An overview of interactions between micronutrients and of micronutrients with drugs, genes and immune mechanisms

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The objective of the present review is to examine critically the consequences of interactions that micronutrients undergo with nutrients and non-nutrients (mainly prescribed medicines) in diets and lifestyle factors (smoking, tea and alcohol consumption). In addition, the review describes recent work on interactions between nutrients and genes, the influence of gene polymorphisms on micronutrients, the impact of immune responses on micronutrients and specific interactions of antioxidant micronutrients in disease processes to minimise potential pro-oxidant damage.

Micronutrients: Medicinal drugs: Genes: Immune function: Antioxidants

Micronutrients are a diverse collection of substances required for intermediary metabolism in varying amounts but usually in amounts less than 1 g/d and sometimes only a few μg/d. Most individuals obtain these substances from their food, but where malnutrition exists or the diet is monotonous and imbalanced, deficiencies can occur. Micronutrient deficiencies may be so severe as to cause clinical symptoms with major metabolic disturbances (Beaton *et al*. 1993; Underwood & Arthur, 1996). Alternatively, an imbalanced diet may only produce a state of sub-optimal nutrition but this may also influence immune responses and the susceptibility to disease (Beaton *et al*. 1993). In such situations metabolic functions may be blunted (Munoz *et al*. 1995) and the body more vulnerable to toxins (Guengerich, 1995) or to physiological stresses; for example, resulting in complications of pregnancy (Keen *et al*. 2003). There is some evidence to suggest that a nutritionally sub-optimal situation may also exist in industrialised countries and is responsible for the high rates of CVD and some cancers (Diet and Health, 1989; World Health Organization, 1990; Beattie & Kwun, 2004).

Poor diets are usually not just deficient in only one nutrient. The nutrient that is least adequate in the diet may cause the main clinical effects but if only that nutrient is replaced, other nutrients may then become critical, preventing any response to treatment. Nevertheless, at the community level, much work has already been done using single nutrient supplements with some striking successes as with vitamin A (Beaton *et al*. 1993) and Zn (Zinc Investigators Collaborative Group, 1999), although there have generally been much poorer outcomes with Fe (Underwood, 2003). Currently, health programmers are trying to develop strategies to give multi-nutrient supplements which contain the major nutrients likely to be low in the diet; commonly vitamin A, folate, Fe and Zn (Christian *et al*. 2003). The objective in giving any supplement is to produce a rapid improvement in nutritional status where dietary improvements may take many years and require complex interventions and close compliance by the recipients.

The potential for adverse consequences from both single- and multi-nutrient supplements through interaction with dietary components should not be overlooked. Supplements usually contain a disproportionately large amount of nutrient relative to normal dietary intakes, as the objective is to improve nutritional status rapidly. Giving large amounts of supplements can have surprisingly adverse effects. Nutrients may have toxic effects themselves on the body as in the case of vitamin A (Hathcock *et al*. 1990; Miller *et al*. 1998; Wiegand *et al*. 1998; Lips, 2003). Even non-toxic β-carotene (β-C) increased mortality from lung cancer in smokers in two large intervention studies (Heinonen *et al*. 1994; Omenn *et al*. 1996). Nutrients can compete with one another and interfere with absorption as happens with Fe and Zn (Lynch, 1997; Sandstrom, 2001). Supplementary nutrients may exacerbate inflammation and thus worsen the effects of disease on the body (Wiegand *et al*. 1998). Fe is potentially a good example of this (Wiegand *et al*. 1998), but there are also reports that supplements of vitamin A can adversely affect respiratory disease (Stephensen *et al*. 2002*a*), and there is one

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Abbreviations: APP, acute-phase protein; APR, acute-phase response; CYP, cytochrome P450; DHAA, dehydro-L-ascorbic acid; GPx, glutathione peroxidase; Hp, haptoglobin; IRP, Fe-regulatory proteins; LPS, lipopolysaccharide; MTHFR, 5,10-methylene-tetrahydrofolate reductase; NOS, NO synthase; PCT, proximal convoluted tubular; RBP, retinol-binding protein; ROI, reactive oxygen intermediates; RXR, retinoid X receptor; Th, T-helper; THF, tetrahydrofolate; VDR, vitamin D receptor; VDRE, vitamin D response element.

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report that vitamin A supplements increase the transmission of HIV to infants from the mother (Fawzi *et al*. 2003).

Physiologically, we adapt to handle nutrients and other substances at the concentrations in our diet and environment. We do have in-built safety mechanisms to handle large amounts of nutrients as well as the many thousands of xenobiotics in our diet. The biotransformation of some such compounds is handled by what are known as phase I and II reactions (Thomas, 1995; Alonso-Aperte & Varela-Moreiras, 2000) (see later sections on interactions between nutritional status, lifestyle and xenobiotics; p. 217). Most endproducts of such biotransformations can be safely excreted by the host but there are some well-known exceptions. Intermediate compounds formed from dietary nitrosamines and aflatoxins are more toxic than the original and, in these cases, they are carcinogenic. The activity of phase I enzymes, however, can be both depressed as well as stimulated by substances in our diet and the actual activity of these enzymes may be a product of the habitual dietary environment in which we live (Guengerich, 1995). Hence individuals regularly consuming diets high in fruit and vegetables may metabolise potential carcinogens differently from others whose diet is low in these foods, and thereby have a lower risk of certain cancers. A well-balanced diet should contain adequate amounts of nutrients to maintain normal metabolism but also a wide range of non-nutrients to optimise phase I and II metabolic processes. The term 'non-nutrient' is used by the writer to indicate the many substances in our diet that have not been identified as being essential for life, but nevertheless can influence, or be influenced by, metabolic processes. Identifying the nature and amounts of relevant non-nutrients is outside the scope of the present review but readers wanting to know more about this fascinating area of nutrition should consult other reviews (Smith & Yang, 1994; Yang *et al*. 1994).

It is proposed to examine a selection of the issues relating to micronutrient interactions. The first two sections will address drug-nutrient interactions and this includes lifestyle factors such as smoking and alcohol use. The next section will look at issues relating to the immune response and micronutrients. The fourth will address some issues relating to gene–nutrient interactions and the last section will address the question of inter-relationships between the antioxidant nutrients and their significance in maintaining health.

Drug–nutrient interactions

Drug–nutrient interactions can be categorised as physicochemical, physiological or pathophysiological (Roe, 1993) and particularly apply to older individuals in the community. Physicochemical interactions are represented by chelation causing the loss of a nutrient and lower activity of a drug. Physiological interactions include drug-induced changes in appetite, digestion, gastric emptying, biotransformation and renal excretion. Alterations in the metabolism, or tissue distribution, of vitamins A and D by female hormones producing higher plasma concentrations of retinol (Amatayakul *et al*. 1989; Ballew *et al*. 2001) and 25-hydroxycholecalciferol (Harris & Dawson-Hughes, 1998) are also examples. Pathophysiological interactions

occur when a drug impairs nutrient absorption or metabolism, or when drug toxicity causes an inhibition of metabolic processes. For example, symptoms of neurotoxicity can arise following the use of isoniazid, the toxicity of which is due to its chelating properties for pyridoxine, eventually depleting systemic pyridoxine concentrations (Lockman *et al*. 2001). Likewise, teratogenic effects of the anticonvulsant drug, valproic acid, may be due to an altered pattern of folate metabolites in the embryo (Wegner & Nau, 1992) (Table 1).

Frequently, drug–nutrient interactions are bi-directional in their effects. For example, while the bioavailability of minerals is reduced by co-administration with some drugs, drug absorption is also reduced by nutrients, especially minerals, thereby reducing the effectiveness of the therapy (Polk *et al*. 1989; Campbell *et al*. 1992). Fe–drug interactions of clinical significance may involve a large number of therapies, for example those involving tetracycline derivatives, penicillamine, methyldopa, thyroxine, etc (Campbell & Hasinoff, 1991). Furthermore, in addition to drug–nutrient interactions influencing plasma nutrient concentrations, disease itself metabolically depresses the circulating concentrations of some nutrients and may increase requirements for nutrients (Thurnham, 1997a). Plasma concentrations of Fe, retinol, ascorbate and Zn are rapidly and almost universally depressed by the inflammatory response and the physiological purpose in these changes should be understood in order to maximise benefits from supplementation (Thurnham & Northrop-Clewes, 2003).

A drug–nutrient interaction refers to the effect of a medication on nutrients in the food we eat or those already in our bodies, or the converse, where food (Thomas, 1995) or dietary treatments (Campbell *et al*. 1992) may have an influence on the effectiveness of a drug. Food ingestion can profoundly affect a drug's pharmacodynamics and can either accelerate or retard drug absorption (Thomas, 1995). Where prolonged medication is prescribed, the potential interaction between food and drugs may be increased and this of course becomes of greater importance in older individuals. In older individuals, a depressed metabolism of drugs or a reduced urinary flow may increase the plasma concentration of a drug and maintain it for longer, so increasing the risk of interaction and toxicity (Bressler, 1993; Blumberg & Couris, 1999). Risk factors which increase the potential for adverse outcomes of drug–nutrient interactions are multiple medications, reduced nutrient intakes, nutrient loss from poor cooking habits, restrictive diets, anorexia, eating disorders, alcoholism, drug dependency or addiction, and renal and hepatic dysfunction (Thomas, 1995).

Common drugs that influence handling or turnover of nutrients

Medications may decrease appetite but usually the effects of drugs operate by altering nutrient absorption, metabolism or excretion (Table 1). The interactions described in this section are those where nutritional status is potentially impaired. However, nutrients may also interfere with drug absorption or metabolism and thereby reduce therapeutic effectiveness.

Table 1. Examples of drug–nutrient interactions*

Altering absorption		Altering nutrient metabolism		Altering nutrient excretion	
Drug	Nutrient malabsorbed	Drug	Nutrient	Drug	Nutrient with increased excretion
Bisacodyl (laxative)	K	Hydralazine (anti-hypertensive)	Vitamin B_6 antagonism	Aspirin (anti-inflammatory)	Vitamin C, K
Cholestyramine (anti-hyperlipaemic)	Fe, carotene, vitamins A, D and K, folate	Isoniazid (anti-tubercular)	Vitamin B_6 antagonism	Furosemide (diuretic)	Vitamin B_1, K, Ca, Mg, Na, Cl^-
Aluminium hydroxide (antacid)	Phosphate, vitamins A and B_1	Methotrexate (anti-neoplastic, anti-psoriatic)	Folate antagonist, malabsorption of vitamin B_{12} and fat	Spironolactone (diuretic)	Na, Cl^-
Colchicine (antigout)	Na, K, carotene, vitamin B_{12}	Penicillamine (chelating agent)	Inhibits vitamin B_6-dependent enzymes, chelates Cu, Fe and Zn	Thiazide (diuretic)	K, Mg and Na but decreased urinary Ca
Mineral oil (laxative)	Carotene, vitamins A, D, E and K, Ca and P	Phenolbarbital (anticonvulsant)	Increased turnover of vitamin D due to hepatic enzyme induction	Tetracycline (antibiotic)	Vitamin C
Phenolphthalein (laxative)	Vitamin D, Ca and other minerals	Phenytoin (anticonvulsant)	As for phenolbarbital and also decreased folic acid levels		
Sulfasalazine (anti-inflammatory)	Folate	Pyrimethamine (antimalarial)	Inhibits dihydrofolate reductase and lowers folic acid levels		
Captopril (anti-hypertensive)	Fe and other minerals	Triamterene (diuretic)	Weak folic acid antagonist		
Olestra (anti-obesity)	Small decreases in carotene and vitamins D and E	Aspirin (anti-inflammatory)	Gastric irritation can cause Fe deficiency		
Stanols and sterols (anti-obesity)	Small decreases in carotene	Corticosteriods	Anti-vitamin D activity can reduce Ca absorption and cause osteoporosis		
		Oestrogen-containing oral contraceptive	Increased turnover of vitamins C and B_6, elevated serum retinol concentrations		

Modified from Smith & Bidlack (1984) with additions from Briggs & Briggs (1972), Windsor et al. (1972), Amatayakul et al. (1989), Jones et al. (1991), Goldman (1997), Harris & Dawson-Hughes (1998), Schaefer et al. (1998), Thomas & Burns (1998), Rieck et al. (1999), Sierksma et al. (1999), and Ballew et al. (2001).

The short-term use of drugs is less likely to have adverse effects on nutritional status than long-term treatments. The problem is that the long-term use of many drugs is escalating. Over the last 40 years, the life expectancy of man has increased by almost 10 years in industrialised countries and by 20 years in parts of the developing world (Russell, 2000). As man lives longer the incidence of chronic diseases rises and the use of long-term drug therapy to fight the effects of chronic diseases such as arthritis, hypertension, coronary artery disease and adult-onset diabetes will continue to increase. Many classes of drugs, including antimicrobials, hypoglycaemics, and hypocholesterolaemic reagents, can be affected by the presence of food, with the geriatric patient being particularly at risk (Bressler, 1993; Thomas, 1995). In a recent survey in The Netherlands, 50 % of individuals over the age of 65 years were using diuretics (van Kraaij *et al.* 1998). Some of the effects that commonly used medicines can have on micronutrients are described below.

Analgesic and anti-inflammatory. Aspirin is probably the best-known analgesic, and such drugs are intended to relieve pain. They commonly cause stomach irritation, and ingestion on an empty stomach is not advised (Thomas, 1995). Aspirin is also reported to lower plasma vitamin C concentrations but the mechanism is not known (Sahud & Cohen, 1971; Alonso-Aperte & Varela-Moreiras, 2000). Severe irritation to the stomach lining can cause bleeding leading to the loss of blood and the risk of anaemia through Fe deficiency (Hodkinson, 1985). In addition, it is suggested that aspirin alters the transport of folate by competition for binding sites on serum proteins, as 70 % of patients with rheumatoid arthritis have low plasma folate concentrations (Alonso-Aperte & Varela-Moreiras, 2000). Low folate concentrations potentially limit the availability of methyl groups derived from one-carbon metabolism. However, attempts to demonstrate the biological importance of low folate concentrations in response to long-term administration of aspirin or acetaminophen in earlier experimental studies found no evidence of any disturbance in DNA methylation or in several markers of the methionine cycle (Varela-Moreiras *et al.* 1993).

Antacids or acid blockers. Antacids neutralise stomach acid and acid blockers reduce the production of stomach acid. These drugs are the largest group of agents recognised to cause interactions along the gastrointestinal tract. Antacids may alter a substance's dissolution by modifying gastric pH and through chelation. Al, a constituent of many antacids, can produce a relaxing effect on gastric smooth muscle that leads to a delay in gastric emptying time. Increased gastric pH leads to a reduced absorption of Ca, Fe, Mg and Zn (Thomas, 1995). Moderate gastric atrophy is common and autoimmune disease, leading to the loss of intrinsic factor in older individuals, can lead to vitamin B_{12} malabsorption. Gastric acid and pepsin are needed to liberate protein-bound cobalamin from food and intrinsic factor is needed to bind cobalamin as part of the absorption process. Furthermore, *Helicobacter pylori* infection increases with age and has been implicated in both peptic ulcer disease and atrophic gastritis. Thus cobalamin malab-

sorption may be exacerbated by anti-ulcer preparations which further lower gastric acidity. A study of outpatients in one hospital found 12–15 % of community-dwelling North American older individuals were B_{12} deficient (Pennypacker *et al.* 1991). And, in a retrospective study, the regular use of anti-ulcer drugs, especially histamine-2 blockers in the elderly, was found by Mitchell & Rockwood (2001) to be significantly associated with the initiation of cobalamin therapy. While the latter is not proof that anti-ulcer preparations cause vitamin B_{12} deficiency, it is suggestive that they might do.

