CONTENT WITH A VEGETABLE LOVE

Plant Foods and Cancer Risk

by
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THE CAROLINE WALKER TRUST

The Trust was set up in memory of the nutritionist and campaigner Caroline Walker, who died in 1988. The Trust's mission is the improvement of public health by means of good food – a cause which Caroline made important to everybody in this country. The Trust, which relies on charitable donations, exists to further her work through research and publications.

INTRODUCTION

Plants are relevant to health and disease; this is a long-held principle across human cultures. The underpinnings of medicine in our culture, and the practice of medicine in many parts of the world today, involve the prescription of specific foods (plants in particular) for a large number of illnesses. Historically, foods ascribed healing properties in and around the Mediterranean and Middle East included cruciferous vegetables (the cabbage family), which were cultivated primarily for medicinal purposes (Fenwick 1983), and members of the allium family (onions, garlic, etc). Pliny declared that consumption of cabbage would cure as many as 87 diseases and that consumption of onions would cure as many as 28 (Kohman 1947). Other therapeutic plants included celery, cucumber, endive, parsley, radish, and legumes (eg peas, beans, pulses). The ancient Romans believed that lentils were a cure for diarrhoea and a curb on emotional disequilibrium (it is not clear whether they related these disorders). A variety of fruits including citrus fruits, raisins, and grapes were incorporated into oral preparations, enemas, inhalations, and topical applications (see Darby 1977).

It has been estimated that from 10 to 70% of all cancer deaths are attributable to diet (Doll 1981). Relevant hypotheses for the diet-cancer link include roles for fat and fibre in the causation of colon cancer; for alcohol in upper digestive tract cancers; and for inadequate intake of various vitamins and minerals, including vitamins A, C, and E and selenium, in several cancers. High intakes of plant foods appear to be protective against cancers at many sites; this is probably better supported by the literature than most of the other dietary hypotheses.

A firm grasp of the role of vegetables and fruit in cancer is important for making dietary recommendations. Current intakes in both the UK and the USA are low. The Dietary and Nutritional Survey of British Adults provides information (*inter alia*) on the eating habits of men and women aged 16 to 64 (Gregory 1990). The most recent data show that the average daily intake of raw and salad vegetables in men is around 33g; in women it is around 35g. For cooked vegetables, excluding potatoes, the intakes for the same groups are 116g and 89g; for chips and fried potatoes, 72g and 41g; other potatoes, 83g and 57g. Fresh fruit intakes

were 60g and 72g respectively. The survey records total intake over 7 days. During that period, just over one half of the respondents reported eating one apple or pear or more and just over a quarter consumed any citrus fruit. Only 62% of those studied consumed any leafy green vegetables in the week of the survey.

It has been estimated from the US National Health and Nutrition Examination Study (NHANES II) that the average American consumes about 0.7 servings/approx 60g of vegetables (1.5 servings/approx 130g when potatoes and salads are included), and 0.7 servings/approx 90g of fruit per day (Lanza 1987; note that serving sizes of vegetables and fruit differ). This is despite US Department of Agriculture statistics showing that annual consumption of fruits and vegetables increased by 23% between 1970 and 1985 (Pearl 1990). If, in fact, consumption of vegetables and fruit reduces cancer risk, public health and economic interventions to increase the current consumption levels would be well justified and, given the currently low intake, relatively effective.

THE EPIDEMIOLOGICAL EVIDENCE

We have recently reviewed the epidemiological evidence regarding the association of cancer risk and vegetables and fruit (Steinmetz and Potter 1991a). We have also undertaken a series of specific studies of the role of plant foods and micronutrients in cancer (Steinmetz 1993a,b, 1994, Bostick 1993a,b). A summary of the literature follows.

Cohort studies

Of the cohort studies, all but one (Phillips 1985) show an inverse association between intake of vegetables or fruits and cancer – that is the higher the consumption, the lower the risk. The bulk of the evidence for such an association covers green and yellow vegetables (Hirayama 1982a, 1982b, 1986, and Colditz 1985) and soya products and legumes (Hirayama 1982a, 1982b, 1986, and Mills 1988). Lung cancer is the cancer for which there is the most cohort study evidence for a lower risk with vegetable and fruit consumption, but that may not signify the strongest link: it is also the most-studied cancer (Hirayama 1982a, 1982b, 1986, Kväle 1983, Long-de 1985, Steinmetz 1993b).

Case-control studies

● *A summary by cancer site:* When we look at the causes of cancer at different sites in the body, each site needs to be considered separately. There are major differences between tissues of the body: in the number and dose of substances, carcinogenic (cancer-causing) or protective, to which they are likely be exposed; in how those substances get there (eaten,

blood-borne, inhaled etc); and in the enzymes that protect each part of the body against cancer-causing substances (the carcinogens), and those that activate them.

Lung cancer Consumption of carrots and green leafy vegetables appears to be particularly associated with a lower risk of lung cancer.

Colon cancer Cruciferous vegetables and carrots appear to be beneficial in relation to colon cancer. Both of these are especially high in anti-carcinogenic substances (see below). They also appear to be more closely associated with a lower risk of colon cancer than other vegetables which have similar amounts of fibre, but are not particularly high in these substances, but that, nonetheless, contain. This suggests that the lower risk associated with vegetables is not explained by fibre alone.

Rectal cancer For rectal cancer, although an association with consumption of vegetables and/or fruits has been consistently reported, no associations with specific foods emerge.

Upper aerodigestive tract cancer Consumption of fruit appears to be associated with lower risk of cancers of the mouth, pharynx, larynx, and oesophagus. This may be related to the common causes of cancers of the head and neck (for which the strongest and most consistent risk factors are tobacco and alcohol). Consumption of vegetables appears to be especially associated with lower risk of cancer of the larynx.

Stomach cancer Several vegetables and fruits have been quite consistently associated with lower risk of stomach cancer, including fruit in general, lettuce, onions, tomatoes, celery, and squash. Of these, fruit and lettuce have been particularly commonly reported. The protective effect of eating vegetables and fruit raw, rather than cooked or preserved, also appears to be a consistent pattern. Canned fruit and potatoes have been positively related to stomach cancer risk in some studies – that is, associated with an increased risk.

