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Spirulina (Arthrospira) as a Nutritional and
Therapeutic Supplement in Health
Management**

Amha Belay, PhD*
Scientific Director, Earthrise Nutritionals Inc., Calipatria, California

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The Potential Application of *Spirulina* (*Arthrospira*) as a Nutritional and Therapeutic Supplement in Health Management

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INTRODUCTION

Spirulina, now named *Arthrospira*, is a microscopic and filamentous cyanobacterium (blue-green alga) that has a long history of use as food. Its name derives from the spiral or helical nature of its filaments (Fig 1). There are reports that it was used as food in Mexico during the Aztec civilization some 400 years ago. It is still being used as food by the Kanembu tribe in the Lake Chad area of the Republic of Chad where it is sold as dried bread called "dihe".¹ *Spirulina* has been produced commercially for the last 20 years for food and specialty feeds.²⁻⁴ Commercial algae are normally produced in large outdoor ponds under controlled conditions (Fig 2). Some companies also produce directly from lakes. Current production of *Spirulina* worldwide is estimated to be about 3,000 metric tons. Sold widely in health food stores and mass-market outlets throughout the world, *Spirulina's* safety as food has been established through centuries of human use and through numerous and rigorous toxicological studies.⁵⁻⁸

Early interest in *Spirulina* focused mainly on its rich content of protein, vitamins, essential amino acids, minerals, and essential fatty acids. *Spirulina* is 60-70% protein by weight and contains a rich source of vitamins, especially vitamin B₁₂ and provitamin A (β -carotene), and minerals,

especially iron. One of the few sources of dietary γ -linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits.

The objectives of this paper are 1) to review the available literature on the potential health effects of *Spirulina* and its extracts, 2) to provide insight into the potential implications of the studies reviewed in the context of possible nutritional and therapeutic applications in health management, and 3) to identify areas of interest for future research.

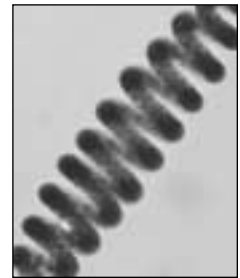


Figure 1. Microscopic view of *Spirulina* (*Arthrospira*) *platensis*

IMMUNOMODULATION EFFECTS

In a 1993 review of the potential health benefits of *Spirulina*, Belay *et al.*⁹ presented the limited published information on this alga and called the attention of researchers to the particular areas of immune enhancement and cancer. Numerous studies have been published since then. The evidence for immune modulation of *Spirulina* in various animal models is so striking that structure function claims have already been applied to some *Spirulina* products.

A summary of the major studies on immunomodulation properties of *Spirulina* is given on Table 1.

Hayashi *et al.*¹⁰ were the first to publish detailed studies on immunomodulatory properties of dietary *Spirulina* in mice. According to their results, 1) mice fed *Spirulina* showed increased numbers of splenic antibody-producing cells in the primary immune response to sheep red blood

* Correspondence:

Amha Belay, PhD

Earthrise Nutritionals Inc.

P.O. Box 270, Calipatria, CA 92233

Phone: 760-348-5027 Fax: 760-348-7258

Email: abelay@cts.com

Table 1. Summary of studies in immunomodulation

Type of Study	Summary of Studies	Reference
Mice	Increased numbers of splenic antibody-producing cells in the primary immune response to sheep red blood cells (SRBC).	10, 11
<i>In vitro</i>	The percentage of phagocytic cells in peritoneal macrophages was significantly increased The proliferation of spleen cells by either concavalin A (Con A) or phytohemagglutinin (PHA) was significantly increased. Hot water extract of <i>Spirulina</i> (SHW) significantly increased proliferation of spleen cells Hot water extract significantly enhanced interleukin-1 (IL-1) production from peritoneal macrophages. Hot water extract enhanced the production of antibodies by spleen cells and macrophage supernatants.	10
<i>In vitro</i>	<i>Spirulina</i> -treated chicken macrophages showed increased spreading and vacuolization with minimal cytotoxicity. Higher percentage of phagocytic macrophages for unopsonized SRBC; higher number of internalized SRBC.	12
Chicken	Significant improvement in PHA-P-induced toe web swelling (CBH assay) at levels of 1,000 ppm and beyond. Increased T-cell proliferation <i>Spirulina</i> supplementation (1,000 ppm) resulted in an almost complete <i>E coli</i> clearance from blood circulation 30 minutes post injection.	14
Chicken	Increased size of thymi. Higher anti-red blood cell antibody titers in the secondary response especially at the higher supplementation (10,000 ppm). Higher PHA-P-mediated lymphoproliferative response especially at 10,000 ppm supplementation. Higher phagocytic potential and NK cell activity in macrophages isolated from the K-strain and broiler chicks.	16
<i>In vitro</i>	Increased phagocytic potential of cat bronchoalveolar lavage macrophages exposed to water extract of <i>Spirulina</i> .	17
Channel catfish	Peritoneal phagocytes from fish fed <i>Spirulina</i> enhanced phagocytosis to zymosan and chemotaxis to <i>E ictaluri</i> exoantigen.	18
Prawns	The activity of granulocytes and hyaline cells was enhanced significantly in tiger prawns (<i>Penaeus monodon</i>) supplied with a feed containing as low as 0.1% (w/w) dry <i>Spirulina</i> .	19

Table 1. Summary of studies in immunomodulation (continued)

Prawns	Prawns fed with <i>Spirulina</i> could clear <i>Vibrio parahaemolyticus</i> , a pathogen of prawns, from the hemolymph at half the time taken by control prawns fed with basal diet.	19
<i>In vitro</i>	In human peripheral blood mononuclear cells (PBMC), <i>Spirulina</i> stimulated the secretion of Interleukin (IL)-1b, IL-4 and interferon (IFN)- γ to nearly 2.0, 3.3, and 13.6 times basal levels, respectively; much higher ratio of IFN- γ to IL-4 favoring cellular immunity.	20
Human	IFN- γ secretion activity and NK-cell cell damage activities were enhanced significantly after two weeks of administration of <i>Spirulina</i> extract to 40 year-old male volunteers. IFN- γ and NK cell activities continued up to 6 months after the administration of extract was discontinued.	21
Mice	Co-administration of <i>Spirulina</i> hot water extract with a cell wall component obtained from <i>Tubercle bacillus</i> (BCG-CWS) resulted in a much higher tumor regression in tumor-bearing mice than with BCG-CWS alone. A 93% reduction in tumor progression in the combined BCG-CWS and <i>Spirulina</i> hot water extract treatment compared to about 48% in the group that received BCG-CWS alone.	22
Human	The above-normal IgE levels observed in 35 pre-school children living in highly radioactive areas were normalized by the administration of 5 grams of <i>Spirulina</i> a day for 45 days.	23
Rats	<i>Spirulina</i> -inhibited compound 48/80-induced anaphylactic shock 100% with doses of 0.5, and 1.0 mg/g body weight. Significant reduction in serum histamine levels induced by compound 48/80. Passive cutaneous anaphylaxis activated by anti-dinitrophenyl (DNP) IgE was inhibited to 69% <i>Spirulina</i> dose-dependently inhibited histamine release from rat peritoneal mast cells (RPMC) by compound 48/80. <i>Spirulina</i> had a significant effect on the anti-DNP IgE-induced histamine release or tumor necrosis factor- α production from RPMC.	24
Rats	Anti-inflammatory effect (reduction in MPO activity) and inhibition in inflammatory cell infiltration by phycocyanin.	26
Mice	IgE antibody levels increased in the mice that were orally immunized with crude shrimp extract as an antigen.	27

Table 1. Summary of studies in immunomodulation (continued)

<p>Prawns</p>	<p><i>Spirulina</i> had no additional effect on the IgE level when it was administered with the antigen. Co-administration of <i>Spirulina</i> with the antigen further enhanced the level of IgG1 in excess of the level found by antigen administration only. IgA antibody level was significantly enhanced by treatment with <i>Spirulina</i> extract concurrently ingested with shrimp antigen compared with the level with shrimp antigen alone.</p>	<p>19</p>
<p>Human</p>	<p>Significant correlation between the total s-IgA level in the saliva and the total amount of <i>Spirulina</i> consumed.</p>	<p>28</p>

cells, 2) the percentage of phagocytic cells in peritoneal macrophages from mice fed a *Spirulina* diet was significantly increased, 3) the proliferation of spleen cells by either Conavalin A (Con A) or phytohemagglutinin (PHA) was significantly increased, 4) addition of a hot water extract of *Spirulina* (SHW) to an *in vitro* culture of spleen cells significantly increased proliferation of these cells with no effect on thymus cells, 5) the hot water extract also significantly enhanced interleukin-1 (IL-1) production from peritoneal macrophages, and 6) addition of the hot water extract to *in vitro* spleen culture and the supernatant of macrophages resulted in enhancement of antibody production. The authors concluded that *Spirulina* and its extract enhance immune function through the modulation of macrophage function, phagocytosis, and IL-1 production. These studies confirmed earlier studies of a preliminary nature reported by Liu *et al.*¹¹

Qureshi *et al.*¹² did similar studies where sephadex-elicited macrophage cultures were exposed to a 10-40 µg/ml (v/v) water-soluble extract of *Spirulina* for 1-16 hours. They found the following: 1) *Spirulina*-treated macrophages showed increased spreading and vacuolization with minimal cytotoxicity, 2) the percentage of phagocytic macrophages for unopsonized sheep red blood cells (SRBC) and average number of internalized SRBC was higher in the *Spirulina*-treated macrophages compared to controls, and 3) culture supernatants from *Spirulina*-treated macrophages contained a factor with tumoricidal potential similar in reactivity to one produced by macrophages after treatment with lipopolysaccharides.¹² Their results support earlier findings by Baojiang *et al.*¹³

Qureshi's group also studied Cutaneous Basophilic Hypersensitivity Response (CBH) and bacterial clearance potential in chicks (B¹⁵ B¹⁵, Cornell K-strain) fed a *Spirulina* supplement diet.¹⁴ CBH was studied by measuring toe web swelling after the injection of PHA-P. Toe web swelling is here used as an index of T-cell proliferation. A significant improvement was observed in PHA-P-induced

peak response in chicks fed *Spirulina* at levels of 1,000 ppm and beyond. It was concluded that *Spirulina* supplementation improved T-cell-mediated response in chickens.

