**Chlorophyllin**

**Chlorophyll**

Chlorophyll is the green pigment which is responsible for the green colour in most plants. Chlorophyll absorbs most in the red and blue portions of the electromagnetic spectrum, thus its intense green color. Chlorophyll is capable of channeling the energy of sunlight into chemical energy through the process of photosynthesis. In photosynthesis, the energy absorbed by chlorophyll transforms carbon dioxide and water into carbohydrates and oxygen. Chlorophyll is a large molecule composed mostly of carbon and hydrogen. At the center of the molecule is a single atom of magnesium surrounded by a nitrogen-containing group of atoms called a porphyrin ring.

The molecular structure of the chlorophylls is similar to that of the heme portion of hemoglobin. Chlorophyll absorbs light in the red and blue-violet portions of the visible spectrum; the green portion is not absorbed and, reflected, gives chlorophyll its characteristic color.

There are several kinds of chlorophyll, the most important being chlorophyll which makes up about 75 percent of the chlorophyll in all green plants. It is also found in cyanobacteria (formerly known as blue-green algae) and in more complex photosynthetic cells. Chlorophyll A is the pigment that participates directly in the light requiring reactions of photosynthesis. Chlorophyll B occurs only in “green algae” and in the plants. Higher plants and green algae, such as chlorella contain chlorophyll A and chlorophyll B in the approximate ratio of 3:1. The difference between the two chlorophylls is that a methyl side-chain in chlorophyll A is replaced by a formyl group in chlorophyll B. Chlorophyll B is an accessory pigment present in plants and other complex photosynthetic cells. It absorbs light energy of a different wavelength and transfers it to chlorophyll A for ultimate conversion to chemical energy. It is an accessory pigment and acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll A.
Photosynthesis

Photosynthesis is a complex process used by many plants and bacteria to build carbohydrates from carbon dioxide and water, using energy derived from light. Photosynthesis is the process of converting light energy to chemical energy and storing it in the bonds of sugar. Photosynthesis is the key initial step in the growth of biomass. In the process oxygen and water are released. Increased levels of carbon dioxide can increase net photosynthesis in some plants. Plants create a very important reservoir for carbon dioxide. From energy produced by sunlight, the leaves combine carbon dioxide from the air and water from the soil to produce carbohydrates. Oxygen is released in the process. Carbohydrates plus fats and proteins are the plant foods necessary for growth and respiration of the tree.

Plants are the only photosynthetic organisms to have leaves (and not all plants have leaves). A leaf may be viewed as a solar collector crammed full of photosynthetic cells. Plants capture light using the pigment chlorophyll, which gives them their green color. This is contained in organelles (compartments within the cells) called chloroplasts. Chlorophyll or closely-related pigments (substances that color the plant) are essential to the photosynthetic process. The cells in the interior tissues of a leaf, called the mesophyll, contain about half a million chloroplasts for every square millimetre of leaf. The surface of the leaf is uniformly coated with a water-resistant, waxy cuticle that protects the leaf from excessive absorption of light and evaporation of water. The transparent, colourless epidermis layer allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

The chlorophyll molecule is the site of “photosynthesis”, the process by which the energy of the sun is converted firstly into the energy of sugars and other carbohydrates. These in turn supply virtually all of the energy needed by the plant and animal world. Chlorophyll is essential in the transformation of light energy to chemical energy in photosynthesis. Plants use chlorophyll to convert the energy of sunlight to food in the process known as photosynthesis.
Chlorophyll health benefits

Chlorophyll has anti-inflammatory, antioxidant, and wound-healing properties. Chlorophyll and chlorophyllin are able to form tight molecular complexes with certain chemicals known or suspected to cause cancer, including polyaromatic hydrocarbons found in tobacco smoke. Chlorophyll is a good source of antioxidant nutrients. Antioxidant nutrients such as vitamins A, C and E help to neutralize harmful molecules (free radicals) in the body that can cause damage to healthy cells. Chlorophyll is an efficient deliverer of magnesium and helps the blood carry oxygen to the cells and tissue. Chlorophyll assists in the chelation of heavy metals. Chlorophyll has been studied for its potential in stimulating tissue growth and in stimulating red blood cells in connection with oxygen supply. Chlorophyll also removes carbon dioxide and carbon monoxide, and has been found to reduce fecal, urinary, and body odor. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs. Chlorophyll may be beneficial in the treatment of calcium oxalate stone disease and that they may have some anti-atherogenic activity. It also breaks down calcium-oxalate stones, which are created by the body to neutralize acid, for elimination. Calcium oxalate stones are better known as kidney stones. Chlorophyll and chlorophyllin may have some antimutagenic and anticarcinogenic potential, may help protect against some toxins, and may ameliorate some drug side effects. Chlorophyll has been used traditionally to improve bad breath, as well as to reduce the odors of urine, feces, and infected wounds.

