Vol 27 No 9 September 1976

Journal of the Society of Cosmetic Chemists

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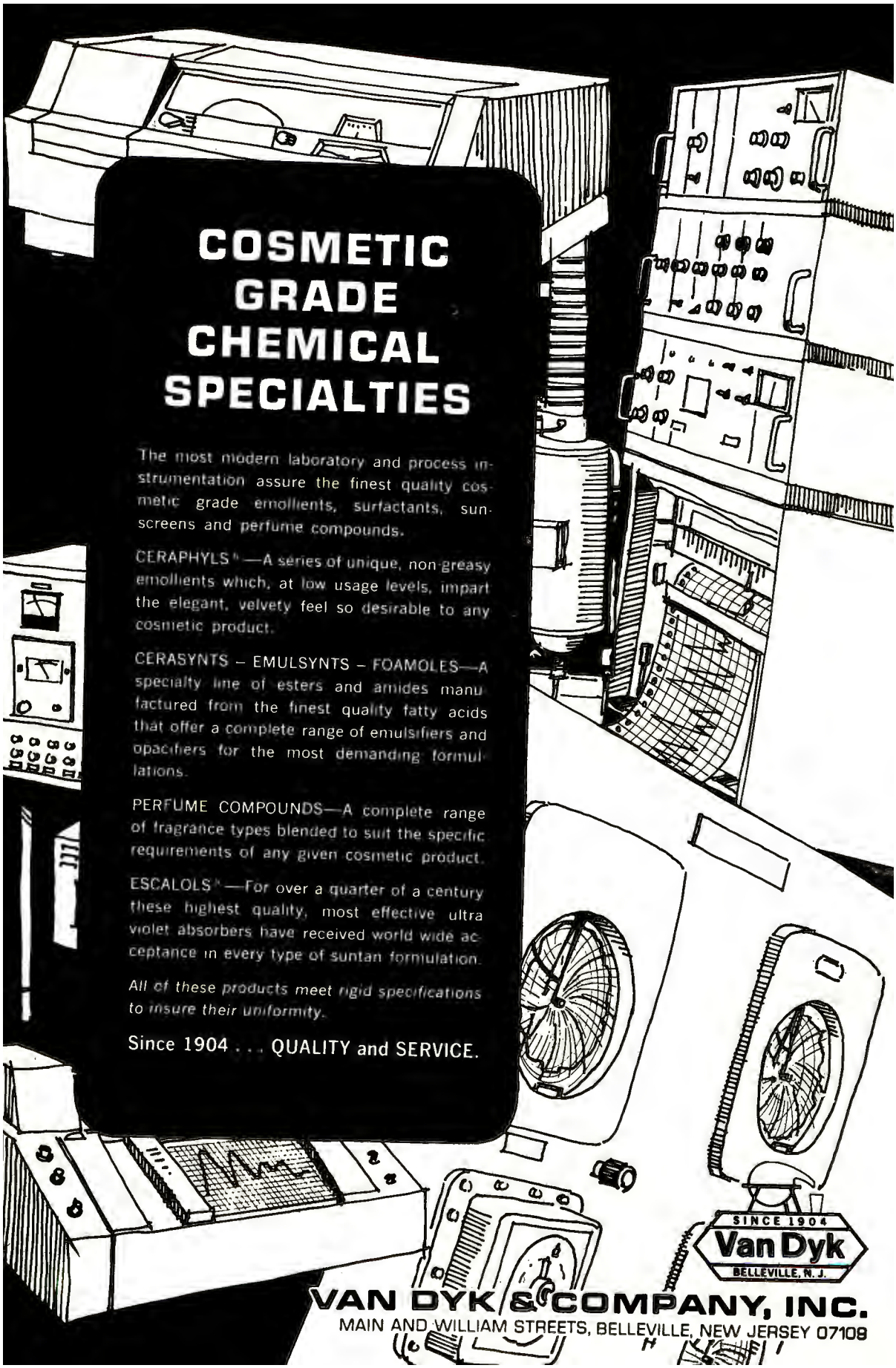
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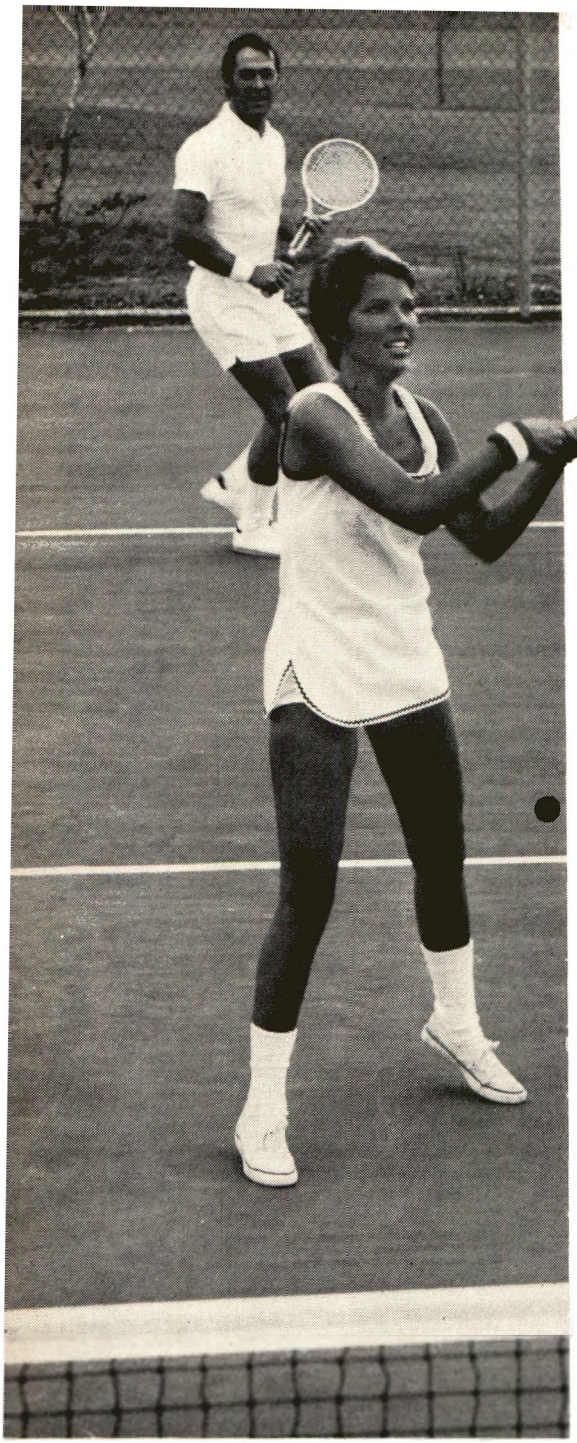

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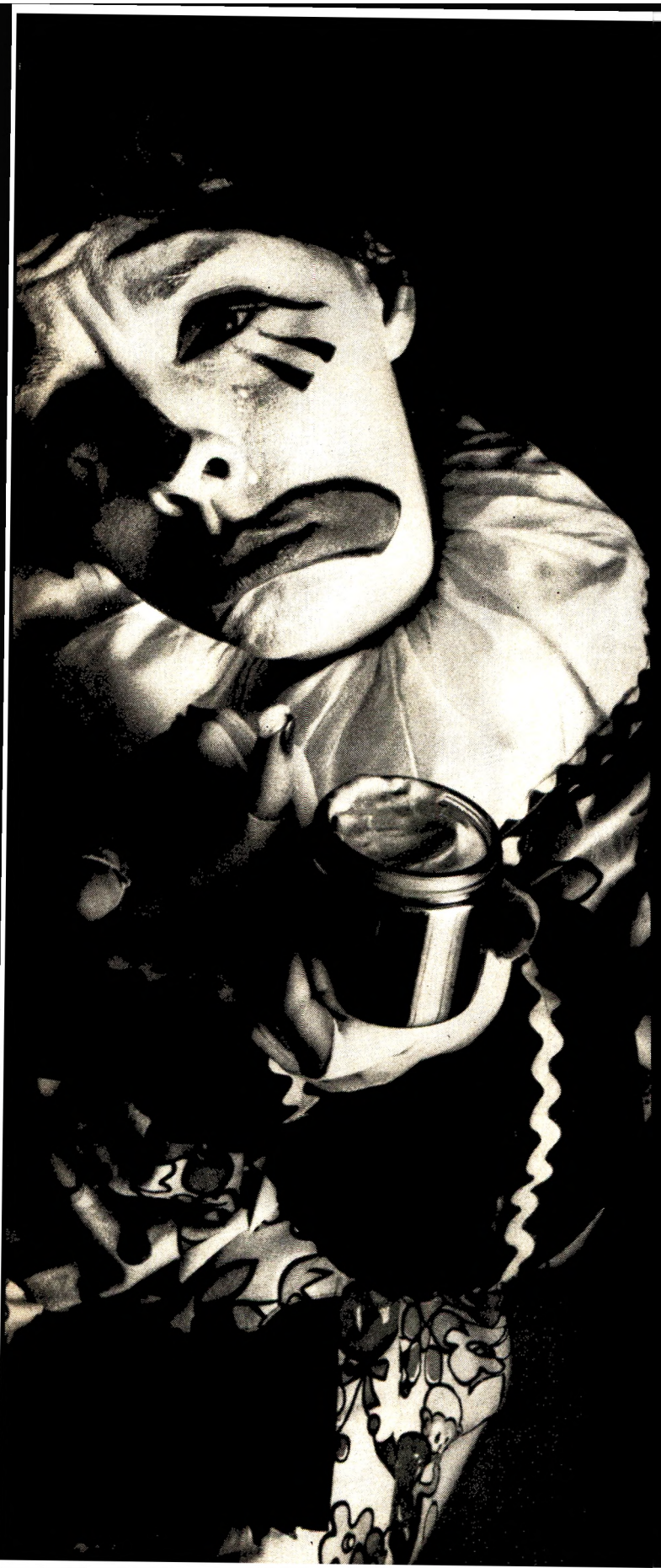

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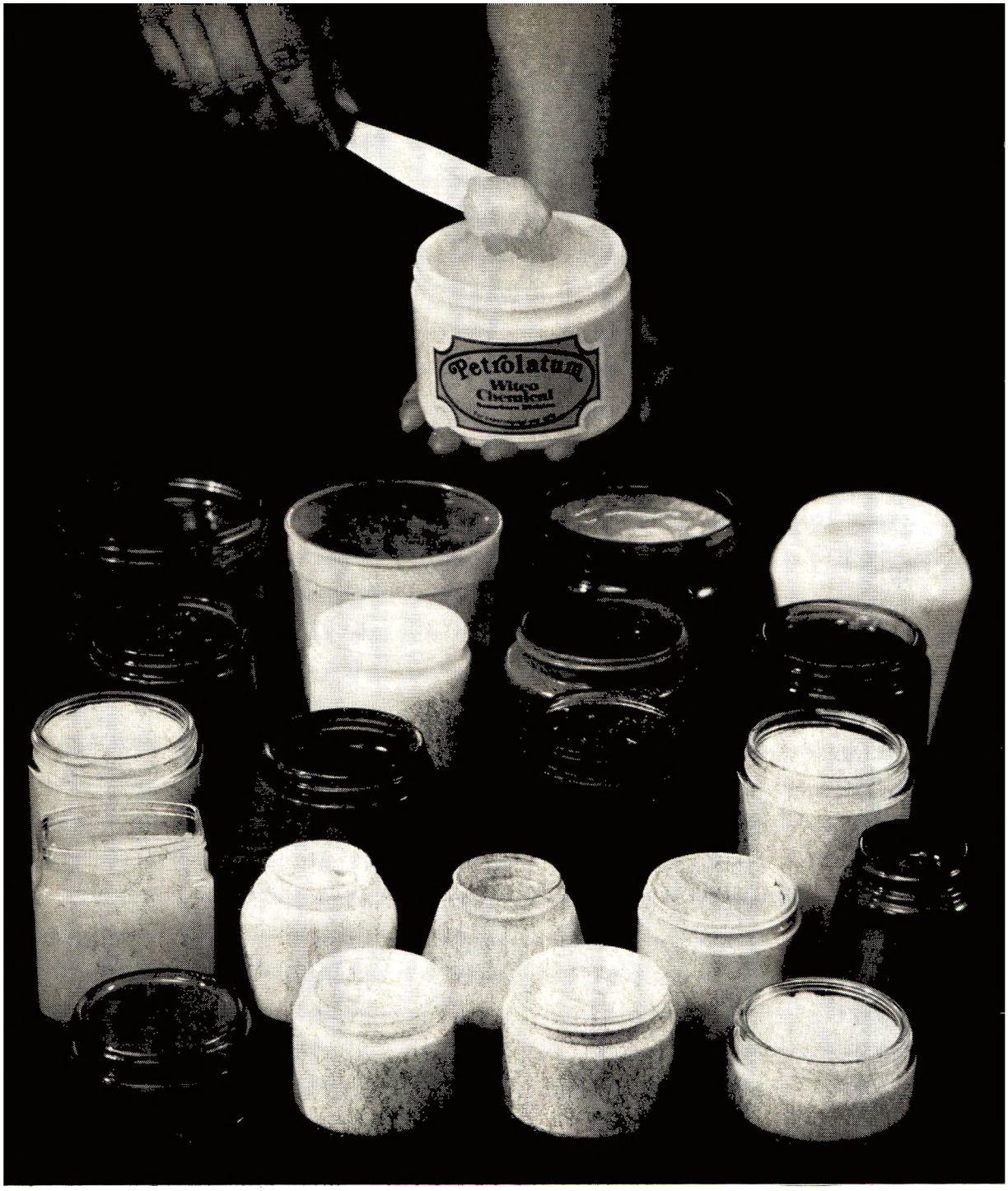

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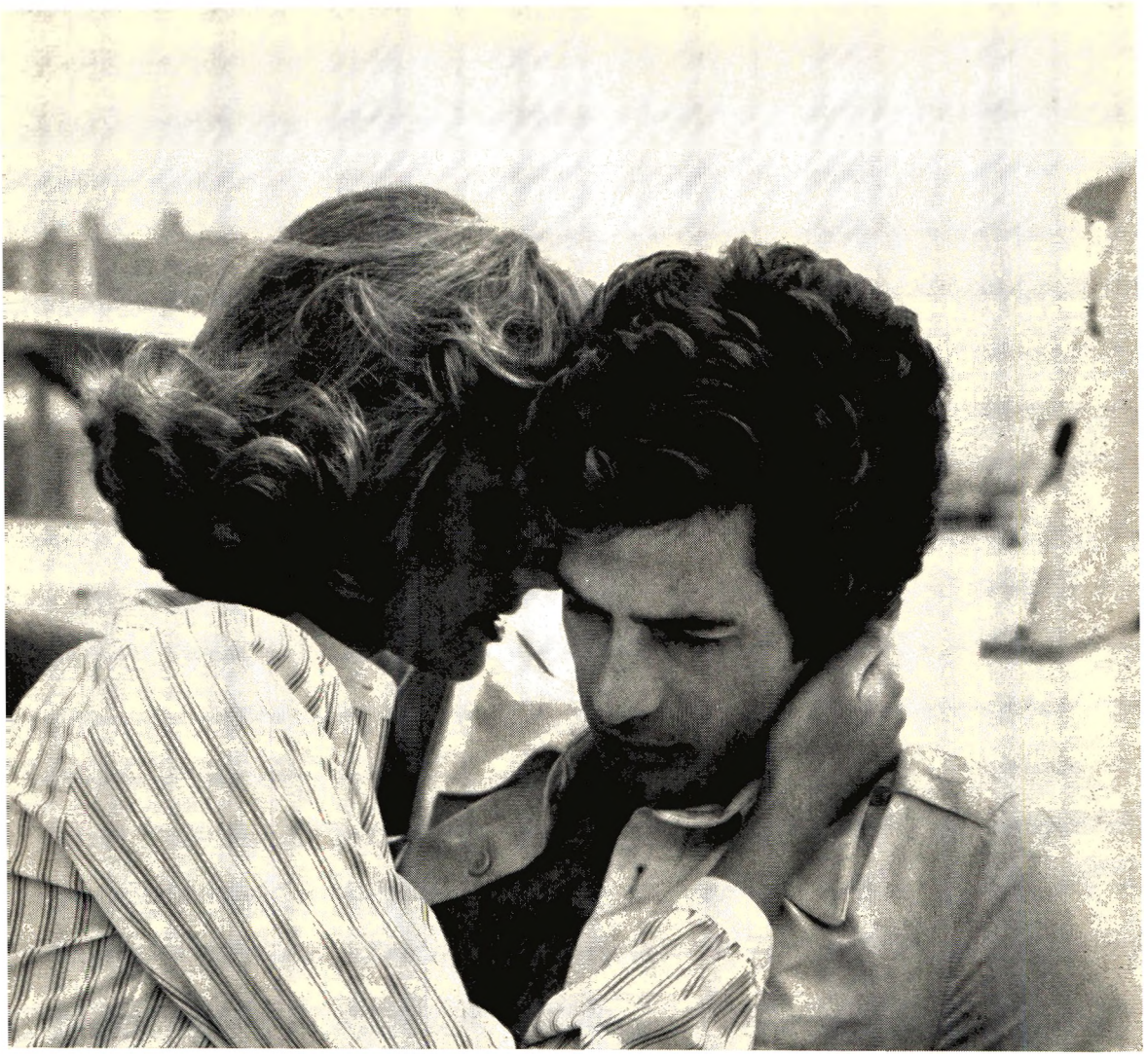

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The following synopses can be cut out and mounted on 3 x 5 in. index cards for reference, without mutilating the pages of the Journal.

Combability measurements on human hair: Mario L. Garcia and Jose Diaz. *Journal of the Society of Cosmetic Chemists* 27, 379 (September 1976)

Synopsis—An instrumental method for measuring the effect of cosmetic products or any other treatment on the combability of human hair has been developed. The required instrumentation, experimental procedure, and interpretation of the data are presented in detail. The method involves the continuous recording of the forces, which oppose the motion of a comb through a swatch of hair. The data thus produced consists of graphs showing the forces opposing (or generated by) combing as a function of the position of the comb along the length of the swatch. Examples of applications are presented.

Testing skin tolerance in the hairless mouse: Christian Gloxhuber. *Journal of the Society of Cosmetic Chemists* 27, 399 (September 1976)

Synopsis—The uv-produced edema in the hairless mouse is an experimental model which may be used for testing the skin tolerance of cosmetic products. It is also suitable as a sunburn model.

Birefringence: polarization microscopy as a quantitative technique of human hair analysis: Roger K. Curtis and Don R. Tyson. *Journal of the Society of Cosmetic Chemists* 27, 411 (September 1976)

Synopsis—An alternative to the conventional method of mechanical stress-strain analysis of human hair condition is presented in this paper. Numerical birefringence is an extremely sensitive measure of molecular orientation. As such, this technique has the potential of determining hair fiber condition as a fundamental molecular level. Basic theories of polarization microscopy are presented and utilized as the basis of a quantitative technique developed for the measurement of birefringence in hair. The theories, morphological origins, and contributions of both the intrinsic and form birefringence components, and the correlation of numerical birefringence with the mechanical properties of hair are discussed. Numerical birefringence, a quantitative measure of the optical anisotropic properties of a hair fiber cortex, as a reflection of the hair strand condition presently observed with mechanical stress-strain testing, is demonstrated.

Intra and extracellular cementing substances: H. P. Baden, L. D. Lee, and J. Kubilus. *Journal of the Society of Cosmetic Chemists*, **27**, 433 (September 1976)

Synopsis—The stratum corneum consists of flattened compacted cornified cells which are filled with cross-linked fibrous proteins. The association of the fibrous proteins with a specific lipid gives rise to the barrier characteristics of the epidermis. Stratum corneum cells are attached to one another by desmosomes and an intercellular cementing substance. The latter material has been rather poorly documented and described. Recent studies concerning diseases associated with hyperkeratosis which employed a keratolytic gel, have suggested that solubilization of this material can result in the loss of adherence of cells to one another. The solubilized material appears to have unique properties, which will be characterized.