Antibiotics. Antibiotics in general can decrease vitamin K synthesis by intestinal bacteria (Stieger *et al.* 1992) and impair clotting mechanisms. Antibiotics are used to treat bacterial infections and, when consumed by mouth, they will reduce the number and type of bacteria found in the intestine. Gut bacteria synthesise a family of compounds known as menaquinones (Mk; vitamin K_2) that have side chains based on repeating 5C (prenyl) units (Mk-n). Man absorbs these in the lower intestine and the human liver normally stores about 90 % vitamin K_2 and the remaining 10 % is phylloquinone (vitamin K_1). Vitamin K_1 is obtained mainly from green vegetables, and is the major form in the circulation. Vitamin K_1 is also believed to be the more physiologically active form of vitamin K and therefore the impact of antibiotics on vitamin K_2 synthesis and on clotting mechanisms is difficult to understand. However, antibiotics may affect vitamin K metabolism by other mechanisms; for example, the cephalosporin antibiotics can contribute to clinical hypoprothombinaemia by the inhibition of hepatic vitamin K epoxide reductase (Schafer *et al.* 1989). Tetracycline antibiotics bind to Ca found in dairy products, and the absorption of most tetracyclines is decreased by milk, antacids and by Ca, Fe and Mg salts. Tetracyclines also bind to Ca in growing bones and teeth, causing staining. Although the interaction between the antibiotic and milk prevents both the absorption of some Ca and the antibiotic, the relative loss of Ca is probably small. Nevertheless in the elderly who are at risk of Ca deficiency and in whom osteoporosis is a common feature, additional Ca losses due to tetracycline therapy may be important. The elderly are also at risk from increasing gastric pH which retards the absorption of Ca, and the increased use of loop diuretics facilitates the loss of Ca as well as other components in the urine (see later; p. 216) (Thomas & Burns, 1998).

Anticoagulants. Drugs such as warfarin slow the process of blood clotting and this can decrease the risk of strokes in individuals whose blood shows tendencies to clot too easily. Such drugs function by interfering in the metabolism of vitamin K in blood clotting. Effective therapy is achieved by balancing the amount of anticoagulant against the usual intake of vitamin K. Hence physicians warn against the consumption of foods high in vitamin K in case an elevated dietary intake of the vitamin impairs the effectiveness of the treatment (Smith & Bidlack, 1984). In addition, haemorrhage caused by the anti-vitamin K action of antacids can be exaggerated in the elderly by taking antioxidants such as tocopherol (Thomas & Burns, 1998) or very large doses of vitamin C (≥ 10 g). However, even with these amounts of

vitamin C, there was no hypothrombinaemic action. Concurrent administration of vitamin C reduced plasma warfarin concentrations by about 17 %, probably due to diarrhoea attributable to the large intake of vitamin C (Feetam *et al*. 1975).

Anticonvulsants. Anticonvulsant drugs help control seizures. Phenytoin, phenobarbital and primidone can cause diarrhoea and decrease appetite, so reducing the availability of many nutrients. The drugs are particularly well known for their stimulatory effects on vitamin D turnover and catabolism by inducing the hepatic microsomal drug-metabolising system (Hahn *et al*. 1972). Vitamin D supplements may be needed to counter the drug effects.

Anticonvulsants also interact with the B vitamin, folate; blood folate concentrations fall shortly following the onset of therapy. Folate supplements will counter the adverse effects on folate status but they also adversely affect the efficiency of anticonvulsant treatment, so folate supplementation has to be monitored carefully. In man, the anti-epileptic drug valproic acid is associated with teratogenicity and folate deficiency through unknown mechanisms, but possibly through effects on the methionine cycle (Alonso-Aperte & Varela-Moreiras, 2000). Wegner & Nau (1992) have demonstrated that valproic acid alters embryonic folate distribution in mice, producing ele-

vated concentrations of tetrahydrofolate (THF) and lower concentrations of both formylated forms of folate. Such effects would seem to suggest that the vitamin B_6-dependent enzyme responsible for the formation of 5,10-methylene-THF may be inhibited by valproic acid (see Fig. 1). Such alterations are partially prevented by the co-administration of folinic acid and *S*-adenosylmethionine, but nevertheless hypomethylation of DNA may be responsible for the teratogenesis (Alonso-Aperte & Varela-Moreiras, 2000).

Anti-hyperlipaemic. Anti-hyperlipaemic drugs reduce blood cholesterol levels. Such drugs work by reducing fat absorption, a side effect of which is a reduction in absorption of the fat-soluble vitamins A, D, E and K and carotenoids. It is also reported that the absorption of vitamin B_{12}, folate and Ca may also be affected.

Cholestyramine was one of the first of the cholesterol-reducing drugs and is still used. Because such drugs cause malabsorption of fat-soluble nutrients, increased recommendations were made to raise the daily intake of vitamin A as long ago as 1985 (Roe, 1985) but cause-and-effect relationships between drugs and vitamin deficiency are difficult to prove, and the research has still not been done (Alonso-Aperte & Varela-Moreiras, 2000).

More recently, the phytostanols were shown to have cho-
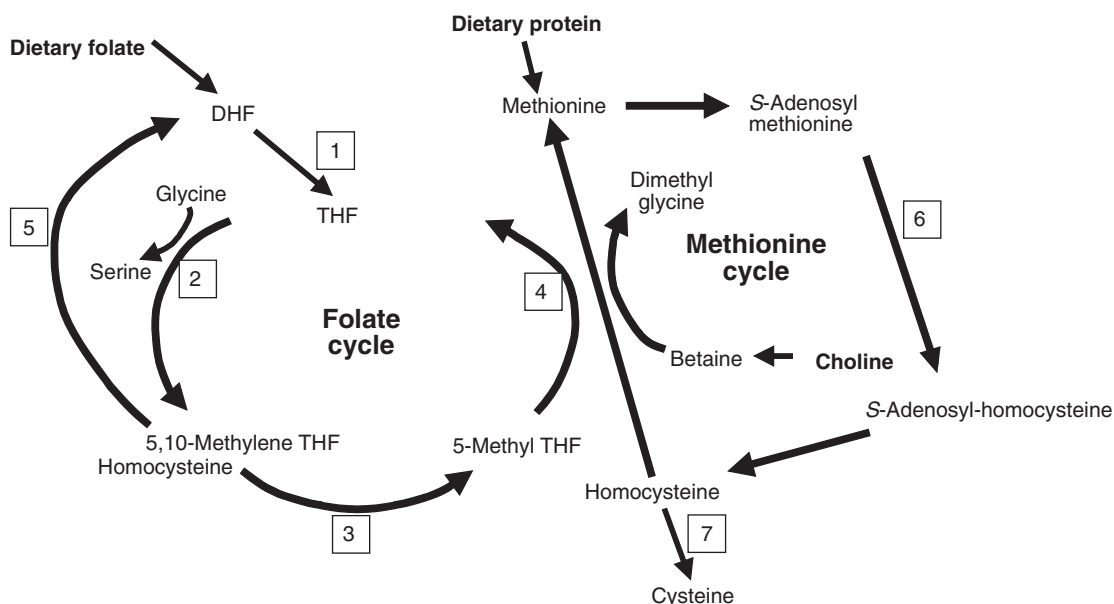
Fig. 1. Influence of vitamin status on folate and methionine cycles. Disturbances can arise from the following nutrient deficiencies: folate, cobalamin, riboflavin, pyridoxine and choline. In addition, drugs which inhibit dihydrofolate (DHF) reductase activity, such as methotrexate, have effects which are similar to folate deficiency. Deficiencies of folate, choline and the use of DHF reductase inhibitors (1) lower circulating metabolites of folate, impair the synthesis of *S*-adenosyl methionine and raise plasma homocysteine concentrations. Cobalamin (4) is needed for 5-methyl tetrahydrofolate (THF)–homocysteine methyl transferase activity. Cobalamin deficiency impairs folate interconversions, lowers THF and methionine and increases homocysteine concentrations. Riboflavin (3) is required for the enzyme 5,10-methylene THF reductase (MTHFR) which provides the methyl donor for methionine synthesis. The C677T polymorphism of the MTHFR gene appears particularly sensitive to riboflavin deficiency, lowering methionine and raising plasma homocysteine concentrations. Pyridoxine deficiency is rare in developed countries. Pyridoxal-5-phosphate (PLP) is required by many enzymes in protein metabolism. Deficiencies of PLP would probably affect folate metabolism by reducing the 5,10-methylene- and 5-methyl-THF metabolites (2) and so raising homocysteine concentrations and/or impairing the removal of homocysteine by inhibiting the transulfuration pathway (7). Pathways (5) and (6) enable purine and pyrimidine synthesis (5) and methyl group donation for lipid, protein and DNA metabolism (6).

lesterol-lowering effects and have been incorporated into spreads for use in the home. Benecol and Flora-Pro-Active are the first products to be recognised as 'functional foods' on the UK market. Also in this category is 'Olestra'. Olestra is a compound formed by the esterification of sucrose and is a non-absorbable, tasteless, fat-like compound. Experience with all these compounds shows that while there are small variable decreases in plasma carotenoid concentrations following use of these substances, detectable effects on serum concentrations of vitamins A, D, E and K are minimal (Goldman, 1997; Hendriks *et al*. 1999).

Anti-hypertensive. Anti-hypertensive drugs are used to control blood pressure. They can affect body levels of K, Ca and Zn. Captopril, a hypotensive drug and an inhibitor of angiotensin-converting enzyme, can bind to Fe in the gut if jointly administered with Fe-containing mineral preparations. When 25 mg captopril was administered orally with 300 mg ferrous sulfate, there was a 37 % reduction in the area under the curve of serum unconjugated captopril concentrations in normotensive human volunteers (Campbell & Hasinoff, 1991; Schaefer *et al*. 1998). Food also interferes with the absorption of captopril, since food usually retards gastric emptying and elevates gastric pH (Mantyla *et al*. 1984). Bioavailability of the drug was reduced by more than 50 %; however the hypotensive effectiveness was no different apart from being delayed from about 1 h to 2 h.

Anti-neoplastic. These agents are used to treat different forms of cancer. Frequently the drugs irritate the lining of the mouth, stomach and intestines and can damage mucosal cells, thus altering digestion. Many cause nausea, vomiting and/or diarrhoea. All of these effects can potentially influence nutrient status.

One particular drug in this group is methotrexate, which competitively inhibits dihydrofolate reductase and the production of THF for intracellular folate metabolism (Fig. 1). This limits the availability of methyl groups for single carbon transfer in the synthesis of purine and pyrimidines (Alonso-Aperte & Varela-Moreiras, 2000). The biochemical perturbations caused by methotrexate are similar to those seen in choline deficiency. Animals on low-dose methotrexate have diminished hepatic folate levels, *S*-adenosylmethionine, methionine and betaine. The action of methotrexate on dihydrofolate reductase inhibits the reduction of pteroyl glutamate (folic acid) to tetrahydropteroyl glutamate (THF) and as a consequence both the supplies of folate co-enzymes and of the tetrahydropteroyl glutamate substrate for the conversion to polyglutamate derivatives (and liver storage) are impaired. Methotrexate also inhibits the transport across the intestine and into hepatocytes and there was a 48 % reduction in hepatic folate content as a result of the methotrexate treatment. The consequence of methotrexate treatment on the distribution of folate metabolites was a decrease in 5-methyl-THF and an increase in formyl-THF. There was no change in the relative concentration of THF. The sharp decline in the concentration of 5-methyl-THF concentrations associated with methotrexate treatment would impede the conversion of homocysteine to methionine. Plasma homocysteine concen-

trations were not measured but methionine was reduced (Selhub *et al*. 1991).

Therapeutic efficiency and adverse effects of methotrexate therapy on folate metabolism will depend on the relative amounts of methotrexate and folic acid in the diet. To reduce the adverse side effects of methotrexate on folate metabolism, folic acid has been given together with the drug. As this potentially decreases the effectiveness of the therapy, the situation has to be monitored carefully. For a further discussion on folate metabolism and micronutrient interactions, see the section on vitamin status and homocysteine concentrations (p. 228).

Anti-tubercular. The anti-tubercular drug isoniazid (isonicotinic acid hydrazide) binds to pyridoxal phosphate and increases the risk of vitamin B_6 deficiency during treatment. In recent years there has been an increase in tuberculosis and with it a corresponding increase in isoniazid-induced neurotoxicity (Temmerman *et al*. 1999). Acute toxic effects of isoniazid can also be precipitated by an overdose of as little as 1·5 g and doses larger than 30 mg/kg often produce seizures (Romero & Kuczler, 1998). Pyridoxal phosphate is a necessary cofactor for the production of the neurotransmitter γ-aminobutyric acid. If isoniazid combines with pyridoxal phosphate it can lead to a reduction in levels of γ-aminobutyric in the brain (Wood & Peesker, 1972). Along with treatment to reduce the seizures, pyridoxine should be administered in a dose equivalent to the suspected amount of drug ingested (Romero & Kuczler, 1998).

Isoniazid can also inhibit hepatic vitamin D 25-hydroxylation and possibly affect metabolism of the vitamin (Bengoa *et al*. 1984). In addition, pellagra is a recognised complication of isoniazid therapy (Darvay *et al*. 1999). Pellagra is the deficiency disease associated with a deficiency of the vitamin niacin (nicotinic acid). There are two main sources of niacin in the diet, niacin and the amino acid tryptophan. The body can convert tryptophan to nicotinic acid through a series of steps, several of which require pyridoxal phosphate as co-enzyme. Hence isoniazid therapy can result in pellagra.

Diuretics. Diuretics stimulate the increased excretion of urine and with it the risk of increased losses of K, Mg and Ca and the water-soluble vitamins. Thiamin status in the elderly is of particular concern in relation to the use of diuretics. Total body thiamin content is rather small and thiamin has a high turnover rate, with a half-life of 10–18 d. Thus to maintain optimal thiamin status, a constant supply of dietary thiamin must be assured (Suter & Vetter, 2000). One particular diuretic, furosemide, has been associated with thiamin deficiency in elderly patients (Pepersack *et al*. 1999). Work has shown, however, that all diuretics lead to increased losses of urinary thiamin (Lubetsky *et al*. 1999; Rieck *et al*. 1999) but furosemide has also been shown to impair the uptake of thiamin by cardiac cells *in vitro* (Zangen *et al*. 1998). However, the clinical importance of the latter effect still needs to be confirmed.

Laxatives. Laxatives speed up the movement of material through the digestive tract, so potentially reducing the time

for nutrient absorption. So, the excessive use of laxatives may deplete vitamins, especially the fat-soluble vitamins A, D, E and K and minerals such as Na and K needed for normal body functions. There may also be increased fluid losses leading to dehydration. Usually electrolytes are reabsorbed in the large intestine; however, laxatives may not allow adequate time for this to occur (Blumberg & Couris, 1999).

