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# Clinical and Non-invasive Evaluation of 12% Ammonium Lactate Emulsion for the Treatment of Dry Skin in Atopic and Non-atopic Subjects

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**Clinical dryness of the leg skin is a common problem among dermatological patients. The efficacy and safety of 12% ammonium lactate emulsion (Keratisdin®) for the treatment of dry skin on the legs of atopic and non-atopic subjects has been assessed by clinical criteria and by five different non-invasive methods. These methods measure biophysical parameters such as electrical capacitance of stratum corneum, skin surface lipids, transepidermal water loss (TEWL), skin surface topography (scanning electron microscopy and image analysis) as well as the biomechanical properties of the skin. Treatment with the test emulsion significantly reduced the severity scores for dryness, desquamation and pruritus when measured 15 days later. All patients tested showed a significant increase in electrical capacitance, skin surface lipids, *extensibility* and firmness of the skin, and an improvement in the skin barrier function and skin surface topography. This study showed that non-invasive techniques are excellent complementary tools in clinical studies. *Key words: Electrical capacitance; Transepidermal water loss; Skin surface lipids; Replica technique; Scanning electron microscopy.***

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The occurrence of dry skin on the legs is common place in patients visiting dermatology clinics.

The chief aim of the present clinical study was to evaluate the efficacy and safety of a moisturizing emulsion containing 12% ammonium lactate, for the treatment of dry skin in atopic and non-atopic patients. Efficacy was assessed according to clinical criteria and by using different parameters of skin physiology and non-invasive biophysical methods.

In 1976, the first international conference on non-invasive skin measurement techniques was held at Miami Beach, U.S.A. Since then rapid advances in skin bioengineering technology have improved the biophysical techniques available on the market.

For this reason the second objective of the present study was to evaluate and criticize different non-invasive techniques (1–5) as complementary tools in daily dermatological practice, using objective parameters to follow the course of a dermatological disease in order to obtain quantitative values amenable to statistical analysis.

The non-invasive techniques used in the present study measure different parameters of skin physiology such as water content, sebum content, biomechanical properties, topography and barrier function of the skin.

## MATERIALS AND METHODS

### *Patients*

Twenty-four female patients, aged 20–50, participated in the study. Twenty-one patients were nurses and 3 were doctors. Nine patients had atopic dermatitis according to the criteria of Hanifin & Rajka (6) and 16 were non-atopic patients.

All of them had 'dry' skin on the legs, defined as a rough, non-inflamed skin surface with fine scaling. All were volunteers, who signed a printed informed-consent form. The nature of the study was explained to them in full.

They were instructed to apply the test emulsion on their legs, twice daily for a month, and they were not allowed to use any other skin care products during the study. Moreover, they were instructed to use a mild, non-irritant syndet for their daily hygiene.

### *Test substance*

Each patient was supplied with two bottles containing the oil-in-water test emulsion (Keratisdin® lotion).

### *Clinical evaluation*

Clinical examinations were performed on the first day (baseline), after 14 days and at the end of the treatment.

The parameters evaluated were dryness, desquamation, folliculitis and pruritus (7) and were scored on a 0–3 (0 = normal) scale.

### *Biophysical non-invasive measurements*

Measurements were performed on the first day, after 14 days and at the end of the treatment, in a clinical room with controlled temperature and humidity (20°C, 40% HR). On the fifteenth and thirtieth days the measurements were performed respectively 3 or 4 h and 12 h after applying the test emulsion.

Physical and mental stress were avoided 15 min before the measurements. We tried to avoid air convection.

### *Measurement equipment*

*Skin hydration state.* The hydration of the stratum corneum was assessed by measuring electrical capacitance of the skin surface by means of a Corneometer 820 PC(8). When the probe was applied to the skin (pressure of 3.5 N, recording time 0.8 s), the capacitance is displayed digitally in arbitrary units (a.u.). The results are expressed as mean values of the measurements performed on three differents sites.

*Skin surface lipids.* The skin surface lipid level was measured with a Sebumeter 810 PC (10). Determination is based on photometric measurement of light transmission through a skin surface imprint obtained by pressure (6 N) applied to the skin for 30 s, using a frosted plastic foil. The Sebumeter readings were converted into µg/ $cm^2$.

