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Near-infrared spectroscopy: A new approach to the characterization of dry skin

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Synopsis

A few years ago, Professor A. M. Kligman posed the question "Is dry skin dry?" Since that time, our knowledge of dry skin has improved considerably, but it is still not clear whether or not the water content of dry skin is lower than that of normal skin. This is, however, a fundamental question that involves one of the most important problems in cosmetics.

In the near infrared, water molecules show two clear absorption bands at 1450 and 1936 nm. The amplitudes are sufficiently high for them to be easily identifiable in the spectrum of the human skin *in vivo*. We have modified a commercial apparatus (Infra-Alyser 500; Bran and Luebbe) in order to obtain a system suited to spectroscopic analyses of all skin sites *in vivo*. Special optic fibers with a very low coefficient of attenuation and an integration sphere have been added to the original apparatus without modifying its capacities of function.

In a first set of *in vitro* experiments, we examined the penetration of IR waves into the various skin layers. The results showed the role of the stratum corneum (SC) in the amplitude of the two water peaks and that the amplitude is proportional to the water content.

The dry skin type known as winter xerosis is characterized by a marked alteration of the appearance and surface state of the SC. This led us to describe the clinical condition in terms of the following criteria: inflammation, roughness (smooth/rough), and presence of flakes and scales. We attributed a score of 0–4, and an overall score was then calculated. The water content of the skin was measured in the same area (external surface of the leg) in a number of subjects, using two biophysical methods—near-infrared spectroscopy and an electrical conductance method that provides only a very indirect measurement of water content. Correlations between the biophysical measurements and the clinical scores showed clearly that the skin judged as being the dryest gave the lowest peak amplitudes in the near infrared. This correlation was much better than that obtained using the clinical scores and the conductance method, particularly for very dry skin. A more thorough analysis of the correlations between the clinical scores and the NIR parameters provides further information on this phenomenon.

From the previous population, three groups of 19 subjects with very dry skin were further studied. The subjects in each group applied a moisturizing preparation to one leg, the other leg serving as a control. The

state of the skin was tested after four weeks of daily application by means of the near-infrared and conductance methods as well as by using the clinical scoring system. The results indicate that the water content of the skin and its conductance changed in the direction opposite to the macroscopic aspect. Near infrared spectroscopy emerged as the most sensitive method for evaluating the efficacy of the preparations. We present the overall results in terms of the clinical description of dry skin, together with the actual efficacy of the moisturizing preparation relative to the expert evaluation.

INTRODUCTION

Despite thorough descriptions of dry skin, its causes are still poorly understood. Several biophysical methods are now available to characterize the state of the skin *in vivo* (1), but although progress has been made in our understanding of it functional properties (2), little is known of its precise nature, particularly with regard to lipid content, the reported variations of which are only minor (3,4). The fundamental question is whether or not dry skin is dehydrated. The lack of informative data is due to the fact that the numerous noninvasive methods used to described skin condition and to determine the efficacy of cosmetic preparations are based on measurements of physical parameters that are themselves dependent on the presence of water, lipids, and other components. In addition, the variation of the physical parameters studied is rarely directly related to differences in water content. In other words, these methods can provide adequate answers to the question "How much?" but not to the question "Why?"

Although water is the main determinant of the overall physical properties of the stratum corneum, other components can play an important role. This is particularly the case of lipids, which modify the relationship between light diffusion, reflection, and transmission, and which also influence the surface properties of the skin; in addition, they can give a smooth or rough feeling according to their nature. Cosmetics are complex compositions of numerous ingredients, but all are composed of water and oil. Most cosmetic products improve the smoothness of the skin and its overall condition, but we are not yet in a position to describe how they do so.

Infrared spectroscopy (5) can, in principle, provide valuable information on the water content of the skin. However, the infrared spectrum of water is wide and poorly defined. The amide I and II bands, the amplitudes of which are modified by the presence of water, are only indirect markers. In practice, measurements are made via a prism within which the rays undergo total reflection and show only poor penetration (less than one micron) into the tissue studied. Furthermore, the measurements take a considerable time, and the occlusive effect of the prism on the skin influences the water content.

The near-infrared spectrum (1100–2500 nm) appears to be more practical, since the water bands are precise. Two types of apparatus—an infrared sensor and a data analyser—are required; in addition, the radiation re-emitted by the skin cannot be determined precisely because of the nature of the applicator (6).

To overcome these difficulties, we have totally modified the optical elements of an integrating sphere of a near-infrared spectrophotometer (Infra-Alyser 500) in such a way as to record spectra at any skin site in a routine manner. We have conducted a study of the true nature of winter xerosis and the efficacy of various treatments, comparing the results with those provided by a simple and widely used electrical conductance method. In addition, the skin was characterized by trained experts in terms of several clinical

criteria, both before and after four weeks of treatment. On the basis of the results obtained, we attempted to answer the following questions:

- Is dry skin insufficiently hydrated, or is it simply rough?
- How good is the agreement between clinical evaluation, and results provided by the physical measurement?
- Are the results of the two physical methods redundant or complementary?

