ANTICARCINOGENIC FACTORS IN PLANT FOODS: A NEW CLASS OF NUTRIENTS?

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INTRODUCTION

In their now famous and widely quoted study of the role of environmental factors in the aetiology of cancer, Doll & Peto (1981) estimated that the proportion of cancer deaths attributable to an adverse effect of diet was approximately 30% in the USA, the same as that due to tobacco. However, unlike tobacco, for which the risk was well defined and, at

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least in theory, easily avoidable, the effects of diet were considered so complex that their mechanisms of action and their means of manipulation could only be guessed at. One of the strongest signals to emerge from epidemiological studies of diet and cancer incidence over the last decade has been the protective effect of diets rich in vegetables and fruits. Block *et al.* (1992) reviewed nearly 200 studies of the influence of fruit and vegetable intake on the risk of cancer in man. In 156 of these studies the results were expressed in terms of relative risk, and of these 128 showed a statistically significant protective effect. The results were particularly consistent for cancers of the alimentary tract, lung, breast and female reproductive organs, and were relatively weak only for cancer of the prostate. It is probable therefore that in industrialized societies many of the important effects of diet are protective, and that dietary strategies for the avoidance of cancer require the maximization of these protective effects. Initiatives such as the '5 A Day for Better Health' campaign, by which the National Cancer Institute has recently sought to promote the consumption of vegetables and fruit in the USA, are a practical reflection of this principle, but the mechanisms through which the various components of these foods may act remain uncertain.

Interest in the anticarcinogenic effects of foods has a relatively long history. Crabtree (1947) defined an anticarcinogen as any factor "which delays or prevents the emergence of malignant characters in any tissue of any species of organism". Such a broad definition encompasses nutrients as well as other biologically active compounds which fall outside any currently accepted definition of nutrients. Certainly vegetables and fruits are a rich source of micronutrients, including several which are intimately involved in cell proliferation and the maintenance of tissue integrity. These include the folates, and the carotenoids which, together with vitamin E and ascorbate, are thought also to protect against oxidative damage to DNA and other cellular components (Diplock, 1991). There is good epidemiological evidence to suggest that levels of carotenoids (Stähelin *et al.* 1991; Ziegler, 1991) and vitamin E in the serum (Murphy *et al.* 1990; Comstock *et al.* 1991) are inversely related to risk of cancer. Nevertheless, the protective effects of these nutrients remain to be established, and it may be that high serum levels are merely markers for a high intake of plant foods.

In this paper we are concerned with the very large number of biologically active 'non-nutrient' compounds in plant foods for which potentially anticarcinogenic effects have been demonstrated experimentally. The growing realization that such compounds exert biochemical and physiological effects in humans raises important theoretical and practical issues. Many of these substances have previously been regarded as potential toxicants. If their biological activity contributes to the protective effects of fruit and vegetables against cancer, what is the balance of risk and benefit? Furthermore, if their protective value is proven, should they be considered for classification as micronutrients in their own right? In this review we will consider these questions in the light of the known mechanisms of action of the biologically active non-nutrients.

MECHANISMS OF CARCINOGENESIS

One difficulty in any discussion of anticarcinogenic components of plant foods is the development of a satisfactory classification scheme. The number of secondary metabolites present in fruits and vegetables is very large and many of them appear to inhibit carcinogenesis by more than one mechanism. They are therefore best classified in terms of their biological effects rather than their chemistry, but such a scheme requires a knowledge of their mechanisms of action which in many cases does not yet exist. Much insight has been gained from studies in which vegetables, fruits, or compounds isolated from them

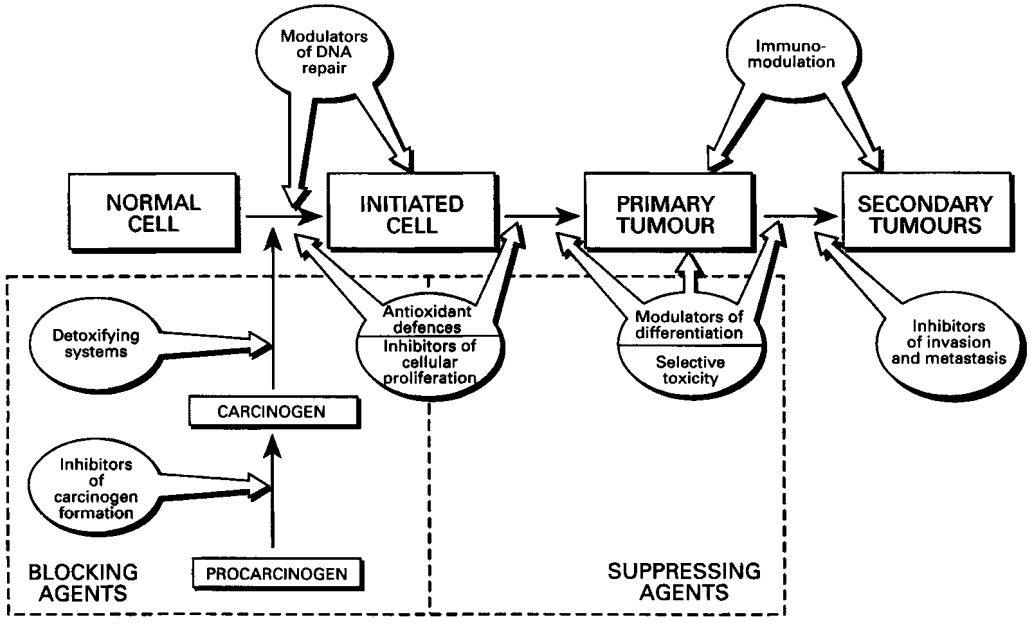

Fig. 1. Mechanisms and sites of interaction whereby protective factors may inhibit the carcinogenic process.

have been administered to experimental animals treated with chemical carcinogens. Before discussing this and other approaches to classification, however, the nature of tumours and the process of carcinogenesis will be briefly reviewed.

Tumour cells are typified by a loss of responsiveness to some or all of the factors regulating cell growth, differentiation and programmed death (apoptosis) in the tissue from which they have arisen. There is usually reduced structural and functional specialization, and malignant tumour cells have the capacity to invade adjacent mesenchyme, and ultimately to migrate to distant sites, giving rise to secondary tumours (metastases). In the simplest model of carcinogenesis the process is assumed to occur in two stages – initiation and promotion. Initiation is the primary event in which cellular DNA undergoes damage which remains unrepaired or becomes misrepaired. The resulting acquired somatic mutation is reproduced at mitosis, giving rise to a clonal population of initiated cells. The initiating event in carcinogenesis is often the result of DNA adduct formation with a genotoxic chemical, but endogenous production of free radicals may be equally important.

Initiated cells do not necessarily give rise to a malignant tumour until they have undergone 'promotion', a process which facilitates their full transformation to an invasive state. Chemical substances which function as promoters are not always genotoxic, but they are often mitogenic, and they may interfere with the expression of genes controlling differentiation and growth. However, some compounds, including polycyclic aromatic hydrocarbons and nitrosamines, are classified as 'complete carcinogens' because they are capable of inducing tumours in experimental animals without the need for exposure to chemical promoters.

Much of the pioneering work on the anticarcinogenic properties of naturally occurring compounds has been carried out by Wattenberg and his coworkers, who proposed a system of classification based on the stage of carcinogenesis at which they act (Wattenberg, 1985). According to this scheme, anticarcinogens are subdivided into two major classes defined

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operationally as 'blocking agents' and 'suppressing agents'. Blocking agents are typically compounds which have been found to be effective when given immediately before or during treatment with chemical carcinogens. They are thought to prevent initiation, either by inhibiting the formation of carcinogens from precursor compounds, or by preventing the active carcinogenic species from acting upon its cellular target. In contrast, suppressing agents are thought to act by preventing the progression of initiated cells to fully transformed tumour cells. Such compounds inhibit the emergence of tumours even when given after treatment with a complete carcinogen or a combination of incomplete carcinogen and promoting agent. We will retain here the general concepts of blocking and suppressing agents, while exploring the extent to which the mechanisms of blocking and suppression have been defined. Fig. 1 summarizes the various possible mechanisms for the inhibition of initiation, promotion and metastasis.

