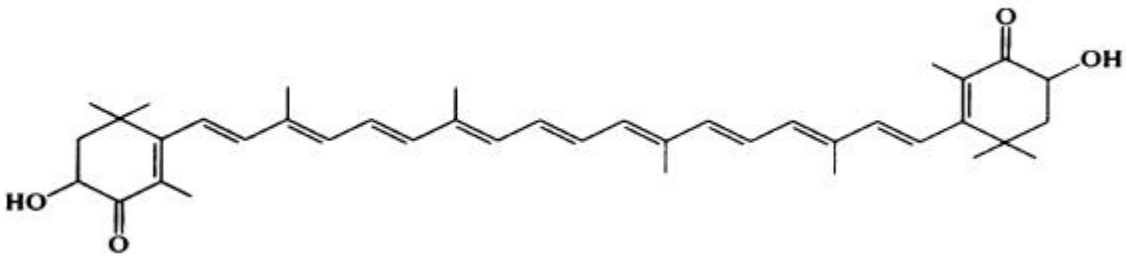
Astaxanthin, Nature's Super Carotenoid



Carotenoids are a family of over 700 natural lipid-soluble pigments that are only produced by phytoplankton, algae, plants, and a limited number of fungi and bacteria. The carotenoids are responsible for the wide variety of colors they provide in nature, most conspicuously in the brilliant yellow and red colors of fruits, vegetables and leaves. In plants and algae, carotenoids are a vital participant in the photosynthetic process along with chlorophyll and other light-harvesting pigments.

While some animals are able to alter carotenoids into other forms, they still must obtain them from their diet. The pink flamingo, for instance, filters *Spirulina* or other algae from bodies of water and converts the yellow carotenoids, beta-carotene and zeaxanthin, into the pinkish-red carotenoids, astaxanthin and canthaxanthin. These red carotenoids are then deposited into the feathered plumage and elicit the striking color of this bird.

Carotenoids, and especially astaxanthin (asta-ZAN-thin), are distinguished by their capacity to interact with chemically reactive species of oxygen known as singlet oxygen and free radicals. Interestingly, animals have adapted to exploit the potent antioxidant properties of carotenoids. One familiar example is seen in the cold-water fish that selectively accumulate astaxanthin from their diet and deposit it in their flesh to protect lipid tissues from peroxidation, a harmful form of oxidation. We recognize this as the healthy pinkish-red glow in the flesh of salmon and trout.

A growing body of scientific literature reveals significant evidence that astaxanthin surpasses the antioxidant benefits of beta-carotene, zeaxanthin, canthaxanthin, vitamin C and vitamin E. Animal cell culture studies have also indicated that astaxanthin can protect skin from the damaging effects of ultraviolet radiation, ameliorate age-related macular degeneration, protect against chemically induced cancers, increase high density lipoproteins and enhance the immune system.

Oxidative Stress

Humans have developed sophisticated systems of arteries, veins and capillaries to deliver and regulate oxygen-rich blood to every cell of the body. The oxygen we utilize from air and require for normal metabolic activity is called 'ground-state' or 'triplet' oxygen and is quite stable. However, it can also be converted to other forms that present severe challenges to cells. Harmful reactive oxygen species such as singlet oxygen, superoxide, peroxyl and hydroxyl radicals are commonly formed as a consequence of photooxidation, physiological stress and normal immune system functions. Singlet oxygen, for example, is an excited form of ground state oxygen that is formed from normal biological activities. It is highly reactive and relatively long-lived and must transfer this excess energy to another molecule to relax again to the ground state of oxygen. Astaxanthin has an especially high propensity to absorb the excess energy from singlet oxygen, release it as heat, and return oxygen and itself back to the

ground state. This neutralization of singlet oxygen is known as 'quenching'. Superoxide radical is produced from ground-state oxygen by gaining an electron, whereby it can then proceed to create more dangerous molecules such as hydroxyl and peroxyl radicals that can attack proteins, nucleic acids and fatty acids.

Free radicals are another family of highly reactive and unstable molecules that have unpaired or missing electrons necessary to fulfill a stable molecular structure. This propels them to rob electrons from other vital cellular molecules to obtain their deficient electron. This stabilizes the original free radical but now transforms the molecular victim (fatty acid, protein, DNA) into a new free radical that seeks more prey. These electron-robbing events often involve the most critical cellular components and can lead to cellular injury such as protein degradation, oxidized lipids and DNA damage. Unfortunately, free radicals have an inordinate affinity to attack electron-rich unsaturated fatty acids, the principle component of cell membranes. These peroxidized fatty acids then create more fatty acid radicals in a chain reaction until an antioxidant or another compatible molecule intercedes and halts the 'falling domino' effect.

A number of theories deduce that an upset oxidative balance can be a contributing factor in such conditions as rheumatoid arthritis, heart disease, Parkinson's disease, Alzheimer's disease, cancer and stroke (Cross et al., 1987). We normally have a balance of free radicals and an arsenal of antioxidants to counter them, such as superoxide dismutase, catalase, and an assortment of peroxidase enzymes. However, a host of conditions such as poor nutrition, stress, air pollution, smoking, ultraviolet light or disease can upset this equilibrium. (Ames and Shigenaga 1992, 1993; Harman 1981;Esterbauer et al., 1992; Steinberg et al., 1989;Moody and Hassan, 1982; Marnett, 1987; Breimer, 1990).

Even strenuous physical exercise leads to an increase in reactive oxygen and nitrogen species that causes oxidative stress and free radical damage to cells (Poulsen et al., 1998; Niess et al., 1999). Enzymatic and non-enzymatic antioxidant systems play a vital role in protecting tissues from excessive oxidative damage during exercise. However, depletion of the antioxidant systems may induce a state wherein the defenses of tissues are overwhelmed by excess reactive oxygen species and are then vulnerable to damage (Ji, 1995; Sen 1995). Reactive oxygen and nitrogen species formed during strenuous exercise can be mediators of skeletal muscle damage, inflammation, and lipid peroxidation as well as a contributing factor in the loss of calcium homeostasis within cells after strenuous exercise (Dekkers, 1996; Witt, 1992; Radaka, 1999; Goldfarb, 1999).

Free radicals generated by oxidative stress of exercise have also been shown to impair immune function and lead to an inflammatory response. Many components of the immune system exhibit adverse change after prolonged, intense exertion. A period of impaired immunity may last from 3 to 72 hours depending on the immune measure (Nieman, 1999). Prolonged stress as a result of excessive exercise can lead to a decline in certain aspects of immune system function such as natural killer cell cytotoxicity of secretory-IgA (Kelly, 1999).

Researchers recommend the use of dietary antioxidants to counteract the oxidative which occurs during exercise (Witt et al. 1992; Ji, 1995; Sen 1995; Kanter, 1998; Niess et al., 1999; Dekkers et al., 1996). Human studies have shown that dietary supplementation with antioxidant vitamins such as carotenoids and vitamins E and C have a favorable effect on lipid peroxidation after exercise (Dekkers, 1996; Goldfarb, 1993; Kanter, 1998). Astaxanthin has been shown to be 100-500 times stronger than vitamin E in preventing lipid peroxidation in rat mitochondria (Kurashige, 1990).

Atherosclerosis develops from deposits of fatty acid and cholesterol plaques in blood vessels and leads to the narrowing and hardening of blood vessels. Ultimately, this disease to coronary heart disease, heart attack or stroke. The lipoprotein carriers of fatty acids and cholesterol are generally classified as LDL (bad cholesterol), HDL (good cholesterol), VHDL and VLDL. The oxidation of the LDL lipoproteins (bad cholesterol) has been implicated as a principal factor in the development of

atherosclerosis. However, clinical evidence also indicates that dietary carotenoids can decrease oxidation of LDL and therefore help defend against this type of cardiovascular disease. Astaxanthin has been shown to accumulate primarily in the blood plasma LDL and VLDL lipoproteins with a slightly lower concentration in the HDL, thereby providing protection against oxidation (Østerlie M. B. et al, 1999a,b)

Astaxanthin, the Super Antioxidant

It is now evident that the antioxidant potential of carotenoids can significantly reduce free radicals and the oxidative load to help the body maintain a healthy state. Carotenoids, and especially astaxanthin, protect cells against oxidation by 1) quenching singlet oxygen and dissipating the energy as heat and 2) scavenging free radicals to prevent and terminate chain reactions. Due to its particular molecular structure, astaxanthin serves as an extremely powerful antioxidant. It has a very effective quenching effect against singlet oxygen, a powerful scavenging ability for lipid and free radicals and effectively breaks peroxide chain reactions (Kurashige et al. 1990; Jorgensen, 1993; Miki, 1991, Di Mascio, 1989, Terao, 1989).