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Combability Measurements on Human Hair

MARIO L. GARCIA, Ph.D. and JOSE DIAZ, B.S.*

Presented May 29, 1975, SCC Seminar, St. Louis, Missouri

Synopsis: An instrumental method for MEASURING the effect of cosmetic products or any other treatment of the COMBABILITY of HUMAN HAIR has been developed. The required instrumentation, experimental procedure, and interpretation of the data are presented in detail. The method involves the continuous recording of the forces, which oppose the motion of a comb through a swatch of hair. The data thus produced consists of graphs showing the forces opposing (or generated by) combing as a function of the position of the comb along the length of the swatch. Examples of applications are presented.

Introduction

Combability can be defined as the subjective perception of the relative ease or difficulty with which human hair can be combed. It depends on the magnitude and on the fluctuations of the forces that oppose combing.

Combability is an important attribute, which is always considered when judging the "condition" of human hair. Improved combability is perceived as the hair being in better condition. Another concept closely associated with combability is that of manageability. Still another factor related to combability is that of the mechanical damage, which is done to hair with the combing process, which is accelerated if the hair is hard to comb or to untangle. It follows that combability, due to its close connection with other desirable hair qualities, is a very important factor in judging the performance of many hair care products.

The method described in this paper was developed in our laboratories for the purpose of quantitatively evaluating combability. It has been extensively tested with a wide variety of hair products and treatments and is now used as a standard test during product development and for claim substantiation in finished products. A number of instrumental methods for evaluating combability have been reported in the literature (1–3). Some of the similarities and differences between those methods and ours will be discussed later. It is our opinion that our method has advantages in its simplicity and in the type of information that can be obtained by using it.

*Clairol Inc. Research Labs., 2 Blachley Road, Stamford, Conn. 06902.

In what follows, the method is first described in full detail so that it can easily be used by any interested laboratory. This description is followed by a selected number of experimental results, interpretation of the data, and a general discussion of the method.

Method

Experimentally, the method consists of suspending a hair swatch from a force-measuring device, inserting a comb close to the root end of the swatch, setting the comb in a straight combing motion through the swatch at a constant speed, and continuously recording the forces that resist its motion during this transit from the point of insertion till it clears the tip end of the swatch. The data resulting from this operation consist of a graph showing the load (in grams) opposing (or generated by) combing as a function of the position of the comb along the length of the swatch. We call this graph a 'Combing Curve.'

Combing curves can be recorded using dry or wet hair. Typical examples of these curves can be seen in Fig. 1 (dry) and Fig. 2 (wet). Dry combing curves are recorded using swatches, which have been previously hand combed. In spite of the precombing, they show gradually increasing combing forces which reach maximum values at or near the tip end of the swatch. Wet combing curves are recorded using swatches which have been purposely tangled by immersing them in water. The resulting curve shows a high incidence of tangles all through the length of the swatch. In some cases, the combing forces are higher close to the tip end of the swatch.

In our method, combability is measured by means of two parameters, which can be directly obtained from the combing curves. The first parameter is 'peak combing load' (PCL). This is the highest load (in grams) that is recorded during the combing of the swatch. Points P in Figs. 1 and 2 are examples of PCLs. If desired, PCL can be converted to peak combing forces (PCF) (in dynes) by multiplying them by the acceleration of gravity ($\approx$980. cm/sec^2). The second parameter is the average combing load (ACL). This is the average load during one combing of the swatch. It is expressed in grams • cm units) by the distance in centimeters traveled by the comb through the swatch.

Both of these parameters give us a quantitative measure of how difficult (or easy) it is to comb a swatch of hair. Our method is based on measuring the changes that occur in such parameters when the hair is treated with a product. Decreases in PCL and/or ACL, which indicate improvements in combability (and vice versa) correlate with what is perceived when the hair is combed by hand.

As could be expected, the absolute values of the PCLs and ACLs depend on a large number of factors such as speed of combing, handling of the hair, dimensions of the hair swatch, curliness of the hair, comb dimensions, comb material, etc., which cannot be totally controlled. It is for these reasons

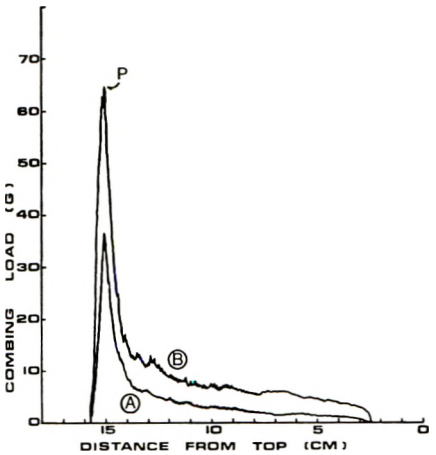

Figure 1. Dry combing curves, B before treatment, A after treatment with commercial creme rinse. Combing loads appear plotted against distance of comb from top end of swatch

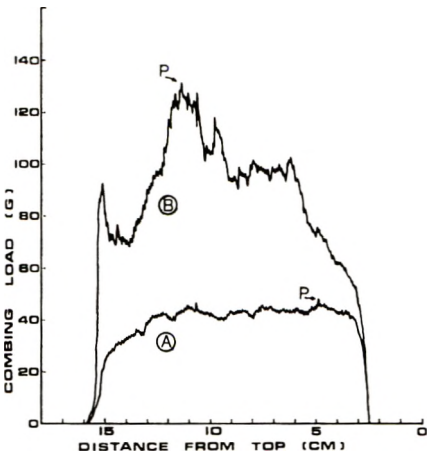

Figure 2. Wet combing curves, B before treatment, A after treatment with commercial creme rinse. Combing loads appear plotted against distance of comb from top end of swatch

that changes in the values of these parameters are more important and more reproducible than their absolute values. It also follows that great care has to be taken so that comparisons—as, for example, before and after the use of a product—are done under experimental conditions that are, insofar as possible, identical. If done carefully, however, this method allows us to measure changes in combing forces of the order of ± 20 per cent. Average changes are calculated by averaging the individual values measured on a set of replicate swatches.

Equipment

The instrumentation consists of an Instron Tensile Tester (Metric Table Model, TM-M°) to which some attachments have been added (see Fig. 3). The Instron load cell B which has a range of 0 to 2,000 g is used. Other recording tensile testing instruments could be similarly adapted.

The attachments to the Instron Tester shown in Fig. 3 are as follows (c) Comb Stand: the comb stand is an L-shaped aluminum part designed to hold different types of combs, it is mounted on the traveling crossbar of the Instron by means of two screws; (b) Comb: the comb used in our measurements consists of 8 cylindrical stainless steel teeth (unpolished), 2.2 mm in diameter, mounted (with an interteeth distance of 1.5 mm) on an aluminum frame. Two removable bars of the same material and diameter as the teeth are

°Instron Corp., Canton, MA.

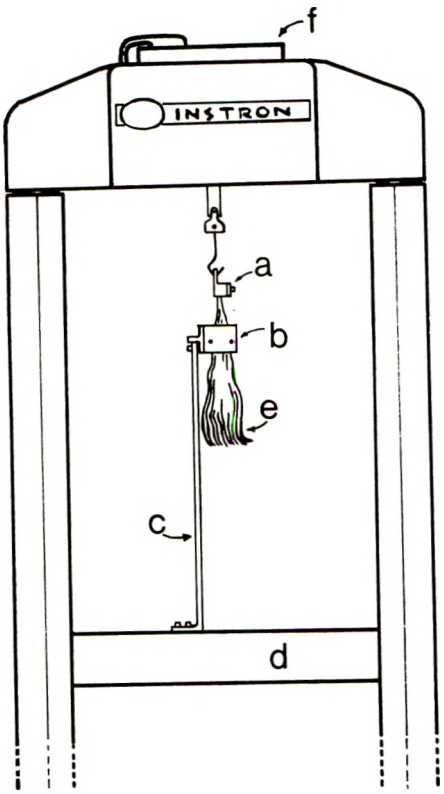

Figure 3. Part of Instron Tensile Tester showing combing attachments: (a) clamp; (b) comb; (c) L-shaped stand; (d) crossbar; (e) hair swatch; (f) load cell

mounted perpendicular to them in order to keep the hair in place during combing (Fig. 4). (a) Clamps: the hair as used for these measurements is mounted on specially designed aluminum clamps (Fig. 4). These clamps produce approximately rectangular cross-sectional swatches .They have a hole on the top from which they are hung on a 1.5 mm diameter metal rod, which is connected to the load cell of the Instron. At the clamp, the dimensions of the cross-section of the hair swatch are 2.8 cm in length and approximately 2 mm in width. At least two regular hand combs should be at hand for each measurement. One of the combs should only be used for clean untreated hair swatches. The other one should be used for the treated swatches. Ordinary hard rubber or nylon combs are suitable.

The measurements are done under standard temperature and humidity conditions ($70 \pm 2°F$, 65 ± 2 per cent RH). This requires the availability of a temperature and humidity controlled room wherein the tensile tester can be located and operated.

Sample Preparation

The preparation of swatches consists of mounting the hair that has been selected to be used in the measurements, on the clamps previously described. The uniformity of this operation is facilitated by proceeding as follows: start by securing enough hair for a complete set of measurements. Six swatches are recommended in routine evaluations of products. If changes in combability are very small, more swatches might be required to ascertain statistically significant changes. About 10 g of hair are required to prepare each swatch. Although if could be desirable to use perfectly straight hair, this is not practical because it is difficult and expensive to obtain such hair. Virgin European hair with its natural soft curl is perfectly suitable for these measurements and should be used.

If possible, all the hair to be used in 1 set of measurements should come from the same batch of commercially purchased hair. Blending the hair is not necessary and is not recommended because the coherence of the hair's natural curl is lost, and this results in excessive tangling.

The length of the individual hairs to be used should be uniform. A good length to start with in preparing swatches is 11 in.

Measure the length of the hair in inches. On a top loading balance weigh (to ± 0.2 g) individual bundles of hair (one for each swatch) so that each

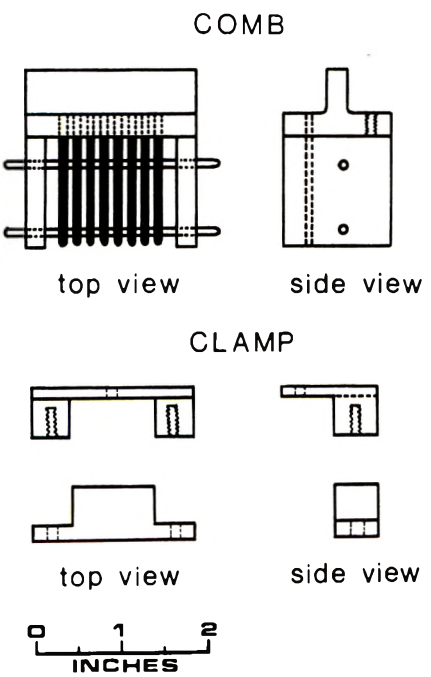

Figure 4. Top and side views of comb and clamp used in measurements

bundle's weight in grams is approximately equal to its stretched length in inches times (0.7); that is, the linear density of each bundle should be approximately 0.7 g/in. Handle the bundles gently so that the hair's natural curl is not unnecessarily disturbed.

Place the rootend section of a bundle in the throat of the clamp and distribute the hair evenly throughout its width. Allow approximately 1 in of hair beyond the throat of the clamp. Secure the male part of the clamp to the other part by means of the 2 screws. Proceed in the same way with the rest of the bundles. Cut the excess (root end) hair close to the top of the clamp's jaws. Stretch the hair swatch with the hands and cut the tip end of the hair at a distance of 6.5 in. from the closest end of the clamp. This can be done conveniently using a laboratory guillotine.* The amount of hair freely hanging from the clamp will weigh 4.5 to 5.0 g.

The hair mounted on the clamps should be cleaned in order to remove dirt, grease, or any foreign material that might be present on the hair when it is purchased. The cleaning is done using a 15 per cent aqueous solution by weight of sodium lauryl ether sulfate. The (unadjusted) pH of this solution is in the range of 7 to 8. The solution is liberally applied twice to each swatch as if it were a shampoo. After the second application, the swatches are thoroughly rinsed under running deionized water (at room temperature) for 30 min. For our purposes, this cleaning treatment is more realistic and less arbitrary than the commonly used precleaning of hair with various organic solvents.

After cleaning, the swatches are combed and hung to dry and equilibrate to the standard conditions of 70°F (21.1°C) and 65 per cent RH for 24 hours in a controlled environment room or chamber.

Experimental Procedure

The experimental procedure consists of measuring the PCLs and/or ACLs of the same hair swatches wet and/or dry before a treatment and after the treatment.

Measurements on Untreated Hair—Wet Measurements as follows. Step 1: turn on the Instron, allow it to warm up, and calibrate, following its operation manual. Step 2: place each of the swatches to be measured in deionized water at room temperature (70°F) for at least 10 min and for no more than 30 min prior to the measurements. This can be done by supporting the swatches by the clamps and allowing them to hang freely inside a large beaker (≈ 3000 ml) full of deionized water. Step 3: take the first swatch out of the water and comb it until no detangling is noticed on further combing. Start this operation using

*Harvard Apparatus Co., Inc., Dover, MA.

the wide-tooth section of a hand comb and finish using the thin-tooth section. Step 4: immerse the hair swatch 3 consecutive times in a separate beaker containing deionized water at room temperature. The purpose of this step is to generate a certain degree of tangling of the swatch under controlled conditions. This step is very important and care should be taken to perform it in the same way each time. It should be done by holding the swatch through the clamp and gently dipping it in and out of the water 3 times. After the third immersion, squeeze out the excess water twice with the fingers. Step 5: with the crossbar sufficiently out of the way (below), hang the hair swatch from the load cell hook and adjust the pen of the recorder so that the swatch plus the clamp read zero. To do this, use the balance control of the recorder, which does not affect its calibration. Step 6: remove the swatch from the load cell and hang it by its clamp close to the Instron. Displace the crossbar upward to the starting position. At this point, the teeth of the comb should be at a distance of 2.5 cm from the lower edge of the hair swatch clamp. Step 7: hang the hair swatch from the load cell, and using two fingers to flatten the swatch, push-guide the hair into the comb. If done carefully, this operation insures a fairly even distribution of the hair between the interteeth spaces. Also, because the width of the swatch at the clamp is 2.8 cm, and the distance between the 2 outer teeth of the comb is 3 cm, it is simple to have all the hair "in" the comb. Place the thin metal rod below the teeth, perpendicular to them, to prevent the hair from coming out of the comb during the measurements. Step 8: once the hair is properly placed in the comb, the actual measurement can be started. This consists of continuously recording the force that is required to move the comb down through the hair swatch at a constant speed. This is done by setting the crossbar in downward motion while continuously recording the load. If a recorder integrator is available, it should be functioning so that the area under the combing curve is measured. The combing speed will be set at 10 cm/min and the chart speed at 10 cm/min. The sensitivity for the recorder will be set according to the values of the forces encountered. Step 9: repeat steps 4 through 8 twice for the same swatch in order to record triplicate runs. Step 10: repeat steps 2 through 9 for the remaining swatches. Step 11: after the wet measurements are completed, comb the swatches using the hand comb, hang them through the clamps and allow them to dry and condition for at least 24 hours at 65 per cent RH and 70°F.

Dry Measurements: Dry measurements comprise steps 12 and 13. Step 12: Start the dry measurements by taking the first conditioned hair swatch and combing it with the hand comb until no detangling is noticed on further combing. Proceed then with steps 5 through 9 as before. Step 13: repeat step 12 for the remaining swatches.

Treatment: Give the treatment to the hair mounted on the clamps following the recommended instructions for the product. Use deionized water whenever

water is needed. The amount of product to be applied to each hair swatch is calculated taking into account the amount recommended for a head of hair, using 100 g of hair as the average weight of hair for adult females, and weight of hair in each hair swatch. The following formula is applied for this purpose.

$$\text{Weight of product to apply per swatch} = \frac{(\text{weight of hair swatch}) \times \left(\begin{array}{c}\text{Amount of product} \\ \text{recommended for a} \\ \text{head of hair}\end{array}\right)}{100}$$

If rinsing with water is the last step of the treatment, care should be given to this operation. Rinsing should be sufficient to eliminate excess product, but not so intense so that the effect of a product could be completely eliminated. The way in which the product is used in actual practice should be followed. For example, shampoos are rinsed until foam is no longer evident; the same should then be accomplished with the rinsing given to the hair swatches. Once the conditions are specified, care should be exercised in rinsing each of the swatches in the same way. Rinsing conditions should specify volume of water, temperature of the water, rinsing time, and method (flowing water or immersion).

Measurements of Treated Hair: Wet measurements on treated hair should be done right after the treatment, preceded only by a 5-min period in which the treated swatch is allowed to relax immersed in water. The rest of the wet treated swatches should be left hanging from their clamps while they wait for the 5-min relaxation period and subsequent measurement. The main reason for doing the wet measurements right after the treatment is because, in practice, the hair has to be combed after any treatment, and it is at that point that the user will associate the product with its effect on wet combability. Obviously, wet measurements can be done at a later time if this will contribute additional information on the effect of the product.

Calculations

Once the measurements are completed the data required to calculate changes in combability are obtained from the combing curves.

The PCL for each run corresponds to the highest load recorded for that run and is read directly from the corresponding combing curve. The load for a full-scale deflection use for recording obviously has to be taken into account.

The ACL for each run is calculated by first measuring the area under the corresponding combing curve (in grams cm • units) and then dividing the value for the area by the distance in centimeters that the comb travels through the hair in that run. This distance is read directly from the curve.

It has been our experience, in developing this method, that per cent changes in PCL are similar in value to per cent changes in ACL. For this reason, and

Table I
Combability Results on Bleached Hair
Before and After the Use of a Semipermanent Dye Product
A

	Wet Measurements 70°F) Peak Combing Load (G)								
	Before Treatment (BT)				After Treatment (AT)				
Swatch	Run Number			Average	Run Number			Average	Per cent
Number	1	2	3	Column	1	2	3	Column	Change
1	700.	1050.	1160.	970.	980.	820.	320.	707.	−27.1
2	1500.	1125.	925.	1183.	366.	360.	664.	463.	−60.9
3	525.	690.	1000.	738.	664.	422.	460.	515.	−30.2
4	1200.	1175.	1210.	1195.	650.	900.	616.	722.	−39.6
5	850.	1950.	825.	1208.	436.	500.	480.	472.	−60.9
6	1845.	1425.	1550.	1607.	440.	350.	530.	440.	−72.7

Average BT = 1150, average AT = 553.

$$\text{Per cent change} = \left(\frac{553. - 1150.}{1150.}\right) \times 100 = -52. \text{ per cent.}$$

B

	Dry Measurements (65 per cent RH, 70°F) Peak Combing Load (G)								
	Before Treatment				After Treatment				
Swatch	Run Number			Column	Run Number			Average	Per cent
Number	1	2	3	Average	1	2	3	Column	Change
1	800.	530.	784.	705.	236.	258.	220.	238.	−66.3
2	416.	390.	304.	370.	200.	247.	140.	196.	−47.0
3	500.	340.	275.	372.	263.	198.	140.	200.	−46.2
4	488.	365.	399.	417.	275.	310.	255.	280.	−32.9
5	1180.	800.	500.	827.	400.	210.	370.	327.	−39.5
6	620.	550.	650.	607.	187.	155.	256.	200.	−32.9

Average BT = 550, average AT = 240.

$$\text{Per cent change} = \left(\frac{240. - 550.}{550.}\right) \times 100 = -56 \text{ per cent.}$$

because they are more readily calculated, the use of PCL values is recommended. The computations are best illustrated by considering examples.

Example I

The data, which appears in Table I are typical and correspond to an experiment that was done for the purpose of determining the effect of an experimental semipermanent dye product on the wet and dry combability of bleached hair.

The columns with headings Run 1–3 contain the PCL values for each of the 3 replicate runs that were recorded for each swatch.

The average column consists of the averages of the runs for each swatch. The average before treatment (BT) and average after treatment (AT) values are the averages of the average values appearing in the average columns. The per cent change in PCL is calculated using the expression

$$\frac{(\text{Average PCL AT} - \text{Average PCL BT})}{\text{Average PCL BT}} \times 100 = \text{per cent change in PCL}$$

If average combing loads are measured instead of peak combining loads, the data are treated in an identical manner.

The results of the above set of measurements are summarized as follows. Effect of experimental direct dye base on combability: average per cent change in dry PCL · · · −56. per cent; average per cent change in wet PCL · · · −52. per cent.

