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Journal of the Society of Cosmetic Chemists

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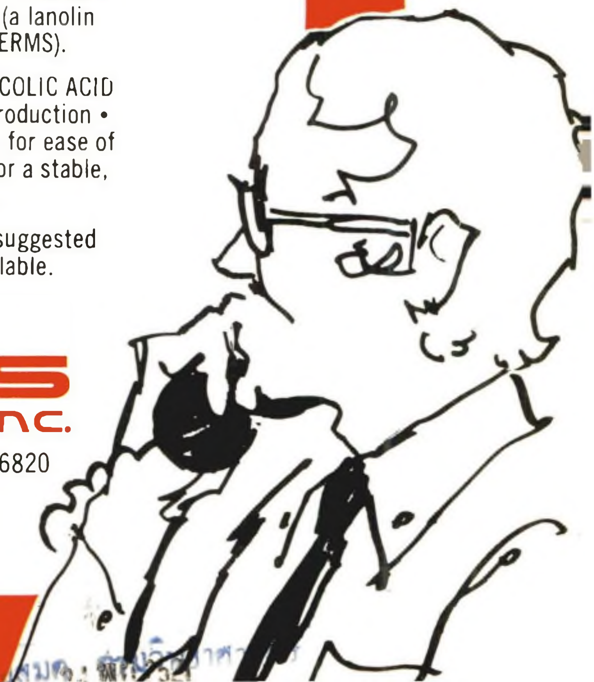

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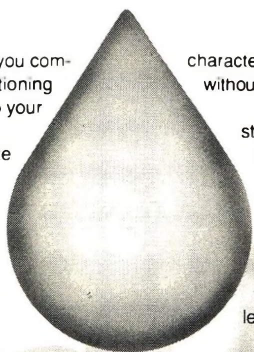

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 3 × 5 index cards for reference, without mutilating the pages of the Journal.

New methods for the in-vivo assessment of skin smoothness and skin softness: Sidney Weinstein. *Journal of the Society of Cosmetic Chemists* 29, 99 (March 1978)

Synopsis—Two systems are described for the assessment, in vivo, of skin softness, and skin smoothness. Tests of validity and reliability are reported, as well as examples of their use in the evaluation of typical cosmetics employed for softening and smoothing skin. A system is described for the assessment of the effectiveness of shaving creams or razors by measuring closeness of shave.

Low-energy emulsification—I—principles and applications: T. J. Lin. *Journal of the Society of Cosmetic Chemists* 29, 117 (March 1978)

Synopsis—The amount of energy normally expended in commercial processing of a cosmetic emulsion is far greater than the amount theoretically required. Whether the emulsion is made by a batch process or semicontinuous process, thermal energy is first supplied to heat the ingredients and mechanical energy is then provided for mixing and emulsification. Additional mechanical energy is expended to cool the product and the heat removed is generally discarded.

A considerable saving in energy is made possible by a more effective usage of thermal and mechanical energy in emulsification. A substantial saving of thermal energy can be achieved by a careful determination of emulsification temperature and by a selective heating of the ingredients. The method discussed here basically involves making an emulsion concentrate which is later diluted with the remainder of the external phase at room termperature.

In addition to conserving energy, the proposed low-energy emulsification technique also offers a great advantage in reducing the processing time and equipment cost. In some instances, the energy cost for processing an emulsified lotion can be reduced by 50 per cent while the production efficiency can be increased by 100 per cent.

Mascara contamination: in use and laboratory studies: D. G. Ahearn, J. Sanghvi and G. J. Haller. *Journal of the Society of Cosmetic Chemists* 29, 127 (March 1978)

Synopsis—Eye area cosmetics are subject to varied levels of microbial contamination during their normal use. Selected mascaras with known preservative formulations were compared for their resistance to microbial colonization during use by study groups and following a laboratory challenge test. A few products supported active growth of microorganisms after less than two weeks of normal use and after laboratory challenge. In study groups, the establishment of reproducing populations of microorganisms in certain mascaras occurred consistently with select individuals. Mascaras containing only parabens or imidazolidinylurea appeared to be less effective in retarding microbial growth than those containing formalin donors or mercurials. Effectiveness of preservative formulations may gradually decrease with the age of the product.

Hydagen, F, ein neuer Hautfeuchtigkeitsregulator—methoden und ergebnisse des wirkungsnachweises: Rainer Osberghaus, Christian Gloxhuber, Hans-Georg van Raay and Siegfried Braig. *Journal of the Society of Cosmetic Chemists* 29, 133 (March 1978)

Synopsis—Screening procedures for the **in vitro** testing of potential regulators of skin moisture are proposed. In addition, FMIR analysis has the potential for comparing the **in vivo** hydration condition of stratum corneum after the application of cosmetic emulsions.

Hydagen F, a partial sodium salt of a polyhydroxycarboxylic acid, performed positively in these tests. Good tolerance for this new cosmetic ingredient is expected in view of extensive toxicological studies.

ห้องสมุด กรมวิทยาศาสตร์

Further experience with a topical cream for depigmenting human skin: Otto H. Mills, Jr. and Albert M. Kligman, *Journal of the Society of Cosmetic Chemists* 29, 147 (March 1978)

Synopsis—Satisfactory lightening of hyperpigmentary skin disorders was obtained by twice-daily application for two to three months of a preparation containing 5.0% hydroquinone, 0.1% Vitamin A acid (tretinoin) and 0.1% dexamethasone, a flourinated corticosteroid.

Excellent results were secured in melasma (chloasma) of white females. Two common pigmentary disturbances in blacks also responded well, namely, the residual hyperpigmentation left by inflammatory acne lesions and in bearded areas affected by ingrown hairs (pseudofolliculitis). Although senile lentigines were not moderated in patients over 65 years of age, good results were observed in patients 40 to 60 years old.

The depigmentation is completely reversible and is not attended by significant local or systemic side effects.

Normal cuticle-wear patterns in human hair: Mario L. Garcia, Joseph A. Epps and Robert S. Yare. *Journal of the Society of Cosmetic Chemists* 29, 155 (March 1978)

Synopsis—A quantitative study of cuticle-wear patterns in human hair from six Caucasian subjects whose hair had only been subjected to normal wear—*i.e.*, no chemically reactive cosmetic treatment—is presented. The data was collected by counting the number of cuticle cell layers at different positions along the length of hair fibers. The counting was done on cross-sectional cuts by means of a Scanning Electron Microscope. Results are analyzed in terms of a mathematical model of cuticle wear. The similarity among the cuticle-wear patterns from the different subjects suggests that, under normal wear conditions, there is a common general pattern of cuticle wear in human hair. A theoretical rate of cuticle wear versus distance from the scalp expression was derived. This expression excludes age *per se* as a major factor in cuticle wear, and points instead to a source of hair surface wear which accelerates as we get closer to the hair ends. An analysis of combing forces showed that the type of damage known to be produced by combing can account for the shape of the observed cuticle-wear patterns. It was also found that beyond a certain length human hair should appear to be growing slower due to a cuticle-loss-fracture mechanism, a consequence of this being that, under otherwise equal conditions, the care with which hair is treated and handled directly affects the maximum length that it can attain.

Measurement of enzyme kinetics on the intact skin—a new method to study the biological effects of cosmetics on the epidermis: Peter T. Pugliese. *Journal of the Society of Cosmetic Chemists* 29, 177 (March 1978)

Synopsis—A new direct fluorometric method allows the measurement of enzyme activity on the intact skin of various body surfaces. This permits normal physiological parameters to operate on the system studied. The pentose phosphate pathway (Entner-Doudoroff), previously shown to operate in the epidermis, provides several enzymes which are used here to assess and compare various dermatological conditions. Glucose-6-phosphate dehydrogenase and lactic dehydrogenase are measured by fluorometric determination of changes in NADPH and NADH. Other enzymes of the Embden Myerhoff and Krebs cycles are measured directly or indirectly by this method. Various cosmetic base ingredients and compounded formulations were studied to determine their effects on epidermal metabolism. Enzyme action was recorded as increased, decreased or not affected. This new method is simple and relatively inexpensive, and allows extremely wide applications.

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J. Soc. Cosmet. Chem., 29, 99–115 (March 1978)

New methods for the in-vivo assessment of skin smoothness and skin softness

SIDNEY WEINSTEIN *NeuroCommunication Research Laboratories, Inc., West Kenosia Ave., Danbury, CT 06810.*

Received June 3, 1977.

Synopsis

TWO SYSTEMS are described for the ASSESSMENT, IN VIVO, of SKIN SOFTNESS and SKIN SMOOTHNESS. Tests of validity and reliability are reported, as well as examples of their use in the evaluation of typical cosmetics employed for softening and smoothing skin. A system is described for the assessment of the effectiveness of shaving creams or razors by measuring closeness of shave.

INTRODUCTION

Blank (1) observed in 1952 that one of the factors which is responsible for skin softness is its moisture content. However, it is clear there are skin treatments which utilize procedures other than moisturization to soften the skin (2).

The term "softness" is also frequently and uncritically used as though synonymous with the term "smoothness," when applied to skin. Yet it is not difficult to distinguish these two terms subjectively and, more importantly, operationally.

Numerous systems have been employed in evaluating the efficacy of various skin treatments in softening and smoothing the skin. Some employ essentially judgmental approaches, *e.g.*, rating scales (3–5). Others, which employ objective systems such as "surfometry" (6), require skin surface biopsies, a procedure which severely limits practical application and which is based upon the assumption of obtaining consistency of intracorneal adhesion.

Skin hardness has been measured (7) by causing "a stylus visibly to scratch the skin." This method requires drawing a stylus repeatedly across the skin, with increasing force applied, until the scratch is visible by its "ability to scatter light." The hardness, defined by the minimal force required to scratch, is detected by an individual's ability to detect the scattering of light. In addition to the indirect nature of using scattering of light, this method also seems to have limited practical utility. Surface topography, utilizing a "silicone rubber replica" of the skin, has also been employed as a means of measuring skin smoothness (7). The disadvantages of a series of procedures which first require making replicas of the skin and then the determination of a surface profile by stylus displace-

ment studies are obvious in routine measures of skin smoothness to assess new cosmetic preparations.

INDENTATION AS A METHOD OF DETERMINING SKIN SOFTNESS

The development of a device for assessing skin softness was initiated by consideration of what consumers operationally do when asked which of various body parts are soft or hard. The majority were found first to prod their faces, palms, etc., with, for example, the tips of their index fingers, a pencil, etc. When questioned concerning what they were attempting to determine, a typical remark was "to see how deep the pencil would go." These observations are not dissimilar to industrial measurements made of hardness of materials.

We therefore designed an instrument comprising a piston, within a cylinder (Figure 1), whose vertical movements are detectable in 10^{-4}-in. units.

The body part to be tested is kept immobile by velcro straps and the tip of the piston is brought to rest very lightly on a section of skin. After a load is placed on the piston, the depth of indentation of the skin, which is measured continuously, asymptotes at about 3 sec. Repeated measures are taken at slightly different areas, since testing the same site has the tendency to modify the skin's elasticity at that point; varying the testing site at points around a 1-cm circle gives consistent readings.

SKIN SOFTNESS: VALIDITY

We applied the device to inert substances varying in softness, *e.g.*, paper, wood, soap, glass, toothpaste, etc., and found that degrees of indentation conformed to our concep-

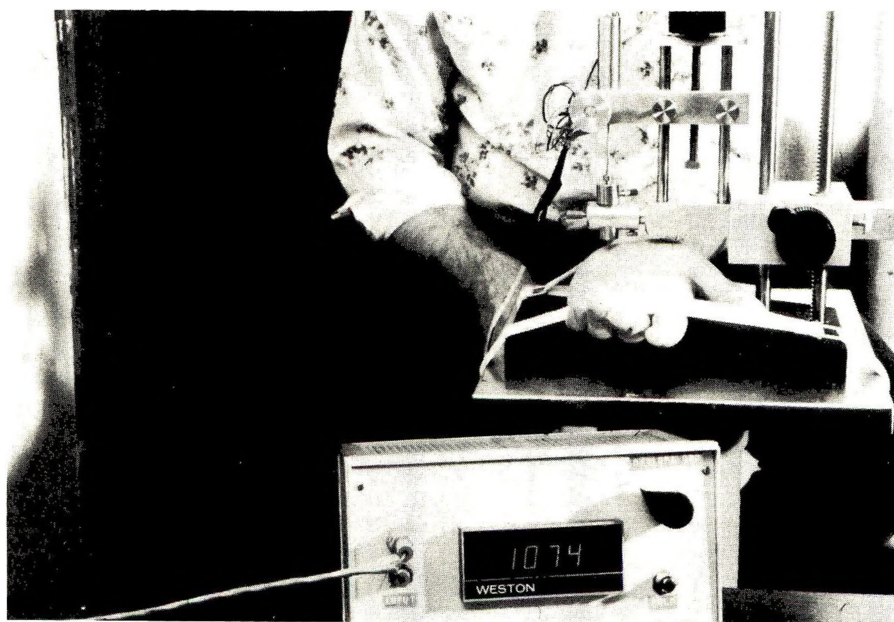


Figure 1. The device for assessing skin softness

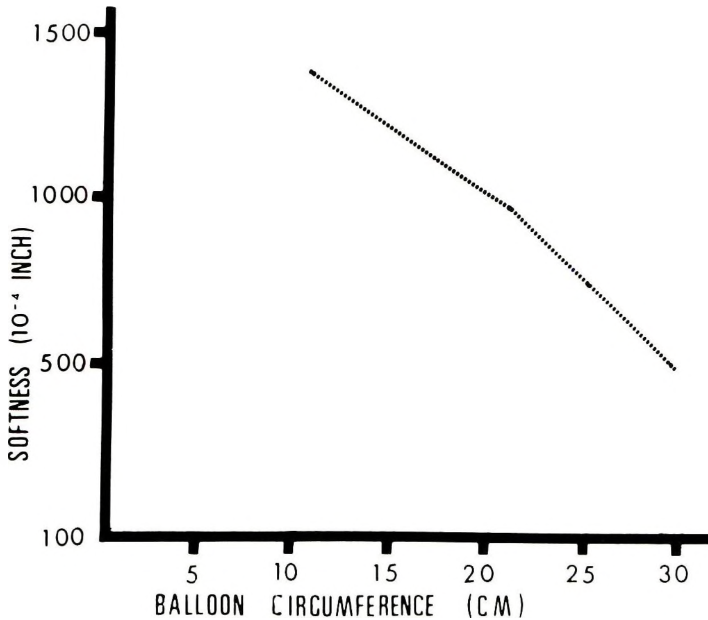


Figure 2. Softness of surface of a balloon inflated to varying degrees

tion of softness. We next employed a quantitative index obtained by testing rubber toy balloons inflated to varying degrees. Figure 2 shows the inverse linear relationship of degree of indentation to the circumference of the balloon. As the balloon is inflated, its turgidity becomes greater, and the degree of indentation diminishes linearly with the increase in circumference.

EXPERIMENT I: SKIN SOFTNESS

Our first study assessed the effects of both immersion in water and application of a skin lotion on softening the glabrous skin of the fingers.

Subjects

Thirty-two subjects, men and women between the ages of 25 and 54, were randomly assigned, 16 to the Water and 16 to the Lotion Group.

Procedures

There were four measures taken in each subject: two preceding and two following the treatment. Following the first test (Pre 1), a retest (Pre 2) was given from 1 to 10 min later. The treatment (Water or Lotion) was then applied, followed by two retests (Post 1) and (Post 2). In subjects with whom water was used as the treatment, a finger, usually the middle one, was immersed in tepid water for 5 min. After it was removed, the excess was gently shaken off and the first posttreatment testing initiated. In those subjects with whom lotion was used, the glabrous surface of the middle phalanx of one finger was covered with lotion that was rubbed into the skin for 1 min. Following four additional minutes during which the lotion remained on the skin, any visible excess was gently removed with cotton wool and the first posttreatment test applied. In both

Table I
Summary of Analysis of Variance of Skin Softness Following Application of Lotion or Water

Source	df	MS	F
Between subjects	31	7,485.85	
Between treatments	1	1,458.00	<1
Between subjects in same treatment group	30	15,373.56	
Within subjects	96	1,328.52	
Between trials	3	12,943.64	14.19**
Interaction treatment × trial	3	2,203.64	2.42*
Interaction pooled subjects × treatment × trial	90	912.17	

*p < 0.07.
**p < 0.001.
Note: The terms "*df*, *MS*, and *F*" refer to "degrees of freedom, mean square, and F-ratio" respectively; df indicates the number of units involved in the comparison; MS is the "error term" and reflects the variance of the variable concerned; the F ratio determines the level of statistical significance according to published tables.

groups (Water and Lotion) the second posttreatment test (Post 2) was given 5 min after Post 1.

Results: Experiment I

Table I gives the results of an analysis of variance comparing the effects of Treatment (Water or Lotion), Trials (2 Pre and 2 Post) and their interaction.

It can be seen that the four trials differed quite significantly and that the interaction of treatment and trial indicated a trend toward significance.

Follow-up *t* tests demonstrated that the slight decrease in softness between the means of both pretreatment trials was not significant; however, the differences between Pre 2 and Post 1 and between Pre 2 and Post 2 were both significant for the average of the two treatments (Table II). The correlation between the measures of softness taken before treatment (between Pre 1 and Pre 2) was 0.754 (30 df, $p < 0.0005$), indicating good test-retest reliability.

Figure 3 shows that there was a significant, immediate increase in softness for both treatments; however, although the degree of softness was maintained in the Lotion Group, it dropped in the Water Group. The *t* tests between the means for the two groups for Pre 1, Pre 2, Post 1 and Post 2 Trials were: 0.73, 0.46, 0.55, and 2.79 respectively. Only the Post 2 means differed significantly ($p < 0.005$).

Table II
t Tests Between Trials for Water and Lotion Treatment Groups

Between Trials	Water	Lotion	Combined Water and Lotion
Pre 1—Pre 2	0.67	1.87	1.80
Pre 2—Post 1	4.34*	4.24*	6.07*
Pre 2—Post 2	1.34	4.58*	4.19*

*p < 0.0005.

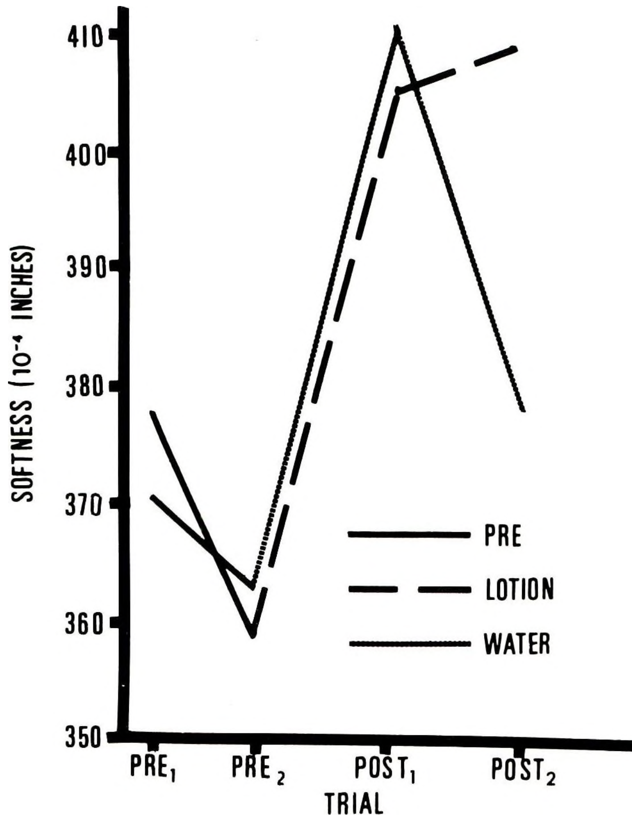


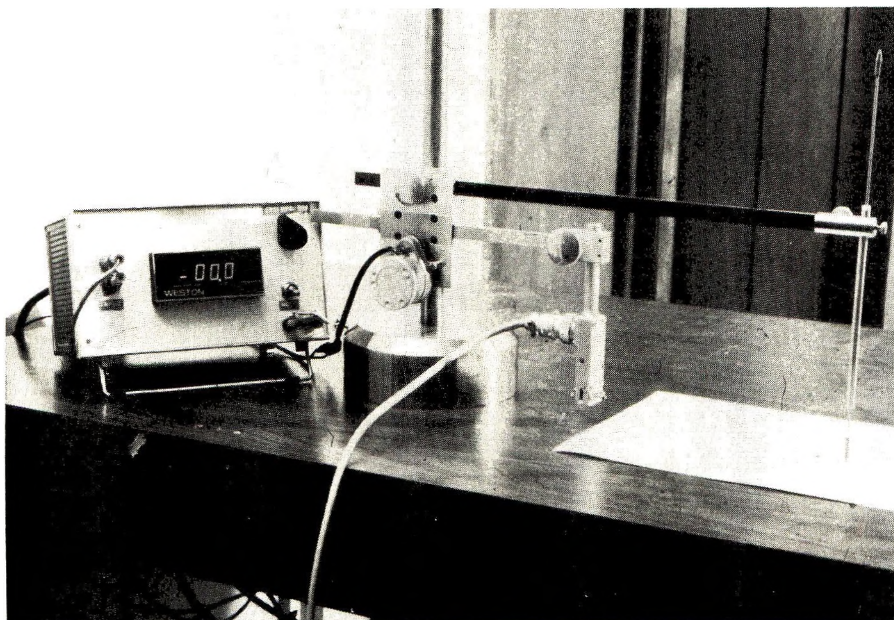
Figure 3. Skin softness in two groups treated prior to and following treatment with lotion or water

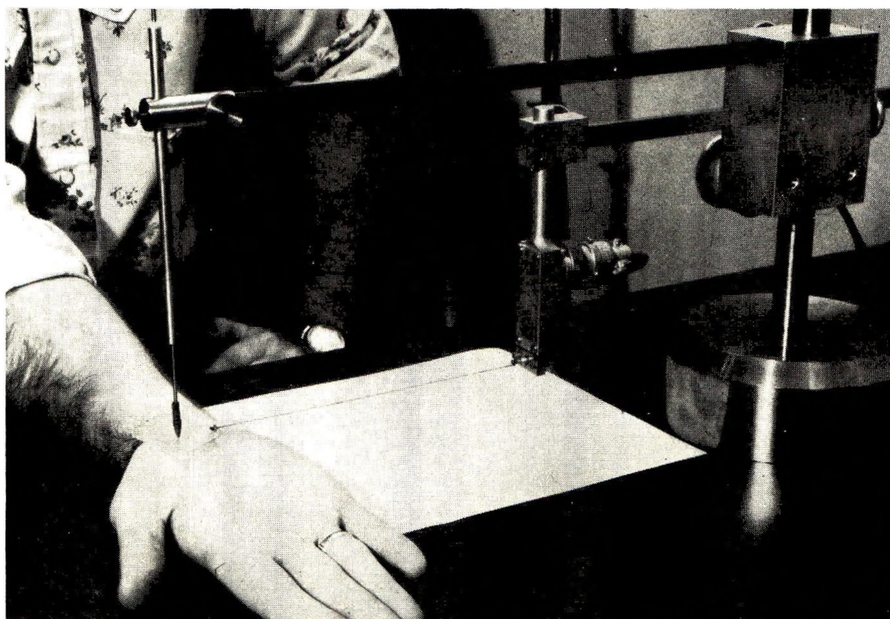
Discussion: Experiment I

It is clear that soaking in water or applying a lotion has an immediate effect in enhancing skin softness. After 5 min, however, the effect of the water had dropped significantly, whereas that of the lotion had remained. Apparently, the moisturization effect produced by the water had been dissipated in 5 min whereas that induced by the lotion lasted at least that long, as measured by this technique.

MEASUREMENT OF COEFFICIENT OF FRICTION AS A METHOD OF DETERMINING SKIN SMOOTHNESS

We applied the concept of the coefficient of friction in producing a device which assesses the smoothness of skin (Figures 4A and 4B). The device comprises a sled of deglazed thermoplastic drawn by a constant-speed (5 mm/min) motor. The force required to move the sled over skin is measured by a force transducer; the resultant voltages from the transducer are amplified and fed to a digital voltmeter, calibrated to read in grams. A rod with a small ball-bearing point rests constantly upon the sled, which is placed upon the area of skin to be tested. The skin and sled are kept horizontal. Measures of the maximum forces required to move the sled are continuously made and recorded. Several, *e.g.*, ten, determinations can be made in the space of a few





Figures 4A and 4B. The device for assessing skin smoothness

minutes and prove to be quite consistent for the same degrees of smoothness. The mean of the determinations is used as the index of smoothness.

SKIN SMOOTHNESS: VALIDITY

To assess the validity of the system, we employed five grades of sandpaper varying in roughness in grains per linear inch as follows (from rough to smooth): 320, 360, 400,

Table III
Mean Forces and Coefficients of Friction for Five Grades of Sandpaper

Sandpaper Grains/inch	Force (g)	SD	Coefficient of Friction C.F.	S.D.
600	5.127	0.076	0.470	0.007
500	7.621	0.110	0.699	0.010
400	9.809	0.112	0.899	0.010
360	11.577	0.155	1.062	0.014
320	12.052	0.130	1.106	0.012

500, 600. Ten determinations were taken of the force required to move the sled over each grade. Table III gives the means of the forces, the coefficient of friction and their standard deviations (Figure 5).

It can be seen that there is a linear relationship between the roughness of the sandpaper and the forces or coefficients of friction obtained. The S.D.'s are only about 1 per cent of their respective means.

The question of whether the system assesses changes in the actual smoothness of skin or the topical lubricating aspect of the lotion, etc. (skin slip), is of interest. We therefore retested the forces and coefficients of friction for the five grades of sandpaper after first applying hand lotion to each sandpaper and then removing it with paper towelling. The changes in force (Pre- to Postlotion) required to pull the sled for the five sandpapers (320 to 600) were: −0.02, 0.01, 0.00, 0.09 and −0.57; and the changes (and percentage changes) of the coefficients of friction were: −0.001 (0.9 per

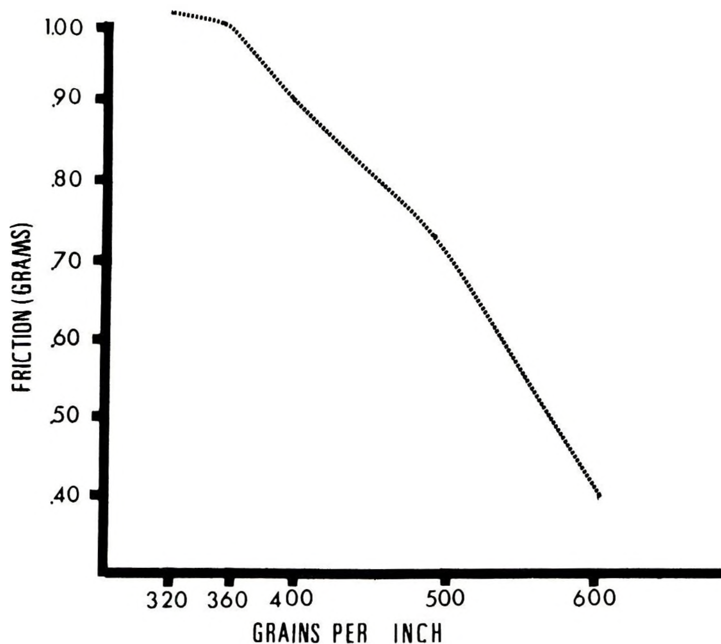


Figure 5. Roughness of five grades of sandpaper assessed by smoothness device

cent), 0.000 (0 per cent), 0.000 (0 per cent), 0.011 (1.3 per cent) and −0.076 (6.9 per cent).