Researchers have developed a variety of methods to measure the antioxidant capacity of carotenoids. Some of these assays are conducted in test tubes (in vitro) to better control conditions or within cells themselves (in vivo). Typically, a chemical that generates free radicals or peroxides is mixed with a substrate such as a fatty acid that can become readily oxidized. When the reaction rate is determined, carotenoids or other antioxidants can then be added to determine how it quenches, or slows the peroxidation rate of the fatty acid. Numerous studies exist demonstrating the potent radical scavenging and singlet oxygen quenching properties of astaxanthin (Haila, 1997;Woodall, 1997;Nakagawa, 1997;Oshima, 1993; Tinkler, 1994). It has been demonstrated that astaxanthin is significantly more effective in neutralizing free radicals than beta-carotene and protects against peroxidation of unsaturated fatty acid methyl esters better than canthaxanthin, beta-carotene or zeaxanthin (Terao, 1989; Jorgensen, 1993). In fact, the antioxidant activities of astaxanthin have been shown to be approximately 10 times stronger than other carotenoids such as zeaxanthin, lutein, canthaxanthin and beta-carotene (Miki, 1991).

Di Mascio utilized a chemiluminescent technique to express the superior singlet oxygen quenching ability of astaxanthin compared to other carotenoids. He also concluded that the effectiveness and potency of astaxanthin was even better expressed at the lower oxygen concentrations found in tissues, as opposed to higher oxygen concentrations normally used with in vitro conditions (Di Mascio, 1989).

Although researchers use different assay systems, astaxanthin has been shown to surpass the antioxidant activity of other carotenoids such as zeaxanthin, lutein, beta-carotene and canthaxanthin. As shown in Figure 1, astaxanthin has a singlet oxygen quenching activity over 500 times greater than alpha-tocopherol, also known as vitamin E (Di Mascio, 1989; Ranby and Rabek 1978; Shimidzu, 1996; Naguib, 2000). Vitamin E (tocopherol) is another key lipid-soluble antioxidant for the body. Interestingly, in vitamin E-deficient rats, astaxanthin can help restore the shortcoming and protect against damage caused by lipid peroxidation. Using a homogenate of rat mitochondria, astaxanthin had a 100-fold greater activity than vitamin E in inhibiting lipid peroxidation. No other compound showed such a strong activity. The authors proposed a role for astaxanthin as a "super vitamin E" in protecting cellular lipids against peroxidation in vivo (Miki, 1991; Kurashige, 1990).

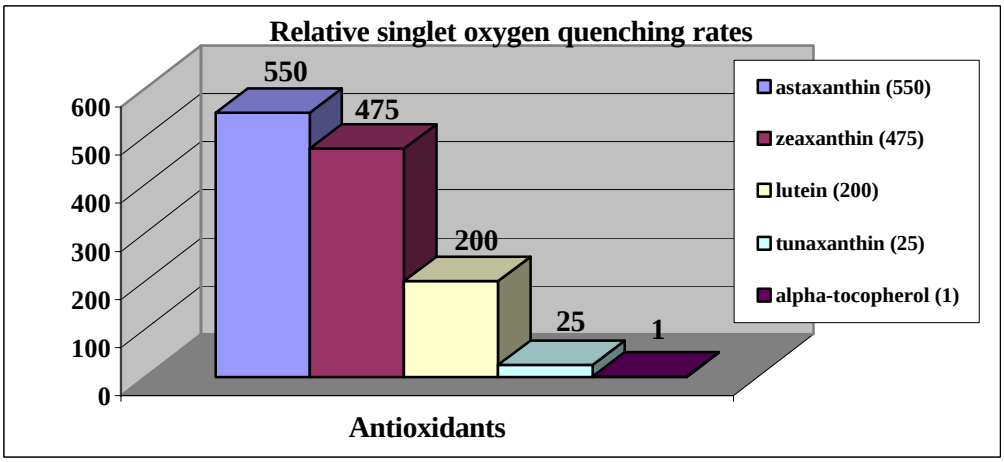


Figure 1. Singlet oxygen quenching activities (Kq $10^{-9}M^{-1}S^{-1}$). Adapted from Shimidzu, 1996

In cell culture studies, similar results demonstrate the efficacy of astaxanthin as an antioxidant against peroxyl radicals. It was shown astaxanthin was more effective than beta-carotene, zeaxanthin or canthaxanthin in protecting membrane phospholipids from peroxidation (Lim, 1992).

In another report, primary cultures of chicken embryo fibroblasts (CEF) were oxidatively stressed by exposure to the herbicide, paraquat, as the radical generator while various levels of astaxanthin were added to ascertain the antioxidant effect. Activities of the antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase were measured as indices of oxidative stress. Without astaxanthin, paraquat increased the activities of SOD and catalase more than two-fold, and decreased the activity of glutathione peroxidase by more than 50% indicating high oxidative stress. Protection against paraquat-induced oxidative stress was observed at all levels of astaxanthin tested and was significantly greater than beta-carotene or vitamin E in this model (Lawlor and O'Brien, N.M., 1994). Other studies show that astaxanthin furnishes more protection to rat liver microsomes undergoing radical-initiated lipid peroxidation than either beta-carotene or vitamin E (Palozza, 1992; Nishigaki, 1994).

Ultraviolet Light

In *Haematococcus* algae, astaxanthin serves a protective role against ultraviolet (UV) light as an adaptive response against oxidative stress. Mature cysts containing astaxanthin have a 6-fold higher tolerance to UVB light than immature cysts. Since astaxanthin is not directly a UV-absorbing pigment, it appears that it functions as a potent antioxidant against the oxygen radicals that are formed from photooxidation (Kobayashi M. and T. Okada, 2000).

When skin is irradiated with ultraviolet light it causes photooxidative damage induced by the formation of reactive oxygen species such as singlet oxygen, superoxide radical and peroxide radicals. This photooxidative stress damages cellular lipids, proteins and DNA and is considered to contribute to erythema, premature aging of the skin, photodermatoses and skin cancers. Beta-carotene has been shown to provide some protection against sunburn (erythema), but should be taken at least 12 weeks prior to exposure. Secondly, it appears that vitamin E (alpha-tocopherol) supplementation together with carotenoids provides additional protection against erythema (Stahl et al., 2000).

Special SKH1 hairless mice sensitive to UV light are often used to understand the effects of antioxidants and light-induced polyamines. Polyamines are central to normal growth and activation of polyamine metabolism (putrescine in particular) is implicated in tumor promotion. In one study, female SKH1 hairless mice were weened at eight weeks and fed six different diets containing 5 ppm beta-

carotene, 10 ppm astaxanthin or retinol. After 4 months, one half of each group was exposed to ultraviolet light, sacrificed, and then putrescine, spermidine and spermine concentrations were measured in the epidermis. After irradiation, astaxanthin alone or in combination with retinol was remarkably effective in preventing increases of free putrescine after damage was induced. The putrescine of the control group increased 4.1-fold whereas the groups fed astaxanthin increased only 1.5-fold. Astaxanthin also had a stronger inhibitory effect on putrescine accumulation than dietary retinol. Additionally, spermidine and spermine concentrations were significantly lower in those groups fed astaxanthin. Taken together, the results indicate that astaxanthin exerts a specific action on transglutaminase enzymes to consume these polyamines in response to skin irradiation (Savoure, 1995).

