Concise Review

Most Free-Radical Injury Is Iron-Related: It Is Promoted by Iron, Hemin, Holoferritin and Vitamin C, and Inhibited by Desferoxamine and Apoferritin

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Abstract. Iron is a double-edged sword. In moderate quantities and leashed to protein, it is an essential element in all cell metabolism and growth, but it is toxic when unleashed [1]. Because of its ability to switch back and forth between ferrous and ferric oxidation states, iron is both a strong biological oxidant and reductant.

The human diet contains a multitude of natural chemicals which are carcinogens and anti-carcinogens, many of which act by generating oxygen radicals, which initiate degenerative processes related to cancer, heart disease and aging (the "oxygen radical hypothesis of aging") [2]. Among these many dietary chemicals are many redox agents, including vitamin C and beta carotene [3].

Free radical damage is produced primarily by the hydroxyl radical (•OH) [4, 5]. Most of the •OH generated in vivo comes from iron-dependent reduction of H_2O_2 [4, 5]. Supporting too much iron as a free radical-generating culprit in the risk of cancer, NHANES I data indicated that high body iron stores, manifested by increased transferrin saturation, are associated with an increased cancer risk [6]. Other data [1] shows an increased heart attack risk.

Sources of Catalytic Iron: Role of Vitamin C

When ferrous iron reduces H_2O_2 to generate •OH, it becomes ferric iron. Vitamin C (ascorbic acid) converts ferric iron back to ferrous iron, itself becoming oxidized ascorbic acid, thus allowing another cycle of •OH generation from renewed ferrous iron [5]. Supplements of vitamin C provide a constant supply of new reduced ascorbic acid, thus turning a sole cycle of iron-dependent •OH generation, in situations of localized iron overload, into a series of cycles, i.e., ascorbate-driven repetitive free radical generation by iron [5, 7]. Heme oxidation is also ascorbate-driven [8-12].

Another endogenous source of catalytic free iron is the iron released when the heme ring is opened by heme oxygenase [8]. The antimalarial, chloroquine, inhibits iron release from heme [9]. The induction of heme oxygenase is itself a cytoprotective response to destroy heme proteins (hemoglobin, myoglobin), which proteins, when released into the extracellular space, can instigate tissue toxicity [10, 11].

Protections Against Catalytic Iron

The intracellular generation of apoferritin is a cytoprotective antioxidant stratagem of endothelial cells [11, 12]. It is probably also a cytoprotective antioxidant stratagem of all proliferating cells, including cancer cells, since serum ferritin is elevated in acute leukemia and many other cancers, including solid tumors, particularly when metastatic [5, 13-15]. These serum ferritin levels may actually rise with chemotherapy [14]. This hyperferritinemia is often mainly ferritin generated in the tumor cells, but malignancy, like chronic inflammation, also

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causes generation of ferritin in the cells of the reticuloendothelial system, since ferritin is an acute phase reactant [1, 14].

Proliferating neuroblastoma cells generate apoferritin [16], presumably as a cytoprotective antioxidant stratagem. High serum ferritin correlates with poor outcome in neuroblastoma [16]. Neuroblastoma cells secreting relatively low levels of ferritin appear most susceptible to destruction by desferoxamine (DSF) [17].

Any trigger which causes cells to proliferate also triggers upregulation of the production of transferrin receptors (TfR) on the outer cell membrane surface; quiescent cells tend to have low TfR expression, and proliferating cells, such as tumor cells, intestinal epithelium and bone marrow progenitor cells, have a high TfR expression [13]. TfR expression is also influenced by cellular differentiation and the phase of the cell cycle [18, 19].

Varani et al. [20] demonstrated that pre-exposure to the potent iron chelator, DSF, protected endothelium from phagocyte-mediated oxidant damage.

Importance of Iron in Tumor Growth

The importance of iron in tumor growth is illustrated by the fact that iron deficiency slows the progression of malignancy [21, 22]. Catalytic iron free radical generation mutates DNA and promotes cancer [2, 3, 23]. Conversely, too much catalytic iron will destroy the cancer cell [5, 13]. Activating the iron within cancer cells to produce cell death is the mechanism of action of some xenobiotic antibiotics, such as the bleomycins and adriamycin and related anthracycline antibiotics, whose cytotoxicity depends on their formation of specific complexes with iron in tumor cells [5, 13, 24].

An increasing body of research is aimed at incorporating knowledge of factors affecting iron status and metabolism in the chemotherapy of cancer. The first international symposium on "Clinical Implications of Iron Metabolism and Cancer Control" was organized in Bormio, Italy, on July 14-15, 1993, by *Lawrence Helson* [25] (of New York Medical College, Valhalla, NY) and *Giovanni Deb* [25] (of Ospedale Bambino Gesu in Rome, Italy). At that symposium, *Donfrancesco et al.* [25] reported that five days of DSF pre-treatment sensitized neuroblastoma cells to alkylating agents, with objective cytoreduction in 23 of 25 patients. *Selig et al.* [17] noted that the neuroblastoma cells that proved most responsive to iron chelating agents like DSF were those secreting relatively low levels of ferritin.

The ultimate aim in all cancer chemotherapy is to selectively kill cancer cells without killing host cells. What is being sought regarding iron and cancer is an effective cancer-inhibiting or -destroying combination of a) dietary iron deficiency [21, 22]; b) iron chelating agents like DSF [25]; c) TfR blockers like monoclonal antibody directed against TfR [26, 27]; and d) intracellular blockers of iron metabolism, such as gallium [13] (which could be delivered to cells coupled to transferrin [Tf]), in an acceptable combination that would produce desirable damage or death to the tumor without unacceptable morbidity for the host. The existence of TfR epitopes on some tumor cells different from host cell TfR [26, 27] raises the hope that "killer" (i.e., coupled to harmful agents) transferrin epitopes can be created which will attach only to tumor, and not host, TfR, such as the cisplatin-transferrin (cisplatin bound to the iron-binding site of transferrin) patented by *Head* [28].

