THE ROLE OF TRACE ELEMENTS AND FREE RADICALS IN THE PREVENTION OF DISEASE

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Evidence is accumulating that most of the diseases that afflict humanity have their origin in deleterious free radical reactions. These diseases include cardiovascular problems, cancer, inflammatory joint disease, senile dementias, and degenerative eye complaints. The basic process of biological aging is also the result of accumulated free radical damage to the organism. The utilization of oxygen in living organisms produces superoxide ($O_2^{\cdot-}$) and hydroxyl OH: radicals, and the activated oxygen species, singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). Metabolism of various organic compounds also yields a range of carbon-centered free radicals. These electrophilic species can damage genetic material and oxidize unsaturated fatty acids in cell membranes. Our natural protection against free radicals and activated oxygen compounds involves a sophisticated multi-layered defence screen of enzymes such as catalase and superoxide dismutase, and molecular antioxidants including ascorbic acid (vitamin C) and α-tocopherol (vitamin E). The antioxidant enzymes have, at their active centres, elements such as copper, zinc, iron, manganese and selenium. These, and other trace elements, play a vital role in maintaining our health. Australians in general are depleted in the essential element selenium. Selenium is in the active centre of the enzyme glutathione peroxidase, the function of which is to catalyze the reduction of lipid peroxides and thus maintain the integrity of cell membranes. For all trace essential elements there is a fairly narrow range between essentiality and toxicity. Too high an intake can be just as damaging as too low. Copper, for example, appears to lead to an increased risk of heart disease if the dietary intake is too low, but a fairly small excess may increase the risk of cancer. Research into the role of trace elements in health and disease is in its infancy, and many important questions remain to be answered.

THE NATURE OF FREE RADICALS

A free radical is any atom or molecule that contains one or more unpaired electrons; an unpaired electron being one that occupies an atomic or molecular orbital on its own. Figure 1 shows that π^* 2p (outer) orbitals of some oxygen species (Halliwell and Gutteridge, 1984). Even ground state molecular oxygen (the type we

breathe) is a free radical, which explains its high reactivity (oxidation) with many compounds (Halliwell and Gutteridge, 1985). However, oxygen cannot normally accept a pair of electrons from a non-radical molecule during oxidation because spin reversal would need to occur before the vacant spaces in the π^* orbital could be filled. Thus oxygen must accept electrons one at a time, which makes the kinetics of many of its reactions slow, unless it gains two single electrons from another free radical (Halliwell and Gutteridge, 1984; Proctor and Reynolds, 1984). By contrast, singlet oxygen ($^1\Delta_g O_2$) can readily accept a pair of electrons, and its reactions with other molecules are very fast. Note that the peroxide ion is not a radical.

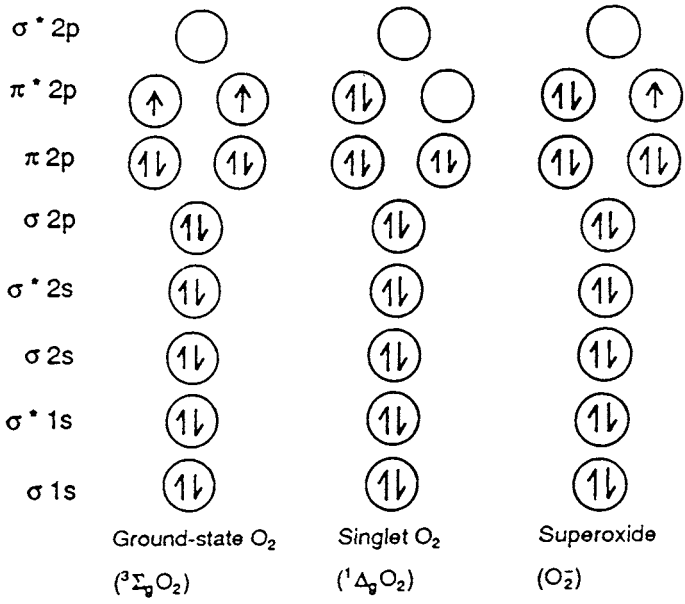

Fig. 1.

Most transition metals are free radicals (Halliwell and Gutteridge, 1985; Gutteridge *et al.*, 1986) (zinc is an exception). Many transition metals can engage in one-electron valency state changes, which makes them excellent redox catalysts, e.g., Fe (II)-(III), Cu (I)-(II), and Mn(II)-(III). Some oxidases use these transition metals as catalysts at their active centres to overcome the sluggish reactions of molecular oxygen (Halliwell and Gutteridge, 1985).