In rat kidney fibroblasts, addition of astaxanthin exhibits superior protection against UVA light-induced oxidative stress compared to lutein and beta-carotene. Cell cultures were grown in differing concentrations of carotenoid-supplemented media and exposed to UVA light for four hours. Subsequently, various parameters were assayed. Catalase (CAT) and superoxide dismutase (SOD) were significantly decreased following the UV insult exposure compared to control cultures, whereas thiobarbituric acid reactive substances (TBARS) were significantly increased. Beta-carotene at a level of 1000 nM and lutein at 100 nM were necessary to protect against UV-induced loss of CAT, whereas it only required 5 nM of astaxanthin. Similarly, levels of 500 nM beta-carotene, 1000 nM of lutein and only 5 nM of astaxanthin were required to protect against loss of SOD activity compared to control cultures. Increases in thiobarbituric acid reactive substances (TBARS) were also measured as indices of oxidative stress. Supplementation of beta-carotene at 100 nM, lutein at 1000 nM and astaxanthin at only 1 nM prevented the UVA-induced increase in TBARS. The authors suggest that carotenoids other than beta-carotene, and particular astaxanthin, may be important biological antioxidants (O'Connor, 1998).
In one conflicting study, SKH hairless mice fed beta-carotene, lycopene or astaxanthin as sole carotenoid sources tended to have higher probability of epidermal tumors. The authors state that it would be prudent to consume foods with mixed carotenoids in addition to vitamins E and C, since they are thought to be intimately involved in the antioxidant cascade with carotenoids (Black, 1998).

Metabolic Effects of Astaxanthin

High-density lipoprotein (HDL) is a complex of lipids and proteins that functions as a transporter of cholesterol in the blood. Higher levels of HDL "good cholesterol" and lower levels of LDL "bad cholesterol" are associated with a decreased risk of atherosclerosis and coronary heart disease. In one study, male Wistar rats fed 0.1% dietary astaxanthin for 30 days had increased HDL cholesterol of 57 mg/dL compared to the control diet with 42.4 mg/dL. Conversely, the LDL bad cholesterol decreased from the control diet of 12.5 mg/dL to 9.6 mg/dL when supplemented with astaxanthin. Neither beta-carotene nor canthaxanthin elicited the same effect. Additional studies are in progress, but it is can be speculated that astaxanthin or other carotenoids can decrease the oxidation of these lipid-carriers and thereby reduce the risk of atherosclerosis (Murillo, 1992).

Astaxanthin may also have a physiological benefit in energy metabolism. Dr. Curt Malmsten conducted a double-blind study at the Paramedical School of Värmdö (Gustavsberg, Sweden) in which 40 healthy students were divided into two groups for a series of physiological tests with and without astaxanthin supplementation. The students were given either a placebo capsule or one with 2 mg of astaxanthin from *Haematococcus* algae. At the end of six months, there was a not a significant difference in the hemoglobin values between the two groups but there was a significant difference in the strength/endurance that was measured by knee bends and a barbell weight. The results showed the

placebo group reached on average score of 21.78 and the experimental group attained 61.74 on average (P=0.047).

It is interesting to note that in stress experiments conducted with shrimp, survival is higher in those fed a diet containing astaxanthin. The positive correlation between survival and pigment concentration of tissues suggests that astaxanthin can function as an intracellular oxygen reserve which permit crustaceans to survive under anaerobic conditions common in pond cultures. After 3 months, shrimp fed astaxanthin at 50 mg/kg diet had an average survival rate of 87%. In contrast, there was 50% survival when shrimp were deprived of carotenoids or supplemented with 50 mg/kg of β-carotene (Chien, 1992). One could surmise that astaxanthin may have positive benefits for endurance during aerobic activities when muscles are demanding oxygen.

Atlantic salmon have the distinction as being the species for which astaxanthin has been shown to be an essential vitamin, with absolute minimum levels being about 5.1 ppm. A recent groundbreaking study in Norway by Christiansen and his colleagues demonstrated that Atlantic salmon fry have a definitive growth and survival requirement for astaxanthin in their diet. Fish fed diets with astaxanthin below 5.3 ppm were found to have marginal growth, those fed levels above 5.3 ppm had significantly higher lipid levels accompanied by lower moisture levels. When fry were fed astaxanthin concentrations below 1 ppm, survival rates plummeted to less than 50% whereas survival of groups receiving higher concentrations had survival rates greater than 90% (Christiansen et al., 1995).

GAP junctions are relatively non-specific pores that connect two cells and are "gated" such that they can open or close in response to certain stimuli. These functions are especially important in the propagation of nerve impulses. Carotenoids are known to protect cells against chemically induced carcinogenic transformations through the enhancement of GAP junctional communication between cells. Connexins are highly homologous proteins that assemble to form gap junctions, small channels which allow for the passage of ions and other small molecules between neighboring cells. Gap junctions play an important role in a variety of cellular processes including homeostasis, morphogenesis, cell differentiation and growth control.

Chemoprevention activity strongly correlates to the expression of the gene, connexin43, coding for a gap junctional protein (Bertram J.S. et al., 1991). GAP junctional communication (GJC) has been linked to increased growth control, chemopreventive carotenoids increase expression of this gene and act as potential chemoprotective agents (Zhang L.X. et al.1991; King T.J. et.al. 1997). This has led to the theory that carotenoids enhance GAP junction communication and thereby serves as a conduit for growth regulatory signals (Bertram, 1991; Zhang et al, 1992). The effect on GAP junctions is also partly explained by the finding that astaxanthin functions as a membrane stabilizer, essentially acting as trans-membrane rivets between lipid bilayers (Woodall, 1997; Milon, 1986). In essence, increased expression of connexin43 GAP protein from astaxanthin may lead to improved cell-to-cell communication, which promotes homeostasis and normal cellular functions.

Support of Eye Health

In humans and other animals, carotenoids are essential for proper health of the eye, a number of studies have demonstrated that dietary carotenoids help to protect the retina against oxidative damage (Snodderly, 1995). The macula is a small central part of the retina encompassing an area of about 2 millimeters in diameter directly behind the lens of the eye. Interestingly, this specialized macular region only occurs in higher primates such as monkeys and man. It consists primarily of cones, which are responsible for color discrimination, and is the region that produces the sharp vision needed to read and see fine details clearly. The photoreceptor cells contain the highest concentration of polyunsaturated fatty acids (PUFA's) of any tissue in the human body and a particularly high level of oxygen that renders it

very susceptible to lipid peroxidation. It is generally agreed that high-energy blue light (400-500 nm) creates excited oxygen species through photooxidation, principally singlet oxygen in the eye, leading to peroxides and other unstable molecules. The cumulative oxidative damage then leads to the degenerative changes seen in the ageing macula (Gerster, 1991).

Carotenoids within the macula are perfectly suited to absorb this high-energy blue light and act as an antioxidant to thereby quench these damaging oxygen species. Clinical studies have indicated that light injury is a major cause of a disease called "age-related macular degeneration" (AMD) because of this cumulative light insult. AMD results in a gradual loss of photoreceptor cells and is the leading cause of irreversible blindness among older Americans that have decreased levels of carotenoids in their eyes. It has been shown that a higher dietary intake of carotenoids is associated with a 43% lower risk of AMD. Specifically, lutein and zeaxanthin, which are primarily obtained from dark and leafy vegetables, were most strongly associated with the reduced risk of AMD (Seddon et al., 1994).

Unlike beta-carotene, astaxanthin is able to readily cross the blood-brain barrier and protect the retina against photo oxidation and loss of photoreceptor cells. Astaxanthin has not been shown to crystallize in the retina, though this has been reported to cause asymptomatic indications with canthaxanthin in the past. Furthermore, astaxanthin has the ability to protect the neurons of the retina as well as those of the brain and spinal cord, from damage caused by free radicals (US Patent 5,527,533).