Voest et al. [24], in a study of nine refractory-to-prior-therapy patients (four non-Hodgkin's lymphoma, two gastric adenocarcinoma, one bladder carcinoma, one small cell lung cancer, one ovarian carcinoma), used a cancer treatment protocol in three stages: first, three days of continuous IV DSF to soak up iron, thereby upregulating transferrin receptors on tumor (and other proliferating) cells; second, one day of iron sorbital citrate (ISC), 100 mg i.m., so the cells will soak up lots of iron; and, third, starting on day 5, adding adriamycin (doxorubicin, as part of a CHOP regimen; CHOP = cyclophosphamide, doxorubicin, vincristine and prednisone) to the ISC, with the doxorubicin mediating free radical generation in the intact human tumor cells, thereby destroying them. The sequence was repeated every 3-4 weeks.

Since adriamycin's anti-cancer activity is iron-dependent, DSF, by soaking up iron, blocks the anti-tumor activity of adriamycin. Of the nine cancer patients treated with this protocol [24] of DSF on days 1-3, ISC on day 4, followed starting on day 5 with a cycle of ISC plus adriamycin, two of four with advanced

refractory-to-prior-therapy non-Hodgkin's lymphoma had significant partial remissions, but both got severe and debilitating DSF-related phlebitis, requiring new peripheral i.v. devices more than once a day. Thus, pre-treatment with DSF and ISC may be of benefit in the treatment of some malignancies with doxorubicin-containing regimens, but severe phlebitis in five of the nine, and ocular toxicity in two of the nine, limits the use of DSF in this approach. *Voest et al.* [24] suggest that attaching DSF to biocompatible polymers may overcome this toxicity.

Since vitamin C drives repetitive generation of destructive free radicals in the presence of high iron levels, it is very likely that future cancer therapies focused on destruction of iron-containing tumor cells by activating that iron will be even more effective if oral or parenteral vitamin C is added to the therapeutic armamentarium. Since supplements of vitamin C can kill patients with iron overload [7, 37], the iron status of the patient as well as of his/her tumor would have to be carefully evaluated before using them.

The Pathways to Catalytic Iron

Figure 1 [29] shows the pathways to catalytic iron. The powerful oxidizing properties of a solution of ferrous salt and hydrogen peroxide were first recognized by *Fenton* a century ago [5]. *Haber* and *Weiss* [5] recognized that free radical intermediates drove the Fenton reaction. The catalytic free radical intermediates formed in the reduction of oxygen to water include superoxide, hydrogen peroxide, and, most catalytic of all, hydroxyl radicals [2, 4, 5]. Superoxide is formed in two steps related to the catabolism of ethanol [30, 31] (Fig. 2). The first involves the metabolism of acetaldehyde by aldehyde oxidase and/or xanthine oxidase. The second involves the catabolism of excess nucleotide by xanthine oxidase [31]. Cimetidine, the H_2-receptor antagonist, can reduce this free-radical injury by scavenging ethanol-induced free radicals [31].

Figure 3 shows in vitro lipid peroxidation by acetaldehyde-xanthine oxidase [30]. Ferritin alone (as a source of iron), or xanthine oxidase alone, produces very little lipid peroxidation, but together they produce massive peroxidation [30]. Figure 4 shows that very little catalytic iron is needed for lipid peroxidation, since μM amounts of DSF prevent the peroxidation [30].

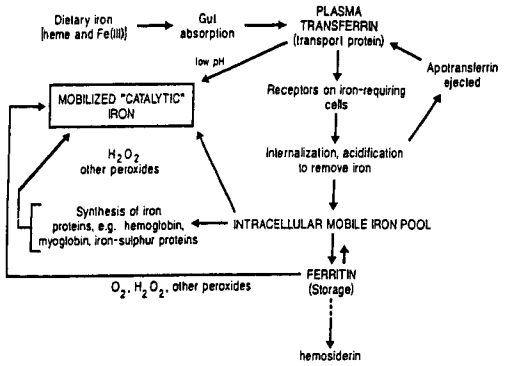

Fig. 1. Figure 1 shows the pathways to catalytic iron [29].

The Importance of Genetics

Each person's genetic blueprint determines what chronic disorders they are predisposed to

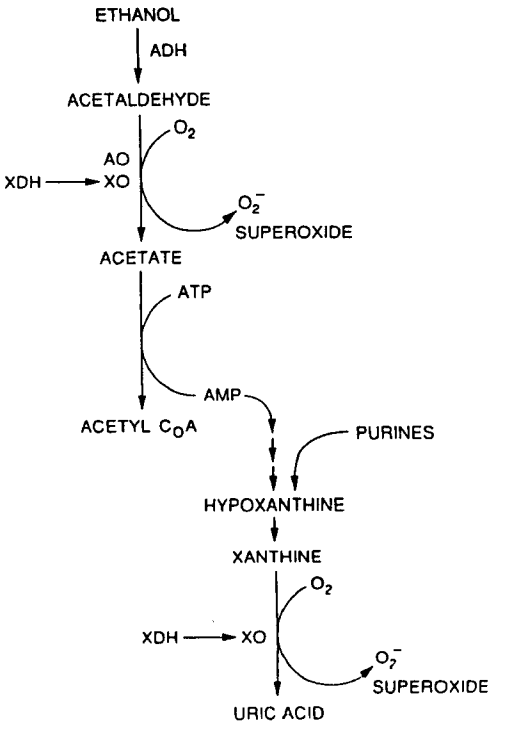

Fig. 2. Figure 2 shows that superoxide is formed in two steps related to the catabolism of ethanol.