The percent change columns on the right hand side of Table I give the per cent changes for the individual swatches. We have chosen not to use these numbers, i.e., their averages, to calculate the total per cent change in PCL, due to the treatment. These numbers, however, give, on inspection, a practical indication of the reproducibility of the experiment and/or treatment effects.

Example II

In most cases, combability measurements involve the comparison of the effects on combability of two or more products. Even when this is not the case, a product whose effect on combability has been previously measured, is normally included in the experiments. This is recommended because it serves as an internal standard which will detect any bias in the results due to differences in the hair. Hair from the same source should always be used in any comparative study.

A comparison between products can be done by measuring the effect on combability of each product individually as in example I and then comparing the average per cent changes. If the effects on combability of two products are sufficiently different, that is, of the order of (per cent change PCL) Product A− (per cent change PCL) Product B ≧ 20 per cent, and if the per cent change in PCL for most of the replicate swatches is uniform, the validity of the observed difference frequently can be decided by simple inspection of the data. If there is any doubt and/or the data are going to be used as part of documentation supporting claims for a product, the statistical significance of the differences must always be established. This is best accomplished by doing an analysis of variance on the data (4). Such an analysis is illustrated as follows with data that were used to compare the effect of shampoo A (Table II) to that of shampoo B (Table III) on the combability of dry human hair. As can be seen in the Tables, 8

Table II

Effect of Shampoo A on the Dry Combability of Untreated Brown Hair

	Peak Combing Load (Grams) 70°F, 65 Per Cent Relative Humidity												
	Before Treatment						After Treatment with Shampoo A						
	Run 1	Run 2	Run 3	Run 4	Run 5	Average Column	Run 1	Run 2	Run 3	Run 4	Run 5	Average Column	Per cent Change
Swatch 1	223.	155.	160.	193.	173.	181.	183.	133.	190.	153.	173.	166.	− 8.
Swatch 2	228.	333.	310.	233.	143.	249.	173.	173.	115.	198.	150.	162.	−35.
Swatch 3	103.	90.	58.	65.	50.	73.	80.	75.	68.	70.	58.	70.	− 4.
Swatch 4	123.	143.	170.	100.	148.	137.	115.	125.	120.	125.	148.	127.	− 7.
Swatch 5	115.	158.	110.	108.	138.	126.	145.	113.	98.	138.	140.	127.	0
Swatch 6	160.	128.	140.	135.	88.	130.	73.	123.	100.	75.	115.	97.	−25.
Swatch 7	90.	170.	125.	193.	213.	158.	123.	138.	100.	253.	93.	141.	−11.
Swatch 8	60.	50.	40.	50.	50.	50.	38.	55.	35.	38.	53.	44.	−12.

Average before treatment = 138.

Average after treatment = 116.8.

$$\text{Per cent change} = \left(\frac{116.8 - 138.}{138.}\right) \times 100 = -15. \text{ per cent.}$$

Table III

Effect of Shampoo B on the Dry Combability of Untreated Brown Hair

	Peak Combing Load (Grams) 70°F, 65 Per Cent Relative Humidity												
	Before Treatment						After Treatment with Shampoo B						
	Run 1	Run 2	Run 3	Run 4	Run 5	Average Column	Run 1	Run 2	Run 3	Run 4	Run 5	Average Column	Per cent Change
Swatch 9	138.	113.	178.	143.	150.	144.	255.	110.	195.	93.	213.	173.	+20.
Swatch 10	173.	148.	165.	175.	108.	154.	118.	200.	145.	155.	108.	145.	− 6.
Swatch 11	170.	165.	215.	268.	190.	202.	133.	155.	145.	240.	230.	181.	−10.
Swatch 12	120.	130.	120.	150.	140.	132.	150.	130.	110.	185.	105.	136.	+ 3.
Swatch 13	100.	70.	95.	90.	95.	90.	65.	60.	105.	90.	100.	84.	− 7.
Swatch 14	80.	60.	85.	80.	75.	76.	95.	65.	45.	65.	60.	66.	−13.
Swatch 15	100.	100.	115.	80.	105.	100.	145.	175.	125.	145.	180.	154.	+54.
Swatch 16	110.	80.	65.	85.	100.	88.	60.	55.	60.	45.	65.	57.	−35.

Average before treatment = 123.2.

Average after treatment = 124.5.

$$\text{Per cent change} = \left(\frac{124.5 - 123.2}{123.2}\right) \times 100 = +1 \text{ per cent.}$$

Table IV
Analysis of Variance for Data in Table II

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between rows	185,415.7	$n_1 - 1 = 7$	26,488.
Between columns	9,052.5	$n_2 - 1 = 1$	9,052.5
Row X column interaction	14,459.6	$(n_1 - 1) \times (n_2 - 1) = 7$	2,065.7
Residual	73,495.6	$n_1 n_2 (n_3 - 1) = 64$	1,148.4
Total	282,423.4	$n_1 n_2 n_3 - 1 = 73$	

n_1 equals number of rows equals 8 (one for each swatch).
n_2 equals number of columns equals 2 (one for each treatment).
n_3 equals number of replications equals 5 (five for each swatch).

swatches were used and 5 replicate runs were done on each swatch. It can be seen in Table II that the use of shampoo A resulted in a decrease of the forces required to comb 7 out of 8 swatches used. On the other hand, Table III shows that of the 8 swatches that were treated with shampoo B, 3 showed an increase and 5 a decrease in their PCLs.

Analysis of Variance for Data in Table II. The statistical parameters needed to perform the analysis are shown in Table IV. The following operations are done to determine the significance of the different components of variance.

Step 1. Significance of the interaction (between rows and columns) against the residual: 2,065.7/1,148.4 = 1.79. For degrees of freedom (df) $N_1 = 7$, $N_2 = 64$ the above ratio is not significant at the 95 per cent confidence level. (Fisher variance ratio test.) This means that the data do not show any detectable statistically significant interaction between the treatment and the PCLs of the swatches. If the interaction had been significant, it would indicate that the effect of the product is a function of a characteristic of some of the swatches, in our case their initial before treatment average PCL. This seldom occurs if all the swatches are prepared from the same homogeneous batch of hair. If it does, it indicates inhomogeneity of the hair, and the best solution is to prepare more swatches and exclude from the set of swatches those that have extremely high values for their initial before treatment PCL. This should be done on the complete set of swatches participating in the experiment. The set would then be randomly divided in half into subsets to be used with each product.

Step 2. Pooling of the sums of squares of the interaction and residual and their degrees of freedom: (14,459.6 + 73,495.6)/(7 + 64) = 1,238.8. This number is now treated as a new mean square for the residual.

Step 3. Significance of the variance due to differences between columns (i.e., due to shampoo A treatment). The value of the ratio of the mean square of the between columns term and that of the new residual determines the significance of the "between columns" variance: 9,052.5/1,238.8 = 7.31. For degrees of freedom $N_1 = 1$ and $N_2 = 71$ the value of the ratio indicates

Table V
Analysis of Variance for Data in Table III

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between rows	133,028.0	$n_1 - 1 = 7$	19,004.0
Between columns	32.5	$n_2 - 1 = 1$	32.5
Row X column interaction	13,401.0	$(n_1 - 1) \times (n_2 - 1) = 7$	1,914.4
Residual	59,972.0	$n_1 n_2 (n_3 - 1) = 64$	937.1
Total	206,433.5	$n_1 n_2 n_3 - 1 = 73$	

n_1 equals number of rows equals 8 (one for each swatch).
n_2 equals number of columns equals 2 (one for each treatment).
n_3 equals number of replications equals 5 (five for each swatch).

statistical significance at the 99 per cent confidence level. This means that the decrease in PCL measured experimentally (−15. per cent) can be considered statistically significant at the 99 per cent confidence level.

Analysis of Variance for the Data in Table III. The parameters needed to perform the analysis appear in Table V.

Step 1. Significance of the interaction: 1,914.4/937.1 = 2.04. For $N_1 = 7$, $N_2 = 64$ the interaction is not significant at the 95 per cent confidence level.

Step 2. Pooling of sums of squares and degrees of freedom: (13,401. + 59,972.)/(7 + 64) = 1,033.4.

Step 3. Significance of the variance due to between columns differences: 32.5/1,033.4 = 0.31. For $N_1 = 1$, $N_2 = 71$ is not significant.

These results confirm that the changes noticed on combing loads (Table III) after the use of shampoo B do not indicate any effect due to this treatment.

The analysis of variance provides us with a criteria to establish the statistical significance of the observed changes in combing forces. In order to compare the effect of shampoo A to that of shampoo B we can proceed as follows.

Step a. Calculate the difference between the average after and average before treatment values for each shampoo, i.e.,

$$\text{average difference for shampoo A} = \overline{D_A} = 116.8 - 138. = -21.2 \text{ g}$$
$$\text{average difference for shampoo B} = \overline{D_B} = 124.5 - 123.2 = 1.3 \text{ g}$$

Step b. Calculate the standard error (standard deviation) of the difference in the means, i.e., D_A and D_B using the formula (5)

$$\text{Standard error} = \frac{\sigma \times \sqrt{2}}{\sqrt{n}} = \sigma_m$$

where σ is the common standard deviation of each of the before and after treatment means, and n (40) is the number of observations used to calculate the means. In our case, σ will be given by the square root of the residual mean square calculated in Step 2 of the analysis of variance. We will have the following:

$$\sigma_{ma} \text{ for shampoo A} = \frac{35.2 \times 1.41}{6.32} = 7.85$$

$$\sigma_{mb} \text{ for shampoo B} = \frac{32.1 \times 1.41}{6.32} = 7.16$$

If desired, these numbers can be used to estimate confidence level limits ($\pm$ L) for the differences, i.e., $\pm L = t \times \sigma_m$. The residual variance used to calculate the standard errors has 74 degrees of freedom. The corresponding value for t for the 95 per cent confidence level is 2.0, hence

$$\pm L_A = \pm 2. \times 7.85 = \mp 15.7 \qquad \pm L_B = \pm 2. \times 7.16 = \pm 14.3$$

and the changes in combing forces for shampoos can be expressed as follows:

Change in PCF shampoo A $= -21.2 \pm 15.7$ g

Change in PCF shampoo B $= \quad 1.3 \pm 14.3$ g

Step c. In order to calculate the significance of the difference between the two average differences for each shampoo we perform a t test. The value for t is given as follows:

$$t = \frac{D_A - D_B}{\sigma_c} \sqrt{\frac{n_1 \times n_2}{n_1 + n_2}}$$

in which σ_c is the combined standard deviation obtained by combining σ_{ma} and σ_{mb}. According to the expression

$$\sigma_c^2 = \frac{\sigma_{ma}^2 \times df_a + \sigma_{mb}^2 \times df_b}{df_a + df_b - 2} = 1151.7$$

$$\sigma_c = 33.9$$

where the degrees of freedom = 74. Also, $n_1 = n_2 = 40$. The calculated value of t equals 2.97. The value of t found on a t-table for the 99 per cent confidence level is 2.6. The difference between changes in PCL produced by shampoos A and B is thus shown to be significant at the 99 per cent confidence level.

In the present example, the analysis of variance would have been sufficient to demonstrate the superiority of shampoo A over B, because it showed that shampoo A had a significant effect while shampoo B did not. In other cases, however, if both products are shown to have a significant effect, the calculations under steps a to c leading to the t-test are required in order to prove the superiority of a product over the other one.

Applications

Effect of commercial hair products on combability. Table VI shows the effect of a selection of commercial products on the combability of originally untreated human hair. It can be noticed from the table that most types of hair products, if formulated correctly, can improve the combability of human hair.

Effect of quaternary ammonium compounds on wet combability. The effect of quaternary ammonium compounds on the combability of human hair is well known (6). Figure 5 shows the effect of increasing amounts of dodecyltrimethylammonium chloride sorpted by bleached hair on its combability. The hair was bleached for 60 min using a commercial lightening product. It was then treated by immersing it in 0.05 g/100 g aqueous solutions of the quaternary for 0, 1, 5, 60, and 120 min at room temperature and then rinsing for 20 sec under running deionized water. The amount of quaternary on the hair was determined by extraction with chloroform and further analysis using the method of G. V. Scott (7). Table VII shows similar data for a set of quaternary ammonium compounds (8). The two levels of uptake were pro-

Table VI
Effect of Commercial Hair Products on Combability

Product	Per Cent Change in Peak Combing Force	
	Wet	Dry
Regular shampoo	+ 4.%	− 19.%
Conditioning shampoo A	− 23.%	− 27.%
Conditioning shampoo B	− 57.%	+ 31.%
Leave-in creme rinse	− 40.%	− 72.%
Rinse-off creme rinse	− 69.%	− 48.%
Semipermanent dye product	− 38.%	− 34.%
Oxidation dye product A	− 55.%	− 15.%
Oxidation dye product B	+741.%	+ 47.%
Oxidation dye product B plus a rinse-off creme rinse	− 65.%	− 25.%
Lightener (15-min treatment)	+265.%	+ 20.%
Lightener (60-min treatment)	+760.%	+110.%
Lightener (60-min treatment) plus a conditioner	+180.%	− 20.%
Conditioning setting lotion	− 63.%	− 72.%

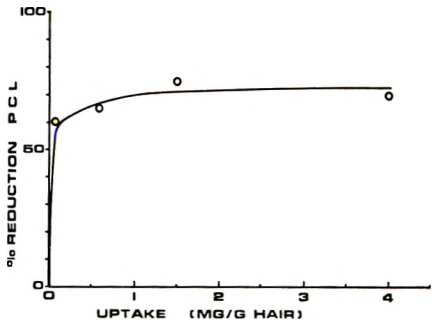

Figure 5. Per cent reduction in wet PCL as function of uptake of dodecyltrimethylammonium chloride on bleached hair

duced by immersing the bleached hair in 1.0 and .05 g/100 g aqueous solutions of the quaternaries for 5 min at room temperature and then rinsing 20 sec as was previously done.

The data showed that only very small amounts of these compounds are needed on the hair in order to produce significant effects on combability, and that increasing the uptake beyond these values does not result in additional benefits. It should be kept in mind that the uptake of these compounds by hair is going to be affected by the pH of the medium, the presence of other compounds in solution (especially anionics), and the type of hair.

Table VII
Effect of Uptake of Quarternary Ammonium Compounds on the Combability of Wet Bleached Hair

	1.0 g/100 g solution 5 min		.05 g/100 g solution 5 min	
Compound	Uptake mg/g Hair	Per cent reduction in PCF	Uptake mg/g Hair	Per cent reduction in PCF
Tetradecyltrimethylammonium chloride	5.9	94. per cent	.2	92. per cent
Decyltrimethylammonium chloride	9.0	83. per cent	1.5	78. per cent
Stearyl dimethylbenzylammonium chloride	2.5	90. per cent	.8	90. per cent
Benzyltrimethylammonium chloride	4.0	54. per cent	.25	50. per cent
Distearyl dimethylammonium chloride	3.6	92. per cent	.2	92. per cent

Discussion

In principle, combability measurements are very simple. In practice, unless great care is taken in the preparation treatment, handling of the swatches, and statistical analysis of the data, the results can lose significance. This is especially true when establishing small differences between products. The main problem arises from the fact that it is difficult to produce a reproducible degree of tangling of the swatches prior to the measurements. In our experience, if the method is followed carefully, changes in combability of the order of ± 20 per cent can be accurately established without having to measure an unpractically large number of swatches. Changes of this order appear to be close to the lower limit of what can be subjectively noticed by combing swatches by hand.

In this method, the before treatment measurements are done on hair which has been cleaned using a detergent solution. This is perfectly justified in testing most products because, in reality, shampooing usually precedes the use of most hair care preparations. In the cases where the effects of shampoos are being measured, it could be argued that the starting point should be unclean hair which, in many cases, has better combability than clean hair. This approach, however, will introduce the unnecessary complication of having to arbitrarily define and reproducibly simulate dirty hair in the laboratory. Although this could be done, we consider that it is justified to start with clean hair and define any effect that a shampoo can have on combability as those effects that can be measured in addition, and beyond the effect produced by shampoos by virtue of just cleaning the hair.

In our method, we chose to quantify combability in terms of PCL and ACL. In particular, PCLs are relevant in terms of what is experienced subjectively while combing hair. This is not only because they correspond to the highest forces, but also because, as shown by the combing curves, they occur abruptly. This characteristic of combing forces has been used by Wedderburn and Prall (3) as the basis for developing a method for measuring combability. It is likely, as pointed out by theso authors, that fast short-term fluctuations in the combing forces, i.e., "tangle noise" or "raspiness" (2), are a factor contributing to the subjective perception of combing resistance. The inclusion of this effect in the evaluation of combability would be most critical in cases where the effect of two products which produce combing curves of similar PCL, but dissimilar "raspiness" were being compared. This situation, however, has not yet arisen in our experience, and we find that qualitatively PCLs increase or decrease simultaneously with the noise level in the combing curves, i.e., smooth curves give small PCLs, while scratchy curves give large ones. Short-term variations in combing forces thus appear to give similar information to that given by PCL. In the absence of a detailed description of the Wedderburn-Prall method, it is not presently possible to do a fair com-

parison with ours. The method developed by Newman *et al.* (1) is similar to ours. It involves the insertion of a comb into a swatch of hair and the measurement of the forces opposing its motion. The authors indicate that after less than a second of comb motion at a rate of 1.5 mm/sec, the combing force reaches a nearly constant value, which is measured. This contrasts sharply with the shape of our combing curves (Figs. 1 and 2), and indicates that these measurements are being done on hair swatches that, either because of their size and geometry and/or because of the way in which they are handled prior to the measurements, do not get tangled before and/or while they are being combed. This approach, although desirable from the point of view of improving reproducibility, is not favored by us, because in reality, tangles are almost always encountered while combing hair, and detangling is an integral part of the function of products developed to improve the combability of hair.

Combability measurements have been reported to be in use at Hoffman-LaRoche Co. Laboratory, but the method has not been described in detail. The method developed by Waggoner and Scott (2), which involves the measurement and analysis of the sound frequencies generated when hair is combed, although it is an interesting approach, suffers in our opinion from the unnecessary complications introduced by the necessity of having to interpret the generation of sound in terms of combing frictional forces which can more easily and directly be measured in the first place.

Conclusion

The increasingly sophisticated product development taking place in our industry is in need of methods which can objectively measure the effect of products on human hair. Although these methods do not completely replace the subjective evaluation of a product's performance, they are obviously of great help in guiding research and in substantiating performance claims made for hair products. Furthermore, by providing quantitative data these methods open the door to the investigation of the underlying physico-chemical phenomena involved in the modification of human hair for cosmetic purposes.

The combability method described in this paper has proven very useful in our laboratory. The authors hope that others will benefit from its use.

Acknowledgment

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J. Soc. Cosmet. Chem., 27, 399-409 (September 1976)

Prüfung von Sonnenbadepräparaten an haarlosen Mäusen

CHRISTIAN GLOXHUBER*

Synopsis — The UV-PRODUCED EDEMA in the HAIRLESS MOUSE is an EXPERIMENTAL MODEL wich may be used for testing the SKIN TOLERANCE of cosmetic products. It is also suitable as a SUNBURN MODEL.

Haarlose Mäuse haben sich in unserem Laboratorium in tierexperimentellen Modellversuchen bei Prüfungen auf Hautverträglichkeit bewährt. Wir haben diese Tierspezies deshalb auch zu Prüfungen herangezogen, wie sie mit Sonnenbadepräparaten durchgeführt werden. Dabei haben wir uns besonders mit folgenden Fragestellungen befaßt:

1. dem Sonnenbrand
2. der Hautverträglichkeit von Sonnenbadepräparaten unter Berücksichtigung einer UV-Vorschädigung der Haut
3. der Hautpigmentierung.

Versuchstiere reagieren unterschiedlich zum Menschen auf eine UV-Bestrahlung. Bei den meisten gängigen Laboratoriumstieren ist das UV-Erythem wenig ausgeprägt. Bei stärkeren Einwirkungen kommt es ohne eine Blasenbildung unmittelbar zu nekrotischen Veränderungen. Wilhelmi (1) und Winder et al. (2) haben sich mit dem UV-Erythem beim Meerschweinchen als Entzündungsmodell befaßt. Eine objektive Quantifizierung bereitet aber Schwierigkeiten. Bei haarlosen Mäusen zeigt sich als Entzündungsfolge einer UV-Bestrahlung in erster Linie ein Ödem, das leicht quantifizierbar ist. Dieses UV-Ödem stellt nach unseren Beobachtungen ein gutes Modell für den Sonnenbrand dar, läßt sich aber auch für verschiedenartige andere Fragestellungen heranziehen (3).