Drugs and tropical diseases. Vector-borne diseases (malaria, sleeping sickness, arbovirus diseases, blood flukes), viral diseases (haemorrhagic fevers), AIDS and illnesses due to malnutrition affect vast areas of the tropics, and there is undoubtedly considerable interaction between the diseases and between the diseases and malnutrition. Such interactions, however, are generally not the subject of the present review. As with many of the drugs used in Western medicine, there are the general effects of food affecting drug efficiency. Thus for the anti-helmintic drug, albendazole, experiments in sheep have shown it is pharmacokinetically 50 % more effective if fed to starved as opposed to fed animals (Lifschitz *et al*. 1997). Likewise, Lumifantrine, one of the antimalarial drugs used when multi-drug resistance is a complication, is almost insoluble in water and administering it with food if possible improves its absorption and effectiveness. Patients receiving the antimalarial drug Fansidar should be adequately hydrated, as the long *in vivo* half-lives of the components increase the risk of crystaluria (Pharmaceutical Information, 2004). However, there appear to be very few reports of specific interactions between drugs used to combat tropical diseases and specific nutrients, but this may be due to the widespread malnutrition and low socio-economic conditions present in many developing countries. Thus, apart from general information on desirable conditions for the administration of drugs used in tropical medicine, there is little specific information on drug–nutrient interactions except for some antimalarial drugs.

Several antimalarial drugs interfere with folate metabolism. The malaria parasite requires *p*-aminobenzoic acid and drugs such as sulfonamides interfere with its ability to obtain this essential nutrient and with the biosynthesis of tetrahydrofolic acid. For example, Fansidar contains sulfadoxine, a structural analogue of *p*-aminobenzoic acid and pyrimethamine, a competitive inhibitor of the enzyme dihyrofolate reductase. The combined use of Fansidar with other drugs having antifolate activity may produce megaloblastic anaemia and such drugs should not be used in patients with megaloblastic anaemia due to folate deficiency.

Two other specific drug–nutrient interactions have been reported in connection with antimalarial drugs. Quinine is reported to potentiate the anticoagulant action of warfarin (Stenton *et al*. 2001), and it is also suggested that the sulfonamide in Fansidar may displace orally administered anticoagulants from their carrier proteins, increasing their anti-coagulant effects (Pharmaceutical Information, 2004). Last, *in vitro* studies with supplements of the B vitamin, riboflavin, report potentiation of the activities of several antimalarial drugs (mefloquine, pyrimethamine and quinine) against the asexual parasite forms of *Plasmodium falciparum* (Akompong *et al*. 2000a). The authors suggest that the reducing properties of riboflavin interfere with the oxidation of Hb in the food vacuole of the parasite, since levels of oxidised Hb, the accumulation of haemozoin (oxidised and polymerised Hb) and the size of the food vacuole in riboflavin-treated parasites were all reduced by approximately 50 % (Akompong *et al*. 2000b).

Influence of nutrients or nutritional status on drug metabolism

The processing or detoxification of drugs and the many foreign compounds that enter our bodies daily in food is done by processes that have been collectively termed phase I and phase II enzyme-catalysed reactions. Phase I reactions include oxidation, hydroxylation, reduction and hydrolysis, leading to changes in the functional groups on molecules. Phase I activity occurs predominantly in the liver and comprises the microsomal or mixed function oxidase systems, NADPH-dependent enzymes and cytochromes P450, the latter in liver, lung and small intestine. The second phase of the transformation is of a synthetic nature and involves conjugation with glucuronate, sulfate or glycine to enable excretion of the conjugate in the urine or bile. In general the intermediate compounds formed by these reactions are safe but there are exceptions, for example, most nitrosoamines, where the products of the phase I reaction are potential carcinogens (Guengerich, 1995).

Cytochrome P450 (CYP) proteins in man are drug-metabolising enzymes but some are also necessary for metabolising important endogenous compounds, for example, cholesterol, prostacyclins, thromoboxane A2, bile acids, steroids, and the degradation of vitamins A and D, etc. The P450 proteins are membrane-bound, haem-containing enzymes found in the endoplasmic reticulum and some are found in the mitochondrial inner membrane. There are two different electron transport chains for the P450 cytochromes depending upon location, but both involve NADPH and flavin co-enzymes. The P450 proteins are categorised into families and subfamilies by similarities in their amino acid structure. Sequences that are more than 40 % identical belong to the same family and those more than 50 % identical, to the same subfamily. In man there are eighteen families and forty-three subfamilies of the CYP genes (Nelson, 2003). Most drugs and other foreign compounds in our diet are metabolised by cytochromes from three families; CYP1, CYP2 and CYP3. Compounds may induce or inhibit specific CYP enzymes and interactions between two or more compounds and the cytochromes are common. The CYP3A subfamily is one of the most important drug-metabolising and abundant P450 proteins in the human liver. It is known to metabolise 120 different drugs, for example, acetaminophen (anti-inflammatory), codeine (analgesic), cyclosporin A (immuno-suppressant), diazepam (anti-depressant), warfarin (anti-coagulant), etc.

Many nutrients and micronutrients can potentially affect phase I enzyme activity, for example niacin and riboflavin are needed for mitochondrial electron transport, Fe and glycine are needed for haem synthesis for CYP, and miner-

als such as Ca, Zn and Mg are needed to maintain membrane integrity. Ca regulation within the cell plays an important role in cell homeostasis (Hoyumpa & Schenker, 1982). Normal intracellular Ca concentration in the cytosol is less than 0·1 μM but is 1·3 mM outside the cell or in the mitochondria or endoplasmic reticulum. The gradients are maintained by ATPase pumps which require normal mitochondrial activity for cellular respiration. Mg is needed for mitochondrial membrane stability and, together with thiamin diphosphate, is an essential co-enzyme for pyruvate decarboxylase which provides the essential substrate, acetyl-CoA, for mitochondrial function. Last, high Zn concentrations (2–10 μM) can induce an increase in inner mitochondrial membrane permeability by opening a high-conductance channel (Wudarczyk *et al*. 1999). Its effects may be caused by interactions with proteins by binding to various mitochondrial sulfhydryl groups and inhibiting the mitochondrial electron transport chain. The effects of Zn are inhibited by Mg at physiological concentrations (Wudarczyk *et al*. 1999). However, cytosolic concentrations of Zn are three times higher than those in mitochondria and it has recently been reported that the transport of Zn across lipid bilayers is substantially increased by lipid peroxidation and that an excessive influx of Zn might be associated with cell death (Pattison *et al*. 2004). In addition, it has recently been suggested that fluctuations in plasma Zn can have a rapid influence on cell metabolism, especially of endothelial cells. Zn-depleted cells become more sensitive to signalling molecules and much more susceptible to oxidant stress (Beattie & Kwun, 2004). It is difficult to establish to what extent low nutritional status influences mixed-function oxidase systems activity *in vivo* but considerable experimental work suggests that the CYP system seems to be sensitive to a number of vitamin deficiencies, including vitamins A, B group, C and E (Guengerich, 1995).

In the case of vitamin C it was reported as early as 1941 that the pentobarbital sleeping time was prolonged in scorbutic guinea-pigs. Zannoni and colleagues showed that the activity of CYP and NADPH cytochrome P450 reductase decreased significantly when microsomal ascorbate had dropped to 30 % of normal. Enzyme activity took about 6 d to return to normal on repletion with ascorbate and most of the effects of vitamin C deficiency occurred in younger animals (200–250 g) (Zannoni & Lynch, 1973). However, there is uncertainty whether human CYP enzymes are also sensitive to vitamin C deficiency, since an attempt to exploit the sensitivity and assess vitamin C status in man by his ability to metabolise ^{13}C-labelled methacetin did not appear to work. This was in spite of the fact that when guinea-pigs in various stages of vitamin C depletion were given [^{14}C]methacetin and breath $^{14}CO_2$ was collected, the amount of label found in the breath was there in proportion to status. However, a human study carried out in adult Gambian males failed to demonstrate any correlation between the metabolism of [^{13}C]methacetin and vitamin C status (Powers, 1987, 1991). The reasons for the failure of the human study may be because the vitamin C deficiency in the guinea-pigs was more severe. Alternatively, the guinea-pigs were young growing animals and it may be that it is only in growing infants and children that the P450

enzymes are sensitive to inadequate vitamin C status. It is also becoming clearer that there are considerable interspecies differences in P450 metabolism of xenobiotics (Nelson, 1999), which might explain the different behaviour between man and guinea-pigs.

Malnutrition, P450 enzymes and cancer

In contrast to vitamin C, however, deficiencies of energy (protein and carbohydrate) reduce P450 enzyme-catalysed oxidations in man. For example, children with kwashiorkor have lower rates of drug metabolism (Guengerich, 1995). There are also many other examples of foods and nutrients influencing human P450 enzyme activity, such as the striking effects of flavonoids. Following a single glass of grapefruit juice, which contained a flavonoid that inhibits a P450 CYP3A4 subfamily enzyme, peak plasma concentrations and areas under the curve of several Ca channel-blocking drugs used to treat essential hypertension were elevated four- to five-fold (Guengerich *et al*. 1986). More recently others have shown that the potency of many more drugs is increased by grapefruit juice, for example drugs to reduce angina, convulsions, blood pressure, etc (Dresser *et al*. 2000; Kane & Lipsky, 2000). Other dietary substances with potentially protective effects against carcinogenic compounds are the S compounds in onions and garlic, the isothiocyanates and indoles in cruciferous plants, capsaicin in capsicum fruits, etc. Experimental work with many of these substances that inhibit phase I or II enzymes show that they block tumour formation, and there is growing evidence of their effectiveness in man. The role of dietary inadequacies and excesses in influencing P450 activity in man may also be of fundamental importance in obtaining a better understanding of the aetiology of several diet-linked cancers. For example, alcohol consumption is associated with head and neck cancers, smoking with lung cancers and a diet high in salt is associated with gastric cancers, etc. In contrast, diets high in fruit and vegetables are associated with a general protection against many cancers and, as indicated earlier (p. 212), several classes of compound are now known to inhibit or induce different P450 enzymes known to metabolise compounds to carcinogenic intermediates. Anyone interested in exploring the potential ramifications and interactions of diet (deficiency and excess), drugs of abuse and the different P450 enzymes which may play a part in the aetiology of these cancers should consult the reviews by Smith & Yang (1994), Yang *et al*. (1994), Guengerich (1995), and Nelson (1999, 2003).

Lifestyle habits and nutrient status

Tea consumption and nutrient status. Polyphenol-containing beverages, such as tea, reduce non-haem-Fe bioavailability (Disler *et al*. 1975; Brune *et al*. 1989); hence several workers have investigated the possibility that tea consumption might interfere with Fe absorption and cause anaemia. Temme & van Hoydonck (2002) examined all studies where tea consumption and Fe status had been examined up to June 2001. Sixteen studies were evaluated and these included all sections of society. All studies were done in Western populations except one in China and another in

Zaire (now Democratic Republic of Congo). The overview concluded that tea consumption does not influence Fe status in Western populations where most individuals have adequate Fe stores. Where individuals had marginal Fe status, a negative association of ferritin or Hb with tea consumption occurred but associations were generally weak, except for in one study where the number of subjects studied was small (Temme & van Hoydonck, 2002). Recent experimental work suggests that flesh foods (beef, poultry and seafood), phytic acid and vitamin C are probably the most important factors determining Fe bioavailability (Reddy *et al.* 2000).

Smoking and nutrient status. Smokers have a higher risk of lung and other cancers than non-smokers. The exact cause of smoking-related cancers is not known but tobacco smoke contains large amounts of reactive species which may well be implicated in tissue damage and the initiation of cancer cell development. In addition, smoking can adversely influence the metabolism of some known dietary carcinogens and thus smokers may have higher risks of cancer from dietary or other environmental factors than non-smokers. CYP2A6 is the principal liver enzyme involved in nicotine clearance and it is also involved in the activation of several hepatotoxins, notably nitrosamines and aflatoxin B_1 (Pelkonen *et al.* 2000). It has been shown that some tobacco-specific nitrosamines have a high affinity for this enzyme (Raunio *et al.* 2001). Heavy smokers are likely to have higher CYP2A6 enzyme activity although there are inter-individual variations in CYP2A6 levels that affect nicotine inactivation rates as well as also potentially influencing smoking patterns. There is a relatively high rate of the CYP2A6 gene deletion in Asian populations resulting in a generally reduced activity in these populations and possibly reduced risks of lung cancer (Raunio *et al.* 2001). The CYP2A6 enzyme is also induced by anticonvulsant drugs but is not altered by acute alcohol ingestion (Pelkonen *et al.* 2000).

Two large studies reported an increased risk of lung cancer in individuals receiving 20–30 mg β-carotene (β-C)/d for 4 or more years (Table 2). Activation of cytochrome enzymes may lie at the centre of the explanation as to why β-C supplements increase the risk of lung cancer in smokers. Paolini *et al.* (1999) reported significant increases in the carcinogen-activating enzymes (CYP1A1/2, CYP3, CYP2B1, CYP2A) in the lungs of rats supplemented with high doses of β-C. In man, correspondingly high enzyme activities of these CYP would predispose tobacco-smoke-exposed individuals to an increased cancer risk from bioactivated tobacco-smoke pro-carcinogens. Furthermore, in the two supplementation studies where there was no increased cancer risk (Physicians' Study, 50 mg β-C every other day; Hennekens *et al.* 1996) or a reduced risk (China study, 15 mg β-C/d; Blot *et al.* 1993), the results do not conflict with the suggestions of Paolini *et al.* (1999). In the Physicians' Study, the majority of the participants were not smokers; therefore exposure to bioactivated, smoke-related pro-carcinogens would have been minimal. In the China Study, most men would have smoked clay pipes and women would have been non-smokers (Thurnham *et al.* 1988). Exposure to pro-carcinogens may have been minimised by

the method of smoking and any gene deletion of CYP2A6, such as that reported in Japanese populations (Raunio *et al.* 2001), would also have reduced risk. Thus the adverse effects of β-C supplements may have been due to the induction of phase I 'carcinogen-activating' enzymes in individuals already exposed to elevated levels of pro-carcinogens.

Smoking is associated with the lower dietary intake of many foods, including fruit and vegetables and dairy products (Rock *et al.* 1999; Morabia *et al.* 2000). In addition, smokers frequently have lower plasma concentrations of nutrients than non-smokers even when dietary intake is taken into account, for example with vitamin C (Faruque *et al.* 1995) and most carotenoids (Thurnham, 1990*a*). In fact others have shown that smoking a single cigarette over a period of 10 min reduced the concentration of plasma nitrate and nitrite (by about 13 %) and of vitamin C (by about 23 %) at 5 min, though in all cases concentrations returned to normal at 60 min (Tsuchiya *et al.* 2002).

There is an increased risk of bone demineralisation, fractures and osteoporosis in women who smoke (Law & Hackshaw, 1997) and a population-based survey of 2319 Swiss women using a validated food-frequency questionnaire reported that smokers consumed significantly less Ca and vitamin D than non-smokers, never-smokers or ex-smokers (Morabia *et al.* 2000). Dietary vitamin D is usually a poor predictor of vitamin D status, as sunlight provides our main supply. Nevertheless, others have also found plasma 25-hydroxycholecalciferol concentrations to be significantly lower in heavy smokers than in non-smokers and this was accompanied by lower Ca absorption, but there was no difference in bone density at any site in the two groups of elderly women studied (Rapuri *et al.* 2000). Whether the P450 enzyme CYP24, which is responsible for vitamin D degradation (Nelson, 2003), is induced by smoking has not to the writer's knowledge been investigated.

