



EXSYMOL

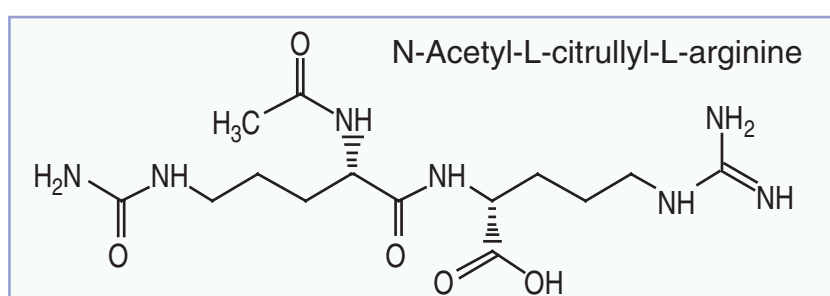
EXSY-ALGINE®

Aqueous solution of N-acetyl-citrullyl-arginine

INCI name: ACETYL CITRULL AMIDO ARGININE (and) WATER

Origin

EXSY-ALGINE® is a stable aqueous solution of an analog of a natural dipeptide produced by a red alga in the actic area



Analytical composition

N-acetyl-L-citrullyl-L-arginine	10.00 %
Sodium methyl paraben	0.14 %
Water sq	100.00 %

Technical characteristics

Limpid, colorless liquid

pH: about 5.5

Density at 20°C: about 1.0

Miscible with water, alcohols and glycols.

Availability

1, 5 or 30 kg drums

Uses

Cutaneous anti-stress

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Stimulates exchanges and nutritious incomes

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Energizing

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Protection during extreme conditions (cold, dryness, pollution)

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Anti-aging

EXSY-ALGINE® : THE DIPEPTIDE WHO CAME IN FROM THE COLD

Design of the product

Arginine and its derivatives are known for their properties in wound healing and anti-oxidation. EXSYMOL's research decided to focus

on the natural derivatives of that particular amino-acid and their possible effects on skin-care. The dipeptide **L-citrullyl-L-arginine**, attracted our attention because of its natural occurrence in red algae (*Chondrus crispus*, *Grateloupia turuturu*) during their vegetative phase that takes place in extreme conditions (few light, cold temperature) in the Arctic or North sea. The alga builds an energy reserve, available at the time of its development, when the conditions are back to normal. At the end of the winter season, **L-citrullyl-L-arginine** represents more than 50% of the total nitrogen content in the plant.

Nutrition and energy for the skin

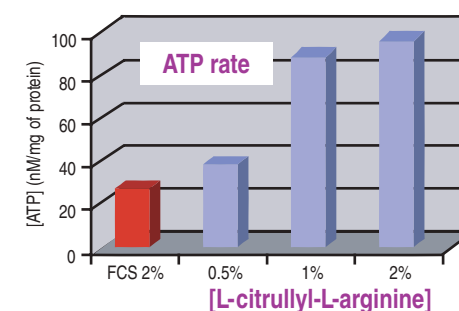
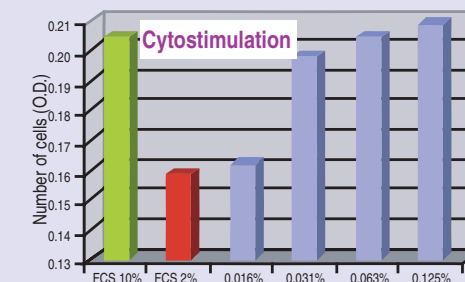
The natural defense of the epiderm and the derm very much depend on factors largely affected by the capacity of supplying the energy, needed by the skin, to keep a satisfactory maintenance of it. However the nutrition provided by the microcirculation is essential, it is very useful to complement with nutrients able to maintain an optimal energy level.

L-citrullyl-L-arginine, or its acetyl derivative synthesized to improve its bioavailability, is able to increase the energetics of the skin, stimulate the cell-growth and reforce the natural metabolism and cutaneous defense. This natural dipeptide liberates arginine and citrulline, which are essential aminoacids for the protein biosynthesis, and in particular for that of collagen. **L-citrullyl-L-arginine** is therefore an important source of useful nitrogen for the skin.