Thus it can be seen that the application of lotion had, at most, a miniscule effect on all but the very smoothest of sandpapers.

SKIN SMOOTHNESS: EXPERIMENT II

Reliability

Twenty subjects of both sexes, ranging in age from 24–55, were tested twice for the smoothness of their palms with a 10-min delay between tests. The Pearson Product Moment Correlation was 0.947, $p < 0.0005$, indicating extremely high retest reliability.

Subjects

Twenty-seven men and women between the ages of 25 and 54 were randomly divided into three groups of nine each and assigned to a Water, Lotion, or Bath Solution group.

Procedure

A pretreatment measure was taken before application of Lotion (A), Bath Solution (B), or Water (C). Immediately following the conclusion of the treatment, the first posttreatment measure was taken (Post 1) and, following an additional 10 min, we recorded the second posttreatment measure (Post 2).

In all groups, one finger (usually the middle) was treated and the middle phalanx tested. For the Water and Bath Solution Groups, the finger was immersed in tepid solutions for 5 min. The Lotion was applied and rubbed over the glabrous skin of the middle phalanx for 1 min and then permitted to remain for an additional 4 min. The excess was then removed with rolling traverses of paper towelling and the visible excesses were removed by gentle rubbing with cotton wool. In all groups, a hair dryer was then employed to blow warm air to dry the skin until it visibly appeared dry.

Results: Experiment II

Table IV gives the summary of an analysis of variance for these data.

It can be seen that there was a significant effect of trials. The *t* tests between Pre and Post 1 and between Post 1 and Post 2 were 1.13 and 1.59, indicating no significant differences between these conditions overall. However, the result of the *t* test between

Table IV

Summary Analysis of Variance for Skin Smoothness Following Treatment with Water, Bath Oil or Lotion

Source	df	MS	F
Between subjects	26	67.74	
Between treatments	2	113.18	1.77
Between S's in same treatment group	24	63.96	
Within S's	54	10.82	
Between trials	2	36.06	3.76*
Interaction treatment × trial	4	12.72	1.32
Interaction pooled s's × treatment × trial	48	9.62	

*$p < 0.05$.

Note: See footnote to Table I for definition of statistical symbols.

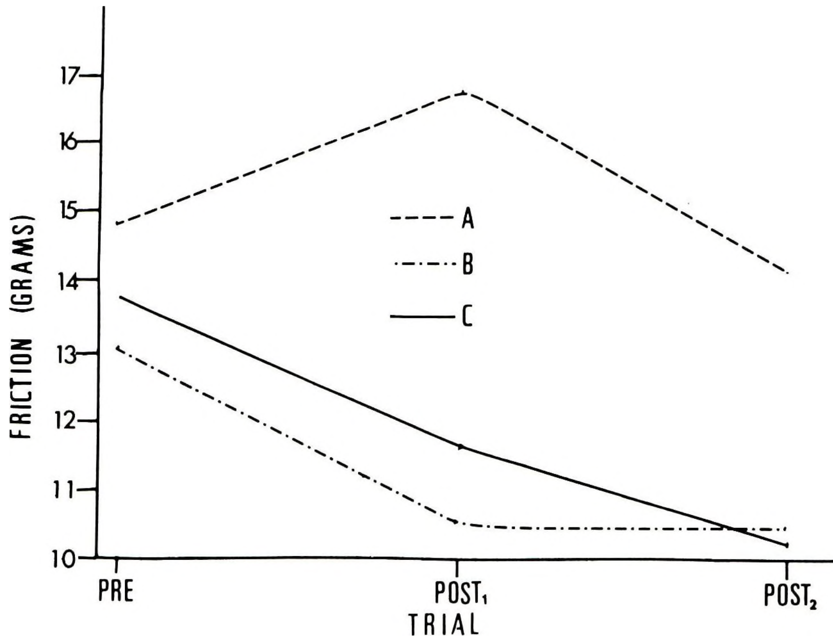


Figure 6. Skin smoothness in three groups tested prior to and following treatment with Lotion (A), Bath Solution (B) or Water (C)

Pre and Post 2 was 2.73 ($p < 0.01$), showing that all treatments combined were effective 10 min after treatment.

Since Figure 6 demonstrates apparent differential effectiveness of the treatments, we looked for *post hoc* trends. We compared the changes in smoothness from pre- to post-treatment for all three treatments. The results indicate a strong tendency for the Water and Bath Solution treatments to produce more skin smoothness immediately after treatment than does the Lotion treatment.

EXPERIMENT III: EFFECT OF THREE TREATMENTS ON SOFTENING AND SMOOTHING SKIN

Subjects

Twenty subjects, 6 men, 14 women, aged 18 to 55 were tested.

Procedure

The glabrous skin of the second phalanx of the index, middle and ring fingers of the preferred hand was selected for treatment and testing.

Pretreatment test. The three fingers were randomly used for the three treatments and were tested in random order. In all cases, softness was measured before smoothness.

Treatments. The three treatments were: (a) soaking the finger for 5 min in a cup of tepid Bath Solution, (b) soaking another finger for 5 min in the same volume of tepid Water and (c) rubbing a small amount of skin Lotion on the finger. Following 5 min of each treatment, the finger was wiped dry and the excess Lotion removed.

Posttreatment tests. The tests were repeated on each finger in the same order: smooth-

Table V
Percentage Increase in Skin Softness After Three Treatments

S	Age	Sex	Lotion	Water	Bath
1	23	M	20	7	24
2	23	M	1	24	22
3	21	F	51	79	22
4	43	F	22	26	32
5	32	F	39	1	13
6	47	F	14	11	34
7	44	F	12	22	4
8	42	F	5	12	6
9	34	F	2	33	38
10	20	M	13	9	19
11	19	F	16	0	0
12	26	M	36	5	18
13	22	F	19	18	48
14	25	M	31	15	26
15	18	F	10	8	8
16	41	M	33	4	19
17	33	F	14	3	−10
18	56	F	6	6	0
19	23	F	13	40	42
20	18	F	33	24	20
Mean			19.5	17.3	19.4

Note: "−" means a decrease in softness.

ness then softness. After testing was completed for a finger, the second finger was treated and then tested and finally the third finger was treated and tested.

Results: Experiment III

Softness. Softness was measured as a percentage change in degree of indentation. The mean percentage change in softness for each treatment is given in Table V.

A Friedman Analysis of Variance was computed to evaluate the relative efficacy of the three treatments ($\chi^2 = 1.68$, $p > 0.05$). The mean relative ranks (from 1–3: low to high effectiveness) were 1.78 for Water, 2.18 for Lotion and 2.05 for Bath Solution.

In view of the lack of significant differences among the treatments, we computed the number of individuals whose skin was softened, remained the same, or made harder by the treatment. Table VI gives the distribution of subjects and the χ^2 for each treatment.

Table VI
Distribution of Subjects Whose Skin Softness Changed After Each Treatment

	Softness				
Treatment	Better	Same	Worse	Total	χ^2 − 2 df
Water	19	1	0	20	34.13*
Lotion	20	0	0	20	39.80*
Bath	17	2	1	20	23.98*

*$p < 0.001$.

Table VII
Mean Percentage Increase in Skin Softness After Three Treatments in Men and Women

Sex	Treatments		
	Lotion	Water	Bath Solution
Men	22.3	12.8	25.6
Women	18.2	20.2	18.3
Difference	4.1	7.4	7.3

It can be seen that all treatments were highly effective in softening the skin over the period employed.

We divided the group into men and women and compared the relative effectiveness of the treatments for each subgroup. Table VII gives the mean increase in skin softness after each treatment for men and women.

It can be seen that men showed a greater softening effect than women for Lotion and Bath Solution: the opposite was true for Water.

In order to assess the effectiveness of the treatments as a function of the initial state of softness of the skin, we divided the group (median split) into those whose initial skin condition was relatively hard and relatively soft.

Table VIII gives the increase in skin softness for these two subgroups.

It can be seen that for Lotion, subjects in the group with relatively soft skin had a slight advantage (3.2%) over those with harder skin in improving their status. However, for Water and for Bath Solution, those with initially harder skins improved much more than those with softer skins. Indeed, whereas the ratio between the groups for Lotion was only 1.18, the ratios between groups was 2.24 for Water and 2.62 for Bath Solution. This difference between groups of different initial status of skin hardness indicates that those with hard skin are affected much more positively after Bath Solution or Water than after Lotion.

Smoothness. The effects for smoothness were measured as a percentage change in the force (in grams) required to move the sled across the skin. Table IX gives the subject number, age, sex and mean percentage increases in smoothness as a function of the three treatments.

The mean increase in smoothness showed that Bath Solution produced a greater mean percentage change (11.6) than Water (7.3) or Lotion (6.7). A Friedman Analysis of Variance was computed to evaluate the relative efficacy of the three treatments ($\chi^2 = 1.90$, $p > 0.05$). The mean relative ranks (from 1–3: low to high effectiveness) were 1.85 for Water, 1.90 for Lotion and 2.25 for Bath Solution.

Table VIII
Mean Percentage Increase in Skin Softness in Subjects with Relatively Hard or Soft Skin

Pretreatment Condition	Lotion	Water	Bath
Relatively Soft	21.1	10.3	11.2
Relatively Hard	17.9	23.1	29.3
Difference	3.2	12.8	18.1

Table IX
Percentage Increase in Skin Smoothness After Three Treatments

S	Age	Sex	Lotion	Water	Bath Solution
1	23	M	22	5	0
2	23	M	1	15	6
3	21	F	1	16	10
4	43	F	0	9	19
5	32	F	22	14	21
6	47	F	9	0	7
7	44	F	4	0	0
8	42	F	10	0	31
9	34	F	0	0	0
10	20	M	20	14	43
11	19	F	20	16	24
12	26	M	0	35	22
13	22	F	15	17	29
14	25	M	25	37	39
15	18	F	0	0	0
16	41	M	8	0	0
17	33	F	0	−13	17
18	56	F	0	−9	−36
19	23	F	−14	0	−6
20	18	F	−5	0	0
Mean			6.7	7.3	11.6

Note: "−" means decrease in smoothness.

In view of the lack of significant differences among the treatments, we computed the number of individuals whose skin was smoothed, remained the same, or roughened by the treatment. Table X gives these distributions and the χ^2 which evaluates them.

It can be seen that Lotion and Bath Solution were significantly effective in improving the skin smoothness of a majority of the group, whereas Water had no such significant effect.

We divided the group into men and women, and compared the relative effectiveness of the treatments for each group. Table XI gives the mean increases in skin smoothness as a function of each treatment for men and women.

It can be seen that men showed a greater skin smoothing effect than women for all three treatments. In order to assess the effectiveness of the treatments as a function of

Table X
Distribution of Subjects Whose Skin Smoothness Changed After Each Treatment

Treatment	Smoothness				
	Better	Same	Worse	Total	χ^2 − 2 df
Water	10	8	2	20	5.17
Lotion	12	6	2	20	7.56*
Bath Solution	13	5	2	20	9.22**

*$p < 0.05$.
**$p < 0.01$.

Table XI
Mean Percentage Increase in Skin Smoothness After Three Treatments in Men and Women

Sex	Treatments		
	Lotion	Water	Bath Solution
Men	12.6	17.6	19.3
Women	4.4	2.8	8.3
Difference	8.2	14.8	11.0

Table XII
Mean Percentage Increase in Skin Smoothness in Subjects with Relatively Rough or Smooth Skin

Pretreatment Condition	Lotion	Water	Bath Solution
Relatively Smooth	8.7	3.5	9.9
Relatively Rough	4.7	9.8	13.3
Difference	4.0	6.3	3.4

the initial state of smoothness of the skin, we divided the group (median split) into those whose initial condition was relatively rough and relatively smooth. Table XII gives the mean increase in skin smoothness for these two groups.

It can be seen that for Water and Bath Solution the subjects with initially rougher skin benefitted more than those with smoother skin; the opposite was true for Lotion.

Relationship between smoothing and softening. We computed a series of Pearson Product Moment Correlation Coefficients to determine the degrees of change for our two measures as affected by the three treatments. (See Table XIII.) It can be seen that the degrees of skin softening and smoothing were highly correlated ($p < 0.001$) after all treatments employed.

Interview data indicated that those individuals who showed little or no effect from the treatments as measured by us tended to utilize hand lotions very frequently, thus making efficacy of the 5-min treatment employed by us relatively minor.

Summary: Experiment III

We found that: 1) Water significantly softens the skin. 2) Lotion significantly softens and smooths the skin. 3) Bath Solution significantly softens and smooths the skin. 4) These effects are nondifferential among the three treatments. 5) The effects of smoothing and softening the skin were highly related after all the treatments. 6) Men benefited more than women for skin smoothing after all treatments, and for skin softening after Lotion and Bath Solution. 7) a–Those whose skin was initially relatively

Table XIII
Correlations (r) Between Percentage Smoothing and Softening for Each Treatment

Treatment	r
Lotion	0.87
Water	0.86
Bath Solution	0.87

harder benefitted more than those with initially softer skin after treatment with Water or Bath Solution, b–Those whose skin was initially relatively rougher benefitted more than those with initially smoother skin after treatment with Water or Bath solution, c–Lotion, paradoxically, benefitted those with initially softer and smoother skin more than those with harder and rougher skin.

EXPERIMENT IV: EFFECTS OF EPIDERMABRASION

One question which is frequently raised in dealing with assessing the skin softening and smoothing effects of various products is whether the measuring instruments are subject merely to the artifactitious result of measuring the properties of the *product* rather than the treated *skin per se.* Thus, we described above (under Skin Smoothness: Validity) the changes found in friction of sandpaper following application and removal of skin lotion. If the lotion were acting as a mere topical lubricant, then sandpaper would be found to be smoother. However, we were able to demonstrate no change in the smoothness of sandpaper, while enhancement of smoothness in skin was obtained, demonstrating that we are indeed measuring the effect of lotion on the skin.

Another approach to the question, which deals with this aspect of validity, is to utilize epidermabrasion. This system employs "deliberate, physical removal of the *stratum disjunctum* and other keratin excrescences of the *statum corneum*" (2) by means of a nonmedicated "polyester fiber web."

The purpose of this study was to determine whether physical abrasion of part of the stratum disjunctum would result in measurably increased smoothing and softening of skin as measured by us.

We studied one subject, a Caucasian female, aged 48. We took two successive measures of softness and smoothness of three regions on her forehead: left, central and right. Following these two pretreatment tests, we left one region untreated, applied a cotton ball soaked with tepid Water to another region for 5 min and rubbed Lotion on the third region for 1 min. Following the minute of Lotion application we waited 4 additional minutes and then removed the visible excess with a cotton ball. Finally, we continuously applied the fiber web to the entire forehead for 30 sec in a circular motion, and retested all sites for smoothness and softness.

Results: Experiment IV

Effects of water and lotion. The Water-treated site showed an immediate, dramatic increase in softness in contrast to the negligible changes for Lotion-treated or Untreated sites. By contrast, Lotion produced an immediate, very large increase in smoothness, whereas Water resulted only in a moderate change, and the Untreated site in no change in smoothness.

Effects of epidermabrasion. The differential results of epidermabrasion superimposed on Untreated, Lotion-treated, and Water-treated skin were not entirely expected. Smoothness and softness measures were not changed by epidermabrasion over the sites previously treated by Water or Lotion. However, the greatest enhancement for both the smoothness and softness measures was obtained when the abrasion was done on the formerly untreated site.

Discussion: Experiment IV

If Lotion or Water had only topical, surface-lubricating effects which our devices detect, then the removal of surface Lotion and the drying of the surface of the skin would have diminished the softening and smoothing effects. The fact that epidermabrasion did not diminish (or enhance) the results when applied to these treated sites indicates that the effects were already incorporated into the stratum corneum and that the instruments were indeed measuring actual changes in the skin and not merely artifacts of local surface lubrication.

This fact is further brought home by the finding that the Untreated site showed that epidermabrasion alone resulted in considerably softer and smoother skin after only one 30-sec treatment.

We thus conclude that our devices are measuring changes in skin and not mere artifacts of surface lubrication and further that, once treated topically, epidermabrasion does not diminish the effect by the removal of the potential surface lubricant.

EXPERIMENT V: AN EVALUATION OF SHAVING

In view of the demonstrated ability of the smoothness evaluation system to assess the enhancement in the smoothness of the skin, we applied a different probe to determine whether we could evaluate the effectiveness of shaving creams or razors in producing close shaves. We produced a new sled (a fine metal mesh) which enables the hair shafts to penetrate it.

The experiment comprised determining the degree of smoothness of the face in a man who hadn't shaved for 24 and 48 hr. We measured his facial smoothness immediately

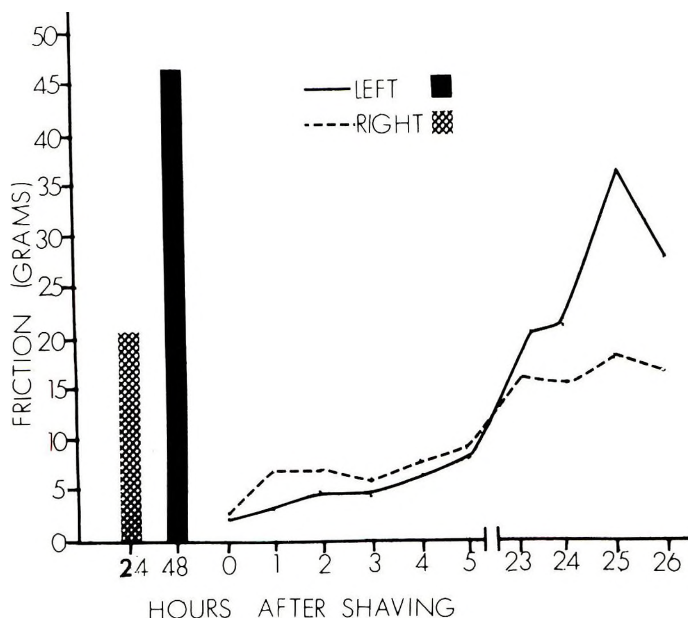


Figure 7. Facial smoothness prior to and following shaving

following shaving and after 1, 2, 3, 4, 5, 23, 24, 25 and 26 hr. The results, which are rather clearcut, are depicted in Figure 7.

This indicates that there is differential hair growth on the two sides of the face, which is monotonic with time. The growth of hair can be measured almost hourly and the effectiveness of shaving can be assessed.

COMPARISON OF SMOOTHING AND SOFTENING SKIN

The series of correlations (see above) has demonstrated that three skin treatments produced smoothing and softening effects on the skin. This finding should not be of surprise. However, since the methods are operationally distinct, one can conceive of producing a smooth skin, *e.g.*, by mechanically removing surface roughness, but not necessarily by softening it. Conversely, one might be able to soften a surface, leave it rough, or indeed, even roughen it additionally in the process. Comparison of the results of Experiments I and II illustrated that such a dissociation may have indeed occurred.

Thus, Figure 3 shows that water had an immediate softening effect which was dissipated after 5 min while the lotion maintained its effect. Figure 6, however, shows that even 10 min after treatment and after forced drying with heated air, the Water Treatment group still showed significant smoothness.

We believe moisturization of the stratum corneum to be one major cause of smoothing and softening the skin; epidermabrasion is another. We are, therefore, considering a study to assess the direct effects of varying *degrees* of moisturization or epidermabrasion on the smoothing and softening of the skin.

CONCLUSIONS

We have described the development of two systems for assessing, *in vivo*, the softness and smoothness of skin. These methods have high retest reliability, precision and validity, and can be applied on various skin surfaces to determine the effectiveness of skin treatments in softening or smoothing the skin. The system for assessing skin smoothness has also been adapted to measure the degree of facial smoothness as a function of efficacy of shaving creams or razors. The two systems are capable of assessing the beneficial effects of various skin treatments and must be considered measures of separate effects.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Norman Orentreich and his staff for helpful criticisms of this report, and for suggesting study of the potential artifact of lotion as an external lubricant. Dr. Orentreich suggested the study of the effect of lotion on sandpaper and the study of the softening and smoothing effects of epidermabrasion. We are grateful to the Orentreich Medical Group for making available a sufficient supply of Buf-Puf, a polyester fiber web, to study the effects of epidermabrasion on softening and smoothing skin. Most of the tests and analyses were capably done by Peter Filosi, Curt

Weinstein and David Canestrari, and the author wishes to express his gratitude to them.

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Low-energy emulsification—I—principles and applications

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Synopsis

The amount of energy normally expended in commercial processing of a cosmetic emulsion is far greater than the amount theoretically required. Whether the emulsion is made by a batch process or semicontinuous process, thermal energy is first supplied to heat the ingredients and mechanical energy is then provided for mixing and emulsification. Additional mechanical energy is expended to cool the product and the heat removed is generally discarded.

A considerable saving in energy is made possible by a more effective usage of thermal and mechanical energy in emulsification. A substantial saving of thermal energy can be achieved by a careful determination of emulsification temperature and by a selective heating of the ingredients. The method discussed here basically involves making an emulsion concentrate which is later diluted with the remainder of the external phase at room temperature.

In addition to conserving energy, the proposed LOW-ENERGY EMULSIFICATION technique also offers a great advantage in reducing the processing time and equipment cost. In some instances, the energy cost for processing an emulsified lotion can be reduced by 50 per cent while the production efficiency can be increased by 100 per cent.

INTRODUCTION

The recent natural gas shortage in the United States has again clearly demonstrated the importance of energy conservation. One area of cosmetic processing that has not been critically examined in terms of energy requirement is emulsion processing. Compared to the energy actually required, a considerable amount of energy is wasted in a typical plant operation of emulsion manufacturing.

In 1965, the author proposed a technique of emulsification referred to as "semicold processing" that was designed to allow manufacturing of emulsion products with a partial heating of the raw materials (1). Since then, the author has tested this technique on numerous emulsified and nonemulsified products in production scale with favorable results. The method allows not only a conservation of thermal and mechanical energies, but also a substantial increase in manufacturing efficiency and a reduction in operating expenses without any compromise in the product quality. In some cases, it is also possible to reduce the capital expenditure on process equipment when planning an expansion of production capacity. The main purpose of this paper is to outline the basic

principle and to point out possible areas of application in practical processing of emulsion products.

ENERGY CONSUMPTION IN EMULSION PROCESSING

In the conventional processing of cosmetic emulsions, the oil- and water-soluble ingredients are usually heated in two separate kettles as illustrated in Figure 1. There are two forms of energy input: thermal energy (TE_1, TE_2) for hearing and mechanical energy (ME_1, ME_2) for mixing and homogenizing (ME_3). It can be shown by energy balance that, in a typical plant production, only a small fraction of the energy input is utilized in actual emulsification, *i.e.*, to break up liquids into small droplets.

If city water is used for cooling the batch, the thermal energy removed during the cooling stage is generally discarded along with the water. If chilled water is used and recycled, additional energy is required by the compressor in the refrigerated system to remove and discard the heat. The majority of mechanical energy supplied dissipates as friction and turns into heat and noise.

An estimate of the total energy utilized *vs.* the energy wasted in a conventional processing of emulsions can be made by calculating the theoretical energy requirement. For illustration, it is assumed that one is making a 1,000 kg batch of a certain O/W emulsion consisting of 25 per cent mineral oil, 5 per cent surfactant and 70 per cent deionized water.

The theoretical energy requirement for emulsification is, of course, dependent on the effectiveness of the surfactant as well as the droplet size distribution of the final emul-

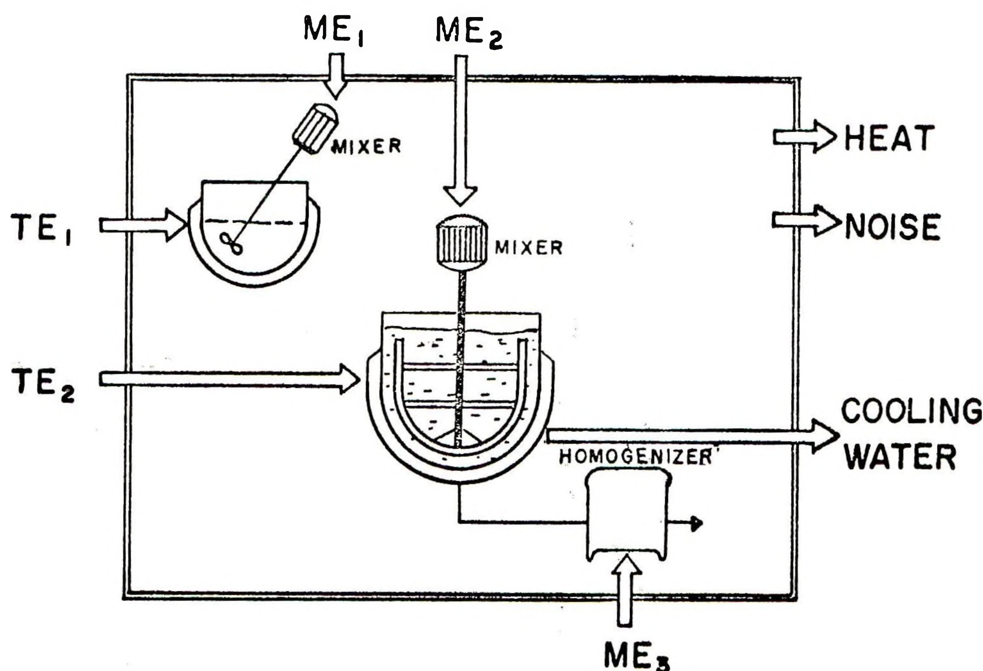

Figure 1. Energy input in emulsion manufacturing

sion. The theoretical amount of work required to break the liquids into droplets can be calculated from the interfacial tension and the change in surface area given by the following equation:

$$W = \gamma \Delta S$$

where W = work done
γ = interfacial tension
ΔS = change in interfacial area

For illustration, it is assumed that the interfacial tension is 2 dyn/cm and the final emulsion droplets are spherical, having a uniform diameter of 1μ. Taking the specific gravity of mineral oil as 0.85, the minimum energy requirement calculated from the above equation is 0.84 Kcal per 1,000 kg of the O/W emulsion. This value represents, of course, a theoretical minimum assuming no internal friction. The actual requirement is expected to be much greater.

The amount of energy consumed in making such an emulsion in a plant scale will be also dependent on emulsification method, emulsification temperature, etc. For this calculation, the following assumptions are made:

Mixer power: one horsepower for each of the two mixers
Mixing time: 90 min per batch for both mixers
Homogenizer power: 5 hp
Homogenizing time: 20 min
Room temperature: 20°C
Emulsification temperature: 80°C
Heat capacity: 1 cal/g, °C for all raw materials and finished emulsion
Batch size: 1,000 kg

The results of the calculation based on the above parameters are given in Table I.

The combined mechanical energy input is close to 3,000 Kcal and the thermal energy consumption is 60,000 Kcal or about 95 per cent of the total. Clearly, compared to the theoretical requirement, a typical plant processing of an emulsion consumes a far greater amount of energy. It should be evident that if one can devise a way to make the emulsion cold, 95 per cent of the energy consumption in this example can be immediately saved.

Hot emulsification is wasteful not only from the energy viewpoint but, even more importantly, from the consideration of production time and efficiency. The time required

Table I
Energy Input

Energy Source	Energy Consumption (Kcal)	Percentage Total
(a) Two Mixers	1,920	—
(b) Homogenizer	1,067	—
Total Mechanical Energy	2,987	5
Total Thermal Energy	60,000	95
Total Energy Input	62,987	100

Table II
Processing Time

Operation	Time (minutes)
Preparation	50
Heating	30
Emulsification	30
Cooling/Mixing	60
Homogenizing/Pumping	30
Clean-up	40
Total	240 = 4 hr

for each operation in a typical batch processing of the emulsion cited in the example is given in Table 2.

