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Diet and Antioxidant/Oxidant Systems

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Free radicals and antioxidants are widely discussed in clinical and nutritional reports and even in the lay press. The assumption is that free radicals are bad and antioxidants good. By contrast, a recent clinical trial suggested that giving the alleged "antioxidant" β-carotene to smokers accelerated the development of lung cancer (1). The purpose of this chapter is to provide some scientific background to help health professionals evaluate such claims and counterclaims.

WHAT IS A FREE RADICAL?

All atoms have a nucleus, containing positively charged protons and neutral neutrons. Electrons orbit around the nucleus, usually associated in pairs. A *free radical* is defined as any species that contains one or more unpaired electrons (2). The presence of unpaired electrons alters the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding nonradical. However, the chemical reactivities of different types of free radicals vary enormously.

WHAT RADICALS ARE MADE IN THE HUMAN BODY?

Humans are exposed to electromagnetic radiation from the environment, both natural (e.g., radioactive radon gas and cosmic radiation) and from man-made sources. Low-wavelength electromagnetic radiation (e.g., τ rays) can split water in the body to generate *hydroxyl radical*, $OH^{\cdot}$. This viciously reactive radical, once generated, attacks whatever it is next to (2).

The human body also makes the *superoxide radical*, $O_2^{\cdot -}$. Some superoxide is made by "accidents of chemistry," in that many molecules in the body react directly with O_2 to make $O_2^{\cdot -}$. Examples are the catecholamines (adrenaline, dopamine), tetrahydrofolates, and some compounds found in mitochondrial and endoplasmic reticular electron transport chains. Such $O_2^{\cdot -}$ generation is an unavoidable result of having these molecules in a body that needs oxygen (2). But in addition, some $O_2^{\cdot -}$ is made *deliberately*. For example, the phagocytic cells that defend the body against foreign

organisms (neutrophils, monocytes, macrophages, eosinophils) generate large amounts of $O_2^{\cdot -}$, as part of the mechanism by which foreign organisms are killed (3). This is an essential defense mechanism against infection. However, it can go wrong: there are several diseases (examples being rheumatoid arthritis and inflammatory bowel disease) in which there is excessive phagocyte activation. This inappropriate phagocyte activation causes tissue damage, to which oxygen radicals contribute (2).

It has been estimated that 1–3% of the oxygen inhaled is used to make $O_2^{\cdot -}$ in the human body. Simple calculations show that more than 2 kg of $O_2^{\cdot -}$ is generated in the human body every year; people with chronic infections may make much more (4).

Another physiologic free radical is *nitric oxide*, $NO^{\cdot}$. This performs many useful physiologic functions such as regulation of the blood pressure and intercellular signaling, but too much $NO^{\cdot}$ (like too much $O_2^{\cdot -}$) can be toxic: excess $NO^{\cdot}$ production is thought to be an important tissue injury mechanism in several diseases, such as ischemic injury and septic shock (5,6).

HOW DO RADICALS REACT?

Radical plus Radical

If two free radicals meet, they can join their unpaired electrons and make a covalent bond (a shared pair of electrons). Thus, superoxide and nitric oxide combine as follows (6):

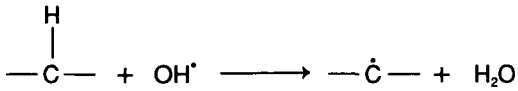
$$O_2^{\cdot -} + NO^{\cdot} \rightarrow \underset{\text{peroxynitrite}}{ONOO^-}$$

At physiologic pH, peroxynitrite can damage proteins directly and will decompose into toxic products that are or closely resemble nitrogen dioxide gas, nitronium ion, and $OH^{\cdot}$ (6). Hence at least some of the toxicity of excess $NO^{\cdot}$ may involve its interaction with $O_2^{\cdot -}$ (6). In addition, excess $O_2^{\cdot -}$ can interact with H_2O_2 plus iron or copper ions, making damaging $OH^{\cdot}$ (7).

