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Guest Lecture

The role of antioxidants in nutritional support

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I am highly honoured to give the guest lecture at this British Association for Parenteral and Enteral Nutrition meeting. I would like to start with a couple of stories. During the late 1970s I held a visiting professorship in Clinical Biochemistry at the Columbia University, New York. My friend and colleague Erich Vinnars and I rented a nice apartment in Fort Lee, New Jersey. Our landlord Patrick, an Irish gentleman, had over his lifetime grown to a substantial 140 kg; whereas his wife Rosalie, born in Sicily, was 'as thin as 6 o'clock', weighing not more than about 45–48 kg. Patrick's genes or his behaviour at table had redoubled themselves in their son Elliot who was a beer-bellied 160 kg. Although all three were charming people, it was a nightmare to be invited for dinner: at least five to six courses accompanied by ample volumes of wine and beer were served; the dessert consisted of enormous cakes, thickly filled with chocolate and cream. On completion of all that gluttony, Rosalie placed a large pot full of pills in the middle of the table and both gentlemen consumed a single capsule each. 'This is lecithin and good vitamins saving our arteries and helping to avoid coronary heart disease', claimed Elliot. 'My dear doctors you should also do something for your health', recommended the father.

After 25 years you may ask what is the current situation. There is no doubt that the issue at hand has passed through a dialectic development. This might be excellently demonstrated by a recent article discovered in the November issue of the famous weekly illustrated journal *Neue Revue* on the occasion of my routine visit to my barber. It seems that all possible dreadful maladies, such as stroke, ischaemic heart disease, hypertension and various kinds of malignancies, may be avoided, minimized or at least considerably postponed by means of sufficient intake of Mg, Se, vitamins A, C, E, carotenoids and other selected substances. These introductory anecdotes may well reflect the complexity of the topic of the present paper.

Very few, if any, clinical investigations have been carried out using antioxidants; probably because of the lack of suitable preparations for use in the clinical setting. The present situation might be simply delineated as follows. Since 1954 we have known that the damaging effects of elevated oxygen concentrations in living organisms are attributable to the formation of free radicals (Gerschman, 1981). We have known about the existence of antioxidants since 1969 (McCord & Fridovich, 1969). We have hypothesized that the damage caused by free radicals is a major contributor to ageing and to many of the degenerative diseases, including cardiovascular disease, cancer, cataracts, age-related

decline in the immune system and degenerative diseases of the nervous system (Aruoma *et al.* 1991; Ames *et al.* 1992). Acute formation of free radicals associated with radiation, reperfusion, trauma, infection and inflammation makes a significant contribution to the pathology of these critical illnesses (Halliwell & Gutteridge, 1989). Finally we have learned that it may be possible to prevent, postpone, treat or limit the severity of these diseases by enhancing the body's antioxidant capacity through improved nutrition with antioxidants (Diplock, 1991, 1994).

In keeping with this background, it is my intention to briefly discuss some essential basic concepts and definitions and hypothesize about future antioxidant therapy as well as speculate on their clinical implications in various human diseases. Indeed, antioxidants have been implicated in more than 100 conditions, from arthritis and haemorrhagic shock to acquired immune deficiency syndrome (AIDS) (Halliwell, 1987; Halliwell & Cross, 1991).

WHAT IS A FREE RADICAL; WHAT IS AN ANTIOXIDANT?

A free radical is simply defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. Biological molecules are usually non-radicals containing only paired electrons, but when a radical gives one electron to, takes one electron from, or simply adds itself to a non-radical, then it becomes a radical; the feature of the reactions is that they tend to proceed as chain reactions (Halliwell & Gutteridge, 1989). Most free radicals are unstable and highly reactive. Some of the free radicals and oxidants found in living organisms are shown in Table 1. Free radicals and various reactive oxygen species might be derived either from normal, essential metabolic processes or from external sources. Examples of both are shown in Table 2.

If free radicals are not inactivated, their chemical reactivity can damage all types of cellular macromolecules, including proteins, carbohydrates, lipids and nucleic acids, resulting in cell injury and atherosclerosis. Superoxide radicals ($O_2^{\bullet -}$) may be converted to H_2O_2 and hypochlorite radicals which can damage membrane proteins and membrane phospholipids. $O_2^{\bullet -}$ can inhibit ATP synthesis and produce breaks in DNA molecules (Cochrane, 1991; Ward, 1991). Indeed, many of the free-radical effects have been implicated in the causation of degenerative diseases as will be discussed during the course of the present paper.