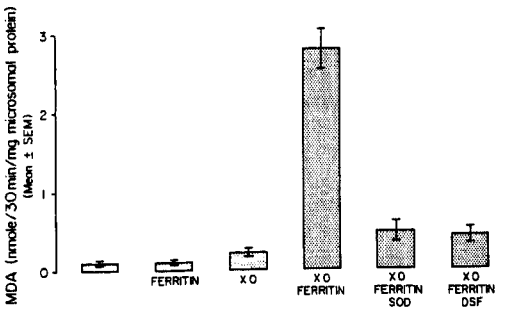

Fig. 3. Figure 3 shows in vitro lipid peroxidation by acetaldehyde-xanthine oxidase. Acetaldehyde added to each test tube. XO = xanthine oxidase; MDA = malondialdehyde; SOD = superoxide dismutase; DSF = desferoxamine [30].

get or not get. Environmental influences, such as the foods we eat, promote or retard the expression of disorders in our blueprint [32-34]. Whether we get cancer or not relates to our genetic blueprint of oncogenes, antioncogenes, and genetically determined iron absorption controls. Whether we get cholesterol-related heart attacks relates to genes that desirably or adversely affect fatty acid, cholesterol and iron absorption, and genes that affect internal cholesterol synthesis, excretion and oxidation [32-34].

Over 10% of Americans have an HLA-linked gene for iron overload, [1] and about 30% of Africans [35] (and African-Americans? [36]) have a non-HLA-linked gene for iron overload. The frequent iron overload among South African blacks drinking from iron-containing stills

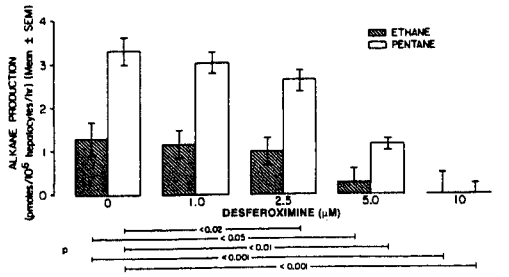

Fig. 4. Figure 4 shows that very little catalytic iron is needed for lipid peroxidation, since μM amounts of DSF prevent the peroxidation [30].

appears to be due to the interaction between the enhanced dietary iron intake and their gene for enhanced iron absorption [35]. In people with genetically enhanced iron absorption, iron and vitamin C supplements can only do harm [1, 7, 37]. Iron absorbed from iron supplements adds to the body's iron burden; vitamin C not only enhances iron absorption, but, worse, both releases catalytic iron from ferritin [1, 5] and drives the cycle of repetitive reduction of ferric to ferrous iron, with repetitive generation of more and more free radicals [5].

Ferritin, Apoferritin and Holoferritin

It is important to note that, although the literature almost exclusively uses the word "ferritin," what is generated within cells in response to an iron challenge is *apo*ferritin, free of iron, which then binds iron that would otherwise be cell-damaging. One ferritin molecule is capable of binding up to 4500 atoms of iron.

A report that someone has a serum "ferritin" of 200 μg/l, for example, is meaningless with respect to the iron content of that ferritin. Some of the "ferritin" may be pure apoferritin with no iron, some may be *holo*ferritin with 4500 atoms of iron per molecule of ferritin, and most of the "ferritin" is probably somewhere between no iron content and 4500 atoms of iron per ferritin molecule.

Serum iron measurements do not measure the iron on ferritin but only the iron on transferrin, plus low molecular weight iron [3] such as might have been absorbed from iron succinate citrate [5].

We assume that a serum ferritin of 200 μg/l, in a patient who has a *low* serum iron and iron-binding capacity, is largely apoferritin with no iron on it, present as an acute phase reactant due to inflammation, since inflammation triggers cells to make and release apoferritin (probably partly to take iron away from bacteria which need iron to grow). Conversely, if a serum ferritin of 200 μg/l is accompanied by a *high* serum iron and iron-binding capacity, then we assume those 200 μg of serum ferritin contain a lot of iron, perhaps even some holoferritin (i.e., ferritin saturated with 4500 atoms of iron per molecule of ferritin).

Except when there is acute serum ferritin elevation in response to inflammation or cancer, the serum ferritin level so closely mirrors body iron stores that multiplying the serum ferritin in μg/l by

10 gives the body iron stores in mg [1]. Therefore, it is logical to assume that serum ferritin in normal people is modest in iron content, ferritin in iron-deficient people is devoid of iron, ferritin in response to inflammation has the same iron content per ml serum as in normal people, and ferritin in the iron-overloaded is loaded with iron.

Because there has been no reliable laboratory confirmation or denial of this logic, we recently asked *Phillip Aisen* to create a method for quantitating the iron content of serum ferritin. We hope to use that method to determine (and compare with serum iron and iron-binding capacity) the range of ferritin iron content in four groups:

1. Normal people
2. Iron-deficient people
3. Iron-overloaded people
4. People with elevated serum ferritin as an acute phase reactant to inflammation, cancer, etc.

Whether or not our logic is correct, laboratory results stating that the serum ferritin is X and the iron content of that serum ferritin is Y will be valuable.

When a macrophage ingests three or more red cells, it dies [38]. If it also ingests vitamin C, the ingestion of only one or two red cells will kill it [38]. This is a dramatic demonstration of the lethality of ascorbate-driven heme oxidation. Since the Kupffer cell is a major site for reclaiming iron from senescent erythrocytes to the circulation, and ascorbate enhances that iron release, ascorbate could well be a major promoter of hepatic failure and death in iron overload [7, 39].

Importance of Assessing Iron Status

Figure 5 shows the stages of iron status [40]. In the United States, over 10% of Americans are in positive iron balance, but only about 6% are in negative iron balance [1]. This 6% is almost all infants and children up to the age of four, children at the onset of puberty, females in the child-bearing years, and pregnant females [1].