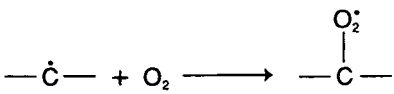
Radical Plus Nonradical

Most molecules found in the human body are not radicals. Hence, any reactive free radical generated is most likely to react with a nonradical. When a free radical reacts with a nonradical, a *free radical chain reaction* results and new radicals are formed. Figures 1 and 2 illustrate (in a very simplified way) two important reactions of this type. Attack of reactive radicals on membranes or lipoproteins starts the free radical chain reaction called *lipid peroxidation* (2). There is growing evidence that lipid peroxidation takes place in human blood vessel walls and contributes to the development of atherosclerosis, raising the risk of stroke and myocardial infarction (8). Atherosclerosis (as "fatty streaks") starts in the first decade of life. It is ironic

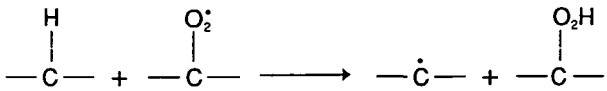
A reactive radical (such as $OH^{\bullet}$) abstracts an atom of hydrogen ($H^{\bullet}$) from a polyunsaturated fatty acid side chain in a membrane or lipoprotein. Monounsaturated fats (such as oleate and its esters) are more resistant, and saturated fats even more resistant. $H^{\bullet}$ abstraction leaves an unpaired electron on the carbon (a hydrogen atom has only one electron, so its removal must leave behind a spare electron)



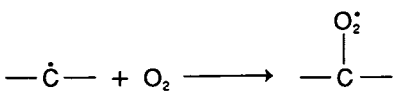
The *carbon radical* reacts with oxygen



The resulting *peroxyl radical* attacks an adjacent fatty acid side chain to generate a new carbon radical



and the chain reaction continues



Overall: Attack of one reactive free radical can oxidize multiple fatty acid side chains to *lipid peroxides*, damaging membrane proteins, making the membrane leaky, and eventually causing complete membrane breakdown. Peroxidation of plasma low density lipoproteins contributes to the development of atherosclerosis (8). Peroxidation of lipids in foods can cause rancidity (25). *This could be a serious problem in baby foods supplemented with highly polyunsaturated fatty acids (e.g., docosahexaenoic acid), especially if attempts were made to simultaneously supplement the foods with iron salts.*

FIG. 1. Radicals beget radicals: the chain reaction of lipid peroxidation.

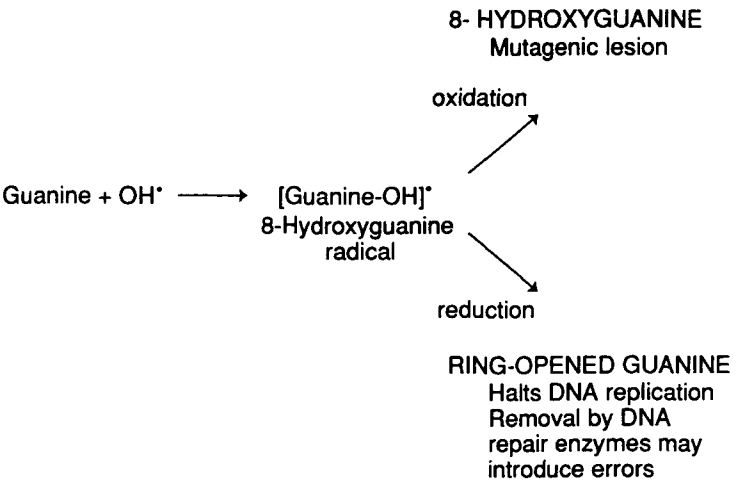


FIG. 2. Radicals beget radicals: attack of hydroxyl radical on the base guanine in DNA.

that polyunsaturated fatty acids (PUFAs), which are essential in the diet, especially for development of the brain and nervous system, are more prone to peroxidation than saturated or monounsaturated fatty acids (Fig. 1): this is a problem in food manufacturing and may also be a problem *in vivo* in that increased PUFA intake may necessitate an increased intake of antioxidants. It will be a special problem in baby foods supplemented with highly polyunsaturated fatty acids (10). Peroxides can also be present in intravenous lipid emulsions and other parenteral solutions used in premature babies (11,12).

If $OH^{\cdot}$ radicals are generated close to DNA, they can attack the purines and pyrimidines and cause mutations. For example, the purine guanine is converted into 8-hydroxyguanine and other products, which can cause errors during DNA replication (9).

