Uterotrophic effect on rats by cutaneous administration of oestrogens

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Key words: Oestrogens, Cosmetics, Uterotrophic effect

Synopsis
Method based on uterine weight increase in immature female rats was devised to better establish the range of inactive-active concentration of several oestrogen alcoholic solutions topically administered. Standardized experimental conditions were carefully the same already published by the Authors. New results presented here, in comparison with those previously obtained, allow to arrange the investigated estrogenic substances according to their decreasing uterotrophic effect as follows (being the inactive concentration indicated for each oestrogen the former number and the minimal active concentration the later): ethinyloestradiol (0.075 µg/ml - 0.125 µg/ml); diethylstilboestrol (0.25 µg/ml - 0.50 µg/ml); 17 β-oestradiol (0.50 µg/ml - 0.75 µg/ml); oestradiol-3-benzoate and oestradiol-17-valerate (0.75 µg/ml - 1.0 µg/ml); oestradiol (2.0 µg/ml - 3.0 µg/ml); oestrone (4.0 µg/ml - 5.0 µg/ml).

Those definitive data confirm the realibility of the adopted method and its possibilities of application in order to detect eventual intentional presence of estrogens both in placental extracts and in cosmetic products.

Riassunto
Il metodo si basa sull'aumento del peso uterino nelle femmine immature di ratto ed è stato elaborato allo scopo di meglio stabilire la varietà di concentrazione attivinaattiva delle diverse soluzioni alcoliche di estrogeni somministrate localmente. Si sono mantenute le stesse condizioni sperimentali tipo già rese note dagli Autori. I nuovi risultati qui presentati, a confronto con quelli precedentemente conseguiti, consentono di classificare le sostanze estrogeniche oggetto della ricerca secondo il decrescente effetto uterotrofico come segue (la concentrazione inattiva viene indicata per ciascun estrogeno dal primo numero, e la concentrazione minima attiva dal secondo): etinilestradiolo (0.075 µg/ml - 0.125 µg/ml); dietilstilbestrolo (0.25 µg/ml - 0.50 µg/ml); 17 β-estradiol (0.50 µg/ml - 0.75 µg/ml); estradiol-3-benzoato ed estradiol-17-valerato (0.75 µg/ml - 1.0 µg/ml); estradiol (2.0 µg/ml - 3.0 µg/ml); estrone (4.0 µg/ml - 5.0 µg/ml).

Tali dati conclusivi confermano l'affidabilità del metodo adottato e le sue possibilità di applicazione nell'individuazione di eventuali presenze intenzionali di estrogeni negli estratti di placenta e nei cosmetici.
Résumé
Pour mieux déterminer la gamme de la concentration - active ou inactive - de plusieurs solutions alcooliques d'oestrogènes qui viennent appliquées localement, on a employé une méthode basée sur le poids utérin des petits rats femelles. On a respecté attentivement les conditions expérimentales qui avaient été déjà appliquées et publiées par d'autres chercheurs. Par rapport aux résultats précédents, ce que nous avons obtenu dans ce cas c'est une méthode qui nous permet de classer, par ordre décroissant, les effets utéotrophiques des oestrogènes que nous avons examinés. Les premières chiffres indiquent, pour chaque oestrogène, la concentration inactive tandis que les autres chiffres indiquent la concentration active minimale: éthinyleoestradiol (0,075 µg/ml - 0,125 µg/ml); diéthylstilboestrol (0,25 µg/ml - 0,50 µg/ml); 17β-oestradiol (0,50 µg/ml - 0,75 µg/ml); oestradiol-3-benzoate et oestradiol-17-valerate (0,75 µg/ml - 1,0 µg/ml); oestriol (2,0 µg/ml - 3,0 µg/ml); oestrone (4,0 µg/ml - 5,0 µg/ml).

Ces données définitives, confirment, tant la fiabilité de la méthode ici présentée, que ses possibilités d'application pour détecter une éventuelle présence intentionnelle d'oestrogènes dans les extraits placentaires et/ou dans les cosmétiques.

