NSP is needed from accurate prospective studies both of diet and associated risk factors.

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