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# Iron, Radiation, and Cancer

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**Increased iron content of cells and tissue may increase the risk of cancer. In particular, high available iron status may increase the risk of a radiation-induced cancer. There are two possible mechanisms for this effect: iron can catalyze the production of oxygen radicals, and it may be a limiting nutrient to the growth and development of a transformed cell *in vivo*. Given the high available iron content of the western diet and the fact that the world is changing to the western model, it is important to determine if high iron increases the risk of cancer.**

## Introduction

Iron is the most abundant transition metal in the human body, and it plays a central role in metabolism. However, a growing skepticism exists of the conventional wisdom concerning the importance of iron repletion. A recent editorial in the *British Medical Journal* decrying iron deficiency as a scourge of even socially advantaged populations (*1*) was followed within months by an editorial in the same journal on the benefit of mild iron deficiency in providing protection from infection (*2*).

The deleterious effects of severe iron deficiency have been well documented. However, the potential dangers of iron excess have been examined only insofar as severe iron overload occurs. Given the high available iron content of the western diet and the fact that dietary practices have been changing throughout the world toward the western model, the biological consequences of moderately elevated iron stores deserve attention. Possible consequences are increased risk of cancer (*3,4*) and sensitivity to radiation injury.

The purpose of this paper is to review the hypothesis that increased available body iron stores may increase the risk of cancer and of radiation-induced tumor formation by one or both of two possible mechanisms. First, excess intracellular iron may increase the ambient concentration of oxygen radicals leading to the depletion of cellular reserves of reducing agents. This condition of increased oxidative stress may thus render the cell more sensitive to the radicals produced by ionizing radiation. Excess intracellular iron may also increase the effective range of radicals produced by radiation. Second, iron may be a limiting nutrient to the growth and replication of a transformed cell in the human body, and thus, high iron stores may increase the chances that a transformed cell will survive to become a clinically apparent neoplasm.

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An effect of body iron stores on sensitivity to radiation injury would have important implications for second malignant neoplasms arising from radiation therapy; diagnostic radiation exposure; occupational radiation and residential radon exposures; and exposure of astronauts and airline crews to cosmic radiation. In all these instances the relative capacity of the host to scavenge oxygen radicals may influence cancer risk associated with radiation exposure, and this capacity may be closely related to iron metabolism. While nutritional antioxidants have been receiving a great deal of attention in this regard, the oxidant iron has received very little.

## Iron and Oxygen Radicals

Oxygen radicals include the hydroxyl radical, the perhydroxyl radical, and their deprotonated forms. They are highly toxic species produced intracellularly by reactions that can be catalyzed by iron. These radicals have been considered to be some of the primary intermediates in the development of radiobiological damage (*5,6*). They have also been implicated in the cellular activation of carcinogens (*6*), the toxicity of several xenobiotics (*7*), and the deleterious effects of aging (*8*). Oxygen radicals can damage DNA extensively by strand breakage and degradation of deoxyribose (*9*), and may be important in carcinogenesis (*10*) and other disease processes (*11*). Halliwell and Gutteridge (*12*) discuss the possible role of oxygen radicals in a variety of disease processes and stress the role of iron. Human phagocytes can cause cytogenetic damage to cultured cells *in vitro* (*13*), and inappropriate stimulation of phagocytes to produce oxygen radicals may lead to tissue damage in the whole organism (*14*).

In tissue at pH 7, the hydroxyl radical exists predominantly in its protonated form (*15*), whereas the perhydroxyl radical exists predominantly in its deprotonated form (*16*) and is called the superoxide radical. This latter radical is formed in almost all aerobic cells,

*in vivo*; it is removed by dismutation to hydrogen peroxide and molecular oxygen. This reaction is catalyzed by the copper-zinc enzyme, superoxide dismutase (SOD). Hydrogen peroxide can then react with ferrous ($Fe^{+2}$) iron to yield hydroxyl radicals via the Fenton reaction:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + {}^{\cdot}OH$$

Transferrin carries ferric iron ($Fe^{+3}$) into the cell where it can react with superoxide radical to form ferrous iron plus molecular oxygen. Thus the net reaction is production of hydroxyl radicals inside the cell.