Table 1 lists some free radicals and activated oxygen species that have been implicated in biological processes, plus their approximate half-lives in a biological system (Harman and Pryor, 1984). Some free radicals and activated oxygen species, e.g., hydrogen peroxide, lipid peroxide and the semiquinone free radical, are sufficiently stable to diffuse some distance in cells, whereas others, such as the hydroxyl radical ($OH^{\cdot}$), will react with the first organic molecule they encounter (Pryor, 1986).

Other biologically important free radicals, not listed in Table 1, are nitrogen dioxide and several carbon-centered free radicals. Ozone (O_3), even though it is not a free radical, is a more powerful oxidizing agent than ground state oxygen.

TABLE 1: Approximate Half-lives of Some Biological Free Radicals and Activated Oxygen Species

Species	Symbol	Half-life(s) at 37°C[a]
Molecular oxygen	O_2	$>10^2$
Hydroxyl radical	$OH^{\cdot}$	1×10^{-9}
Superoxide radical	$O_2^{\cdot-}$	1×10^{-6}
Singlet oxygen	$^1O_2$	1×10^{-6}
Hydrogen peroxide	H_2O_2	10^b
Lipid peroxide	ROOH	$>10^2$
Alkoxyl radical	$RO^{\cdot}$	1×10^{-6}
Peroxyl radical	$ROO^{\cdot}$	1×10^{-2}
Semiquinone radical	$Q^{\cdot-}$	$>10^2$

[a] In biological system.

[b] Short lifetime in presence of catalase or glutathione peroxidase.

However, like many highly reactive non-radical species, most of the reactions of ozone with organic compounds involve free radical production (Pryor, 1986; 1986a).

Photochemical smog contains ozone and NO_x ($NO + NO_2$). Both NO and NO_2 are stable free radicals that react rapidly with biological compounds such as thiols and hemoglobin. Whereas smog usually contains less than 1ppm of NO_x, undiluted cigarette smoke has several hundred ppm; in fact gas-phase tobacco smoke contains 10^{17} reactive oxyradicals per puff (Cross, 1987; Hoffmann, 1987)!

Carbon-centered free radicals are also common in biological systems. Here the odd electron is located on a carbon, rather than an oxygen, atom. Similarly, nitrogen (e.g., amines) and sulfur (e.g., thiols) centered free radicals are commonly encountered.

Lipid peroxidation

Lipid peroxidation is one of the most important free radical-mediated biological processes, and involves both oxygen- and carbon-centered free radicals (Gutteridge *et al.*, 1986; Halliwell, 1987; Halliwell and Gutteridge, 1985). Lipid peroxidation

involves attack on a polyunsaturated fatty acid molecule (e.g., linoleic acid) in a biological membrane, leading to decreased membrane fluidity, increased non-specific membrane permeability ("leaky" membranes) and inactivation of some membrane-bound enzymes. Accelerated lipid peroxidation can result from lack of free radical scavengers in the membrane (e.g., vitamin E) or increased oxidative stress as a result of the intake of xenobiotic drugs (e.g., antimalarial drugs) or breathing air with a higher than normal concentration of oxygen (DiGuiseppi and Fridovich, 1984; Fridovich, 1984; Emanual, 1985). The aging process almost certainly involves accelerated lipid peroxidation, and lipid peroxides have been called the "ultimate" toxin because of their long biological lifetime and their highly damaging nature (DiGuiseppi and Fridovich 1984; Emanuel, 1985; Slater *et al.*, 1984).

Lipid peroxidation is a chain reaction. A hydoxyl radical initiates the process by abstracting a hydrogen atom from a polyunsaturated fatty acid (PUFA) side chain (LH) to form water and a carbon-centered free radical ($L^{\cdot}$). This carbon radical then undergoes an internal rearrangement to yield a conjugated diene, which then reacts with molecular oxygen to give a peroxy radical ($LOO^{\cdot}$). The peroxy radical then abstracts a hydrogen atom from a second PUFA side chain to yield another $L^{\cdot}$ and continue the chain reaction, forming a lipid peroxide (LOOH) in the process [DiGuiseppi and Fridovich (1984) and Emanuel (1986)]. A single hydroxyl radical can thus cause a calamitous cascade of membrane oxidation. The antioxidant vitamin E (α-tocopherol, EH) terminates this chain reaction by donating a hydrogen atom and trapping peroxy radicals (Wefers and Sies, 1988; Willson, 1983).