The total processing time in this example is 4 hr, including clean-up and preparation. It is to be noted that 30 min is spent on introducing the thermal energy and 60 min is spent on removing it at a latter stage. Therefore, a total of 90 min of valuable processing time can be saved by adopting the use of a cold method.

LOW-ENERGY EMULSIFICATION

Even though the advantages of a cold process are quite obvious, the popular use of waxy raw materials in cosmetic emulsions presents a serious problem in practical processing. Even with the use of a homogenizer, stearic acid and most waxy substances would not emulsify properly in cold water. Hence, a completely cold emulsification is feasible only with limited emulsions consisting solely of liquids or liquid-soluble ingredients.

However, in most cosmetic emulsions the total amount of waxy material is generally below 20 per cent and sometimes only 3 or 4 per cent; a question can thus be raised as to the necessity of heating the entire 100 per cent of the ingredients in order to obtain a good emulsion. The basis of low-energy emulsification is to combine the advantage of cold emulsification with the practical necessity of hot emulsification by selectively applying heat to a part of the ingredients.

Figure 2 illustrates the common batch processing of a cosmetic emulsion. If the final emulsion is an O/W type, the internal phase generally consists of oils and waxes. The external phase is made up of water and water-soluble components. In this figure, h and H represent the heat supplied to the internal and external phases respectively. After emulsification, the batch is generally cooled to room temperature by either circulating cooling water in the kettle jacket or passing the warm emulsion through a heat exchanger. Neglecting the small amount of heat lost to the atmosphere, the frictional heat and the heat of mixing, the heat that must be removed from the emulsion is $h + H$.

In a low-energy method illustrated in Figure 3, instead of heating the entire external phase, only half of the water phase is first heated to make a concentrated emulsion; the remaining half is then added at room temperature. The energy supplied to the external phase is now half of the original value and the heat which must be removed later is only $h + 0.5\,H$.

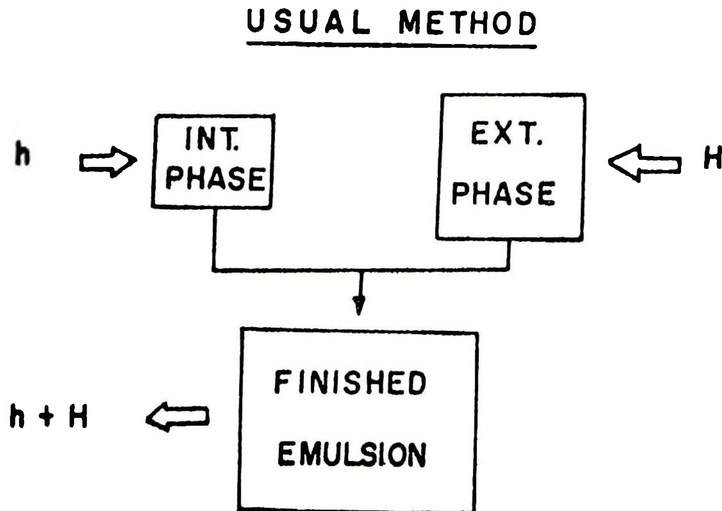

Figure 2. Conventional emulsion processing

In this example, only 50 per cent of the external phase was withheld for cold dilution, but there is no reason why one cannot withhold 70 per cent or more to achieve an even greater energy conservation. In some instances, a portion of the oil phase can be withheld and added cold later if it consists of mostly liquid materials such as mineral oil. One limitation is that one cannot withhold too much so as to make the processing of the concentrate difficult or the subsequent dilution impossible.

There are many different ways to apply the low-energy emulsification technique. One method (Figure 4) uses two kettles and an automatic metering valve. The entire oil phase is heated in one of the kettles and the first portion of water and water-soluble ingredients is heated in the other kettle. Depending upon the desirability of a phase

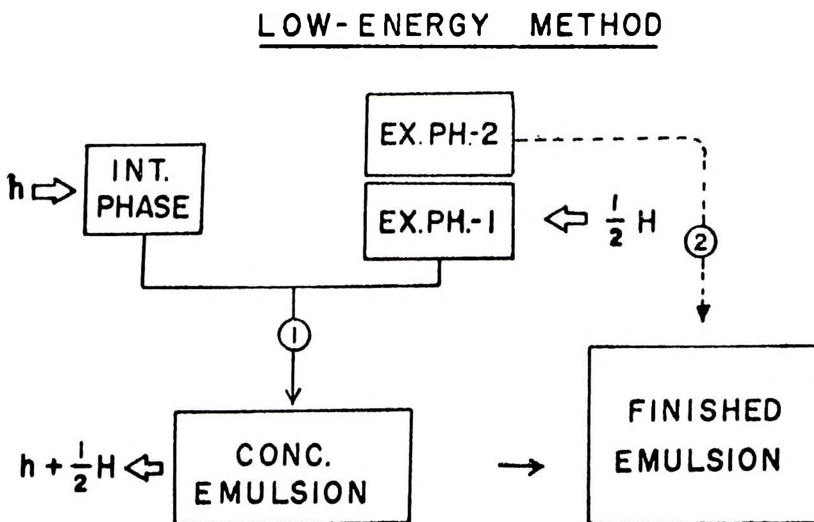

Figure 3. Low-energy emulsion processing

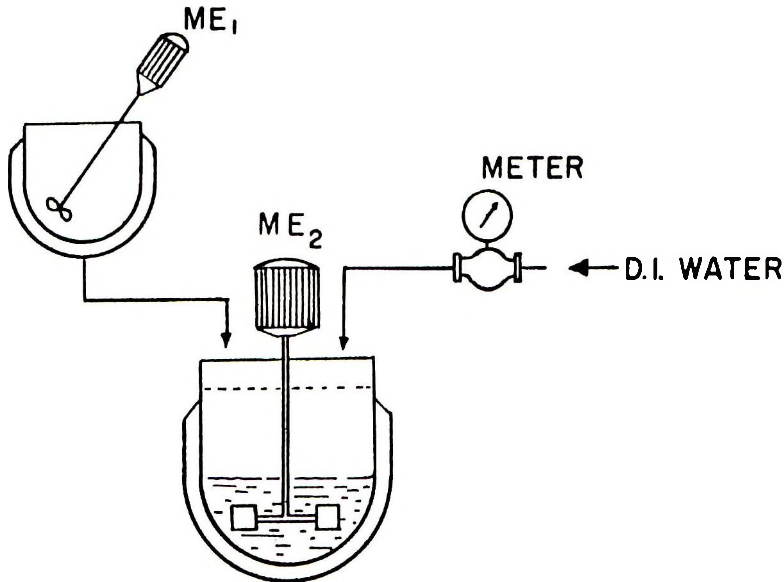

Figure 4. Two-kettle low-energy emulsion processing

inversion, the oil phase can be added to the aqueous phase or *vice versa* to form the emulsion concentrate. While the concentrate is being cooled, the remainder of the cold deionized water can be metered in to complete emulsification.

The saving of thermal energy is quite obvious in such a technique. The mechanical energy, ME_1, supplied to the upper kettle remains unchanged, although ME_2, supplied to the lower processing kettle, may be somewhat different. In the low-energy method, the initial emulsion is more concentrated and generally more viscous. On the other hand, there is less material in the kettle in the first stage so that the total mechanical energy consumption will not be much different. If anything, the over-all mechanical energy consumption will be less for the new process since the cooling time is shortened considerably by the addition of the cold water.

The low-energy technique illustrated here involves a two-step operation. It may appear, at first, that it would take longer to process a batch in comparison to the conventional, one-step procedure. According to the author's experience, the low-energy method actually requires much less processing time and in many situations this benefit may be even more desirable than the conservation of energy. The reason is that the most time-consuming part of making a commercial emulsion is often the cooling of the batch. Particularly if the product is very viscous or if the cooling water is not very cold, the time required may be very long. In the new process, the addition of less energy at the beginning means removal of less heat during the cooling period, resulting in a substantial shortening of the heating and cooling time. Moreover, the dilution step can be carried out during the cooling stage by simply metering the cold, deionized water so that there is no extra time consumed in the second stage.

APPLICATION OF THE LOW-ENERGY METHOD IN PRODUCTION EXPANSION

In addition to saving energy and processing time, the low-energy technique described here may be applied in some instances to save capital expenditure in planning a produc-

tion expansion. Since only a portion of the batch is heated in the low-energy method, it is possible to use a smaller kettle for processing or, in some instances, use the existing kettle to make larger batches to save capital expenditure on expensive, jacketted kettles.

The economy of such a technique should become apparent by considering the following examples. Consider a company which now has a 200-gallon tank to make a certain, low-solids, O/W moisturing lotion. For illustration, it is assumed that the time required for each compounding operation is the same as the time presented in Table II. Suppose that it is now desired to increase the production capacity of this product by 2.5 times to meet the increased sales demand. One proposal calls for purchasing a new 500-gallon process tank and increase the batch size by 2.5 times. However, a careful engineering study would soon reveal that the purchase of a new kettle alone would not guarantee a proportional increase in the actual production if the conventional emulsification technique is used.

The reason should become apparent if the time required for each operation (Table II) is carefully examined. The preparative time includes the time required for weighing the ingredients and for metering or weighing the deionized water in the aqueous phase. Since the batch size is now 2.5 times greater, it will take longer to catch the deionized water. The heating and cooling times will be much greater, not only because more material is involved but also because of the fact that the larger the kettle, the smaller will be the heat transfer surface per unit volume of the material. The time spent on homogenizing and pumping will increase proportionally to the batch size.

If it now takes 4 hr to complete a 200-gallon batch, it will likely take six or more hours to process a 500-gallon batch. This means that only one batch can be produced in an 8-hr work day and one can expect only a 25 per cent increase in the actual production capacity.

Naturally, there are ways to speed up each compounding step to allow completion of the 500-gallon batch within the 4-hr limit, but this will require more equipment. For example, the time required to catch the deionized water can be shortened by installing a larger ion-exchange unit. The cooling time can be shortened by using a rotary, scraper heat exchanger. The homogenizing/pumping time can be shortened, but larger equipment will be needed. It is evident that a considerable capital expenditure will be required for the proposed production increase.

By far the more economical way of meeting the need would be to adopt a modified, low-energy technique (Figure 5). The idea presented here is to make an emulsion concentrate in the existing 200-gallon kettle and then dilute it in a 500-gallon storage tank equipped with a mixer.

The method is particularly ideal if the emulsion has a relatively small internal/external phase ratio. Since only 200 gallons of the material is heated, the heating and cooling times will not increase. The time needed for pumping and homogenizing will be the same. The only operation that may require extra time is the metering of 300 gallons of deionized water. However, a relatively inexpensive, automatic metering valve can be conveniently used to meter the water while the concentrate is being processed in the kettle so that no extra time will be required in this operation. It should not present any difficulty to complete two 500-gallon batches of this emulsion in an 8-hr period using such a technique. The only new equipment required for this process is a 500-gallon storage tank. A storage tank, however, is much less expensive than a jacketted, stain-

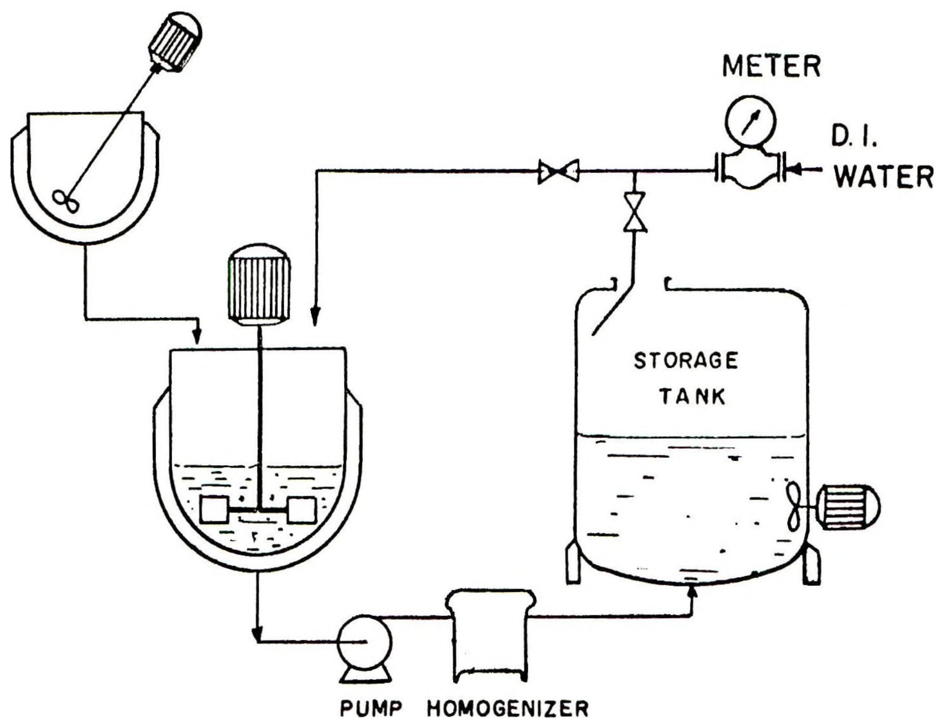

Figure 5. Modified low-energy emulsion processing

less steel kettle of the same capacity. Therefore, from both the production efficiency and equipment cost viewpoints, the low-energy method is far superior to the conventional method of emulsification.

LIMITATION OF LOW-ENERGY TECHNIQUE

There are, naturally, some limitations on the application of the proposed low-energy technique. One of the important points to be considered is that mixing becomes difficult if the viscosity of the concentrate is too high. There is therefore a limit as to how much of the external phase one can withhold. Fortunately, most emulsions, even fairly concentrated, are flowable at the elevated temperature at which emulsification is normally carried out.

The second limitation is that, upon dilution, a stable emulsion of desired properties must be obtainable. The desired properties may be the correct texture, opacity or certain rheological properties. This requirement is, of course, very important from the marketing viewpoint. However, since emulsion is an extremely complex system with so many physical factors which can affect its properties, it is not easy to define the conditions which will satisfy this requirement.

The only reliable way to determine if the method works satisfactorily would be to conduct carefully controlled pilot batch experiments. It is important to remember that there is no set way to carry out low-energy emulsification. There are many variations possible and with some imagination many advantages can be derived.

In some instances, for example, the presence of a thickener can make the concentrate very thick and cause difficulty in mixing. Sometimes such a problem can be solved by dispersing the thickener in the diluting water. If the viscosity of the thickener is pH-dependent, it may also be possible to avoid the problem by carefully controlling the pH of the concentrate. Phase inversion of the concentrate can cause a problem if the emulsion does not invert to the desired type upon dilution. Sometimes such a problem can be prevented by changing the order of phase combination or the surfactant location (3).

It should be cautioned that since cold deionized water is used in the second stage, this water must be sterilized to avoid microbial contamination.

CONCLUSION

Although the technique described here lends itself readily to the processing of low-solids, O/W emulsions, there is no reason why it would not apply to W/O emulsions or nonemulsified products. In fact, the method works very nicely on processing most shampoos and even some make-up preparations. The author has successfully tested a W/O system by withholding parts of both the oil and aqueous phases. However, such a technique becomes rather involved and requires experience to handle properly.

In using the technique, it is well to reexamine the emulsification temperature used. In many instances it is possible to lower the emulsification temperature without affecting the emulsion quality. In some emulsions stabilized with nonionic surfactants, it may be desirable to keep the emulsification temperature above the PIT (phase inversion temperature) in order to facilitate emulsification.

In some cases, considerable savings in mechanical energy used in mixing or homogenizing can be achieved by changing emulsification method. Elimination of energy-consuming high-shear equipment can be frequently accomplished by optimizing emulsification conditions. For example, without changing formulation, it is sometimes possible to significantly facilitate emulsification by controlling the emulsifier location or presolubilizing the oil phase (4). In some instances, one can obtain a finer emulsion using the low-energy technique than using a conventional method.

The author has conducted considerable basic work on variables affecting droplet size distribution and stability of emulsions prepared by this low-energy technique. A paper dealing with these aspects will be published in the future.

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Mascara contamination: in use and laboratory studies

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Synopsis

Eye area cosmetics are subject to varied levels of microbial CONTAMINATION during their normal use. Selected MASCARAS with known preservative formulations were compared for their resistance to microbial colonization DURING USE by study groups and following a laboratory challenge test. A few products supported active growth of microorganisms after less than two weeks of normal use and after LABORATORY challenge. In study groups, the establishment of reproducing populations of microorganisms in certain mascaras occurred consistently with select individuals. Mascaras containing only parabens or imidazolidinylurea appeared to be less effective in retarding microbial growth than those containing formalin donors or mercurials. Effectiveness of preservative formulations may gradually decrease with the age of the product.

INTRODUCTION

Most mascaras contain antimicrobials to maintain the integrity of the product and to protect the consumer from the development of potentially harmful contaminants. Nevertheless, certain mascaras examined during use have been found to be overgrown with microorganisms and in some instances to be associated with eye infections (1–3). Ramp and Witkowski (4) have reviewed typical procedures for testing preservative systems in cosmetics. These involve the introduction of microorganisms, usually about 1×10^6 cells, into a 1-ml or 1-g sample of product. Effectiveness is determined by the recovery of less than 0.1 per cent of the inoculum after seven days. Sampling intervals may be extended for several months. Bruch (5) expressed concern about the lack of comparative data published on the various testing procedures. Yanagi (6) recommended that the challenge test for cosmetics be carried out in the commercial containers in which they are sold to the consumer. Laboratory evaluation of the effectiveness of the preservatives in mascaras by the usual microbiological procedures is difficult as many formulations are not readily solubilized by water. Dilution procedures used for enumerating microorganisms in mascaras may require the use of solvents or emulsifiers which may be toxic to the challenge microorganisms (7,8). At present, there is no standard procedure for evaluating the resistance of mascara formulations to microbial degradation. This report compares "in use" study-group analysis with labora-

tory challenge tests to determine the effectiveness of preservative formulations in mascaras.

MATERIALS AND METHODS

MASCARAS

Mascaras were purchased at retail or secured directly from the manufacturer between 1975 and 1977. They were semi-solid, oily products of poor miscibility with water. The preservatives included in the mascaras are presented in Table 1. Concentrations of the microbial inhibitors were provided by the manufacturer and varied for the different brands. The total parabens per mascara ranged from 0.15 per cent to 0.5 per cent, imidazolidinylurea from 0.3 to 0.4 per cent and Dowicil 200 from 0.15 to 0.2 per cent. None of the mascaras contained over 60 ppm of mercury. Several mascaras were stored at room temperature for up to one year and at 37 and 56°C for seven days prior to laboratory challenge.

CHALLENGE ORGANISMS

Mascaras were challenged separately and sequentially with *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* initially isolated from used mascaras. The bacteria were grow at 37°C in tryptic soy broth (Difco) for 18 to 24 hr, harvested by centrifugation and washed three times in phosphate-buffered saline (PBS; NaCl 8.0 g, KCl 0.20 g, KH_2PO_4 0.12 g, Na_2HPO_4 0.91 g, deionized water 1 l, pH 7.2). The final pellet was resuspended in 10 ml of PBS, vortexed for 15 sec, sonicated at maximum power for 15 sec (Ratheon 5120A) and vortexed again for 15 sec. The bacterial suspension was diluted to an optical density (OD) between 0.4 and 0.7 at 600 nm for *S. epidermidis* and at 500 nm for *P. aeruginosa.* Appropriate dilutions of these solutions were made from a standard OD-colony-forming-unit curve to give approximately 10^6 colony-forming units (cfu) per ml. Viable counts were determined by spreading 0.1 ml of appropriate serial dilutions in triplicate on tryptic soy agar using PBS as a diluent. One ml containing 10^6 cfu was diluted to 6 ml with PBS. The 6-ml cell suspension was drawn into a syringe fitted with a Swinny filter holder (Millipore Corporation) and slowly forced through a 13-mm diameter membrane with an average pore size of 0.22 μm.

Table I
Preservative Content of Mascaras

Preservatives	Mascara Code							
	A	B	C	D	E	F	G	H
Methyl paraben	−	+	+	−	−	+	+	+
Propyl paraben	+	+	+	+	−	−	−	−
Butyl paraben	−	−	−	−	−	+	−	−
Dehydroacetic acid	−	+	−	−	+	−	+	−
Imidazolidinylurea	+	+	+	−	−	−	−	−
Dowicil 200	−	−	−	−	+	+	+	−
Phenylmecuric acetate	+	−	+	−	−	−	+	−

MEMBRANE CHALLENGE

Mascara was removed with aseptic technique from its container and 300 to 400 mg was packed into a sterile glass cylinder 3 mm deep with an inner diameter of 13 mm. Two membranes were placed on the mascara. The membrane in direct contact with the mascara had an average porosity of 8 μm. This membrane served to retain the mascara in the well and to facilitate successive challenges with various microorganisms. The second membrane (0.22 μm porosity), containing the challenge organism (side with organism facing upwards), was placed over the first membrane. Periodically, the upper membrane was removed, replaced in the Swinny and back-flushed with 6 ml of buffered saline. The membrane was removed from the Swinny, placed in the 6 ml of saline, agitated and treated with mild sonication. The saline suspension was serially diluted and plated on appropriate media for enumeration of microorganisms. The same cylinder of mascara was challenged sequentially with membranes containing different organisms. Details of this procedure have been described elsewhere (3).

CONTAINER CHALLENGE

Suspension of microorganisms (1×10^6 cfu/g) was introduced directly into the original cosmetic container. The mascara was cultured periodically.

STUDY GROUPS

The study groups, each utilizing 15 to 25 college students, were conducted as previously described (2). Briefly, a single study group used a mascara of an identical lot of formulation for 9 to 11 weeks. The mascaras were cultured weekly for aerobic microorganisms and the history (number of times used, time elapsed since last use, individual habits, etc.) of use was recorded. If a mascara yielded over 50 cfu of the same contaminant on three consecutive samplings, it was withdrawn and a new mascara was issued. An incidence of contamination (IC) was recorded for each product. A single IC was defined as four or more colony-forming units of microorganisms per 3 to 8 mg of mascara present at least 4 hr after the last use. The IC was calculated from the total samplings of all mascaras used in the study groups.

Table II
Contamination of Mascaras During 9 to 11 Weeks of Use

Mascara	Incidence of Contamination	Number Established Contaminants/ Number Mascaras*
B	61**	6/13
C	6	0/17
D	33	0/22
F	7	0/17
G	9	0/18
H	23	0/21

*Established contaminants defined as presence of microorganisms at concentrations of at least 10^3 cells/mg ten days after last use.

**Percentage of samples yielding 4 cfu per 3 to 8 mg of mascara, at least four hours after last use.

RESULTS AND DISCUSSION

An analysis of study-group results with selected mascaras is presented in Table II. Eight containers of mascara B yielded high numbers of *S. epidermidis* after an average of 22 uses. In excess of 10^5 cells/g was isolated from six of the mascaras ten days after their last use. In addition to *S. epidermidis,* three of these six each contained high numbers of either *Klebsiella pneumoniae, Pseudomonas putida* or *Candida parapsilosis.* These three mascaras were used by participants wearing hard contact lenses. These same participants, in study groups with other mascaras, usually demonstrated an IC above 20. The two mascaras (D, H) containing only parabens frequently yielded bacteria 4 to 8 hr after use, but no bacteria were recovered from these containers after they were stored for ten days.

The membrane challenge tests are summarized in Table III. As with the study groups, mascaras B, D, and H appeared to have relatively ineffective preservative systems. Mascaras B and D supported growth of the challenge organisms, whereas extended survival of the inoculum was found on mascara H. Direct challenge of organisms into the containers of these three mascaras with culture after seven days gave positive results only for mascara D with *P. aeruginosa.*

Two new lots of mascara B, one obtained just after production and one fortified with Dowicil 200 instead of imidazolidinylurea, and one new lot of mascara D supplemented with thimersol were obtained. These mascaras were given to small study groups of six to eight individuals and challenged with the double membrane procedure. The study group IC for the three was less than seven after 25 uses with none showing established contaminants. No organisms were recovered at three days with the membrane challenge test. Containers of these mascaras were stored (closed) for seven days at 37 and 56°C and then challenged with the membrane test. Only mascara B containing parabens and imidazolidinylurea (Table I) that had been kept at 37 and 56°C supported growth of bacteria. The other mascaras showed a slight decrease in inhibitory activity after heat treatment with 2 to 3 per cent of the challenge inocula recoverable after one day. A loss of 1 to 3 per cent in inhibitory activity was observed for mascaras A and F after one year of storage at room temperature. The exact age of mascara B tested in Table III is unknown. Mecurials and the formaldehyde preservative appeared more stable to heat than imidazolidinylurea.

Table III
Recovery of Challenge Organisms with Membrane Test

Challenge Organisms (1×10^6)	Mascaras A	B	C	D	E	F	G	H	WP*
S. epidermidis	<0.1**	51	0	6	0	0	<0.3	9	37
P. aeruginosa	0	3	0	>100	0	0	0	2	14
*P. aeruginosa****	—****	>100	0	>100	0	0	0	46	15

*WP, white petrolatum control.

**Percentage of inoculum recovered after three days, mean of ten tests.

$$S^2 = \Sigma \frac{(x_i - \bar{x})^2}{n - 1}.$$

***Sequential challenge, initial challenge with *S. epidermidis*.

****—not done.

Ramp and Witkowski (4) defined a well preserved cosmetic as one which would reduce a microbial challenge by at least 99.99 per cent in a relatively short period of time. If introduction of inocula directly into mascara containers is used to determine preservative effectiveness, the 99.99 per cent guideline may be obtainable as was shown in this study. This test, however, may not reflect the stability of the preservative system under conditions of use. In this preliminary analysis, the membrane test (Table III) appeared to correlate more closely with the in-use, study-group results (Table II) than with the container challenge test.

The membrane test was designed specifically for anhydrous products, but it appeared also to function with emulsion mascaras. The test permitted the sequential challenge of an aliquot of mascara with different microorganisms. Previous studies have suggested that contamination with *S. epidermidis* may make mascaras more susceptible to subsequent attack by pseudomonads (3). In this study, *P. aeruginosa* grew on mascara B following but not prior to challenge with *S. epidermidis*. Mascaras are applied frequently in humid bathrooms and condensation may occur in the mascara container. Cidal activity in this moisture layer appears necessary for the proper preservation of emulsion and anhydrous mascaras. Rapid death of the challenge organisms on the membrane may reflect the ability of the microbial inhibitors to diffuse and function in the microlayer of water on the surface of the cosmetic.

ACKNOWLEDGEMENT

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Hydagen F, ein neuer Hautfeuchtigkeits-regulator - Methoden und Ergebnisse des Wirkungsnachweises

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Synopsis — Screening procedures for the **in vitro** testing of potential regulators of skin moisture are proposed. In addition, FMIR analysis has the potential for comparing the **in vivo** hydration condition of stratum corneum after the application of cosmetic emulsions. Hydagen F, a partial sodium salt of a polyhydroxycarboxylic acid, performed positively in these tests. Good tolerance for this new cosmetic ingredient is expected in view of extensive toxicological studies.

1. Einleitung

Die Erforschung der Grundlagen der Hautfeuchtigkeitsregulation hat Anfang der 50er Jahre durch die Dermatologie entscheidenden Auftrieb erhalten. Es konnte nachgewiesen werden, daß durch ein Gemisch wasserlöslicher Verbindungen, das als Hauptkomponenten 2-Pyrrolidon-5-carbonsäure, Aminosäuren, Lactate, Harnstoff und verschiedene Zucker enthält, Feuchtigkeit in den obersten Hautschichten festgehalten wird. In der Kosmetikliteratur ist dieses System unter dem Begriff Natural Moisturizing Factor (NMF) bekannt.