$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + {}^{\cdot}OH$$

When cells are exposed to ionizing radiation, oxygen radicals such as superoxide and hydroxyl are formed. The amount of damage caused by the superoxide and hydroxyl radicals depends on their chances of diffusing to critical tissue sites. Both radicals will react with themselves to form hydrogen peroxide; but while the hydroxyl radical will also react rapidly with most biochemical structures (*17*), the superoxide radical is more selective (*18*). The recombination product, hydrogen peroxide, is generally even less reactive and can thus diffuse farther than the superoxide or hydroxyl radicals. Catalase and peroxidases reduce the concentration of the diffusing hydrogen peroxide in the cell by catalyzing its disproportionation.

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

$$2H_2O_2 + 2RH_2 \xrightarrow{\text{Glutathione peroxidase}} 4H_2O + R_2$$

However, if ferrous iron is present,hydrogen peroxide can be decomposed to reform hydroxyl radicals by the Fenton reaction. The net effect of ferrous iron is to increase the probability that a reactive hydroxyl radical can reach a critical tissue site.

Although neither free ferrous nor ferric ions are thought to exist in tissue in significant concentrations, iron can exist in a variety of iron complexes, e.g., $Fe^{+3}$ ADP (*19,20*). These complexes react rapidly with superoxide radical to form ferrous iron. This ferrous iron is then available for the Fenton reaction. Graf et al. (*20*) analyzed the ability of 12 different iron complexes to catalyze the Fenton reaction. They found that there is a requirement for at least one free iron coordination site for the iron complex to successfully produce hydroxyl radicals from hydrogen peroxide. For example, while iron complexes of adenosine di- and triphosphate were efficient, iron bound to phytate or Desferal (desferrioxamine B methanesulfonate) was ineffective.

Although mice administered superoxide dismutase show decreased radiosensitivity, Marklund et al. (*21*) found no correlation of superoxide dismutase activity and radiosensitivity in seven cancer cell lines. These authors did find evidence for increased repair of radiation damage with higher concentrations of glutathione peroxidases. Indirect evidence for a role of superoxide dismutase in defense against oxidative damage comes from the observations of increased longevity correlated with higher SOD activity when comparing many disparate animal (*22*) and primate species (*23*).

## Iron Distribution

About 60% of the 3 to 4 g of iron in a human is bound in hemoglobin (*24*). The remainder is used in cellular metabolism or is in storage in the reticuloendothelial system, spleen, or liver. The distribution of this iron is accomplished by transport of transferrin in the serum; it is stored in cells as ferritin (*24–26*).

Transferrin is the iron transport protein in serum, and it distributes iron to the cells. It is highly polymorphic with variants determined by multiple alleles at a single gene locus (*27*). Transferrin is a glycoprotein with a molecular weight of about 77,000 and two iron binding sites (*28*). It attaches to a transferrin receptor on the cell surface (*29*). The transferrin-iron complex is taken by endocytosis into the cell (*30,31*) where the iron is transferred to other complexing agents. The apotransferrin is ejected from the cell and recycled for use (*32*). The iron atoms can go to a number of places inside the cell, including iron-containing enzymes; ferritin, the tissue iron-storage protein; or nonspecific iron complexes with, for example, ADP (*19,20*). Transferrin is normally about 30% saturated with iron. The more saturated it is, the more iron complexes might be available for Fenton reaction.

Excess iron stimulates synthesis of ferritin by the polyribosomes (*33*). Ferritin is composed of 24 subunits with a total molecular weight of approximately 440,000 (*34*). Typically, 2500 atoms of iron are stored inside the ferritin structure as hydrous ferric-oxide-phosphate micelles (*35–37*). Though the ferric micelles grow like crystals, the ferritin-iron complex has a relatively uniform magnetic moment of 3.8 Bohr magnetons (horse spleen ferritin). It is not clear how iron gets into and out of ferritin (*38*). It involves an oxidation-reduction with flavin mononucleotide (*39*), but what enzymes, if any, are involved is unknown. In the presence of superoxide radical, the ferric iron stored in ferritin may be reduced to ferrous iron and again be available for the degradation of hydrogen peroxide to form hydroxyl radicals (*40*). The physical chemistry of the hydrous ferric phosphate complex in ferritin (*41*) will be important in coming to understand this process.