Synopse
Eine auf dem Zuwachs des Utergewichts in jungen weiblichen Ratten gegründete Methode wurde verfolgt, um eine tiefgreifende Bewertung zu ermöglichen, was die tätig-und-untätige Konzentration verschiedener topisch eingegebenen östrogen und Alkoholösungen betrifft. Die standardisierte Forschungsbedingungen waren die selben die von den Forschern veröffentlicht wurden. Die neuen hier gegebenen Ergebnisse ermöglichen (im Vergleich zu den schon erlangten Daten) die folgende Klassifizierung der ausprobierten östrogenstoffe nach ihrer abnehmenden uterotrophischen Auswirkung (die erste Nummer zeich die untätige Konzentration jedes östrogenstoffes und die zweite die am wenigsten tätige Konzentration): Ethinyleoestradiol (0,075 µg/ml - 0,125 µg/ml); Diéthylstilboestrol (0,25 µg/ml - 0,50 µg/ml); 17β-Oestradiol (0,50 µg/ml - 0,75 µg/ml); Oestradiol-3-Benzoat und Oestradiol-17-Valerat (0,75 µg/ml - 1,0 µg/ml); Oestriol (2,0 µg/ml - 3,0 µg/ml); Oestrone (4,0 µg/ml - 5,0 µg/ml).

Diese Daten bestätigen die Glaubwürdigkeit dieser Methode und ihre Anwendungsmöglichkeiten, um die eventuelle absichtliche Anwesenheit von östrogenstoffen in Plazentaextrakten und kosmetischen Produkten zu entdecken.

Resumen
El procedimiento se basa sobre el incremento del peso uterino en ratas hembras y se ha ejecutado para establecer mejor la gama de concentración activa-inactiva de varias soluciones alcohólicas de estrógenos de administración tópica. Las condiciones experimentales standard fueron precisamente las mismas que se publicaron antes por los Autores. Los nuevos resultados que se presentan aquí, en comparación a los que se consiguieron previamente, permiten relacionar las sustancias estrogéneas investigadas con su efecto uterotrófico decreciente de la manera siguiente (siendo la concentración, inactiva para cada estrógeno indicada con el primer número y la concentración mínima activa con el segundo número): etinileodradiol (0,075 µg/ml - 0,125 µg/ml); dietilstilbestrol (0,25 µg/ml - 0,5 µg/ml); 17β-oestradiol (0,5 µg/ml - 0,75 µg/ml); estradiol-3-benzoato y estradiol-17-valerato (0,75 µg/ml - 1 µg/ml); estróliol (2 µg/ml - 3 µg/ml); estrón (4 µg/ml - 5 µg/ml). Esos datos conclusivos confirman la fiabilidad del procedimiento que se ha adoptado y sus posibilidades de aplicación para detectar la posible presencia intencional de estrógenos tanto en extractos de placenta como en cosméticos.
Introduction

In a previous publication the Authors (Salvatore et al., 1987) investigated the detection of uterotrophic effect induced in immature female rats topically treated both with natural and synthetic oestrogens. The existence of a dose-response relationship, particularly in the case of 17β-oestradiol (see Figure 1), was demonstrated. Moreover, the minimal dose capable of causing uterotrophic effect or the inactive dose under precise standardized experimental conditions were established only for a few of the oestrogens under examination.

Thus the aim of the present work is to further explore the range of inactive-active concentrations of alcoholic solutions of oestradiol-17-valerate, oestradiol-3-benzoate, oestrone, oestriol and ethinylestradiol, for which the previous data was inadequate.

Since the use of oestrogens in cosmetics, even those naturally present in placenta or in its extracts, is not permitted under Law No. 713, October 11, 1986 (recently updated by D.M. No. 530 November 24, 1987), our research is essential in arriving at a valid general screening method suitable even for the analysis of commercial samples with unknown oestrogen content.

Experimental

For each oestrogen, the following working solutions were prepared in 95% ethanol: oestradiol-17-valerate, 0.75 µg/ml and 1.0 µg/ml; oestradiol-3-benzoate, 0.75 µg/ml and and 1.0 µg/ml; oestriol 2.0 µg/ml; ethinylestradiol, 0.07 µg/ml; oestrone, 4-5-7 µg/ml respectively; and 17β-oestradiol 0.75 µg/ml. The last substance was used as reference to test the repro-

Fig. 1: Uterus of untreated immature female rat in comparison with uterus of immature female rat topically treated with 17β-oestradiol.
ducibility of dose-response relationship previously established (Salvatore et al., 1987).

The animals were chosen from a group of homogeneous size thereby reducing the possibility of error and individual variation; they were then housed in single disposable cages.

