EPIDEMIOLOGY AND MECHANISMS RELATING DIET TO RISK OF COLORECTAL CANCER

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CONTENTS

INTRODUCTION

Colorectal cancer is the second most common cancer in Western societies, affecting up to 6 % of men and women by the age of 75. It ranks second after lung cancer in men, and breast cancer in women. Table 1 shows age standardized incidence rates for men and women for the major cancers in England and Wales. Incidence is increasing slightly, particularly in men, but mortality is decreasing owing to improvements in detection and treatment. Like most cancers, risks increase with age and Fig. 1 shows rates for colon and rectal cancer at different ages for men and women in England and Wales (Muir *et al.* 1987).

When age is taken into account, there remains at least a 15-fold range in age standardized incidence throughout the world. Countries with the highest risk include Australia, New Zealand, the USA and parts of Northern Europe, and those with the lowest risk include rural Africa, China and India (Parkin *et al.* 1992). In low risk countries, the majority of colorectal cancers are situated in the right side of the colon, whereas in high risk countries the majority of cancers are located in the lower bowel, near the rectosigmoid junction.

Both migrant studies and secular changes in incidence rates show that environmental factors are the main reason for the geographical differences in colorectal cancer incidence. Migrants from low risk areas rapidly adopt the incidence rates of a high risk population, for example Japanese migrants to Hawaii, Southern Europe migrants to Australia (McMichael *et al.* 1980; Haenszel *et al.* 1973). In Japan itself, there have been striking changes. Whereas rates were once low, age specific colorectal cancer incidence rates have increased markedly since 1960, and are approaching those recorded in Britain (Fig. 2). Death rates from large bowel cancer in younger (30–40-year-old) Japanese are falling, however (Boyle *et al.* 1993).

The strong evidence that the majority of large bowel cancers are attributable to environmental factors means that it is a potentially preventable disease. In addition, cancer is generally accepted to be a disease of genes, occurring as a result of mutations in or loss of genes in the large bowel epithelium, leading to malignant growth. In comparatively rare

Table 1. *Age standardized rates per 100000 for major cancers excluding skin in men and women in England and Wales 1983–1987* (*Parkin* et al. *1992*)

Site	Male	Site	Female
Bronchus, lung	65·4	Breast	56·1
Colorectal	30·8	Colorectal	22·4
Prostate	23·1	Bronchus, lung	20·5
Bladder	17·7	Uterus	19·9
Stomach	16·9	Ovary	11·4
Pancreas	7·4	Stomach	6·8
Oesophagus	6·5	Bladder	4·9
Kidney	5·6	Pancreas	4·9
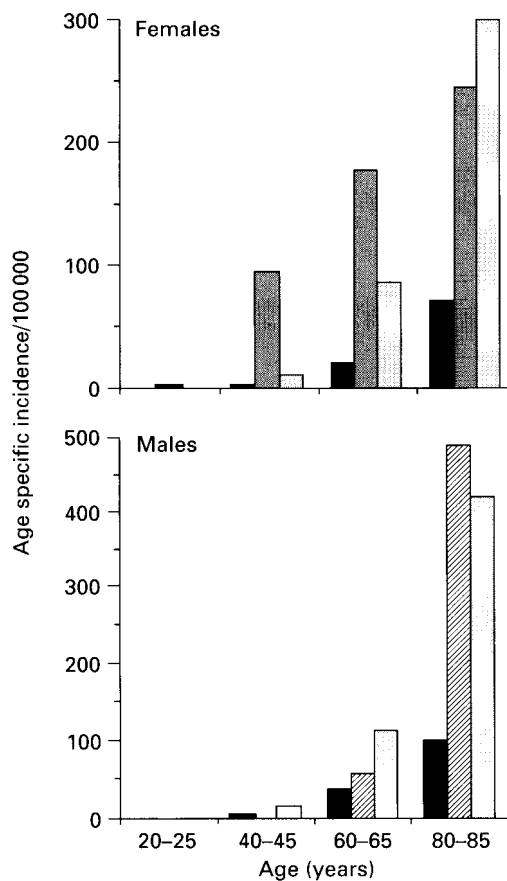
Fig. 1. Age specific incidence rates for different cancers (■), pancreas; (⊠), breast; (▨), prostate; (▦), large bowel; in England and Wales from ages 20 to 85 in 1983–1987 (Muir *et al.* 1987).

cases, these genetic defects are inherited, but the majority of large bowel cancers in societies where it is common are sporadic because affected patients have no known family history. The investigation of tumours from patients with inherited types of colorectal cancer has provided insight into the sporadic form of the disease because the same mutations occur in
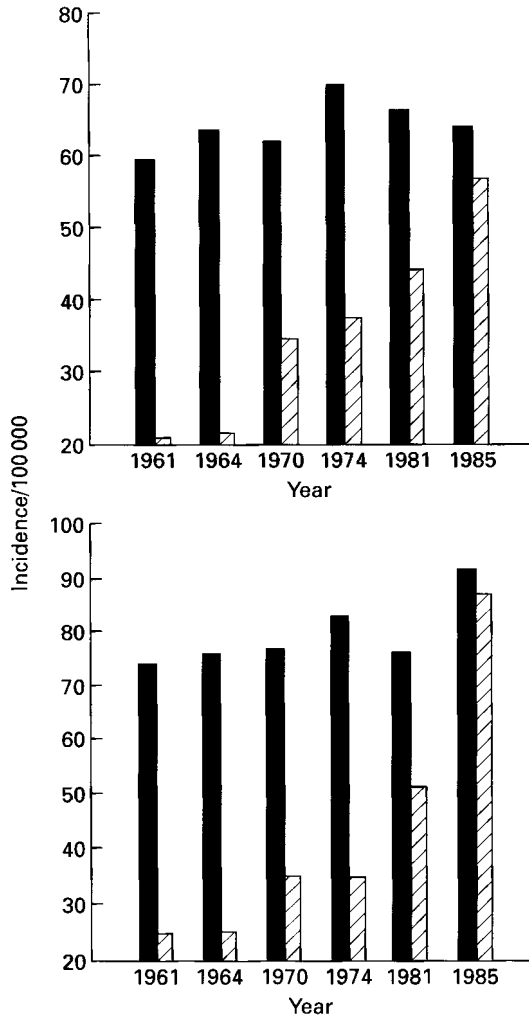
Fig. 2. 25 year secular trends in age specific colorectal cancer incidence rates in women and men aged 55–60 years in Japan (Miyagi registry) and in the UK (Birmingham registry). Solid columns UK, Hatched columns, Japan (Parkin *et al.* 1992 and preceding volumes Cancer Incidence in Five Continents, vols I–V, e.g. Muir *et al.* 1987).

both types to a significant extent (Bodmer *et al.* 1987; Solomon *et al.* 1987). This leads to the supposition that environmental factors are involved in somatic alterations in sporadic cancer. At present, the extent to which diet is capable of causing somatic alterations in genes known to be involved in the causation of cancer, or is able to prevent or mitigate these alterations, is an emerging area of research (see pp. 201–202).

For the time being, evidence linking diet with colorectal cancer is limited to epidemiological associations with support from experimental studies and plausible hypotheses. This review details the epidemiological evidence on pp. 203–214 and potential mechanisms in pp. 216–227. Overall conclusions linking the two are then summarized (pp. 227–228). Pp. 214–216 summarize the effect of diet found in animal models of colorectal cancer.

GENETIC FACTORS AND DIET

The development of cancer is generally thought to have a number of stages – primarily initiation, promotion and progression. Until recently, diet was not implicated in initiation but high fat diets, for example, were shown to promote or increase the numbers of tumours initiated by large doses of carcinogens in animal models (see pp. 214–216). With the development of new techniques, genetic models for human cancer have been proposed, and those for colorectal cancer are the most advanced.

Benign tumours or polyps (adenomas) occurring in the wall of the large bowel are premalignant lesions, known to confer increased risks of malignant tumours developing at a later stage. The risk of cancer increases with the size and numbers of polyps (Lotfi *et al.* 1986; Schofield & Jones, 1992). Vogelstein *et al.* (1988) were the first to demonstrate stepwise accumulation of chromosome deletions and mutations as disease progressed through adenomas to carcinoma. If diet does have a direct effect on somatic mutations, different dietary factors are likely to affect different stages of this sequence. Fig. 3 shows the original data on which present models are based. In these studies, few mutations or deletions were shown in small adenomas, but over 40 % of larger adenomas and carcinomas had *ras* (see below) mutations, indicating that they occurred at an early stage. By the carcinoma stage, there were multiple deletions, 70 % with deletions on chromosomes 18 and 17, later associated with loss of the p53 gene (see below). Fig. 4 is a later model, showing progression from normal epithelium through benign polyps to malignant tumours, with the progressive accumulation of these and other genetic mutations or chromosome deletions.

The first event is attributed to a mutation on chromosome 5, which is strongly associated with polyp development. The gene concerned (APC) is also a tumour suppressing gene, and was initially discovered in patients with a rare inherited syndrome known as familial adenomatous polyposis coli (Bodmer *et al.* 1987). Deletions on chromosome 5 also occur in sporadic tumours (Solomon *et al.* 1987).

Mutations of the *ras* gene, located on chromosome 12 and involved in the control of cellular proliferation and differentiation, occur in about half of colon cancers. As shown above, they occur less frequently in adenomas, indicating that *ras* mutation occurs before the conversion to malignant carcinoma. Particular types of mutations are most commonly involved (G-A transitions at the second G of a GG pair at codon 12 or 13 of K *ras*) and these are characteristic of alkylating agents such as *N*-nitroso compounds (NOC) (Bos, 1989). Faecal levels of NOC are elevated after a high meat diet (see p. 216).

Deletions on chromosomes 18 and 17 are common in colorectal carcinomas and are associated with loss of tumour suppressing genes. The DCC (deleted in colon cancer) gene is located on chromosome 18, and the gene on chromosome 17 encodes for the nuclear phosphoprotein *p53*, which controls a checkpoint in the cell cycle, is a trigger for programmed cell death (apoptosis) and is part of a pathway responsible for DNA damage repair (Friend, 1994). Mutant *p53* has a prolonged half life and, as shown above, has been detected in 70 % of colorectal carcinomas (Vogelstein *et al.* 1988). *p53* mutations commonly occur in cancers of other organs, but the type of the mutations in the genes is specific to particular sites. *p53* mutations in large bowel cancers for example are most commonly found at codons 175, 248, 273 at CpG hotspots, with G:C and A:T transitions found in 79 % (Hollstein *et al.* 1991). Cytosine could be deaminated, leading to a transition to thymine, either spontaneously through oxidative damage, by relative deficiency of methyl groups or in the presence of nitrite (Harris, 1993). These latter factors could be affected by diet (see pp. 222–223, 226). Butyrate (see pp. 217–218) induces apoptosis in colonic cell lines.
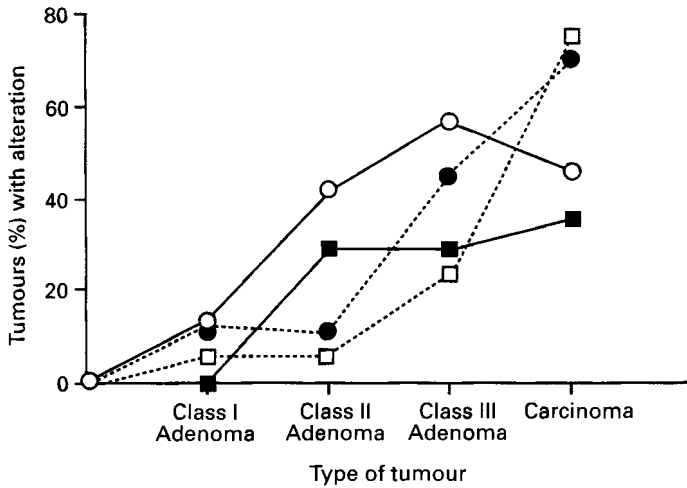
Fig. 3. Genetic alterations during colorectal tumour progression (○), *ras* mutation; (■), 5q allelic deletion; (●), 18q allelic deletion; (□), 17p allelic deletion (reproduced with permission from Vogelstein *et al.* (1988).
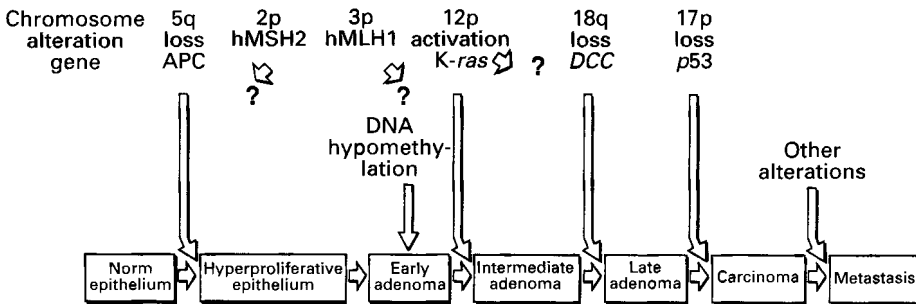
Fig. 4. Genetic model for colorectal cancer, adapted from Fearon & Vogelstein (1990), showing possible interactions with diet.