In a recent review of the evidence for the effects of smoking and alcohol use on micronutrient requirements in pregnancy, it was suggested that vitamin C requirements increase for pregnant smokers and that plasma concentrations of β-C, vitamin B_{12}, vitamin B_6 and folate concentrations are lower in pregnant than non-pregnant smokers. The authors were unclear, however, whether the lower concentrations were due to increased requirements, lower dietary or supplement intakes, haemodilution or other factors (Cogswell *et al.* 2003). The authors also reported that Fe supplementation partially ameliorates impaired fetal growth caused by Cd intake from cigarette smoke in experimental studies.

Ethyl alcohol consumption and nutritional status. The consumption of ethyl alcohol impairs the absorption of thiamin in both man and the rat (Thomson *et al.* 1970; Hoyumpa *et al.* 1977; Ibner *et al.* 1982). Ethyl alcohol inhibits the active but not the passive process of thiamin absorption. Note, this is different from the mechanism of action of thiaminases in raw fish, bracken and tea, where potentially all dietary thiamin is vulnerable to degradation (Cathcart & Thurnham, 1998). The active process of thiamin absorption occurs as a result of the ATPase found in the brush-border membrane in the jejunum in the rat. Ethanol at 0·5 M, added *in vitro* to brush-border membrane

Table 2. Adverse effects from nutritional supplements

Supplement	Route of administration and references	Recipients	Consequences	General comments
Vitamin C	Oral (Barton et al. 1998)	Patients with haemochromatosis	To avoid complications of Fe overload, dietary management includes avoidance of medicinal Fe, mineral supplements, excess vitamin C and uncooked seafoods	In situations where erythrocytes may be fragile or transferrin saturation may be high, there is an increased risk of free Fe ('high transferrin saturation) or pro-oxidant Fe (haemoglobin) being present in the plasma or other body fluids (for example, synovial fluids in the joints). Ferric Fe is reduced to ferrous Fe by vitamin C, which in combination with superoxide and peroxide can produce hydroxyl radicals and potentially cause free radical damage (see p. 232)
	Oral (3–4 g) in soft drinks over 4–6 h (Mehta et al. 1990)	G6PD deficiency in two boys	Erythrocyte lysis. Two boys admitted to hospital stuporose, pale, icteric, with tachypnoea, tachycardia and haemoglobinuria	
	Adult male who received 2 g vitamin C on consecutive days (Cambell et al. 1975)	G6PD deficiency in a black man who was admitted with second-degree burns of his hand	Intravascular haemolysis and acute renal failure. Hb in urine found to be 58 g/l. Became comatose with respiratory distress and right-sided hemiparesis. Died on day 22	
	Oral, megadose treatment (Halliwell, 1994)	Adults with terminal haemorrhagic cancers	Four patients died of haemorrhagic tumour necrosis soon after vitamin C treatment was started	
Fe	Oral, pharmacological doses (McFarlane et al. 1970)	Malnourished infants	Worsening disease and death	Administering Fe may facilitate increased bacterial or parasitic growth and worsen disease outcome and, in infants with malnutrition, overwhelming infection was the most important cause of death. The Fe load within the body may also influence disease severity directly by exacerbating inflammatory damage. Such effects may be that much greater where the body depends more on cell-mediated than humoral immunity to protect it from infection, as in the infant. Cell-mediated immunity involves the greater use of free radicals to control infection than humerol immunity, and the presence of Fe could exacerbate such activity. Thus infants would be at a greater risk of toxicity from Fe than children over 12 months
	Iron dextran intravenously (Oppenheimer et al. 1986; Oppenheimer, 1989)	Healthy neonates in Papua New Guinea	Higher prevalence of malaria and more severe malaria in those receiving iron dextran. Even infants in the control groups with higher birth Hb levels (and thus higher total body Fe content) were significantly more likely to have malaria at follow-up and more likely to be admitted to hospital with malaria	
Zn	Oral, 0–30 mg Zn/d for 3 d (Braunschweig et al. 1997)	Patients with a diagnosis of catheter sepsis or pancreatitis on home parenteral nutrition	A significantly higher temperature was recorded in those on extra Zn	These results suggest that Zn supplements both at the RDA level and higher may impair monocyte function. The mechanism of action is not immediately obvious. Additional Zn may stimulate the growth of invading pathogens; alternatively, high concentrations of plasma Zn may be pro-inflammatory in certain circumstances. See section on Zn and the immune response (pp. 224–225) and Wellinghausen et al. (1997)
	Oral, 100–300 mg/d (Chandra, 1984)	Adults	At high Zn dosages (i.e. ten times the daily requirements), diminished lymphocyte proliferation, neutrophil chemotaxis and phagocytosis were reported	
	Oral for 105 d; follow up at 60 and 105 d (Schlesinger et al. 1993)	Marasmic infants	A decrease in the number of infants able to phagocytose one or more Candida buds was observed after 60 d of Zn supplementation compared with admission (P<0.03). The number of infants with depressed fungicidal activity increased	

		significantly after 105 d of nutritional rehabilitation in the Zn-fortified groups as compared with the controls ($P<0.04$). The number and duration of impetigo episodes was significantly greater in the group fed the Zn-fortified formula. These results suggest that Zn supplements at the RDA level may impair monocyte function	A possible explanation for the toxic effects of β-carotene has been proposed by Paolini et al. (1999). These authors suggest that β-carotene is able to induce the activity of several cytochrome P450 enzymes with potential pro-carcinogenic activities (see p. 219)
β-Carotene	20 mg β-carotene/d for 5 to 8 years (Heinonen et al. 1994)	A randomised, double-blind, placebo-controlled primary prevention trial in adult smokers	An excess cumulative incidence of lung cancer was observed after 18 months and increased progressively thereafter, resulting in an 18 % difference in incidence at the end of the study (95 % CI 3, 36 %; $P=0.01$)
β-Carotene and retinol	30 mg β-carotene and 25 000 IU of retinol palmitate/d for 4 years (Omenn et al. 1996)	A total of 18 314 smokers, former smokers and workers exposed to asbestos	The treatment group had a relative risk of lung cancer of 1.28 (95 % CI 1.04, 1.57; $P=0.02$) as compared to the placebo at the end of the study

G6PD, glucose-6-phosphate dehydrogenase; RDA, recommended dietary allowance; IU, international units.

reduced ATPase activity in the jejunum by 41 % (Hoyumpa *et al.* 1977). At low dietary thiamin concentrations (<1·0 μmol/l), transport is a saturable, Na-dependent active process that proceeds against an electrochemical gradient and is adversely affected by anoxia, low temperature, metabolic inhibitors and structural analogues. At concentrations above 1·0 μmol/l, thiamin transport is unaffected by these factors and appears to be a passive process.

Likewise, in human subjects, parenteral alcohol administration reduces the absorption of thiamin. ^{35}S-labelled thiamin was given to twelve healthy subjects and thirteen patients with fatty liver and a history of chronic alcoholism (Thomson *et al.* 1970). Subjects were given 200 mg thiamin to saturate deposits before the experiment. Labelled thiamin was absorbed via the portal venous system. A mean increase in blood thiamin of 15 μg/l occurred in the healthy subjects 90 min after 5 mg thiamin HCl and about 35 % of the labelled thiamin was recovered from urine over a 72 h period. Oral ethanol (1·5–2 g/kg) caused a 40 % or more reduction in blood and urine thiamin concentrations in 25 % of the normal subjects while a mean decrease of 60 % of blood and urine thiamin concentrations occurred in the alcoholics. Reduced absorptive capacity for thiamin persists despite the receipt of thiamin and other vitamins for 3–4 d but the effects of the alcohol were reversible since thiamin absorption, general nutriture and hepatic morphology had returned to normal after a 2–3-month period of adequate nutrition and abstinence from alcohol (Thomson *et al.* 1970).

In addition, animal studies suggest that chronic alcohol consumption at levels of 20–50 % of energy intake during pregnancy may mobilise fetal vitamin A from the liver and result in increases in vitamin A in fetal organs and subsequent defects (Cogswell *et al.* 2003). A deterioration in the mother's vitamin A stores may also jeopardise the amount of vitamin A in her milk. This could possibly influence the vitamin A status of the breast-fed infant since the human infant is born with very little hepatic vitamin A; it depends on its mother's milk in the early months of life for vitamin A. Chronic alcohol abuse is known to interfere with the storage of liver vitamin A in adults (Leo & Lieber, 1982), and poor control of plasma retinol concentrations could have adverse consequences for the fetus at an earlier stage of development, as excess vitamin A is known to have teratogenic effects.

Alcohol abuse results in a striking depletion of hepatic vitamin A (Leo & Lieber, 1982). Ethanol is oxidised to acetaldehyde by two enzyme systems; alcohol dehydrogenase and the microsomal ethanol oxidising system. The predominant enzyme in the latter system is CYP2E1 (McCarver, 2001). Isozymes of alcohol and other dehydrogenases convert ethanol and retinol to their corresponding aldehydes *in vitro*. In addition, new pathways of retinol metabolism have been described in hepatic microsomes that involve, in part, CYP enzymes, which can metabolise various drugs. In view of these overlapping metabolic pathways, it is not surprising that multiple interactions between retinol, ethanol and other drugs occur. Accordingly, the prolonged use of alcohol, drugs, or both, results not only in a decreased dietary intake of retinoids and carotenoids, but also accelerates the breakdown of retinol through cross-

induction of degradative enzymes. In addition, acetaldehyde interacts with liver stellate cells, the main storage sites of retinol in the body, stimulating their capacity to produce fibrous tissue and impairing the ability to store retinol (Leo & Lieber, 1999).

Micronutrients and the immune response

The immune response is part of an orchestrated series of responses by the body known as the acute-phase response (APR) that follows infection or trauma. The APR is essentially a protective response of the body against the danger posed by disease and is designed to facilitate both the inflammatory and repair processes, and to protect the organism against the potentially destructive action of inflammatory products (Steel & Whitehead, 1994). For example, tissue damage can be controlled or reduced by limiting cytokine production, neutralising reactive oxygen intermediates, inhibiting proteinases, etc (Tilg et al. 1997). Reductions occur in the concentration of several plasma nutrients with the onset of the immune response and these may be misinterpreted as nutrient deficiencies (Thurnham, 1997a). These falls in circulating nutrient concentrations can occur very rapidly and initially they are probably a consequence of increased endo- and epithelial permeability which is an early effect of the APR. Subsequently, the low nutrient concentrations may be maintained by other factors (described later; pp. 222–226), indicating that lower plasma concentrations are part of the APR and may have a protective function. If such 'apparent-deficient states' are protective, then administering large quantities of micronutrients at such times may upset this homeostasis. Table 2 illustrates examples of some adverse effects of nutrient supplementation in situations where an inflammatory environment may exist in vivo. This section will attempt to examine some of these issues and suggest explanations for the changes seen. Low plasma nutrient concentrations also occur in true nutrient-deficient states when the body maximises mechanisms for nutrient economy and possibly the same mechanisms operate in disease states.

Vitamin A

Hyporetinolaemia is an early feature of many infections (Reddy et al. 1986; Thurnham & Singkamani, 1991; Louw et al. 1992), predominantly due to a reduced hepatic synthesis of retinol-binding protein (RBP; Rosales et al. 1996). RBP, like transthyretin, albumin and transferrin, is a negative acute-phase protein (APP) and the rapidity of the response following infection suggests that the changes in RBP synthesis accompany those of the other APP initiated by cytokines IL-1 and IL-6. The rapid fall in plasma retinol associated with infection may initially be due to an increased capillary permeability facilitating quicker distribution of retinol to tissues where it is needed to counter the infection. Labelling studies suggest that vitamin A in the extravascular pool is returned only slowly (37 d) to the plasma (Green & Green, 1994); hence the recovery of plasma retinol concentrations following infection is dependent on a resumption of RBP synthesis and mobilisation from the liver.

Vitamin A has been known as the anti-infection vitamin for more than 50 years but its role in immune mechanisms is somewhat non-specific. Meta-analyses of eight major community vitamin A supplementation studies showed an overall reduction in mortality of 23 % (Beaton et al. 1993) and vitamin A supplements have also been shown to reduce the severity of severe diarrhoea and to be vitally important for the treatment of measles (Barklay et al. 1987; Hussey & Klein, 1990). However, the precise role of vitamin A in the immune response is still not known with certainty. As indicated elsewhere, retinoic acid is important for the control of cellular differentiation and this has important implications regarding the maintenance of epithelial tissues as barriers to infection and in the cellular expansion of immune cells following antigenic stimulation. Vitamin A is also involved in the activation of macrophages and may have a more specific role in T-cell-mediated responses and indirectly in the humoral response, i.e. the synthesis and secretion of immunoglobulins (Ross & Hammerling, 1994; Ross, 1996, 1998). It was recently shown that 9-cis-retinoic acid specifically enhances T-helper (Th) 2 development and that vitamin A deficiency biases the immune response in the Th1 direction (Stephensen et al. 2002b). It should be noted, also, that the active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), has also been shown to be a potent inducer of monocytic differentiation (Kreutz & Andreesen, 1990) and in this and many of its other functions, calcitriol appears to need retinoic acid to assist its binding to DNA and transcriptional functions (Gianna et al. 1996). For more detail, see the paragraphs later (p. 226) on vitamin D, and on vitamin A in the following section (p. 226).

Vitamin C

Evidence for changes in blood vitamin C concentrations associated with infection was first reported in the early 1970s when rapid falls in leucocyte ascorbate concentration were documented within 24 h of the onset of the common cold (Hume & Weyers, 1973) or following surgery (Irvin et al. 1978). Leucocyte ascorbate concentrations normalised over the next 3–6 d and the changes were shown to be due to the rapid influx into the circulation of newly synthesised leucocytes, most of which were neutrophils and contained very little vitamin C. Normalisation in leucocyte ascorbate concentrations can be accompanied by lower plasma concentrations (Vallance et al. 1978). The leucocytosis is of course a feature of the APR (Sipe, 1985) and the dilution of the resident polymorphonuclear leucocytes by the newly released, vitamin C-depleted polymorphonuclear leucocytes accounts for the apparent fall in leucocyte ascorbate concentration associated with stress conditions.

Plasma ascorbate concentrations are strongly linked to dietary intake and limited by a renal threshold of approximately 80 μmol/l (Friedman et al. 1941). In patients with disease, concentrations of plasma ascorbate are frequently lower than those seen in well-nourished subjects, but of course this may well be due to the anorexia of infection (Thurnham, 1990b). Nevertheless, administration of therapeutic doses of IL-2 to patients with malignancies produced a profound fall (80 %) in plasma ascorbate concentrations but the fate of the vitamin C in these patients is not known

and plasma ascorbate concentrations returned to pre-treatment values when treatment was stopped (Marcus *et al*. 1991). While these changes in concentration may be due to a redistribution of vitamin C within the tissues, it would be interesting to know where the vitamin C was localised during treatment with IL-2.

