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1.

Free radical scavenging action of Bio-Catalyzer No.11 (Immun'Age) and its by-product

Santiago, LA, Osato, JA, Hiramatsu, M, Edamatsu, R and Mori, A

Free Radical Biol. Med. 11 (4): 379-383, 1991

(Presented at the International Conference on Bioradicals detected by ESR Spectroscopy,
Yamagata, Japan, 12-16 June 1994)

Oxygen free radicals are active chemical substances which have been known to play an important role in the occurrence of a variety of disorders such as epilepsy, ischemia, trauma, rheumatoid arthritis, cancer as well as in the natural process of aging. Among the reactive oxygen intermediates, hydroxyl free radicals ($\cdot\text{OH}$) are the most potent in damaging proteins and deoxyribonucleic acid (DNA) through the oxidation of these biological materials. They also strongly enhance destruction of lipids through a process called peroxidation. Sources of $\cdot\text{OH}$ include ionizing radiation, ultrasound, lithotripsy, lyophilization, ozone and ethanol metabolism, iron solutions and guanidino compounds in the brain.

Using electron spin resonance (ESR) spectrometry, our findings proved that Bio-Catalyzer and its by-product, at a concentration of 45.45 mg/ml are effective antioxidants, being able to scavenge 95% $\cdot\text{OH}$ generated by the Fenton reaction which is an iron-induced oxidation. On the other hand, 5% superoxide ($\cdot\text{O}_2^-$) generated by hypoxanthine-xanthine oxidase system and 11% of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 25 mg/ml Bio-Catalyzer. Bio-Catalyzer by-product was less potent, scavenging 5% $\cdot\text{O}_2^-$ and none of DPPH radicals. Moreover, the results of *in vivo* tests demonstrated that 1g/kg body weight of Bio-Catalyzer administered 30 minutes prior to iron injection significantly inhibited formation of thiobarbituric acid reactive substances (TBARS), which is an index of lipid peroxidation.

The above-mentioned findings suggest that Bio-Catalyzer or its by-product may be useful in the management of lipid peroxidation, traumatic epilepsy and aging.

2.

Bio-Catalyzer No.11, Bio-Catalyzer 2B, Bio-Catalyzer No.66 quenched free radicals and inhibited lipid peroxidation in iron-induced epileptic focus in rats

Santiago, LA, Osato, JA, Hiramatsu, M and Mori, A, *Oxygen Radical.*: 405-408, 1992

Chemical studies on the reactions involved in epilepsy caused by injuries implicate the most potentially damaging hydroxyl radicals ($\cdot\text{OH}$), which are known to initiate lipid peroxidation in the lining of the brain. In our previous *in vitro* study, it was documented that Bio-Catalyzer and its by-product Bio-Catalyzer 2B possess scavenging activity against $\cdot\text{OH}$. In addition, using an experimental rat model of iron-induced epilepsy, Bio-Catalyzer was found to inhibit formation of thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation.

In this experiment, using the same *in vitro* experimental procedure, it was found that the new health product Bio-Catalyzer No.66 appeared to be the most potent as a free radical scavenger, compared to Bio-Catalyzer and Bio-Catalyzer 2B, requiring only 25 mg/ml to quench approximately 95% $\cdot\text{OH}$ while the latter two required twice as much to elicit the same effect.

To further analyze the scavenging activity of Bio-Catalyzer No.66, rat brain homogenate was induced to generate the hydrogen and carbon-centered radicals by the addition of a mixture of iron solution and ascorbic acid (vitamin C). Bio-Catalyzer No.66 was also found to scavenge the resulting free radicals.

Moreover, in *in vivo* models of iron-induced epilepsy, Bio-Catalyzer 2B and Bio-Catalyzer No.66 were found to decrease the TBARS level in the brain while samples taken from rats which were not treated with Bio-Catalyzer 2B nor Bio-Catalyzer No.66 had high amounts of TBARS.

These data suggest that Bio-Catalyzer, Bio-Catalyzer 2B and Bio-Catalyzer No.66 may act as natural antioxidative health foods due to their free radical scavenging activity, as well as a protective barrier against injury due to reactive chemical species.

3.

Stability of the hydroxyl radical scavenging components of the health food "Immun'Age"

Santiago, LA, Osato, JA and Mori, A

Med. Sci. Res. 20: 27-28, 1992

Studies on Immun'Age have suggested that its free radical scavenging activity and its inhibitory action against the formation of thiobarbituric acid reactive substances (TBARS) are the mechanisms to which the purported therapeutic value of this health food can be attributed. In an attempt to establish the stability of the components of Immun'Age that are responsible for the observed activities, the product was processed to separate the alleged active ingredients. The four water-soluble fractions obtained had molecular weights of over 200,000; between 50,000 and 200,000; between 10,000 and 50,000, and; below 10,000. These isolated materials were subjected to conditions such as treatment with 60% perchloric acid which gives a final pH of 1.2, and heating to 100°C for one hour which would normally inactivate biological materials. Immun'Age granules were also tested for stability two years after storage at 4°C. Electron spin resonance (ESR) spectrometry, a method of measuring the presence and activity of chemical species, was then used to analyze the free radical scavenging activity of the isolated components and Immun'Age granules. It was found that all samples retained their hydroxyl radical scavenging activity.

Results confirmed that Immun'Age can remain stable and active even after acid treatment, boiling and long-term storage.

4.

Decreased release of monoamine metabolites in iron-induced epileptogenic focus in the rat following administration of Bio-Catalyzer

Santiago, LA, Osato, JA, Kabuto, H and Mori, A

Med. Sci. Res. 21: 139-141, 1992

In previous studies, Bio-Catalyzer was claimed to have prevented iron-induced free radical reactions that are believed to cause damage in the brain, particularly its membrane and the blood vessels in it. This action of Bio-Catalyzer was attributed mainly to its property as a hydroxyl radical scavenger and as an inhibitor of the peroxidation of lipids that are present in large quantities in the brain. In this experiment, the effects of Bio-Catalyzer on the release of neurotransmitters were determined.

