Body stimulation and firming (bust and body)

BIO-BUSTYL

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CONTENTS

1. DESCRIPTION OF BIO-BUSTYL

The bust is one of the most universal elements of corporal beauty of women.
It thus demands particular care and attention.

Whereas various major problems of the breast (hypertrophy, underdevelopment, relaxation of the muscles) can only be treated by methods of plastic surgery, certain small defects may be corrected by adequate cosmetic treatment.

The stimulation of tissue metabolism, the increase in cellular respiration and in tonus, the firming of cutaneous tissue are all elements that contribute to improved bearing of the bust.

BIO-BUSTYL

A product combining biotechnological origin and peptide synthesis, is designed to respond to these demands of stimulation.

BIO-BUSTYL contains three components:

- a biotechnological bacterial filtrate rich in stimulation and growth factors

- the Amphiphilic Biopeptide **Palmitoyl-Glycyl-Histidyl-Lysine (fundamental peptide sequence of collagen)**

- and the Amphiphilic Biopeptide **Palmitoyl-Valyl-Glycyl-Valyl-Alanyl-Prolyl-Glycine (so called "ELASTIC" sequence of the elastin).**

These three components act in synergy in order to stimulate cell metabolism and to strengthen the tissue:

* The bacterial biofiltrate is obtained by the culture of aerobic microorganisms (they require great amounts of oxygen) on a specific nutritive medium. This culture medium allows the germs to grow very rapidly.

  At the end of fermentation, the germs are eliminated by tangential microfiltration, the filtrate rich in stimulation and growth factors is standardised and preserved.

* The Amphiphilic Biopeptide **Palmitoyl-Glycyl-Histidyl-Lysine** is obtained by peptide synthesis from the individual free amino acids. This molecule was chosen on the basis of our knowledge of collagen metabolism; in fact, this tripeptide, a naturally occurring fragment of collagen, is able to stimulate the synthesis of collagen in fibroblasts, even at very low concentrations (10^{-8} M/l) [1]. We have attached palmitic acid to this tripeptide in order to increase the substantivity of the peptide to epidermis [2]. The presence of this Biopeptide in **BIO-BUSTYL** assures the stimulation of collagenic cellular metabolism in the dermis.

* The Amphiphilic Biopeptide **Palmitoyl-Valyl-Glycyl-Valyl-Alanyl-Prolyl-Glycine** is also of synthetic origin (chemical peptide synthesis). This sequence of amino acids was chosen within the structure of elastin, another important macromolecule of skin tissue. This hexapeptide represents the repetitive "elastic" chain link of elastin; furthermore, it possesses its own biological activity: namely chemotaxis, that is the capacity to attract repair cells (fibroblasts) to specific sites of action (wound healing, tissue regeneration) [3].

The Biopeptide participates thus in tissue restructuring and firming.

2. EFFICACY TESTS

2.1. *in vitro* Tests

2.1.1. Stimulation of cell respiration (OXYGRAPHY)

The principle and the details of the method have been described in detail previously. The basal rate of oxygen consumption in a liver cell suspension is measured using a CLARK type electrode and a GILSON oxygraph. The addition of the active product to the suspension stimulates mitochondrial respiration which results in an increase of the oxygen consumption. The recording of the concentrations of dissolved oxygen in the medium as a function of time generates slope curves as shown in figure 1.

The increase of the slope is expressed as a percentage, according the following formula:

