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Meat or wheat for the next millennium?
Plenary Lecture

High-meat diets and cancer risk

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Up to 80 % of breast, bowel and prostate cancers are attributed to dietary practices, and international comparisons show strong positive associations with meat consumption. Estimates of relative risk obtained from cohort investigations are in the same direction, although generally weak, and red and processed meats rather than white meat seem to be associated with elevated risk of colon cancer. In breast cancer, there are consistent associations with total meat intake and there is evidence of a dose response. Despite these associations with meat, existing studies suggest that vegetarians do not have reduced risk of breast, bowel or prostate cancer, but there are no quantitative estimates of amounts of meat consumed by meat eaters in these cohort studies. Possible mechanisms underlying epidemiological associations include the formation of heterocyclic amines in meat when it is cooked. These heterocyclic amines require acetylation by P450 enzymes, and individuals with the fast-acetylating genotype who eat high amounts of meat may be at increased risk of large-bowel cancer. NH_3 and *N*-nitroso compounds (NOC) formed from residues by bacteria in the large bowel are probably also important. NH_3 is a promotor of large-bowel tumours chemically induced by NOC, and some of the chromosomal mutations found in human colo-rectal cancer are consistent with effects of NOC and heterocyclic amines. However, the type, amount, and cooking method of meat or protein associated with increased risk are not certain. The effects of high levels of meat on NH_3 and NOC output are not reduced by increasing the amount of fermentable carbohydrate in the diet, but interaction between meat, NSP and vegetable intakes on the risk of cancer has not been studied comprehensively. The interaction between dietary low-penetrance genetic polymorphic and somatic mutation factors has also been investigated to a limited extent. Current Department of Health (1998) recommendations are that meat consumption should not rise, and that consumers at the top end of the distribution should consider a reduction in intakes.

Meat: Ammonia: *N*-Nitroso compounds: Cancer

Cancer is now the most common cause of death in the UK, largely because of the high incidence of breast, lung, bowel and prostate cancers. These cancers are virtually absent in some countries of the developing world, and the overall cancer burden in these countries is strikingly lower (Parkin *et al*. 1992). Nevertheless, rates change to match those of the host population within one to two generations in migrants who move from low-risk to high-risk areas, and there are also rapid secular changes within single populations (Bingham *et al*. 1996). Thus, the majority of cancers common in Western populations are caused by environmental factors and, hence, are largely preventable. Up to 80 % of breast, bowel and prostate cancers are currently attributed to diet (Willett, 1995).

Cancers occur as a result of a number of different abnormalities which occur when the process of cell division during turnover and renewal goes out of control. Typically, control over the rate of cell division is lost, so that cells

Abbreviations: NOC, N-nitroso compounds.

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proliferate to form a tumour, and cells do not differentiate into their specialized tissues. It is generally accepted that this process of malignant growth occurs as a result of mutations in, or loss of, genes which are then passed to later generations of cells. In comparatively rare cases, these genetic defects are inherited, but the majority of common cancers are 'sporadic' because affected patients have no known family history. Of large-bowel cancers for example, 80 % are sporadic. Neverthless, the investigation of tumours from patients with some inherited colon cancers has provided insight into the sporadic form of the disease, because the same mutations occur in 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. Appropriate modification of these factors would mean that the cancer could be avoided.

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. A genetic model for colon cancer is the most well established (Fearon & Vogelstein, 1990), but the somatic mutations involved at other sites have not been as well characterized. In colon cancer, the ras mutations most commonly involved are 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). 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 the diet. Butyrate, formed during fermentation of carbohydrates in the large bowel, has well-known effects on gene expression, DNA repair enzymes and apoptosis (Hague *et al*. 1993).

Particular genotypes have also been found to be at increased risk of cancer, e.g. those who are glutathione S transferase (*EC* 2.5.1.18) competent or null (Strange *et al*. 1991). The Japanese population contains a higher proportion of fast acetylators, which may account for the striking rise in colon cancer associated with Westernization and, for example, meat consumption. High-meat-eating fast-acetylator phenotypes are at increased risk of acquiring colonic adenomatous polyps and cancers (Roberts-Thompson *et al*. 1996).

At present, evidence linking diet with cancer is limited to epidemiological associations with diet, with support from experimental studies, and plausible hypotheses. A major problem in epidemiological studies of cancer is the absence of easily-accessible intermediate risk markers, 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. Information being collected from all participants in the European Prospective Investigation of Cancer 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 (Riboli, 1992; Day *et al*. 1998).

