HAIR CARE

HAIR GROWTH

Pronalen fibro-actif

PROVITAL GROUP

For a beautiful life from cells to the skin

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Pronalen fibro-actif

Germs of Soy (*Glycine soja Siebold and Zucc*.) and Wheat (*Triticum vulgare Vill*.).

Bioactive peptides and oligosaccharides.

It is made up of bioactive molecules which provide sufficient energy to increase the proteinic synthesis of the collagen, elastin and the glycosaminoglycans and benefit the skin by preventing and delaying the appearance of the clear signs of cutaneous aging (wrinkles). The product can also be incorporated into hair products as it revitalises hair growth, and is a vegetable alternative to Placenta extract.

PRONALEN FIBRO-ACTIF HSC

Standardised hydrosoluble extract.

Titred 0.04-0.20 %of total nitrogen, 0.20-0.85% of reducing sugars and 0.10-0.40% of non-Reducing sugars.

BOTANY AND CHEMISTRY

Germination involves a sequence of physiological processes which occur in the seeds of higher plants and of which the final result is the transformation of the embryo into an adult plant.

The most important metabolic aspects in germination are respiration and the mobilisation-transformation of the reserve substances. The main aim of the respiration is to form ATP and the pyridine nucleotides needed for the metabolic activity to take place inside the seeds, which will cause the reserve macromolecules to be transformed into simpler molecules or biomolecules.

The germination process begins when conditions are optimal for the reserve material to be used suitably and the expected biomolecules to be obtained.

The following reserve substances are available:

- **Glucides**

  Starch is the reserve polysaccharide in most seeds and once transformed, gives glucose as a final product. Hemicellulose, amyloids and galactomannans can also be found.

- **Proteins**

  Hydrolysis of these compounds gives rise to an accumulation of peptides and free amino acids in the cotyledons, and particularly proline, glutamine, glutamic acid and asparagine.

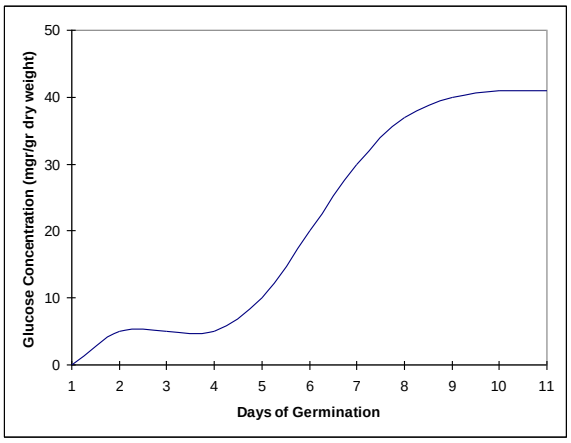
- **Lipids**

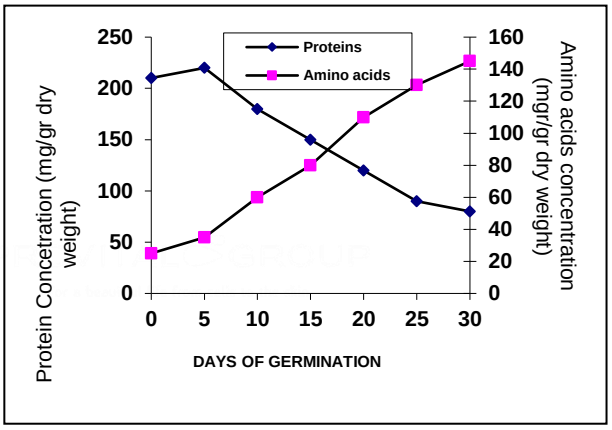
  The main seed reserves are the triglycerides which, when hydrolysed, become glycerol, fatty acids and may finally end up as Acetyl-CoA which enters the Krebs cycle as a substrate. Unsaturated fatty acids such as oleic, linoleic and linolenic acids are basically predominant.

- **Minerals elements**

  Phosphate is found as a reserve substance in this mineral element in the form of phytine, and also other cations (potassium, calcium and magnesium) which comprise the salt.

The following figures show the main changes in the levels of the main actives occurring in the seeds during germination. The levels of macromolecules fall whereas the levels of the bioelements rise until such a time (depending on the type of seed) that they also fall due to the degradation caused by the growth of the plant. It is therefore very important to know when to stop the process and when the maximum levels of active biomolecules may be achieved.