Energizing and nourishing

Cytostimulation: The nourishing effect of **EXSY-ALGINE®** has been substantiated through its cyto stimulation property of **L-citrullyl-L-arginine** on human fibroblasts submitted to a deprived nutrition medium and supplemented with the dipeptide. The number of cells is determined by the technique of Neutral Red incorporation and O.D. (optical density) measure.

When the fibroblasts are cultured with 2% FCS (deprived medium) the O.D. (optical density) is about 0.15 and with 10% FCS (optimal condition), the O.D. is 0.20. With concentrations of **L-citrullyl-L-arginine** as low as 0.04%, the O.D. has already recovered the optimal result of 0.20.

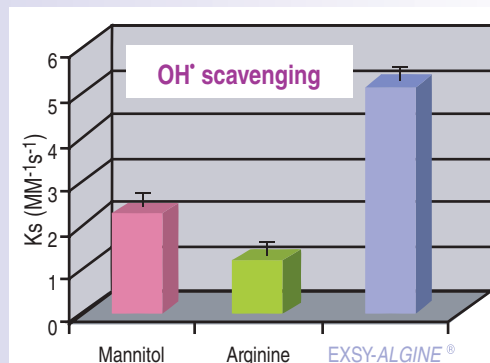
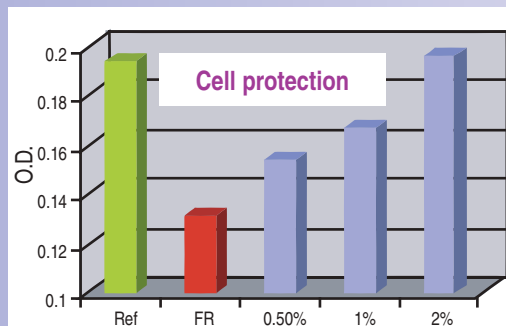


Energetic metabolism : ATP is the universal source of energy for all cells in order to activate most biological processes. In cold conditions the energy resources are highly important and therefore, we studied the effect of **L-citrullyl-L-arginine** on energetic metabolism.

The procedure consisted in the measurement of ATP in a culture of fibroblasts in deprived medium in comparison with a medium supplemented with **L-citrullyl-L-arginine** at various concentrations. A stimulation of ATP rate, possibly partially in relation with cyto stimulation, is observed.

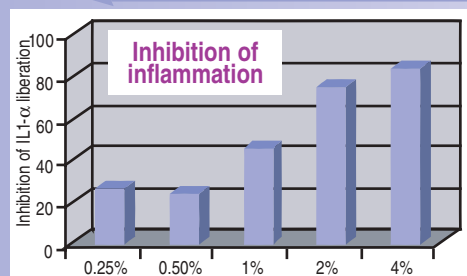
Anti-oxidant activity / Anti-inflammatory

Anti-oxidant activity / cell protection: a culture of human keratinocytes is submitted to free radicals generated by the enzymatic system hypoxanthine / xanthine-oxidase. The cell viability is measured by UV (O.D.) after incorporation of neutral red. The graph here below shows that a concentration of 2% of **L-citrullyl-L-arginine** was able to fully protect the keratinocytes submitted to free-radicals.



The OH• scavenging activity of **EXSY-ALGINE®** is evaluated in a biochemical test by measure of the hydroxyl radical scavenging constant Ks, of the deoxyribose oxidation by OH• generated from ascorbate - Fe³⁺ in presence of hydrogen peroxide.

The free-radical scavenging constant Ks is 3.5 times more important than that of arginine and more than twice that of mannitol, a reference scavenger in that reaction.