MATERIALS AND METHODS

MATERIALS

Near-infrared spectrophotometer. Diffuse-reflection infrared spectroscopy has been considerably developed since the initial work by Norris in the 1970s (7). This rapid, noninvasive method can be used to obtain spectra of solid, opaque samples. In the agro-alimentary field, it is mainly used for *in vitro* analysis of water, lipids, and proteins (7). Apart from applications aimed at identifying raw materials and analyzing finished products, most uses in cosmetology (e.g., the assessment of hydration and the efficacy of moisturizing agents) involve *in vivo* measurements. The apparatus we have adapted to spectral measurements *in vivo* is an integrating sphere spectrophotometer (Infra-Alyser 500) coupled to a PC AT microcomputer. An external integrating sphere has been added (Figure 1) so as to acquire a full energy spectrum and to optimize the signal-to-noise ratio, without modifying the basic functions.

The external integrating sphere is identical to the internal sphere and is connected to the optical elements of the Infra-Alyser 500 via two special optical fibers with a very low attenuation coefficient (<0.5 db/m) throughout the study spectrum. The reference beam and the analytical beam are collected by the optical elements through a group of

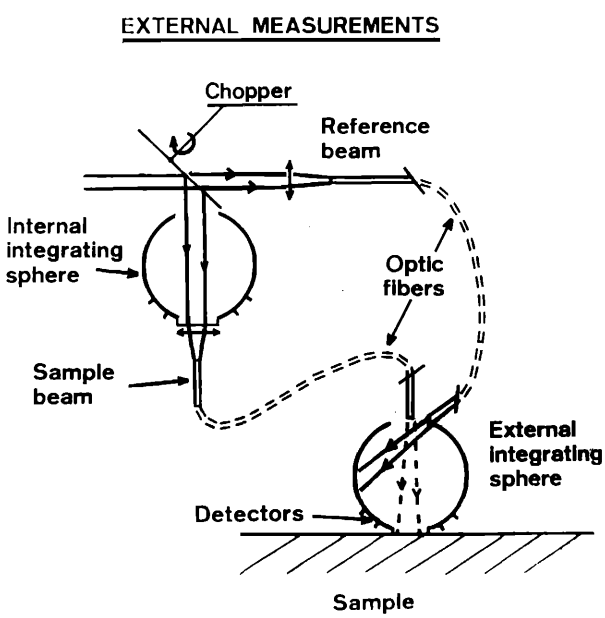

Figure 1. Modification scheme of the Infra-Alyser 500 to obtain *in vivo* measurement on all body sites.

adapted lenses, as well as under the internal sphere by a special optical system. The beam geometry (solid angles and incidence) in the external sphere is identical to that in the internal sphere. In preliminary studies, these modifications did not lead to a wavelength shift or change the absorption spectra of test materials. The fibers permit measurements to be made over the range 900–2500 nm with sufficient sensitivity, something that has not always been the case (6).

The spectra were acquired from 1100 to 2500 nm in 4-nm steps and expressed as absorbance (log 1/R), where R is the ratio between the energy reflected on the walls of the sphere and that retrodiffused by the sample. For homogeneous samples with little absorbance and no diffusion, the concentration of an analyte can be considered directly proportional to the absorbance (Ai) at a given wavelength (λi) according to an equation based on Beer-Lambert's law:

$$C = KAi = K \log (1/Ri)$$

In practice, particularly for measurements of the skin, the phenomena are far more complex. This law is no longer applicable because of interference due to analytes other than that under study and, above all, to diffusion due to the granulometry or surface state of the skin. The effects of this diffusion can be partly eliminated by calculation based on the difference in the absorbance at two wavelengths. The resulting equation is as follows:

$$C = K (A2 - A1) = K \log (R1/R2) \text{ (equation 1)}$**Conductance measurements.** Skin conductance was determined using a DermoDiag® apparatus (8), which operates at 10 MHz; the result is related to the degree of hydration, the surface state (contact impedance between the skin and the electrode), and the thickness of the stratum corneum.

EXPERIMENTAL STUDIES

In vitro. Samples of stratum corneum isolated by heat-trypsin treatment were equilibrated at various degrees of relative humidity and analyzed by means of near-infrared spectroscopy. The difference in absorbance at 1936 and 1100 nm was recorded, and the water content was deduced from the sorption isotherms determined by differential calorimetry and weighing (9). Spectroscopic analysis of the skin *in vitro* was performed before and after removal of the epidermis by heat treatment, in order to study the influence of the most superficial layers of the skin.

In vivo.