Our genetic nutrition pyramid [34] (Fig. 6), created by simply putting a genetic base and appropriate sidebars on the U.S. Government's nutrition pyramid of the five basic food groups, converts the government's pyramid, usable for the theoretical average American, into one usable by each individual American, allowing the consumer to talk intelligently with the nutrition expert [41]. The genetic blueprint of each individual determines whether that individual should eat more or less than the "theoretical average" American should eat daily from each of the five food groups.

For example, those with a gene for increased iron absorption should be vegetarians [34, 42], because the average iron absorbability by "normal" people averages only 3% from plant foods, but five times as much (15%) from animal foods [1]. Assuming a single gene for iron overload increases this [43, 44] by about 25%, that would only mean about 4% iron absorption from a vegetarian diet, but about 20% absorption from an animal protein diet. The average American, who is an omnivore, absorbs about 10% of the iron in his/her mixed plant and animal food diet, which, with one gene for iron absorption, could increase to about 13%.

Alcohol or vitamin C, each of which enhances iron absorption [1, 7, 42], would each act like a "second gene" for iron absorption, further enhancing iron absorption from food. Alcohol and vitamin C have the further undesirable effects that alcohol, in its normal catabolism, releases catalytic iron [23, 30], and catalytic iron is released from ferritin by vitamin C [5, 7], so much so that thalassemic patients with iron overload have died from a single dose of vitamin C releasing so much catalytic iron that a fatal cardiac arrythmia was produced [7]. Vitamin C supplements can cause rapid progression to death of the otherwise more slowly progressive congestive cardiomyopathy of hemochromatosis [39]. Three young athletes so died [7]; in none of them had anyone thought to assess iron status before letting them take vitamin C supplements. In fact, out of ignorance [45, 46] many coaches push vitamin, mineral, amino acid, herbal, and other supplements, all of which can only do harm and no good for their athletes, since their athletes are already getting all the nutrients they need in their food.

Transferrin can be a potent growth factor for cancer cells [26] and for prostate cancer metastases [27]. One can probably predict whether a prostate cancer will metastasize to bone by measuring numbers of transferrin receptors on the surface of each prostate cancer cell [27]. If a small number, it will not metastasize; if a large

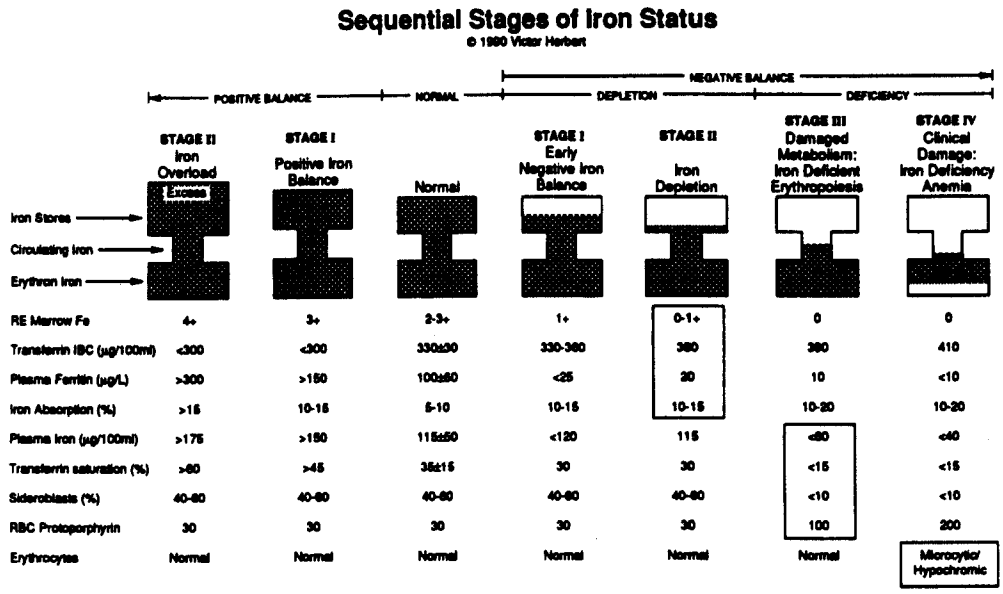

Fig. 5. Stages of iron status.

