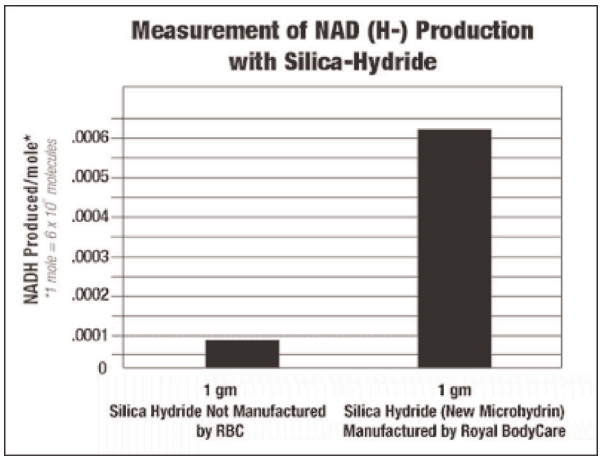
# Three independent studies prove that new Microhydrin is even more effective! (October 22, 2002)

**Why do you take a silica hydride supplement?**

You take it to increase your energy, your hydration, and to reduce dangerous free radicals in your body. You take it to improve your health in ways that can be demonstrated only in tests of biological activity.

In order to obtain a true measure of biological activity of New Microhydrin, three different independent studies have recently been completed.

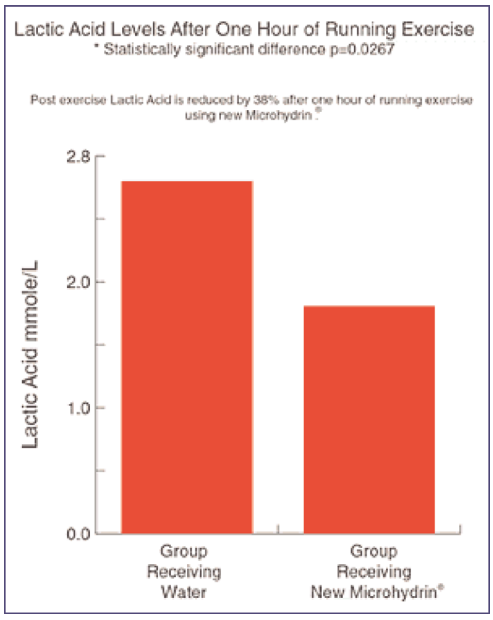



## 1. Increasing Your Energy

On September 17, 2002 a study was conducted at the University of Colorado Health Science Center comparing silica hydride manufactured by RBC (New Microhydrin ) with the previous form of silica hydride not manufactured by RBC.
Dr. Joe McCord, an internationally recognized and highly published scientist, who specializes in the study and evaluation of antioxidants, conducted the study. He chose one of the most important functions in your body, the reduction of NAD to NADH. The H-ions supplied by NADH are necessary for you to make ATP, the source of your energy. NADH also supports cellular enzymes that result in your metabolism of nutrients and your elimination of toxins.
Dr. McCord reported that New Microhydrin reduced seven times more NAD to NADH than the earlier silica hydride that was not manufactured by RBC. This helps explain the great testimonials we are receiving on the increased energy people feel after consuming New Microhydrin and New Microhydrin Plus.

## 2. Reducing Your Lactic Acid - A Source of Muscle Cramps

A study was conducted by Dr. Allan Goldfarb at the University of North Carolina in the Department of Exercise and Sport Medicine. Subjects that received New Microhydrin equivalent to 4 capsules per day in fresh distilled water consumed for five days showed 38% less lactic acid buildup after one hour of strenuous running. No other antioxidant reported in the peer reviewed literature has shown this type of reduction of lactic acid after exercise.




## 3. Reduction Of Your Most Common Free Radicals

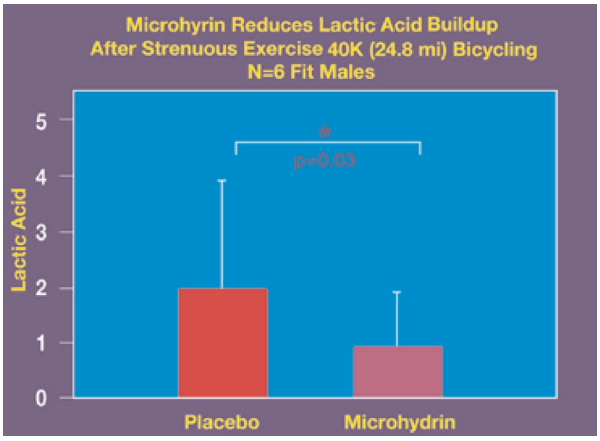
Your body has more peroxyl free radicals than any other free radical. They are particularly damaging because they cause chain-breaking reactions in your cells that collapse your cell membranes. Dr. Joe McCord of the University of Colorado Health Science Center conducted a study comparing two forms of silica hydride. Dr. Mc Cord reported that New Microhydrin, manufactured by RBC, was 828% more effective in scavenging these dangerous peroxyl free radicals than the silica hydride not manufactured by RBC.

# Abstracts of Microhydrin Evaluation Studies

***Clinton H. Howard and Kimberly Lloyd***
Revised January, 2000

Royal BodyCare is conducting an ongoing series of laboratory and clinical studies designed to evaluate the nutritional characteristics and benefits of Microhydrin (250 mg per capsule) as a mineral antioxidant. The following is a brief summary of the results of studies completed as of January 2000.

## Microhydrin Effectively Lowered Blood Lactic Acid Levels During Strenuous Exercise



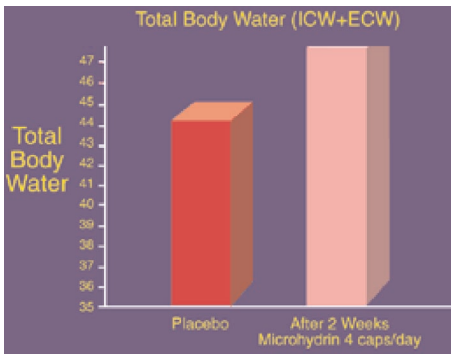

The Exercise Physiology Department of the University of North Texas Health Science Center at Fort Worth, Texas conducted a double blind, placebo controlled crossover study in 6 male cyclists for blood lactic acid (lactate) clearance during a 40K (24.8 mi) timed bicycle ride at maximum speed. Subjects received 4 capsules of Microhydrin or placebo daily taking one in the morning, two at noon and one in the evening during the week prior to testing and during the week of testing. Subjects refrained from all other non-prescribed supplements during the testing period. Additionally, subjects took 2 capsules with water 30 min. before the start of exercise. Blood lactate levels was measured before and 5 min. after each exercise session. Microhydrin significantly decreased blood lactic acid (lactate) levels as compared to placebo during strenuous exercise ($p = 0.03$). (*Unpublished data Peter Raven Ph.D. 1999 & Wendy Wasmund, B.S. University of North Texas Health Science Center at Fort Worth, 1999.)*

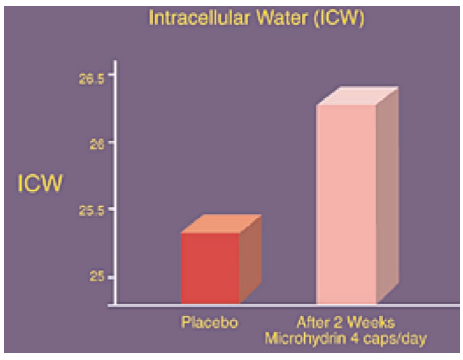
Lactic acid accumulates during strenuous or prolonged exercise. It is a common problem for athletes, people who work out, play sports or do physical exercise for an extended time.