	Day 1	Day 10
Nucleic acids (mg/100g)	3,2	12,0
Vitamins (mg/g)		
B_2	1,3	5,4
PP	62,0	103,0
H	0,2	0,4
B_5	7,6	12,6
B_6	2,6	4,6
B_1	7,0	9,0
Folic acid	28,0	106,0
Minerals (mg/100g)		
P	423	1050
Mg	133	342
Ca	45	71

Table 1. Changes in the seed composition during germination (wheat)

PRONALEN FIBRO-ACTIF is obtained from seeds with only water used in their germination and no pesticides and other chemical additives. The process is stopped after 3 days as the active biomolecules (peptides, amino acids, oligosaccharides, glucose) have reached their maximum levels.

COSMETIC PROPERTIES

Hair activity

Fundamentally, the common type of baldness especially male-pattern baldness, is inherited. Improvement and prevention of baldness, hair growth, and prevention of hair loss have been studied for many years and a great many kinds of measures, products, devices and instruments have been introduced. Public attention has been focused on cosmetic products influencing hair growth.

The hair cycle: phases and regulation

Hair growth is a highly controlled process that follows a characteristic cycle. Every hair follows its own cycle, independently of others.

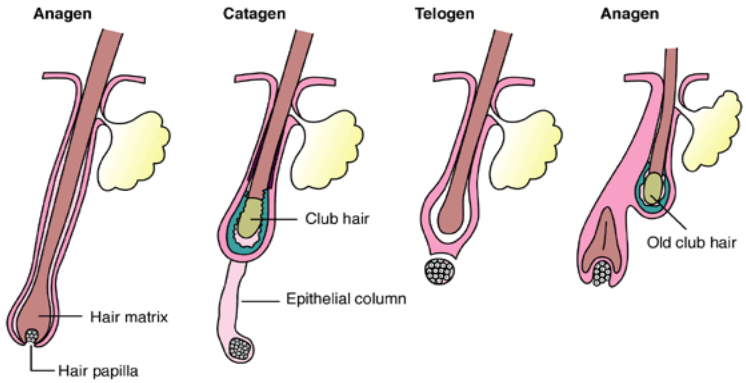


Figure 1. Phases of the hair cycle.

- **Anagen phase**. The growing phase. During this phase, follicular cells show highly active metabolism, which results in hair growth. Studies carried out on human legs revealed a proportion of 50-60% hairs in the anagen phase, which grow about 0.20 mm/day (Van Neste, 2003).

  During this phase, hairs become visible on the skin surface. The duration of the anagen phase and the hair length depend on the body area under consideration.

- Scalp 2 - 6 years
- Leg: 19 - 26 weeks
- Arm: 6 - 12 weeks
- Moustache: 4 - 14 weeks

- **Catagen phase**. The shortest phase of the hair cycle; it lasts for about 14 days. The number of hair follicles in this phase is very reduced, approximately 1%. This is the regression phase, where mitosis in the germinal matrix stops, the bulb is separated from the papilla and the follicle becomes shorter and contracted.

- **Telogen phase**. The resting phase of the hair cycle. As a hair reaches this phase, it definitely stops growing. The duration of this phase depends on the body area under consideration; in the leg follicles, it lasts about 19 weeks, with a proportion of telogen hairs of 45% (Van Neste, 2003). Hairs entering the telogen phase, evetually fall and a new matrix gradually develops, which gives rise to a new hair.

Considering hair cycle, it is desirable that, for the improvement and prevention of male pattern baldness, the active ingredient should have the ability to maintain a longer period of anagen hairs and a shorter period of telogen hairs, or an ability to change the hair cycle from the telogen to the anagen phase smoothly

Various types of products are expected to promote hair growth by different means:

- Supplying nutrients with vasodilators and stimulants for improving blood circulation.
- The potential antagonistic action of female hormones.
- Nourishing the hair follicle with such nutrients as amino acids and/or vitamins.

There is a study where the authors tried to get a trichogram of the hair-root in human volunteers with various degrees of baldness. Figure 2 shows the correlation between the degree of progress of alopecia and the level of glucose 6 phosphate dehydrogenase (G6PDH) activity of the follicle. In this figure, there is a high mutual relation between decline in the ratio of anagen hairs and the decline in the activity of G6PDH.