Pancreas cancer Almost every case-control study of pancreas cancer has reported that consumption vegetables and/or fruits is associated with lower risk, but as with rectal cancer, no specific patterns are apparent.

Bladder cancer Consumption of vegetables and fruit in general, and of carrots in particular, appears to be associated with lower risk.

Hormone-dependent cancers By contrast to cancers at each of the

epithelial sites above, the hormone-dependent cancers appear less closely associated with vegetable and fruit consumption. There is some evidence for an inverse relationship with cancers of the breast and uterus, but the evidence is not as consistent as that for the above cancers. For ovarian and prostate cancers, virtually no evidence exists for an association.

● *A summary by types of vegetables and fruits*

A site-specific review of the vegetable and fruit data is useful, particularly for understanding mechanisms of cancer. But what's most important for dietary recommendations is an overall assessment of which vegetables and fruits are associated in the most consistent way with cancer at all sites. To this end, the Table shows a summary of the cohort and case-control studies of all cancer sites, and presents the number of studies finding increased risk, decreased risk, or no association between cancers and specific types of vegetables and fruits.

Overall, the evidence strongly suggests that consumption of the following vegetables and fruits is lower in people who get cancer: raw and fresh vegetables, leafy green vegetables, cruciferous vegetables, carrots, broccoli, cabbage, lettuce, raw and fresh fruit, and citrus fruit. Raw-and-fresh-vegetables is the outstanding food category: more than 80% of studies show an inverse association, and just one study shows a positive association. The following categories were reported linked to a lower risk in more than 70% of all case-control studies: raw or fresh vegetables, leafy green vegetables, carrots, broccoli, and lettuce.

In addition, for all categories except potatoes and legumes, more than 80% of the associations reported were either in the direction of reduced cancer risk, or null. This shows that there is very little likelihood of harm arising from consumption of these foods. For broccoli, 100% of the associations showed a lower risk or no association. The evidence for consumption of potatoes and legumes is somewhat more equivocal; an inverse or null relationship found in 67% and 54% of studies respectively.

Even the 'no-associations' should not necessarily be interpreted as evidence against a lower risk, as a variety of factors tend to ensure that the associations seen in epidemiological studies - whether with increased or decreased risk - are conservative.

A comment must be made regarding the positive associations presented in this table. More than one-third of the positive associations listed (excluding those for legumes) come from studies by Tajima (1985), who consistently found positive associations for consumption of almost all

Table: Summary of the studies in the epidemiological literature that have reported on the relationship between vegetrables and fruit and risk of cancer at any site

Type of vegetable, fruit, etc	Total number of studies	Number of studies showing a reduced risk[a]	Number of studies showing no association[a]	Number of studies showing a higher risk[a]
Raw or fresh vegetables[b]	30	25 (83%)	4 (13%)	1 (3%)
Leafy green vegetables[c]	62	49 (79%)	6(10%)	7(11%)
Cruciferous vegetables	42	28 (67%)	10 (23%)	4 (10%)
Allium vegetables[d]	18	11 (61%)	4 (22%)	3 (17%)
Carrots[e]	44	33 (75%)	8 (18%)	3 (7%)
Broccoli[f]	10	7 (70%)	3 (30%)	0 (0%)
Cabbage[g]	22	13 (59%)	5 (23%)	4 (18%)
Lettuce[h]	23	19 (83%)	1 (4%)	3 (13%)
Potatoes	30	9 (30%)	11 (37%)	10 (33%)
Legumes[i]	24	7 (29%)	6 (25%)	11 (46%)
Raw or fresh fruit	43	22 (51%)	17 (40%)	4 (9%)
Citrus Fruit[j]	29	17 (59%)	9 (31%)	3 (10%)

NOTES TO THE TABLE

a number in parentheses is the percentage of total studies
b includes categories titled raw vegetables, fresh vegetables, uncooked vegetables, salad vegetables, & raw yellow-green vegetables
c includes categories titled leafy green vegetables, green leafy vegetables, dark green vegetables, green vegetables, raw green vegetables, kale, chicory, & spinach
d includes categories titled allium vegetables, onions, raw onions, & cooked onions
e includes categories titled carrots, raw carrots, & cooked carrots
f includes categories titled broccoli & raw broccoli
g includes categories titled cabbage & raw cabbage
h includes categories titled lettuce, lettuce & endive, lettuce salad, & romaine lettuce
i includes categories titled legumes, dried beans, beans, kidney beans, peas & beans, seeds & legumes, & soybeans
j includes categories titled citrus fruit & fresh citrus fruit

vegetables and fruits studied in a series of methodologically similar case-control studies in Japan. Such positive associations were not limited to vegetable and fruit consumption, but were seen for almost all foods studied. In these studies, the control group included patients with gastritis, ulcers, polyps, and other diseases; only 48% of the control group did not have abnormal clinical findings. Thus, the case-control differences are likely to have been affected by a decreased intake of food and energy in general, or of vegetables and fruit specifically, by the diseased persons in the control group. Tajima and colleagues found their own results 'unexpected' in light of 'the results of most previous epidemiological and experimental studies'.

Strength of the associations: half the risk (at least)

Most of the studies show no more than a halving of risk of cancer with high consumption of vegetables and fruit. As noted above, a variety of factors ensure that the strength of the associations seen in epidemiological studies – whether increased or decreased – are conservative. So if odds ratios consistently suggest a halving of risk, they may be signalling stronger underlying relationships. Even if the risk of cancer were only halved by increasing vegetable and fruit consumption, this would still represent a major modifiable influence on the overall burden of cancer in the community: because low vegetable and fruit consumption is so widespread, and because dietary behaviour is changeable.

MECHANISMS

Phytochemicals - plant chemicals that protect us

There are many biologically plausible reasons why consumption of vegetables and fruit might reduce the likelihood of cancer. These include the presence of potentially anti-carcinogenic substances such as carotenoids, ascorbate, tocopherols, selenium, dietary fibre, dithiolthiones, isothiocyanates, indoles, phenols, protease inhibitors, allium compounds, plant sterols, limonene, and others. These are increasingly being called, collectively, phytochemicals or bioactive compounds – chemicals of plant origin that play a crucial role in our metabolism. In a companion paper to our review of the epidemiology, we have outlined what is known, at present, about some of these bioactive compounds (Steinmetz and Potter 1991b).