The decreased lactic acid (lactate) levels immediately after strenuous exercise is another indicator of the ability of Microhydrin to help in providing a direct energy source (ATP production) to cellular function. An ergogenic energy function is when a substance enhances biochemical energy without introducing additional carbohydrates or calories to the diet.

## Microhydrin in Intra and Extra Cellular Hydration

A double blind placebo controlled pilot study was conducted using the RJL Bioelectrical Impedance Analyzer that measures hydration of the body based on nutritional status developed by R. J. Liedtke. Seven subjects received 4 capsules of Microhydrin per day for two weeks and were crossed over receiving 4 capsules per day of a placebo (rice bran flour). Averaged values showed increased intracellular and extracellular hydration due to the consumption of Microhydrin as compared to the placebo group.

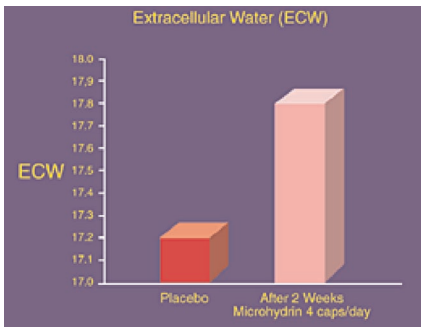


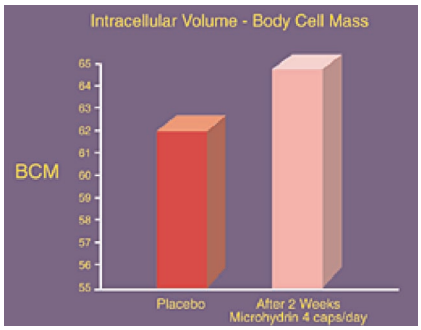




When consuming Microhydrin, the value for Total Body Water (intracellular and extracellular water) increased by 2.7%. The changes observed in TBW when subjects consumed Microhydrin, compared to the placebo, showed statistical significance ($p < 0.05$) using a student's test for small sample groups.

Intracellular Water, the most sensitive indicator of nutrition and metabolic state, increased by 2.7%.

Body Cell Mass (intracellular volume), another indicator of water within cells, also showed a 2% increase during supplementation with Microhydrin.

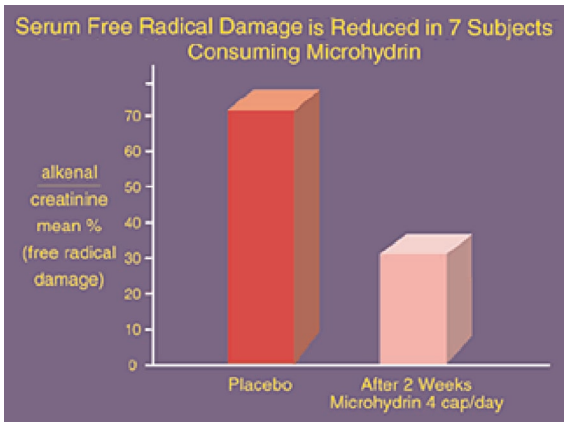



The increase observed in Extracellular Water values were statistically significant ($p < 0.05$) when consuming Microhydrin as compared to taking the placebo. *(Unpublished data Gary Osborn R.Ph. & Heriberto Salinas, MD Texas Institute of Functional Medicines, 1999)*

Body Cell Mass (BCM) and Intracellular Water (ICW) are assessments of intracellular volume and water inside the cell, respectively. Extracellular Body Water, water that bathes the cells, also showed increased volume. Intracellular water makes up approximately 60% of the total body water of healthy adults. Intracellular water, as an indicator of cell integrity, is found to be higher in babies but decreases as adults age or lose body cell mass. Healthy tissue cells hold water within the cells and have higher anabolic (building up) function rather than catabolic (breaking down) function.

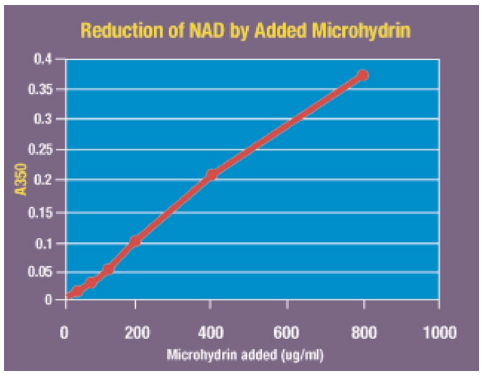
## Microhydrin Protection Against Oxidative Stress




A double blind placebo controlled crossover pilot study was conducted on 7 subjects who received 4 capsules/day of Microhydrin for two weeks, and received 4 capsules/day of a placebo for two weeks. Urine alkenal/creatinine ratios were measured. During Microhydrin supplementation, a 43% increase in free radical protection was observed as compared to the placebo group. Microhydrin was observed in this study to protect against serum alkenals. Alkenals are the oxidative products of serum lipid peroxides occurring from free radical attacks on cellular lipid membranes and lipoproteins. They are indicators of free radical damage in the body associated with a higher risk of age related diseases. *(Unpublished data Gary Osborn R.Ph. & Heriberto Salinas, MD Texas Institute of Functional Medicines, 1999)*

## Evaluation of Microhydrin in the Production of NADH in Vitro

ATP production, the principal source of energy in the mitochondria, depends on the production of NADH. Reduced hydrogen, generated by the Kreb's Cycle, is carried by NADH into the mitochondria for the electron transport chain reactions that will ultimately create a molecule of water and ATP.



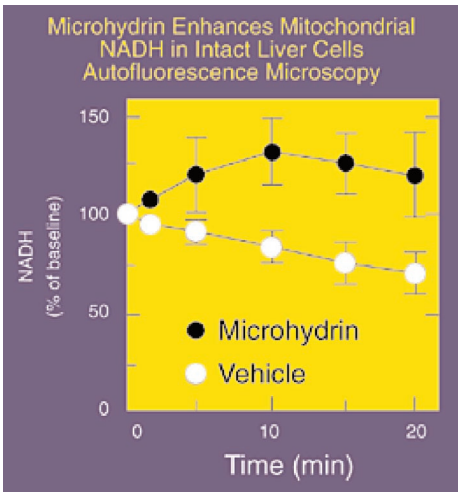

Hydrogen is one of the most important elements donating an electron, an electron pair or its proton to reduction/oxidation reactions of numerous enzymes and intermediates within the cell's metabolic pathways. In vitro assays measured the direct conversion of NAD+ to

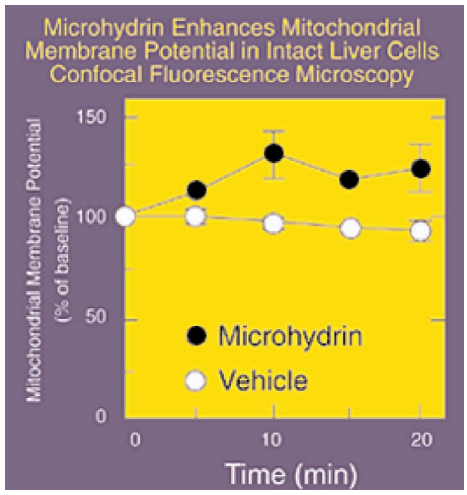
NADH by the addition of Microhydrin. The sequential addition of increasing concentrations of Microhydrin to NAD+ showed a linear increase in NADH production as measured by its absorption at 350 nm. (*Unpublished data Joe McCord, Ph.D University of Colorado Health Sciences Center, 1998).*

## Microhydrin Increases Mitochondrial NADH Production and Enhances Mitochondrial Membrane Potential in Intact Liver Cells

Microhydrin (200 ug/ml) was introduced to cultured 90% viable rat hepatocytes (500,000cells/ 4ml medium). Blue autofluorescence of mitochondrial NADH was visualized by a Zeiss LSM 410 inverted

laser scanning confocal microscope using a 40X water immersion lens and 356/365 nm excitation light from a UV argon laser. Under the conditions used, autofluorescence arises primarily from mitochondrial NADH. Oxidation of NADH to NAD causes loss of fluorescence since only NADH is fluorescent.