*Transepidermal Water Loss (TEWL).* Water evaporation from the skin surface was measured quantitatively with an Evaporimeter (Servo Med EP1). This device uses the method of vapour pressure gradient calculation described in detail by Nilsson (9). The evaporimeter probe consists of a cylindrical chamber 12 mm in diameter that contains two thin capacitive film transducers for measuring relative humidity above the skin. The probe is protected by an open cylindrical Teflon capsule (chimney) which is placed over the test area in order to prevent air

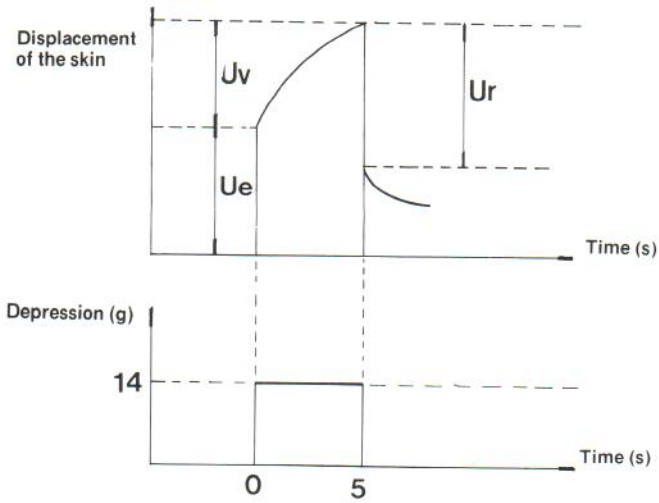


*Fig. 1.* Displacement of skin, versus time.

convention. TEWL is calculated automatically and displayed digitally in $g/m^2$ h.

Readings at a stable level were performed 30 s after application of the probe of the skin.

The TEWL values recorded during 30 s were submitted to treatment by a computer.

*Skin surface topography.* (*a*) Negative skin replicas. At the baseline and on days 15 and 30, a skin replica of the lower leg was made in order to describe the skin surface microtopography in greater detail. These replicas were formed from silicone rubber material as used for dental impressions. A thin layer of freshly prepared material was spread with a spatula over 2 $cm^2$ of the skin surface and allowed to polymerize. The material set in about 2-4 min and could be gently peeled from the skin. Each specimen was coded and stored in an individual glassine envelope until measured (11).

(*b*) Scanning electron microscopy. Positive skin replicas were prepared with Araldite® (hard epoxy resin) from the negative skin replicas. These positive replicas were mounted on aluminium SEM stubs, coated with gold (100 Å) and then viewed in a scanning electron microscope (Cambridge Stereoscan S-120) using an accelerating voltage of 15 kV (12).

(*c*) Image analysis. Negative skin replicas were studied by image analysis (13, 14). This technique projects lateral light onto the 'lunar craters' and crevices of the skin, using a fiberoptic illuminator set at a fixed incident angle of light (38⊖).

The images were recorded with a high-resolution black and white video camera and fed in to a computer with image-processing hardware and software specially designed for evaluating the shades in degrees of grey. The resulting image was then digitalized into a 512 × 512 pixel matrix with 256 degrees of brightness in grey.

The images were analysed from different aspects. First of all a graphic representation was made in the form of histogram of the frequency distribution of pixel light intensity. This distribution gives information about the roughness of the skin. By selecting three arbitrary interval values of grey level and calculating the corresponding percentage of the total area in the frequency distribution, three parameters can be defined: % of valleys (1–100 pixels), % of passes (101–180 pixels) and % of crests (181–256 pixels). These parameters give information about the roughness of the skin. When the roughness increases, the percentage of valleys increases and the percentage of crests decreases.

By plotting the grey level values along a horizontal segment of this digitalized image, we obtained a profile that depicts the surface features. Three horizontal segments were selected for each replica.

When the roughness decreases, the profile becomes smoother and flatter.

Table I. *Mean scores of skin dryness, desquamation, folliculitis and pruritus*

| | Initial | 15 days | 30 days |
|---|---|---|---|
| Patients (*n*) | 24 | 24 | 22 |
| Dryness | 2.0 | 0.04 | 0 |
| Desquamation | 0.9 | 0 | 0 |
| Folliculitis | 0.21 | 0 | 0 |
| Pruritus | 0.85 | 0 | 0 |

*Biomechanical properties of the skin*

The biomechanical properties of the skin (extensibility, firmness) were measured with a new suction device designed by Courage & Khazaka (Cutometer SEM 474) (15, 16). This evaluates the mechanical properties by recording the strain on the skin surface, which is drawn into the opening of a special measuring probe by means of a defined degree of vacuum. The penetration depth of the skin into the probe is measured without any friction or mechanical effect, by using two optical lenses positioned at the opening of the measuring probe.