In another inherited syndrome, the Lynch syndrome (also called Hereditary Non-Polyposis Colorectal Cancer), in which polyps do not occur but affected individuals may develop cancer in other organs in addition to the bowel, another mutant gene has been found on chromosome 2 (Peltomäki *et al.* 1993). This familial colorectal cancer gene appears to be responsible for maintaining accurate DNA replication in many different locations in chromosomes. Mutation of this gene leads to multiple small changes, or microsatellite instability, that would induce greater susceptibility to colon (and other) cancers in patients with the Lynch syndrome. Microsatellite instability also occurs in another gene on chromosome 3 in hereditary non-polyposis colorectal cancer (Bronner *et al.* 1994). In rats one of the heterocyclic amines found in cooked meat is able to induce microsatellite instability (see pp. 223–224).

Particular genotypes have also been found to be at increased risk of colon cancer, for example those who are glutathione S-transferase competent or null (Strange *et al.* 1991) (see p. 225). Fig. 4 shows possible interactions between dietary factors and the genetic model of colorectal cancer.

EPIDEMIOLOGY OF DIET AND COLORECTAL CANCER

MAIN FACTORS (FAT, MEAT, STARCH, NON-STARCH POLYSACCHARIDES, VEGETABLES)

Cross-sectional comparisons

A number of comparisons between varying colorectal cancer rates worldwide and diet have been made. Higginson & Oettle and Burkitt and coworkers were among the first to document differences in colorectal cancer incidence among 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" (Higginson & Oettle, 1960; Burkitt, 1969). In Japan, the secular trends in colorectal cancer incidence have been accompanied by increasing westernization of the diet, so that meat intakes have increased 9-fold since 1950 and fat intakes 3-fold. Rice (and probably starch) consumption fell by one third, but there was little change in non-starch polysaccharide (NSP; dietary fibre) consumption over this time (Minowa *et al.* 1983; Kuratsune *et al.* 1986). However, owing to the low content of NSP in rice, Japanese intakes of NSP have never been high.

In the UK there was a decline in colorectal cancer rates during the 1940s and 1950s, which has been attributed to the 12 g per day wartime increase of NSP intakes (McMichael *et al.* 1979; Powles & Williams, 1984).

Armstrong & Doll (1975) attributed much of the international variation in large bowel cancer incidence between countries to dietary differences, especially meat and fat consumption. The relationship with meat is shown in Fig. 5. Other ecological studies of this type have found similar strong positive associations between fat and animal protein consumption and bowel cancer mortality (Drasar & Irving, 1973; Howell, 1975; Thind, 1986). McKeown-Eyssen & Bright-See (1985) correlated dietary fibre intakes in addition with colon cancer mortality rates in 38 countries. They reported higher estimates of dietary fibre intake in low colon cancer risk countries ($r = -0.66$). However, because of the strong correlation with meat and fat ($r = 0.88$ and 0.74 respectively), the fibre correlations became non-significant when partial correlation analysis taking into account meat and fat was performed ($r = -0.18$, -0.36).

Within the UK, where meat (150 g per day) and fat (100 g per day) intakes are high, partial correlation controlling for fat, beef and protein suggested that the protective associations with NSP were independently related to bowel cancer (Bingham *et al.* 1979, 1985). Significant inverse relations between bowel cancer and intakes of NSP and dietary fibre were also obtained from two studies of geographical areas at differing risk of colorectal cancer within Scandinavian populations, and in Germany (IARC, 1982; Boing *et al.* 1985).

When accurately measured, the amount of NSP found in diets worldwide is much less than the other major polysaccharide in food, starch. Information on starch intake is rarely reported in the literature, because most papers confine their reports to total carbohydrate estimates. Older food tables estimated carbohydrates 'by difference' and direct measurement of sugars and starches was not undertaken. In a recently published study of individual surveys of food consumption in 12 countries, starch consumption was assessed (Cassidy *et al.* 1994). A positive association with protein and fat ($r = 0.60$ and 0.62 respectively) was confirmed, and weak negative associations with NSP ($r = -0.23$). There were, however, strong ($r = -0.70$) inverse associations between colorectal cancer incidence and starch intake. These were maintained after partial correlation controlling for meat and fat consumption.

Cross-sectional examinations to date have therefore concentrated on relations with fat,
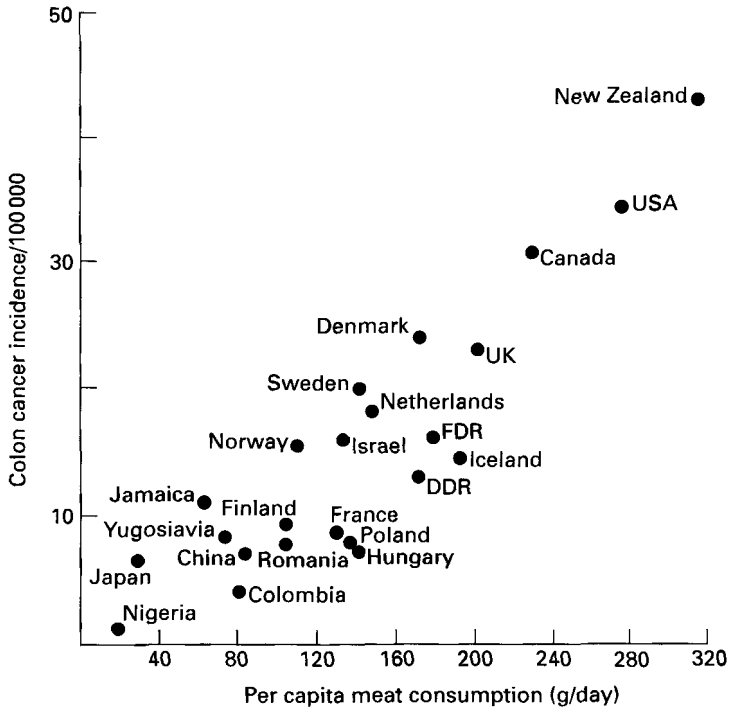
Fig. 5. The relationship between meat consumption and colorectal cancer in various countries, adapted from Armstrong & Doll (1975).

meat and NSP (fibre). High meat and fat intakes seem to be prerequisites for high colorectal cancer incidence, and within these high risk populations increased levels of NSP are protective. Vegetarians are generally at lower risk (Johansson, 1990; Frentzel-Beyme & Chang-Claude, 1994). Vegetables and starch also have strong protective associations against colorectal cancer. In the UK, regional differences in colorectal cancer mortality are strongly related to consumption of vegetables excluding potatoes ($r = -0.94$) (Bingham et al. 1979).

Retrospective case-control studies

Analytic epidemiological studies offer major advantages over cross-sectional population studies because individual exposure to a dietary variable can be related to individual outcome. The most common type of study is the retrospective case-control, whereby patients with bowel cancer are studied after diagnosis and the results compared with those of controls who are healthy, or hospitalized for other conditions.

Reviews of some 30–35 existing case-control studies, conducted in widely varying circumstances and populations, including the USA, Japan, Canada, Australia, France and Belgium, have all concluded that the majority of studies of this sort indicate an increased risk of colorectal cancer in individuals who reported consuming more meat, protein and fat, and an increased risk for those reporting a lower consumption of fibre or fibre-containing foods (Bingham, 1990 a, b; Trock et al. 1990; Margetts, 1994; Potter et al. 1994).

There is also a consistent inverse association with protection from vegetables. According to Potter et al. (1994), 23 out of 28 studies reporting results for vegetables showed a significant inverse association. In a meta-analysis of case-control studies, relative risk

estimates for vegetables alone (0·48) were only slightly more convincing than those based on fibre intake (0·58) (Trock *et al.* 1990). Attributable population risk estimates from case-control studies suggest that 25–35 % of colorectal cancers might be prevented by high intake of vegetables and fibre and that 15–25 % could be attributed to a high fat intake (Tomatis *et al.* 1990). Measurement of starch consumption has only rarely been attempted in case-control studies; one study showed a significant reduction in risk with starch, and two others showed no effect (Tuyns *et al.* 1987; Slattery *et al.* 1988; Zaridze *et al.* 1993).

A major drawback of case-control studies is that the most common symptoms in large bowel disease are pain and altered bowel function (Cummings, 1981 *a*), 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. This procedure is difficult to validate with certainty, but circumstantial and other evidence suggests that recall of past diet is strongly influenced by present diet, since reports of past dietary consumption are more closely related to present consumption, and the discrepancy is greater the longer the period of recall (Friedenreich *et al.* 1992). Recall bias has not been assessed in case-control studies of large bowel cancer, but relative risks for breast cancer were higher in relation to high fat intake in two case-control assessments compared with those found in the same individuals assessed prospectively (Friedenreich *et al.* 1991; Giovannucci *et al.* 1993 *b*).

Cohort studies

Cohort studies are designed to eliminate bias arising from consequence rather than cause of disease, but need to be large to accumulate sufficient power. Results of studies set up several years ago are now beginning to appear in the literature, but the need for accuracy in dietary assessment has only recently been recognized, hence very crude assessments of dietary intake, mainly based on short lists of foods (food frequency questionnaires) were used in most of these. These assessments would have been associated with a substantial degree of measurement error not amenable to correction. The major drawback with these types of methods is that hypotheses are likely to change over the course of a 10-year prospective study and the list of foods devised for a food or nutrient concerned with one hypothesis (for example fat consumption) is unlikely to be suitable for another, such as NSP or vegetables. There is currently much interest in minor constituents of the diet (see below) and factors such as cooking practices may become increasingly important; prospective studies that include detailed estimates of food consumption (or biomarkers of intake) are more likely to be able to investigate any relationship between these factors and cancer risk. Equally, risks from diet are more likely to be measurable in cohorts of individuals with widely differing dietary habits, for example in the European Prospective Investigation of Cancer; this is a cohort of 400000 individuals in nine European countries, from northern countries such as Sweden and the UK to Mediterranean countries such as Greece and Spain. Information being collected from all participants includes not only estimates of diet but also the collection of biological specimens which will be used to link diet and cancer registrations with intermediate markers of risk such as hormonal status, DNA adducts, biomarkers of diet and genotypic risk factors.

No biomarker of colon cancer risk is currently available, but the usefulness of biomarkers in characterizing individual risk is illustrated in a follow-up study of markers of aflatoxin exposure in relation to liver cancer. The range of aflatoxin contamination of foods is very great, so that use of food tables of average levels of contamination is unlikely to pick up individual exposure. Relative risks of cancer from aflatoxin consumption were only 0·9 and insignificant (95 % confidence intervals (CI) 0·4–1·9) for individuals classified as having had high dietary exposure, as assessed by an interview on the frequency of con-

Table 2. *Trends in risk of large bowel cancer in prospective studies by quantile of fat and meat*

Total number	Significant positive association	Significant inverse association	Effects not significant
Fat			
8	Morgan *et al.* 1988	Stemmermann *et al.* 1984	Garland *et al.* 1985
	Willett *et al.* 1990		Thun *et al.* 1992
			Bostick *et al.* 1994
			Giovannucci *et al.* 1994
			Goldbohm *et al.* 1994
Total meat			
3		Hirayama, 1981	Phillips & Snowdon, 1993
			Goldbohm *et al.* 1994
White meat			
6		Willett *et al.* 1990	Heilbrun *et al.* 1989
			Thun *et al.* 1992
			Bostick *et al.* 1994
			Goldbohm *et al.* 1994
			Giovannucci *et al.* 1994
Red meat			
6	Willett *et al.* 1990		Heilbrun *et al.* 1989
	Giovannucci *et al.* 1994		Thun *et al.* 1992
			Bostick *et al.* 1994
			Goldbohm *et al.* 1994
Processed meat			
4	Willett *et al.* 1990		Bostick *et al.* 1994
	Goldbohm *et al.* 1994		Giovannucci *et al.* 1994

sumption of 45 foods. However, aflatoxin exposure biomarkers in urine samples obtained from individuals in the cohort were able to detect substantial significant relative risks for liver cancer in the order of 6–10. Relative risks were 59·4 (16·6–212·0) in individuals positive for urine biomarkers of both aflatoxin and hepatitis B (Quian *et al.* 1994). Unfortunately the accessibility of suitable samples for biomarker analysis is likely to be more difficult in colon cancer, owing to the need to sample either faecal matter or mucosal tissue.