In animal tests, seven albino Lewis rats were first fed a normal diet and placed on a twelve hour cycle of light and darkness for 14 days. Four rats were then administered intraperitoneal injections of astaxanthin corresponding to 37.5 mg astaxanthin/kg of body weight at 12 hour intervals. All seven rats were then exposed to 180-200 ft-candle (1800-2000 lux) green-filtered fluorescent light at 490-580 nm for 24 hours. The rats were then kept in the dark for a two-day recovery period and euthanized for analysis of the retinas. By measuring the thickness of the outer nuclear layer (ONL) of the retina, a quantitative determination of the photoreceptor cell degeneration could be made. It was found that control rats without treatment or photic injury had an ONL measurement of 45 microns, whereas the group receiving photic injury without astaxanthin supplementation had an ONL measure of 32 microns. The ONL measurement of rats receiving astaxanthin and photic injury had an ONL measurement of 42 microns, which showed that administration of the carotenoid provides a significant protection to receptor cells from photic injury. The astaxanthin protected the photoreceptors in each of the four quadrants and in the whole eye as well.

A similar follow-up study was conducted with oral dosing of astaxanthin to measure the effects of photic injury on rhodopsin levels in the eye. It was found that rhodopsin levels in the retinas of control rats fell for six days following photic injury and then began to recover. After 6 hours of photic injury, the rhodopsin level of control rats was 0.75 nmol, and continued to decrease to 0.5 nmol after 6 days. The level improved to 0.8-0.85 nmol after 13 days from the initial photic insult. In contrast, the astaxanthin-fed rats had a rhodopsin level of about 1.15-1.2 nmol at the 6-hour post injury stage. Additionally, the rhodopsin did not decrease over the subsequent 6 days, but increased to a level of about 1.25 nmol and remained essentially constant through day 13 after photic injury. The authors state that the astaxanthin not only protects the receptor cells from photic injury but also ameliorates the effects of the damage since the rhodopsin levels never decrease, but rather increase over the recovery period (US Patent 5,527,533).

Cancer Deterrence

Epidemiological studies have demonstrated a correlation between carotenoid intake and the reduced incidence of coronary heart disease and certain cancers, macular degeneration, and increased resistance to viral, bacterial, fungal and parasitic infections (Seddon, 1994; Zhang, 1999, Rao, 1999; Rumi, 1999; Batieha, 1993). Studies indicate that the mechanism for this protective attribute is partly due

to the direct enhancement of the immune response by carotenoids. Anticarcinogenic effects of carotenoids are likely attributable to its antioxidant effect, insofar as oxygen radicals are related to the process of cancer initiation and propagation.

A synopsis of these studies demonstrates that supplementation with carotenoids increases the number of circulating lymphocytes (T-helper cells), enhances T and B lymphocyte proliferation, improves rejection of foreign tissue, increases killer cell destruction of tumor cells and neutrophil killing of *Candida* fungi, and inhibits loss of macrophage receptors (Bendich, 1990). Mice fed carotenoids had significantly reduced tumor growth when the primary lesion was excised and then re-challenged with the same tumor (Tomita, 1987). Virus-induced tumors such as murine sarcoma are slowed by carotenoids, as well as adenocarcinoma, squamous cell carcinoma, fibrosarcoma, and chemically induced tumors (Bendich, 1990). These studies present strong evidence that orally administered carotenoids can directly affect the immune responses to cancerous tumors and lead to a lower tumor burden.

Typically, various chemicals are used to induce specific cancers in rats or mice and different dietary supplements are added or left out to test their effects. In rats, the chemical azoxymethane (AOM) has been used to induce colon cancer and study the effect of anticancer agents. In a recent study, animals were treated with AOM by 3 weekly injections and then fed diets with or without astaxanthin at 100 and 500 ppm for a further 34 weeks. At the end of the 37-week study, 63% of the chemically induced group had cancerous intestinal neoplasms with an average 0.97 neoplasms per rat. However, the chemically induced group that was subsequently treated with 100 ppm astaxanthin had a 41% incidence of neoplasms with an average of 0.48 per rat. Groups treated with 500 ppm of dietary astaxanthin had a reduced cancer rate of 31% with an average of 0.41 neoplasms per rat. The authors state the results clearly indicated that administration of astaxanthin during the post-initiation phase significantly inhibited AOM-induced colon carcinogenesis in a dose-dependent manner. They speculate that the significant antitumor properties of astaxanthin may be partly due to its antiproliferative effect on carcinogen-exposed epithelium as well as enhanced immune response (Tanaka, 1995a).

In another study, mice were given a chemical carcinogen in drinking water for 20 weeks and then water with 50 ppm astaxanthin was administered for 20 more weeks. At the end of the 41-week study, the chemically induced control mice had a 42% incidence of bladder carcinomas. Of these 11 tumors were transitional cell carcinomas and the remaining were squamous cell carcinomas with keratinization. However, the chemically induced mice that were post-treated with 50 ppm astaxanthin had a significantly lower rate of 18% bladder carcinomas. Of these only 1 was a transitional cell carcinoma and the remaining were squamous cell carcinomas. The researchers suggest that astaxanthin is a possible chemopreventive agent for bladder carcinogenesis and such an effect is partly due to antioxidant effects and suppression of cell proliferation (Tanaka, 1994).

Researchers have also given rats 20 ppm of a carcinogen in their drinking water for 8 weeks to induce oral cancer. The animals were fed diets with and without 100 ppm astaxanthin either during or after the induction phase. At the end of the 32-week study, the incidences of tongue squamous cell carcinomas was 54% in the non-treated control group, while both groups treated with astaxanthin had none. The control group had a 17% incidence of squamous cell papilloma whereas the astaxanthin groups had zero. The incidence of severe dysplasia (pre-cancer indicator) was 58% in the non-treated control group, 0% in those fed astaxanthin with the carcinogen, and only 5% in the group fed astaxanthin after induction with the carcinogen. The authors comment that the results clearly indicate that dietary administration of astaxanthin significantly depressed the development of the chemically-induced tongue neoplasms. This was seen when astaxanthin was fed either during the chemical insult or subsequently. The authors speculate that the inhibitory effects of astaxanthin may be partly due to its antiproliferative effect on epithelium cells and enhanced immune response. Additionally, increased expression of a major

gap junction gene called “connexin43” may explain the suppressing effects of astaxanthin on carcinogenesis (Tanaka, 1995b).

In another applied study, mice were fed benzopyrene to induce stomach tumorigenesis and subsequently fed various concentrations of astaxanthin-enriched egg yolks. Those fed the astaxanthin-rich egg yolks developed only one third as many neoplasms per animal and fewer incidences compared to the control when stomach tumorigenesis was initiated with chemicals. The authors conclude that astaxanthin-enriched egg yolks inhibited tumorigenesis of mouse forestomach induced by benzopyrene (Lee Sang, 1997).

Mice fed synthetic diets containing 0, 0.1, or 0.4% beta-carotene, canthaxanthin or astaxanthin were injected with tumor cells into the mammary fat pad after three weeks to determine the anticancer activities of the carotenoids. After 66 days of feeding, the concentration of astaxanthin in the plasma was 135-145 fold higher than that of beta-carotene and 4-6 fold higher than that of canthaxanthin at both doses. By the end of the study, all mice had tumors but they varied in size considerably. Final tumor weights of the unsupplemented mice (1.9 grams) were similar to those groups fed beta-carotene at the two dosages (1.8 and 2.1 grams). Mice fed canthaxanthin had tumor weights of 1.3 and 1.5 grams for the two diets, respectively. However, mice supplemented with astaxanthin had the lowest tumor weights of 1.2 and 1.0 grams for the two diets. Final tumor volume in mice fed astaxanthin was 50-60% smaller than that of the control mice. Dietary astaxanthin inhibited mammary tumor growth in a dose-dependent manner, whereas this effect was not observed with beta-carotene or canthaxanthin. TBARS activity was also significantly smaller in mice supplemented with 0.4% astaxanthin compared with the control indicating the lower lipid peroxidation activity in the mammary tumors (Chew, 1999).