Whiting et al. (*40*) showed that chromosome damage to Chinese hamster ovary cells by ferritin increased in proportion to its concentration in culture medium, and Gutteridge et al. (*42*) reported that ferritin at all levels of iron loading increased lipid peroxidation of bovine brain liposomes, particularly in the presence of ascorbate. O'Connell et al. (*43*) examined this further and found that ascorbate did not enhance the effect of ferritin at pH 4.5. In contrast, the iron-binding protein lactoferrin inhibits peroxidation of bovine brain lipo-

somes (*44*), as does the iron complexing agent, desferrioxamine (*45*).

Young et al. (*46*) studied the uptake of radiolabeled transferrin iron by rat hepatocytes. They found that about 70% was taken into ferritin after a 2 hr incubation with transferrin. This result is in keeping with the liver as a primary storage site for iron. A residual iron pool, possibly bound in iron complexes, increased very rapidly at the start of incubation, then declined. The authors estimated the steady-state concentration of the residual iron pool to be about 23 pg/10,000,000 cells. It may also be the iron most able to catalyze production of radicals. Iron-ADP is particularly effective in reacting with hydrogen peroxide to form the hydroxyl radical (*19,20*). Superoxide dismutase, catalase, and glutathione peroxidase all work against the production of the hydroxyl radical. Iron available for the Fenton reaction works for the production of that radical.

## Mechanisms of Radiosensitization

Chemical modification of radiation sensitivity has been of keen interest to clinicians for many years (*47*). Hypoxic cells are radioresistant, and hyperbaric oxygen has been used to increase the effectiveness of radiation in treatment of cancer. Chemical agents that might mimic the effects of oxygen have been introduced and used with varying degrees of success. This class of agents is electron-affinic, and well-known examples include misonidazole and metronidazol. These and a number of other newly synthesized hypoxic cell radiosensitizers are themselves capable of transforming C3H10T1/2 cells *in vitro* (*48*). The transformation is inhibited by superoxide dismutase, suggesting that production of oxygen radicals accounts for at least a portion of the effect of these drugs both as radiosensitizers and transformers. A variety of new radiosensitizing drugs are being tested (*49*), and metal complexes that bind to DNA are also being examined (*50*). The radiosensitizer RSU-1069 binds to DNA possibly at phosphate groups (*51*). The tumor promoter TPA has also been shown to increase transformation of C3H10T1/2 cells by gamma rays (*52*).

Greenstock and Whitehouse (*53*) studied the reaction mechanism of electron-affinic radiosensitizers and described a model in which the sensitizers irreversibly affix radiation damage in critical molecules such as DNA by further oxidizing radical sites on those molecules formed by attack of oxygen radicals. In the absence of sensitizer, much of this damage would be repaired by natural cellular reducing agents. Oxygen added 5 msec after irradiation of hypoxic cells does not radiosensitize them (*54*); this is consistent with the view that rapid reactions are involved in sensitization. Greenstock and Whitehouse (*53*) state "Sensitizer efficacy is determined by the ability to oxidize the radiation target and is found to increase exponentially with increasing electron affinity." They also concluded that the sugar-phosphate backbone of DNA is the primary site of damage, not the purine and pyrimidine bases.