ANTIOXIDANT DEFENCES

In 1954 Gerschman and Gilbert (Gerschman, 1981) proposed that most of the damaging effects of elevated oxygen concentration in living organisms could be attributed to the formation of free radicals. This idea, however, did not capture the interest of many biologists and clinicians until the discovery of an enzyme specific for the catalytic removal of a free radical in 1969. That enzyme was superoxide dismutase (*EC* 1.15.1.1; SOD; McCord & Fridovich, 1969). The pioneering work of McCord and Fridovich (McCord & Fridovich, 1969; Fridovich, 1988) represents the first piece in the mosaic explaining the nature of antioxidant defences and has led to many fundamental scientific discoveries. These include the explanation of the mode of action of phagocytosis (Babior *et al.* 1988),

Table 1. *Selected reactive oxygen species in living organisms*

Non-radicals		Free radicals	
Hydrogen peroxide	H_2O_2	Superoxide radical	$O_2^{\bullet-}$
Singlet oxygen	$^1O_2$	Lipid peroxyl radical	$LOO^{\bullet}$
Hypochloric acid	HOCl	Hydroxyl radical	$OH^{\bullet}$
Ozone	O_3	Nitric oxide radical	$NO^{\bullet}$

Table 2. *Selected sources of free radicals*

Internally generated
Mitochondria
Phagocytes
Xanthine oxidase (*EC* 1.1.3.22)
Reactions with Fe and with other transition metals
Arachidonate pathways
Peroxisomes
Exercise
Inflammation
Ischaemia and reperfusion
External sources
Cigarette smoke
Radiation
u.v. light
Certain drugs, reagents and industrial solvents
Pollution

understanding of the adaptation of bacteria during the transition from anaerobic to aerobic life, clarification of how toxins (e.g. paraquat, alloxan, 6-hydroxydopamine) damage cells, and of how human tissues may be injured by inflammation, injury or reperfusion (Cohen, 1988; Halliwell & Gutteridge, 1989).

Today we know that antioxidant defences consist of enzymes and water- and lipid-soluble substances. The line of enzymic defence includes glutathione peroxidases, SOD and catalase (*EC* 1.11.1.6). Selected actions of these enzymes are shown in Fig. 1. SOD are a family of antioxidant enzymes catalysing the decomposition of $O_2^{\bullet-}$ to H_2O_2 and O_2. Glutathione peroxidases contain Se and reduce hydroperoxides arising from lipid oxidation. Catalase specifically catalyses the decomposition of H_2O_2.

The second line of defence consists of low-molecular-weight compounds, e.g. vitamins E and C, carotenoids (Frei, 1994) and so-called non-nutrient antioxidants, e.g. phenolic and polyphenolic compounds (Birt & Bresnick, 1991).

Special attention has been paid to the importance of removing both $O_2^{\bullet-}$ and H_2O_2 *in vivo* (Halliwell *et al.* 1994). Indeed, $O_2^{\bullet-}$ can accelerate the production of $OH^{\bullet}$ from H_2O_2 in the presence of transition metal ions by donating an electron, and thereby reducing Fe^{3+} and Cu^{2+} to Fe^{2+} and Cu^{+} respectively (Halliwell, 1987; Halliwell & Gutteridge, 1989). Subsequently, further reaction with H_2O_2 may yield Fe^{3+} and Cu^{2+} simultaneously with the formation of more $OH^{\bullet}$ (Halliwell & Gutteridge, 1992). The interaction between $O_2^{\bullet-}$ and $NO^{\bullet}$ is notable and represents a dangerous event (Beckman *et al.* 1990). $O_2^{\bullet-}$ together with $NO^{\bullet}$ can generate the non-radical peroxynitrite which is directly cytotoxic

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$$2\ O_2^{\bullet -} + 2\ H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\ H_2O$$

$$LOOH + 2\ GSH \xrightarrow{\text{Glutathione peroxidase}} LOH + H_2O + GSSG$$

Fig. 1. Selected actions of enzymes involved in antioxidant defences. L, lipid. Superoxide dismutase, *EC* 1.15.1.1; catalase, *EC* 1.11.1.6; glutathione peroxidase, *EC* 1.11.1.9.

(Beckman *et al.* 1990; Radi *et al.* 1990). Decomposition after protonation yields numerous reactive species, e.g. $OH^{\bullet}$ and $NO_2^{\bullet}$ (Beckman *et al.* 1990; Hogg *et al.* 1992). Transition metal ions also accelerate the decomposition of lipid hydroperoxides into cytotoxic products such as aldehydes, alkoxyl radicals and peroxyl radicals as well as peroxynitrite (Esterbauer *et al.* 1988; Halliwell & Gutteridge, 1989). Traumatic injury, inflammation and sepsis are associated with excessive production of NO and $O_2^{\bullet -}$, resulting in severe oxidative damage due to the production of highly reactive $OH^{\bullet}$ (Nava *et al.* 1991). Another view is that NO could be protective by removing $O_2^{\bullet -}$ and preventing metal-independent $OH^{\bullet}$ generation (Halliwell, 1989; Harbrecht *et al.* 1992).