ANTIOXIDANT DEFENSES

Because free radicals are made constantly and in large amounts in the human body, antioxidant defenses have evolved to protect us. *Superoxide dismutase* enzymes remove $O_2^{\cdot-}$, converting it to hydrogen peroxide (H_2O_2), which is mostly removed by *catalase* and *glutathione peroxidase* enzymes (2). These enzymes have not fully developed in the lungs of preterm babies, which predisposes them to oxygen toxicity (13).

Iron and copper ions are powerful promoters of free radical damage, accelerating lipid peroxidation (Fig. 1) and causing $OH^{\cdot}$ formation. To minimize this, the adult human body has a complex system of metal ion transport and storage proteins to ensure that these essential metals are rarely allowed to be in the "free" state. For example, iron bound to the plasma protein transferrin will not catalyze damaging free radical reactions (14). The same is true of iron bound to the protein lactoferrin (14), found in secretory fluids, including milk (15). However, in a high percentage of preterm babies, and a lower percentage of apparently normal full-term babies, transferrin is iron-saturated and plasma contains iron that can catalyze damaging free radical reactions, such as $OH^{\cdot}$ formation (16–18).

The human body also contains molecules that remove free radicals by reacting directly with them in a noncatalytic manner—*reduced glutathione (GSH), α-tocopherol, and ascorbate*. Premature babies often have low α-tocopherol levels, which may predispose them to retrolental fibroplasia and intracranial hemorrhage (see 19).

α-Tocopherol and ascorbate are normally obtained from the diet. Many other dietary constituents may be important free radical scavengers, but the evidence is not always good. Table 1 summarizes the author's views. In addition, GSH and ascorbate can be pro-oxidant in the presence of iron ions (Table 1; also see below), which can sometimes be present in the plasma of neonates (16–18).

Despite all of these antioxidants, some free radicals still escape to do damage in the human body. Thus DNA undergoes constant "oxidative damage" and has to be repaired. Free radicals attack proteins and the damaged products have to be degraded. Endproducts of lipid peroxidation are measurable in human body fluids, in atherosclerotic lesions, and in the "age pigments" that accumulate in old tissues (4).

OXIDATIVE STRESS

Because antioxidant defenses are not 100% efficient, increased free radical formation in the human body is likely to increase damage. The term *oxidative stress* is often used to refer to this. If mild oxidative stress occurs, tissues often respond by making extra antioxidant defense enzymes (13). However, severe oxidative stress can cause cell injury and death (4).

One way of imposing oxidative stress is by the action of certain toxins—those that produce free radicals or deplete antioxidant defenses. Examples are the herbicide paraquat, the solvent carbon tetrachloride, and the analgesic acetaminophen (2). One particular area of interest is the possibility that the side effects of several drugs involve increased oxidative damage (20). Exposure of neonates to oxidative stress, e.g., by exposure to increased O_2 or infection (causing increased phagocytic release of $O_2^{\cdot-}$ and H_2O_2) may be particularly damaging because of the "catalytic" iron that can be present in their plasma (16–18,21).

OXIDATIVE STRESS AND HUMAN DISEASE—PREVENTION

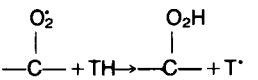
There is growing evidence that the major "killers," cardiovascular disease and cancer, can be prevented or delayed to some extent by dietary changes, such as

TABLE 1. *Dietary antioxidants: where are we now?*

A diet rich in fruits, nuts, grains, and vegetables seems to be protective against several human diseases, possibly because of the antioxidants they contain and/or the many other compounds present.

1. KNOWN TO BE IMPORTANT AS ANTIOXIDANTS

Vitamin E (fat-soluble) — General name for a group of compounds, of which α-tocopherol is the most important. Found in membranes and lipoproteins. Block the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicals (8).



The tocopherol radical (T•) is much less reactive in attacking adjacent fatty acid side chains and can be converted back to TH by vitamin C. Important in protection against atherosclerosis and, in babies, retrolental fibroplasia and possibly intracranial hemorrhage (8). Severe deficiency causes neurodegeneration.

2. WIDELY THOUGHT TO BE IMPORTANT AS AN ANTIOXIDANT

Vitamin C (ascorbic acid) — Has multiple metabolic roles, e.g., in collagen synthesis and hormone production. Inhibits the carcinogenicity of dietary nitrosamines (22). Probably assists α-tocopheral in inhibiting lipid peroxidation by recycling the tocopherol radical (2).

$$T^{\bullet} + \text{ascorbate} \rightarrow TH + \text{ascorbate}^{\bullet}$$

Good scavenger of many free radicals and may help to detoxify certain inhaled air pollutants (ozone, oxides of nitrogen, cigarette smoke) in the respiratory tract (23). Some potential pro-oxidant effects (see text).