Each experiment had four animal groups (including the control group), each of which numbered 10 animals (7 animals in the case of the 0.07 µg/ml ethinyl oestriol solution). Before treatment, the backs of the rats were shaved using an electric razor (AESCULAP Favorita II CT 104; clipping height 1/20 mm) and then the animals were weighed. With each solution of a known oestrogen concentration, three topical applications were made (0.25 ml each) at 0, 8 and 24 hours. Thus, each treated rat received a total dose of 0.75 ml of alcohol solution at a known oestrogen concentration. Control animals received the same volume of 95° ethanol.

**Results and discussion**

Table 1 shows the experimental schema of this study. 17β-oestradiol at a concentration level of 0.75 µg/ml was used as a reference substance. This concentration proved to be the minimal active concentration confirming the data of our previous study and showing a good reproducibility of the adopted method. A concentration of 5.0 µg/ml for oestrone represented the minimal active concentration while at 4.0 µg/ml this hormone was inactive. Oestradiol was inactive at a concentration of 2 µg/ml. On investigating synthetic oestrogens, we found that the concentration dose of 0.07 µg/ml had no effect on ethinyl oestradiol. This hormonal substance proved to be the most active of the oestrogens tested to date with a minimal active concentration of 0.125 µg/ml. The 17β-oestradiol esters showed a similar biological response since a concentration of 0.75 µg/ml resulted inactive while 1.0 µg/ml was the minimal active concentration.

In Table 2 the final results of minimal active concentrations and inactive concentrations for each oestrogen are given in their order of decreasing uterotrophic effect.

In conclusion, the data obtained demonstrated the reliability of this biological test as a screening method for detecting any deliberate addition of oestrogenic substances in commercial products of unknown oestrogen content.
Table I
Experimental design and results obtained as to the effects on rat uterine weight variations for the oestrogens investigated. The tested concentration and the correspondent relative uterine weight for each oestrogen are reported in the first and the second line, respectively, for each assay (* P < 0.01).

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Treated groups</th>
<th>Control groups</th>
</tr>
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<tbody>
<tr>
<td><strong>Assay No.</strong></td>
<td><strong>Total number of rats</strong></td>
<td><strong>17 β-oestradiol</strong></td>
</tr>
<tr>
<td>1</td>
<td>40 (10 per group)</td>
<td>0.65 µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>47 (10 per group)</td>
<td>0.75 µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>47 (12 per group)</td>
<td>0.75 µg/ml</td>
</tr>
</tbody>
</table>

(11 rats)
Table II
Definitive results of each oestrogen investigated arranged in decreasing uterotrophic effect order (P < 0.01)

<table>
<thead>
<tr>
<th>Oestrogens</th>
<th>Inactive concentration (µg/ml)</th>
<th>Minimal concentration with uterotrophic activity (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethinyloestradiol</td>
<td>0.075</td>
<td>0.125</td>
</tr>
<tr>
<td>Diethylstilboestrol</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>17 β-oestradiol</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Oestradiol-3-benzoate</td>
<td>0.75</td>
<td>1.0</td>
</tr>
<tr>
<td>Oestradiol-17-valerate</td>
<td>0.75</td>
<td>1.0</td>
</tr>
<tr>
<td>Oestriol</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Oestrone</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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Computerized morphometric analysis of acne lesions

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Key words: Acne, acne evaluation, computerized analysis

Synopsis
An evaluation system of acne lesions by means of morphometric computerized analysis is presented. The method allows (even in relation to a treatment): a quantitative evaluation which is effective even in the case of a high number of lesions; the evaluation of the dimensions of individual lesions and of the area they occupy even in relation to a skin area which has been chosen as sample (area %).

Riassunto
Sistema di valutazione delle lesioni da acne attraverso analisi morfometrica computerizzata. Questa metodica consente (anche in relazione al trattamento) una valutazione quantitativa efficace anche nel caso di un gran numero di lesioni, nonché la valutazione delle dimensioni delle lesioni individuali e dell'area occupata, in relazione anche alla zona di epidermide scelta a campione (area %).

Résumé
Nous illustrons une méthode d'évaluation des lésion acneéiques basée sur une analyse morphométrique informatisée. Cette méthode nous permet d'obtenir (même lorsque associée à des soins de la peau) une évaluation quantitative que est est valable même en présence de beaucoup de lésions; en outre elle nous permet de mesurer les dimensions de chaque lésion et de la portion de tissu épithelial qu'elles occupent même en relation avec une zone de la peau prise comme échantillon.