Also studied was the effect of *Spirulina* supplementation on blood clearance profile and splenic bacterial load. Leghorn chicks fed a diet containing 1,000 ppm of *Spirulina* exhibited numerically reduced bacterial load in both circulation and spleen. *Spirulina* supplementation at 1,000 ppm showed an enhanced *E coli* clearance from blood circulation reaching almost negligible levels 30 minutes post injection.¹⁴ The effect was ascribed to improvement in the activity of phagocytic cells as reported in their earlier study.¹² According to the authors, the reduction of viable bacteria in spleen tissue over time is suggestive of an immunopotentiating effect of a particular dietary compound on mononuclear phagocytic system cells. The antibacterial effect of macrophages activated by *Spirulina* has also been reported elsewhere.¹⁵

In another study involving Cornell K-strain White Leghorn and broiler chicks, Qureshi *et al.*¹⁶ found that K-strain chicks had larger thymi over controls. They also found high anti-red-blood-cell antibody titers in the secondary response, especially at the higher supplementation group (10,000 ppm) compared to the control group (0 ppm). Chicks in the 10,000 ppm *Spirulina* group also had higher PHA-P-mediated lymphoproliferative response over the controls. Macrophages isolated from both K-strain and broiler chicks had higher phagocytic potential and NK cell activity. The authors concluded that a dietary inclusion of *Spirulina* at a level of 10,000 ppm might enhance disease resistance potential in chickens.¹⁶ Qureshi's group did similar studies with cat macrophages exposed to *Spirulina* extract. They found increased phagocytic potential using red blood cells and *E coli*.¹⁷

In a study involving the effect of *Spirulina* in channel catfish (*Ictalurus punctatus*), Duncan and Klesius¹⁸ found that fish fed *Spirulina* had a lower percentage of erythro-

cytes and a higher percentage of lymphocytes than fish fed a control diet. There was no difference in thrombocytes and macrophages in the *Spirulina* and control diet. However, peritoneally-elicited phagocytes from fish fed *Spirulina* showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiella ictaluri* exoantigen (5.9 times). *Spirulina* also enhanced production of antibodies to key-hole limpet hemocyanin (KLH) but not to *E ictaluri*. Their results are similar to those by Hayashi *et al.*¹⁰ who showed in mice that *Spirulina* increased the antibody response to a thymus-dependent antigen but not to a thymus-independent antigen.¹⁰ In a similar study by Lee¹⁹ the activity of granulocytes and hyaline cells was enhanced significantly in tiger prawns (*Penaeus monodon*) supplied with a feed containing as low as 0.1% (w/w) dry *Spirulina*. The increase in phagocytic activity of hemocytes was a function of *Spirulina* content and time. Prawns fed with *Spirulina* could clear *Vibrio parahaemolyticus*, a pathogen of prawns, from the hemolymph at half the time taken by control prawns fed with basal diet.

A group of researchers from University of California-Davis²⁰ reported in 2000 the results of a study that adds evidence for the immunomodulatory effects of *Spirulina*. Using human peripheral blood mononuclear cells (PBMC), they demonstrated that *Spirulina* stimulated the secretion of Interleukin (IL)-1 β , IL-4, and interferon (IFN)- γ to nearly 2.0, 3.3, and 13.6 times basal levels, respectively. Induction of (IFN)- γ by *Spirulina* was found to be comparable to that seen after phytohemagglutinin (PHA) stimulation while being much less mitogenic than PHA in the induction of IL-4. The preponderance of (IFN)- γ over IL-4 is believed to show that *Spirulina* is more effective in stimulating a Th-1-type response and hence potentiates cell-mediated immunity. The moderate stimulation of the production of IL-4, that may be due to the antagonistic activity of (IFN)- γ on IL-4, may mean that *Spirulina* helps balance the production of Th-1 and Th-2 cytokines. Thus *Spirulina* may provide strong protection against intracellular pathogens and parasites, and may also be effective in fighting extracellular parasites.²⁰

In a paper presented at the 30th annual meeting of the Japanese Society for Immunology, Saeki *et al.*²¹ presented the results of a human study using *Spirulina* extract. The immune modulation property of the extract in 40-year-old male volunteers was investigated using IL-12/IL-18-dependent IFN- γ secretion induction activity and cell damage activity by NK cell as indices. 50 ml of a commercial *Spirulina* drink (Dainippon Ink & Chemicals, Inc.) containing 40% *Spirulina* hot water extract was administered to over 40-year-old male volunteers every day, and the IL-12/IL-18-dependent IFN- γ secretion induction activity of lymphocyte was chronologically analyzed by ELISA. Natural killer cell damage activity in blood was analyzed simultaneously. The analyses of the above parameters were done before and after administration of the extract. IFN- γ

secretion activity and NK cell damage activities were enhanced significantly after two weeks of *Spirulina* extract administration. Surprisingly, the IFN- γ and NK cell activities continued up to 6 months after administration of extract was discontinued.²¹

In another study reported at the 59th annual meeting of the Japanese Cancer Association, the same group, Saeki *et al.*,²² reported the results of an investigation on the adjuvant effect of a hot water extract of *Spirulina* in the regression of tumors in tumor-bearing mice. Co-administration of *Spirulina* hot water extract with a cell wall component obtained from *Tubercle bacillus* (BCG-CWS) resulted in a much higher tumor regression in tumor-bearing mice than with BCG-CWS alone or in the untreated control mice. In this study BCG-CWS was administered in the mice, which were then immunized with inactivated B-16 melanoma. Following this, active melanoma cells were implanted subcutaneously. It is believed that microbial cell wall components like BCG-CWS act as inducers of dendritic cells, which play a major role in the induction of tumor-specific cytotoxic T-lymphocytes (CTL). The latter are believed to target high MHC-expressing tumor cells but fail to attack low MHC-expressing cells that are eliminated by natural killer cells. Since *Spirulina* has been found to be an effective activator of NK cells in previous studies, they suggested tumor cells that escaped attack by CTL cells were probably killed by NK cells. Thus both BCG-CWS and *Spirulina* extract effectively controlled the tumor. The combined administration of BCG-CWS and *Spirulina* extract resulted in more than 90% regression.²²

The effect of *Spirulina* or its extracts on allergic reactions caused by food and other factors has been the subject of research recently. According to a Russian patent, the above-normal IgE levels observed in children in highly radioactive areas were normalized by the administration of 5 grams of *Spirulina* a day for 45 days. The level of IgE in the blood was taken as an index of allergy. The study was conducted with 35 preschool children living constantly in highly radioactive environments. (2-5 kv km²).²³

Yang *et al.*,²⁴ did extensive studies on the effect of orally-administered *Spirulina* on anaphylactic reaction. The following is a summary of their findings: 1) *Spirulina* inhibited compound 48/80-induced anaphylactic shock 100% with doses of 0.5 and 1.0 mg/g body weight, 2) *Spirulina* significantly reduced serum histamine levels induced by compound 48/80 in rats, 3) passive cutaneous anaphylaxis activated by anti-dinitrophenyl IgE was inhibited to 69%, 4) *Spirulina* dose-dependently inhibited histamine release from rat peritoneal mast cells by compound 48/80, and 5) *Spirulina* had a significant effect on the anti-DNP IgE-induced histamine release or tumor necrosis factor α production from RPMC. The authors postulate that the effects observed are possibly due to inhibition of anaphylactic degranulation of mast cells by *Spirulina*.²⁴

Subsequent studies by Kim *et al.*²⁵ on the effect of *Spirulina* on mast-cell-mediated immediate-type allergic reactions in rats also showed similar results. In this study *Spirulina* dose-dependently inhibited the systemic allergic reaction induced by compound 48/80 in rats. Compound 48/80-induced allergic reaction was inhibited 100% with intraperitoneal doses of 100-1,000 µg/g body weight. In rats treated with intraperitoneal dosages of *Spirulina* at concentrations ranging from 0.01 to 1,000 µg/g body weight, serum histamine levels were reduced in a dose-dependent manner. *Spirulina* also dose-dependently inhibited histamine release from rat peritoneal cells activated by compound 48/80 or anti-DNP IgE. *Spirulina* also had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor α production.²⁵

In a similar study published in 1999, Gonzalez *et al.*²⁶ reported anti-inflammatory activity of phycocyanin extract in acetic-acid-induced colitis in rats using myeloperoxidase activity and histopathological and ultrastructural observations. Phycocyanin substantially reduced myeloperoxidase activity, and histopathological studies showed inhibition in inflammatory cell infiltration. The protection against inflammation was believed to be due to antioxidative and oxygen scavenging properties of phycocyanin.²⁶

Hayashi *et al.*²⁷ investigated the influence of dietary *Spirulina platensis* on different classes of antibodies, including IgA, IgE, and IgG1 in mice as possible evidence of the protective effect of *Spirulina* toward food allergy and microbial infection. IgE antibody levels increased in mice orally immunized with crude shrimp extract as an antigen (Ag group). *Spirulina* had no additional effect on the IgE level when administered with the antigen. However, co-administration of *Spirulina* with the antigen enhanced the level of IgG1 in excess of the level found by antigen administration only. IgA antibody level was significantly enhanced by treatment with *Spirulina* extract concurrently ingested with shrimp antigen compared with the level of shrimp antigen alone. In mice treated with *Spirulina* for 4 weeks before antigen stimulation, an enhancement of IgA was observed in lymphoid cells, especially in the spleen and the mesenteric lymph node.²⁷

More recently, Ishii *et al.*²⁸ studied the influence of *Spirulina platensis* on IgA levels in the saliva of 127 human subjects. Their results showed that total s-IgA levels were significantly higher when the subjects took *Spirulina* for over a year compared to those who took *Spirulina* for less than a year. They also found a significant correlation between the total s-IgA level in the saliva and the total amount of *Spirulina* consumed.