Neurotransmitters are chemical substances that are elicited by stimulated nerve cells. These include 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), among others. Epileptic seizure is an example of an oxidative stress-stimulated reaction of the nerve cell as induced by iron. The results of the experiment revealed that after iron injection and the subsequent onset of epileptic seizure, there was an increase in the release of the afore mentioned neurotransmitters by the nerve cells, except that of HVA. However, with Bio-Catalyzer administration, which was injected into the abdomen simultaneously with iron injection in the brain, such reaction was suppressed.

This study confirms that Bio-Catalyzer can provide protection to the brain against oxidative stress and consequently, protection from damages arising from such insult

5.

Age-related increases in superoxide dismutase activity and thiobarbituric acid-reactive substances: Effect of Bio-Catalyzer in aged rat brain

Santiago, LA, Osato, JA, Liu, J and Mori, A

Neurochemical Research. 18 (6): 711-717, 1993

One of the explanations for the normal life process of aging is the involvement of reactive oxygen species (ROS) associated with the observed increase in the level of the antioxidant superoxide dismutase (SOD) which is produced as a normal body defense against the damaging Reactive Oxygen Intermediates (ROI). In addition, studies have revealed that during aging the index of lipid peroxidation, thiobarbituric

acid reactive substances (TBARS), are increased. In this study, the effect of Bio-Catalyzer on aging of the brain was investigated by allowing rats to grow old, after which, SOD activity and TBARS level in different parts of the brain were measured. The results showed that corresponding to the increased life span and improved physical conditions observed after intake of Bio-Catalyzer, SOD activity increased while the TBARS decreased.

These findings suggest that Bio-Catalyzer can enhance the protective action of SOD while it can suppress the age-related damage in the brain due to peroxidation of lipids that are abundant in the brain.

6.

**Antigenotoxic potential of Bio-Catalyzer α p No.11
(Immun'Age against somatic cell genotoxic agents)**

de Castro-Bernas, G, Remo, GM, Abrigo, RB, San Miguel, MM, Osato, JA,
Santiago, LA and Mori, A
Med. Sci. Res. 21 (3): 107-108,1993

Deoxyribonucleic acid (DNA) is a material within the body which carries genetic information that is responsible for the development of individual characteristic traits. Disruption of the normal process of development and maturation due to damage to DNA is called mutation. This mutation leads to cancer formation. Substances causing DNA damage are called mutagens. Genotoxic agents are chemical mutagens that induce heritable changes in the DNA. The alleged therapeutic action of Immun'Age on a number of cancers and other human diseases that are free radical-mediated has been attributed to its hydroxyl radical action and to its inhibitory property against thiobarbituric acid reactive substances (TBARS) formation. In this experiment, the potential antigenotoxic effect of Immun'Age against cyclophosphamide and dimethylnitrosamine (DMN) was studied. Cyclophosphamide is both a carcinogen and an anti-tumor drug while DMN is a carcinogen. It was found that Immun'Age was able to inhibit the DNA-damaging effects of the chemicals introduced as manifested by the observed reduction in the number of micronuclei in polychromatic red blood cells.

The results of this experiment provide clues to the probable mechanism(s) of action of Immun'Age in cases of disorders due to DNA damage.

7.

**Antioxidant protection of Immun'Age in cerebral
ischaemia-reperfusion injury in the gerbil**

Santiago, LA, Osato, JA, Ogawa, N and Mori, A
NeuroReport. 4: 1031-1034, 1993

Cerebral ischaemia-reperfusion injury (IRI) is a kind of damage that is caused by an increase in the production of superoxide radicals ($\cdot O_2^-$) as a consequence of a loss (ischaemia) and sudden upsurge (reperfusion) of blood supply in the brain. The $\cdot O_2^-$ undergo further chemical reaction to produce the potent hydroxyl radicals ($\cdot OH$). The resulting $\cdot OH$ then initiates peroxidation of lipids which leads to the formation of another type of free radical called carbon-centered radicals (CCR). This experiment used gerbils to demonstrate the occurrence of lipid peroxidation following IRI and the inhibition of the same in animals which were fed with Immun'Age prior to the induction of IRI. It was found that the levels of CCR and TBARS in the different parts of the brain were significantly reduced.

The results suggest that Immun'Age, through its antioxidant and free radical scavenging activities, can provide protection against the harmful effects of IRI.

8.

Effect of Immun'Age on the alcohol-induced changes in blood substances in humans

Koide, Y, Miyagawa, F, Osato, JA, Santiago, LA and Mori A
Neurosciences. 19s.1: 85-88, 1993

The amount of glucose and superoxide dismutase (SOD) in the blood has been known to undergo changes during alcohol intake. To determine the effects of Immun'Age on these substances, a group of male subjects was allowed to drink alcohol. In addition, half of the group was given Immun'Age and the other half was not. Measurement of the afore mentioned substances was made at three time points: before alcohol intake and then 40 and 120 minutes after drinking. The blood sugar level of those who did not receive Immun'Age was significantly reduced 120 minutes after alcohol intake while the activity of the protective antioxidant enzyme SOD was found to decrease 40 minutes after drinking. However, during the second measurement, the SOD activity was found to be increased. On the other hand, those who received Immun'Age had no change in glucose level after alcohol intake. The SOD activity was initially high, most probably due to the enhancing action of Immun'Age on this enzyme, a finding supportive of what we found in our previous study. Moreover, the activity level 40 minutes after drinking remained high and was significantly higher than that shown without Immun'Age. This indicates that Immun'Age has a potential to increase the SOD activity and to prevent the decrease of SOD activity due to alcohol drinking.

These findings provide evidences about the protective action of Immun'Age against alcohol-induced changes in blood substances in humans.

9.

Studies on biological activities of Immun'Age

Okuda, H, Ominami H, Zhou, A, Matsuura, Y, Osato, JA and Santiago, LA
The Clinical Report. 27 (11): 4249-4258, 1993

A series of experiments using laboratory animals was conducted to determine the biological activities of Immun'Age.

Immun'Age was found to reduce the lipid peroxide content in the liver and serum of rats which were given heat-treated corn oil. This implies protection rendered by Immun'Age against liver injury induced by lipid peroxides.