Weiterhin ist erwiesen, daß die Erscheinungsform der im kosmetischen Sinne trockenen, rauhen und rissigen Haut nicht vom Fettgehalt, sondern in besonderem Maße vom Wassergehalt des Stratum corneum abhängt.

Nachdem dieser Zusammenhang erkannt war, hat es zahlreiche Versuche gegeben, durch kosmetische Wirkstoffe in diesen Mechanismus einzugreifen. Auf dem Markt wurden Präparate mit „Feuchtigkeitsfaktoren" und "moisturizing cremes" angeboten, deren Anzahl eine ständig steigende Tendenz aufweist.

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Das Hauptproblem bei der Entwicklung und Prüfung von Hautfeuchtigkeitsregulatoren liegt darin, daß die Messung ihrer Effekte schwierig ist. Da die meßtechnische Erfassung von Effekten jedoch Voraussetzung zum Auffinden von Wirkstoffen ist, befaßten auch wir uns zunächst mit der Entwicklung von Prüfmethoden. Über die Resultate dieser Arbeiten und die Entwicklung eines neuen Wirkstoffes wird im folgenden berichtet.

2. Meßmethoden

2.1 In vitro-Nachweise

Für ein Screening potentieller Hautfeuchtigkeitsregulatoren wurden die folgenden in vitro-Modelle entwickelt:

2.1.1 Ermittlung der Gleichgewichtsfeuchte

Proben der zu untersuchenden Substanzen (ca. 300-500 mg) wurden mit einer definierten Menge Wasser angefeuchtet und bei 23° 24 Stunden verschiedenen Luftfeuchtigkeitsgehalten (1%, 30%, 47%, 65%, 89% und 100% relative Feuchtigkeit) ausgesetzt. Die Werte der aufgenommenen bzw. abgegebenen Wassermengen wurden gravimetrisch bestimmt und graphisch aufgetragen. Aus den hieraus resultierenden Kurven kann diejenige relative Feuchtigkeit ermittelt werden, bei der weder Wasserabgabe noch Wasseraufnahme erfolgt. Dieser Wert, den wir als Gleichgewichtsfeuchte bezeichnen, ist ein Maß für das Wasserretentionsvermögen einer Substanz. Je niedriger der Wert liegt, um so positiver ist das Produkt zu beurteilen. Aus der Steilheit der Kurve läßt sich weiterhin das Wasseraufnahmevermögen (Hygroskopizität) der Substanz ablesen.

2.1.2 Messungen an der Schweineepidermis

Als Modell wurde Schweineepidermis gewählt, weil sie der menschlichen Haut in ihrem Aufbau ähnlich ist.

2.1.2.1 Gewinnung der Schweineepidermis

Unmittelbar nach dem Töten der Schweine wurden die Borsten der Haut mittels einer Haarschermaschine (Scherkopf 0,1 mm) abgeschnitten. Die Schweine wurden in 50-60° warmem Wassser ca. 3-5 Minuten gebrüht, die Epidermis anschließend abgeschält und bei −20° bis zum Gebrauch gelagert.

2.1.2.2 Bestimmung der Wasserretention sowie der Rehydratation von Schweineepidermis

Ausgestanzte Epidermisstückchen (1 x 2 cm) wurden 2 Stunden in eine 10-prozentige Lösung der Prüfsubstanz gelegt, unter standardisierten Bedingungen mittels einer kleinen Presse abgetupft und 24 Stunden zwischen 2 Klammem frei hängend in einem 100 ml Erlenmeyerkolben bei 23° und 30% bzw. 50% relativer Feuchtigkeit (eingestellt durch Schwefelsäure-Wasser-Mischungen) getrocknet. Die Austrocknung der imprägnierten Probe auf X-% des Anfangsgewichtes wurde mit dem entsprechenden Wert einer in reines Wasser gelegten Epidermis (Blindwert) verglichen. Die Verbesserung der Wasserretention sowie der Rehydratation gegenüber dem Blindwert wurde in △ % H_2O angegeben. Die Abweichungen betrugen bei den jeweiligen Doppelversuchen maximal ± 2 absolute Einheiten. Bei größeren Abweichungen wurde der Versuch wiederholt. Die Rehydratation wurde durch 24-stündiges Trocknen der imprägnierten und abgetupften Schweineepidermis bei 30% relativer Feuchtigkeit und anschließende 24-stündige Inkubation bei 90% relativer Feuchtigkeit analog bestimmt.

2.1.2.3 Elastizitätsmessungen von Schweineepidermis

Das elastische Verhalten der Epidermis wird durch den Hydratationszustand des Stratum corneum maßgeblich beeinflußt. Wird die mit Feuchtigkeitsregulatoren imprägnierte Schweineepidermis bei konstanter Luftfeuchtigkeit getrocknet, so kann die hydratisierende Wirkung der Prüfsubstanzen durch vergleichende Elastizitätsmessungen mit der in Wasser gelagerten Epidermis bestimmt werden. Hierzu werden ausgestanzte Epidermisstückchen (1 x 6 cm) zwei Stunden in 10-proz. wäßrigen Lösungen des zu prüfenden Produktes gelegt und unter standardisierten Bedingungen abgetrocknet (vgl. 2.1.2.2). Die Proben wurden zwischen zwei Klammern frei hängend bei 90% relativer Feuchte 24 Stunden inkubiert und in einer Zugprüfmaschine* bei 0-50 p Belastung mit einem Vorschub von 10 mm/min gedehnt. Als Maß für die Elastizität wurde die Dehnung in mm angegeben, die im linearen Teil der Kraft-Dehnungskurven bei einer Belastung zwischen 5-30 p gemessen wurde.

2.2 In vivo-Nachweis

Entscheidend für den Wirkungsnachweis ist der Nachweis der Feuchtigkeitsretention am Menschen. Bei diesen Prüfungen muß unbedingt darauf

* Fa. Zwick & Co., (Type 1402), Einsingen/Donau

geachtet werden, daß auch tatsächlich am Wirkungsort gemessen wird, d.h., daß bei Bestimmungen des Wassergehaltes nur die Feuchtigkeit des Stratum corneum erfaßt wird. Hierfür ist z.B. die von N.A. Putnam 1972 (1) und von der Dow Chemical Corp. 1974 (2) beschriebene FMIR-Analyse* prinzipiell geeignet.

Die Methode beruht darauf, daß die menschliche Haut bei 1645 cm^{-1} und 1545 cm^{-1} zwei charakteristische starke Absorptionsbanden zeigt, von denen die sog. Amid I-Bande des Hautproteins mit der OH-Deformationsschwingung des Wassers zusammenfällt, während die andere — Amid II-Bande — dem Protein allein zuzuordnen ist. Durch Anwesenheit von Wasser wird das Intensitätsverhältnis Amid I/II entsprechend erhöht. Das beobachtete Intensitätsverhältnis Amid I/II ist somit ein Maß für die Feuchtigkeit der Haut.

Wir verwendeten einen "Skin Analyzer" der Wilks Scientific Corp., Norwalk/USA, mit weit herausgeführter Kristallhalterung und zwei spiegelbildlich angeordneten FMIR-Einheiten, die auf einem Rahmen in einem IR-Gitterspektrometer (Perkin-Elmer, Mod. 621) montiert waren. Bei der praktischen Durchführung der Messung wurde die Unterarminnenseite der Testpersonen mittels einer entsprechenden Vorrichtung mit einem konstanten Druck von 0,3 kp/cm^2 gegen den Germaniumkristall (Außenfläche 2 x 5 cm) dieser FMIR-Anordnung gepreßt.

An der mit der Haut des Unterarms in direkter Berührung stehenden Germaniumoberfläche wird bei den Frequenzen, die die Probe zu absorbieren vermag, die innere Totalreflexion vermindert und dadurch der IR-Strahlung das Spektrum der Hautoberfläche „aufgeprägt".

Da die Eindringtiefe der Strahlung bei Verwendung des Kristalls nur einige Mikron beträgt, beziehen sich die Ergebnisse ausschließlich auf die oberste Hautschicht (Stratum corneum), die Ziel der Untersuchung ist. Das charakteristische Hautspektrum in diesem IR-Bereich zeigt die folgende Abbildung 1.

* FMIR = Frustrated Multiple Internal Reflection

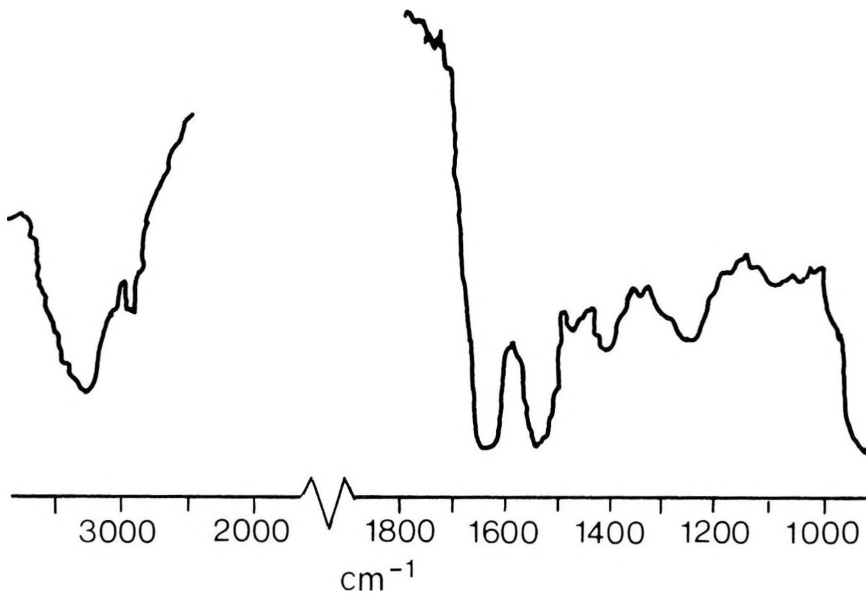
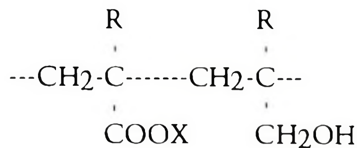


Abb. 1: IR-Spektrum der Haut

3. Ergebnisse der Wirksamkeitsprüfung von Hydagen F

Bei der Suche nach geeigneten Hautfeuchtigkeitsregulatoren für kosmetische Präparate gelangten wir zu einem Partial-Na-Salz einer Polyhydroxycarbonsäure, deren Struktur in idealisierter Form wie folgt angegeben werden kann:



wobei R = H, -CH_2OH oder -COOX bedeuten
und X = H oder Na sein kann (3).*

Die Ergebnisse der Wirksamkeitsprüfung sind im folgenden zusammengefaßt:

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* Dieses Produkt ist der Firma Henkel KGaA inzwischen unter der Bezeichnung Hydagen F warenzeichenrechtlich geschützt.

3.1 In vitro-Tests

3.1.1 Messung der Gleichgewichtsfeuchte

Hydagen F wurde im Vakuumexsikkator über konz. H_2SO_4 bis zur Gewichtskonstanz getrocknet. Zur Messung wurden jeweils 20 Proben mit einer definierten Wassermenge angefeuchtet und bei verschiedenen Feuchten, wie unter 2.1.1 beschrieben, gelagert. Die Mittelwerte und Standardabweichungen der Wasseraufnahme bzw. -abgabe, die sich nach 24 Stunden einstellten, sind aus Abbildung 2 ersichtlich.

Hieraus ergibt sich eine „Gleichgewichtsfeuchte" von 41 % rel. Feuchte, d.h., oberhalb dieser rel. Feuchte nimmt Hydagen F Wasser auf und unterhalb dieses Wertes gibt das Prudukt allmählich Wasser ab.

Der entsprechende Wert betrug für Kochsalz ca. 75 % rel. Feuchte und für ein dem NMF nacherstelltes synthetisches Produkt ca. 58 % rel. Feuchte.

J.H. Blank (4) stellte fest, daß bei rel. Feuchten von 60% ein Gleichgewichtszustand besteht, bei dem der Feuchtigkeitsgehalt der Hornhaut nicht unter 10mg/100mg Trockengewicht abfallen kann, und daß oberhalb dieser rel. Feuchte das Stratum corneum weich und elastisch bleibt. Unterhalb von 60% rel. Feuchte kann die Hornhaut austrocknen und brüchig werden.

Demnach würde Kochsalz, obwohl es bei hohen Feuchten extrem viel Wasser aufzunehmen vermag, bei durchschnittlichen Luftfeuchtigkeiten von 40-60% rel. Feuchte eher austrocknend als hydratisierend wirken. Da die Gleichgewichtsfeuchte von Hydagen F bei ca. 41% rel. Feuchte liegt, ist die Voraussetzung, der Austrocknung des Stratum corneum entgegenzuwirken, prinzipiell gegeben.

3.1.2 Bestimmung der Wasserretention und Rehydratation von mit Hydagen F behandelter Schweineepidermis

Nach der in Abschnitt 2.1.2.2 beschriebenen Methode wurden Wasserretention und Rehydratation von Schweineepidermis bestimmt, die mit wäßrigen Hydagen F-Lösungen behandelt worden waren. In Tabelle 1 sind die Ergebnisse von jeweils 20 Einzelmessungen zusammengestellt. Auch in diesem Modell zeigt Hydagen F gute wasserretinierende sowie rehydratisierende Eigenschaften.

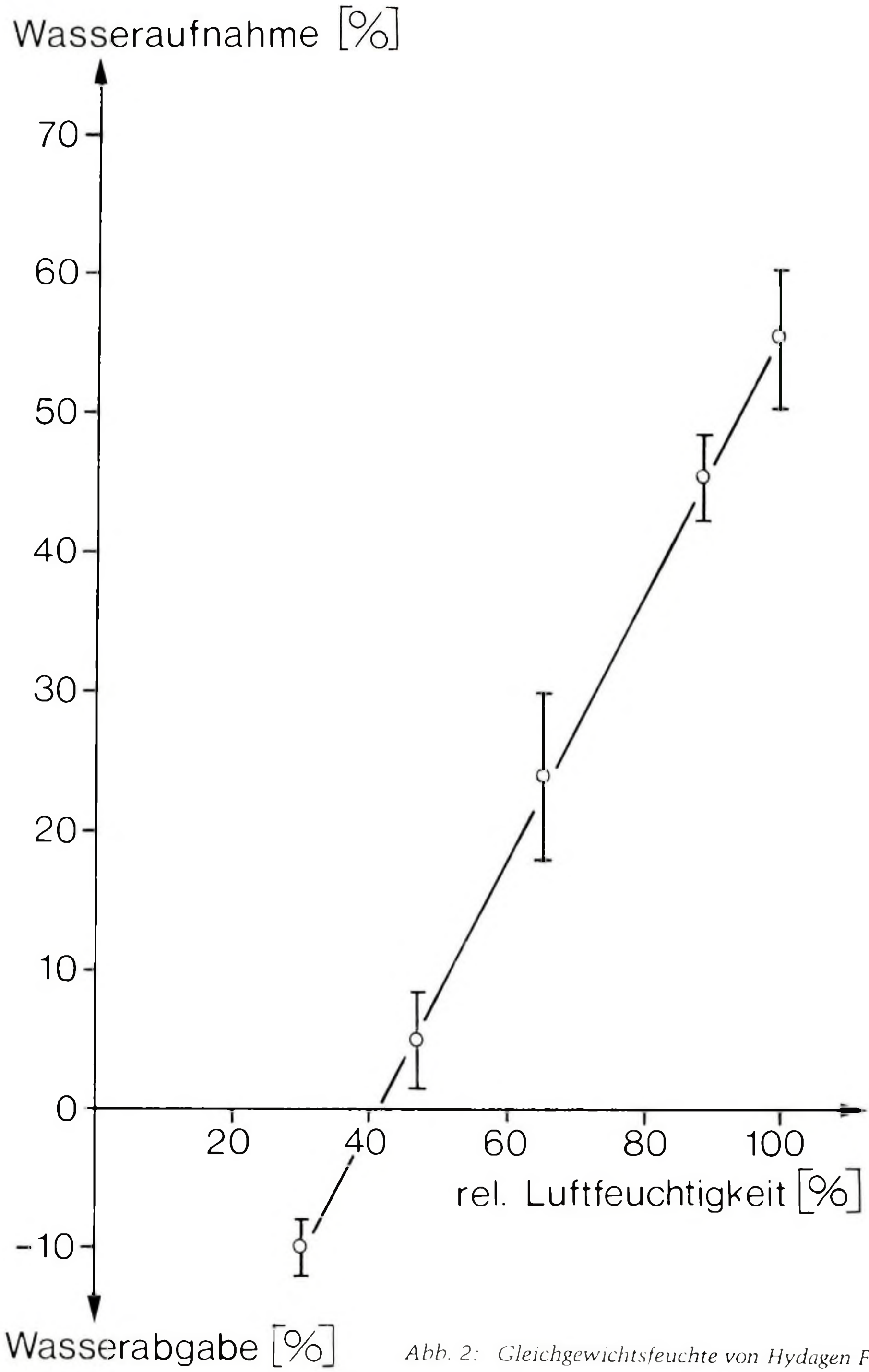

Abb. 2: Gleichgewichtsfeuchte von Hydagen F

Produkt	Wasserretention Δ% H_2O nach Austrocknung bei		Rehydratation Δ% Wasseraufnahme bei
	30% r.F.	50% r.F.	90% rel.F.
Wasser (Vergleichswert)	0	0	0
Hydagen F (10-proz. Lsg.)	10,2 ± 2,1	16,9 ± 3,5	27,6 ± 7,9

Tabelle 1

Ergebnisse der Wasserretention und Rehydratation von Schweineepidermis, die mit Hydagen F behandelt wurde.

3.1.3 Ergebnisse der Dehnungsmessungen der mit Hydagen F behandelten Schweineepidermis

Da Dehnungsmessungen mit biologischem Material stets uneinheitlich sind, ist eine größere Zahl von Messungen erforderlich. Aus jeweils 40 Einzelmessungen resultierten für die Wasserbehandlung (Vergleichswert) Dehnungswerte von 0,3 - 0,5 mm; für die mit Hydagen F behandelte Epidermis hingegen Dehnungswerte von 3,3 ± 0,7 mm. Typische Kraft-Dehnungsdiagramme einer lediglich mit Wasser und einer mit 10-proz. wäßriger Hydagen F-Lösung behandelten Epidermis zeigt Abbildung 3.

Die nur mit Wasser behandelte Epidermis ist unelastisch und trocken, während die mit Hydagen F behandelte Haut noch genügend Wasser enthält und daher elastisch und dehnbar ist.

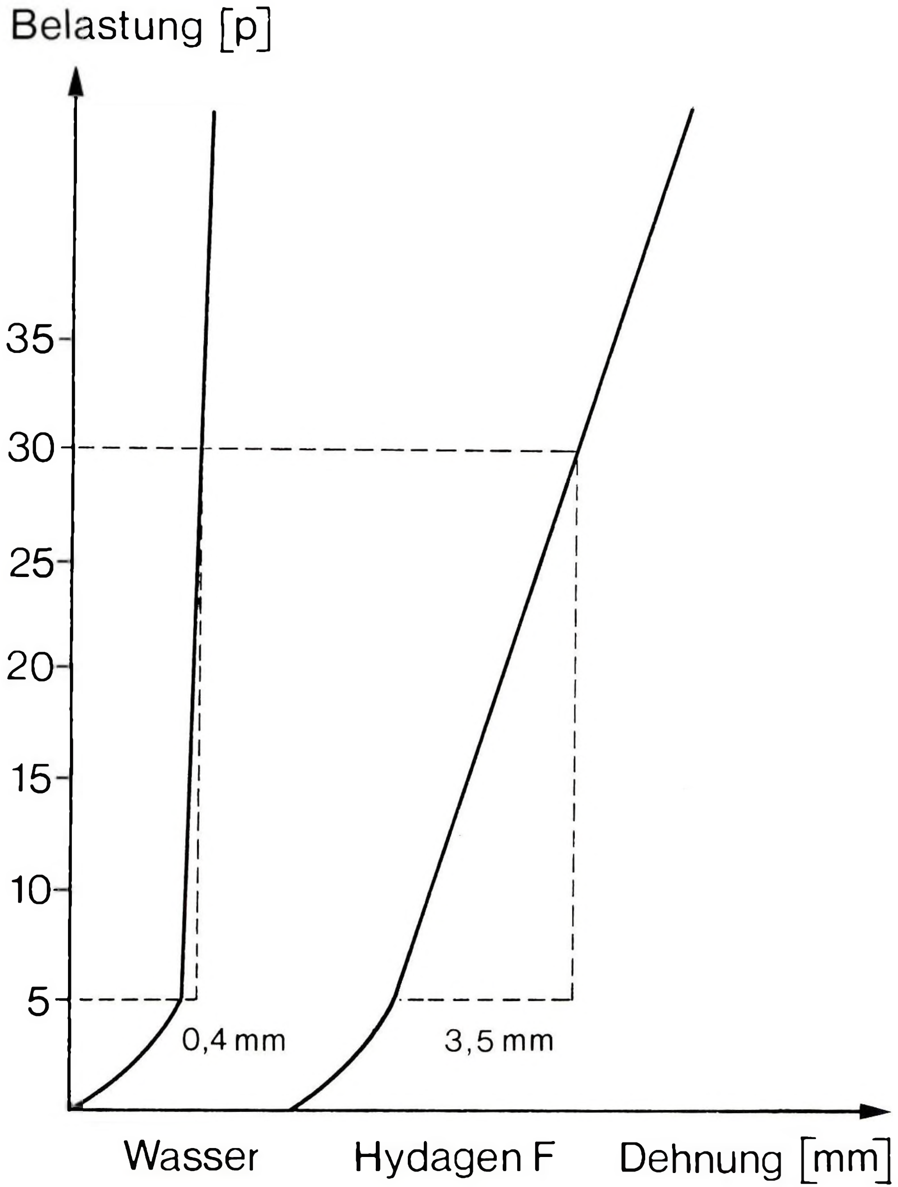

Abb. 3: Kraft-Dehnungsdiagramme von Schweineepidermis nach 24 Stunden Inkubation bei 90% rel. Feuchte.

3.2 In vivo-Prüfung von Hydagen F

Zur in vivo-Prüfung der hauthydratisierenden Wirkung wurden 3% Hydagen F in folgende O/W-Rahmenrezeptur eingearbeitet und an 11 weiblichen Probanden im Vergleich zur entsprechenden wirkstofffreien Emulsion getestet:

Paraffinöl 40 cP	17,0 %
Myritol 318® (Henkel)	2,0 %
Lanette 0® (Henkel)	2,5 %
Stearinsäure	2,8 %
Carbopol 940® (Goodrich)	0,5 %
Hydagen F®	3,0 %
Triisopropanolamin	3,16%
Phenonip® (Nipa Lab.)	0,1 %
Wasser	68,94%

3.2.1 Applikation der Cremes

Die Unterarminnenseite der Testpersonen wurde mit Äther entfettet, mit einer Syndet-Seife intensiv gewaschen, 1 Minute mit Leitungswasser gespült, dann noch zweimal mit der Syndet-Seife für jeweils 15 Sekunden gewaschen und sorgfältig mit Wasser abgespült. Zur Trocknung wurde der Arm abgetupft und 5 Minuten gefönt. Nach weiteren 5 Minuten erfolgte die erste Messung an einer zuvor markierten Teststelle von 10 cm^2 der Unterarminnenseite. Darauf wurde eine eingewogene Menge Creme mit einem stumpfen Plexiglasspatel auf die markierte Fläche 2 Minuten lang einmassiert und die überschüssige Creme sorgfältig abgestrichen und zurückgewogen. Die aufgetragene Crememenge betrug 10 ± 2 mg/cm^2. Nach Einziehen der Creme (2,5 Minuten) erfolgte die zweite Messung. Weitere Messungen wurden 0,5 Stunden und 1,5 Stunden nach der ersten Messung durchgeführt. Für die vergleichenden Untersuchungen wurde an einem Arm die wirkstoffhaltige und an dem anderen die wirkstofffreie Creme appliziert.

3.2.2 Vorversuche zur FMIR-Analyse Hydagen F-haltiger Cremes

Bei den Messungen können sich aufgrund von Überlagerungen der charakteristischen IR-Banden durch Bestandteile der Emulsionsgrundlagen sowie durch den Wirkstoff selbst Schwierigkeiten ergeben. Diese Faktoren mußten daher zunächst überprüft werden.

a) Creme ohne Hydagen F

Das Spektrum der mit Äther und Seife gereinigten und anschließend getrockneten Haut des linken Armes einer Versuchsperson zeigte im betreffenden IR-Bereich praktisch nur die Amid I-Bande (1645 cm^{-1}), die Amid II-Bande (1545 cm^{-1}) sowie einen geringen Carboxylgehalt (1400 cm^{-1}) an. Das Intensitätsverhältnis Amid I zu Amid II (Auswerteverfahren nach der Basislinienmethode) betrug ca. 1,3.

Die Haut wurde sodann mit der wirkstofffreien Creme behandelt (3.2.1) und 2,5 Minuten, 30 Minuten sowie 90 Minuten nach Applikation der Creme vermessen. Alle drei Spektren zeigten praktisch ein identisches Bild.

Die ermittelten Intensitätsverhältnisse, Amid I zu Amid II, ergaben folgende Werte:

Messung nach 2,5 min.: 1,2
Messung nach 30,0 min.: 1,4
Messung nach 90,0 min.: 1,4

Innerhalb der Fehlergrenzen sind die Ergebnisse von unbehandelter Haut und behandelter Haut gleich, d.h., es erfolgte keine meßbare Erhöhung des Wassergehaltes der Hautoberfläche. Die Bestandteile der Emulsionsgrundlage störten bei der Messung nicht.

b) Creme mit 3% Hydagen F

Wiederum wurde die zuvor gereinigte, unbehandelte Haut sowie die mit Hydagen F-Creme behandelte Haut am rechten Arm vermessen.

Das Spektrum des unbehandelten rechten Arms war praktisch identisch mit dem des unbehandelten linken Arms. Das Intensitätsverhältnis Amid I zu Amid II betrug wiederum ca. 1,3.

Die Amid II-Bande wurde nun von der asymmetrischen C-O-Valenzschwingung der vorhandenen Carboxylat-Anionen unterlagert, weshalb eine entsprechende Korrektur der Intensität der 1545 cm^{-1}-Bande erfolgte. Dies geschah durch Substraktion der bei 1400 cm^{-1} gemessenen Intensität des gleichen Spektrums, multipliziert mit dem natürlichen Intensitätsverhältnis der asymmetrischen und symmetrischen C-O-Valenzbande (1500 bzw. 1400 cm^{-1}) des Carboxylat-Anions. Für diesen Faktor wurde in unserem Fall der Wert 1,5 festgelegt, der einen guten Mittelwert für verschiedene Carboxylate darstellt; danach ergaben sich folgende Intensitätsverhältnisse für Amid I zu Amid II:

Messung 2,5 min. nach Applikation der Creme: 2,1
Messung 30,0 min. nach Applikation der Creme: 3,3
Messung 90,0 min. nach Applikation der Creme: 2,7

Das Verhältnis der Amid I- zur Amid II-Bande hatte folglich gegenüber der Creme ohne Hydagen F deutlich zugenommen und zeigte damit einen höheren Wassergehalt des Stratum corneum an.

Quantitative (prozentuale) Aussagen sind jedoch nur bedingt möglich, da die Auswertung der Bandenintensitäten mit der Basislinienmethode nach subjektivem Ermessen erfolgen muß.