Before applying the probe to the skin, the following parameters must be specified: vacuum level, duration of action, and post-action time. The values of these parameters in this experiment were 400 mbar, 5 s and 2 s respectively.

The deformation curve as a function of time is presented in graphical form in Fig. 1. The following parameters were calculated in mm from this curve (5):

Ue: Peak of signal for elastic strain, represents the *extensibility* of the stratum corneum (*Extensibility*)

Ur: represents the ability of the skin to recover its equilibrium after the removal of suction (Tonicity)

Ur/Ue: Ratio represents the strength of the skin under stress (Skin Firmness)

*Statistical methods*

Statistical analysis was performed using Student's *t*-test for paired observations. The results on days 15 and 30 were compared with the initial values.

## RESULTS

*Clinical evaluation*

The mean results of the clinical parameters evaluated are shown in Table I.

Treatment with test emulsion had significantly reduced the severity scores for dryness, desquamation and pruritus when measured 15 days later. The scores on day 30 was not significantly different from that on day 15.

The grading of the signs is illustrated in Fig. 2 by photographs of patient no. 15 at the start and at the end of treatment.

No adverse reactions such as stinging or irritation were reported during the study.

All the subjects subjectively assessed the efficacy and cosmetic acceptability of the test emulsion. The results were 8.8 (SD 1) and 8 (SD 0.9) respectively on an arbitrary scale (0–10).

*Electrical capacitance*

All the patients showed a significant increase in electrical capacitance (moisture content) after 15 days of treatment, and maintained these values until the end of the experiment. The results are shown in Table II.

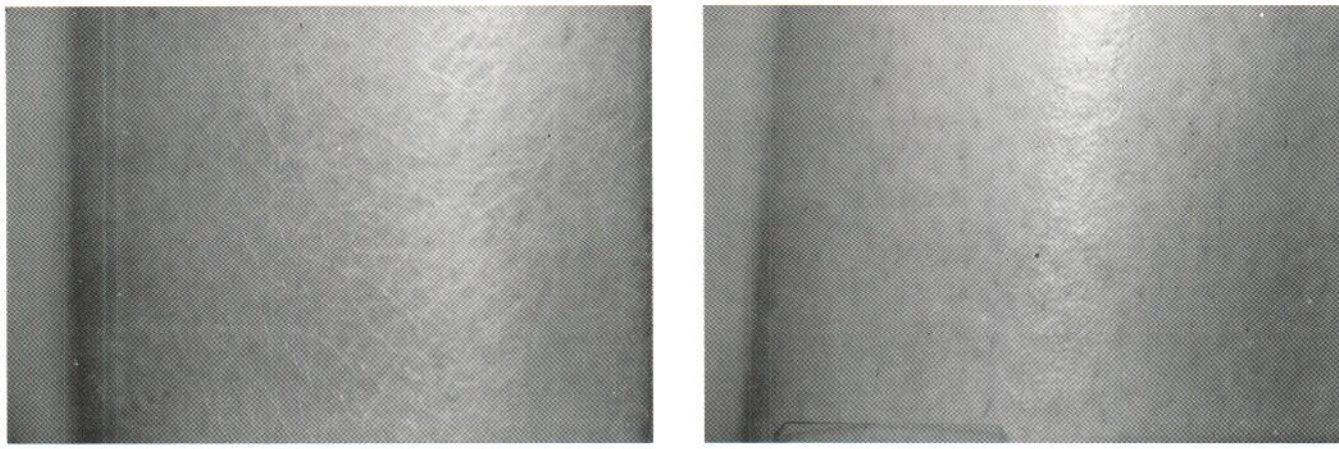

*Fig. 2.* (*a*) Patient photograph before treatment; (*b*) at conclusion of treatment.

*Skin surface lipids*

All the patients showed at baseline a lower content of skin surface lipids (casual level) (9.5 μ/cm$^2$).

After 15 and 30 days of treatment the Sebumeter values were 22.4 and 12.9 μg/cm$^2$, respectively.

All the subjects experienced a significant increase in their casual lipid content ($p$ ‹0.005).