Epidemiology of meat and cancer

Armstrong & Doll (1975) attributed much of the international variation in cancer incidence between countries to dietary differences, especially meat and fat consumption. High correlations between meat consumption and cancer of the colon (0·85–0·89), breast (0·78), uterus (0·78), prostate (0·60), and kidney (0·70) were reported (Armstrong & Doll, 1975). These findings could have arisen from confounding by other environmental factors, but they did stimulate investigation into more detailed epidemiological studies of diet and cancer. Results of cohort 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 food, food-frequency questionnaires, were used in most of these studies. These assessments would have been associated with a substantial degree of measurement error, not amenable to correction (Bingham & Day, 1997). For example, if meat is associated with increased risk, lower rates for cancer would be expected in vegetarians. In a recent meta analysis of five cohorts, meat eaters were not at greater risk than non-meat eaters, although the standardized mortality ratios for all cohorts were low, and the amount of meat consumed by meat eaters could not be established (Key *et al*. 1998). Meat is particularly associated with cancer of the breast, prostate and bowel, although there are some epidemiological associations also with cancer of the lung and pancreas (Department of Health, 1998).

Colon cancer

Bowel cancer is the second most common cancer in the UK, after breast cancer in women and lung cancer in men. Up to the 1960s it was rare in Japan and Far East countries, but the incidence has increased rapidly, so that age-specific large-bowel cancer rates in males are now greater in Japan than in the UK (Bingham *et al*. 1996). These changes are attributed to Westernization of the Japanese diet, including increased meat consumption. Various indices of meat consumption have been measured in prospective studies of colon cancer. 'Red' meat is usually taken to include beef, lamb and pork, including that in meat products. Although the majority of studies have wide CI and, therefore, non-significant results, there is a trend for red and processed meats to elevate colon cancer risks, and some evidence that white meat is associated with either no effect, or reduction in risk. In the recent Department of Health (1998) report, the evidence was classified as 'moderately consistent' of a positive association between red and processed meat consumption and colon cancer. Two of three cohort studies show a dose response, with relative risks in the order of 2 associated with ten to twelve portions of red meat per week (Department of Health, 1998). The report also found that there was moderately consistent evidence that poultry (white meat) and fish consumption are not associated with risk of colon cancer.

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Breast cancer

Breast cancer is the commonest cancer of women living in Western populations, and incidence is rising in the UK (Coleman *et al*. 1993). The risk of breast cancer increases markedly with age, but its development is highly dependent on the hormones associated with ovarian function (Bernstein & Ross, 1993). Dietary factors which might affect hormonal status are therefore of particular interest in breast cancer, but there are consistent associations also with meat. All cohort studies have shown an elevated risk for total meat, and this was significant in five of the ten studies (Department of Health, 1998). Furthermore, there is evidence of a dose response in those studies which have reported quantitative estimates of meat consumption, with highest estimates of relative risk associated with daily meat consumption (Department of Health, 1998). The report found moderately consistent evidence that higher meat consumption is associated with increased risk of breast cancer, particularly red or fried or browned meat (Department of Health, 1998).

Prostate cancer

Prostate cancer is the most common hormone-related cancer in men, and incidence has been rising rapidly by about 3–4 % per year in the UK (Coleman *et al*. 1993). As with breast cancer, meat consumption has been linked with increased risk, with significant findings in five of eight prospective studies (Department of Health, 1998). The Department of Health (1998) report found weakly consistent and moderately consistent evidence respectively that total and red meat were associated with increased risk.

Mechanisms relating meat to cancer

The association between meat consumption and cancer is usually attributed to the formation of heterocyclic amines in meat when it is cooked. In rodents, heterocyclic amines are carcinogenic in a wide variety of organs, mainly liver, but including skin, lung, colon and mammary gland. One, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine, 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 colo-rectal cancers (Canzain *et al*. 1994). Furthermore, G–C base pair mutations are produced in cell lines, which are similar to those produced in *APC* gene mutations of colon tumours in rodents (Gooderham *et al*. 1996).

Present estimates of risk from these compounds are rather low when extrapolated from animal carcinogenicity data. Calculated daily intakes of total heterocyclic amines are in the order of 1000–5000 less than that found to induce carcinogenicity (on a w/w basis) in rodents (Sugimura, 1986). Hence, comparisons between the amount required for carcinogenicity in animals and amounts found in diets suggest that the relative contribution of heterocyclic amines to the incidence of colon cancer may be small, e.g. 0·25 % of all colon cancers (Layton *et al*. 1995).