Thiol depletion can have a radiosensitizing effect (*55–57*), whereas exogenous glutathione (*58*) and other aminothiols (*59*) can act as radioprotectors. Buthionine sulfoximine inhibits the enzyme gamma-glutamylcysteine synthetase and leads to the rapid depletion of reduced glutathione (*55*). Cells are viable and apparently otherwise unaffected at exposure to concentrations sufficient to deplete glutathione. Glutathione-depleted V79-379A cells (a Chinese hamster fibroblast line) were radiosensitized at all oxygen tensions examined though the effect was least in air and greatest in anoxia (*55*). Radiosensitization of hypoxic cells by misonidazole is enhanced by glutathione depletion (*55,60,61*). Bump et al. (*62*) conclude that "In general, radiosensitization [by glutathione oxidizing agents] correlates with the rate of reaction with cellular reducing agents and occurs only when the reductive capacity of the cell is exceeded." Glutathione depletion radiosensitizes even when induced after irradiation consistent with a role for glutathione in radiation damage repair (*62*). There may be important differences in the cross-link repair process between cycling and noncycling tumor cells (*63*), and oxygenation of tumor tissue affects cell turnover.

Calcium homeostasis is important in maintenance of cellular reducing agents, in particular glutathione (*64*). Smith et al. (*65*) found that incubation of freshly isolated hepatocytes in calcium-free media greatly increased the toxicity of carbon tetrachloride, bromobenzene, and ethyl methanesulfonate. Reed and Fariss (*64*) speculated that calcium efflux led to depletion of glutathione and consequently greater susceptibility to oxidative damage by the hepatotoxins.

There is evidence that electromagnetic radiation can affect calcium homeostasis at certain combinations of frequency and intensity (*66–68*). This leads to the intriguing possibility that nonionizing radiation may, under certain circumstances, act as a radiosensitizer and that exposure to nonionizing radiation might increase toxicity of carbon tetrachloride to hepatocytes *in vitro*. So-called window effects of nonionizing radiation on fibroblast protein synthesis (*69*) and photochemical reactions (*70*) have been reported. There is also a report that a 2.45 GHz microwave affects mouse-embryo fibroblast transformation by X-ray and phorbol ester (*71*).

One of the important late effects of radiation is on the bone marrow (*72*). Leukemia, and in particular acute nonlymphocytic leukemia, is strongly related to ionizing radiation exposure (*73,74*). The high concentration of iron in the marrow may account, in part, for the high radiation sensitivity of hemopoietic tissue. Since nonlymphocytes such as neutrophils and macrophages generate oxygen radicals during normal functioning (*9,75*), nonlymphocytes may rely more heavily on cellular reducing agents and may be at greater radiation sensitivity than lymphocytes. The evidence is not adequate to determine whether electromagnetic fields influence risk of leukemia, but this has been suggested from occupational studies (*76*), and again nonlymphocytic leukemia has been cited as the cancer most strongly associated

with exposure in adults. However, a small study of residential exposure to electric and magnetic fields failed to provide support for the hypothesis (*77*). Age at exposure to either ionizing or nonionizing radiation would be expected to be important since the baseline risk of lymphocytic and nonlymphocytic leukemias changes greatly with age (*78*).

Radiation leukemogenesis is, no doubt, very complex. The interaction of chemicals and irradiation in T-cell leukemogenesis depends on the dose of each (*79*). Other factors such as marrow iron concentration and calcium homeostasis may also be involved.

Certain cancer therapeutic drugs such as mitomycin C apparently produce oxygen radicals, yet are not radiosensitizers (*80*). However, the differential toxicity of mitomycin C is greatest to hypoxic cells at low concentrations. Calculations based on the electron affinity of the drug show that detectable radiosensitization would require concentrations far in excess of those tested and that they would be highly cytotoxic (*80*).

Radiosensitizers such as paraquat are thought to increase the DNA damaging capability of ionizing radiation by virtue of the additional production of superoxide radical that leads to the depletion of reduced glutathione (*81*). Reduced glutathione is necessary for the activity of the seleno-enzyme glutathione peroxidase that scavenges peroxides and thereby works against hydroxyl radical formation (*82*). Since the principal mechanism by which ionizing radiation damages DNA is thought to be by the hydrolysis of water to produce oxygen radicals (*83,84*), iron-catalyzed oxygen radicals may sensitize a cell to radiation damage by depleting the cell of its radical scavenging capabilities. This would be analogous to the mechanism by which paraquat acts as a radiosensitizer. Such a cell would then be more susceptible to radiation damage. Teicher et al. (*85*) provided evidence that certain cyclopentadienyl complexes of cobalt and iron are radiosensitizers of hypoxic EMT6 cells. The possible mechanisms for this effect discussed included metal binding to DNA and increased oxidative cleavage.