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Methodik

In den von uns durchgeführten Untersuchungen verwenden wir haarlose Mäuse eines selbstgezüchteten hr-hr-Stammes. Zur Erzeugung eines UV-Ödems werden die Tiere in speziellen Haltevorrichtungen fixiert und aus einer Entfernung von 50 cm mit einer Osram-Ultravitalux-Lampe bestrahlt*. Zur Ermittlung der Ödemstärke wird im Bestrahlungsbereich eine Hautfalte in ihrer Dicke gemessen, wobei wir in Anlehnung an Schütz (4) und Schmid (5) einen etwas modifizierten Schnelltaster der Fa. Kröplin (649 Schlüchtern) verwenden. Die Details dieser Methode sind an anderer Stelle beschrieben (3).

1. Abhängigkeit der Ödemstärke von der Bestrahlungszeit

Durch Variation der Bestrahlungszeit läßt sich die Stärke der UV-Reaktion gut dosieren. In einer ersten Versuchsreihe wurde der Verlauf der Ödemreaktion in Abhängigkeit von der Bestrahlungsdauer ermittelt. Gruppen von 10 bzw. 12 Tieren wurden 1, 2,5, 5 bzw. 10 Minuten in der angegebenen Weise bestrahlt und im Anschluß daran das Verhalten des Hautödems bis 96 Std. verfolgt.

2. Wirkung von Lichtschutzpräparaten

Die Wirkung der UV-Strahlung auf die Haut wird in den handelsüblichen Lichtschutzpräparaten durch Lichtfilterstoffe so weit reduziert, daß ein stärkerer Sonnenbrand vermieden wird. Das vorliegende Modell gibt die Möglichkeit, solche Filterstoffe oder Sonnenschutzcremes praktisch zu erproben, wenn sie vor der UV-Bestrahlung auf die Haut appliziert werden. Wir haben drei Produkte, die als Lichtschutzpräparate auf dem Markt angeboten werden, in unserem Modell auf ihre Schutzwirkung geprüft. Die Präparate waren mit den Lichtschutzfaktoren 2, 3 und 5 ausgewiesen. In diese Prüfung haben wir außerdem eine Creme einbezogen, der wir als Lichtfilterstoff 1,4 % 2-Äthylhexyl-p-methoxyzimtsäureester zugesetzt hatten. Die Präparate wurden 1 Stunde und unmittelbar vor der Bestrahlung in dünner Schicht mit dem Finger aufgetragen (Versuchsgruppen 5, 9 bzw. 10 Tiere). Die Bestrahlungsdauer war 10 Minuten. Im Anschluß daran wurde das Verhalten des Hautödems über 144 Std. beobachtet.

3. Hautverträglichkeitsprüfungen an UV-geschädigter Haut

Die Haut haarloser Mäuse ist in ihrer Reaktionsfähigkeit ein empfindliches Instrument gegenüber irritierenden Substanzen. Derartige Wirkungen

* Die erzielte Reaktionsstärke ist bei diesen Bestrahlungen stark von der dem Untersucher oft nicht bekannten vorangegangenen Brenndauer der Lampe abhängig. Der Ausfall der Reaktion muß deshalb häufig durch Kontrollversuche überprüft werden.

lassen sich ebenfalls in der angegebenen Weise messend verfolgen. Dieses Verfahren kann zur vergleichenden Prüfung von Stoffen auf Hautverträglichkeit Verwendung finden. Es hat sich gezeigt, daß die Vorschädigung der Haut, wie sie beim Sonnenbrand vorliegt, ebenfalls simuliert werden kann, d. h. man kann an diesem Modell auch leicht Sonnenschutzcremes etc. auf lokale Verträglichkeit untereinander unter den Bedingungen des Sonnenbrandes vergleichen. Zum Studium dieser Wirkungen wurden verschiedene Hautcremes auf die intakte Haut von haarlosen Mäusen aufgebracht und das Hautverhalten messend verfolgt. Die gleichen Hautcremes wurden im Anschluß daran Mäusen appliziert, die eine durch UV-Strahlung vorgeschädigte Haut aufwiesen.

In diesen Versuchen gelangten verschiedene Cremetypen zur Untersuchung. Sie sind mit I—VI bezeichnet. Die Applikation der Produkte erfolgte unmittelbar im Anschluß an die Bestrahlung (Bestrahlungsdauer 10 min.). Jede Versuchstiergruppe bestand aus 5 Mäusen. Gleiche Tiergruppen wurden auch ohne Bestrahlung analog als Vergleich behandelt. Bei allen Tieren wurde das Verhalten des Hautödems über 144 Std. verfolgt.

4. Untersuchungen über phototoxische Wirkungen

Produkte, die beim Sonnenbaden Verwendung finden, müssen frei sein von Bestandteilen mit phototoxischen Eigenschaften. Das UV-Ödem-Modell an haarlosen Mäusen läßt sich auch für derartige Untersuchungen heranziehen. Allerdings ist es dabei erforderlich, die Bestrahlungsstärke so schwach zu wählen (2,5 min.), daß nur ein geringes Ödem entsteht und eine Verstärkung durch den phototoxischen Stoff erkannt werden kann. In den Versuchen wurde Limetteöl und Bergamotteöl den Tieren 2 Std. und 30 min. vor der Bestrahlung konzentriert in dünner Schicht appliziert. Eine Kontrollgruppe wurde ohne Bestrahlung analog behandelt. Im Anschluß daran wurde das Ödem messend verfolgt.

5. Hautpigmentierung nach UV-Bestrahlung

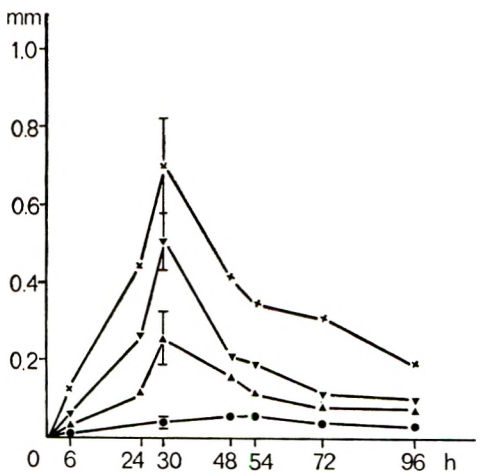
Das Sonnenbaden ist meist mit dem Wunsch verbunden, eine braune, „gesunde" Hautfarbe zu bekommen. Zum Studium von Pigmentierungsvorgängen nach UV-Bestrahlung ist unseres Wissens bisher kein tierexperimentelles Modell beschrieben, das mit der Hautpigmentierung beim Menschen vergleichbar wäre und zu Pigmentierungsstudien herangezogen werden kann. Im Gegensatz dazu werden verschiedene Untersuchungen zur Frage der Depigmentierung beschrieben, z. B. Denton et al. (6), Bleehen et al. (7) und Gellin et al. (8). Der verwendete Stamm von hr-hr-

Mäusen zeigt die Eigenschaft der Pigmentierung nach UV-Bestrahlung und eignet sich zu Pigmentierungsstudien. Auf den Effekt der Pigmentierung haben erstmalig wohl Forbes und Urbach (9) hingewiesen. Die Pigmentierung ist bei diesen Tieren dann gut auslösbar, wenn man sie mit einer schwachen Ödemdosis täglich ca. 14 Tage lang bestrahlt. In den Versuchen wurden die Tiere täglich 10 Minuten in der beschriebenen Weise mit einer Lampe bestrahlt, die schon eine etwas längere Brenndauer aufwies.

Ergebnisse

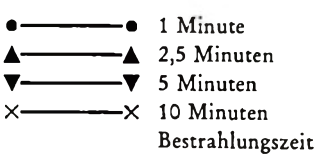
1. Abhängigkeit der Ödemstärke von der Bestrahlungszeit

Die Resultate der Ödemstärke unter verschiedenen Bestrahlungszeiten sind in der graphischen Abb. 1 wiedergegeben. Es zeigt sich, daß das Ödem ein Maximum bei 30 Stunden aufweist und im Anschluß daran eine Normalisierung des Hautzustandes sich wieder einstellt. Bei noch stärkeren Bestrahlungen kommt es zur Ausbildung einer Hyperkeratose, in Extremfällen zu Nekrosen, die hier aber außerhalb der Betrachtung bleiben sollen.



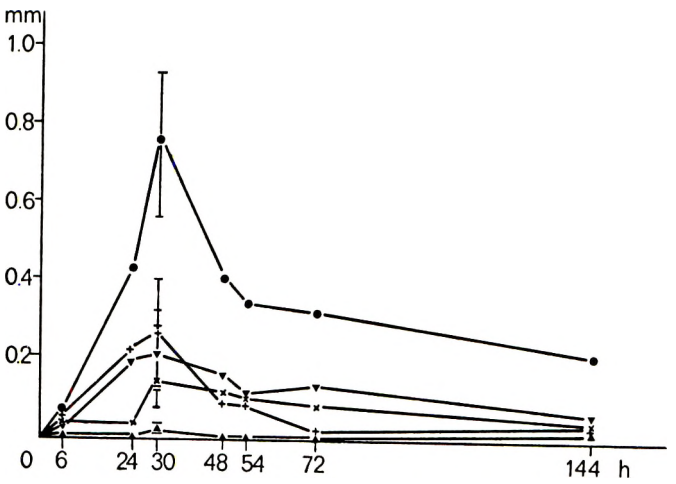
Graph. Abbildung 1

Abhängigkeit der Hautödemstärke von der Bestrahlungsdauer



2. Wirkung von Lichtschutzpräparaten

In der graphischen Abb. 2 sind die Ergebnisse einer Kontrollbestrahlung im Vergleich zu Ergebnissen beim vorherigen Auftragen von Lichtschutzpräparaten wiedergegeben. Die Lichtschutzwirkung ist deutlich aus der Graphik abzulesen.



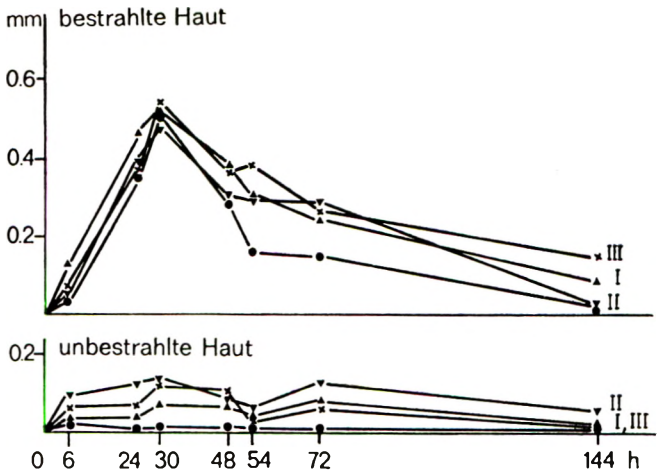
Graph. Abbildung 2

Verhinderung des UV-Ödems durch Applikation von Lichtschutzmitteln

▲——▲ Präparat mit angegebenem Lichtschutzfaktor 5
▼——▼ Präparat mit angegebenem Lichtschutzfaktor 2
×——× Präparat mit angegebenem Lichtschutzfaktor 3
+——+ Creme mit 1,4 % 2-Äthylhexyl-p-methoxy-zimtsäureester
●——● Kontrolle unbehandelt (18 Tiere)

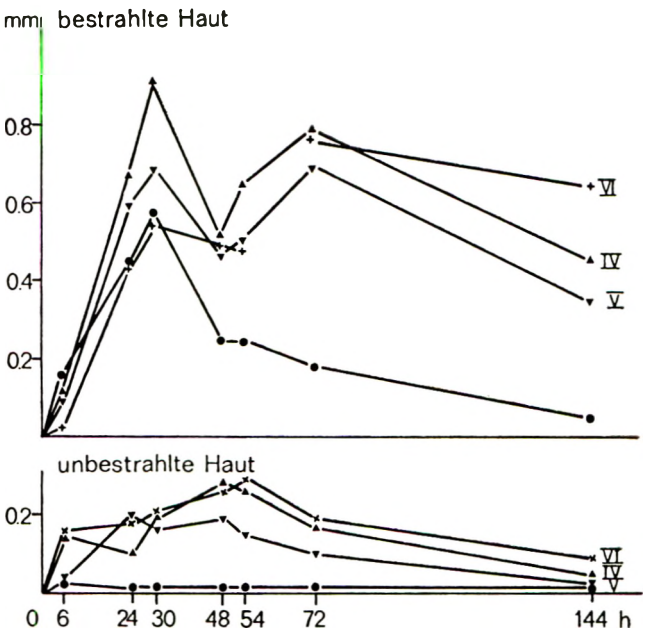
3. Hautverträglichkeitsprüfungen an UV-geschädigter Haut

Das Verhalten von 3 Hautcremetypen (I—III) an intakter Mäusehaut und nach UV-Vorbestrahlung ist in der graphischen Abb. 3 wiedergegeben. Es zeigt sich, daß diese Präparate weder an unbestrahlter noch UV-bestrahlter Haut eine Wirkung erkennen ließen. Die Meßwerte streuen innerhalb der Werte der Kontrollgruppen. In der graphischen Abb. 4 sind weniger ideal verträgliche Cremetypen (IV—VI) in ihrer Wirkung dargestellt. Solche Produkte zeigen bei der Ödemmessung schon an unbestrahlter Haut einen geringen Effekt. Ganz besonders ausgeprägt ist der Effekt bei UV-vorgeschädigter Haut.



Graph. Abbildung 3

Prüfung von Cremetypen an unbestrahlter und bestrahlter Mäusehaut
Teil I Standardabweichungen der 30-Std.-Werte vgl. Tabelle 1



Graph. Abbildung 4

Prüfung von Cremetypen an unbestrahlter und bestrahlter Mäusehaut
Teil II Standardabweichungen der 30-Std.-Werte vgl. Tabelle 1

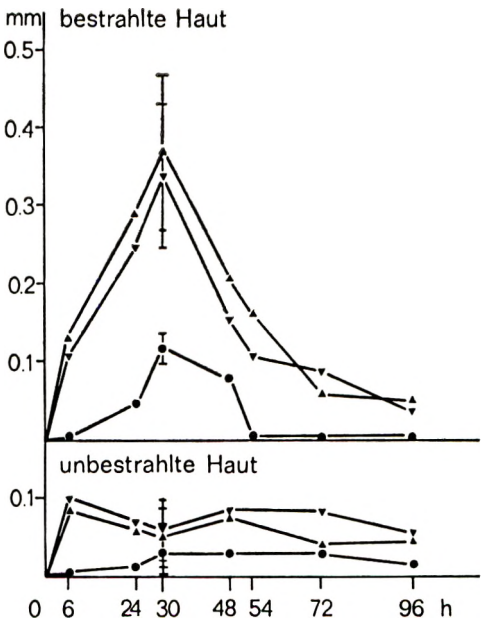
Tabelle 1

Standardabweichungen der 30-Stunden-Werte der Zunahme der Hautfaltendicke

Graph. Abbildung 3	unbestrahlte Haut	bestrahlte Haut
Kontrolle	± 0 mm	± 0,05 mm
Creme Typ I	± 0,1 mm	± 0,06 mm
Creme Typ II	± 0,01 mm	± 0,02 mm
Creme Typ III	± 0,02 mm	± 0,02 mm
Graph. Abbildung 4		
Kontrolle	± 0 mm	± 0,22 mm
Creme Typ IV	± 0,03 mm	± 0,034 mm
Creme Typ V	± 0,03 mm	± 0,17 mm
Creme Typ VI	0,002 mm	± 0,14 mm

4. Untersuchungen über phototoxische Wirkungen

Der phototoxische Effekt von Substanzen läßt sich, wie die graphische Abb. 5 zeigt, mit der Versuchsanordnung erkennen. Bergamotteöl und



Graph. Abbildung 5

Untersuchung von Bergamotteöl und Limetteöl auf phototoxische Wirkungen an der Haut haarloser Mäuse

●——● Kontrolle
▲——▲ Bergamotteöl
▼——▼ Limetteöl

Limetteöl zeigen als unverdünnte Substanzen eine so gute Hautverträglichkeit bei haarlosen Mäusen und lösen unter den Versuchsbedingungen nur so geringe Veränderungen aus, daß sie noch innerhalb der Streuung der unbehandelten Leerkontrolle liegen. Die Bestrahlungsstärke war unter den gewählten Versuchsbedingungen ebenfalls so gering, daß sie ein eben erkennbares Hautödem auslöste. In Gegenwart der beiden Substanzen trat jedoch bei Zusammenwirken von Bestrahlung und Substanzeinwirkung eine deutliche Ödemwirkung auf, die als phototoxischer Effekt gedeutet werden darf, zumal die phototoxischen Eigenschaften beider Substanzen bekannt sind.

5. Hautpigmentierung nach UV-Bestrahlung

Das Ergebnis des beschriebenen Bestrahlungsversuches zeigt die Abb. 1. Das bestrahlte Tier zeigt auf seiner Rückenhaut im Gegensatz zum un-

Abbildung 1

Pigmentierung nach UV-Bestrahlung, Vergleich mit einem Kontrolltier

bestrahlten Kontrolltier eine deutliche Pigmentierung. Diese Pigmentierung blaßt im Laufe der Zeit, wie das auch beim Menschen bekannt ist, wieder ab.

Diskussion der Versuchsergebnisse

Die Haut haarloser Mäuse zeigt bei UV-Bestrahlung in mancher Hinsicht ein qualitativ ähnliches Verhalten wie die menschliche Haut, so daß haarlose hr-hr-Mäuse als Versuchsmodelle für verschiedene Experimente mit Sonnenbadepräparaten herangezogen werden können. Dies gilt vor allem dann, wenn man als Folge der UV-Einstrahlung kein Erythem als Schädigungsfolge erwartet und als solche das Hautödem heranzieht. Ein Hautödem gehört auch zum Bild des Sonnenbrandes beim Menschen.

Die Versuche haben ergeben, daß sich das UV-Ödem der haarlosen Maus nach UV-Bestrahlung gut dosieren läßt. Es ist deshalb möglich, Reaktionen verschiedener Stärke zu messen, wie das für die Ermittlung von Lichtschutzfaktoren nötig ist. Andererseits ist eine geeignete Vordosierung des Schadens nötig, je nachdem, ob man phototoxische bzw. sonnenbrandmildernde Wirkungen untersucht.

Es ist seit langem bekannt, daß die Resorptionsfähigkeit der Haut unterschiedlich ist, je nachdem, ob sie sich im Normalzustand oder in einem Zustand der Entzündung befindet. Man hat diesem Umstand bislang bei der Verträglichkeitsprüfung dergestalt Rechnung getragen, daß man die Produkte nicht nur an intakter, sondern auch skarifizierter Haut prüfte. Eine solche Prüfung von Produkten ist aber im Falle von kosmetischen Präparaten, die allenfalls auf die UV-geschädigte Haut aufgetragen werden, unbefriedigend, und wir glauben, daß das beschriebene Modell sehr viel zuverlässigere Aussagen machen wird, zumal im Modell die gleiche Schädigung wie in der Praxis vorliegt. Produkte, die in dieser Versuchsanordnung insbesondere an UV-vorgeschädigter Haut nicht völlig reaktionslos vertragen werden, können in der Praxis bei Personen mit normaler Hautbeschaffenheit durchaus zufriedenstellend vertragen werden.

Die Möglichkeit, Pigmentierungsstudien an einem tierexperimentellen Modell durchführen zu können, eröffnet der Kosmetikwirkstofforschung eine Reihe interessanter Möglichkeiten, auf die zunächst nicht näher eingegangen werden soll.

Frl. Rosi Südkamp danke ich für die gewissenhafte Durchführung der experimentellen Untersuchungen.

Zusammenfassung

Das UV-Ödem bei der haarlosen Maus ist ein Prüfmodell, das bei der Hautverträglichkeitsprüfung kosmetischer Produkte verwendet werden kann. Es ist auch als Sonnenschutzmodell geeignet.

