BARNET

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Presents

NATURAL AMINO ACID

"TORCH OF LIFE"*
SUPPLIER AND PROTECTOR

THIOTAINE

AGI Dermatics

*Torch of Life terminology first used by Lavoisier, 1789)

INTRODUCTION - MITOCHONDRIA, OXYGEN, ENERGY & WELL-BEING

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INTRODUCTION: MITOCHONDRIA, OXYGEN, ENERGY AND WELL-BEING

1. Oxygen/ATP in Mitochondria

Energy is the moving force of life. It is a fundamental and indispensible element in cellular activity. All cells must produce energy to survive, and oxygen consumption is fundamental to the process. Lavoisier understood this in 1789, and dubbed mitochondria the "torch of life."

In our daily life we associate oxygen with "outdoor activity." We go to the mountains for fresh air. In trendy shopping areas we can visit oxygen bars. Oxygen is associated with health, with looking and feeling good.

It is also known that Olympic long-distance runners train in the mountains to increase the oxygen levels in their blood to help give them a competitive edge. Respiration also takes place in the body, not only in the lungs. At the cell level the energy is made by hundreds to thousands of mitochondria per cell (Figure 1), the powerhouse of the cells.

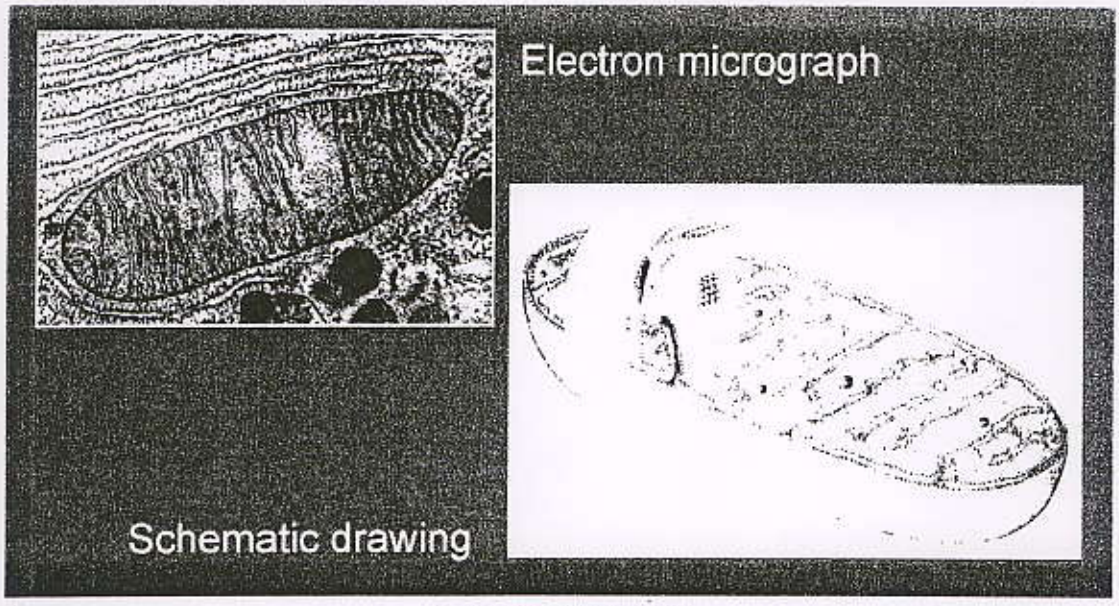


Figure 1: Mitochondria

Energy and oxygen are related and Thiotaine is a molecule that helps assure efficient use of oxygen for more efficient energy production. Thiotaine is also an anti-oxidant, reduces 8-oxo-guanine synthesis and reduces MMP-1 release, according to Obayashi et. al (1), all assuring the well-being aof the cells and mitochondrias.

Skin energy production declines from youth as there is a decrease in ATP (*Greco et al., FASEB J. 17:1706, 2003*) and in increase in lactic acid (*Goldstein et al., J. Cell Physiol. 112:419, 1982*).

Mitochondria created a symbiotic relationship with the host cells billions of years ago. Mitochondria breathes; they use fatty acids and oxygen to produce CO_2 and ATP.

ATP is the "currency energy" (Figure 2).

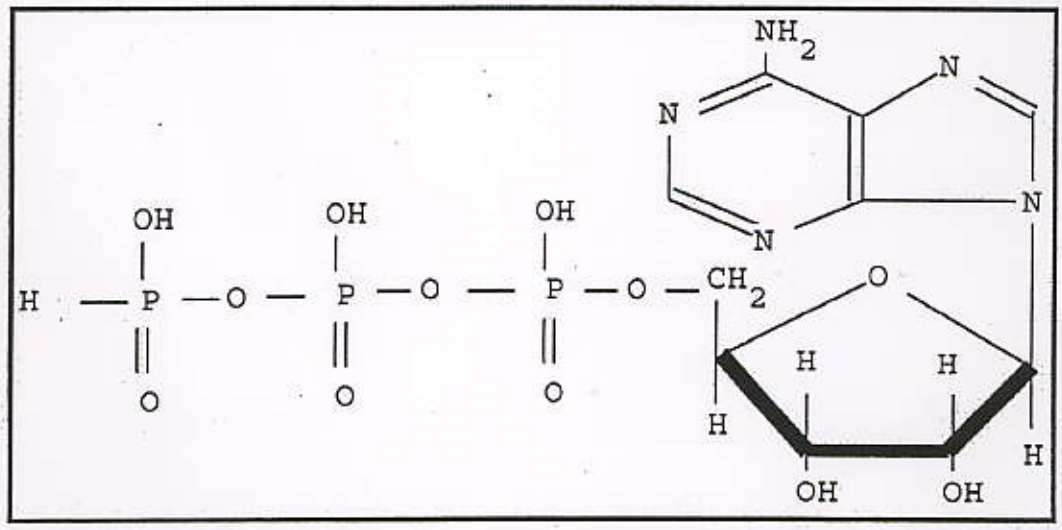


Figure 2: ATP Chemical Structure
(energy currency)

The corresponding process is:

$C_6H_{12}O_6 + 6O_2 \Rightarrow 6H_2O + 6CO_2 + ATP$
(carbohydrate)

2) DNA in Mitochondria

Mitochondria have 1-2% of the total DNA. This circular DNA is important because mitochondrial DNA code for sub-units of

--- ATP Synthase (linked to ATP synthesis)
--- NADH dehydrogenase (linked to respiration)
--- Cytochrome Oxidase

...for a total of 13 units.

NADH Hydrogenase is involved in the process of NADH recycling and is key to respiration (Figure 3).

Figure 3: Respiration Process

Integrity of mitochondrial DNA is therefore important.

OK: $O_2 \longrightarrow H_2O$

WRONG: $O_2 \longrightarrow O^*_2$

$O_2 \longrightarrow H_2O_2$

H_2O_2 leads to DNA damage in the form of 8-oxo-guanine and in increase in MMP-1 release.

3) UV-A and UV-B and Aging

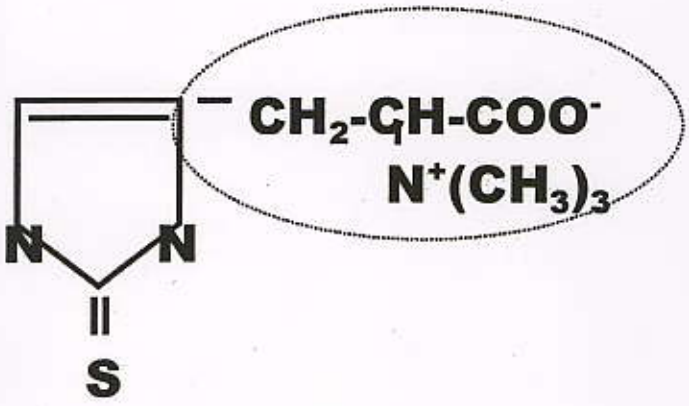
Recently, the increase in the aged population and the increase in UV at the earth's surface (2) have focused on the public's concern on the long-term effects of UV-A (320 nm - 400 nm) and UV-B (290 nm -320 nm), especially the acceleration of premature skin aging. Photoaged facial skin is characterized by the appearance of deep wrinkles at the corner of the eyes and around the mouth. Many studies have demonstrated that the alterations of the extracellular matrix at the papillary dermis, collagen and elastin substantially contributes to the formation of photoaged skin (3-9). The decrease of collagen fibers and the disappearance of elastin fine fiber and oxytalan fiber has been observed in photoaged skin. These alterations are caused by repeated UV exposure.

4) Thiotaine - Natural Amino Acid

Thiotaine (Ergothioneine) is a natural antioxidant and an amino acid not incorporated into protein, whose sulfur is predominantly in the thione form (Figure 4). Thiotaine is a fungal metabolite that cannot be endogenously synthesized by mammals; it must be taken up in the diet (10). Is is found in many mammalian tissues in millimolar quantities (10). Thiotaine is generally regarded as an antioxidant, although results are conflicting. Some regard it as a scavenger of hydrogen peroxide (11), while others contend that it does not readily react with hydrogen peroxide but does scavenge hydroxyl radicals (12). Also, some date indicate that Thiotaine quenched O_2 by monitoring 1270-nm phosphorescence derived from O_2 (13).

In this study, we examined the scavenging abilities of Thiotaine against O_2^- and $^1O_2$ using chemical and biological systems to identify antioxidative characters. Also, the effects of Thiotaine on UV-induced cellular responses such as expression of TNF-Alpha and MMP-1 were evaluated.

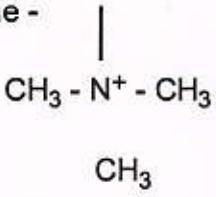
Figure 4: L-ergothioneine chemical structure



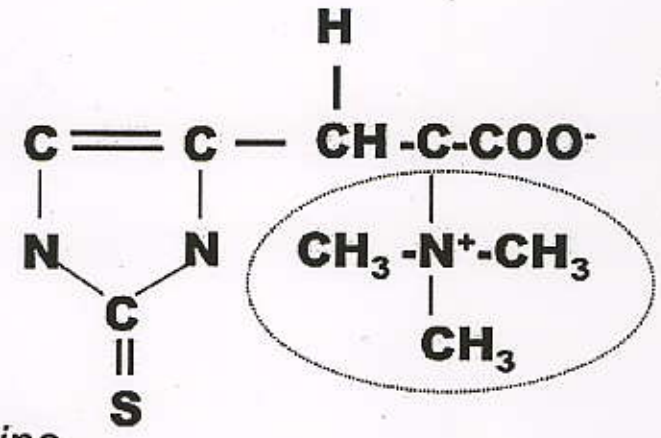
PART ONE - THIOTAINE: ENERGY in MITOCHONDRIAS

Role in Energy Production and Fatty Acid Transport

The transferring group of Thiotaine -

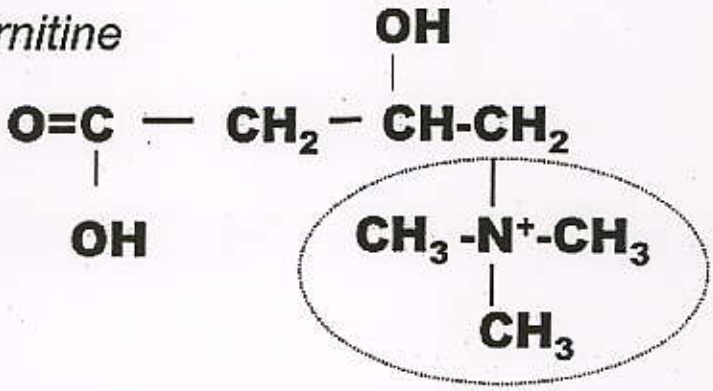


- is also present in Carnitine



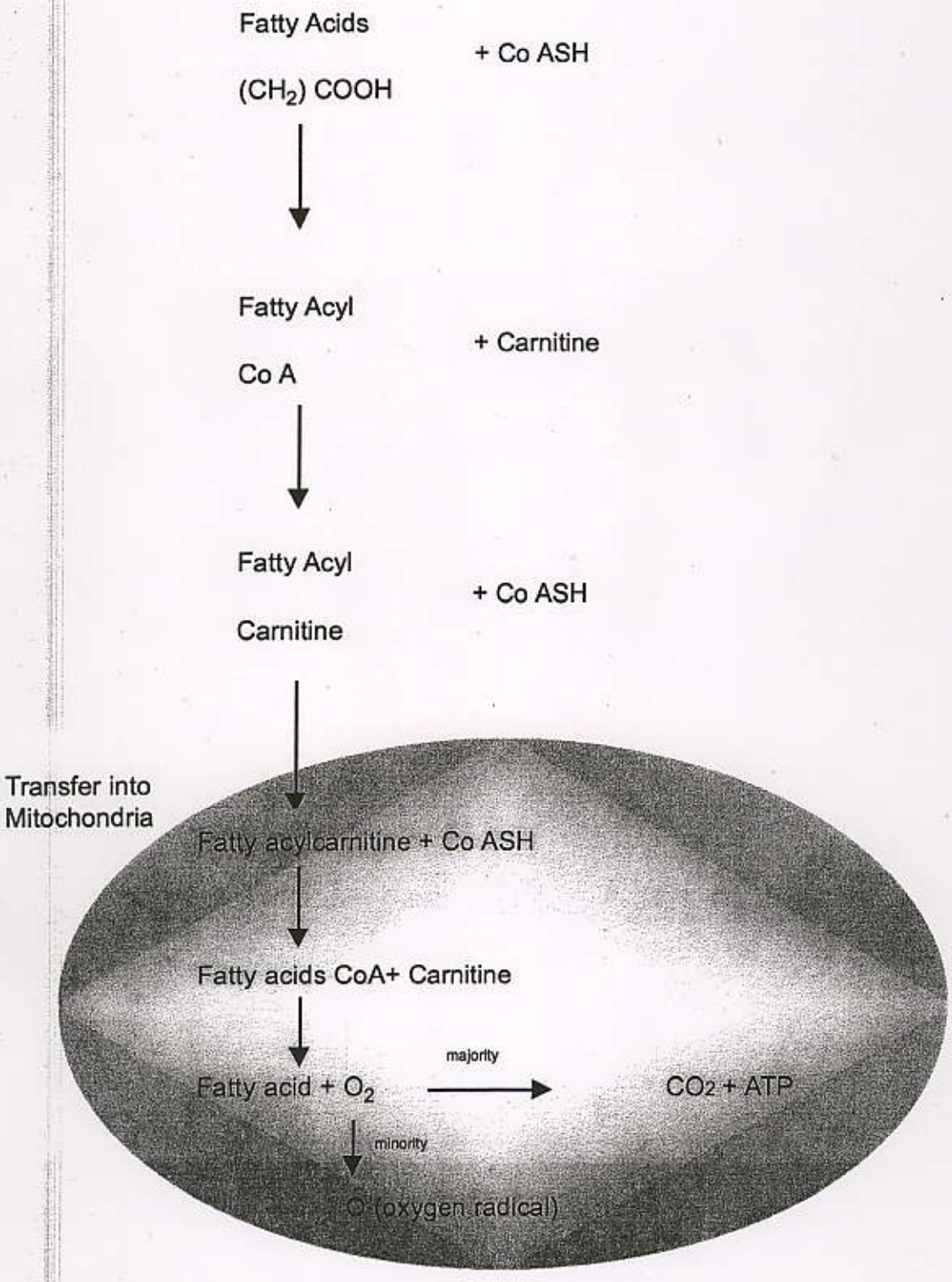
Thiotaine

Carnitine



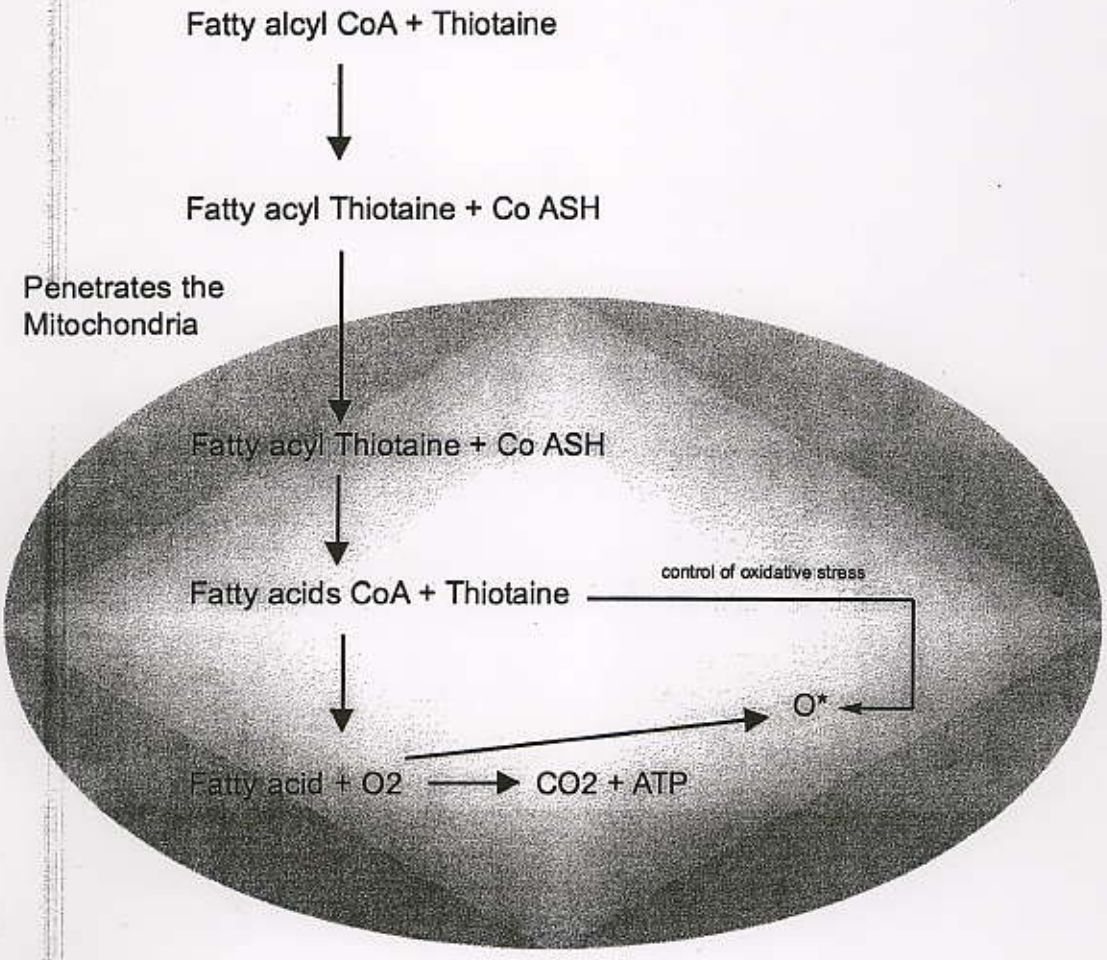
Carnitine is used in slimming products. It helps to transfer fatty acid in the mitochondria and the oxygen present will burn the fatty acid.

Carnitine's mode of action is described below.



Consequence: Less Fatty Acid; More Energy

Thiotaine's mode of action is similar.



Consequence: Less Fatty Acid, More Energy

But Also: Control of Oxidative Stress by the second group of Thiotaine:
The Thiol

PART TWO: THIOTAINE IS AN ANTI-OXIDANT

1) Role as Anti-Oxidant in UV-A attack

UV-A radiation generates singlet oxygen (O_2) (type II photosensitization) through photosensitization reactions with several intracellular chromophors such as NADH, NAPDH, and flavine protein (14). It has been reported that O_2 generated by UV-A mediates the induction of MMP-1 through the pathway of IL-6 and IL-1 (15,16).