IMPLICATIONS OF THE RESULTS OF THE STUDIES

Our immune systems are our defense against pathogenic organisms like bacteria, viruses, cancer cells, and parasites,

and against a whole series of compounds that are recognized as “foreign” or “non-self”. Any cell or molecule recognized as non-self is attacked by immune system cells and the antibodies they produce. The immune system is a complex system that involves specialized cells that communicate with each other via chemical messengers called cytokines. The immune system is also intricately tied up with the nervous and endocrine systems. Hence, impairment of the immune system has far-reaching consequences in the body.

It is now well established that nutrient deficiency is associated with consistent changes in immune responses such as number of T-cells, lymphocyte response to mitogens and antigens, phagocyte function, secretory IgA antibody response, complement activity, NK cell activity, and production of cytokines.²⁹ Nutrient excesses are also associated with impaired immune function. For example, dietary intake of large quantities of fats impairs immune response. In addition, the immune system can be positively or negatively affected by certain phytochemicals found in conventional foods and foods derived from other plants like algae, mushrooms, and some herbs.

The findings of the studies summarized above are significant in the context of natural and nutritional therapeutic intervention in the prevention of infection by pathogenic organisms. Non-specific cell-mediated immunity of the type found in these studies is the first line of defense against invading organisms. Enhancement of this aspect of the immune system will therefore have far-reaching advantages in body defense. Moreover, *Spirulina* appears to have a balancing effect on important immune cells and cytokines. Although cytokine-induced responses are generally protective in nature, an excess production and/or activity of cytokines can be harmful. It is known that the body possesses an elaborate system of checks and balances to control the production and activity of individual cytokines and that this process requires proper nutrition. *Spirulina* may provide such a nutritional role in modulating immune system function favorably.

The role of *Spirulina* in the activation of INFγ and NK cells observed in the human clinical study and the adjuvant effect seen in the regression of implanted mouse tumors are important in relation to the potential nutritional and therapeutic use of *Spirulina* in cancer immunotherapy. The anti-allergy effects observed in these studies are also significant in relation to natural therapeutic intervention in allergic situations. *Spirulina* neither induced nor enhanced allergic reactions dependent on IgE. On the contrary, it was found to enhance IgA production when ingested both concurrently with antigen and before antigen stimulation, providing protection against allergic reactions. The observation that secretory IgA production was found to correlate with *Spirulina* consumption may point to the potential role of *Spirulina* in mucosal immunity. The salivary glands are recognized as part of the common mucosal immune system,

and saliva is commonly used to study the effect of various parameters on the human mucosal immune system.³⁰ Recently there is a focus on research in the therapeutic use of antigen feeding for immunization and/or oral tolerance induction.³¹ It is now well established that oral or intranasal immunization confers protection against a variety of viral and bacterial mucosal pathogens. On mucosal surfaces, secretory IgA antibodies elicit a whole series of biological responses such as agglutination of microorganisms, neutralization of bacterial enzymes, toxins, and viruses, and immune exclusion and inhibition of antigen or allergen absorption.³² Though conjectural, *Spirulina* may have a role in modulating these beneficial effects that result in the killing or inactivation of pathogens, antigens, and allergens, in addition to protection offered through the stimulation of cell-mediated immunity.

ANTIOXIDANT EFFECTS

A summary of studies related to the antioxidant effects of *Spirulina* is given in Table 2.

The antioxidant properties of *Spirulina* and its extracts have attracted the attention of researchers recently. In one of the earliest studies, Manoj *et al.*³³ reported that the alcohol extract of *Spirulina* inhibited lipid peroxidation more significantly (65% inhibition) than the chemical antioxidants like α -tocopherol (35%), BHA (45%), and β -carotene (48%). The water extract of *Spirulina* was also shown to have more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). An interesting aspect of their findings is that the water extract had a significant antioxidant effect even after the removal of polyphenols.

In another study, by Zhi-gang *et al.*,³⁴ the antioxidant effects of two fractions of a hot water extract of *Spirulina* were studied using three systems that generate superoxide, lipid, and hydroxyl radicals. Both fractions showed significant capacity to scavenge hydroxyl radicals (the most highly reactive oxygen radical) but no effect on superoxide radicals. One fraction had significant activity in scavenging lipid radicals at low concentrations.

In a study by Miranda *et al.*,³⁵ the antioxidant activity of a methanolic extract of *Spirulina* was determined *in vitro* and *in vivo*. The *in vitro* antioxidant assay involved a brain homogenate incubated with and without the extract at 37°C. Peroxidation of rat brain homogenate was inhibited by almost 95% with 0.5 mg of the methanolic extract. The IC₅₀ of the extract in this system was found to be 180 μ g. The *in vivo* antioxidant capacity was evaluated in plasma and liver of animals receiving a daily dose of 5 mg for 2 and 7 weeks. Plasma antioxidant activity in brain homogenate incubated at 47°C showed that the antioxidant capacity of plasma was 97% and 71% for the experimental group and 74% and 54% for the control group after 2 and 7 months. The antioxidant effect was attributed to beta carotene, tocopherol, and phenolic compounds working individually or in synergy.³⁵

In what appears to be the first report on antioxidant and anti-inflammatory properties of c-phycoyanin, Romay *et al.*³⁶ showed that phycoyanin was able to scavenge hydroxyl (IC₅₀ = 0.91 mg/ml) and alkoxy (IC₅₀ = 76 μ g/ml) radicals with activity equal to 0.125 mg/ml of dimethyl sulfoxide (DMSO) and 0.038 μ g/ml of trolox, specific scavengers of those radicals respectively. Phycoyanin also inhibited liver microsomal lipid peroxidation (IC₅₀ = 12 mg/ml). It is interesting to note that the oxygen-scavenging activity of c-phycoyanin was only 3 times lower than that of superoxide dismutase (SOD). The addition of SOD to the phycoyanin did not alter the antioxidant activity of the phycoyanin, suggesting a different mechanism of action.³⁶ Further studies by the same group³⁷ revealed the anti-inflammatory activity of phycoyanin in some animal models of inflammation. Phycoyanin reduced significantly and in a dose-dependent manner the ear edema induced by arachidonic acid and tissue plasminogen activator in mice, as well as carageenan-induced rat paw edema. Phycoyanin also showed anti-inflammatory activity in a sub-chronic cotton pellet granuloma test where sterile cotton pellets were implanted in the axillae of rats. Oral administration of phycoyanin resulted in significant anti-inflammatory activity in all models tested. The anti-inflammatory activity observed was attributed to antioxidant and oxygen scavenging activity of phycoyanin and perhaps also due to its inhibitory effect on arachidonic acid metabolism. When compared with indomethacin, a standard anti-inflammatory drug, phycoyanin showed a weaker activity (50-300 mg/kg, p.o.) compared to 3-10 mg/kg, p.o., for the former. However, the LD₅₀ of indomethacin was 12 mg/kg in rats and 50 mg/kg in mice, p.o., and induces many side effects in patients under treatment. The LD₅₀ of phycoyanin in rats and mice was greater than 3g/kg, p.o. In fact, no mortality was observed even at 3 g/kg, p.o.³⁷

Vadiraja *et al.*³⁸ studied the effect of c-phycoyanin from *Spirulina platensis* on carbon tetrachloride and R-(+)-pulegone-induced hepatotoxicity in rats. In this study a single dose (200 mg/kg) of phycoyanin was administered intraperitoneally to rats one or three hours prior to R-(+)-pulegone (250 mg/kg) or carbon tetrachloride (0.6 ml/kg) challenge. Phycoyanin significantly reduced the hepatotoxicity caused by these chemicals. Both chemicals are believed to cause hepatotoxicity due to the formation of free radicals. The hepatoprotective effect of phycoyanin was therefore attributed to the inhibition of reactions involved in the formation of reactive metabolites and possibly due to its radical scavenging activity. Duran *et al.*³⁹ also found a similar hepatoprotective effect in experiments where rats were fed an oil extract of *Spirulina* or its defatted fraction.³⁹ Recently, Bhat and Madayastha⁴⁰ reported that c-phycoyanin from *Spirulina* effectively inhibited CCl₄-induced lipid peroxidation in rat liver *in vivo*. The inhibition by both native and reduced phycoyanin was