In a separate set-up, fat cells were taken from rats and were induced to undergo breakdown of fats (lipolysis) by treatment with the hormone epinephrine. When given in combination with Bio-Normalizer, epinephrine was able to strengthen its ability to induce lipolysis. Immun'Age alone, however, was not able to stimulate lipolysis. This enhancing ability of Immun'Age for epinephrine-induced lipolysis may be a suitable approach for the reduction of body fat in obese patients.

Toxohormone-L is another lipolytic substance that has been detected in the abdominal fluid of patients with cancers. It has also been found to suppress food intake. These could be the reasons for the observed weight loss and poor appetite among cancer patients. In the experiment using fat cells taken from rats to which sarcoma (a type of cancer) cells and Toxohormone-L have been introduced, Immun'Age was able to reduce lipolysis. This suggests that Immun'Age can help cancer patients in preventing weight loss as well as in increasing their appetite.

Another aspect of this study dealt with mice which were induced to develop tumors by injecting sarcoma cells under their skin. It is an established fact that one of the cells of the immune system, natural killer (NK) cell, has the ability to readily recognize and subsequently destroy a wide variety of cancer cells. In this experiment, mice which received Immun'Age after the introduction of sarcoma cells, demonstrated an increase in NK cell activity. This provides clues about the anti-cancer activity of Immun'Age.

10.

The biological activities of Bio-Catalyzer α : ρ No.11 (Immun'Age)

de Castro-Bernas, G, Remo, GM, Abrigo, R, Cuadra, MS, Urbano,
G, Santiago, LA, Osato, JA and Mori, A

Trends in Traditional Medicine Research, Chan, K, *et. al*,

The School of Pharmaceutical Sciences, University of Science, Malaysia: 366-375, 1993

Bio-Catalyzer α : ρ No.11 (Immun'Age), a fermented natural health food product, was found to be a potent scavenger of hydroxyl radicals and has some ability to quench superoxide and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Experiments also showed that Immun'Age significantly inhibited thiobarbituric acid reactive substances (TBARS) formation, an index of lipid peroxidation among animals which were induced to develop epilepsy by iron injection into the brain. The hydroxyl radical scavenging components of Immun'Age were found to be resistant to heat (100 °C for one hour), and to high acidity (pH1.2). Immun'Age granules can also be stored for two years at 4 °C without loss of activity. Studies to determine the potential of Immun'Age to fight against invading bacteria revealed that Immun'Age can inhibit the growth of commonly encountered microorganisms in the digestive tract. In addition, Immun'Age was also found to inhibit the damaging effect of genotoxic agents such as dimethylnitrosoamine (DMN) and cyclophosphamide when injected into the abdomen or when fed to experimental mice.

11.

Antioxidant protection of Immun'Age against post-traumatic epilepsy and ischemia-reperfusion injury

Santiago, LA, Osato, JA and Mori, A

Frontiers of reactive oxygen species in biology and medicine. 471-472, 1994

Aspartate is an excitatory amino acid (EAA) which, when present in large quantities, causes rapid relay of information among nerve and brain cells. This excitation of cells can be very fast as demonstrated in cases of convulsions, ischemia-reperfusion injury (IRI) and epileptic seizures. The release of aspartate from the junctions between nerve cells has been observed to be stimulated by a chemical substance called potassium ion (K⁺).

To support the results of our previous findings regarding the neuroprotective antioxidant property of Immun'Age, slices of mouse brain were used in this study wherein Immun'Age was administered together with the solution containing K⁺. The amount of aspartate was then subsequently measured. It was observed that Immun'Age was able to cause a significant reduction in the K⁺ -stimulated aspartate release.

As a functional food, Immun'Age appears to suit the purpose of blocking the production of EAA's like aspartate, thus, it may be considered a new type of neuroprotective agent.

12.

Effects of Immun'Age on serum components and immunological functions in humans

Santiago, LA, Uno, K, Kishida, T, Miyagawa, F, Osato, JA and Mori, A

Neurosciences. 20s: 149-152, 1994

The effect of one-month administration of Immun'Age on the immunological, biochemical and antioxidant functions of the blood and serum of 14 healthy and unhealthy subjects was studied. There was an observed increased mean rate of gamma (γ)-interferon production in both groups. The interferon- γ production slightly decreased after the second week then went up to the original level on the fourth week of Immun'Age intake. Interferon- γ and - β correspond to the non-specific and specific immunological functions, respectively. The exceptionally high levels of GOT and GPT (liver function markers) and the lipid components in three unhealthy subjects were significantly decreased after 14 days of treatment. In addition, the renal integrity such as uric acid level was maintained and other biochemical parameters such as the phospholipids concentration declined significantly ($p < 0.05$).

These results suggest that Immun'Age has the capacity to improve the general condition and the quality of life of patients with impaired immune competence or malfunctioning liver.

13.

Effects of dietary supplements on IFN producing capacity in humans

Kishi, A, Uno, K, Matsubara, Y, Osato, JA and Kishida, T

Journal of Interferon Research .14s.1: s179, 1994

(Presented at the 1994 ISICR Annual Meeting on the Interferon System,
Budapest, Hungary, 2-7 October 1994)

Interferons are substances produced by immune cells as a normal defense reaction to fight, mainly against virus infections. The interferon-producing capacity of blood cells is considered to be a useful parameter in evaluating immunological functions. Since Immun'Age has been documented to possess immunomodulating activity, its effect on the interferon-producing capacity of human whole blood was investigated in this experiment. Immun'Age was able to enhance the production of interferon.

This finding suggests that Immun'Age, being an enhancer of interferon production, can help improve the general condition and the quality of life of patients with impaired immune competence without any side effects.

14.

**Antimicrobial potential of Bio-Catalyzer α : ρ No.11 (Immun'Age)
against enteric microorganisms**

Osato, JA, de Castro-Bernas, G, Remo, GM, Cuadra, MS, Urbano, G,
Abrigo, MRB, Santiago, LA and Takamizawa, K

Acta Manilana. 42: 9-14, 1994

Bio-Catalyzer α : ρ No.11 (Immun'Age) has been found to be a potent and stable free radical scavenger. It can also help the body cope with infection and diseases by enhancing the activity of immune cells. In this study, the effect of Immun'Age on a number of opportunistic and pathogenic bacteria was determined. Opportunistic bacteria are micro-organisms that are normally found in the human body but can become causes of disease when the immune function of the body is impaired. Pathogenic bacteria, on the other hand, are those which are naturally disease-causing organisms. The bacteria on which the activity of Immun'Age was tested were propagated in the laboratory from specimens taken from the digestive tract of patients. The experiment revealed that when the test microorganisms were exposed to Immun'Age, the proliferation of organisms was minimal, seen as a thin layer of bacterial growth on the medium.