Of the existing studies which have reported, total fat intake has been measured in eight prospective cohort studies of large bowel cancer (Stemmermann *et al.* 1984; Garland *et al.* 1985; Morgan *et al.* 1988; Willett *et al.* 1990; Thun *et al.* 1992; Bostick *et al.* 1994; Giovannucci *et al.* 1994; Goldbohm *et al.* 1994). In two (Morgan *et al.* 1988; Willett *et al.* 1990) there was a significant positive association between trends in fat consumption and large bowel cancer (Table 2). The majority of studies showed no significant effects, and one suggested a significantly lower risk with higher fat intake (Stemmermann *et al.* 1984). However, most studies in which relative risks have been reported suggest an elevation of relative risk for individuals at the upper ends of the distribution in fat consumption, although confidence intervals generally embrace 1·00 and relative risks are less than 2 (Fig. 6). Available evidence to date, therefore, is weakly supportive of an increased risk of bowel cancer with high fat diets, although results from more accurate ongoing prospective studies are awaited.

Various indices of meat consumption have been measured in eight prospective studies (Hirayama, 1981; Phillips & Snowdon, 1985; Heilbrun *et al.* 1989; Willett *et al.* 1990; Thun *et al.* 1992; Bostick *et al.* 1994; Giovannucci *et al.* 1994; Goldbohm *et al.* 1994). Table 2 shows that few studies have reported risks from total meat consumption in colorectal
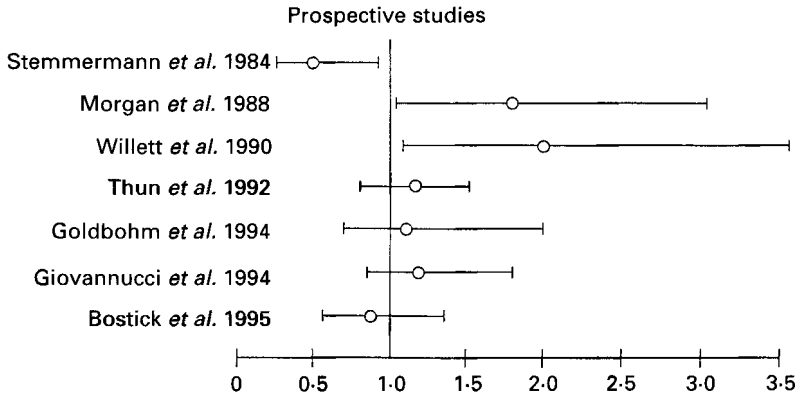
Fig. 6. Relative risks for colon cancer and 95% confidence intervals for the highest *v.* the lowest quartile of fat consumption in prospective studies.
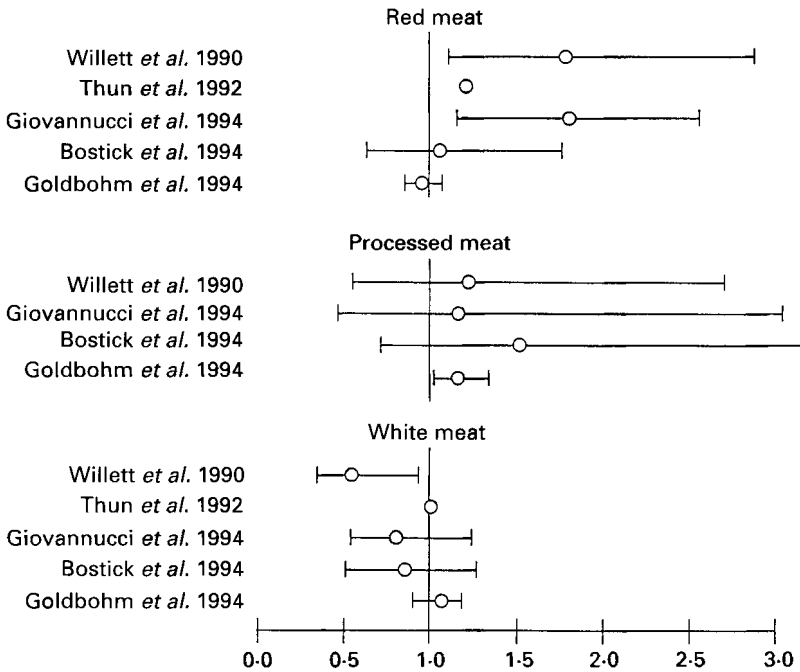
Fig. 7. Relative risks for colon cancer and 95% confidence intervals for the highest *v.* the lowest quartile of meat consumption in prospective studies.

cancer. Two studies (Willett *et al.* 1990; Giovannucci *et al.* 1994) have shown significant evidence of increased trends of colorectal cancer risk with red meat consumption and one a significant reduction in risk from white meat (Willett *et al.* 1990). Table 2 shows that a significant elevated risk for processed meat consumption and large bowel cancer was shown in two studies (Willett *et al.* 1990; Goldbohm *et al.* 1994).

Fig. 7 shows relative risks for highest *v.* lowest classifications of red, processed and white meat consumption, and confidence intervals where these are published. Though the majority of studies have wide confidence intervals and therefore non-significant results,

Table 3. *Trends in risk of large bowel cancer in prospective studies by quantile of fibre and vegetables*

| Total number	Significant positive association	Significant inverse association	Effects not significant
Fibre			
7	none	Heilbrun *et al.* 1989	Morgan *et al.* 1988
		Thun *et al.* 1992	Willett *et al.* 1990
			Giovannucci *et al.* 1994
			Goldbohm *et al.* 1994
			Steinmetz *et al.* 1994
Vegetables			
6	none	Morgan *et al.* 1988	Hirayama, 1981
		Shibata *et al.* 1992 (women)	Shibata *et al.* 1992 (men)
		Thun *et al.* 1992	Giovannucci *et al.* 1994
		Steinmetz *et al.* 1994	

Fig. 8. Relative risks for colon cancer and 95% confidence intervals for the highest *v.* the lowest quartile of fibre consumption in prospective studies.

there is a trend for red and processed meats to increase colon cancer risks, and some evidence that white meat is associated with either no effect, or reduction in risk.

Seven studies have assessed fibre or fibre-containing foods, and two (Heilbrun *et al.* 1989; Thun *et al.* 1992) detected a significant inverse protective trend between dietary fibre and colorectal incidence. No study demonstrated an enhanced risk (Table 3). Five did not detect significant trends, but in one (Willett *et al.* 1990) fibre was protective in high fat consumers and in two a non-significant inverse trend (Giovannucci *et al.* 1994; Steinmetz *et al.* 1994) was shown. The lack of association with trends in bowel cancer in the Male Health Professionals Study of Giovannucci *et al.* (1994) occurred despite higher fibre consumption in the lowest risk group (Fig. 8). Relative risks for this and other studies which report them also tend to be less than 1·00 and range from 0·9 to 0·5 in individuals classified in the upper ends of the distribution of fibre intake (Fig. 8). No study has reported bowel cancer risk using analyses of dietary fibre as NSP. No prospective study has attempted to measure starch consumption, although cereals appeared to be inversely associated in the study of Hirayama (1981).
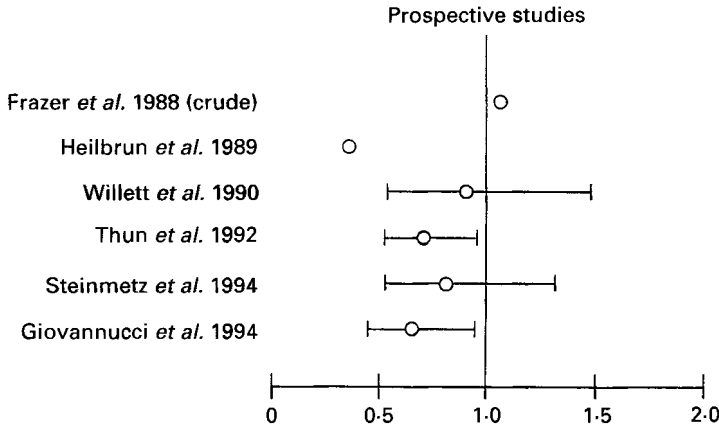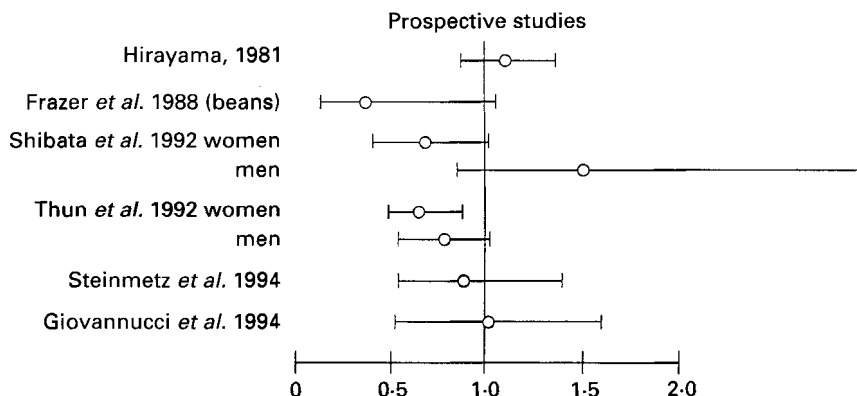
Fig. 9. Relative risks for colon cancer and 95% confidence intervals for the highest *v.* the lowest quartile of vegetable consumption in prospective studies.

Six prospective studies have investigated vegetable consumption. None have reported increased trends in risk with increased consumption, and three have shown significant protective effects (Morgan *et al.* 1988; Thun *et al.* 1992; Steinmetz *et al.* 1994). In one, significant protective effects were shown in women and a non-significant higher risk in men (Shibata *et al.* 1992). A non-significant higher risk was also shown in the study of Hirayama (1981), Table 3. Willett *et al.* (1990) did not report vegetable consumption, although a failure to find significant effects with 'vegetable fibre' probably indicates no significant effects. No significant findings were shown in the study of Giovannucci *et al.* (1994). Fig. 5 shows relative risks in the individuals classified in highest *v.* lowest categories of consumption; these are generally in the direction of lower risk with increased consumption and relative risks in the order of 0·9–0·5.

Of the few prospective studies published so far, results to date are therefore not entirely consistent with those obtained from cross-sectional and case-control studies, suggesting a markedly increased risk for greater total meat and fat consumption. Only the studies of US health professionals (Willett *et al.* 1990; Giovannucci *et al.* 1994) are strongly supportive of a role for meat, and that for red meat, although the evidence so far suggests that processed meat consumption also appears to increase the risk. The US health professional studies have not detected an inverse role for vegetables and no role for fibre was detected in women, although generally the evidence relating vegetable and NSP intake is more consistently associated with protection from large bowel cancer. Overall, there is suggestive evidence of a slightly increased risk for fat, red meat and processed meat and a reduced risk for vegetables and NSP. However, the magnitudes of the relative risks are low and generally non-significant, in the order of 1–2 for increased risk and 1–0·5 for reduced risk (Figs 6–9). This compares with relative risks of 15 for smoking and lung cancer. More good quality data are required, and will not be forthcoming for 5–10 years.

Studies in patients with adenomatous polyps

The dietary habits of patients with polyps are probably related to risk of cancer, although the risk of transformation for an individual adenoma is low. There are a number of case-control studies of dietary factors (Table 4). The majority have yielded non-significant associations. Two prospective studies examining macronutrients have so far been conducted (Stemmermann *et al.* 1988; Giovannucci *et al.* 1992) and a number of intervention studies are in progress (see p. 212). The prospective study of Giovannucci *et al.* (1992), on 7284 male

Table 4. *Summary of trends in risk from adenomatous polyps case-control and prospective* studies by quantile*

Item	Total number	Positive association	Inverse association	Not significant
Meat	7	*Giovannucci et al. 1992 (red) Kono et al. 1993	Neugut et al. 1993 (chicken)	Neugut et al. 1993 (red) Hoff et al. 1986 Macquart-Moulin et al. 1987 Sandler et al. 1993 Benito et al. 1993
Protein	6			Hoff et al. 1986 Macquart-Moulin et al. 1987 *Giovannucci et al. 1992 *Stemmermann et al. 1988 Neugut et al. 1993 Sandler et al. 1993
Fat	6	Hoff et al. 1986 Sandler et al. 1993 (females) *Giovannucci et al. 1992 Little et al. 1991 (polyunsaturates)	Neugut et al. 1993 (males)	Neugut et al. 1993 (females) Sandler et al. 1993 (males) Macquart-Moulin et al. 1987 *Stemmermann et al. 1988
Fibre	5		Neugut et al. 1993 (males) Little et al. 1991 *Giovannucci et al. 1992	Neugut et al. 1993 (females) Hoff et al. 1986 Sandler et al. 1993
Vegetables	5		Hoff et al. 1986 *Giovannucci et al. 1992 Sandler et al. 1993 (women) Benito et al. 1993	Macquart-Moulin et al. 1987 Neugut et al. 1993 Sandler et al. 1993 (men)

health professionals, showed positive associations with red meat and fat (including saturates and monounsaturates but not polyunsaturates), and inverse associations with total carbohydrates, dietary fibre, and dietary fibre from cereals and vegetables.