Bleomycin is a cancer therapeutic drug that cleaves DNA. Its action is dependent on ferrous iron and oxygen, and it is believed that production of oxygen radicals at the DNA molecules accounts for its efficacy (*86*). Hoffmann et al. (*87*) examined differences in sensitivity to DNA strand breaks by exposure to hydrogen peroxide. They found that a given amount of hydrogen peroxide produced 5 to 10 times more strand breaks in human DNA than in hamster DNA, and 2 to 4 times more in mouse DNA. The ability to scavenge hydrogen peroxide was similar. They hypothesized that differences in chromatin-bound iron accounted for the species-specific sensitivity, although they did not measure iron content in the cells.

Willson (*88,89*) has discussed the importance of iron in radiation-induced injury as well as in potential effects in treatment of cancer with radiation. He has also stressed the possible importance of zinc in the safe sequestration of intracellular iron; low zinc may increase the danger that iron will catalyze radical production.

## Hydrogen Peroxide

Ito et al. (*90*) reported that oral administration of hydrogen peroxide to mice induced hyperplasia and stomach cancer. They later reported (*91*) that there was a strong negative correlation of tissue damage by hydrogen peroxide and catalase activity of the mouse. Ionic iron or iron-saturated ferritin effectively catalyzes the production of hydroxyl radicals from hydrogen peroxide (*92*), and this was significantly inhibited by apotransferrin in the cell-free system used by the authors. It should be noted that the method of implicating the hydroxyl radical must be chosen with care as certain assays may themselves alter the yield of the radical (*93*).

Ward et al. (*94*) provided evidence that double-strand breaks in DNA are required to cause cell death. They used hydrogen peroxide at 0°C to produce single-strand breaks and asserted that these breaks resulted from a metal catalyzed oxidation of the hydrogen peroxide to the hydroxyl radical. At the cold temperature, the reduction of ferric back to ferrous was slow, and thus only one molecule at a time could cause damage. At 37°C, hydrogen peroxide was much more lethal. The authors suggest a mechanism whereby hydrogen peroxide diffuses to DNA where it can be oxidized by a DNA-ferrous iron adduct to a hydroxyl radical (*95*); the ferric iron can be reduced once again to ferrous by another hydrogen peroxide molecule, and then react with a third to produce a double strand break and cell death. Spitz et al. (*96*) showed that hydrogen peroxide resistant variants of Chinese hamster fibroblasts had increased catalase activity.

Imlay and Linn (*97*) have studied the effects of DNA-bound iron on the formation of hydroxyl radicals from hydrogen peroxide and the influence of NADH and repair enzymes on the toxicity of these reactions. These reactions are efficient enough to suggest a new technology for DNA cleavage. Iron adducts of EDTA were used to bind to DNA and cleave at sequence-specific sites nonenzymatically by exploiting Fenton chemistry (*98*).

Halliwell and Gutteridge (*12,99*) have suggested that hydrogen peroxide may be a particularly dangerous molecule due to the fact that it is not highly reactive itself, and it is nonpolar and can diffuse through cell membranes. If produced in the cytoplasm, it may then diffuse to nuclear DNA where, if a DNA-iron adduct is encountered, it can be readily oxidized to the hydroxyl radical. Many of the dangerous products of radiation hydrolysis of water in the cell may use themselves up rapidly. Since radiation killing is primarily the result of DNA damage (*100,101*), hydrogen peroxide, though mild-mannered itself, may serve to preserve a portion of the radiation insult long enough for it to do its maximal damage directly at the DNA molecule.

Adams and Jameson (*54*) and Chapman and Gillespie (*100*) discuss three periods in the time course of radiation effects. The first is the physical period during which primary interactions of fast electrons or high energy photons with cellular atoms occur. The second is the

chemical period during which secondary interactions of resulting ions (e.g., hydrated electrons) with cellular molecules occur. These reactions are virtually complete within one second of irradiation. The chemical period continues through secondary biochemical reactions for up to hours. Diffusion of hydrogen peroxide to DNA-iron adduct sites may occur during this period. The third period is the biological, during which long-term consequences such as cancer are experienced. There is also evidence for two different time periods for radiation damage repair processes in eukaryotic cells (*102*).