WHAT IS OXIDATIVE STRESS?

Oxidative stress might be defined as a condition of imbalance between pro-oxidants and antioxidants. Pro-oxidant elements include all factors which play an active role in the enhanced formation of free radicals or other reactive oxygen species. In this instance cellular mechanisms (defects in mitochondrial respiration, specific enzymes) as well as exogenous mechanisms (smoking, polyunsaturated fatty acids, air pollution, drugs, etc.) may contribute.

WHAT IS ANTIOXIDANT CAPACITY?

The antioxidant capacity is an individual measure and might be defined as the sum of available endogenous and exogenous defence mechanisms which ensure the oxidative balance. The activity of the endogenous antioxidant mechanisms can partially adjust to the changes in oxidative stress. However, the activity of the exogenous system is largely dependent on the individual intake of antioxidants (Biesalski, 1995). Antioxidants are to be found, as previously mentioned, among both nutrients and non-nutrients.

VITAMIN E

The term vitamin E is a combined name for various tocopherols and tocotrienols which share similar biological activity. α-Tocopherol, the major antioxidant in cell membranes, is a so-called chain-breaking antioxidant, located in biological membranes and lipoprotein particles. Attached to the hydrophobic structure of α-tocopherol is an OH group, whose H atom is very easy to remove. Peroxy radicals generated during lipid peroxidation extract H from the tocopherol molecule:

$$CO^{\bullet}_2 + TOH \rightarrow CO_2\,H + TO^{\bullet}.$$

The tocopherol radical is poorly reactive, being able to attack adjacent fatty acid chains, so that the chain reaction is stopped. It is widely thought that the tocopherol radical can migrate to the membrane surface and be converted back to α-tocopherol by reaction with ascorbic acid (Burton & Traber, 1990).

A naive overstated belief in the magic effect of vitamin E on health and disease is not new. The Rodale Organization, publishers of *Prevention Magazine*, pursued the cause of vitamin E mysticism, or fanaticism, in an exceptional way. J. I. Rodale himself rediscovered the apparent potential of vitamin E when he discovered he had heart disease. It is said that Mr Rodale (during a dramatic television interview with Dick Cavett in 1974) proclaimed that he expected to live to 120 by taking vitamin E supplements. Unfortunately, he succumbed to a heart attack during the broadcast (Rynearson, 1974).

VITAMIN C

Vitamin C is a water-soluble substance and is the most important antioxidant in extracellular fluids. Its role as an antioxidant is based on the reaction with aqueous peroxyl radicals producing ascorbyl radicals (Weber *et al.* 1996). Thus, vitamin C is both an antioxidant in itself and it serves, as already mentioned, as a co-antioxidant by interacting with vitamin E (Burton & Traber, 1990).

Anecdotes recalling the wonder-working effects of vitamin C are numerous. The first one might be the observation of the dramatic effects of a tea-like extract from conifers on scurvy. The French explorer Cartier recorded in his ship's log that '. . . the juice of tree cured not only scurvy in ailing men but all of the diseases they ever had . . .' (Lövenberg *et al.* 1968).

Food fanatics do not clearly differentiate food and drugs. This is due to the proposed magical power of a given food the use of which is thought to fulfil a persistent human desire. The push–pull of fear and hope, originating from the unique ability of the human subject to contemplate his own inevitable death, is indeed an indefatigable aspect of food fanaticism. This is suggested in the introduction of the late Nobel Laureate Linus Pauling's (1970) book *Vitamin C and the Common Cold*. Pauling states: 'In April 1966 I received a letter from Dr Irwin Stone, a biochemist . . . He mentioned in his letter that I had expressed a desire to live on for the next fifteen or twenty years. He said he would like to see me remain in good health for the next fifty years, and that he was accordingly sending me a description of his high-level ascorbic-acid regimen'. Pauling's (1970) book was a significant force in promoting 'orthomolecularism' and thereby inspired widespread excessive vitamin C supplementation (Pauling, 1970).

CAROTENOIDS

The carotenoids are a group of red, orange and yellow pigments (β-carotene, α-carotene, lycopene, lutein, zeoxanthin, β-cryptoxanthin). Vitamin E can protect β-carotene from oxidation. Some carotenoids can act as precursors of vitamin A. However, this property is unrelated to their antioxidant activity (Krinsky, 1989).

NON-NUTRITIVE ANTIOXIDANTS

During the last decade data from both experimental and epidemiological studies have accumulated showing that vegetable grains and fruits contain a large variety of potentially therapeutic substances termed plant chemicals or phytochemicals (Pratt, 1992). Technically, the term 'phytochemical' refers to every naturally-occurring chemical substance present in plants, especially to those phytochemicals which are biologically active (Caragay, 1992). Usually, these phytochemicals are present only in small amounts. Natural antioxidant phytochemicals occur in all higher plants and in all parts of plants: wood, bark, stems, pods, leaves, fruits, roots, flowers, pollen and seed (Pratt & Hudson, 1990; Pratt, 1992).