Table 2. Summary of studies on antioxidant effects of *Spirulina* or extract

Type of Study	Summary of Studies	Reference
<i>In vitro</i>	The alcohol extract of <i>Spirulina</i> inhibited lipid peroxidation more significantly than the chemical antioxidants like α -tocopherol, BHA and β -carotene. Water extract showed more antioxidant activity than gallic acid and chlorogenic acid; antioxidant activity remained high even after removal of polyphenols.	33
<i>In vitro</i>	Two fractions of a hot water extract showed significant capacity of scavenging hydroxyl radicals (the most highly reactive oxygen radical); one of the fractions had significant activity in scavenging lipid radicals at low concentrations.	34
<i>In vitro</i>	Peroxidation of rat brain homogenate was inhibited by almost 95% with 0.5 mg of the methanolic extract. The IC ₅₀ of the extract in this system was found to be 180 μ g.	35
Rat	Plasma antioxidant activity in brain homogenate incubated at 47°C showed that the antioxidant capacity of plasma was 97% and 71 % for the experimental (<i>Spirulina</i> extract) group and 74% and 54% for the control group after 2 months and 7 months, respectively.	35
<i>In vitro</i>	Phycocyanin was able to scavenge hydroxyl (IC ₅₀ = 0.91 mg/ml) and alkoxy (IC ₅₀ = 76 μ g /ml) radicals. The activity was equal to 0.125 mg/ml of dimethyl sulfoxide (DMSO) and 0.038 μ g/ml of trolox. Phycocyanin also inhibited liver microsomal lipid peroxidation (IC ₅₀ = 12 mg/ml).	36
Mice, Rats	Oral administration of phycocyanin reduced significantly and in a dose-dependent manner the ear oedema induced by Arachidonic Acid (AA) and tissue plasminogen activator (TPA) in mice, as well as carageenan-induced rat paw oedema.	37
Rats	Phycocyanin also showed anti-inflammatory activity in a sub-chronic cotton pellet granuloma test where sterile cotton pellets were implanted in the axillae of rats. When compared with indomethacin, a standard anti-inflammatory drug, phycocyanin shows a weaker activity (50-300 mg/kg, p.o.) compared to 3-10 mg/kg, p.o for the former. However the LD ₅₀ of indomethacin was 12 mg/kg in rats and 50 mg/kg in mice, p.o. The LD ₅₀ of phycocyanin in rats and mice was greater than 3g/kg, p.o.	37
Rats	Carbon tetrachloride (0.6 ml/kg) and R-(+)-pulegone (250 mg/kg)-induced hepatotoxicity in rats was reduced significantly when phycocyanin was administered intraperitoneally to rats one or three hours prior to the challenge.	38
Rats	A similar hepatoprotective effect as above was seen in actual feeding experiment in rats with an oil extract of <i>Spirulina</i> or its defatted fraction.	39

Table 2. Summary of studies on antioxidant effects of *Spirulina* or extract (continued)

Rats	Oral administration of c-phycoyanin (100 mg/kg) in rats prevented kainic -acid-induced behavioral and glial reactivity in the rat hippocampus, crossing the hemato-encephalic barrier. The study also shows that phycocyanin reduces experimental status epilepticus. Authors postulate potential use of phycocyanin in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's, diseases induced by oxidative-stress-induced neuronal injury.	40, 41
<i>In vitro</i>	Phycocyanin inhibited 2,2'-azobis(2midinoprapane)dihydroxychloride (AAPH)-induced human erythrocyte haemolysis in the same way as trolox and ascorbic acid, two well known antioxidants. Based on IC ₅₀ values phycocyanin was found to be sixteen times more efficient as an antioxidant than trolox and about 20 times more efficient than ascorbic acid.	42
<i>In vitro</i>	The antioxidant activity of phycocyanobilin (a component of phycocyanin) was greater than that of alpha tocopherol, zeaxanthin and caffeic acid on a molar basis.	43
	Phycocyanin from spray-dried <i>Spirulina</i> had a similar antioxidant activity as phycocyanin from fresh <i>Spirulina</i> .	43
Rats <i>In vitro</i>	C-phycoyanin from <i>Spirulina</i> effectively inhibited CCl ₄ -induced lipid peroxidation in rat liver <i>in vivo</i> Phycocyanin showed a potent peroxy radical scavenger capacity with a rate constant-ratio of 1.54 compared to 3.5 for uric acid (a known peroxy radical scavenger).	40
<i>In vitro</i>	Isolated enzyme assays and whole blood assays show that C-phycoyanin from <i>Spirulina platensis</i> is a selective inhibitor of cyclooxygenase -2 (COX-2) with a very low IC ₅₀ COX-2/IC ₅₀ COX-1 ratio (0.04).	44
<i>In vitro</i>	IC ₅₀ value obtained for COX-2 inhibition by phycocyanin was much lower (180 nM) as compared to those for celecoxib (255 nM) and rofecoxib (401 nM), the well-known selective COX-2 inhibitors.	44
<i>In vitro</i>	Potential role in hepatoprotection, anti-inflammatory and anti-arthritic action postulated.	44

concentration-dependent with an IC₅₀ of 11.35 and 12.7 μM, respectively. Their studies have shown unequivocally that phycocyanin is a potent peroxy radical scavenger with a rate-constant ratio of 1.54 compared to 3.5 for uric acid (a known peroxy radical scavenger).

A recent interesting and elaborate study shows that oral administration of c-phycocyanin (100 mg/kg) in rats prevents kainic-acid-induced behavioral and glial reactivity in the rat hippocampus suggesting a corresponding protective effect on neurons. The study showed that phycocyanin reduced experimental status epilepticus, suggesting possible therapeutic intervention in the treatment of some forms of epilepsy. According to the authors,⁴¹ kainic acid (KA) triggered excitotoxicities resulted in the production of reactive oxygen species. It is therefore postulated that the protective effect of phycocyanin in neuronal damage may be due to its free-radical scavenging and antioxidant properties.

An interesting aspect of this study is the finding that oral administration of phycocyanin exerts its effect in the hippocampus, crossing the hematoencephalic barrier. According to the authors, these findings and the virtual lack of toxicity of phycocyanin suggest that this phytochemical could be used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's, diseases brought on by oxidative stress-induced neuronal injury.⁴¹

Moreover, Romay's group⁴² has recently reported that phycocyanin inhibited 2,2'-azobis(2midinoprapane)dihydrochloride (AAPH)-induced erythrocyte haemolysis in the same way as trolox and ascorbic acid, well-known antioxidants. Based on IC₅₀ values (concentration of the additive that gave the 50% inhibition of peroxidative damage), phycocyanin was found to be 16 times more efficient as an antioxidant than trolox and about 20 times more efficient than ascorbic acid.⁴² These findings were supported by a more recent study⁴³ that showed that the antioxidant activity of phycocyanobilin (a component of phycocyanin) was greater than that of alpha tocopherol on a molar basis. The antioxidant effect of phycocyanobilin was evaluated against oxidation of methyl linoleate in a hydrophobic system or with phosphatidylcholine liposomes. The study also showed that phycocyanin from spray-dried *Spirulina* had a similar antioxidant activity as phycocyanin from fresh *Spirulina*. The results suggest that the antioxidant activity of phycocyanin is attributable to phycocyanobilin, a prosthetic group in phycocyanin since the apoprotein component may be denatured upon drying.⁴³ The fact that the dried phycocyanin showed the same level of activity as the intact protein makes the preparation and utilization of phycocyanin commercially feasible.

According to Reddy *et al.*,⁴⁴ c-phycocyanin from *Spirulina platensis* is a selective inhibitor of cyclooxygenase -2 (COX-2) with a very low IC₅₀ COX-2/IC₅₀ COX-1 ratio (0.04). Interestingly, their study showed that the

IC₅₀ value obtained for COX-2 inhibition by phycocyanin was much lower (180 nM) as compared to those for celecoxib (255 nM) and rofecoxib (401 nM), the well-known selective COX-2 inhibitors. The apoprotein component of phycocyanin was responsible for the inhibition of COX-2 since reduced phycocyanin and phycocyanobilin were found to be ineffective. The authors suggest that the hepatoprotective, anti-inflammatory, and anti-arthritis properties of phycocyanin reported in the literature might be due, in part, to its selective COX-2 inhibitory property, though they did not exclude a similar effect of phycocyanin through its ability to efficiently scavenge free radicals and inhibit lipid peroxidation.

IMPLICATIONS OF THE STUDIES

The relationship between antioxidant intake and incidence of chronic diseases such as cancer, cardiovascular disease, cataracts, and premature aging is now well established through many epidemiological, intervention, and clinical studies. This strong association between diet and cancer led the National Cancer Institute to initiate the 5-A-Day Program in 1991,⁴⁵ recommending 5 servings of fruits and vegetables daily. The United States Department of Agriculture recommends 5-9. Surveys show that only 23% of adult Americans consume the recommended level, and the median level was about 3 servings a day. People whose lifestyle choices preclude eating properly may want to improve their diets by adding nutritional supplements rich in antioxidants.