Thus, increased available iron may increase oxidative damage to cells in the absence of radiation exposure and may also increase the damaging capability of radiation in two ways: react with radicals produced by radiation to increase their tissue reactivity and effective range, and increase oxidative stress and deplete the cell of its radical-scavenging capabilities.

## Iron As Limiting Nutrient

There is another mechanism by which iron level may influence development of clinically apparent cancer. Iron may be a limiting nutrient in humans to the growth and replication of invading pathogenic microorganisms. By analogy, successful establishment of a malignant clone from one or a few transformed cells may depend on the availability of iron. Three lines of evidence for this idea are discussed by Weinberg (*103*). First, bacteriostatic effects of conalbumin in egg white, lactoferrin in milk, and transferrin in human serum were all shown to be the result of their avidity for iron. Second, the human body's response to bacterial invasion or cancer often incudes several mechanisms that could reduce the availability of iron: intestinal iron assimilation is restricted, plasma iron level decreases, and fever develops. Third, a variety of epidemiological observations are consistent with the notion that reduced iron level plays a role in defense against infection.

Weinberg (*103*) has developed the argument that neoplastic cells may behave like invading microorganisms with regard to iron needs and acquisition. There is evidence that neoplastic cells have altered transferrin binding capability and that they may produce other iron-binding polypeptides that may enhance growth in iron-restricted environments (*104*). There are similarities in the host response to invading microorganisms and to neoplastic cell growth. In addition, neoplastic cells have been found to be killed by heating at temperatures 1 to 1.5 degrees less than their normal counterparts (*105*). This effect has formed the basis for hyperthermia treatment of cancer (*106*) and may result from affects on iron acquisition (*107*).

Konopka and Neilands (*108*) found that human serum albumin reduces the ability of certain bacterial siderophores to bind iron. These authors concluded that these results indicate that serum albumin may act synergistically with other factors in the serum, such as transferrin, to limit iron supply and in this way restrict the growth of invading microorganisms. If albumin can affect iron acquisition by transformed cells in a similar manner, then low albumin might be associated with increased risk of cancer. This was found to be the case in a study of Chinese government workers (*3*) and in a study of the U.S. adult population (*4*).

Bergeron et al. (*109*) injected a leukemia cell line, L1210, in two groups of mice. One group received iron dextran injections and the other iron-free dextran. Both groups were fed nonpurified Rodent Lab Chow from Ralston Purina. The group receiving iron died sooner and had greater tumor burden of cells (peritoneal harvest prior to death). The authors concluded that their results were consistent with the hypothesis that iron may be a limiting nutrient to the growth of a cancer cell. Hann et al. (*110*) induced iron deficiency in three strains of mice through dietary restriction and found that transplanted colon adenocarcinoma, hepatoma, and mammary adenocarcinoma cells grew more slowly in these iron deficient mice than in normal controls.

Cavanaugh et al. (*111*) found that the bacterial iron chelators Parabactin and Compound II inhibited the growth of L1210 cells in culture, and they concluded that the mechanism involved inhibition of the iron-containing enzyme, ribonucleotide reductase, and consequently DNA synthesis. Basset et al. (*112*) reported that in addition to blocked DNA synthesis, iron chelation by desferrioxamine also blocked other systems critical for cell growth.

A surface receptor for transferrin on malignant cells has been identified (*113,114*). This receptor segregates with malignant behavior in somatic cell hybrids between normal and malignant cells. A monoclonal antibody to the transferrin receptor stops growth of a T leukemia cell line *in vitro* (*114*), and the mechanism is thought to be by restricting iron uptake by the cell (*115*). Monoclonal antibodies can also inhibit the normal growth of erythroid bursts (*116*). Increased transferrin receptor concentration may confer an important growth advantage to malignant cells in an iron restricted host environment. Transferrin receptors are expressed in detectable amounts on normal dividing cells and on certain differentiated cells as well (*117*), and they are found in greater numbers on immature murine erythroid cells than on mature cells (*118*).