Pro-carcinogen to cancer-prone cell: how cancers develop

When the body is first exposed to specific cancer-causing substances (or carcinogens), many are not in their active form. The steps between exposure to the pro-carcinogen and the conversion of a normal cell into a cancer-prone cell (transformation) can be considered as follows.

- The pro-carcinogen is activated to the ultimate carcinogen form by the body's own P450 enzymes. It is worth keeping in mind that the body is not trying to make carcinogens - it is trying to make soluble certain insoluble foreign compounds, and thereby enable their excretion in the urine. But the body's job is complicated by the fact that the same enzyme can often make one compound less carcinogenic and another more carcinogenic.
- Either form of the carcinogen - pro-carcinogen or ultimate carcinogen - may be converted by Phase II enzymes into a form that is relatively inert and even more easily excreted. These Phase II enzymes are typified by glutathione *S*-transferase.
- If not excreted, the carcinogen can pass through the cell membrane and the nuclear membrane; the carcinogen can then interact with the DNA in a cell nucleus – forming adducts and/or producing mutations. DNA synthesis and replication (or DNA repair) subsequently occur. The repair has varying degrees of fidelity to the original cells; if the DNA is not repaired accurately, cell replication goes on to produce daughter cells with copies of the mutated DNA.
- These cells with mutated DNA then synthesize an abnormal protein (or fail altogether to synthesize a protein) crucial to normal cell function – or even a protein crucial to controlling cell replication itself. (This last is almost certainly what happens when a tumour suppressor gene, such as p53, mutates or is deleted.)

This sequence of stages brings a cell a step closer to becoming a cancer cell. It's not inevitable: even with abnormal DNA, the cell may cease to replicate and then undergo either differentiation (in which it becomes a specialised cell) or cell death (apoptosis). DNA damage probably has to occur several times before a cell becomes completely free of growth restraints and turns into a fully cancerous cell. Finally, the abnormal cells obtain a growth advantage over the normal cells and steadily increase in numbers (promotion) – often becoming more malignant and better able to spread (progression). These are steps that themselves involve further changes in the cellular DNA.

Specific anti-carcinogenic phytochemicals

At almost every one of the above stages, known phytochemicals (bioactive compounds) can alter the likelihood of carcinogenesis – occasionally in a way that enhances risk, but usually in a favourable direction. For example, such substances as glucosinolates and indoles, isothiocyanates and thiocyanates, phenols, and coumarins can stimulate the body's production of solubilizing and (usually) inactivating enzymes. Ascorbate and phenols block the formation of carcinogens such as

nitrosamines. Flavonoids and carotenoids can act as anti-oxidants, essentially disabling carcinogenic potential. Lipid-soluble compounds such as carotenoids and sterols may alter membrane structure or integrity. Some sulphur-containing compounds can suppress DNA- and protein-synthesis. Carotenoids suppress DNA-synthesis and enhance differentiation (the process by which cells mature into their final functioning state and stop replicating). More details are presented below.

The variety of substances in vegetables and fruits for which protective mechanisms have been postulated and, in many cases, shown experimentally, lends plausibility to the view that a diet high in plant foods is protective against cancer. Because some of these substances are the subject of entire fields of research themselves, a complete reference list would be intolerably long: review articles are quoted as the major sources of data for the more intensively investigated substances.

Carotenoids

Investigation of the relationship between carotenoids, retinol and cancer has produced an extensive literature. Reviews have been presented by Fontham (1990), Willett (1990), Ziegler (1989), Olson (1986), Graham (1984), Hennekens (1986), Krinsky (1991), the National Research Council (1989). More than 500 carotenoids occur in nature. They are present in dark green leafy vegetables and in yellow/orange vegetables and fruits. ß-carotene is found in most of these; it is not the predominant carotenoid in most vegetables, but carrots, sweet potatoes, and red palm oil are especially high. The predominant carotenoids in green leafy vegetables are the oxygenated carotenoids (xanthophylls), of which the major representative is lutein. Beta-carotene, alpha-carotene, and lycopene are hydrocarbon carotenoids. Lycopene is found in large amounts in tomatoes and red palm oil, but is otherwise relatively scarce. Carotenoids are vulnerable to destruction by heat, particularly the xanthophylls. Micozzi (1990) has compiled a useful list of vegetable carotenoid values.

One of the mechanisms by which carotenoids may reduce risk of cancer is via conversion to vitamin A. In the US, one-third or less of dietary intake of vitamin A comes from carotenoids (Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board 1989); the majority is retinol, which is found in animal products. Functions of retinol include a role in epithelial cell differentiation. Since de-differentiation (the failure of growing cells to reach fully-functioning maturity) is a feature of cancer cells; low serum vitamin A may plausibly be invoked as a relevant agent. But pro-vitamin A activity is a property of only a subset of carotenoids. A

different plausible mechanism derives from the capacity of carotenoids to quench singlet oxygen molecules, generated as by-products of normal metabolic processes, and to trap free radicals. ß-carotene may also enhance immune function (Krinsky 1991, Bendich 1988).

In vitro, retinoids can reverse or prevent malignant changes in cells exposed to a number of carcinogens (see Hennekens 1986). Retinoic acid has anti-proliferative capacity, can induce differentiation, and may influence expression of oncogenes (See Prasad 1990. Oncogenes are growth promoting genes; abnormalities in them can increase the likelihood of cancer.). Most animal research on cancer and vitamin A has also involved retinoids, not carotenoids. Retinol deficiency predisposes animals to pre-malignant changes and to enhance the development of chemically-induced tumours; however, a few workers have reported that retinoids may enhance tumour production. (See Hennekens 1986, Bertram 1987, Graham 1984).

Protective effects of carotenoids without vitamin A activity (Mathews-Roth 1985) and a mechanism of inhibition for carrots that is unrelated to ß-carotene (Rieder 1983) have been reported. The epidemiological evidence showing an inverse association between green vegetable consumption and cancer risk suggests that xanthophylls (approximately 90% of carotenoids in green vegetables) act as potential anti-carcinogens. The finding that consumption of raw vegetables appears to be associated more often with lower risk than consumption of cooked vegetables is consistent with a role for these heat-sensitive carotenoids.