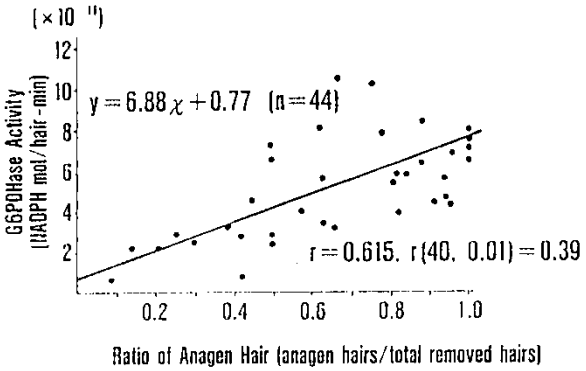


Figure 2. Ratio of Anagen hair and G6PDH activity

This clearly means that the level of energy metabolism becomes lower in the hair follicles of advanced alopecia and that the depression of the energy metabolism in the follicle is an important major factor for the progress of alopecia.

In addition to this work, they also studied biochemical changes in the hair follicle at the time of change of hair cycle from telogen to anagen. They used guinea pigs which have a specific character in that their hairs go into the anagen phase in a shorter time by artificial clipping.

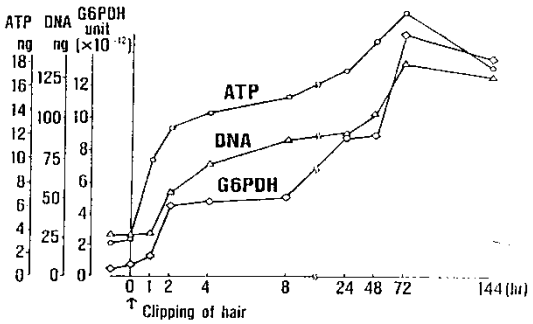


Figure 3. Changes in the ATP, DNA and G6PDH levels

Using this disposition, adenosine triphosphate (ATP: a high energy material in the hair follicles), deoxyribonucleic acid (DNA: as an index of hair follicles proliferate) and G6PDH (as a main enzyme in the energy production) were measured after the activation, the amount of ATP went several times higher and the GPDH activity reached a high level.

From these findings it is concluded that, in order to promote hair growth, the most important subjects for research would be the cause of depression of the energy producing system and measures to provide an efficient energy supply to the hair follicles.

The pilous follicles proliferate in the bulb, where they receive the nutrients necessary for their development. As mentioned before it has been shown that molecules such as ATP and enzymes such as glucose-6-phosphatase (G-6P) enhance hair growth, as the actives are a source of energy on the one hand, and the enzyme G-6-P hydrolyses glucose and glycogen into molecules capable of entering the Krebs cycle to finally give ATP, energy.

PRONALEN FIBRO-ACTIF has been shown to increase mitochondrial respiration (as it has been demonstrated in the realized tests) by increasing the cell oxidative metabolism, thus bringing about an increase in the synthesis of ATP, the provider of the energy necessary for hair growth, development and maintenance. The mechanism can be explained as glucose carried by the blood stream is decomposed through glycolysis and the TCA cycle and gives the energy material ATP. (See Figure 4).

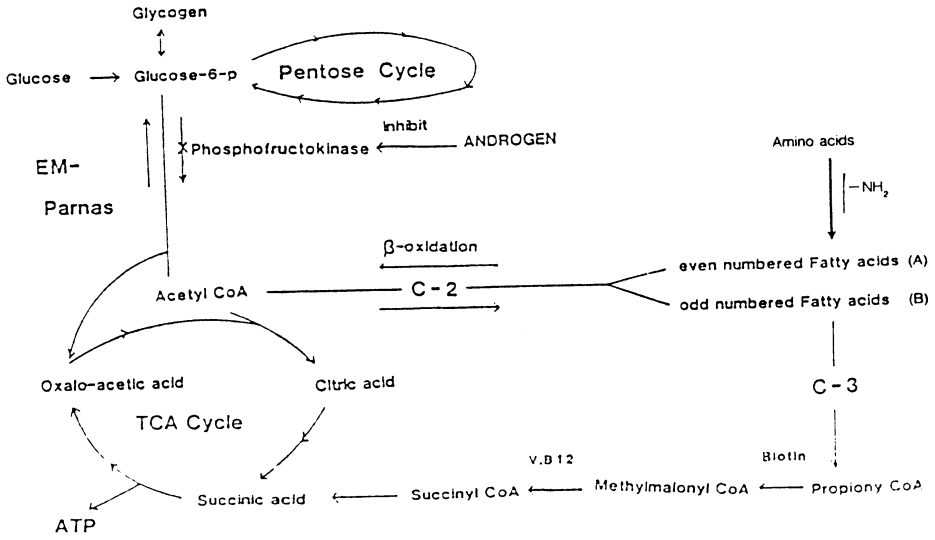


Figure 4. Metabolism of nutritional elements

However, in hair follicles of the male pattern alopecia, inhibition of phosphofructokinase (PFK), a major enzyme of glycolysis, would be induced by androgenic hormone, and the energy supply might be supressed.