OTHER DIETARY FACTORS

Calcium

Interest in calcium arose as a result of the hypothesis that high intakes (2 g/d or more) would inhibit the toxic effects of free fatty acids entering the large bowel (Newmark et al. (1984), see p. 219). UK average intakes of calcium from food are about 700 mg/d, so supplements are required to attain these high levels. Several intervention studies with calcium are in progress to assess recurrence of polyps in patients at high risk of colon cancer but no results have yet been reported.

Potter et al. (1994) have reviewed the epidemiological evidence and suggested that a protective association with calcium is evident. To date, six cohort studies have reported calcium intake in relation to risk of colorectal cancer. All are suggestive of a reduced risk with high intakes although relative risks were not universally significantly reduced (Fig. 10).
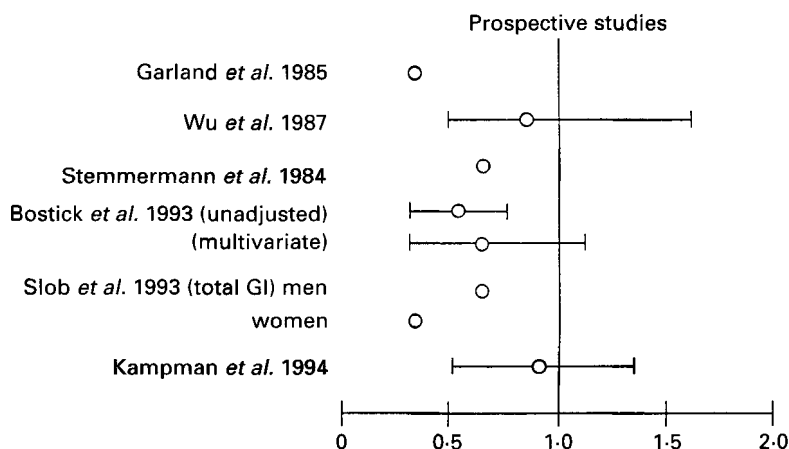
Prospective studies

Fig. 10. Relative risks for colon cancer and 95% confidence intervals for the highest *v*. the lowest quartile of calcium consumption in prospective studies.

Relative risks in the study of Slob *et al.* (1993) refer to all gastrointestinal (including stomach) cancer because there were too few cases for a separate analysis. Women who died of colorectal cancer had, however, consumed less calcium than the rest of the population. Bostick *et al.* (1993) were unable to detect significant differences in multivariate analyses of a cohort study. Univariate results for calcium in food did not show a significant protective effect, but cases had consumed 209 mg calcium/d from supplements whereas controls had consumed 283 mg/d from supplements, and these differences were significant. Overall therefore, results are suggestive but effects may arise from supplements rather than food.

Iron

Free iron is a well accepted catalyst in the Fenton reaction which yields hydroxyl radicals and it has been proposed that oxidative damage occurs in the colon, which can be suppressed by the presence of phytic acid, a known chelator of iron (Graf & Eaton, 1993). Animal studies have shown that very high doses of iron (580 mg/kg diet, ten times greater than human intakes) induce tumours, and that tumour yield is reduced if phytate is added to the diet (Nelson *et al.* 1989; Ullah, 1990). However, subsequently it has been shown that increased iron does not increase lipid peroxidation products in a rodent colon cancer model (Kuratko & Pence, 1995).

Of three epidemiological studies that have measured iron stores only the study of Nelson *et al.* (1994) assessed iron status from serum ferritin levels, which reflect body stores. In this case-control study there was no significant increase in relative risk for cancer patients (who may have reduced stores due to gastrointestinal bleeding), but adenoma patients at the upper end of the distribution in ferritin levels had a significant elevated relative risk of 4·3 (CI 2·00–10·1). The results remained significant after adjusting for alcohol intake and excluding individuals with serum ferritin levels in excess of 400 ng/ml. Two studies have measured transferrin saturation, although levels can be elevated secondarily to chronic inflammation. The study of Stevens *et al.* (1994) was based on a relatively small cohort and separate analyses for colon cancer were not possible. Elevated transferrin saturation levels were associated with increased risk of all cancer, either incidence or mortality. In a large Finnish cohort, elevated risks of 3·04 (CI 1·64–5·62) for colorectal cancer and cancer at all sites (1·43, CI 1·16–1·77) were evident in those individuals classified as having higher levels of transferrin saturation (Knekt *et al.* 1989).

Iron intake was measured in four case-control studies, three in polyp patients and one in rectal cancer patients. They have generally shown no significant effects or inverse trends (Hoff *et al.* 1986), but supplemental intakes may not have been recorded (quoted from Nelson *et al.* 1994). In summary, although high iron stores are associated with increased risk, these may not be related to increased intake, and data on intake from prospective studies are required to confirm a role for iron in colorectal cancer.

Vitamin D

Vitamin D is classically associated with calcium homeostasis but a more fundamental role in controlling cell growth and differentiation has more recently emerged. Garland *et al.* (1985, 1989) found significant protective effects against colon cancer for both dietary vitamin D and serum 25-hydroxyvitamin D in two separate cohorts. Later epidemiological evidence is less consistent, with a non-significant effect for vitamin D shown in one cohort and in two case-control studies (Potter *et al.* 1994).

Vitamins E and C

One reason often put forward for the protective effect of vegetables against cancer is that vegetables and fruits are the major source of vitamin C (70% in British diets) and of vitamin E. These are able to inhibit the formation of the surrogate for carcinogenic N-nitroso compounds, N-nitrosoproline, and possibly the mutagen isolated from faeces, fecapentaene. Like β-carotene, they are part of the antioxidant defence, which may be important in preventing spontaneous point mutations.

Using pooled data from five prospective cohorts, however, relative risks for the top quartile of serum vitamin E adjusted for serum cholesterol were not significant (0·7; 95% CI 0·4–1·1), and there were no significant trends in risk across quartiles (Longnecker *et al.* 1992). Relative risks were significantly reduced (0·3; CI 0·1–0·8) when only those patients with an interval of between 5 and 7·5 years from blood collection to diagnosis were included. At longer intervals the relative risk increased to 1·2 (CI 0·4–3·3). The authors concluded that the evidence for any protective effect was weak, although evidence from larger prospective studies was required to determine whether vitamin E has a modest protective effect in colorectal cancer (Longnecker *et al.* 1992). A later cohort (Bostick *et al.* 1993) reported a significant reduction in relative risk of colon cancer for women less than 60 years old who had taken vitamin E supplements, although no effects were found for dietary vitamin E alone. Logistically, there is little information on prospective levels of plasma vitamin C in relation to cancer and only the Basel prospective study (Stahelin *et al.* 1991) has assessed plasma vitamin C levels in relation to cancer, and the cohort size is too small to permit a separate analysis for colon cancer.

There are many other constituents of plants (folate, flavonoids, NSP, glucosinolates, carotenoids) and these could be of more relevance than these vitamins in colorectal cancer (see pp. 212–214, 218, 224–226).

Folate

Folate and vitamin B_{12} are critical elements in $-CH_3$ group metabolism and thus in thymidine and DNA repair. In women using oral contraceptives, localized folate deficiency was the suggested reason for the presence of dysplasia in the cervix, and intervention with folate has been shown to improve dysplasia scores (Whitehead *et al.* 1973; Butterworth *et al.* 1982). Bronchial metaplasia has also been shown to be improved in smokers given supplements of folate and vitamin B_{12} (Heimberger *et al.* 1988).

Connexions with large bowel dysplasia and hence cancer risk began with a retrospective case-control study in which folate supplementation was associated with a non-significant

lower incidence of dysplasia in ulcerative colitic patients, who are at high risk of colon cancer (Lashner *et al.* 1989). In a later study, risk of dysplasia in ulcerative colitis was inversely associated with red cell folate levels (Lashner, 1993). No prospective study has reported on the role of folate in colorectal cancer, but relative deficiency has been implicated in another large bowel disorder that carries increased risk of cancer, adenomatous polyps. Giovannucci *et al.* (1993*b*) found significant ($P = 0.02$) inverse trends in risk with increasing intake, with relative risks of 0·71 (CI 0·56–0·89) for colorectal polyps in individuals in the top quintile of folate intake. The median levels of energy adjusted folate intakes in men and women (847 and 711 μg/d) would not have been achievable from dietary sources (average UK intake 220 μg/d) and must have been largely derived from supplements. Folate from food alone was not significantly related to adenomas.

Meat is the major source of methionine which is the precursor of S-adenosylmethionine, the methyl donor for DNA methylation. S-adenosylmethionine is regenerated using folate. Despite their earlier positive associations found between adenomas and meat consumption (Giovannucci *et al.* 1992) the authors demonstrated inverse associations with methionine intake. No information on the contribution of supplements to total methionine intake is given in the paper, but energy adjusted intakes in the top quintile were 2·5–2·7 g/d, compared with WHO requirements of approximately 1·0 g/d for methionine and cysteine combined (WHO, 1973). Positive associations with alcohol, capable of diminishing hepatic S-adenosylmethionine, were also found, and the authors proposed that all three were related to colorectal cancer risk *via* hypomethylation of DNA (Giovannucci *et al.* 1993*b*).

Alcohol

In vitro studies suggest that alcohol is not a direct carcinogen, although acetaldehyde, the main metabolic product in humans, is a known carcinogen. Alcohol free extracts of some beverages are also genotoxic (IARC, 1988). Alcohol consumption is particularly linked with high risk of rectal, rather than colon, cancer. The prospective Kaiser Permanente study, for example, showed a stronger ($P = 0.03$) trend for alcohol use in rectal cancer than in colon cancer ($P = 0.11$). Relative risks in people consuming three or more drinks per day were 3·17 (1·05–9·57) for rectal cancer but non-significantly elevated in colon cancer (1·17; 0·92–3·19) (Klatsky *et al.* 1988). The IARC working group was unable to draw conclusions about the role of alcoholic beverages in the causation of colon cancer, and found the evidence linking rectal cancer and consumption of alcohol, mainly beer, suggestive but not conclusive (IARC, 1988). Potter *et al.* (1994) suggested that alcohol consumption is independently linked to colon cancer, and noted that seven out of fifteen general population studies showed positive associations. However, the meta-analysis of Longnecker *et al.* (1990) led to an overall relative risk of only 1·1 (1·05–1·14). More recently, Doll *et al.* (1993) also found no consensus on the interpretation of existing observations, except that if a risk does exist for colorectal cancer it is less than 2-fold, even with high levels of alcohol consumption.

Energy balance and exercise

There is an independent effect of total energy restriction on chemically induced carcinogenesis of the large bowel in rodents (Rogers *et al.* 1993). However, there appears to be little effect of energy intake in human cohort studies, where this is reported (Willett *et al.* 1990; Giovannucci *et al.* 1994). In humans, energy intake and expenditure, and hence energy balance, are interrelated, so that alterations in body size or expenditure might be expected to be related to colon cancer risk. Potter *et al.* (1994) were unable to establish a consistent effect of body size on colon cancer risk, but there were consistent protective effects of increased energy expenditure, in case control and cohort studies assessed from

both reported occupations and recreational activity (Potter *et al.* 1994). Direct evidence is available from a cohort study of Japanese migrants, in which physical activity and heart rate were measured. There were significant ($P = 0.03$) inverse trends in relative risk with resting heart rate, and significantly reduced relative risks in individuals classified in the upper third of physical activity (0·71; 0·51–0·99) (Severson *et al.* 1989).

Although the effect of exercise is usually attributed to reduced transit time through the large gut (Gerhardsson de Verdier *et al.* 1990), there is no effect of exercise on transit time in studies in which food intake has been controlled (Bingham & Cummings, 1989). Since relative risks for cancers at sites other than the large bowel are also reduced by exercise, a more general mechanism for a protective effect is likely. Increased serum triglycerides and glucose have been suggested to be the common factors linking diet, obesity and lack of exercise to increased risk of colon cancer. Higher levels of serum triglycerides were found to be associated with increased risk of polyp recurrence, and higher levels of circulating insulin or glucose may be associated with increased neoplastic cell growth (McKeown-Eyssen, 1994).