The line graph summarizes data from 3 Microhydrin and 3 vehicle (control) experiments. In the Microhydrin group NADH increased 20% over 20 minutes while the vehicle group showed a decrease in NADH fluorescence by about 30%. These preliminary experiments suggest that Microhydrin promotes electron transfer to NAD in intact living hepatocytes. Moreover, Microhydrin prevented the spontaneous oxidation (or bleaching) of NADH that generally occurs during an incubation of this type (see vehicle plot) thereby indicating a continuous recharging of the pyridine nucleotide (NADH).

Mitochondrial membrane potential was monitored using overnight cultured hepatocytes similar to the NADH experiment and which were loaded for 20 min. with the fluorescent probe tetramethylrhodamine methylester (TMRM). The medium was adjusted to pH 7.4 to assure that the previously noticed increase in membrane potential was not due to a pH effect. The TMRM-loaded cells were imaged with a Zeiss 410 inverted laser scanning confocal microscope through a 63X objective lens. In these experiments, an increase of the mitochondrial fluorescence of TMRM represents an increase of mitochondrial depolarization (more negative membrane potential). The line graph summarizes data from 3 Microhydrin and 4 vehicle experiments. In the vehicle group, TMRM fluorescence decreased by about 6% over 20 minutes. In the Microhydrin group, TMRM increased about 25%. These preliminary experiments suggest that Microhydrin enhances mitochondrial membrane potential in intact living hepatocytes. The combination of increased mitochondrial membrane potential and increased NADH suggests an enhancement of bioenergetic capacity of the mitochondria when Microhydrin is present in the cell suspension (Unpublished data 1999). Microhydrin appears to be providing electrons or H- available to the cofactors that are able to utilize these for cellular energy production. NADH provides electrons to the mitochondria electron transport chain directly producing H20 and ATP, the primary cellular energy source for numerous biochemical reactions throughout the cell.

### Evaluation of Microhydrin as a Scavenger of Free Radicals, Report I

Tests on Microhydrin were conducted using electron spin resonance techniques (ESR) by a scientist who specializes in the evaluation of antioxidants at a major university. The following is quoted from the test report:

"We have made a thorough investigation of the antioxidant activity of Microhydrin by several experimental methods. Hydroxyl radical scavenging activity was found in two different Microhydrin preparations provided."

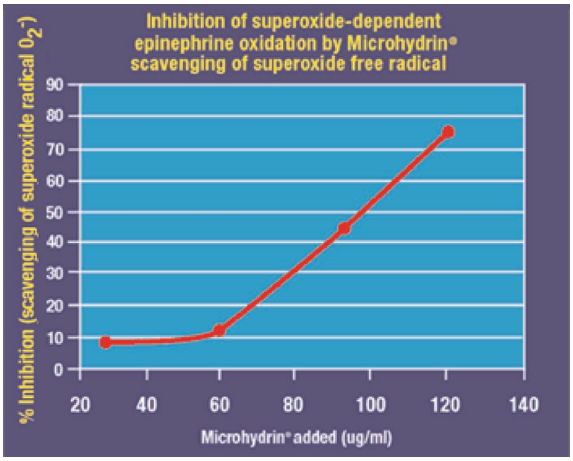
"Our conclusions are that Microhydrin has antioxidant activity towards hydroxyl radicals. Hydroxyl radicals are among the most dangerous of oxygen free radicals that occur in biological systems. They are the same types of radicals that can be produced by exposure to ionizing radiation. Therefore, it can be stated that Microhydrin has antioxidant activity in this regard." *(Personal Communication: Lester Packer, Ph.D., University of California at Berkley, 1999)*

### Evaluation of Microhydrin as a Scavenger of Free Radicals, Report II

Tests on Microhydrin were conducted by a scientist who specializes in the evaluation of antioxidants at a separate major university. The following is quoted from the test report: "When assayed in the

'standard' assay for super oxide dismutase activity based upon the reduction of cytochrome c by xanthine (see J. Biol. Chem. 244: 6049-6055, 1969), Microhydrin showed two characteristics:

1. **Microhydrin can directly reduce cytochrome c, showing that it is a reducing agent (or an antioxidant).**
2. **Microhydrin can inhibit the superoxide-mediated reduction of cytochrome c, indicating that it can scavenge the superoxide free radical."**

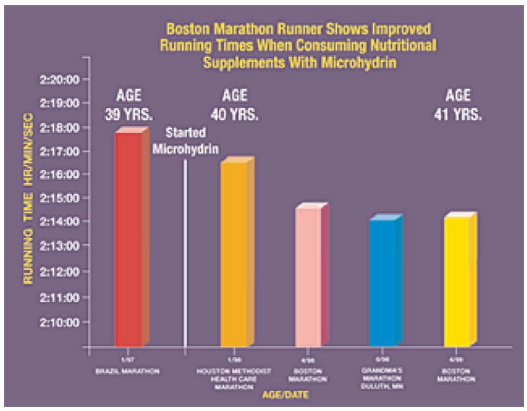



"When assayed with an alternative method based on the ability of superoxide to oxidize epinephrine to adrenochrome, Microhydrin also showed its ability to scavenge the superoxide radical and inhibit the process." *(Personal Communication: Joe McCord, Ph.D., University of Colorado Health Sciences Center, 1998). An abstract of this in vitro antioxidant data was presented and published in the Proceedings of the National Hydrogen Association 10th Annual Meeting. pg. 595-610; 1999)*

## Physician Using Microhydrin Climbs Mount Everest Without Oxygen

In 1991, at age 39, Denis Brown M.D. of British Columbia attempted to climb Mt. Everest without supplemental oxygen. He reached 26,000 feet. Again in 1994 he made the climb and achieved 28,000 feet before he surrendered to the extreme conditions and turned back. In 1999, at age 47, he added Microhydrin to his supplement regime. Microhydrin helps to reduce the build-up of free radicals and lactic acid, and supports production of ATP. He succeeded in reaching the peak of the South Summit at 28,750 ft. without supplemental oxygen and reported that this time he felt stronger and had more energy than on previous climbs.

## Aging Marathon Runner On Microhydrin Improves Time




In January 1997, at age 39, Andrey Kusnetzov ran the Brazil Marathon in 2 hr. 17 min. and 52 sec. The following December, he added Microhydrin to his daily supplement regime, taking two capsules with a third capsule opened into his glass of water each morning and again each evening. In January, he ran 2 minutes faster, winning first place in the Masters Division (Age 40+) at the Houston Methodist Marathon. The following April 1998, he continued to improve his time and won the Boston Marathon Masters. One year later, at age 41, in a field of 12,000 runners, he again won the Boston Marathon Masters in a faster time of 2 hr. 14 min. and 20 sec., despite higher wind and heat which caused other top runners to finish with slower times than in the previous year. Following the race, RBC's medical director entered the recovery room where exhausted runners collapsed on cots and were treated for fainting, hyperthermia, and vomiting. Andrey remained standing, relaxed, and appeared unaffected by the run.