The accumulation of ascorbate by neutrophils in response to stress (Moser & Weber, 1984) is clearly a necessary part of neutrophil function as the ability is absent in the neutrophils of patients with chronic granulomatous disease and whose cells are unable to produce superoxide when stimulated (Washko *et al*. 1993). However, large supplements of vitamin C (≥ 600 mg/d with and without vitamin E) can inhibit superoxide production (Herbaczynska-Cedro *et al*. 1994, 1995; de la Fuente *et al*. 1998) while smaller amounts (200 mg/d) enhance it (Jayachandran *et al*. 2000). Thus, large vitamin C supplements may be counter-productive to neutrophil free radical generation and cyto-toxicity, although both high and low doses of supplementary vitamin C have been found to increase neutrophil adherence and phagocytic capacity (de la Fuente *et al*. 1998; Jayachandran *et al*. 2000). The 'respiratory burst' is the index of phagocytic capacity, and is associated with increased NADPH oxidase (to generate superoxide) and myeloperoxidase activity (to convert H_2O_2 to hypochlorite). Bourgeois (2003) has suggested that ascorbate taken up by neutrophils protects NADPH oxidase and potentially increases its activity since it can react with superoxide to generate H_2O_2. Ascorbate does not destroy peroxide (Anderson & Lukey, 1987); therefore ascorbate assists in the generation of hypochlorite by neutrophil meloperoxidase. The serum concentrations of ascorbate that Bourgeois (2003) referred to, however, ranged from 5 to 50 µg/ml (i.e. 30–300 µmol/l). Such serum concentrations cover the normal range and include concentrations likely to occur for a short time in individuals taking vitamin C supplements. The potential *in vivo* activity of ascorbate provides a rationale for its rapid uptake by the phagocyte at the start of infection.

Large doses of vitamin C administered at the time of inflammation will potentially aggravate oxidant damage within tissues (see the section on pro-oxidant properties of vitamin C; p. 231) and the uptake of ascorbate by neutrophils may provide a safe store for an important antioxidant and lower the risk of increasing inflammation at the site of trauma. However, in convalescent states, there are suggestions that vitamin C supplements may have beneficial effects (Herbaczynska-Cedro *et al*. 1995; Gokce *et al*. 1999; Wilkinson *et al*. 1999). Evidence suggests that vitamin C alters the redox state of arterial smooth muscle guanyl cyclase, thereby altering arterial sensitivity to NO (Murphy, 2002). Also, flow-mediated dilation of the brachial artery has been found to show a significant improvement post-treatment with vitamin C (Gokce *et al*. 1999). A progressive neutrophil leucocyte infiltration of damaged myocardium has been observed during the first 24 h after infarction. This is initially a beneficial process designed to remove damaged tissue. However, if the process continues it may exacerbate myocardial injury due to the over-production of reactive oxygen intermediates (ROI), and endothelial cells can be the primary target of immunological injury resulting in vasculopathy and organ dysfunction (Grech *et al*. 1996). As indicated earlier, vitamin C and E supplements increase neutrophil adherence, chemotactic and phagocytic capacity, coupled with a reduction in superoxide production (de la Fuente *et al*. 1998). Furthermore, 250 mg vitamin C daily for 6 weeks was found to significantly reduce monocyte adhesion to endothelial cells in subjects with low compared with high concentrations of plasma ascorbate (mean 32 and 67 µmol/l respectively). Thus vitamin C (with or without vitamin E) supplements in convalescent states such as coronary artery disease appear to improve blood flow through damaged tissues, reduce infiltration by monocytes and possibly reduce the net superoxide production from infiltrating neutrophils. That is, vitamin C supplements in the post-traumatic stage may assist the healing process, by promoting endothelial function.

Vitamin D

The active metabolite of vitamin D_3, $1\alpha,25$-dihydroxyvitamin D_3 or calcitriol, has a central role in Ca homeostasis and most of the biological actions of calcitriol are mediated through its ability to bind to specific genes and modulate gene transcription. Calcitriol also displays profound antiproliferative, pro-differentiating and immunosuppressive properties (Lemire, 1995; Christakos *et al*. 1996) and, in many of its effects on gene transcription, calcitriol is believed to interact with retinoic acid, the active form of vitamin A. Vitamin A has long been known as the 'anti-infective vitamin' and emerging literature suggests that more attention should perhaps be directed at its interactions with vitamin D in understanding the immunological functions of vitamin A.

Calcitriol has prominent immunomodulatory properties (Lemire, 1992) and receptors for calcitriol have been identified on many cells of the haematolymphopoietic system (Hannah & Norman, 1994). More than fifty gene products have been identified indicating metabolic involvement by vitamin D in mineral homeostasis, autoregulation of vitamin D metabolism, cell differentiation and proliferation, bone and extracellular matrix proteins, growth factors, hormones and others (Hannah & Norman, 1994). Of particular interest are the interactions between vitamins D and A in gene transcription. The vitamin D receptor (VDR) binds to the vitamin D response element (VDRE) in the 5' flanking region of target genes but a number of reports have suggested that a nuclear accessory factor is required to assist binding to DNA and transcriptional function. Retinoid X receptor (RXR), which binds 9-*cis*-retinoic acid, is reported to assist binding of VDR to VDRE in some genes and evidence suggests that the VDR-RXR heterodimer is the functional transactivating species (Yu *et al*. 1991; Christakos *et al*. 1996). For more detail, see also the section on vitamin A and gene regulation (p. 226).

The primary source of calcitriol is the proximal convoluted tubular (PCT) cells in the kidney (Brunette *et al*. 2003). Studies using vitamin D-depleted animals support the endocrine role of parathyroid hormone-stimulated PCT in maintaining circulating levels of 1,25-dihydroxyvitamin D_3 during vitamin D insufficiency. The renal synthesis of calcitriol is primarily responsible for Ca homeostasis by

stimulating dietary Ca uptake by the gastrointestinal tract. In addition, since the PCT cells are the major site of Ca and phosphate absorption, local expression of vitamin D-1α-hydroxylase probably also up regulates Ca receptor and sodium phosphate co-transport protein expression (Bland *et al*. 2001).

However, vitamin D-1α-hydroxylase activity has also been shown to be present in other tissues, for example, mature monocytes and macrophages, activated T and B lymphocytes (Provvedini *et al*. 1983) and cells in the more distal areas of the nephron (Bland *et al*. 2001). Experimental studies using cells from the distal region, namely a human cortical collecting duct line, have demonstrated that vitamin D-1α-hydroxylase activity is increased under normocalcaemic conditions by added calciotropic regulators forskolin, calcitonin and parathyroid hormone, and that there is potent induction by bacterial lipopolysaccharide (LPS; Bland *et al*. 2001). It has also been shown that relatively high concentrations of extracellular Ca at sites of injury and infection may modify immune responses and enhance monocyte chemotaxis (Olszak *et al*. 2000). The synthesis of calcitriol may also be increased in such conditions.

Calcitriol has been reported by many workers to inhibit the proliferation of several human or murine promyelocytic leukaemia cells and promote the differentiation of the cells to the monocyte or macrophage phenotype (Abe *et al*. 1981; Miyaura *et al*. 1981; Nakamura *et al*. 1996). Such cells express the CD14 antigen, non-specific esterase activity and become plastic-adherent (Nakamura *et al*. 1996). The CD14 antigen is characteristic of monocytes and mediates the binding of LPS (Testa *et al*. 1993). It has been suggested that transforming growth factor-β may potentiate the calcitriol-induced differentiation whereas alone it is largely ineffective (Testa *et al*. 1993). Following the culture of leukaemic cells lines treated with both calcitriol and transforming growth factor-β, CD14 antigen was comparable with that found on normal monocytes, while the production of adhesion molecules of the β2-integrin family (CD11a, b and c) exceeded those produced by cells treated with calcitriol only.

Thus the production of calcitriol at sites other than the PCT cells appears to be responsive to infection and promote maturation and differentiation of the monocyte or macrophage. However, the clinical use of calcitriol or its analogues tends to be for its anti-inflammatory properties, for example, in the treatment of psoriasis (Kejian & Mrowietz, 1998). Calcitriol and analogues of vitamin D have been shown to have immunosuppressive properties in autoimmune disease and organ transplantation (Lemire, 1995). This may be partly attributable to an inhibition of pro-inflammatory cytokine production (TNF-α and IL-6) by peripheral blood mononuclear cells (Shibaki *et al*. 1998) and a reduction in phagocytic activity (Spittler *et al*. 1997). However, others have reported calcitriol to promote phagocytosis and intracellular killing by macrophages (Bar-Shavit *et al*. 1981). In addition, evidence suggests that calcitriol inhibits the expression of B7·2 molecules, expressed on antigen-presenting cells to engage with the counter receptor, CD28, on T-cells. In this way calcitriol may inhibit the ability of monocytes and macrophages to induce T-cell activation (Lemire, 1995; Clavreul *et al*. 1998).

Iron

Nutritional deficiency of Fe results in anaemia, and growth may also be impaired. Both anaemia and poor growth are common in many developing countries, where both exposure to and rates of infection are particularly high. There are rapid falls in the plasma concentrations of Fe and Zn following the onset of infection and even before the onset of any fever. The greatest falls occur in those who subsequently develop fever (Pekarek *et al*. 1969; Beisel, 1976). The hypoferraemia of infection is accompanied by changes in plasma concentrations of several Fe-binding proteins (Thurnham & Northrop-Clewes, 2003) that facilitate the uptake of Fe by the reticulo-endothelial system or removal and re-utilisation of Hb from effete erythrocytes. To understand the potential role of infection in contributing to Fe and Zn deficiencies, it is important to understand the purpose and possible functions of those pathological responses to infection that affect these minerals. For a discussion on the pro-oxidant properties of Fe and the potentially protective role of hypoferraemia, see p. 227 and 231.

Fe affects lymphocyte activation and proliferation and how macrophages handle Fe. The proliferative phase of lymphocyte activation is an Fe-requiring step as Fe is essential for enzymes such as ribonucleotide reductase, which is involved in DNA synthesis. Hence a large number of clinical studies have found reduced T-cell function *in vivo* as manifest by impaired skin-test reactions and reduced *in vitro* proliferation of T-cells in Fe-deficient individuals (Brock, 1993). The extent to which mild anaemia, the major problem in developing countries (Stoltzfus, 1997), impairs lymphocyte proliferation and impairs immune responses is more difficult to evaluate.

Zinc

Zn is an essential element for the functioning of more than 300 metalloenzymes, and the highly proliferative immune system is reliant on Zn-dependent proteins involved in general cellular functions such as replication, transcription and signal transduction (Wellinghausen *et al*. 1997). In addition, experimental data suggest that Zn deficiency depresses the recruitment and chemotaxis of neutrophils, impairs natural killer cell activity, impairs phagocytosis by macrophages and neutrophils and impairs the generation of the oxidative burst (Rink & Gabriel, 2000). Severe Zn deficiency, as described in the rare autosomal recessive disease acrodermatitis enterohepatica, is accompanied by thymic atrophy and a high frequency of bacterial, viral and fungal infections if not treated (Nelder & Hambidge, 1975). Zn influences all immune cell subsets, but Zn is especially important in the maturation and function of T lymphocytes because Zn is an essential cofactor for the thymus hormone, thymulin (Wellinghausen *et al*. 1997). It is suggested that Zn deficiency influences Th1 more than Th2 cells. The production of interferon-γ, IL-2 and TNF-α by Th1 cells is decreased, whereas the production of IL-4, IL-6 and IL-10 by Th2 cells is not affected (Prasad, 1998). Th1 lymphocytes are important in cell-mediated immunity and responsible for IL-2 and interferon-γ release while Th2 cells are linked to antibody-mediated immunity and the production

of IL-4, IL-6, IL-10 and IL-13 (Prasad *et al*. 1997). The control of T-lymphocyte activation is delicately regulated by Zn, and the physiological plasma Zn concentration of 12–16 µmol/l is optimally balanced for T-cell function (Wellinghausen *et al*. 1997).

With the onset of infection, plasma Zn concentrations fall rapidly; in febrile illness the fall can be as much as 70 % (Beisel, 1976). About 65 % of plasma Zn is bound to albumin and there is also a prompt fall in albumin and other proteins of a similar size after injury, due to an increase in microvascular permeability. However, albumin alone cannot account for the fall in plasma Zn concentrations, as the Zn:albumin concentration ratio falls by almost 50 % (Fleck & Myers, 1985). The main reason for the fall in plasma Zn concentrations is probably a consequence of monocyte stimulation by trauma or bacterial products, because experimental IL-1 infusions decrease plasma Zn concentrations and increase metallothionine transcription in hepatocytes (Moncada *et al*. 1991). During this APR, Zn is redistributed from the plasma to the liver and to lymphocytes (Beisel, 1995). The advantage to the host of this response may be deprivation of Zn from invading pathogens (Clohessy & Golden, 1996). It is also important to note that while 12–16 µmol/l is the optimal plasma Zn concentration for T-cell function in healthy subjects, higher concentrations can be inhibitory, thus a reduction in plasma Zn may be anti-inflammatory (Wellinghausen *et al*. 1997). Workers have also shown that the mitogenic properties of Zn, i.e. the direct induction by Zn of cytokine production in polymorphonuclear leucocytes, is enhanced by bacterial LPS and phytohaemagglutinin at concentrations of Zn which would not normally be mitogenic (Wellinghausen *et al*. 1997). Hence, while the early fall in plasma Zn concentrations in response to injury might be considered an immunological disadvantage, the presence of bacterial antigens together with low plasma Zn may in fact optimise T-cell stimulation.

Low plasma Zn concentrations (<10·7 µmol/l) have been associated with not only reduced growth and development, but also impaired immunity and increased morbidity from infectious diseases (Brown *et al*. 1998). The response to Zn supplements, however, is variable and may be dependent on whether plasma Zn concentrations reflect a true Zn deficiency or an infection-associated depression of plasma Zn. A meta-analysis of supplementation studies in children below 13 years showed that overall there was a highly significant positive impact of Zn on change in weight (Brown *et al*. 1998). However, a review of eight randomised controlled intervention trials performed recently in less-developed countries found no evidence that maternal Zn supplementation promotes intra-uterine growth. There was evidence to suggest beneficial effects on neonatal immune status, early neonatal morbidity and infant infections. However, evidence was conflicting with respect to labour and delivery complications, gestational age at birth, maternal Zn status and health and fetal neurobehavioural development (Osendarp *et al*. 2003). However, Zn supplements have been shown to provide beneficial effects against diarrhoea (Zinc Investigators Collaborative Group, 2000) and this is possibly the best evidence of the widespread nature of Zn deficiencies in developing countries, as described below.

There is some evidence to suggest that an overproduction of intestinal NO may be responsible for the diarrhoea associated with Zn deficiency. Experimental studies show that inducible NO synthase (NOS) is elevated in the intestinal cells of Zn-deficient compared with Zn-adequate rats. A challenge with IL-1α increased intestinal inducible NOS activity in all groups but inducible NOS expression was more marked and prolonged in the Zn-deficient groups compared with other groups (Cui *et al*. 1997). Subsequently, the same workers (Cui *et al*. 1999) also showed that intestinal microvascular permeability (demonstrated by extravasation of Evans Blue) was greater in Zn-deficient compared with Zn-adequate rats. The administration of an inhibitor of inducible NOS for 2 weeks in the drinking water inhibited the extravasation of Evans Blue but not inducible NOS mRNA of the different groups of rats (Cui *et al*. 1999). Thus preventing the production of NO prevented extravasation produced by increased intestinal microvascular permeability, but not the increase in inducible NOS activity produced by Zn deficiency.