Population studies of dietary carotenoids include prospective studies by Shekelle (1981) and Paganini-Hill (1987) and a large number of case-control studies, particularly of lung cancer; see Le Marchand (1989), Ziegler (1989), Fontham (1990). Each of these has shown an inverse association between carotenoid intake and risk. Two studies of lung cancer (Hirayama 1982b, 1986) have attempted to distinguish which vegetables, associated with which carotenoids, are most closely related to risk. The authors reported the consumption of dark green vegetables (high in lutein), tomatoes (high in lycopene), and total vegetables to be at least as strong as ß-carotene or carrots themselves as risk predictors. Howe's 1990 meta-analysis of eight breast cancer case-control studies showed a 20% reduction in risk of post-menopausal breast cancer ($P<0.003$) from high ß-carotene intake.

All five prospective studies (Stähelin 1991, Nomura 1985, Menkes 1986, Wald 1988, Connett 1989) examining blood ß-carotene levels and lung

cancer risk reported an inverse association (four of them statistically significant). Three studies (Stähelin 1991, Nomura 1985, Wald 1988) of stomach cancer showed similar results (only one statistically significant). Associations between cancer risk and blood retinol have not been as consistent as the links those with ß-carotene. Burney (1989), Helzlsouer (1989) and Comstock (1990) reported studies of pancreas, bladder, and rectum cancer showing lycopene associated with lower risk. Hsing (1990) and Stryker (1988) found no associations between a variety of carotenoids and melanoma or prostate cancer.

Clinical trials, using carotenoids and retinoids both as treatment and chemoprevention, (see Bertram 1987, Hennekens 1983, Stich 1984a, 1984b, Hong 1990) should begin to clarify the role of these compounds in the overall inverse association between vegetables and fruit and the risk of epithelial cancers.

Vitamin C

There are a large number of recent reviews of the literature on vitamin C and cancer: National Research Council (1989), Bertram (1987), Willett (1984a), Chen (1988), Colditz (1987), Block (1991). Vitamin C is found in highest quantities in fruits and vegetables (particularly citrus fruits and juices, broccoli, peppers, tomatoes, strawberries, melons, cabbage, and green leafy vegetables). Vitamin C is destroyed by heat, easily oxidized and water-soluble, and therefore readily lost in cooking. Substantial amounts of can be provided by supplements and fortified foods.

Wattenberg (1985) has categorized vitamin C as preventing the formation of carcinogens from precursors. An important mechanism by which the vitamin may reduce cancer occurrence, particularly stomach cancer, is through the capacity to reduce nitrite; nitrite reacts readily with secondary amines to form nitrosamines (National Research Council 1989). Vitamin C is an anti-oxidant and can enhance immune response. It is also involved in collagen synthesis; a deficiency may therefore disrupt connective tissue integrity and thus help to allow tumour growth, or prevent effective tumour encapsulation (Cameron 1979).

In vitro, vitamin C lowers the mutagenicity of gastric juice (O'Connor 1985). It has also been shown to induce regression (partial disappearance) of malignant changes in hamster lung cells and to increase survival of radiation-exposed ovarian tumour cells (see Willett 1984a). Feeding vitamin C to animals pre-treated with carcinogens results usually in a reduction of tumours or no evidence of effect. Some ascorbate salts have been shown to promote bladder cancer in rodents: see National Research

Council (1989) for references to animal studies. Guinea pigs, like humans, require vitamin C in their diets (unlike rats, neither humans nor guinea pigs can make their own) but vitamin C shows either no effect or a growth-promoting effect following induction of sarcomas in guinea pigs (see Bertram 1987).

Case-control studies of cancer in some directly-exposed epithelial tissues shows risk to be inversely related to vitamin C intake (see Bertram 1987, Willett 1984a, Fontham 1990). Howe (1990) reported a similar finding for breast cancer. Chemoprevention trials are underway (Bertram 1987).

Vitamin E

Although less-studied than vitamins A and C, there are several reviews of the area available: National Research Council (1989), Chen (1988), Bertram (1987), Colditz (1987), Willett (1986), Fiala (1985), Diplock (1991). Major dietary sources of vitamin E are vegetable oils and margarine. Other sources include whole grains, wheat germ, seeds, nuts, and green vegetables such as lettuce and asparagus. Its most active form in foods is alpha-tocopherol, but other tocopherols and tocotrienols have vitamin E activity.

Vitamin E is a significant intra-cellular anti-oxidant. It protects cell-membrane polyunsaturated fatty acids from oxidative damage. Further, it maintains selenium in the reduced state and has been shown to inhibit nitrosamine formation, especially at low pH (see Bertram 1987 and Fiala 1985). More speculative mechanisms have been proposed (see National Research Council 1989). Wattenberg (1985) characterizes vitamin E as a cancer inhibitor, its major effect being prevention of carcinogen formation from precursors.

In vitro, tocopherols have been shown to modify the expression of the oncogenes – *myc* and H-*ras*. See Prasad (1990). Overall, animal experimental results have been inconclusive (see National Research Council 1989). Epidemiological studies of dietary vitamin E are inconsistent, perhaps because of methodological limitations: see National Research Council (1989), Bertram (1987). We have recently shown that higher levels of tocopherol are associated with lower risk of colon cancer in the Iowa Women's Health Study (Bostick 1993b); supplemental rather than dietary tocopherol appears to explain this finding. Of 13 studies where blood was collected prospectively, seven showed a statistically significant difference between cases of all cancers, or of site-specific cancers, and controls (see Knekt 1988). Chemoprevention studies are under way (Bertram 1987).

Folic Acid

Folic acid (or folate) is an essential vitamin found in high concentrations in green leafy vegetables, asparagus, broccoli, beets, and a variety of beans. Oranges and orange juice contain moderate amounts, and are major contributors of folate to the US diet because of high consumption. Mild to moderate folate deficiency is surprisingly prevalent. MacGregor (1990) has recently discussed a possible anti-carcinogenic role.

At least one epidemiological study has shown a lower risk of colon cancer with a higher consumption of folate. This is an association that the authors plausibly attribute to the effects of low folate on DNA hypomethylation (Giovanucci 1993) – an early change in cell DNA observed in the sequence of events leading to colon cancer (Fearon and Vogelstein 1990).