Major phytochemicals are phenolic acids, flavonoids, flavones, isoflavones, flavonols etc., cumarine derivatives, phytoalexane derivatives, cinnamic acid, and many other polyphenols. The antioxidant activities of these phytochemicals range from extremely slight to very great. The mode of action of the natural antioxidants might be related to one or more of the following functions: (a) free-radical scavengers, (b) reduction agents, (c) potential complex formation with pro-oxidant metals, (d) quenching the formation of singlet-oxygen (Pratt & Hudson, 1990). Consequently, phytochemical dietary components may actively contribute to the control of oxidative reactions and provide protection *in vivo*. It is conceivable that the mixtures of these compounds found in fruits and vegetables can, at the appropriate doses, perform as effective free-radical quenchers, protective antioxidants and/or protectors and regenerators of antioxidants (Steinmetz & Potter, 1991). In an effort to foster a greater understanding of how non-nutritive phytochemicals may aid cancer prevention, the US National Cancer Institute has recently embarked on a major initiative. The final goal is to develop experimental preparations or 'designer foods' that are supplemented with food ingredients or extracts rich in cancer-preventing substances (National Academy of Sciences, 1989).

ANTIOXIDANT THERAPY: FACTS AND FANCY

The role of free radicals in human disease is excellently presented in Robin Cook's (1994) novel entitled *Fatal Cure*:

'. . . Part of Sam's good fortune had always been his health, yet at half past four on February seventeenth, something strange began to happen. Numerous water molecules within many of his cells began to split apart into two fragments: a relatively inoffensive hydrogen atom and a highly reactive, viciously destructive hydroxyl free radical.
As these molecular events transpired, Sam's cellular defenses were activated. But on this particular day those defenses against free radicals were quickly exhausted; even the antioxidant vitamins E, C and beta-carotene which he diligently took each day could not stem the sudden, overwhelming tide.
The hydroxyl free radicals began to nibble away at the core of Sam Flemming's body. Before long, the cell membranes of the affected cells began to leak fluid and electrolytes. At the same time some of the cells' protein enzymes were cleaved and inactivated. Even many DNA molecules were assaulted, and specific genes were damaged . . .'.

Robin Cook (1994) describes the consequences of a radiation injury; the patient being

assaulted by criminal and corrupt physicians and hospital administrators. Indeed, radiation generates $OH^{\bullet}$ directly by splitting water molecules (Von Sonntag, 1987).

$$H_2O \rightarrow H^{\bullet} + OH^{\bullet}$$

damaging lipids, proteins and DNA.

For most human diseases, however, the oxidative stress is secondary to the primary disease. Indeed, the uncontrolled propagation of toxic radicals often leads to more cellular injury than that caused by the original insult, and it certainly contributes to the generalized changes in capillary permeability and diffuse tissue injury characteristic of multi-organ failure.

MODE OF ANTIOXIDANT ACTION

The exact underlying mechanisms for antioxidant actions are multifold and have not yet been specified in all their facets. Although certain mechanisms for the individual antioxidant nutrients and nutrient components are known, their complex interplay might be established initially after a study of their combined nutritional effects in suitable amounts and adequate proportions. Further research is needed in order to understand the multifold activities and interactions with other nutrients (considering, for example, absorption, distribution, cellular effects, synergism and antagonism). Importantly, in animals, supplementation with one micronutrient can relieve symptoms caused by deficiency of another, but not fully replace it. It is easy to speculate that as a result of these interactions, a combination of antioxidants may be more effective than larger quantities of a single antioxidant. This also applies to other aspects of antioxidant action, for example the influence on immune function or the impact on gene expression. The question of the distribution of individual antioxidants and their cellular availability is of particular importance for efficient activity.

THERAPEUTIC IMPLICATIONS OF ANTIOXIDANTS

It is tempting to speculate about the clinical usefulness of therapeutic intervention using antioxidants. According to Halliwell *et al.* (1994), future attempts to use antioxidants in the treatment of human disease might be divided into three main areas: (1) administration of antioxidants that occur naturally in the human body (α-tocopherol, GSH or SOD); (2) administration of synthetic antioxidants, e.g. probucol, or chelating agents that suppress Fe^{2+}–Fe^{3+}-dependent free-radical reactions, or xanthine oxidase (*EC* 1.1.3.22) inhibitors; (3) investigation of the possibility that drugs developed to protect against other mechanisms of tissue injury may have additional benefits.