BLOCKING AGENTS

The first line of defence against chemical carcinogenesis is the ability of tissues such as those of the liver and intestinal mucosa to intercept and detoxify potentially damaging environmental substances. Many carcinogenic compounds enter cells by passive diffusion (Landers & Bunce, 1991), and are detoxified by several mechanisms. Fig. 2 charts the detoxification pathways which lead to an excretable metabolite of a carcinogen which is represented in this example by a quinone. This figure will form the basis for much of the subsequent discussion. For convenience the metabolic pathway is usually divided into Phase I (which often involves carcinogen activation), Phase II (conjugation) and Phase III (transport out of the cell: Prochaska *et al.* 1985; Ishikawa, 1992). Paradoxically, the metabolic pathways involved in detoxification may also serve to generate potentially damaging chemical species. Products of phase I reactions in particular may lead to free radical mediated damage of lipids, protein, carbohydrate and DNA, and many of the activated compounds can form DNA adducts. One of the earliest blocking agents to be identified was disulfiram, which inhibits induction of large bowel tumours by 1,2-dimethylhydrazine in rodents by preventing its metabolic conversion to an active form (Fiala *et al.* 1977). Subsequently, a variety of naturally occurring non-nutrient compounds, including aromatic isothiocyanates and indoles from cruciferous vegetables and organo-sulphur compounds found particularly in allium species, have been found to block chemical carcinogenesis in animals (Wattenberg, 1993). In this section we will review both non-nutrient and some nutrient blocking agents, and show how they play a vital role in regulating the efficiency of carcinogen metabolism. We will also review mechanisms whereby the adverse effects of endogenous free radicals may be blocked by naturally occurring plant constituents.

PHASE I METABOLISM – ACTIVATION BY MONOOXYGENASES

Hydrophobic carcinogens partition into the cell membrane, where they are activated by membrane bound monooxygenase enzymes, primarily cytochrome P450 (Black & Coon, 1987; Guengerich, 1992*a*), and flavin dependent enzymes (Ziegler, 1991). The product of this reaction is an oxygenated compound such as an epoxide which forms a substrate for further metabolism by phase II enzymes (Guengerich, 1992*a*). The final product of the P450 catalysed reaction may itself be highly carcinogenic (McManus & McKinnon, 1991). For example, aflatoxin B_1 is metabolized to aflatoxin-B_1-8,9-epoxide, which is capable of forming an adduct with the N-7 atom of guanine in DNA (Ishikawa, 1992). Benzo(*a*)pyrene,

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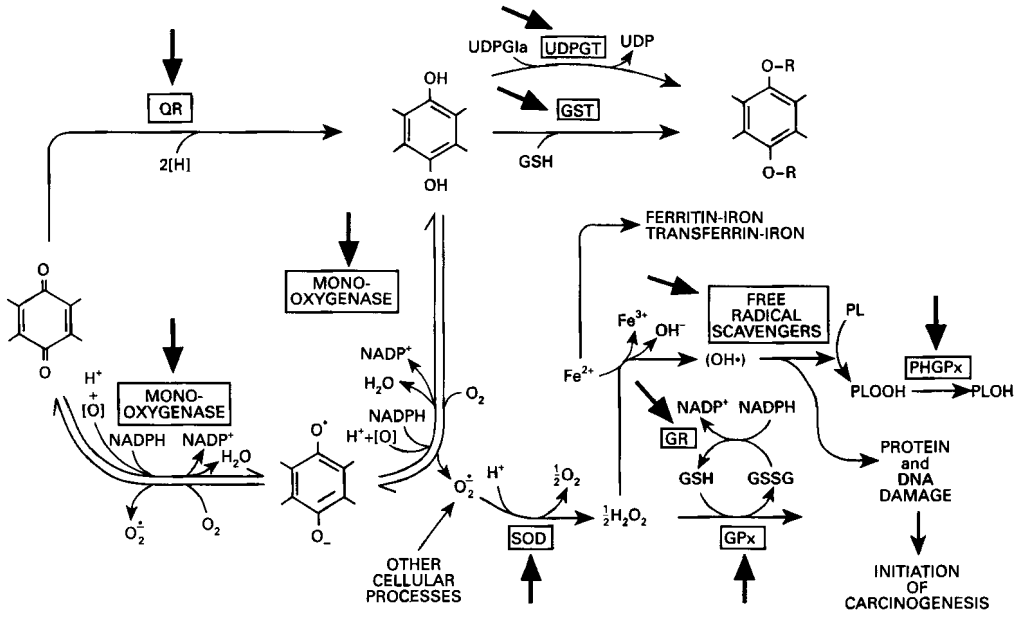

Fig. 2. The inter-relationship between detoxification pathways and free radical mediated oxidative damage, using a quinone xenobiotic as an example. Possible sites for dietary intervention in the detoxification of 2, 3, 5, 6-tetramethyl-*p*-benzoquinone are shown by filled arrows. UDPGla, UDP-glucuronic acid; PL, phospholipid, its hydroperoxide (PLOOH) and alcohol (PLOH).

a polycyclic aromatic hydrocarbon, is converted to many genotoxic products including phenols and epoxides (Guengerich, 1992*a*).

Phase I reactions may also give rise to problems for the cell by the production of free radicals. The role of cytochrome P450 in free radical damage has been investigated by several groups (Castillo *et al.* 1992; Dai *et al.* 1993; Ohmori *et al.* 1993). With reconstituted enzyme systems, in the absence of substrate, reduction of P450 by NADPH or NADH causes the production of reactive oxygen species (Aust *et al.* 1985). Even in the presence of substrate, coupling is so loose that 50% of the reducing equivalents appear as superoxide and peroxide, rather than causing oxidation of substrate (Hochstein *et al.* 1988). These results indicate that the P450 monooxygenase system may be responsible for the production of reactive oxygen species as a consequence of normal cellular metabolism, although such extreme conditions may not occur *in vivo*. Certain cytochrome P450 isoenzymes are more prone to the production of reactive oxygen species than others. Thus in animal studies, P450 2E1, an enzyme involved in glycerol oxidation, is poorly coupled, and liver microsomes prepared from animals treated with P450 2E1 inducers exhibit higher rates of H_2O_2 production (Clejan & Coderbaum, 1991). P450 are therefore often considered as pro-oxidants (Dai *et al.* 1993).

The number of P450 isoenzymes so far found in human tissues exceeds 30 (Guengerich, 1992*a*) and a full discussion is beyond the scope of this review. The nomenclature of the P450 isoenzymes is updated in Nelson *et al.* (1993). The P450 which metabolize xenobiotics belong to classes I, II and III (Guengerich, 1992*b*). Nutrient status influences phase I metabolism by affecting the transcription, translation or protein stability of cytochrome P450 (Yang *et al.* 1992). In rats, vitamin E deficiency gives a slight decrease in total P450 (Williams *et al.* 1992), whereas in the case of vitamin C, both high doses and a deficiency lead to lowered monooxygenase activities (Yang *et al.* 1992).

Many dietary non-nutrients bring about much greater changes in P450 levels. Indole-3-carbinol, a compound found in cruciferous vegetables, gives rise to a 7·5-fold increase in P450 1A1 in the liver, and a 14-fold increase in the small intestine, when fed to rats at a very high level (500 mg/kg body weight) for 14 d (Wortelboer *et al.* 1992). Quercetin, a flavonoid which occurs widely in the diet, has been shown to induce P450 levels *in vitro*. For example it can give rise to increases in P450 1A1 as high as 12-fold in mouse hepatoma cells in culture (De Long *et al.* 1986). The relevance of such effects in the context of real human diets remains to be established.

There are many examples of drugs and xenobiotics (Forrester *et al.* 1992), including caffeine (Gandhi & Khanduja, 1992) and ethanol (Lucas *et al.* 1992), which increase some P450 involved in phase I metabolism. Other non-nutrient components of human foods which have been shown to have this effect include the flavonoids tangeretin, flavone and nobiletin (Yang *et al.* 1992). Induction of P450 may, in theory, increase the risk of carcinogenesis. On the other hand diallyl sulphone, a metabolite of diallyl sulphide which is a major flavour component of garlic oil, irreversibly inhibits P450 2E1 (Brady *et al.* 1991), and this has been proposed as a mechanism for the blocking activity of diallyl sulphone (Brady *et al.* 1991; Horie *et al.* 1992). Phenethyl isothiocyanate, a breakdown product from many cruciferous vegetables (Yang *et al.* 1992), with demonstrated blocking activity (Morse *et al.* 1989), and naringenin, from grapefruit juice (Fuhr *et al.* 1993), also inhibit P450 2E1 and 1A2 respectively, and thus both have the potential to decrease phase I metabolism. It has been suggested that inhibition of P450 activity by phenethyl isothiocyanate occurs both through chemical inactivation and by a competitive mechanism (Smith *et al.* 1993). Again, it must be emphasized that the significance of results obtained *in vitro* or at very high doses *in vivo* remains to be properly assessed.