3.2.2 Tests mit mehreren Probanden und Zusammenfassung der Versuchsergebnisse

Nach den unter 3.2.2 beschriebenen orientierenden Versuchen wurde die O/W-Creme an 10 weiteren Testpersonen vermessen. Die Ergebnisse sind der Tabelle 2 zu entnehmen.

Nach den vorliegenden Ergebnissen kann festgestellt werden, daß bei Anwendung der Creme ohne Hydagen F in allen Fällen bereits nach 0,5 Stunden bzw. 1,5 Stunden ein starker Rückgang des Wassergehaltes des Stratum corneum zu verzeichnen war. Der Mittelwert des Intensitätsverhältnisses Amid I/Amid II betrug 1,4. Dieser Quotient war bei den entsprechenden Versuchen mit Hydagen F deutlich erhöht (Mittelwert: 2,7).

Die Differenz der Mittelwerte ohne und mit Hydagen F ist signifikant. Somit darf eine erhöhte Wasserretention der Haut durch Anwendung der Hydagen F-Creme als sichergestellt gelten.

3.3 Toxikologische Untersuchungen

Als potentieller kosmetischer Wirkstoff wurde Hydagen F umfangreichen toxikologischen Prüfungen unterzogen. Die akute Toxizität wurde an Mäusen bei oraler Applikation untersucht und ergab einen LD_{50}-Wert von > 5 g/kg. Zur Prüfung der subakuten Toxizität wurde ein 90-Tage-Test an Ratten unter SPF-Bedingungen durchgeführt. Bei Versuchsende wurden bei allen Ratten zahlreiche Parameter des Blutes und Harns untersucht. Die inneren Organe wurden nach Tötung der Tiere gewogen und histologisch untersucht. Selbst bei einer Dosis von 5000 ppm wurden keine Befunde beobachtet, die auf eine toxische Wirkung der Testsubstanz zurückzuführen wären. Weiterhin wurde die Hautverträglichkeit an haarlosen und weißen Mäusen sowie an Albino-Kaninchen und an Meerschweinchen

getestet. Die Schleimhautverträglichkeitsprüfung erfolgte am Kaninchenauge.

In keinem dieser Tests wurden toxische Reaktionen festgestellt.

Tabelle 2:

Amid I / Amid II — Bandenverhältnis als Kreterium der Feuchtigkeitsretention.

Proband Nr.	gereinigte und getrocknete Haut		2,5 Minuten n. Applikation der Creme	0,5 Stunden n. Applikation der Creme	1,5 Stunden n. Applikation der Creme
1	links 1,3 rechts 1,3	ohne Hydagen F mit Hydagen F	1,2 2,1	1,4 3,3	1,4 2,7
2	links 1,5 rechts 1,7	ohne Hydagen F mit Hydagen F	1,9 2,1	1,3 2,5	1,4 3,2
3	links 1,7 rechts 1,5	ohne Hydagen F mit Hydagen F	1,8 2,0	1,6 2,3	1,7 2,6
4	links 1,1 rechts 1,1	ohne Hydagen F mit Hydagen F	1,2 3,9	1,3 2,0	1,3 2,4
5	links 1,1 rechts 1,7	ohne Hydagen F mit Hydagen F	2,0 2,0	1,0 3,7	0,9 2,0
6	links 1,4 rechts 1,3	ohne Hydagen F mit Hydagen F	1,2 1,9	1,8 ••	1,6 ••
7	links 1,1 rechts 1,1	ohne Hydagen F mit Hydagen F	3,0 3,6	1,9 3,4	1,3 2,8
8	links 1,2 rechts 1,6	ohne Hydagen F mit Hydagen F	1,9 4,0	1,2 5,7	1,8 2,1
9	links 1,1 rechts 1,4	ohne Hydagen F mit Hydagen F	3,6 2,7	1,6 ••	2,1 ••
10	links 1,2 rechts 1,2	ohne Hydagen F mit Hydagen F	3,8 2,5	1,2 3,3	1,7 2,1
11	links 1,2 rechts 1,2	ohne Hydagen F mit Hydagen F	3,9 3,5	1,4 1,9	1,5 3,0

•• Auswertung wegen sehr kleiner Intensitäten nicht möglich.

Zusammenfassung

In der vorliegenden Arbeit werden in vitro-Screening-Modelle zur Prüfung potentieller Hautfeuchtigkeitsregulatoren vorgestellt; weiter wird über die FMIR-Analyse als Möglichkeit zur vergleichenden in vivo-Messung des Hydratationszustandes des Stratum corneum nach Applikation kosmetischer Emulsionen berichtet.

Hydagen F, ein Partial-Natriumsalz einer Polyhydroxycarbonsäure, zeigte in diesen Tests ein besonders positives Verhalten. Die Verträglichkeit dieses neuen kosmetischen Wirkstoffs wurde durch umfangreiche toxikologische Untersuchungen abgesichert.

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Further experience with a topical cream for depigmenting human skin

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Synopsis

Satisfactory LIGHTENING of HYPERPIGMENTARY SKIN disorders was obtained by twice-daily APPLICATION for two to three months of a PREPARATION containing 5.0% hydroquinone, 0.1% Vitamin A acid (tretinoin) and 0.1% dexamethasone, a flourinated corticosteroid.

Excellent results were secured in melasma (chloasma) of white females. Two common pigmentary disturbances in blacks also responded well, namely, the residual hyperpigmentation left by inflammatory acne lesions and in bearded areas affected by ingrown hairs (pseudofolliculitis). Although senile lentigines were not moderated in patients over 65 years of age, good results were observed in patients 40 to 60 years old.

The depigmentation is completely reversible and is not attended by significant local or systemic side effects.

INTRODUCTION

In a previous work, we showed that a formulation containing Vitamin A acid (tretinoin), hydroquinone and a corticosteroid could bring about complete loss of melanin from the skin of normal blacks and was highly beneficial in disorders of hyperpigmentation, notably melasma (chloasma), freckles and excess pigmentation following inflammation (1).

Bleaching occurred despite an increase in the density of pigment-forming cells (melanocytes). Each of the three components was essential for effectiveness. Hydroquinone is known to interfere with the tyrosine–tyrosinase pathway of melanin synthesis. This drug also causes subcellular membrane damage and inhibits the formation of melanosomes, the organelles in which melanin is packaged (2). By itself, its effectiveness is too limited. How tretinoin and corticosteroids contribute to the depigmenting action is conjectural. The latter may inhibit melanin production by melanocytes in the same way that it suppresses collagen synthesis by fibroblasts, presumably through a repression of the general metabolic activity of the cell; steriods are known to be cytostatic to the epidermis (3). Tretinoin, on the other hand, stimulates cell turnover (4)

and it seems a likelihood that the rapid outward migration of cells might interfere with the transfer of pigment granules from melanocytes to keratinocytes. Thus the triad of active components collaborate to curtail the synthesis of melanin, reduce the production of membrane-bound melanosomes in which the pigment is aggregated, impair the donation of melanosomes to keratinizing cells and promote the more rapid loss of pigment *via* increased epidermopoesis.

Before a combination of such pharmacologically potent drugs can come into general use, there must be extensive clinical evaluation to learn the advantages and limitations in the therapy of hyperpigmentary disorders. The latter are misery-inducing conditions which cause great emotional suffering; they should not be viewed as mere cosmetic nuisances. In this paper we shall report further experiences and a new indication for this depigmenting formulation.

MATERIALS AND METHODS

COMPOSITION AND SUBJECTS

The subjects were out-patients of the Hospital of the University of Pennsylvania and the test formulation was:

Tretinoin	0.1 per cent
Hydroquinone	5.0 per cent
Dexamethasone	0.1 per cent
Hydrophilic Ointment U.S.P. q.s.	

and material was never more than two months old.

CLINICAL STUDIES

MELASMA

The subjects were 19 young adult females whose facial pigmentation was linked to the taking of contraceptive pills. The cream was applied before retiring once daily for the first week. In all but a few who experienced too much discomfort from peeling and dryness, the exposure was increased to twice daily to speed up the response.

Lightening was generally clear-cut by the third to sixth week. By 12 weeks, the hyperpigmented areas had virtually blended with the surrounding normal skin in 16 of the 19 patients. In these, the results were excellent. The normal skin of white persons is comparatively resistant to the lightening effect. The results were only moderately good, however, in two patients, though both expressed satisfaction with the result. One patient was inexplicably resistant and did not achieve a satisfactory result even after four months. Once-daily application was sufficient for maintaining the lightening at the desired level.

POSTINFLAMMATORY HYPERPIGMENTATION

Hyperpigmentation is a very common residium of inflammatory disorders on the skin of blacks. Intense pigmentation may follow a variety of pathologic changes: *via* insect bites, contact allergy, abrasions, burns, etc. (Figures 1, 2). Damage to the skin, no mat-

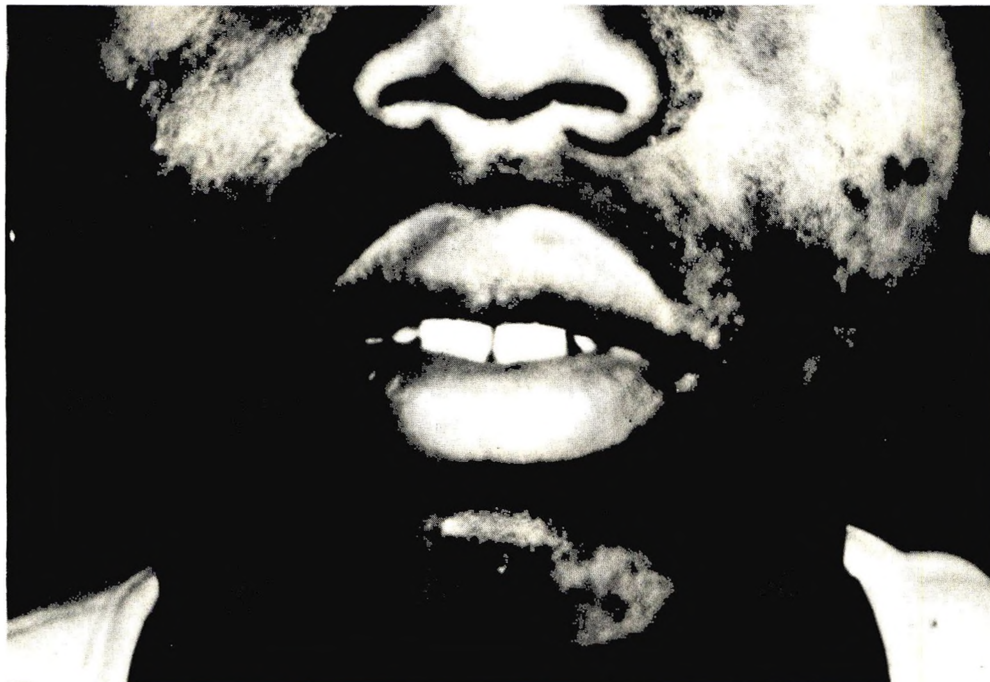

Figure 1. Postinflammatory hyperpigmentation following patient's use of abradant and anti-acne lotion to treat acne vulgaris

Figure 2. After 8 weeks of twice-daily application of depigmenting formula, hyperpigmentation was eliminated and acne vulgaris was under good control

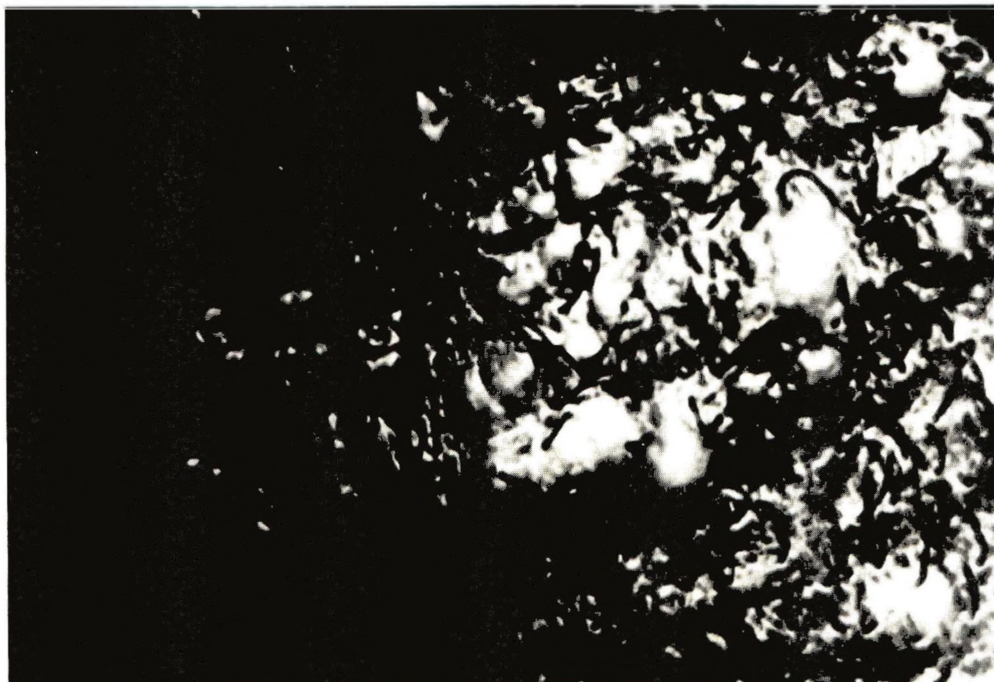

Figure 3. Papular lesions of pseudofolliculitis located on the neck of a 23 year old black male. Condition had persisted for approximately seven years

ter how induced, characteristically leads to an increase in the quantity of hyperfunctioning melanocytes.

Because of our interest in acne, we have come to appreciate how commonly inflammatory lesions leave conspicuous spots of hyperpigmentation on the face of blacks. Picking and squeezing of papulo-pustules, a pernicious habit of many acne patients, black and white, is particularly likely to induce excessive pigmentation. Another hyperpigmenting disorder, especially common in black males, is pseudofolliculitis of the beard in which ingrowing hairs produce inflammatory papules and pustules. The beard area shows a splotchy, mottled pattern of intense melanization. Speckling is prominent around the follicles. These are distressing, embarrassing afflictions with which we have long been concerned, ineffectively for the most part. The depigmenting formulation was adequately evaluated in 25 black acne patients, mainly females, and in 11 black males with pseudofolliculitis. These subjects completed three months of therapy. The patients were urged to use "spot" therapy, applying the medication with cotton-tipped applicators to each hyperpigmented site. The medication was applied twice daily using more of the material on the darkest spots. Peeling often occurred at the start, but generally abated after a few weeks.

Lightening was generally apparent by five to six weeks. Although the response was slower than with melasma, a satisfactory degree of lightening occurred in practically all acne patients by 12 weeks and in eight of the males with pseudofolliculitis (Figures 3 and 4). Color balance was more difficult to achieve in blacks. The best blending was achieved by patients who were concerned and adroit enough to confine the medicament precisely to the hyperpigmented spots. A small wisp of cotton wound around the tip of a toothpick was an effective applicator for spot therapy.

Figure 4. After 5 weeks of twice-daily application of formulation

A secondary benefit in some acne patients was a more rapid resolution of hyperpigmented papules. Probably both the tretinoin and the corticosteroid contributed to the healing effect of these long-lasting, hyperpigmented, inflammatory lesions.

SENILE LENTIGINES

Earlier trials in treating these lesions produced disappointing results. This was the outcome in patients who were applying the formulations as many as three or four times daily. Despite these results, we continued to use the formulation, noting an occasional success. In one review of the data, we realized that depigmentation was occurring in patients aged 50 to 60 years. The original study had included patients 65 years and older with the majority being over 70 years. We have since concentrated the use of the formulation in patients under 65 years of age. Thus far, we have tested 11 patients (40 to 60 years), recording good results in nine. The favorable response is apparent at one to four months of treatment. Applications are once to twice daily, dependent on the rate of resolution. This group is being expanded and a more detailed report of the findings will be published.

DISCUSSION

Adverse reactions to this combination have been very limited, the most common being mild to moderate irritation. We encourage patients to use enough medication to produce some scaling and dryness for the first few weeks of treatment. By deliberately provoking a mild irritant reaction, the patient is assured that sufficient drug has

penetrated. Actually, the skin becomes accommodated within a month or so of daily use and greater amounts can be applied without signs of irritation.

There have been no instances of contact sensitization or photosensitization. The skin may be more vulnerable to sunburning radiation during the irritative phase. Apart from this enhanced reactivity (*not* phototoxicity), there is a very important reason for avoiding midday erythemogenic sunlight or, alternatively, to use sunscreens, namely, that this radiation strongly antagonizes the lightening effect. Furthermore, after depigmentation has been secured, sun exposure may lead to rapid repigmentation within seven to ten days. In fact, there may be a genuine rebound in which the pigmentation becomes even greater than originally.

The depigmenting effect is always transient. Applications must be continued on a maintenance basis so long as the melanizing stimulus persists. Unlike substituted phenolic compounds such as monobenzyl ether of hydroquinone, hydroquinone itself does not destroy pigment-forming cells and the danger of permanent depigmentation does not exist. Indeed, we have demonstrated that the density of melanocytes is about doubled after depigmentation is achieved. (The added quantity of enzymically active melanocytes underlies the rebound phenomenon.) Again, in contrast to substituted phenols, we have never observed depigmentation in any region outside the area of application.

We have strenuously warned against the persistent use of flourinated steroids on the

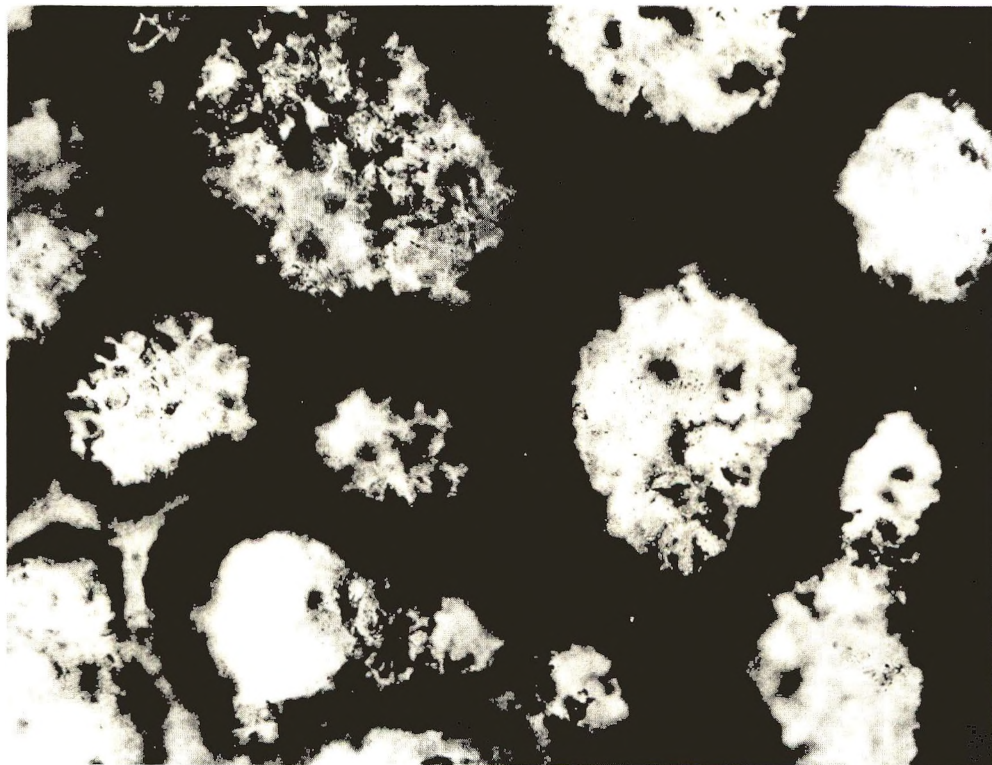

Figure 5. Pretreatment: Melanin granules are prominent in the basal layer, often in the form of caps over the nuclei. Pigment granules are visible in the horny layer of black skin (× 400)

face. All too often, middle-aged women will apply potent steroids for months or years to control some minor skin abnormality with the frequent result, usually without awareness of cause, of a steroid-induced eruption. The latter takes three forms: steroid acne, steroid rosacea and peri-oral dermatitis (5). Varying amounts of atrophy and dilated blood vessels are also well known steroid effects.

It is necessary to explain why we have never encountered adverse steroid effects in chronic users of the depigmenting cream. We think it exceedingly unlikely that steroid eruptions will ever turn up owing to the presence of tretinoin in the formulation. The biologic effects of tretinoin are virtually opposite to those of steroids. Tretinoin, for example, stimulates mitoses while steroids are inhibitory (3). Tretinoin promotes wound healing while steroids delay wound healing (6). The former is comedolytic (7), the latter enhances the formation of closed and open comedones (8). And so the potential damages that steroids could exert on skin structure, especially atrophy, are completely nullified by tretinoin.

A common condition which responds well to this formulation is freckles. Here there is no hope of restricting applications to the hyperpigmented spots. The whole area must be treated. This brings up the question of color blending—won't the normal skin between the freckles also become depigmented? One might end up with an ivory white landscape which would be a lot more noticeable than the original mottling. The fact is that normal skin is relatively consistant; the rule is that the greater the pigmenting, the

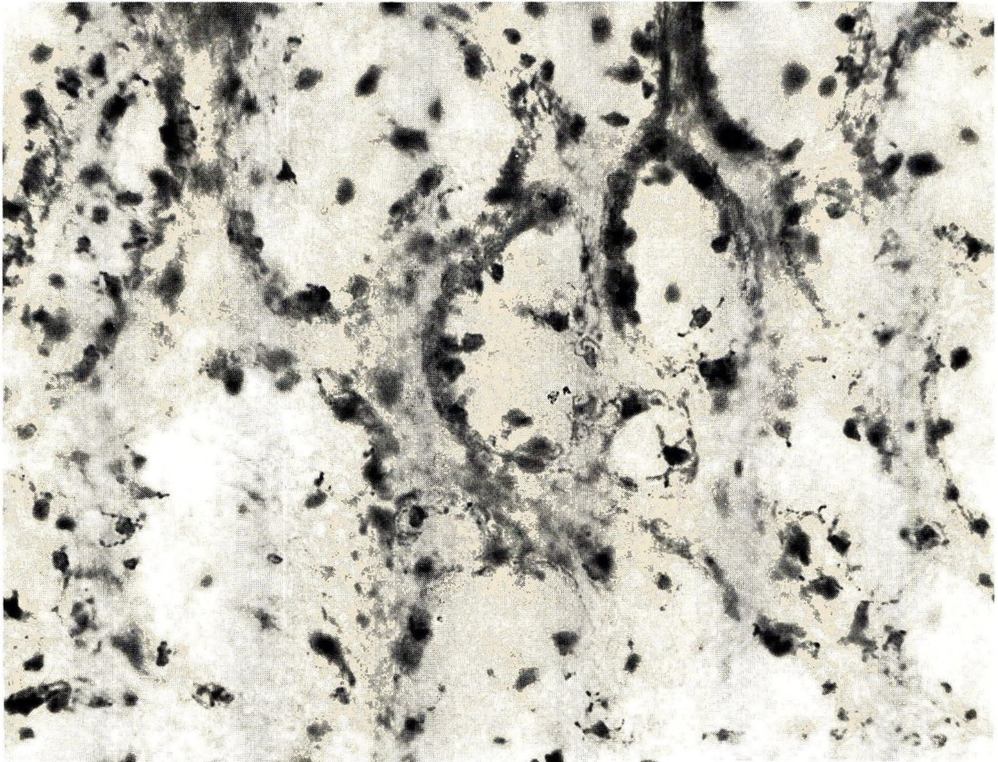

Figure 6. After 8 weeks of treatment, pigment granules, mainly in basal cells, can barely be made out ($\times$ 400)

easier it is to achieve depigmentation. The skin of blacks, paradoxical as it seems, is more readily lightened than the skin of whites (Figures 5 and 6). Complete depigmentation in comparison to hypopigmentation, can be attained in deeply pigmented blacks but not in whites. Hyperpigmented areas are more responsive than the surrounding terrain. Fortunately, the blending problem thus rather takes care of itself with some individual exceptions.

The reason for poor results in older patients with senile lentigines is presently under investigation. The clinical appearance of the lesions in the fourth and fifth decade may be a time when they are more susceptible to treatment. The dynamics of older skin functions may not be receptive to pharmacological intervention designed to alter melanin synthesis and transfer.

Modifications of the basic formulation are now being evaluated. It is already clear that good results can be secured, albeit more slowly, with half the concentration of steroid and tretinoin. A less greasy vehicle will certainly be developed; a gel form has much to recommend it.

Finally, there is one type of hyperpigmentation in which lightening is an impossibility, namely, when some sharp inflammatory process has caused melanin granules to be dumped into the dermis, literally a melanin tatoo. The formulation is not a bleaching agent in the sense that hydrogen peroxide is. Already formed pigment is not affected. It is the inhibitors' effect on the synthesis and transfer of melanin that underlies the depigmenting action.

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Normal cuticle-wear patterns in human hair

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Synopsis

A quantitative study of CUTICLE-WEAR PATTERNS in HUMAN HAIR from six Caucasian subjects whose hair had only been subjected to normal wear—*i.e.*, no chemically reactive cosmetic treatment—is presented. The data was collected by counting the number of cuticle cell layers at different positions along the length of hair fibers. The counting was done on cross-sectional cuts by means of a Scanning Electron Microscope. Results are analyzed in terms of a mathematical model of cuticle wear. The similarity among the cuticle-wear patterns from the different subjects suggests that, under normal wear conditions, there is a common general pattern of cuticle wear in human hair. A theoretical rate of cuticle wear versus distance from the scalp expression was derived. This expression excludes age *per se* as a major factor in cuticle wear, and points instead to a source of hair surface wear which accelerates as we get closer to the hair ends. An analysis of combing forces showed that the type of damage known to be produced by combing can account for the shape of the observed cuticle-wear patterns. It was also found that beyond a certain length human hair should appear to be growing slower due to a cuticle-loss-fracture mechanism, a consequence of this being that, under otherwise equal conditions, the care with which hair is treated and handled directly affects the maximum length that it can attain.

INTRODUCTION

It is well known that the cortex of human hair is surrounded by layers of cuticle cells. These cells, which are flat and very thin, are wrapped around the cortex, building up a system of concentric layers that overlap in telescopic fashion. This assembly of cells is referred to as the cuticle. By virtue of being the outermost section of the hair, the cuticle is subjected to a multitude of environmental influences which cooperate towards its gradual destruction. Many of these, such as exposure to the sun and washing, are the same ones that contribute to the deterioration of the surface of fibers in fabrics. Others, such as combing, brushing, setting and cosmetic chemical treatments, are more unique to hair. Fortunately, the multilayer structure of the cuticle is such that, when under normal mechanical and chemical wear small fractions of the cuticle cells fracture and separate from the hair, they leave behind a fresh uneroded surface belonging to the cell below. This clever scheme of nature allows hair that has been on a living head for two or three years to still have an appealing unworn surface.

From an aesthetic point of view, the importance of the cuticle cannot be overemphasized. Since it serves as the optical surface through which hair interacts with light, the structure and state of preservation of the cuticle determine the extent to which incident light is reflected, scattered, and transmitted, thus determining to a great degree the pleasing appearance of human hair (1,2). As the surface through which hair makes physical contact with other bodies and with itself, its frictional properties determine how hair feels to the touch and how it combs, handles and styles. Structurally, the cuticle contributes critically to the preservation of the physical integrity of hair fibers. Without the radial mechanical constraint of the cuticle, the microfibers in the cortex would rapidly break apart just from the everyday handling of the hair. The potentially important contribution of the cuticle to the mechanical properties of human hair and its effect on the overall rates of chemical treatment of the hair have been brought to our attention by Wolfram and Lindeman (3).