The result suggests that Immun'Age has the potential to protect the body from infections in the stomach and intestines by stopping the growth of commonly encountered opportunistic and pathogenic microorganisms found in the digestive tract.

15.

Effect of Immun'Age on macrophage chemotaxis in naturally-occurring gingivitis rat

Shinohara, M, Morikawa, Y, Ogata, K, Osato, JA and Ohura, K

Canadian Journal of Physiology and Pharmacology, Volume 72, Supplement 1, 1994.

(Presented at the XIth International Congress of Pharmacology,
Montreal, Canada, 24-29 July 1994)

Macrophages are cells of the immune defense mechanism which are responsible for "eating" and the subsequent killing of disease-causing foreign materials that gain entry into the body. Macrophages accumulate at the site of infection by a process called chemotaxis. In this experiment, rats that are genetically manipulated so as to develop gingivitis were used to examine the effect of Immun'Age on

macrophage chemotaxis. Gingivitis is the inflammation of the gums due to infection. It was found that 2% Immun'Age solution given to the rats by mouth can significantly reduce chemotaxis.

This is an implication that Immun'Age plays a role in the functioning of the immune system.

16.

Immun'Age modulates free radicals in brain, blood and macrophage

Osato, JA, Afanas'ev, IOB, Korkina, LG, Santiago, LA, Horitsu, H, and Mori, A
Journal of Free Radicals in Biology & Medicine. Volume 2, Number 3 (F/G9): 1994

To provide for the scientific basis of the purported therapeutic and preventive actions of Immun'Age (IMMUN'AGE), a fermented functional health food made from papaya, we studied by electron spin resonance/spin rapping and chemiluminescence (CL) methods, its effects on the free radical production in different systems. IMMUN'AGE inhibited hydroxyl, peroxy, carbon-centered, and lipid peroxides in various rat brain regions; suppressed oxygen radicals in cell-free systems such as the Fenton reaction, xanthine-xanthine oxidase, $H_2O_2 + NaClO$, $H_2O_2 +$ horseradish peroxidase; reduced spontaneous and menadione-induced superoxide release from human erythrocytes: decreased luminol-amplified CL but increased lucigenin-dependent CL; and enhanced superoxide dismutase activity in inflamed murine macrophage. While IMMUN'AGE prevented the formation of hydroxyl and peroxy radicals, it induced the production of intracellular superoxide radicals by dormant and activated phagocytes, human neutrophils, and rat peritoneal macrophage.

17.

Effects of Immun'Age (A Food Supplementation) on Free Radical Production by Human Blood Neutrophils, Erythrocytes, and Rat Peritoneal Macrophages

Osato, JA, Korkina, LG, Santiago, LA, Afanas'ev, IB
Supplement to Nutrition. Volume 11, Number 5: 568-572, 1995.

Immun'Age, a natural Japanese health food prepared by the fermentation of *Carica papaya*, exhibits therapeutic properties against various pathologies including tumors and immunodeficiency. To understand the mechanism of Immun'Age's therapeutic effects, we studied its action on the production of active oxygen species in cell-free systems (the Fenton reaction, the xanthine-xanthine oxidase system, and the hydrogen peroxide-hypochloride or hydrogen peroxide-horseradish peroxidase systems) and by human blood neutrophils and erythrocytes and rat peritoneal macrophages. Immun'Age efficiently inhibited the formation of oxygen radicals in cell-free systems and partially decreased spontaneous and menadione-stimulated superoxide production by erythrocytes, but manifested both stimulatory and inhibitory effects on oxygen radical release by dormant and activated phagocytes (neutrophils and macrophages). We suggest that Immun'Age is able to enhance the intracellular production of innocuous superoxide ions and, at the same time, to diminish the formation of reactive hydroxyl radicals, perhaps by the inactivation of ferrous ions, the catalysts of the superoxide-driven Fenton reaction. We also propose that the normalization of an organism's levels is one of the molecular mechanisms of Bio-Normalizer activity.

18.

Antioxidant Defenses of Immun'Age

Osato, JA, Santiago, LA, Mori, A and Horitsu, H

Magnetic Resonance in Medicine. 6:306-308, 1995

(Proceedings of the International Conference on Bioradicals Detected by ESR Spectroscopy in conjunction with the 16th Annual Meeting of the Japanese Society of Magnetic Resonance for Life Science)
Yamagata, Japan, 12-16 June 1994

The antioxidant properties of Immun'Age have been previously documented. In an attempt to better understand its mechanism of action, this study involved the detection of non-enzymatic and enzymatic components of Immun'Age which could be responsible for its ability to react with active oxygen species and their derivatives. Results revealed that among the non-enzymatic constituents of Immun'Age are glutathione and the amino acids methionine, arginine, threonine, tyrosine, and lysine which are known to possess antioxidant properties due to the hydroxyl, thiol and sulfhydryl groups that they contain. The enzymes found in Immun'Age include SOD, catalase, and peroxidase.

With these results, it can be concluded that Immun'Age is effective in protecting cells against the toxicity and mutagenicity of active oxygen species by virtue of its capacity to scavenge free radicals.

19.

Antioxidant Activity of Japanese Skin Care Product Runn-Runn

Osato, JA, Santiago, LA, and Mori, A

Magnetic Resonance in Medicine. 6:324-326, 1995

Runn-Runn, a brown granular face wash and body skin care made by the fermentation of rice bran and Immun'Age, was found to scavenge 99.0% of DPPH radicals at 100mg/ml, 99.2% of hydroxyl radicals generated from the Fenton reaction at 50mg/ml, 97.03% of superoxide radicals produced by the hypoxanthine-xanthine oxidase system at 50mg/ml, 85.0% of carbon-centered radicals generated in rat homogenate at 10mg/ml. This activity may be ascribed to its two major antioxidative components, Immun'Age and rice bran.