The mechanism of control of P450 1A1 transcription has been extensively studied (Saatcioglu *et al.* 1990; Fujii-Kuriyama *et al.* 1992; Gonzalez *et al.* 1993; Wu & Whitlock, 1993). The key protein is the Ah receptor, which binds to xenobiotics including dietary compounds, translocates into the nucleus, and then binds to a specific sequence of DNA (the xenobiotic responsive element) upstream of the P450 1A1 gene, which enhances transcription (Gonzalez *et al.* 1993). The increase in transcription is directly related to the specificity of the Ah receptor protein as shown for indole-3-carbinol, 3,3′-diindolylmethane (the main gastric conversion product of indole-3-carbinol) and dioxin (Jellinck *et al.* 1993).

Free radical mediated damage

Free radical mediated damage occurs as a consequence of normal cellular metabolism, and is exacerbated by poorly coupled redox reactions, including those mediated by cytochrome P450, by free iron released from storage proteins (Minotti *et al.* 1991; Winterbourn *et al.* 1991; Reif, 1992), by tissue injury (Powell & Tortollani, 1992), by reperfusion (Kilgore & Lucchesi, 1993), and by u.v. light (Godar *et al.* 1993). Free radical mediated damage is one of the mechanisms involved in initiation of carcinogenesis and such damage is reduced by antioxidants. Nutrient antioxidants such as vitamin E and carotenes play an important role in protecting the membrane and LDL (Bowry *et al.* 1992; Packer, 1991, 1992; Rousseau *et al.* 1992; Krinsky, 1993). The amount of vitamin E in membranes is apparently low: about 1 molecule per 1000–2000 phospholipids (Packer, 1992). Vitamin C is also usually considered to be an antioxidant, and may be involved in regeneration of vitamin E from tocopheryl radicals (Buettner, 1993). Vitamin E and C are chain breaking antioxidants since they react very poorly with oxygen, can be regenerated by enzymic systems, and their respective radicals are relatively harmless (Buettner, 1993). Oxidized vitamin C (dehydroascorbic acid) is converted back to vitamin C by protein disulphide-isomerase (*EC* 5 . 3 . 4 . 1) and thioltransferase, also called glutaredoxin (Wells *et al.* 1990),

by a reaction which results in oxidized glutathione. This provides an important metabolic link between vitamin C and glutathione, which plays a vital role in both detoxification (phase II) reactions (see later) and in protection against free radical mediated damage (Meister, 1991).

The properties of non-nutrient food borne antioxidants have been studied *in vitro* by several groups, but few have been proven to work *in vivo*. In assessing a potential biological antioxidant, several methods are necessary (Halliwell, 1990), and all compounds classed as antioxidants, including vitamins E and C, can be pro-oxidant under certain conditions (Halliwell, 1990; Maiorino *et al.* 1993; Mukai *et al.* 1993). There are many examples in the literature of non-nutrient antioxidants (for a review see Pratt, 1992). Some examples from a long list of candidate antioxidants are carnosol and carnosic acid from the herb rosemary (Aruoma *et al.* 1992), caffeine (Shi *et al.* 1991), cinnamic acids, especially ferulic, *p*-coumaric and caffeic acids which are found in many plant foods (Howie *et al.* 1990; Scott *et al.* 1993), the flavouring agent, vanillin (Liu & Mori, 1993), flavonoids, especially catechin, found in high concentration in tea, and quercetin, found in many plant foods (Morel *et al.* 1993; Scott *et al.* 1993), and diallyl polysulphides from 'aged' garlic extracts (Horie *et al.* 1992). Pro-oxidant activities of some non-nutrients have also been described. Examples include the flavour cinnamaldehyde (Raveendran *et al.* 1993) and carnosol/carnosic acid (Aruoma *et al.* 1992).

In addition to compounds which act as antioxidants by virtue of their redox chemistry, there are also endogenous antioxidant systems (Fig. 2). These include the enzymes glutathione peroxidase (*EC* 1 . 11 . 1 . 9; Ladenstein, 1984), the α form of glutathione transferase (GST, *EC* 2 . 5 . 1 . 18; Mannervik & Danielson, 1988), glutathione reductase (*EC* 1 . 6 . 4 . 2; Chow, 1988), superoxide dismutase (*EC* 1 . 15 . 1 . 1; Hirose *et al.* 1993), catalase (*EC* 1 . 11 . 1 . 6; Deisseroth & Dounce, 1970), phospholipid hydroperoxide glutathione peroxidase (Schuckelt *et al.* 1991), and (see Fig. 2) metal binding proteins such as ferritin and transferrin (Bomford & Munro, 1992; Testa *et al.* 1993). The relative importance of some of these enzymes has been estimated (Remacle *et al.* 1992). In addition, there are enzymes which remove the products of free radical mediated damage such as macro-oxyproteinase and other proteinase systems, which remove damaged proteins (Pacifici & Davies, 1990), DNA repair enzymes (see below), and the glutathione transferases and peroxidases which inactivate lipid hydroperoxides (Ladenstein, 1984; Ursini *et al.* 1985; Ursini & Bindoli, 1987; Mannervik & Danielson, 1988) and remove lipid breakdown products (Mannervik *et al.* 1985).

Diet exerts influence over many of these repair mechanisms (Fig. 2). For example the nutrient selenium is essential for glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase (Schrauzer, 1992) and copper, zinc and manganese are essential for synthesis of CuZn superoxide dismutase and Mn superoxide dismutase respectively (Donnelly & Robinson, 1991). The prosthetic group of glutathione reductase is a flavin, and so dietary riboflavin is essential for the active enzyme. Indole-3-carbinol decreases the levels of superoxide dismutase and glutathione peroxidase in rat liver (Shertzer & Sainsbury, 1991) but, with the exception of GST discussed below, generally little is known about the effect of non-nutrients on the expression of these enzymes.

Alternative route to phase I metabolism – quinone reductase (QR)

QR (*EC* 1 . 6 . 99 . 2) is an enzyme which catalyses the 2-electron reduction of many quinones, without a 1-electron reduced free radical intermediate (Lind *et al.* 1982), and consequently there is less likelihood of free radical mediated damage (Fig. 2). Theoretically QR is able to compete with phase I enzymes for substrates, but being mainly a cytosolic enzyme its activity toward hydrophobic substrates *in vivo* is probably limited. The main

isoenzyme of QR requires a flavin prosthetic group (Edwards *et al.* 1980), and so dietary riboflavin is essential. However, its expression is markedly influenced by many non-nutrient compounds.

A rapid and convenient assay for QR has been developed (Prochaska *et al.* 1985; Prochaska & Santamaria, 1988) and a range of inducers has been identified. These include quercetin, coumarin, α-angelicalactone (De Long *et al.* 1986), benzyl isothiocyanate (Talalay & Prochaska, 1987) and sulphoraphane (Zhang *et al.* 1992). Extracts from many vegetables, especially brassicas, also induce QR. This is dependent on processing (Kore *et al.* 1993; Tawfiq *et al.* 1994) because endogenous plant enzymes in the vegetables break down glucosinolates into more biologically active species such as isothiocyanates (Fenwick *et al.* 1989). Experiments on animals have also indicated a wide range of inducers of QR, such as phenethyl isothiocyanate (Guo *et al.* 1993), eugenol (Verhagen *et al.* 1993) and erucin (Zhang *et al.* 1992). Diet can intervene at more than one level in the induction process, including the stabilization of protein or mRNA, but it is most effective at the transcription stage. Certain inducers of QR, including aromatic isothiocyanates and flavonoids, are known to possess blocking activity (Wattenberg, 1993).

QR is often considered as a phase II enzyme, and a unifying theory for induction of phase I and phase II enzymes has been presented (Prochaska *et al.* 1985), postulating that inducers fall into two classes – bifunctional (induce phase I and II enzymes) and monofunctional (induce phase II only). The former include compounds such as benzo(*a*)pyrene and aflatoxin (Talalay & Prochaska, 1987). The latter include diphenols such as hydroquinone and isothiocyanates (Talalay & Prochaska, 1987). Bifunctional inducers require activation by phase I enzymes to redox labile molecules before they are able to induce phase II enzymes. This hypothesis suggests that the redox signal is the most important for induction of QR, but does not satisfactorily explain induction by compounds such as isothiocyanates (Daniel, 1993).