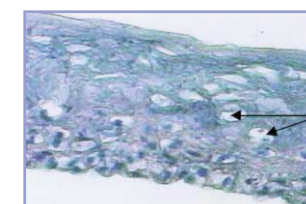


Any cutaneous stress is a disturbing element interfering with the health of the skin. Oxidative stress, and in particular photo-induced oxidation, generates inflammatory reactions hence regenerative disturbance. The anti-inflammation property of **L-citrullyl-L-arginine** is tested on a culture of keratinocytes submitted to UV irradiation (50 mJ/cm²). The inflammation is monitored through the quantification of the interleukine IL-1α liberated. The results obtained are displayed in the graph besides. The results show that **L-citrullyl-L-arginine** has a dose-dependent anti-inflammation property evidenced at concentrations as low as 0.25%.

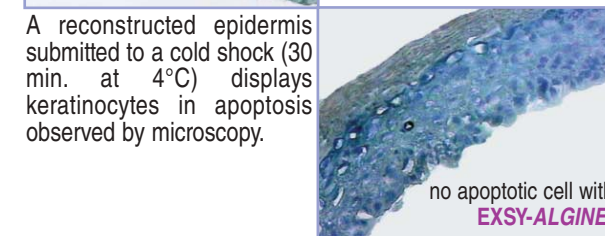
Skin protection at low temperatures

The spectacular survival capacity of the alga in the winter season, and the sudden recovery, suggested that **EXSY-ALGINE®** could be involved in the process of protection at low temperature or during a cold shock. Therefore, the effect of that natural dipeptide was studied on **reconstructed epidermis** submitted to a cold shock (4°C during 30 min).

The dipeptide is able to protect the skin from the stress generated by the cold shock



apoptotic keratinocytes after cold-shock

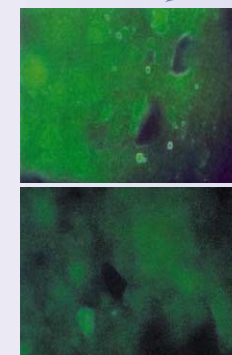


no apoptotic cell with EXSY-ALGINE®

The same epidermis, treated with **EXSY-ALGINE®** does not show any apoptotic cell.

Mechanism

It is reported in the literature that, during cold shocks, stress proteins, HSPs, are expressed from the keratinocytes. This is the reason why we studied by immunofluorescence the expression of the HSPs in a reconstructed skin submitted to a cold shock (4°C during 30 min).



Without **EXSY-ALGINE®**, HSPs are expressed during a cold shock (yellow fluorescence).

No significant expression of HSPs is evidenced when the epidermis submitted to a cold shock, is treated with **EXSY-ALGINE®**.

EXSY-ALGINE® was able to protect the skin from the cold shock and the related stress without inducing an overexpression of the HSPs : the dipeptide takes over from the HSPs (HSP's like activity).



Tolerance study

Clinical tests have been performed to evidence the safety of **EXSY-ALGINE®** for cutaneous irritation, sensitization, phototoxicity and photoallergy.

The tolerance of N-acetyl-L-citrullyl-L-arginine has also been studied *in vitro* by non-animal alternative methods. The ocular tolerance is evaluated by studying the cytotoxicity on a culture of fibroblasts isolated from rabbit cornea and also by Het Cam techniques. The cutaneous tolerance is evaluated on human skin explants. The results observed indicate that :

- **EXSY-ALGINE®** is not irritant.

The potential mutagenic activity of N-acetyl-L-citrullyl-L-arginine has also been studied by Ames test and **no mutagenic activity** has been induced on selected bacterial strains.

Formulation

EXSY-ALGINE® is a very stable solution. The product can be formulated in the aqueous phase without restriction nor constrain. It has no incompatibilities of common knowledge. The recommended concentration is about 1%.

Existing studies

(available upon request)

N-ACETYL-L-CITRULLYL-L-ARGININE : THE DIPEPTIDE WHO CAME IN FROM THE COLD:

Cytostimulation

Activity of **L-citrullyl-L-arginine** on energetic metabolism

Effect of **L-citrullyl-L-arginine** on HSP's expression in case of cold shock

Anti-oxidation and related activities

Anti-free radical

Anti-inflammation activity

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Toxicity - Tolerances