The antioxidant nutrients and disease prevention—what do we know and what do we need to find out?[1,2]

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This has been a spectacularly successful conference. I say that not only because of the quality of the papers that were presented, reflecting the very real advances in our knowledge in this area, but also because of the unusual breadth of the subjects covered.

Chemistry

The symposium started with several lectures that outlined and explained the mechanistic background necessary to understand free radical reactions, and especially those reactions that are important in free radical biology. These lectures stressed the widespread nature of radical-mediated oxidative processes in normal biochemistry as well as in pathology and also the role of antioxidant nutrients in protecting biological systems against oxidative stress.

Some speakers addressed the difficulty in proving that a given biological effect results from free radical-mediated processes. Free radicals are so reactive that they cannot be directly observed in biological systems. Thus, we are on the trail of an elusive species, and we must look for its "footprints." Although a number of footprints that mark free radical activity in biological samples have been reported (1, 2), there is a need both to validate those that we have and to develop newer methods that are more sensitive and more specific.

The task of proving radical involvement in chronic disease conditions is often difficult. For example, smokers do not have a reduced level of vitamin E in blood plasma, but they do have a strikingly lower level of vitamin E in their pulmonary lavage fluid (3). Also, smokers have blood plasma that gives very nearly normal values of thiobarbaturic acid reactive materials (TBARS), but smokers exhale elevated amounts of ethane and pentane (4). Thus, cigarette smoke puts the greatest oxidative burden on the lung, as seems reasonable. Therefore, methods for detecting radical involvement must be targeted to, and sensitive to, oxidative damage to the lung when probing oxidative stress in smokers.

Several speakers provided insights into the rates at which vitamins E and C are oxidized in model systems, ranging from simple phospholipids to human blood serum and the LDL particle. More studies are needed of the mechanisms by which antioxidant nutrients protect model systems against oxidation. For example, Professor Niki presented data on the oxidation of human blood serum, showing that vitamin E in the LDL particle disappears very much faster than does vitamin E in the red blood cell membrane. While reasons for this can be hypothesized, it is an interesting observation that is worthy of further work.

Professor Esterbauer presented studies of the oxidation of the LDL particle, a process that may be involved in the development of fatty streaks in atherosclerosis. Esterbauer's group has shown, for example, that oxidation of polyunsaturated fatty acids (PUFAs) leads to the production of a number of aldehydes, most notably malonaldehyde and 4-hydroxy-2-nonenal (HNE) (5). These aldehydes can diffuse farther in the cell than can reactive radicals, and HNE therefore may be implicated in damage to LDL.

β-Carotene and related compounds

At present, the strongest evidence for an anticarcinogen effect for the antioxidant nutrients appears to exist for β-carotene. The anticarcinogenic effect of β-carotene is often ascribed to its antioxidant properties, either explicitly or by implication. However, this conference brought out the paucity of studies on the antioxidant properties of β-carotene both in vitro and in vivo. There are several published studies showing that β-carotene has antioxidant properties in some model systems, but the evidence is incomplete. In chlorobenzene solution, β-carotene is most effective as an antioxidant at low oxygen tensions (6). In contrast, at normal oxygen concentrations in aqueous micelles β-carotene does not behave as an antioxidant at all (7). Studies on the antioxidant properties of β-carotene in an aqueous system at low oxygen tensions have not been reported; these studies should be done since many organs (and most tumors) are oxygenated at much lower levels than are in vitro solutions exposed to an atmosphere of air.

To probe whether β-carotene behaves as an anticarcinogenic compound *because* of its antioxidant properties, it would be useful to know the products that are formed when β-carotene acts as an antioxidant. If a marker molecule could be identified that is produced when β-carotene traps radicals, this species could be searched for in cell culture or animal studies where β-carotene displays anticarcinogenic properties.

Several speakers also stressed the large number of carotenoids; more than 600 have been characterized (8). Virtually nothing

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is known about the antioxidant properties of the other carotenoids.

WF Malone pointed out that the National Cancer Institute currently lists over 1000 compounds of all types that are worthy of testing for anticarcinogenic effects. Eventually data relating the antioxidant properties of many of these compounds with their anticarcinogenic potential will allow a test of the hypothesis that antioxidant properties are a useful predictor of anticarcinogenic activity.

Diseases that involve radical-mediated processes

A remarkable number of diseases were discussed that appear to be partly mediated through free radical intermediates. Impressive new data on the potential involvement of oxidized LDL in atherosclerosis were presented. The area of ischemia/reperfusion injury continues to be an active field for research, and data involving postischemic injury to gastrointestinal mucosa and heart tissue were presented.

Extensive data on cataracts and shorter discussions on rheumatoid arthritis, intraventricular hemorrhage, and Parkinson's disease were presented. The role of free radicals in aging and improvements in immunocompetence caused by dietary antioxidant nutrients also were discussed.

Antioxidants and reducing agents

It must be stressed that any one of these nutrients can play a role as a *reducing agent* without necessarily behaving either as an *antioxidant* or a *free-radical scavenger*. An example of this is the inhibitory effect of vitamin C against stomach cancer that was reviewed at this conference. This inhibitory effect is most likely due to ascorbate trapping a nitrosating agent derived from nitrite and preventing the nitrosation of amines to nitrosamines. In this reaction, vitamin C acts as a reducing agent by becoming sacrificially nitrosated, but radicals are not involved. Similarly, as discussed above, β-carotene may or may not possess antioxidant and/or radical scavenging properties in systems in which it acts as an anticarcinogenic compound.