In addition to these DNA hypomethylation effects (which may in any case be the underlying mechanism), *in vitro* and animal studies show that folate deficiency causes increased formation of micronuclei and chromosomal damage. Cancer-related breakpoints, including certain fragile sites associated with oncogenes, have also been shown to be increased. Dysplasia (an abnormal and disordered cell growth) in cervix and lung cells can be reversed by administration of folate. Finally, caffeine consumption may act in concert with folate deficiency to produce these effects (MacGregor 1990). For references to animal and in vitro studies, see MacGregor (1990).

Selenium

Reviews of the association between dietary selenium and cancer include those by Bertram (1987), Willett (1986), the National Research Council (1989), Diplock (1991). Selenium is found in foods in amounts proportional to soil-content and is often present as an amino-acid compound. Selenium is a co-factor for glutathione peroxidase, an enzyme that protects against oxidative damage. At high levels, it suppresses cell proliferation. It may also enhance immune response (see National Research Council 1989, and Colditz 1987).

In vitro, selenium decreases the mutagenicity of many compounds (see Willett 1986). Conversely, several selenium compounds, specifically selenites, are known to damage DNA (National Research Council 1989). Animal experiments on both deficiency and high dietary intake of selenium are inconsistent. Where very high doses of selenium have been given – many times the nutritional requirement and, in some cases, near toxic doses – the result is an inhibition of induced carcinogenesis. Ip

(1985), in a review, noted that 31 of 35 studies found such a result (see also Bertram 1987, Fiala 1985, Milner 1985, Willett 1984).

There is an inverse relationship between selenium level in crops and cancer mortality (see National Research Council 1989 for references). In 9 of 13 cohort studies with prospectively-collected blood, low selenium was a risk factor for cancer (see Helzlsouer 1989, Knekt 1988). This relationship is found more often in men than in women, and seen most consistently for stomach cancer.

Dietary fibre

Jacobs (1988), the National Research Council (1989), Potter (1990), Trock (1990), Greenwald (1986, 1987) have recently reviewed the evidence for an association between cancer and dietary fibre particularly in relation to colorectal cancer.

The area is made particularly difficult by the lack of a universally-accepted definition of 'dietary fibre' (see Potter 1990, and Pilch 1987), and by a lack of consistent, well-accepted methods of food-fibre analysis. Dietary fibre is found in vegetables, fruits, legumes, nuts, seeds, and unrefined grains. (For fibre values of foods, see Lanza 1986.)

The data appear to support a protective effect of high faecal bulk, but not rapid transit time of faeces through the gut. In general, insoluble fibres tend to decrease transit time and increase faecal bulk; soluble fibres are less effective (see National Research Council 1989 for references). Several specific mechanisms have been proposed for dietary fibre in colon carcinogenesis (see Jacobs 1988). Fermentation by colonic bacteria leads to more acid (ie lower) pH in the colon as the production of short-chain fatty acids increases; this inhibits the conversion of primary to secondary bile acids – which are made by bacteria in the colon, and are potential co-carcinogens. Further, at low pH, free bile acids are less soluble and thus less available as co-carcinogens. Fermentation may also release bound calcium, which is then free to bind bile acids and fatty acids. Finally, fermentation in the proximal colon leads to production of butyrate, a short-chain fatty acid with tumour-inhibiting properties.

The results of case-control studies on fibre and colon cancer are inconsistent. Of 13 case-control studies, 5 provide strong support and 4 provide moderate support for a reduced risk. Two studies give support for no association and 2 equivocally suggest increased risk (for references to case-control studies see Potter 1990 and Trock 1990). Howe (1992) completed a formal meta-analysis of 13 case-control studies where dietary

fibre was measured, and concluded that there is a lower risk with higher consumption; the data show a dose-response (p for trend < 0.0001) and an approximate halving of risk for the uppermost 20% of the population versus the lowest 20%.

The prospective study of Willett (1990b) examined the associations with fibre intake on incidence of colon cancer over a six-year period among female nurses. A dose-related decrease in risk was associated with fruit fibre (expressed as crude fibre); for the 20% of nurses with the highest intake of crude fibre, an odds ratio of 0.6 (0.4-1.1) was reported versus the lowest 20%. None of the other fibre variables – total dietary fibre, total crude fibre, vegetable fibre, and cereal fibre – were associated with risk of colon cancer. Data from the prospective Iowa Women's Health Study (Steinmetz 1994) suggest inverse associations with particular vegetables. In that study overall, the energy-adjusted risk of the 25% of women with the highest dietary fibre intake however, was only 0.80 times that of the lowest quarter (95% confidence limits: 0.49-1.31).

Dithiolthiones

Bueding (1986) has reviewed the data on the effect of dithiolthiones – found particularly in cruciferous vegetables – on carcinogenesis. Studies of animals given a synthetic dithiolthione, oltipraz, show reduced numbers of carcinogen-induced tumours of lung and forestomach. Oltipraz reduces DNA-aflatoxin adducts in liver and kidney (Kensler 1985, 1987) in rats, and chromosome breaks and lipid peroxidation in elderly mice (Stohs 1986). Other experiments show that oltipraz and other dithiolthiones increase levels of glutathione and activities of a variety of Phase II enzymes (see Bueding 1986). Wattenberg 1985 has classified dithiolthiones as agents that protect against cancer by blocking the reaction of electrophilic carcinogens with cellular macromolecules. No clinical trials have been undertaken.

Glucosinolates and Indoles

The effects of indoles on carcinogenesis were reviewed by Hocman (1989). Glucosinolates are found in cruciferous vegetables; brussels sprouts, rutabaga (swede), mustard greens, and dried horseradish are particularly high. More than 20 different glucosinolates have been isolated. Glucobrassicin and sinigrin each make up approximately 30% of the glucosinolates in cruciferous vegetables. Glucobrassicin is found in plant cells in a separate compartment from the enzyme myrosinase; when plant cells are damaged this enzyme converts the glucobrassin to indoles. The average daily intake of glucosinolates in Japan is estimated to be over 100 mg (Bailey 1987); in Britain, daily intakes of glucobrassicin and of

indoles are about 30 mg and 10 mg respectively (Wattenberg 1986).