$$LOO^{\cdot} + EH \longrightarrow LOOH + E^{\cdot} \quad \ldots (1)$$

$$LOO^{\cdot} + E^{\cdot} \longrightarrow \text{non-reactive product} \quad \ldots (2)$$

Vitamin E is the most efficient known terminator of lipid peroxidation; one molecule of α-tocopherol can protect 1,000 lipid molecules (Pauling, 1986).

Source of biological free radicals

Free radicals in biological systems can originate from a variety of sources. Molecular oxygen can react with a range of small organic molecules, including thiols, hydroquinones, flavins and catecholamines, to yield superoxide radical (Byczkowski and Gessner 1988).

$$O_2 + e^{-} \longrightarrow O_2^{\cdot-} \quad \ldots (3)$$

Hydrogen peroxide is a product of $O_2^{\cdot-}$ dismutation:

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^{+} \longrightarrow H_2O_2 + O_2 \quad \ldots (4)$$

Superoxide dismutase catalyses this rather slow reaction, making it about 10^4 times faster (Halliwell and Gutteridge 1984).

Numerous enzymes generate superoxide radical during their catalytic cycling (Halliwell and Gutteridge, 1984; Byczkowski and Gessner, 1988). These include xanthine oxidase, aldehyde oxidase, and dihydroorate dehydrogenase (Byczkowski and Gessner, 1988). Peroxisome enzymes such as urate oxidase and D-amino-acid oxidase are potent, direct sources of cellular H_2O_2, and the cytochrome P-450 (mixed function oxidase) system, responsible for the metabolism of foreign hydrophobic chemicals, produces both $O_2^{\cdot}$ and H_2O_2 (Freeman and Crapo, 1982; Naqui and Chance, 1986). Microsomal and plasma membrane-associated enzymes such as lipoxygenase and cyclooxygenase are utilized in arachidonic acid metabolism, a reaction that involves carbon-centered free radicals and a heme-bound hydroxyl radical (Freeman and Crapo, 1982; Naqui and Chance, 1986).

The mitochondrial electron transport chain reduction of oxygen proceeds via oxygen free radicals and H_2O_2:

$$O_2 + e^- \longrightarrow O_2^{\cdot} \quad \text{... (5)}$$

$$O_2^{\cdot} + e^- + 2H^+ \longrightarrow H_2O_2 \quad \text{... (6)}$$

$$H_2O_2 + e^- + H^+ \longrightarrow H_2O + OH^{\cdot} \quad \text{... (7)}$$

$$OH^{\cdot} + e^- + H^+ \longrightarrow H_2O \quad \text{... (8)}$$

Net: $$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O \quad \text{... (9)}$$

This reaction is catalyzed by the heme-copper enzyme cytochrome oxidase but, despite the high efficiency of this enzyme, some $O_2^{\cdot-}$ and H_2O_2 escape into the mitochondrion and the cytosol (Greenwood and Hill, 1982).

Hydroxyl radical can be produced directly as the result of the reaction between H_2O_2 and a suitable reductant such as glutathione or NADH (Florence, 1984; Florence, 1986):

$$NADH + 2H_2O_2 + H^+ \longrightarrow NAD^+ + 2H_2O + 2OH^{\cdot} \quad \text{... (10)}$$

In addition, the iron- or copper-catalyzed Haber-Weiss reaction can also produce $O_2^{\cdot-}$ and H_2O_2:

$$Fe\ (III) + O_2^{\cdot-} \longrightarrow Fe\ (II) + O_2 \quad \text{... (11)}$$

$$Fe\ (II) + H_2O_2 \longrightarrow Fe\ (III) + OH^- + OH^{\cdot} \quad \text{... (12)}$$