The Sebumeter values after 30 days were lower than after 15 days because these readings were made at different times after application of the emulsion.

On day 15, the Sebumeter readings were taken 3–4 h after applying the emulsion, these readings including skin surface lipids and emulsion oil, in accordance with Serup et al. (17). However, on the thirtieth day the Sebumeter readings were taken 12 h after application of the moisturizing lotion; they included only the skin surface lipids (17).

The readings were made at different times in order to ascertain whether the emulsion lipids penetrate into the outer epidermis. Provided that the Sebumeter values are lower after 30 days than after 15 days, we can assume that the emulsion lipids probably do penetrate into the outer epidermis.

*Transepidermal water loss*

The TEWL values obtained are shown in Table III. There is no significant increase in transepidermal water loss after the treatment.

The TEWL results are consistent with an improvement in skin barrier function. The great increase in the stratum corneum water content (Corneometer values) is not accompanied by an increase in TEWL values, such as would have occurred if the horny layer had not been modified.

*Skin surface topography*

*Scanning electron microscopy.* Analysis by scanning electron microscopy of positive skin replicas shows that at the baseline the skin is characterized by intense desquamation and loss of regular skin pattern.

At the end of the treatment the skin shows a significant decrease in desquamation and a regular pattern consisting of major furrows running parallel and, between these, geometric figures formed by minor furrows.

Fig. 3 a shows SEM micrograph of skin replicas before and after the treatment.

*Image analysis.* After treatment, all the patients showed a significant shift in the frequency distribution histogram maximum to higher values of grey level, and a narrower histogram. These results demonstrate that the treatment does improve skin surface topography. Fig. 4 shows histograms before and after the treatment.

The treatment with moisturizing lotion reduces the proportion of valleys and increases that of crests. Thus after 15 and 30 days of treatment, the proportion of valleys had decreased by 19.4% and 19.8%, respectively, while that of crests had increased by 31.0% and 44.2%, respectively.

The profiles at baseline and at the end of the clinical trial are presented in Fig. 5. These show that the treatment reduces the skin's roughness and makes it smoother and flatter.

*Biomechanical properties of skin*

At the end of the experiment, all patients showed a significant improvement in biomechanical properties (*extensibility* and firmness). The values for Ue (*extensibility*) and Ue/Ur (firmness) are presented in Table IV.

Table II. *Mean values of electrical capacitance (arbitrary units)*

| | Initial | 15 days | 30 days |
|---|---|---|---|
| Electrical capacitance | 76.3 | 109.1* | 103.9* |
| SD | 7.6 | 7.9 | 6.5 |

*$p$ ‹ 0.001

Table III. *Results of transepidermal water loss*

| | Initial | 15 days | 30 days |
|---|---|---|---|
| TEWL | 3.4 | 3.7* | 5.7* |
| SD | 1.03 | 2.75 | 3.3 |

*Not significantly different.