In animal experiments, continuous infusion of SOD and catalase enhanced the recovery of function after reperfusion injury (Myers *et al.* 1985). Importantly, neither SOD alone nor catalase alone influenced the function of the stunned myocardium, only their combination administration (Jeroudi *et al.* 1990). Unfortunately, the clinical application of SOD and catalase in patients with post-ischaemic dysfunction is limited by the need for parenteral administration, and by the large molecular weight which hinders intracellular penetration. Thus, these enzymes are not ideal antioxidants for therapeutic use (Bolli, 1991).

Available strategies have been recently reviewed (Schiller *et al.* 1993) and a wide range

of substances with therapeutic potential have been listed. In addition to nutritive and non-nutritive antioxidants, antioxidant drugs are also available. These substances include allopurinol (which blocks free-radical proliferation), scavengers (desferal Mn, Cu^{2+} 3,5-di-isopropylsalicylic acid, cyclic nitroxides, mannitol dimethyl sulphoxide and dimethylthiourea), metal chelators (such as deferoxamine) and substances, e.g. altipraz and ebselen, which enhance endogenous antioxidant defences (Table 3). Indeed, there is an impressive catalogue of compounds, sufficient for many years of future investigations (Youn *et al.* 1991; Grimble, 1994).

It is noteworthy that the recovery of the stunned myocardium can be enhanced by mercaptopropionylglycine (MPG). This free-radical scavenger enters the intracellular space and is active against $OH^{\bullet}$ (Myers *et al.* 1986). MPG is well tolerated during long-term therapy and could be given prophylactically as an oral capsule to patients at high risk (Bolli, 1991). Parenteral MPG alleviates post-ischaemic dysfunction regardless of whether it is administered before ischaemia or just before reperfusion injury. However, infusion of MPG started minutes after reflow of blood was ineffective, suggesting that the critical radical-mediated injury occurred in the first 10 min after reperfusion injury (Bolli *et al.* 1989).

Sulphydryl antioxidants

R. F. Grimble (1994) raised the question 'to what extent might nutritional intervention be effective?', and he proposed the possibility of interactions of free radicals, antioxidants, cytokines and exogenous substances of a nutritional nature. Sulphydryl antioxidants are supposed to be especially important in this context.

There are some particularly interesting studies elucidating the potential role of S-containing antioxidants. Macrophages act as cysteine transporters under the action of inflammatory stimuli such as endotoxin and tumour necrosis factor (TNF). The uptake of cysteine in macrophages is competitively inhibited by glutamate (Grimble, 1994). During episodes of immunosuppression or in diseases with compromised immunocompetence, e.g. AIDS, and malignancy, increased extra- and intracellular glutamate concentrations are observed (Fürst *et al.* 1981; Ollenschläger *et al.* 1988).

Cysteine also enhances a number of lymphocyte functions, e.g. cytotoxic T-cell activity (Dröge *et al.* 1991). A high glutamate:cysteine value is associated with a low proportion of T-helper cells (Dröge, 1993). N-acetyl-cysteine, reduced glutathione and cysteine inhibit the expression of the nuclear transcription factor in stimulated T-cell lines (Mihm & Dröge, 1990; Mihm *et al.* 1991). This observation might provide an interesting approach in the treatment of AIDS since the transcription factor enhances human immunodeficiency virus (HIV) mRNA expression. In fact, *in vitro* studies demonstrate that the stimulatory effects of TNF, induced by free radicals, on HIV replication in monocytes can be inhibited by S-containing antioxidants (Grimble, 1994). These basic studies indicate that treatment of inflammatory diseases and AIDS with sulphydryl antioxidants may be beneficial, and powerful arguments have been advanced in favour of such treatment (Roederer *et al.* 1992; Dröge, 1993). Clinical studies using this strategy are not yet available. One reason might be the lack of suitable preparations. The use of N-acetyl-cysteine in human subjects is not appropriate because of the lack of tissue acylases, except in the kidney (Fürst, 1994). Thus, following its administration this compound will accumulate and subsequently be excreted via the urine (Magnusson *et al.* 1989; Fürst, 1994).

Table 3. *Selected antioxidant drugs with therapeutic potential*

Drug	Property
Allopurinol	Blocks free radical proliferation
Desferal Mn Cu^{2+} 3,5-di-isopropyl salicylic acid Cyclic nitroxides Mannitol Dimethyl sulphoxide Dimethyl thiourea	Non-enzymic scavenger
Deferoxamine etc.	Metal chelators
Altipraz Ebselen	Enhances endogenous antioxidant defences

Cysteine is unstable, and commercial GSH, sufficiently pure for clinical use is extremely expensive. We are in the process of synthesizing stable and highly-soluble cysteine dipeptides (Fürst, 1994; Fürst & Stehle, 1994). The peptides are easily available, the half life being less than 3 min and the disappearance of the dipeptide is associated with simultaneous equimolar liberation of the constituent amino acids. When glutamine- and tyrosine-containing dipeptide preparations have been registered and are commercially available, the role of cysteine peptides may be followed, not only as nutrients but also as antioxidant and intracellular regulators.