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Im April 1975 wurde in Oberursel das Deutsch-Schwedische Symposion „Photochemotherapie - Grundlage, Technik und Nebenwirkungen" „Photochemotherapy - Basis Technique and Side Effects" abgehalten, auf dem über Probleme der Photomedizin gesprochen wurde. Insbesondere wurden Vorzüge und Nachteile der *Photochemotherapie der Psoriasis* eingehend diskutiert (UV-Strahlung und UV-Sensibilisatoren). Im Anschluß an diese Tagung wurde auf dem Kongreß der Deutschen Dermatologischen Gesellschaft in Nürnberg mit besonderer Blickrichtung auf das o. g. Thema eine Erklärung verabschiedet, die von Prof. Dr. E. Fahr, Mainz, verlesen wurde. Sie hat folgenden Wortlaut:

Erklärung auf dem Dermatologen-Kongreß in Nürnberg 1975

Es muß nachdrücklich betont werden, daß die Psoralen/Licht-Therapie von größter Bedeutung für die Dermatologie, aber auch für andere Zweige der Medizin und Biologie werden kann. Bevor diese Methode jedoch in die allgemeine dermatologische Praxis übernommen werden sollte, bedarf es noch sehr detaillierter dermatologischer, chemischer und molekularbiologischer Untersuchungen.

J. Soc. Cosmet. Chem., **27**, 411-431 (September 1976)

Birefringence: Polarization Microscopy as a Quantitative Technique of Human Hair Analysis

ROGER K. CURTIS, B.A. and DON R. TYSON, B.S.*

Presented May 29, 1975, SCC Seminar, St. Louis, Missouri

Synopsis: An alternative to the conventional method of mechanical stress-strain analysis of HUMAN HAIR condition is presented in this paper. NUMERICAL BIREFRINGENCE is an extremely sensitive measure of molecular orientation. As such, this technique has the potential of determining hair fiber condition at a fundamental molecular level. Basic theories of POLARIZATION MICROSCOPY are presented and utilized as the basis of a quantitative technique developed for the measurement of birefringence in hair. The theories, morphological origins, and contributions of both the intrinsic and form birefringence components, and the correlation of numerical birefringence with the mechanical properties of hair are discussed. Numerical birefringence, a quantitative measure of the optical anisotropic properties of a hair fiber cortex, as a reflection of the hair strand condition presently observed with mechanical stress-strain testing, is demonstrated.

Introduction

The traditional method of detemining the condition of a human hair fiber is by measuring its mechanical stress-strain characteristics. The parameters determined, including: Young's modulus; force at yield point; ultimate tensile strength; and either break point extension, or various parameters of elastic recovery hysteresis; (1) are abstract or secondary effects of the basic chemical-molecular occurrences in cosmetic treatment and conditioning. What is needed is an additional system of analysis, which by looking at hair on a foundation molecular level, is more specific.

Crystalline, or molecular chain-sheet substances of a nonisometric nature, exhibit the optical property of birefringence when they are placed in a field of plane polarized light (2). This phenomenon, due to a condition known as

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optical anisotropism, is a result of electron orbit polarizability and/or a refractive index differential between a crystal and its surrounding medium in which the crystal is embedded (2, 3, 4). The quantitative measure of birefringence, referred to as the numerical birefringence, is an extremely sensitive measure of optical anisotropism, which arises from molecular orientation (2, 5).

The cortical region of a human hair is optically anisotropic (3, 6). In its function as the major fiber component (7), the cortex contributes 92 per cent to the elasticity of the hair. This is borne out by an analysis of the correlation matrix of a multiple linear regression model that explains the factors contributing to elasticity in hair (8, 14).

In the course of a cosmetic treatment, be it chemical or mechanical in nature, the condition of a hair fiber is changed. The parameter of elasticity, or the resistance to and recovery from deformation by force, plays a major role in the final fiber condition after treatment(1). The change of condition during cosmetic treatment is due to molecular bonding changes, which occur mainly in the cortex (7, 9).

Therefore, the action of cosmetics, which affect condition and elasticity, should also affect the optical anisotropism of the cortex. Thus numerical birefringence, as a very sensitive measure of molecular orientation, emerges as having the potential to very accurately determine hair condition.

This paper presents a quantitative system of analysis of human hair condition, based upon the optical phenomenon of numerical birefringence.

Theory of Birefringe

Plane polarized light is utilized to observe the phenomenon of birefringence. Ordinary light vibrates in waves, traveling in random planes perpendicular to the direction of propagation. By placing a polarization plate in front of a light source, only those waves tarveling in planes parallel to the axis of polarization of the plate are transmitted; the others being absorbed (Fig. 1A).

An anisotropic material has two unique optical properties as follows: (1) any one plane of light waves striking an anisotropic material is split into two wave-planes, traveling 90° relative to each other; and 45° each relative to the original plane of propagation (Fig. 1 B). Hence the term double refraction or birefringence; (2) a plane of light passing through an anisotropic material encounters a path of a different refractive index, and thus travels at a different velocity, in each different direction of traverse (2, 5).

Therefore, a wave of plane polarized light strikes an anisotropic material, is split into two waves, one of which, the ordinary or simply fast wave, is traveling through a path of lesser refractive index, and thus faster than the extraordinary or slow wave, which is traveling at a perpendicular angle in a more difficult path of higher refractive index (2, 5).

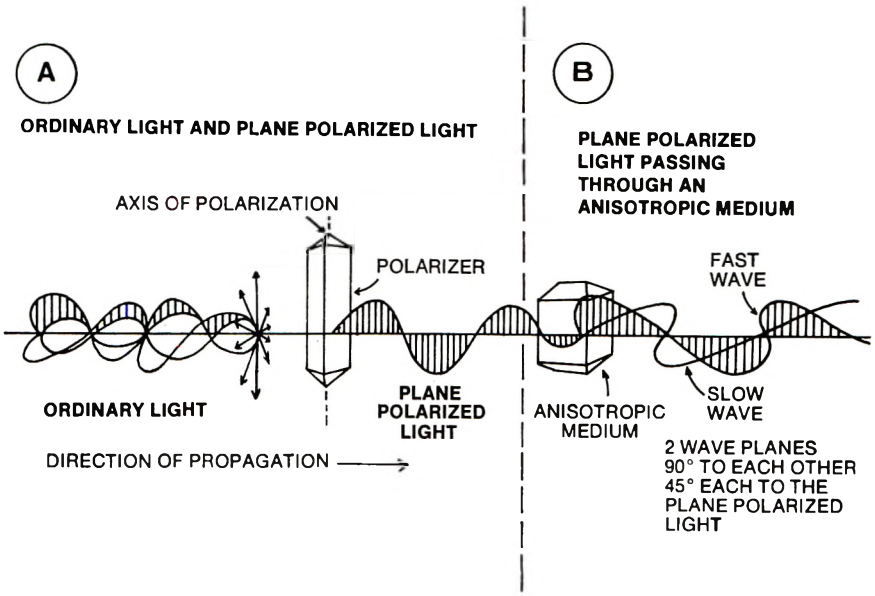


Figure 1. (A) Ordinary light waves being polarized; and (B) ordinary light waves further split into two perpendicular waves, each traveling at different velocity, while traversing anisotropic medium

The difference in refractive index between the two paths of the fast and slow wave ($n_2 - n_1$) equals the numerical birefringence (2).

As the 2 waves advance through the material, the slow wave increasingly lags behind the fast wave in direct proportion to the numerical birefringence (the difference in path velocities) and to the distance or thickness traveled. This quantity of "lag," measured in units of distance, is termed retardation. Hence the formulation, retardation equals numerical birefringence x thickness; or $\Delta = (n_2 - n_1)d$. The numerical birefringence is determined by measuring the retardation and the thickness (2).

The retardation lag of the slow relative to the fast wave causes the two waves to go out of phase with each other (Fig. 2). These two waves, exiting the anisotropic material, interfere with each other, causing an elliptical interference pattern. In Fig. 3(A) the two waves are coming at you in phase with each other, the retardation distance having been some full multiple of the wavelength ($\Delta = n\lambda$). They are vibrating in unison, i.e., each reaching points 1-20 at the same time from points A, A′ to B, B′. The elliptical interference pattern is centered around an axis, formed by connecting the points of intersection of consecutive lines, drawn from the wave position at a particular instant, perpendicular to the wave axis. In Fig. 3(B), the slow wave has been

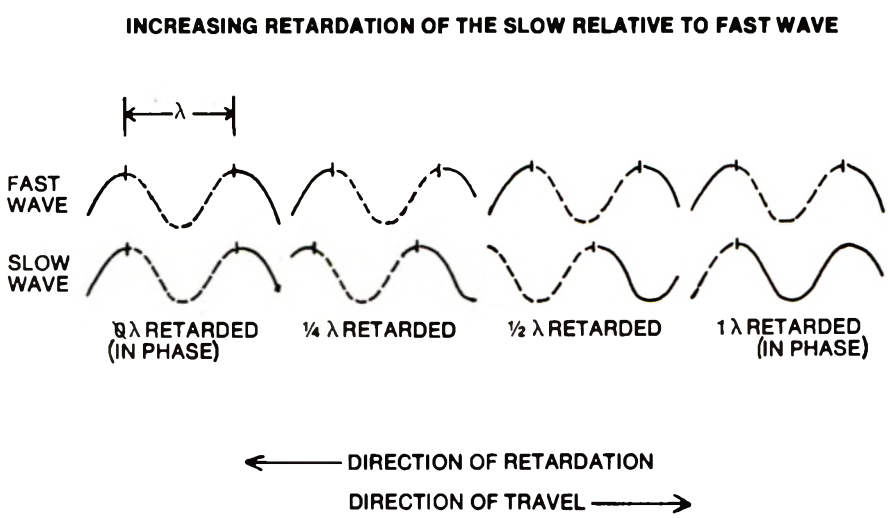


Figure 2. Two parallel waves of equal length λ, traveling at different velocities. As slow wave increasingly retards from fast wave, two go in and out of phase with each other

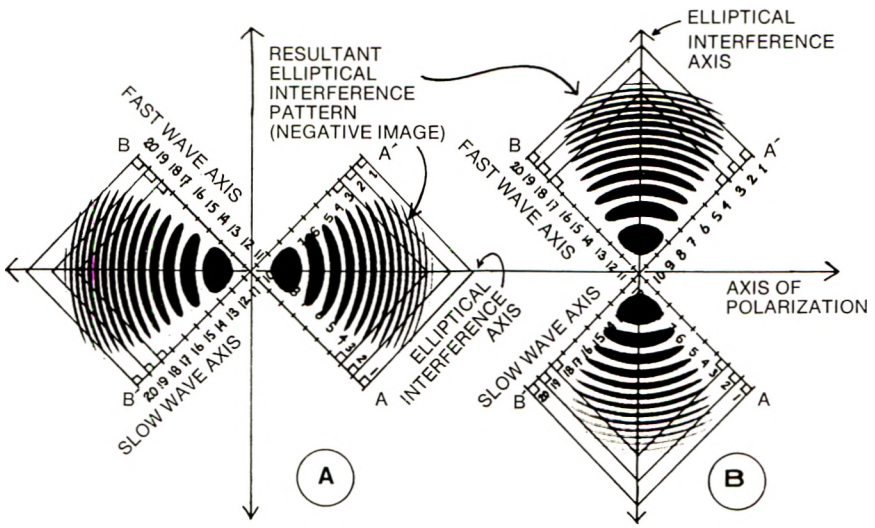


Figure 3. Orientation of elliptical interference pattern formed by two interfering polarized waves; (A) in phase; and (B) ½ λ out of phase (see text)

retarded some full multiple of the wavelength plus one-half ($\Delta = n + \frac{1}{2}\lambda$). In this case, as the fast wave advances from point A, through points 1-20, finally to point B, the slow wave, being ½λ out of phase, advances from point

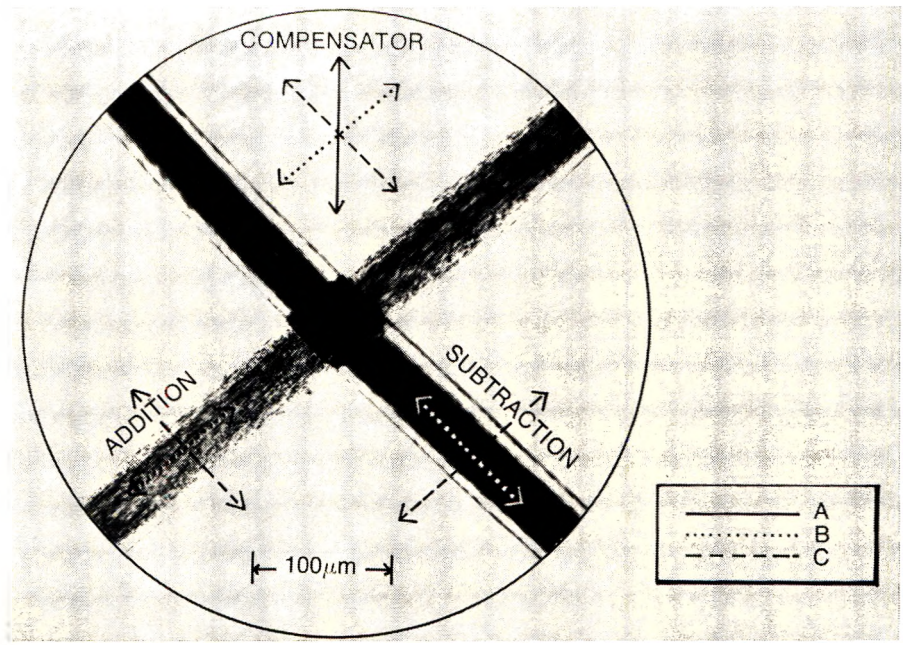


Figure 4. (A) Axis of polarization; (B) slow wave axes of compensator and hair; (C) fast wave axes of compensator and hair. In wave axes of hair and background retardation compensator: parallel alignment produces *summation* of their retardations—perpendicular alignment produces *subtraction* of their retardations

B′, through points 20-1, finally to point A′; the elliptical interference pattern has shifted 90° (2).

An important concept, utilized in the measurement of numerical birefringence, is that of addition and subtraction of retardation. If anisotropic objects are placed in line (i.e., one on top of another) in the path of light of a polarization system with their slow wave axes parallel, the total amount of retardation in the system equals the sum of the retardation of the individual objects. If, however, the slow wave axes are aligned perpendicular to each other, the total retardation is equal to the difference between the objects as they tend to cancel each other out (Fig. 4) (2).

The anisotropic material being illuminated as has been described (Fig. 1(B)) is viewed through a second polarizing plate, termed the analyzer, whose axis of polarization is 90° to that of the "bottom" polarizer. Only when a portion of the elliptical interference pattern lies in the same axis as that of the analyzer, does light pass; thus, light due to retardation, which in turn is caused by the product of numerical birefringence and thickness, can be seen and measured. No light is passed, a condition referred to as extinction, when $\Delta = n\lambda$; maximum light is passed when $\Delta = n + \frac{1}{2}\lambda$ (Figs. 5 and 6) (2, 5).

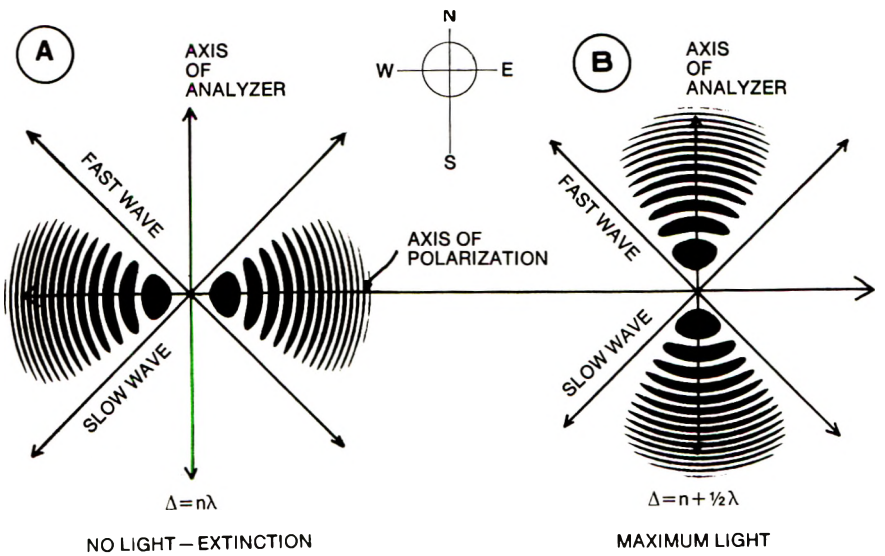
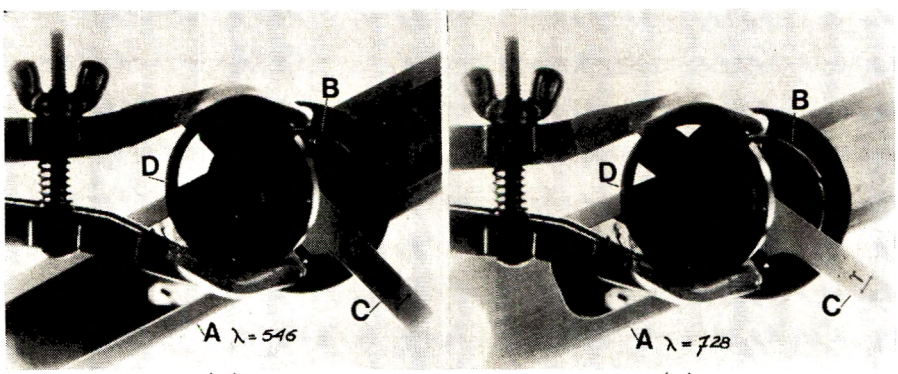


Figure 5. Fast and slow waves exist anisotropic medium to form elliptical interference pattern: (A) $\Delta = n\ \lambda$: the interference pattern is aligned 90° to the analyzer axis; no light can pass; (B) $\Delta = n + \frac{1}{2}\ \lambda$: the pattern is in alignment with analyzer axis; maximum light is transmitted



(A) (B)

Figure 6. (A) (A) Light passing through monochrometer of wavelength λ; (B) polarizer; (C) 546 nm retardation compensator; (D) analyzer. (A) compensator causes 1 λ of retardation; no light; (B) [2 compensators (C) are stacked together equaling 1092 nm retardation]. Compensator causes 1½ λ of retardation; maximum light transmission

Until now, only monochromatic light, or light of one particular color and having only one wavelength, has been considered in the theory of birefringence that has been presented. White light is made up of a combination of all wavelengths of light in the visible spectrum, from about 400 to 700 nm. The same phenomena of birefringence and retardation occur, as has been previously described, for monochromatic light with one exception: retardation of a particular distance value will cause some wavelengths or colors of light to be

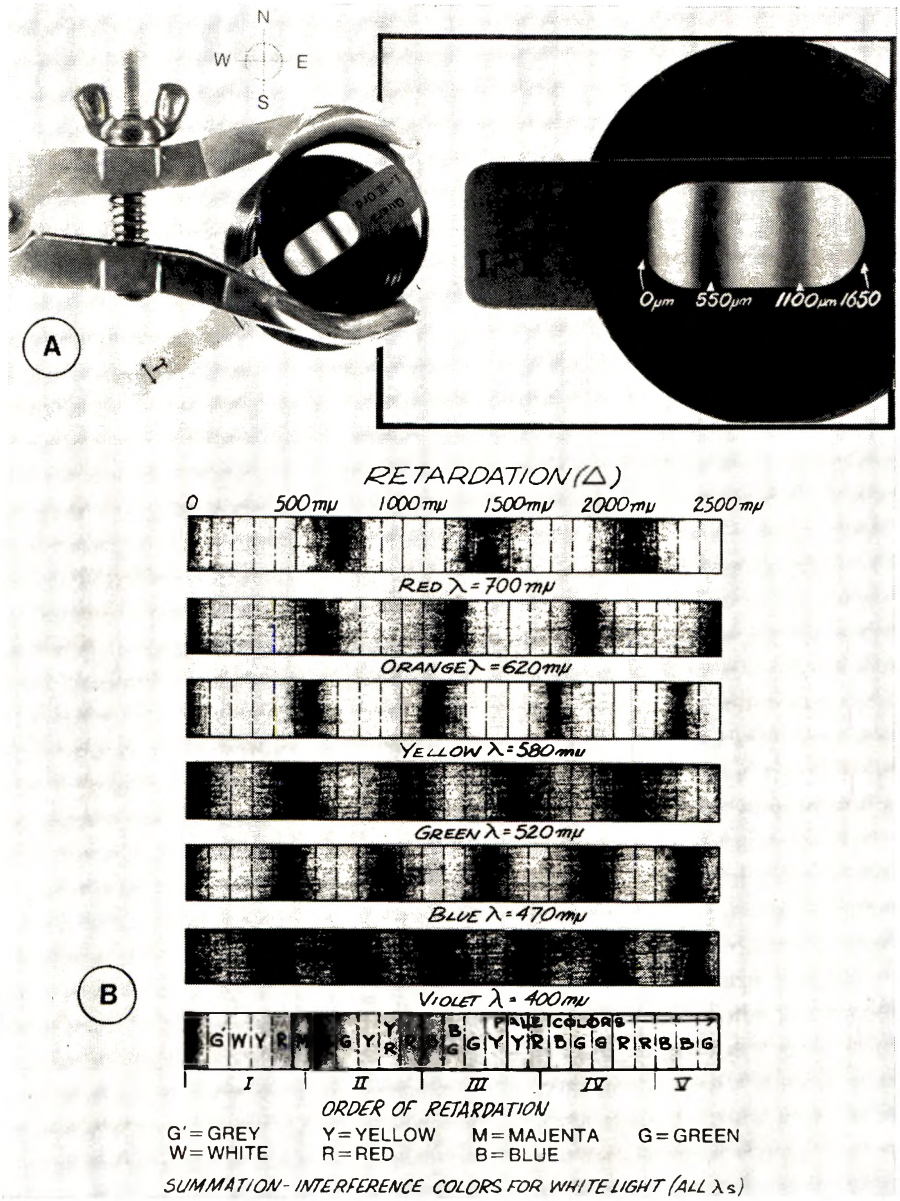


Figure 7. (A) Wedge of aniosotropic quartz causing increasing retardation from 0 μm to 1.650 μm. Note repeating dark bands (representing order of retardation) at intervals of the λ of white light: 550 nm. (B) [Adapted with permission of the publisher (2)], from *Mineral Optics: Principles and Techniques* by Wm. Revell Phillips, W. H. Freeman and Company, copyright © 1971. Retardation of particular value transmitting each color λ differentially. The summation of these λs causes repeating series of colors

in phase, some one-half out of phase, and others out of phase in varying degrees in between. Each of the individual wavelengths will, therefore, pass the top analyzer in a differential amount where they summate, giving a single color or wavelength. Figure 7 is a black-and-white representation of this phenomenon. For a color interpretation of the summation of retardation, refer to Michael Levy's birefringence chart (2).