Synopse
Hier wird ein Forschungssystem für Aknebeschädigungen gezeigt, das sich auf eine morphometrischen Analyse durch Datenverarbeitungsmaschinen stützt. Diese Methode ermöglicht (auch in Zusammenhang mit einer Therapie) eine grössenmässige Bewertung durchzuführen, die auch im Falle mehrerer Beschädigungen wertvoll sein kann, und dann auszuwerten wie hoch die individuellen Beschädigungen und ihre Ausdehnung auf die Haut im Zusammenhang mit der Hautfläche, die als Muster genommen wurde, sind (Hautflächerprozentsatz).

Resumen
Se presenta aquí un sistema para la evaluación de las lesiones de acné por medio de un análisis morfométrico computarizado. Ese procedimiento permite (también con referencia al tratamiento) una evaluación cuantitativa eficaz también en caso de un gran número de lesiones y la evaluacion del tamaño de lesiones individuales y de la área que ocupan con referencia también a la área de epidermis que se ha elegido como muestra (area %).
Introduction

The course of acne and its response to therapy is usually evaluated by means of clinical observation and measurement. Clinical evaluation can be accomplished either by specific lesion counting or global evaluation: in both cases the judgement is subjective and therefore it does not allow exact quantification.

The evaluation of individual lesions requires that specific lesions in specific sites be counted so that minimum and maximum numbers be established: too few lesions would make it impossible to judge improvement, too many would make counting difficult (8).

Kligman and Plewig (2) suggested a method to simplify counting by means of grade assignment (ex. grade 1 = less than 10 comedones, grade 2 = 10-25 comedones, etc.). Comparison among groups with the same acne-grade is easier (ex. in relation to treatment) but lesions must be partly counted anyway. There are some advantages in limiting the number of lesions to be counted by using masks with gaps on correctly pre-determined face areas.

In global evaluation, individual lesions are not counted and the grading is based on some numerical scale, such as the Pillsbury 4-grade scale (6) or the Cook 9-grade scale (1) which requires compa-

Graph 1: Pre-treatment.
ring the patient to a set of 5 standard photographic reproductions of acne. This method, adapted to office use (9), has been recently employed (3) evaluate to the effectiveness of two different antibiotic treatments (7).

Even if the global evaluation method is easier than counting specific lesions, it is less precise. In both cases the margins of error after successive visits or different observations could not be reduced sufficiently.

Since 1985 (4, 5) we have been checking the possibility of using computerized morphometric analysis in evaluation of acne lesions.

Material and methods

35 mm color slides of skin areas containing acne lesions were taken through macro objective Pentax 50 mm, f 1:4 lens. These photographs were then scanned by means of a high geometric linearity TV-camera (d<0.3% over the whole recording field of 512×512 pixels²) connected to a computerized image analyzer Kontron Zeiss IBAS 2.

A suitable program, maintained the same for all the subjects was utilized. With this program the image was interactively elaborated by electronic filters and densito-
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metrically analyzed over 256 grey levels, in order to obtain a binary image suitable to the quantitative evaluation of the most significant morphometric parameters. After standardization of the primary subject image (directly taken from the color transparency), on a scale over 256 levels, the image itself was submitted to interactive elaboration in order to define the areas to analyze quantitatively. A measuring frame was placed over the image so as to de-limit a homogeneous area which avoids surfaces with different lightings. Amplitude and coordinates were memorized in the program, for a successive and exact application on the same skin area after treatment. In this image were accurately analyzed the frequency of distribution of the various grey levels, by defining inside the 0-256 range a threshold for the grey interval corresponding to the examined lesions. If the illuminating conditions of the examined areas of the subject were the same before and after a treatment we chose the same grey interval values, otherwise it was necessary to modify that value in order to obtain the best possible correspondence between the electronic image and the original color transparency.

Fig. 1: Pre-treatment (black and white from color transparencies).
In this way it was possible to discriminate the lesions inside the measuring frame. The grey levels of such an interval were converted to white (level 255), obtaining a binary image suitable to quantitative evaluation of lesions acne. This evaluation was automatically performed after identification with pseudocolours. Morphometric analysis of acne lesions in a patient before and after a treatment (systemic antibiotic therapy) was performed.