The control of QR by redox signals is *via* a sequence of DNA in the 5′ flanking region of the gene, which is called the ARE (antioxidant responsive element; Rushmore *et al.* 1991). Depending on the species, these responsive elements may or may not contain within them an element responsive to phorbol-12-*O*-tetradecanoate-13-acetate (Li & Jaiswal, 1992; Xanthoudakis *et al.* 1992; Daniel, 1993; Rushmore & Pickett, 1993) which binds to the transcription factors *fos* and *jun*. These are regulated *via* a protein called *ref*-1, which controls the redox state of sulphydryl residues, essential for DNA binding, on *fos* and *jun* (Abate *et al.* 1990; Xanthoudakis *et al.* 1992). The sulphydryl–disulphide redox state of the cell is communicated *via* thioredoxin. This implies that a wide range of antioxidants will influence expression of QR *via* thioredoxin, and indeed butylated hydroxyanisole, butylated hydroxytoluene, and ethoxyquin do affect expression (De Long *et al.* 1986; Derbel *et al.* 1993). However, there is a growing body of evidence that transcription factors other than *fos* and *jun* are involved in controlling expression (Rushmore & Pickett, 1993). Nguyen & Pickett (1992) have identified two transcription factors of 28000 and 45000 Daltons, that are constitutive and bind to the ARE sequence. In identifying the ARE, Pickett's group showed that this element responded to redox cycling phenolics, such as hydroquinone and catechol, but not to resorcinol which cannot redox cycle (Rushmore *et al.* 1991). By implication, redox cycling phenolics in the diet may affect binding to the ARE. It is clear that control of expression *via* the ARE is a subject for future research. Indeed, a variety of chemicals with a large range of structures is able to induce QR, and this property may correlate with their reactivity as Michael acceptors (Riley & Workman, 1992).

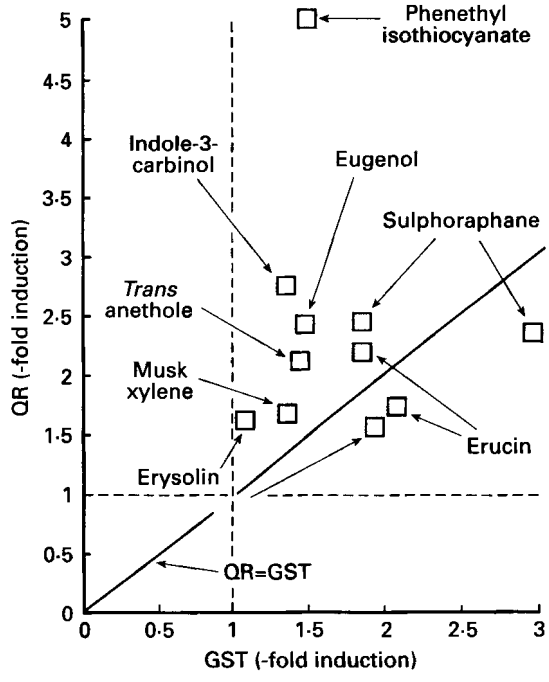

Fig. 3. Co-ordinate induction of GST and QR in animals? Induction (-fold) of GST and QR (equal induction represented by ——) is compared for several dietary compounds using animal models: sulphoraphane, erucin and erysolin in mouse liver and stomach (Zhang *et al.* 1992); phenethyl isothiocyanate in rat liver (Guo *et al.* 1993); indole-3-carbinol in rat liver (Wortelboer *et al.* 1992); eugenol and transanethole in rat liver (Verhagen *et al.* 1993); musk xylene in rat liver (Iwata *et al.* 1993).

CONJUGATION BY PHASE II ENZYMES

Conjugation results in a xenobiotic which is more hydrophilic, and can therefore be transported more readily out of the cell. Metabolites from the action of monooxygenase or QR reduction are conjugated with glutathione by GST, with UDP-glucuronic acid by UDPglucuronosyltransferase (UDPGT, 2.4.1.17; Mannervik, 1985; Brierley & Burchell, 1993), with 3′-phosphoadenosine-5′-phosphosulphate by sulphotransferases (*EC* 2.8.2.x) or by amino acid transferases (commonly with glycine). The route of conjugation depends on the chemical nature of the compound and varies from one species to another. GST and UDPGT are the most studied systems. GST requires no nutrients as prosthetic groups, but requires glutathione as a cofactor: synthesis of glutathione requires dietary cysteine (Meister, 1991). There are a large number of isoforms of GST in humans, and all GST can be classified as α, μ, π, θ or microsomal (Black & Coon, 1987; Mannervik & Danielson, 1988; Black & Wolf, 1991). The isoforms have individual but overlapping specificity profiles for a wide range of electrophilic carcinogens. Only GST α is able to reduce organic hydroperoxides (but not H_2O_2) and so contributes to reducing free radical mediated damage (Mannervik & Danielson, 1988). Most of the work on control of expression of GST has been performed on animal models, and many compounds have been tested for their ability to induce GST in rats (Nijhoff *et al.* 1993 and Fig. 3). In one study, quercetin, flavone, ferulic acid, ellagic acid, coumarin, α-angelicalactone and curcumin all induced GST α in rat liver, although in contrast to other studies (Bradfield *et al.* 1985) large doses of Brussels sprouts (20% of diet) did not. All of the above, except for quercetin and curcumin, also increased liver glutathione levels, as did Brussels sprouts (Nijhoff *et al.*

1993). Although the genes have been sequenced (Klone *et al.* 1992; Rozen *et al.* 1992), control of GST α in humans is poorly understood. The rat and mouse α classes are controlled *via* the ARE/EpRE/TRE mechanism (EpRE, electrophile responsive element; TRE, phorbol-12-*O*-tetradecanoate 13-acetate responsive element) as described above for QR (Friling *et al.* 1992; Nguyen & Pickett, 1992; Daniel, 1993), and there is some evidence for coordinate induction of GST and QR in these species (Talalay *et al.* 1988). Although many compounds which are inducers of QR are also inducers of GST, Fig. 3 shows that the induction is not quantitatively coordinated for a range of non-nutrient compounds. It will be very interesting to see if regulation of QR and GST are coordinately controlled in humans. Compounds with the ability to induce both QR and GST in humans would presumably exhibit potent activities as blocking agents, especially if this were combined with antioxidant properties.

UDPGT also exists as a family of enzymes (Brierley & Burchell, 1993), again with different but overlapping specificities. The induction of UDPGT by drugs and other xenobiotics has been relatively well studied (Sutherland *et al.* 1993), and compounds such as dipyridyls induce UDPGT as well as GST, but not sulphotransferase in rats (Franklin, 1991). Dietary lipid affects the UDPGT activity. In a study in which rats were fed a diet supplemented with fish oil there was a 3-fold increase in UDPGT after 4 weeks compared to a control group given a fat-free diet (Dannenberg & Yang, 1992). Cytochrome P450 1A2 and 2E1 are also induced by fish oils (Yoo *et al.* 1990), and regulation of expression of UDPGT, like P450 1A1/2, is influenced by the Ah receptor (Brierley & Burchell, 1993). Expression is also influenced by the peroxisome proliferator receptor (Brierley & Burchell, 1993). The effect of non-nutrients on UDPGT expression has been poorly studied in humans. Subjects fed a diet high in cabbage and Brussels sprouts showed a modest increase in UDPGT (Miners & Mackenzie, 1991). Given the wide range of drugs that induce UDPGT, it will be of interest to determine which, if any, non-nutrients affect the expression of UDPGT in humans.

In the final phase of detoxification (Phase III) glutathione conjugates are actively removed from the cell by the glutathione-S-conjugate export pump, which is dependent on ATP (Ishikawa, 1992). Some carcinogens are removed by P-glycoprotein, without the need for conjugation and this reaction also requires ATP (Ishikawa, 1992). We are unaware of any non-nutrient food constituents which influence this process.

INDIRECT EFFECTS *VIA* ENTERIC BACTERIAL METABOLISM

Mallett & Rowland (1990) have pointed out that bacteria present in the gut lumen express enzymes which may influence the metabolism of xenobiotics in host tissues. For instance, when potentially carcinogenic compounds are detoxified by UDPGT (Dutton, 1980), the conjugates are secreted *via* the bile into the gut lumen. The activity of bacterial β-D-glucuronidase (*EC* 3.2.1.31; Cole *et al.* 1985; Gadelle *et al.* 1985) may result in the hydrolysis of the conjugates, and their consequent reabsorption into the circulation in their active, deconjugated form (Rowland *et al.* 1985). β-Glucuronidase activity has been implicated in the activation of benzo(*a*)pyrene and 1,2-dimethylhydrazine conjugates in the colon (Nanno *et al.* 1986; Mallett & Rowland, 1990). It has been demonstrated that the yield of colorectal tumours is lower in germ free rats exposed to 1,2-dimethylhydrazine than in similarly treated conventional rats (Reddy *et al.* 1974) and that a β-glucuronidase inhibitor can protect rats with a conventional flora from the induction of tumours by azoxymethane, a metabolite of 1,2-dimethylhydrazine (Takada *et al.* 1982).