## Tests On The Functions of Microhydrin

Microhydrin is a mineral (silica, potassium, magnesium) formed into extremely small negatively charged colloids (nanocolloids) that have been saturated with hydrogen. The minerals have been found to be in the range of 1-5 microns in size when analyzed with Electron Scanning Micrographs. Its function is to provide literally trillions of hydrogen anions capable of donating electrons into body fluids. Electrons, which Albert Szent-Gyorgyi called the "fuel of life", are abundantly available in organically grown raw vegetables, fruits, and grains, but are deficient in our modern diet of over-cooked, acidic, or highly oxidized foods, beverages, and drinking water. Tap water and bottled waters are oxidized, acidic, and do not provide a source of electrons. When consumed, the body must substantially alter their chemical characteristics in order to convert water into optimal extracellular and intra-

cellular body fluids. Three such characteristics are conductivity, oxidation/reduction potential (ORP) and surface tension.

### Conductivity

Conductivity is critical to the optimal function of body fluids. When conductivity was assessed, 250 mg of Microhydrin in 8 oz of tap water increased from 10 to 895 µMhos/cm. Total dissolved solids also increased.

**CONDUCTIVITY TABLE**

| | 8 oz water | 8 oz water plus 250 mg Microhydrin® |
|---|---|---|
| Surface Tension | 73 dynes | 45 dynes |
| Conductivity | 10 µMhos/cm | 895 µMhos/cm |
| Total Dissolved Solids | 30 ppm | 400-450 ppm |

*Values provided by Environmental Technology (E/I T), Fullerton, CA.*


### Oxidation/Reduction Potential

To measure the level of electrons available in Microhydrin, a 250 mg capsule was dispersed into 8 oz of tap water and into each of twelve 8 oz glasses of different brands of bottled water. The results shown were measured before, and one hour after the addition of Microhydrin:

## Water Values With Microhydrin

| | Range of pH | Range of ORP |
|---|---|---|
| Tap Water | 6-6.5 | +250 to + 600 mv |
| Tap Water + 250 mg Microhydrin | 9-10 | -350 to - 650 mv |
| 12 brands Of Bottled Water | 6.4 - 8.2 | +60 to + 490 mv |
| 12 brands Of Bottled Water + 250 mg Microhydrin | 9.2 - 9.8 | -500 to -700 mv |

The ph and Oxidation Reduction Potential (ORP) were measured in various types of bottled and tap waters. Microhydrin decreases the ORP and increses the ph of water.

| ORP Comparison of Antioxidants | | |
|---|---|---|
| Liquids | pH | ORP |
| Pycnogenol (50 mg) | 4.20 | +145 |
| Green Tea (tea bag) | 6.00 | +86 |
| CoQ 10 (30 mg) | 6.20 | +49 |
| Vitamin C (500 mg) | 4.50 | +47 |
| Wheat Grass | 6.20 | +36 |
| Exta - Cee (RBC) | 5.80 | -31 |
| Phycotene Microclusters (RBC) | 9.00 | -56 |
| Coral Calcium | 10.30 | -112 |
| Organic Carrot Juice | 6.56 | -120 |

*This table shows a comparison of various types of antioxidant products.*
*Products are single servings dissolved in 100 ml. of deionized water.*

### Biological Oxidation-Reduction Reactions

Metabolic oxidation-reduction reactions are extremely important to the cell. Oxidation-reduction reactions (ORP or redox) involve the loss of electrons by one chemical species, becoming oxidized, and the gain of electrons by another species, becoming reduced. The flow of electrons in redox reactions is responsible, directly or indirectly, for all of the work done by living organisms. The tendency of these reactions to occur depends upon the relative affinity of the electron acceptor for electrons. Electron flow is spontaneous and exergonic (releases energy) in the cell because oxygen, the final electron acceptor, has a higher affinity for electrons than do the intermediates that donate electrons.

ORP is a measure of oxidation-reduction potential with standard meters, such as the Electronmeter™, that measure negative ions in solution. The Nernst equation relates standard reduction potential (Eo) to potential (E) at any concentration of oxidized and reduced species in a system. Another value derived from the Nernst equation utilizing pH, measures relative hydrogen (rH2) and is used as a redox indicator for biological fluids as seen in the Biological Terrain Assessment study. Various types of water depending on mineral salt content and water treatments will show variability in ORP readings with standard meters. ORP measurements with Microhydrin vary depending on water purity, dilution, the time it has remained in solution, proper cleaning of the meter probes and maintenance of the meter.

### Surface Tension

The surface tension of tap water is approximately 73 dynes. The surface tension of extracellular fluids is much lower at approximately 45 dynes. This low surface tension is critical to cellular function, absorption of nutrients, and to the removal of toxins. A 250 mg capsule of Microhydrin dispersed into an 8 oz glass of tap water has been shown to reduce surface tension to approximately 45 dynes, the same surface tension of extracellular fluids.

## Safety Studies With Microhydrin

### Accute Oral Toxicity Study

Microhydrin has been evaluated using standardized tests at a toxicology laboratory and was found to be a nontoxic substance. Microhydrin (250 mg in rice bran flour) was administered, with corn oil as an oral carrier, to rats at a concentration of 5 gm/kg body weight. The LD50 was found to be greater than 5 gm/kg which meets the U.S. Food and Drug Administration's limit tests and the European Regulatory Standards (2 gm/kg) for a nontoxic substance.

### Pre-Clinical Trial With Human Subjects Taking Microhydrin

A controlled clinical trial showed that in eight normal adult subjects, seven of the nine Biological Terrain Assessment parameters improved. Saliva pH, saliva rH2, blood resistivity, and urine resisti-

vity showed statistically significant improvement ($p < 0.05$) for these eight subjects after 18 days of supplementation with 4 Microhydrin per day on a schedule of one in the morning, two at midday and one in the evening. Three other parameters (urine pH, urine rH2, and saliva resistivity) also showed improvement over the 18-day supplementation period when their averaged changes were calculated. *Presented at the American College of Nutrition Symposium on Advances in Clinical Nutrition. Albuquerque, New Mexico, October, 1998.*

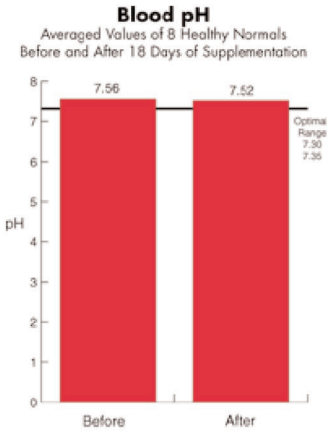
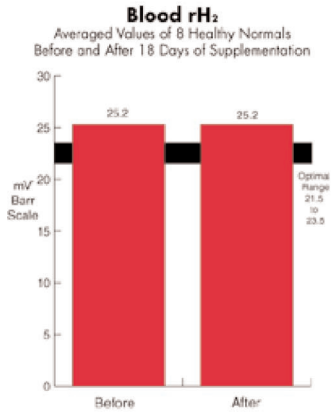


Figure 1



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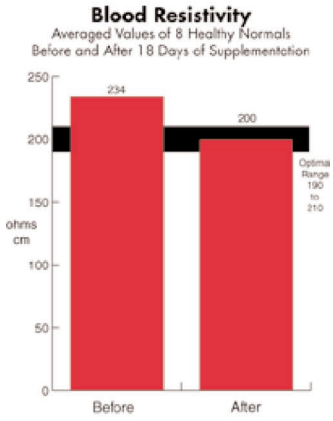