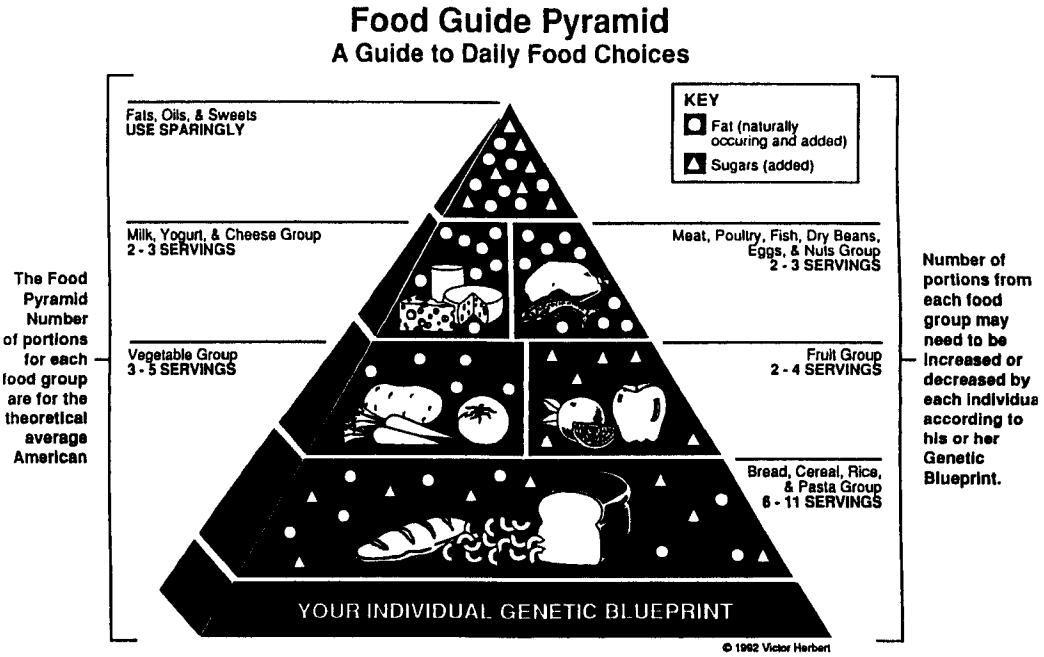

Fig. 6. Figure 6 is our Genetic Nutrition Food Guide Pyramid [34], created from the 1992 U.S. Government "USDA Food Guide Pyramid" by adding a base consisting of "your genetic blueprint," and sidebars pointing out that the food pyramid number of portions are for the theoretical average American, which may need to be increased or decreased by each individual according to his/her genetic blueprint.

number, it will. In addition, free iron inhibits the tumoricidal activity of macrophages [47].

Everyone needs to be tested for iron status. It may be that close to 20% of Americans have a gene for iron overload, since *Gordeuk et al.* [36] noted that about 20% of adult American males have a mean transferrin saturation of 41% as compared to 26% for the other 80% of adult American males. We agree with *Lauffer* [42], who wrote:

> "I also worry about the effects of excess vitamin C on people with even moderately high iron levels. Since iron may contribute to major killers such as heart disease and cancer, vitamin C could accelerate this destructive process by increasing iron absorption and exacerbating iron-induced tissue damage."
>
> "The concern that undiagnosed hemochromatosis victims could be harmed by vitamin C was one reason the 1980-1985 RDA Committee of the National Academy of Sciences tried to decrease the RDA for this vitamin from 60 milligrams a day to 40 milligrams for men and 30 for women [48]. While their main concern was that there was no good evidence that higher amounts were beneficial, these experts explicitly mentioned the possibility of iron toxicity from vitamin C. Unfortunately, the attempt to decrease the RDA was bulldozed by 'pro-vitamin C' NAS staff members who rewrote these recommendations and, in 1989 issued the tenth edition of the RDAs with the RDA for vitamin C set back at its original value" [49].

Kemp et al. [26] reported inhibiting hematopoietic tumor growth with DSF plus monoclonal antibody to transferrin receptors. Similar treatment could well be effective for metastatic prostate cancer and metastatic breast cancer, both of which have increased transferrin receptors on their proliferative state cells. As recently reviewed [13], in 1966 French workers first showed that chemotherapy produces a sharp rise in serum iron; cytoxan, methotrexate, and many other cytotoxic chemotherapy drugs cause a sharp increase in serum iron levels very rapidly [13]. Iron responsive elements trigger transferrin receptor upregulation in cancer cells.

Vitamin C is a double-edged sword, essential for health and antioxidant in physiologic amounts, but pro-oxidant and cancer-promoting in pharmacologic amounts [3, 7]. Whether the antioxidant or pro-oxidant actions of ascorbate will predominate depends upon the bodily system and the concentration within it of iron, ascorbate and other reactants [3, 7]. In isolated biological systems in vitro, lower concentrations of added ascorbate promote massive free radical generation by producing ascorbate-driven iron recycling with continuous generating of highly oxidant free radicals; in similar in vitro systems, higher concentrations of added ascorbate are primarily antioxidant by scavenging hydroxyl radicals [3, 7]. In iron overload in the living human, the highly oxidant recycling of iron with massive free radical generation is driven by ascorbate [5, 7].

For all these reasons, in February 1993 we filed a Citizen Petition with the Food and Drug Administration (FDA) [37] asking that every supplement containing iron and/or vitamin C have on its label a notice stating that "this product can only be harmful to that over 10% of Americans who are born with a gene for iron overload; nobody should take supplements of iron and/or vitamin C unless they have first had their iron status determined by a responsible health professional."

The ubiquitous and pernicious—"these bodyguards help arrest harmful free radicals"—ads promoting sales of supplements of vitamin C and beta carotene sell billions of pills, but deceive by fraudulent concealment of the truth that these two vitamins are in fact redox agents, antioxidant in some circumstances and pro-oxidant in others [3, 7].

Antioxidant Vitamins May Do As Much Harm As Good

Recent papers by *Stampfer et al.* [50] and *Rimm et al.* [51] provide significant epidemiologic support for the concept that vitamin E reduces the risk of coronary disease in Americans. Unfortunately, neither paper discussed whether this coronary benefit was balanced by non-coronary harm.

In an accompanying editorial, *Steinberg* [52] indicated that supplements of "antioxidant" vitamins E, C and beta carotene (the latter two are in fact not "antioxidant," but "redox") [3, 7] "are generally considered not to be toxic." In reality, whether they are likely to do more good than harm or more harm than good is

determined in significant part by one's genetic predisposition to oxidized LDL cholesterol-related heart disease versus other diseases [33, 34]. Vitamin E supplements have been associated with a number of harms, and, by "enhancing" immune function, may promote progression of a wide variety of genetically predisposed autoimmune disorders of endocrine, connective, neural and dermal tissue [53]. If there is genetic predisposition, supplements of vitamin C can maim or kill [7]. Supplements of beta carotene can produce liver damage [54].

Kim et al., from the Centers for Disease Control (CDC), recently reported [55] that *all-cause* mortality of Americans was not improved by supplements. The likelihood is therefore strong that less heart disease means more other disease. *Kim et al.* [55] concluded, "We found no evidence of increased longevity among vitamin and mineral supplement users in the United States. Considering the wide use of supplements in the general population, the cost-effectiveness and the safety of supplement use need to be better defined."