Taurine offers protection against oxidant damage in experimental lung inflammation (Banks *et al.* 1992). This interesting substance received very little attention until 1993, although there are numerous early indications of its clinical relevance. We and others (Askanazi *et al.* 1980; Pathirana & Grimble, 1992; Fürst, 1994) found low extra- and intramuscular taurine concentrations after trauma and infection. Low taurine concentrations in plasma and platelets have been described in adult and paediatric patients receiving taurine-free total parenteral nutrition (TPN) (Vinton *et al.* 1987; Paauw & Davis, 1990), and low intramuscular taurine concentrations are a typical feature in chronic renal failure (Bergström *et al.* 1989). Interestingly, as early as 1972 a marked increase in taurine excretion was observed after total body irradiation (Dilley, 1972). This finding and a profound taurine deficiency was confirmed recently in patients receiving intensive chemotherapy and/or radiation (Desai *et al.* 1992). Experimental depletion of tissue taurine concentrations, especially in the lung, produces inflammation; administration of pro-oxidants results in severe lung oedema and interstitial fibrosis. Taurine administration ameliorates the symptoms (Gordon & Heller, 1992). The underlying mechanism of taurine action may be due to its interaction with H_2O_2 and Cl^- in the myeloperoxidase reaction thereby producing taurine chloramine, an oxidant with very low reactivity, partially quenching free-radical generation (Grimble, 1994). Actually, taurine chloramine may exert a potent anti-inflammatory effect by suppressing TNF and NO production in endotoxin- and interferon-α-stimulated macrophage cell lines (Park *et al.* 1993).

Free crystalline taurine is available for inclusion in intravenous or enteral preparations. However, we hypothesized that the extremely high intracellular–extracellular trans-

membrane gradient (250:1) might limit cellular uptake of taurine. We proposed that enhanced transmembrane transport might be facilitated by binding taurine to a suitable amino acid carrier (Dressel *et al.* 1994). Accordingly, we synthesized three taurine conjugates, L-alanyl-taurine, L-phenylalanyl-taurine and L-tyrosyl-taurine, and investigated intestinal uptake and utilization in a perfusion model after vascular administration (Hummel *et al.* 1995). Apparently, taurine conjugates are efficiently hydrolysed by membrane-bound peptidases and the liberated taurine is subsequently transported. Consequently, taurine conjugates may assist in the support of taurine-deficient target tissues.

CLINICAL IMPLICATIONS

As outlined previously, vitamin E and vitamin C are important antioxidant nutrients which may have a role in the nutritional support of critically-ill patients. Nevertheless, the clinical importance of supplementary vitamins E and C remains controversial and whether or not pharmacological doses of these vitamins will prove beneficial in reducing oxidative damage in critically-ill patients is unknown (Kelly, 1994). Decreased circulating levels of antioxidants are a frequent finding during catabolic stress. Plasma concentrations of vitamins A, C and E, as well as that of β-carotene, are profoundly decreased in patients with adult respiratory distress syndrome and this pattern is associated with greatly increased thiobarbituric acid-reactive substances. The concentrations of antioxidant vitamins are reduced in patients with cystic fibrosis; inflammation further aggravates the pathological pattern. In these patients the activity of glutathione peroxidase (*EC* 1.11.1.9) and plasma Se level are depleted compared with healthy controls (I. Elmadfa, personal communication). The combined results indicate that catabolic conditions are associated with increased oxidative stress and decreased antioxidant capacity. This line of reasoning leads to the conclusion that delivery of adequate amounts of antioxidants might be essential to counteract free-radical action and subsequent oxidative damage.

Wolf & Seeger (1982) administered vitamin E to patients with acute respiratory failure as early as 1977. More recently, it has been shown that supplementation with vitamin E enhances cell-mediated immunity and reduces the incidence and severity of infectious diseases in healthy elderly subjects (Meydani *et al.* 1992). Tocopherol increases delayed hypersensitivity as well as lymphocyte proliferation and IL-2 production in response to mitogens (Odelye & Watson, 1991).

Operative trauma, injury and infection are conditions associated with a decline in plasma vitamin E concentration (Goode *et al.* 1995). We measured the plasma concentration of vitamin E and β-carotene in 180 multiple-injury patients at commencement of TPN and repeatedly during the 20–40 d observation period (Biesalski *et al.* 1994). Nutritional support comprised 0·15–0·28 g N/kg and energy corresponding to 1·2 BMR (50% carbohydrates, 30% fat). After 3–5 d on TPN, vitamin E and retinol concentrations decreased markedly corresponding to 34 and 43% of the initial values respectively. Daily supplementation with a multi-vitamin preparation, including 15–40 mg vitamin E and 7·5–20 mg vitamin A in fifty-three patients resulted in a considerable increase in plasma vitamin E concentrations, while vitamin A levels were not affected (Fig. 2). The decrease in vitamin A concentration in plasma was accompanied by a profound decline of retinol-binding protein (RBP) levels indicating an impairment of its synthesis in the liver.