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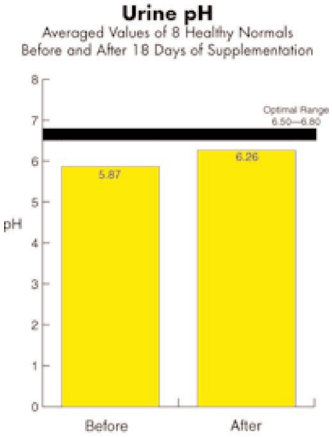
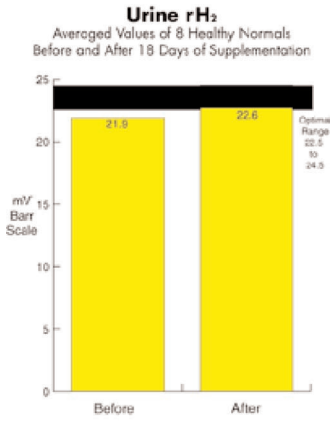


Figure 3



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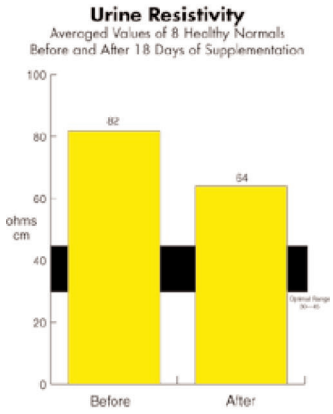

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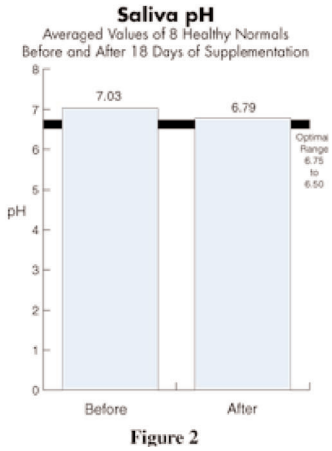

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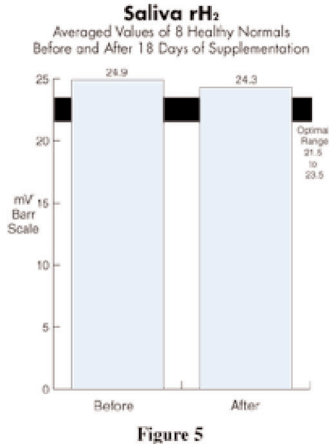


Figure 5

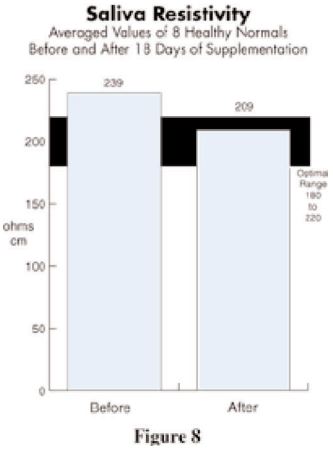


Figure 8

Blood pH and blood rH2 were the only parameters that did not change during the supplementation period. These values may not change as readily due to the heterogeneous and stable nature of blood. The nutritionists and physicians who monitored the study with Biological Terrain Assessment results and other clinical data observed no adverse side effects in these subjects during the supplementation period.

*Presented at the American College of Nutrition Symposium on Advances in Clinical Nutrition. Albuquerque, New Mexico, October, 1998.*

# Biological Terrain Assessment Results of 14 Subjects Before and After Testing with a Supplement Containing Silicon Bonded To Reduced Hydrogen Ions

A sample of the Ultar glacier water in Pakistan when analyzed contained 369 mg/ml of suspended rock flour and was composed of 70-80% biotite mica, 15-20% plagioclase feldspar, 5% vermiculite mica, traces of other soluble silicates, minerals (predominantly Ca+2, SO4-2, Mg+2, Na+, K+, Cl-, and HCO3-) and trace minerals [1,3]. Cooperative knowledge shared about water content from geologists, combined with the knowledge of nutritionists concerning optimum health needs has provided the impetus for upgrading the role of drinking-water in human health and longevity. A supplement has been developed which provides the qualities of glacial water containing silicate mineral crystals with a high specific surface area and ion exchange capacity [3,11].

A preliminary trial utilizing Biological Terrain Assessment (BTA), a historical method developed by Claude Vincent, a French hydrologist, and revised with modern technology, measures the pH, reduction/oxidation potential (rH2) and resistivity (r) of blood, saliva and urine of the subjects tested. BTA analysis is a non-invasive, inexpensive procedure that clinicians use to enhance their knowledge about the biochemistry of the body. Although BTA is not meant as a diagnostic tool, these parameters provide clinicians with important information concerning the effects of nutritional or therapeutic programs that tend to normalize these values important to health. A preliminary trial utilizing biological terrain assessment values provided a unique tool to assess possible short-term alterations due to the supplement.

## METHODS

A group of physicians and clinicians that utilize the BTA S-2000 analyzer in their clinical practices selected subjects for the trial. Signed informed consent was obtained from each individual. Two males with an average age of 49 yr. and six females with an average age of 45 yr. and who were not diagnosed with clinical pathologies, consumed 4 capsules a day on a schedule of one in the morning, two in the afternoon and one in the evening for 18 days. Three other subjects (#8, #9, and #12) who were clinically diagnosed with pathological medical conditions, were monitored closely and completed 18 days of the supplement program. Subject #8 was a 65-yr. old female with conditions indicating metal poisoning. Subject #9 was a 40 yr. old female diagnosed with chronic fatigue syndrome. Although she had undergone medical tests such as MRI and routine blood tests, the origin of her chronic fatigue and muscle weakness was unknown. Subject #12 was a 58 yr. old female diagnosed with fibromyalgia and chronic fatigue syndrome. These subjects' initial BTA values and other clinical diagnostic information indicated that they were more physically compromised than the other group.

All capsules administered in this study contained 250 mg of naturally occurring food grade silicon dioxide, potassium, magnesium sulfate and fatty acids developed by a proprietary process in a base of rice powder and were made available by Royal BodyCare, Inc. of Dallas, Texas as its product Microhydrin™. The instrument utilized for biological fluid analysis was a BTA S-2000™ produced by Biological Technologies International, Payson, Arizona. Blood was drawn from subjects using a non-heparin syringe and assayed within 2 minutes. Other fluids were collected and subjected to analysis according to the procedure described in the BTA S-2000 instruction manual.