The structure of individual cuticle cells and of the cuticle as a whole has been extensively studied and considerable progress has been made (4–12). In most of these studies, however, with some exceptions [for example, Swift and Brown (14,16), Bottoms, *et al.* (13, 15,17)] the cuticle has been treated as an unchanging structural component of the hair; the subject of its wear receiving little attention. At an average rate of growth of 5 in. per year, the tip ends of the hairs on a female head can typically be two to three years old. During this time, and just due to normal handling, the five to ten cuticle cell layers that hair has when it emerges from the scalp will have gradually been worn away, and we frequently find that very close to its tip the hair shows an exposed cortex with very few or no cuticle cells left. This phenomenon is very likely accelerated if the hair has been subjected to strong chemical treatments.

Because of the important roles of the cuticle, a study of its wear has potential use in understanding and improving the performance of cosmetic hair products. This, coupled with the scant information on the subject, initiated this work in our laboratories. The results of the first step of this investigation, consisting of a study of cuticle-wear patterns on human hair from six female subjects whose hair had only been subjected to normal wear—*i.e.*, no chemically reactive cosmetic treatments—are presented in this paper. Also included is a mathematical analysis of the data which rendered some interesting insights into the phenomena of cuticle wear and its likely connection with combing damage.

EXPERIMENTAL

The experimental work consisted of examining hair fibers longitudinally and cross-sectionally at predetermined distances from their root ends by means of a Scanning Electron Microscope. Hair from six Caucasian subjects was used. The participating females had been letting their hair grow long for at least two or three years prior to our sampling. That is, they had not been cutting their hair with the possible exception of occasional trimmings near the ends to eliminate split ends, etc. The ends of their hair were approximately even. Ten fibers per subject were examined. For each subject, the longer hairs in the scalp were chosen for our work. This normally meant hair with root ends in the uppermost parietal regions of the scalp. The hair samples were obtained by cutting the hair as close as possible (1 to 2 mm) to the scalp. Distances along hair shafts from where this cut was made are indiscriminately referred to in what follows as either distances from root end or distances from the scalp. None of the subjects had treated

their hair with chemically reactive cosmetic products (lighteners, permanent waving products, oxidation dyes, etc.) for at least five years prior to sample gathering. The hair had thus only been subjected to what we call normal wear, which includes shampoos, soap, water, sea water, water settings, hair dryers, hot rollers, combing, brushing, sun exposure and exposure to the atmosphere.

Table I contains general information on the subjects participating in this study.

Table I
Subject Summary

Subject	Sex	Age (Years)	Approximate Maximum Hair Length (cm)	Hair Color
1	F	25	60	Blonde
2	F	30	60	Blonde
3	F	15	40	Blonde
4	F	29	40	Brown
5	F	8	30	Brown
6	F	20	30	Brown

After sampling, the hair was shampooed with a commercial shampoo[1], rinsed with distilled water at room temperature for 10 min, allowed to dry at ambient temperature and prepared for examination. The hair fibers that were 30 cm long were sampled at 15-cm intervals, and the fibers 40 and 60 cm long were sampled at 10-cm intervals from the root end. Sample preparation consisted of cutting 1.5-cm-long segments from the fibers at the above intervals and then from each segment cutting $\approx$50-μm-thick cross sections for internal examination, using hand-held, single edge industrial razor blades and aided visually with an American Optical Model 46 stereoscopic microscope. The remaining portion of the segment was used for external examination. The hair specimens were then mounted on aluminum sample stubs 1.4 cm in diameter, using conductive silver paint.

The specimens were metal coated with a 10 to 20 nm layer of Au-Pd (60 per cent Au—40 per cent Pd). This continuous uniform coating suppresses charging of the non-conductive biological specimen and increases electron emission from the sample surface. A JEOLCO Model JEE-4B vacuum evaporator was used to metal coat the specimens at a vacuum of 4×10^{-5} torr.

The specimens were examined by secondary electron emission in a JEOLCO Model JSM U-3 Scanning Electron Microscope at an accelerating voltage of 15 kv. Photomicrographs of the image displayed on the cathode ray tube were taken with Polaroid 4 × 5 in., Type 52 film using a JEOLCO Model SMU 3-CS1 camera. Using these photomicrographs, the following information was obtained for each of the hair sections examined.

NUMBER OF CUTICLE CELL LAYERS

Cuticle cell layers were counted, using photomicrographs taken at the edge of cross sections at magnifications of the order of 10,000 X. At least five cross-sectional cuts were

[1] Clairol herbal essence shampoo for normal to dry hair.

Figure 1. Photomicrograph of edge of a cross-sectional cut of a hair fiber at the root end taken at the magnification (10,000X) that was used to count number of cell layers. Eight cuticle cell layers can be counted

examined at each of the distances from the scalp for each hair. The value recorded as the number of cuticle cell layers for that distance was the maximum number of layers that could be unequivocally counted in any of the cuts among the five examined. Figures 1 and 2 are photomicrographs of the edges of cross-sectional cuts typical of the ones used to count the number of cell layers.

MINOR AND MAJOR AXES, ELLIPTICITY AND CROSS-SECTIONAL AREA

The major axis was determined by measuring the length of the longest possible straight line drawn from one side of the photomicrographed cross section to the other passing through the center of the cortex. The minor axis was determined by measuring the length of a straight line drawn perpendicular to and bisecting the major axis and extending from one side of the photomicrographed cross section to the other. Obviously, the magnification used is taken into account. The ellipticity was calculated by dividing the minor axis by the major axis.

The cross-sectional areas were determined by measuring the areas of the cross-sectional views on the photomicrographs. This was done by cutting the outline of the cross-

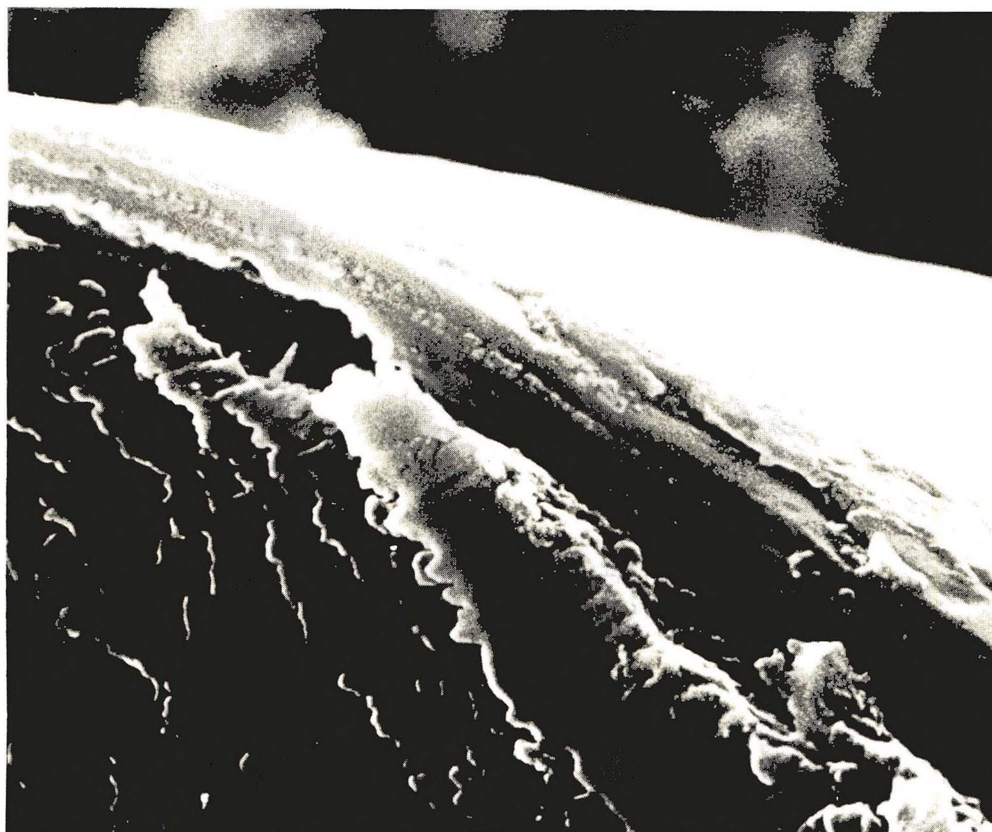

Figure 2. Photomicrograph of edge of a cross-sectional cut of a hair fiber at 40 cm from the root end of a 60-cm-long hair (taken at 10,000X). Four cuticle cell layers can be counted

sectional views and weighing the cutouts with an analytical balance. The actual areas were then calculated, taking into account the magnification used (usually 1,000 X) and the weight per unit area of the Polaroid print paper. This method was chosen because it is more accurate than the one involving the use of the major and minor axes of the cross sections and the formula for the area of an ellipse. The S.E.M. magnification on the photomicrographs was checked using a calibrated grid.

RESULTS

The data in Table II corresponds to three of the ten hairs that were examined from Subject 1. It illustrates the type of natural variability found from hair to hair within a subject. Table III contains the average value for the ten hairs examined for each subject.

CROSS-SECTIONAL AREA AND ELLIPTICITY

The cross-sectional area values are of interest because they do not show a reduction in going from the root to the tip end of the fibers as could be expected due to cuticle loss if the cortex dimensions did not change in the process. Actually, small increases were observed in the hairs of some of the subjects examined.

Table II
Subject 1

Hair	Distance From Root End (cm)	Number of Cuticle Cell Layers	Major Axis Length (μm)	Minor Axis Length (μm)	Ellipticity	Cross-Sectional Area (μm^2)
2	0	8.	99.	63.	0.636	5.15×10^3
	10	8.	102.	81.	0.794	6.49×10^3
	20	6.	96.	66.	0.687	5.09×10^3
	30	5.	97.	67.5	0.696	5.33×10^3
	40	4.	101.	75.	0.743	6.19×10^3
	50	1.	102.	73.3	0.718	6.33×10^3
	60	0.5	97.7	73.3	0.750	5.83×10^3
3	0	7.	93.5	69.5	0.743	5.38×10^3
	10	6.	95.5	73.3	0.767	5.23×10^3
	20	6.	115.	104.	0.904	6.33×10^3
	30	4.	104.3	69.9	0.670	6.02×10^3
	40	2.	95.	74.	0.779	5.70×10^3
	50	2.	86.	54.	0.628	4.34×10^3
	60	0.	83.	70.	0.843	4.66×10^3
5	0	7.	93.	73.	0.784	5.37×10^3
	10	7.	94.	73.	0.776	5.80×10^3
	20	6.	102.	71.	0.696	5.99×10^3
	30	5.	113.	76.6	0.678	7.14×10^3
	40	4.	100.	65.	0.650	5.55×10^3
	50	1.	95.	69.	0.726	5.29×10^3
	60	0.	92.	60.	0.652	4.95×10^3

A calculation was made of the percentage of the hair volume (or cross-sectional area) which corresponds to the cuticle layer at the root end of the hair. This was done by measuring the areas corresponding to the cuticle and cortex on cross section photomicrographs. The average results for the ten hairs for each subject appear in Table IV.

The fact that percentage reductions in cross-sectional area values of the order of magnitude shown in Table IV are not observed shows that the cortex of human hair expands radially during its lifetime on a human head. The reason is likely to be a reduction in radial constraining forces due to cuticle loss, coupled with internal mechanical and chemical damage to the cortex through normal wear. The average percentage increase in the cross-sectional area of the cortex for all our subjects' hairs can be calculated to be approximately 15.0 per cent. This expansion of the cortex should give rise to a reduction in its bulk density of the same order of magnitude. This phenomenon is probably very closely associated with "overporous" behavior towards dyeing and other treatments in human hair.

The cross-sectional area values *vs.* distance from the scalp for each subject appear to follow a pattern characterized by an initial increase in area followed by decreasing values. No simple explanation can be offered for this pattern unless it is due to hair diameter growth variations.

The ellipticity of the hair used in our study was in the range of 0.635 to 0.853. No trends could be seen for the changes in ellipticity along the hair length. Neither were trends detected for the values of the minor and major axes along the length of the fibers.

Table III
Average Results (Ten Hairs per Subject)

Subject	Distance From Root End (cm)	Number of Cuticle Cell Layers*	Major Axis Length (μm)	Minor Axis Length (μm)	Ellipticity**	Cross-Sectional Area (μm^2)
1	0	7.0 ±0.58	97.0	73.3	0.758	5.69×10^3
	10	6.7 ±0.59	97.7	76.8	0.781	5.79×10^3
	20	5.6 ±0.96	104.1	75	0.714	5.95×10^3
	30	5.0 ±0.67	99.8	80.5	0.739	5.96×10^3
	40	3.7 ±0.90	98.5	74.1	0.758	5.87×10^3
	50	2.4 ±0.75	97.6	74.0	0.732	5.58×10^3
	60	0.4 ±0.48	94.1	70.0	0.741	5.33×10^3
2	0	7.1 ±0.79	85.1	53.4	0.635	3.64×10^3
	10	6.5 ±0.51	83.4	53.0	0.683	3.97×10^3
	20	6.0 ±0.34	84.4	54.4	0.653	3.95×10^3
	30	5.5 ±0.61	85.5	55.3	0.647	3.75×10^3
	40	4.5 ±0.77	82.5	54.1	0.656	3.76×10^3
	50	2.6 ±0.96	82.9	52.9	0.647	3.58×10^3
	60	1.2 ±1.25	82.9	54.0	0.661	3.67×10^3
3	0	6.4 ±0.37	74.3	46.8	0.698	3.12×10^3
	10	5.7 ±0.59	78.7	51.4	0.654	3.32×10^3
	20	4.8 ±0.56	73.6	48.3	0.604	3.31×10^3
	30	3.3 ±0.48	81.2	50.2	0.617	3.40×10^3
	40	0.4 ±0.33	78.1	49.4	0.632	3.24×10^3
4	0	8.7 ±0.48	67.3	48.1	0.733	3.01×10^3
	10	7.7 ±0.68	66.1	51.2	0.777	3.13×10^3
	20	6.8 ±0.56	71.5	50.0	0.713	3.32×10^3
	30	5.5 ±0.61	67.1	54.0	0.816	3.40×10^3
	40	2.5 ±1.22	63.2	51.9	0.756	3.23×10^3
5	0	6.4 ±0.50	64.2	53.7	0.853	2.79×10^3
	15	3.5 ±0.84	69.5	56.1	0.804	3.27×10^3
	30	0.3 ±0.25	67.7	53.6	0.815	2.93×10^3
6	0	6.0 ±0.39	76.4	57.9	0.763	4.03×10^3
	15	4.3 ±1.02	82.0	64.5	0.768	4.30×10^3
	30	1.3 ±0.93	80.9	59.2	0.711	4.06×10^3

*The ± values correspond to the 95 percent confidence level limits of the averages.
**These values are the average of the individual ellipticity values for each of the ten fibers.

CUTICLE-WEAR PATTERNS (C.W.P.)

External observation of the hair fibers revealed the same findings of previous studies (13, 14); that is, the edges of the cuticle cells which are smooth and rounded close to the root ends gradually become sharp, irregular and jagged as we move toward the tip ends.

Table IV

	Sub. 1	Sub. 2	Sub. 3	Sub. 4	Sub. 5	Sub. 6
Percentage of volume (or of Cross-Sectional Area) corresponding to cuticle layer at root end	13.5	12.1	11.4	19.1	17.1	14.4

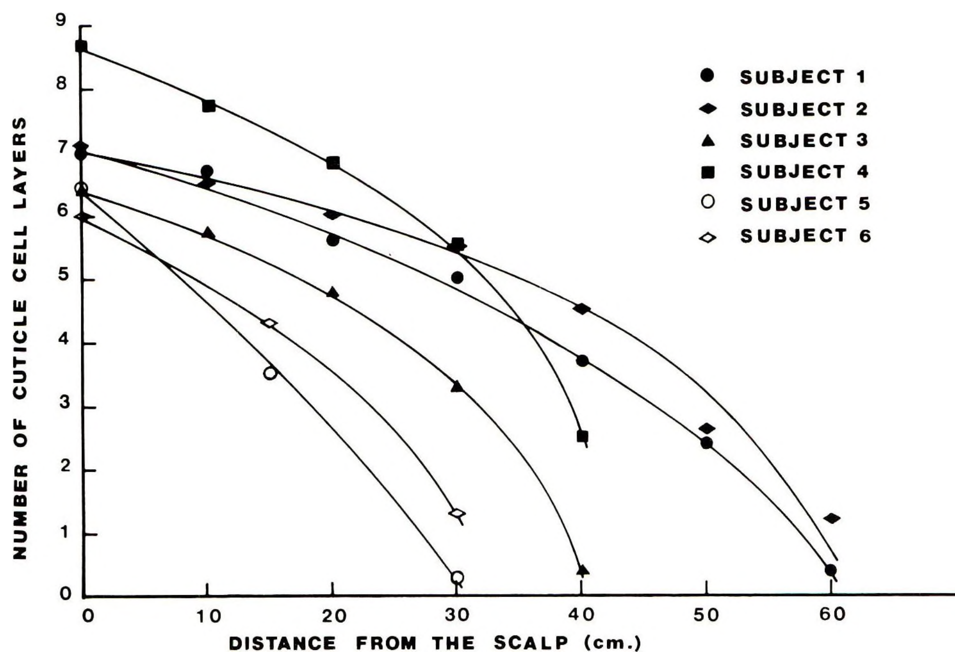

Figure 3. Average number of cuticle cell layers *vs.* distance from the scalp for hair of each of the six subjects. Solid lines were visually fitted to the data points

If we analyze the number of cuticle cell layers (C) *vs.* distance from the scalp (x) data (Table III), we find interesting similarities among the hairs from different subjects (Figure 3). These patterns are the net result of years of cuticle wear. If we assume that there can be something in common among them, the common factor does not appear to be the age of the specific sections of the hair at different distances from the scalp. If this were the case, we would expect that cuticle-wear patterns from different subjects could, if corrected for the different number of cuticle cell layers at the scalp level, fall approximately on top of each other. This, however, is not the case with our data. That is, if cuticle-wear patterns are displaced vertically so that the number of cuticle cell layers at the scalp level are arbitrarily made to coincide for all of the subjects, it can easily be seen, for example, that hairs that are 30 cm long have lost many more cuticle cell layers at their ends than 60-cm-long hairs at a distance of 30 cm from the scalp.

In thinking of ways of analyzing this data, we found that if the number of cuticle cell layers is plotted against the distance from the scalp (x) divided by the length of the hair (L) and if the curves are displaced vertically so that they all start with the same number of cuticle cell layers at the scalp, the similarity among wear patterns for different subjects becomes more apparent (Figure 4). We became interested at this point in determining what type of relationship must exist between rates of cuticle wear and distance from the scalp in order to generate the apparent common cuticle-wear pattern observed in our data. For this purpose, we had to develop a mathematical model for cuticle wear. This was facilitated by fitting an empirical equation to the data from the six female subjects' hair using a least-squares method with the aid of a computer. The curve fitting was done considering the data from the six subjects as belonging to the same population. The result was

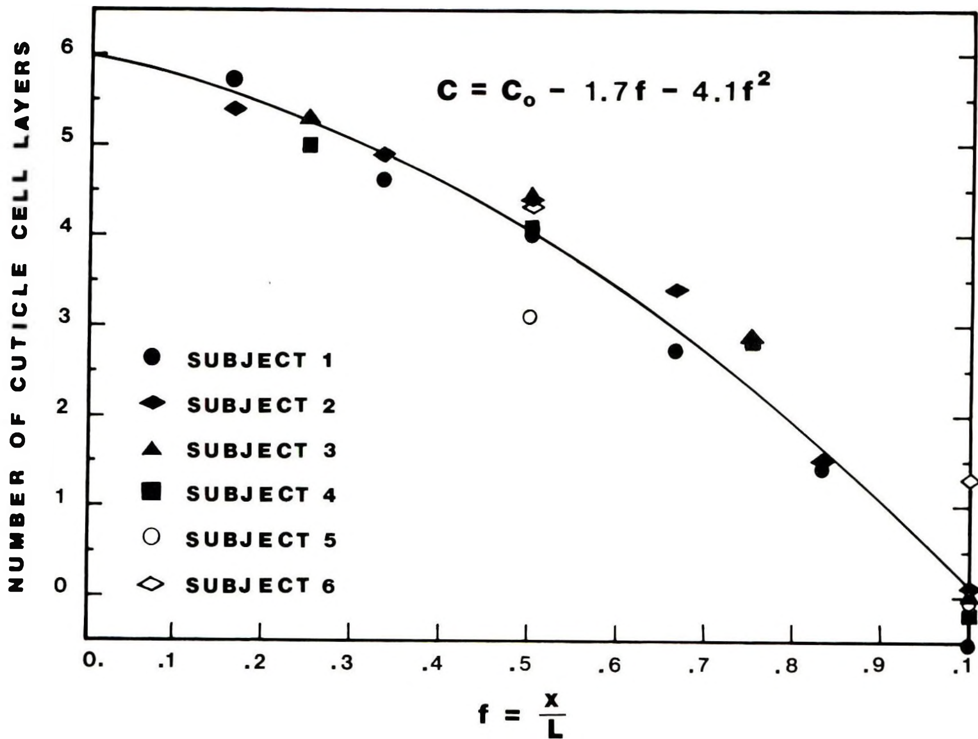

Figure 4. Average number of cuticle cell layers (corrected for the differences in the number of cuticle cell layers at the root end) *vs.* fractional distance from the scalp (f). Solid line is a graph of the empirical equation (shown in figure) statistically fitted to the data from all subjects

$$C = C_0 - 1.7f - 4.1f^2 \qquad [1]$$

where C_0 = number of cuticle cell layers at the scalp

$f = \frac{x}{L}$

L = observed length of the hairs

and 1.7 and 4.1 = empirical parameters.

Equation [1] (solid line, Figure 4) fits the data to within approximately ±0.5 cuticle cell layers, which is of the order of the uncertainty in the number of cuticle cell layer average values shown in Table III arising from the natural variability of this parameter from hair to hair. It should be emphasized that this is only an empirical equation and that other mathematically different functions can be fitted to this data.

At any specific time, not all of the hairs on a scalp are growing or are being subjected to cuticle wear at the same rate. Differences in rates of hair growth result from the different life cycles of the individual follicles. Variations in the instantaneous rates of cuticle wear result from the different position that each hair occupies on the scalp. Even more important is the variation due to the intrinsically random nature of the wear phenomena of a large number of fibers constantly changing spatial distribution and configuration. However, at any specific time, for any one person, a value will exist for the average rate

of growth of all of the fibers on the scalp and also for the average rate of cuticle wear at any distance from the scalp for all of the fibers. The analysis that follows was done by focusing on one hypothetical hair fiber which is assumed to be growing at the average rate and experiencing the average rate of cuticle wear of the large population of fibers to which it belongs. The cuticle-wear dynamics of this hair thus describes that of the parent hair population. The minimal amount of available data on rates of human hair growth as a function of the different stages of the follicle life cycles, hair length, etc., precludes a more detailed analysis that would take those factors into consideration.

If we consider the changes in C taking place simultaneously in an element (H) of this hypothetical hair located within the fixed distances of x and $x + \Delta x$ from the scalp, and assume that

a) the average rate of hair growth (for all fibers) is constant and
b) the average rate of cuticle loss (for all hairs) at any time t and distance from the scalp x can be treated as varying in a continuous fashion and corresponds to the time averages of all factors affecting cuticle wear,

we can derive the following differential equation describing the dynamics of cuticle wear (see Appendix for details).

$$\left(\frac{dC}{dt}\right)_N = -\frac{dC}{dx} \cdot \frac{dx}{dt} - \left(\frac{dC}{dt}\right)_W \qquad [2]$$

where $\left(\frac{dC}{dt}\right)_N$ = Net rate of change per unit time of number of cuticle cell layers C at time t, and distance x resulting from cuticle wear and hair growth.

$\frac{dC}{dx}$ = Change in C along hair shaft (x) at time t and distance x.

$\frac{dx}{dt}$ = Rate of hair growth.

$\left(\frac{dC}{dt}\right)_W$ = Rate of change per unit time of number of cuticle cell layers C at time t, and distance x, resulting just from cuticle wear.

Rate of Cuticle Wear at Different Positions Along Hair Shaft

The fitness of our data to equation [1] suggests that the C.W.P. for any of the subjects (corrected for C_0) will have the same shape as would be observed for any other subject when the former's length coincides with the latter's at some time t. That is, for example, Subject 1's C.W.P. would become like Subject 5's when the former's hair gets to be that long (see Figure 3). If this assumption is correct, it remains to be explained how, for example, the ends of Subject 1's hair could still retain 0.5 cuticle cell layers during the time that it would take to grow from 30 to 60 cm. An unlikely explanation would be that the ends were practically undamaged during that time. A more logical one, however, is that when the number of cuticle cell layers falls below a critical range (≤ 1.5) the cortex becomes so vulnerable to handling that that section of the hair eventually breaks off. This could explain why we rarely observed any significant length of hair without cuticle, even on hairs which were shorter than the average (perhaps trimmed) length of the bulk of the hairs.

This argument is introduced into our analysis as follows: If L_c (critical length) is the length at which breakage begins to happen, we can write for $L_0 > L_c$:

$$L_n = L_0 + L_L \text{ (if } L_0 \leq L_c \text{, then } L_0 = L_n)$$

where L_0 = observed length of hair (previously referred to as L)
L_L = lost length of hair
L_n = natural length of hair if it did not break or was not cut

and equation [1] can then be written

$$C = C_0 - 1.7\frac{x}{L_0} - 4.1\frac{x^2}{L_0^2} \qquad [3]$$

Equation [3] can then be used in conjunction with the differential equation [2] to analyze some aspects of cuticle-wear dynamics. Differentiating [3] with respect to time (x constant)

$$\left(\frac{dC}{dt}\right)_{NET} = \frac{k_0}{L_0}(1.7f + 8.2f^2) \qquad (\text{where } k_0 = dL_0/dt) \qquad [4]$$

Differentiating with respect to x (L_0 constant)

$$\frac{dC}{dx} = \frac{1}{L_0}(-1.7 - 8.2f) \qquad [5]$$

Substituting [4] and [5] in [2] and rearranging

$$-\left(\frac{dC}{dt}\right)_W = (k_0 f - k_n)\frac{(1.7 + 8.2f)}{L_0} \qquad (\text{where } k_n = dx/dt = dL_n/dt) \qquad [6]$$

at the end of the hair f = 1, hence

$$-\dot{C}_{W,1} = (k_0 - k_n)\frac{9.9}{L_0} \qquad \text{where } \dot{C}_{W,1} = \frac{dC}{dt}_{W,(f=1)} \qquad [7]$$

Equation [7] predicts the length at which k_0 will be zero (*i.e.*, no apparent hair growth) for any $\dot{C}_{W,1}$. For example, making $k_0 = 0$ in [7] we obtain:

$L_0 = 86.$ cm for $\dot{C}_{W,1} = 0.004$ for cuticle cell layers/day (C.C.L./day),
$L_0 = 58.$ cm for $\dot{C}_{W,1} = 0.006$ C.C.L./day,
$L_0 = 43.$ cm for $\dot{C}_{W,1} = 0.008$ C.C.L./day
and $L_0 = 35.$ cm for $\dot{C}_{W,1} = 0.01$ C.C.L./day.