3. PROBABLY IMPORTANT IN HUMAN HEALTH, BUT NOT NECESSARILY BY ACTING AS ANTIOXIDANTS

β-carotene, other carotenoids, related plant pigments — Increasing epidemiologic evidence that high body levels are associated with diminished risk of cancer and cardiovascular disease, particularly in smokers (38). Often simplistically grouped with vitamins E and C as antioxidants. Although many carotenoids exert antioxidant effects *in vitro* under certain conditions (38), it remains to be proved that this mechanism of action exists *in vivo* (4).

4. POSSIBLY IMPORTANT AS ANTIOXIDANTS

Flavonoids, other plant phenolics — Plants contain many phenolic compounds that inhibit lipid peroxidation and lipoxygenases *in vitro* (e.g., flavonoids) (26,39,40), although (like ascorbate) they can sometimes be pro-oxidant if mixed with copper or iron ions *in vitro* (26). It has been speculated that flavonoids in red wine could partly explain the "French paradox" (40). We do not know how many of these products are absorbed from the gut or become available *in vivo* to act as antioxidants. Many of them are being considered as antioxidant food additives.

reduction in fat intake and increased consumption of fruits, grains, and vegetables. There is also growing evidence that free radical damage is involved in the development of these diseases as well as in neurodegenerative disease (8,9,22). We obtain several compounds from a healthy diet that act (or may act) to diminish oxidative damage *in vivo*: Table 1 summarizes the author's view of our current state of knowledge. Since our endogenous antioxidant defenses are not 100% efficient, it seems reasonable to propose that dietary antioxidants are particularly important in diminishing the cumulative effects of oxidative damage over the long human life span and that they account for some of the beneficial effects of fruits, grains, and vegetables. For example, if continuous free radical damage to DNA, perhaps not always efficiently repaired, is involved in the development of spontaneous cancers (9), then more dietary antioxidants might help. There is considerable evidence that an increased dietary intake of vitamin E will decrease death from myocardial infarction (8). The dietary vitamin E requirement is probably raised if the percentage of polyunsaturated fatty acids in the diet is increased, a point worth bearing in mind in the design of baby foods rich in polyunsaturates.

CAN ANTIOXIDANTS BE PRO-OXIDANT? VITAMIN C, PLANT PHENOLICS, AND QUESTIONS OF IRON NUTRITION

Vitamin C has many antioxidant properties *in vitro*, several of which are relevant *in vivo*. The author believes that the major antioxidant activities of vitamin C *in vivo* are its ability to regenerate vitamin E (in cell membranes and especially in low-density lipoproteins; Table 1), so facilitating its action in inhibiting lipid peroxidation, and the ability of ascorbate to scavenge inhaled air pollutants such as ozone, oxides of nitrogen, and noxious free radical constituents of cigarette smoke (23). *In vitro*, however, vitamin C can also be *pro-oxidant*. Mixing ascorbate with iron ions causes $OH^{\cdot}$ generation, and iron/ascorbate mixtures have been used for decades to induce lipid peroxidation (2). Indeed, it has been claimed that the mixture of metal ions and ascorbate in some vitamin pills might even generate $OH^{\cdot}$ once the pills dissolve (24). A mixture of ascorbate and copper rapidly inactivates the H_2O_2 degrading enzyme catalase, and several investigators have described cytotoxic and mutagenic effects of ascorbate on isolated cells, probably involving interaction with iron or copper ions added to (or contaminating) the media surrounding the cells. This pro-oxidant effect of ascorbate is also well known to food scientists. For example, Porter (25) has referred to the actions of ascorbate in foods in these terms: "Of all the paradoxical compounds, ascorbic acid probably tops the list. It is truly a two-headed Janus, a Dr. Jekyll–Mr. Hyde, an oxymoron of antioxidants."

Hence, when metal ions are present, ascorbate can *stimulate* free radical damage. For example, a copper ion/ascorbate/H_2O_2 mixture devastates the bases of DNA by generating $OH^{\cdot}$. Several flavonoids and other plant phenolics can exert similar pro-oxidant effects *in vitro*: they can inhibit lipid peroxidation but, when mixed with iron

or copper ions, they can accelerate damage to other biological molecules such as DNA and proteins (26).