Astaxanthin has been shown to reduce the carcinogenicity of aflatoxin by inducing enzymes called “CYP1A” and “CYP1A2” which enhance diversion of toxic byproducts towards detoxification pathways (Gradelet, 1997). At dietary levels of 300 ppm, astaxanthin is a strong inducer of CYP1A1 and CYP1A2. (Gradelet, 1996b). In contrast to lycopene or vitamin A, astaxanthin was very efficient in reducing the number and size of liver preneoplastic foci in aflatoxin-induced carcinogenesis (Gradelet, 1998).

Astaxanthin may exert antitumor activity through the enhancement of particular immune responses. Mice were fed 40 mcg/kg body wt/day starting zero, one and three weeks before subcutaneous injection with Meth-A tumor cells. It was subsequently found that the astaxanthin-fed mice had significantly lower tumor size and weight than controls when the supplementation was started one or three weeks prior to tumor inoculation but not at zero time. Furthermore, these astaxanthin groups with the greatest antitumor activity also had elevated cytotoxic T lymphocyte (CTL) activity and interferon-γ (IFN-γ) production. The authors note that their results indicate dietary astaxanthin at biological levels can suppress Meth-A tumor cell growth and stimulate immunity against these wayward cells during early tumor development (Jyonouchi et al., 2000).

Immune Support

Singlet oxygen is also cytotoxic to the immune system by virtue of its ability to catalyze production of free radicals. This action can facilitate degradation of macrophage cell membranes resulting in dysfunction and reduced efficiency of phagocytosis (Bendich, 1991). Carotenoids have been shown to enhance both the non-specific and specific immune system and protect cell membranes and cellular DNA from mutation (Bendich A. 1989). Carotenoids have a significant stimulatory effect on the immune system, as seen by the proliferative response of spleen cells and thymocytes during antibody response of mice. Astaxanthin enhances the release of interleukin-1 alpha and tumor necrosis factor alpha

in mice greater than canthaxanthin and beta-carotene. The conclusion of one study was that astaxanthin had the best cytokine-inducing activity and may provide an immunomodulating role (Okai, 1996).

A key regulator of the immune system is T-helper (Th cells) which are mainly activated by antigens or foreign bodies presented by APC (antigen presenting cells). IgM antibody is produced during the early response to a foreign microorganism or antigen and is restricted to the bloodstream. IgG is the most abundant type of circulating antibody and can transverse blood vessels. To understand the effects on these cells, astaxanthin was added at very low concentrations (10^{-8} mol/L) to unprimed spleen cells collected from mice such that various parameters could be studied. Subsequently, the Th1 and Th2 cells were stimulated with specific antigens for each clone and compared to the control. In Th1 cells, astaxanthin significantly enhanced the number of IgM antibody-secreting cells from about 130 to 350, per million cells. In Th2 cells, astaxanthin boosted production from 85 to 160, per million cells. Other carotenoids such as zeaxanthin, lutein, lycopene and canthaxanthin did not have the same effect. Using primed spleen cells, astaxanthin significantly enhanced the numbers of IgM antibody-secreting cells in Th1 cells from about 80 to 130 (per million cells) and from 40 to 110 in Th2. Astaxanthin was also the only carotenoid to significantly increase the number of IgG antibody-secreting cells in both Th clones, from about 120 to 220 and 80 to 180, respectively. The authors conclude that astaxanthin has a significant enhancing action on antibody production when cells are presented with antigens (Jyonouchi, 1995a, 1996).

Companion studies also show that astaxanthin at physiological levels (10^{-8} mol/L) enhanced T-dependent antigen specific humoral immune responses. Using a slightly different mouse system as the above model, astaxanthin significantly augmented the number of IgM antibody-secreting cells when unprimed B cells were incubated with Th1 or Th2 cells and stimulated with suboptimal doses of antigen. Astaxanthin also enhanced the number of IgG antibody-secreting cells under the same conditions. The importance of these studies is that at the initial stage of a pathogen invasion, doses of a particular antigen may be suboptimal to elicit an effective immune reaction, and astaxanthin appears to enhance the response (Jyonouchi, 1995b).

Another study indicates a significant immunomodulating action of astaxanthin for humoral immune responses to T-dependent antigens and the authors suggest that carotenoid supplementation may be beneficial in restoring humoral immune responses in older animals. Furthermore, it was speculated that dietary carotenoids could reduce the chance of developing autoimmunity and malignancies by enhancing T-helper functions and promoting specific antibody responses (Jyonouchi, 1994).

Autoimmune mutants of mice known as MRL/l develop generalized lymphadenopathy, proteinuria and a lupus-like syndrome characterized by production of various antibodies, hypergammaglobulinemia and glomerulonephritis. The MRL/l strain has a short life span and typically dies at age 6-8 months due to renal failure and lymphoid abnormality. A study was designed to determine whether carotenoids could suppress the development of proteinuria and lymphadenopathy in this strain of mice. The development of lymphadenopathy was delayed three weeks in mice fed a calorie-restricted diet (CR) of 10-11 kcal/day compared to those on full-fed diets of 16-18 kcal/day. Total cell numbers spleen and axillary lymph nodes were 3-fold lower in mice fed the CR diet and peritoneal exudative (PE) cells were reduced by about 18-fold. Interestingly, supplementation of dietary carotenoids also delayed the development of lymphadenopathy about 2 weeks compared to the full-fed group and decreased the total spleen and axillary lymph node cells. Astaxanthin exerted more prominent suppressive actions than did beta-carotene. Furthermore, full-fed MRL/l mice supplemented with carotenoids displayed much lower proteinuria. Dietary carotenoids were about 50% as effective as the CR diet in suppressing the development of proteinuria by the age of 10 weeks and astaxanthin elicited a more prominent effect than beta-carotene (Tomita et al., 1993).

Helicobacter pylori is a bacteria that colonizes the human gastric epithelium, causing type B gastritis, peptic ulcer disease and gastric cancer. In the United States, *Helicobacter* affects about 20% of persons below the age of 40 years, and 50% of those above the age of 60 years. The pathogenesis of the disease partially caused by the body's own immunological response to the bacteria. Upon gastric mucosa infection, the immune system reacts with T-helper-1 (Th1) cell-mediated response and the release of IFN-γ. This in turn activates phagocytic cells that contribute to mucosal inflammation and damage. A recent study demonstrated that mice infected with *H. pylori* and then treated with *Haematococcus* algae showed a significantly decreased bacterial load and inflammation in the stomach. Treatment of the infected mice with a *Haematococcus* algae extract reduced the mean bacterial load from 407 CFU to 101 CFU and mean gastric inflammation score from 1.85 to 1.25. This mediation is associated with a shift of the T-lymphocyte response from a predominant Th1-response dominated by IFN-γ (interferon-γ) to a Th1/Th2 combined response with IFN-γ and IL-4 (interleukin-4). These results suggest that the initial polarized Th1 response with IFN-γ is associated with the pathogenesis of these gastric diseases and that the secondary Th2 response with IL-4 elicited by *Haematococcus* algae is associated with control of *H. pylori* infection and gastric mucosal repair. Additionally, it is presumed that another mechanism of action is the neutralization of reactive free oxygen metabolites that may attenuate the inflammation.

Interestingly, this is the first report in the literature in which a substance can cause an immune shift from a damaging cytokine associated with infection to a cytokine providing protective functions. (Bennedsen et al., 1999). Since whole *Haematococcus* algae was used in these experiments, the immune shift effects cannot be definitively attributed to astaxanthin, though it is presumed to be the active agent. A second paper clearly demonstrates that astaxanthin-rich *Haematococcus* algae meal can drastically reduce *Helicobacter pylori* bacteria from the stomachs of infected mice resulting in significantly decreased gastric inflammation and lipid peroxidation. Furthermore, the *Haematococcus* algae meal had a direct antimicrobial effect against *Helicobacter pylori* when cultured in vitro (Wang X. et al., 2000)

Haematococcus Algae is Natures' Richest Source of Astaxanthin

Astaxanthin was first characterized and termed in 1938 from an extract of the lobster, *Homarus astacus*. The pigment in *Haematococcus* was called "haematochrom" until 1944 when Tisher correctly identified the principal carotenoid as astaxanthin. Astaxanthin is quite common in nature, especially in the marine environment and is probably best known for eliciting the pinkish-red hue to the flesh of salmon and trout, as well as shrimp, lobsters and crayfish. These animals obtain astaxanthin in their diet from zooplankton, insects or crustaceans that have accumulated astaxanthin from phytoplankton.