UV-A exposure to dermal fibroblasts leads to the reduction of collagen synthesis (17) and the excess elevation of matrix metalloproteinase-1 (MMP-1)/interstitial collagenase (18). MMP-1 is a member of the MMP's, a superfamily of endopeptidase that is capable of degrading extracellular matrix components (19). Excess expression of MMP-1 by skin fibroblasts causes subsequent damage of dermal connective tissue. The imbalance between the synthesis and degradation of collagen critically contributes to the process of matrix alteration (20) and leads to photoaging.

a) Quenching Activity Against O_2

The quenching activity of Thiotaine was measured by using the ESR spin-trapping method and lipid peroxidation (LPO) initiated by O_2. In general, hematoporphytin produces O_2 during UV-A irradiation. As a source of O_2 the hematoporphytin and UV-A system was used. The ESR spectrum of the $^1O_2$ is shown in Figure 5. The addition of Thiotaine showed a decrease of O_2^- derived TEMP radicals in a dose-dependent manner. These results indicated that Thiotaine effectively quenched O_2.

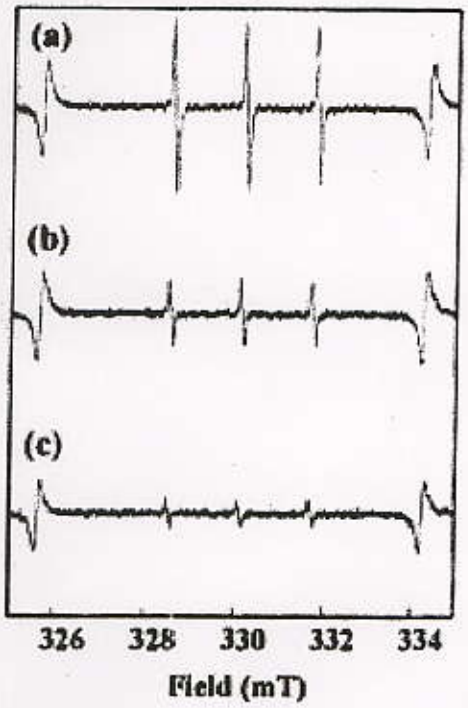


Figure 5. Singlet oxygen quenching effect of Thiotaine by ESR study
(a) Control (without Thiotaine)
(b) Thiotaine 10 mg/ml
(c) Thiotaine 20 mg/ml

The results for LPO initiated by $^1O_2$ are shown in Table 1. Rose bengal plus visible light was used as the source of $^1O_2$. The LPO level of control liposomes was 23.81 nmol/ml, and the exposure to O_2 increased LPO to 91.84 nmol/ml. The addition of Thiotaine reduced LPO to 26.53 nmol/ml, a 96% reduction.

Table 1. Inhibition of Thiotaine on Lipid Peroxidation Initiated by Singlet Oxygen

	nmol LPO/ml
Liposomes alone	23.81
Liposomes + rose bengal	91.84
Liposomes + rose bengal + 20 µM Thiotaine	26.53

As a source of singlet oxygen, the photo-irradiated rose bengal system was used. Liposomes prepared from phosphatidyl choline with 10 mM rose bengal were irradiated using a Sylvania 150W slide projector. Oxidation products in the liposomes were assayed with K-Assay™ LPO-CC from Kaniya Biomedical Company (Seattle, WA). Data are expressed as mean value from dependent examinations in duplicate.

b) MMP-1 mRNA Expression

Fibroblasts exposed to UV-A enhance MMP-1 production with up-regulation of MMP-1 mRNA expression. Thus, we examined the effect of Thiotaine on MMP-1 mRNA expression in cultured normal human fibroblasts, exposed to UV-A. MMP-1 mRNA in human fibroblasts was elevated 1.25-fold at 24 h post UV-A irradiation. Thiotaine reduced MMP-1 mRNA expression levels in a dose-dependent manner (Figure 6). The results indicated that Thiotaine down-regulated MMP-1 mRNA expression of fibroblasts induced by UV-A irradiation.

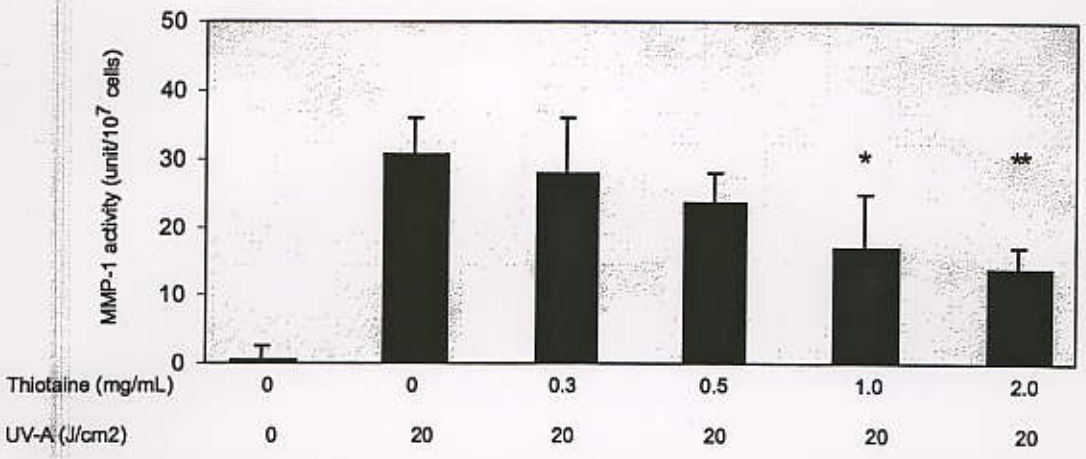


Figure 6. Thiotaine-suppressed MMP-1 production induced by UCV-A irradiation. Human fibroblasts were exposed to UV-A at a dose of 20 J/cm2 in the presence of various concentrations of Thiotaine in HBS. MMP-1 activity and cell numbers were measured after UV-A irradiation for 24 hours. n = 4. Significance: *p< 0.05; **p < 0.01.

2) Role in Anti-Oxidation in UV-B attack

UV-B radiation creates superoxide anion (O_2^-) (type I photosensitization) due to reaction with water, activation of mitochondrial function and release of peroxides by inflammatory cells (21). UV-B causes acute damage in the skin, such as DNA damage and apoptosis of keratinocytes, even in dermal cells. In addition, UV-B induces the production of cytokines, hormones and chemical messengers IL-1, TNF-alpha, propiomelanocortin-derived hormones and prostaglandin E2, which consequently leads to erythema and inflammation in the dermis (22).

a) Scavenging Ability Against O_2^-

The scavenging ability of Thiotaine against O_2^- was evaluated using the hypoxanthine and xanthine oxidase system as a source of O_2^-. Thiotaine showed scavenging activity against O_2^- in a dose-dependent manner in the micromolar range (Figure 7).

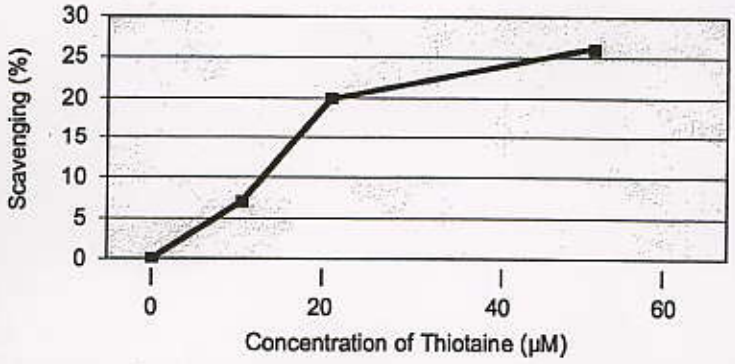


Figure 7. Scavenging the effect of Thiotaine against superoxide anion generated by hypoxanthine-xanthine oxidase system. The OD_{560nm} in the absence of Thiotaine was set as 0% scavenging. The scavenging percent was calculated as the reduction in OD divided by the starting OD X 100.

In addition, we examined the effects of Thiotaine on lipid peroxidation (LPO) of liposomes initiated by O_2^- generated by alloxan. The base level of LPO in the control liposome was 17.37 nmol/ml, and the addition of alloxan to the system was increased to 50.31 nmol/ml. Thiotaine (20 µM) reduced LPO to 22.12 nmol/ml, an 85% reduction, and exhibited superior effects among other sulfur-containing antioxidants that were tested at the same concentration (Figure 8, following page).

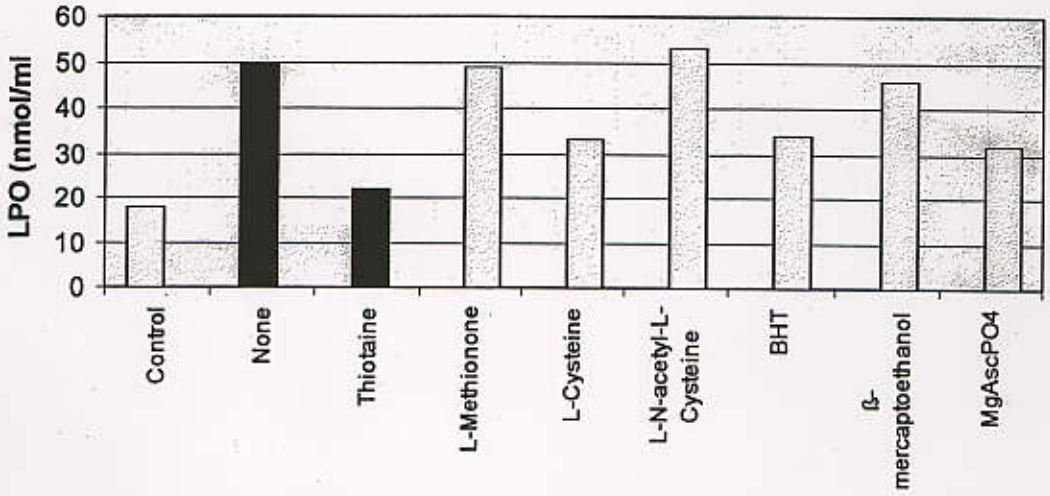


Figure 8. Scavenging effect of antioxidants against superoxide anion generated by alloxan. Lipid peroxides (LPO) were generated in liposomes by alloxan without addition (none). The level of LPO in samples with 20 µM antioxidants was measured after 60 minutes.

b) TNF-alpha Expression by UV-B Irradiation

To examine the effects of Thiotaine on UV-B induced TNF-alpha expression, we carried out a reporter assay using fibroblast cell line XPTNF2, which is an SV40 fibroblast line deficient in DNA repair and carrying the TNF-alpha promoter chloramphenicol acetyl-transferase (CAT) reporter gene. UV-B irradiation of these cells increased the promoter activity, and as a result exhibited a CAT activity of 39.87 nmol/mg/h. Twenty µM and 50 µM Thiotaine reduced CAT activities to 15.97 and 22.07 nmol/mg/h, respectively (Table II).

Table II
Induction of TNF-alpha by UV-B in Fibroblasts

Treatment	Net CAT Activity (nmol/mg/h)
UV-B (100 J/m^2)	39.87
UV-B + 20 µM Thiotaine	15.97
UV-B + 50 µM Thiotaine	22.07

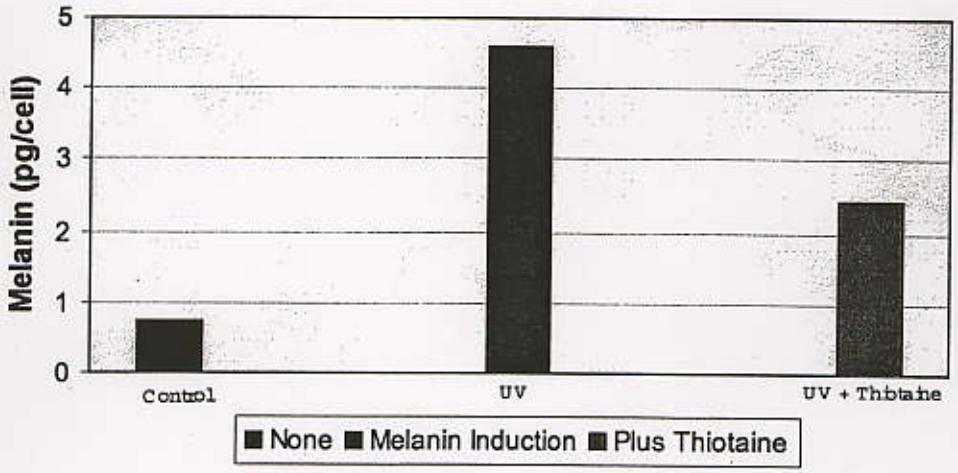
Fibroblast line XPTNF2, which is an SV40 fibroblast line deficient in DNA repair and carrying the TNF-alpha promoter chloramphenicol acetyltransferase (CAT) reporter gene. Assay of TNF-alpha promoter activity as described in text. Assays in duplicate, background subtracted, and results averaged.

PART THREE: THIOTAINE AS A CLARIFIER

Clarifier:

Thiotaine acts like a clarifier because it is:

* A metal chelator like Kojic Acid
* An antioxidant like Vitamin C
* It inhibits tyrosinase and also inhibits melanin in cell culture at 1% use level



Thiotaine

PRODUCT SPECIFICATIONS

INCI Name: L-Ergothioneine
CAS #: 497-30-3

Test	Specifications
HPLC ANALYSIS: L-ERGOTHIONINE	1.8 - 2.2 MM
APPEARANCE	CLEAR AND COLORLESS SOLUTION
ODOR	CHARACTERISTIC
BACTERIA AND FUNGI	<100 ORGANISMS/GRAM
PATHOGENS	<1 CFU/GRAM
PHENOXYETHANOL	1.0 - 1.2%
PH	7.0 - 8.0

MATERIAL SAFETY DATA SHEET

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1. CHEMICAL PRODUCT IDENTIFICATION

Product Name:	Thiotaine
Manufacturer's Name	AGI Dermatics
Common Chemical Name:	Ergothioneine & Water
INCI Name:	Ergothioneine & Water

2. COMPOSITION/INFORMATION ON INGREDIENTS

Substance/Preparation:	Preparation
Information on hazardous ingredients	N/A

Chemical Name	%	EINECS No.	CAS Number
Ergothioneine			497-30-3
Water			7732-18-5

Preservative: Phenoxyethanol 1%

3. HAZARD IDENTIFICATION

Human Health Hazards:	None known

4. FIRST AID MEASURES

Effects and Symptoms:

Ingestion:	No adverse effects known. Low toxicity.
Inhalation	No adverse effects known.
Skin Contact	No adverse effects known.
Eye Contact	No adverse effects known.

First Aid Measures:

Ingestion:	Induce vomiting if large amounts are ingested and seed medical attention.
Inhalation:	Remove to fresh air. Seek medical attention if breathing is labored.
Skin contact:	Wash thoroughly with soap and water. Call a doctor if irritation develops.
Eye contact:	Flush with water for 15 minutes. Seek medical atten tion.

5. FIRE FIGHTING MEASURES

Extinguishing Media

Suitable:	Not flammable
Special Firefighting Procedures:	None
Hazardous Thermal (de)composition Products:	N/A
Protection of Firefighters:	N/A

6.ACCIDENTAL RELEASE MEASURES

Personal Precautions:	Avoid contact with skin and eyes.
Environmental Precautions:	No special precautions necessary.
Methods of cleaning up	Remove excess. Wash area with detergent and water.

7. HANDLING AND STORAGE

Handling:	Avoid contact with skin and eyes.
Storage:	Store at 20-25° C. Do not freeze. Keep away from light.

8. EXPOSURE CONTROL/PERSONAL PROTECTION

Respiratory System Protection:	No special respirator necessary.
Skin and Body Protection:	Wear suitable protective clothing.
Hand Protection:	Wear impervious gloves.
Eye Protection:	Wear goggles with side shields.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	Liquid
Color:	Clear
Odor:	Characteristic
pH:	7.0 - 8.0
Flash Point	>200° C
Solubility:	
Water	Soluble

10. STABILITY AND REACTIVITY

Conditions to avoid:	None expected if stored and handled properly.
Materials to avoid:	None expected if stored and handled properly.
Hazardous Decomposition Products:	N/A

11. TOXICOLOGICAL INFORMATION

Skin Irritation:	Low irritation
Eye Irritation:	Low irritation
Sensitization:	Not tested

12. ECOLOGICAL INFORMATION

13. DISPOSAL CONSIDERATIONS

Method of Disposal:	As per federal, state and local regulations.

14. TRANSPORT INFORMATION

Land - Road/Railway	Non-hazardous material
Inland Waterways	Non-hazardous material
Sea	Non-hazardous material
Air	Non-hazardous material
National Transport Regulations	Non-hazardous material

15. REGULATORY INFORMATION

Label Name:	Thiotaine

16. OTHER INFORMATION

History	
Date of issue	March 20, 2002

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UNICHONDRIN ATP

THE MODERN ACTIVE AGENT CONCEPT FOR LASTING PRESERVATION OF YOUTHFUL SKIN

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UNICHONDRIN ATP

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UNICHONDRIN ATP

1. Introduction

The main task of modern skin care cosmetics is without doubt the preservation of a youthful and healthy condition of the skin. In addition, it can also be expected of effective skin care products that they eliminate minor deviations from ideal conditions due to stress and environmental factors and that they ensure an optimal equilibrium between all the skin functions. Nevertheless, despite regular daily care, the natural ageing process with its undesirable consequences cannot be arrested. However, premature ageing of the skin due to our modern stressful lifestyle and detrimental environmental factors can nevertheless be specifically counteracted with modern active agent cosmetics. According to recent biochemical findings, the visible consequences of the inexorable ageing process can be demonstrably and increasingly effectively reduced with potent active agents.

The external appearance of the skin depends very considerably on the connective tissue. The extremely complex processes in the connective tissue of the skin determine its hydration capacity, elasticity, pliability, and not least the presence or absence of wrinkles. The age-related changes affecting specifically the connective tissue of the skin is the subject of continuous research, and this is constantly discovering new findings. This is the scientific basis of modern cosmetics.

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UNICHONDRIN ATP

2. Concept of the active agent complex Unichondrin ATP

The connective tissue of the skin consists of connective tissue cells or fibroblasts and an extra cellular matrix that occupies the space between the cells. This matrix consists of a complex network or reticulum of a wide variety of macromolecules called biopolymers. It is these biopolymers that determine the mechanical properties of the tissue. The matrix consists partly of amorphous gelatinous substances and partly of structural fiber proteins. The biosynthesis of the constituents takes place in the fibroblasts. The amorphous biopolymers consist of glycosaminoglycans (GAG) (also called glucosaminoglycans or mucopolysaccharides), glycoproteins (also called glucoproteins or mucoproteins) and proteoglycans.The fiber proteins are mainly collagen, elastin and reticulin.

Glycosaminoglycans (= mucopolysaccharides) are formed from aminosaccharides and uronic acids. The best known of the glycosaminoglycans is without doubt hyaluronic acid. Hyaluronic acid is used widely in cosmetics and has the following structure:

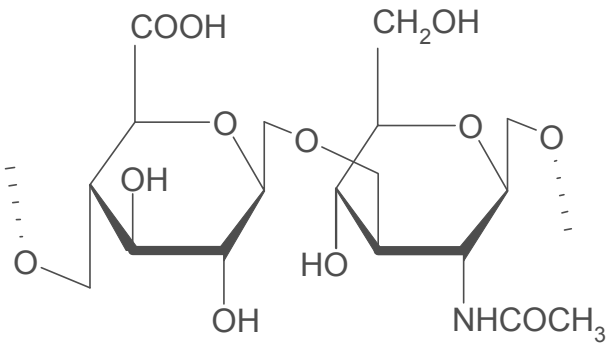

Figure 1: Hyaluronic acid

Hyaluronic acid is formed from N-acetylglucosamine and glucoronic acid in the ratio 1:1 and has a molecular mass of between 1 and 15 million depending on the conditions.