When all the speculation that large intakes of vitamins C, E and A are protective against cancer is replaced by actual study, they appear to have no protective value (except when the diet is low in vitamin A, and then only RDA amounts of vitamin A are needed) [56]. Partly because of genetic variation [33, 34], every supplement with a theoretical up-side has a real down-side [1, 7, 37, 40, 45, 46, 57]. Perhaps similarly, sulfites in wine may inhibit generation of superoxide and reduce breast cancer frequency [58], but wine nevertheless may perhaps somehow increase risk of cancer of the lung [59], thereby having no protective value against *overall* mortality from cancer, depending on what else individuals eat, drink and smoke [58]. The huckstering of vitamin C, resulting in 40% of all Americans taking vitamin C supplements in the false belief those supplements are doing them good, is a classic example of the technique of repeating a deception over and over so that it comes to be accepted not only as truth, but as catechism. Thus, the public, and even health professionals, fall victim to the gurus, the hucksters and the arrogance of ignorance [45, 46, 60, 61].

Because of what the media and the vitamin profiteers' public relations agencies have taught us (and our teachers) as catechism, it's not what we don't know about nutrition that hurts us; it's what we know for sure that's dead wrong [46].

The Bottom Line

Both oxidants and antioxidants are needed in the biochemical economy of human cells. We need to inhale oxygen because it is the fundamental oxidant we use; without it we would die. Cells walk a balance between essential oxidant and essential antioxidant processes. That is the fundamental reason moderate antioxidation is helpful and excessive antioxidation is harmful. Free radicals are the price we pay for breathing [62]. Iron and vitamin C are both redox agents, usually antioxidant in the moderate quantities found in food, but often oxidant in the large quantities found in many supplements [7]. As supplements, they can be antioxidant or oxidant, depending on circumstances, but act particularly as oxidants when body iron stores are high, as is true for over 10% of Americans, and/or when the patient has a hemolytic anemia, such as thalassemia or sickle cell disease, or when the patient has deficiency of vitamin B_{12} or folic acid producing a megaloblastic anemia, or a hypoplastic anemia. In those anemias, iron from the missing red cells piles up in the bone marrow and other reticuloendothelial cells, producing iron overload in them, which equilibrates with a high serum ferritin [7, 40]. When total body iron is high, vitamin C supplements can kill by sudden release of massive amounts of catalytic iron [7, 37]. Conversely, if tumor iron is high but host iron is low, vitamin C supplements theoretically can selectively kill the tumor.

Addendum

On November 1-3, 1993, the FDA sponsored a *Public Conference: Antioxidant Vitamins and Cancer and Cardiovascular Disease,* to which several dozen scientists doing relevant research were invited to make presentations. It was held at the National Academy of Sciences. The purpose was to advise the FDA on what its posture should be with respect to advertising of "antioxidant" vitamins.

There was unanimous agreement that labeling and advertising should be for consumer education, should be consistent with scientific evidence about which there was substantial agreement in the scientific community, and should be neither deceptive nor misleading. It, for example, would be misleading to present

only the up-side and deceive the consumer by leaving out the down-side.

Scientific agreement was essentially unanimous among the invited nutrition scientists that vitamins C, E, and beta carotene are mischaracterized by describing them solely as "antioxidant" (*fighter against harmful free radicals*), since they are in fact redox agents, antioxidant in some circumstances (often so in the physiologic quantities found in food), and pro-oxidant (*producing billions of harmful free radicals*) in other circumstances (often so in the pharmacologic quantities found in supplements). Vitamin C is a special case, since, in the presence of iron, it is violently pro-oxidant, and, for genetic reasons, over 10% of American whites and perhaps up to 30% of American blacks have high blood iron.

Therefore, representing the above vitamins as *"antioxidant"* in either labeling or advertising is a *consumer deception,* since it tells the consumer only the up-side and deceives the consumer by omitting the down-side. To protect the public, the FDA and the Federal Trade Commission (FTC) should forbid using the word "antioxidant" to describe the above vitamins in labeling or advertising.

Further, as *Dr. Herbert* stated at the close of the Conference (which was audio taped by the FDA, and transcripts of which are available from the FDA), *for consumer protection it is mandatory that every advertisement and label for vitamin C supplements must include the following:*

> "NOTICE: Do not take this product until your blood iron status has been determined. Six percent of Americans are in negative iron balance, and this product may help them. Twelve percent of Americans are in positive iron balance and this product may hurt them."

All labeling and advertising for vitamin C supplements should also bear the following:

> "NOTICE TO HEALTH PROFESSIONALS: Iron status is determined by measuring serum ferritin and, if it is high, also measuring serum iron. Alternatively, it is measured by determining percent saturation of the serum iron binding capacity."

At the Conference, we noted that in 1961 our group published in the *New England Journal of Medicine* (265:1033-1038) a study of a patient with scurvy and high body iron stores (in his case, because he had folic acid deficiency, which raises body iron stores [as vitamin B_{12} deficiency also does]). His blood iron shot so high as to saturate his iron-binding capacity after we gave him vitamin C, demonstrating that vitamin C can mobilize into the bloodstream enormous quantities of iron from elevated stores. *Tom Bothwell et al.* had reported the same in *South African Medical Journal* (1959;24:144).

It is well known among hematologists who study iron overload due to transfusion therapy of patients with thalassemia or sickle cell disease that vitamin C can mobilize such an enormous amount of iron from high body iron stores as to overwhelm the iron-binding capacity of iron-binding proteins, with the resultant free iron producing death within minutes to hours from iron-induced cardiac failure.