Metabolism and Bioavailability

Little is known about the bioavailability and metabolism of chlorophyll or chlorophyllin. The lack of toxicity attributed to chlorophyllin led to the belief that it was poorly absorbed. However, significant amounts of copper chlorine was measured in the plasma of humans taking chlorophyllin tablets in a controlled clinical trial, indicating that it is absorbed. More
research is needed to understand the bioavailability and metabolism of natural chlorophylls and chlorin compounds in synthetic chlorophyllin.

**Biological Activities**

**Complex Formation with Other Molecules**

Chlorophyll and chlorophyllin are able to form tight molecular complexes with certain chemicals known or suspected to cause cancer, including polyaromatic hydrocarbons found in tobacco smoke, some heterocyclic amines found in cooked meat, and aflatoxin-B1. The tight binding of chlorophyll or chlorophyllin to these potential carcinogens may interfere with their absorption from the gastrointestinal tract and reduce the amount that reaches susceptible tissues.

**Antioxidant Effects**

Chlorophyllin can neutralize several physically relevant oxidants in vitro, and limited data from animal studies suggest that chlorophyllin supplementation may decrease oxidative damage induced by chemical carcinogens and radiation.

**Modification of the Metabolism and Detoxification of Carcinogens**

In order to initiate the development of cancer, some chemicals (procarcinogens) must first be metabolized to active carcinogens that are capable of damaging DNA or other critical molecules in susceptible tissues. Since enzymes in the cytochrome P450 family are required for the activation of some procarcinogens, inhibition of cytochrome P450 enzymes may decrease the risk of some types of chemically induced cancers. Studies in vitro indicate that chlorophyllin may decrease the activity of cytochrome P450 enzymes. Phase II biotransformation enzymes promote the elimination of potentially harmful toxins and carcinogens from the body. Limited data from animal studies indicate that chlorophyllin may increase the activity of the phase II enzyme quinone reductase (14).

**Disease Prevention**

**Aflatoxin-Associated Liver Cancer**
Aflatoxin-B1 (AFB1) is a liver carcinogen produced by certain species of fungus and found in moldy grains and legumes, such as corn, peanuts and soybeans. In hot, humid regions of Africa and Asia with inadequate grain storage facilities, high levels of dietary AFB1 are associated with increased risk of hepatocellular carcinoma. Moreover, the combination of hepatitis B infection and high dietary AFB1 exposure increases the risk of hepatocellular carcinoma still further. In the liver, AFB1 is metabolized to a carcinogen capable of binding DNA and causing mutations. In animal models of AFB1-induced liver cancer, administration of chlorophyllin at the same time as dietary AFB1 exposure significantly reduced AFB1-induced DNA damage in the livers of rainbow trout and rats, and dose-dependently inhibited the development of liver cancer in trout.

Because of the long time period between AFB1 exposure and the development of cancer in humans, an intervention trial might require as long as twenty years to determine whether chlorophyllin supplementation can reduce the incidence of hepatocellular carcinoma in people exposed to high levels of dietary AFB1. However, a biomarker of AFB1-induced DNA damage (AFB1-N7-guanine) can be measured in the urine, and high urinary levels of AFB1-N7-guanine have been associated with significantly increased risk of developing hepatocellular carcinoma. In order to determine whether chlorophyllin could decrease AFB1-induced DNA damage in humans, a randomized placebo-controlled intervention trial was conducted in 180 adults residing in a region in China where the risk of hepatocellular carcinoma is very high due to unavoidable dietary AFB1 exposure and a high prevalence of chronic hepatitis B infection. Participants took either 100 mg of chlorophyllin or a placebo before meals three times daily. After 16 weeks of treatment, urinary levels of AFB1-N7-guanine were 55% lower in those taking chlorophyllin than in those taking the placebo, suggesting that chlorophyllin supplementation before meals can substantially decrease AFB1-induced DNA damage. Although a reduction in hepatocellular carcinoma has not yet been demonstrated in humans taking chlorophyllin, scientists are hopeful that chlorophyllin supplementation will provide some protection to high-risk populations with unavoidable dietary AFB1 exposure.
It is not known whether chlorophyllin will be useful in the prevention of cancers in people who are not exposed to significant levels of dietary AFB1, as is the case for most people living in the U.S. Many questions remain to be answered regarding the exact mechanisms of cancer prevention by chlorophyllin, the implications for the prevention of other types of cancer, and the potential for natural chlorophylls in the diet to provide cancer protection. Scientists from the Linus Pauling Institute’s Cancer Chemoprotection Program have been awarded a grant from the National Cancer Institute to study these questions and others aimed at identifying dietary phytochemicals effective in reducing the risk of lung, stomach, and breast cancers induced by polyaromatic hydrocarbons, colorectal cancer induced by heterocyclic amines, as well as liver cancer induced by AFB1.