Hyaluronic acid is degraded by the enzyme hyaluronidase.

Another building block no less important for the structure of the extracellular matrix is chondroitin sulfate. This glycosaminoglycan consists of N-acetylgalactosamine and glucuronic acid. It is known to exist as various isomers, and it is mainly types A and C with the structures described below:

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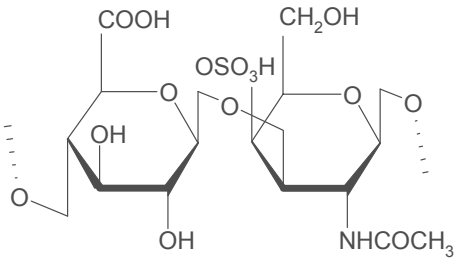

Figure 2: Chondroitinsulfat A

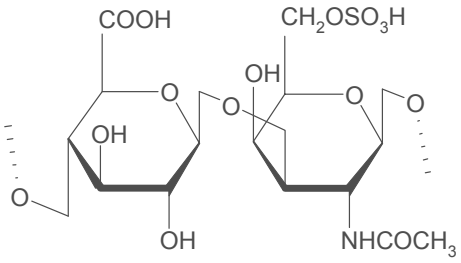

Figure 3: Chondroitinsulfat C

The molecular mass is between 5,000 and 50,000 and is thus several orders of magnitude smaller than that of hyaluronic acid.

The free sulfate groups give chondroitin sulfate a pronounced anionic character and thus very considerably improve the substantivity. Furthermore, chondroitin sulfate is not degraded by hyaluronidase. Chondroitin sulfates reduce the activation energy for the formation of collagen fibers and thus assists their generation.

Chondroitin sulfates are the most important constituents of the proteoglycans. Proteoglycans are structurally similar to glycoproteins, i.e., glycosaminoglycans are bound as side chains to peptide chains („core protein“) of various lengths. Proteoglycans form a three-dimensional reticulum of varying density in the extracellular space, and the other connective tissue structures are embedded into this reticulum. These proteoglycans show a wide range of structural variation, and not all details of their functions are yet known. The most important functions definitely include, for example, the water-binding ability, the binding of positively charged substances, ion exchange, and cohesion of the matrix.

Recent investigations also show that proteoglycans not only have mechanical functions but also perform important biochemical tasks that influence cell and tissue behavior.

The points of special interest to cosmetics chemists are the possible changes occurring in the connective tissue in response to every day stress and skin ageing and how these changes can be delayed.

Various investigations have shown that the fibrils in the connective tissue become thinner with increasing age and the proportion of soluble collagen decreases because the intermolecular cross-linkages lower the solubility. The degree of cross linking is partly a function of age, but cross-linking is also accelerated by environmental factors such as exposure to UV radiation and free radicals. The moisture retention capacity decreases as the degree of cross linking increases, and this leads to the visible changes in ageing skin. With increasing age there is also a reduction in the regeneration capacity of the fibroblasts and thus also of the extracellular matrix.

The content of chondroitin sulfates in particular also decreases markedly as skin ages. Unfortunately, it is the glycosaminoglycans and thus also the chondroitin sulfates that counteract cross linking of the collagen. A chondroitin sulfate deficit in the skin thus accelerates the ageing process.

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In view of the above results, it is rational to use chondroitin sulfates in cosmetics. Although the molecules of chondroitin sulfate are considerably smaller than those of, for example, hyaluronic acid, it cannot be assumed that they can be absorbed through the skin and thus made available to metabolism of the fibroblasts. Instead, the macromolecular chondroitin sulfates form a film of high substantivity on the surface of the skin, and as a result of the outstanding water-binding capacity of these molecules, brings about an improvement in the moisture retention capacity of the skin and thus an immediate effect that can be both felt and seen.

The lasting preservation of a healthy and youthful condition of the connective tissue requires optimal cellular metabolism of the fibroblasts. The extracellular matrix is the result of an extremely complex system of interactive anabolic processes of the intermediate metabolism. The maintenance of these processes requires energy. In order to meet this energy requirement, nature has devised an extraordinarily efficient system that is present in every living creature. The energy supplied to the body through the food chain is stored in the form of adenosine-5'-triphosphate (abbreviation: ATP), which is a very energy-rich molecule. During the energyconsuming biosynthesis of the glycosaminoglycans (= mucopolysaccharides) in the fibroblasts, ATP is cleaved into adenosine diphosphate and orthophosphate in a process that releases energy:

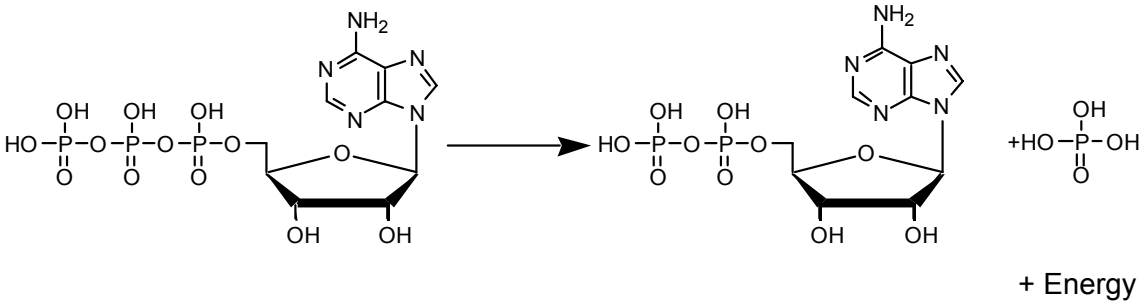

Figure 4: Biosynthesis

A variety of in-vitro studies on cell cultures have shown that addition of ATP actually stimulates the cellular metabolism and promotes the formation of extracellular matrix substances. For this reason, ATP is often called a „biocatalyst“ in specialist literature.

The high energy content of ATP has the consequence that, particularly in aqueous solution, ATP is not very stable. However, the presence of protein hydrolysates reduces the rate of breakdown of ATP very considerably. It therefore appears rational to use ATP in cosmetic products only in conjunction with protein hydrolysates. Protein hydrolysates are also biopolymers and are used in cosmetics to improve the moisture retention capacity of the skin.

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The points described above form the scientific basis that explain the rational and clear route to the concept of the active agent complex Unichondrin ATP:

- Chondroitin sulfate is a biopolymer with a pronounced moisture retention comparable to that of hyaluronic acid but with a higher substantivity that occurs in the connective tissue of the skin and thus, as a component of the active agent complex
  Unichondrin ATP, brings about an immediate effect on the skin that can be both felt and seen.

- ATP is a biocatalyst that stimulates the cellular metabolism and thus assists the fibroblasts in the formation of the extracellular matrix of the connective tissue and forms the basis for the lasting effect of the active agent complex Unichondrin ATP.

- The protein hydrolysate contained in Unichondrin ATP not only assists the hydrating properties of chondroitin sulfate but also stabilizes the ATP present in the active agent complex.

- The greatest possible hydration is the best precondition for penetration of the hydrophilic ATP molecules through the skin. Unichondrin ATP therefore contains a high proportion of 1,3-butylene glycol.

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UNICHONDRIN ATP

3. Efficacy

3.1 General

The aim of the active agent complex Unichondrin ATP is to delay the ageing processes in the connective tissue of the skin and to preserve its youthful appearance, as manifested by improved hydration and better skin topography (wrinkle depth, wrinkle density, wrinkle length, roughness). These assessment criteria can be measured objectively with a high degree of reliability on living human skin. For the assessment of the efficacy, Unichondrin ATP was blended into a neutral base cream to form a test cream with a concentration of 2.5%. The same base cream without addition of the active agent was used as the placebo comparison product.

Experience has shown that, despite comparative investigations with placebo products, the efficacy of a cosmetic active agent can be influenced by the composition of the base cream used to generate the test cream. It is therefore of little benefit to perform investigations of this type on as many subjects as possible. The validity can, however, be increased by lengthening the application and assessment periods. The efficacy studies described below were performed on 4 subjects according to the established study designs of the Consumer Product Testing in the USA.

3.2 Skin moisture

The efficacy of the active agent complex Unichondrin ATP was tested for skin moisturization using the electrical conductivity of the skin of the forearm. The subjects were forbidden to use any cosmetics other than soap during the 7 days preceding the test phase. The test phase lasted 28 days, during which the test cream and the placebo cream were applied twice daily. The electrical conductivity of test patches on the skin was determined on day 1 (before the start of treatment), on day 15 (after 2 weeks) and on day 29 (after 4 weeks). The results can be summarized as follows:

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The skin moisture content increased by 12% more after two weeks of application of Unichondrin ATP test cream than placebo cream, and by 62% more after four weeks of application of Unichondrin ATP test cream than placebo cream.

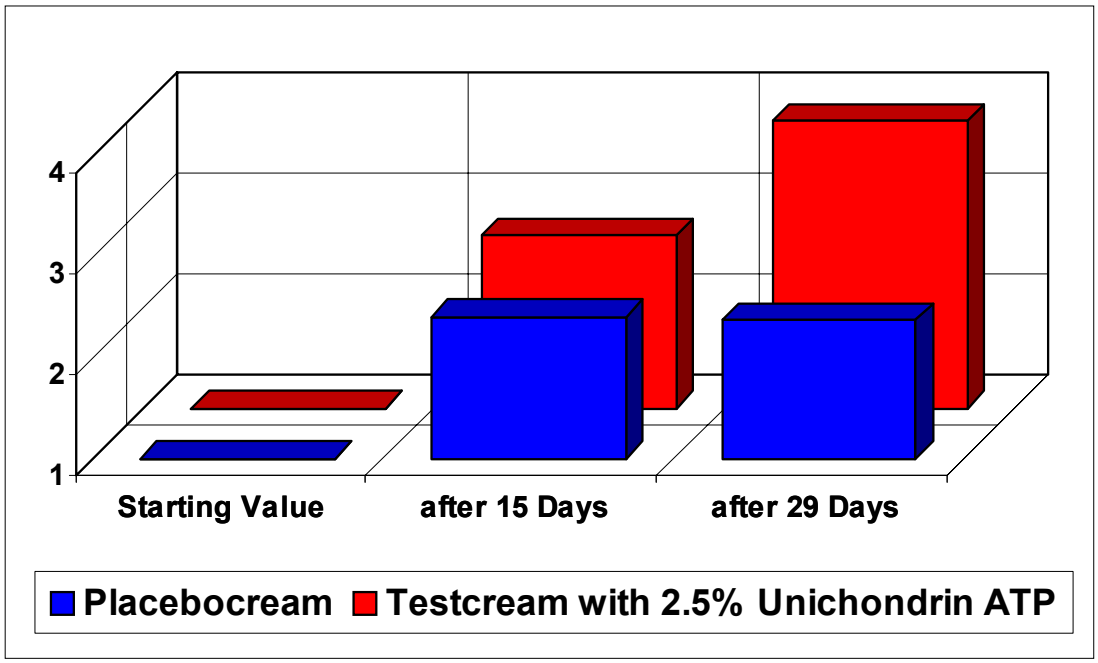

Figure 5: Comparison placebo cream with test cream with 2.5% Unichondrin ATP

The skin moisture content did not increase further during further treatment with the placebo cream after 2 weeks. By contrast, a marked additional improvement was achieved with the test cream during a further two weeks of treatment. From this, it may be concluded that the active agent complex Unichondrin ATP already brings about a clear immediate effect after a short duration of application, and also shows a lasting long term effect.

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UNICHONDRIN ATP

3.3 Skin topoqraphy

In order to assess the influence of the active agent complex Unichondrin ATP on the skin topography, silicone replicas of the outer canthus of the right and left eyes were made before and after the treatment phase. The subjects were forbidden to use any cosmetics other than soap during the 7 days preceding the test phase. The test phase lasted 28 days, during which the test cream was applied twice daily to one side of the face and the placebo cream applied twice daily to the other side. The silicone replicas were analyzed using computer aided image analysis. The results can be summarized as follows:

Average amplitude (RZ):	Test cream:	46% reduction
	Placebo cream:	35% reduction
Number of peaks (RN):	Test cream:	67% reduction
	Placebo cream:	29% reduction
Residual length (RS):	Test cream:	58% reduction
	Placebo cream:	54% reduction
Primary wrinkle (RT):	Test cream:	42% reduction
	Placebo cream:	25% reduction
Roughness:	Test cream:	42% reduction
	Placebo cream:	30% reduction

These results can be seen as plots below as well as digitized image analyses of the silicone replicas before and after the 28 days of treatment with Unichondrin ATP.

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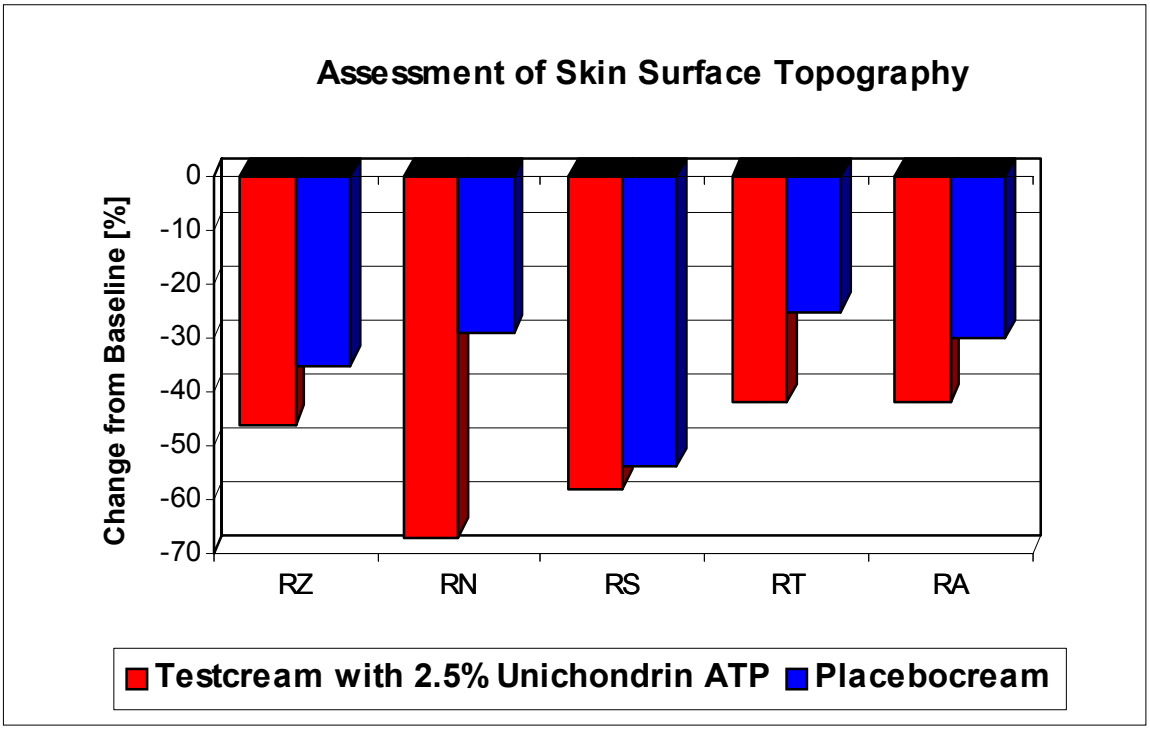

Figure 6: Assessment of skin surface topography

RZ: Average amplitude
RN: Number of peaks
RS: Residual length
RT: Primary wrinkle
RA: Roughness

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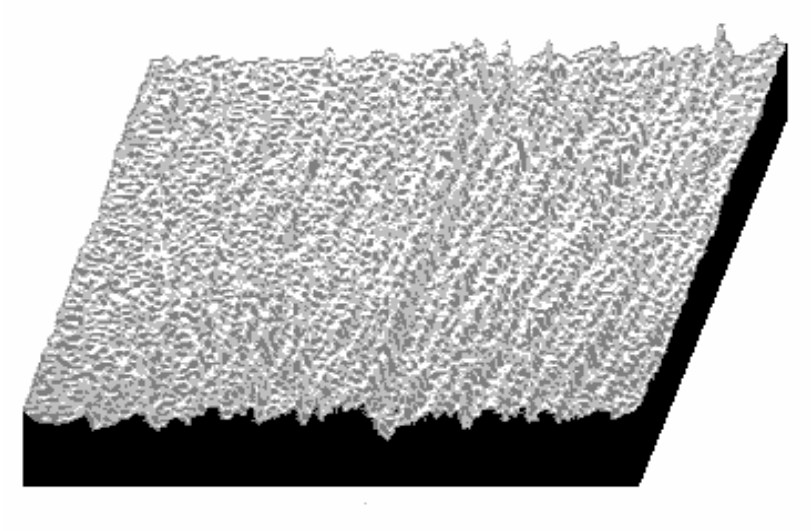

Figure 7: 3-D representation of digitized image - Basisline

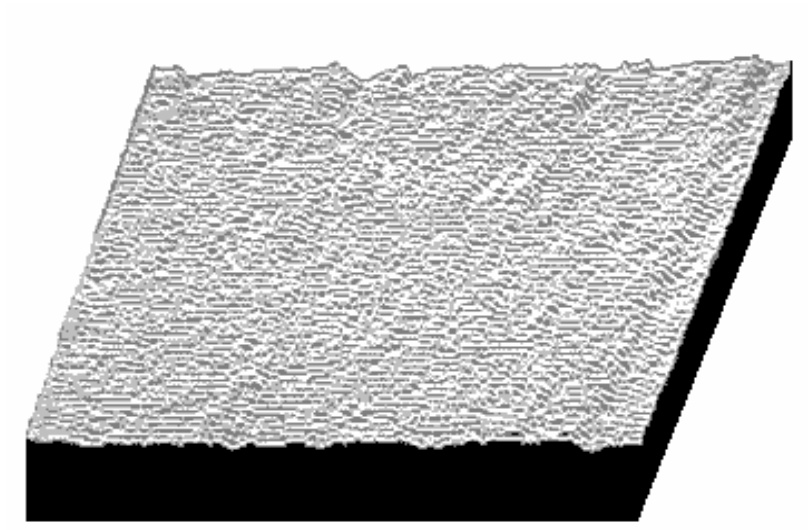

Figure 8: 3-D representation of digitized image - Final

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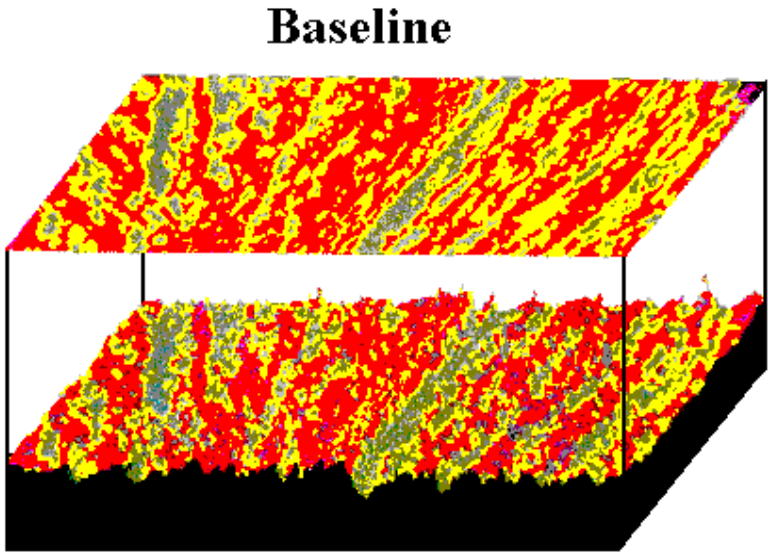