Gut bacteria also express β-glycosidase activity, one of the effects of which is the liberation of biologically active flavonoid aglycones from their glycoside conjugates

(Brown, 1988). It has been shown that the presence of the dietary flavonoid rutin (itself a substrate for β-glycosidase) in the diet of rats leads to a 20-fold increase in the level of β-glycosidase activity in the caecum and that this is associated with changes in the ability of hepatic microsomes obtained from those rats to activate bacterial promutagens *in vitro* (Mallett *et al.* 1989). Modulation of β-glucuronidase and β-glycosidase activities in enteric bacteria remains a hypothetical blocking mechanism and in theory would remove the requirement that a compound taken orally would need to traverse the gut wall and enter the circulation in an active form in order to modulate the expression of xenobiotic metabolizing enzymes.

INDUCERS OF DNA REPAIR

Wattenberg does not include agents that modify DNA repair in his classification scheme but Morse & Stoner (1993) classified such compounds as blocking agents. Clearly they cannot be described as suppressors for they exert their influence prior to promotion. However, they do not share the common feature of all other blocking agents, which act to prevent the occurrence of DNA lesions rather than to reduce the deleterious effects of such lesions. Furthermore, while DNA repair may be involved in the conversion of various forms of DNA damage into mutagenic lesions, and thus could be said to be involved in initiation, certain lesions such as O^6-alkylguanine adducts are thought to be capable of inducing mutations without further modification. Thus their repair could be considered a postinitiatory event. Given these complicating factors, we propose that modifiers of DNA repair should be included as a category in their own right, acting after blocking agents, as strictly defined, but prior to suppressing agents.

Much of the evidence for the existence of naturally occurring inducers of DNA repair has been obtained using prokaryotic cells. For example it has been reported that the natural flavouring vanillin can inhibit the induction of mutations in *Escherichia coli* by the promotion of *RecA* dependent recombinational repair (Ohta *et al.* 1986, 1988). Similar antimutagenic properties have been reported for the flavouring agents cinnamaldehyde and coumarin (Ohta *et al.* 1983*a*, *b*) while Shimoi *et al.* (1985) have reported that tannic acid, which is found in tea, protects *E. coli* from the mutagenic effects of u.v. light. The fact that protection was only conferred by tannin components (gallic acid, (-)-epicatechin gallate, (-)-epigallocatechin and (-)-epigallocatechin gallate) to uvr^+ but not $uvrA^-$ strains led them to propose that the antimutagenic activity was due to stimulation of *uvrA* dependent excision repair (Shimoi *et al.* 1986).

The relevance of these findings to human nutrition may be questioned, given that prokaryotic and eukaryotic DNA repair processes are known to differ markedly in their sensitivity to modifying agents such as caffeine. However, vanillin, anisaldehyde, cinnamaldehyde and coumarin have been shown to protect CHO cells from the clastogenic and mutagenic effects of u.v. light and X-rays *in vitro* (Sasaki *et al.* 1990*a*). Vanillin has also been shown to protect CHO cells from the clastogenic effects of mitomycin C (Sasaki *et al.* 1987) and from the mutagenic effects of u.v. and X-rays (Imanishi *et al.* 1990), and has been shown to have protective activity against the induction in mice of micronuclei by mitomycin C or by X-rays (Inouye *et al.* 1988; Sasaki *et al.* 1990*b*) and of mutations by ethylnitrosourea (Imanishi *et al.* 1990). It has been proposed that vanillin promotes DNA repair *via* DNA nucleotidyltransferase β (*EC* 2.7.7.7; Sasaki *et al.* 1990*a*). The complexity of this field is illustrated by the work of Imanishi *et al.* (1991) who showed that components of tea tannin can inhibit mammalian DNA repair when unmodified, but that metabolites resulting from pretreatment with a metabolically active hepatic microsome extract (S9) can actually promote excision repair activity in the same test systems.

SUPPRESSING AGENTS

The concept of a suppressing agent was introduced by Wattenberg to account for the ability of compounds such as sodium cyanate, tert-butylisocyanate and benzyl isothiocyanate to inhibit the appearance of tumours, even when fed considerably after the chemical treatment which initiated the neoplasia (Wattenberg, 1981). Benzyl isothiocyanate is found in cruciferous vegetables, and Wattenberg's group went on to feed brassicas to rats previously treated with 7,12-dimethylbenz(*a*)anthracene in order to test their ability to inhibit the emergence of mammary tumours. Cabbage or broccoli was fed to rats for an 18 week period, starting 1 week after cessation of treatment with the carcinogen. For both vegetables the numbers of animals with mammary tumours in the treatment group were 40%–50% lower than in the control groups and the average number of tumours per rat was about 60% lower (Wattenberg *et al.* 1989*a*, *b*). Similar observations were made for other foods including green coffee beans, green Brazilian cocoa beans and orange oil (Wattenberg, 1983). Recently many more naturally occurring suppressing agents have been identified (Wattenberg, 1993).

Any logical classification of suppressing agents must be based upon the identification of their mechanisms of action, but in most cases this is unknown. Even for the aromatic isothiocyanates, first identified as suppressing agents by Wattenberg, the mechanism of action remains uncertain and is probably multifactorial (Wattenberg, 1993). For example, Sugie *et al.* (1993) have reported that oral administration of benzyl isothiocyanate and benzyl thiocyanate to male F344 rats resulted in the suppression of proliferative activity in hepatocyte primary cultures derived from them. However Musk & Johnson (1993*b*) have shown that aromatic isothiocyanates are selectively toxic against transformed colorectal tumour cells. Thus the same compounds may exert quite different effects at different stages of carcinogenesis. The difficulty of achieving a satisfactory classification of suppressing agents reflects our current incomplete understanding of tumour biology. By definition, suppressing agents act by inhibiting tumour promotion. In Fig. 1 we have used a simple classification which reflects the possible mechanisms whereby compounds may inhibit the progression of cells initiated by genotoxic damage.

INHIBITORS OF CELL PROLIFERATION

Cellular hyperproliferation is now recognized as a potentially important mechanism of carcinogenesis (Preston-Martin *et al.* 1990). Cell populations undergoing a high rate of division are more susceptible to DNA damage, both by endogenous oxidative mutagenesis and by exogenous chemical mutagens, than quiescent cells (Tong *et al.* 1980). Single stranded DNA is intrinsically more vulnerable to damage than double stranded, and the time available for DNA repair is reduced in rapidly proliferating cells (Ames & Gold, 1991). Furthermore, rapid proliferation is thought to favour somatic recombination, allowing cells which are heterozygous for a mutation favouring transformation to become homozygous and give rise to neoplastic progeny (Groden *et al.* 1990). Somatic recombination may be an important aspect of carcinogenesis during both initiation and promotion. Environmental factors which can inhibit proliferation could therefore be classified as either blocking or suppressing agents, depending upon the tissue concerned, and the particular mechanism of carcinogenesis which is being inhibited. This illustrates once more the difficulty of fitting the growing list of food borne protective factors into any rigid system of classification. Those cellular control mechanisms that have been identified as potentially susceptible to modification by food borne protective factors will now be

described briefly and examples will be given. In view of the incomplete understanding of promotion, the relationships between the mechanisms remain undefined and, in the case of multifunctional agents, their relative importance cannot yet be assessed.

Modification of intracellular signalling

The proliferation of normal cells is regulated by a complex system of membrane receptors and intracellular signal pathways, some of which may be susceptible to modulation by plant cell constituents derived from food. Receptor mediated hydrolysis of membrane bound phosphatidylinositol-4,5-biphosphate yields two intracellular second messengers, inositol 1,4,5-triphosphate and 1,2-diacylglycerol. This sequence of events forms part of a signal transduction pathway which is involved in the regulation of cellular proliferation in a range of mammalian tissues (Berridge & Irvine, 1984). Under normal circumstances, release of 1,2-diacylglycerol leads to activation of protein kinase C (PKC, *EC* 2 . 7 . 1 . 37), a step which is known to stimulate cell proliferation by mechanisms which include activation of ornithine decarboxylase (ODC), while inositol 1,4,5-triphosphate mobilizes intracellular calcium.