Rice bran is rich with known antioxidants such as alpha-tocopherol, oryzanol, isovitexin, squalene, phenolic compounds, and superoxide dismutase-like activity. Immun'Age contains malic and citric acids, vitamin C, simple sugars, SOD, sulfhydryl amino acids, glutathione, peroxidase, catalase and yeast.

Cancer of the skin implicates free radicals generated by ultraviolet irradiation. These harmful species can cause accelerated aging, excessive oxidants, increased collagen synthesis and lipid peroxidation, thereby damaging the skin. Runn-Runn may be used as an antioxidant for skin care treatment as it protects against potentially harmful effects of ultraviolet irradiation that can cause accelerated aging and excessive collagen synthesis of the skin.

20.

Nature's Food Antioxidant in Health and Disease Prevention

Santiago, LA, Osato, JA and Mori, A

Magnetic Resonance in Medicine. 6:327-329, 1995

(Proceedings of the International Conference on Bioradicals Detected by ESR Spectroscopy in conjunction with the 16th Annual Meeting of Japan Society of Magnetic Resonance for Life Science)
Yamagata, Japan, 12-16, June 1994

The human body is highly endowed with antioxidant defenses which, however, are depleted when tissues are continuously attacked and damaged by free radicals. Supplementation with food dietary antioxidants is thus regarded as an important yet conservative approach in natural remedies against free radical-induced diseases. This study focused on the detection of free radical scavenging activity of some food antioxidants such as papaya, bakers yeast, soybean paste (miso), rice bran, horseradish (wasabi)

and Immun'Age, using electron spin resonance (ESR) spectroscopy. The samples tested exhibited varying degrees of potency in scavenging superoxide anion, DPPH, and hydroxyl free radicals. Immun'Age had a weak scavenging action against DPPH and superoxide anion, but gave a relatively strong activity against hydroxyl free radicals.

Considering their intrinsic nutrient values and antioxidative protection, these foods are envisaged as double-pronged approaches in nutrition, health and medicine.

21.

**Radioprotective and Antioxidant Effects of Zinc Aspartate and Immun'Age
in Children with Acute Myeloleukemia and Lympholeukemias**

Korkina, LG, Osato, JA, Chivilyeva, I, Samochatova, E, Cheremisina, Z and Afanas'ev, I

Supplement to Nutrition 11 (5): 555-558, 1995

(Presented at the 4th Western Pacific Congress on Chemotherapy and Infectious Diseases,
Manila, Philippines, 4- 7, December 1994)

It is well known that a large amount of free radicals are formed in cases of leukemia. The treatment regimen for this cancer of the blood includes chemotherapy and irradiation which are mediated by oxygen radicals and which are known to have side effects that can lead to encephalopathy or diseases of the brain. This study was carried out to determine the efficiency of Immun'Age as an antioxidant in preventing irradiation-induced brain diseases. In addition, zinc aspartate was used as a source of the trace element zinc, which has been known to possess antioxidant properties.

In this study it was found that Immun'Age was able to suppress the development of encephalopathy. The liver functions, too, which are usually adversely affected during chemotherapy and irradiation were improved by Immun'Age treatment. Moreover, Immun'Age was able to restore the level of the protective antioxidant glutathione which was initially decreased after irradiation treatment while the activities of the main antioxidants superoxide dismutase (SOD) and catalase were stimulated. The number of immune cells called granulocytes usually decrease following chemotherapy and irradiation therapy. This leads to insufficient oxygen radical production, thus disabling the immune system in exerting its protective functions. In this study, Immun'Age was able to stimulate oxygen radical production by monocytes to compensate for the granulocyte deficiency. More importantly, there were no adverse side effects observed among the patients. Zinc behaved similarly as the Immun'Age in inhibiting oxygen radical overproduction by circulating white blood cells.

It can be inferred from the results of this experiment that Immun'Age can be a promising medicine as a supportive care for patients undergoing intensive courses of chemotherapy and irradiation. It can help maintain a high quality of life for ailing cancer patients.

22.

Protective action of Immun'Age against oxidative stress and brain disorders

Santiago, LA, Osato, JA and Mori, A

Oxidative Stress and Aging. Cutler, RG, Packer, L, Bertram, J and Mori, A, eds. Birkhauser Verlag : 245-257, 1995 [Presented at the:(1) 1st International Meeting on Oxidative Stress and Aging, Hawaii, USA, 22-26 March 1994; (2) 24th Annual Meeting of the American Aging Association, Washington, DC, USA, 14-

18 October 1994; (3) International Symposium on Natural Antioxidants: Molecular Mechanisms and Health Effects, Beijing, China, 20-24 June 1995, and; (4) International Conference on Society, Stress and Health in Countries

Undergoing Rapid Transition,
Moscow, Russia, June 27-July 2, 1995]

Oxidative stress or the cell killing effect of reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radical, superoxide anion and hydrogen peroxide leads to cell degeneration in the brain resulting in various disorders like epilepsy, ischemia and aging. Antioxidants, on the other hand, can retard or even abort these oxidation reactions. This study on experimental rats, was carried out to determine the effect of Immun'Age on methylguanidine (MG), a chemical compound which has been shown to induce

convulsions due to its hydroxyl radical-generating property. This study also investigated gamma (γ)-aminobutyric acid (GABA)-mediated neurotransmission. GABA is a major inhibitory transmitter in the brain which when produced in low quantities can lead to epileptic seizures. Oxidative stress indeed hastens aging and enhances the release of stimulatory transmitters in the brain which can result in epileptic seizures and nerve cell damage. Following oral administration of Immun'Age, the activity of the antioxidant superoxide dismutase (SOD) increased among aging rats, whereas the indicators of lipid peroxidation called thiobarbituric acid-reactive substances (TBARS) decreased. These corresponded to increased life span and improved physical conditions of the experimental animals. However, no correlation between oxidative stress and MG was found, both as a function of age and Immun'Age supplementation in the diet of the animals. On GABA, Immun'Age was found to modulate its release from inhibitory nerve cells suppressed by high concentrations of potassium ion (K^+) and oxidative stress.