While the connection between the consumption of fruits and vegetables and the incidence of disease cannot be attributed to the antioxidant components alone, many studies suggest that antioxidants play the major role. Fruit and vegetable antioxidants are derived from the carotenoid pigments. *Spirulina* provides an adequate amount of a spectrum of carotenoid pigments, especially beta carotene (associated with cancer prevention) and zeaxanthin (associated with prevention of age-related macular degeneration (AMD)). In this respect *Spirulina* is a "microvegetable" that can provide some of the antioxidants needed. Many studies have also revealed that antioxidants like the carotenoids in fruits, vegetables, and *Spirulina* have a synergistic effect. Thus whole-food supplements are expected to provide more antioxidant protection compared to individual components. *Spirulina* also contains phycocyanin and polysaccharides, both known to have antioxidant properties. In addition, antioxidants that have a direct effect on reactive oxygen species, *Spirulina* contains an important enzyme, superoxide dismutase, that acts indirectly by slowing down the rate of oxygen radical generating reactions.

The results of the studies summarized above point to the potential use of *Spirulina* together with other conventional approaches in a nutritional supplementation strategy

geared toward the prevention and mitigation of health problems like cancer, heart disease, and premature aging that are associated with free radical damage. In the context of using *Spirulina* as an antioxidant, it is worth noting that it contains about 7% phycocyanin (dry weight basis) and a relatively high content of superoxide dismutase (1,700 units/g) in addition to a high content of mixed carotenoids. A significant aspect of these studies is that orally-administered phycocyanin is bioavailable and can even pass the blood-brain barrier. The role of phycocyanin in COX-2 inhibition may also result in the potential application of *Spirulina* in the management of inflammatory conditions and toxicity due to chemicals and drugs.

ANTICANCER EFFECTS

The antioxidant and immune modulation effect of *Spirulina* and its extracts discussed above are to a certain extent associated with *Spirulina*'s cancer prevention potential. In this summary we report only those studies targeted directly at cancer research. Table 3 summarizes the studies on anti-cancer effects of *Spirulina*.

The only human study on the effect of *Spirulina* on chemoprevention of cancer is that by Mathew *et al.*,⁴⁶ who studied the effect of *Spirulina* on oral leukoplakia (a precancerous lesion) in pan tobacco chewers in Kerala, India. In a study involving 44 subjects in the intervention group and 43 in the placebo group, they found that supplementation with *Spirulina* at 1 g/day for 1 year resulted in complete regression of lesions in 45% of the intervention group and 7% in the control group. The effect was more pronounced in homogeneous lesions. Since supplementation with *Spirulina* did not result in increased serum concentrations of retinal beta carotene, the authors concluded that other constituents in *Spirulina* may be responsible for the regression of lesions observed.⁴⁶ The results of this study are significant because, even in developed countries, tobacco use is the cause of 30% of the incidence of cancer, with the greatest influence on lung and oral cancer.⁴⁷ As recommended by the authors, it is worth investigating this potentially useful aspect of *Spirulina* in more rigorous human trials. If *Spirulina* proves to have such effect, it can easily be incorporated in the daily diet as a therapeutic agent.

The human oral cancer study corroborates the results found in earlier studies on the effect of *Spirulina* and *Dunaliella* extract on experimental cancer in hamster buccal pouches. Schwartz and Shklar⁴⁸ studied the effect of administration of 250 µg of the extract in 0.1 ml of MEM (minimum essential medium) directly to DMBA (7,12-dimethylbenz(a)-anthracene)-induced squamous cell carcinoma of hamster buccal pouches. Other treatments included injection of beta carotene, cantaxanthin, 13 cis-retinoic acid and sham-injected controls. All treatments involved 250 µg in 0.1 ml MEM twice weekly for 4 weeks. After four

weeks of treatment, total tumor regression was found in 30% of the animals treated with extract, 20% of the beta carotene-treated animals, and 15% of canthaxanthin-treated animals. Partial tumor regression was found in the remaining 70% of the extract-treated animals.⁴⁸ An interesting observation of this study is that the algae extract appears to be more effective than beta carotene alone, suggesting a synergistic effect between the various components of the algae. In another study Schwartz *et al.*⁴⁹ have shown that algae-derived phycocyanin had a cytostatic and cytotoxic activity against squamous cell carcinoma (human or hamster). Phycocyanin may have played a synergistic effect here as well as in the human study reported above. In subsequent studies, Schwartz *et al.*⁵⁰ were able to demonstrate that an extract of *Spirulina* and *Dunaliella* administered orally (140 µg every 3 days for 28 days) prevented tumor development in hamster buccal pouches. Carcinomas that were beginning to develop were destroyed in what appeared to be an immune response. This was surmised from the dense lymphocytic-monocytic infiltrate observed. The monocytes were found to be cytotoxic to tumor target cells *in vitro* and the lymphocytes were found to be T-cells. Thus the algae extract was believed to prevent cancer development by stimulating an immune response to selectively destroy small initial foci of developing malignant cells.⁵⁰ It is worth noting that the algae extract was not cytotoxic to normal cells in all experiments performed.

In a murine model, Lisheng *et al.*⁵¹ found that a polysaccharide extract of *Spirulina* inhibited the proliferation of ascitic hepatoma cells of mice injected at a dose of 200 mg/kg. In this study 36 healthy female mice were injected with 5×10^6 of ascitic hepatoma cells, and the controls were injected with distilled water. On the second day after injection of the hepatoma cells, 200 mg/kg/day of polysaccharide from *Spirulina platensis* was injected for 6 days. Another group (prevention group) was injected with the polysaccharide for 5 days before the injection of the hepatoma cells. Quantification of the ascites was done 6 days after injection of the hepatoma cells. Compared to the control group, those treated with the extract after the transplantation of the tumor showed a 54% reduction in tumor progression, while those where the extract was administered five days before tumor transplantation showed a 91% decrease in tumor progression.⁵¹ According to Chen *et al.*,⁵² the number of aberrant crypts formed in the colon of rats by a single or multiple injection of 1,2-dimethyl hydrazine (DMH) was reduced significantly ($p < 0.01$) in rats fed *Spirulina* particularly during weeks 13 and 16 after injection.

Recently, Mishima *et al.*⁵³ have elegantly demonstrated inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel polysaccharide isolated from *Spirulina platensis*. Seven intermittent i.v. injections of 100 µg of Ca-SP in mice caused a marked decrease of lung tumor colonization of B-16-BL6 cells in a spontaneous lung metastasis

Table 3. Summary of studies on anticancer and antiviral effects of *Spirulina* or its extracts

Type of Study	Summary of Studies	Reference
Human	In a study involving 44 subjects in the intervention group and 43 in the placebo group supplementation with <i>Spirulina</i> at 1 g/day for 1 year resulted in complete regression of oral leukoplakia in pan tobacco chewers in India. Complete regression was observed in 45% of the intervention group compared to only 7% in the control group.	46
Hamsters	Administration of 250 µg of a <i>Spirulina/Dunaliella</i> extract (in 0.1 ml of minimum essential medium, MEM) directly to 7,12 dimethylbenz(a)-anthracene (DMBA)-induced squamous cell carcinoma of hamster buccal pouches resulted in total tumor regression in 30% of the animals treated with extract 20% of the beta-carotene-treated animals and 15% of canthaxanthin-treated animals showed complete regression . Partial tumor regression was found in the remaining 70% of the extract-treated animals.	48
Human and Hamsters	Algae-derived phycocyanin had a cytostatic and cytotoxic activity against squamous cell carcinoma (human or hamster).	50
Hamsters	An extract of <i>Spirulina</i> and <i>Dunaliella</i> administered orally (140 µg every 3 days for 28 days) prevented tumor development in hamster buccal pouches. The dense lymphocytic-monocytic infiltrate observed suggested that the anticancer effect could be due to an immune response. The algae extract was not cytotoxic to normal cells in all experiments performed.	49
Mice	A polysaccharide extract of <i>Spirulina</i> inhibited the proliferation of ascitic hepatoma cells of mice injected at a dose of 200 mg/kg. The group treated with the extract after the transplantation of the tumor showed a 54% reduction in tumor progression. The group where the extract was administered five days before tumor transplantation showed a 91% decrease in tumor progression.	51
Rats	The number of aberrant crypts formed in the colon of rats by a single or multiple injection of 1,2-dimethyl hydrazine (DMH) was reduced significantly ($p < 0.01$) in rats fed <i>Spirulina</i> .	52
Mice	Seven intermittent i.v. injections of 100 µg of Ca-SP in mice caused a marked decrease of lung tumor colonization of B16-BL6 cells in a spontaneous lung metastasis model.	53

Table 3. Summary of studies on anticancer and antiviral effects of *Spirulina* or its extracts (continued)

