Vol. 28 No. 2 February 1977

Journal of the Society of Cosmetic Chemists

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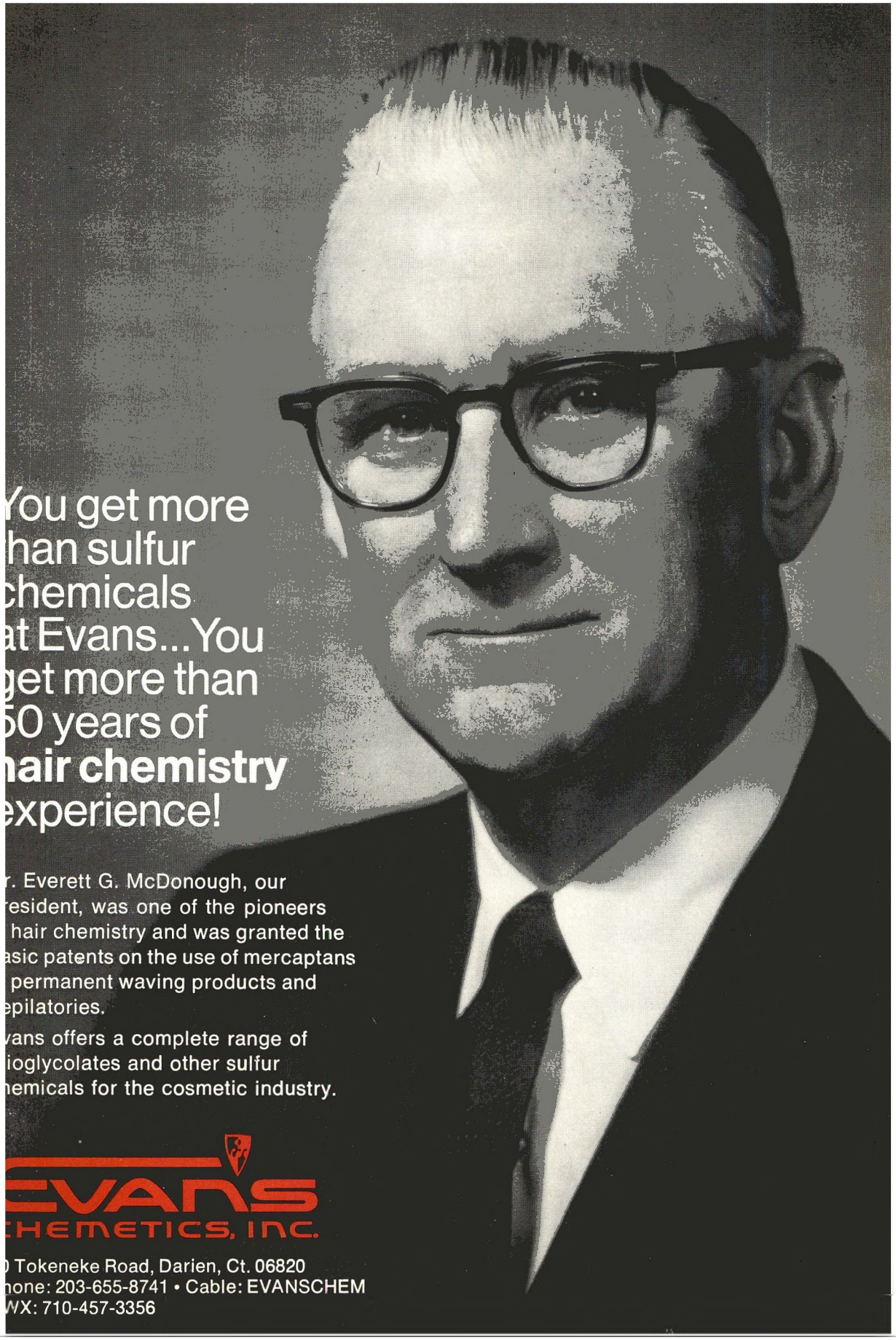
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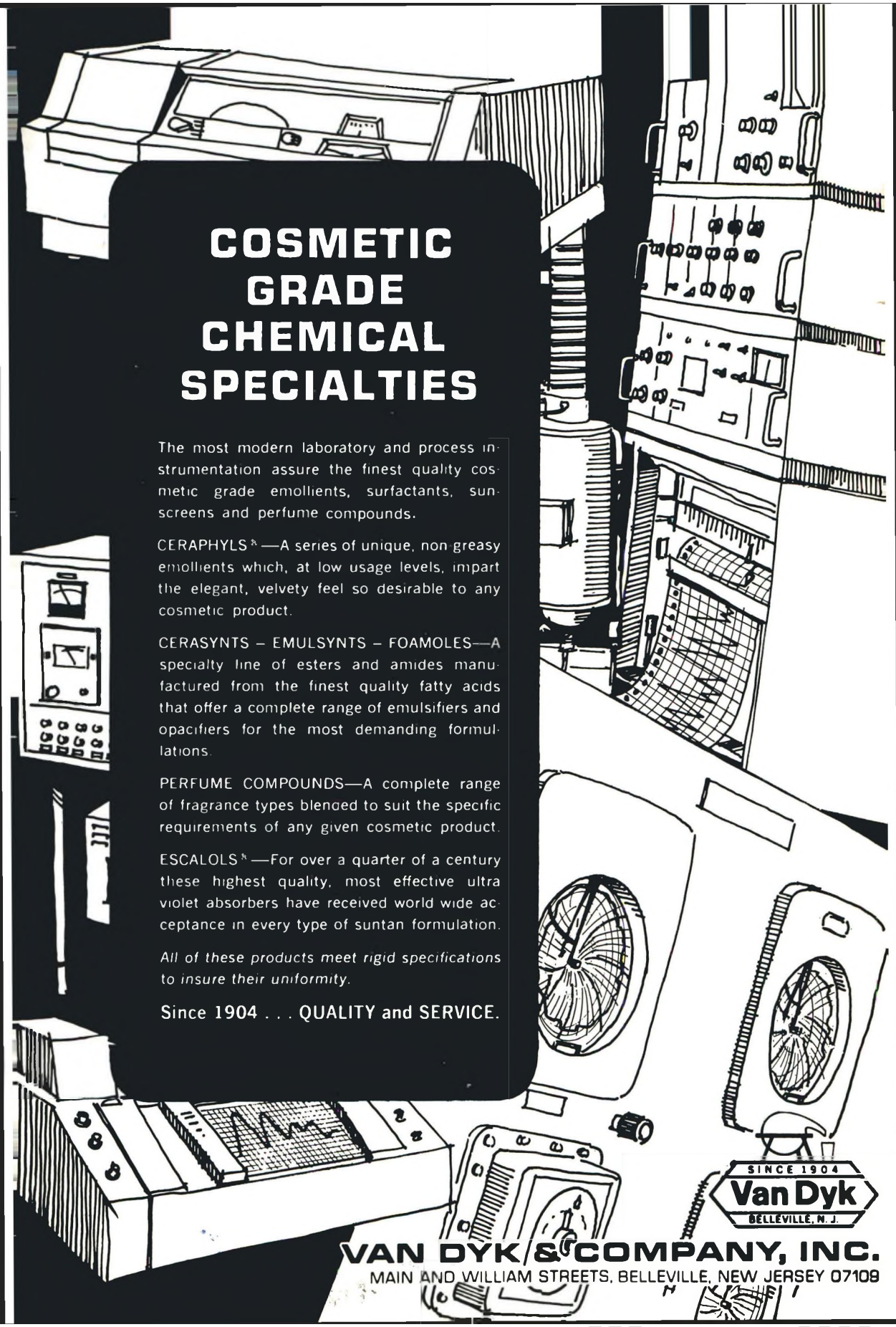