Inositol hexaphosphate, usually referred to as phytate, is widely distributed in plant foods and is present at particularly high levels in cereals and legume seeds. It has been proposed that the supposed anticarcinogenic properties of diets rich in cereals may be due to the presence of phytate rather than dietary fibre, and evidence for a protective effect of isolated inositol hexaphosphate has been obtained in animal experiments (Shamsuddin & Sakamoto, 1992). When the compound was administered orally to rats 2 weeks or 5 weeks after treatment with the chemical carcinogen azoxymethane, a significant reduction was observed in the number of animals with tumours and the number and size of tumours in the affected animals (Shamsuddin & Ullah, 1989). This is strong evidence to suggest that exogenous inositol hexaphosphate can function as a suppressing agent, perhaps by entering and modifying the inositol phosphate metabolism pathways of the cell. Further work will be needed to clarify the mechanisms involved and assess the significance in relation to the human diet.

The direct inhibition of PKC might also provide a mechanism for suppression as stimulation of PKC leads to the activation of proteins that affect cellular proliferation (Blumberg, 1988). Glycyrrhetic acid is a breakdown product of glycyrrhizin, a sweet constituent of licorice. This compound has been demonstrated both to inhibit PKC (O'Brian *et al.* 1990) and to possess antitumorigenic activity, preventing the inflammatory and toxic effects of 12-*O*-tetradecanoylphorbol-13-acetate (Wang *et al.* 1991; Nishino, 1992). PKC may also be involved in the control of intercellular communication in that tumour promoters which have been shown to activate PKC have also been demonstrated to inhibit the transfer of fluorescent dye between adjacent cells in culture (Enomoto & Yamasaki, 1985). It has been proposed that compounds that inhibit such communication may function as tumour promoters by interfering with cell–cell growth regulatory mechanisms and thereby encouraging cellular replication (Trosko & Chang, 1984). Epidemiological evidence suggests that green tea may be protective against cancer (Oguni *et al.* 1989). An extract of green tea consisting largely of various catechins (Maeda & Nakagawa, 1977) previously shown to possess antimutagenic and anticarcinogenic properties (Cheng *et al.* 1986; Ruch *et al.* 1989) apparently prevents the inhibition of intercellular communication induced in cultured mammalian cells by the promoters phenobarbital and 12-*O*-tetradecanoylphorbol-13-acetate (Ruch *et al.* 1989).

Proteinase inhibitors have been reported to suppress tumour promotion in rodents at a variety of sites including skin, colon, breast and liver (Yavelow *et al.* 1983; Troll *et al.* 1984, 1992; Billings *et al.* 1990). They inhibit the transformation of mammalian cells *in vitro* by

radiation and by oncogenes (Kennedy & Little, 1981; Yavelow *et al.* 1983, 1985; Garte *et al.* 1987) and inhibit the production of oxygen radicals by tumour promoters (Frenkel *et al.* 1987; Troll *et al.* 1987). Troll *et al.* (1992) have proposed that proteinase inhibitors might exert their antitumorigenic effect by inhibiting the proteolytic modification of PKC which is necessary for its activation as a tumour promoter (Murray *et al.* 1987). Many experiments using proteinase inhibitors have involved the topical application of inhibitors to skin. However, it has been demonstrated that inclusion of a soyabean extract containing the Bowman–Birk proteinase inhibitor in the diet can protect mice against the induction of lung tumours by 3-methylcholanthrene (Witschi & Kennedy, 1989) and against induction of colonic adenomas by 1,2-dimethylhydrazine (Weed *et al.* 1985). Furthermore, epidemiological evidence suggests that diets containing high levels of foods rich in proteinase inhibitors are protective against cancers of the breast, colon and prostate (Correa, 1981). It has been proposed by Schelp & Pongpaew (1988) that chemoprevention may be possible by a nutritionally induced increase in the production of endogenous inhibitors.

Inhibition of oncogene expression

The progressive loss of proliferative control which characterizes cellular transformation is accompanied by an accumulation of acquired genetic defects involving proto-oncogenes, many of which code for proteins involved in the regulation of cell proliferation. One mechanism by which dietary agents might suppress promotion is by inhibiting the post-translational modification of oncoproteins. For example the *ras* family of proto-oncogenes code for a group of small guanosine 5′-triphosphatases, including $p21^{ras}$, which regulate various aspects of cellular growth and differentiation. *Ras* mutations are associated with many types of tumour. To exert its transforming properties the mutated $p21^{ras}$ must be translocated to the plasma membrane, and for this to occur it must undergo farnesylation (Casey *et al.* 1989; Kato *et al.* 1992). Limonene is a monoterpene component of orange peel oil which inhibits the development of mammary carcinomas at the promotional stage of induction by the carcinogen 7,12-dimethylbenz(*a*)anthracene in the rat (Elegbede *et al.* 1986) and has also demonstrated antitumorigenic properties in a murine model (Wattenberg *et al.* 1989*b*). It has been proposed that the antitumorigenic properties of *d*-limonene reflect its ability to reduce the farnesylation of $p21^{ras}$ (Crowell *et al.* 1991). Although exposure to other monoterpenes has been shown to bring about a decrease in expression of an enzyme involved in the synthesis of farnesyl moieties (3-hydroxy-3-methylglutaryl coenzyme A reductase; *EC* 1.1.1.88) in the liver of rats (Clegg *et al.* 1982), this reduction does not seem to play a role in influencing the farnesylation of $p21^{ras}$ (Crowell *et al.* 1991). This mechanism may be relevant to other aspects of cellular control mediated by isoprenylated proteins. It is interesting to note that limonene is one example of a substance which, though it is capable of suppressing tumorigenesis in certain models, can also be shown to be carcinogenic in other circumstances. In this instance, the induction of kidney tumours in the rat by limonene appears to be species and sex specific (Dietrich & Swenberg, 1991).

Polyamine metabolism

The intracellular level of polyamines plays a central role in the control of proliferation (Williams-Ashman & Canellakis, 1979; Pegg, 1988). Tumour promoters such as 12-*O*-tetradecanoylphorbol-13-acetate have been found to increase the activity of ODC, which catalyses the formation of the polyamine precursor putrescine from ornithine (McCann *et al.* 1992), and so increases the concentration of polyamines in affected tissues (Slaga, 1983). It has been demonstrated that an increase in ODC activity and polyamine levels is an

absolute requirement for the proliferation of a variety of human tumour cells both *in vitro* and *in vivo* (Luk, 1992), and inhibitors of ODC have been shown to deplete polyamine levels and block proliferation in cultured cells (Mamont *et al.* 1976) and in tumours *in vivo* (Pegg, 1988). It has been proposed that the ability of the flavonoid apigenin to suppress skin tumorigenesis in mice, and of curcumin, a constituent of turmeric, to suppress early markers of tumorigenesis in the rat colon, may be related to reduced ODC levels in the target tissues (Wei *et al.* 1990; Rao *et al.* 1993). Other flavonoids that have been reported to inhibit promoter induced increases in ODC include kaempferol, luteolin, morin and fisetin (Nakadate *et al.* 1984; Fujiki *et al.* 1986).

Inhibition of arachidonic acid metabolism is closely linked to the direct inhibition of ODC. Increased metabolism of arachidonate is commonly seen in experimental models of tumour promotion (Earnest *et al.* 1992). Both the cyclo-oxygenase and the lipoxygenase (*EC* 1.13.11.12) pathways of arachidonic acid metabolism (Moncada *et al.* 1980) are implicated in this promoting effect, which may operate *via* the up-regulation of ODC activity (Yamamoto & Kato, 1992). Blocking the metabolism of arachidonate has been shown to inhibit the growth of human cells both *in vitro* and *in vivo* (Levine *et al.* 1972; Hial *et al.* 1977; Bayer *et al.* 1979; Sato *et al.* 1983; Goodlad *et al.* 1989). Curcumin, an inhibitor of promotion induced by croton oil and 12-*O*-tetradecanoylphorbol-13-acetate in the murine skin model (Huang *et al.* 1988; Soudamini & Kuttan, 1989), has been shown to inhibit the activities of cyclo-oxygenases and lipoxygenases (Huang *et al.* 1991; Rao *et al.* 1993). The ability of the flavonoids quercetin, fisetin, kaempferol and morin to suppress promotion (Kato *et al.* 1983; Nakadate *et al.* 1984) may correlate with their ability to inhibit lipoxygenase (Nakadate *et al.* 1984).