Suitable catalysts for this fairly slow reaction are iron-EDTA and copper-1,10-phenanthroline (Florence, 1984). Vanadium, titanium and cobalt redox couples can also act as catalysts (Halliwell and Gutteridge, 1985). It is debatable, however, if effective iron and copper catalysts exist *in vivo* except in unusual diseases,

since the presence of "free" (non-protein bound) iron or copper in biological fluids has never been proven (Cross, 1987; Florence, 1984). Ferritin has been suggested (Halliwell and Gutteridge, 1985; 1986) as a source of free iron, but iron may only dissociate from aged or degraded protein (Florence, 1984). However, by administering salicylate to volunteers, and measuring hydroxylated salicylate derivatives in urine, Halliwell *et al.*, (1985) provided some evidence that hydroxyl radical is produced *in vivo*. A hydroxyl radical bound to iron in a heme molecule ("crypto" hydroxyl radical) may be involved in biological oxidations by cytochrome C and heme oxidases (Florence, 1985; Halliwell and Gutteridge, 1985; Youngman, 1984). An intriguing theory to explain the high rate of cardiovascular diseases in developed countries is based on the much higher iron status of people in affluent societies (Weinberg, 1984).

Singlet oxygen ($^1O_2$) is a potent oxidizer of lipid membranes (Halliwell and Gutteridge 1985; Naqui and Chance, 1986). It can arise in biological systems from photochemical reactions involving prophyrins, flavins, and chlorophylls. It is also formed when hypochlorite reacts with H_2O_2 (Halliwell and Gutteridge, 1986).

$$OCl^- + H_2O_2 \longrightarrow Cl^- + H_2O + {}^1O_2 \qquad \ldots (13)$$

Since hypochlorite is formed during phagocytosis with the enzyme myeloperoxidase, reaction (13) may be relevant *in vivo* (Cross 1987; Halliwell and Gutteridge, 1985).

Hydroxyl radical and singlet oxygen are the most reactive of the activated oxygen species. Their very reactivity, however, may limit the damage they can produce, since they can be scavenged so easily (Cross, 1987; Florence, 1983). For example, intracellularly-produced $OH^{\cdot}$ was extremely toxic to the marine diatom *Nitzschia closterium* whereas extracellularly-produced $OOH^{\cdot}$ was completely innocuous (Florence and Stauber, 1986). Apparently, organic molecules in the growth medium or on the exterior of the cell membrane scavenged the hydroxyl radical before any damage could be done. The less reactive, but much more stable, H_2O_2 molecule, on the other hand, can cross cell membranes as readily as water, and could diffuse unimpeded to a vulnerable part of the cell where it could react with a suitable reductant to produce $OH^{\cdot}$ (reaction 10), leading to damage of, for example, genetic material. Lipid peroxides are similarly very dangerous because of their relative stability and selective reactivity (DiGuiseppi and Fridovich, 1984; Emanuel, 1985).

It should be pointed out that oxygen free radicals, despite being so dangerous if generated in the wrong place or at too high a concentration, do perform several life-sustaining tasks in living organisms (Halliwell and Gutteridge, 1985; Florence, 1983). They are essential to respiration (reactions 5-9) and they are the lethal agents in phagocytosis, where a circulating leucocyte engulfs a pathogenic microorganism and destroys it with a burst (the "respiratory burst") of $O_2^{\cdot-}$, H_2O_2, OCl^- and, possibly $OH^{\cdot}$ (Halliwell and Gutteridge, 1985). Prostaglandin synthesis and the metabolism of alcohol also involve free radicals, and free radical modification of DNA may be necessary for heritable change (Tomlasoff *et al.*, 1980).

FREE RADICAL SCAVENGERS

Utilization of oxygen

All aerobic organisms have an impressive array of free radical scavengers. Many of these scavengers are designed specifically to protect the organism from oxygen-derived free radicals and other activated oxygen species. Man has the longest maximum lifespan potential of all mammals because he has superb free radical defenses (Tomlasoff *et al.*, 1980; Cutler, 1984; Ono and Okada,1985).

Life apparently arose spontaneously 3.5 billion years ago from basic chemicals produced from the primitive oxygen-free atmosphere by free radical reactions initiated by ionizing radiation from the sun (Harman, 1981). About one billion years later, blue-green algae appeared, and some 1.3 billion years ago the concentration of atmospheric oxygen, produced by the photosynthesizing algae, had reached 1% of the present value, the toxic level for the fermentative anaerobes (Greenwood and Hill, 1982). The anaerobic procaryotes disappeared, except for a few in oxygen-deficient areas, and the sturdier, more complex, and more energy efficient eucaryotes became the dominant cells. Up to 18 times more energy in the form of ATP can be extracted from glucose by oxidizing it to CO_2, compared with anaerobic glycolysis (Greenwood and Hill, 1982).