Pro-oxidant Effects: Biologically Relevant or Laboratory Artifacts?

Are these pro-oxidant effects relevant *in vivo*? We do not really know. In fact, this question is related to another important nutritional question: what is the optimal intake of iron? Iron is essential for human health (e.g., for hemoglobin, cytochromes, hydroxylase enzymes), but could too much iron cause harm? This is an important question in relation to the iron supplementation of foods.

In the healthy adult human body, although not in premature and some full-term babies (16–18), most transition metal ions are safely bound to transport and storage proteins and are not available to catalyze free radical reactions (2). Hence, the antioxidant properties of ascorbate (and any plant phenolics that are absorbed through the gut; see Table 1) probably predominate over any pro-oxidant effects in healthy people. There is a debate (27–29) over whether ascorbate in the plasma of premature babies is good or bad in terms of free radical reactions: it could be either, perhaps depending on whether or not catalytic metal ions are present.

Another factor to bear in mind is the possible presence of diseases that can lead to iron overload. Twice as many men in the USA have the inborn disease idiopathic hemochromatosis as have real iron deficiency anemia (30). This disease disturbs the regulation of iron absorption from the diet, so that the body becomes iron-overloaded and iron catalytic for free radical reactions is present in large amounts *in vivo*. Giving vitamin C to iron-overloaded patients without giving an iron-chelating agent (such as deferoxamine) has produced serious and sometimes lethal clinical consequences (31). Could ascorbate then be deleterious to some premature babies (28,29)?

A second caveat is that injury to human tissues causes release of iron and sometimes copper ions (32,33). As we all get older, we get sicker. In advanced human atherosclerotic lesions, metal ions catalytic for free radical reactions can be measured; indeed, the contents of such lesions (taken from cadavers) *stimulate* $OH^{\cdot}$ formation in the presence of H_2O_2 and ascorbate *in vitro* (34). There are repeated (although controversial) suggestions that high body iron and copper stores are associated with increased risk of cancer and cardiovascular disease (35,36). Could this be because the more iron or copper there is in a tissue, the more it can be mobilized to catalyze free radical reactions after an injury? If this is the case, then the *in vitro* pro-oxidant effects of ascorbate and flavonoids might become relevant *in vivo*. The optimal intake of iron in babies, adolescents, and adults may well be very different (37): iron is *essential* for brain development, but do adults need all the fortification of food with iron that is currently undertaken in many countries?

WHAT DO WE KNOW?

Perusal of Table 1 shows that many unanswered questions remain. Vitamin E seems protective against cardiovascular disease, retinopathy of prematurity, and possibly

intracranial hemorrhage, but what is the optimal dietary intake? Does it relate to the PUFA content of the diet? Carotenoids may be important anticancer agents, but is this action really related to an antioxidant effect? A good daily intake of vitamin C probably helps protect against cardiovascular disease and some forms of cancer (e.g., stomach cancer), but could much higher intakes do good or harm? Is ascorbate good for adults but bad for some babies? Is there too much iron supplementation? Fortunately, experimental tools to answer these questions are now becoming available. Thus, methods exist for measuring both ongoing and steady state (i.e., the balance between damage and repair) oxidative damage to DNA, proteins, and lipids in the human body (4,32). These methods may help us to gain information about optimal nutritional intakes. If the major "killers," cancer and vascular disease, could be delayed by even a few years by dietary changes, the social and economic benefits would be enormous.

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DISCUSSION

Dr. Haschke: You did not mention the role of zinc as a component of enzymes which act as antioxidants. Can you comment on that?

Dr. Halliwell: There is evidence for increased oxidative damage in zinc deficiency, and of course zinc is important for some of the antioxidant enzymes like the copper/zinc superoxide dismutase. However, the exact molecular mechanism of zinc action is unknown. Zinc seems to stabilize membranes in some unspecified way. So I think it is important, but I am not sure exactly how it acts.

Dr. Haschke: There is a hypothesis that selenium, as part of glutathione peroxidase, is responsible for a general protection of the cells, whereas zinc, as part of certain specific enzymes, is only responsible for the protection of specific cells in the body.