In these conditions, lipids are apt to be utilized as an energy source. When the fatty acids having an even number of carbon atoms are metabolized, the carbon chain is decomposed into two carbon units through β-oxidation to give acetyl-coA. But, if too much acetyl-CoA is generated, it cannot be utilized further without oxaloacetic acid, and eventually it is accumulated as a form of acetoacetic acid. Therefore, it cannot be decomposed because of the feedback route and acetyl-coA does not produce an efficient energy supply.

On the other hand, as shown in figure 5(B), a fatty acid having an odd number of carbon atoms is also decomposed by β-oxidation process to form acetyl-CoA. Then, at the end of β-oxidation, propionyl-CoA having three carbon atoms is formed. Propionyl-CoA is converted to succinic acid under presence of biotin and vitamin B12. This succinic acid is converted to oxaloacetic acid briefly in the TCA cycle.

Therefore, this bioactive complex can also be used in hair products due to its revitalising action and its enhancement of proteic synthesis. As mentioned above, it acts as an efficient material for energy supply to hair follicles of the male pattern alopecia under supressed energy production, and activates the metabolism in the hair follicles, and exhibits hair growing effects.

Due to this, **PRONALEN FIBRO-ACTIF** is highly recommended for cosmetic products with a hair activity (growth and anti-hair loss).

Anti-aging activity

The skin contains fibrous proteins which are the structural elements of the tissue. The synthesis of these proteins (collagen and elastin) takes place in the fibroblasts under the influence of the oxygen, ATP and other necessary nutrients.

The synthesis of new molecules takes place more and more slowly as the number of fibroblasts falls with age, protein synthesis loses momentum and the compounds necessary for such synthesis are not so accessible. In the same way, the structure of the fibers which already exist is altered, causing the appearance of cutaneous flaccidity and wrinkles in the skin.

Until now the main products used to prevent skin aging, improve its look and avoid wrinkles were Placenta hydrolyzed. Today, animal products are being replaced by vegetable products which act in the same way as Placenta extract.

PRONALEN FIBRO-ACTIF comprises a set of bioactive molecules which are capable of penetrating the most inner layers of the skin and provide the necessary energy to increase the proteic synthesis of the collagen, elastin and glycosaminoglycans thus having beneficial effect on the skin by preventing and delaying the appearance of the clear signs of aging (wrinkles).

Due to this, **PRONALEN FIBRO-ACTIF** is highly recommended for cosmetic products with an anti-aging activity.

EFFICACY TEST

Introduction

Through the process of respiration the aerobic cells obtain energy from the oxidisation of biomolecules (amino acids, glucose, fatty acids) by the molecular oxygen. This process comprises three different stages the first being the cycle of the tricarboxylic acids in which the biomolecules already degraded into compounds of two carbon atoms turn into carbon dioxide and hydrogen atoms. In the further two stages (electronic transport and oxidative phosphorylation), the hydrogen atoms are reconducted to eventually give ATP and reduce the oxygen to water.

Oxygen Consumption

Two models were used to assess the action of **PRONALEN FIBRO-ACTIF** on the cell metabolism; liver mitochondria and culture of human fibroblasts.

Mitochondrias are considered “energy piles” of the cell, where the whole of the cell oxidative metabolism and ATP synthesis take place. This synthesis in conjunction with cell respiration is called oxidative phosphorylation and can be set out in the following reactions:

$$NADH + H^+ + 3ADP + 3P_I + 1/2\ \mathbf{O_2} \longrightarrow 6\ NAD^+ + \mathbf{H_2O} + 3\mathbf{ATP}$$

$$FADH_2 + 2ADP + 2Pi + 1/2\ \mathbf{O2} \longrightarrow 6\ FAD^+ + H2O + \mathbf{2ATP}$$

The working of the respiratory chain can be followed by measuring the OXYGEN CONSUMPTION of the process, which will indicate which falls in oxygen levels have been caused by the mitochondria working correctly and/or the presence of actives providing the necessary biomolecules for the oxidative phosphorylation to be caused and consequently an increase in the working of the mitochondria itself.

Material and methods

Details of material and methods used for the proteic quantification and oxygen consumption of this product are at your disposal, in case it is needed.