Figure 9: 3-D representation of digitized image with 2-D projection – Basisline

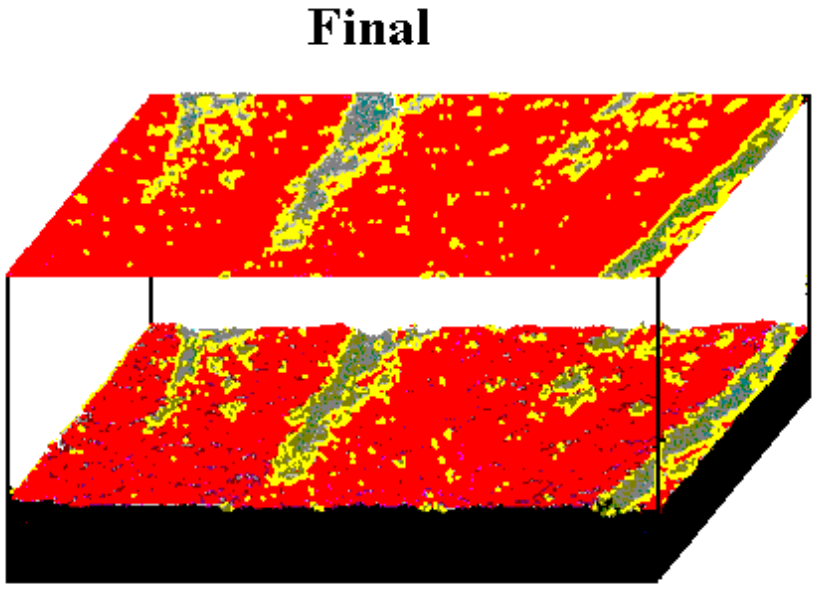

Figure 10: 3-D representation of digitized image with 2-D projection - Final

Yellow :	Peaks
Red :	Transitions
Green :	Valleys

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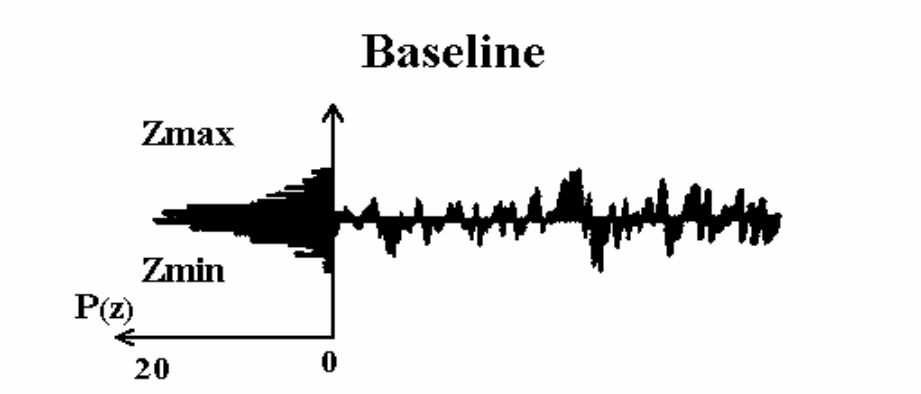

Figure 11: Line density graph (*) – Basisline

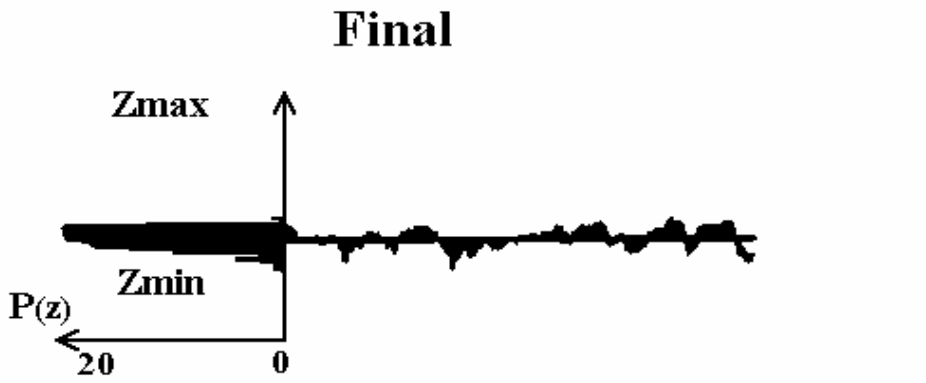

Figure 12: Line density graph (*) - Final

* Profile of one line selected from two dimensional digitized image and quantitated (planar) density of same line.

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UNICHONDRIN ATP

4. Skin tolerance

Unichondrin ATP has been investigated extensively in comprehensive patch tests, and is well tolerated by the skin without problems.

5. Use

Unichondrin ATP is a potent active agent complex for high quality cosmetic skin care products for lasting preservation of youthful skin. Unichondrin ATP is water soluble and therefore suitable for blending into all types of emulsions, lotions, gels, etc.
On the basis of the efficacy studies described in Section 3, we recommend a concentration for Unichondrin ATP of between 2.5 and 5%.

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UNICHONDRIN ATP

6. Characteristics

Composition	Unichondrin ATP is a water soluble active ingredient complex consisting of chondroitin sulfate (sodium salt), adenosin triphosphate, hydrolyzed vegetable protein and butylene glycol. Unichondrin ATP contains no preservative (self-preserving).
Appearance	Slightly viscous liquid.
Analytical data	See specifications.
Solubility	Miscible in all proportions with water Soluble in propylene glycol. Soluble in aqueous ethanol. Insoluble in lipids
Storage	Storage conditions: see safety data sheet Shelf life: see specifications
Processing	Unichondrin ATP is relatively stable and can easily be processed under conditions common in the production of cosmetics. However, temperatures over 70°C should be avoided. Intolerance reactions may occur with cationic substances.

Identification	**INCI name**	**CAS-No**
.	Butylene glycol	107-88-0
	Hydrolyzed vegetable protein	100209-45-8
	Adenosine triphosphate	56-65-5
	Sodium chondroitin sulfate	9007-28-7

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VITACELL®

SKIN

ACTIVE INGREDIENT FOR COSMETOLOGY

CELL REVITALIZING FACTOR
REGENERATOR OF EPIDERMAL ENERGY = ATP

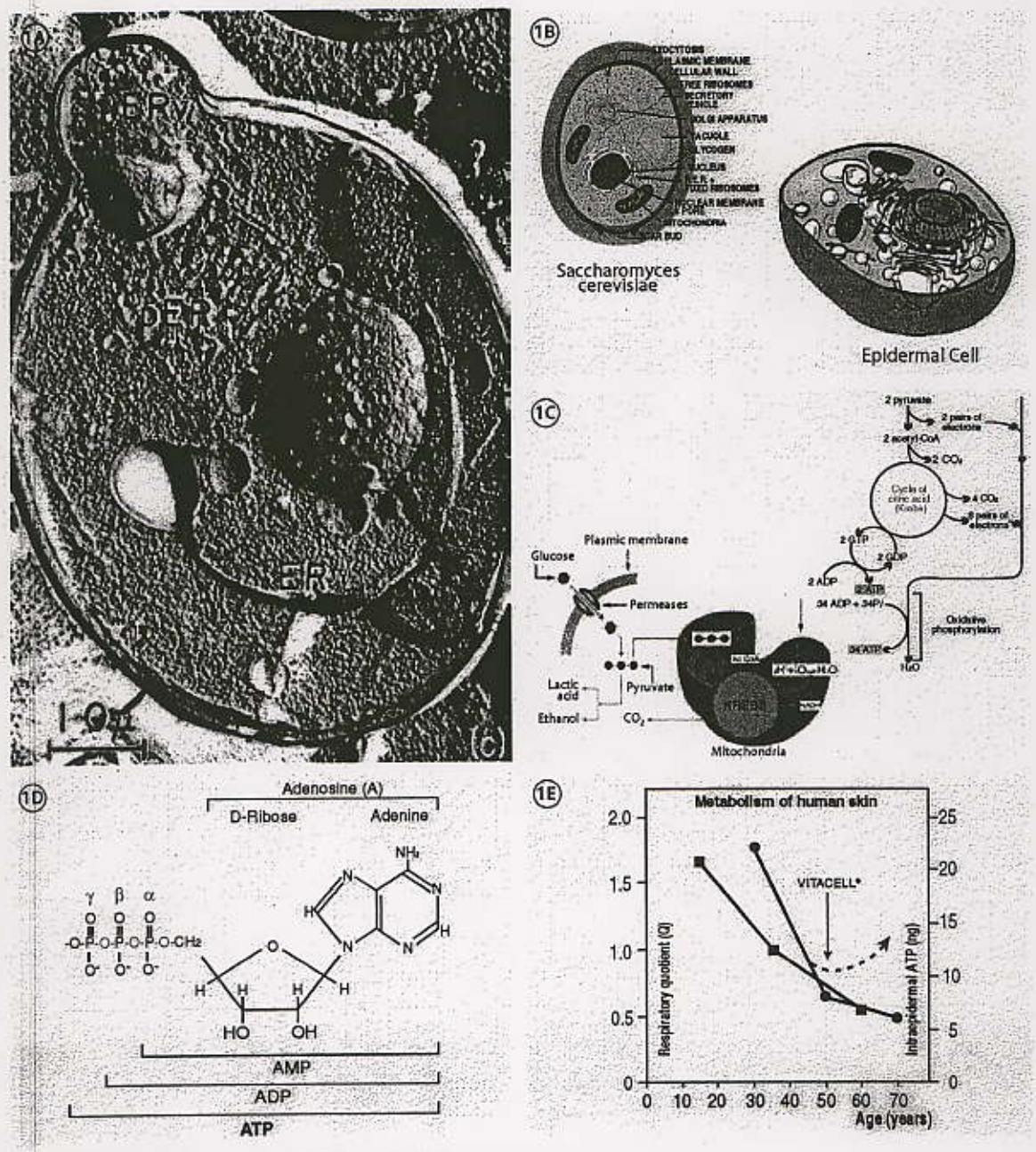

Fig. 1 – VITACELL®: origin, energy metabolism, ATP and functions.

1A - Saccharomyces cerevisiae (The Yeasts. AH Rose and JS Harrison, Ed. Vol 1: Biology of Yeasts. 1987).

1B - VITACELL® is obtained from the purified cytosolic fraction of Saccharomyces cerevisiae. Yeast and epidermal eukaryote cells (keratinocytes) have similar ultrastructural (organelles such as mitochondria) and thus functional metabolic characteristics.

1C - Energy metabolism reaches its full capacity in the mitochondria (Krebs' cycle and chain of electron carriers).

1D - VITACELL® regenerates ATP (a central energy carrier) used for various forms of biological work.

1E - During the aging process, there is a sharp decline in the metabolism of human skin due to a lower respiratory quotient (work by Goldschmiedt) and reduced potential for generating ATP (work at L.S.). In mature or stressed skin, VITACELL® reactivates the energy potential of the epidermis so it can restore ATP to the level observed in young skin.

ATP and skin care

1. ATP, energy and work

• Seven simultaneous properties are necessary and sufficient for a cell to live and persist. One of them is the cell's capacity to extract energy from its surrounding medium and to transform this energy into the various forms of the work required for its survival.

• The energy required to elaborate cell constituents (such as proteins, polysaccharides, nucleic acids...) is supplied directly or indirectly by hydrolysis of ATP into ADP or AMP+Pi.

• ATP is therefore a **central energy carrier.** ATP has two high-energy terminal pyrophosphate bonds (β and γ). When these bonds are broken, energy is supplied to almost every biosynthetic assembly process as well as many other forms of biological work including mechanical work, ion and molecule transportation, osmotic work, electron energization...

• In most cases, hydrolysis of ATP implies the activation of hydrolyzing enzymes (ATPases). ATPases are controlled by special regulatory mechanisms so that, when a specific kind of work is accomplished, for example when a structural element is shortened or inflected, ATP is hydrolyzed at the same time only as needed... (Fig. 2).

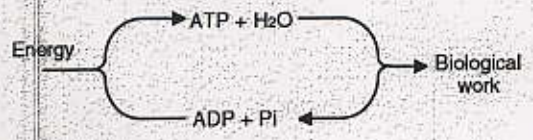

Fig. 2 – ATP cycle.

2. ATP, skin and aging

• Skin is a living tissue made of highly developed eukaryotic type cells containing many mitochondria, specialized cytoplasmic organelles where certain Krebs' cycle reactions, high-energy aerobic oxidative metabolism, lead to the biosynthesis of ATP.

• The epidermis is an exclusively cellular tissue where most of these bioreactions occur.

• Skin aging and bioenergetics:
- a decline in the metabolism of living cells is characteristic of skin aging. H. Goldschmiedt showed that the respiratory quotient of human skin (Q = volume CO_2 expired / volume O_2 inspired), an expression of oxidative metabolism, decreases with aging (Fig. 1E),
- LS scientific works (unpublished) have shown that the intracellular ATP (an excellent tracer of energetic metabolism and of global cell vitality) dramatically and regularly decreases in the human epidermis with increasing age (Fig. 1E).

VITACELL®

DEFINITION / COMPOSITION

• VITACELL® is a natural active substance. This biotechnological product is isolated and purified from a unicellular eukaryote microorganism (yeast): ***Saccharomyces cerevisiae.***

• VITACELL® is:
- a strong activator of bioenergetic metabolism,
- a vitalizing agent,
- an ATP regenerator for living skin cells.

• VITACELL® has no growth factor effect and does not contain any ATP.

Main components:
- Amino-acids and peptides } from yeast
- Nucleosides } cytoplasm

SKIN BENEFITS

1. Cell vitalizing agent - ATP regenerator - Anti-aging agent.

• Cells draw their energy from ATP. This energy is used to transport molecules and ions, for electron transfer, and for biosynthesis, biodegradation, and differentiation processes... Because it is the universal bioenergy mediator, ATP is often called "the fuel of life". ATP is present in cells in catalytic quantities and must be endlessly regenerated to play its role as an energy mediator.

• VITACELL® stimulates the ability of skin cells to biosynthesize and regenerate ATP. It helps the skin recover its energy potential, similar to the potential of young skin. VITACELL® may be considered as a strong anti-aging co-active agent.

2. Stimulator of respiratory metabolism in skin cells, by activating enzymes in the bioenergetic pathways.
VITACELL® works closely with epidermal cellular physiology, helping it direct substrates to oxidative respiratory pathways (Krebs' cycle) predominantly over anaerobic (glycolytic) pathways.

3. Cell repair
By favoring oxidative and bioenergetic cellular metabolism, VITACELL® plays a key role in:
- capture and elimination of excess cellular toxins,
- activation of intracellular exchanges,
- neutralization of environmental pollutants.

COSMETIC USE

- Designed for tired, dull, atonic skin.
- For preventing or reducing early skin aging: especially in areas exposed to attacks from environmental or other types of stress.
- For stimulating preparations (face, body...).
- For regenerating and repairing care: anti-aging, anti-stress, after-sun preparations.

DOSAGE / SOLUBILITY / MODE OF INCORPORATION

1. Dose of use:
VITACELL® LS 8430 (liquid): 2 to 5%.
VITACELL® POWDER LS 7979: 1 to 2%.
VITACELL® POWDER LS 7979 is twice as concentrated as VITACELL® LS 8430.

2. Solubility: VITACELL® is soluble in water, insoluble in fat.

3. Mode of incorporation:
VITACELL® LS 8430 is incorporated during the finishing process at 50°C, or at room temperature for cold processing.
VITACELL® POWDER LS 7979: prepare extemporaneously an aqueous mother solution. Then add it to the cosmetic preparation during the final phase at 50°C.

ANALYTICAL CHARACTERISTICS

1. Aspect:
VITACELL® LS 8430: limpid light yellow liquid, with a weak odor.
VITACELL® POWDER LS 7979: white fine powder, with a characteristic odor.

2. Specifications: upon request.

TOLERANCE

Good.

EFFICACY

Test summaries overleaf.

STORAGE

In their original packaging, at 15 - 25°C.

INCI NAME

VITACELL® LS 8430: Yeast Extract.
VITACELL® POWDER LS 7979: Mannitol (and) Yeast Extract.

MANUFACTURER

Laboratoires Sérobiologiques S.A.

EFFICACY TESTS

STIMULATION OF THE EPIDERMAL ATP SYNTHESIS (EX VIVO)

Aim
To show the stimulating effect on cell vitality and the cutaneous energizing effect of a topical application of VITACELL® POWDER LS 7979 versus placebo, using fluorescent enzymatic assay.

Protocol
Experiments were conducted in skin biopsies taken from 6 female volunteers, 21 to 55 years old.
Standardized single topical application on 2 adjacent areas of a skin biopsy:
- one area treated with an emulsion containing 2% VITACELL® POWDER LS 7979,
- the other area with a placebo emulsion.

The energizing activity was identified and quantified by assaying epidermal intracellular ATP (bioluminescence).

Results
The level of epidermal ATP (ng/million of cells) increased by +65% with the emulsion containing 2% VITACELL® POWDER LS 7979 in comparison with the placebo emulsion.

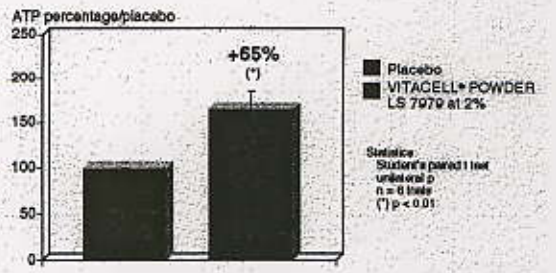

Fig. 3 – VITACELL® POWDER LS 7979 at 2%. Illustration of its stimulating effect on epidermal intracellular ATP stimulation following a topical application.

Conclusion
[redacted]

STIMULATION OF CELL VITALITY (IN VITRO - PRIMARY CULTURES)

Aim / Protocol
The stimulating activity of VITACELL® LS 8430 on cell vitality was measured in human cells:
- in keratinocytes (e
mis),
- in fibroblasts (derm
in growth and in survival.

The intracellular ATP was measured by bioluminescence (increase in % of treated cells ATP/control medium cells ATP).

The kinetics of the effect were determined from different incubation times for 3 concentrations of the active substance.

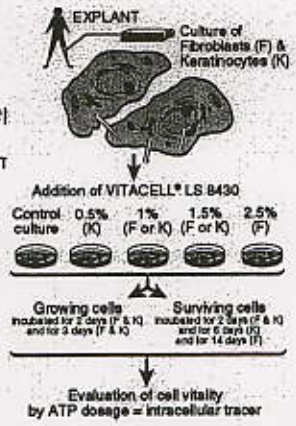

Fig. 4 – Experimental protocol.

Results
Similar results were obtained for fibroblasts and keratinocytes. For keratinocytes for example (Fig. 5), 1.5% VITACELL® LS 8430 induced:
- in growing cells, a +47% increase (after 3 days),
- in surviving cells, a +46% increase (after 6 days).

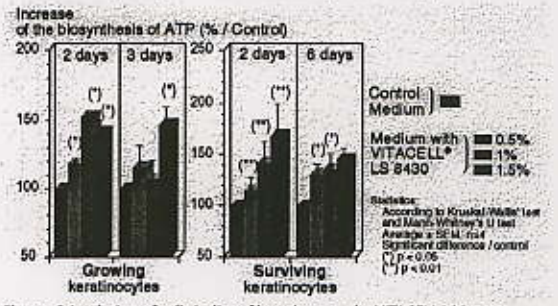

Fig. 5 – Stimulation of cell vitality of keratinocytes by VITACELL® LS 8430.

Conclusion
[redacted]
vitality.