Although natural sources of astaxanthin are numerous, nearly all are found in very low concentrations. By far, the green algae *Haematococcus pluvialis* provides the most concentrated natural source of astaxanthin known, from 10,000-40,000 ppm (mg/kg) astaxanthin in addition to other important carotenoids such as beta-carotene, lutein and canthaxanthin. As a comparison, the flesh of wild Atlantic salmon on average contain 5 ppm of astaxanthin, Coho salmon about 14 ppm astaxanthin and sockeye salmon average 40 ppm (Turujman, 1997). Since astaxanthin from *Haematococcus* is typically provided at 1-mg dosages in dietary supplements, each gelcap has the same amount of astaxanthin as 200 grams of Atlantic salmon.

A relative of *Haematococcus* known as "snow algae" can occasionally be seen as the blood-red layer on icebanks and snowfields in springtime. *Haematococcus* occurs in nature worldwide, but is most often found in cooler pools of fresh water. In fact, cultures are often isolated from backyard birdbaths or rock quarries. Under these conditions, *Haematococcus* is motile and utilizes the available nitrate, phosphate, and other nutrients to grow and reproduce (Figure 2). However, when nutrients become

limiting or the pool begins to dry the alga form a protective cell wall and encyst. Massive amounts of astaxanthin are produced, and the cells undergo a dormant stage until the next influx of water and nutrients (Figure 3). Cells can remain viable in this encysted stage with its protective astaxanthin for decades. Red cysts are significantly more resistant to photoinhibition and oxygen radicals than green cells, suggesting significant protective roles for astaxanthin (Kobayashi et al., 1992a).

Figure 2- Green, motile stage of *Haematococcus* algae, when nutrients are abundantly available. The flagella "whips" can be seen which allow motility in search of the best environmental conditions (Photo, 400x magnification).

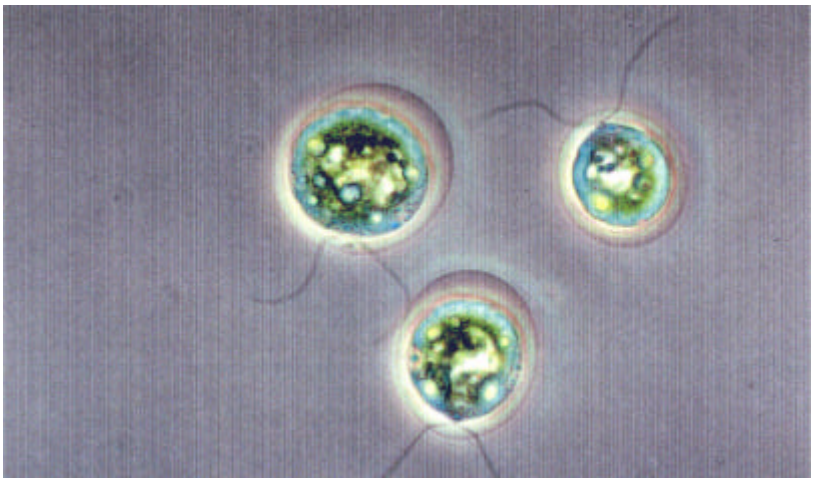
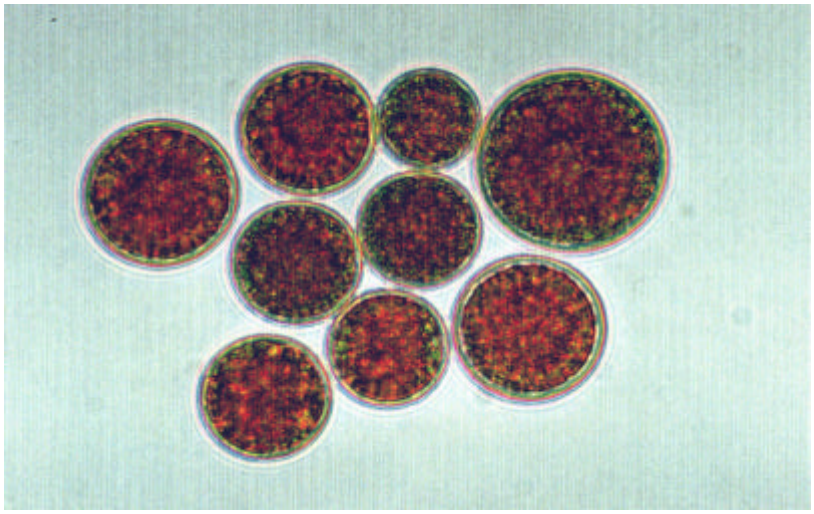


Figure 3-Red encysted stage of *Haematococcus* algae. When nutrients become limiting, cells produce massive amounts of astaxanthin to protect against UV and oxidative damage (Photo, 400X magnification).



Advanced technology has now been developed to grow natural strains of *Haematococcus* in closed culture systems and harnesses the unique properties of the algae to produce very high

concentrations of natural astaxanthin. All media ingredients for the cultivation of the algae are food grade or higher quality and the algae is pasteurized to prevent microbial contaminants. No solvents, pesticides, herbicides or toxic substances are used during cultivation or manufacturing of the product. Lots are standardized to contain from 1.5% (15,000 ppm) astaxanthin, predominately in the esterified form that provides the highest stability. Other beneficial carotenoids such as β-carotene, canthaxanthin, and lutein are also present in lesser amounts. Most importantly, the production process includes a technique which "cracks" greater than 95% of the cells to enable maximum bioavailability, resulting in a fine dark red powder.

Safety Studies of *Haematococcus* Algae Meal

Animal studies have proven the safety of consuming *Haematococcus* algae, it has never been associated with any toxicity in the reported literature or in field studies. *Haematococcus* algae has been reviewed by the US FDA and cleared for marketing as a new dietary ingredient by means of the DSHEA (21 CFR 190.6). It is also been approved in Japan for use in both foods and animal feeds. A different formulation of *Haematococcus* algae has already gained wide acceptance in the aquaculture markets as a pigmentation and vitamin source for salmon, trout, shrimp and ornamental fish and has been approved as a feed additive for salmonids by the Canadian Food Inspection Agency and US Food and Drug Administration. Similar registrations are in progress in the European Union.

A number of standard toxicity and safety studies have been conducted with *Haematococcus* algae. Acute oral toxicity studies conducted on Charles River CD rats with a dosage level of 5 grams of *Haematococcus* algae/kg for 13 days. Groups were evaluated for mortality, pharmacotoxic signs, body weights, and necropsy examinations during the 13-day study. The results demonstrated that the LD_{50} value of each lot was greater than the administered dose of 5 grams/kg. No visible abnormalities were observed, nor differences in body weights during the study. The postmortem examination did not reveal any abnormalities in the organs at the end of the study.

An oral toxicity study in rats was conducted in rats by administering 6 grams/kg of *Haematococcus* by oral route for 14 days. No treatment-related deaths occurred during the course of the study. Routine clinical and laboratory observations did not show any adverse changes in the test animals of either sex. The post-mortem examination showed no changes in organ weight or gross pathology. It was concluded that *Haematococcus* algae administered by oral route at the maximum dosage of 6 grams/kg/day was well tolerated and caused no adverse effects. An acute toxicity study was conducted in which rats were administered 12 grams/kg of *Haematococcus* algae. At the end of the 14-day observation period, there were no mortalities, adverse clinical signs or behavioral alterations noted in the animals. Body weight gain was unaffected by the treatment and a post-mortem pathology showed no appreciable macroscopic findings at the end of the 14 days. It was concluded that the LD_{50} value was higher than 12 grams/kg with no pathological changes.