*Table 1. Average Changes and Statistical Data for 8 Healthy Normals Before and After 18 Days of Supplementation*

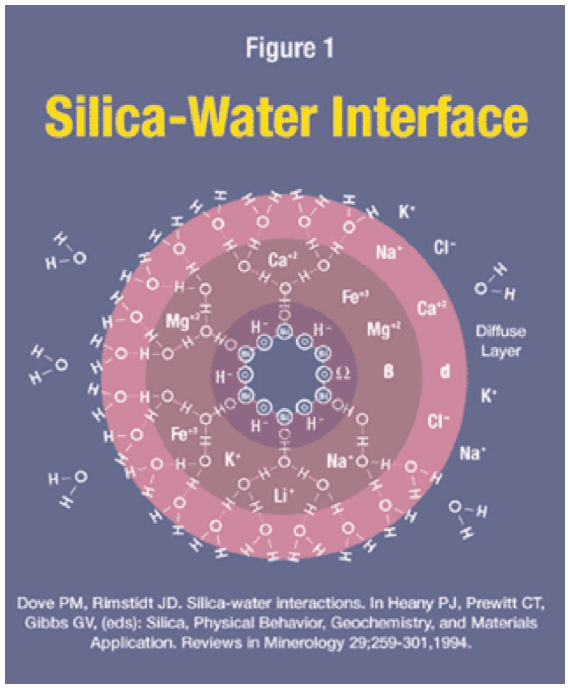
| Test | Average % Change | Average Unit Change | Mean + SD | SE | p value |
|---|---|---|---|---|---|
| Blood pH | 0.94 | 0.07 | -0.0337 ± 0.0855 | 0.0229 | 0.1833 |
| Saliva pH | 4.10 | 0.29 | -0.2488 ± 0.3056 | 0.0817 | 0.0187* |
| Urine pH | 11.70 | 0.65 | 0.2588 ± 0.8848 | 0.2365 | 0.3101 |
| Blood rH2 | 0.88 | 0.20 | -0.0250 ± 0.2188 | 0.0585 | 0.6818 |
| Saliva rH2 | 2.84 | 0.70 | -0.6125 ± 0.6813 | 0.1821 | 0.0120* |
| Urine rH2 | 5.92 | 1.26 | 0.6125 ± 1.8849 | 0.5037 | 0.2634 |
| Blood r | 19.10 | 52.00 | -34.1250 ± 52.8100 | 14.1164 | 0.0462* |
| Saliva r | 23.80 | 53.60 | -29.3750 ± 54.3980 | 14.5383 | 0.0830 |
| Urine r | 24.70 | 64.00 | -17.5000 ± 22.6337 | 6.0491 | 0.0232* |

**Statistically significant difference from baseline value within group ($p < 0.05$), using student t test for a small sample group. Abbreviations: rH2, reduction/oxidation value measured in millivolts, Barr scale; r, resistivity value measured in ohms cm; pH = -log 10-7 [H+] Values are means ± Standard Deviation (SD), Standard Error (SE)*

# A Silicate Mineral Supplement, Microhydrin®, Traps Reduced Hydrogen Providing In Vitro Biological Antioxidant Properties

## Introduction

It was the purpose of this investigation to study the effects of the reduced hydrogen silicate mineral towards several standard procedures that would indicate its antioxidant properties (reducing potential) in biological molecules. Since the silicate mineral supplement analog is saturated with reduced hydrogen and provides reducing potential in water of approximately -650 mV, a study was undertaken to observe oxygen free radical scavenging ability using several methods reported in the current literature. It was also analyzed for the ability to act as a reducing agent (antioxidant) in several standard assays (direct reduction of cytochrome c and NAD+).




## Hydrogen

Hydrogen is vital to life processes because of its unique atomic structure. It is one of the most important elements donating an electron, an electron pair or its proton to reduction/oxidation (redox) reactions of numerous enzymes and intermediates within metabolic pathways throughout the cell. Numerous biochemical reactions depend on nicotinamide adenine dinucleotide (NAD, NADH) and flavin adenine dinucleotide (FAD, FADH, FADH2) and their respective reduced forms for hydrogen electron transfer. Final transfer of electrons provided by hydrogen occurs in the energy cycle during the breakdown of glucose through glycolysis, the citric acid cycle and the mitochondria respiratory electron transfer (transport) chain (11) (Figure 5).

## Hunza Water

The Ultar Glacier of Hunza, West Pakistan has received interest in this century by both geologists and medical professionals as to the unique association towards unusual health and longevity of the people who consume the glacier stream water (4-7). Research has revealed that drinking the glacially pulverized rock flour of the Hunza river, eating a favorable diet, and community participation have been critical factors in achieving renowned longevity, a low rate of heart disease, and exceptionally good to excellent health in this community (4-7).

In analyzing the correlation to health and the use of glacial waters, it is apparent that not only do glacial waters have an abundance of important trace minerals but also the amorphous silicate derivatives themselves have unique characteristics in their ability to structure water and transport minerals and electrolytes (1,8,9). Although the structural studies are complex as to the kinetics of the silanol (SiOH) groups formed in different silica (SiO2) complexes, the initial adsorbed water adjacent to the surface is oriented and has properties (e.g., entropy, mobility, and dielectric constant) different from those of bulk water (1).

## Silicates

Silicates comprise a mineral family and are among the most abundant components of the earth's surface. Natural amorphous and crystallized silicates line rivers, glacier streams and marine environments. Weathering, pressure, ionic conditions and temperatures through the ages produce a variety of soluble silicate derivatives. Numerous water sources including glacial rock suspensions have been analyzed for their silicate structures and mineral compositions (1,2). In particular, various mineral water sources and glacier regions have been linked to the health of the inhabitants who have depen-

ded on them for their drinking water (3,4). Some of the relationships between geochemical environment, health and disease are well documented such as iodine deficiency leading to diseases of the thyroid (i.e. goiter, hypothyroidism, cretinism, and increased risk of thyroid cancer) (3). Numerous minerals are now realized to perform vital functions as cofactors and provide numerous structural and functional roles within cells and tissues. Recent evidence suggests that geochemical environments of water sources have a profound influence on the level of health in humans and animals (3-5).

Silicates tend to structurally arrange water molecules up to three layers (wetting). In the presence of molecular water, the silanol groups of small silicates ionize, producing mobile protons that associate/dissociate with the surface to impart an electrical conductivity to the surface that attracts minerals and ions as shown in Figure 1 (1). These layers have been further described as the omega (o-innermost water layer), beta (ß- second water layer) and delta (d-outermost water layer) (Figure 1). The resulting surface-solution interface that exists at wetted mineral surfaces is called the electrical double layer or zeta potential. It is this characteristic that tends to transport small ions, minerals and electrolytes (i.e. hydrogen, iron, magnesium, calcium, and sodium, etc.) (1,8,9). These SiOH groups and the resulting water arrangements tend to cage or sieve minerals. They also hold hydrogen atoms within these structures (1,9,10). When hydrogen is further reduced a biological antioxidant property is maintained in the silicate mineral particle.

## Methods

All capsules used in antioxidant and reduction analyses contained 250 mg of the naturally occurring food grade silica, potassium carbonate, magnesium sulfate and fatty acids developed by a proprietary process in a base of rice powder and were made available by Royal BodyCare, Inc. of Irving, Texas as its product Microhydrin®.

## Reduction Assays With Microhydrin

When the reduced hydrogen silicate mineral (Microhydrin) was dissolved or suspended in phosphate-buffered saline, pH 7.4, it directly reduced both cytochrome c and NAD+ indicating its strong reducing capacities (antioxidant). When assayed in the standard assay for superoxide dismutase activity based upon the reduction of cytochrome c by xanthine oxidase/xanthine, it reduced cytochrome c. These overall reactions, the reduction of cytochrome c (Cyt c) and the reduction of NAD+ by the reduced hydrogen silicate mineral (Mic) are shown below. NAD+ reduction will be presented in detail later.

$$Mic(H:^-) + Cyt\ c(Fe^{+3}) \rightarrow Mic + Cyt\ c(Fe^{+2}) + H^+$$

$$and\ 2Mic(H:^-) + NAD^+ \rightarrow 2Mic + NADH + H^+$$

## Super Oxide Scavenging Assays With Microhydrin

The superoxide mediated reduction of cytochrome c showed that the reduced silicate mineral inhibited the superoxide-mediated reduction of cytochrome c, indicating that the reduced silicate is reducing, or scavenging, the superoxide radical also:

$$Mic\ (H:^-) + O_2^- + H^+ \rightarrow Mic + H_2O_2$$

In this assay Microhydrin was found to inhibit and prevent the reduction of cytochrome c by superoxide:

$$O_2^- + Cyt\ c(Fe^{+3}) \rightarrow O_2 + Cyt\ c(Fe^{+2})$$

An alternative assay to further elucidate the activity of the reduced silicate mineral, observed the oxidation of epinephrine to adrenochrome by superoxide (unpublished data).