**Therapeutic Uses of Chlorophyllin**

**Internal Deodorant**

Observations in the 1940s and 1950s that topical chlorophyllin had deodorizing effects on foul-smelling wounds led clinicians to administer chlorophyllin orally to patients with colostomies and ileostomies in order to control fecal odor. While early case reports indicated that chlorophyllin doses of 100-200 mg/d were effective in reducing fecal odor in ostomy patients, at least one placebo-controlled trial found that 75 mg of oral chlorophyllin 3 times/d was no more effective than placebo in decreasing fecal odor assessed by colostomy patients. Several case reports have been published indicating that oral chlorophyllin (100-300 mg/d) decreased subjective assessments of urinary and fecal odor in incontinent patients. Trimethylaminuria is a hereditary disorder characterized by the excretion of trimethylamine, a compound with a “fishy” or foul odor. A recent study in a small number of Japanese patients with trimethylaminuria found that oral chlorophyllin (60 mg 3 times/d) for 3 weeks significantly decreased urinary trimethylamine concentrations.

**Wound Healing**

Research in the 1940s indicating that chlorophyllin solutions slowed the growth of certain anaerobic bacteria in the test tube and accelerated the
healing of experimental wounds in animals led to the use of topical chlorophyllin solutions and ointments in the treatment of persistent open wounds in humans. During the late 1940s and 1950s, a series of largely uncontrolled studies in patients with slow-healing wounds, such as vascular ulcers and pressure (decubitus) ulcers, reported that the application of topical chlorophyllin promoted healing more effectively than other commonly used treatments. In the late 1950s, chlorophyllin was added to papain and urea-containing ointments used for the chemical debridement of wounds in order to reduce local inflammation, promote healing and control odor. Chlorophyllin-containing papain/urea ointments are still available in the US by prescription.

**Sources**

**Chlorophylls**

Chlorophylls are the most abundant pigments in plants. Dark green leafy vegetables like spinach are rich sources of natural chlorophylls. The chlorophyll contents of selected vegetables are presented in the table below.

<table>
<thead>
<tr>
<th>Food</th>
<th>Serving</th>
<th>Chlorophyll (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>1 cup</td>
<td>23.7</td>
</tr>
<tr>
<td>Parsley</td>
<td>½ cup</td>
<td>19.0</td>
</tr>
<tr>
<td>Cress, garden</td>
<td>1 cup</td>
<td>15.6</td>
</tr>
<tr>
<td>Green beans</td>
<td>1 cup</td>
<td>8.3</td>
</tr>
<tr>
<td>Arugula (rocket)</td>
<td>1 cup</td>
<td>8.2</td>
</tr>
<tr>
<td>Leeks</td>
<td>1 cup</td>
<td>7.7</td>
</tr>
<tr>
<td>Endive</td>
<td>1 cup</td>
<td>5.2</td>
</tr>
<tr>
<td>Sugar peas</td>
<td>1 cup</td>
<td>4.8</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>1 cup</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Chlorophyllin is a semi-synthetic mixture of water-soluble sodium copper salts derived from chlorophyll. Chlorophyllin is water-soluble.

Chlorophyllin, the water-soluble analogue of the ubiquitous green pigment chlorophyll has been shown to be a potent antioxidant and radio protector under both in vitro and in vivo conditions. It is a potent protector of membrane damage and prevents protein oxidation, lipid peroxidation besides depletion of thiols induced by radiation, photosensitization and other oxidants. To analyze the possible mechanisms involved, we have studied the reaction of chlorophyllin with biologically relevant radical species in the form of linoleic acid peroxyl radical (LOO'), chloroperoxyl radical (CCl3O2'), azide radical (N3') and thiol radical (RS')). The summary of results obtained are given below: Pulse radiolysis studies show that these radicals (CCl3O2', N3', RS') react with chlorophyllin in a similar way producing transient absorption bands at 370 nm and in the region of 430-450 nm with bleaching at 410 nm. The one electron oxidized species was long lived without any decay upto 1 millisecond. The high rate constant values reveal that chlorophyllin is able to scavenge these radicals effectively. However, the reaction of LOO radical with chlorophyllin was slow and the yield of one-electron oxidized species of chlorophyllin was also small. This indicates that inhibition of lipid peroxidation observed with chlorophyllin may involve other mechanisms.

Introduction

In recent years there has been an increased interest in areas related to newer developments in the prevention of disease especially those involving natural compounds with antioxidant activities. These antioxidants neutralize free radicals or their activities. Oxidative stress involving the enhanced generation of reactive oxygen species (ROS) including free radicals and other related reactive species has been implicated in a variety of toxicities and ailments. ROS have been implicated as possible causative factors for more than 100 diseases. These include cardiovascular ailments, neural disorders, diabetes, various forms of cancer, hepatitis, urolithiasis, tissue injury and immune disorders. Hence compounds with potent antioxidant activity can help in the prevention/cure of diseases as well as in toxicities. Such compounds, especially derived from natural sources and
capable of minimizing oxidative damage in situ, have potential uses to control human diseases. These compounds, capable of interacting with DNA, cellular membranes and other biomolecules have potential protective properties against deleterious free radicals.