Results

All examples of the evaluation system are presented. Two color transparencies (before and after treatment; photo 1, 2) are submitted to TV-recording connected to the computerized image analyzer. After the normalization of the primary subject image an electronic measuring frame is superimposed (photo 3, 4) and the enlargement index is calculated. The images are densitometrically analyzed and a binary image is obtained (photo 5, 6). Evaluation of lesions is automatically performed:

1) a quantitative evaluation: before treatment the lesions are 59 and after treatment 43;
2) a quantitative evaluation of each lesion area. The lesions are distributed according to their extension in 25 predetermined classes. If elements are below 0.2 mm² they are eliminated for calculation and graphs: a verification has shown that below said value a number of misleading elements can be included, such as scales, debris and ostia. Before treatment the average area is mm² 2.289 (graph 1). After treatment the average area is mm² 2.179 (graph 2).

3) An evaluation of the skin area covered by lesions in relation to the measuring frame area (area %) that before treatment is 12.14% and after treatment is 8.445%.

Discussion and conclusions

The evaluation methods of acne lesions and of their regression, in relation to treatment, which have been presented up to now allow, both in vivo or in photographic reproductions, the simple counting of a number of lesions, which is generally limited, and whose global evaluation is insufficiently precise.

The method presented here has allowed (in the same pre- and post-treatment sample area): a quantitative evaluation, effective even with a high number of lesions, a quantitative evaluation of individual lesion areas, and an evaluation of the lesions as a percentage of a chosen skin area. It is a method that minimizes subjectivity in the evaluation of the smallest differential quantitative modifications of selected parameters, induced by a therapy, in a single subject. Nonetheless there are some drawbacks: it is impossible to automatically distinguish elementary lesion types and the interactive determination of a densitometric threshold is required.

Fig. 3: Pre-treatment: *normalized electronic image with a superimposed measuring frame (electronic window).
Fig. 4: Post-treatment, «normalized» image with the same measuring frame.

Fig. 5: Pre-treatment: interactive determination of densitometric threshold (segmentation).
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Fig. 6: Post-treatment.

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Enhancement of normal hair growth by topical treatment

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Key words: Hair growth, Topical treatment, Vasodilators, Vasoactive drugs, Hair density, Trichosaccharides

Synopsis
The effect on hair growth of a hair lotion containing trichosaccharides and vasodilators was assessed on 13 healthy volunteers in an open experiment. On one side of the scalp was treated, the other was used as a control. In a first study the right side was treated; in a second one, 9 months later, the left side was treated. After 30 days of treatment, on the treated side a significant (p<0.001) increase in the number of hair shafts per cm² of scalp area was found together with a higher hair length (p<0.001).

Riassunto
Durante un esperimento aperto si è valutato su un campione di 13 volontari sani l'effetto di una lozione per capelli contenente tricosaccharidi e vasodilatatori sulla crescita dei capelli. È stato trattato un lato del cuoio capelluto, usando l'altro come riferimento. In un primo studio è stato trattato il lato destro, e in un secondo, 9 mesi dopo, il sinistro. Dopo 30 giorni di trattamento, sulla parte trattata si è riscontrato un aumento significativo (p<0.001) del numero di capelli per cm², nonché un allungamento (p<0.001) dei capelli.

Résumé
L'effet d'une lotion qui contient des trichosaccharides et des vaso-dilatateurs, a été testé sur 13 volontaires sains. On a traité une partie seulement du cuir chevelu des sujets, à fin de pouvoir établir une comparaison entre les deux parties. Le test a été divisé en deux phases: la première fois on a soumis au test la partie gauche, après deux mois on a traité la partie droite du cuir chevelu. Après 30 jours de traitement, la partie traitée a révélé une augmentation importante du nombre (p<0.001) et de la longeur (p<0.001) des cheveux pour chaque cm² du cuir chevelu.
Synopse
Die Auswirkung eines aus Trichosaccaryd und gefäßerweiternden Stoffen zusammengesetzten Haarwassers wurde im Lauf eines offenen Experiments auf 13 gesunde Menschen analysiert, die sich freiwillig solchen Experimenten unerziehen. Nur eine Seite der Kopfhaut wurde mit diesem Haarwasser behandelt, und die andere wurde als Kontrollmuster benutzt.
Zuerst wurde die rechte Seite und dann (nach 9 Monaten) die linke Seite behandelt. Nach 30 Tagen Behandlung konnte man einen bedeutenden Haarzuwachs ($p < 0,001$) und Haarlänge ($p < 0,001$) bemerken, was die behandelte Haufläche anbelangte.