The utilization of oxygen is, however, not without its problems. The *in vivo* reduction of oxygen produces $O_2^{\cdot-}$, $OH^{\cdot}$ and H_2O_2 (reactions 5-9) which are highly damaging to the cell, and this toxicity increases rapidly if the oxygen concentration becomes much higher than the ambient 20% of the atmosphere. Our margin of safety is narrow. We possess defenses against oxygen toxicity which are sufficient to meet ordinary demands, but which can be easily overwhelmed (Halliwell and Gutteridge, 1984).

Enzymatic defenses

Superoxide dismutase (SOD) removes $O_2^{\cdot-}$ via catalysis of reaction (6) (Ansher *et al.*, 1986). There are three forms of SOD; copper-zinc, manganese and iron (Cutler, 1984; Oberley *et al.*, 1980; Totter, 1980). Copper-zinc and manganese SOD are found in eucaryotic cells (including human), while iron-SOD occurs in bacteria (Tomlasoff *et al.*, 1980; Ono and Okada, 1985).

Copper-zinc SOD consists of two identical sub-units, each having a single intramolecular S-S bond (Florence, 1980). These bonds are essential to the stability of the protein, and are unusually resistant to radiation and chemical attack, a property doubtlessly essential to the efficient functioning of SOD as a free radical scavenger (Florence, 1980).

Two enzymes, glutathione peroxidase and catalase, are used to catalyze the decomposition of H_2O_2. Glutathione peroxidase has four atoms of selenium/mole, and uses glutathione as substrate to reduce H_2O_2 and lipid peroxides (Shamberger, 1984). Catalase is a hemoprotein, and has the advantage that it does not require an

auxiliary reductant to destroy H_2O_2. These two enzymes, together with SOD, work in a synergistic fashion to protect lipid membranes and protein sulfhydryl groups, especially in the mitochondrion, from attack by O_2^- and H_2O_2 (Griffin, 1979). Glutathione peroxidase also removes lipid peroxides, and thus inhibits the chain reaction of lipid peroxidation.

Non-enzymatic defenses

The free radical dissociating enzyme defense system is backed up by an array of non-enzymatic defenses (a "strategic reserve"), consisting of small nucleophilic molecules that constitute a multi-layered defense array against activated oxygen species, and which also scavenge carbon-centered free radicals (Halliwell and Gutteridge, 1986; Slater, 1984; Wayner *et al.*, 1987). Some of these antioxidants, with approximate average concentrations in human blood plasma (mgL^{-1}) are: ascorbic acid (vitamin C), 10; reduced glutathione, 400 (whole blood); α-tocopherol (vitamin E), 10; uric acid, 50; β-carotene, 2; and ceruloplasmin, 340 (Ames, 1983; Oliver *et al.*, 1984). Albumin and glucose also have free radical scavenging properties (Halliwell and Gutteridge, 1986).

Vitamin E, like β-carotene, is lipid soluble and occurs where it is most needed, in the cell membrane, where it performs the critical task of terminating the potentially calamitous chain reaction of lipid peroxidation. Ascorbic acid is not lipid soluble, but is a versatile reductant, and reacts synergistically with vitamin E and reduced glutathione (GSH) to produce a powerful antioxidant system (Wefers and Sies, 1988; Florence, 1983; Lathia *et al.*, 1988).

Selenium

Selenium may be the most important antioxidant element in the human body (Shamberger, 1984; Clark and Combs, 1986; Dreosti, 1986). In addition to being essential to the functioning of glutathione peroxidase, it appears to have other more subtle roles, such as enhancing DNA repair mechanisms while delaying cell mitosis (Ip, 1985). This role may allow initiated cancer cells to repair themselves before division, so that the progeny are not malignant. An international study of the selenium content of the diet showed an excellent inverse correlation with cancer incidence (Clark, 1985), as did blood selenium and cancer incidence in Provinces across China (Yu *et al.*, 1985). China is an ideal country to study the epidemiology of cancer and selenium, because the soil (and hence crops) in some areas of China are so low in selenium that a specific type of cardiomyopathy (Keshan Disease) occurs (Shamberger, 1984; Yu *et al.*, 1985), while soil selenium is so high in other Provinces that chronic seleniosis sometimes occurs (Yu *et al.*, 1985).