INTERVENTION STUDIES

Intervention studies so far have mainly been confined to assessing the effect of intervention on recurrence of polyps in high risk groups of patients with familial adenomatous polyposis, and of patients with adenomatous polyps. There are several large intervention studies in progress and dietary items being tested include supplements of wheat bran, ispagula, resistant starch, β-carotene and other antioxidant vitamins found in vegetables, calcium, and reduction in fat.

Very large supplements of vitamin A (30000 i.u.), C (1 g) and E (70 mg) reduced proliferation in upper crypt compartments in 23 patients compared with 23 patients given placebo, but there was no significant effect on polyp recurrence in patients given 400 mg doses of vitamins C and E compared with a placebo of lactose (McKeown-Eyssen *et al.* 1988; Paganelli *et al.* 1992). In an intervention study of patients with familial adenomatous polyposis, rectal polyp recurrence was inhibited to a greater extent by supplements of bran with vitamins C and E than by supplements of these vitamins alone (Decosse *et al.* 1989). In 300 polyp patients studied in Australia, supplements of 20 mg β-carotene increased polyp recurrence, particularly large polyps (MacLennan *et al.* 1991, 1995). Reduction in fat intake was not associated with an overall reduction in polyp numbers, although the combination of a low fat diet and 25 g wheat bran for 4 years did lead to a significant reduction in the frequency of large adenomas (MacLennan *et al.* 1995). A large multicentre trial from the USA has shown that supplements of 25 mg β-carotene, 1 g vitamin C or 400 mg vitamin E had no effect on polyp recurrence (Greenberg *et al.* 1994). A trial of Finnish smokers also showed no effect of large supplements of 20 mg β-carotene or 50 mg vitamin E per day on the incidence of large bowel cancer (ATBC, 1994). So far, therefore, any protective effect of vegetables in colon cancer cannot be attributed to antioxidant vitamins, and supplements of β-carotene may increase the risk. In the two studies that have so far reported results, supplements of NSP as bran seemed to reduce risk.

DIET AND CHEMICALLY INDUCED CARCINOGENESIS

NON-STARCH POLYSACCHARIDES, STARCH

The effect of purified sources of dietary fibre on chemically initiated colorectal cancer in animals has been investigated in a large number of studies. Owing to heterogeneity of experimental protocols, coupled with incorrect or non-existent statistical analysis, Klurfeld

(1990) was unable to interpret the existing evidence to support a protective effect for 'insoluble' fibre (bran, cellulose etc.) in chemically initiated colorectal cancer. In contrast, a collation of overall findings of experimental studies by the Federation of American Societies for Experimental Biology (Pilch, 1987) showed that bran appeared to have a consistently protective effect against chemical carcinogenesis. Bran decreased the number of tumours in 13 out of 17 studies, and increased the number of tumours compared with control levels in only one study. Cellulose also appeared protective in six of nine studies, with no significant difference in three. However, 'soluble fibres' are associated with tumour enhancement (Jacobs, 1990). This may be related to the fact that soluble fibres are rapidly fermented in the caecum, whereas insoluble fibres survive for longer (see pp. 224–227). Pectin also appears to have differing effects in humans to rodents, at least on bacterial enzyme activity (see pp. 219–220). In addition, the relevance of these studies to normal human diets containing comparatively low levels of mixed NSP is uncertain.

The effect of starch on chemical carcinogenesis has not been investigated so intensively, and there is only one report in the literature as yet. Using potato starch granules as the source of resistant starch, tumorigenesis induced by dimethylhydrazine was increased in rodents, but suppressed when wheat bran was added (Young et al. 1996).

FAT, ENERGY

Energy restriction has a powerful effect in reducing tumorigenesis at most sites via a number of postulated mechanisms such as reduced growth in all tissues, alteration of carcinogen metabolism, and reduction in oxidative damage to DNA. The restrictions required are > 10% of total intake, and the converse (increased tumorigenesis with increasing intake) is not clearly demonstrated because rodents will not voluntarily ingest excess energy (Rogers et al. 1993).

There is some debate as to whether high fat diets or high energy diets (usually a consequence of high fat diets) are more important. For example, in a combined analysis of fat, protein and energy intake, there were no significant effects of total fat on either initiation or promotion on azoxymethane induced tumours of the large intestine. Only energy intake was significantly associated (Clinton et al. 1992). In contrast, independent effects of fat and energy were proposed in a study where a significant increase in the number of azoxymethane induced tumours was found in animals fed high fat (as maize oil) ad lib. diets, compared with animals fed low fat restricted, low fat ad lib. and high fat restricted (by 20%) diets (Steinbach et al. 1993).

Any effect of total fat is also likely to be modulated by the effects of NSP and starch, in addition to energy and type of fat, and to depend on the use of direct acting carcinogens (e.g. N-methyl-N'-nitro-N-nitrosoguanidine) or indirect ones, such as azoxymethane. No consistent effects of total fat on N-methyl-N'-nitro-N-nitrosoguanidine tumours at high levels of fibre were found, for example, whereas tumour incidence increased with high fat, low fibre diets (Sinkeldam et al. 1990).

n-3 AND n-6 FATTY ACIDS

The type of fat consumed seems to be important in colorectal tumorigenesis, n-6 fatty acids being required to at least 5% energy to satisfy tumour requirements. Higher levels may induce a greater number of tumours (Reddy & Sugie, 1988). At equivalent levels, n-3 fatty acids are associated with lower tumour incidence. Reddy & Sugie (1988) and Reddy et al. (1991), for example, found higher levels of tumours in rats fed 24% maize oil than in rats fed 18% menhaden oil plus 5% maize oil. However, the effect of n-3 and n-6 fatty acids in

reducing and increasing tumorigenesis respectively is not as consistent in the animal models of colon cancer as it is in models of mammary cancer (Rogers *et al*. 1993).

PROTEIN AND MEAT

Visek (1978) proposed a role for high protein diets in colorectal cancer and showed that tumour yield is increased in animals fed high protein diets (Topping & Visek, 1976). However, there have been few investigations in animals and one later study has found no effect of protein on either the total number of tumours or percentages of animals with tumours, apart from an increase in polyploid types of tumour (Clinton *et al*. 1992). Cooked casein (and sugar) has been shown to increase colonic microadenoma in rodents (Corpet *et al*. 1990) and low protein diets are known to reduce the activity of phase I enzymes (see pp. 224–225). There are conflicting reports of the effects of high meat diets in modulating promotion of colon carcinogenesis in animal models (Reddy *et al*. 1976; Pence *et al*. 1995) but meat may be more important in initiation rather than promotion (see pp. 222–224). No effect of raw or grilled beef has been shown compared with soya protein (Clinton *et al*. 1979).

POTENTIAL MECHANISMS

At least 10 different dietary factors are linked with colorectal cancer. Plausible hypotheses involving dietary factors in the aetiology of large bowel cancer mainly concern events in the lumen of the large bowel and the metabolism of the colonic flora, which in turn is controlled by diet. In addition, genotoxins are modified by phase I and II enzymes and colonic epithelial cells are exposed to systemic factors such as n-3 fatty acids.

THE COLONIC FLORA AND FERMENTATION

The colon is host to a large and diverse commensal flora of anaerobic bacteria. With more than 400 species (Feingold *et al*. 1983) the colonic flora has considerable flexibility and potential for metabolic transformations. Enzymes are readily induced by changes in the metabolic environment and a wide variety of substances can be hydrolysed, reduced, degraded, or synthesized. 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. Lipids in the form of bile acids, phospholipids and fatty acids are also metabolized by the flora. Sulphate and nitrate arising directly or indirectly from diet are also of considerable current interest.

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 (biomass). The SCFA are absorbed by the intestinal mucosa where they stimulate sodium absorption and bicarbonate production (Cummings, 1981*b*). Sugars such as lactose in lactase deficient individuals, and oligosaccharides such as fructooligosaccharides, and inulin are substrates for fermentation, but normally it is the polysaccharides that are quantitatively most important. Of these, only 12 g NSP ('fibre') is available in western diets, an amount that is insufficient to account for the known amounts of SCFA produced (Cummings, 1981*b*). However, studies in man have shown that a significant amount of starch escapes digestion in the small gut, depending on the physical form of the food eaten, the granule type, and how it is cooked and processed (Englyst *et al*. 1992). This starch, resistant starch, reaches the large bowel and is also a substrate for fermentation (see also Chapter 1).

Dilution, stool weight and transit time

Carbohydrate fermentation has a number of implications for protection against large bowel cancer. The stimulation of bacterial growth, together with water binding to residual unfermented NSP, leads to an increase in stool weight, dilution of colonic contents and faster transit time through the large gut (Cummings, 1981 *a*; McBurney *et al.* 1985; Phillips *et al.* 1995). This may reduce contact of genotoxins with mucosal cells, and supplements of bran have been found to reduce faecal mutagenicity (Venitt *et al.* 1986; Reddy *et al.* 1987). Long transit time has not been related to large bowel cancer risk, but there is a strong inverse association between high stool weight and colorectal cancer incidence (Cummings *et al.* 1981, 1992). Low stool weight leads to constipation, which together with use of cathartics are risk factors for colorectal cancer. Odds ratios are 1·48 (1·32–1·66) and 1·46 (1·33–1·61) respectively, with attributable risks for colon cancer of 4·4% in the US population (Sonnenberg & Muller, 1993). High stool weight is also associated with less crosslinking during passage of a DNA surrogate through the gut (Bingham *et al.* 1996). The association between low stool weight and bowel disease and the linear relationship between NSP consumption and stool weight, with a 5 g increase for every 1 g NSP consumed, is the basis for UK and WHO recommendations of an 18 g population average intake of NSP, a 50% increase for the UK and most Western populations (World Health Organization, 1990; Department of Health, 1991)

Butyrate

During fermentation, approximately 60, 20 and 20% molar ratios of acetate, propionate and butyrate are formed in the large gut (Cummings, 1981 *b*). Molar ratios can however be varied according to the substrate. In *in vitro* batch cultures, 29% of butyrate can be produced from starch, compared with 2–8% from NSP sources (Englyst *et al.* 1987), and *in vivo* molar ratios of butyrate can be made to increase by 50% by feeding the glucosidase inhibitor acarbose (Scheppach *et al.* 1988). Starch may therefore be a better source of butyrate than NSP.

SCFA are absorbed from the large gut (Cummings *et al.* 1987), enhancing the functional capacity of the epithelium (Scheppach *et al.* 1990). Less butyrate is found in the portal vein, and in the isolated human colonocyte butyrate accounts for about 75% of oxygen consumption. Glucose is able to replace butyrate to a lesser extent in the proximal colon compared with the distal colon (Roediger, 1980). This suggests that the sigmoid and distal colon are particularly dependent on adequate supplies of butyrate. These are the areas of the gut where most tumours arise.

Because of this nutritional SCFA function, indices of proliferation induced by resistant starch or NSP are necessarily greater than levels found on baseline semi-purified diets in some rodent studies. Incubation of caecal biopsies with SCFA increased the labelling index in the lower crypt compartments of human caecal biopsies from patients maintained on low dietary fibre diets (18 g, equivalent to 12 g NSP). Butyrate and propionate were more effective than acetate. However no expansion of the proliferative compartment to the crypt surface was observed (Scheppach *et al.* 1992). NSP and resistant starch, which are slowly fermented because of their stereochemistry, are more likely to reach the distal colon before being fermented into SCFA. Hence slowly fermented resistant starch and NSP sources are likely to be physiologically more important in the distal bowel than NSP sources such as pectin and guar gum which are rapidly fermented in the caecum.

Butyrate was suggested as a protective agent against colon cancer in 1981 (Cummings *et al.* 1981). Two studies using direct acting carcinogens in rodents have not shown a protective effect against carcinogenesis of large amounts of butyrate added to drinking

water (Freeman, 1986; Deschner *et al.* 1990). These experiments were conducted with rodents fed on Purina chow which, with its 4·5% crude fibre content, would have been equivalent to 20% by weight NSP or an intake of 100–200 g NSP per day in human terms. This would have already provided butyrate markedly in excess of rat or human needs.