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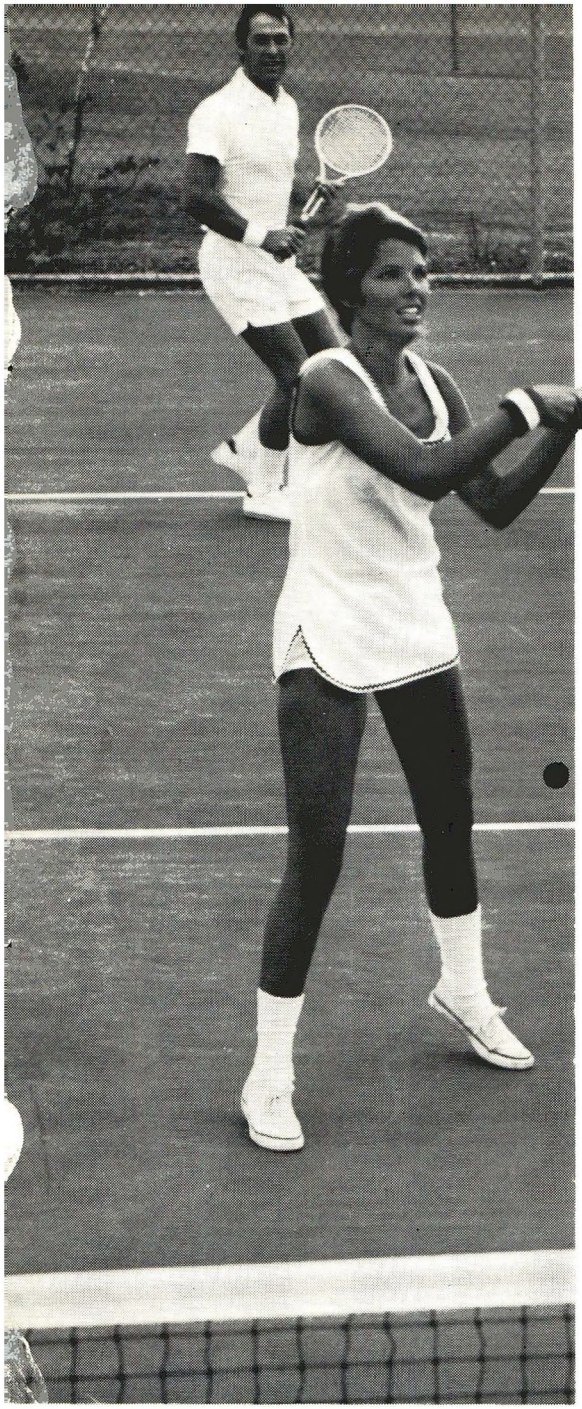