Because of the potential lethality of vitamin C supplements in persons with iron overload, the "1992 Management Protocol for the Treatment of Thalassemia Patients," distributed by the Thalassemia International Federation, states the following (page 15) concerning the role of vitamin C:

"Iron-loaded patients usually become vitamin C deficient, probably because iron oxidizes the vitamin. When this is the case, administration of vitamin C increases excretion of iron in response to Desferal, a brand of DSF. Vitamin C increases the availability of iron, and so may increase its toxicity if large doses are taken without simultaneous Desferal infusion. Therefore the following precautions are recommended:

a. Start treatment with vitamin C only after an initial month of treatment with Desferal.

b. Give vitamin C supplements only if the patient is receiving Desferal regularly.

c. Do not exceed a daily dose of 200 mg. The minimum effective dose of vitamin C is about 2-5 mg/kg (*N. DiPalma, A. Piga* unpublished data). In general, 50 mg suffice for children under 10 years of age, and 100 mg for older children. Vitamin C should be given only on days when Desferal is taken, ideally when the pump is set up."

The protocol is available in the United States from the Cooley's Anemia Foundation, Box CEP, 105 East 22nd Street, New York, NY 10010.

The pro-oxidant effects of beta carotene, and its many other properties totally unrelated to antioxidant action, were delineated at the Conference by *James Allen Olson,* including that the anti-cardiovascular disease and anti-cancer actions of beta carotene very likely have

to do with chemical properties of beta carotene entirely distinct from antioxidant properties.

Relevant Exhibits presented to the FDA at the close of the Conference by *Dr. Herbert* (an invited participant) included:

Exhibit A:

First page of a chapter by *Borg* and *Schaich* entitled "Pro-oxidant Action of Antioxidants," from Volume 1 of Handbook of Free Radicals and Antioxidants in Biomedicine, CRC Press, 1989. (*Borg* and *Schaich* were not aware when they wrote that chapter (probably in 1988), of convincing evidence of the pro-oxidant effects of vitamin E. Such convincing evidence was published by *Mukai et al*).

Exhibit B:

First page of a *Mukai et al.* paper entitled, "Kinetic study of the pro-oxidant effect of tocopherol. Hydrogen abstraction from lipid hydroperoxides by tocopheroxyls in solution," in the journal *Lipids* (1993;28:747-752).

Exhibit C:

A one-page letter and page 4 of a four-page statement "Elementary Comments On Vitamin E" by *M. K. Horwitt,* dated October 18, 1993, addressed to FDA's *John N. Hathcock,* from *Max Horwitt.* The letter and statement were distributed to all attendees at the November 1-3, 1993, FDA Conference. On page 4 of his statement, *Dr. Horwitt* wrote:

"Any toxicity of vitamin E has, so far, not proved to be a problem but I remain cautious. Very high levels of antioxidants in blood and tissues may make them pro-oxidants. A number of reports have shown that the tocopherols can inhibit platelet adhesion and aggregation.... My position on health claims on labels is that they should be absent or stated very conservatively."

Exhibit D:

V. Herbert paper in the December 1992 *Journal of the American Dietetic Association* discussing negative and positive iron balance, and citing *Gordeuk et al.* in *New England Journal of Medicine* (1992;326:95-100) that there may be heterozygous hemochromatosis in up to 30% of black Americans.

Exhibit E:

Our paper presented at the *Eighth International Symposium on Molecular Biology of Hematopoiesis* in Basel, Switzerland, on July 12, 1993, on ascorbate-driven free radical generation from iron (and ascorbate-driven oxidation of heme).

Exhibit F:

Relevant chapter "The Truth About Iron," in the three author (*Simoupolis, Herbert, Jacobson*) 1993 Macmillan book *Genetic Nutrition: Designing a Diet Based on Your Family Medical History: Your Genes Can Tell You What to Eat —And Avoid— To Live a Longer, Healthier Life.*

Exhibit G:

V. Herbert article in the January 1993 issue of *Nutrition Today*, further delineating harms from vitamin C supplements.

Exhibit H:

V. Herbert letter published in the October 29, 1993 *CNI Nutrition Week,* delineating that the study in China published in the September 15, 1993 *Journal of the National Cancer Institute* did not show that supplements of vitamin E and beta carotene protected against cancer, but rather that when one corrected vitamin E and beta carotene deficiencies, rampant in that Chinese population but almost unknown among Americans, the frequency of esophageal and gastric cancer was reduced.

Exhibit I:

The two abstract summary, published in November 1993 in *Blood,* of our studies in the north of China, where vitamin B_{12} deficiency and folic acid deficiency are rampant, showing that providing the missing vitamin B_{12} and folic acid reduced the frequency of esophageal cancer, and even partially reversed esophageal dysplasia, the intermediate lesion between normality and cancer.

As *Dr. K. Lewin* pointed out at the November 1-3 Conference, that part of dysplasia consisting of cells already DNA damaged to become committed cancer cells is not reversed; the uncommitted cells revert to normal.

As we suggested at the Conference, where vitamin C appears to act against cancer, it may be that it is acting in its role of preserving folic acid against oxidative destruction. It may be that it is the folic acid that suppresses the development of cancer by methylating DNA. Methylation of DNA "switches off" cell proliferation. For

three decades, we have been trying to methylate malignancy DNA to "switch it off."

Misled by a November 11 headline and press release from the American Heart Association, entitled "Round 2: autopsy study appears to refute link of excess iron to heart disease risk," *The Wall Street Journal* on November 12, 1993, published an equally misleading story under the equally misleading headline, "U.S. Study Questions Link of Heart Disease to Body's Iron Level."

The cited autopsy study, by cardiologist *Michael Miller,* of University of Maryland Medical Center, and *Grover M. Hutchins,* professor of pathology and director of autopsy services at the Johns Hopkins Hospital, found that only three of 65 patients with homozygous hemochromatosis (H-H disease, severe body iron overload, present in about one of 250 Americans) had a high-cholesterol-related coronary artery blockage of 90% or more. Only four of the H-H patients had cholesterol levels over 240 mg/dl. This is usual in H-H, because the iron-overload-associated liver damage reduces liver cholesterol synthesis, which normally accounts for about 85% of circulating serum cholesterol, with absorbed food cholesterol accounting for only about 15%.