Mice	C-phycoyanin from <i>Spirulina</i> inhibited the growth of K562 leukemia cells in a dose-dependent manner with statistically significant inhibition observed at 80 and 160 mg l ⁻¹ . The IC50 value of C-PC was found to be 72.5 mg l ⁻¹ in XTT dye reduction assay.	54
<i>In vitro</i> Hamsters	Water extract of <i>Spirulina platensis</i> inhibited the replication of <i>Herpes simplex</i> virus type (HSV-1) in HeLa cells within the concentration range of 0.08-50 mg/ml. Extract did not have virucidal effect but interfered with the adsorption and penetration into host cells. The ID50 for cytotoxicity of the extract to HeLa cells was 26.3 mg/ml, while the ED50 for antiviral activity was found to be 0.173 mg/ml, giving an <i>in vitro</i> therapeutic index of 152. In an experimental study of HSV-1 corneal infection of hamsters, food containing the extract effectively prolonged the survival time of infected hamsters at doses of 100 and 500 mg/kg.	65
<i>In vitro</i>	Calcium spirulan (Ca-SP) from <i>Spirulina platensis</i> found to inhibit the replication of several enveloped viruses including <i>Herpes simplex</i> virus type I (HSV-1), human cytomegalovirus (HCMV), measles virus, mumps virus, influenza A virus, and HIV-1 virus.	66
<i>In vitro</i>	The anti-HIV-1 activity of Ca-SP was found to be comparable to dextran sulfate, a known potent anti-HIV-1 agent, and its anti-HSV-1 activity was four to five-fold higher than that of dextran sulfate. The anti-HIV-1 activity of Ca-SP or DS was five and four times higher in cultures treated with Ca-SP or DS during infection when compared with that in cultures treated after infection. Ca-SP was considered superior to DS in possible therapeutic application.	67
<i>In vitro</i>	An aqueous extract <i>Spirulina (Arthrospira) platensis</i> inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells. The extract inactivated HIV-1 infectivity directly when pre-incubated with virus before addition to human T-cell lines.	68

model. Their studies of the invasion of B16-BL6 melanoma, colon 26 M3.1 carcinoma, and HT-1080 fibrosarcoma cells through reconstituted basement membrane (Matrigel) shed light on the mechanism of the inhibition of the invasion. The authors suggest that Ca-SP could reduce lung metastasis of B16 - BL6 melanoma cells by inhibiting the tumor invasion of basement membrane, probably through heparanase activity and through the prevention of the adhesion and migration of tumor cells to laminin substrate. Their results showed that Ca-SP strongly inhibited the degradation of heparan sulphate by purified heparanase. According to these workers, this remarkable anti-heparanase activity might provide a promising basis for improved therapeutic approaches to tumor invasion and metastasis.⁵³

In a very recent study⁵⁴ (2000), c-phycocyanin (C-PC) from *Spirulina platensis* inhibited the growth of human leukemia K562 cells. The effect of phycocyanin was at first studied following the growth of the K562 cells in semi-solid agar culture at concentrations of 20, 40, 80, and 160 mg⁻¹. The results showed that phycocyanin inhibited the growth of the K562 leukemia cells in a dose-dependent manner with statistically significant inhibition observed at 80 and 160 mg⁻¹. The effect of phycocyanin was further studied using cell viability measurement using XTT dye reduction assay. Phycocyanin was again found to inhibit cell viability in a dose- and time-dependent manner. The IC₅₀ value of C-PC was found to be 72.5 mg⁻¹. Furthermore, flow cytometric assay based on DNA content analysis revealed that accumulation of K562 cells occurred in the G-1 phase when cells were incubated in C-PC for 6 days. There were higher percentages of cells in the G-1 phase at phycocyanin concentrations of 40 and 80 mg⁻¹. DNA fragment analysis did not show the ladder formation typically observed in apoptosis, indicating that a different mechanism may be at play in the inhibition process.⁵⁴

According to a Japanese patent,⁵⁵ oral administration of phycocyanin extracted from *Spirulina* was found to increase the survival rate of mice that had been injected with liver tumor cells. The lymphocyte activity of the treatment group was significantly higher than that of the control group, suggesting some sort of stimulation of the immune system.

Another study of interest is that by Qishen *et al.*⁵⁶ who reported enhancement of endonuclease activity and repair DNA synthesis by polysaccharides of *Spirulina platensis*. The effect of the extract was studied by means of endonuclease assay and radioautography. The results showed that the presence of the extract significantly enhanced both the repair activity of radiation-damaged DNA excision and the unscheduled DNA synthesis.⁵⁶ The same group⁵⁷ also found that an extract of *Spirulina platensis* caused a significant reduction of micronucleus frequencies induced by gamma-radiation in mouse bone marrow cells.

IMPLICATIONS OF THE STUDIES

Doll and Peto⁵⁸ did the landmark study establishing that 35% of all human cancer deaths appear to be associated with diet and nutrition. Since then numerous experimental, epidemiological, and clinical studies have proved this connection.⁵⁹⁻⁶⁰ These studies have demonstrated conclusively that numerous nutrient and non-nutrient constituents in foods in our diet have the potential to confer chemopreventive properties or enhance conventional therapy. For example, there is evidence that vegetables and fruits, rich sources of antioxidants like vitamin C, E, and beta carotene, might protect against different forms of cancer. There is also a recent body of evidence to suggest that physiological aging of the immune system may affect cell-mediated immunity that in turn results in cancer development, autoimmune disease, and susceptibility to infection.⁶¹

Radiation, chemotherapy, and surgery therapies cause side effects often worse than the cancer itself. Preventive and mitigation methods utilizing natural products directly or as adjuvant to conventional cancer treatment are therefore the focus of recent research.⁶² The studies summarized above suggest such a role for *Spirulina*. *Spirulina* may offer some degree of protection against certain forms of cancer through its effect on the immune system, through a direct effect in the repair of DNA, and antioxidant protection from reactive oxygen species generated during normal or abnormal metabolism and from toxic substances in the environment. Further research along these lines is recommended to validate these assumptions.

ANTI-VIRAL EFFECTS

A summary of the studies on the anti-viral effects of *Spirulina* is given in Table 3.

Interest in the study of antiviral effects of *Spirulina* was triggered by a report from researchers at the National Cancer Institute about the discovery of potent antiviral compounds from extracts of blue-green algae. Gustafson *et al.*⁶³ found that sulfonic-acid-containing glycolipids isolated from *Lyngbya lagerheimii* and *Phormidium tenue* were active against HIV-1 in cultured human lymphoblastoid CEM, MT-2, LDV-7, and C3-44 cell lines. At the time, these sulfolipids were given high priority by the NCI for further preclinical investigations and for evaluation of feasibility as candidates for clinical testing.⁶³ In subsequent studies this group screened about 600 strains of cultured cyanobacteria representing some 300 species. Approximately 10% of the cultures produced substances that caused significant reduction in cytopathic effects normally associated with viral infection.⁶⁴

According to Hayashi *et al.*,⁶⁵ the water extract of *Spirulina platensis* inhibited the replication *in vitro* of *Herpes simplex* virus type (HSV-1) in HeLa cells within the concentration range of 0.08-50 mg/ml. The extract did not have virucidal effect but interfered with the adsorption and

penetration of virus into host cells. Addition of the extract (2 mg/ml) 3 hours before infection resulted in inhibition of virus-specific protein synthesis without affecting host cell protein synthesis. The ID₅₀ for cytotoxicity of the extract to HeLa cells was 26.3 mg/ml, while the ED₅₀ for antiviral activity was found to be 0.173 mg/ml, giving an *in vitro* therapeutic index of 152. In an *in vivo* study involving experimental HSV-1 corneal infection of hamsters, they found that food containing the extract effectively prolonged the survival time of infected hamsters at doses of 100 and 500 mg/kg. From the data obtained in the *in vivo* study the authors postulated that *Spirulina* supplementation might prevent herpetic encephalitis.

Hayashi *et al.*⁶⁶ isolated from *Spirulina platensis* a novel sulfated-polysaccharide, calcium spirulan (Ca-SP), that inhibits the replication *in vitro* of several enveloped viruses including Herpes simplex type I (HSV-1), human cytomegalovirus (HCMV), measles virus, mumps virus, influenza A virus, and HIV-1 virus.⁶⁶ In a later study, Hayashi *et al.*⁶⁷ found that the anti-HIV-1 activity of Ca-SP is comparable to that of dextran sulfate (DS; a known potent anti-HIV-1 agent), while its anti-HSV-1 activity was four- to five-fold higher than that of dextran sulfate. The anti-HIV-1 activity of Ca-SP or DS was five and four times higher in cultures treated with Ca-SP or DS during infection when compared with that in cultures treated with these substances after infection. Ca-SP was found to be superior to DS in possible therapeutic application because 1) enhancement of viral replication at low concentrations, a usual phenomenon in DS, was not observed with Ca-SP, 2) Ca-SP was found to have a much lower anticoagulant effect than DS, 3) Ca-SP was found to have a much longer half-life in the blood of mice compared to DS, and 4) Ca-SP was four to five times more effective in inhibiting HSV-1 compared to DS.⁶⁷

More recently, Ayehunie *et al.*⁶⁸ reported that an aqueous extract of *Spirulina (Arthrospira) platensis* inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells. Depending on the cell type used, therapeutic indices (EC₅₀/IC₅₀) ranged between 200 and 6,000. The extract inactivated HIV-1 infectivity directly when pre-incubated with virus before addition to human T-cell lines. These authors found antiviral activity both in the polysaccharide fraction as well as in a fraction depleted of polysaccharides and tannins. The authors believe that the aqueous extract of *S. platensis* might be of potential clinical interest.⁶⁸

IMPLICATIONS OF THE STUDIES

Abuse and misuse of antibiotics has resulted in the emergence of new antibiotic-resistant bacteria and viruses. The most prominent viral disease, HIV/AIDS, has now claimed the lives of millions of people. In Africa and Asia, HIV/AIDS has become pandemic. Conventional anti-HIV drugs are

beyond the reach of ordinary people. Vaccines are still a long way to come. It is apparent that alternative sources be sought. Therapeutic use of herbs and algae products with known bactericidal and virucidal properties may offer alternative approaches. Whole-plant products and crude extracts often have ingredients that may work synergistically to effect viral or bacterial killing or inactivation. Resistance to these varied substances may not be as easy as to one single antibiotic. It is also known, at least for some natural products, that they exert their protective effect through stimulation or modulation of the immune system. Therefore instead of trying to find the magic chemical bullet to kill viruses or bacteria, the body's immune system is activated to protect against infection. Such an approach will also be useful even after infection sets in. For example, HIV/AIDS sufferers are usually affected by opportunistic infection once the virus debilitates the immune system. Enhancement of the immune system through therapeutic intervention with natural products may offer protection against such infection.