Chlorophyllin is a water-soluble analogue of chlorophyll, the ubiquitous photosynthetic green pigment present in food materials of plant origin as well as in nutritional supplements such as extracts from Spirulina and Chlorella vulgaris. Chlorophyll has been credited with several beneficial properties. Chlorophyllin (CHL) has proved to be better than the parent compound as evident from studies employing model systems. It possesses highly potent antioxidant activity. It has been used, without apparent toxic side effects, to treat a number of human ailments. As a food pigment it can be widely used in cakes, beverages, sweets, ice creams etc. On a daily basis, it is used in green toothpaste and cosmetics. It has been found to exhibit anticarcinogenic and antimutagenic properties. Our earlier studies have shown chlorophyllin to be a potent antioxidant against oxidative stress induced by radiation, photosensitization and peroxynitrite as measured by inhibition of lipid peroxidation, protein oxidation, DNA damage and restoration of endogenous antioxidants.

Though its scavenging ability of primary radicals such as hydroxyl radical, superoxide and singlet oxygen has been established, its ability to react with biologically important secondary radicals such as peroxyl radicals and thyl radicals has not been examined. The present investigation is towards this objective. The secondary radicals studied in the present investigation are lipid peroxyl radicals (LOO.), chloroperoxyl radical (CCl3O.) and thiol radicals (RS.). Peroxyl radicals are able to abstract hydrogen atom from another lipid molecule (adjacent fatty acid), especially in the presence of metals such as copper and iron, thus causing an autolytic chain reaction. LOO- can combine with H to give LOOH and this reaction characterizes the propagation stage of lipid peroxidation. Chloroperoxyl radical has been used as a representative peroxyl radical and also for its inherent simplicity in performing experiments. The action of cytochrome P450 system on toxicants like CCl4 generates trichloromethyl radical which is able to react with oxygen to give chloroperoxyl radical. Thiols are present in living
systems which in addition to participating in cellular redox processes also take part in scavenging free radicals. This reaction is known as an antioxidant reaction. The thiol radicals thus generated in living systems are considered to be reactive oxidants as they are able to initiate lipid peroxidation by abstracting bisallylic hydrogen from poly-unsaturated fatty acids. The thiol radicals can also add to the double bond leading to efficient cis/trans isomerization of mono and polyunsaturated fatty acids.

**Materials and Methods**

The pulse radiolysis system using 7 MeV electrons has been described earlier. The dosimetry was carried out using an air-saturated aqueous solution containing 5 x 10^-2 mol dm^-3 KSCN (G = 23,889 dm3 mol^-1 cm^-1 per 100 eV at 500 nm). The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The optical path length of the cell was 1.0 cm. The width of the electron pulse was 50 ns. Time-dependent absorbance differences were recorded on a digital oscilloscope. High purity (> 99.9 %) N2O, from BOC India Pvt. Ltd. was used.

Lipid peroxyl radical was generated by pulse radiolysis carried out in aerated aqueous solution containing 2 x 10^-2 mol dm^-3 linoleic acid.

\[ LH + \cdot OH \rightarrow L^- + H_2O \]

Thiol radicals were generated on pulse radiolysis of cysteine (10^-3 M) in N2O saturated solution containing chlorophyllin (6.5 x 10^-5 M).

\[ RSH + \cdot OH \rightarrow RS^- + H_2/OH \]

**Azide radicals were generated on pulse radiolysis of N2O-saturated aqueous solution of NaN3 (0.1 M).**

\[ N_3^- + OH \rightarrow Nb"^- + OH^- \]

The bimolecular rate constants were calculated by plotting pseudo-first order rate of formation of the transient against the concerned solute concentration. The uncertainty in the measurement in bimolecular rate constant is <10%. The transients obtained in the pulse radiolysis study were used to characterize the product radical. The rate constants determined and presented in the text are not mere radiation chemical
parameters, but they reflect on the efficiency of scavenging free radicals and the ease with which competing reactions occur.

**Results and Discussion**

Figure 1 show the transient optical absorption spectrum obtained on reaction of N3. with chlorophyllin, One-electron oxidized species is observed to have transient absorption bands at 320, 370 and in the region of 430-450 nm with bleaching at 410 nm. The one-electron oxidized species is observed to be stable at least upto 1ms and recovery of bleaching is also not observed in this time scale.

Fig. 1

![Figure 1](image1)

Fig. 2

![Figure 2](image2)
Chloroperoxyl radicals are also observed to react with chlorophyllin with a bimolecular rate constant of 7x10^8 M^{-1}S^{-1} and the transient absorption spectra was similar to that obtained on reaction with azide and thiol radicals. Azide radicals are observed to react with chlorophyllin with a bimolecular rate constant of 1x10^9 M^{-1}S^{-1} as determined from the growth of the transient band at 360 nm and bleaching of ground state absorption at 400 nm.