New Zealanders are particularly low in selenium, and suffer high rates of cancer, cardiovascular disease, asthma, and sudden infant death syndrome (SIDS). Low dietary selenium has also been linked (Robinson, 1988) with inflammatory diseases such as rheumatism, arthritis, and repetitive strain injury, a not unlikely situation considering

the free radical scavenging properties of selenium. A study has been initiated in Christchurch Hospital on the relationship between dietary selenium and SIDS (C.C. Winterbourn, private communication).

Selenium yeast is a cheap and convenient dietary selenium supplement for those who are not yeast sensitive.

PROTECTION AGAINST CANCER AND AGING BY DIETARY CONTROL OF FREE RADICAL FORMATION

The possibility that cancer can be minimized and, the rate of aging reduced by the intake of antioxidants (free radical scavengers) has intrigued nutritionists for the past 20 years (Florence, 1983; Gey *et al.*, 1987; Young, 1983). If cancer and aging are indeed caused by oxidizing free radicals, an increase in the concentration of antioxidant in tissues and the circulatory system may offer some protection. Certainly, many serum antioxidants show a decline with age (Young, 1984), and an increase in dietary antioxidants usually brings about an increase in mean lifetime (but not maximum lifespan) in experimental animals (Harman, 1984). Prospective human studies have shown that lower rates of cardiovascular disease and cancer are associated with a high status of serum vitamins A and E, β-carotene and selenium. Several epidemiological studies have indicated that the cruciferous vegetables, cabbage, broccoli, brussel sprouts and cauliflower, protect against cancer (Florence, 1983; Ansher *et al.*, 1986). The active compound in these vegetables is believed to be a dithiolthione (Ansher *et al.*, 1986).

Based on the ascorbic acid content of a primitive vegetarian diet, Pauling (1970) estimated that modern man needs a diet with an average of 2.3g of vitamin C per day. The degree of supplementation required will, of course, depend on individual diet and lifestyle, but 2.3g of vitamin C per day will lead to an ascorbic acid serum concentration about three times the unsupplemented level (Pauling, 1970). Tobacco smoking lowers serum vitamin C (each cigarette destroys about 25mg of ascorbic acid), and some smokers suffer from chronic, sub-clinical scurvy (Pauling, 1986).

Nitrosamines, formed from dietary amines and nitrite (present in preserved food or produced naturally from nitrate), are perhaps the most universal and potent class of carcinogen (Hoffmann, 1987; Lathia *et al.*, 1988; Shamberger, 1984). We are exposed to them continuously, and they are probably the specific cause of gastric (stomach) cancer (Shamberger, 1984). Their formation is catalyzed by dietary compounds such as thiocyanate, iodide and polyphenols, and several nitrosamines are present in tobacco and tobacco smoke (Hoffmann, 1987). Ascorbic acid destroys nitrosamines rapidly and completely, and a combination of vitamins C and E is even more effective (Lathia *et al.*, 1987). A high concentration of free vitamin C in the stomach, intestines, bladder and tissues would seem to be desirable for protection against nitrosamines.

The argument is often put forward that intakes of vitamin C greater than about 150 mg per day are unnecessary because, when more than this is taken, vitamin C often appears in the urine. However, despite its appearance in the urine, much more

than 150 mg per day is usually needed to ensure tissue saturation of the vitamin (Pauling, 1986). There are no known ill effects of vitamin C when taken at the rate of 1 to 3 grams per day (Pauling, 1986). The frequently-raised connection between oxalate renal stones and vitamin C is false, and arose from inadequate analytical methods, where urinary vitamin C interfered in the determination of oxalate (Pauling, 1986; Cathcart, 1985).

Australia is one of the few countries in the world where the over-the-counter sale of dietary supplements containing selenium is prohibited. In excess, selenium, like many freely available drugs such as aspirin, is toxic (Shamberger, 1984). But this is not sufficient reason for making it unavailable to the public. The Chinese cancer study (Yu *et al.*, 1985) suggests that the optimum serum concentration of selenium is 250-300 mg/L. In most countries, supplementation, e.g., by low-cost selenium yeast tablets, would be necessary to achieve this serum concentration. If the tablets were prepared to contain, say, 50 μg selenium per tablet, a huge excess over the recommended dosage would be needed to cause even low-grade chronic selenium toxicity (Shamberger, 1984).