As summarized in the studies above, the antiviral activity of the aqueous extract of *Spirulina* has been demonstrated in various *in vitro* and animal models. The active component of the extract appears to be Ca-SP. This compound could be a good candidate for therapeutic intervention against HIV-1 and other viruses because of its low anticoagulant activity, long half-life in the blood, and dose-dependent bioactivity without stimulation of viral replication at low concentration.⁶⁹⁻⁷¹ Moreover Ca-SP has been shown to inhibit a host of other viruses that cause opportunistic infection by HIV-1 like cytomegalovirus and *Herpes simplex* virus. As far as therapeutic use is concerned, the aqueous crude extract has been shown to have similar effects. The crude extract has a potential to be used as a dietary supplement to offer an adjuvant effect to conventional treatment of viral infections. Since the polysaccharide extract is known to boost the immune system, the antiviral effect (inhibition of replication or attachment) may be augmented by the enhancement of the overall immune response of the body. This potential needs to be investigated further in more animal models and humans and under different modes of administration before conclusions are drawn about the effectiveness of such use.

EFFECTS ON HYPERLIPIDEMIA

A summary of studies on the cholesterol-regulatory properties of *Spirulina* is given in Table 4. One of the earlier studies on the reduction of serum cholesterol by *Spirulina* was that done on rats by Devi and Venkataraman.⁷² Since then several workers have confirmed this in studies involving animals and humans.

In an elaborate study involving feeding rats with high cholesterol diets with and without *Spirulina* supplementation, Kato *et al.*⁷³ found that the elevation of total chole-

Table 4. Summary of studies on cholesterol reduction and related effects

Type of Study	Summary of Studies	Reference
Mice	<p>The elevation of total cholesterol, LDL+VLDL cholesterol and phospholipids in the serum was reduced significantly when the high experimental cholesterol diet was supplemented with 16% <i>Spirulina</i>.</p> <p>The reduction in HDL cholesterol caused by the high cholesterol diet was also reduced in the mice fed the high cholesterol diet in the presence of <i>Spirulina</i>.</p> <p>Adipohepatosis induced by a high fat and high cholesterol diet was also reduced rapidly when the mice were shifted from the high fat high cholesterol diet to a basal medium supplemented with <i>Spirulina</i>.</p>	73
Rats	<p>Liver levels of triglycerides and phospholipids in rats fed a diet supplemented with 5% <i>Spirulina</i> were significantly lower than rats fed either 60% glucose or 60% fructose.</p>	74
Rats	<p>Levels of triglycerols and cholesterol were found to be significantly lower in rats on a diet supplemented with <i>Spirulina</i> compared to those on control diets. Prior to the measurement, fatty liver was induced by a single intraperitoneal injection (1 ml/kg) of carbon tetrachloride to both the control and experimental rats.</p>	75
Rats	<p>Feeding <i>Spirulina</i> at 5%, 10%, and 15% of the diet resulted in a significant inhibition of total and HDL-cholesterol ($p < 0.01$), triglyceride ($p < 0.05$) and phospholipid ($p < 0.01$) in fructose-induced hypolipidemic rats. The <i>Spirulina</i> diet group showed a statistically significant ($p < 0.01$) increase in the activity of LPL than the high fructose diet group suggesting role of LPL in lowering triglycerides.</p>	76, 77
Rats	<p>Rats fed 20% water-soluble fraction of <i>Spirulina</i> were found to have a high HDL to LDL ratio and low fasting serum glucose levels compared to rats fed a water-soluble fraction or casein.</p>	78
Mice	<p>Compared to the control group the <i>Spirulina</i>-supplemented mice showed significantly lower levels of total HDL and LDL cholesterol (31%, 20%, and 54% reduction, respectively) after 14 days of feeding. Further reduction in cholesterol levels was observed after 35 days of feeding.</p> <p>The HDL to LDL cholesterol ratio was found to be significantly higher (2 to 2.4-fold increase) in mice fed <i>Spirulina</i>.</p> <p>In mice fed <i>Spirulina</i> for 35 days, plasma levels of triglycerides decreased by 44% compared to the control group.</p>	80

Table 4. Summary of studies on cholesterol reduction and related effects (continued)

Human	<p>In the group (15 male volunteers) taking 4.2 g/day <i>Spirulina</i> continuously for 8 weeks, a statistically significant reduction of LDL-cholesterol ($p < 0.05$) was observed after 8 weeks.</p> <p>In the group taking the same amount for 4 weeks only, a statistically significant reduction in LDL cholesterol was found after 4 weeks ($p < 0.05$), but thereafter increased to its baseline value after the administration of <i>Spirulina</i> was discontinued.</p> <p>The atherogenic effect (a measure of fat deposition in arteries) declined significantly in the former group ($p < 0.01$).</p> <p>No significant difference in the level of HDL.</p>	81
Human	<p>In ischemic heart disease patients <i>Spirulina</i> supplementation significantly lowered blood cholesterol, triglycerides, LDL and VLDL cholesterol, and higher HDL cholesterol. Supplementation with 4 g/day <i>Spirulina</i> showed a higher effect in reducing serum total cholesterol and LDL levels than 2 g/day.</p>	82

terol, LDL+VDL cholesterol, and phospholipids in the serum was reduced significantly when the experimental high cholesterol diet was supplemented with 16% *Spirulina*. The fall in HDL cholesterol caused by the high cholesterol diet was also reduced in mice fed the high cholesterol diet in the presence of *Spirulina*. Adipohepatosis induced by a high fat and high cholesterol diet was also reduced rapidly when the mice were shifted from the high fat, high cholesterol diet to a basal medium supplemented with *Spirulina*. Their findings were supported by more recent studies. De Rivera *et al.*⁷⁴ measured liver levels of triglycerides and phospholipids in rats fed a diet supplemented with 5% *Spirulina*, and either 60% glucose or 60% fructose. The control animals were fed either 60% glucose or 60% fructose. Rats fed the *Spirulina* diet had lower levels of liver triglycerides and phospholipids compared to control groups.⁷⁴ Torres-Duran *et al.*⁷⁵ studied the effect of *Spirulina* on liver and serum lipid levels of rats where fatty liver was induced by a single intraperitoneal injection (1 ml/kg) of carbon tetrachloride. Liver lipid concentrations did not change in rats fed the purified diet with or without *Spirulina*. However, after carbon tetrachloride treatment, liver triglycerols and cholesterol were significantly lower in rats fed the *Spirulina*.⁷⁵

In a study involving rats, Iwata *et al.*⁷⁶ found that feeding *Spirulina* at 5%, 10%, and 15% of the diet resulted in a significant inhibition of total and HDL-cholesterol ($p < 0.01$), triglyceride ($p < 0.05$) and phospholipid ($p < 0.01$) in fructose-induced hypolipidemic rats. However, no differ-

ence in liver lipids was found between the high fructose diet and *Spirulina* diet groups. In a subsequent study Iwata *et al.*⁷⁷ attempted to elucidate the mechanism of the hypotriglyceridemic effect of *Spirulina*, by studying the activities of two kinds of lipases, lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL). The *Spirulina* diet group showed a statistically significant ($p < 0.01$) increase in the activity of LPL than the high fructose diet group. There was no significant difference in the activity of H-TGL in the two groups. Since LPL is a key lipolytic enzyme in the metabolism of TG-rich lipoproteins, it was postulated that the hypotriglyceridemic effect of *Spirulina* might be through its effect on the metabolism of lipoproteins.⁷⁷

Rats fed a 20% water-soluble fraction of *Spirulina* were also found to have a high HDL to LDL ratio and a low fasting serum glucose level compared with rats fed a water insoluble fraction or casein.⁷⁸ A significant increase in HDL cholesterol was also found in another study.⁷⁹ Additional evidence for cholesterol-regulating effect comes from Fong *et al.*⁸⁰ who studied the effect of *Spirulina* on plasma cholesterol and triglyceride levels in mice. Compared to the control group the *Spirulina*-supplemented mice showed significantly lower levels of total, HDL and LDL cholesterol (31%, 20%, and 54% reduction, respectively) after 14 days of feeding. Further reduction was observed after 35 days of feeding. The HDL to LDL cholesterol ratio was found to be significantly higher (2- to 2.4-fold increase) in mice fed *Spirulina*. In mice fed *Spirulina* for 35 days, plasma levels of triglycerides decreased by 44% compared to the control group.