Study population. Dry skin was characterized by studying the external surface of both legs (just below the middle of the calf) in a panel of 159 women with a mean age of 40 years (range, 18–67), who had given their informed consent.

The efficacy of five cosmetic preparations was determined by studying five groups of about 20 women per group matched for age and the dryness score (>2). The treatment was applied twice daily for four weeks to one leg, the other serving as a control. Products were different O/W (products B, C, D) and W/O (products A, E) formulations containing various proportions of moisturizing agents except product D. The measurements

and clinical evaluations were made at the beginning (T0) and end of the treatment period (T4).

Clinical scores. The aspect of the dry skin was evaluated by a trained expert on the basis of the following five criteria:

- "papyracé" state of the skin ("cigarette paper" aspect)
- roughness (tactile evaluation)
- presence of squames
- presence of scales ("snakeskin" aspect)
- irritation (subclinical inflammation: redness)

Each criterion was scored from 0 to 4 according to the degree of severity; half points were permitted. The average of the five criteria has been taken as the overall score of each individual. The score for each item was also calculated.

Statistical analysis. Correlations between the experimental values were sought by using Spearman's test for nonparametric variables and Pearson's test for parametric variables. Regression lines were constructed from the mean scores (half-point steps), together with the corresponding standard error of the mean (SEM). Three-factors analysis of variance, including a hierarchical analysis [time, product; subject (product)], was used to determine the statistical significance of the treatment effects, followed by the Newman-Keul test. The results are presented as means ± SEM.

RESULTS

IN VITRO STUDIES

The absorbance of the dermis was greater than that of total skin (Figure 2), the difference, i.e., the spectrum of the epidermis, showing the two characteristic water peaks at 1450 and 1936 nm. As expected, the absorbance of the skin increased after removal of the epidermis, partly because of the removal of the least-hydrated layer, the stratum corneum, and partly because of an increase in the volume analyzed due to the greater infrared penetration.

Figure 3 shows the correlation ($r = 0.98$, $p < 0.001$) between the difference in absorbance at 1936 and 1100 according to the water content of the stratum corneum. The difference in absorbance at the wavelengths used clearly increased with water content, as did the water peak (1936 nm). This difference in absorbance is computed according to the modified Beer-Lambert's law (equation 1) and is better than any other one computed using the water peak wavelengths and references.

CHARACTERIZATION OF DRY SKIN *IN VIVO*

Figure 4 shows the mean spectra obtained for the study population according to the overall clinical score. Absorbance fell gradually with the increase in the clinical score, and this was particularly marked for the two water peaks (1450 and 1936 nm) and less marked in the region between 2000 and 2500 nm.

Finally, a multiple correlation study has shown that the absorbance (1936–1100 nm) was better correlated to the dry skin score than the 1450–1100 nm one ($r = 0.789$

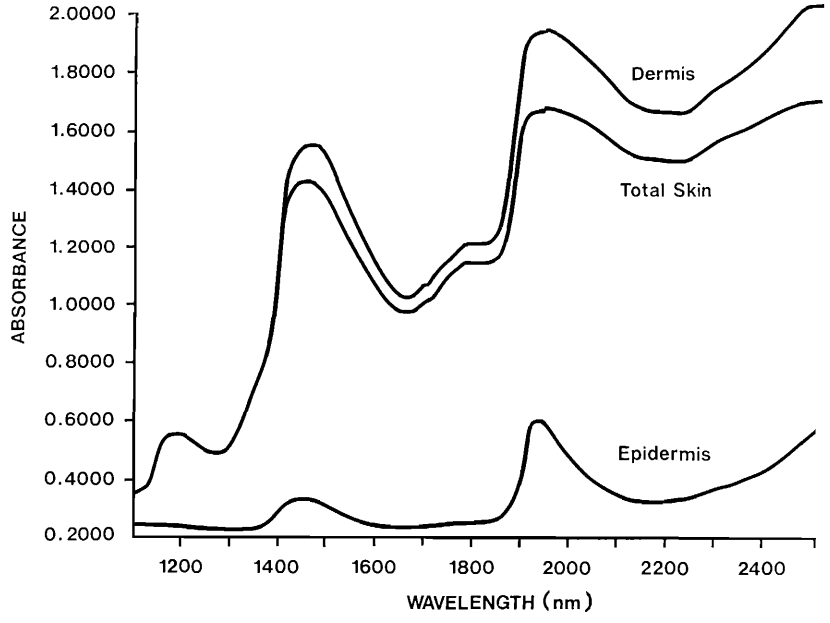

Figure 2. NIR absorption spectra of total skin, dermis, and epidermis.

versus r = −0.720 for n = 106). The band at 1100 nm is the wavelength at which the absorbance of skin is minimal. We therefore used the difference in absorbance at 1100 and 1936 nm in the rest of the study, which is, as in the *in vitro* experiment, better than other ones.