It must be noted that in workers exposed to plutonium, high iron stores might serve to reduce the risk of plutonium absorption. Plutonium binds to ferritin and transferrin and when iron stores are high, plutonium absorption is lowest (*119,120*).

## Diet and Iron Status in Humans

Severe iron-deficient diet leads to iron deficiency anemia, and severe iron-overloaded diet can lead to iron-overload (*121*). Within the normal range of iron intake, however, the relationship between intake and iron stores is unclear. Human iron exchange is restricted to about 10%/year (*122*), and typically much less than 10% of ingested iron is absorbed (*26*). Serum ferritin level is thought to be the best single indicator of iron stores

in otherwise healthy individuals,and it varies widely in the population (*123*). The mean concentration is higher in men than in women (*124*). However, the First National Health and Nutrition Examination Survey concluded that the prevalence of anemia was not closely related to dietary iron intake in the United States (*125*).

Evidence that intake does affect iron status is provided by a study in Africa of two closely related groups of one Turkana tribe (*126*). The diets of the two groups were similar in total calories, but one group consumed fish and milk, whereas the other consumed primarily milk and thus had lower available iron intake. The two groups were very similar in height, weight, and age. The fish eaters, however, had hemoglobin of 12.7 g/dL, transferrin saturation of 26%, and serum iron of 59.7 μg/dL, whereas the milk drinkers had values of 11.6, 14 and 38.2, respectively. Lower iron intake led to lower iron stores. Conversely, Olsson et al. (*127*) reported that in Sweden where food has been iron fortified for over 30 years, there was an unusually high prevalence of high serum iron and early stage hemochromatosis in men.

## Inorganic Iron

Inorganic iron salts have not been found to be remarkable in transformation or carcinogenesis (*128–130*). Stokinger (*131*) concluded that there is little evidence for an increased cancer risk in humans exposed to iron-oxide (in mining, for example). However, Turver and Brown (*132*) have shown that iron found in asbestos fibers can generate oxygen radicals and induce lipid peroxidation and DNA-strand breaks in C3H10T1/2 cells. Desferrioxamine inhibited the damage. They concluded that iron-catalyzed damage may play an important role in asbestos pathogenicity in humans. Weinberg (*133*) has hypothesized that the difference in the carcinogenicity between amphibole asbestos (high cancer risk and high iron content) and serpentine asbestos (low cancer risk and low iron content) can be explained by the iron content of these respective silicates.

Much current research on diet and cancer has focused on the protective effects of such nutrients as tocopherol, carotenoids, selenium, and ascorbic acid (*134–137*). While each undoubtedly has many effects on cellular metabolism, they share the fact that they are all antioxidants. Thus, iron may catalyze the production of proximate carcinogens (oxygen radicals) while these antioxidants may destroy them. In a recent review article Cerutti (*138*) discusses the evidence for an effect of prooxidants on increased cancer risk. The prooxidant states (e.g., hyperbaric oxygen tension, radiation, ferrous iron) are mediated through the production of oxygen radicals, in particular the hydroxyl radical. Uric acid has been suggested as another antioxidant defense mechanism and has been shown to bind ferric iron in such a way as to inhibit its oxidation by ascorbate (*139*). Stocker and Ames (*140*) have also suggested that bilirubin and copper are important to consider as antioxidants. Iron binding by phytate has been argued to account for a reduced colon cancer risk for those consuming a high fiber diet (*141*).

## Conclusions

The two broad mechanisms by which iron may increase risk of cancer or of radiation-induced transformation are depicted in Figure 1. Iron may increase oxidative stress to a cell and thereby the risk of transformation. And iron availability may influence the chances that a cancer cell will survive. These possibilities could be tested by controlled experiments. Radiation exposures of cells from the same strain but with different iron contents may show whether or not the iron content in the cells had an effect on their radiation-induced mutation or death.