However, there was no difference in triglyceride levels in the *Spirulina* and control groups after 14 days of feeding.⁸⁰

To date, there are three published reports of cholesterol-reduction effects of *Spirulina* in humans. In the first study by Nakaya *et al.*,⁸¹ 30 male volunteers with mild hyperlipidemia and mild hypertension were divided into two groups. Group A subjects were given *Spirulina* at 4.2 g/d⁻¹ and group B subjects were given the same amount of *Spirulina* for 4 weeks and were observed for the next 4 weeks without giving *Spirulina*. The results showed a statistically significant reduction of LDL-cholesterol ($p < 0.05$) in group A subjects after 8 weeks. The LDL-cholesterol also fell significantly in group B subjects after 4 weeks ($p < 0.05$), but thereafter increased to its baseline value after the administration of *Spirulina* was discontinued. No significant difference was observed in the level of HDL cholesterol. On the other hand, the atherogenic index (a measure of fat deposition in arteries) declined significantly in group A subjects ($p < 0.01$) after 4 weeks.⁸¹

Ramamoorthy and Premakumari⁸² studied 30 ischemic heart disease patients with blood cholesterol levels above 250 g/dl. Divided into three groups of 10, group A and B subjects were given 2 g and 4 g *Spirulina* per day, respectively, for 3 months, and group C served as control. The group with *Spirulina* supplementation had significantly lower blood cholesterol, triglycerides, LDL and VLDL cholesterol and higher HDL cholesterol. Supplementation with 4 g per day *Spirulina* showed a higher effect than a 2 g dose in reducing serum total cholesterol and LDL.

In a human clinical study involving 15 diabetic patients, Mani *et al.*⁸³ found a significant reduction in total lipids, free fatty acids, and triglyceride levels. A reduction in LDL/HDL ratio was also observed.

IMPLICATIONS OF THE STUDIES

Collectively the results of the animal and human studies summarized above provide support for the cholesterol-lowering activity of *Spirulina*.

A vast amount of experimental and epidemiological evidence shows the connection between diets high in fat and cholesterol and the incidence of cardiovascular disease. There is also an increased awareness among Americans that diets high in cholesterol present a risk of cardiovascular disease. Despite this, cardiovascular disease is the number one killer in the United States, claiming about one million lives a year and totaling 41% of all deaths. It is often said that a fast lifestyle makes it difficult for many Americans to make proper food choices. Supplementation with natural food supplements like *Spirulina* may contribute, in part at least, to an overall strategy to manage this serious health problem.

OTHER EFFECTS

This section sheds light on less extensively studied

potential applications of *Spirulina*.

Probiotic effects

Tsuchihashi *et al.*⁸⁴ found that an intake of *Spirulina* at 5% of the diet increased the population of *Lactobacillus* in the caecum of rats by 3 times over a control group of rats not fed *Spirulina*.⁸⁴ Similar results were obtained by de Mule⁸⁵ in *in vitro* studies with *Lactobacillus lactis* and *Candida albicans*.⁸⁵ More recently Parada *et al.*⁸⁶ have reported a stimulatory effect of extracellular products from algae on lactic acid bacteria including *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus*.

In humans, *Lactobacillus* is believed to have three functions: to improve digestion and absorption of foods, to protect from infection, and to stimulate the immune system. In patients with an Acquired Immune Deficiency Syndrome (AIDS), nutrient malabsorption associated with opportunistic infections from microorganisms like *Candida albicans* can speed expression of disease symptoms. One recommended strategy for halting the progression of AIDS is based on nutrient supplementation as well as supplemental *Lactobacillus*.⁸⁷ *Spirulina* might offer such a nutritional and therapeutic strategy.

Effects against diabetes, obesity, and blood circulation

According to Takai *et al.*,⁸⁸ a water-soluble fraction of *Spirulina* was found effective in lowering the serum glucose level at fasting while the water-insoluble fraction suppressed glucose level at glucose loading.⁸⁸ Similar results were found in other studies.⁷⁸⁻⁷⁹ In a human clinical study involving 15 diabetics, a significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day *Spirulina* supplementation.⁸³ In a double-blind-crossover study versus placebo, Becker *et al.*⁸⁹ have found that a supplementary diet of 2.8 g of *Spirulina* 3 times d⁻¹ over 4 weeks resulted in a statistically significant reduction of body weight in obese outpatients. *Spirulina* has also been found to suppress high blood pressure in rats.⁹⁰ A vasodilating property of rat aortic rings by *Spirulina* possibly dependent upon a cyclooxygenase-dependent product of arachidonic acid and nitric oxide has been reported by Paredes-Carbajal *et al.*⁹¹

Cheng-Wu Z *et al.*⁹² did a preliminary study on the effect of polysaccharides and phycocyanin on peripheral blood and hematopoietic system of bone marrow in mice. Their studies showed that C-phycocyanin and polysaccharides from *Spirulina* had a high erythropoietin (EPO) activity.

Effects against toxicities from heavy metals and other compounds

According to Yamane *et al.*,⁹³ rats with high mercury dosage showed rising blood urea nitrogen (BUN) and serum creatinine, both indicators of acute nephritis. The addition of 30% *Spirulina* in the diet resulted in a significant decrease in BUN and serum creatinine levels. Rats

given 3 pharmaceuticals, para-aminophenol (anodyne), gentamicin (antibiotic), and cis-dichlorodiamino-platinum (anti-cancer), showed similar kidney improvement on a *Spirulina* diet.⁹³ In a follow-up study, Fukino *et al.*⁹⁴ found similar effects of *Spirulina* on renal toxicity induced by inorganic mercury and cisplatin. In addition to BUN and serum creatinine, urinary excretion of alkaline phosphatase (ALP) and glutamic oxaloacetate transaminase (GOT) were measured as further indicators of renal function. The activities of both enzymes were significantly reduced in the group fed 30% *Spirulina*. The effective component was found in the water-soluble fraction of the *Spirulina* extract, within which the substances with a molecular weight of more than 100,000 were believed to be responsible. From this observation it was suggested that phycocyanin might be responsible in the suppression of renal toxicity.⁹⁴ Hepatoprotection from toxic compounds has already been discussed above under antioxidant effects of *Spirulina*. Shastri *et al.*⁹⁵ did studies on the protective role of dietary *Spirulina* on lead toxicity in Swiss albino mice. They observed a significant increase in the survival time in pre- and post-treated *Spirulina* compared with a control group without *Spirulina*. Lead-induced toxicities were reduced as suggested by the higher testes weight, animal weight, and tubular diameter in the *Spirulina* treated group.

Radiation protection effects

The radioprotective effect of a crude ethanol precipitate (CEP) of *Spirulina platensis* was studied using the micronucleus test in polychromatic erythrocytes (PCE) of mouse bone marrow. In this system the extract caused a significant reduction of micronucleus frequencies induced by γ -radiation. Gamma-radiation followed by treatment with CEP led to about the same radioprotective effect as CEP treatment followed by γ -radiation. From this the authors concluded that the protective compound probably acts as a DNA-stabilizing factor, and they ruled out the possibility of a radical scavenging mechanism.⁵⁷ The ability of CEP to reduce the incidence of micronucleated bone marrow cells is believed to reflect its antimutagenic and repair-stimulating capacities much as has been postulated by Schwartz *et al.*⁴⁹

Mazo *et al.*⁹⁶ subjected rats to gamma irradiation and followed intestinal barrier permeability to polyethylene glycol 4000. Addition of *Spirulina* to the diet led to near complete normalization of permeability. Feeding phycocyanin extract from *Spirulina* to rats exposed to x-rays (5 Gy) resulted in the normalization of decreases in dehydrogenase activity, energy-rich phosphate level, and efficiency of antioxidant defense observed in rats without phycocyanin supplementation.⁹⁷

Implications of the Results of the Studies

There are not many studies in the areas mentioned under this section, nor are the studies as rigorous as those

done on immune modulation, anti-cancer/anti-viral effect, and cholesterol-reduction effects. Nevertheless they offer insight into the potential of *Spirulina* to offer diverse health benefits. These areas deserve more research.

CONCLUSION

Despite the few human studies done so far on the health benefits of *Spirulina*, the evidence for its potential therapeutic application is overwhelming in the areas of immunomodulation, anti-cancer, anti-viral, and cholesterol-reduction effects. Traditional therapies always rely on the use of natural products and have been the source of information for the discovery of many drugs we have today. Currently, increased cost of health care has become a driving force in the shift towards interest in wellness, self-care, and alternative medicine, and a greater recognition between diet and health care. *Spirulina* is already in use in these new health care approaches. Further clinical research will help solidify the merit of its use.

Systematic screening for therapeutic substances from algae, particularly cyanobacteria (blue-green algae), has received greater attention recently.^{64, 98} Should therapeutic application be established for it, *Spirulina* would offer the following unique advantages and possibilities: (1) the technology for mass cultivation and harvest of *Spirulina* is well-established, (2) *Spirulina* has undergone two decades of toxicity testing in addition to its known centuries of human use, (3) microbiological and other safety standards have been established for *Spirulina* products, (4) the two most commonly grown species *Spirulina (Arthrospira) platensis* and *Spirulina (Arthrospira) maxima* are free from cyanobacterial toxins and can be grown (under controlled conditions) free of contaminant cyanobacteria by virtue of their adaptation to a highly alkaline environment. Indeed, it is this same property that makes it possible to grow *Spirulina* in land unsuitable for conventional agriculture, and (5) *Spirulina* has already been popularized through two decades of commercialization as a food and dietary supplement.

In a similar review article published in the *Journal of Applied Phycology* in 1993, we called the attention of researchers to further research in the areas of immunomodulation and anti-cancer effects of *Spirulina*. A comparison of the number of papers reviewed then and now clearly shows the great attention that is being given to research on the potential health benefits of *Spirulina*. The future holds great promise for more socially and professionally beneficial research. It is hoped that a careful evaluation of the results of the studies summarized above will provide a guide to future research and a basis for current therapeutic use of *Spirulina*.

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