Some indoles increase certain of the body's enzymes in dose-response fashion (see Loub 1975, Salbe 1986 and reviews by Bailey 1987 and Hocman 1989). The aggregate effect is probably anti-carcinogenic (Carr 1985). A further mechanism against the development of hormone-related cancers involves oestrogen metabolism directly. In rodents and humans, at approximately 50 times the US average daily intake, indoles increase the capacity of the liver to inactivate oestrogens (Michnovicz 1990). To the degree that indoles are able to do this with more normal intakes, they may reduce risk of oestrogen-related cancers (Michnovicz 1986, 1988, Schneider 1983, Bradlow 1985, 1986, Fishman 1984). Animal studies suggest that indoles are protective against a variety of tumours (Wattenberg 1978, 1986, Morse 1988, Bailey 1987, Dashwood 1989) but not against all (Salbe 1986, Morse 1988).

Isothiocyanates and Thiocyanates

Isothiocyanates are found in large amounts in cruciferous vegetables. Others have been made synthetically. Animal experiments show that the naturally-occurring and synthetic compounds inhibit both DNA-methylation and the early and late stages of carcinogenesis; see Wattenberg (1978b, 1977, 1981, 1987), Morse (1989). Wattenberg (1985) has defined benzyl isothiocyanate as an agent that prevents the reaction of carcinogens with critical sites. One relevant mechanism involves the capacity of isothiocyanates to induce Phase II solubilizing enzymes, including glutathione *S*-transferase (Sparnins 1982).

Coumarins

These compounds are found in vegetables and citrus fruits. Their mechanism of action of coumarins may also involve induction of Phase II enzymes (Sparnins 1982). Wattenberg (1985) classified coumarins as blocking agents. Animal experiments show tumour inhibition (see Wattenberg 1978b).

Flavonoids

These include quercetin, kaempferol, myricetin and chrysin, and are found in most fruits and vegetables. Tangeretin, nobiletin, and rutin are found in citrus fruits. Berries, tomatoes, potatoes, broad beans, pea-pods, and coloured onions are relatively high in quercetin; radishes and horseradish are relatively high in kaempferol. Food values for specific flavonoids have been published by Hermann (1976). The average daily intake of flavonoids for Americans is approximately 1g (Brown 1980).

Flavonoids are, to varying degrees, anti-oxidants. Some flavonoids induce mixed-function oxidase activity – with potentially mixed results for carcinogenesis. *In vitro* and animal experiments have also produced mixed results; see Brown (1980), Jansen (1982), Friedman (1984), Fiala (1985).

Phenols

Stich (1984c) has published a review of the anti-carcinogenic properties of phenols. Some of these compounds, alpha-tocopherol and quercetin, have been discussed above. Caffeic, ferulic, and ellagic acids are phenols that are widely distributed in plants. Chlorogenic acid (a form of caffeic acid) is the most prevalent phenolic compound and is found in almost all fruits and vegetables. Phenol levels are highest in freshly harvested fruits and vegetables; phenolic compounds are readily oxidized during processing and storage. Values for the amounts of phenolic compounds in some fruits have been published by Hermann (1973) and Stich (1984c). It has been estimated that humans consume approximately 1g of plant phenols daily (Hocman 1989). Their probable mechanism of action involves the induction of Phase II enzymes. They also inhibit *N*-nitrosamine production (Stich 1984c). Wattenberg (1985) classified caffeic and ferulic acids as agents that prevent carcinogen formation and that block the reaction of carcinogens with macromolecules. *In vitro*, phenolic compounds inhibit mutagenicity and, in animals, decrease lung and skin tumours (see Fiala 1985).

Isoflavones

Messina (1991a,1991b) and Adlercreutz (1990) have proposed that isoflavones are important cancer-preventive agents. They are found in soya beans and at least 300 other plants (Messina 1991a). Genistein and daidzein are soya isoflavones that are found at high levels in human urine (Messina 1991a). Equol is found in mammalian urine and is derived from plant precursors (Messina 1991a).

One proposed anti-carcinogenic action of isoflavones is a consequence of their weak oestrogenic activity. They bind to oestrogen receptors, blocking binding by more potent oestrogens, and eliciting only a minor oestrogenic response – generally about 0.1% of that of conjugated endogenous oestrogens (Messina 1991b, 1991a). But isoflavones also stimulate production of sex hormone-binding globulin, resulting in a decrease in free oestrogen (Adlercreutz 1990). These twin effects, blocking and binding, may thwart the progression of the hormone-dependent cancers – breast, endometrium, ovary, even prostate. These phyto-oestrogens could be considered as a class of naturally-occurring tamoxifens, to which some isoflavones are structurally similar. Other mechanisms have been

proposed: genistein may help to contain uncontrolled cell growth by inhibiting production of the signalling chemicals called tyrosine kinases (Messina 1991b, Adlercreutz 1990). Some cytochrome P450 enzymes - those which activate pro-carcinogens to become carcinogens – may also be inhibited (Messina 1991a).

Studies in rodents have shown a decreased incidence of carcinogen-induced breast tumours with diets high in soya beans. One study reported that the effect was seen for both cooked and raw soya beans, ruling out protease inhibitors as the relevant agents, since they are destroyed by cooking (see below; see also Messina 1991a, Adlercreutz 1990). Human observational data are also consistent with a preventive role for isoflavones. Vegetarians excrete higher levels of isoflavones – implying higher blood levels, probably from soya – and have lower risk of many cancers. Asian women generally consume more soya than Americans and have lower rates of breast cancer – both in their countries of origin and, for one or two generations, as immigrants. Seventh-day Adventist men and Japanese men living in Hawaii, both of whom consume relatively large amounts of tofu and legumes, have lower rates of prostate cancer. Of course, differences in other factors may also contribute to the explanation of these observations (see Messina 1991a, Adlercreutz 1990). Pre-menopausal women fed large amounts of soya had longer menstrual cycles, suggesting alterations in hormone metabolism in a manner likely to decrease the risk of hormone-related cancers (see Messina 1991b).