The regression line in Figure 5, constructed using the overall data obtained at T0 (before beginning treatment), is a mathematical representation of the decrease in the spectra illustrated in Figure 4. Despite the large number of values (n = 310), the correlation coefficient (r = −0.536, $p < 0.001$) remained very high.

For the overall data at T0, the changes in the overall clinical score (Figure 6) as a function of the individual "scaling" and "roughness" scores correlated strongly with the presence of scales (r = 0.76), but weakly with roughness (r = 0.43). The roughness score no longer changed above an overall score of 3, a phenomenon reflected by the correlations between absorbance and these two individual scores: the "scaling" score was still strongly correlated (r = −0.409) with IR absorbance (Figure 7a), while the "roughness" score was only weakly correlated (Figure 7b) (r = −0.344). It is worth noting that the three other descriptive scores ("papyracé," squames, irritation) are not correlated either to the difference in absorbance (r = −0.15, −0.19, −0.13) or to the overall score (r = 0.25, 0.20, 0.28).

The correlation between electrical conductance and the clinical score was significant but linear only for scores below 2.5 (Figure 8). Beyond the conductance is independent of the score. The correlations between descriptive scores values and conductance concern the "papyracé" scores (r = −0.331, $p < 0.01$) and roughness (r = −0.370, $p < 0.01$).

Figure 9 compares the overall results obtained with the five cosmetic preparations, comparing the treated and untreated legs, for the three following parameters: change in

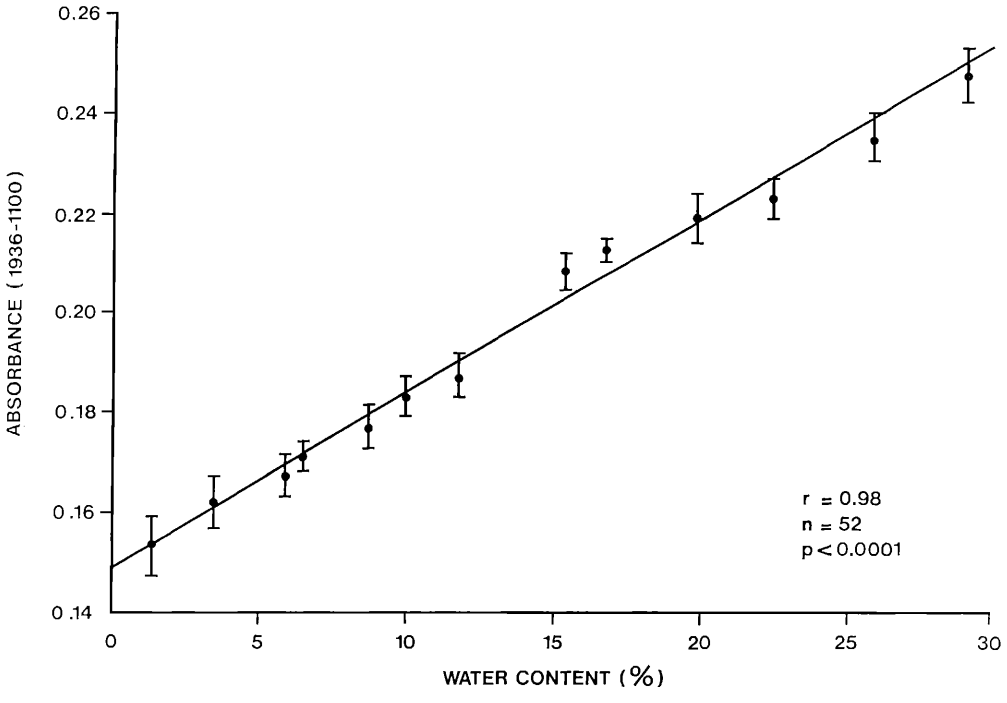

Figure 3. *In vitro* experiment: Influence of the water content of stratum corneum samples on the NIR absorption measured at 1936 nm and 1100 nm.

near-infrared absorbance, change in conductance, and change in clinical dryness score. The change in the near-infrared parameter (Figure 9a) showed that all the treatments except preparation D had statistically significant activity. Products A, C, and E were significantly more effective than product B, which, in turn, was more effective than product D. In terms of conductance, product D was again statistically ineffective, but products A, C, and E were significantly more effective than products B and D, which had comparable effects (Figure 9b). Finally, the reduction in the dryness score (Figure 9c) was significant for products A, B, C, and E, which showed similar activity, but not for product D. On the basis of near-infrared absorbance, which correlated with the clinical score, product B was distinguished from products A, C, D, and E.