There are two types of birefringence which occur in an anisotropic material: intrinsic and form (2, 3, 11). Intrinsic birefriengence is a function of the anisotropic polarizability (nonisometric molecular orientation) of electron orbits, and not dependent upon any particular morphology. Form birefringence, however, occurs when crystallites of one refractive index are immersed in a medium of another refractive index. Intrinsic birefringence occurs on a molecular level, whereas form birefringence occurs more on a macromolecular or structural level. The measured quantity, numerical birefringence, is the summation of the intrinsic birefringence and form birefringence (2, 3, 4).

Experimental

Apparatus

Microscope: A Reichert Zetopan research microscope is outfitted for polarization as follows (Fig. 8(A)):

(1) 100 W quartz-iodine lamp house*; (2) strain free, 0.95 N.A., dual-diaphragm condenser;* (3) calibrated-rotating polarizer and analyzer;* (4) 25 X, 0.60 N.A. Neofluar N.A.† objective, checked to be strain free; (5) KPL 8 X Pol Ocular†, modified to contain a measuring reticle; (6) 360° rotating stage*; (7) Gips Rot 1 Ord Compensator* (Fig. 8(B)); and (8) Ehringhaus Compensator with quartz plates† (Fig. 8(B)).

Rotary Device: This device enables a hair fiber to be rotated 360° on its own axis for microscopic viewing and to evaluate structure and measure dimensions (Fig. 8(C)). Two 25x75 mm glass micro slides (i.e., Corning 2948‡), one cut longitudinally with a standard glass cutter, are needed to make 1 reuseable device. The 3 pieces are held together with an epoxy resin glue. It is necessary to use only high quality glass is all parts of the system as any inherent strain present will cause "background" birefringence. A capillary tube ~ 0.5 mm i.d. ~ 1.0 mm o.d., and 75–90 mm long (i.e., Scientific Products B4195-2**), to which a short piece of plastic tubing (Corning Silastic®‡ medical grade tubing 0.035 in. i.d.) is attached, holds the hair for rotation.

The capillary tube is filled with immersion oil, n_D = 1.515, of a light to medium viscosity (Carl Zeiss Immersionsoel†). The holding channel is filled with oil of the same refractive index, but of a higher viscosity to dampen tube movement (i.e., Ziess EinschluBmittel W15†). With a coverslip in place (Corning 22 x 40 mm Number 1-½‡), a nearly homogenous refractive

*Reichert Division of American Optical Scientific Instruments, Buffalo, N.Y. 14215.
†Carl Zeiss, Inc., New York, N.Y. 10018.
‡Corning Glass Works, Scientific Glassware and Equipment Dept., Corning, N.Y. 14830.
**Scientific Products, McGaw Park, Illinois.

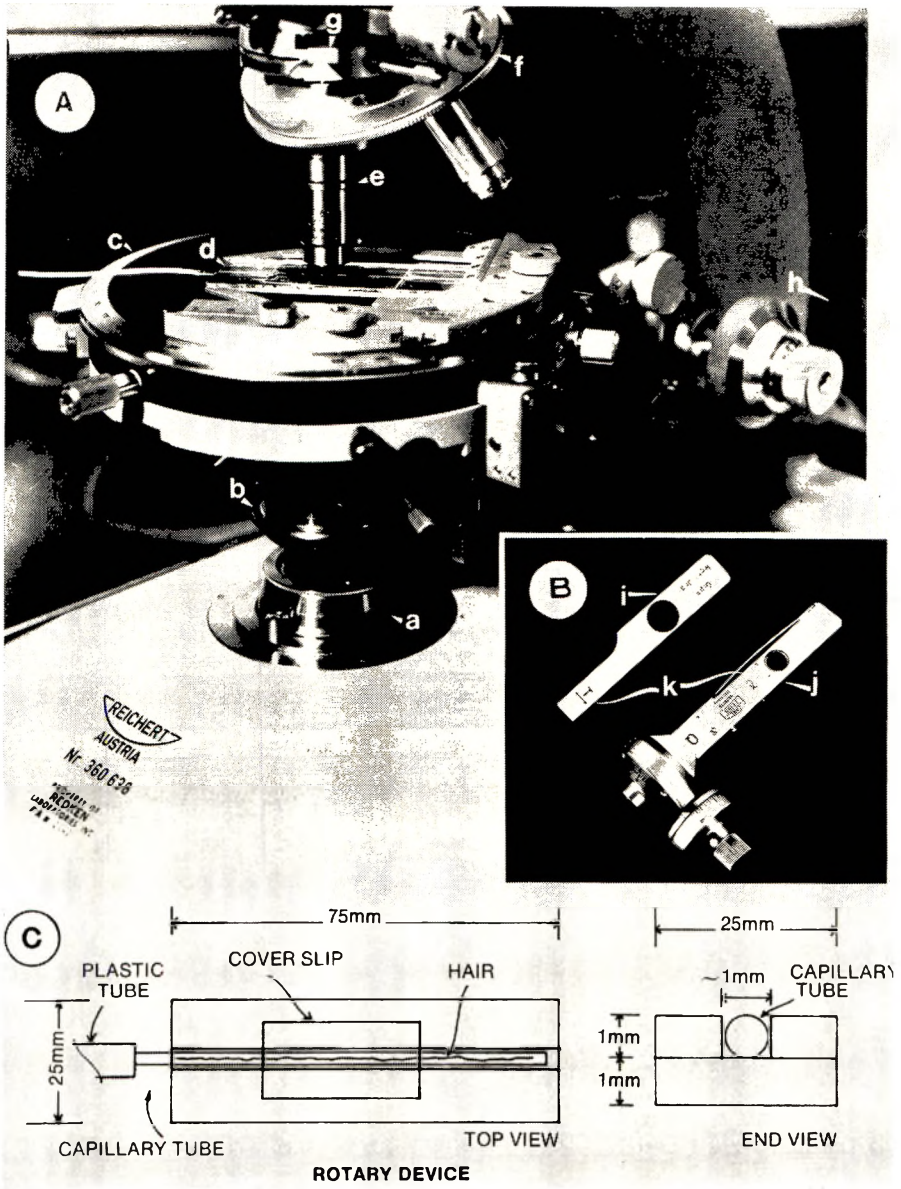


Figure 8. Reichert Zetopan microscope apparatus for determination of numerical birefringence in hair: (A) microscope with (a) polarizer, (b) strain free condenser, (c) rotary stage, (d) rotary device, (e) strain free Neofluar objective, (f) analyzer, (g) compensator, (h) quartz-iodine light source; (B) detail of quartz first order red (i) and Ehringhaus rotary quartz (j) compensators. [Note slow wave axis orientation (k).]; (C) detail of hair rotary device

index path having plane outside surfaces is formed. This arrangement virtually eliminates any distortion, due to the convex surfaces of the capillary tube. Another version of this device has been utilized in previous studies (12).

Stress-Strain Tester

In this study, stress-strain measurements are used as a reference to which the numerical birefringence is compared and correlated. A single hair fiber, 1.9 cm long, is suspended between a set of clamps. Force, applied to one end by a constant speed motor, elongates the fiber at a rate of 1.5 per cent/sec, while being monitored on the other end by a strain gauge transducer.* Stress versus strain graphs are plotted on XY Recorder.†

Procedures

1. *Microscope Alignment;* With the microscopic system having achieved a condition of Kohler illumination, the polarizer and analyzer are inserted and rotated to the correct position. This is best done by first rotating the bottom polarizer to a position of either ϕ, 90, 180, or 270°, and then, while focusing on an illuminated microslide, by rotating the top analyzer until maximum extinction (minimal light transmission) has been reached. If the fixed first-order red compensator plate is now inserted into the compensator slot, a deep red background will appear in the microscope field. This is indicative of a N-S, E-W, polarizer-analyzer alignment, with a diagonal slow-fast wave alignment of the anisotropic compensator (2, 5).

2. *Measurement of Numerical Birefringence in Human Hair Shafts:* To recall, retardation equals numerical birefringence x thickness ($\Delta = (n_2 - n_1)d$). In trying to determine the numerical birefringence, this can be rearranged to read: numerical birefringence equals retardation/thickness ($(n_2 - n_1) = \Delta/d$) (See Fig. 11(B) later on) (2).

A hair shaft of approximately 4 cm length is inserted into the glass capillary tube, into which the medium weight immersion oil is drawn from a reservoir. The capillary can then be plugged and stored in a microhemocrit tube sealer-holder.

*Stathan Instruments, Inc., Oxnard, California 93030.
†Hewlett Packard, Inc., Palo Alto, California 94303.

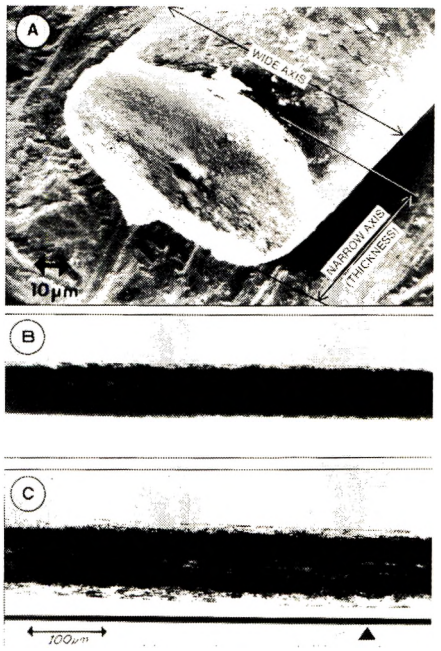


Figure 9. (A) Scanning electron micrograph showing elliptical axes of human hair; (B) hair inside rotary device for measurement on its narrow axis; and (C) on its wide axis. Black line is edge of capillary tube (arrow)

To begin the actual measurement, with the rotary device in place on the microscope stage, a few drops of the heavy weight immersion oil is inserted into the slot, together with the loaded capillary tube, and a coverslip is placed on top. This basic procedure is repeated each time a hair is viewed, as miniature air bubbles, churned by capillary rotation, become trapped under the coverslip, disrupting the homogeneous refractive index, introducing distortion and loss of resolution.

The rotary microscope stage is positioned so that the hair is aligned with its longitudinal axis parallel to the slow wave axis etched on the first order red compensator.

In this position, the slow wave axis of the hair is in the same axis as that of the compensator, giving the hair added retardation providing enhanced viewing ability.

Hair is essentially elliptical in shape, with a wide and narrow axis (Fig. 9(A)). The narrow axis is equivalent to the thickness when the hair is resting on its wide axis, the position used for evaluating the numerical birefringence of the fiber.

The hair is placed exactly on its narrow axis. At that point, both hair edges

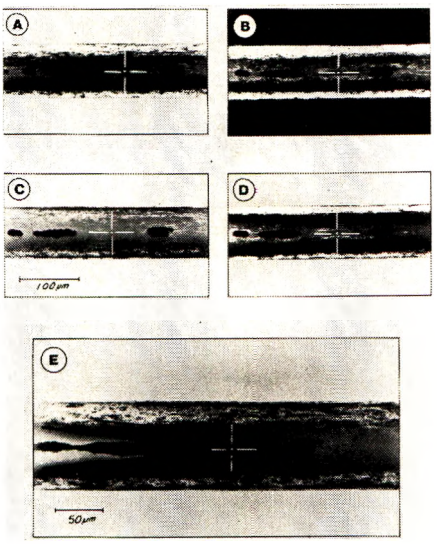


Figure 10. Determination of retardation with Ehringhaus rotary compensator. (Hairs B, C, D, and E are oriented 90° to the compensator.) In series from A through E, colors in hair go down in order i.e., in left to right direction on summation-interference color chart (Fig. 7). (A) hair oriented with quartz first order compensator; (B) Ehringhaus compensator set at ϕ (note extinction background); (C) retardation added to field by compensator with equal amount subtracted from hair; (D) increasing retardation from compensator brings color bands to background and decreased hair center color; (E) all of retardation of hair has been subtracted out in centered black extinction "arrow." From degree of compensator rotation, retardation can be calculated

will appear sharp, the retardation colors will be more or less symmetrically running down the shaft, and the hair will be at its thinnest point (Fig. 9(B)). Record the measurement of the axis with the viewing reticle.

The hair is then rotated 90° where again, the edges will be sharp, the retardation colors symmetrical, and will now be at its widest point (Fig. 9(C)). A measurement of the hair wide axis "diameter" in this position, together with that of the narrow axis, can be put into the formula for the area of an ellipse, 0.5 wide x 0.5 narrow x π, to determine the cross-sectional area. This information is useful in swelling and/or stress-strain analysis.

Next, the retardation must be measured to calculate the numerical birefringence. To do this, the quartz rotary compensator is put in place of the first order red. The hair is to remain on its wide axis, aligned as above. When the rotary compensator is set at ϕ, it is lying flat and has no effect on the system. When it is turned in either direction from ϕ, it adds retardation to the microscopic field by getting effectively thicker to the traversing light. The slow wave axis of the rotary compensator is 90° to that of the first order red (Fig.

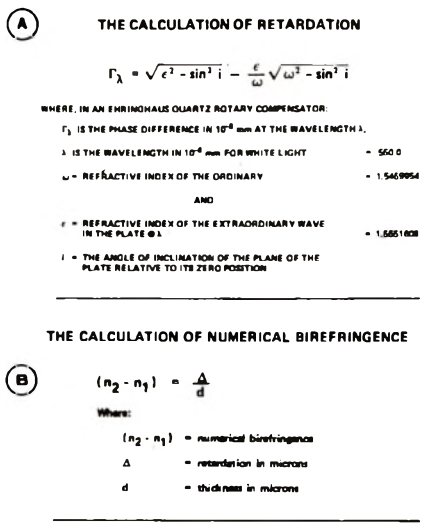


Figure 11.

8(B)). By switching compensators, without changing the orientation of the hair, the slow and fast wave axes of the compensator are now opposed 90° relative to those of the hair, rather than being aligned. Therefore, beginning from ϕ, any retardation added to the microscopic field by rotation of the compensator, is subtracted from the hair. By rotating the compensator, until no birefringence or light is present in the center of the hair or at the edge of the medulla (if present), the retardation can be calculated. Figure 10 portrays this procedure. The compensator reads in degrees of rotation. This has to be converted into microns of retardation through tables supplied with the compensator, or more accurately from a mathematical formula (Fig. 11(A)) (13).

The numerical birefrigence is then determined from the measurements taken (Fig. 11(B)). A programmable microcomputer is used for the various calculations.

The retardation calculation is based upon the use of white light (monochromatic light is not necessary in this system), having a conventional gravity point of $\lambda = 550$, with the Ehringhaus quartz plate compensator fast and slow wave refractive indices being 1.5459954 and 1.5551609, respectively. Precise readings are based upon the assumption that the optical system and the measured object are centered and aligned. In taking readings, the

compensator should be tilted in both directions from ϕ, with their average used in the calculation of retardation. This averaging process tends to cancel out any error caused by eccentricity (13).

Experimentation

Two experiments were conducted to test the significance of numerical birefringence as a measure of hair conditions as follows.

(1) A total of ~ 150 strands from a female Caucasian volunteer with virgin hair were picked at random. Each of these hairs was measured for the following parameters: wide and narrow axis diameter, cross-sectional area, microns of retardation, the numerical birefringence, and stress-strain curve characteristics. Any hairs showing obvious mechanical/chemical damage to the cortex were not used.

A prediction model was set up, utilizing an Olivetti P652 microcomputer* and Olivetti stepwise multiple linear regression program no. 3.03* to look at the contribution of numerical birefringence to the force at yield point (Fig. 12). Utilizing the model, a prediction of the dependent variable, force at 10 per cent elongation, is made from the measured numerical birefringence, the cross-sectional area, and their interaction (the product of the two). The actual prediction is accomplished by putting the three measured values into the solution vector of the model (Table I) (14).

(2) Two hairs, approximately 8 in. long, were cut into 3 parts each and placed in vacuo at ~ 1×10^{-5} Torr overnight. Each of the three parts was picked at random with respect to distance from the scalp, and placed in either 100 per cent glycerol, distilled water, or a solution formulated of the two, to simulate the conditions of 0, 100, and 50 per cent relative humidities (3, 15). Mechanical stress-strain measurements together with diameter and numerical birefringence measurements, were taken within 30 min after immersion into the solution.

Results and Discussion

The results of experiments 1 and 2 are summarized in Table I and Fig. 13, respectively.

An attempt is being made to answer two questions through these experiments as follows: (1) what is the value of numerical birefringence in describing the static condition of a hair fiber; and (2) how does the change of numerical birefringence in a hair fiber relate to the effect of a particular treatment or conditioner? Thus, two proposed uses of numerical birefringence are tested as a measure of human hair condition.

*Olivetti of America, Inc., New York, N.Y. 10022.