Adverse consequences of Zn supplementation have also been reported (Table 2). Fever was greater in patients on home parenteral nutrition with catheter sepsis who were given Zn supplements (30 mg/d) compared with those given 0 or 23 mg/d (Braunschweig *et al*. 1997). Depressed immune responses were observed in another study where patients were given 100–300 mg/d (Chandra, 1984). Likewise, the phagocytic and fungicidal activity of monocytes was impaired, and the duration of episodes of impetigo was increased in a group of marasmic infants who were given a Zn-fortified formula in a double-blind controlled trial (Schlesinger *et al*. 1993). The results were seen between 60 and 150 d of Zn supplementation and suggest that Zn supplements even at the recommended dietary allowance level may impair monocyte function.

Selenium

Se status is assessed by the concentration in plasma or the activity of erythrocyte glutathione peroxidase (GPx). The latter is the better measure of functional status, while plasma concentrations reflect differences in the dietary intake as seen for example in Japanese individuals consuming different Se intakes in Japan and Brazil (Karita *et al*. 2001) and the increase in plasma Se in response to Se supplements (Greenman *et al*. 1988). Serum Se is also reported to be low in disease (Galloway *et al*. 2000; Maehira *et al*. 2002) and to be a sensitive predictor of survival in individuals with HIV (Baum *et al*. 2002). While low serum Se may indicate anorexia or low dietary intake during infection, recent experimental work suggests that as part of the APR to disease, there is a redistribution of body Se from the plasma and liver to the muscles and other tissues such as spleen and thymus (Maehira *et al*. 2002). Using hepatocytes, these workers also showed that the concentration of plasma Se found in human diseases (mean 0·82 (range 0·36–1·28) µmol/l was optimal for stimulation of the transcription factor NF-κB, and synthesis of the APP, C-reactive protein (0·5–1·0 µmol/l). Se at physiological levels (1·54 (range 0·94–2·14) µmol/l) inhibits NF-κB transcription (Maehira *et al*. 2003). Later in the section on pro- and

antioxidants, the central role of NF-κB in coordinating the innate immune response is more fully discussed (p. 229).

However, not all the immunological properties of Se would appear to be optimal at the plasma concentrations found in disease. Others have shown that Se supplementation in patients with HIV is associated with an up regulation of IL-2 and increased activation, proliferation, differentiation and programmed cell death of Th cells. IL-2 is important for the generation of cytotoxic T-lymphocytes and for the activation and proliferation of natural killer cells. Cytotoxic T-lymphocytes are important for the recognition and removal of virus-infected cells, while natural killer cells are important for the non-specific killing of tumour cells (Baum et al. 2002). However, the apparently different requirements for Se for activation of NF-κB and T-cell function may both be met by the redistribution of Se which takes place as part of the APR (Maehira et al. 2002). Concentrations of Se fall in the liver and plasma but increase in the spleen and thymus, tissues where Se is normally concentrated (McKenzie et al. 1998). The benefits of Se supplementation in HIV may be due to the prolonged nature of the disease and a high utilisation of Se.

Se is a constitutive component of the enzyme GPx. The enzyme exists in several isoforms, with slightly different functions, but is found in all tissues (Halliwell & Gutteridge, 1985). One of the main functions of GPx is to convert lipid peroxides to the hydroxy acid and H_2O_2 to water. Thyrodoxin is a specific seleno-peroxidase found in the thyroid where it removes peroxides (Howie et al. 1998). Se deficiency partially blunts the thyroid response to I supplements (Zimmermann et al. 2003). The clinical effect of this may be that in children with both deficiencies, the combined deficiencies may worsen the goitrous condition and/or lead to its earlier appearance, although oral iodised oil is an effective method of I repletion in goitrous children. Experimental work has also shown that when Se is deficient, a high I intake may allow thyroid tissue damage due to a lack of Se-dependent GPx activity during thyroid stimulation (Hotz et al. 1997; Howie et al. 1998).

Disease and trauma are linked with increases in redox stress within tissues (Evans & Halliwell, 2001) and experimental studies indicate that certain viruses may take advantage of compromised antioxidant status. Keshan disease in China is geographically associated with Se deficiency, but temporal fluctuations in incidence suggest that other factors are involved in its aetiology. Chinese workers suspected that enteroviruses, and particularly coxsackieviruses, were responsible for the cardiomyopathy; experimental studies showed that Se-deficient mice were more susceptible than Se-supplemented mice to the cardiotoxic effects of coxsackievirus B4 that had been isolated from the blood of a Keshan disease victim (Beck et al. 1994). These initial observations were explored by Beck and colleagues who found that susceptibility to these viruses was not specifically associated with Se deficiency but could also be increased by vitamin E deficiency or a combination of vitamin E deficiency and PUFA excess. Their work showed that a previously avirulent strain of coxsackievirus CVB3/0 was changed to a virulent phenotype when passaged through vitamin E-deficient animals (Beck et al. 1994). More recently, these workers have also reported that similar

observations had been made in mice given excess Fe and in GPx 'knock-out' mice (Beck, 1999). Analysis of the genomic structure of the newly developing viruses led Beck and colleagues to suggest that increased oxidative stress in disease facilitates the enhanced growth rate of the invading virus, increasing the probability of development of more virulent mutations (Beck & Matthews, 2000). That is, it was not Se deficiency specifically which promoted the development of more virulent viruses, but impaired antioxidant status in appropriate tissues and cells.

Micronutrients in gene expression

Vitamin A and gene regulation

The process of cell differentiation takes place in all tissues through the body. It has been known for a long time that epithelial tissue differentiation was sensitive to vitamin A deficiency, as the normal mucous-secreting cells were replaced by keratin-producing cells. This is the basis of the pathological process termed xerosis that leads to the drying of the conjunctiva and cornea of the eye. The process can be reversed by treatment with vitamin A and it has become clear that vitamin A plays a hormone-like role in controlling the differentiation of cells in tissues and organs throughout the body.

Within tissues all-trans-retinol is in association with cellular RBP and can be oxidised to all-trans-retinoic acid and to 9-cis-retinoic acid. These oxidised forms of retinol are transported by cellular retinoic acid-binding proteins to the nucleus where they tightly bind to one or more nuclear receptors. There are two families of retinoic acid receptors (RAR and RXR) and three members of each family have been identified (α, β and γ). Both the 9-cis- and all-trans-retinoic acids bind to the RAR members, while the most important ligand for RXR is the 9-cis isomer. When retinoic acid binds to the nuclear receptor, the complex regulates gene expression by binding to short sequences on DNA known as retinoic acid response elements or enhancers. The expression of the six receptors varies between cells but most, if not all, cells express at least one of the receptors. Several hundred genes have been shown to be induced or repressed by retinoids (Blomhoff, 1994).

Vitamin D and gene regulation

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 or calcitriol, also exerts its effects on gene transcription through the VDR. There are several different VDR alleles which are strongly linked to bone mineral density (Morrison et al. 1994). The calcitriol–VDR complex migrates to the nucleus where it binds to the VDRE in the 5' flanking region of target genes to elicit the appropriate transcriptional response. There are reports, however, that for many of the vitamin D-responsive genes, calcitriol works in association with vitamin A. RXR in accompaniment with 9-cis-retinoic acid is reported to assist the binding of VDR to VDRE and that the VDR-RXR heterodimer is the functional transactivating species (Yu et al. 1991; Christakos et al. 1996). The RXR and VDR are members of a super-family of transcription factors that become active

after binding to their cognate ligands. The ligands for these receptors are small lipophilic molecules that include the steroid and thyroid hormones and the vitamins A and D (Mangelsdorf, 1994).

It is interesting to speculate whether the possible need for vitamin A to assist vitamin D-linked Ca absorption can explain the effect of oestrogen therapy in the prevention of bone loss in post-menopausal women (Gallagher, 2001). It was recently reported that while calcitriol up regulated trans-cellular Ca transport across Caco-2 cells, 17 β-oestradiol had no effect by itself and did not increase the effects of calcitriol when the two compounds were added together (Cotter & Cashman, 2003). However, as mentioned earlier (p. 212), hormone-replacement therapy significantly increases plasma retinol by 15–20 % (Ballew et al. 2001). Concentrations of plasma retinol are normally tightly controlled and such an increase in circulating concentrations may have important effects at the cellular level, one of which might be an increase in tissue retinoic acid concentrations. Such effects in the elderly might be to increase the effectiveness of vitamin D in promoting Ca absorption and preventing bone loss. It would be interesting to see what effects the addition of 9-cis-retinoic acid might have on vitamin D-stimulated transepithelial Ca transport in Caco-2 cells.

Genetic regulation of vitamin C transport

The evidence discussed earlier (p. 222) suggests that vitamin C has an important role in immune function and the rapid changes in tissue concentrations associated with infection suggest that mechanisms of tissue uptake are under the control of specific, infection-related factors. Man lacks the capacity to synthesise vitamin C because a non-functional gene is carried for the enzyme L-gulonolactone oxidase, which is required for the final step in the synthesis of ascorbate from glucose. However, it has recently been shown that, in common with the rat (an animal which does synthesise vitamin C), we possess two genes which encode L-ascorbic acid transporter enzymes SVCT1 and SVCT2 (Wang et al. 2000). Both enzymes are extremely substrate specific and do not transport glucose. SVCT1 transport activity was found to be sensitive to extracellular pH (optimum pH about 7·5) that may affect the binding activity for L-ascorbic acid. Of particular interest also was the fact that tissue distribution tended to be organ specific. SVCT1 had a limited distribution, mainly confined to the bulk transporting epithelia such as the kidney and small intestine while SVCT2 displayed a much broader distribution in the brain, retina, spleen, leucocytes and in several endocrine and neuroendocrine tissues. The reason for the different distribution of the two forms is not yet known, but their distribution may suggest they are differentially regulated.

For example, recent work suggests that Zn induces osteoblastic differentiation by stimulating the expression of SVCT2 enzymes to enable vitamin C to traverse the membrane of the immature osteoblast from the extracellular space (Wu et al. 2003). The differentiation of osteoblasts requires the secretion of a type I collagen-containing extracellular matrix by osteoblast precursors and vitamin C is an essential vitamin for prolyl and lysyl hydroxylation necessary for the maturation of collagen peptide.

A further interesting aspect of the gene for SVCT1 is its localisation on chromosome 5, within a region that is commonly deleted in malignant myeloid diseases. It is suggested that its localisation raises the possibility that SVCT1 may play a role in tumour suppression, as there is evidence that L-ascorbic acid has cytotoxic effects on leukaemia cells in vitro (Iwasaka et al. 1998; Roomi et al. 1998).

In contrast to L-ascorbic acid, the oxidised form of vitamin C, dehydro-L-ascorbic acid (DHAA), enters cells via GLUT where it is then converted back to L-ascorbic acid (Root-Bernstein et al. 2002). Transport of DHAA by the GLUT mechanism is obviously influenced by the prevailing concentrations of plasma glucose. Root-Bernstein et al. (2002) suggest that diabetic neuropathy, retinopathy and nephropathy are a consequence of elevated DHAA concentrations, when plasma glucose concentrations rise so high that transport of plasma DHAA into cells is blocked. They suggest that cells in nerves, retina and kidney preferentially take up DHAA rather than L-ascorbate and, that in the face of elevated glucose concentrations, the uptake of DHAA is prevented. The cells are thus deprived of an essential antioxidant and pathologies develop. Interestingly, it was reported over 30 years ago that injections of DHAA into rats stimulated the release of insulin and lowered blood glucose. That is, DHAA may interact with insulin and glucose in a negative-feed-back manner to ensure that vitamin C activity is regenerated (Edgar, 1970). However, even if insulin production were impaired, DHAA is unstable and would be rapidly catabolised to 2,3-diketo-gulonic acid. The latter has no vitamin C activity and its formation could lead to a gradual reduction in vitamin C status. Low plasma concentrations of ascorbate have been reported recently in newly diagnosed diabetics in two large surveys, and, while Will et al. (1999) maintain that the differences in vitamin C concentrations from control subjects disappear after correction for important covariates such as sex, age and dietary intake, others found that the differences still remained after correction (Sargeant et al. 2000). Further research in this area of cellular vitamin C transport is awaited with interest.

Control of iron metabolism

The regulation of Fe metabolism is normally under the control of Fe-regulatory proteins (IRP). These are cytoplasmic proteins that coordinate cellular Fe traffic by binding to sequences on mRNA known as Fe-responsive elements and protecting them from degradation. The mRNA involved encode proteins responsible for Fe uptake, storage and utilisation. As a consequence, in Fe deficiency IRP bind to mRNA and promote the expression of transferrin receptor protein while ferritin synthesis is repressed. Hence the utilisation and absorption of Fe is increased. When Fe is adequate, ferritin synthesis is promoted and Fe storage occurs (Hesketh et al. 1998).

TNF-α has an important role in Fe homeostasis since it: (a) induces expression of apoferritin mRNA and the synthesis of ferritin in adipocytes and muscle cells; (b) induces increased transferrin receptor mRNA and transferrin receptor protein in fibroblasts; (c) inhibits the release of Fe from peritoneal macrophages; (d) reduces the incorporation of plasma Fe into newly synthesised erythrocytes. The admin-

istration of TNF-α to experimental animals leads to reduced serum Fe concentrations (Gordeuk *et al*. 1992), and a reduction of plasma Fe in the incubation period of most generalised infectious processes also occurs in man (Beisel, 1976).

In infection, the cytokines TNF-α and IL-1 are increased and these reorganise the normal control of Fe metabolism. Plasma ferritin levels increase in spite of the hypoferraemia since ferritin mRNA is sensitive to both Fe and cytokines. It has been found that rat hepatoma cells exposed to IL-1 and TNF-α double the amounts of ferritin released into the medium over 24 and 48 h (Tran *et al*. 1997), and others have reported that TNF-α in particular promotes ferritin translation, resulting in increased Fe storage in a human monocytic cell line (Fahmy & Young, 1993). NO is also increased in infection and recent data suggest that NO may also have a direct role in the post-transcriptional gene regulation mediated by IPR. NO is produced both by macrophages *in vivo* as a physiological response to infection and by a variety of cell types as an intracellular messenger (Arroyo *et al*. 1992). In a low-Fe environment, interferon-γ, TNF-α, IL-1 or LPS induce macrophage NOS, and NO is central to macrophage-mediated cytotoxicity (Moncada *et al*. 1991). NO activates the mRNA-bonding activity of IRP and in this regard mimics the consequences of Fe starvation (Pantopoulos *et al*. 1994). NO-induced binding of IRP to Fe-responsive elements specifically represses the translation of transfected Fe-responsive element-containing indicator mRNA as well as the biosynthesis of the cellular Fe-storage protein, ferritin. These findings illustrate a regulatory connection between the NO-NOS pathway and cellular Fe metabolism (Weiss *et al*. 1993).