These findings support the earlier propositions of Immun'Age being a useful preventive antioxidant against oxidative stress and epilepsy.

23.

**Bio-Catalyzer α - ρ No.11 (Immun'Age) Supplementation:
Effect of Oxidative Stress to Isolated Rat Hearts**

Haramaki, N, Marcocci, L, D'Anna, R, Yan, LJ, Kobuchi, H and Packer, L
Biochemistry and Molecular Biology International. 36 (6): 1263-1268, 1995

[Presented at the: (1) Conference of the Oxygen Club of California on Oxidants and Antioxidants in Biology, Novel Antioxidants and Health Effects of Antioxidant Nutrients, Oxidative Stress and Signal Transduction, Sir Francis Drake Hotel, San Francisco, CA, USA, 22-24 March 1995, and; (2) Conference of the Oxygen Club of California on Biological Oxidants and Antioxidants, Fess Parker's Red Lion Resort, Santa Barbara, CA, USA, 8-10 February 1996]

Heart tissue is a well recognized target of oxidative stress. In particular, ischemia-reperfusion conditions, such as those occurring during a heart attack or myocardial infarction, are known to increase cellular damage as a result of free radical generation. Immun'Age has been previously found to possess antioxidant properties and as such, may provide a protective action against heart disease. To better define the antioxidant properties of Immun'Age, the effects of orally supplemented Immun'Age on oxidative damage in rat hearts were investigated. It was found that Immun'Age was able to lower the leakage of the enzyme lactate dehydrogenase (LDH) from the heart tissue which has been known to be otherwise increased in cases of ischemia-reperfusion injury. To further study the antioxidant effect of Immun'Age, oxidative stress was induced by chemically stimulating the production of peroxy radicals. The resulting levels of thiobarbituric acid-reactive substances (TBARS) and protein carbonyl derivatives in experimental rats which were not fed with Immun'Age were found to be significantly high whereas they were found to be reduced in those which received the food supplement.

The data indicates that orally supplemented Immun'Age can help protect the heart against injury caused by ischemia-reperfusion.

24.

Free radical regulatory and immunomodulatory effects of Immun'Age

Osato, JA, Afanas'ev, I, Korkina, L, Santiago, LA, Mori, A and Takamizawa, K

Nutrition, Lipids, Health, and Disease. Ong, ASH, Niki, E and Packer, L, eds. 4: 45-58; 1995

[Presented at the: (1) UNESCO/COSTAM SFRR-Asia Workshop on Nutrition, Lipids, Health, and Disease, Penang, Malaysia, 1-3 September 1994, and;
(2) 7th Biennial Scientific Meeting of the International Society for Free Radical Research, Sydney, Australia, 6-10 November 1994]

Phagocytosing cells such as circulating white blood cells and macrophages, being the most powerful sources of reactive oxygen species (ROS) in the body, are commonly used to evaluate the free radical scavenging activity of potential antioxidants like Immun'Age.

Since Immun'Age has been previously documented to possess antioxidant properties, this study was carried out to determine its effects on free radical production in cell-free systems, as well as oxygen radical production by inflamed and normal rat macrophages and neutrophils. In this study, Immun'Age was found to inhibit the production of active oxygen species (hydroxyl and superoxide) in a concentration-dependent manner in cell-free test system. On the other hand, superoxide production by macrophages was found to be enhanced while the production of the very active hydroxyl and hydroxyl-like radicals was decreased. Simultaneously, Immun'Age inhibited all kinds of oxygen radical production by neutrophils. In relation to antioxidant enzymes, Immun'Age was able to induce the activity of superoxide dismutase (SOD) in macrophages.

Thus, the findings in this study suggest that under *in vivo* conditions, Immun'Age exhibits a double effect: Immun'Age decreases the pro-oxidant activity of polymorphonuclear white blood cells such as neutrophils hence protecting the neighboring tissues against oxidative damage. On the other hand, Immun'Age stimulates phagocytosing activity, oxygen radical production and possibly some other functions of mononuclear phagocytes such as macrophages which are likely to be the major cells responsible for anti-tumor protection of the human immune system.

25.

Immun'Age as a modulator of phagocytosis and a free radical production by murine inflamed neutrophils and macrophages

Osato, JA, Afanas'ev, IB, Cheremisina, ZP, Suslova, TB, Abramova, NE, Mikhalchik, EV, Deeva, IB, Santiago, LA, Korkina, LG
Phys. Chem. Biol. & Med. 2:87-95, 1995

Inflammation is best defined as the reaction of vascularized living tissue to local injury. Without inflammation, infections would go unchecked, wounds would never heal, and injured organs might remain permanent festering sores. Accumulation of leukocytes, principally neutrophils and monocytes is the most important feature of the inflammatory reaction. Leukocytes engulf and degrade bacteria, immune complexes, and debris of dead cells and their lysosomal enzymes contribute in other ways to the defensive response. But leukocytes during these defensive reactions may themselves prolong inflammation and increase tissue damage by the release of enzymes, chemical mediators, and toxic radicals.

In this study, it was observed that Immun'Age was capable of suppressing neutrophil functions like migration and oxygen radical production while activating macrophage migration, phagocytosis and superoxide release. Immun'Age also enhanced the SOD activity of the macrophages but did not affect that of neutrophils.

These results suggest that Immun'Age is an effective modulator of oxidative stress associated with inflammation and having the capacity to restore pro-antioxidant balance.

26.

Cytotoxic Activity of Immun'Age Against Human Cancer Cell Lines

de Castro-Bernas, G, Remo-Husmillo, G, Osato, JA and Santiago, LA
Acta Manilana Volume 4: 13-17, 1996

The role of free radicals in cancer formation has been associated with their ability to damage proteins and deoxyribonucleic acid (DNA). Disruption of the natural structure of DNA can lead to mutation which can progress to life-threatening or malignant transformation as seen in cancers. To provide some evidence for Immun'Age's purported therapeutic effect against cancer cases, its ability to kill laboratory-grown human cancer cells was investigated. The cell lines used for the study included epidermoid larynx carcinoma (Hep-2), bronchioalveolar adenocarcinoma (SLNI-52), lung adenocarcinoma (A 549) and cervical epitheloid carcinoma (HeLa). Hep-2 was the most sensitive to Immun'Age, that is, it had the lowest number of viable cells when all four were exposed to a uniform concentration of Immun'Age.