In cultured cell lines, butyrate is a well recognized antiproliferative agent, arresting cell growth in G_1, and inducing differentiation. Histone deacetylase is inhibited and other SCFA are much less active in this respect (Kruh, 1982). Alterations in gene expression occur and chromatin accessibility to DNA repair enzymes is altered (Smith, 1986). Rodent studies have shown that luminal butyrate levels are inversely associated with colonic cell proliferation, and positively associated with histone acetylation (Boffa *et al.* 1992). High starch diets, providing substrates for increased amounts of butyrate in the lumen, have been shown to reduce proliferative activity in the colon of mice and in humans (Caderni *et al.* 1989; Van Munster *et al.* 1994).

In addition to its effect on cell growth and differentiation, butyrate stimulates cytoskeletal organization, and alters gene expression. In colonic cells, butyrate induction of placental-like alkaline phosphatase is regulated by increased message levels which occur with differentiation. In LS174T cells, the 5′ flanking regions of the placenta-like alkaline phosphatase gene were found to contain *cis* acting elements which regulate placenta-like alkaline phosphatase expression (Kim *et al.* 1994). In Swiss 3T3 cells, arrest of cell growth and differentiation by butyrate is associated with a reduction in c myc, p53, thymidine kinase and induction of cfos and aP2 (Toscani *et al.* 1988). Paraskeva and colleagues (Hague *et al.* 1993) have suggested that butyrate induces apoptosis, or programmed cell death, which may account for its role in reducing proliferation.

Fat, bile acids, pH

High fat diets increase faecal mutagenicity in humans (Nair *et al.* 1990) and lead to increased levels of bile acids in the colonic lumen (Cummings *et al.* 1978). The secondary bile acid deoxycholic acid is known to be damaging to the mucosa and a promoter of bowel cancer in rodent systems (Narisawa *et al.* 1974). Bile acids alone are unlikely to affect colon cancer risk because there is no difference in faecal bile acid excretion either between cases and healthy matched controls, or between individuals living in high risk areas compared with low ones (Setchell *et al.* 1987). However, because of their insolubility, bile acids are less damaging at low pH (Rafter *et al.* 1986). During fermentation, the SCFA produced reduce luminal pH. Bacterial 7α-dehydroxylase activity and hence conversion of primary to secondary bile acids deoxycholic and lithocholic acids is also inhibited (Midvedt & Norman, 1968; Van Munster *et al.* 1994).

Diacylglycerol

A role for bile acids in promotion may be through diacylglycerol (DAG), one of two intracellular messengers formed from phosphatidyl inositol. DAG increases the affinity of protein kinase C for calcium and renders it active at physiological levels of this ion, phosphorylating serine and threonine residues in many target organs (Nishizuka, 1986). Phorbol esters are well known promoters because they resemble DAG but are not degraded.

The fatty acid composition of DAG is modified by intake of dietary lipid, and it is possible that a change in the type of DAG may affect activation of protein kinase C. Methyl group deficiency is also able to increase DAG levels, at least in the liver (Rogers *et al.* 1993). Increased levels of protein kinase C have been reported in colonic tumour tissue (Guillem *et al.* 1987) and this group of workers has also shown that DAG production from

phosphatidylcholine is enhanced in human fermentation systems by the presence of deoxycholic acid (Morotomi *et al.* 1990). Total faecal DAG levels have been shown to be reduced by a supplement of 15 g wheat bran in women (Reddy *et al.* 1994).

Calcium

Another proposal is that damage to the colonic mucosa by free fatty acids and bile acids arising from a high fat diet can also be ameliorated by supplements of calcium, which form insoluble soaps in the colon, and prevent the consequent epithelial cell proliferation (Newmark *et al.* 1984). The authors calculated that 1·5–2 g of calcium per day are needed to neutralize the estimated 32 mM of fatty acids and phosphorus entering the colon. A number of relatively short term intervention trials with these large doses of calcium, measuring proliferation by labelling index with tritiated thymidine from rectal biopsies, have been started, usually in patients with polyps from whom biopsies can be more easily obtained. There have been inconsistent effects on overall mucosal cell proliferation, with some studies showing increased labelling indices, others reduced indices, and in others normalized turnover rates in the upper crypt (Kleibeuker *et al.* 1993; Zimmerman, 1993; Kubben *et al.* 1994; Bostick *et al.* 1995; Weisgerber *et al.* 1995).

Fecapentaenes

Fecapentaenes, compounds with a central pentaene structure and a chain length of 12 or 14 C, are direct acting alkylating mutagens (Gupta *et al.* 1984). They are known to be produced by *Bacteroides* spp., the commonest species in the human colon, probably from plasmalogen phospholipids (Van Tassell *et al.* 1989) which are structural components of lipid bilayers and which have their own unique pathway of biosynthesis. Cells of the heart, brain and muscle are especially rich in plasmalogens (Garg & Haerdi, 1993). Two studies have shown that colorectal cancer cases have lower fecapentaene excretion than controls (Schiffman *et al.* 1989; De Kok *et al.* 1993) and synthetic fecapentaene-12 is not a rodent large bowel carcinogen (Ward *et al.* 1988; Weisburger *et al.* 1990). However, it is able to induce colonic proliferation (Hinzman *et al.* 1987) and is a promoter in tumours induced with *N*-methyl-*N*-nitrosourea (Zarkovic *et al.* 1993). There have been few studies of the effect of diet on fecapentaene excretion in humans although no relationship was detected in a questionnaire study with dietary fibre. High excreters were less likely to be consuming vitamins C and E (Schiffman, 1987).

Bacterial deconjugation

Conjugation with glucuronide is an important part of hepatic 'phase II' enzyme activity rendering foreign compounds more water-soluble for urine excretion and less likely to be absorbed after reaching the gut in bile. Other phase II enzymes form sulphate and glutathione conjugates. Deconjugation, however, is part of the metabolic role of the large bowel flora, but as a result reabsorption occurs and an enterohepatic circulation is established. This is important in bile acid and oestrogen metabolism, and β-glucuronidase activity may be relevant to the metabolism of some genotoxic compounds (see pp. 223–224) in human diets. In plants, most flavonoids and sulphur containing compounds (see pp. 224–226) are conjugated with sugars; deconjugation by bacterial glycosidase will release the parent molecule. Nitrate reductase activity is required for dissimilatory reduction of nitrate to nitrite and therefore NOC formation within the colon (see p. 222).

There is extensive evidence of the effect of dietary modification on rodent bacterial enzyme activity (Rowland, 1991). The relevance to human microflora is uncertain since for example caecal β-glucuronidase levels are reported as 156 μmol/h per g in rats, but lower

in mice (43 μmol) and in human faeces, 36 μmol (Rowland *et al.* 1986). β-Glucosidase and β-glucuronidase responses to dietary pectin are different in humans compared with rats and mice (Rowland & Mallett, 1990). In human studies, Reddy *et al.* (1974) suggested that removal of meat from the diet reduced faecal β-glucuronidase levels, and Goldin *et al.* (1980) reported lower levels in vegetarians compared with omnivores. Johansson *et al.* (1990) and Ling & Hänninen (1992) found a reduction in faecal levels when omnivores changed to a vegetarian or vegan diet. Total outputs are unlikely to be changed since faecal weight when reported is generally greater than that observed with high meat diets. None of these studies was highly controlled and differences could have arisen from a number of differences in diet, for example an increase in starch and NSP consumption.

Goldin *et al.* (1980) could not detect a significant change with a more direct trial in which either red meat was eliminated or a supplement of bran was given. However, supplements of 18 g/d of pectin and 30 g bran halved β-glucuronidase activity in six volunteers (Mallett *et al.* 1988 *a*). In rats with an introduced human faecal flora, high fat human diets induced a significantly higher caecal β-glucuronidase activity (Rumney *et al.* 1992, 1993), but in humans Mallett *et al.* (1988 *a*) found no effect of a supplement of fat, and Cummings *et al.* (1978) found no difference in a controlled study in which dietary fat was increased from 62 to 152 g per day. In humans therefore, any effect of diet on bacterial β-glucuronidase is likely to be brought about by fermentation of starch and NSP, rather than changes in fat intake. Kadlubar (1994) suggests that bacterial glucuronidase deconjugation, at least in relation to heterocyclic amines, is probably unimportant since there is little effect of bile duct ligation on DNA adduct formation (see p. 223) with heterocyclic amines in the colon and other tissues.

β-Glucosidase stimulation, liberating flavonoids and sulphur-containing compounds during fermentation from increased starch and NSP, might be important in humans but activity per g faeces was halved when 18 g pectin were fed and significantly reduced with 30 g bran per day (Mallett *et al.* 1988 *a*). Johansson *et al.* (1990) found no change in glucosidase activity with a change to vegetarian diets. The importance of the effects of diet on β-glucosidase activity in humans remains to be established. Contrary to expectations, quercetin is better absorbed when conjugated as the glucoside than when not (Hollman *et al.* 1996).

Johansson *et al.* (1990) found reductions in arylsulphatase activity in individuals placed on vegetarian diets, but there have been no other investigations of the effect of diet in humans on bacterial arylsulphatase activity, nor of glutathione deconjugases. In animals, bran and fructooligosaccharides inhibit faecal nitrate reductase activity (Mallett *et al.* 1986; Rowland & Tanaka, 1993), but there has been little investigation of the effect of diet on activity of this enzyme in humans.

Sulphite

Sulphite is a widely used preservative in foods such as jams, potato products and wines. Some water supplies have high sulphate levels and sulphur-containing amino acids in meat are major sources in western diets. On absorption into the blood stream, sulphite is oxidized to sulphate. Some sulphate then reaches the large bowel, where it acts as an electron acceptor for sulphate reducing bacteria, which are able to succeed in competition with methanogenic bacteria for hydrogen produced during fermentation. Western populations are therefore less frequent carriers of methanogens, and more frequent carriers of sulphate reducing bacteria than rural African populations (Gibson *et al.* 1988; Segal *et al.* 1988). The end product of the activities of sulphate reducing bacteria is hydrogen sulphide, which is highly toxic, inhibits butyrate oxidation, and which has had a role proposed for it in colitis and large bowel cancer (Roediger *et al.* 1993).

Nitrogen metabolism

Approximately 2 g nitrogen, equivalent to 12 g protein, enter the large bowel daily, mainly in the form of protein, peptides and amino acids (Gibson *et al.* 1976; Chacko & Cummings, 1988). The amount can be increased by increasing protein intake (Gibson *et al.* 1976; Silvester & Cummings, 1995), by heat treatment of dietary proteins (Porter & Rolls, 1971; Corpet *et al.* 1994), and by the physical form of food (Chacko & Cummings, 1988). The proteins in pulses, for example, are poorly digested in the small gut when eaten whole, but 90 % digested when homogenized before consumption (Chacko & Cummings, 1988).

Many different types of proteolytic bacteria are found in the large gut; extracellular proteinases such as those of the clostridia are related to pathogenicity but intracellular or cell associated proteinases are likely to contribute to general proteolysis in the colon. pH optima are alkaline to neutral, and production varies according to species, probably in response to different fermentation conditions. Production may therefore respond to active carbohydrate fermentation in the right colon, but when readily fermented carbohydrates, such as pectin, are exhausted other proteolytic enzymes may respond to protein released from bacterial cell lysis in the left colon (Macfarlane & Cummings, 1991).

Peptides are particularly important, having been shown to stimulate the growth of many intestinal bacteria. Uptake is rapid, and since there are no specific transport mechanisms in the colonic mucosa, unlike in the small bowel, significant host utilization is unlikely. Most bacteria are unable to obtain much energy from amino acid fermentation, but some are more versatile and deaminate to form ammonia, SCFA, and a variety of other products including phenols and branched chain fatty acids (Macfarlane & Cummings, 1991). Phenols increase in urine in response to increase meat consumption (Cummings *et al.* 1979) and, as promoters, have been implicated in bowel cancer (Bone *et al.* 1976). However, there is no clear association epidemiologically between urinary phenol excretion and cancer incidence, and urinary phenol is probably a non-specific indicator of nitrogen fermentation in the gut (Bingham, 1988). When carbohydrate fermentation is active, ammonia is assimilated into glutamine or glutamate when the amino group can be distributed to other amino acids as required (Macfarlane & Cummings, 1991).

Ammonia

When carbohydrate supplies are limited ammonia concentrations accumulate *in vitro* (Macfarlane *et al.* 1986) but in the presence of fermentable carbohydrate, ammonia is used for bacterial protein synthesis, and faecal ammonia concentration falls (Cummings *et al.* 1979). The finding in rodents that rapidly fermented high pectin diets induce higher levels of ammonia in the distal colon and lower levels in the caecum (Lupton & Marchant, 1989) is to be expected, since carbohydrate fermentation in the distal colon will be exhausted at this site with a rapidly fermented carbohydrate such as pectin.