Hereditary haemochromatosis is a disease characterised by progressive Fe overload which, if undetected, can lead to cirrhosis, diabetes mellitus, cardiac disease, arthritis or hepatocellular carcinoma or a combination of these. The basic deficit appears to be an increase in Fe absorption, decrease in Fe excretion and production of preferential deposits of Fe in hepatic parenchymal cells rather than Kupffer cells. Recent evidence suggests that TNF-α may be involved in the aetiology of the disease because of its location on chromosome 6 and its effect upon Fe transport. The haemochromatosis gene is tightly linked to the HLA complex on the short arm of chromosome 6. TNF-α, which is also located on chromosome 6, down regulates Fe absorption and a lower production of TNF-α occurs in stimulated monocytes from patients with haemochromatosis in comparison with controls (Gordeuk *et al*. 1992; Conrad *et al*. 1994). Changes in Fe metabolism in haemochromatosis can be contrasted with those which occur during inflammation but authors caution that the lack of TNF is not necessarily opposite to the effects of increased TNF (Gordeuk *et al*. 1992).

Implications of polymorphisms in the haptoglobin gene on vitamin C and iron

Serum haptoglobin (Hp) comprises two protein chains, and there are two forms of the α chain that differ by one amino acid giving rise to three variants of the Hp molecule in serum. Hp 1-1 comprises two α-1 and two β chains. Hp 1-2 comprises five proteins with different combinations of the three chains and Hp 2-2 comprises four variants of the α-2 chain and one β chain. It seems that Hp 1-1 has the best Hb-binding capacity and because Hp 2-2 binds Hb less well, certain aspects of the immune response are increased (see below) (Delanghe *et al*. 1998). Hp 2-2 appears to have spread from India and the distribution of the three forms is still not stable. Caucasians tend to have approximately 10–20 % Hp 1-1, blacks 30–50 % and Asians <10 %. The Hp 2-2 is most common in Asians (>50 %), lowest in blacks and intermediate in Caucasians (Langlois & Delanghe, 1996).

One phenotype of the Hp gene has been reported to influence vitamin C metabolism. The results indicate a lower stability of vitamin C (higher rate of ascorbic acid oxidation) in Hp 2-2 carriers than the other two types, namely Hp 1-1 and Hp 2-1. It has been shown there was less Hp in the blood of Hp 2-2 individuals. On the basis of this it has been suggested that there would be potentially more Hb-Fe from effete erythrocytes present in serum causing the oxidation of ascorbate (Langlois *et al*. 1997).

Likewise, the same phenotype has also been reported to influence Fe metabolism. It was reported that both uninfected and HIV-infected individuals exhibiting the Hp 2-2 phenotype accumulated more Fe and had greater serum ferritin concentrations than those of the Hp 1-1 or 2-1 phenotypes (Delanghe *et al*. 1998). Thus possession of the Hp 2-2 phenotype may make individuals more susceptible to disease by lowering plasma vitamin C concentrations (although this might be viewed as protective; see the later section on pro-oxidants (p. 231)) and increasing potential inflammatory damage as a result of increased tissue Fe concentrations.

Vitamin status and plasma homocysteine concentrations

Recent work has shown that plasma concentrations of folic acid and vitamin B_{12} and, to a lesser extent, riboflavin can influence plasma homocysteine concentrations. Folate intermediary metabolites are the primary source of one-carbon units for methylation and DNA synthesis and repair. Homocysteine is an important intermediate in the one-carbon cycle. Intracellular homocysteine is either converted to cysteine via the vitamin B_6-dependent trans-sulfuration pathway or is re-methylated to methionine by the cobalamin-dependent methionine synthase (*EC* 2.1.1.13). The latter enzyme requires 5-methyl-THF as a methyl donor. If methionine synthase activity falls through a lack of vitamin B_{12} or of folic acid, the concentration of homocysteine increases (Fig. 1). This explains why vitamin B_{12} and folic acid are major determinants of plasma homocysteine.

The formation of 5-methyl-THF is catalysed by the enzyme 5,10-methylene-THF reductase (MTHFR; *EC* 1.7.99.5) (Selhub, 1999). A commonly occurring C677T polymorphism of the MTHFR gene confers reduced enzyme activity and is associated with moderate increases in homocysteine, particularly in subjects with folate deficiency. Supplementation with folate in doses from 0·2 to 10 mg/d has been shown to reduce both normal and elevated plasma homocysteine concentrations (Ward, 2001).

Riboflavin status has also been shown to influence plasma homocysteine concentrations, but the relationship is essentially confined to subjects with the C677T polymorphism of the MTHFR gene. Riboflavin is a precursor of FMN and FAD, which serve as cofactors for enzymes involved in the metabolism of vitamin B_6, folate and cobalamin. Both riboflavin cofactors are involved in cobalamin metabolism through the enzyme methionine synthase reductase (*EC* 2.1.1.135). FMN is a cofactor for pyridoxine-5'-phosphate oxidase (*EC* 1.4.3.5) through which the active form of vitamin B_6 is generated (pyridoxal-5'-phospate), whereas FAD is a cofactor for MTHFR. MTHFR catalyses the formation of 5-methyl-THF, the methyl donor for methionine synthase. Pyridoxal-5'-phospate is the cofactor for the enzyme which generates 5,10-methylene-THF, the latter being the substrate for both the MTHFR enzyme as well as thymidylate synthase. Riboflavin status was shown to influence homocysteine concentrations in healthy adult Norwegian blood donors (Hustad *et al*. 2000). These workers showed that plasma homocysteine concentrations were 1·4 μmol/l higher in the quartile with the lowest compared with highest riboflavin concentrations. This compared with a 2·8 μmol/l difference between the highest and lowest quartiles for folate and 1·0 μmol/l in the case of vitamin B_{12}. However, the riboflavin–homocysteine relationship was essentially confined to subjects with the C677T polymorphism of the MTHFR gene. Riboflavin status was rated on the basis of plasma riboflavin concentrations, but no erythrocyte glutathione reductase stimulation tests were done. Erythrocyte glutathione reductase stimulation is a better discriminator of poor riboflavin concentrations than plasma riboflavin concentrations; it would be interesting to determine the effects of riboflavin supplements on plasma homocysteine concentrations in individuals or a population with riboflavin deficiency as assessed by erythrocyte glutathione reductase stimulation.

Micronutrients as pro- and antioxidants

Antioxidant nutrients

Cellular integrity.   Disruptions to cellular integrity lead to the rapid release of cytokines by non-specific immune cells of the innate or natural immune system distributed through the body. The cytokines help to mount an inflammatory response and to recruit specialised cells such as mononuclear phagocytes, natural killer cells, neutrophils, etc to the site of damage or infection. It is now known that the rapid induction of the synthesis of these cytokines is coordinated by a common cellular element, a transcription factor known as NF-κB.

NF-κB is critical for the inducible expression of many genes involved in the immune and inflammatory responses including IL-1, -2, -6, -8, TNF-α, TNF-β, serum amyloid A protein, etc. It is reported that NF-κB exists in almost all cells but that it remains in the cytoplasm bound to an inhibitory protein, inhibitory κB. Exposure of cells to various inducers leads to the dissociation of the cytoplasmic complex and the translocation of the free NF-κB to the nucleus. *In vitro* studies have shown that phosphorylation accompanies the dissociation of the NF-κB-inhibitory κB complex with a rapid degradation of the inhibitory κB protein. Inducers, such as TNF-α, can cause significant activation of NF-κB within minutes, allowing NF-κB to function as an effective signal transducer and rapidly connect events in the cytoplasm to response genes in the nucleus. One such response is the rapid up regulation of inhibitory κB-α synthesis, which then helps to shut down the NF-κB response and provide a uniquely suited feedback loop to sensitively control a transient inducer of responsive genes (Kopp & Ghosh, 1995). However, a unique feature of signalling through NF-κB is the diversity of signalling molecules (viruses, ROI, mitogens, cytokines, etc) and situations that activate NF-κB and the types of genes responsive to NF-κB. Nevertheless, the common feature of the inducers is that they all signal situations of stress, infection or injury to the organism (Kopp & Ghosh, 1995).

NF-κB can be activated by ROI (Schreck *et al*. 1992) and common inducers can be inhibited by antioxidants (Schreck *et al*. 1991). Thus the NF-κB mechanism is of particular interest to those wishing to account for the health advantages associated with antioxidant-rich fruit and vegetable diets. *N*-Acetyl-L-cysteine is a precursor of the antioxidant GSH and a scavenger of ROI, and suppresses the activation of NF-κB by many agents. This supports the idea that the redox state of the cell plays a general role in the activity of NF-κB (Schreck *et al*. 1991, 1992; Kopp & Ghosh, 1995). However, *in vitro* studies with micronutrients which influence the redox state have to be carefully interpreted (Erickson *et al*. 2000). In neutrophils, deficiencies of Fe can reduce myeloperoxidase activity (Keith & Jeejeebhoy, 1997) and supplements of vitamins C and E suppress the production of oxygen free radicals (de la Fuente *et al*. 1998). Thus potentially both dietary deficiency and dietary excess can reduce the oxidant activity of neutrophils, thereby disrupting the coordination of the inflammatory response, impairing the killing of bacteria and/or reducing tissue damage. Among the questions that need to be answered, are: is there an optimal redox state *in vivo* to enable efficient bacterial killing with minimal damage to surrounding tissues (Erickson *et al*. 2000) and are certain antioxidant nutrients more important than others in regulating this state? (See earlier discussion on Se pp. 225–226.)

Vitamin E.   Vitamin E falls into the class of conventional antioxidants, which are generally phenols or aromatic amines. The amount of vitamin E in membranes is several thousand times less than the amount of potentially oxidisable lipid (Kornbrust & Mavis, 1979). Under oxidative stress, vitamin E undergoes a very rapid transfer of phenolic hydrogen to the recipient free radical with the formation of a resonance-stabilised phenoxyl radical from the vitamin E. The phenoxyl radical is relatively unreactive towards lipid or oxygen and therefore does not propagate the chain reaction. However, the phenoxyl radical is no longer an antioxidant and to maintain the antioxidant properties of membranes, the vitamin E must be regenerated (Chaudiere & Ferrari-Iliou, 1999). Water-soluble vitamin C is the popular candidate for this role (Packer *et al*. 1979), but thiols and particularly GSH can also function *in vitro* (Niki *et al*. 1982; Wefers & Sies, 1988; Chaudiere & Ferrari-Iliou, 1999).

Vitamin C. Ascorbic acid is a powerful reducing agent and many, if not all, of the biological properties of vitamin C are linked to its redox properties. For example, the essential defects in scurvy such as the breakdown of connective tissue fibres (Myllyla *et al*. 1978) and muscular weakness (Hulse *et al*. 1978) are both linked to hydroxylation reactions, the activity of which requires ascorbate to maintain loosely bound Fe in the ferrous form. Oxidation of the Fe to its ferric form inactivates the enzymes (Bates, 1981). The possible role of Zn status in these interactions may also need to be considered in view of its role in inducing SVCT2 enzyme activity in osteoblast differentiation (Wu *et al*. 2003). In the eye, vitamin C concentrations are about fifty times higher than those in the plasma and may protect against the oxidative damage of light (Koskela *et al*. 1989). Vitamin C displays redox functions in catecholamine biosynthesis (Bates, 1981) and spermatogenesis may also need ascorbate to protect DNA from oxidative damage (Fraga *et al*. 1991). Spermatogenesis needs many more cell divisions than oogenesis, and it is suggested that DNA damage varied inversely with the intake of vitamin C between 5 and 250 mg/d (Fraga *et al*. 1991). Frei (1991) has also shown that vitamin C was superior to all other biological antioxidants in protecting plasma lipids exposed *ex vivo* to a variety of sources of oxidant stress. Last, plasma vitamin C may protect plasma folate concentrations; thus it should be inversely associated with plasma homocysteine concentrations. Jacques *et al*. (1995) reported that dietary vitamin C intakes were inversely associated with plasma homocysteine concentrations in older individuals in the Framingham study and suggested that this is probably largely due to a strong positive association between vitamin C intake and plasma folate concentrations.

Carotenoids. The carotenoids have a general structure comprising two six-membered C rings separated by eighteen C atoms usually in the form of a conjugated chain of double bonds. The chemical properties of the carotenoids closely relate to the extended system of conjugated double bonds and the various functional groups on the terminal ring structures. Although there are many hundreds of carotenoids found in nature, there are relatively few found in human tissues, the five main ones being β-C, α-carotene, lycopene, β-cryptoxanthin and lutein (Thurnham, 1994*a*). The ROI scavenged by carotenoids are peroxyl radicals (Burton & Ingold, 1984; Palozza & Krinsky, 1992), and carotenoids in general, and lycopene specifically, are very efficient at quenching singlet oxygen (Foote & Denny, 1968; Di Mascio *et al*. 1989). Singlet oxygen is generated during photosynthesis, therefore carotenoids are important in protecting plant tissues, but there is limited evidence for this role in man. However, β-C has been used in the treatment of erythropoietic protoporphyria with some success, which is a light-sensitive condition where singlet oxygen might be involved in the pathogenesis (Mathews-Roth, 1986). Otherwise, results from studies suggesting that β-C provides protection against solar radiation are somewhat equivocal. There was no benefit reported when large amounts of β-C were used to treat individuals with a high risk of non-melanomatous skin cancer (Greenberg *et al*. 1990). However, two carotenoids (lutein and zeaxanthin) which occur specifically associated with the rods and cones in the eye (Bone *et al*. 1988) may protect the retinal pigment epithelium against the oxidative effects of blue light (Gerster, 1991).

The antioxidant properties of carotenoids depend on oxygen tension in the surrounding tissue. At low oxygen tension, β-C acts as a chain-breaking antioxidant whereas at high oxygen tension it readily auto-oxidises and exhibits pro-oxidant behaviour (Burton & Ingold, 1984). Palozza (1998) reviewed much of the evidence and suggested that β-C has antioxidant activity between 2 and 20 mmHg oxygen tension, but in air or when oxygen tension is >150 mmHg, it is much less effective and can show pro-oxidant activity as the oxygen concentration increases. The widespread distribution of carotenoids in plants, and the considerable epidemiological evidence that consumption of fruit and vegetables was protective against heart disease, led to three major β-C intervention studies (World Health Organization, 1990). In two of these (ATBC, CARET) the subjects were smokers or individuals who had previously been exposed to asbestos (Heinonen *et al*. 1994; Rowe, 1996). In both there was excessive mortality from lung cancer in the β-C-treated groups. In the third study (Physicians' Study), the subjects were not primarily smokers and the overall conclusion was that β-C caused neither benefit nor harm (Hennekens *et al*. 1996). The evidence that β-C may induce P450 enzymes that activate known pro-carcinogens (Paolini *et al*. 1999) is discussed in the section on smoking (p. 219).

Flavonoids and polyphenols. Polyphenols are compounds which, by definition, are made up from multiple phenol rings. They can be split into two groups; flavonoids and non-flavonoids. Flavonoids are the most common and widely distributed group of phenolics with over 4000 individual flavonoid compounds known to occur in nature (Harbourne, 1994). Structurally they comprise a minimum of at least two phenol moieties linked via a pyran ring. They can be free or polymerised or linked to sugars or other non-flavonoid phenols. Dietary sources of flavonoids are predominantly fruits and vegetables, or products derived from these foods, such as wines and fruit juices. The simpler flavonoids tend to be water soluble and are usually conjugated with various sugars in the form of glycosides. Cooking usually has little effect on the glycosides but colonic bacterial β-glycosidases will hydrolyse the glycosidic link, releasing the aglycone. The aglycones tend to be insoluble and, to be absorbed, must be conjugated to glucuronide or sulfate groups by phase II enzymes (see the section on nutrients and metabolism of drugs; p. 217).