Thiol radicals generated on pulse radiolysis of cysteine (10-3 M) in N2O saturated solution of chlorophyllin (6.5 X 10^{-5} M) showed the formation of a transient band ~370 nm with bleaching at 410 nm with a bimolecular rate constant of ~ 2 X 10^8 m^{-1} s^{-1}. The spectra is similar to that obtained on reaction with azide radicals, but only the rate constant value was lower.

Table 1: Reaction rate constants of various radicals studied with chlorophyllin determined using pulse radiolysis

<table>
<thead>
<tr>
<th>Reaction of CHL with</th>
<th>Rate constant (dm3 mol^{-1} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3-</td>
<td>1 x 109</td>
</tr>
<tr>
<td>CCl3O2-</td>
<td>7 x 108</td>
</tr>
<tr>
<td>RS-</td>
<td>2 x 108</td>
</tr>
</tbody>
</table>

Pulse radiolysis studies reveal that chloroperoxyl, azide and thiol radicals react with chlorophyllin in similar way. The high rate constant values reveal that chlorophyllin is able to scavenge chloroperoxyl, azide and thiol radicals effectively.

However the reaction of LOO- radical with chlorophyllin was slow and the yield of one-electron oxidized species of chlorophyllin was also small. Our earlier studies have indicated chlorophyllin to be a potent protector of lipids and proteins in rat liver mitochondria (Table 2).

Table 2 : Protective effect of chlorophyllin against radiation-induced lipid peroxidation and inactivation of superoxide dismutase as compared to established antioxidants at equimolar concentration of 50 ^iM
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid peroxidation (nmoles LOOH/mg protein)</th>
<th>Superoxide dismutase (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.38 ± 0.17</td>
<td>6.37 ± 0.10</td>
</tr>
<tr>
<td>Rad</td>
<td>43.55 ± 1.54</td>
<td>1.50 ± 0.17</td>
</tr>
<tr>
<td>Rad+CHL</td>
<td>20.15 ± 3.71</td>
<td>5.25 ± 0.14</td>
</tr>
<tr>
<td>Rad+Asc</td>
<td>40.21 ± 1.47</td>
<td>3.00 ± 0.28</td>
</tr>
<tr>
<td>Rad+GSH</td>
<td>25.50 ± 1.83</td>
<td>4.80 ± 0.10</td>
</tr>
<tr>
<td>Rad+Mann</td>
<td>35.66 ± 2.01</td>
<td>2.62 ± 0.21</td>
</tr>
<tr>
<td>Rad+t-BuOH</td>
<td>34.39 ± 1.25</td>
<td>2.25 ± 0.43</td>
</tr>
</tbody>
</table>

This indicates that inhibition of lipid peroxidation observed with chlorophyllin may involve other mechanism apart from radical scavenging.

**CHLOROPHYLLIN and LIVER CANCER**

Study Shows Chlorophyllin Intervention Could Prevent Some Environmental Cancers.

*November 27, 2001 edition of Proceedings of the National Academy of Sciences*

Chlorophyllin is a derivative of chlorophyll and is used as an over-the-counter diet supplement and as a food colorant. Taking chlorophyllin greatly reduces the levels of aflatoxin-DNA damage byproducts in the
body, which are indicators of exposure to carcinogenic aflatoxins and increased risk of liver cancer.

“Our study shows that taking chlorophyllin three times a day reduced the amounts of aflatoxin-DNA damage by 55 percent, compared with taking a placebo,” says Thomas Kensler, PhD, professor of environmental health sciences at the Johns Hopkins Bloomberg School of Public Health. “Taking chlorophyllin or eating green vegetables, like spinach, that are rich in chlorophyll may be a practical way of reducing the risk of liver cancer and other cancers caused by environmental triggers,” explains Dr. Kensler.

Dr. Kensler and his colleagues conducted a double-blind study among residents of Qidong, China. The people of the region have an extraordinarily high rate of liver cancer, which is due in part from routinely eating foods contaminated with carcinogenic aflatoxins. The aflatoxin is produced by molds found in foods like corn, peanuts, soy sauce, and fermented soybeans. For the study, researchers recruited 180 healthy adults. Half of the group was given 100 mg tablets of chlorophyllin to take three times a day with meals for four months. The other half was given a placebo. Urine and blood samples were taken over four months to determine the effects of chlorophyllin on excretion of aflatoxin-DNA damage products.

According to the study’s results, the people who took chlorophyllin showed a 55 percent reduction in aflatoxin-DNA damage, compared to the placebo group.

“Studies conducted by our co-author, George Bailey of Oregon State University, have suggested that chlorophyllin acts as an ‘interceptor molecule’ to block the absorption of aflatoxins and carcinogens in the diet,” explains John Groopman, PhD, professor and chairman of the Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health. “Our study shows that chlorophyllin can effectively reduce aflatoxin levels, which should reduce the risk of liver cancer. Since chlorophyllin is found in many foods or can be easily added to the diet, it could be a safe and effective prevention method. The study
adds to the evidence that green vegetables contain effective anti-
carcinogens,” adds Dr. Groopman.
Follow up studies are planned to determine whether this early protective
action of chlorophyllin extends to either delay the onset or reduce the
incidence of liver cancer.