Ammonia in drinking water enhances cell proliferation at levels of 5–10 mmol found in the human colon, promotes N-methyl-N'-nitro-N-nitrosoguanidine induced adenocarcinomas in rodents (Tsujii *et al.* 1992, 1993) and has been implicated in large bowel carcinogenesis (Visek, 1978). In humans, high meat or protein diets increased faecal ammonia concentration (from 10 to 30 mmol/l faecal dialysate) and greater starch and NSP intakes reduced it (Cummings *et al.* 1979; Bingham *et al.* 1996). There is no effect of unfermentable bran, nor of resistant starch (Silvester *et al.* 1995; Bingham *et al.* 1996). Altered faecal ammonia levels may therefore be important in promotion of carcinogenesis; patients with uterosigmoidostomies who have luminal ammonia concentrations as high as 100 mM have a greatly increased risk of developing tumours distal to the site of ureteric implantation (Tank *et al.* 1973; McConnell *et al.* 1979).

N-nitroso compounds

Preformed NOC have not consistently been associated with large bowel cancer; oral doses of nitrosamines in animal feeding studies, for example, mainly result in liver and oesophageal tumours (Peto *et al.* 1984). Nitrosated amides are direct acting carcinogens, tending to cause tumours near to the site they are produced, whereas nitrosated amines require hydroxylation *via* cytochrome P450 enzymes and can initiate tumours at distal sites (Shuker, 1989). Nitrosamines are efficiently metabolized in the liver, little of an ingested dose of *N*-nitrosodimethylamine reaching the circulation (Bartsch & Montesano, 1984). Preformed NOC occur in food but technological changes in the production of the major sources, beer and nitrite cured meat products, have halved dietary consumption of volatile nitrosamines to about $0.5 \mu g/d$ (Preussman, 1984). The major non-dietary exogenous source is tobacco, the mainstream smoke from one cigarette containing up to $65 \mu g$ volatile nitrosamines and the side stream smoke $1000 \mu g$ (Preussman, 1984).

Endogenous formation of NOC also occurs, since the colonic lumen is rich in amines and amides produced primarily by bacterial decarboxylation of amino acids. In the presence of a nitrosating agent, these can be *N*-nitrosated to a large variety of NOC. Several mechanisms are involved, and chemical *N*-nitrosation may occur at low pH, under neutral or alkaline conditions (as in the small and large intestine), and be enhanced by the presence of various catalysts such as minerals, including iron, and formate. The nitrosating agents are nitric oxide $(NO^{\bullet})$ for amides and N_2O_3 for amines, which is produced from nitrite at low pH. At higher pH, when nitrosation proceeds at a faster rate, N_2O_3 is also produced from nitric oxide $(NO^{\bullet})$ by reaction with molecular oxygen (Leaf *et al.* 1989). In the anaerobic large bowel, nitrate entering the body partly in food and water is reduced to nitrite in the colon during dissimilatory nitrate metabolism by the colonic flora. Supplements of nitrate have therefore been shown to elevate faecal NOC levels (Rowland *et al.* 1991). A number of facultative and anaerobic colonic bacteria are also able to catalyse the formation of NOC at an optimum pH of 7.5 (Suzuki & Mitsuoka, 1984; Calmels *et al.* 1985).

There is another significant source of endogenous nitrate production, which has been deduced for some time, since nitrate excretion exceeds that consumed in food and water (Witter *et al.* 1979). Wagner *et al.* (1983) showed that nitrate synthesis is enhanced during immunostimulation, and Stuehr & Marletta (1985) showed that nitrite and nitrate are produced from macrophages. Studies with ^{15}N established that the source is dietary arginine, used to produce NO, which together with superoxide cause oxidative injury and cell death (Iyengar *et al.* 1987). Other fields of research established that NO accounted for the biological activity of endothelium-derived relaxing factor and inducible nitric oxide synthase produces continuous amounts of NO from arginine (Palmer *et al.* 1987; Änggård, 1994). Increased arginine from protein might be expected to increase urine nitrate excretion, an effect which has been shown in animals (Mallett *et al.* 1988*b*; Ward *et al.* 1989). Thus, NO from stimulated macrophages in the large bowel mucosa, together with nitrite produced from reduced nitrate diffusing into the gut, are therefore available for NOC formation.

In the 1980s it was established that faecal samples contain negligible amounts of volatile NOC (Archer *et al.* 1981), but since that time newer methods to measure total NOC by chemiluminescence have been developed. Rowland *et al.* in 1991 detected an average of $13 \mu g/$faecal sample on a low (11 mg) nitrate diet and found a marked increase to $60 \mu g/$sample with a 300 mg/d supplement of nitrate in humans. High protein diets have also been shown to increase urine *N*-nitrosoproline levels in animals (Mallett *et al.* 1988*b*; Ward *et al.* 1989). In humans, a 3–4-fold increase in protein, as meat, has been

shown to increase faecal NOC levels 4-fold (Silvester *et al.* 1995; Bingham *et al.* 1996). NOC are alkylators, and alkylative DNA adducts of O^6methylguanine have been detected in human colonic tissue (Hall *et al.* 1991). Very low levels are found, because the adduct is efficiently repaired (Margison & O'Connor, 1990). Experiments with the direct acting carcinogen *N*-methyl-*N*-nitrosourea in rats, known to induce G to A transitions, have detected mutations in K *ras* in codons 12 and 13, but only in 30% carcinomas and 2% adenomas (Jacoby *et al.* 1992). 1,2-dimethylhydrazine, a carcinogen requiring activation, does induce a high percentage of K *ras* mutations in codons 12 and 13 of rat tumours (Jacoby *et al.* 1991). The effect of high meat diets in inducing alkylative mutations typical of NOC and relevant to colon cancer (see p. 201–202) is under active investigation in humans.

Heterocyclic aromatic amines

Over 20 mutagenic heterocyclic amines (HAA) have been isolated from cooked fish, beef, chicken, pork, soyabeans, and isolated proteins. All except one (Lys-P-1) isolated so far have an exocyclic amine (Eisenbrand & Tang, 1993). The type of cooking, time, temperature, type and content of fat, all affect the amount and type of HAA found. Grilled or fried rare to medium rare meat contains less than well done meat; a standard protocol of grilling or frying for 6 min at 200 °C surface temperature is generally used to produce experimental amounts. Mutagenicity can be reduced by deep frying and boiling, and increased in beef if butter rather than oil is used in cooking. Generally, frying, grilling and barbequeing generate more HAA than stewing, steaming, microwaving or poaching (Barrington *et al.* 1990; IFT, 1993; Layton *et al.* 1995). The mutagens are formed on the outside and pan residues contain significant amounts. They are formed probably from Maillard reactions between a hexose such as glucose and an amino acid with linkage of the resulting aldehyde with creatinine (Jagerstaad *et al.* 1986).

In rodents, heterocyclic amines are carcinogenic in a wide variety of organs, mainly liver but including skin, lung, and mammary gland. Three, MeIQ (2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline), IQ (2-amino-3-methylimidazo[4,5-f]quinoline), and PhIP (2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine) are large bowel carcinogens (Takayama *et al.* 1984; Kato *et al.* 1989; Ito *et al.* 1991). PhIP, although relatively less mutagenic than other HAA, is as carcinogenic at the same levels of administration, 0·04% in rodent diets. It is also the most abundant in cooked food; beefsteak fried for 6 min at 190 °C for example contains 23·5 ng/g PhIP and 5·1 ng MeIQx (Gross, 1990). Calculated daily intakes of total HAA range from 0·4 to 16 μg/d (Wakabayashi *et al.* 1992), but this is in the order of 1 to 1000 to 5000 less than that found to induce carcinogenicity (Sugimura, 1986). Maximum to minimum risks of between 1 to 1000 and 1 to 10000 have been calculated and these have been taken to suggest that HAA are relevant carcinogens in human cancer (Eisenbrand & Tang, 1993). Comparisons between the amount required for carcinogenicity in animals and amounts found in diets therefore suggest that the relative contribution of HAA to colon cancer incidence may be small, for example 0·25% of all colon cancers (Layton *et al.* 1995).

For conversion to the carcinogenic form, the exocyclic amine of IQ, PhIP and MeIQ is hydroxylated by cytochrome P450 enzymes (see p. 224–225), mainly CYP1A2. Covalent binding to deoxyguanosine in DNA then occurs. There are organ, species, and individual differences in P450 enzymes so that direct extrapolation from rodent experiments to human risks may be misleading. Comparatively high levels of PhIP adducts have been found in human colonic tissue (Friesen *et al.* 1994).

The large bowel flora and dietary NSP may also be important since in rats low fat, low beef, high NSP diets have been shown to enhance excretion of IQ sulphamate and sulphate metabolites, and to reduce irreversible binding of IQ to a surrogate of DNA, polyethyleneimine, compared with a high meat, high fat, low NSP diet (O'Neill *et al.* 1992).

This suggests that these diets alter IQ metabolism so that more is detoxified and less is hydroxylated to the active metabolite. Bacteria are able to convert IQ to another metabolite, 7OH-IQ, and beef dripping and human high NSP diets have been shown in rats with human faecal flora to enhance this metabolism (Rumney *et al.* 1993). Weisburger *et al.* (1994) have shown that this metabolite is not carcinogenic in rodents, and it is possible that this is another detoxification metabolite whose excretion is enhanced by high NSP diets.

Owing partly to the comparatively small amounts of HAA found in human diets in comparison with the amount required for carcinogenicity, the importance of HAA in large bowel cancer is uncertain. The mutations established so far in colon cancer (see pp. 201–202) are mainly point mutations, of single bases, rather than the deletion or addition of a nucleotide, a frameshift. However, in mutagenicity tests, the HAA tend to produce frameshifts rather than transitions (Hatch *et al.* 1988). No obvious mutations in *ras* or p53 have been shown in colon cancers induced in rats by PhIP or IQ (Weisburger, 1993). Nevertheless, PhIP has attracted particular attention because it tends to be the most abundant, and colon tumours that are produced from it in rats have a high frequency of microsatellite instability which is similar to that seen in human inherited and sporadic colorectal cancers (see pp. 201–202) (Canzian *et al.* 1994).

SYSTEMIC FACTORS

n-*3 and* n-*6 fatty acids*

Animal studies have indicated a reduction in colon cancer incidence with increased levels of fish oils (see pp. 217–218), reduced cell proliferation (Steinbach *et al.* 1993), and reduced aberrant crypt formation (Takahashi *et al.* 1993). The polyunsaturated $\omega 3$ and $\omega 6$ fatty acids are metabolized to a range of compounds that have many physiological activities at very low concentrations, including an inflammatory response. Arachidonic acid ($\omega 6$) is metabolized to leukotriene B4 which is more inflammatory than leukotriene B5 metabolized from eicosapentaenoic acid ($\omega 3$) in fish oils (Leaf & Weber, 1988). Two studies in humans have also indicated reduced rectal cell proliferation rates with supplements of fish oils, one in volunteers and one in patients with polyps. In the patients with polyps, a placebo of olive oil was used, and the difference occurred in the upper crypt compartments associated with higher risk of adenomas, whilst in the volunteers there was a general reduction (Anti *et al.* 1992; Bartram *et al.* 1993). Prostaglandin E2, another inflammatory prostaglandin produced from arachidonic acid, was also reduced (Bartram *et al.* 1993). Low doses of fish oils are as effective as high doses (Anti *et al.* 1994). Non-steroidal anti-inflammation drugs such as piroxicam, indomethicin and aspirin also inhibit prostaglandin and chemically induced large bowel tumours (Reddy *et al.* 1990, 1993). In humans, several studies show that use of aspirin is associated with reduced risk of colorectal cancer (Marnett, 1992).

Effects of diet on Phase I and II enzyme activity

Drugs and other xenobiotics are known to be metabolized by a variety of enzyme catalysed reactions. These have been classified into phase I and phase II reactions: phase I reactions (oxidation, hydroxylation, reduction, hydrolysis) generally render compounds more reactive, whereas phase II (conjugations) generally, but not exclusively, decrease biological activity and water solubility, hence facilitating their excretion (Williams, 1978). In human large bowel cancer, these enzyme activities could have a role.