Resumen
Se ha valorado el efecto que una loción para el pelo con tricosácarides y vasodilata-doresha ejercitado sobre el crecimiento del pelo de 13 voluntarios sanos durante un experimento abierto. Se ha tratado un lado del cuero cabelludo y se ha utilizado como control el otro lado. Durante un primer estudio se ha tratado el lado derecho, y en un segundo, 9 meses después, se ha tratado el lado izquierdo. Después de 30 días de tratamiento, sobre el lado que se había tratado se ha encontrado un incremento significativo ($p < 0,001$) del número de pelos por cm² junto a un alargamiento del pelo ($p < 0,001$).
Introduction

For decades hair lotions have been claimed to promote hair growth, but without any scientific evidence. The main reason for that was the lack of reliable methods to assess hair growth and hair density of the scalp. The trichogram technique (1, 2) based on the study of the bulbs of pulled hairs was an important advance and allowed interesting progress in the understanding of pathological hair conditions. But its use is limited, being this technique mostly qualitative. Furthermore, there are strong variations in «hair formula» depending on scalp areas and seasons (3). A more quantitative, though still uneasily practicable, method is the phototrichogram described by Saitoh (4) which allows the measurement of hair density and hair growth through a macrophotography.

Using the latter technique, we have measured the influence of a topical lotion on hair growth in normal people.

Material and methods

13 healthy volunteers, aged 16-30 years, without any sign of male pattern alopecia or scalp disease were selected for the experiment.

The tested lotion* was a mixture in an base of nicomethanol tartrate and ethyl nicotinate, both well-known vasodilators, and mucopoly saccharides extracted from mammalian gut (trichosaccaridesR+) (Table I). It was applied once every other day by gently spreading on the scalp without any rubbing, for at least one month. In a first study, the hair lotion was applied only on the right side of the scalp (late winter 1984). In a second study only the left side was treated (Autumn 1985). In each experiment, the untreated side served as a control. Each study involved 10 volunteers but 7 people participated in both studies. The statistical study was made using the analysis of variance and the paired t test comparisons, for each study separately, as an interaction was found between treatment and side effect.

Hair growth parameters were assessed as follows: two symmetrical areas, of about 4 cm², selected on each side of the scalp, were shaved and photographed four days later with a Polaroid CU5 close-up hand camera. After enlargement of the picture, hair shafts were counted on an area of 1 cm² using a network (see photograph). Hair length was measured before, during (at regular intervals), and at the end of treatment, using magnified photographs according to the Saitoh’s method.

Results

1 - Hair density

On treated areas, the mean hair densities
(Table II and III) had increased by day 34 as compared to day 4, while on control areas, hair densities had clearly decreased in both studies. The analysis of variance on the whole data showed a significant difference due to the treatment \((F=18.5 \, 1-18 \, df-p<0.001)\) but a weak interaction between side and treatment effect \((F=6.2 \, 1-18 \, df-p<0.05)\). Consequently, a paired t test was made on each study separately. Due to the large range of data obtained, the result of the first study was not statistically significant. The number of subjects displaying an increase in hair density by more than 2% on the treated side was 14/20 as compared to 3/20 on the control side \((p<0.01, \chi^2 \text{ test})\).

**2 - Hair length after 30 day's growth**

By the 30th day following shaving (Table IV), the mean length of hair was clearly (1st study) or barely (2nd study) higher in the treated side. Under paired t test comparison, the difference between treated and not treated sides highly significant \((p<0.001)\).

In the first study (where the treatment had been maintained up to 68 days), a significant increase in hair length was found on the treated side on days 4 and 30, but not on day 64. In the second study, a greater hair length was found by days 20 and 30 but this was not statistically significant. There were only intraindividual differences, hair length being at a time significantly higher on treated si-

<table>
<thead>
<tr>
<th>Table II</th>
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<tr>
<td><strong>Number of hair shafts per cm² (mean and standard deviation) in 10 normal subjects</strong></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Treated side</td>
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<tr>
<td>Control side</td>
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<tr>
<td>Total difference</td>
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<table>
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<th>Table III</th>
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<tbody>
<tr>
<td><strong>Number of hair shafts per cm². Difference between D 34 (end of treatment) and D 4 (before treatment)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Treated side</td>
</tr>
<tr>
<td>Control side</td>
</tr>
<tr>
<td>Treated minus control side</td>
</tr>
</tbody>
</table>
Discussions in 6 subjects and on the control side in one subject (Table V).