Dr. Halliwell: The only two roles we know of in humans for selenium are its involvement in thyroid hormone metabolism and its role in glutathione peroxidases. But we also know now that there is a family of glutathione peroxidases with different metabolic roles. Selenium also may play a role in carcinogen detoxification.

Dr. Dominguez: We have studied the aluminum content of various infant formulas and we have found that some have high aluminum levels. Do you think that the presence of aluminum could induce autoxidation, especially in baby foods supplemented with high polyunsaturated fatty acids?

Dr. Halliwell: It is possible but I would think that manufacturers of foods and fluids are now very careful to control the aluminum content. Aluminum itself will not induce free radical damage directly. We have made experimental observations that if you have both iron and aluminum ion or both iron and lead ion, the presence of those other metals will aggravate iron-dependent free radical damage. The problem with polyunsaturates in baby food is, of course, that highly polyunsaturated fatty acids are essential for the development of the nervous system but they also make the food extremely difficult to handle—it has a propensity to go rancid. You have to think about antioxidants and about whether you really need to put iron in the formula.

Dr. Guesry: You mentioned that vitamin C played a role in restoring the antioxidative capacity of α-tocopherol and that being the case, many people would like to increase their intake of antioxidants like vitamin E and vitamin C. But too much may have a deleterious effect. Could you tell us under what circumstances antioxidants may become pro-oxidants?

Dr. Halliwell: I believe vitamin C is an important antioxidant in humans, firstly because of its interaction with vitamin E, and secondly because in the respiratory tract, it helps in the detoxification of a whole series of inhaled oxidants such as ozone and nitrogen dioxide. Its pro-oxidant effects arise when you mix it with transition metal ions, particularly iron and copper. Ascorbate/iron and ascorbate/copper are very much used *in vitro*, e.g., to induce lipid peroxidation. In healthy human subjects, the body takes a great deal of care to ensure that we don't have free iron or copper around. So those pro-oxidant effects are less significant than the antioxidant effects. However, in clinical situations where you do have free iron, the balance may of course change. For example, there are studies on patients with iron overload disease in whom giving mega dosage of ascorbic acid caused all kinds of problems, particularly exacerbation of cardiac injury. In the manuscript I wrote for this meeting, I listed some papers which debate the point of whether vitamin C in premature babies is good or bad. Some people believe it is an important antioxidant; others believe they have evidence that because there is an iron overload in these babies, there are undesirable pro-oxidant effects, so vitamin C is contraindicated. Although we don't have any direct data on this, it may be a bad thing to give vitamin C to children being given chemotherapy for leukemia. I think vitamin C is not good for anyone who has iron overload. What I am not sure about is the optimal intake of vitamin C. I certainly don't endorse the consumption of 10 g of vitamin C per day. I think maybe 100 or 200 mg would be quite sufficient.

Dr. Guesry: I would like to have your interpretation of the Finnish study that was published about one year ago on heavy smokers who received β-carotene and α-tocopherol for the prevention of lung cancer.

Dr. Halliwell: The Finnish study showed that vitamin E at the doses used did not do anything much. The interesting result was with β-carotene. The data were in line with previous studies in showing that a high plasma β-carotene is associated with a low risk of cancer and *vice versa*. But of course this does not mean the effect is caused by β-carotene. If you have a lot of β-carotene in the plasma, it means you eat plenty of vegetables, and we know

vegetables are protective against cancer for all kinds of reasons. So the real question was whether β-carotene was a marker of diet or whether it was directly protective. What the Finns appeared to show was that it is not the β-carotene, it is something else; β-carotene is only a marker. One thing that has always intrigued me is that the effects of β-carotene seem to show up particularly well, both good and bad, in smokers. So there is some interaction between cigarette smoking and β-carotene. Our group at UC Davis is now studying the chemistry of the interaction of β-carotene with cigarette smoke to see if a toxic product can be identified.

Dr. Whitehead: In this meeting we have been challenged by Nestlé to concentrate on 1- to 18-year-old people and I think that we are finding it remarkably difficult. However, I would appreciate your comments on the age-related response to DNA damage. From a graph you showed, there seems to be a massive increase in DNA damage between age 60 and 80; does this mean this is the critical time for diet intervention? We seem to be concentrating very little on young people. Does that mean they are less important from this point of view?