Saponins

Messina (1991a) and Oakenfull (1989) have discussed saponins as potential anti-carcinogenic agents. These compounds are found in a number of plants and in particularly significant amounts in soya: some 5% of the dry weight of soya beans is accounted for by saponins. A suggested mechanism of action is the ability of saponins to bind bile acids and cholesterol (Messina 1991a). Animal and *in vitro* studies show that saponins reduce cell proliferation in the gut and decrease growth-rate and rate of DNA synthesis in some tumour cells (see Messina 1991a).

Protease Inhibitors

Seeds and legumes are especially rich sources of protease inhibitors. Soya beans contain at least 5 types of these compounds. Although protease inhibitors in soya are destroyed by heat, some survive the processing into tofu. Others, in kidney beans and chickpeas, remain unchanged after canning. Grains, including barley, wheat, oats, and rye, also contain protease inhibitors - these enzymes comprise 5 to 10% of the water soluble protein and are concentrated in the embryo and endosperm.

Trypsin inhibitors are the most studied, and are found in the highest levels in potatoes and sweet corn; they are found also in spinach, broccoli, cucumbers, brussels sprouts and radishes (see Richardson 1977 and Fiala 1985). Synthesis also occurs within the body; this is increased in cancer and other conditions (Schelp 1988).

Most protease inhibitors are proteins of between 70 and 90 amino acids. Their action involves competitive inhibition of proteases, via the formation of complexes (see Richardson 1977). A possible mechanism on cancer involves an effect on proteases produced by cancer cells: the actions of cancer-associated proteases include the destruction of the extracellular matrix, cellular detachment, and subsequent local invasion (Schelp 1988). *In vitro*, they inhibit growth of transformed cells (see Schelp 1988). Animal studies show that protease inhibitors reduce the occurrence of tumours (see Schelp 1988, Wattenberg 1985, 1987). No human research has yet been undertaken on protease inhibitors.

Plant Sterols

Vegetables are rich sources of plant sterols, including ß-sitosterol, campesterol, and stigmasterol. Together, these make up about 20% of dietary sterols. Intake of plant sterols has been estimated to be approximately 250 mg per day in the US diet. They are similar in structure to cholesterol but pass through the gastrointestinal tract almost completely unabsorbed (see Raicht 1980 and Fiala 1985).

Raicht (1980) found that inclusion of 0.2% ß-sitosterol in the diet resulted in decreased occurrence of NMNU-induced colonic tumours in rats, by a mechanism that probably does not involve the body's enzymes. Faecal concentrations of cholesterol and bile acids in this study were not affected by ß-sitosterol. Because of their structural similarity to cholesterol, plant sterols may affect cellular membranes. No human research has been done on the effects of plant sterols but it has been reported that vegetarians have higher levels of faecal ß-sitosterol (see Raicht 1980).

Allium Compounds

Onions, garlic and chives, are allium vegetables. Diallyl sulfide and allyl methyl trisulfide are components of garlic oil. The anti-carcinogenic mechanism may involve stimulating the production of detoxification systems. Activation of microsomal mono-oxygenase enzymes and the Phase II enzyme, glutathione *S*-transferase, has been observed in response to allium compounds (see further below). Anti-bacterial properties of onions and garlic may suggest a mechanism for protection against

stomach cancer, specifically by inhibiting the bacterial conversion of nitrate to nitrite.

In vitro work has shown that onion extracts decrease the proliferation of tumours (see You 1989). Several animal studies have shown reduced occurrence of tumours at several alimentary tract sites, and increased activity of enzymes such as glutathione *S*-transferase, as a result of dosing with allium compounds (Wargovich 1988, Wattenberg 1986) and onion extracts (You 1989). No experimental human research has been undertaken on allium compounds in humans. But ecologic studies have shown that an area of northern China, where garlic production is high, has the lowest rate of mortality from stomach cancer in a country where gastric cancer, in general, is high; and that in an area of Georgia, USA, where vidalia onions are grown, the stomach cancer mortality rate is about one half the national US level (itself among the lowest rates in the world). Case-control studies in Greece, China, and Hawaii, have shown that high consumption of allium vegetables is associated with reduced risk of stomach cancer (see You 1989). One cohort study of women has shown an inverse association between garlic consumption and colon cancer risk (Steinmetz 1994).

Limonene

The major component of citrus fruit oils is D-limonene. Wattenberg (1983) has shown that, in mice, citrus oils added to a semi-purified diet induce glutathione *S*-transferase activity, and inhibit tumours of forestomach, lung, and breast (see Wattenberg 1986 and Hocman 1989).

ADAPTATION AND MALADAPTATION

The relationship between eating plant foods and cancer risk is probably most usefully considered in the context of evolution and adaptation. For the purposes of understanding the vegetable, fruit, and cancer data in the broader context, this paper takes as a starting point the existence of a seasonally-variable diet to which humans are well adapted. I will focus especially on intakes of substances for which we are dependent on the environment, and intakes of substances to which we have little or infrequent exposure. The following argument is developed in more detail elsewhere (Potter and Graves 1991, Potter 1992).

The diet we evolved with?

We cannot be certain to what kind of diets humans are best adapted (although the length and structure of the digestive tract, dental structure, and enzyme patterns provide some clues). Nonetheless, it is reasonable to attempt some tentative speculation on our early dietary patterns.

Significant variability in details must have existed – in the same way that extensive geographic and temporal variability are seen today.

Some probable common features of our early diet include:

- A high intake of a wide variety of plant foods – roots, leaves, nuts, seeds and fruit. Grains can only have become a staple in the last 10 to 15 thousand years, although before that they were probably gathered regularly in season.
- Sporadic intake of lean meat low in saturated fat, with a more secure and regular supply of fish and seafood for coast dwellers.
- An intake of insects, grubs, bone marrow, and organ meats.
- Very low intake of alcohol - and this largely the result of finding over-ripe fruit before birds did.
- Little refining or fractionation of food.
- Low and irregular intake of eggs, and probably no non-human milk.
- Seasonal variations, both in total amount of food available and in kinds of foods available, resulting in a variable intake of particular nutrients season by season.