Consequently, extrahepatic target tissues are not sufficiently supplied with vitamin A, a severe disturbance in keeping with the known increased vitamin A requirement during episodes of catabolic stress, including compromised epithelial renewal. Supplementation with vitamin A failed to increase plasma concentration presumably due to both the homeostatic regulation of vitamin A and the impaired RBP synthesis. Intravenous supplementation with retinyl ester might be more efficient, facilitating its extrahepatic incorporation in various tissues (Gerlach *et al.* 1989). Again, the marked reduction in two major antioxidants in the immediate post-injury phase suggests increased oxidative stress and also indicates that antioxidant capacity is not sufficient to compensate for the increased requirement. We may speculate that the reason why vitamin E declines in the absence of supplementation is the result of an increased vitamin E influx, transported by lipoproteins, chiefly LDL, into the sub-endothelial space. The increased influx may be due to the ischaemia-induced leakage of the endothelial barrier. In this situation vitamin E might contribute to the stabilization of the endothelial barrier and thereby serve as a potential therapeutic tool in counteracting reperfusion-induced tissue damage.

There are obvious indications that in contrast to the patients mentioned or healthy subjects, ingestion of a high load of supplementary vitamin E in certain patients fails to increase serum vitamin E concentration (Seeger *et al.* 1987; Kelly, 1994). Reduced vitamin E absorption is claimed to be the probable explanation for the marked difference in response to oral loading. This drawback, due to poor bioavailability, can be overcome by the use of a parenteral preparation. I am aware of at least three preparations available for clinical trials.

An extensive body of literature emphasizes the clinical and physiological importance of glutamine (for references, see Pathirana & Grimble, 1992). Supplemental glutamine increases protein synthesis, improves N balance, enhances intestinal adaptation, attenuates intestinal and pancreatic atrophy, reduces bacterial translocation and exerts many other beneficial effects. Less attention is directed towards the highly interesting function of this amino acid as the precursor of GSH. Glutamine supplementation has been shown to preserve hepatic GSH. Following glutamine supplementation, stores of GSH in liver and other tissues increased above normal concentrations (Hong *et al.* 1992; Harward *et al.* 1994; Rouse *et al.* 1995). In a current report combined therapy with vitamin E and glutamine was successful in the treatment of severe veno-occlusive disease following bone-marrow transplantation (Nattakom *et al.* 1995). The administration of 400 mg vitamin E and 20 g glutamine daily was associated with reversal of the clinical and biochemical signs of severe hepatic dysfunction. After 65 d of treatment, bilirubin and body weight were normalized and the ascites and pedal oedema had resolved. This report strikingly demonstrates the potential usefulness of antioxidants in critically-ill patients (Nattakom *et al.* 1995). Also, in a current double-blind randomized treatment study, glutamine-supplemented TPN significantly improved survival (Griffiths *et al.* 1996). Early TPN treatment with a glutamine dipeptide in intensive care may be beneficial after severe illness.

Antioxidant cocktail?

It is becoming more and more clear that the major antioxidants, vitamins E, C and GSH may act in a synergistic or complementary fashion (Kelly, 1994). Ascorbate scavenges a wide variety of oxidants, including myeloperoxidase-derived hypochlorite, and regenerates vitamin E (Doba *et al.* 1985). A network of antioxidants for defence against oxidative stress