Dr. Halliwell: The graph you are referring to is not a measure of DNA damage, it is a measure of the incidence of cancer with age, and of course makes the point that cancer is mainly a disease of old people. Biologically, the really interesting cancers are those of young people such as leukemia or, to a lesser extent, testicular cancer, because they do not follow that pattern. In the usual age-related cancers, it looks as though it takes time to accumulate all the changes in DNA, and the question is whether the constant free radical damage to DNA is a contributor to this. If it is a contributor, then anything that diminishes the steady-state level of free radical damage to DNA should be beneficial in delaying the incidence of cancer; it is not going to prevent it, but I think it will delay it. One approach is to try to measure DNA damage in the human body and see if by manipulating the diet, we can make a difference. It is the same with cardiovascular disease. If by manipulating the diet, you could delay the incidence by 5 or 10 years, you would abolish a lot of human misery. But the question is, how do we measure it?

Dr. Ballabriga: Current recommendations concerning lipids in children after 2 years of age suggest a total fat intake of around 30% of total daily energy, equally divided between polyunsaturated, monounsaturated, and saturated fatty acids. In southern European countries, in which the so-called Mediterranean diet is traditional, the supply of monounsaturates plays the main role in the supply of fat in the diet. We also know that the composition of low-density lipoprotein can change according to the diet, especially if the intake of polyunsaturates is very high, and in this case the possibility of autoxidation theoretically increases. Do you think that a combination of increased monounsaturates with a lesser proportion of polyunsaturates and a theoretical increase of antioxidants in the diet could be a good combination?

Dr. Halliwell: I hesitate when someone asks me if something is correct or not because the nutrition field is so complicated. It makes a difference, for example, if when looking at the effect of diet, you measure atherosclerosis or you measure death from myocardial infarction, because a lot of people with advanced atherosclerosis don't die. If you take aspirin after one heart attack, it delays your chance of getting another by diminishing blood clotting, but that probably has nothing to do with free radicals. And it seems to me that polyunsaturates are going to do all kinds of things. For example, they are going to diminish blood cholesterol and the tendency for thrombosis, but they will also make low-density lipoproteins more sensitive to peroxidation in the test tube at least. Of course, if at the same time you increase the intake of antioxidants like vitamin E, you may cancel out that effect. So I think you need to look at the overall effect of diet. One study we are involved in at the moment is to switch people from a meat-rich to a vegetable-rich diet to see what happens to LDL peroxidizability

and indices of lipid peroxidation and DNA damage. I am not keen on people taking huge amounts of polyunsaturates, like fish oil capsules, because one third of the fish oil capsules we have analyzed contain peroxidized lipid. With more polyunsaturates, you need more antioxidants, whether in the form of vitamin E or these other things I have discussed, or just more vegetables.

Dr. Gruskin: As you know, free cytosolic calcium is related to vascular resistance, and you have mentioned that oxidative stress increases cytosolic calcium. Can you relate oxidative stress to the hypertensive process?

Dr. Halliwell: We haven't done any research on this but various groups are looking at the interaction between nitric oxide (NO) and superoxide because superoxide can be produced by vascular endothelium. But to get large amounts, you have to damage endothelium, e.g., subject it to shear stress, high blood pressure, ischemia/reperfusion, and so on. Normally the vascular endothelium makes NO all the time, and NO reacts with superoxide, so they cancel each other out. Some scientists have claimed that in animal models of hypertension, superoxide dismutase normalizes blood pressure by getting rid of from the damaged vascular wall and allowing NO to work properly.

Dr. Kostopoulou: Could you comment on the significance of the free plasma iron in full term babies? Is it related to iron supplementation during pregnancy? How long does it persist?

Dr. Halliwell: We haven't done many studies on how long it persists. The problem is with a normal preterm baby, you can get cord blood, and you may be allowed to take a blood sample from the heel, but if you ask the mother for permission to take several blood samples a day for the next few days, you are not likely to get it. And there are ethical questions as well. But we have been able to study a couple of babies and free iron seems to persist for 2–4 days. As far as we can tell, looking at what happens to the babies in the first 1 or 2 years of life, it does not seem to do any harm. The hypothesis would be that it could make them more sensitive to environmental oxidative stress or cigarette smoke, or they might respond more badly if they develop an infection, so that their phagocytes generate O_2^- and H_2O_2.