Epidemiological evidence

The epidemiologists who spoke at this conference stressed that establishing a linkage between the intake of a particular nutrient and a beneficial health outcome requires several studies by several independent groups. Only when a majority (but not necessarily all) of the studies support the hypothesis of a health effect from a particular nutrient can the hypothesis be taken seriously. Using these guidelines, the epidemiological evidence for a health benefit appears to be strongest for the inverse relationship between intakes of β-carotene and the risk of getting lung cancer (8). In addition there are one or more reports of positive correlations between high levels of vitamin E and vitamin C and protection against certain cancers and/or ischemic heart disease. Clearly much more work in this area will be done and reported in the coming years.

I was particularly struck by comments made during lectures or from the floor regarding the overall impact of the epidemiological data already in hand. The following remarks, quoted as nearly verbatim as my note-taking allows, illustrate the sentiment of the group:

Richard Peto: ". . . with regard to β-carotene, we may be onto something extremely important here [in the prevention of human cancer]"

Max Horwitt: "Those of us who served on the Food and Nutrition Board of the National Research Council had a bias against vitamin supplements, but we are changing our view."

H Garewal: "Based on all the evidence, carotenoids are very active in preventing oral cancer."

C Schorah: "We may have a simple dietary component [vitamin C] that can prevent gastric cancer."

Daily intake of vitamins

It remains very controversial to suggest that taking vitamin pills, as opposed to eating a healthy diet, might be beneficial. Yet a number of our speakers (eg, Gladys Block) presented data showing that substantial population groups, in America and elsewhere, are not getting even the US Recommended Dietary Allowance (RDA) level (9) of some of these nutrients. Furthermore the intervention trials sponsored by the National Cancer Institute of the National Institutes of Health that are currently underway use levels of these micronutrients that are considerably higher than the RDA, daily intakes that can only be conveniently achieved by taking supplements. Thus, an implicit assumption is made that the amount of a nutrient necessary to prevent well-recognized vitamin deficiency diseases (the RDA) may be less than that which might provide protection against cancer or heart disease. In this regard, the very low toxicity of these nutrients, and especially β-carotene, vitamin C, and vitamin E, was discussed. These nutrients appear to have no toxic side effects for the general population, even at levels that are at least one order of magnitude higher than the RDA. One exception is that very high levels of vitamin E should not be recommended for persons on anti-coagulant therapy.

Different intakes of a nutrient may be necessary to prevent different pathological conditions

I want to point out a feature of nutrient intakes and disease that is not generally discussed (10). **Figure 1** shows the situation for three different hypothetical pathologies that all respond to the presence of a vitamin or nutrient. Pathological condition A responds in such a way that the RDA of this vitamin completely protects humans from this condition; in fact, even levels somewhat lower than the RDA already maximize the protective effect due to this nutrient, providing as much protection as can be achieved. In contrast, pathological condition B requires the full RDA amount of protection; an intake only slightly less than the RDA results in a measurably greater pathology of type B. And finally, the amount of pathological condition C that can be detected continues to decrease as the amount of the vitamin increases, even up to levels very much higher than the RDA.

This description may seem vague and theoretical, but it is quite real. For example, Bendich et al (11) have shown in a study of vitamin E in rats that myopathy responds as shown in panel A; ie, rats are protected against myopathy by a baseline (ie, an RDA-like) amount of vitamin E and further amounts of vitamin E provide no additional protection. On the other hand, red blood cell hemolysis follows pattern B: levels much below a baseline level do not provide complete protection. Most strik-

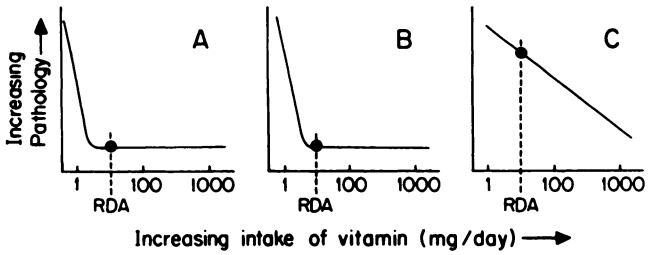

FIG 1. The differing response to a micronutrient is shown for three different pathological conditions. The micronutrient provides the maximum protection it can afford against condition A at an intake either at, or even slightly below, the RDA (9). For condition B, the RDA provides complete protection, but amounts slightly less are not sufficient. For condition C, however, the protection afforded by the micronutrient against this pathology continues to increase as long as the intake of the nutrient is increased, so that more than RDA-like amounts are of increased benefit. Conditions probably vary in their response to micronutrients in this way, as shown, eg, by the study of rats and vitamin E by Bendich et al (11).

ingly, improvements in immune competence follow pattern C. Increasingly higher doses of vitamin E, even up to levels very much higher than a baseline amount, continue to improve immune responsiveness. In fact, immunocompetence correlates with the serum levels of vitamin E over an enormously wide range, from 0.04 to 18 μg/mL (11).

Conclusions

The participants in the congress went away with a very optimistic feeling. The disease pattern in developed countries reflects the fact that the classic infectious diseases that killed most of our citizens in 1900 have almost been eradicated. Now 75% of Americans die of heart and blood vessel diseases along with cancer (12). Evidence was presented that strongly suggests that free radical-mediated injury plays a role in the etiology of these diseases, as well as others such as cataract formation. Micronutrients such as vitamins E and C and β-carotene may reduce the incidences of these diseases.

The overall tone of this conference suggests that we have passed a watershed with regard to our attitude toward the use of micronutrients and the antioxidant vitamins. A number of speakers suggested that it may not be many years before the concept of the RDA is broadened, with one daily intake of a nutrient being recommended to prevent known vitamin deficiency diseases and a substantially higher value recommended to optimize the disease-preventing properties of these nutrients. Thus, there is reason for considerable hopefulness about our ability to reduce the incidences of the most life-limiting of the chronic diseases within the coming decades, with the consequent improvement in health, life span, and well-being of our citizens.

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