FUTURE RESEARCH

The association between disease, especially age-related diseases such as cancer, senile dementias, cardiovascular diseases and arthritis, and free radical formation is rapidly achieving more credence (Cross, 1987; Marx, 1987). Dietary antioxidants may offer protection against these free radical diseases, but research in this area is hindered by lack of funding and by skepticism from parts of the medical profession. With the escalating costs of medical treatment and of caring for an aging population, any simple preventive scheme such as dietary supplementation would have tremendous economic benefits. However, since deterioration from these age related diseases can take place over 30-50 years, supplementation should, ideally, be carried out for the whole of the adult life.

Ideally, all the vitamins and minerals needed for maximum protection from disease would be obtained from food alone. In a modern society, however, this is unlikely to occur because of variations in the nutrient content of food (e.g., soils) are becoming deficient in selenium (Frost, 1983), and because of the increasing addiction of most of the population to "fast" and packaged foods.

Research is urgently needed into the *in vivo* formation and detection of free radicals in humans (Ip, 1985), the existence in biological systems of factors such as free iron and copper that can catalyze the formation of hydroxyl radical, and the role of natural free radical scavengers. Previously unsuspected protective factors such as germanium which is present in garlic and onions (Kidd, 1987), and the dithiolthiones in cruciferous vegetables (Ansher *et al.*, 1986) may be important. There may be many potent, naturally-occurring free radical scavengers that could profitably be used as dietary supplements. Large-scale prospective epidemiological studies on disease and antioxidants should be initiated to establish the role of dietary free radical scavengers, and to determine their optimum intakes for protection against the degenerative diseases.

Preventive medicine, sadly neglected in Western countries, must be taken much more seriously.

TRACE ELEMENTS IN HEALTH AND DISEASE

The first half of this century saw the rapid development of an understanding of the role of vitamins in human health. The second half of the century belongs to trace elements.

There are now 15 trace elements known to be essential to human health and development — arsenic, chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc — and four others —cadmium, lead, lithium and tungsten — may be essential. Because life developed in the presence of *all* the elements, it is likely that more will, eventually, be found to be essential.

While a deficiency of these elements will cause ill health and even death, an excess may be just as dangerous. For every element there is a window of safe intake, between essentiality and toxicity. For some major elements, e.g., calcium, this window is wide but, for most trace elements, e.g. selenium, it is quite narrow. In this respect, trace elements are different to vitamins, especially the water-soluble vitamins, where a large excess over the RDA is usually harmless. Antagonistic effects between trace elements are also common; an excess of one element may severely inhibit the uptake of another. For these reasons, the random use of trace elements as dietary supplements should be viewed with caution, and any dosage well in excess of the RDA should be prescribed only by a professional qualified in this area.

Although research into the role of trace elements in health and disease is expanding rapidly, we are still profoundly ignorant about this vital and fascinating area of biology. Some recent developments are now described.

Iron—In a typical Western diet of meat and vegetables, over 80 percent of the iron absorbed by the body comes from meat. Whereas the bioavailability of iron from grains and vegetables is very low, typically 1-5 percent, heme iron in meat is nearly 40 percent available (Monsen *et al.*, 1978). In addition, heme promotes the absorption of non-heme iron. For this reason, with the exception of some pre-menopausal women and vegetarians, aenemia is rare in Western countries. In third world countries, however, where meat intake is low, aenemia is so prevalent that it is probably the world's most common disease.

Cardiovascular disease is the most common cause of death in Western society, and the rate is very much higher than in the underdeveloped countries. This is usually explained by our higher intake of fat and lack of exercise. Blood cholesterol certainly correlates with the incidence of cardiovascular disease, but it has been apparent for some time that there must be another important contributor to heart disease that has not yet been discovered (Buist, 1989).

Sullivan (1981; 1989) proposed that iron overload is a significant factor in ischemic heart disease, and that the high iron status of Westerners (high ferritin, low unsaturated iron binding capacity) is the cause of their excess rate of heart disease

compared with people in underdeveloped countries. This theory would also explain why pre-menopausal women have a much lower rate of heart disease than men, and why the rate after menopause approaches that of men. Iron loss by menstruation or blood donation may be protective against heart disease. This hypothesis would be readily checked by a retrospective epidemiological study of cardiovascular disease in long-term blood donors.