Results

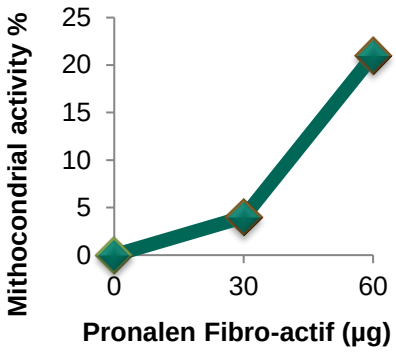


Figure 5.Mitochondrial activity-Pronalen quantity relation

- **Mitochondrial model**

1.-The activation by **PRONALEN FIBRO-ACTIF** depends on the concentration. The first graph shows how mitochondrial activity increases with greater concentrations of **PRONALEN FIBRO-ACTIF** (µg). In this case the concentration of the mitochondrial protein was 7.43 mg and the process was carried out without incubation.

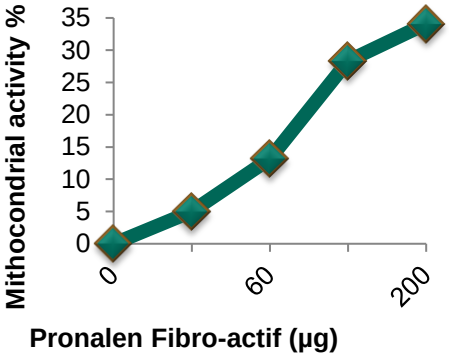


Figura 6. Mithocondrial activity

2.-The velocity with which the oxygen was consumed in the various mitochondrial preparations obtained is related to the proteic content, therefore the lower the protein concentration of the purified of liver mitochondria preparation, the slower the oxygen consumption. Under these conditions the activating effect of the **PRONALEN FIBRO-ACTIF** is far more obvious.

Figure 6 shows us the increase in the mitochondrial activity with greater concentration of **PRONALEN FIBRO-ACTIF** (µg), as the oxygen levels detected in the cell fall progressively. In this case, the mitochondrial protein concentration was in the order of 12.23 mg and the process was carried out after 15-18 hours of incubation.

As shown in figures 1 and 2, the final results under these conditions are not affected by previous incubation.

- **Human skin fibroblasts model**

The velocity of the oxygen consumption depends on the proteic content of the cell preparation in use. As in the tests carried out with mitochondrial suspensions, the activating effect of **PRONALEN FIBRO-ACTIF** is greater when the control cells consume less oxygen.

Figure 7 presents the increase in the mitochondrial activity of the fibroblasts with greater concentrations of **PRONALEN FIBRO-ACTIF** (µg). In this case the concentration of the mitochondrial protein was 5.45 mg. The concentration of the substrates was 5mM for glutamate and malate, while the ADP concentration was 50 µM.

Figure 8 shows the same test but with previous incubation. Thus, shows that the previous incubation of the fibroblast cultures with **PRONALEN FIBRO-ACTIF** slightly increased the percentage of activation.

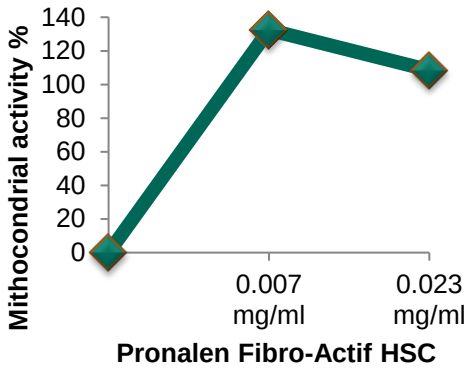


Figura 7. Mithocondrial activity

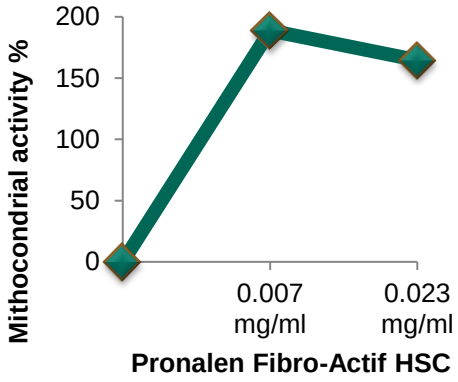


Figura 8. Mithocondrial activity

RECOMMENDED DOSE

From data obtained in-vitro, the suggested dosage is as follows:

- ✓ Hair treatments: 1.0 - 2.0%
- ✓ Anti-aging treatments: 1.0 - 2.0%

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