What this means is that for any of the above pairs of L_0, $\dot{C}_{W,1}$ values the hair will not grow any longer unless the rate of cuticle wear (defined by the parameter $\dot{C}_{W,1}$) is reduced. Minimizing cuticle wear is thus essential for growing long hair. Solving for k_0 and substituting in [6] we have

$$-\left(\frac{dC}{dt}\right)_W = \frac{(1.7 + 8.2f)k_n(f - 1)}{L_0} - \dot{C}_{W,1} \cdot \frac{(1.7 + 8.2f)f}{9.9} \qquad [8]$$

Equation [8] gives values for $(dC/dt)_W$ as a function of f and L_0 (which are known) and $\dot{C}_{W,1}$ and K_n which were not measured in our study. Note that if for any subject the rates

of hair growth k_n and k_0 were measured, equation [7] would give $\dot{C}_{W,1}$, and [8] would then completely define the rate of cuticle wear at any distance from the scalp for that subject. In addition, any of the infinite number of damage *vs.* x curves generated by this equation for different values of k_n and $\dot{C}_{W,1}$ will result in a C.W.P. pattern given by [3]. In other words, different rates of hair growth and cuticle wear could still produce C.W.P. similar to the ones observed in our study as long as the cuticle wear *vs.* x pattern follows, or is very similar to, any of the curves predicted by [8]. Different k_n and $C_{W,1}$ values will have the effect of influencing the development in time of C.W.P., but not its basic shape. In order to observe the form of [8], a set of values can be given to the parameters in this equation. An accepted average value for k_n is $\approx$ 0.035 cm/day. The order of magnitude of $\dot{C}_{W,1}$ can be estimated with the data for two shorter hair female subjects, 5 and 6. For Subjects 5 and 6, the tip ends of their hair (assuming that $L_0 \approx 30$ cm) have lost an average of $6.2 - 0.8 = 5.4$ cuticle cell layers (Table II) in $t = 30/0.035$ days. That is, the average rate of cuticle loss was approximately 0.0063 C.C.L./day. Considering that it is likely that when the hair was much shorter the rate of cuticle damage at the hair ends was less than the average value, it is probable that $\dot{C}_{W,1}$ is larger than 0.0063 C.C.L./day for the 30-cm-long hairs.

Figure 5, curves A, B and C, shows the hypothetical rate of cuticle wear versus x pattern predicted by [8] for hairs 30, 45 and 60 cm long respectively, $k_n = 0.03$, and $\dot{C}_{W,1} = 0.008$. Curves D, E and F are similar curves calculated using $\dot{C}_{W,1} = 0.006$. An in-

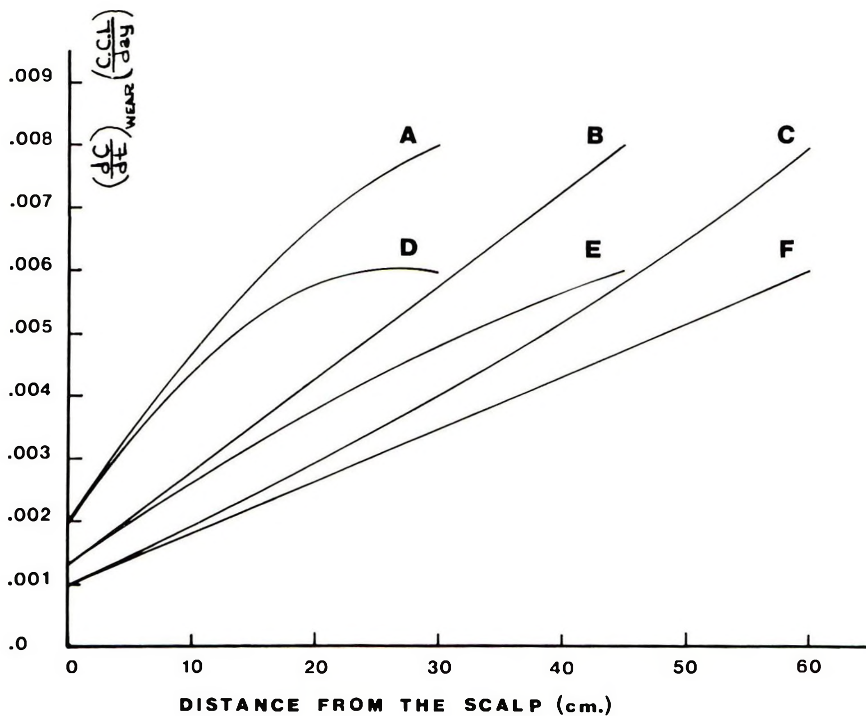

Figure 5. Rates of cuticle wear *vs.* distance from the scalp calculated using equation [8] in text. Curves A, B and C were calculated using k_n (natural rate of hair growth) = 0.03 cm/day, and $\dot{C}_{W,1}$ (rate of cuticle wear at tip end of hair) = 0.008 cuticle cell layers/day. For curves D, E and F, k_n = 0.03 cm/day and $\dot{C}_{W,1}$ = 0.006 cuticle cell layers/day

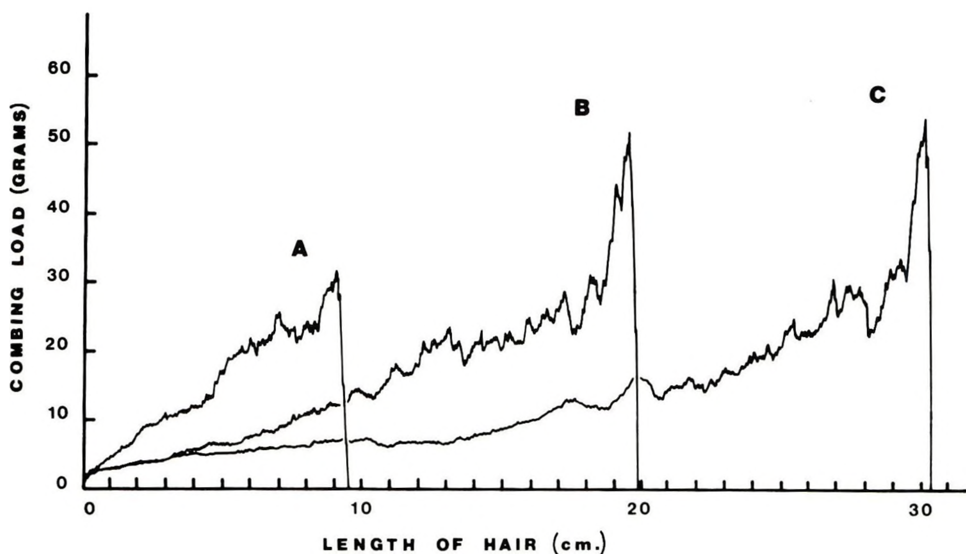

Figure 6. Combing loads *vs.* position of comb (measured in cm from root end) on dry hair swatches 10.(A), 20.(B) and 30.(C) cm long. In looking at the graph, the comb should be considered to have been moving from left to right

teresting characteristic of the curves predicted by [8] is that the rate of cuticle damage is always greater toward the tip ends of the hair, increasing in some cases almost linearly with x. Also, at any fixed distance from the scalp, the longer the hair the lower the rate of damage. This pattern of damage *vs.* distance from the scalp excludes the age of the hair *per se* as a major factor in cuticle wear, and points instead at a source of damage that is related for any one segment of hair to the position (distance from the scalp) of that segment relative to the total length of the hair. The type of damage that is produced during combing appears to fulfill these requirements.

Combing curves recorded in our laboratory with an instrument which allows us to measure the forces encountered by a comb as it moves through a swatch of hair can be seen in Figures 6 and 7 (19). Figure 6 shows such curves for dry hair swatches tangled prior to combing (conditioned and measured at 65 per cent R.H., 70°F); the swatches were of increasing lengths (curve A ≈ 10 cm, curve B ≈ 20 cm, curve C ≈ 30 cm). Figure 7 shows the corresponding curves for the same swatches (also tangled prior to combing) recorded while wet. These curves are plots of the loads (forces) encountered by the comb, against its position along the length of the swatch. In Figures 6 and 7 the comb was moving from left to right.

The recorded combing forces are the sum of all the frictional forces generated at each hair-to-hair and hair-to-comb point of contact by the moving comb. Frictional forces between two sliding surfaces always produce some surface damage, and A. C. Brown and J. A. Swift (16) have convincingly demonstrated the extensive amount of cuticle damage that can be produced during combing, especially when there is some degree of hair tangling (18). Higher combing forces values are the result of more points of contact and/or higher normal loads at each contact point, and can be expected to produce higher levels of surface damage. Cuticle wear during combing should thus be directly related to the magnitude of the combing forces. If combing damage is a major factor in

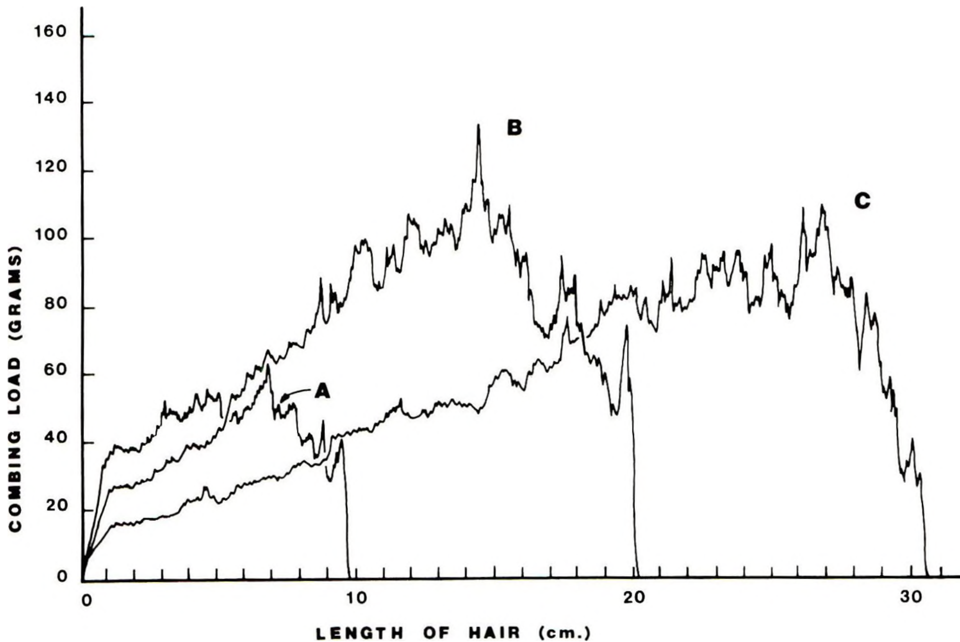

Figure 7. Combing loads *vs.* position of comb (measured in cm from root end) on wet hair swatches 10.(A), 20.(B), and 30.(C) cm long. In looking at the graph, the comb should be considered to have been moving from left to right

cuticle wear, there should be some similarity between the shape of the combing curves (especially the ones for wet hair because hair is then mechanically weaker and more susceptible to damage) and the rate of cuticle wear *vs.* x hypothetical curves predicted by equation [8]. This can be observed to be the case by comparing Figures 6 and 7 with Figure 5. Practically all of the numerous combing curves which we have observed are characterized by initial low combing forces which, on the average, increase as the comb moves towards the tip end of the hair bundles. Even more important is the fact that as the hair gets longer (Figures 6 and 7) combing forces continue to be large closer to the tip ends and diminish near the root ends. This is precisely the general shape of the curves that can be plotted using expression [8] (Figure 5). It can therefore be concluded that the frictional forces *vs.* distance from the scalp pattern characteristic of combing is very likely a major factor in generating C.W.P. of the shape we have observed.

Another source of damage that should be given serious consideration and which, on first sight, appears likely to contribute to the generation of C.W.P. similar to ones observed is exposure to the sun. R. Beyak *et al.* have shown that a correlation exists between the amount of radiation and the mechanical weakening of hair fibers as measured by yield forces at 15 per cent elongation (20). Interestingly, these results show the changes in tensile properties increasing exponentially with increased amounts of radiation. If it is assumed that some weakening of the cuticle is occurring concurrently with the weakening of the fiber as a whole, that such weakening is also accelerated by increased exposure and that weakening leads to cuticle loss, simple exposure to the sun could, in principle, produce C.W.P. such as those observed. However, the evolution in time of C.W.P. produces an increase in C at any distance x as the hair grows longer. In

other words, the rate of cuticle damage at any fixed distance x decreases as the hair length increases. This could only occur if sun exposure is gradually reduced as the hair gets longer. Although this situation could occasionally happen in reality, as a general explanation it is arbitrary. Sun damage, however, is a very real effect experienced by beach attendants during the summer and which continues during the rest of the year to a degree depending on sun exposure. Its manifestations, *i.e.*, split-end formation, dull, coarse looking hair, indicates that sun radiation produces and/or accelerates cuticle damage. It can be concluded that although sun damage by itself does not appear likely to generate C.W.P. such as the ones observed, it must contribute to it. Its main role in cuticle wear is probably that of increasing the amount of damage produced by combing (or brushing).

Still another source of damage which could contribute to the generation of C.W.P. of the observed shape is setting the hair in rollers. Although we do not believe that this operation is nearly as damaging as combing, any damage produced by it is likely to be concentrated at the ends of the hair, as is the case in combing. This will occur because (a) the hair ends will be in direct contact with the potentially damaging surface of some roller types, (b) hair ends can easily be sharply bent and twisted when hair is wrapped around rollers and (c) the radius of curvature increases as we move away from the ends of hair wrapped around a roller, *i.e.*, the degree of bending increases towards the end.

Observed Rates of Hair Growth

It was previously mentioned that it appears likely that beyond a certain length, a cuticle-loss, hair-breakage mechanism becomes operative. A direct result of this would be that hair will appear to grow at a rate less than the natural rate of hair growth out of the scalp. Mathematically, this situation can be described as follows. Equation [7] can be written

$$k_0 = \frac{dL_0}{dt} = k_n - \frac{\dot{C}_{W,1} L_0}{9.9}$$

and integrating, assuming that k_n is constant we have

$$L_0 = \frac{9.9}{\dot{C}_{W,1}} \left[k_n - e^{-\frac{\dot{C}_{W,1}(t+A)}{9.9}} \right] \qquad [9]$$

A is an integration constant which should be evaluated considering that [9] is only valid for $t \geq t_c$ where t_c (critical time) is the time at which the hair breakage due to cuticle-loss mechanism starts to operate. We can then write:

for $t < t_c$ $\quad L_0 = k_n t$

for $t = t_c$ $\quad L_n = k_n t_c = \frac{9.9}{\dot{C}_{W,1}} \left[k_n - e^{-\frac{\dot{C}_{W,1}(t_c+A)}{9.9}} \right] \qquad [10]$

and for $t > t_c$ $\quad L_0$ is given by [9]

Solving for A in [10] we have:

$$A = -\left[t_c + \frac{9.9}{\dot{C}_{W,1}} \ln \left(k_n - \frac{\dot{C}_{W,1} L_c}{9.9} \right) \right] \quad \text{(where } L_c = k_n t_c\text{)} \qquad [11]$$

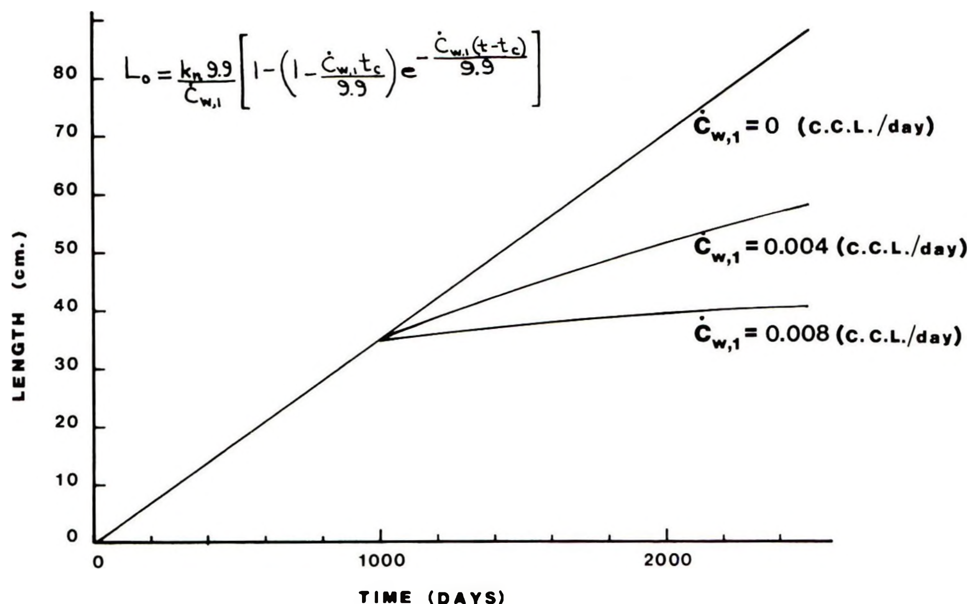

Figure 8. Calculated observed (L_0) length of hair *vs.* time predicted by the equation [12] shown in figure for three different values of $\dot{C}_{w,1}$

Substituting [11] in [9] we obtain

$$L_0 = \frac{k_n 9.9}{\dot{C}_{w,1}}\left[1 - \left(1 - \frac{\dot{C}_{w,1} t_c}{9.9}\right) e^{-\frac{\dot{C}_{w,1} t'}{9.9}}\right] \quad (\text{where } t' = t - t_c) \qquad [12]$$

Equation [12] describes the effect of k_n and $\dot{C}_{w,1}$ on L_0 (observed length) with time. Figure 8 shows the effect of time on L_0 predicted by [12] for k_n = 0.035 cm/day and three different values of $\dot{C}_{w,1}$. The curves were calculated assuming L_c equal to 35 cm (t_c = 1000 days).

Effect of Hair Cutting on Cuticle-Wear Patterns

Our analysis so far has been based on the fact that the subjects participating in our study had not cut their hair for at least three to four years prior to the experiment (with the exception of perhaps occasional trimming to remove split ends). It is of interest to consider what would happen in cases that differ from the above situation.

In the first place, let us consider the case in which hair that has been allowed to grow freely up to a certain length L_0 is then kept at that length by its owner through frequent cuttings. For a hair of length L_0, the rate of damage at any x will be given by [8] for the correct values of $\dot{C}_{w,1}$ and k_n. If k_n does not change and $\dot{C}_{w,1}$, is not significantly changed by any changes in hair treatment and handling habits, the form of [8] will not change. For such a hair prior to cutting, the number of C.C.L. at any distance from the scalp was continuously increasing as the hair grew longer. This occurred because the rate of supply of hair with a larger number of C.C.L. (given by the first term on the right of [2] was bigger than $(dC/dt)_w$ at any x. After cutting, however, this increase in the number of C.C.L. will continue only until in [2]

$$\frac{dC}{dx} \cdot \frac{dx}{dt} = \left(-\frac{dC}{dt}\right)_{W} \qquad [13]$$

which will eventually occur due to the decrease in (dC/dx) produced by a positive $(dC/dt)_{NET}$ at any x. At this point, a steady state is achieved and the C.W.P. will not change with time. Steady state cuticle-wear patterns can be calculated for any value of k_n and $\dot{C}_{W,1}$, by means of [13]. Thus, combining [13] with [8]

$$\frac{dC}{dx} = \frac{(1.7 + 8.2f)(f - 1)}{L_0} + \frac{\dot{C}_{W,1}}{9.9\,k_n}(1.7 + 8.2f)f$$

and integrating (for constant L_0)

$$C = C_0 - 1.7f - \left(3.2 + 0.1\frac{\dot{C}_{W,1}L_0}{k_n}\right)f^2 + \left(2.8 - 0.3\frac{\dot{C}_{W,1}L_0}{k_n}\right)f^3 \qquad [14]$$

Figure 9 shows two steady state cuticle-wear patterns for k_n = 0.035 cm/day, L_0 = 60 cm, C_0 = 6 C.C.L. and $\dot{C}_{W,1}$ = 0.002 and 0.004 C.C.L./day, compared to the normal C.W.P. of a growing hair. It illustrates the interesting result that hair kept at a constant length by cutting will be in better condition than would be that same hair arriving at the same length by growing freely.

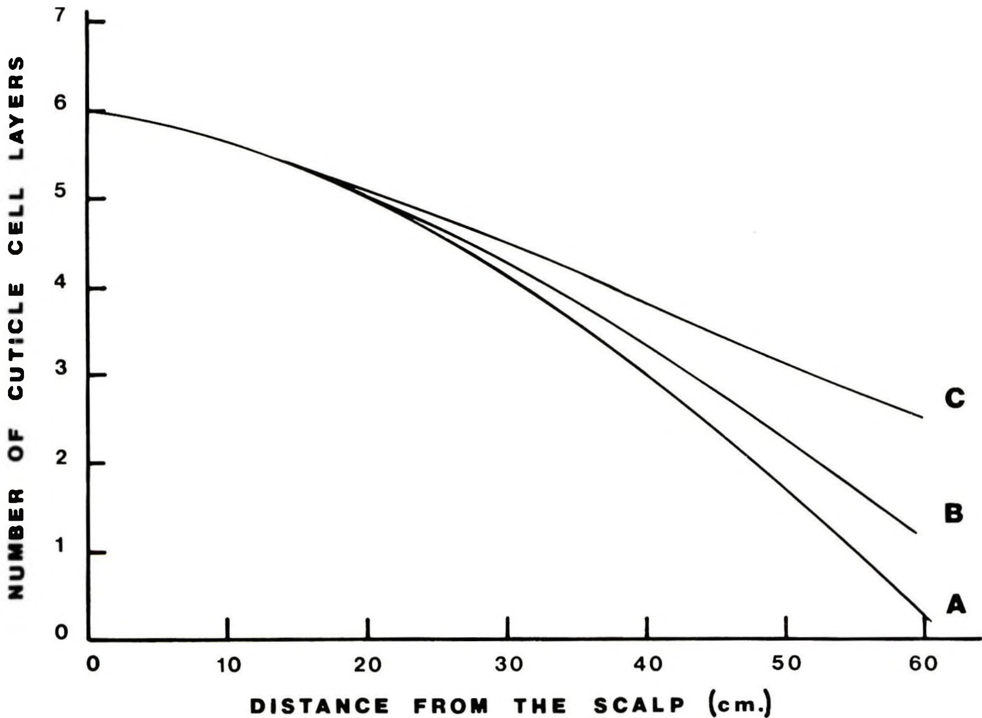

Figure 9. *Curve A:* cuticle-wear pattern of 60-cm-long hair that has arrived at this length by growing freely. *Curve B:* steady state cuticle-wear pattern for hair kept at a length of 60.cm by frequent cutting, calculated using equation [14] in text, $\dot{C}_{w,1}$ = 0.004 C.C.L./day. *Curve C:* same as Curve B but $\dot{C}_{w,1}$ = 0.002 C.C.L./day

CONCLUSIONS

1. Determining the number of cuticle cell layers at different positions along the hair shaft by means of Scanning (or Transmission) Electron Microscopy is a good method for quantitating and analyzing the degree and type of wear that hair has been subjected to. We believe that this approach will prove more productive than previous ones which have been based on the qualitative description of observations of changes in the appearance of the outermost cuticle cell layers along the hair shaft.
2. Although well aware that the number of subjects included in our study was very small, the similarity of the cuticle-wear patterns among subjects and the fact that all of the data can be reasonably well superimposed by plotting the number of cuticle cell layers versus the fractional distance from the scalp suggest that, under normal wear conditions, there is a common, general pattern for cuticle wear in human hair. An empirical equation describing this pattern was presented.
3. An expression for the rate of cuticle wear versus distance from the scalp, which would generate the type of cuticle-wear patterns shown by our data, was mathematically derived. This expression excludes age *per se* as a major factor in cuticle wear and points instead to a source of hair surface wear which accelerates as we move closer to the hair ends. An analysis of combing curves shows that the type of damage known to be produced by combing (or brushing) can very well be responsible for the shape of cuticle-wear patterns that our data reveals.
4. The rate of cuticle wear *vs.* x function [8] will have a different shape depending on the value of its parameters k_n and $\dot{C}_{W,1}$. Any member of this family of curves will, however, satisfy the differential equation [2] thus producing a C.W.P. described by [3]. It is therefore possible to have different rates of damage *vs.* x patterns for different subjects and still end up with C.W.P. which have the same shape.
5. Our data shows that, at any common distance from the scalp x, the state of preservation of the cuticle is better for a long-hair subject than for a short-hair one.
6. As the cuticle wears during hair growth, the cortex appears to gradually expand, reaching an expansion of the order of 15 per cent in its cross-sectional area as we approach the tip ends.
7. It was found reasonable to assume that beyond a certain length, which we refer to as the critical length (L_c), human hair will appear to be growing slower due to a cuticle-loss-fracture mechanism. If the hair is cut, it will then appear to be growing faster. This apparent faster growth will continue until a new L_c is reached.
8. Hair that is kept at a constant length will be in better condition in regard to its cuticle than hair of the same length which is growing freely.
9. Last, but most important, the care with which hair is treated and handled directly affects the maximum length that it can attain. The use of products which reduce combing damage should effectively enable a person to grow longer hair. Faster rates of hair growth, more cuticle cell layers on the hair at the follicle and longer follicle growth cycles will also contribute to increasing the maximum attainable length.

The authors are well aware that the number of subjects participating in our study was relatively small. Our assumptions and results should therefore be tested further by gathering more extensive data of the type presented in this paper. It is hoped, however, that our conclusions, some of the questions they pose, and the mathematical tools proposed for their analysis will stimulate additional research on the subject of cuticle wear and, what is even more important, on its prevention.