Dr. Dalmau: There are a lot of preparations containing vitamin C and iron to treat iron deficiency anemia. Do you think it is a good combination or it can be dangerous because vitamin C can be pro-oxidant? Does this depend on the total amount of vitamin C or on the proportion?

Dr. Halliwell: It seems to me that the human gut is designed to keep iron out, not to let iron in. It is actually quite difficult to get a lot of iron through the gut at all except in people with idiopathic hemochromatosis. So most people will excrete most of the iron that they take in the form of supplements; they will only absorb what they need, and there is a very careful regulation of iron absorption. One paper has been published saying that if you grind up pills containing iron and ascorbate *in vitro*, they will make hydroxyl radicals. We have attempted to repeat that finding with a number of different pills but have been unsuccessful to date, I suspect because the iron is chelated in some way to substances like citrate. If you do have iron plus ascorbate in the gut, then there is a potential for pro-oxidant effects, and this has been demonstrated in several studies where both iron and ascorbate were instilled into the stomach with measurable pro-oxidant effects. Charles Babbs in the United States showed that human feces are remarkably pro-oxidant. He suspended feces in water and measured hydroxyl radical formation and found a great deal. An important factor was unabsorbed iron in the feces. So in terms of iron supplementation, there is the question of what gets in and of what stays in the colon. Of course, this is a somewhat artificial situation because when you suspend feces in water, they are under highly aerobic conditions, while in the colon they are probably anaerobic, but with heavy iron supplementation, there is a theoretical potential for causing damage to the colon by generating oxygen radicals in that environment. So I do

not recommend heavy iron supplementation at all. We know that iron is essential for the growing child and for maintaining adequate iron balance during pregnancy in adults. But apart from that, we really are not very clear about the optimal dietary intake of iron or whether having too much exerts pro-oxidant effects.

Dr. Ballabriga: Peroxides can be generated in food lipids according to the conditions of transport, storage, etc., and some of these peroxides can escape the digestion of fats. Do you think that these kinds of peroxides can be absorbed afterward and then act as oxidants of LDLs?

Dr. Halliwell: The literature on this is very controversial but I have seen some good papers which suggest that small amounts can be absorbed. The intestine has a very active glutathione peroxidase system which metabolizes all kinds of peroxides. My instinct is that peroxides are not absorbed very well because the intestine can detoxify them, but I think traces can get in.

Dr. Soriano: Glucose has been shown to be an oxidative substance. In fact, glycated products are supposed to play an important role in the changes in diabetes mellitus through oxidative stress. Could you comment on this?

Dr. Halliwell: Patients with diabetes do seem to be under oxidative stress. They have a rapid turnover of vitamin C and may have raised plasma lipid peroxides. I am not sure what the mechanism is; it may be related to glycation, it may be something else. A number of diabetologists now recommend their patients to take extra vitamin E. I am not sure there has been a good controlled clinical trial on this but there is a growing feeling that vitamin E supplementation is to be recommended in diabetes.

Dr. Bonjour: You have mentioned that antioxidants could be beneficial in cancer and in cardiovascular disease. I wonder whether they could also be useful to protect against progression of chronic renal failure. I ask this question because we have been experimenting with a compound—a so-called free radical scavenger—that was developed during the Second World War to protect the U.S. army against atomic radiation. This compound has now been marketed for protection against adverse effects of radiotherapy. In several experiments with this compound, we found we could protect against deterioration of renal function in chronic renal failure. Do you have some recent information on where free radicals could be involved in this deterioration of kidney tissue and whether antioxidants could in fact be effective?

Dr. Halliwell: What I have talked about almost entirely today is dietary antioxidants and their role in disease prevention, to delay or prevent cancer or cardiovascular disease by changes in the diet. There is also a second field, in which we use antioxidants as therapeutic agents and of course a number of companies have developed antioxidant drugs. For example, probucol has been evaluated for prevention of restenosis after angioplasty. Several antioxidants are being tested in various diseases: there is one under trial for rheumatoid arthritis, one under trial for inflammatory bowel disease, and so on. As far as the specific question of the kidney is concerned, there is an excellent recent article on free radicals in the kidney where the evidence is reviewed that free radicals play a role in some forms of nephritis, in nephrotoxicity, and so on (Annv Rev Physiol. 1995; 57: 245–262). So there are reasons for supposing that antioxidants might be useful.