Oestrogen metabolism

Certain tumour types are responsive to oestrogens and this presents another mechanism by which promotion could theoretically be inhibited. 16β-Hydroxyoestrone functions as a promoter of mammary cell transformation (Telang *et al.* 1992) and enhances the expression of oncogenes in human cancer cells (Hsu *et al.* 1991), whilst 2-hydroxyoestrone is anti-oestrogenic in cell culture (Schneider *et al.* 1984). Thus the induction of enzymes that increase the 2-hydroxylation of oestrogens, relative to their 16β-hydroxylation, may be anticarcinogenic (Jellinck *et al.* 1993). Such induction has been reported in mouse and rat liver following feeding with indole-3-carbinol, which, as we have seen, is derived from cruciferous vegetables (Baldwin & LeBlanc, 1992; Jellinck *et al.* 1993). In human volunteers the rate of 2-hydroxylation of oestradiol in an *in vivo* radiometric assay was increased by 50% after daily exposure to 6–7 mg indole-3-carbinol/kg for 7 d and the urinary excretion of 2-hydroxyoestrone was increased relative to that of oestriol (Michnovicz & Bradlow, 1990, 1991). Indole-3-carbinol forms condensation products under acid conditions; the ability of these products to induce similar effects to indole-3-carbinol and the inactivity of the parent compound when administered intraperitoneally has led to the suggestion that the condensation products formed in the acid milieu of the stomach might be the active moieties in this induction (Bradfield & Bjeldanes, 1991).

Caffeine has been shown to reduce the number of mammary tumours induced in mice by a combination of 17β-oestradiol and progesterone (VanderPloeg & Welsch, 1991). In other studies however, caffeine has been shown to enhance the development of mammary tumours in mice, spontaneous or induced by 7,12-dimethylbenz(*a*)anthracene (Nagasawa & Konishi, 1988; Welsch *et al.* 1988), and to enhance the development of pancreatic tumours in hamsters exposed to *N*-nitroso-bis-(2-oxopropyl)amine at a postinitiation stage (Nishikawa *et al.* 1992). The mechanism(s) by which caffeine exerts its effects remain a matter for conjecture, though Alldrick & Rowland (1988) have demonstrated that it can

inhibit the ability of mouse hepatic microsomes to metabolize 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, Trp-P-2 and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline to bacterial mutagens and Welsch *et al.* (1988) have observed an enhancing effect of caffeine on the responsiveness of murine mammary organ cultures to mammotrophic growth hormone.

Human blood, faeces and urine commonly contain varying quantities of oestrogenic compounds derived from precursors found in plant foods. Lignans are compounds with a dibenzylbutane structure that were first identified as secondary metabolites of plants. The two most important mammalian lignans, enterodiol and enterolactone, are derived from lignans of plant origin by the activity of bacteria in the alimentary tract (Axelson *et al.* 1982). Isoflavonoids are a group of plant diphenols, of which many have oestrogenic activity. They occur extensively in plants used as human foods, and many of them have been detected in human and animal urine. These compounds include equol, methylequol, daidzen, dihydrodaidzen, 3′,7-dihydroxyisoflavan and others (Adlercreutz, 1990).

The mammalian lignans and the isoflavonoids exhibit weak oestrogen-like activity, together with a variety of other physiological activities including antiproliferative effects and cytotoxicity (Setchell & Adlercreutz, 1988). Many of these effects are apparently mediated *via* the interaction of phyto-oestrogens with mammalian oestrogen receptors. For example lignans and isoflavonoids compete with endogenous oestrogens for type II oestrogen binding sites and may thereby regulate the growth of oestrogen dependent tissues (Markaverich *et al.* 1988). A second important effect of these compounds is the stimulation of endogenous production of sex hormone binding globulin (SHBG) which modulates the biological activity of oestrogen in humans. Adlercreutz and co-workers have observed that enterolactone causes a dose dependent stimulation of SHBG synthesis in HepG2 cells in culture. A positive relationship between plasma SHBG levels and urinary lignan and isoflavonoid excretion in humans has also been observed, and ascribed to stimulation of hepatic SHBG synthesis by dietary phyto-oestrogens (Adlercreutz *et al.* 1993).

Epidemiological evidence can be interpreted in favour of a protective role for the phyto-oestrogens against sex hormone dependent tumours, principally carcinoma of the breast and prostate cancer. Urinary excretion of these compounds is reported to be higher in vegetarians than in omnivores, and lower in breast cancer patients. Japanese women consuming a traditional diet have both a low incidence of breast cancer and a relatively low mortality from this disease (Nomura *et al.* 1978), and Japanese men experience relatively low mortality from prostate cancer, although the incidence of this common but often slow growing tumour is apparently similar to that of western populations. Adlercreutz *et al.* (1991) proposed that the slow growth of both breast and prostate cancers in Japanese patients consuming traditional diets may be due to inhibitory effects of isoflavanoids derived primarily from soya products. This proposal is supported by the observation that a high intake of soya products is associated with reduced risk of breast cancer amongst women in Singapore (Lee *et al.* 1991), but there is little direct experimental evidence for the putative mechanism.

DIRECT ACTING MODULATORS OF CELL DAMAGE

Suppression of free radical production

Oxidative damage by free radicals appears to be important at the promotion stage as well as the initiation stages of carcinogenesis discussed earlier. Many food borne compounds that can inhibit the generation of oxidative damage may exert an antipromotional effect. For example epigallocatechin gallate, which is found in teas, has been shown to reduce both

the induction of lung tumours, and the formation of 8-hydroxydeoxyguanosine (a marker for oxidative DNA damage) in mouse lung tissue exposed to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, without having any effect on the initial level of alkylation in the DNA (Xu *et al.* 1992). It has also shown antipromoter activity in skin, duodenum and liver (Fujiki *et al.* 1992). The catechin-rich extract of green tea referred to above as an inhibitor of intercellular communication has also been shown to protect cultured cells against cytotoxicity induced by oxygen radicals (Ruch *et al.* 1989).

Selective cytotoxins

Chemotherapy and radiotherapy involve exposure to high levels of cytotoxic agents with the aim of inhibiting the growth of tumour cells and ultimately of killing them. Ideally therapeutic agents would be, if not absolutely specific for tumour cells, then at least highly selective in their mode of action. It is not conceivable that the human diet normally contains biologically significant concentrations of cytotoxic agents of a similar non-specificity to commonly used therapeutic agents but many secondary plant metabolites are cytotoxic, and some of these compounds may selectively target tumours or precancerous cells.

Evidence for selective toxicity against transformed cells has been presented for polyunsaturated fatty acids (Bégin *et al.* 1986), and also for the compounds 1-cyano-2-hydroxy-3-butene, which is found in cruciferous vegetables (Wallig *et al.* 1993) and quercetin (Larocca *et al.* 1991), the latter having been shown to be active *in vivo* (Castillo *et al.* 1989). We have recently shown that the dietary compound allyl isothiocyanate, again derived from cruciferous vegetables and a major constituent of mustard, is more toxic towards transformed HT29 human colorectal adenocarcinoma cells than towards cells which have been experimentally detransformed *in vitro* (Musk & Johnson, 1993*a*). A similar selective effect of quercetin has also been observed (S. R. R. Musk & I. T. Johnson, unpublished observations). It will clearly be of interest to determine whether other dietary compounds with demonstrated cytotoxic activity (Mori *et al.* 1988; Babich *et al.* 1993) might also be selective and if so, whether such compounds might have a practical role to play as food borne protective factors in chemoprevention, or as chemotherapeutic agents.

INDUCERS OF CELLULAR DIFFERENTIATION

Tumour promoters are known to influence cell differentiation, either by inhibiting normal differentiation or by inducing inappropriate differentiation programmes (Yamasaki, 1984). The second class of suppressing agent comprises compounds which act by modulating the differentiation of tumorous or pretumorous cells, thereby slowing or even halting their growth. Retinoids, calcium and vitamin D have been shown to modify cell differentiation both *in vitro* and *in vivo* (Lotan 1992*a*; Moon *et al.* 1992), and to exert antipromotive effects in animal models of tumorigenesis (Lotan, 1980; Dawson & Okamura, 1990) although it has also been proposed that these compounds may act by modifying the expression of ODC (Dawson *et al.* 1987) or by enhancing intercellular communication (Hossain *et al.* 1989). Certain dietary carotenoids such as astaxanthin, canthaxanthin and fucoxanthin, which do not demonstrate provitamin A activity in mammals, are known to exhibit anticarcinogenic properties in animal models (Mathews-Roth, 1982; Grubbs *et al.* 1991; Okuzumi *et al.* 1993; Tanaka *et al.* 1994). An effect on differentiation remains a theoretical mode of action for these compounds. Fucoxanthin suppressed the growth of, and expression of N-*myc* in, tumour cells *in vitro* (Okuzumi *et al.* 1990) and canthaxanthin inhibited the growth of tumour cells and the transformation of 10T1/2 cells (Pung *et al.* 1988; Huang *et al.* 1992).