$$Microhydrin + O_2^- + epinephrine \rightarrow H_2O_2 + adrenochrome$$

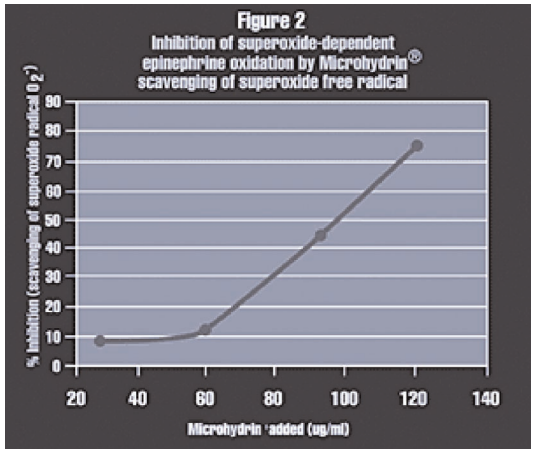
$$Superoxide\ free\ radical \rightarrow Hydrogen\ peroxide$$

Data is shown in Table 1 for superoxide dismutase-like (SOD) activity based on the oxidation of epinephrine by superoxide.

In this assay the reduced hydrogen mineral silicate (Microhydrin) scavenged the superoxide ion again. Quantitatively, half-maximal inhibition was achieved at a Microhydrin concentration of 90 µg/ml (Table 1). When concentrations of Microhydrin were at 60 µg/ml or When concentrations of Microhydrin were at 60 µg/ml or greater, epinephrine oxidation was inhibited showing that the silicate mineral reduced the superoxide free radical. The assay conditions utilized superoxide dismutase at a concentration of 50µg/ml with a conversion rate of 0.15 µg/ml (unpublished data) (Table 1, Figure 2).

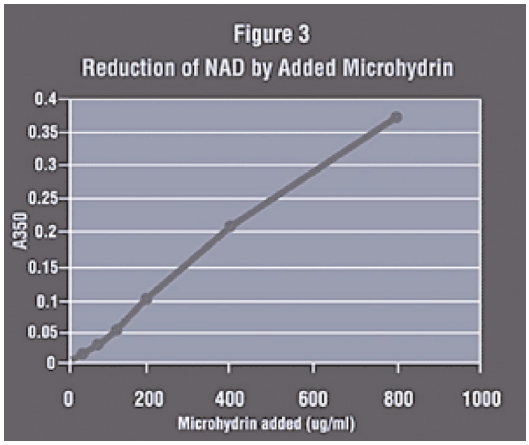
Table 1
Inhibition of Superoxide-Dependent Epinephrine Oxidation by Microhydrin

| Control rates | Microhydrin added µg/ml | Rate w/SOD | % Inhibition (superoxide scavenging) |
|---|---|---|---|
| 1.21 | 30 | 1.16 | 11.04 |
| 1.25 | 60 | 1.13 | 13.34 |
| 1.46 | 90 | 0.77 | 40.95 |
| 1.37 | 120 | 0.31 | 76.23 |
| 1.23 | 150 | 0.59 | 54.75 |
| Ave. 1.304 | | | |




## NAD Reduction With Microhydrin

The ability of Microhydrin to reduce $NAD^+$ to NADH was also examined. The redox potential for this reaction is –0.32 V (– 320 mV), requiring a fairly strong reducing agent. Microhydrin reduced $NAD^+$ to NADH. The reduction of $NAD^+$ was verified by scanning the absorption spectrum from 200 nm to 500 nm. In all subsequent studies $NAD^+$ reduction was monitored as the difference between two wavelengths (340 nm to 500 nm).

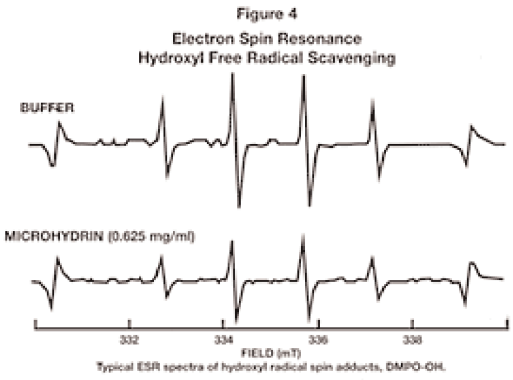



The 500 nm wavelength served as a reference (independent of $NAD^+$ reduction, but controlling for turbidity changes due to the light scattering effect of the silicate) (Figure 3). $NAD^+$ could be titrated by sequential addition of aliquots of Microhydrin in a linear fashion.

Microhydrin was suspended for 30 min. at 10mg/ml. Microhydrin was added incrementally to 60µM $NAD^+$(Figure 3).

## Scavenging of Hydroxyl Free Radical with Microhydrin

When the reduced hydrogen silicate mineral supplement (Microhydrin) was analyzed for antioxidant activity by Electron Spin Resonance (ESR) techniques, the reduced silicate mineral supplement showed activity toward the scavenging of hydroxyl free radical (OH) at a concentration of 0.625 mg/ml (Figure 4). Hydroxyl radicals were generated by the Fenton reaction. All reagents were dissolved in 0.1M potassium phosphate buffer, pH 7.4, with the exception of FeSO4 which was dissolved in distilled water. Fifty microliters of sample solution, 0.18 M DMPO (spin trap, 5,5-dimethyl-pyrroline-N-oxide) (50 µl), 2mM H2O2 (50 µl) and 0.2mM FeSO4 (50 µl) were mixed for 10 seconds and quickly transferred into a quartz flat cell (200 ml capacity, JEOL, Tokyo, Japan). Exactly 30 seconds after the addition of FeSO4, ESR spectra of the DMPO-OH spin adducts were recorded (unpublished data) (figure 4).

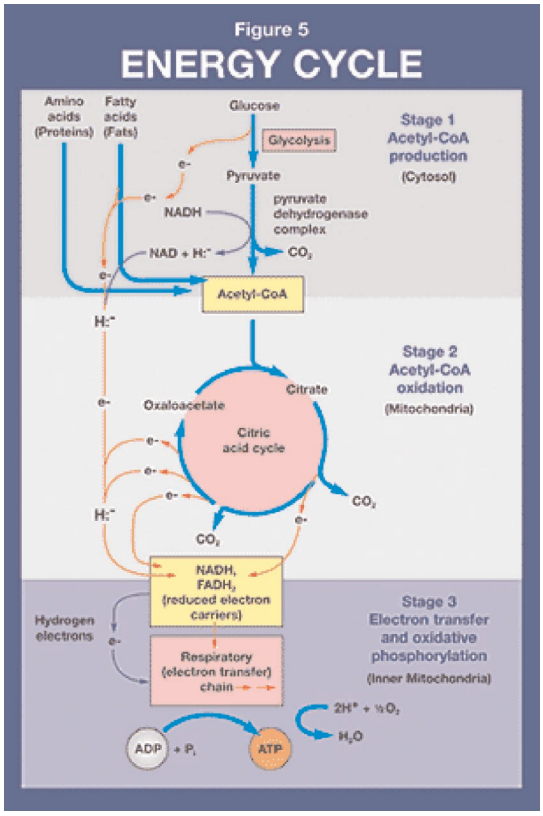



Two samples of the supplement were evaluated one showing hydroxyl free radical scavenging at 0.19 ± 0.05 EPC-K1 µmol-equivalent/mg and another sample at 0.15 ± 0.03 EPC-K1 µmol-equivalent/mg (unpublished data).