MULTIPLE LINEAR REGRESSION MODEL CORRELATING THE OPTICAL/MECHANICAL CHARACTERISTICS OF HUMAN HAIR

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \epsilon$$

WHERE:
- Y = FORCE IN GRAMS @ 10% DEFORMATION
- X_1 = AREA IN SQUARE MICRONS
- X_2 = NUMERICAL BIREFRINGENCE
- $X_1 X_2$ = INTERACTION OF X_1 AND X_2
- ϵ = UNEXPLAINED RANDOM ERROR

$\beta_0 \ldots \beta_3$ ARE THE WEIGHTS THAT ARE ASSOCIATED WITH EACH OF THE MODEL PARAMETERS

Figure 12. Model used to test for contribution and significance of independent parameters

Table I

	1 AREA μm^2	2 NUMERICAL BIREFRINGENCE $(n_2 - n_1)$	3 INTERACTION OF 1 & 2	4 GRAMS FORCE
1		-0.537301	0.896277	0.848987
2			-0.145358	-0.331718
3				0.85443

CORRELATION MATRIX

	$\bar{X}$	σ
1	3743.57	896.57
2	.008763	.000909
3	32.4061	6.1874
4	44.2762	9.2478

PARAMETER DISTRIBUTIONS

SOLUTION VECTOR: N = 143

FORCE (IN GRAMS) = 4.9191 + 0.004361 (area) + 0.7106 (area x numerical birefrigence)

	SE = 0.000952	SE = 0.1380
	t = 4.58	t = 5.084
	df = 140	df = 140
	P < 0.01	P < 0.01

COEFFICIENT OF DETERMINATION = 0.7652

OVERALL F = 228.1795

df = 2,140 P < 0.001

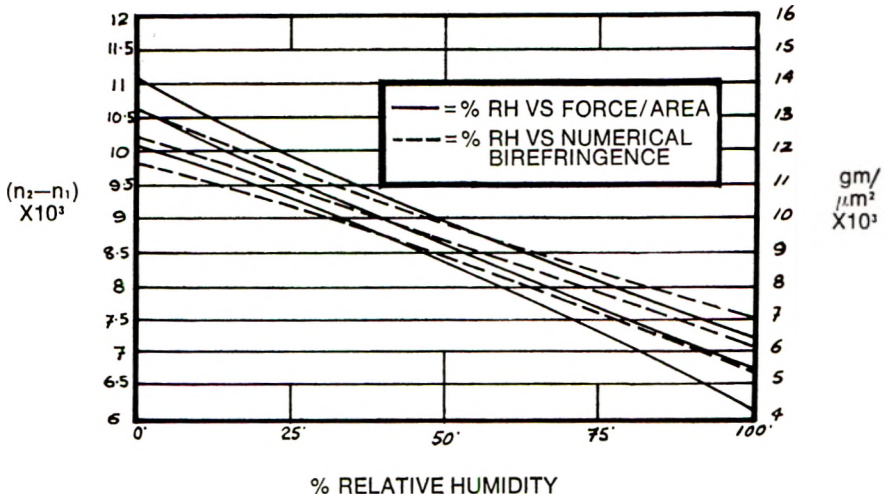


Figure 13. Linear regression with 95 per cent confidence interval of force/area and numerical birefringence versus per cent relative humidity

In the context of this paper, the term condition is used synonymously with the parameter descriptions body and manageability. These, in turn, are more specifically defined as elasticity or the resistance to and recovery from deformation induced by external force. It can be seen by the nature of this definition, that hair condition is traditionally thought of in a mechanical light (1, 9). Obviously, there is a molecular basis to this mechanical behavior (7, 9, 16). The α-helix molecular chain arrangement, basis of the keratin fibril system, is a symmetrically ordered configuration mainly responsible for this longitudinal stability in hair. In addition, both surrounding and infiltrating the fiber system is an amorphous cement-like matrix, which is high in cystine cross-linking, and has both α and β keratin chains (7, 16, 17, 18).

It is this relationship between the fiber system and matrix which becomes important as the contributing factor to both optical and mechanical properties of the hair (3, 7, 9, 16, 17, 18) (Fig. 14).

The mechanical parameters of this relationship can be explained in terms of a two-phase model, which becomes apparent through experimentation with the aqueous swelling of hair (9, 16).

A hair fiber undergoes various changes due to absorbed moisture between the relative humidity levels of 0 to 100 per cent. Approximately 2.7 times less force is required to stress a hair to yield point in a saturated versus dry state. At the same time, swelling occurs at a rate of 16 per cent radially but only

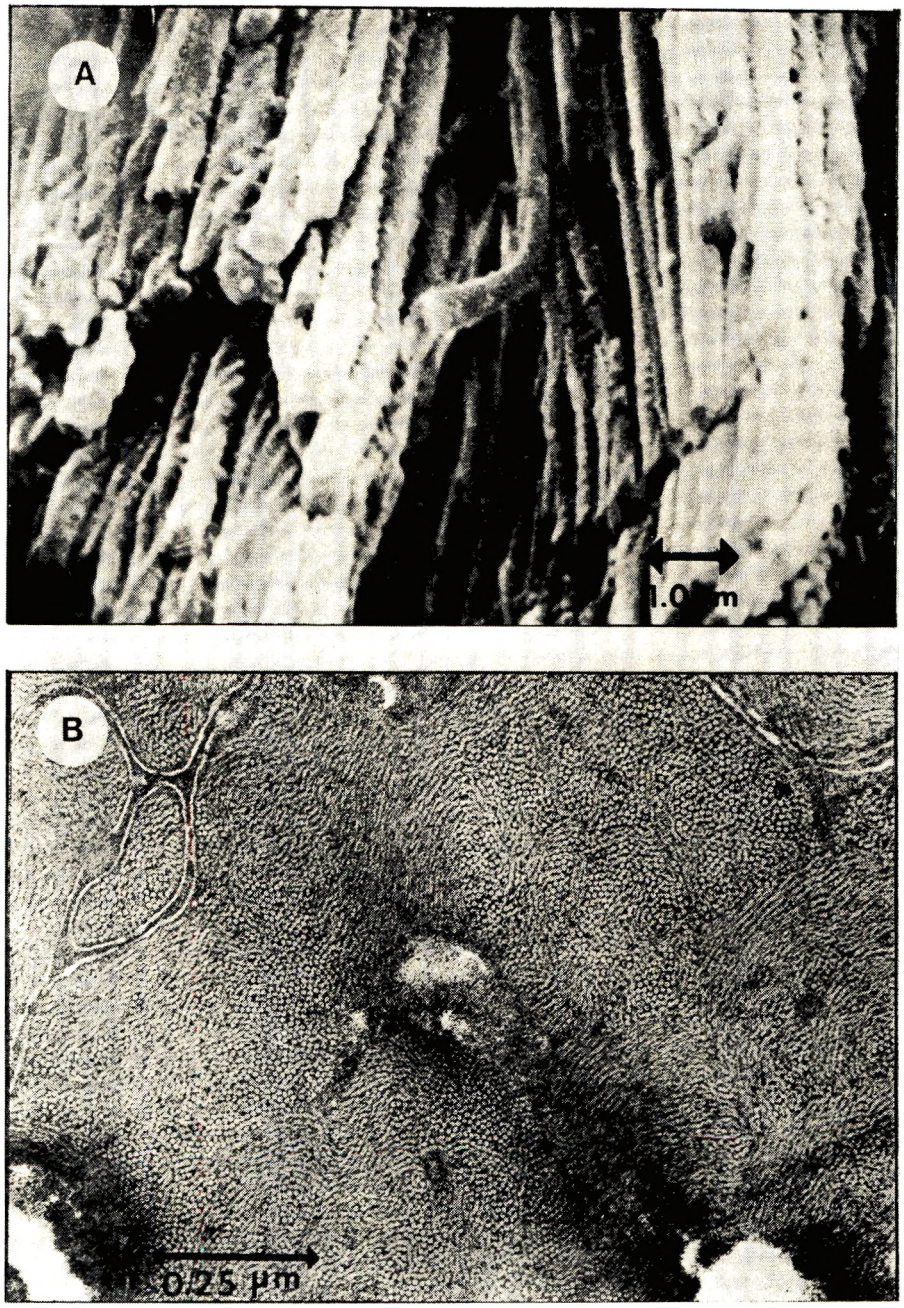


Figure 14. (A) Scanning and (B) transmission electron micrographs of fibril and matrix system in human hair cortex. Note fiber bundles in (A) made up of small fibers, and in (B) the 80 Å protofibrils (small white circles), surrounded by an amorphous matrix (stained dark)

1.2 per cent longitudinally. Equally, there are changes in the torsional-longitudinal modules ratio (9, 16).

The two-phase model is one in which a system of rodlets or fibrils, hydrophobic in nature, are imbedded in an amorphous hydrophilic matrix. Water entering this system affects the hydrogen and salt bonds of the matrix, while the rodlet-fibrils remain relatively unaffected. The swelling experiments bear this out: the rapid rate of radial swelling is indicative of matrix bond breakdown, while the low rate of longitudinal swelling is indicative of the fibril system molecular stability. A reduction in the longitudinal Young's Modulus is indicative of a change in the summation of the fiber matrix relationship, contributing to the total elasticity (3, 7, 9, 16).

These changes are caused by variance in the water content of the fiber as a result of varied relative humidity. Water, in effect, is used as a model conditioning agent for the purpose of these experiments.

Within the realm of cosmetic conditioning and treatment, there also exist more severe agents (i.e., permanent waving solutions and bleaches), which not only affect the hydrogen and salt linkages, as is the case with water, but also the disulphide and even, at the extreme, the fibril backbone peptide bonds. These agents also have an effect equal in magnitude on the two-phase system and ultimately the mechanical-physical properties of hair (1, 7, 20).

This two-phase mechanical model has a rather unique correlation: it emerges surprisingly similar to the classic Frey-Wyssling optical model of composite body-rodlet birefringence (Fig. 15) (19). In this model, rodlet or micellar structures of a crystalline nature are imbedded in a surrounding medium of a different refractive index, as is the case in hair. The rodlet structures themselves are responsible for a contribution to the birefringence of the total system in the mode of intrinsic birefringence. In addition, however, the relationship between the refractive index of the rodlets and their surrounding medium produces form birefringence, which is directly proportional to the difference between the 2 refractive indices of the components. The summation of intrinsic and form birefringence equals the total: numerical birefringence (3, 4, 11 19).

In Table I, the significance of a numerical birefringence system to determine the static condition of a hair strand can be seen. Previous work correlating the molecular orientation of fibrous material, as measured by numerical birefringence, with sonic pulse velocity, which is very well correlated with Young's Modulus in hair, encourages this (21, 22). The correlation matrix explains the contributions of the various parameters to the dependent variable, grams force at 10 per cent deformation, as well as any interactions which occur berween parameters. Cross-sectional area offers the greatest single contribution to the force; a large hair is expected to require more force than a small one. In addition, information about the orientation or integrity of the

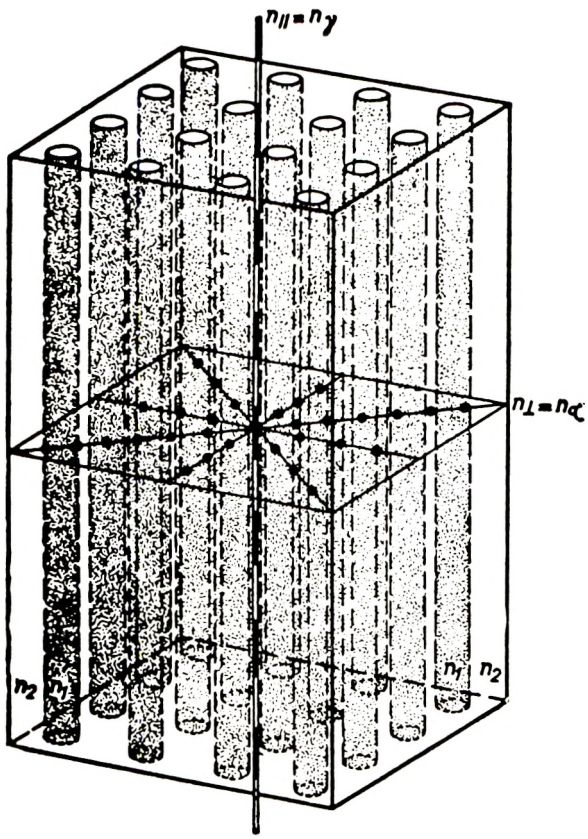


Figure 15. Classical Frey-Wyssling optical model of composite body-rodlet birefringence [with permission of the publisher (19)]

fiber-matrix system can be sought from the numerical birefringence value. This correlation, however, is low and not significant. (It does not appear in the solution vector.) This is due to the inherent characteristics of human hair being such that the numerical birefringence has a tendency to be inversely proportional to the cross-sectional area (the correlation of parameters 2 with 1) and, thus, is not independent. In order to get additional information about orientation and integrity, the interaction term of the cross-sectional area and numerical birefringence must be looked at to correct for nonindependence. This parameter has a correlation of > 0.85 with the grams force.

The solution vector is based upon the 143 hairs placed into the model. It can be seen by the statistical t-values that the 2 parameters, area and the interaction of area and numerical birefringence, are highly significant: $P < 0.01$.

If one takes the 2 measurements from a hair and places them into the solution vector formula, a predicted value of the force in grams at 10 per cent deformation can be obtained. With this model, the coefficient of determination is > 0.76. Thus, more than 76 per cent of the variance of the dependent variable, force in grams, can be explained. This value is significant as is expressed by the high overall F-ratio (228, df 2, 140), and low P-value (<0.001). With the future insertion of additional pertinent variables, as well as a greater sample size base, an additional amount of the unexplained random error can be explained. For more detail about this type of model see (14).

From Fig. 13, it can be seen that both Young's Modulus and numerical birefringence are similarly correlated negatively with relative humidity. A change in relative humidity, or more precisely, a change in water content, inversely changes both the mechanical elasticity and optical anisotropic properties of the hair.

Cosmetic conditioning, or treatments which affect the condition of hair, change the hydrogen and salt bonding arrangements of the matrix, and even the more thermodynamic bonds of the entire cortex, in the case of harsher available cosmetic treatments (7). As a result, there is a change in the mechanical stress-strain characteristic, which is the traditional parameter of measurement, as well as a change in the refractive index of the matrix (1, 3, 7, 9, 16, 20). This causes a change in the form birefringence, in addition to a possible change in the fibrils themselves, causing a change in the intrinsic birefringence (3, 4, 11, 19). Again, numerical birefringence is an extremely sensitive measure of molecular orientation in an anisotropic material (3, 5, 21).

Changes in mechanical elasticity or condition are a result of changes in molecular bonding and orientation (1, 7, 9, 16, 20). In that light, stress-strain analysis appears to be measuring a secondary parameter.

The mechanical method of testing is inherently obtrusive. Required are both an optical device for size determination and a tensile device for mechanical determination. Any inconsistency or flaw over the span of material analyzed tends to bias the results (1).

To build a system of analysis, it is necessary to use certain known parameters of evaluation as a tangible reference to which a correlation can be established. In the case of numerical birefringence, mechanical stress-strain analysis has served this function.

Numerical birefringence is presented as an alternative quantitative system of analysis of human hair condition at the molecular level. With this technique, one is able to determine, unobstrusively, utilizing only one instrument, at specific areas on the shaft, molecular occurrences associated with hair condition and conditioning.

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A MESSAGE FROM THE EDITOR OF THE JSCC

As you know, The Journal of the Society of Cosmetic Chemists, along with all other publications, has been faced with increasing production costs over the past year. The cost of paper along with printing costs have increased to the point where some action must be taken in order to insure the continued scientific and technical integrity of The Journal. The Publications Committee has been considering this problem for the past several years and has found it necessary to increase its subscription rate to non-members, increase its advertising rates as well as to increase the total number of pages of advertising copy. We have now reached the point where we can no longer increase the number of advertising pages without proportionally increasing the number of pages devoted to scientific and technical articles. To do so would drastically change the nature of our Journal to the point where it would lose much of its professional status.

Therefore, in order to increase the number of pages devoted to scientific and technical papers, the Board of Directors have approved the institution of a modest page charge to be assessed each author of a published paper. While these page charges will be waived by the business office in cases of undo hardship, it is expected that sufficient income will be received so as to insure the continued viability of scientific and professionals journals.

If we recognize that publication is one of the goals of research, then the cost of publication should be included as part of the research funding.

Sincerely,
John J. Sciarra, Ph.D.
Editor

J. Soc. Cosmet. Chem., 27, 433-441 (September 1976)

Intra and Extracellular Cementing Substances

H. P. BADEN, M.D., L. D. LEE, Ph.D. and J. KUBILUS, Ph.D.*

Presented May 1975, SCC Seminar, St. Louis, MO.

Synopsis: The STRATUM CORNEUM consists of flattened compacted cornified CELLS which are filled with cross-linked FIBROUS PROTEINS. The association of the fibrous proteins with a SPECIFIC LIPID gives rise to the barrier characteristics of the epidermis. Stratum corneum cells are attached to one another by desmosomes and an intercellular cementing substance. The latter material has been rather poorly documented and described. Recent studies concerning diseases associated with hyperkeratosis which employed a keratolytic gel, have suggested that solubilization of this material can result in the loss of adherence of cells to one another. The solubilized material appears to have unique properties, which will be characterized.

Introduction

The epidermis is a complex tissue, which by means of a variety of mechanisms, acts as a protective barrier for the body. Our understanding of how it operates at the molecular level is gradually expanding, but some aspects have proved more difficult to investigate. This paper will deal with those factors responsible for maintaining the integrity of the tissue, these being the cement materials. This term is used in a very broad sense, since a number of the structural components appear to play some role. We can divide these substances into materials which hold a single cell together and those which hold groups of cells together.

A major component of the epidermal cell is the α fibrous protein, which appears as filaments in electronmicrographs of the skin (Fig. 1). These filaments are first observed in the basal layer and go through a series of changes as the cells ascend into the stratum corneum. It is thought that these 70-80 Å filaments extend across the cell from one wall to another and hook on to attachment plates of desmosomes. Since it has been estimated that the basic fibrous protein has a length which is only a fraction of the width of a cell, the filaments must result from an aggregation of fibrous proteins. The fibrous pro-

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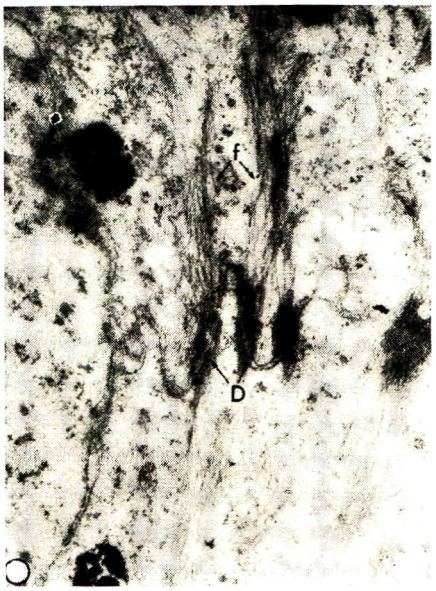

Figure 1. Electromicrograph of cell in stratum spinosum: (D) its desmosome and (F) shows filaments inserting into it

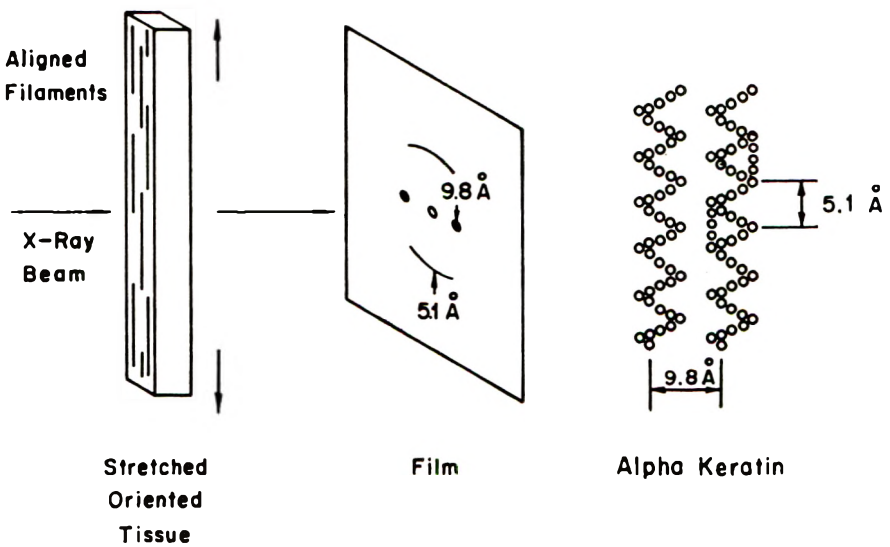

Figure 2. α helix of keratin showing X-ray diffraction pattern

tein has been shown to be a helical molecule, similar, but not identical to the classical α helix (Fig. 2).