Discussion

In the experiment, acceptable variation coefficients were obtained both for mean hair density (15.3% to 23.9%) and hair growth (7.6% to 16.7%). As expected, control sides demonstrated spontaneous trends with time. The reduction in hair density was statistically significant and occurred mostly during the second study, thus partly explaining the lower increase in hair shaft number as compared to the first study. Conversely, the rate of hair growth was significantly higher on both sides in the second study than in the first one. Despite these physiological variations, statistically significant increases in hair density and hair growth ranging from 8.1% to 12.6% for hair density and from 2.3% to 20.1% for growth rate, were observed on treated sides. It is unlikely that a mechanical effect on hair growth has occurred as the lotion was applied only every other day and without rubbing. The used formula comprised known vasodilators which have long been used for promoting hair growth. Their effectiveness has never been quantitatively assessed, although they have been commonly used, sometimes successfully, in alopecia areata.

Other ingredients, such as mucopolysaccharides, may also have an effect as their increase in the dermis has been shown to be associated with hair growth (5, 6). Penetration studies should be performed together with further experiments to substantiate this point. Nevertheless, the above experiment demonstrates that the Saitoh's method is sensitive enough to allow the identification of changes in hair growth parameters even in normal people and to assess the stimulating effect of a lotion applied every other day only.

Acknowledgement: The authors wish to thank Deglaude Laboratories for providing the lotion, and Mrs Roche for typing the manuscript.

Table IV
Hair length (mm) on day 30 (mean and standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>1st study</th>
<th>2nd study</th>
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<tbody>
<tr>
<td>Treated side</td>
<td>12.3 ± 1.5</td>
<td>13.5 ± 1.9</td>
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<tr>
<td>Control side</td>
<td>10.2 ± 1.7</td>
<td>13.2 ± 1.0</td>
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<tr>
<td>Treated minus</td>
<td>2.1 ± 1.1</td>
<td>0.3 ± 1.1</td>
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<tr>
<td>control side</td>
<td>(+20.1%)</td>
<td>(+2.3%)</td>
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<tr>
<td></td>
<td>(p&lt;0.001)</td>
<td>(NS)</td>
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Enhancement of normal hair growth by topical treatment

<table>
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<th>Subject number</th>
<th>Day</th>
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<th>p</th>
<th>treated (T) versus control (C) side</th>
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<td>1</td>
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<td>1.42</td>
<td>NS</td>
<td></td>
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<td></td>
<td>D30</td>
<td>1.39</td>
<td>NS</td>
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<td>&lt;0.05</td>
<td>T&lt;C</td>
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<td>NS</td>
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<tr>
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<td>&lt;0.001</td>
<td>T&gt;C</td>
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<td>NS</td>
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<td></td>
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<td>0.24</td>
<td>NS</td>
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<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>D20</td>
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**REFERENCES**

Lead-time

We need a lot of this! There are many of you who have wonderful ideas for meetings. There are ideas for location, for theme, for time of year. There are ideas for subjects you would like to hear discussed and for subjects upon which you would like to speak.

Of course, some of the ideas will not be wonderful. Nevertheless, they are there — you do have them. What I would now like you to do is share them. Once I have the ideas, we can explore using them. There is a remote chance that they might fit into this year’s plan. Possibly into next year’s. Probably they can become the plan for a few years hence.

That is an explanation of «lead-time». In order to have your ideas utilized, I must have them well in advance. For us to place a meeting in a location of choice may require up to five years advance planning and reservations in peak seasons. A program with depth in a single area of interest requires twelve to eighteen months of preparation. And so on - and on.

The simple fact is, we want all of our programs to be excellent in site, topic, scope and presentation. We need your help in the form of ideas and comments. We need the help with a lot of «lead-time».

We have opportunities for regional and joint meetings around the world. We have possibilities for cooperation in on-going educational programs in Europe and in the USA. In order to develop such potential benefits, we must know your wishes — how you can help — what you want to learn.

So, please send me your ideas. And let me have some lead-time.

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Perrysburg
Ohio 43551
USA