# Conclusion

## Reduced Hydrogen In The Energy Cycle

These preliminary investigations showed that the reduced hydrogen silicate mineral was an antioxidant (reducing agent) using several different methods. It was shown to directly reduce NAD+ to NADH effectively. NAD+ and its reduced form, NADH, are the primary cofactors participating in numerous biochemical reactions for metabolism and energy production in the cell including the mitochondria's electron transport chain and the coupled production of ATP (adenosine triphosphate). In the case of NAD-linked dehydrogenase enzymes such as glyceraldehyde-3-phosphate dehydrogenase, reduced hydrogen (H:-) is generated which can directly enter the electron transport chain (11). Two reduced hydrogen ions are generated from a complete cycle of glycolysis with both these donating a total of four electrons that enter the mitochondria's electron transport chain (Figure 5) (11).




The reduced silicate mineral was observed to be a strong reducing agent by the direct reduction of cytochrome c. Cytochrome c is a crucial iron containing electron carrier protein in the respiratory electron transport chain of human cells. Cytochrome c is coupled to the production of ATP in the mitochondria where most ATP is created. ATP will participate in numerous critical reactions throughout the cell.

Energy in the form of ATP production by the three linked processes of glycolysis, the citric acid cycle (Kreb's cycle) and mitochondria respiration rely on the transfer of electrons from hydrogen and reduced hydrogen in these pathways by the important intermediates NAD, NADH, FAD, FADH, and FADH2. Numerous other metabolic processes in the cell depend on ATP and these hydrogen containing cofactors for fatty acid utilization, protein synthesis, DNA synthesis and repair, gene regulation, and deamination of amino acids for the excretion of ammonia in the form of urine, to name a few (11).

## Antioxidant Function of Microhydrin

An antioxidant (reducing agent) is a compound that has an electron available to give to another molecule that needs one in order to stabilize its reactivity. Electrons orbiting around atoms prefer being paired or in even numbers (2, 4, 6, 8). Certain molecules, like the hydroxyl ion or superoxide ion, if not soon reduced (gain an electron) will have a strong enough affinity to attract electrons randomly from other molecules that will suffer structural and functional damage as this occurs. As the bio-molecules of cells are damaged in this way they begin to "age" or are not as normal as healthy cells.

The reduced silicate mineral supplement was also shown to scavenge the superoxide radical and the hydroxyl free radical. Hydroxyl radicals are among the most dangerous of oxygen free radicals that occur in biological systems and are the same as those formed by exposure to ionizing radiation. Both of these free radicals cause extensive oxidative damage to biological macromolecules such as DNA, membrane polyunsaturated fatty acid chains, and enzymes (12,13). Free radical damage (oxidative stress) is now considered to play a causative role in certain diseases such as Alzheimer's, rheumatoid arthritis, adult respiratory disease, cancer, heart and blood vessel diseases and aging (12,13).

## Hydrogen as an Antioxidant (electron donor)

Recent studies have shown that hydrogen in its reduced form scavenges active oxygen species and protects DNA from damage by oxygen free radicals (13). Reduced hydrogen is considered to be an ideal reactive species to counter active oxygen free radicals. Since water is the medium in which all organisms are born, composes the matrix of life and supports all molecular interactions, it is now understood that this is also part of its biological role (13,15). Various functions of water chemistry, its geological chemistry, even its genetically determined transport across the cell membrane and biochemical functions are now being investigated more extensively.

Hydrogen in its ground state holds one electron. It can also be found in nature to hold a second electron, known as the hydride (H:-) ion or reduced hydrogen. Hydrogen can be reduced (H:-) such as when water is treated with electrolysis, or when other catalytic procedures are used (13-15). The hydrogen produced in the process is called active hydrogen because of its activated reducing potential as an antioxidant and it has a redox potential of –350 mV (13,15). Reduced water, the dissolved supplement, and Hunza water have reduction/oxidation potential values of –350 mV or lower indicating low dissolved oxygen and high dissolved hydrogen in solution which indicate the exclusive properties of reduced hydrogen and electron availability unique to these systems. Blended fresh fruit and vegetable juices also show negative redox values (16).

Other antioxidants (i.e. vitamin E, vitamin C, etc.) do not tend to display such negative electron availability, as measured by standard redox measurements, because of the various structures of the molecules, their individual chemical characteristics and functional proximity in reactions which tend to determine their antioxidant role. Ascorbic acid, vitamin C (+80 mV) has a relative redox potential much higher than NADH (-320 mV) or the reduced silicate supplement (-650 mV), therefore, an equal amount of ascorbic acid will not reduce the same amount of NAD+ at the same rate as Microhydrin (a stronger reducing agent) will.

Ascorbic acid however is required to act as an electron donor in specific enzymatic reactions that only will recognize it in order to function, as is the case with other antioxidant vitamins. Although many vitamins have shown antioxidant function in and beyond their role as enzymatic cofactors, antioxidants are now realized to act towards random free radicals generated by metabolism or detrimental intermediates and are preventing otherwise damaging free radical reactions.

Since the reduced silicate can provide electrons for numerous reactions as an antioxidant it is now being further investigated as to these important antioxidant functions. Since biological molecules can be prevented from oxidation (loss of electrons) when reduced hydrogen is present to provide electrons the reduced silicate mineral is providing another function in addition to mineral and electrolyte transport. This antioxidant function may be an important factor in the silicate mineral system found in glacier waters renowned for longevity and enhanced health in communities that consume water with these properties (4-7).

## Biological Function of Silica

Silicon (element name) has been discovered to be an important trace element necessary for cartilage and bone tissue formation (18). Silicates are essentially non toxic when taken orally as evidenced by its use for over forty years as an over-the-counter ant-acid (magnesium trisilicate) for stomach indigestion. Silica compounds have also been used as pharmaceutical preparations (methylsilanetriol salicylate) for circulatory ischemias and osteoporosis (18). Silica compounds have unique buffering capacities shown often in their use in experimental biological systems (i.e.stabilizing biomolecules in column chromatography). A recent study found silica to provide a protective effect against high levels of aluminum in drinking water associated with cognitive impairment in the elderly especially when the pH was high (17). Solutions containing colloidal silicates have been used to successfully preserve canine hearts and kidneys for transplantation (19,21).

When the silicate mineral supplement was taken orally in an initial clinical study using human subjects it began showing an effect on urine pH. Most values showed changes to a more normal alkaline pH, considered beneficial towards health. A continuing state of blood and urine acidosis can indicate compromising health conditions. In this same study electrolyte and mineral transport were observed to opti-

mize as seen by statistically significant ($p < 0.05$) results in resistivity (1/conductivity) values in blood and urine in eight subjects after taking 4 capsules of the supplement per day for 18 days (20).
Some nutritional investigators speculate that a silica mineral deficiency is involved in the causation of several human disorders including atherosclerosis, osteoarthritis, and hypertension, as well as, the aging process (18). Those speculations demonstrate the critical need for studying the importance of silica nutrition and function, especially in aging humans (18). Due to the unique chemical properties of silicate minerals, their antioxidant properties, long-term use, safety and recent research the scientific and nutritional communities continue to investigate the possibilities of these minerals as to their varied nutritional benefits.

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