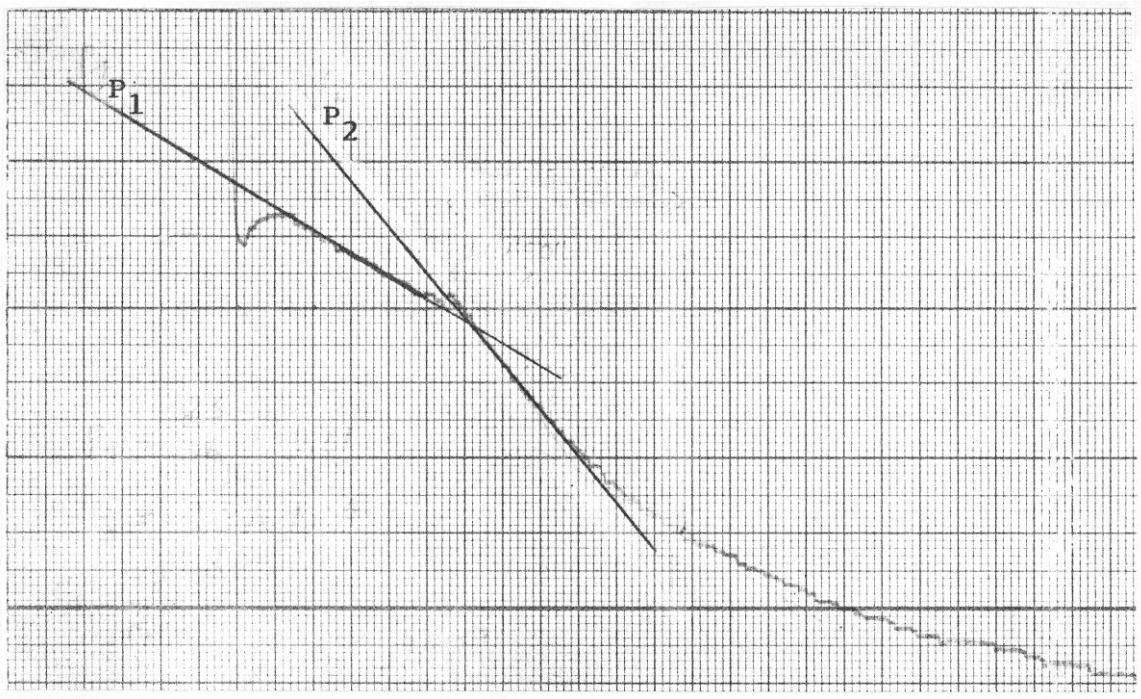
$$\frac{n_e - n_t}{n_t} \times 100$$

Where:

n_e : is the number of moles of oxygen consumed per minute and per mg of fresh cells in the control batch.

n_t : is the number of moles of oxygen consumed per minute and per mg of fresh cells in the test batch.

Figure 1: OXYGRAPHY Recording



$$P_1 = \frac{23}{38} = 0.61$$

$$\frac{P_2 - P_1}{P_1} \times 100 = 106\%$$

$$P_2 = \frac{23}{18.5} = 1.24$$

The dose of 2% BIO-BUSTYL in these experiments leads to a mean increase of cell respiration of 103 ± 4%.

2.1.2. Stimulation of cell growth

We have chosen the following protocol for measuring the stimulating activity of **BIO-BUSTYL** *in vitro*.
This test is carried out with fibroblasts of 3T3 lineage. We use 96-well microplates and measure the cell viability by MTT coloration.

Protocol

5000 cells are seeded in each well, covered with DMEM medium which is supplemented with 2% fetal calf serum (FCS) and which contains increasing concentrations of **BIO-BUSTYL (0, 0.5, 2.0 and 5.0%).**

Incubation lasts 72 hours at 37°C in an incubator. The controls contain DMEM medium with 10% FCS.

After reaction with MTT reagent, the absorption is read at 570 nm in a Dynatec microplate reader.

Results:

The following table shows that **BIO-BUSTYL stimulates cell growth** to a remarkable degree. This can be explained by the richness of the nutritive substances contained in the biotechnological product.

Medium	Number of cells (5600 initially)	Growth compared to 2% FCS
DMEM 2 % FCS	20,000	–
DMEM 10 % FCS	29,000	45 %
0.5% BIO-BUSTYL	22,000	10 %
1% BIO-BUSTYL	28,500	42.5 %
5% BIO-BUSTYL	35,500	77.5 %

These two experiments show that BIO-BUSTYL possesses a very high potential of cell stimulating activity.

2.2. *in vivo* Tests

Taking into account the biological activities of the Amphiphilic Biopeptides contained in **BIO-BUSTYL** (stimulation of collagen synthesis by **Palmitoyl-Gly-His-Lys** and tissue restructuring by **Palmitoyl-Val-Gly-Val-Ala-Pro-Gly),** we undertook a study of the **Skin Elasticity Parameters** by the method of Fermometry.

Principle

The probe of the Fermometer generates repeated depressions of 300 mbar and sucks in a small zone of skin. The deformation of the tegument is followed by the deviation of a light beam and recorded as a curve in time. Several successive aspirations are effected at the same site; from their recorded curves different parameters can be extracted (extensibility, plasticity, tonicity, elasticity, firmness, skin fatigue).