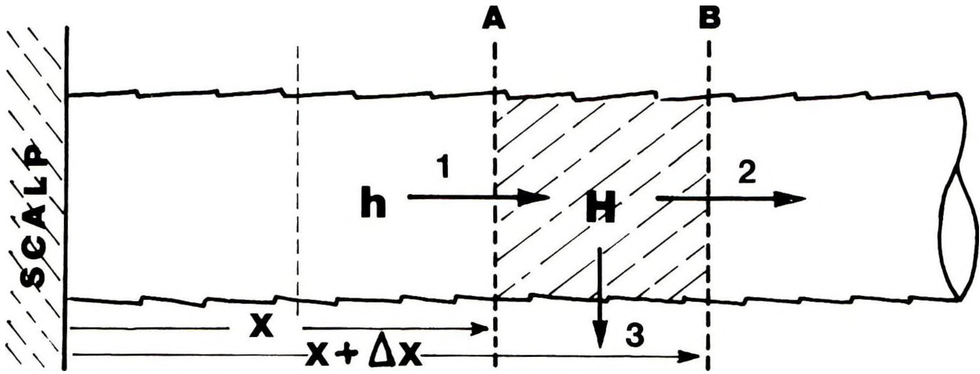

Figure 10. Flow diagram of cuticle into and out of a hypothetical hair element H located within the fixed distances of x and x + Δx from the scalp

APPENDIX

Let H be an element of a hypothetical hair located within the fixed distances of x and x + Δx from the scalp (Figure 10). Let C′ (proportional to C) stand for mass of cuticle (in grams). Let [C′] be the concentration of cuticle, expressed as mass of cuticle per unit volume of hair. Assume that (a) the average rate of hair growth (for all fibers) is constant and (b) the average rate of cuticle loss (for all hairs) at any time t and distance from the scalp x can be treated as varying in a continuous fashion, and corresponds to the time averages of all factors affecting cuticle wear. Then, during a time interval Δt, the following changes in C′ will be taking place:

$$\begin{bmatrix}\text{Change in } C' \text{ per unit time due to} \\ \text{cuticle entering H through boundary} \\ \text{A due to hair growth (Flow ① Figure 10)}\end{bmatrix} = \left[\frac{\Delta C'}{\Delta t}\right]_{G.I.} = \left[\overline{C'_h}\right] \cdot A \cdot \frac{\Delta x}{\Delta t}$$

where $\left[\overline{C'_h}\right]$ = Average concentration (during Δt) of C′ in element h (adjacent to H) which moves into position H during Δt

A = Cross-sectional area of hair (assumed constant)

Δx = Length of elements h and H

G.I. = Stands for growth of hair into H

If Δt approaches 0 (*i.e.*, dt) and Δx approaches 0 (*i.e.*, dx), then $\left[\overline{C'_h}\right]$ approaches $\left[C'_x\right]$ (*i.e.*, concentration of C′ at x), and the above expression becomes

$$\left(\frac{dC'}{dt}\right)_{G.I.} = \left[C'_x\right] \cdot A \cdot \frac{dx}{dt} \qquad [15]$$

In the same manner:

$$\begin{bmatrix}\text{Change in } C' \text{ per unit time due to} \\ \text{cuticle leaving H through boundary} \\ \text{B due to hair growth (Flow ② Figure 10)}\end{bmatrix} = \left[\frac{\Delta C'}{\Delta t}\right]_{G.O.} = \left[\overline{C'_H}\right] \cdot A \cdot \frac{\Delta x}{\Delta t}$$

As before, if $\Delta t \rightarrow 0$ and $\Delta x \rightarrow 0$ then $\left[\overline{C'_H}\right] \rightarrow \left[C'_{(x+dx)}\right]$, and:

$$\left(\frac{dC'}{dt}\right)_{G.O.} = \left[C'_{(x+dx)}\right] \cdot A \cdot \frac{dx}{dt} \qquad [16]$$

where G.O. stands for growth of hair out of H. The change in C′ within H due to cuticle wear can be expressed as

$$\begin{bmatrix}\text{Change in C}' \text{ per unit time due to} \\ \text{cuticle leaving H due to wear} \\ \text{(Flow ③ Figure 10)}\end{bmatrix} = \left[\frac{\Delta C'}{\Delta t}\right]_W = \left(\frac{dC}{dt}\right)_W \quad [17]$$

where W stands for wear. The net change in C′ within H per unit time can then be expressed as:

$$\left(\frac{dC'}{dt}\right)_{NET} = ① - [② + ③]$$

or

$$\left(\frac{dC'}{dt}\right)_{NET} = \left(\frac{dC'}{dt}\right)_{G.I.} - \left[\left(\frac{dC'}{dt}\right)_{G.O.} + \left(\frac{dC'}{dt}\right)_W\right] \quad [18]$$

Substituting [15] and [16] in [18] we have:

$$\left(\frac{dC'}{dt}\right)_{NET} = [C'_x] \cdot A \cdot \frac{dx}{dt} - [C'_{(x+dx)}] \cdot A \cdot \frac{dx}{dt} - \left(\frac{dC'}{dt}\right)_W \quad [19]$$

noting that

$$[C'_x] = \frac{C'_{(x)}}{A \cdot dx}, [C'_{(x+dx)}] = \frac{C'_{(x+dx)}}{A \cdot dx}$$

[19] becomes

$$\left(\frac{dC'}{dt}\right)_{NET} = \frac{C'_x \quad C'_{(x+dx)}}{dx} \cdot \frac{dx}{dt} - \left(\frac{dC'}{dt}\right)_W$$

or

$$\left(\frac{dC'}{dt}\right)_{NET} = -\frac{dC'}{dx} \cdot \frac{dx}{dt} - \left(\frac{dC'}{dt}\right)_W$$

C′ is (to a good approximation) proportional to C; therefore, dC′ = KdC hence

$$\left(\frac{dC}{dt}\right)_{NET} = -\frac{dC}{dx} \cdot \frac{dx}{dt} - \left(\frac{dC}{dt}\right)_W$$

which is equation [2] in the text of the paper.

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Measurement of enzyme kinetics on the intact skin—a new method to study the biological effects of cosmetics on the epidermis

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Synopsis

A new DIRECT FLUOROMETRIC method allows the MEASUREMENT of ENZYME ACTIVITY on the INTACT SKIN of various body surfaces. This permits normal physiological parameters to operate on the system studied. The pentose phosphate pathway (Entner-Doudoroff), previously shown to operate in the epidermis, provides several enzymes which are used here to assess and compare various dermatological conditions. Glucose-6-phosphate dehydrogenase and lactic dehydrogenase are measured by fluorometric determination of changes in NADPH and NADH. Other enzymes of the Embden Myerhoff and Krebs cycles are measured directly or indirectly by this method. Various COSMETIC base ingredients and compounded formulations were studied to determine their EFFECTS on epidermal metabolism. Enzyme action was recorded as increased, decreased or not affected. This new method is simple and relatively inexpensive, and allows extremely wide applications.

INTRODUCTION

The need for new methods to study the biochemistry of the epidermis *in situ* is becoming more apparent. Methods involving extrapolation from animal models are not always applicable to the human epidermis because of species differences. Excised skin from human volunteers has certain inherent drawbacks including pain, disfigurement and the problem of an isolated specimen removed from its normal milieu. The use of an *in vivo* and *in situ* method would obviate many of these problems. This paper describes such a method.

The skin is the largest body organ, representing approximately one-sixth of the body weight. Far from being a mere barrier to the internal and external environment, the skin is proving to be a dynamic organ with a profound effect on the internal metabolism. A review of the extensive literature on the metabolic functions of the epidermis reveals carbohydrate metabolism to be unique (1–4). For this reason we chose epidermal carbohydrate metabolism as our biochemical system. Previous studies have demonstrated enzyme activity in histochemical sections (5, 6), cell homogenates (7) and epidermal stripping (8). These experiments have been both comprehensive and

elegant with conclusive demonstration of enzyme activity in the upper layers of the epidermis. Recently Schalla *et al.* (9) demonstrated enzyme activity in the intact epidermis by perfusion of a glass chamber attached to the surface of the skin *in situ*. Their measurements were recorded as a change in absorbance of the perfusate in a flow-through spectrophotometer.

Early studies by Chance *et al.* (10) have shown that fluorescence changes can be detected in the intact organ and that these changes reflect a state of oxidation-reduction within the organ cells. This study and prior studies by Chance *et al.* (11) indicate that tissue irradiated at 366 μ emitted fluorescence characteristic of the reduced pyridine nucleotides. The preponderance of nucleotide fluorescence in the tissues precludes the measurement of other cellular fluorophores with similar fluorescence characteristics. It was further concluded that the assay of reduced pyridine nucleotides is insensitive to the state of oxygenation of hemoglobin and thus this system can be used to follow oxidation-reduction ratios in anoxic states. Based on these previous studies, we designed a system that would measure fluorescence changes of the pyridine nucleotides involved in carbohydrate metabolism in the epidermis.

METHODS AND MATERIALS

THE FLUOROMETER

Our system employs a sensitive fluorometer that measures the change in fluorescence of nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) when they are in the reduced state, NADH or NADPH, respectively. Excitation is at 340 to 365 nm and emission is at 460 nm. In oxidized form (NAD or NAPD) they are not fluorescent. The emission intensity is directly proportional to the amount of reduced nucleotide. Measurements of changes in fluorescence, either increased or decreased, can be utilized with this system. Our instrument employed the electronic components of the Metabolite Fluorometer E704, designed and built at the Johnson Foundation of the University of Pennsylvania. Essentially, this instrument employs a GEF4T4 Germicidal UV lamp with a E062 socket. The ultraviolet light from this source is filtered through a Corning No. 5840 filter or Wratten 18A Kodak filter. The visible emitted light is detected by a photomultiplier tube (PMT) EM19524B (supplied by EMI Gencom Inc., 80 Express Street, Plainview, New York). Ultraviolet light is filtered out by inserting a UV filter 2B (Kodak) or a Corning No. 3389 filter at the face of the PMT. Power supply to the PMT is provided by a Model 6515A DC Power Supply from Hewlett Packard. The recorder is a Honeywell Electronik 19 with 100 mv sensitivity, usually set at a speed of 0.5 in./min. The amplifier and output circuit diagram for the E704 is available from Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.

For our instrument, we designed the metabolite chamber and detection systems to allow direct measurement on the skin as follows (Figure 1). An aluminum block (D) was machined to allow insertion of the ultraviolet light source (E) and photomultiplier (F) with respective filters (G and H) at an angle of 22.5° from the perpendicular. The bottom of the block was machined to accommodate a closure mechanism (B) and a reaction chamber (A). The slide closure mechanism (C) and the reaction chamber are of stainless steel. By closing the reaction chamber, *in vitro* enzymatic reactions can be

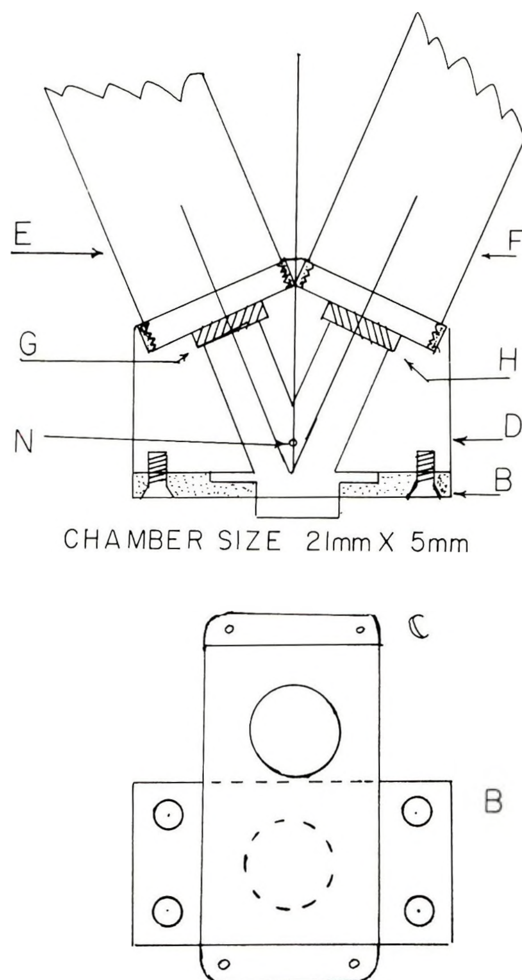

Figure 1. Design of instrument for direct measurement of enzymes on skin

conducted to calibrate the instrument. The closure also protects the PMT when the instrument is being moved or adjusted on the subject's skin. Provision to introduce reactants onto the skin is accomplished by holes (N) drilled at 22.5° through the block to accommodate 22-gauge needles.

All enzyme substrates and control enzymes used in this study were purchased from Sigma Chemical Company. Reagents were analytical grade.

CALIBRATION OF THE INSTRUMENT

The instrument was calibrated to give a full-scale deflection with 1 millimicromole (μM) of NADPH when added in 0.1-ml volume to 2 ml of reaction media. After the instrument is allowed to warm up, 2 ml of distilled water is added to the reaction chamber and the base line is allowed to stabilize. NADPH is then added and adjustments made to allow maximum sensitivity of 1 μM for a full-scale deflection. This is

Table I
Concentration of Reactants

Enzyme	Reactants
1. Glucose-6-Phosphate Dehydrogenase	5.8×10^{-3}M glucose-6-phosphate in 0.063M, Tris $MgCl_2$ 0.1M pH 7.4, NADP 1 mg/5 ml
2. Isocitric Dehydrogenase	Isocitrate 3×10^{-4}M Manganese Cl_2 0.1M$MnCl_2$ in NACl 0.15 per cent, Tris buffer 0.063M pH 7.5, NADP 1 mg/ml
3. Lactic Dehydrogenase	Pyruvate Na 2×10^{-5}M, Phosphate buffer 0.05M pH 8.5, $NADH_2$ 0.5 mg/ml
4. Uridine 5-diphospho-glucose dehydrogenase	UDPG 6.28×10^{-5}M Phosphate buffer 0.05M pH 7.0, NAD 1 mg/ml

generally a much greater sensitivity than needed for most measurements. We found many kinetic measurements could be made at a sensitivity of 20 to 60 μM full scale.

After sensitivity is established, the chamber is thoroughly cleansed with distilled water. The instrument is then ready for an *in vitro* assessment of the conditions necessary. In the case of glucose-6-phosphate dehydrogenase, we used the following concentrations and quantities: glucose-6-phosphate, 5.8×10^{-3}M, in tris buffer with $MgCl_2$ at pH 7.4; glucose-6-phosphate dehydrogenase from Bakers Yeast (Sigma #G-6378), 0.05 unit in distilled water; and NADP, 0.04 mg/ml. The coenzyme, NADP, is added incrementally by 0.1-ml aliquots (0.004 mg) until a maximum velocity (Vmax) is obtained. (The absorption of this reaction mixture without added enzyme is 0.01). Measurements of skin reactions are taken as rates rather than Vmax and the yeast enzyme is not used as an internal standard. Measurements are made at 1-min intervals for a total of 5 to 20 min.

The instrument is calibrated each time a new enzyme system is studied. Before the *in vivo* measurements are made, the chamber is thoroughly cleansed and an additional aliquot of substrate and cofactor is added to ensure that no residual enzyme remains

Table II
Summary of Enzyme Activity*

Subject	Sex	Age	G-6-PD	LDH**	ICD	UGDPD
1. L.M.	F	20	2.0	0.5		0.5
2. M.R.	F	20	1.6	8.0		
3. M.H.	F	20	0.9	0.1	2.7	3.0
4. G.G.	F	20	1.0	0.7		
5. J.P.	F	45	2.9	3.5	1.5	2.0
6. T.A.	M	31	1.0	3.2		
7. S.S.	F	41	2.1	7.1		
8. P.P.	M	49	2.4	5.0	0.5	
9. M.S.	M	36	1.9	2.7		
10. S.G.	F	31	1.5	2.5		
11. B.B.	M	22	1.2	3.0		3.4
12. J.C.	F	37	1.9	1.4		

*Calculated in μM/min cm^2.

**Random group of people studied without regard to fasting or nonfasting states. Note marked variations in LDH activity.

Values represent an average of three determinations on the dorsal surface of the forearm. Reproducibility $\pm 0.4 \mu$M/min/cm^2.

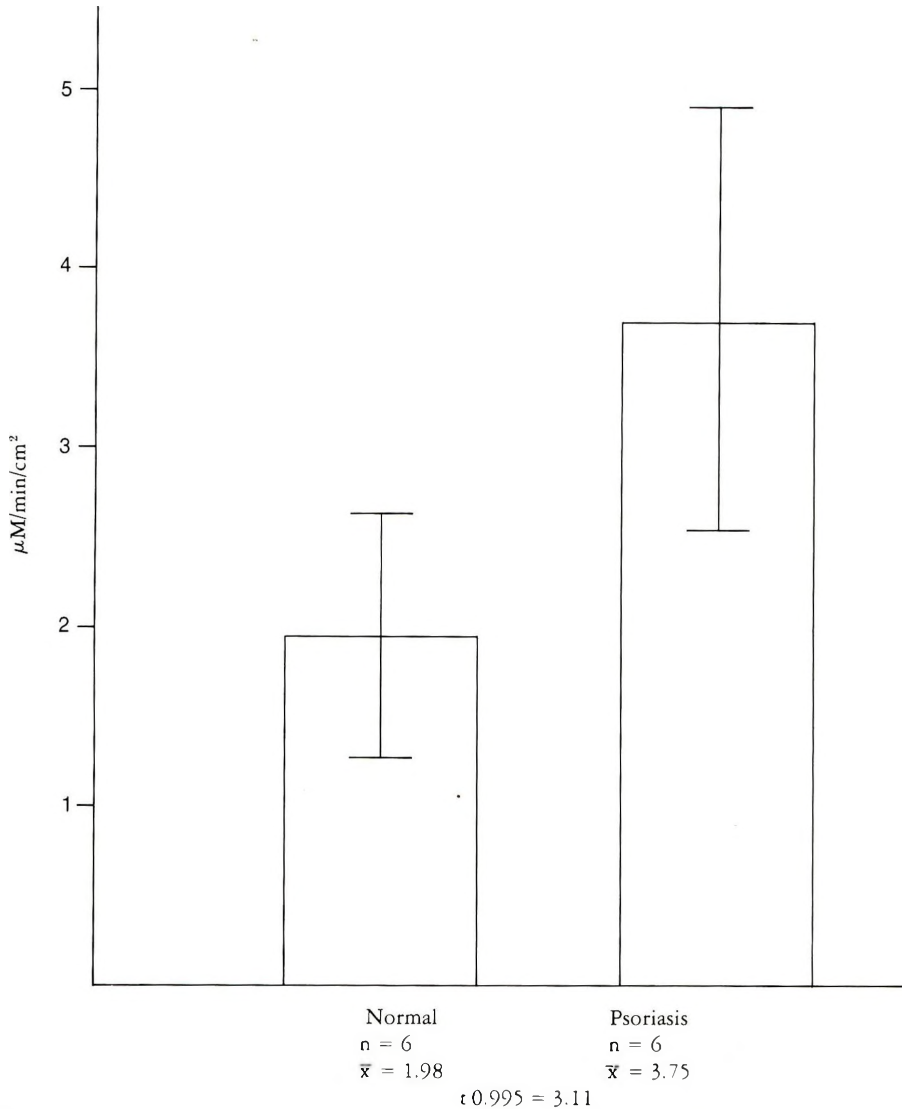

Figure 2. Epidermal glucose-6-phosphate activity in normal subjects and subjects with active psoriasis

within the chamber. The system, after cleansing again with distilled water, is ready for *in vivo* measurements. Enzyme activity was calculated in μM/min/cm$_2$ (milli-micromoles per minute per square centimeter of skin surface). This is equivalent to mU/min/cm^2 (miliunits per minute per square centimeter) which is the method used by Schalla *et al.* (9). The concentration of the reactants used in these experiments is given in Table I.

The results of the glucose-6-phosphate dehydrogenase activity measurement on six normal subjects and six subjects with psoriasis are presented in Figure 2. Table II summarizes the activity of the enzymes measured on 12 normal subjects. Activities are recorded for the dorsal surface of the forearm as considerable variation is noted in enzyme activity from different areas of the body (see Table III). We were unable to

Table III
Comparison of G-6-PD Activity on Various Parts of the Body

Site	G-6-PD Activity* Normal	Psoriasis
Arm: palmar surface	2.2	3.9
Arm: dorsal surface	1.5	3.5
Calf	0.6	1.0
Thigh	—	—
Lower back	—	—

*Averages calculated in μM NAPDH/3min/cm^2.
Six normal subjects.
Six subjects with psoriasis.
Average of 3 measurements per area, per patient. Reproducibility $\pm 0.6\ \mu$M.

get consistent measurements of isocitric dehydrogenase in normal subjects and not at all in individuals with active psoriasis.

STUDIES WITH COSMETIC RAW MATERIALS

Using the values obtained in Table II as a base line, we studied the effects of certain common cosmetic ingredients on the enzyme activity of the epidermis. The dorsal surface of the forearm was used throughout the study. The test material was placed on the test site after a base line enzyme activity was obtained; care was exercised to place the material in a preselected area of 3.8 cm^2. Subject had been instructed not to wash arm for 24 hr prior to the test. Material was placed on the test site with a microliter syringe delivering 10 μl, followed by gentle rubbing into the epidermis. Repeat enzyme determinations were made 4 hr later. Anoxic states were induced by applying a standard 1-in.-wide rubber tourniquet for 3 min above the elbow. Table IV summarizes the results reported as an increase or decrease of enzyme activity over the control rate.

DISCUSSION OF RESULTS

There is an immediate question of what is really being measured in these studies. It is believed by the author that we are actually measuring the oxidation-reduction state of

Table IV
Effect of Various Cosmetic Agents on the Reaction of Two Enzymes in Normal Subjects*

Agent	G-6-PD	G-6-PD Anoxic state	LDH	LDH Anoxic state
Mineral oil (70 visc.)	−80	No effect	−>80	No effect
Lanolin alcohol	−10 to −20	+50	+50	+>80
Isopropyl palmitate	−10 to −20	+50	+50	+>80
**Bath oil	−10 to −20	+50	+50	+>80
Lactic acid/sodium lactate	−90	No effect	+>100	+>100
***Sodium lauryl ether sulfate	−>100	Inhibited	Not studied	Not studied

*Activity expressed as % increase (+) or decrease (−) over control rate.
**Experimental bath oil containing no isopropyl myristate, isopropyl palmitate, or mineral oil.
***The skin was tested 4 hr after application of 10 μl of the agents used, with the exception of the sodium lauryl ether sulfate, which was tested after 0.5 hr.

the pyridine nucleotides in the first few layers of the epidermis, probably in the upper granular layer. It was shown by Schalla *et al.* (9) in studies carried out with C^{14} labelled NAD that less than 1 per cent of the NAD crossed the cell wall (10^{-11} mol/mg), compared to 10^{-7} mol/mg of hydrogen. It is obvious therefore that the hydrogen ion is the actively moving ion that causes the change in fluorescence.

The question of bacteria activity can be raised also as a source of fluorescence. The studies of Marples (13) on skin flora indicate a figure of 1.05 million bacteria per m^2 on the forearm. Our own values on the 12 subjects used in the study yield an average value of 10.6 colonies over 120 cm^2 area for the unwashed arm and a value of 1.1 colonies per 120 cm^2 for the water-washed arm. Since less than 4 cm^2 were used in the study, the bacteria effect may be discounted. Finally, the question of absorbancy of liquids from the chamber by the epidermis may fairly well be ruled out by the studies of Scheuplein (14) on percutaneous absorption. The changes observed in this study are almost instantaneous and precluded diffusional rates mechanisms.

While the enzyme rates reported in this study are far below those reported for cell-free extracts and whole-cell suspensions, they represent an entirely different class of enzyme kinetics. Possibly they follow the kinetics outlined by Blum and Jender (15) and by O'Sullivan (16) which deal with geometrically constrained enzyme systems and slowly diffusing substrates. Since we were unable to calculate an actual Km value for glucose-6-phosphate dehydrogenase, we calculated an "apparent KM" of 2.9×10^{-4} for the conditions used.

One very interesting observation on several patients with psoriasis was the unexpected *increase* in fluorescence in the presence of partial epidermal anoxia induced by local

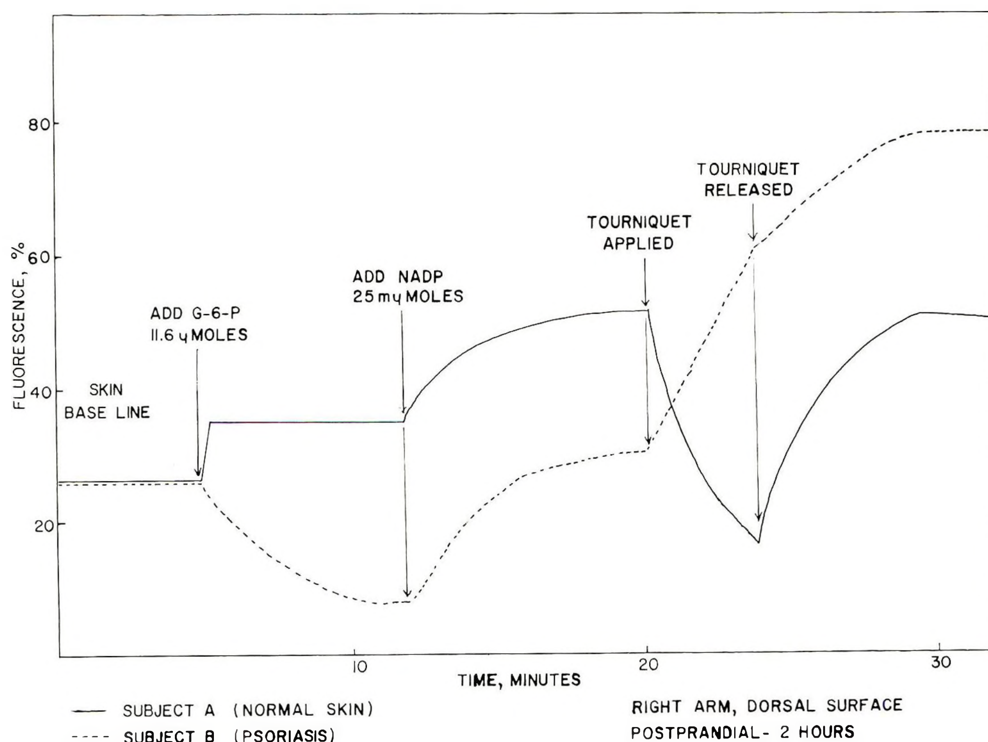

Figure 3. Comparison of effect of partial anoxia in normal individuals and individuals with psoriasis

venous occlusion. Partial anoxia causes a decrease in fluorescence in the normal individual. This is seen in Figure 3. At present, we have no explanation for this phenomenom. It is found only in patients with active psoriasis and quickly returns to normal when the skin is treated with steroids, or exposed to sunlight for 4 to 6 hr. The effect appears to be related to the severity of the disease, though we have observed it in individuals who have inactive psoriasis and are not under treatment of any type.

A great deal of additional work needs to be done on this system before it will become a routine procedure. The ease of performing the test, the lack of epidermal invasion, and the use of human subjects should make the procedure generally applicable for the study of epidermal effects of applied agents.

CONCLUSIONS

1. A new *in vivo* and *in situ* method for studying epidermal biochemistry has been outlined.
2. The method is reproducible and correlates with similar methods—that is—perfusion and stripping techniques.
3. Classical Michaels-Menten kinetics do not appear to hold for this technique. Precise kinetic studies are needed.
4. Applications appear almost limitless, since the epidermis is accessible and the method adaptable to any reaction in which fluorescence or absorption changes can be measured.

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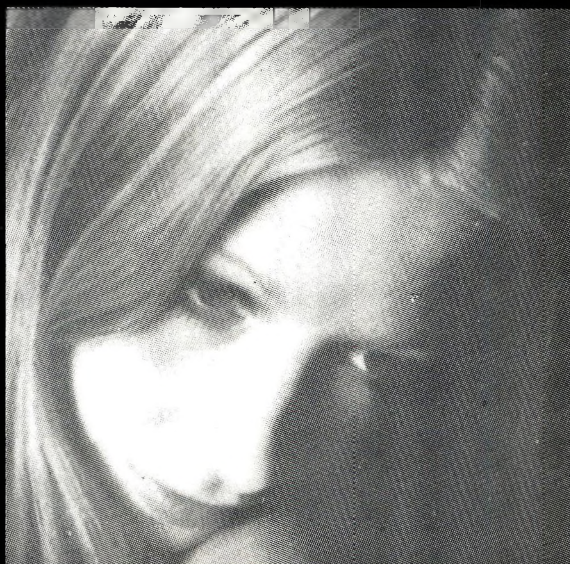

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CALL FOR PAPERS

1978 ANNUAL SCIENTIFIC MEETING SOCIETY OF COSMETIC CHEMISTS

Prospective authors are asked to submit abstracts of papers of 200 words or less **immediately** for the 1978 Annual Scientific Meeting of the Society of Cosmetic Chemists to be held November 30th and December 1st, at The Waldorf-Astoria in New York City.

The Program Co-Chairmen, Robert Raymond and Dr. Peter Sgaramella, have issued a Call for Papers; the subject for the papers is open. All abstracts of papers should be sent to either the Program Co-Chairmen or the SCC office at: Society of Cosmetic Chemists, 50 East 41st Street, New York, NY 10017.

The Society of Cosmetic Chemists Award sponsored by Perry Brothers, Division of Mallinckrodt, will be awarded to the best paper presented at the Annual Scientific Meeting. The award will be $1,000. In addition, if the special panel choosing the awardee feels that other papers are deserving of special recognition, they may, at their discretion and with the approval of the Board of Directors of the Society, present one or two additional awards of $500 each to papers designated as Honorable Mentions.

To be considered for the awards, all papers must be submitted to the award committee in manuscript form at least 4 weeks prior to the actual presentation. November 1st is the deadline for the 1978 Annual Meeting. The award committee will consider relevance to our industry, originality and presentation in choosing an awardee.

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