The iron overload theory is consistent with the well known ability of free iron to catalyze biological free radical reactions. Takkunen *et al.*, 1989 however, have criticized the suggestions that high iron status leads to heart disease and cancer (Weinberg, 1984) and claimed instead that high body iron stores may actually *decrease* morbidity and mortality.

Zinc—Zinc is one of the most important trace elements, being involved in the functioning of over 50 enzymes in the human body. Yet it is extremely difficult to find a measurable index of zinc deficiency; the zinc "taste test" is probably as good as any (Stevens *et al.*, 1988).

Some severe cases of anorexia nervosa respond dramatically to zinc supplementation (Stevens *et al.*, 1988; Jones, Bryce-Smith and Simpson, 1984; Dinsmore *et al.*, 1984). Originally it was believed that zinc deficiency in these patients was a result of fasting, but it now appears that low zinc intake may actually contribute to the onset of anorexia (Schauss and Bryce-Smith, 1987).

Copper—Although several studies have set the RDA of copper at 2 mg/day, the role of this trace element in human health is a complete enigma. While some studies have shown unequivocally that a deficiency of copper can lead to high serum cholesterol and an increased risk of cardiovascular disease (Reiser *et al.*, 1987) other work suggests that excess copper causes neurological complaints, hypertension, liver and kidney disfunctions, cancer, and accelerated aging (Pfeiffer and Mailloux, 1987). Certainly, copper is a potent catalyst of lipid peroxidation and free radical reactions. It has been claimed that a typical Western diet does not provide sufficient copper (Klevay *et al.*, 1980) but tapwater from a copper pipe water service typically contains 0.5-1.0 mg copper/litre, so most people would obtain much of the RDA from drinking water alone.

Vitamin C and zinc significantly reduce the bioavailability of copper (Finley and Corklewski, 1983).

Selenium—Selenium has emerged as a vital trace element for protection against cancer, heart disease, and inflammatory joint disease. A large epidemiological study in China, where soil (and hence crop) selenium concentrations vary widely, showed an excellent inverse correlation between serum selenium and cancer rate (Yu *et al.*, 1985). This study suggests that in Australia we could lower cancer rates by as much as 40% if we increased our serum selenium from the present 0.09 mg/litre to 0.25 mg/litre, a value typical of Japan and some South American countries where the rates of colorectal, breast and lung cancer are very low. Recent research indicates that RSI, cot death, and arthritis may also be responsive to selenium supplementation.

Manganese—Although manganese is an essential trace element, an excess can cause a neurological complaint similar to Parkinson's disease (Bell, 1988). Aborigines

living on Groote Eylandt, Northern Australia, where the soils are high in manganese, suffer from a Parkinson-like disease (Bell, 1988). Aborigines have a "close-to-the-earth" lifestyle and, on Groote Eylandt, are exposed to high levels of manganese. Manganese appears to exert its neurotoxic effect by catalyzing the oxidation of the neurotransmitter, dopamine (Florence and Stauber, 1988).

Lead—It has always been assumed that only organolead compounds (e.g., tetraethyl lead) can be adsorbed through the skin. Recent work in our laboratories has shown, however, that all lead compounds, such as lead oxide, lead metal powder and lead nitrate, will dissolve in sweat and be transported rapidly through the skin and into the circulatory system (Florence *et al.*, 1988; Beckmann, 1989). Skin adsorption therefore represents another potential hazard when working with this, and possibly other, heavy metals.

Aluminium—The neurotoxic effects of aluminium are now well known. What is not so well known, however, is that some drugs and dietary substances greatly enhance the bioavailability of aluminium. Citrate, for example, increases aluminium absorption nearly 10-fold (Alfrey, 1987). Some deaths have occurred in the USA when patients being treated with large amounts of citrate for kidney stones, have taken aluminium hydroxide gel preparations for indigestion or as an anti-diarrhoeic (Alfrey, 1987). Since citrate is common in many foods, it is important that aluminium intake should be kept to a minimum.

Dietary trace elements play a vital role in human health, yet there is obviously a great deal of confusion about the optimum intake and the safe range of each element. Much research remains to be done, and new essential elements will certainly emerge. For example, in our laboratories we have recently found that human blood contains 1-2 micrograms/litre of platinum. This is an extraordinarily high concentration of such a rare metal. Does it have a biological function? In many chemical reactions, platinum is an excellent catalyst, so perhaps Nature has made use of it in living organisms. These and other unanswered questions ensure that biological trace element research will continue to expand.

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