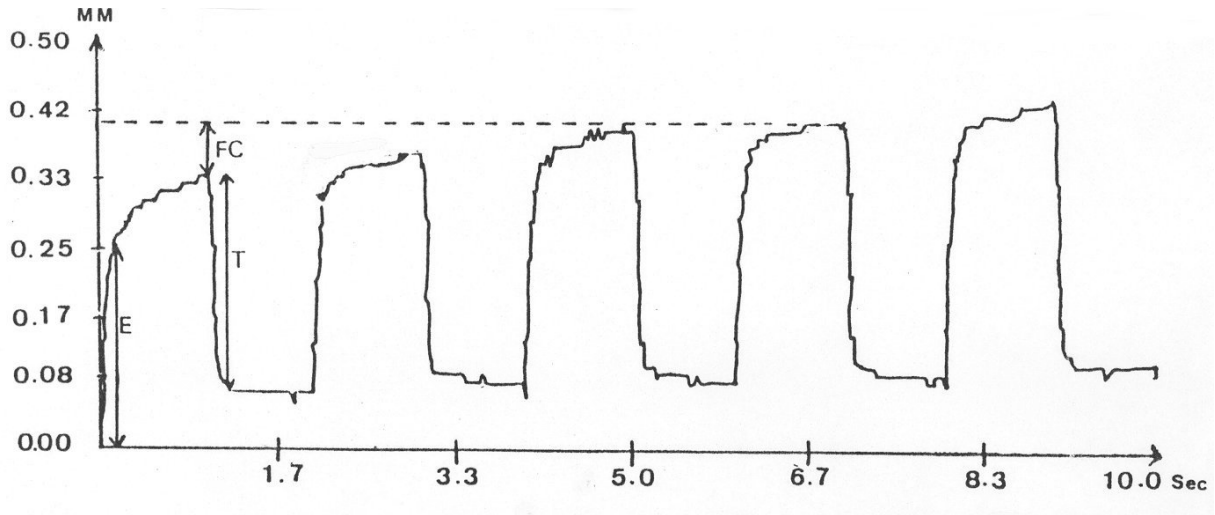
Panel

Fourteen female volunteers aged 30 to 55 years participated in the test. The usual inclusion and exclusion criteria were applied (ability to follow the protocol, free consent; illness, pregnancy, cutaneous afflictions).

BIO-BUSTYL was tested at 4% concentration in a light emulsion; the test was carried out against a placebo (emulsion without **BIO-BUSTYL)** in double blind mode.
The products were applied to the bust, morning and evening, during 30 days, at amounts of 1 to 2 mg/cm^2.

Results:

Figure 2 shows a typical recording obtained in this study. Note the locations on the graph that allow us to study the parameters which describe the elastic behaviour of the skin.



Legend: E = Elasticity (elastic extension)
T = Tonicity
FC = Skin fatigue

Habitually, the termes **Elasticity E** (more precisely Elastic Extensibility = immediately reversible extension) and **Tonicity T** (relaxation) are used to calculate the cutaneous **Firmness F** from the ratio T/F (generally < 1).

The evaluation of **Skin Fatigue FC** (variation of the total extensibility between successive aspirations) is a means to refine the study.

The measured values on each participant at days D0 and D30 are analysed by statistical treatment using a Student's T-test (paired values).

Table 1 resumes the values compiled and averaged from the recordings.

Table 1

Parameters	D0	D30	Difference (%)
Elasticity x100			
Placebo	48.99	49.48	1 Non significant
BIO-BUSTYL	51.14	49.85	– 2.6 Non significant
Tonicity x100			
Placebo	35.08	35.0	– 0.2 Non significant
BIO-BUSTYL	36.06	40.9	+ 13 Highly significant (p<0.01)
Firmness x10			
Placebo	7.08	7.03	– 0.7 Non significant
BIO-BUSTYL	6.98	8.27	+ 18.5 Highly significant (p<0.01)

Thus, we observe a strong increase in **Firmness (+18.5%)** that results from a cumulative effect of a decrease in **elastic extensibility E (-2.7%)** and an increase in **Tonicity T (+ 13%).**

Figure 3 shows the effect of the treatment with the cream containing 4% **BIO-BUSTYL** on the **Skin Fatigue FC parameter** (difference in total extensibility between the fourth and the first aspiration).

"Skin Fatigue" FC regresses by 16.2%, whereas it remains unchanged at the sites of placebo application.

These changes are statistically significant (p <0.01).