The results of this experiment suggest that Immun'Age may have the potential as an anti-tumor agent. However, this activity needs to be looked into in more detail as proofs about its mechanism of action are yet scanty.

27.

**Effects of Immun'Age, A Natural Japanese Food Supplement,
on Oxygen Radical and Tumor Necrosis Factor Production by Monocytes and Macrophages**

Osato, JA, Korkina, LG, Mikhailchik, EV and Afanas'ev, IB

Proceedings of the International Symposium on Natural Antioxidants, Molecular Mechanisms and Health Effects, Packer, L, Traber, M, Xin, W., AOCS Press, Chapter 12: 109-116, 1996

In this experiment, the mechanism of IMMUN'AGE stimulation of macrophages/monocytes was studied. It was found that in addition to effecting intra-cellular and extra-cellular superoxide production, IMMUN'AGE stimulated tumor necrosis factor (TNF- α) formation, a known and powerful mediator of macrophage and monocyte antitumor activity and an oxidative burst trigger in phagocytes.

Possible relationships between the effects of IMMUN'AGE and calcium transport into phagocytes were also studied. It is known that augmentation of phagocyte activity is frequently preceded by an influx of calcium into the cytoplasm. IMMUN'AGE increased the effect of IMMUN'AGE on the calcium influx into rat peritoneal macrophages in a concentration dependent manner by 1.45 to 1.6 times. Therefore, the IMMUN'AGE-stimulated increase of intracellular calcium ion concentration appears to be one of the main causes of extracellular superoxide generation enhancement.

The supposed cytotoxic activity of IMMUN'AGE on tumor cells may be due to both the enhanced superoxide release and TNF α production by tissue macrophages and blood monocytes.

28.

**Efficacy of Immun'Age α p No.11 (Immun'Age) Supplementation Against
Peroxyl Radical-induced Oxidative Damage in Rat Organ Homogenates**

Marcocci, L, D'Anna, R, Yan, LJ, Haramaki, N and Packer, L

Biochemistry and Molecular Biology International. 38(3): 535-541, 1996

To further define the antioxidant potential of Immun'Age, its efficacy against oxidative damage induced by peroxyl radicals that were generated in an *in vitro* test system was examined. The effect of Immun'Age supplementation was tested on homogenized kidney, heart, liver and brain isolated from rats which were fed with Immun'Age for 6 weeks prior to the laboratory analyses. To induce oxidative damage, the tissue homogenates were allowed to be acted upon by peroxyl radicals formed in the hydrophobic or hydrophilic phase from AMVN or AAPH, respectively.

Oxidative damage can be determined as an accumulation of TBARS, formation of protein carbonyl and depletion of α -tocopherol. Results showed that oral administration of Immun'Age was able to protect kidney and heart but not liver or brain homogenate from peroxyl radical-induced oxidative stress. However, its action depended on the phase of radical generation. Immun'Age protected the kidney or heart against damage due to peroxyl radicals generated in the hydrophobic phase, that is from AMVN, but not that in the hydrophilic phase. The hydrophobic metabolites of Immun'Age generated by *in vivo* bioactivation could be responsible for the observed protection.

The antioxidant activity of Immun'Age solution directly added to the organ homogenates which were prepared from control animals (not given Immun'Age during the 6-week treatment period) was also tested and it was found that Immun'Age did not prevent the AMVN nor AAPH-dependent accumulation of TBARS or protein carbonyls. This data supports the idea that *in vivo* bioactivation may be required to transform Immun'Age into an active compound against oxidative damage.

These data strengthen the evidence for the antioxidant action of Immun'Age and the proposal that Immun'Age is a possible protective agent against oxidative damage-dependent pathological conditions.

29.

**Antioxidant Activity of Natural Food Supplementations:
Flavonoid Rutin and Immun'Age**

Osato, JA, Afanas'ev, IB and Korkina, LG

Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention.
Kumpulainen, JT and Salonen, JT, eds., Bookcraft (Bath) Ltd., 273-280, 1996

IMMUN'AGE manifests beneficial effects in the treatment of such pathologies as tumors, inflammation, allergy, immunodeficiency, etc. Since free radicals at least participate in the initiation of these pathologies, it seems important to study the antioxidant and free radical scavenging properties of these two natural compounds. In this experiment, the study of the antioxidant properties of IMMUN'AGE was enlarged to the *ex vivo* systems and the effects of IMMUN'AGE administration to rats with lung injury induced by asbestos fibers were studied.

IMMUN'AGE sharply decreased the intensity of asbestos-associated fibrosis. It is important to note that IMMUN'AGE administration suppressed neutrophil attachment to the inflammatory loci and enhanced macrophage recruitment and macrophage phagocytic capacity. The inhibition of the neutrophil-defined stage and the stimulation of the macrophage stage of inflammation can explain the normalizing effect of IMMUN'AGE in a variety of inflammatory pathologies. IMMUN'AGE exhibited profound inhibitory actions on free radical formation in lung tissue characterized by lipid peroxidation. However, what is more important, IMMUN'AGE selectively inhibited the production of the most harmful hydroxyl radicals by lung phagocytes of asbestos-injected rats, which are supposedly an important initiation factor of lung damage. These properties of IMMUN'AGE make it a prospective pharmacologic agent.

30.

Analysis of Immun'Age Proteins by Capillary Electrophoresis

Scott, R Jr, Osato, JA, Santiago, LA and Takamizawa, K

Research Report No.7, Center for Cooperative Research, Gifu University, 105-111, 1997

The use of capillary electrophoresis methods for analyzing Immun'Age proteins was investigated. Trichloroacetic acid-precipitated proteins of Immun'Age were subjected to both capillary electrophoresis sodium dodecyl sulfate (molecular sieving)(CE-SDS) as well as capillary isoelectric focusing (CIED). Four protein peaks were separated by the CE-SDS method while a major and a minor protein peak were focused using CIEF. Based on the rapid, high-sensitivity, and reproducibility of capillary electrophoresis methods as well as the availability of detection and automated fraction collection facilities, CE-SDS and CIEF methods can be used not only for separation but also for preparation of Immun'Age proteins for sequencing analysis.