Although the definitive laboratory experiments await execution, four epidemiological studies have been done that are consistent with the hypothesis that high iron stores are associated with increased risk of general mortality and of cancer. Sera were tested from two populations for ferritin and transferrin. Ferritin was higher and transferrin was lower in Solomon Islanders who subsequently died over a 10-year period (*142*) and in Chinese government workers who developed cancer (*3*) than in suitably chosen controls. Selby and Friedman (*143*) reported a reduced lung cancer risk associated with anemia and with high total iron binding capacity of serum in women in the Kaiser-Permanente Medical Plan in California. Stevens et al. (*4*) reported that cancer risk was higher in men with evidence of high iron stores in the United States. Four sites appeared strongly related: lung, colon, bladder, and esophagus. On the basis of the idea that iron may be a limiting nutrient to invading pathogens and cancer cells (*103*), Weinberg and Weinberg (*144*) suggested giving blood as possibly reducing risk of disease. This is being tested in a population of blood donors.

According to the schema proposed in this paper, excess iron itself might increase cancer risk. In addition, iron elevated short of a level that would by itself increase cancer risk, might nonetheless act as a radiosensitizer by virtue of the mechanisms previously described. Very few experiments have been performed that have addressed directly this iron hypothesis. Ra-

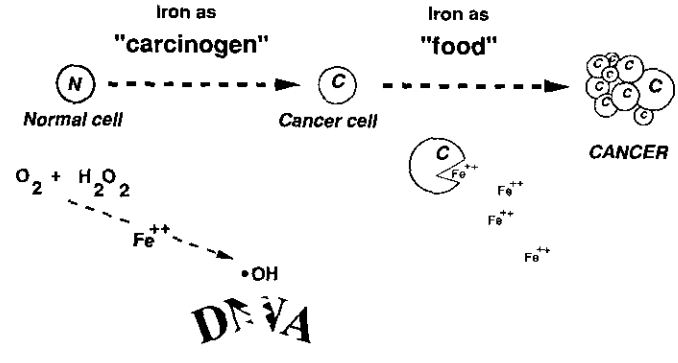


FIGURE 1. Two mechanisms by which iron may increase cancer or radiation-induced transformation.

diation transformation experiments in which antioxidants have been tested as protectors (*59,136*) could easily be modified to determine if transferrin-iron or ferritin-iron complexes added to the growth medium act to increase the effectiveness of radiation. In addition, the protocol of Skov (*84*) in which the mechanisms by which the radiosensitizers misonidazole and 2,2,6,6-tetramethyl-1,4-piperidone-N-oxyl enhance radiation-induced DNA damage could be modified to examine the effects of organic iron (bound to ferritin, transferrin, or ADP). A mouse leukemogenesis experiment in which dietary anemia is induced in one group, iron excess by injection is induced in another group, and a normal iron level is maintained in a third group would address directly the hypothesis that iron level affects the tumor burden after a specified radiation dose.

Increased cancer risk has been associated with screening, diagnostic, or therapeutic radiation exposure to certain human populations: those given radiation therapy for a first cancer (*145–147*), women exposed to mammographic screening (*148*), those given Thorotrast (*149*) and radium (*150*) patients, and radiation-treated ankylosing spondylitics (*151*). It is the thesis of this paper that the iron status of the exposed individuals may be one of the factors determining level of risk: the higher the iron stores, the higher the risk.

In addition to affecting risk of cancer, iron nutriture may be implicated in prognosis after cancer diagnosis and in the site of distant metastases. A malignant neoplasm must acquire iron in order to thrive, therefore clinical manipulations of iron status may be of value in management of cancer patients. In addition, the higher iron level of a tissue, the greater may be the chance it will be the site of a metastasis; the liver is a common metastatic site and is iron-rich.

Iron-deficiency anemia is a serious problem worldwide, and iron repletion has been a primary goal of nutritional programs. Possibly for this reason, physiological variation in iron availability has not received scrutiny as a possible carcinogen or radiosensitizer. Excess iron over a minimum necessary to avoid anemia may in fact have adverse long-term health consequences including an increase in cancer risk and increased susceptibility to radiation-induced cancer.

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