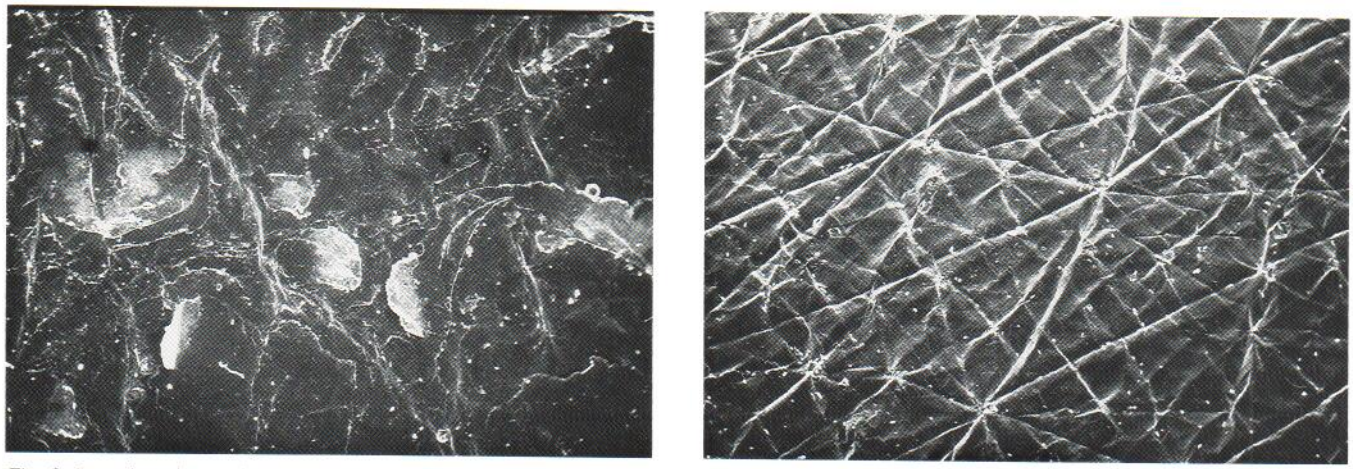

*Fig. 3.* Scanning electronic micrograph of skin replica, (*a*) before treatment, (*b*) after treatment.

## DISCUSSION

This clinical study has shown that treatment with 12% ammonium lactate emulsion (when coupled with excellent patient compliance) can effectively and safely relieve the symptomatic severity of dry skin in both atopic and non-atopic subjects. The clinical assessment was consistent with the non-invasive biophysical evaluation. Thus the SEM results show that the skin evidences a significant decrease in desquamation and regains its regular pattern after treatment. These findings can all be attributed to the keratolytic and humectant action of the lactate. This humectant action also explains the large increase in stratum corneum water content found after the treatment (18, 19, 20).

The increase in water content (Corneometer values) and restoration of the skin regular pattern (SEM) after the treatment are corroborated by the improvement in biomechanical properties of stratum corneum (*extensibility* and firmness) measured with the Cutometer.

Powers & Fox (21) and Rietschel (22, 23) have demostrated that transepidermal water loss ought to increase when the skin is treated with moisturizers based on humetant agents. In our experiment, however the TEWL did not increase significantly, which can be explained by the improvement in skin barrier function following the treatment.

The intercellular lipid-rich compartment of the epidermis is of great importance for the skin barrier function (24, 25, 26). After considering the Sebumeter values, we believe that the test emulsion restores the skin barrier by lactate keratolytic action and because the emulsion lipids probably penetrate into the outer epidermis and merge into his lipid-rich compartment.

The correlation shown in this study between clinical and non-invasive results prompts us to suggest that the best way to gain a fuller understanding of skin dryness in atopic and non-atopic subjects is an approach based on clinical studies and the use of a battery of non-invasive biophysical techniques.

We advocate that non-invasive techniques should be used in conjunction with – not instead of – clinical studies.

## ACKNOWLEDGEMENTS

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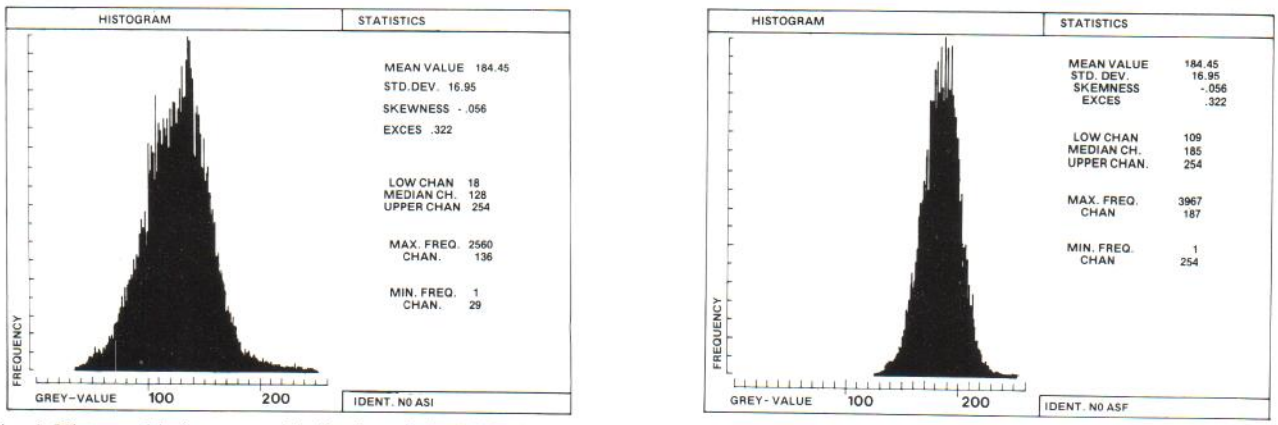


*Fig. 4.* Histographic frequency distribution of pixel light before left and after right treatment.