DISCUSSION

With the equipment described, the near-infrared radiation penetrated deeply into the skin, although the superficial layers had a clear influence, as shown in Figures 2 and 4. The study of isolated stratum corneum samples with varying degrees of relative humidity showed that the difference in absorbance at 1936 and 1100 nm indeed reflected water content, since, for values between 0 and 30% (at which water is bound), there was a linear relationship between absorbance and water content. It was also this difference in absorbance that was best correlated with the overall skin dryness score, which, as mentioned above, was the sum of several clinical parameters (roughness, scaling, "cigarette paper" aspect, etc.).

The correlation was far better than that between conductance values and the degree of

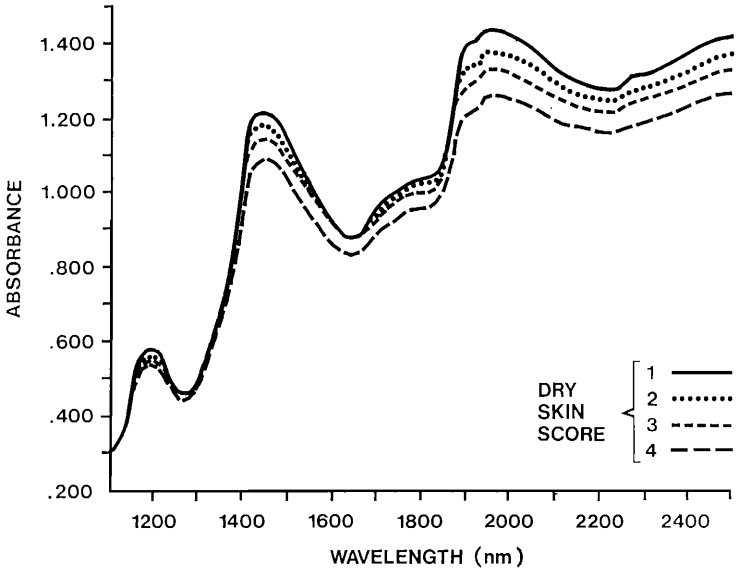

Figure 4. NIR absorption spectra of skin showing different scores of dryness (intermediate scores not presented).

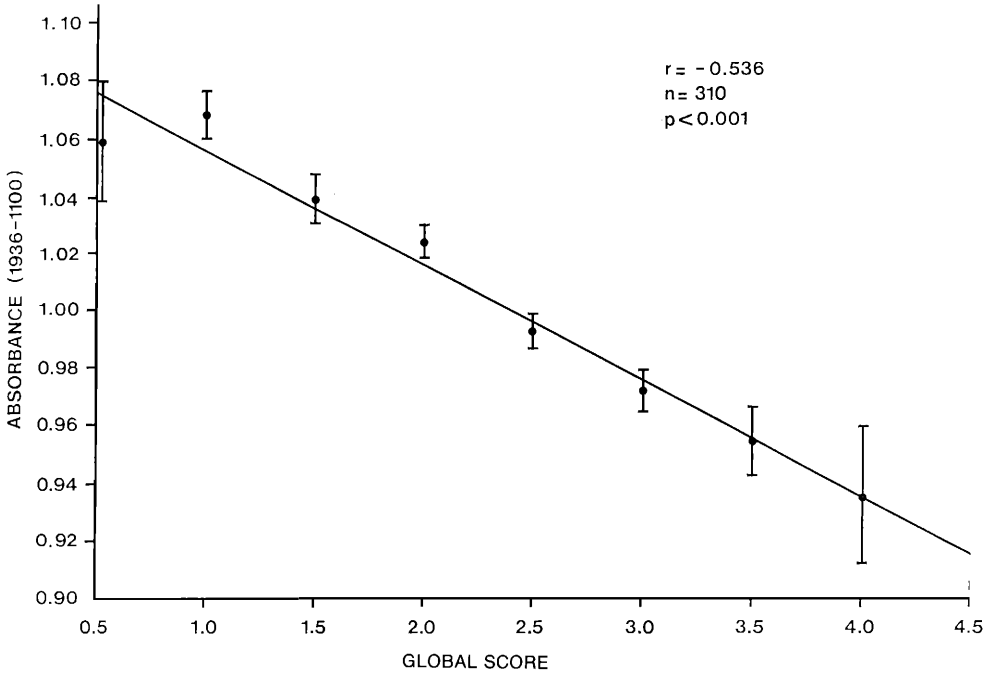

Figure 5. Linear correlation between the NIR absorbance (measured at 1936–1100 nm) and the global score.

skin dryness: above an overall score of 3, no further changes in conductance were observed. A closer examination of the clinical data shows that electrical conductance was related to the roughness of the skin: skin roughness no longer varied above an overall