The average cholesterol level in the H-H patients was only 160-170 mg/dl, well below the level of the average American. As would be expected if iron increases the severity of high-cholesterol-associated coronary artery disease, the one with a high cholesterol of 329 mg/dl had severe coronary artery disease.

The Baltimore researchers also found, as would be expected in people with cholesterol levels below 200 mg/dl, that the degree of coronary blockage in the H-H patients was half that in age- and sex-matched controls (i.e., non H-H people) with higher serum cholesterols.

Thus, the Baltimore workers in essence confirmed the *Salonen et al.* study from Finland published in *Circulation* in September 1992, which reported that, when one divides patients with high LDL cholesterol into two groups, those with elevated serum ferritins in the range above 200 μg/l due to the moderately increased body iron stores of heterozygous hemochromatosis (only one H gene, as present in over 10% of Americans) have more than twice the coronary artery disease risk of those with serum ferritins in the range of 100 μg/l. The logical mechanism, an extension by *Salonen et al.* of that proposed by *Dan Steinberg et al.* of University of California at San Diego School of Medicine, is that high LDL cholesterol *per se* is relatively harmless, but high iron oxidizes it to the coronary-artery-damaging oxidized LDL cholesterol.

Our group has shown that vitamin C supplements (taken daily by over 40% of Americans) increase the iron-associated oxidation of LDL cholesterol [67].

The Baltimore workers blundered in hypothesizing that "if iron is an important risk factor, we would find coronary heart disease when we look at the most severe cases of iron overload." This is only true when there is a high LDL cholesterol for the high iron to act upon. They then compounded their blunder by concluding that their "results indicate that iron overload patients are not at increased risk for coronary artery disease (CAD) and suggest that other factors may account for the enhanced CAD rate previously reported."

In fact, for the reasons stated above, their conclusion is correct for the severe iron overload of H-H disease, which afflicts only about one American in 250. However, their conclusions in no way contradict the *Salonen et al.* study, despite *Dr. Miller's* claim in the press release that they do.

The *Salonen et al.* study from Finland does not relate to H-H disease, but rather to heterozygous hemochromatosis, which afflicts over 10% of American whites, and perhaps as many as 30% of American blacks. These individuals have moderately elevated body iron, manifested by a high serum iron-containing ferritin. The millions of them with high LDL cholesterol are at risk from iron supplements (or supplements of vitamin C, which enhance both iron absorption and iron toxicity). Headlines suggesting that such supplements are no problem are a disservice to them.

Note Added in Proof

It has been known since the excellent study by *Moertel et al.* at the Mayo Clinic, published more than a decade ago in the *New England Journal of Medicine,* that cancer patients treated with megadoses of vitamin C did much worse than cancer patients who did not get vitamin C supplements. Despite that

study, and despite the fact that mega-C promoter *Linus Pauling* (who wrote that, because of the mega-doses of vitamin C he took every day, he expected to be cancer-free until age 120), now has prostate cancer in his 90s (as his genetic history would have predicted) [34], mega-C is still being touted for cancer by "alternative" practitioners [63].

One must recognize that there are three kinds of alternatives: genuine, questionable, and fradulent [61, 64].

We have pointed out [32, 37, 45] that, for two reasons, smokers who take supplements of vitamin C will probably get lung cancer much faster. Vitamic C supplements are promoted to smokers on the grounds that they have lower blood levels of vitamin C than nonsmokers. While this is true, it is a non-sequitur. Nonsmokers have average blood vitamin C levels 11 times the minimum needed to sustain normality with respect to vitamin C, and smokers have blood levels 9 times the minimum level needed to sustain normality; this is hardly undernourishment requiring vitamin C supplements. The two reasons that vitamin C supplements should promote faster lung cancer development in smokers are:

1. Vitamin C rapidly drives nicotine from the blood into the urine, causing the nicotine-addicted smoker to reach for the next cigarette (with its carcinogens) that much faster, in order to sustain his/her "nicotine high" [32, 37].
2. As reviewed above, free radicals promote cancer by mutating DNA, most free radicals are generated by catalytic iron, and catalytic iron generation of free radicals is ascorbate-driven.

Dovetailing perfectly with our perception that "antioxidant" vitamin C promotes cancer rather than inhibiting it is the recently reported study of 29,133 Finnish male smokers aged 50 to 69 [65]. That double-blind, placebo-controlled, primary-prevention trial divided the subjects randomly into 4 regimens: alpha-tocopherol (vitamin E) (50 mg per day) alone, beta carotene (20 mg per day) alone, both alpha-tocopherol and beta carotene, or placebo. Follow-up continued for five to eight years.

Compared to the placebo, the "antioxidant" provitamin, beta carotene, produced an 18% *higher* incidence of lung cancer and an 8% *higher* total mortality. Alpha-tocopherol had no apparent effect on total mortality, but did produce more deaths from hemorrhagic stroke. (We had predicted this because of the pro-hemorrhagic action of large doses of alpha-tocopherol) [66].

Our most recent research on ascorbate-driven free radical generation from iron was presented at the 1994 annual meetings of the Federation of American Societies for Experimental Biology [67] and the Association of American Physicians [68].

Four decades ago it was shown that vitamin C oxidizes hemoglobin (producing Heinz bodies) [69], and that vitamin C oxidatively destroys reduced glutathione in red cells [70].

Our perception that methylation of DNA "switches off" cell proliferation, and therefore folate deficiency promotes cancer [71, 72] recently received strong support from the Harvard Physician Study [73]. That study found that 47,931 US male health professionals who were free of diagnosed colon cancer at the study's start had a strong association of low consumption of folate and methionine, and high alcohol intake, with colon cancer [73].

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