Heterocyclic amines and *N*-nitrosamines are activated by hydroxylation (phase I) by P450 enzymes present in the liver and small intestinal mucosa. The P450 enzyme CYP1A2, which *N*-oxidizes aromatic amines, also catalyses the demethylation of caffeine, and

caffeine is also acetylated before excretion in urine. Using a standard dose of caffeine as a surrogate to phenotype individuals into fast or slow oxidizers, patients with large bowel cancer have been shown to be faster oxidizers and acetylators than healthy matched controls (Kadlubar *et al.* 1992). However, genotyping of the polymorphic *N*-acetyltransferase 2 in colonic tissue does not show a difference between cases and controls and it is suggested that aromatic amine acetylation in the colon is largely determined by NAT1, which is monomorphic and does not segregate individuals into fast and slow acetylators (Rodriguez *et al.* 1993). Phenotypic studies of cases and controls in large bowel cancer have not been controlled for diet, and a large number of dietary variables are known to affect hepatic and gastrointestinal phase I and II enzyme activity, mainly in animals and to a limited extent in humans.

Acetylation (phase II) also occurs in activation of PhIP. Acetylation of PhIP occurs in the colon, but not in liver (Turesky *et al.* 1991). The phase II enzyme glutathione S-transferase(u) may be also important, because a greater number of glutathione S-transferase(u) null compared with competent patients have been found amongst cases of colorectal cancer than in healthy matched controls (Strange *et al.* 1991).

Nutrients

There is an extensive literature showing that most P450 enzyme system activity is decreased when protein intake is reduced, probably because protein synthesis and liver cell proliferation are retarded (see Yang & Yoo, 1991). Hayes *et al.* (1978) found that changes occurred within 1–2 weeks of a change in protein intake in rats. Phase II enzyme activity may also be reduced, so that the net result may be an increase or decrease in toxicity of xenobiotics in protein deficient animals (Yang & Yoo, 1991). Both the total amount and type of fat seem to be important in maintaining hepatic high levels of P450, although at levels of 10–20 % by weight saturates and monounsaturates seem to be less effective than polyunsaturates (Yang & Yoo, 1991). In the small intestine there seems to be little effect of fat, but high corn oil diets increase P450 enzymes in rat colon microsomes with little effect in germ free rats (Noordhoek & van Bladeren, 1991). Phase II enzyme activity may also be decreased, for example glutathione transferase activity arising from a deficiency of cysteine, so that toxicity of pesticides in animals may be further increased (Yang & Yoo, 1991).

In rodents, riboflavin is essential for flavoenzymes in P450 enzymes, and deficiency causes marked reductions in activity in both the liver and intestine (Yang & Yoo, 1991; Noordhoek & van Bladeren, 1991). Iron deficiency seems to increase hepatic activity but decrease small intestinal hydroxylase, an effect which requires intraluminal supplies to return to normal levels as epithelial cells migrate from the crypts to tips of the mucosal villi (Hoensch *et al.* 1976). Deficiencies of vitamins A and E also alter P450 enzyme activity.

These effects in animals are likely to account for some of the known effects of nutrients on carcinogenesis in animal models (see pp. 214–216). However, to what extent it is possible to extrapolate to human cancer is unknown, for example in the metabolism of HAA, since there has been little investigation of the effect of nutrients in humans. A change from a 40 % to 10 % protein diet decreased theophylline clearance, and semisynthetic diets also reduce small intestinal P450 monooxygenase activity in humans (Anderson *et al.* 1982; Hoensch *et al.* 1984).

Vegetables

Using animal models, Wattenberg (1971) was the first to show that vegetable constituents such as indole-3-carbinol have a profound effect on intestinal P450 activity. On the basis of extensive evidence showing inhibition of carcinogenesis at a variety of sites (though not

the large bowel) Wattenberg proposed a classification of the numerous constituents of fruits and vegetables, based on their ability to prevent carcinogenesis. Examples were vitamin C, which inhibits N-nitrosamine formation, and blockers, for example phenols and isothiocyanates, which have effects on phase I and phase II enzyme activity (Wattenberg, 1985).

Indoles and isothiocyanates are derived from glucosinolates of which there are about 120 found in brassica vegetables such as broccoli, cabbage and Brussels sprouts (Fenwick et al. 1982). They may be either phase I or phase II enhancers, for example, and broccoli contains indoles, which have been shown to enhance hepatic and small intestinal phase I enzymes and to elevate large bowel tumour production (Loub et al. 1975; Pence et al. 1986). Sulphoraphane, identified in broccoli, has been shown to be an inducer of the phase II enzymes quinone reductase and glutathione S-transferase (Zhang et al. 1992). All of the above studies have been conducted in animals given large quantities of chemical carcinogens or in cell culture lines, and the applicability of these findings to human cancer and predictability at different organs is uncertain. Diallyl sulphide in garlic induces glutathione S-transferase and suppresses colon tumours induced by dimethylhydrazine, but in another model it enhances hepatocarcinogenesis. The related diallyl disulphide was, however, an inhibitor of colon carcinogenesis (Wargovich, 1987; Sparnins et al. 1988; Takahashi et al. 1992). Furthermore, the effect of these compounds on hepatic phase I and II enzyme activity may not predict their effect in the large bowel mucosa which may be more relevant to colon cancer (O'Neill et al. 1996).

Nevertheless, genotypes who are glutathione S-transferase null are at increased risk of colon cancer (Strange et al. 1991). Large quantities of Brussels sprouts (3 portions per day) have been found to elevate liver glutathione S-transferase activity in humans and to reduce oxidative DNA damage (Bogaards et al. 1994; Nijhoff et al. 1995; Verhagen et al. 1995). There is much current interest in constituents of vegetables and fruit that can act as chemopreventers, particularly in those which induce phase II enzymes, but not phase I (Talalay et al. 1991).

Folate

Vegetables are a major source of folate in human diets and folate, vitamin B_{12}, methionine and choline are critical elements in methyl group metabolism and therefore in DNA metabolism and replication. There is some epidemiological support for a role for DNA hypomethylation in increasing risk of colon cancer, although not from diet (see pp. 212–213). In an animal model of colon cancer, rats fed folate deficient diets had a greater incidence of dysplasia and carcinoma and significantly fewer of them were free from neoplastic lesions than the control animals (Cravo et al. 1992). In a small supplementation study of patients with resected neoplasms, rectal DNA methylation increased with folate (Cravo et al. 1994). However, the importance of folate and hypomethylation is uncertain since intestinal neoplasia has been found to be suppressed by DNA hypomethylation in a DNA methyltransferase deficient mouse, and folate depletion did not reduce colonic DNA methylation in rats (Laird et al. 1995; Kim et al. 1995).

Flavonoids

A very large class of natural compounds, widespread in plants, mainly as the glycosides, is the flavonoids. They participate in photosynthesis and they and their breakdown products are yellow and orange pigments and, as anthocyanins, blue and red pigments. At least 1 g per day of flavonoids are present in human food, and tea and wine contain large quantities. In the 1920s they were classified by Szent-Györgyi as 'Vitamin P' since they were found to cure the haemorrhagic symptoms of scurvy (Havsteen, 1983; Roger, 1988).

The flavonoids have strong antioxidant properties, as they are metal ion chelators, scavengers of superoxide and OH⁻, and able to terminate lipid peroxidation chain reactions (Yang & Wang, 1993). Early reports suggested that they would enhance phase I enzyme activity (IFT, 1993). Rutin and quercetin have been known for some time to be mutagenic, but more recent studies suggest them to be protective experimentally. However, although these compounds appear to have a protective effect in epidemiological studies of cardiovascular disease, no effects have been observed in one prospective study of cancer (Hertog *et al.* 1993; Goldbohm *et al.* 1996; Keli *et al.* 1996).

Green tea, and to a lesser extent black tea, contain flavonols such as epigallocatechin which are also part of the flavonoid group. Tea polyphenols increase phase II enzymes and inhibit phase I enzymes (Khan *et al.* 1992; Mukhtar *et al.* 1992). Deschner *et al.* (1991, 1993) have also found them to inhibit hyperproliferation, colonic abnormalities and tumour incidence in mice, regardless of the level of fat in the diet. Green tea has been consistently shown to inhibit carcinogenesis in the lung, oesophagus, forestomach, liver, duodenal and small intestine, in addition to the large bowel (Yang & Wang, 1993). One of four rodent studies demonstrated no inhibition of colon carcinogenesis by green tea, but in mice green tea extracts significantly inhibited dimethylhydrazine-induced intestinal (mainly colon) cancer, as did epigallocatechin-3-gallate (Yin *et al.* 1994). Low doses per rat of green tea extracts (0·05, 0·01, or 0·002% in drinking water) also inhibited N-methyl-N-nitrosourea induced colon carcinogenesis (Narisawa & Fukaura, 1993). Oral postinitiation doses in rats of 0·01% green polyphenol extracts reduced azoxymethane tumour numbers by 60% and tumour incidence by 51%, with a greater inhibition (53 and 38% respectively) at a higher, 0·1%, dose (Yamane *et al.* 1991). The effect of black tea, which is more commonly consumed by humans in western societies, has not been investigated so intensively for chemoprevention in bowel cancer, but limited studies suggest that it may have a similar effect. However, there is inconclusive epidemiological support for a role for either green or black tea drinking in large bowel cancer protection (Yang & Wang, 1993; Goldbohm *et al.* 1996).

Other flavonoids, the isoflavones, inhibit models of breast cancer and have also been suggested to be important in the genesis of bowel cancer (Barnes *et al.* 1990; Setchell & Adlercreutz, 1988). There is an extensive literature showing that one, genistein, inhibits NO synthase activity *in vitro*, but effects *in vivo* in relation to endogenous NOC formation (see pp. 222–223) have not been investigated.

SUMMARY AND CONCLUSIONS

Cross-sectional comparisons, case-control studies and trends in food intakes show high rates of colorectal cancer in populations consuming diets high in meat and fat, and low in starch, NSP and vegetables. These studies suggest that the potential for prevention of colorectal cancer by diet is very great. Attributable population risk estimates from case-control studies suggest that 25–35% of colorectal cancers might be prevented by high intake of vegetables and fibre and that 15–25% of colorectal cancers could be attributed to a high fat intake (Tomatis *et al.* 1990). There are plausible physiological reasons for a protective effect of starch and NSP in large bowel cancer. Cross-sectional comparisons suggest that the recommendation (Department of Health, 1991) to increase starch and NSP consumption in the UK by 50% from 12 to 18 g/d will increase stool weight by 25% and reduce large bowel cancer incidence on a population level by 15%.

Existing results from cohort investigations show only weak associations and more data are required from large studies using accurate dietary assessment techniques in populations where there is extensive dietary variation, and which have collected biological samples in

order that interaction between diet, biomarkers of diet, and different genotypes that may determine risk can be examined. A major problem in epidemiological studies of large bowel cancer is the absence of an easily accessible intermediate risk marker, known to alter in response to diet in metabolic studies, that can be used to link dietary intake and the presence of the disease in either intervention or prospective studies.

To meet the Department of Health (1991) recommendations for NSP intake, vegetable and fruit consumption needs to double in the UK. Vegetables may have added benefits in reducing colorectal cancer rates because they contain antioxidant nutrients and flavonoids, sulphur-containing compounds, and folate, all of which can be shown to favourably affect factors thought to be important in reducing risk. Intervention studies are generally held to be the most robust way of testing hypotheses but those already conducted with β-carotene and vitamin E have not reduced risk of either large bowel cancer or recurrence of precursor lesions such as adenomatous polyps. Results from other interventions with calcium, NSP and resistant starch are awaited.

A protective effect of starch and NSP probably arises from their marked effect on bacterial metabolism in the large bowel, which leads to an increase in stool weight, and in butyrate production, and reduced pH, and levels of secondary bile acids, diacylglycerol, and free ammonia. In rodents given known carcinogens, 'insoluble' sources of NSP are generally protective, and high fat (or energy) diets increase tumorigenesis. Tumorigenesis may be reduced by n-3 fatty acids, but the effects of fat in rodent models of colon cancer are less consistent than those found in mammary tumorigenesis. In humans, a role for fat in increasing risk *via* increased bile acid excretion has been proposed. A risk from red and processed meat seems to be emerging from prospective studies, and high levels of meat increase faecal ammonia and NOC concentrations, and intakes of HAA. Some NOC and HAA are known carcinogens and some of the chromosomal mutations found in colorectal cancer are consistent with effects of NOC and HAA. Meat consumption should not increase. There has also been some interest in the past in the effect of bacterial enzymes in modifying carcinogen metabolism but the relevance of this to human diets and carcinogenesis is uncertain. The effect of diet on fecapentaene excretion is unknown. There are numerous different compounds in vegetables actively under investigation for their effect on carcinogenesis at different sites, including the large bowel, but intervention studies already conducted with β-carotene and vitamin E suggest that the active ingredients in vegetables involved in colorectal cancer protection are not antioxidant nutrients. As these attempts to alter risk with supplements have so far not been successful, they are not recommended for the general population.

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