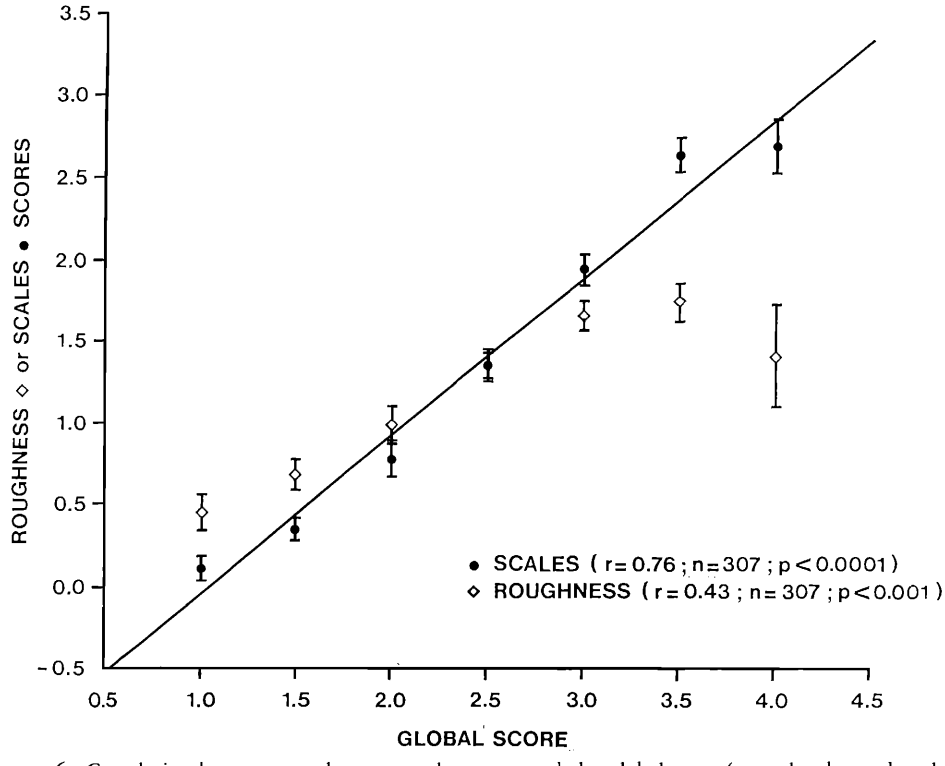

Figure 6. Correlation between roughness or scales scores and the global score (note that beyond a global score of 3, the roughness score no longer increases).

score of 3; higher scores were mainly due to marked scaling. The lack of correlation between conductance and roughness was clearly due mainly to the poor quality of the electrical contact between the skin surface and the measurement electrode. Needless to say, this poses a problem for the precise interpretation of the results of electrical conductance measurements. Such difficulties have previously been pointed out (8), but our data provide a concrete illustration of the problem.

The use of an integration sphere that collects all the radiation re-emitted probably explains why the NIR absorbance method was so sensitive. Above a clinical score of 3, scaling probably corresponds to a reorganization of the stratum corneum or the epidermis in response to relatively deep dehydration, which the Infra-Alyser recorded linearly up to scores of about 4.5.

With regard to the efficacy of the cosmetic preparations, product D, which does not contain moisturizing agents, was totally ineffective, regardless of the parameter considered. The results for the other products varied according to the measurement techniques used. The clinicians considered them to be equally effective, whereas product B was ranked between the group A/C/E and product D in the NIR method and on a level with product D in the electrical conductance method. This latter result was probably erroneous since, despite a degree of subjectivity and a lack of accuracy, the clinical scores could not have been so far from the real situation. It is more likely that the unexpected classification of product D by the impedance method was due to its lack of sensitivity above clinical scores of 3.