STIMULATION OF CELL METABOLISM ON EPIDERMAL KERATINOCYTES (IN VITRO)

Aim / Protocol
• ATP is a transitory high-energy element. It is an intermediate between energy producing structures (mitochondria) and energy consuming systems (protein synthesis enzymes, DNA, sodium or calcium pumps, detoxification, differentiation...).
• ATP synthesis depends on the respiratory chain which, thanks to nutrients provided by VITACELL® POWDER LS 7979, reduces oxygen into H_2O to form a proton gradient in the mitochondrial intermembrane space.

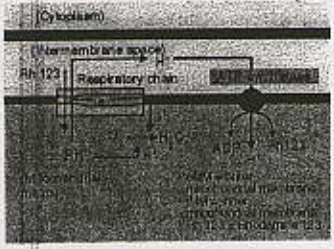

Fig. 6 – ATP synthesis depends on the intramitochondrial respiratory chain.

• The proton gradi
causing ATP-synthetase to produce ATP may be quantified with a specific fluorescent test: Rhodamine Rh 123 (Fig.6).
• Keratinocytes at
fluence were incubated for 48 hours either in presence of the DMEM* control medium, or in presence of the control medium containing increasing concentrations of VITACELL® POWDER LS 7979.
• After 48 hours, the cells are counted and the ATP rate quantified.

Results
VITACELL® POWDER LS 7979 applied on keratinocytes at the concentration of 0.30% showed a strong stimulating effect on cell metabolism. No growth effect was observed.

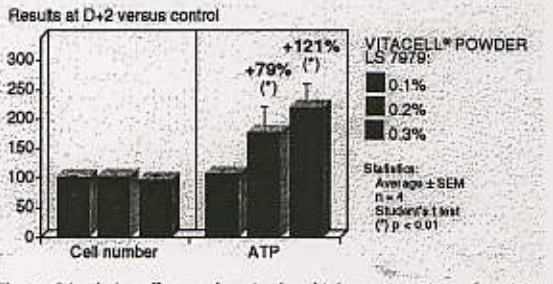

Fig. 7 – Stimulating effect on the mitochondrial enzyme activity of surviving keratinocytes incubated 48 hours in presence of VITACELL® POWDER LS 7979.

* DMEM = Dulbecco's Minimum Essential Medium

STIMULATION OF METABOLIC ACTIVITY (CLINICAL STUDY)

Aim
Evaluation based on the measurement of the partial pressure of transcutaneous oxygen ($TcpO_2$), a parameter which is closely linked to the O_2 supply in epidermal cells and therefore to their cellular metabolism.

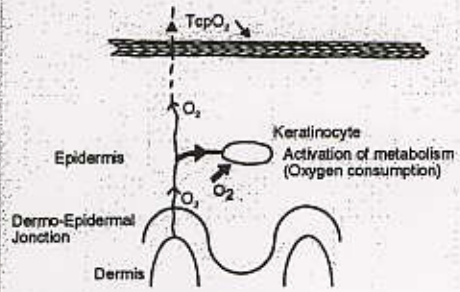

Fig. 8 – Principle of the measurement.

Protocol
Double blind clinical study on 12 volunteers during 3 weeks.
Randomized treatment on the internal side of the forearm with:
- a placebo cream,
- a cream containing 5% VITACELL® LS 8430.

Measurement of the partial pressure before and after the treatment.

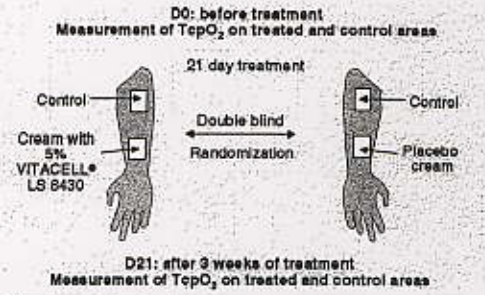

Fig. 9 – Experimental protocol.

Results
Treatment with VITACELL® LS 8430 significantly improved the partial pressure of transcutaneous oxygen.

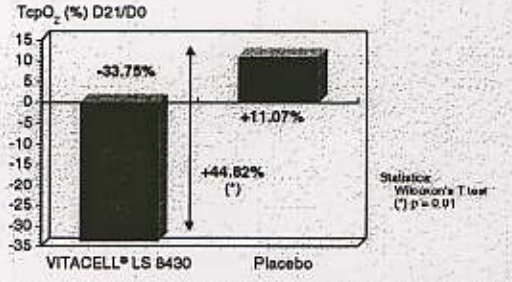

Fig. 10 – Time course of transcutaneous O_2 partial pressure of ($TcpO_2$), compared with the control area, after 3 weeks of treatment with VITACELL® LS 8430. Results in 12 volunteers.

Conclusion
The emulsion containing 5% VITACELL® LS 8430 induced good metabolic activity, significantly higher than with placebo.

STIMULATION OF O_2 CONSUMPTION OF EPITHELIAL CELLS (IN VITRO)

1. Oxygraphy (Polarography)
Polarographical measurements of the O_2 consumption of an epithelial cell homogenate, in presence of various concentrations of VITACELL®.

Determination of the Efficient Dose 50 (ED50), inducing a + 50% increase of the O_2 consumption.

Results
Adding VITACELL® provided a strong increase in oxygen consumption by epithelial cells:
- for VITACELL® LS 8430: ED50 ~1.00%,
- for VITACELL® POWDER LS 7979: ED50 ~0.55%.

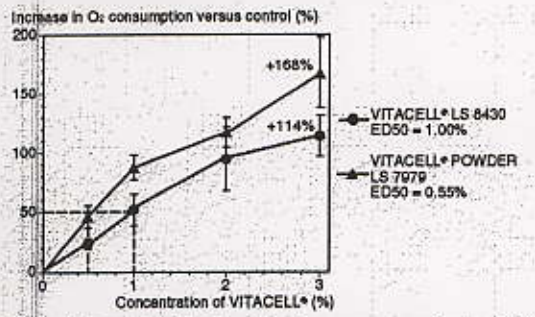

Fig. 11 – In vitro study of the stimulation of oxygen consumption by epithelial cells with increasing doses of VITACELL® LS 8430 and VITACELL® POWDER LS 7979. ED50 = Efficient Dose 50%.

2. Respirometry (Warburg Manometry)
Evaluation of cell breathing by measuring the quantities of gas exchanged. Human skin biopsies are taken and placed in the Warburg's respirometer, made of containers connected to capillary manometers.

The consumption of oxygen is evaluated according to the change of pressure in the system corresponding to the volume of oxygen used.

Results
[redacted]

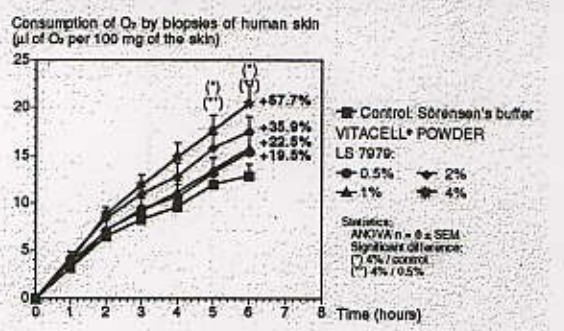

Fig. 12 – O_2 consumption by human skin biopsies in presence of VITACELL® POWDER LS 7979.

LABORATOIRES SÉROBIOLOGIQUES

Member of cognis

VITACELL®

SKIN

CELL REVITALIZING FACTOR

REGENERATOR OF EPIDERMAL ENERGY = ATP

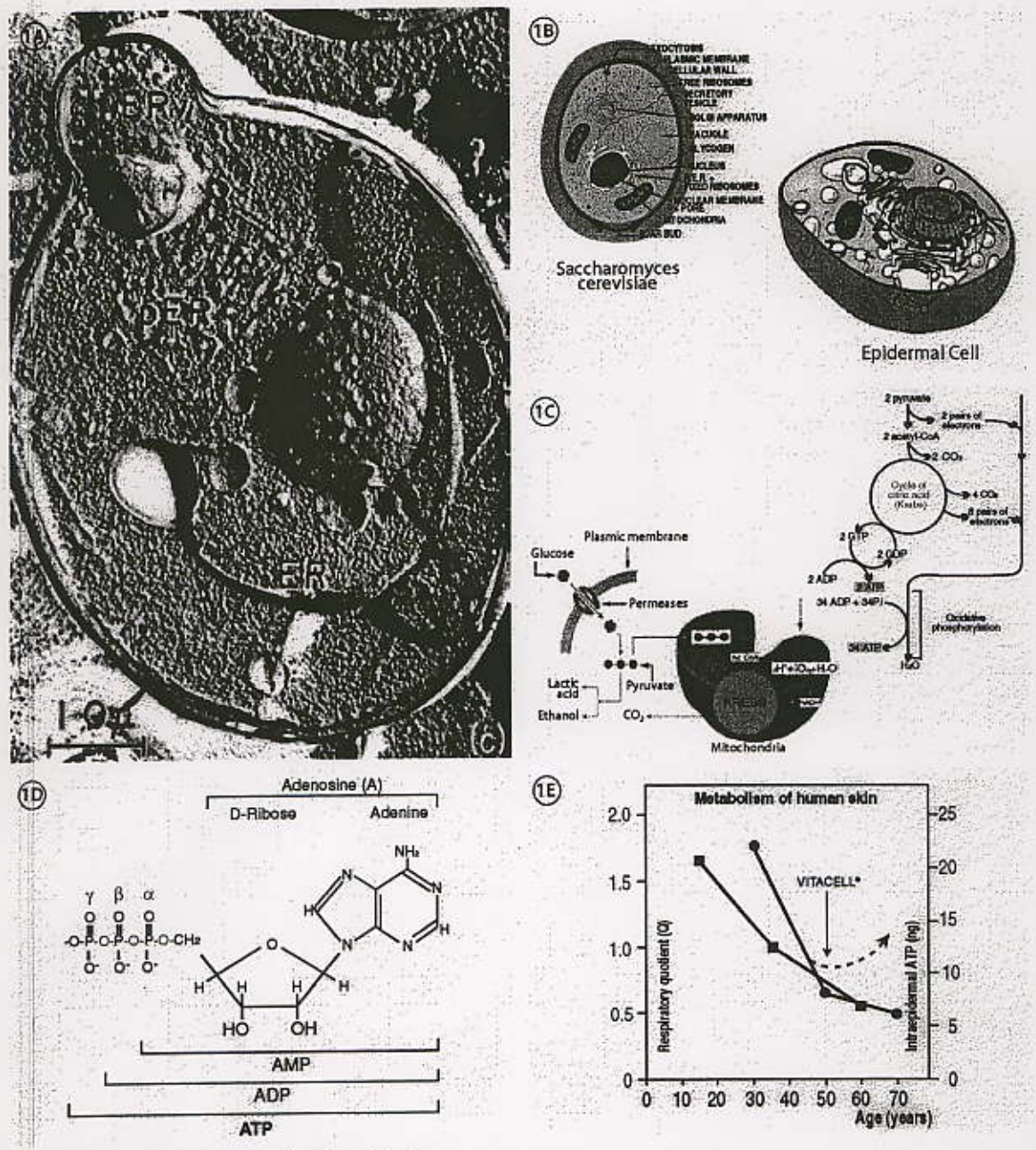

Fig. 1 – VITACELL®: origin, energy metabolism, ATP and functions.

1A - Saccharomyces cerevisiae (The Yeasts. AH Rose and JS Harrison, Ed. Vol 1: Biology of Yeasts. 1987).

1B - VITACELL® is obtained from the purified cytosolic fraction of Saccharomyces cerevisiae. Yeast and epidermal eukaryote cells (keratinocytes) have similar ultrastructural (organelles such as mitochondria) and thus functional metabolic characteristics.

1C - Energy metabolism reaches its full capacity in the mitochondria (Krebs' cycle and chain of electron carriers).

1D - VITACELL® regenerates ATP (a central energy carrier) used for various forms of biological work.

1E - During the aging process, there is a sharp decline in the metabolism of human skin due to a lower respiratory quotient (work by Goldschmiedt) and reduced potential for generating ATP (work at L.S.). In mature or stressed skin, VITACELL® reactivates the energy potential of the epidermis so it can restore ATP to the level observed in young skin.

ACTIVE INGREDIENT FOR COSMETOLOGY

ATP and skin care

1. ATP, energy and work

• Seven simultaneous properties are necessary and sufficient for a cell to live and persist. One of them is the cell's capacity to extract energy from its surrounding medium and to transform this energy into the various forms of the work required for its survival.

• The energy required to elaborate cell constituents (such as proteins, polysaccharides, nucleic acids...) is supplied directly or indirectly by hydrolysis of ATP into ADP or AMP+PI.

• ATP is therefore a **central energy carrier.** ATP has two high-energy terminal pyrophosphate bonds (β and γ). When these bonds are broken, energy is supplied to almost every biosynthetic assembly process as well as many other forms of biological work including mechanical work, ion and molecule transportation, osmotic work, electron energization...

• In most cases, hydrolysis of ATP implies the activation of hydrolyzing enzymes (ATPases). ATPases are controlled by special regulatory mechanisms so that, when a specific kind of work is accomplished, for example when a structural element is shortened or inflected, ATP is hydrolyzed at the same time only as needed... (Fig. 2).

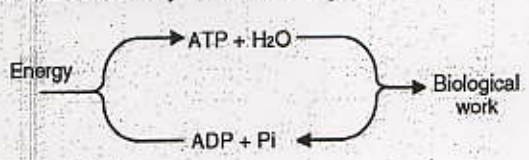

Fig. 2 – ATP cycle.

2. ATP, skin and aging

• Skin is a living tissue made of highly developed eukaryotic type cells containing many mitochondria, specialized cytoplasmic organelles where certain Krebs' cycle reactions, high-energy aerobic oxidative metabolism, lead to the biosynthesis of ATP.

• The epidermis is an exclusively cellular tissue where most of these bioreactions occur.

• Skin aging and bioenergetics:
- a decline in the metabolism of living cells is characteristic of skin aging. H. Goldschmiedt showed that the respiratory quotient of human skin (Q = volume CO_2 expired / volume O_2 inspired), an expression of oxidative metabolism, decreases with aging (Fig. 1E),
- LS scientific works (unpublished) have shown that the intracellular ATP (an excellent tracer of energetic metabolism and of global cell vitality) dramatically and regularly decreases in the human epidermis with increasing age (Fig. 1E).

VITACELL®

DEFINITION / COMPOSITION

• VITACELL® is a natural active substance. This biotechnological product is isolated and purified from a unicellular eukaryote microorganism (yeast): ***Saccharomyces cerevisiae.***

• VITACELL® is:
- a strong activator of bioenergetic metabolism,
- a vitalizing agent,
- an ATP regenerator for living skin cells.

• VITACELL® has no growth factor effect and does not contain any ATP.

Main components:
- Amino-acids and peptides } from yeast cytoplasm
- Nucleosides

SKIN BENEFITS

1. Cell vitalizing agent - ATP regenerator - Anti-aging agent.

• Cells draw their energy from ATP. This energy is used to transport molecules and ions, for electron transfer, and for biosynthesis, biodegradation, and differentiation processes... Because it is the universal bioenergy mediator, ATP is often called "the fuel of life". ATP is present in cells in catalytic quantities and must be endlessly regenerated to play its role as an energy mediator.

• VITACELL® stimulates the ability of skin cells to biosynthesize and regenerate ATP. It helps the skin recover its energy potential, similar to the potential of young skin. VITACELL® may be considered as a strong anti-aging co-active agent.

2. Stimulator of respiratory metabolism in skin cells, by activating enzymes in the bioenergetic pathways.
VITACELL® works closely with epidermal cellular physiology, helping it direct substrates to oxidative respiratory pathways (Krebs' cycle) predominantly over anaerobic (glycolytic) pathways.

3. Cell repair
By favoring oxidative and bioenergetic cellular metabolism, VITACELL® plays a key role in:
- capture and elimination of excess cellular toxins,
- activation of intracellular exchanges,
- neutralization of environmental pollutants.

COSMETIC USE

- Designed for tired, dull, atonic skin.
- For preventing or reducing early skin aging: especially in areas exposed to attacks from environmental or other types of stress.
- For stimulating preparations (face, body...).
- For regenerating and repairing care: anti-aging, anti-stress, after-sun preparations.

DOSAGE / SOLUBILITY / MODE OF INCORPORATION

1. Dose of use:
VITACELL® LS 8430 (liquid): 2 to 5%.
VITACELL® POWDER LS 7979: 1 to 2%.
VITACELL® POWDER LS 7979 is twice as concentrated as VITACELL® LS 8430.

2. Solubility: VITACELL® is soluble in water, insoluble in fat.

3. Mode of incorporation:
VITACELL® LS 8430 is incorporated during the finishing process at 50°C, or at room temperature for cold processing.
VITACELL® POWDER LS 7979: prepare extemporaneously an aqueous mother solution. Then add it to the cosmetic preparation during the final phase at 50°C.

ANALYTICAL CHARACTERISTICS

1. Aspect:
VITACELL® LS 8430: limpid light yellow liquid, with a weak odor.
VITACELL® POWDER LS 7979: white fine powder, with a characteristic odor.

2. Specifications: upon request.

TOLERANCE

Good.

EFFICACY

Test summaries overleaf.

STORAGE

In their original packaging, at 15 - 25°C.

INCI NAME

VITACELL® LS 8430: Yeast Extract.
VITACELL® POWDER LS 7979: Mannitol (and) Yeast Extract.

MANUFACTURER

Laboratoires Sérobiologiques S.A.

EFFICACY TESTS

STIMULATION OF THE EPIDERMAL ATP SYNTHESIS (EX VIVO)

Aim
To show the stimulating effect on cell vitality and the cutaneous energizing effect of a topical application of VITACELL® POWDER LS 7979 versus placebo, using fluorescent enzymatic assay.

Protocol
Experiments were conducted in skin biopsies taken from 6 female volunteers, 21 to 55 years old.
Standardized single topical application on 2 adjacent areas of a skin biopsy:
- one area treated with an emulsion containing 2% VITACELL® POWDER LS 7979,
- the other area with a placebo emulsion.

The energizing activity was identified and quantified by assaying epidermal intracellular ATP (bioluminescence).

Results
The level of epidermal ATP (ng/million of cells) increased by +65% with the emulsion containing 2% VITACELL® POWDER LS 7979 in comparison with the placebo emulsion.

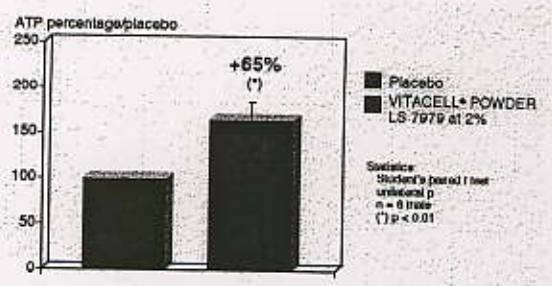

Fig. 3 – VITACELL® POWDER LS 7979 at 2%. Illustration of its stimulating effect on epidermal intracellular ATP stimulation following a topical application.

Conclusion
Topical application of a 2% VITACELL® POWDER LS 7979 emulsion has a clear stimulating effect on the biosynthesis of epidermal intracellular ATP.
This effect favors maintenance of good quality epidermis (structure, properties, functions) and, if needed, cell repair or increased potential for resisting stressful attacks.

STIMULATION OF CELL VITALITY (IN VITRO - PRIMARY CULTURES)

Aim / Protocol
The stimulating activity of VITACELL® LS 8430 on cell vitality was measured in human cells:
- in keratinocytes (epidermis),
- in fibroblasts (dermis)

in growth and in survival.

The intracellular ATP was measured by bioluminescence (increase in % of treated cells ATP/control medium cells ATP).

The kinetics of the effect were determined from different incubation times for 3 concentrations of the active substance.