However, for conversion to the carcinogenic form, the exocyclic amine of heterocyclic amines is hydroxylated by cytochrome P450 enzymes, mainly CYP1A2. Covalent binding to deoxyguanosine in DNA then occurs. There are organ, species, and individual differences in P450 enzymes, so direct extrapolation from rodent experiments to human risks may be misleading. Using another heterocyclic amine, 2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline, which was radioisotopically labelled at low doses with ^{14}C, and accelerator mass spectrometry, deoxyguanosine adducts were shown in the human colon, but at approximately ten times greater levels than in rodents at the same dose and time point following exposure (Turteltaub *et al*. 1997). The human colon, therefore, may be more susceptible to the effects of these compounds than the colon of rodents.

Meat and nitrogen metabolism in the colon

Meat also alters N metabolism and enhances the production of endogenous promotors and possible carcinogens such as NOC within the colon. The amount of N entering the large bowel (mainly in the form of protein, peptides and amino acids) can be increased by increasing protein intake (Silvester & Cummings, 1995). There are many different types of proteolytic bacteria found in the large bowel which, depending on pH and substrate availability, may respond to active carbohydrate fermentation in the right colon, or to protein released from bacterial cell lysis in the left colon when readily-fermented carbohydrates, such as pectin, are exhausted (Macfarlane & Cummings, 1991). Some versatile bacteria deaminate proteins and their components to form NH_3, short-chain fatty acids, and a variety of other products including phenols and branched-chain fatty acids. When carbohydrate fermentation is active, NH_3 is assimilated into glutamine or glutamate and the amino group is distributed to other amino acids as required (Macfarlane & Cummings, 1991).

In human subjects, the increase in N entering the colon as a result of consuming high-meat diets increases faecal NH_3 concentration. NH_3 is a promoter of carcinogenesis induced by NOC in rodent models (Clinton *et al*. 1988), and patients with ureterosigmoidostomies who have very high lumen NH_3 concentrations have a greatly increased risk of developing tumours distal to the site of ureteric implantation (Tank *et al*. 1973). In *in vitro* fermentation studies, and in some *in vivo* studies, carbohydrate in the form of starch and NSP reportedly reduces faecal NH_3 concentration (Macfarlane & Cummings, 1991). However, the results of studies in which high amounts (300–600 g/d) of meat were fed, showed that bran, resistant starch and vegetables do not reduce faecal NH_3 levels (Cummings *et al*. 1979; Bingham *et al*. 1996; Silvester *et al*. 1997; Murphy, 1998).

NOC are also found in the colon, and they are formed endogenously because the amines and amides produced primarily by bacterial decarboxylation of amino acids can be *N*-nitrosated in the presence of a nitrosating agent. Chemical *N*-nitrosation may occur under neutral or alkaline conditions (as in the small intestine and large bowel). 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

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(Allison & Macfarlane, 1988). Supplements of nitrate, therefore, have been shown to elevate faecal NOC levels (Rowland *et al*. 1991). A number of facultative and anaerobic colonic bacteria are able to catalyse the formation of NOC at an optimum pH of 7·5 (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 causes oxidative injury and cell death (Iyengar *et al*. 1987). Other fields of research have established that NO accounts for the biological activity of endothelium-derived relaxing factor, and inducible NO synthase (*EC* 1.14.13.39) produces continuous amounts of NO from arginine (Angaard, 1994). Increased levels of arginine, from protein, might be expected to increase urine nitrate excretion, an effect which has been shown in animals (Mallett *et al*. 1988; Ward *et al*. 1988). Thus, NO from stimulated macrophages in the large-bowel mucosa, together with nitrite produced from reduced nitrate diffusing into the gut, are available for NOC formation.

In the 1980s it was found that faecal samples contain negligible amounts of volatile NOC (Archer *et al*. 1981), but since then newer methods to measure total NOC by chemiluminescence have been developed, and the presence of NOC in faeces in animals and man is now well established (Massey *et al*. 1988; Rowland *et al*. 1991; Bingham *et al*. 1996; Silvester *et al*. 1997; Hughes *et al*. 1999).

In human subjects, we have been investigating the hypothesis that high-meat diets increase faecal NOC levels, and that an increase in fermentable carbohydrate reaching the colon could be expected to reduce them. All these studies were carried out in a metabolic suite, where diet could be carefully controlled and all specimens collected over prolonged periods. All diets were isoenergetic and contained equal amounts of fat, and were matched to each individual's energy expenditure. Nitrate-free water was used for drinking and cooking. We first studied the effect of red-meat consumption on faecal NOC levels in eight male volunteers who consumed diets low or high in meat (60 or 600 g as beef, lamb or pork/d) whilst living in a metabolic suite. Increased intake of red meat induced a significant ($P < 0·024$) three-fold increase from 40 (SE 7) μg NOC/d to an average of 113 (SE 25) μg NOC/d, mainly as acidic and basic nitrosamines (Bingham *et al*. 1996). Subsequent studies have confirmed this effect of red meat, and shown that there is a dose response to 0, 60, 240 and 420 g meat/d (Hughes *et al*. 1999).