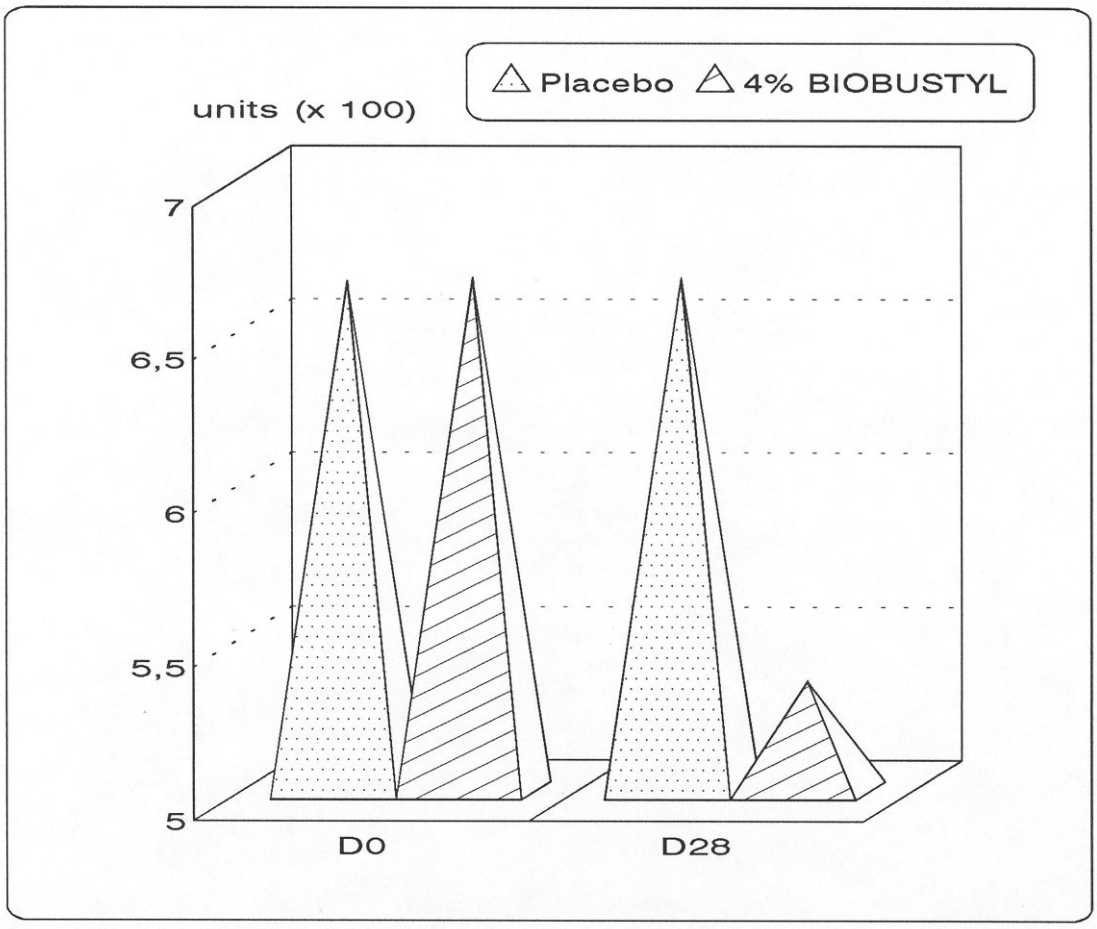


Fig.3

3. CONCLUSION AND COSMETIC IMPORTANCE

The results of the studies described show the impact of BIO-BUSTYL for cosmetic care.

- **Stimulation of tired metabolism**
- **Synthesis of dermal collagen**
- **Restructuring activity of the elastic peptide fragment**
- **Increase in tonus and firmness contribute to improvement of the beauty of the bust.**

We recommend the use of BIO-BUSTYL between 3 and 6%.

4. PHYSICO-CHEMICAL CHARACTERISTICS

4.1. Provisional values

Aspect	:	Fluid gel
Odor	:	characteristic
pH	:	≈ 6.0
Specific weight	:	≈ 1.09
Refractive index	:	≈ 1.385
Water content (K. Fischer)	:	≈ 61.5%
Total germs count	:	< 100 germes/g

REFERENCES

1. **MAQUART F.X. et al. 1988, FEBS Letters 282, 343-346**

2. **SEDERMA BROCHURE: Amphiphilic Biopeptides**

3. **SENIOR R.M. et al. 1984, J. Cell biol. 99, 870-874**