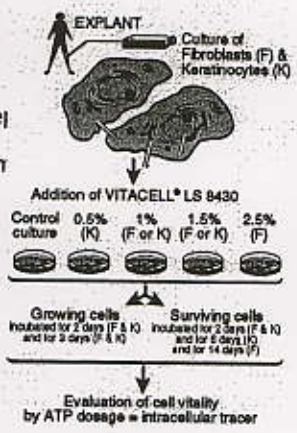

Fig. 4 – Experimental protocol.

Results
Similar results were obtained for fibroblasts and keratinocytes. For keratinocytes for example (Fig. 5), 1.5% VITACELL® LS 8430 induced:
- in growing cells, a +47% increase (after 3 days),
- in surviving cells, a +46% increase (after 6 days).

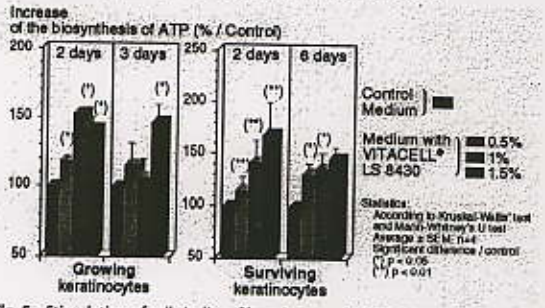

Fig. 5 – Stimulation of cell vitality of keratinocytes by VITACELL® LS 8430.

Conclusion
VITACELL® LS 8430 showed a good stimulating effect on cell vitality.

STIMULATION OF CELL METABOLISM ON EPIDERMAL KERATINOCYTES (IN VITRO)

Aim / Protocol
• ATP is a transitory high-energy element. It is an intermediate between energy producing structures (mitochondria) and energy consuming systems (protein synthesis enzymes, DNA, sodium or calcium pumps, detoxification, differentiation...).
• ATP synthesis depends on the respiratory chain which, thanks to nutrients provided by VITACELL® POWDER LS 7979, reduces oxygen into H_2O to form a proton gradient in the mitochondrial intermembrane space.

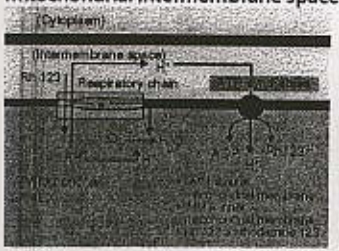

Fig. 6 – ATP synthesis depends on the intramitochondrial respiratory chain.

• The proton gradient causing ATP-synthetase to produce ATP may be quantified with a specific fluorescent test: Rhodamine Rh 123 (Fig. 6).
• Keratinocytes at confluence were incubated for 48 hours either in presence of the DMEM* control medium, or in presence of the control medium containing increasing concentrations of VITACELL® POWDER LS 7979.
• After 48 hours, the cells are counted and the ATP rate quantified.

Results
VITACELL® POWDER LS 7979 applied on keratinocytes at the concentration of 0.30% showed a strong stimulating effect on cell metabolism. No growth effect was observed.

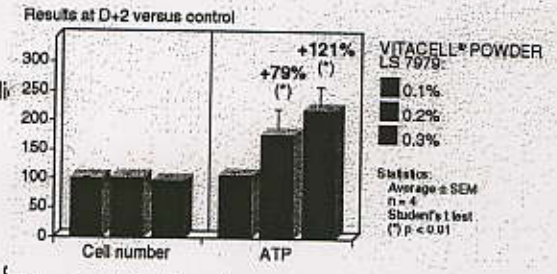

Fig. 7 – Stimulating effect on the mitochondrial enzyme activity of surviving keratinocytes incubated 48 hours in presence of VITACELL® POWDER LS 7979.

* DMEM = Dulbecco's Minimum Essential Medium

ACTIVE INGREDIENT FOR COSMETOLOGY SKIN

STIMULATION OF METABOLIC ACTIVITY (CLINICAL STUDY)

Aim

Evaluation based on the measurement of the partial pressure of transcutaneous oxygen ($TcpO_2$), a parameter which is closely linked to the O_2 supply in epidermal cells and therefore to their cellular metabolism.

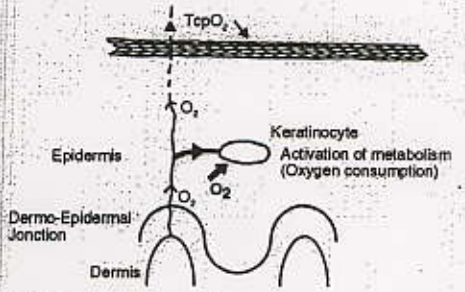

Fig. 8 – Principle of the measurement.

Protocol

Double blind clinical study on 12 volunteers during 3 weeks.
Randomized treatment on the internal side of the forearm with:
- a placebo cream,
- a cream containing 5% VITACELL® LS 8430.

Measurement of the partial pressure before and after the treatment.

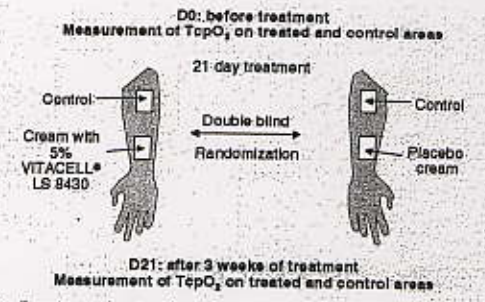

Fig. 9 – Experimental protocol.

Results

Treatment with VITACELL® LS 8430 significantly improved the partial pressure of transcutaneous oxygen.

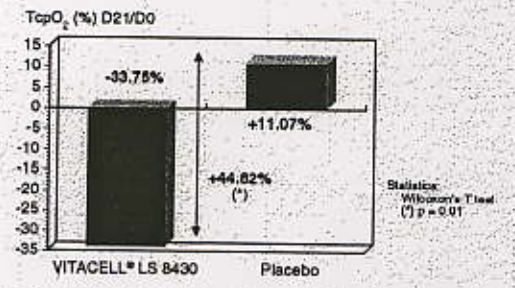

Fig. 10 – Time course of transcutaneous O_2 partial pressure of ($TcpO_2$), compared with the control area, after 3 weeks of treatment with VITACELL® LS 8430. Results in 12 volunteers.

Conclusion

The emulsion containing 5% VITACELL® LS 8430 induced good metabolic activity, significantly higher than with placebo.

STIMULATION OF O_2 CONSUMPTION OF EPITHELIAL CELLS (IN VITRO)

1. Oxygraphy (Polarography)

Polarographical measurements of the O_2 consumption of an epithelial cell homogenate, in presence of various concentrations of VITACELL®.

Determination of the Efficient Dose 50 (ED50), inducing a + 50% increase of the O_2 consumption.

Results

Adding VITACELL® provided a strong increase in oxygen consumption by epithelial cells:
- for VITACELL® LS 8430: ED50 ~1.00%,
- for VITACELL® POWDER LS 7979: ED50 ~0.55%.

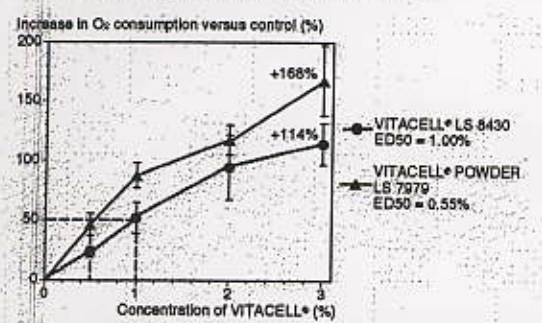

Fig. 11 – In vitro study of the stimulation of oxygen consumption by epithelial cells with increasing doses of VITACELL® LS 8430 and VITACELL® POWDER LS 7979. ED50 = Efficient Dose 50%.

2. Respirometry (Warburg Manometry)

Evaluation of cell breathing by measuring the quantities of gas exchanged. Human skin biopsies are taken and placed in the Warburg's respirometer, made of containers connected to capillary manometers.

The consumption of oxygen is evaluated according to the change of pressure in the system corresponding to the volume of oxygen used.

Results

Clearly significant increases in oxygen consumption from +19.5% to 57.7% according to the tested concentrations.

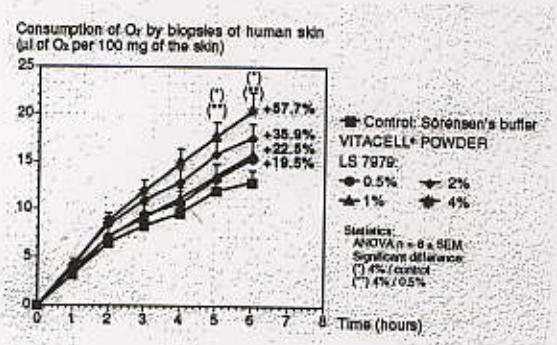

Fig. 12 – O_2 consumption by human skin biopsies in presence of VITACELL® POWDER LS 7979.

HEADQUARTERS: LABORATOIRES SÉROBIOLOGIQUES S.A. - 3, RUE DE SEICHAMPS - BP40 - F-54425 PULNOY (FRANCE) - FAX. 03.83.29.98.65 - TEL. 03.83.29.97.91

8430-801-119/7979-801-144

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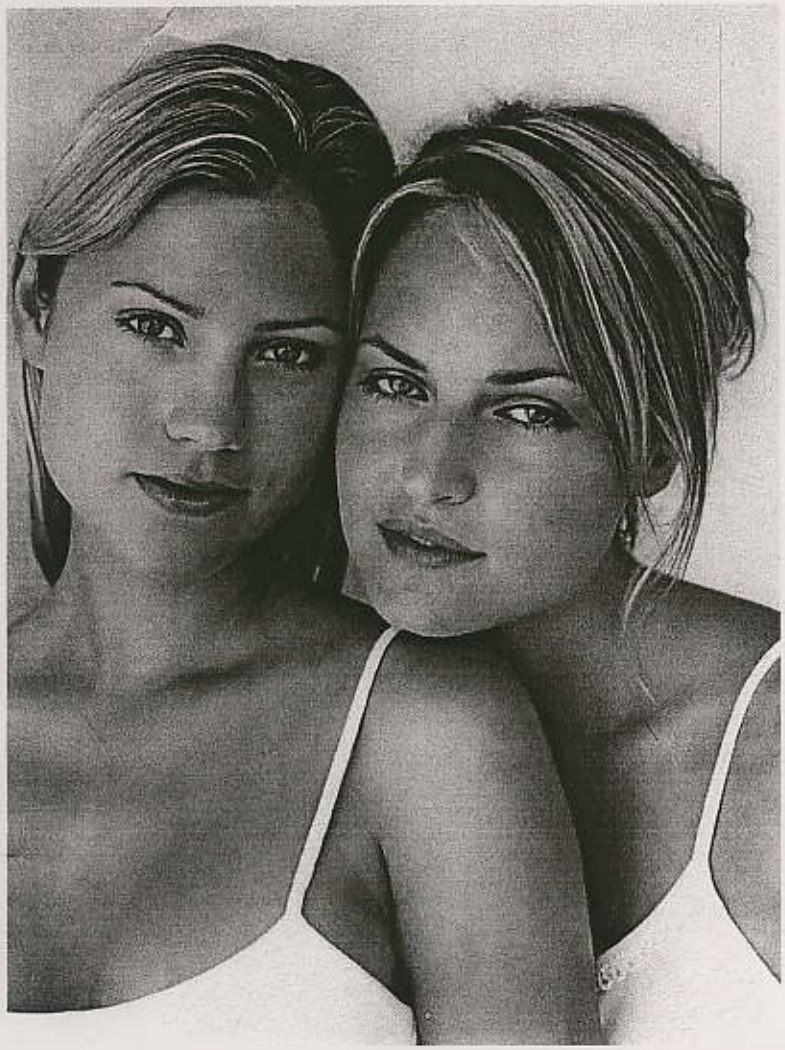
t: 908.561.5200 f: 618.258.6808
www.archpersonalcare.com
archpc@archchemicals.com

Cosmetic Ingredients & Ideas®



Biodynes® O_3

Urban Defense and Age Defense

Biodynes® O_3 meets the standards for ecological and organic cosmetics according to ECOCERT (www.ecocert.com).

INCI Name:
Water & Saccharomyces Ferment Filtrate Lysate

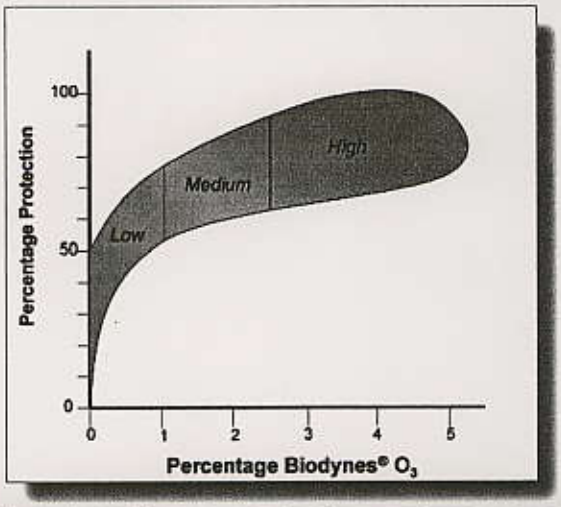
Preservation System:
0.9 - 1.1% Phenoxyethanol

SAP Code#: 137180

Key Claims:
- Enviromental pollution defense
- DNA Protection
- Skin firming and anti-wrinkle
- Anti-glycation / AGE prevention

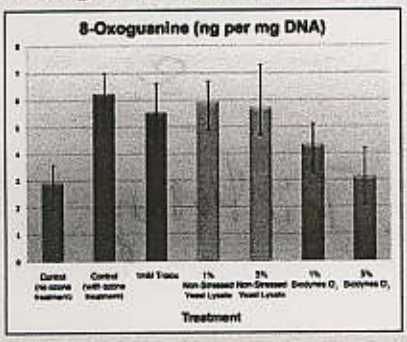
Use Level:
1 - 5%

Urban Defense Protection Factor with Increasing Concentrations of Biodynes® O_3



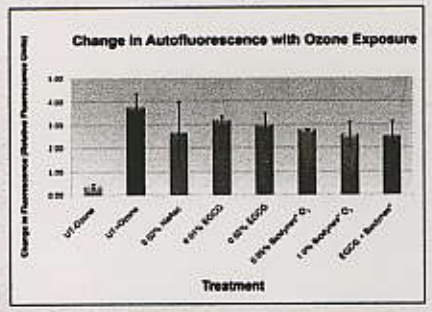
In Vitro

In Vitro DNA Degradation Study Calculating the Urban Defense Factor



- MatTek® full thickness tissue exposed to ozone (1.2 ppm) for 1 hour [equivalent to 8 hours exposure of skin on a highly polluted day]
- Extracted DNA was analyzed for 8-oxoguanine (the end-product of the oxidation of guanine)
- **Biodynes® O_3** effectively offers DNA protection against oxidative stress, as shown by decreasing levels of 8-oxoguanine

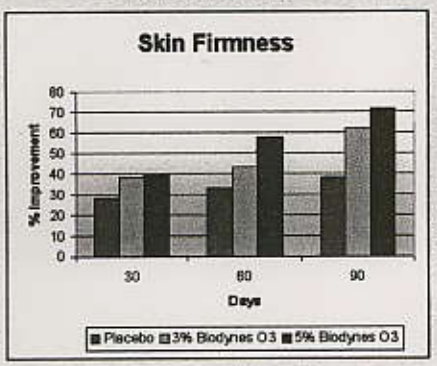
In Vitro AGE Analysis: Quantifying Advanced Glycation Endproducts



- MatTek® full-thickness tissue
- Tested against Vitamin C and 70% EGCG at the same concentration
- **Biodynes® O_3** performed better than Vitamin C and EGCG isolated from Green Tea

In Vivo

In Vivo Dermatological Assessment: Skin Firmness

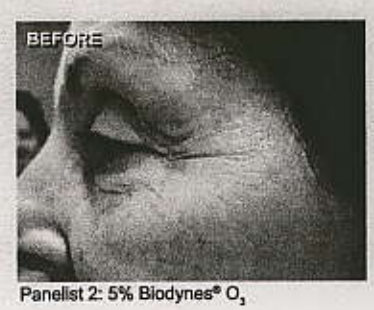


- 90-Day Study conducted on 60 volunteers
- 3% Biodynes® O_3 offered a **61% improvement**
- 5% Biodynes® O_3 showed a **72% improvement**

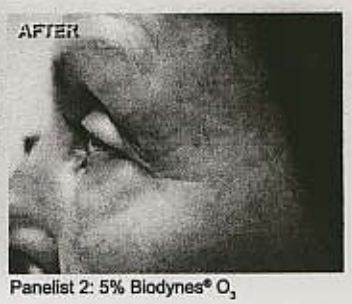
In Vivo Anti-Wrinkle Results

Panelist 1: 5% Biodynes® O_3

Panelist 1: 5% Biodynes® O_3



Panelist 2: 5% Biodynes® O_3

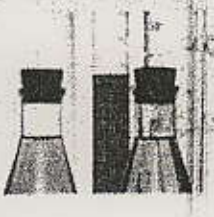


Panelist 2: 5% Biodynes® O_3

- After a 90-day treatment with 5% **Biodynes® O_3**, a statistically **significant anti-wrinkle effect** was observed in 34% of the participants compared to placebo-treated controls

ARCH™ **Product Data**

Biodynes® O_3 (3 oxygen atoms) • OZONE IS CREATED NATURALLY AND BY HUMAN POLLUTION (patent pending)
Ozone Stressed Yeast Lysate
SAP Code#: 137180
Assigned INCI Designation: Water (and) Saccharomyces Ferment Filtrate Lysate



03/21/05 v1.3

Product Information

Ozone (O_3) is a highly reactive form of molecular oxygen in which normal molecular oxygen combines with highly reactive oxygen radicals to form transitory, but somewhat stable molecules containing three oxygen atoms.[1] Sources for ozone can be completely natural such as the generation of ozone due to lightning strikes or formation of ozone in the upper atmosphere through reaction of high energy solar plasma with oxygen in the troposphere. However, ozone can also be generated from more manmade sources including, in particular, urban smog, and many cities actually monitor urban ozone levels as an indicator of smog.[2] Because ozone is a key component of human-produced smog, it has been studied extensively for its respiratory health effects, particularly for people prone to asthma and people living in urban populations. More recently, it has now been recognized that ozone has detrimental effects on skin.[3-11] In particular, it has been established that ozone, while it can only penetrate a few microns into the stratum corneum, will almost instantly react with vitamin C and vitamin E reservoirs in the skin, rapidly depleting these important antioxidants. In addition, it has been shown that ozone will also rapidly convert skin and cellular lipids into lipid peroxides that lead ultimately to membrane and skin degradation. Studies have also demonstrated that ozone can oxidize important proteins and nucleic acids.[12-14] To date, however, there has only been one study conducted which looked directly at the effects of ozone *in vivo* on human skin.[11] In this study, significant reduction of vitamins C and E were noted and lipid peroxides were also significantly increased.