Chlorophyllin and Cancer Prevention

Preventing cancer is a lot easier than trying to stop it once it occurs. Research shows that there are several different ways of preventing the disease, which occurs in stages that take years to unfold. The first stage involves damage to DNA. Cancer cells are essentially the body’s own cells. However, they contain a corrupted version of normal DNA. DNA damage is caused by a loss of methylation patterns, and from attacks on the structure itself. Corrupted DNA gets replicated over and over as new cells are made. That is why it is critically important to prevent the damage from occurring in the first place.

The human body is constantly bombarded with damaging free radicals, not only from the environment, but from the body itself. The immune system, for example, creates free radicals to help kill pathogens. Mechanisms have evolved to counteract the damage and protect DNA. Antioxidants are the main defense. They are found both in food, and the body makes its own.
Because they protect DNA, antioxidants are an important defense against cancer. They block free radicals that can damage DNA. The degree to which they are able to neutralize free radicals can be detected in a laboratory, and many compounds have been tested for their ability to stop various types of free radicals. However, not all antioxidants are good at stopping all types of free radicals.

Researchers from the Johns Hopkins School of Public Health are in the process of doing a study to see if chlorophyllin supplements will make a difference. So far they have determined that if chlorophyllin is taken three times a day with meals, it reduces aflatoxin carcinogens in the body by 55%. They estimate that this simple, cost-effective approach will delay the development of liver cancer.

The Johns Hopkins study relates to aflatoxin, but chlorophyllin prevents liver cancer caused by other things as well. It has been proven in rodents that chlorophyllin also prevents liver cancer caused by a nitrosamine found in beer and hard liquor. Nitrosamines are extremely potent carcinogens that also turn up in “lunch meat,” hotdogs and the like.

**An anti-cancer supplement with power**

Chlorophyllin is a derivative of chlorophyll, the natural green pigment in plants. It is obtained from natural chlorophyll through a hydrolysis process that converts fat-soluble chlorophyllin into a water-soluble dye that can be used commercially, for instance, as a food coloring agent and deodorant. There is a long history of chlorophyllin use in the health care industry.

Natural chlorophyll is very unstable, and it deteriorates very quickly after isolation and purification. With the chlorophyllin derivative, through hydrolysis and replacement of magnesium—the metal ion that’s naturally found in chlorophyll—with copper, you get a very stable compound that does not oxidize or degrade as quickly as chlorophyll. You might think that replacing magnesium with copper would make the compounds more reactive, but the copper is very tightly bound, so it is not available to engage in detrimental reactions.
One of the most potent antioxidants that have been discovered is a form of chlorophyll known as chlorophyllin. Chlorophyllin is a semi-synthetic form of the natural chlorophyll that makes plants green. It has better effects than chlorophyll, and has been extensively tested for many years. Chlorophyllin, it turns out, is very good at blocking two types of free radicals that occur in food: heterocyclic amines and aflatoxin. Aflatoxin is a toxin from a fungus that grows on grains such as corn and rice. It is highly carcinogenic, and causes liver cancer. It cannot be seen on food, but if present, can harm a person without them knowing it.

Heterocyclic amines are chemicals created when food is cooked, particularly at high temperature. According to the Cooked Food Mutagen Reference List and a report in Food Chemistry and Toxicology, food exposes the human body to as many carcinogens as cigarette smoke. Grilled meat, fried potatoes, broiled fish-these foods contain heterocyclic amines. Ironically, when the body metabolizes these by-products in an attempt to get rid of them, it converts them into carcinogens.

One way heterocyclic amines and aflatoxin cause cancer is by generating DNA-damaging free radicals. Vitamin C is an antioxidant most people are familiar with. While it is very good for many things, vitamin C does not have what it takes to stop this kind of damage. Chlorophyllin and I3C (a phyto-chemical from cruciferous vegetables) have been proven the most powerful against aflatoxin and heterocyclic amines. It appears that they work in different ways, and may also work in a complimentary way. They are very powerful-more powerful, even, than the body’s own antioxidant, glutathione.

Chlorophyllin also goes after free radicals generated by other things such as x-rays and sunlight. It reduced a type of DNA damage from x-rays 90% in one study. This is remarkable protection. Chemotherapeutic drugs are another source of free radicals. Free radicals from chemotherapy damage healthy tissue; sometimes a chemo drug cannot be used because of this side effect. In a study on cyclophosphamide, chlorophyllin significantly reduced bone marrow damage without interfering with the drug’s anticancer effect.

**Neutralizes carcinogens**
But that is not all chlorophyllin can do. Studies show that in addition to being a powerful antioxidant, chlorophyllin can actually latch onto carcinogens such as heterocyclic amines and prevent them from binding to DNA. For this reason, it has been called an “interceptor molecule”. When chemicals attach themselves to DNA, it causes an “adduct”. Adducts prevent DNA from being replicated normally when cells divide. It is important to prevent this type of damage so that DNA will replicate normally and not create the potential for cancer. Chlorophyllin is able to intercept cancer-causing agents because of its unique chemical structure.