Other variations in this pattern would have been defined by climate – in turn affected by time and geography – including the consumption of high-fat (but not high saturated-fat) diets in extreme northern populations – as animal foods would necessarily replace absent or markedly reduced plant foods in winter. In general, until very recently, saturated fat and alcohol intake would have been low, intakes of plant foods (but not grains) high, and food sources highly varied and their availability seasonally variable. By contrast, the late 20th-century industrial diet is often characterised by high intakes of saturated fat and alcohol, and low intakes of plant foods.

The new essential nutrients?

There's an argument that helps to elucidate the diet-cancer connection, in the light of a postulated 'original' diet, and human adaptation to it. The essential nutrients – both energy-bearing and micro-nutrients – are available to varying degrees in nature; they have important functions in growth, development, and reproduction; the human organism is dependent on their ready availability; deficiencies or excesses impair growth, development, and reproduction. In order to survive as individuals and as a species, our diet necessarily has to include them. This is the way we already understand the role of essential nutrients - vitamins, fatty acids, and amino acids.

But there is a plausible analogy in relation to other substances which may

be necessary for the maintenance of the organism – including, especially, dietary patterns that reduce the risk of cancer. It is this: the normal long-term functioning of cells depends on the presence of a variety of widespread dietary constituents including, *but not confined to*, those nutrients essential for growth and development.
In their long-term absence, cells malfunction. This malfunctioning state may make the cells more susceptible to exposure to carcinogens; it may impair some specific protective mechanisms, such as the responsiveness of enzyme systems that detoxify and eliminate carcinogens. It may also be characterized by higher cell replication rates, as somatic cells seek to adapt to the new (unprotected) conditions. Some of the phytochemicals described above, while not yet usually thought of as 'essential nutrients', are probably best described in this way. They are the naturally-occurring chemicals that, in concert with our own detoxifying enzymes, we rely on to keep us cancer-free.

A converse argument applies to those dietary constituents that are rare in nature: if substances are normally met with only occasionally (or not at all), a high intake may have detrimental consequences. This applies both to very rare exposures that produce acute poisoning; and to unaccustomed high intakes over time that overwhelm the metabolic processes which normally handle them. Bacterial, plant, and fungal toxins are members of the first class. There are several examples of the second: a high fat/high calorie intake, which has consequences for cholesterol and insulin metabolism, adipose storage, and sex steroid hormone production; a high grain diet, found particularly in agricultural communities, which is often associated with a reduced intake of other plant foods (and of animal foods) and which contains large amounts of abrasive material that may increase cell replication rates, particularly in the upper digestive tract; and a high intake of alcohol, which, together with a generally reduced range of foods, is associated with a wide variety of metabolic abnormalities. In particular, alcohol can act as a solvent (carrying smoking-related carcinogens into cells) and as a chronic irritant (encouraging rapid cell turnover) especially in the upper digestive and respiratory epithelium. There may be differing degrees of adaptation in long-exposed versus unexposed populations in each of these cases.

Why would humans need to avoid cancer?

It's possible to object to this adaptation argument in relation to chronic diseases like cancer. Natural selection influences reproductive success; chronic diseases are largely diseases which strike after the reproductive years; so there was no evolutionary need for dietary adaptation to ensure long-term survival of the human species. There are four responses to this.

The first is to argue that humans have a long period of infant and juvenile dependence; thus survival of parents in a healthy state is likely to favour the survival of offspring to reproductive years. Second, consider the survival of tribes or bands. The tribes that had the best chance of survival would have been those with sufficient elders who knew how to respond to infrequently met hazards – food or water shortage, epidemic disease, and natural hazards such as fire or extreme weather. The tribal wisdom maintained by the old would have meant survival of the tribe. Tribes without elders, and without knowledge, would be more likely to perish. So tribes in which people lived longer (eg by avoiding diseases such as cancer) would, in turn, have survived other threats to pass on their wisdom, their adaptive eating habits, their adapted metabolisms, their genes, and their tendency to longevity. Third, by arguing that chronic diseases are a phenomenon of older age, and therefore cannot have been selected for, one ignores the fact that they are *not* a phenomenon of younger age – which implies that some resistance (at least to the point of postponing them to older ages) has been selected for. Fourth, a diet that reduces risk of cancer may also be a diet that improves reproductive success. There are a wide variety of substances that increase – and some compounds that reduce – both teratogenicity (occurrence of birth defects) and carcinogenicity. Thus, selection for a diet that improved reproductive success could directly select for reduced risk of cancer.

SUMMING UP

There are a variety of ways in which diet may influence the development of human cancers. What we propose here is a theoretical framework for understanding why a high plant food intake lowers cancer risk – and indeed for understanding diet and cancer more generally. It is expressed in the form of an argument that, although not yet fully developed, has the following features.

- There is a dietary pattern to which humans are well-adapted – an 'original diet'.
- This original dietary pattern had specific features that included regular exposure to a variety of phytochemicals – bioactive compounds found in plants – on which human metabolism is dependent in the long run. These constitute another class of 'essential nutrients' – essential to maintenance of a cancer-free organism, in just the same way that vitamins, fatty acids and amino acids are essential to growth and development.
- Conversely, the original dietary pattern was low in highly abrasive cereal products with less resultant damage, and less need for frequent cell repair, particularly to the upper gastrointestinal tract.
- The diet involved variability in intake season by season, which was accompanied by variability in cell replication rates, particularly in the

lower gastrointestinal tract. Lean seasons ensured a low risk of obesity.
- There was little intake of alcohol and therefore little capacity for its solvent and chronic cell damage capacities.
- Abandonment of each of these aspects of diet to which we are adapted has consequences for carcinogenesis. A high intake of fat, of grains, and of alcohol, and increased obesity are each associated with an identifiable pattern of cancers.

Reduced consumption of vegetables and fruit means a reduced intake of a wide variety of substances: substances that keep enzyme systems 'tuned' to handle occasional high intakes of carcinogens, that block the activation of other carcinogens, that act to help the body in producing anti-carcinogens, that reduce the capacity of transformed cells to proliferate, and so on. Further, these plant-derived substances may be at their most beneficial when the metabolism is exposed to high levels of carcinogenic products and endogenous co-carcinogenic compounds – most of which are associated with eating a diet high in cooked animal foods, living in an industrial society, and using tobacco and alcohol.

Vegetables and fruit contain the anti-carcinogenic cocktail to which we are adapted. We abandon it at our peril.

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