Very recently, it was suggested that ozone may be created naturally in the human body as a potential defense mechanism by macrophages circulating in the blood stream although these findings remain somewhat controversial.[15-16] In this study, it was suggested that ozone may attack vascular cholesterol deposits which causes a conversion of the cholesterol into oxidation products that ultimately result in plaque buildup. The possibility that ozone destroys vascular cholesterol raises the very distinct likelihood that topically contacted ozone may destroy or diminish the important reservoirs

Key Product Attributes

- **Lipid Barrier Protection and Repair**
- **DNA Protection**
- **Antioxidant**
- **Protection of antioxidants (vitamins)**
- **Free radical protection**

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of cholesterol in the skin's lipid bilayer. It is already well established that ozone will oxidize cellular cholesterol and cholesterol *in situ*.[17-18] The stratum corneum contains only about 5% cholesterol and cholesterol esters on a total weight basis, but this tiny amount of material is critical for proper skin lipid function.[19]

Arch Personal Care Products has been manufacturing and selling unique yeast fermentation lysates for many years for cosmetic applications.[20] Of late, the possibility has been explored that ozone might influence the way yeast grow if it is applied in a sublethal fashion to the growing microorganisms. Certainly, the possibility exists that ozone should place a stress on growing yeast such that the yeast would need to respond with protective agents that help it survive against the stress.[21-24] It was anticipated that isolation of the key proteins generated by the stressed yeast would provide a new form of yeast lysate that could protect the skin against the ravages of ozone. **Arch Personal Care** is pleased to announce the launch of their newest stressed yeast technology, **Biodynes® O_3**.

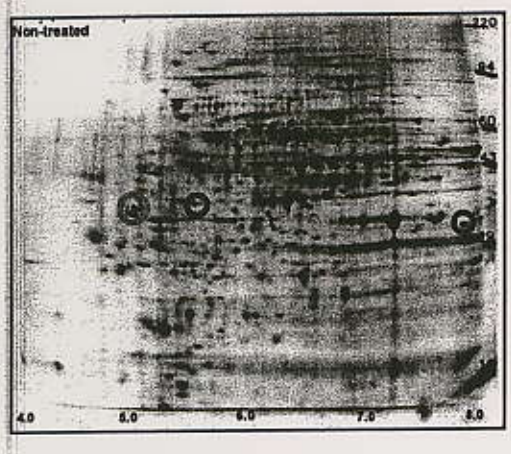
Manufacturing Process

Biodynes® O_3 is manufactured using fermentation biotechnology in which yeast are fed a nutrient media essential for development of the yeast. During the cellular growth phase of the yeast, a sublethal dose of ozone is applied to the yeast generated via a commercial ozonator. The ozone is applied for a specific duration of time while maintaining a viable cell count in the fermentation process. Upon completion of the ozone application, the yeast cells are then lysed to break open the cellular membrane and the key constituents of the interior of the cell, including material from the cytoplasm and the nuclear materials, are isolated.

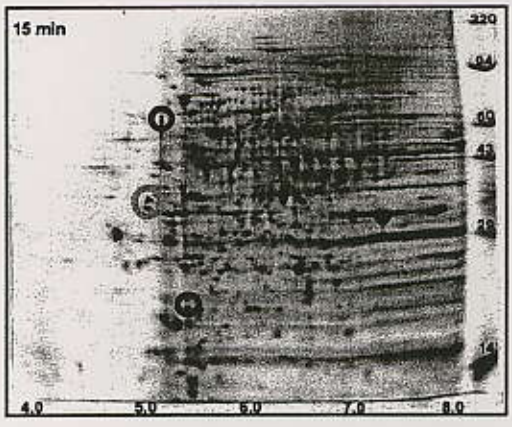
2-D Electrophoresis of the Lysate

After isolation of the lysate, the changes that occurred in the protein composition of the yeast were examined using 2-Dimensional Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (2D SDS-PAGE).[25] The images of an unstressed (left) and an ozone stressed yeast lysate (right) are shown below (Figure 1). Careful examination of the two gels side by side shows that the application of the ozone does indeed cause up-regulation and down-regulation of certain proteins.

Figure 1



Unstressed Yeast Lysate



Ozone Stressed Yeast Lysate

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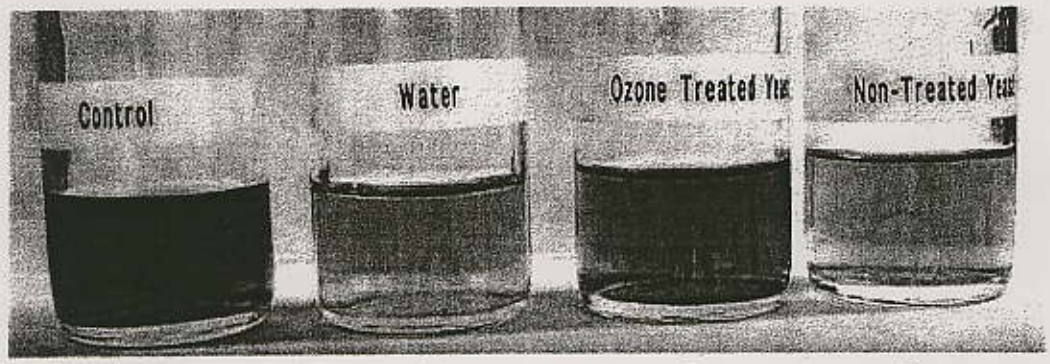
In Figure 1, visually obvious changes in protein composition as a result of ozone stress are noted in the green and red circles (the yellow circle represents an internal standard that is used in SDS PAGE to indicate reproducibility of the electrophoresis). The gel on the left was not stressed, the one on the right was stressed for fifteen minutes with ozone. The green circles show examples of proteins that were present in the unstressed yeast (left) that visually faded as a result of ozone stress, (right, a down regulation of these proteins). The red circles in the ozone-stressed yeast lysate indicate proteins that are upregulated as a result of the ozone stress compared to the unstressed gel on the left. Many other less obvious examples exist in these two gels, the examples above are only intended to demonstrate that indeed ozone stress can influence the production of proteins within a living system. More in-depth computer aided analysis of these gels can help to identify the bulk of the changes that have occurred.

In situ testing

It has been reported that it is possible to use an ozone sensitive dye called indigo trisulfonic acid as an indicator of the presence of ozone.[15] *In situ*, ozone reacts with a double bond in this dye and cleaves the dye into two molecules. Upon cleavage of the dye's double bond, the brilliant blue color of the dye is changed to colorless, water white. The dye is, interestingly, not reactive to hydrogen peroxide, but is very sensitive to the presence of ozone.

Using the dye as an indicator, two lysates were tested for their ability to control ozone attack. In Figure 2, the control container (A) has just water and the dye and has not been treated with ozone. It is shown to provide an indication of the intense blue color of the dye prior to ozonolysis. The container labeled (B) contains water and dye and has been treated for 10 minutes with a controlled ozone purge. The degradation of the blue color clearly indicates the effect of the ozone on the dye. The third container (C) was treated with 2% of **Biodynes® O_3** and then treated with ozone for 10 minutes. The final container (D) was treated with 2% of a similar non-ozone stressed yeast lysate and then ozone purged. The presence of the much stronger blue color in container C compared to container D indicates that something that has been produced in **Biodynes® O_3** is working to diminish the oxidative effects of the added ozone. The effect is pronounced and most likely related to the production of protective proteins by the yeast grown in the presence of sublethal doses of ozone.

Figure 2. *In situ* Dye Testing

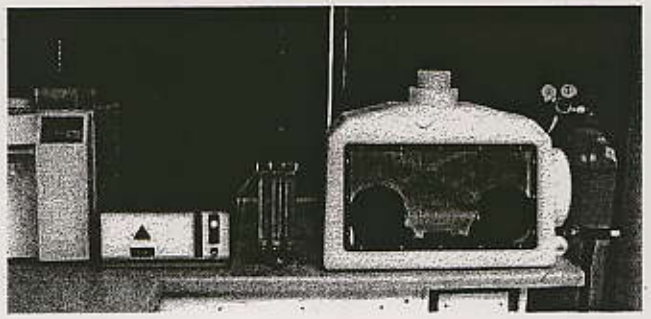


***In vitro* studies**

Skin DNA damage protection

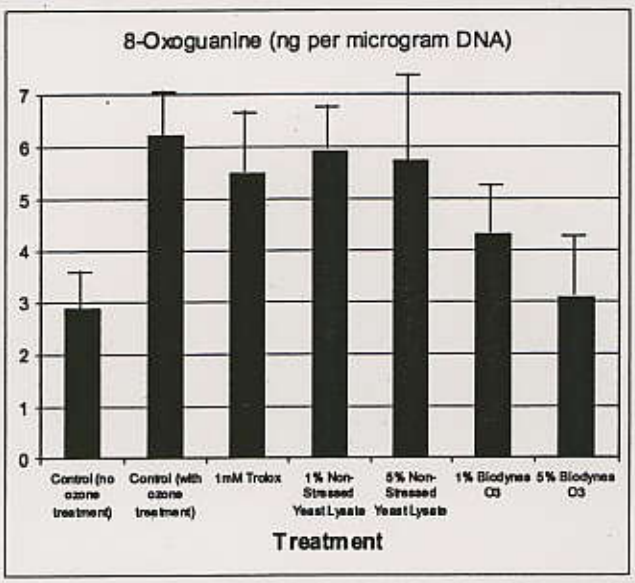
Using a specially designed cell culture system (see Figure 3), we were able to bathe Epiderm® Full Thickness tissue in an atmosphere of ozone.

Figure 3. Cell Culture System for Ozone Analysis



It has been recognized that ozone will oxidize guanine nucleic acids into 8-oxoguanine, a well defined mechanism of cellular aging.[13, 26-27] Analysis of 8-oxoguanine accumulation can be an indicator of cell deterioration.[26] It is possible to analyze skin cells for their 8-oxoguanine content which results from oxidation of guanine in the nuclear and mitochondrial DNA. Epiderm tissue was exposed to 10ppm ozone for one hour. This is indicative of an eight hour exposure of approximately 1.2ppm ozone per hour, which would be considered a polluted day of exposure. Analysis of the tissue for 8-oxoguanine demonstrated that compared to a non-stressed yeast lysate control and to a Trolox (a water soluble vitamin E derivative) positive control, **Biodynes® O_3** was able to protect DNA against oxidative damage, Graph 1. In fact, at a concentration of 5% **Biodynes® O_3**, was able to essentially return the detrimental oxidative effects of ozone back to nominal levels (i.e., those of untreated tissue controls).

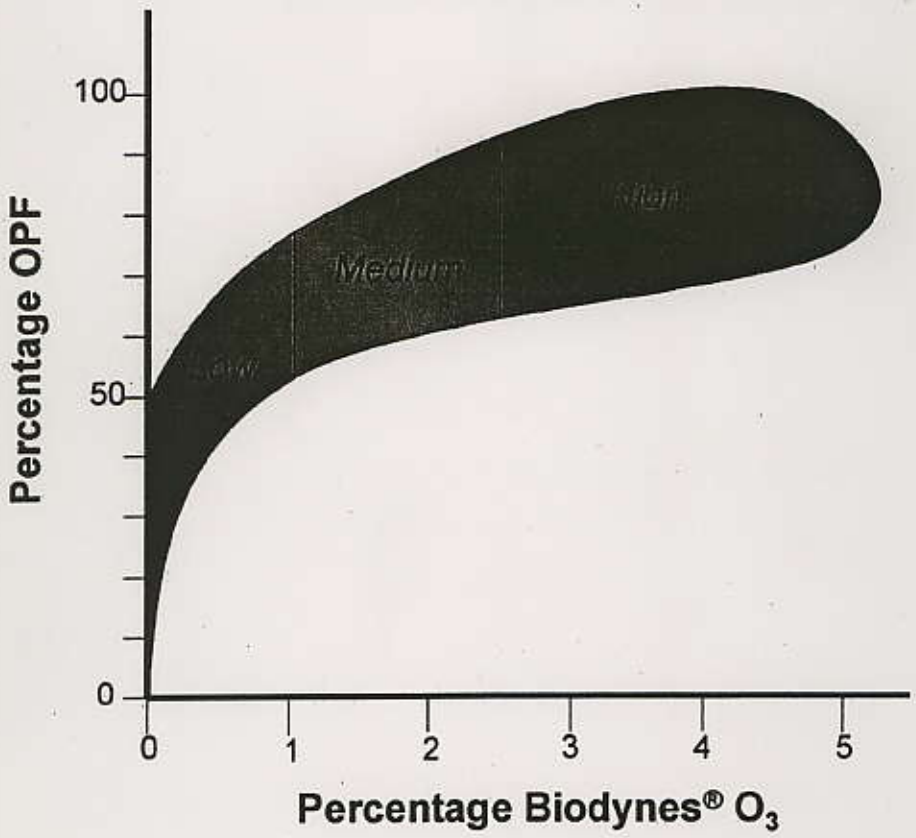
Graph 1. Skin DNA damage protection measured by 8-oxoguanine accumulation.



Ozone Protection Factor (OPF)

The data generated from the DNA Protection Study provides us with an opportunity to develop a new definition of skin protection called the "Ozone Protection Factor" or OPF. The results of this study can be directly correlated to the ability of **Biodynes® O_3** to protect skin from environmental ozone exposure. The control cells that were not treated with **Biodynes® O_3** represent an OPF of 0%; as a significant increase in 8-oxoguanine results from direct ozone exposure. Incorporation of 1% **Biodynes® O_3** offers the cells a moderate OPF in the range of 50%; as the presence of **Biodynes® O_3** effectively reduces the level of 8-oxoguanine compared to the control. Likewise, 5% treatment of **Biodynes® O_3** offers a high OPF in the range of 100%. These values are plotted below as the anticipated performance of various concentrations of **Biodynes® O_3** to protect the skin from a day of ozone exposure, Graph 2.

Graph 2. Anticipated Ozone Protection Factor For Various Concentrations of Biodynes® O_3



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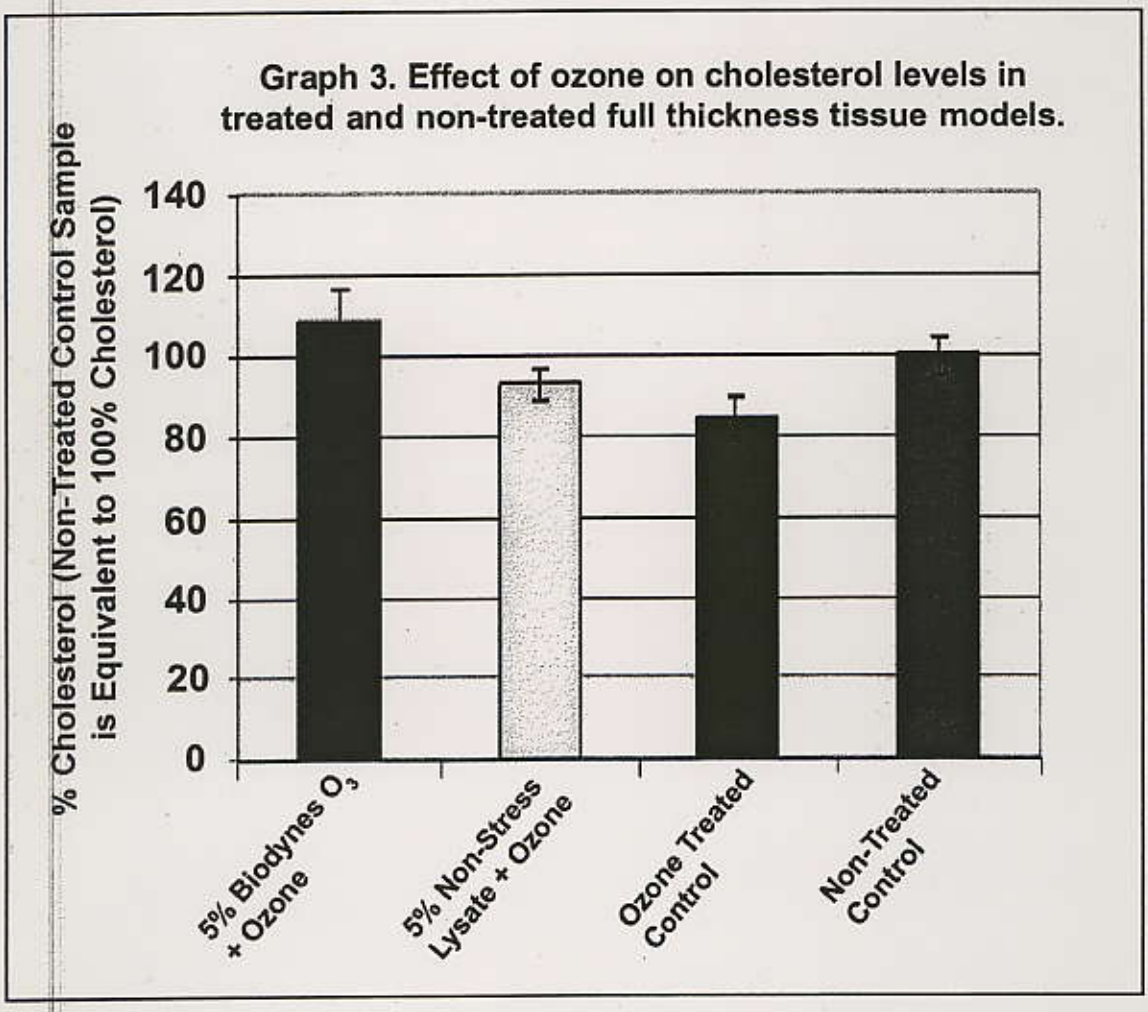
In Vitro

Skin Cholesterol Damage Protection

In order to investigate whether or not ozone has a detrimental effect on the cholesterol comprising the lipid bilayer of the skin, Thin Layer Chromatography (TLC) was employed to examine cholesterol levels in Epiderm full thickness tissue. Scanning densitometer measurements of the intensity of the TLC bands provides graphical data of the levels of cholesterol in each tissue sample. Samples of tissue were exposed to two hours ozone and the cholesterol was isolated by extraction. Examinations of the levels of cholesterol present in the tissue after exposure to ozone are shown below in Graph 3. Treatment of the tissue with 5% **Biodynes® O_3** (dark blue) maintained cholesterol levels similar to those found on the untreated control (green) and significantly superior to tissue that was unprotected and treated with ozone (red) or tissue that was treated with a yeast lysate that was not stressed with ozone (light blue).

Graph 3. Effect of ozone on cholesterol levels in treated and non-treated full thickness tissue models.



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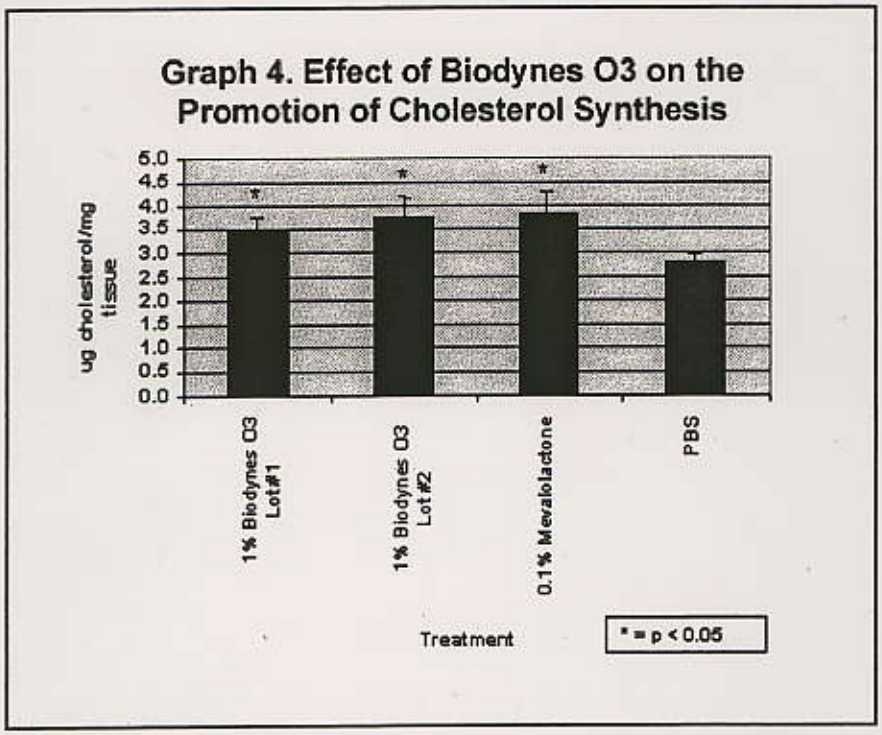
In Vitro

Cholesterol Synthesis – Reparative Effect of Biodynes O_3

From Graph 3, it appeared that **Biodynes O_3** not only protected the skin against ozone damage, but also stimulated the skin to produce cholesterol. These results, however, were not statistically significant. In order to confirm whether or not this effect was indeed real, a follow-up study was conducted in which we simply applied 1% **Biodynes O_3** to the Epi-Derm tissue for eight days without applying an ozone stress to the tissue. The lipids were extracted and analyzed to determine the amount of cholesterol present in the samples.