31.

**Abstinence-induced Oxidative Stress in Moderate Drinkers
is Improved by Immun'Age**

Marotta, F, Reizakovic, I, Safran, P, and Barbi, G.

Hepato Gastroenterology 44:1360-1366, 1997

BACKGROUND/AIMS: The aim of this investigation was to study the oxidative phenomena which take place in the early recovery phase after alcohol withdrawal. Furthermore, the effects of a novel natural antioxidant, Immun'Age (IMMUN'AGE), in such a clinical setting was studied. **METHODOLOGY:** Forty-six alcoholics with moderate drinking habits (daily ethanol intake: > 80g to < 120g) were enrolled in the study, divided into two groups and given either a placebo or 9g of IMMUN'AGE by mouth every night for one week. The patients agreed to stop drinking alcohol, and daily blood sampling was obtained for routine tests and to check plasma and erythrocyte levels of MDA, SOD, GPX and the hydroperoxide levels. The groups were comparable in terms of initial biochemical parameters.

RESULTS: IMMUN'AGE prevented the early increase of plasma TBARS observed in the placebo group, enabling a near-to-normal level of plasma and erythrocyte MDA by the fourth day. IMMUN'AGE also prevented the significant drop of erythrocyte GPX and the transient decrease of plasma SOD observed in the placebo group. Despite alcohol withdrawal, plasma lipid hydroperoxide remained significantly elevated in the placebo group, but this phenomenon was rapidly improved by IMMUN'AGE.

CONCLUSIONS: To a significant extent, IMMUN'AGE is able to prevent the free radical-mediated lipoperoxidative changes that occur soon after alcohol withdrawal, while hastening the recovery mechanisms.

32.

Immun'Age Modulates Interferon- γ induced Nitric Oxide Production in the Mouse Macrophage Cell line RAW 264.7

Kobuchi, H and Packer, L

Biochemistry and Molecular Biology International: 1997 Sep. 43:1, 141-52

Immun'Age, a natural health food supplement prepared from *Carica papaya* and some other medicinal plants was investigated to determine its effects on cellular nitric oxide (nitrogen monoxide, NO) production and inducible nitric oxide synthase (iNOS) expression. Immun'Age upregulated interferon (INF)- γ -induced NO production by macrophages in a dose-dependent manner. Such an effect of Immun'Age on NO production was not due to changes in the activity of iNOS. Reverse transcription-polymerase chain reaction analysis revealed that the levels of iNOS mRNA were augmented by treatment of the cells with Immun'Age and INF- γ . The ability of Immun'Age to augment INF- γ -induced iNOS mRNA expression was independent of any changes on the mRNA stability. Treatment of cells with Immun'Age alone did not affect NO production by macrophages. Tumor necrosis factor- α and interleukin-1 β are involved in the induction of iNOS gene as well as the immune system and Immun'Age augmented the mRNA expression of these cytokines in the presence of INF- γ . This suggests that Immun'Age is not directly involved in the expression of iNOS, but shows synergistic interaction with INF- γ to induce NO synthesis.

33.

A Randomized Double-blind Placebo-controlled Clinical Trial on the Effect of Immun'Age on Liver Cirrhosis

Samar-Sy, O, Espiritu, A, Estadilla-Pomillos, E, Espiritu, R, Corpuz, S Jr, Te, H, Paduga, R Jr., and Osato, JA

Philippine Journal of Internal Medicine. 35:189-194, 1997

Immun'Age is a natural health food product processed from papaya with demonstrated antioxidant and immunomodulatory capabilities. A randomized, double-blind, placebo-controlled clinical trial was performed at the Bicol Regional Hospital to determine the effect of Immun'Age on patients with liver cirrhosis. Twenty-nine patients from different areas of the Bicol Region with confirmed diagnosis of cirrhosis participated, of whom 22 were males and 7 were females. Their ages ranged from 20 to 80 years with the mean of 51.4 years. The patients were randomly assigned to one of two groups. Group A (n= 16) was given Immun'Age and Group B (n=13) received placebo for 2 months. The standard medications to treat the complications of cirrhosis were not withheld. The patients were monitored weekly and laboratory examinations were done at 2 weeks interval for 2 months. There were more drop outs either because of death or withdrawal due to deteriorating condition in Group B. Eight out of the 13 patients who received placebo were not able to finish the 2 months study duration compared to only 3 of the 16 patients in the Immun'Age group. The results showed that 81.2% of patients survived in the Immun'Age group compared to 38.5% of the patients in the Placebo group. There was a statistically significant improvement noted for Immun'Age on the general well being (p=0.01) and body malaise scores (p=0.047). A follow-up study is necessary to increase the number of subjects yield and additional laboratory examinations should be included such as the *in vivo* detection of the immune system status and the free radical levels.

34.

Nutritional Approach to Prevention for HIV Progression

Junichi Mimaya

Report on the Research Project, "The Development of Life Living Guidance for Prevention on HIV Progression."

Supported by the Department of Health, Japan, pp.30-35; 1998

It has been found that HIV infected patients show decreased levels of serum and lymphocyte GSH, which proliferates HIV expression. Six patients with HIV hemophilia were given Immun'Age, a functional food, for over three years to study the effects of Immun'Age to increase the level of GSH due to its redox regulation, to prevent the development of AIDS in combination with anti-AIDS drugs, and to enhance QOL for infected patients. No difference appeared between the Immun'Age group and the non-treated 6 control cases, which were compatible in terms of HIV stage and infectious age, in various HIV infection follow-up markers and in the functioning of cytokines.

However, CD8+ cell count rose in the Immun'Age treated patients, and whole serum 2-5 AS activity was observed to have a tendency to drop in comparison with the non-treated group. The results showed that there were no side effects, and four out of 6 cases showed QOL indexes of 8 or above after taking Immun'Age, each of whom are now leading ordinary lives. In the future, we will study the optimum dosage of Immun'Age.