It is one thing to know chlorophyllin has anti-cancer effects at the molecular level; it is another to know that these effects will translate into something visible. Will an “excess of chlorophyllin in the diet” prevent cancer, as researchers at the New York Medical College predict it will?

**Other cancers**

Another cancer that has been studied in conjunction with chlorophyllin is colon cancer. Both chlorophyllin and I3C are powerful at stopping this type of cancer in the early stages. Research shows that chlorophyllin inhibits a precancerous condition known as “aberrant crypt foci” by about 90% in rats treated with the most prevalent heterocyclic amine in fried beef (i.e., hamburgers). I3C was 100% effective in the same study. Both work by keeping the heterocyclic amine from being metabolized. Stopping the metabolism of dangerous chemicals so that they don't become carcinogenic is another way that chlorophyllin works against cancer-in addition to stopping free radicals and intercepting toxins.

Chlorophyllin has been tested in human breast cancer cells. Researchers at the University of Kentucky tested eight cancer-preventive agents, including chlorophyllin, against chemically-induced DNA adducts. Chlorophyllin was one of the top three DNA defenders, inhibiting adducts by greater than 65%. Genistein and ellagic acid came in second at greater than 45% inhibition.

The effective dose of chlorophyllin needed to protect DNA is low, making it the most potent DNA protection available in such a small package.
According to the studies done so far, a person should take 2 to 4 mg per kilogram of body weight. (Note: a kilogram is 2.2 pounds).

Our human studies are all done with purified chlorophyll or chlorophyllin, not with spinach, which is rich in chlorophyll. When the spinach was fed after colon cancer was initiated in the rats, it had a protective effect against carcinogenesis. It was pretty impressive, and we did not expect it. We expected to see a protective effect when the spinach was given at the same time as the carcinogen, but that was not observed. So we have to sort it out. Is it because the chlorophyll is not bioavailable? Was it given appropriately with the carcinogen dose? There are a lot of variables to examine when you get a surprising finding like that.

Nobody is aware of any toxicity issues for natural chlorophyll. The FDA allows a level of intake of chlorophyllin that is in the range of 100-150 milligrams per tablet. No toxicity has ever been reported, and we have not observed any in our short-term studies with humans. In future long-term human trials, there is a potential risk of tumor promotion by chlorophyllin based on the rat colon carcinogenesis model. So, under certain circumstances, at certain doses of chlorophyllin and exposure to certain carcinogens, there may be some risk. Interestingly, we observed protection in both very low and very high doses. In those experiments, chlorophyllin was given after cancer initiation—after the carcinogen was administered.

**Chlorophyllin is both a positive and negative modifier of mutagenicity**

The mechanism responsible for the modification of mutagenicity by chlorophyllin has been investigated using mutagenic compounds with different mechanisms of action, including the mono-functional alkylating agents, \(N\)-methyl-\(N'\)-nitrosourea (MNU) and ethyl-methane-sulphonate (EMS); nitrosamines related to tobacco products, i.e. dimethyl-nitrosamine (DMN), \(N\)-nitro-sonornicotine (NNN) and 4-(\(N\)-methyl-\(N\)-nitrosoamino)-1-(3-pyridinyl)-2-butanone (NNK); the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) and two of its metabolites, i.e. (−)-\(7\beta, 8\alpha\)-dihydroxy-7,8-dihydrobenzo[a]pyrene (7,8-diol) and (+)-\(7\beta, 8\alpha\)-dihydroxy-
\(9_{\alpha},10_{\alpha}\)-oxy-7,8,9,10-tetrahydrobenzo[\(\alpha\)]pyrene (BPDE): and a complex mutagenic mixture, an extract and subfractions of Swedish moist oral snuff (SMOS). Mutagenicity was monitored with the Ames Salmonella/microsome assays (STY) and \(hprt\) V79 point mutation assay (V79). The effects of chlorophyllin on the mutagenicity of the nitrosamines in the STY assays were found to be complex. In the presence of either NNN or NNK, low concentrations of chlorophyllin actually potentiated the mutagenicity > 2-fold. However, at higher, but still non-toxic concentrations, chlorophyllin decreased the mutagenicity of both compounds. The same type of dose-response relationship for chlorophyllin was indicated in the V79 assay system with DMN, although the effect was much weaker. The results with STY were further confirmed by replacing chlorophyllin with another porphyrin compound, hemin. In contrast, biliverdin, a porphyrin structure without the central metal ion, was unable to potentiate the mutagenicity of NNK in STY. These findings suggest that chlorophyllin interferes with electron transport, which was further supported by the finding that glucose-6-phosphate can be excluded from the bio-activating NADPH-generating S9 mixture in the presence of chlorophyllin without altering the mutagenicity. The mutagenicity of MNU or EMS was not influenced in either of the assays, which is consistent with the hypothesis that the modification of nitrosamine mutagenicity may involve changes in metabolic activation. The potentiating effect of chlorophyllin was further investigated using both B[\(\alpha\)]P and BPDE in the STY assay and 7,8-diol and BPDE in the V79 assay. The results show a complete suppression of the mutagenicity of all three compounds in both systems by non-toxic levels of chlorophyllin. There was no enhancement of PAH-induced mutations with low concentrations of chlorophyllin. Since enhancement only occurs with nitrosamines we suggest that chlorophyllin specifically interferes with the form of cytochrome P450 catalyzing DMN-demethylase activity. Findings with the complex mixtures SMOS were similar to these with PAH, indicating that the major mutagenic activity in these extracts is not caused by nitrosamines. To date, only speculations, about the mechanism by which chlorophyllin exerts its antimutagenic activity have been presented. Scavenging of radicals or suppression of metabolic activation, as well as inactivation due to complex formation with either the metabolically activated mutagen or a non-activated precursor, has
all been suggested. We conclude that chlorophyllin has several mechanisms of action, including antimutagenic activity arising from complex formation with PAH; no effect on monofunctional alkylating agents; and, finally, interference with electron transport to a specific form of cytochrome P450.