Indeed, it turns out that a 1% treatment of **Biodynes O_3** does stimulate the production of cholesterol in this *in vitro* model. A summary of the results is shown below in Graph 4. Phosphate buffered saline was used as the negative control and mevalolactone was used as the positive control for this study at a level of 0.1%. Treatment of the tissue with two separate batches of **Biodynes O_3** show a statistically significant increase in cholesterol production compared to the PBS control and equivalent compared against a sample of mevalolactone, a product that has been demonstrated to stimulate production of cholesterol when applied topically.[28] An increase in epidermal cholesterol content has been associated with improved barrier function, thus topically applied materials that can promote cholesterol synthesis can provide a beneficial effect.



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Conclusions

Biodynes® O_3 is a new yeast extract that offers one of the first, scientifically-designed, topically applied defenses against ozone, which is rapidly becoming a well recognized threat against skin health, aging and well being. **Biodynes® O_3** is effective at protecting fragile cellular nucleic acids, including nuclear and mitochondrial DNA and RNA, and skin lipids, critical for proper functioning of the skin's lipid structure, from the detrimental effects of ozone. **Biodynes® O_3** allows a formulator to literally "dial in" a factor of ozone protection (OPF) into their product depending on anticipated exposure times that the consumer may need the protection. **Biodynes® O_3** can help assure the consumer that they are receiving steps towards proper care and safeguards for the skin from harmful effects of the environment.

Typical Properties

Appearance	Clear yellowish liquid*
pH	4.0 - 7.0
(Direct @25° C)	
Non-Volatile Matter	1.0 - 4.0%
(1g - 1hr - 105° C)	
Residue on Ignition	1.5% Maximum
(800° C)	
Nitrogen	0.1 - 1.0%
(Kjeldahl)	
Microbial Content	100 opg Maximum
	No Pathogens
Phenoxyethanol	0.9 - 1.1%
(HPLC)	
Recommended Use Level	1 - 5%

* *May sediment on standing. Mix well before use. Protect from freezing.*

Product Safety Review

Epi-Ocular – MTT Viability Assay	Minimal/to Mild
(Products tested at 100%)	
Epi-Derm – MTT Viability Assay	Minimal/to Mild
(Products tested at 100%)	
RIPT	Non-Sensitizing

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ENVIRO$_3$SCREEN BODY PROTECTION LOTION
ST-53

Environmental aggressors such as ozone not only damage the surface of the skin but also cellular DNA. **Biodynes® O_3** is an ozone stressed yeast lysate that offers DNA and lipid barrier protection and repair. **NAB® Grass Roots Extract** is an excellent source of anti-oxidants, photoprotective and genoprotective materials. **Gel Base BSM5** and **Liquiwax™ DICDD** provide a non-greasy, non-oily silky feel. **Surfhope® SE C1816** improves product application and attributes a creamy feel to the product. Microbiological product integrity is protected with the **Biovert™ System.**

Ingredient	INCI Nomenclature	Supplier	%
Phase A			
Deionized Water	Water	-	66.05
Disodium EDTA	Disodium EDTA	-	0.10
Propylene Glycol	Propylene Glycol	-	4.00
Pemulen TR-1	Acrylates/C_{10-30} Alkyl Acrylate Crosspolymer	Noveon	0.35
Surfhope SE C1816*	**Sucrose Stearate**	**Arch Personal Care Products**	**0.50**
Phase B			
Demoblock OMC	Ethylhexyl Methoxycinnamate	Alzo	7.50
Escalol 567	Benzophenone-3	ISP	3.00
Escalol 587	Ethylhexyl Salicilate	ISP	6.00
Liquiwax DICDD**	**Diisocetyl Dodecanediolate**	**Arch Personal Care Products**	**2.00**
Gel Base BSM-5	**Cyclomethicone & Dimethicone & Phenyl Trimethicone**	**Arch Personal Care Products**	**2.50**
GMS 165	**Glyceryl Sterate & PEG-100 Stearate**	**Arch Personal Care Products**	**1.40**
Stearic Acid	Stearic Acid	-	3.00
Cetyl Alcohol	Cetyl Alcohol	-	0.20
Phase C			
TEALAN 99%	Triethanolamine	RITA	1.25
Phase D			
Biodynes O_3	**Water & Saccharomyces Ferment Filtrate Lysate**	**Arch Personal Care Products**	**1.00**
NAB GrassRoots Extract	**Lophophyrum Elongatum (wheatgrass) Extract & Oryzopisis Miliciea (ricegrass) Extract & Collinsonia Canadensis (stoneroot) Extract**	**Arch Personal Care Products**	**1.00**
Phase E			
Biovert System	**Glucose & Glucose Oxidase & Lactoperoxidase**	**Arch Personal Care Products**	**1.05**

* Manufactured by Mitsubishi Industries and distributed in the U.S. by Arch Personal Care Products.
** Manufactured by Innovachem Inc. and marketed by Arch Personal Care Products.

Specifications:

pH 6.50 – 7.50 Viscosity: 15,000 – 25,000 cps (#6, 10 rpm@ 25°C)

Procedure:

1. Combine Phase A and heat to 75°C. Mix until uniform.
2. Combine Phase B and heat to 75°C. Mix until uniform.
3. While mixing, add Phase A to Phase B and mix until homogeneous.
4. Add Phase C and mix until homogeneous.
5. Remove the heat and continue to mix, cool to below 40°C.
6. Add **Biodynes O_3** and **NAB GrassRoots Extract** from Phase D, and mix until uniform.
7. Add **Biovert Substrate** and **Biovert Enzyme** from Phase E, and mix until uniform.
8. Slowly mix until cooled to room temperature.

Arch Personal Care Products L.P. 70 Tyler Place South Plainfield, NJ 07080 USA
Tel. 908.561.5200 Fax. 908.561.9174 email. archpc@archchemicals.com

METROTECTION FACE AND BODY
ST-54

Ozone, smog and other urban pollutants attack the lipid barrier of our skin. **Biodynes® O_3** is an ozone-stressed yeast lysate that offers both DNA and lipid barrier protection. This lotion uses **Liquiwax™ PolyEFA,** proven to be a highly effective wetting agent for pigments such as zinc oxide enhancing the SPF. Due to its linoleic acid content, it also provides significant moisturizing benefits. **Gel Base BSM-PE** enhances product aesthetics by providing a powdery feel. Microbiological product integrity is protected with **Mikrokill™ PCC.**

Ingredient	INCI Nomenclature	Supplier	%
Phase A			
Water	Water	-	61.00
Glycerin	Glycerin	-	3.00
Sodium Chloride	Sodium Chloride	-	1.00
Phase B			
Liquiwax PolyEFA*	**Octyldodecyl/PPG-3 Myristyl Ether Dimer Dilinoleate**	**Arch Personal Care Products**	**4.00**
Z-Cote	Zinc Oxide	BASF	6.00
SF 1328	Cyclomethicone & Dimethicone Copolyol	GE/Kobo	10.00
Gel Base BSM-PE	**Cyclomethicone & Dimethicone & Phenyl Trimethicone & Polyethylene**	**Arch Personal Care Products**	**2.00**
Cyclomethicone	Cyclomethicone		4.00
Dermoblock OMC	Ethylhexyl Methoxycinnamate	Alzo	7.50
Phase C			
Mikrokill PCC	**Phenoxyethanol & Caprylyl Glycol & Chloroxylenol**	**Arch Personal Care Products**	**0.50**
Biodynes O_3	**Water & Saccharomyces Lysate Extract**	**Arch Personal Care Products**	**1.00**

* Manufactured by Innovachem and marketed by Arch Personal Care Products.

Specifications:

pH: N/A
Viscosity: 27,500 – 35,000 cps

Procedure:

1. In side kettle combine Phase A and mix until uniform.
2. In main kettle add Phase B ingredients **Liquiwax PolyEFA** and Z-Cote HP-1, mix until uniform. Add SF 1328, Cyclomethicone, **Gel Base BSM-PE** and Ethylhexyl Methoxycinnamate and mix until uniform.
3. Slowly add Phase A to Phase B under prop agitation and mix until uniform.
4. Add Phase C ingredients **Mikrokill PCC** and **Biodynes O_3** and mix until uniform.

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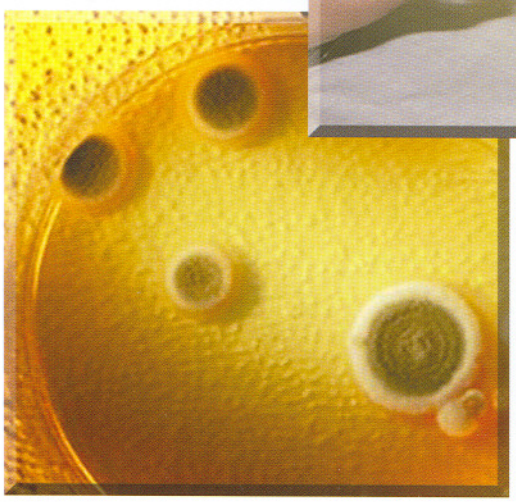
Botanistat PF-64

The Ideal Alternative to Paraben Preservatives



DISTRIBUTED BY
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(818) 349-4149 FAX (818) 349-4017

Botanigenics, Inc.

The Ideal Alternative To Paraben Preservatives:

- Paraben and formaldehyde-free preservative
- Broad spectrum antimicrobial protection at low usage levels
- Globally approved
- Stable and effective over a wide pH range (3.0-10.0)
- Easy and versatile to use in formulations
- Compatible with essentially all cosmetic materials
- Emolliency and skin-conditioning properties
- Excellent safety and toxicological profile
- Cost effective and readily available
- Suggested use level 0.75-1.5 %

Botanistat PF-64

INCI DESIGNATION: Phenoxyethanol (and) Caprylyl Glycol (and) Ethylhexylglycerin (and) Hexylene Glycol

Botanistat PF-64 is a globally approved, paraben and formaldehyde-free preservative system for the personal care and toiletry industries. This proprietary ingredient is based on a carefully balanced blend of highly effective biocide components in an emollient base for optimized preservation in a variety of applications and products. Botanistat PF-64 provides comprehensive protection from microbial contamination, including Gram-positive and Gram-negative bacteria, fungi, yeasts and molds. In addition, Botanistat features skin conditioning properties that enhance the product's mildness and aesthetics during and after application.

As a broad spectrum antimicrobial agent, Botanistat PF-64 can be used alone as the primary preservative in a product. This minimizes the total amount of preservative needed to ensure proper preservation while simplifying the formulation process. It is safe, non-toxic and non-irritating and there is no evidence that it causes skin or eye sensitization. Botanistat PF-64 is compatible with most personal care and cosmetic ingredients, including complex molecules such as proteins and surfactants. Botanistat PF-64 is a versatile, easy to use liquid, highly stable and effective over a broad pH range. It can be incorporated into products under a wide range of temperatures(15°C - 85°C). Botanistat PF-64 is effective in anionic, cationic, and non-ionic systems, and can be used synergistically with auxiliary preservatives, glycols and chelating agents. Overall, Botanistat PF-64 is a unique, and economical preservative complex, which helps provide safe and stable finished products for the health and beauty market.

Applications

Botanistat PF-64 can be used to preserve a wide range of personal care products including:

Hair Care Applications:	**Shampoos, leave-in and rinse-off conditioners, styling gels and sprays**
Skin Care Applications:	**Face and body moisturizers, eye products, creams, lotions, masks, gels and toners**
Toiletry Products:	**Deodorants, antiperspirants, body washes, after shaves, toners, face balms**
Sun Care and Tanning Applications:	**Sunscreen creams and lotions, daily SPF moisturizers and sunless tanning products**
Color Cosmetic Applications:	**Foundations and eye makeup, lipsticks and lip glosses**

Microbiological Effectiveness

Botanistat PF-64 offers comprehensive protection from microbial contamination, including Gram-positive and Gram-negative bacteria, fungi, yeasts and molds.

The following summary of challenge test results demonstrates the long term effectiveness of the Botanistat preservative system.

An emulsion base high in polysorbates and proteins was tested at both ambient and elevated temperatures, in addition to a surfactant base.

Test Article 1- Lotion Base 5574-1

An oil in water emulsion containing 3% polysorbates plus 1.5% wheat and silk proteins preserved with 1% Botanistat; pH of 6.23

Challenge test results (in CFUs)

S. Aureus	ATCC 6538	$9.7X10^5$
E. Coli	ATCC 8739	$1.1x10^6$
P. Aeurginiosa	ATCC 9027	$1.0 x10^6$
C. Albicans	ATCC 10231	$8.5 x10^5$
A. Niger	ATCC 16404	$8.5x10^5$

Total Bacterial Inoculum level: $1.1x10^6$

Total Bacterial Count in Sample

Day 0	$2.60x10^5$
Day 3	<10
Day 7	<10
Day 14	<10
Day 21	<10
Day 28	<10

Pass

Total Mold/Yeast Inoculum level: $8.4x10^5$

Total Mold/Yeast Count in Sample

Day 0	$4.1x10^5$
Day 3	<10
Day 7	<10
Day 14	<10
Day 21	<10
Day 28	<10

Pass

Test Article 2- Lotion Base 5574-1

An oil in water emulsion containing 3% polysorbates plus 1.5% wheat and silk proteins. The formula was preserved with 1% Botanistat and stored at 50ºC for 30 days; pH of 6.23

Challenge test results (in CFUs)

S. Aureus	ATCC 6538	$8.2 x10^5$
E. Coli	ATCC 8739	$1.2 x10^6$
P. Aeurginiosa	ATCC 9027	$9.0 x10^5$
C. Albicans	ATCC 10231	$6.5 x10^5$
A. Niger	ATCC 16404	$8.5 x10^5$

Total Bacterial Inoculum level: $9.3x10^5$

Total Bacterial Count in Sample

Day 0	$5.0x10^5$
Day 3	<10
Day 7	<10
Day 14	<10
Day 21	<10
Day 28	<10

Pass

Total Mold/Yeast Inoculum level: $8.0x10^5$

Total Mold/Yeast Count in Sample

Day 0	$5.4x10^5$
Day 3	$1.7x10^5$
Day 7	$3.9x10^4$
Day 14	$3.4x10^3$
Day 21	$3.4x10^3$
Day 28	$3.7x10^2$

Pass

Test Article 3- Surfactant Base 5575-1

Surfactant base preserved with 1% Botanistat, stored at 50ºC for 30 days; pH of 5.50

Challenge test results (in CFUs)

S. Aureus	ATCC 6538	$8.2 x10^5$
E. Coli	ATCC 8739	$1.2 x10^6$
P. Aeurginiosa	ATCC 9027	$9.0 x10^5$
C. Albicans	ATCC 10231	$6.5 x10^5$
A. Niger	ATCC 16404	$8.5 x10^5$

Total Bacterial Inoculum level: $9.3x10^5$

Total Bacterial Count in Sample

Day 0	$4.7x10^5$
Day 3	<10
Day 7	<10
Day 14	<10
Day 21	<10
Day 28	<10

Pass

Total Mold/Yeast Inoculum level: $8.0x10^5$

Total Mold/Yeast Count in Sample

Day 0	$1.4x10^5$
Day 3	<10
Day 7	<10
Day 14	<10
Day 21	<10
Day 28	<10

Pass

BOTANISTAT PF-64 was independently tested by Consumer Products Testing Co.
All samples meet CTFA mixed inocula test for Antimicrobial Preservative Effectiveness using mixed cultures

Chemical Composition

Botanistat PF-64 is an optimized proprietary complex of phenoxyethanol, caprylyl glycol, ethylhexylglycerin and hexylene glycol, all non-animal derived. This proprietary complex functions as a highly effective, broad spectrum preservative that also contributes to the formula's solvency and emolliency, with additional skin softening benefits.

Product Specifications

Appearance @ 25°C:	Clear, pale yellow liquid
Odor:	Faint, characteristic
Moisture (KF), %:	3.0% MAX
Specific Gravity:	0.984-1.044

Safety And Toxicological Profile

Skin Irritation:	RIPT a formula containing 5% Botanistat PF-64 did not indicate a potential for dermal irritation
Eye Irritation:	A HET-CAM Assay of a formula containing 1.5% Botanistat PF-64 indicated virtually no ocular irritation potential in vivo
Delayed Contact Sensitization:	RIPT of a formula containing 5% Botanistat PF-64 did not indicate a potential for allergic contact sensitization

Botanistat PF-64 has not been tested on animals

Formulation Guidelines

Botanistat PF-64 offers ease-of-use and versatility to the cosmetic formulator and production team. It can be added to aqueous, anhydrous, or emulsion systems, which are cold or hot process preparations. In emulsions, Botanistat PF-64 can be added to the oil or water phase prior to emulsification. It can also be added after emulsification at or below 80°C. In aqueous systems an additional solubilizer may be needed, like Polysorbate 80 (Botanisol P80). **Botanistat PF-64 is compatible with essentially all raw materials and it is stable over a broad pH range of 3.0 to 10.0.**

Suggested use level: 0.75-1.5%

Solubility Chart - 1% solution

	Room Temperature	*50°C*
Water	d	d
Butylene Glycol	s	s
Caprylic/Capric Triglyceride	s	s
Safflower Seed Oil	s	s
Cyclopentasiloxane	i	s
Dimethicone	i	d
Ethanol (200 proof)	s	s
Isododecane	s	s
Isoprene Glycol	s	s
Isopropyl Myristate	s	s
Mineral Oil	s	s
PEG-8	s	s
Phenyltrimethicone	s	s
Propylene Glycol	s	s
Shampoo Base*	s	s

s=soluble
i=insoluble
d=dispersible
**Ammonium Laureth Sulfate, Ammonium Lauryl Sulfate, Cocamidopropyl Betaine, Cocamide DEA, Water*

Regulatory Status

INCI DESIGNATION: Phenoxyethanol (and) Caprylyl Glycol (and) Ethylhexylglycerin (and) Hexylene Glycol

CAS #:	Phenoxyethanol: 122-99-6, Caprylyl Glycol: 1117-86-8, Ethylhexylglycerin: 70445-33-9, Hexylene Glycol: 107-41-5
EINECS #:	Phenoxyethanol: 204-589-7, Caprylyl Glycol: 214-254-7, Hexylene Glycol: 203-489-0, Ethylhexylglycerin: 408-080-2

- BOTANISTAT PF-64 is approved for use in personal care products globally.
- The EU's maximum concentration limit is 2%
- All components of BOTANISTAT PF-64 are approved for use in Japan

Handling And Safety

Material Safety Data Sheets are available upon request from Botanigenics. Similar information for solvents and other chemicals used with Botanigenics should be obtained from your suppliers. When solvents are used, proper safety precautions must be observed.

Buying Guide

INCI DESIGNATION: Phenoxyethanol (and) Caprylyl Glycol (and) Ethylhexylglycerin (and) Hexylene Glycol

Package Size:	180 kilo drums / 18 kilo pails
Storage Conditions:	Store in a cool, dry place tightly sealed, away from sunlight
Shelf Life:	1 yr