Higher dosage studies of acute oral toxicity have been conducted with both male and female mice ranging from 10.4-18.0 grams *Haematococcus* algae per kg of body weight with no mortalities or abnormalities were observed at the end of the study. Mutagenicity tests under standard conditions are negative for *Haematococcus* algae. A published study with rats fed 400 ppm astaxanthin for 41 days showed no harmful effects on body/organ weight, enzyme activities, pregnancy, or litter size (Nishikawa et al., 1997).

A human bioavailability study showed that following a single oral administration of 100 milligrams of astaxanthin, an average peak level of about 1.2 milligrams appeared in the blood plasma after 6 hours and then slowly declined. The majority of the astaxanthin was associated with VLDL and LDL cholesterol carriers with a lesser amount in the HDL (Østerlie et al. 1999 a,b).

Conclusions

Haematococcus algae is a safe and natural form of astaxanthin that has been shown to have excellent antioxidant properties beyond other carotenoids. Positive outcomes in cancer deterrence, immune enhancement, carpal tunnel syndrome and macular degeneration studies are likely related to these superior antioxidant properties as well as yet unknown mechanisms.

References

Astorg P., S. Gradelet, R. Berges, and M. Suschetet. 1997. Dietary lycopene decreases the initiation of liver preneoplastic foci by diethylnitrosamine in the rat. Nutr. Cancer. 29(1):60-68.

Ames B.N. and M.K. Shigenaga. 1992. Oxidants are a major contributor to aging. Ann. N.Y. Acad. Sci. 663:85-96.

Ames B.N., M.K. Shigenaga and T.M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. 90(17):7915-7922.

Batieha et al. 1993. Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiology, Biomarkers and Prevention. 2:335-339.

Bennedsen, M., X. Wang, R. Willen, T. Wadstrom and L. P. Andersen. 1999. Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. Immunology Letters 70(3): 185-189.

Bendich A. 1989. Carotenoids and the immune response. J. Nutrition 119: 112-115.

Bendich A. 1990. Carotenoids and the immune system. In: Carotenoids Chemistry and Biology. Ed. N.I. Krinsky, Plenum Press, New York, pp. 323-335.

Bendich A. 1991. Non vitamin A activity of carotenoids: immunoenhancement. Trends in Food Science and Technology. May, pp. 127-129.

Bertram J.S., A. Pung, M. Churley, T.J. Kappock, L.R. Wilkens and R.V. Cooney. Diverse carotenoids protect from chemically-induced neoplastic transformation. Carcinogenesis (Lond.). 12:671-678

Bertram J.S. 1991. Cancer prevention by retinoids and carotenoids: proposed role of GAP junctional communication. In: Vitamins and Minerals in the Prevention and Treatment of Cancer. M.M. Jacobs, ed. CRC Press, Boca Raton, pp. 31-50.

Black H.S. 1998. Radical interception by carotenoids and effects on UV carcinogenesis. Nutrition and Cancer. 31(3):212-217.

Breimer L.H. 1990. Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage. Mol. Carcinog. 3:188-197.

Chien Y-H and S-C Jeng. 1992. Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levls and feeding regimes. Aquaculture. 102: 333-346.

Christiansen R., O. Lie, and O.J. Torrissen. 1995. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First-feeding fry. Aquaculture Nutrition. 1:189-198.

Chew B.P., J.S. Park, M.W. Wong and T.S. Wong. 1999. A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice *in vivo*. Anticancer Research. 19:1849-1854.

Cross et al. 1987. Oxygen radicals and human disease. Ann. Intern. Med. 107:526-545.

Dekkers J.C., L.J.P. van Doornen and H.C.G. Kemper. 1996. The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med. 21(3):213-238.

Di Mascio P., M.E. Murphy, and H. Sies. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 274: 532-538.

Di Mascio P.D., M. Murphy, and H. Sies. 1991. Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. Am. J. Clin. Nutr. 53:194S-200S.

Esterbauer H. J Gebicki, H. Puhl and G. Jurgens. 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Rad. Biol. Med. 13:341-390.

Gerster H. 1991. Antioxidant protection of the ageing macula. Age & Ageing 20:60-69.

Goldfarb A.H. 1999. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Can. J. Appl. Physiol. 24(3):249-266.

Gradelet S., P. Astorg, J. Leclerc, J. Chevalier, M.F. Vernevaut and M.H. Siess. 1996b. Effects of canthaxanthin, lycopene and lutein on liver xenobiotic-metabolizing enzymes in the rat. Xenobiotica. 26:49-63.

Gradelet S., P. Astorg, A.M. Le Bon, R. Berges, and M. Suschetet. 1997. Modulation of aflatoxin B1 carcinogenicity, genotoxicity and metabolism in rat liver by dietary carotenoids: evidence for a protective effect of CYP1A inducers. Cancer Lett. 114(1-2):221-223.

Gradelet S., A.M. Le Bon, R. Berges, M. Suschetet, and P. Astorg. 1998. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. Carcinogenesis 19(3):403-411.

Harman D. 1981. The aging process. Proc. Natl. Acad. Sci. 78:7124-7128.

Haila K.M., B.R. Nielsen, M.I. Heininen, and L.H. Skibsted. 1997. Carotenoid reaction with free radicals in acetone and toluene at different oxygen partial pressures. An ESR spin-trapping study of structure-activity relationships. Zeit. Fuer Leben-Unters. Forsch. 204(2):81-87.

Hughs D.A., A.J. Wright, P.M. Finglas et al. 1997. The effect of beta-carotene supplementation on the immune function of blood monocytes from healthy nonsmokers. J. Lab. Clin. Med. 129:309-317.

Ji L.L. 1995. Oxidative stress during exercise: implication of antioxidant nutrients. Free Rad. Biol. Med. 18:1079-1086.

Jorgensen K. and L. Skibsted. 1993. Carotenoid scavenging radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. Z. Lebensm. Unters Forsch. 196:423-429.

Jyonouchi H., L. Zhang, M. Gross, and Y. Tomita. 1994. Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. Nutrition and Cancer 21(1):47-58.

Jyonouchi H., S. Sun, and M.D. Gross. 1995a. Astaxanthin, a carotenoid without vitamin a activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen. J. Nutr. 125(10):2483-2492.

Jyonouchi H, S. Sun, and G. Myron. 1995b. Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: Astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. Nutr. Cancer. 23(2):171-183.

Jyonouchi H., S. Sun, M. Mizokami, and M.D. Gross. 1996. Effects of various carotenoids on cloned, effector-stage T-helper cell activity. Nutr. Cancer 26(3):313-324.

Jyonouchi H., S. Sun, K. Iijima and M. Gross. 2000. Antitumor activity of astaxanthin and its mode of action. Nutrition and Cancer. 36(1):59-65.

Kanter M. 1998. Free radicals, exercise and antioxidant supplementation. Proc. Nutr. Soc. 57:9-13.

King T.J., F. Khachik, H. Bortkiewicz, L. Fukushima, S. Morioka and J.S. Bertram. 1997. Metabolites of dietary carotenoids as potential cancer preventive agents. Pure and Appl. Chem. 69(10):2135-2140.

Kobayashi M. and T. Okada. 2000. Protective role of astaxanthin against u.v.-B irradiation in the green alga *Haematococcus pluvialis*. Biotechnology Letters 22:177-181.

Kobayashi. M. et al. 1992a. Effects of light intensity, light quality, and illumination cycle on astaxanthin formation in green alga, *Haematococcus pluvialis*. J. Ferm. Bioeng. 74(1): 61-63.

Kurashige M., Okimasu M. and K. Utsumi. 1990. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol. Chem. Phys. Med. NMR 22(1):27-38.

Lawlor S.M. and O' Brien N.M. 1995. Astaxanthin: Antioxidant effects in chicken embryo fibroblasts. Nutrition Research 15:1695-1740.

Lee Sang H, W. Park Cherl, S. Park Wong, C. Lee Young, S. Choi Eui, and L. Ha Yeong. 1997. Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by astaxanthin containing egg yolks. Agric. Chem. Biotechnol. 40(6):490-494.