It has been estimated that the DNA in each cell of our body suffers 10,000 oxidative hits per day, leading to the formation of more than 20 different oxidative DNA lesions. Human studies show lifestyle and other environmental influences may profoundly modify outcomes of aging.

It is not just environmental toxins (e.g., cigarette smoke, coal dust, and diesel emission particles) that pose a concern. Foods cooked at high temperature also inflict cellular damage. Deep-fried foods along with well-done beef steak, ham-burgers, and bacon cause the formation of gene-mutating heterocyclic amines. Even so-called healthy foods contain small amounts of undesirable substances.

Chlorophyllin is the modified, water-soluble form of chlorophyll that has been shown to have DNA protective and antioxidant properties, inhibiting DNA adduction. Chlorophyllin also quenches all major oxygen species and acts to protect mitochondria. Chlorophyllin also has a role in preventing dietary unavoidable exposure to aflatoxin, a naturally occurring mycotoxin, by reducing its oral bioavailability.

Chlorophyllin with Zinc provide consistent protection. The copper that is a naturally occurring part of chlorophyllin is tightly bound to the chlorophyllin molecule and not easily available to the body. However, because zinc competes with copper for absorption into the bloodstream, 10 mg of zinc if added will guard against any free copper that might be present.

**Supplements**

**Chlorophyll**

Green algae like chlorella are often marketed as supplemental sources of chlorophyll. Because natural chlorophyll is not as stable as chlorophyllin
and is much more expensive, most over-the-counter chlorophyll supplements actually contain chlorophyllin.

**Chlorophyllin**

Oral preparations of sodium copper chlorophyllin (also called chlorophyllin copper complex) are available in supplements and as an over-the-counter drug used to reduce odor from colostomies or ileostomies or to reduce fecal odor due to incontinence. Sodium copper chlorophyllin may also be used as a color additive in foods, drugs, and cosmetics.

**Safety**

Natural chlorophylls are not known to be toxic, and no toxic effects have been attributed to chlorophyllin despite more than 50 years of clinical use in humans. When taken orally, chlorophyllin may cause green discoloration of urine or feces or yellow or black discoloration of the tongue. There have also been occasional reports of diarrhea related to oral chlorophyllin use. When applied topically to wounds, chlorophyllin has been reported to cause mild burning or itching in some cases. Oral chlorophyllin may result in false positive results on guaiac card tests for occult blood. Since the safety of chlorophyll or chlorophyllin supplements has not been tested in pregnant or lactating women, they should be avoided during pregnancy and lactation.

**Summary**

* Chlorophyll a and chlorophyll b are natural, fat-soluble chlorophylls found in plants.

* Chlorophyllin is a semi-synthetic mixture of water-soluble sodium copper salts derived from chlorophyll.

* Chlorophyllin has been used orally as an internal deodorant and topically in the treatment of slow-healing wounds for more than 50 years without any serious side effects.

* Chlorophylls and chlorophyllin form tight molecular complexes with some chemicals known or suspected to cause cancer, and in doing so, may block their carcinogenic effects. No carefully controlled studies have been
undertaken to determine whether a similar mechanism might limit uptake of required nutrients or minerals.

* Supplementation with chlorophyllin before meals substantially decreased a urinary biomarker of aflatoxin-induced DNA damage in a Chinese population at high risk of liver cancer due to unavoidable dietary aflatoxin exposure.

* Scientists are hopeful that chlorophyllin supplementation will be helpful in decreasing the risk of liver cancer in high-risk populations with unavoidable dietary aflatoxin exposure. However, it is not yet known whether chlorophyllin or natural chlorophylls will be useful in the prevention of cancers in people who are not exposed to significant levels of dietary aflatoxin.