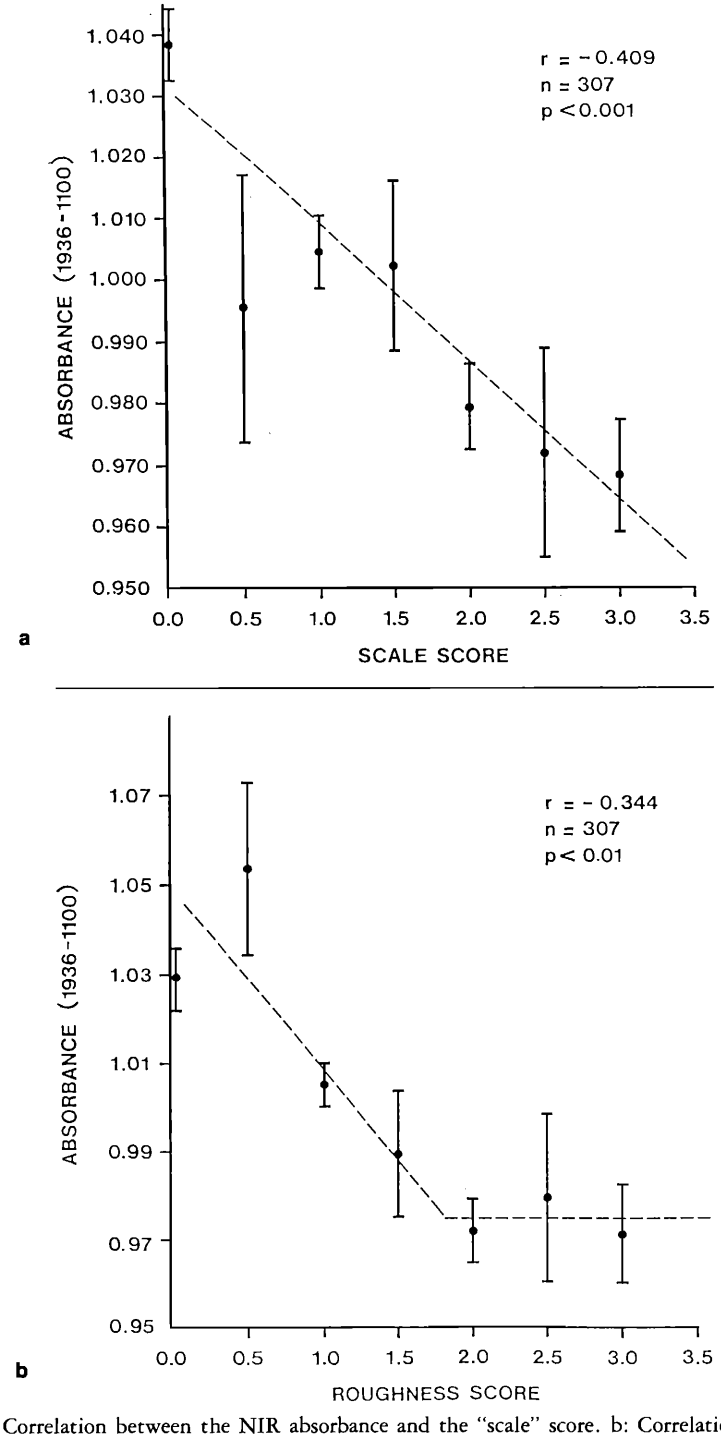

Figure 7. a: Correlation between the NIR absorbance and the "scale" score. b: Correlation between the NIR absorbance and the "roughness" score.

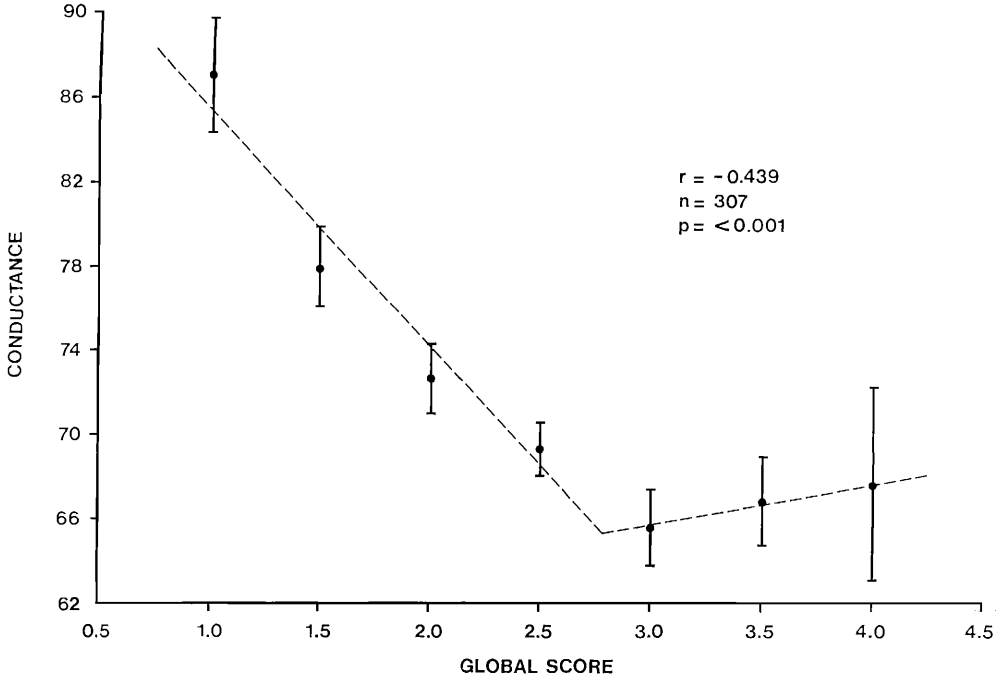

Figure 8. Correlation between the electrical conductance in voltage and the global score. The statistical parameters correspond to a linear correlation obtained with the whole data.

The correct classification of products in terms of their efficacy on dry skin was therefore given by the NIR absorption method and the clinical scoring technique. As the statistical analysis showed a significant difference between NIR results for product B and products A, C, and E, this is probably the most sensitive technique for distinguishing between different cosmetic preparations.