The α proteins have been most extensively studied in cow snout epidermis (1, 2, 3). The α protein of the viable epidermis, prekeratin, can be solubilized by urea or buffers of organic acids below pH 2.7. Purification from acid buffers can be achieved by isoelectric precipitation. This material is insoluble in pH range 3-10, but can be maintained in solution at neutral pH by the addition of urea, guanidine, or sodium dodecyl sulfate (SDS). The analysis of amino acid reveals a high content of the acidic amino acids and glycine (Table I). The cystine content is quite low, unlike the α protein of hair and nail. In SDS electrophoresis has shown that a number of components (Fig. 3), and immunologic studies indicate that the A and B families are distinct from one another, but both are necessary to form an α helix. These results are best interpreted as the prekeratin molecule consisting of 3 polypeptides, 2 A chains and 1 B chain. It would appear that a major prekeratin exists with the A, A', B chains and a minor one exists with an A, A', B' chain. No cystine cross-links occur between the polypeptides of prekeratin.

As the cells of the viable epidermis become cornified at the base of the stratum corneum, the α fibrous proteins become cross-linked and can only be solubilized by alkaline buffers that contain a denaturing agent such as urea and a reducing agent (4, 5). This process is irreversible, and the stratum corneum fibrous proteins become cross-linked when the reducing agent is removed. The amino acid composition of the stratum corneum reveals a one-half cystine content of 2 residues/100 residues indicating that no one-half cys-

Table I

Amino Acids	Prekeratin
Lysine	5.1
Histidine	1.0
Arginine	6.1
Aspartic acid	9.1
Threonine	4.0
Serine	11.1
Glutamic acid	14.1
Proline	1.4
Glycine	16.4
Alanine	6.7
Valine	4.0
Methionine	1.3
Isoleucine	3.5
Leucine	9.2
Tyrosine	2.8
Phenylalanine	3.6
Half cystine	0.6

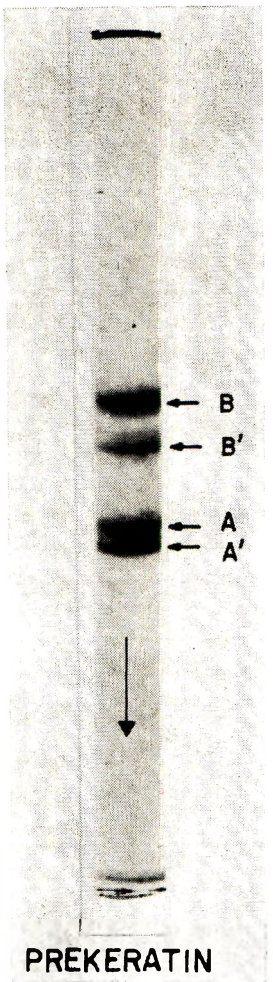

Figure 3. Polyacrylamide SDS electrophoresis of prekeratin. There are two groups of polypeptides

tine rich matrix proteins are present as has been the case with hair and nail (6).

Thus, the system for maintaining the integrity of the epidermal cells in the stratum corneum involves filamentous protein, which is attached to the cell wall, and, which shows interchain disulfide cross-linkage (another structural protein complex, keratohyalin, which is unique to the epidermis is also involved.) This material is newly synthesized in the granular layer and has been thought to coat the filaments and stabilize them. The controversy, which

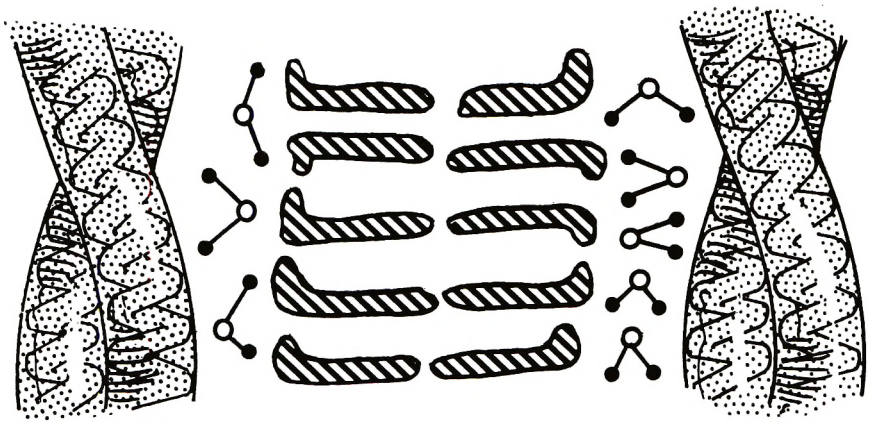

Figure 4. Polar lipid is shown between polypeptide chains with its long axis perpendicular to fibre

exists concerning the chemical nature of this material, probably has as its basis the complex nature of the material and the different methods used by several investigators to isolate it (7, 8). It has been difficult, however, to accept this concept of keratohyalin, since in a number of conditions, including ichthyosis vulgaris, no keratohyalin is formed, yet the filaments and stratum corneum appear to be normally stabilized. We feel that more work is necessary to determine the exact role of keratohyalin.

A final unique feature of the keratinization process is the deposition of a lipid material between the filaments (9). X-ray diffraction studies have indicated that a polar lipid, with its long axis perpendicular to that of the filaments, appears as cornification procedes (Fig. 4). It is likely that this protein lipid complex functions as the barrier. Extraction of the stratum corneum with lipid solvents removes the lipid, and at the same time, the barrier function of the stratum corneum is lost.

What has been described may be called the intracellular cement materials and probably is the major barrier of the stratum corneum. Our preliminary work with human and animal epidermis indicates that, what has been found in bovine snout epidermis, is generally applicable to both. As research continues in this area, new facts will be added to complete the picture.

Information on intercellular cement is far less complete. In the viable epidermis, no irreversible linkage between cells can be present, since cells move up from the basal layer to the stratum corneum. The desmosomes of the epidermis are clearly important in holding cells together, and when these are disturbed, as in certain diseases, acantholysis or cell separation and blistering occurs (10). As a cell rises in the epidermis, these attachments must constantly be broken and reformed. The mechanism for this process has not been clarified.

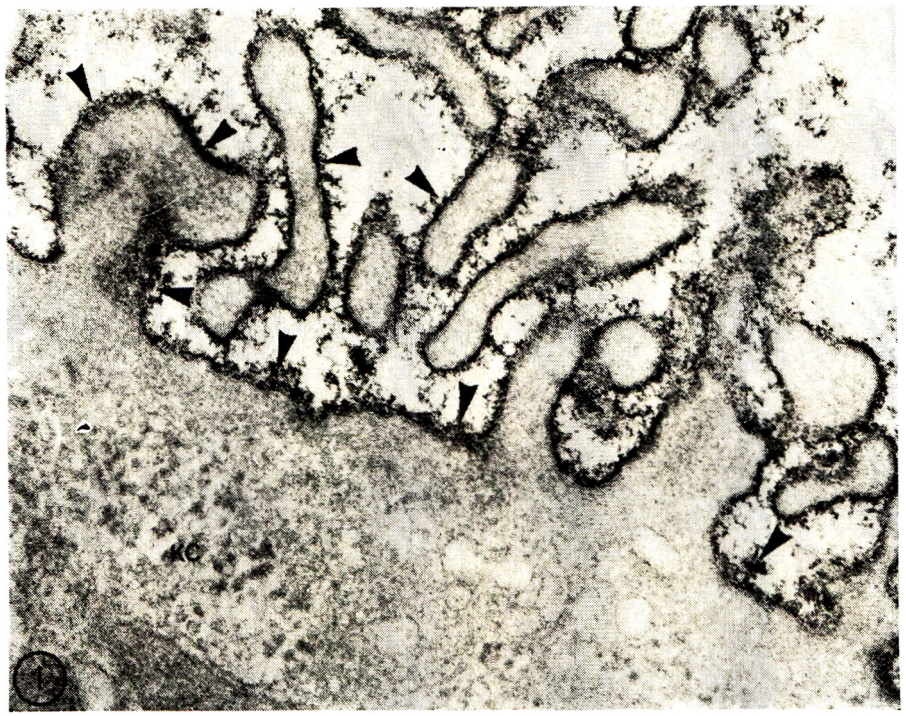

Figure 5. The glycocalyx is shown between epidermal cells in culture. The material is stained with Ruthensum red and has fuzzy appearance

A carbohydrate material called glycocalyx (11) has been described as coating keratinocytes in the viable epidermis (Fig. 5). This material may be the antigen which reacts with the antibody found in the sera of patients with pemphigus. The role that this material plays in holding cells together remains to be demonstrated.

In the stratum corneum, a firm attachment between cells is formed. This is in part, a result of the stacking (which has been observed), which permits careful overlapping of cell and maximum use of cell surfaces (12). The thickness of the stratum corneum is almost certainly related to the capacity of cells to stick together. Eventually, at the skin surface, loss of cell adhesion occurs and desquamation results. By inference from what has been observed in certain forms of ichthyosis, cell separation is easier to achieve at a higher water content of the stratum corneum. Thus, the common type of winter dry skin frequently ameliorates when the individual is exposed to a high humidity environment.

Recent studies have perhaps indicated new approaches for looking at the cement material of the stratum corneum (13, 14). In studies designed to im-

prove the therapy of ichthyosis, it was discovered that mixtures of propylene glycol in the 40 to 80 per cent range in water, under plastic occlusive dressings, resulted in rapid shedding of the stratum corneum. This appeared to be true for ichthyosis vulgaris and sex-linked ichthyosis. A marked increase in the effectiveness of the treatment resulted from the addition of salicylic acid. A gel containing salicylic acid and propylene glycol, which worked quite effectively has been finally developed. Use of this preparation under occlusive plastic dressings overnight resulted in rapid and dramatic loss of the thickened stratum corneum. Not only could this effect be observed in ichthyosis vulgaris and sex-linked ichthyosis, but in lamellar ichthyosis as well (Fig. 6). One could observe true keratolysis that is separation of the stratum corneum in sheets, which could be removed by rubbing the skin when the dressings were removed.

This action of propylene glycol and salicylic acid is not peculiar to the stratum corneum of ichthyosis, but can also be observed in hyperkeratosis as-

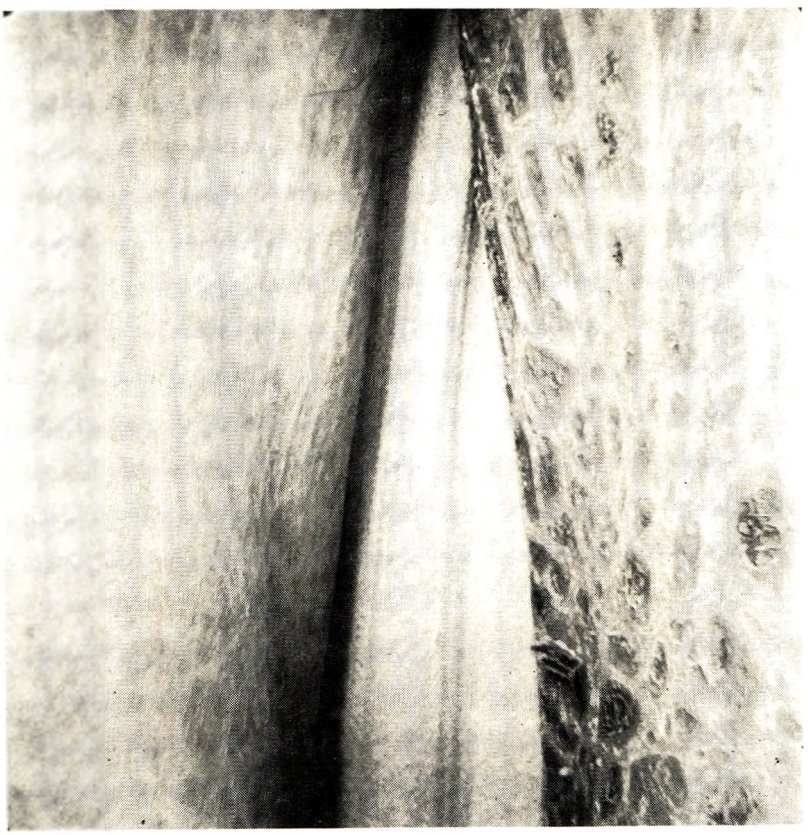

Figure 6. Appearance of skin of individual with lamellar ichthyosis after treatment with salicylic acid propylene glycol gel: untreated on right and treated on left

sociated with psoriasis and eczema (15). It effects the stratum corneum of all body surfaces including the palms and soles. This can also be observed with normal skin, indicating that factors involved in thickening of the stratum corneum may be an exaggeration of normal mechanisms for holding the stratum corneum together.

The nature of the cementing substances in the stratum corneum remains to be demonstrated. Although they are difficult to visualize, still the desmosomes are present and may have been modified to become very resistant cross-links by dessication. In addition, intercellular material has been described as appearing above the granular layer. This material has been poorly defined, but has been proposed by some authors as a cementing substance. It is not known how this material relates to the glycocalyx in the viable layers of the epidermis, and some have suggested that it comes from the membrane coating granules. Finally, as yet unrecognized materials may play a major role.

A firm fact of some significance is that hair and nail must have quite different mechanisms for holding cells together. The propylene glycol solutions and propylene glycol and salicylic acid gel do not cause keratolysis of nail and hair even after prolonged use. If mechanisms similar to stratum corneum were involved in holding cells together, these tissues would not be as resistant.

A reasonable approach to this problem is to treat stratum corneum with agents known to produce keratolysis and to determine the nature of the solubilized products. Recent work has begun in our laboratory, which uses solutions of propylene glycol and salicylic acid. For technical reasons, materials with molecular weights below several thousand are not amenable to investigation in our preliminary study. However, we have identified solubilized polypeptides in the molecular weight range 5,000 to 15,000 using electrophoretic techniques. Identification of their chemical composition is in progress, and these may give clues to importance in cell cement.

Acknowledgments

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December 6-7, 1976

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Participation by noted experts in each of these fields is anticipated. The four moderators will be as follows:

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Session III. Augie Grueneberg
Session IV. Sharon Reich

AMERICANA HOTEL

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(2) S. Rothman, *Physiology and Biochemistry of the Skin,* The University of Chicago Press, Chicago, Ill., 1954, Pp. 494-560.

References to books containing contributions from numerous authors appear as follows:

(4) S. D. Gershon, M. A. Goldberg, and M. M. Rieger, *Permanent Waving,* in M. S. Balsam and E. Sagarin, *Cosmeticss Science and Technology,* vol. 2, 2nd ed., Wiley Interscience, New York, N.Y., 1972, Pp. 167-250.

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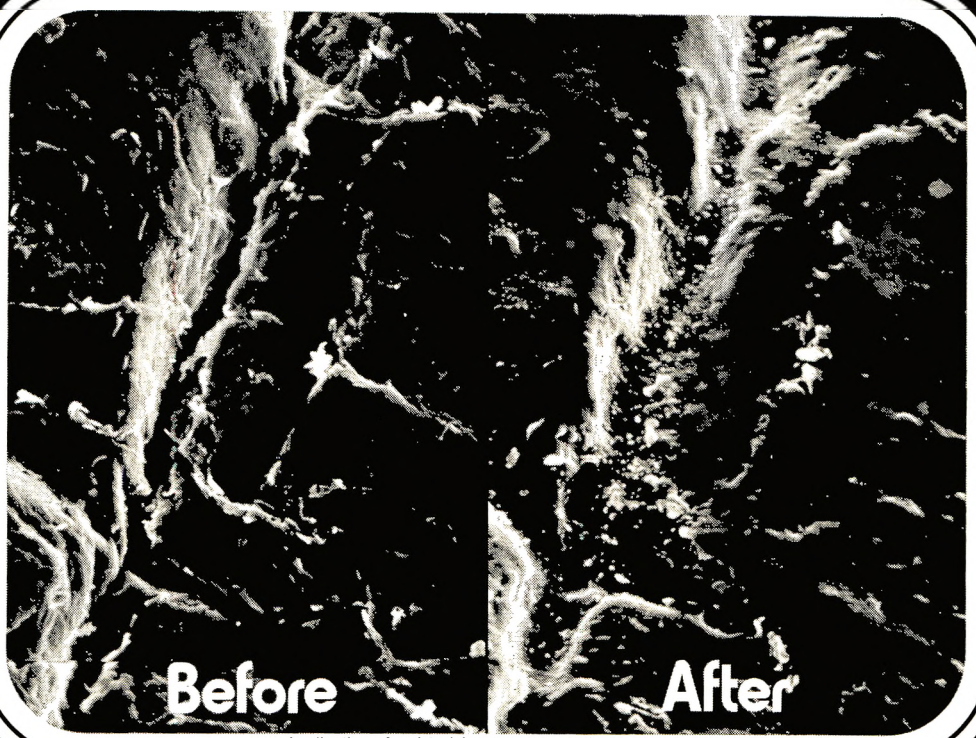

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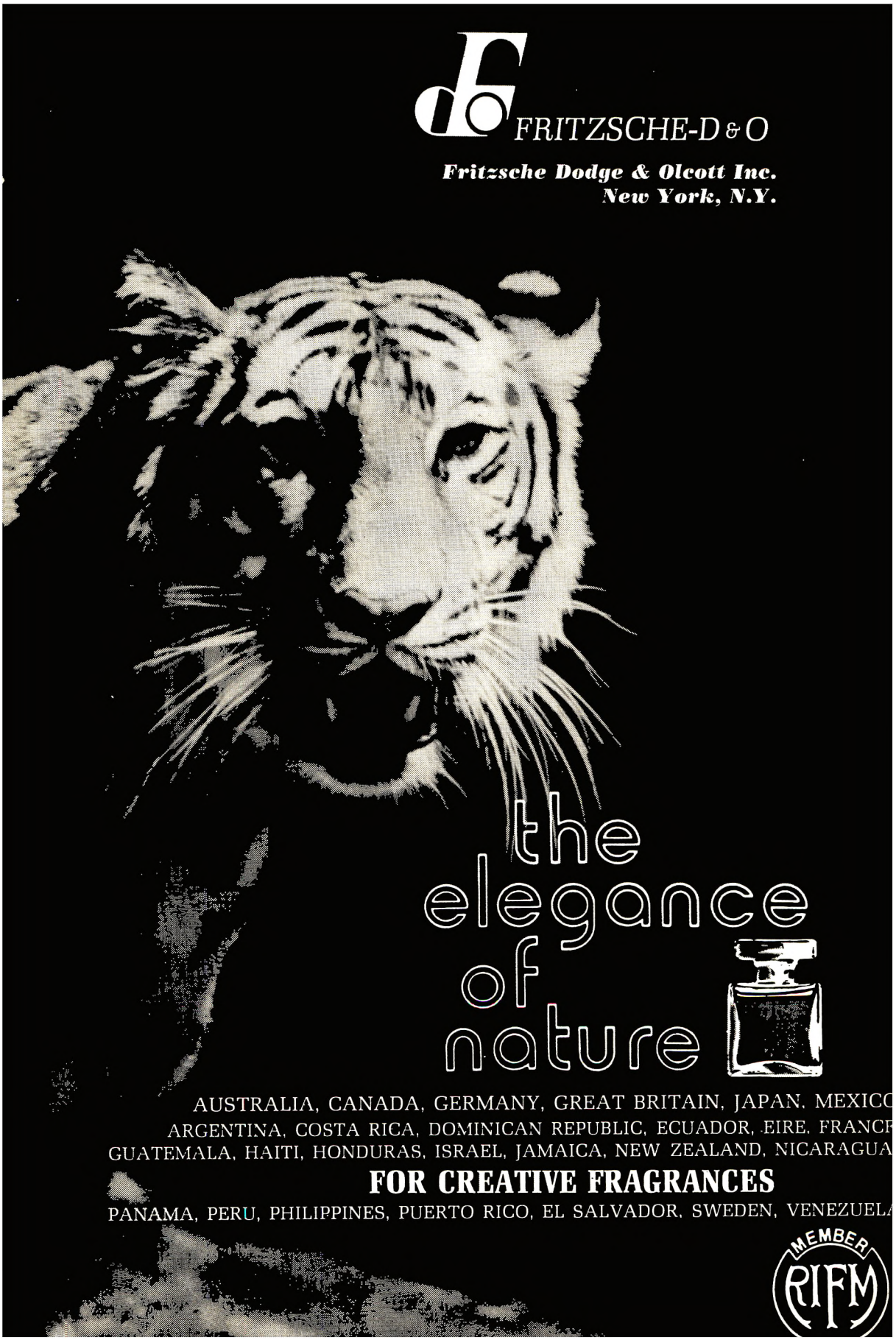
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