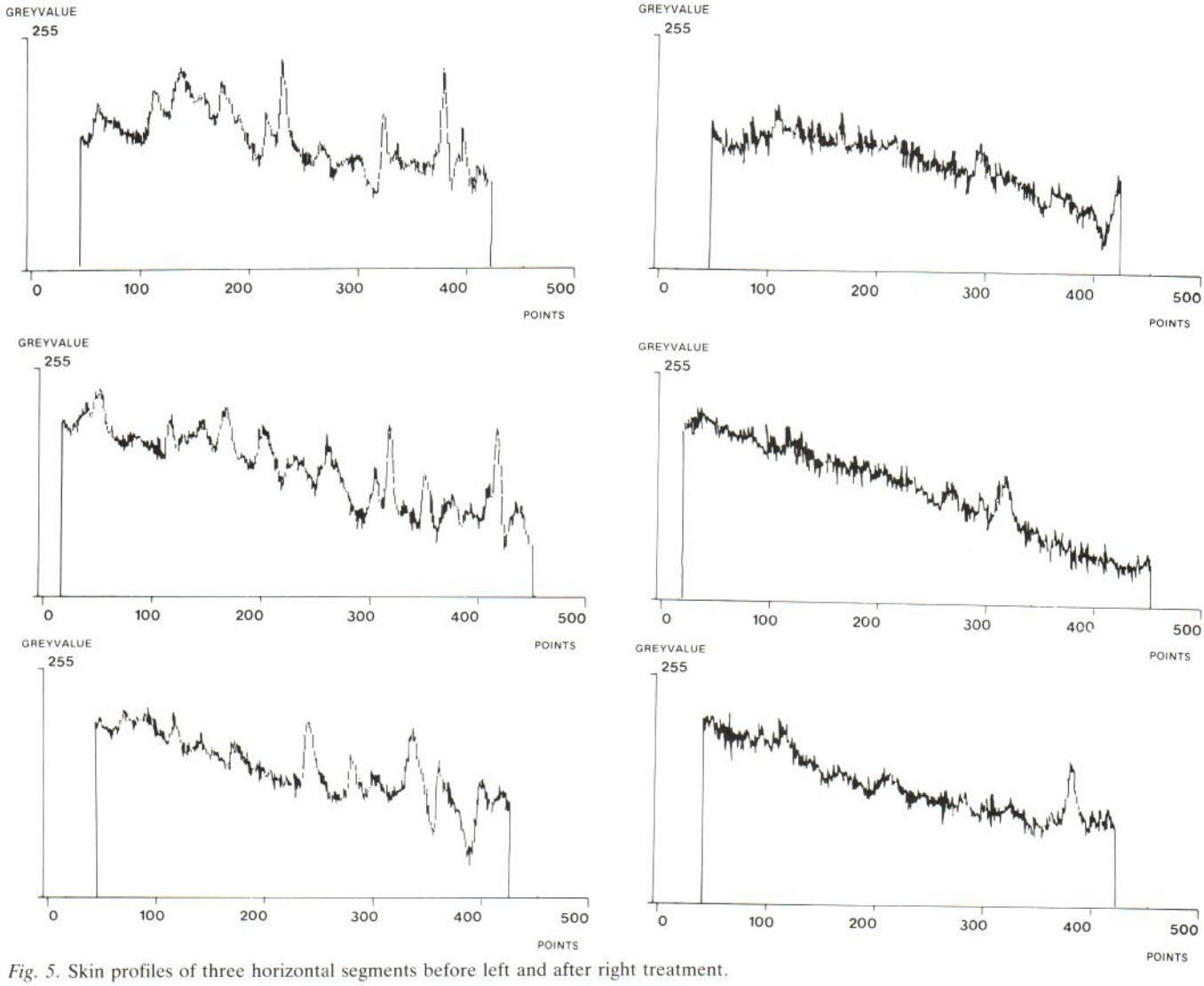


*Fig. 5.* Skin profiles of three horizontal segments before left and after right treatment.

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Table IV. *Results of biomechanical properties*

| | Initial | 15 days | 30 days |
|---|---|---|---|
| Ue | 0.038 | 0.16* | 0.19* |
| SD | 0.014 | 0.05 | 0.1 |
| Ue/Ur | 0.68 | 0.83** | 0.88** |
| SD | 0.23 | 0.13 | 0.1 |

$^{*}p < 0.001$ $^{**}p < 0.01$

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