Lim B.P., A. Nagao, J. Terao, K. Tanaka, T. Suzuki, and K. Takama. Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. Biochim. Biophys. Acta. 1126(2):178-184.

Marnett L.J. 1987. Peroxyl free radicals: potential mediators of tumor initiation and promotion. Carcinogenesis 8:1365-1373.

Miki W. 1991. Biological functions and activities of animal carotenoids. Pure and Applied Chem. 63(1):141-146.

Milon A., G. Wolff, and Y. Nakatani. 1986. Organic carotenoid-phospholipid bilayer systems. Incorporation of zeaxanthin, astaxanthin and their C50 homologous dimyristoylphosphatidylcholine vesicles. Helv. Chemica. 69:12-24.

Moody C.S. and H.M. Hassan. 1982. Mutagenicity of oxygen free radicals. Proc. Natl. Acad. Sci. 79:2855-2859.

Murillo E. 1992. Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats. Arch. Latinoam Nutr. 42(4):409-413.

Naguib Y. 2000. Antioxidant activities of astaxanthin and related carotenoids. J. Agric. Chem. 48:1150-1154.

Nakagawa K., S. Kang, D. Park, G. Handelman and T. Miyazawa. 1997. Inhibition by beta-carotene and astaxanthin of NADPH-dependent microsomal phospholipid peroxidation. J. Nutrit. Sci. and Vitamin. 43(3):345-355.

Niess A.M., H.H. Dickhuth, H. Northoff and E. Fehrenbach. 1999. Free radicals and oxidative stress in exercise-immulogical aspects. Exercise Immun. Rev. 5:22-56.

Nishigaki I., A. Dmitrovskii, W. Miki and K. Yagi. 1994. Suppressive effect of astaxanthin on lipid peroxidation induced rats. J. Clin. Biochem. Nutrit. 16(3):161-166.

Nishikawa Y., Y. Minenaka and M. Ichimura. 1997. Physiological and biochemical effects of carotenoid (beta-carotene and astaxanthin) on rat. Kashien Daigku Kiyo. 25:19-25.

O'Connor I. and N. O'Brien. 1998. Modulation of UVA light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts. J. Dermatol. Sci. 16(3):226-230.

Okai Y. and K. Higashi-Okai. 1996. Possible immunomodulating activities of carotenoids in in vitro cell culture experiments. Int. J. Immunopharmacol. 18(12):753-758.

Oshima S., F. Ojiima, H. Sakamoto, Y. Ishiguro, and J. Terao. 1993. Inhibitory effect of beta-carotene and astaxanthin on photosensitized oxidation of phospholipid bilayers. J. Nutrit. Sci. Vitaminol. 39(6):607-615.

Østerlie M. B., Bjerkeng and S. Liaaen-Jensen. 1999a. On bioavailability and deposition of bent Z-isomers of astaxanthin. *In* Mosquera, M.I.M., Galán, M.J. and Méndez, D.H. (Eds.): Pigments in Food Technology, Proceedings of the 1st International Congress on Pigments in Food Technology. Seville, Spain, March 24.-26, 1999, pp. 157-161.

Østerlie M. B., Bjerkeng and S. Liaaen-Jensen. 1999b. Blood appearance and distribution of astaxanthin E/Z isomers among plasma lipoproteins in humans administered in a single meal with astaxanthin. Abstract 2A-13. Presented at the 12th International Symposium on Carotenoids in Cairns, Queensland, Australia, July 18-23, 1999.

Palozza P. and N.I. Krinski. 1992. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys. 297(2):291-295.

Park J.S., B. P. Chew and T.S. Wong. 1997. FASEB 11:A447, #2586.

Poulsen H.E., S. Loft and K. Vistisen. 1996. Extreme exercise and oxidative DNA modification. J. Sports Sci. 14:343-346.

Ranby B. and J.F. Rabek, editors. 1978. Singlet Oxygen. Wiley, Chichester, England.

Rao et al. 1999. Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case-control study. Nutr. Cancer. 33(2):159-164.

Rumi et al. 1999. Decrease in serum levels of vitamin A and zeaxanthin in patients with colorectal polyp. Eur. J. Gastroenterol. Hepatol. 11(3):305-308.

Savoure N., G. Briand, M.C. Amory-Touz, A. Combre, M Maudet and M. Nichol. 1995. Vitamin A status and metabolism of cutaneous polyamines in the hairless mouse after UV irradiation: action of beta-carotene and astaxanthin. Int. J. Vitam. Nutr. Res. 65(2):79-86.

Seddon J.M. et al.. 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 272(18): 1413-1420.

Sen C.K. 1995. Oxidants and antioxidants in exercise. J. Appl. Physiol. 79(3):675-686.

Shimidzu N., M. Goto, and W. Miki. 1996. Carotenoids as singlet oxygen quenchers in marine organisms. Fisheries Science. 62(1): 134-137.

Snodderly D.M. 1995. Evidence for portection against age-related macular degeneration by carotenoids and antioxidant vitamins. Amer. J. Clinical Nutr. 62(S):1448S-1461S.

Stahl W., U. Heinrich, H. Jungmann, H. Sies and H. Tronnier. 2000. Carotenoids and carotenoids plus vitamin E protect against iltraviolet light-induced erythema in humans. A. J. Clin. Nutr. 71:795-798.

Steinberg G.S. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Eng. J. Med. 320:915-924.

Tanaka T. Y. Morishita, M. Suzui, T. Kojima, A. Okumura, and H. Mori. 1994. Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoids astaxanthin. Carcinogenesis. 15(1):15-19.

Tanaka T., T. Kawamori, M. Ohnishi, H. Makita, H. Mori, K. Satoh, and A. Hara. 1995a. Suppression of azomethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase. Carcinogenesis 16(12):2957-2963.

Tanaka T., H. Makita, M. Ohnishi, H. Mori, K. Satoh, and A. Hara. 1995b. Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. Cancer Res. 55(18):4059-4064.

Terao J. 1989. Antioxidant activity of beta-carotene related carotenoids in solution. Lipids. 24:659-662.

Tinkler, J.H., F. Bohm, W. Schalch, and T.G. Truscott. 1994. Dietary carotenoids protect human cells from damage. J. Photochem. Photobiol B. 26(3):283-285.

Tomita Y., K. Himeno, K. Nomoto, and H. Endo. 1987. Augmentation of tumor immunity against synergistic tumors in mice by beta-carotene. J. Natl. Cancer Inst. 78: 679-681.

Tomita Y., H. Jyonouchi, R.W. Engelman, N.K. Day and R. A. Good. 1993. Preventive action of carotenoids on the development of lymphadenopathy and proteinuria in MRL-lpr/lpr mice. Autoimmunity. 16:95-102.

Turujman S.A., W.G. Wamer, R.R. Wei and R.H. Albert. 1997. Journal of AOAC International. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. 80(3):622-632.

Wang X., R. Willen and T. Wadstrom. 2000. Astaxanthin-rich algal meal and Vitamin C inhibit *Helicobacter pylori* infection in BALB/cA mice. Antimicrob. Agents and Chemother. 44(9):2452-2457.

Witt E.H., A.Z. Reznick, C.A. Viguie, P. Starke-Reed and L. Packer. 1992. Exercise, oxidative damage and effects of antioxidant manipulation. J. Nutr. 122:766-773.

Woodall A., G. Britton and M. Jackson. 1997. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: Relationship between carotenoid structure and protective ability. Biochim. Biophys. Acta. 1336(3):575-586.

Zhang L.X., R.V. Cooney, and J.S.Bertram. 1991. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis (Lond.). 12:2109-2114

Zhang L.X., R.V. Coonery, and J.S. Bartram. 1992. Carotenoids up-regulate Connexin-43 gene expression independent of their provitamin A or antioxidant properties. Cancer Res. 52: 5707-5712.

BioAstin™ Technical Bulletin #062 Revision Date: October 2, 2000

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