In two volunteers, there was no effect of 600 g white meat and fish on faecal NOC; low-white-meat diet, 68 (SE 10) μg NOC/d, high-white-meat diet 56 (SE 6) μg NOC/d (Bingham *et al*. 1996). This suggests that the increase in faecal NOC and nitrosating products is brought about by a specific effect of red meat not seen with white meat. A major difference between red and white meat is in their content of Fe, which is poorly absorbed from the small intestine. This difference in effect may be due to the fact that Fe and Mo are integral components of nitrate reductase (*EC* 1.7.99.4) and are essential for enzyme activity (Stouthammer, 1976). Faecal nitrate reductase is a key step in determining the levels of production of nitrosating agents such as nitrite from nitrate and *N*-nitrosation (Calmels *et al*. 1985). Further studies are in progress, but so far differences in faecal NOC concentration in response to red and white meat have been inconclusive (Hughes, 1999).

Interrelationship with vegetable foods and fermentation in the colon

Carbohydrate entering the large bowel stimulates anaerobic fermentation, leading to the production of short-chain fatty acids (acetate, propionate and butyrate), gas, and an increase in microbial cell mass (biomass). The short-chain fatty acids are absorbed by the intestinal mucosa, where they stimulate Na absorption and bicarbonate production (Cummings *et al*. 1981). 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 (Burkitt, 1969; Cummings *et al*. 1981). Long transit time has not been related to large-bowel cancer risk, but there is a strong inverse association between high stool weight and colo-rectal cancer incidence (Cummings *et al*. 1992). Low stool weight leads to constipation, which together with the use of cathartics is a risk factor for colo-rectal cancer; odds ratios are 1·48 (95 % CI 1·32, 1·66) and 1·46 (95 % CI 1·33, 1·61) respectively, with attributable risks for colon cancer of 4·4 % in the US population (Sonnenberg & Muller, 1993). In addition, butyrate has anti-proliferative and differentiating effects, and was suggested as a protective agent in colon cancer in 1981 (Cummings *et al*. 1981). In cultured cell lines, Paraskeva and colleagues (Hague *et al*. 1993) have shown that butyrate induces apoptosis, a mechanism of probable importance in the chemoprevention of cancer.

Contrary to expectation that NOC would be reduced when high-red-meat diets are supplemented with bran, there was no reduction in NOC levels (Bingham *et al*. 1996). However, faecal weight increased, and hence the contents of the lumen were diluted. Transit time is inversely related to faecal weight. The net result with the high-bran diet would have been less contact between NOC (arising from the high-red meat content) and the colonic mucosa. Later studies with resistant starch and with vegetables have also shown no reduction in faecal NOC levels, but similar effects on faecal weight, transit time, and hence contact of NOC with the large-bowel mucosa (Silvester *et al*. 1997; Hughes, 1999). The lack of effect on faecal NH_3 levels has been noted previously (p. 245). These effects would suggest an indirect mechanism for any protective effect of plant foods against cancer, but this has not been investigated epidemiologically.

Conclusion

Despite interesting possibilities, a direct link between the epidemiology of most dietary factors, intermediate risk markers and the end points of cancer in human subjects has yet to be established. The study of diet in relation to different genotypes at risk for cancer is likely to emerge as a

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key area, perhaps explaining low estimates of relative risk within populations despite strong relationships internationally between dietary habits such as high meat consumption and the occurrence of this disease. One preliminary study has shown an increase in relative risks for mutations in the K-*ras* gene and meat consumption in colon-cancer cases (Freedman *et al*. 1997). It is likely that more studies of this type, linking somatic mutations, diet and cancer will be published, allowing a much stronger case to be made for or against meat being related to cancer. Meanwhile, there are public health recommendations to increase vegetable and NSP consumption in order to increase stool weight and reduce risk of large-bowel cancer and other large-bowel disorders (Department of Health, 1998). The type, amount, processing, cooking and dose responses of meat or protein responsible for increasing the risk of cancer are uncertain; hence, the current recommendation for a 140 g limit is statistical, based on the distribution of meat in the UK population (Department of Health, 1998).

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