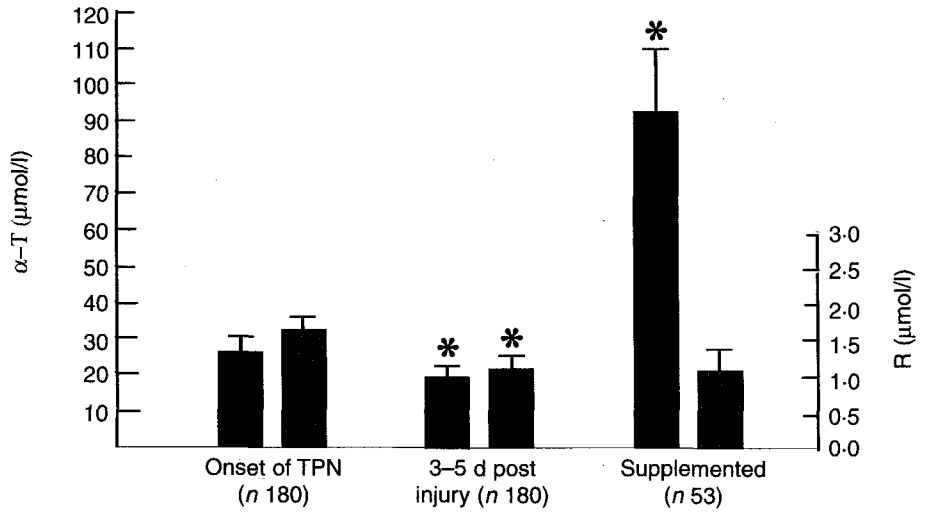

Fig. 2. Changes in plasma α-tocopherol (α-T; ■) and retinol (R; ▩) concentrations following multiple trauma, and the effect of supplementation with α-T and R. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those at the onset of total parenteral nutrition (TPN): *P<0·001. (From Biesalski *et al.* 1994.)

may have the benefit of functional overlap which will ensure that any individual component will be available in sufficient amounts on demand (Kelly, 1994). Thus, it may be more beneficial to provide the patients with an 'antioxidant cocktail'. We propose tentative proportions of supplementary vitamin E, C and β-carotene as 1:2:0·1 (Biesalski *et al.* 1995). We have no basis yet on which to suggest a value for GSH.

There are major technical difficulties to be solved before such a cocktail can be put together and prepared, and it is essential that the efficacy of each individual component is identified alone and in combination. Trials are in progress to facilitate the production of suitable preparations.

OXIDANTS BAD: ANTIOXIDANTS GOOD?

In association with the impressive list of the beneficial properties of antioxidants McCord (1995) raises the following question. Is it possible to have too much of a good thing? Most would answer affirmatively. It may be more provocative to ask whether it may be possible to have too little of a bad thing? Paradoxically, there may be several situations in which $O_2^{\bullet-}$ may be beneficial. Production of $O_2^{\bullet-}$ by activated polymorphonuclear lymphocytes and other phagocytes is an essential component of their bactericidal action (Babior, 1978). $O_2^{\bullet-}$ production may also trigger cell division, and serve as a physiological regulator of that process (Murrell *et al.* 1989). $O_2^{\bullet-}$ are terminators of lipid peroxidation via the liberation and redox cycling of cellular Fe stores (Nelson *et al.* 1994). Thus, the verdict 'good' or 'bad' is an oversimplification of a difficult inquiry. On the other hand we do not have data showing that intake of large doses of antioxidants will never be toxic. There is some experimental evidence that high levels of a single antioxidant in the presence of low levels of other micronutrients and a high oxidative state may result in cell injury (Bowry *et al.* 1992). Recently published data from clinical trials indicate that in many instances high

doses of antioxidant supplements do not reduce the incidence of tumours or affect the number of deaths from ischaemic heart disease (Greenberg *et al.* 1994; The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994). The ingestion of excessive amounts of antioxidants is presumed to shift the oxidant–antioxidant balance toward the antioxidant side. This is supposed to be beneficial; however, this may also adversely affect key physiological processes that, as mentioned previously, are dependent on free radicals. As time elapses, more and more knowledge will emerge. Presumably, we have seen only the tip of the iceberg (McCord, 1995).

FINAL THOUGHTS

The present review covers only some aspects of a highly specialized, extensive topic. Future work should advance our understanding of the critical role of antioxidants in various fields of clinical nutrition. The challenge of integrating an imperfect background of widely diverse but pertinent knowledge is also important in the investigation of the role of antioxidants during episodes of critical illness.

The present accumulated knowledge about antioxidants undoubtedly represents a great challenge for physicians, nutritional scientists, pharmacists, food technologists, and food chemists. The increasing number of investigations in this field will presumably raise more questions than solutions:

1. which antioxidants possess true disease-preventing and/or health-promoting properties and therapeutical potentials?
2. once identified, which limitations are to be considered in order to avoid risks to health?
3. are potential side effects likely to emerge following high intakes of nutritive and non-nutritive antioxidants?
4. are combinations of antioxidants more effective than larger quantities of single substances?
5. are phytochemicals to be considered as future intravenous components; 'the green parenteral'?
6. do additions of large amounts of antioxidants influence the taste and structure of enteral products?
7. will the beneficial properties of natural nutritive and non-nutritive antioxidant-enriched preparations be maintained during routine technical processing?

Nevertheless, it is certain that future success in putting acquired knowledge into practice will be rewarded by improved patient survival. Surprisingly, exciting medical progress of yesterday will become the common daily medical exercise of tomorrow. Continued rigorous critical evaluation of assumptions and hypotheses about relationships between diet, nutrition, health and disease will eventually provide us with reliable knowledge of what can and what cannot be achieved through clinical nutrition.

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