The conclusions of our study concern both the nature of dry skin and its assessment (and consequently the efficacy of topical agents). The results clearly show that dry skin is indeed dehydrated, and confirm hypotheses based on indirect methods showing that the stratum corneum of extremely dry skin is almost two times less elastic than that of normal skin (2). This fundamental observation should now form the basis for studies of the physiological phenomena that give rise to abnormal keratinization, and should also lead to the development of new and more effective moisturizers.

The results of the NIR absorption method, which can be applied *in vivo* to all skin sites, were strongly correlated with the clinical scores; the intrinsic property it measures can be directly interpreted in terms of water content, something that was not previously possible. Indeed, although the electrical conductance method is simple and informative, the present study clearly shows its limitations.

We have often insisted on the complementary nature of the various methods used to study the nature of dry skin and the efficacy of cosmetic products (10); the results of this work confirm our view but illustrate the exceptional value of the NIR absorption method for measuring a parameter fundamental to cosmetology, i.e., skin water content.

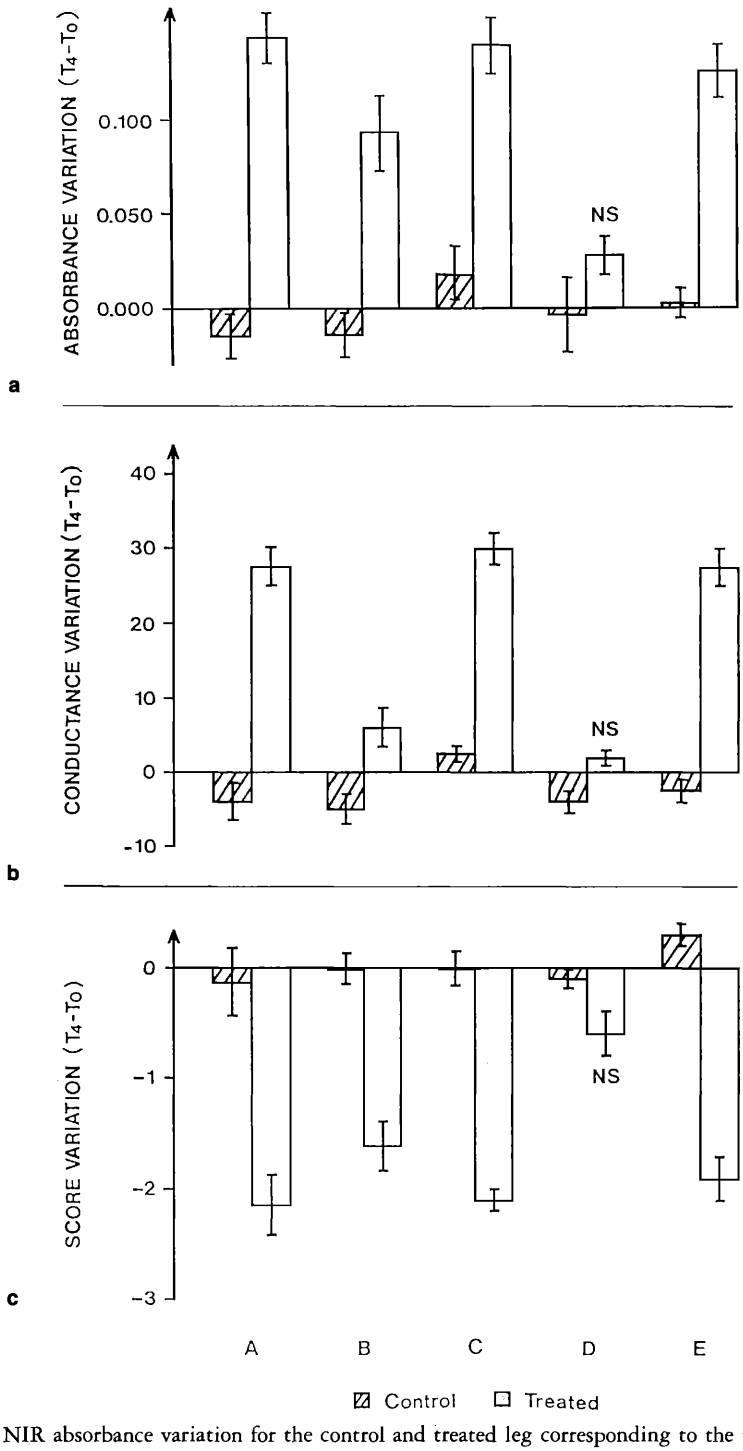

Figure 9. a: NIR absorbance variation for the control and treated leg corresponding to the treatment for 4 weeks by the five products A, B, C, D, and E. b: Conductance variation in the same conditions. c: Score variation in the same conditions.

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