ANTIMETASTATIC AGENTS

The final stages of epithelial tumorigenesis involve invasion of the basal lamina by fully transformed cells and migration, *via* the blood or lymph systems, to new tissue sites where secondary tumours can develop. Bracke *et al.* (1989) have reported that the invasive behaviour of MO_4 cells (fetal mouse cells transformed with Kirsten murine sarcoma virus) towards embryonic chick heart fragments *in vitro* can be greatly reduced by exposure of the cultures to the flavonoid tangeretin. Inhibition was noticeable at a concentration of 0·01 mM and was completely reversible after removal of the tangeretin. Similar observations have been reported for another flavonoid, (+)-catechin (Bracke *et al.* 1984, 1987) and for retinoids (Lotan, 1992*b*). Such an effect might contribute to the inhibitory action of dietary cabbage on the yield of pulmonary metastases in mice injected with BALB/c tumour cells (Scholar *et al.* 1989). Thus dietary components may modify the carcinogenic process even after the completion of promotion.

IMMUNOMODULATION

Certain compounds that possess antitumorigenic properties have also been shown to enhance the immune response. Examples include β-carotene and the carotenoids canthaxanthin and astaxanthin (Bendich & Shapiro, 1986; Jyonouchi *et al.* 1991, 1993). It has been proposed that the antitumorigenic action of these compounds may be causally linked to their immunoenhancing properties (Shklar & Schwartz, 1988; Bendich, 1989). Given that high levels of prostaglandins E_1, E_2, A_1 and A_2 have been shown to suppress the killing of tumour cells by natural killer cells and that inhibitors of prostaglandin synthesis can restore natural killer functions to mice with experimentally depressed activity (Brunda *et al.* 1980; Taffet & Russell, 1981), it is also possible that inhibitors of arachidonic acid metabolism might exert an antitumour effect *via* this mechanism (Goodwin, 1984).

A NEW CLASS OF NUTRIENTS?

Clearly, a variety of biologically active constituents other than those conventionally recognized as nutrients may contribute to the protective effects of diets rich in fruits and vegetables against cancer. There is still much to be done to clarify the mechanisms by which plant constituents act, and there is an urgent need to assess their relative importance so that the practical implications for human diets can be properly assessed. Although an unprecedented variety of plant foods is now available to modern western consumers, the bulk of fruit and vegetable consumption involves relatively few species and varieties. Moreover the varieties most readily available tend to be determined by commercial factors related to agricultural production and distribution. It is inevitable that the increasing application of molecular biology will lead to the manipulation of food crops to improve pest resistance, yield, keeping qualities and flavour. The implications for health of such trends can only be assessed if the ensuing compositional changes and their biological significance are properly understood.

One obvious practical issue which must be addressed is the balance of risk and benefit conferred by the consumption of compounds which in many cases express more than one type of potent biological activity. Many of the substances that we have described as potential anticarcinogens have also been shown to possess potentially hazardous properties. For example, indole-3-carbinol has been shown to promote carcinogenesis in certain models (Pence *et al.* 1986; Bailey *et al.* 1987) and its toxicology will need to be thoroughly

Table 1. *Examples of dietary anticarcinogens with genotoxic or tumorigenic effects*

Compound	Assay	Reference
Allyl isothiocyanate	Positive in Ames test	Yamaguchi, 1980
Allyl isothiocyanate	Induction of aberrations in mammalian cells	Kasamaki *et al.* 1982
Allyl isothiocyanate	Induction of bladder tumours by feeding	Dunnick *et al.* 1982
Allyl isothiocyanate	Transformation of mammalian cells	Kasamaki *et al.* 1987
Anisaldehyde	Induction of aberrations in mammalian cells	Kasamaki *et al.* 1982
Benzyl isothiocyanate	Positive in Ames test	Yamaguchi, 1980
Benzyl isothiocyanate	Induction of aberrations in mammalian cells	Musk & Johnson, 1993*b*
Benzyl isothiocyanate	Induction of SCE in mammalian cells	Musk & Johnson, 1993*b*
trans r-Cinnamaldehyde	Induction of aberrations in mammalian cells	Kasamaki *et al.* 1982
trans r-Cinnamaldehyde	Transformation of mammalian cells	Kasamaki *et al.* 1987
Coumarin	Induction of bile duct tumours by feeding	Griepentrog, 1973
Curcumin	Induction of aberrations in mammalian cells	Ishidate *et al.* 1988
Diallyl sulphide	Enhancement of DEN induced hepatocarcinogenesis	Takahashi *et al.* 1992
Indole-3-carbinol	Promotion of AFB_1 induced carcinogenesis	Bailey *et al.* 1987
Indole-3-carbinol	Enhancement of DMH induced carcinogenesis	Pence *et al.* 1986
Kaempferol	Induction of mutations in mammalian cells	Maruta *et al.* 1979
Phenethyl isothiocyanate	Induction of aberrations in mammalian cells	Musk & Johnson, 1993*b*
Phenethyl isothiocyanate	Induction of SCE in mammalian cells	Musk & Johnson, 1993*b*
Quercetin	Positive in Ames test and SOS chromotest	Rueff *et al.* 1992
Quercetin	Induction of aberrations in mammalian cells	Ishidate *et al.* 1988
Quercetin	Induction of recombination in mammalian cells	Suzuki *et al.* 1991
Tannic acid	Induction of liver tumours by s.c. administration	Korpássy & Mosonyi, 1950
Vanillin	Induction of aberrations in mammalian cells	Jansson & Zech, 1987
Vanillin	Induction of SCE in mammalian cells	Jansson & Zech, 1987

studied before an increase in its consumption can be recommended (Bradfield & Bjeldanes, 1991). Other examples of mutagenic, clastogenic, carcinogenic and cocarcinogenic effects of dietary constituents previously referred to are given in Table 1. Clearly the message of epidemiology is that vegetables and fruits are overwhelmingly beneficial in their effects, but the prospect of manipulating the composition of commercial varieties inevitably raises issues of safety. It remains unclear whether any of the compounds which have been identified experimentally as potential anticarcinogens would represent a real hazard to humans if their intake were increased, but the caveat of Bradfield & Bjeldanes (1991) regarding the need for a thorough investigation of the toxicology of indole-3-carbinol should be extended to the other putatively anticarcinogenic agents.

Can it be argued that any of the biologically active substances discussed in this review should be classified as micronutrients? The Oxford English Dictionary defines a *nutrient* simply as a substance serving as nourishment. *To nourish* is "to supply (a thing) with whatever is necessary to promote its growth or formation or to maintain it in proper condition". The last part of this definition would certainly encompass the prevention of cancer, but nutritional science tends to regard nutrients more narrowly as substances which are essential in the sense that a specific deficiency disease results if they are absent from the diet. It is improbable that any of the biologically active substances discussed here meet this criterion, but it is conceivable that human beings have become adapted to a cocktail of food borne plant metabolites which help to maintain resistance to neoplasia. The fact that many of these compounds are potentially toxic if consumed above a threshold dose need not necessarily preclude their classification as nutrients. At first sight this is something of a paradox but several recognized micronutrients have been shown to be mutagenic under appropriate conditions *in vitro* (Chow, 1990).

Although human beings are highly adaptable omnivores, it is reasonable to assume that a high intake of fruits and vegetables is a biological norm. Hunter-gathering has probably been the dominant means of food provision throughout most of human evolutionary history. Modern hunter gatherers obtain more than half of their food energy from plant sources (Lee, 1967) and it has been calculated that even in industrialized societies human beings are exposed to about 1·5 g of potentially toxic plant constituents/d (Ames & Gold, 1990). Many of these compounds are themselves mutagenic, and may be carcinogenic at high doses. Natural selection has probably ensured that the chemical defence mechanisms of the human body are both highly effective and inducible (Ames & Gold, 1991). It would then follow that the maintenance of such defence mechanisms is a normal, diet dependent physiological function, and it may be appropriate to regard the constant provision of the necessary food borne factors as an aspect of normal nutrition. If this hypothesis survives further rigorous research it will be necessary to recognize the existence of a class of dietary substances which, although distinct from micronutrients defined in the conventional sense, are necessary for the maintenance of optimum health. The term 'dietary phytoprotectants' may be suitable as a collective term for such substances.

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