Quercetin is a major flavonol (a sub-class of the flavonoids) which is found ubiquitously in the diet (Hertog *et al*. 1993). There is much evidence to suggest that it is a powerful bioactive constituent of the human diet with powerful antioxidant activity and free-radical scavenging properties (Ramanathan *et al*. 1994; Terao *et al*. 2001). However, most of the work carried out on quercetin has been done on the aglycone as substrate. It is only recently that the identification of quercetin metabolites has been reported, namely quercetin-3-glucuronide, 3'-methyl-quercetin-3-glucuronide and quercetin-3'-sulfate (Day *et al*. 2001). In this report, about 80 mg of metabolites (expressed

as the aglycone equivalent) were recovered after feeding 200 g onion to four volunteers. Only about 0·01 % quercetin was found in plasma as the aglycone. While some of the metabolites found will retain the biological properties of the aglycone, others will not and much work needs to be done to re-evaluate the previous experimental work done with flavonoid aglycones to characterise the important metabolites in man and their biological activity.

As indicated earlier, an adequate dietary intake of polyphenolics is important for their potential beneficial effects in reducing chronic disease. Such effects may be exerted, for example, via their influence on P450 enzymes, potentially preventing the bioactivation of dietary pro-carcinogens. However, there are disadvantages in vegetarian diets in that some phenolic compounds will bind mineral ions, for example Fe, Zn, Ca, etc, and prevent absorption (Hallberg & Hulthen, 2004). In addition, the converse is probably also true, that mineral supplements such as Fe and Ca that bind to polyphenols will also prevent the absorption of polyphenols (R Welsh, unpublished results).

Pro-oxidant nutrients

Most biological antioxidants are potentially pro-oxidants. When an antioxidant molecule accepts an unpaired electron from a free radical, the intermediate formed by the antioxidant becomes itself a free radical. Fortunately, this is mainly a problem for food chemists (Pokorny *et al.* 2001) and does not appear to be a major problem in the human body. However, in the case of vitamin C, changes in plasma concentrations occur which may be linked to its potential to be a pro-oxidant in inflammatory conditions. Likewise, Fe in healthy subjects is carefully controlled by binding to a variety of proteins in plasma and tissues and is unlikely to have pro-oxidant effects. However, in inflammation and disease, changes occur in the handling of Fe which can be interpreted as a way of minimising potential pro-oxidant effects, and these will be described later (p. 232).

Inflammation and vitamin C. During inflammation, several metabolic changes are activated which depress the concentration of some vitamins with antioxidant properties, especially vitamin C. Within 24 h of surgery (Irvin *et al.* 1978; Vallance, 1986) or following an attack of influenza (Hume & Weyers, 1973), leucocyte vitamin C concentrations are depressed. This apparent depression is due to the mobilisation of new neutrophils from bone marrow, which enter the circulation with low to absent concentrations of ascorbate (Irvin *et al.* 1978; Vallance, 1986). The depression continues for 3 to 5 d while the cells gradually acquire vitamin C, probably from the plasma. Several workers have shown that granulocytes will actively take up ascorbate *in vitro* (Evans *et al.* 1982; Moser & Weber, 1984; Wang *et al.* 1997) and where residual inflammation remains, plasma ascorbate concentration tends also to be low (Vallance *et al.* 1978; Riemersma *et al.* 2000). It has been suggested that granulocytes require the ascorbate to protect them from free radical products that they produce during phagocytosis (Hemila *et al.* 1984).

An alternative reason why plasma ascorbate concentrations fall in the presence of inflammation may be to reduce the risk of oxidation of transition metals. *In vivo*, ascorbic acid is an antioxidant but some transition metals, for example the higher valency states of Fe and Cu, are thermodynamically more easily reduced than ascorbate, hence any non-protein-bound Fe^{3+} or Cu^{2+} are reduced to Fe^{2+} and Cu^+ respectively by the presence of ascorbate. However, non-protein-bound transition metals rarely occur in healthy blood and tissues, but in damaged tissues such metals can occur in the emerging fluids (Chevion *et al.* 1993), and the formation of the ferrous ion is potentially very damaging for tissues. Ferrous Fe in particular is a powerful catalyst of the Haber–Weiss and Fenton reactions that form hydroxyl radicals with potentially damaging consequences for any molecule in the vicinity (reactions 4 and 5; p. 232).

Ascorbate frequently catalyses damage in tissues *in vitro* probably because any tissue preparation is likely to be contaminated with unbound Fe. This may be the explanation for the recent suggestion that ascorbate and many of its derivatives have anti-cancer properties (Roomi *et al.* 1998). *In vitro* experiments with a malignant leukaemia cell line (P388D1) suggested that the concentration of ascorbate that inhibited cell growth by 50 % was approximately 17 μmol/l. Mean concentrations of plasma ascorbate tend to fall in the range of 11–20 μmol/l in populations where there is a risk of chronic disease, for example, the elderly (Department of Health and Social Security, 1979). Mean concentrations of plasma ascorbate also tend to fall where there is an increased exposure to disease, for example in the rainy season in developing countries (Bates *et al.* 1982; Knowles *et al.* 1991). Of course, plasma ascorbate concentrations are also strongly correlated with the dietary intake of vitamin C, but healthy populations do tend to have higher concentrations of plasma ascorbate than those exposed to sickness or trauma or who are sick (Thurnham, 1994*b*).

Other workers have suggested that the cytotoxic properties of the ascorbate derivatives against human tumour cell lines is due to their ability to generate H_2O_2 (Iwasaka *et al.* 1998) and have shown that the cytotoxic activity of sodium ascorbate was almost completely inhibited by the addition of catalase to the assay. In any case, the generation of H_2O_2 by sodium ascorbate is probably due initially to the reaction of ascorbate with transition metals in the medium, i.e. it is an artefact of the experimental conditions (see also p. 223. Nevertheless, the treatment of cancer is often aggressive and likely to cause inflammation as indicated by the usefulness of Fe chelators to lessen the side-effects of chemotherapy (Beare & Steward, 1996). However, the use of large amounts (up to 45 g) of vitamin C to treat cancer has not been successful (Cameron & Campbell, 1974); it was later pointed out that the deaths of four patients from haemorrhagic tumour necrosis soon after treatment was started may have been due to the pro-oxidant effects of vitamin C (Halliwell, 1994) (Table 2).
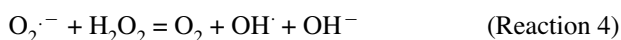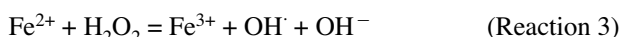
Transition metals. In the absence of inflammation, Zn, Cu, Mg and Se are intimately involved in protecting the body against oxidative stress. Superoxide dismutase is found in all aerobic cells and is responsible for the dismutation of the free radical superoxide (reaction 1). In the cytoplasm, Zn and Cu are cofactors, while in the mitochondria, Zn and Mg work together to produce H_2O_2.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ = H_2O_2 + O_2 \qquad \text{(Reaction 1)}$$
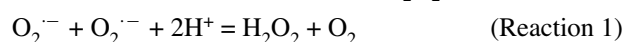
The H_2O_2 produced as a product of the dismutation reaction is removed by GPx, a Se-dependent enzyme. To reduce H_2O_2 to water, the enzyme uses GSH as a source of hydrogen (reaction 2). Cellular concentrations of glutathione are maintained by the enzyme glutathione reductase, which is a riboflavin-dependent enzyme.

$$H_2O_2 + 2GSH = GSSG + 2H_2O \qquad \text{(Reaction 2)}$$
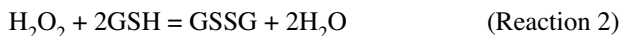
Superoxide dismutase and GPx are widely distributed in aerobic tissues and, if no catalytic metal ions are in the vicinity, endogenously produced superoxide and H_2O_2 at physiological concentrations have limited, if any, damaging effects (Halliwell *et al*. 1992). However, during inflammation and disease, tissue structures break down, and in these conditions transition metals which are normally tightly bound to proteins and other subcellular structures are freed (Chevion *et al*. 1993; Beare & Steward, 1996) and are potentially able to generate the hydroxyl radical (OH·) by the Haber–Weiss reaction (reaction 3) or the Fenton reaction (reaction 4). Pathological conditions greatly increase the concentrations of both superoxide and NO, and the formation of the toxic intermediate peroxynitrite (ONOO) with the reactivity of the hydroxyl radical (reaction 5) has been demonstrated in macrophages, neutrophils and cultured endothelium (Koppenol *et al*. 1992; Carreras *et al*. 1994).

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^. + OH^- \qquad \text{(Reaction 3)}$$

$$O_2^{.-} + H_2O_2 = O_2 + OH^. + OH^- \qquad \text{(Reaction 4)}$$

$$NO + O_2^{.-} = ONOO \qquad \text{(Reaction 5)}$$

Depressed serum Fe concentrations develop abruptly during the incubation period of most generalised infectious processes (Beisel, 1976). This can be viewed as a measure by the body to reduce Fe availability for pathogenic bacteria and counter potential pro-oxidant damage. The body alters the transport and distribution of Fe by blocking Fe mobilisation and stimulating Fe uptake from plasma by the liver, spleen and macrophages (Thurnham, 1990*b*, 1995; Weiss *et al*. 1995). In addition, NO has been shown to play a role in the coordination of Fe traffic by mimicking the consequences of Fe starvation and leading to the cellular uptake of Fe (Pantopoulos *et al*. 1994). These changes in the control of Fe traffic are part of a generalised response known as the APR which in the short term is considered protective (Thompson *et al*. 1992). For further discussion on the control of Fe traffic, see the earlier section on micronutrients in gene expression (p. 227).

It was initially suggested that the hypoferraemia of infection protected the host by reducing the Fe available for bacterial growth (Weinberg, 1984), and certainly the overwhelming infections resulting in deaths in a number of malnourished children who received Fe would support that view (McFarlane *et al*. 1970) (Table 2). However, while some bacteria may benefit from Fe supplements, other pathogenic bacteria have powerful siderophores enabling them to compete quite successfully against the Fe-binding proteins in the plasma (Bullen, 1981). The importance of the hypoferraemia therefore may be more closely linked with its potential influence on the redox status in the tissues (Thurnham, 1997*b*). Hypoferraemia may protect against the potential pro-oxidant properties of Fe and the exacerbation of tissue damage at sites of inflammation (Thurnham, 1990*b*). In this connection it is interesting to point out that lactoferrin is secreted by neutrophils at sites of inflammation. Lactoferrin has a higher affinity for Fe than transferrin and can also bind Fe under acid conditions, such as those found at sites of inflammation (Bullen, 1981). Thus a reduction in plasma ascorbate and Fe can be viewed as a physiopathological response to the potential pro-oxidant conditions that can arise as a result of inflammation and disease. Reactive oxygen species generation is an important part of human defences in malaria and other diseases (Reibnegger *et al*. 1987) and malaria is possibly a good example of where pro-oxidant conditions coupled with Fe supplements may worsen the disease outcome (Table 2).

Withholding Fe from the circulation will eventually cause anaemia, which is very common in developing countries. Both dietary and infection-related factors contribute to this (Walter *et al*. 1997). Frequent infections reduce circulating Fe and increase the risk of anaemia. Infection reduces appetite and fever blocks Fe absorption (Beresford *et al*. 1971). In Papua New Guinea, infants whose Hb at birth was highest and those given iron dextran at birth were more likely to be admitted to hospital with severe malaria than infants not given Fe and with a low Hb at birth (Oppenheimer, 1989). These observations were made during a study on Fe supplementation to infants at birth, living in an area where there was a high prevalence of malaria (Oppenheimer *et al*. 1986); yet, a similar study in the same area in schoolchildren did not find any adverse consequences of an Fe supplement on malaria prevalence (Harvey *et al*. 1989). Age may be an important factor to consider in the case of Fe, since cell-mediated immunity is the first form of defence against disease in infants and other non-immune individuals (Reibnegger *et al*. 1987), while schoolchildren living in a malaria-endemic area would be able to mount a humoral response, and cell-mediated immunity may be less active in such children. Thus the undesirability of high levels of Fe in its various forms in plasma may be of far greater importance in the infant where anti-malarial defence is more dependent on generating reactive oxygen species by phagocytes, than in the schoolchild.

Thus the hypoferraemia of infection may be a defensive strategy developed initially to protect the non-immune infant and, because of its success, it continues into adulthood even though its importance becomes superseded by humoral immunity as we get older.

Conclusions

Many micronutrients are potentially vulnerable to the effects of prescribed medicines when consumption is regular and sustained. Thus the elderly are particularly at risk of some micronutrient deficiencies such as thiamin, folate and vitamins C and D, whilst the intake of nutrients such as vitamin K, Fe and folate may have to be regulated carefully to avoid reducing the efficiency of some therapies. Lifestyle factors such as smoking, alcohol and tea consumption and general dietary habits certainly influence micronutrient status and may have a conditioning effect on health status.

Alcohol abuse can specifically induce thiamin deficiency but the consumption of any substance, especially if consumed regularly and in large amounts, can influence the biotransformation of dietary substances and have a much wider impact on the metabolism of xenobiotic compounds that may influence our risks of chronic disease.

There are profound effects of infection on certain micronutrients. Our bodies respond to infection by a rapid series of changes over 24 to 48 h that are collectively termed the APR. The concentrations in the blood of several nutrients including vitamin A, vitamin C, Fe and Zn fall acutely during this period. Changes occurring during the APR are generally considered to be beneficial to the host and there are possible advantages resulting from the falls in micronutrient concentrations.

There are many interactions between micronutrients and genes. Nutrients such as vitamin A have been recognised for some time to exert metabolic effects through a direct effect of all-*trans*- or 9-*cis*-retinoic acid on response elements in DNA, alone or in concert with other substances, and influence a number of different genes. Vitamin D also exerts its effects through response elements and, in some if not all of its actions, it works in concert with vitamin A. Not only does vitamin D influence Ca homeostasis through this mechanism, it also has important effects on immune mechanisms. While we may have lost the ability to synthesise vitamin C, we possess two genes which exert control over vitamin C transport in different tissues and may have important implications in the movement of vitamin C. Fe uptake, storage and utilisation is very tightly controlled by IRP which bind to and stabilise mRNA. Infection, however, can very powerfully override the control of Fe metabolism and produce a state which is akin to Fe deficiency. Polymorphisms of different genes can also influence vitamin C, Fe and folate metabolism.

Antioxidant nutrients are potentially pro-oxidant nutrients but the latter properties are not evident *in vivo* unless tissue integrity is compromised, as in disease. The redox status of the cell is one of the important factors influencing the transcription factor NF-κB which coordinates cytokine release and the recruitment of specialised immune cells to the site of damage or infection. There is a growing body of evidence that antioxidant status is closely linked to cellular integrity and that it is an important regulator of cellular function, especially in disease.

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