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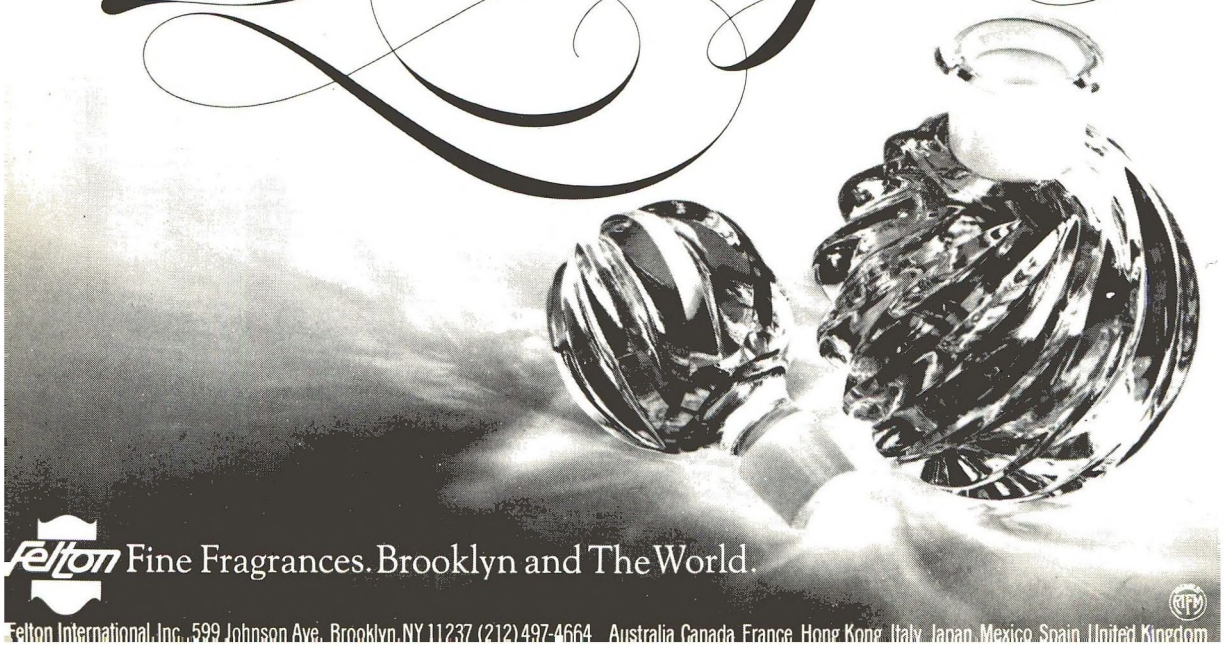

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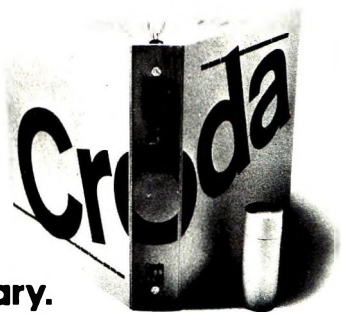

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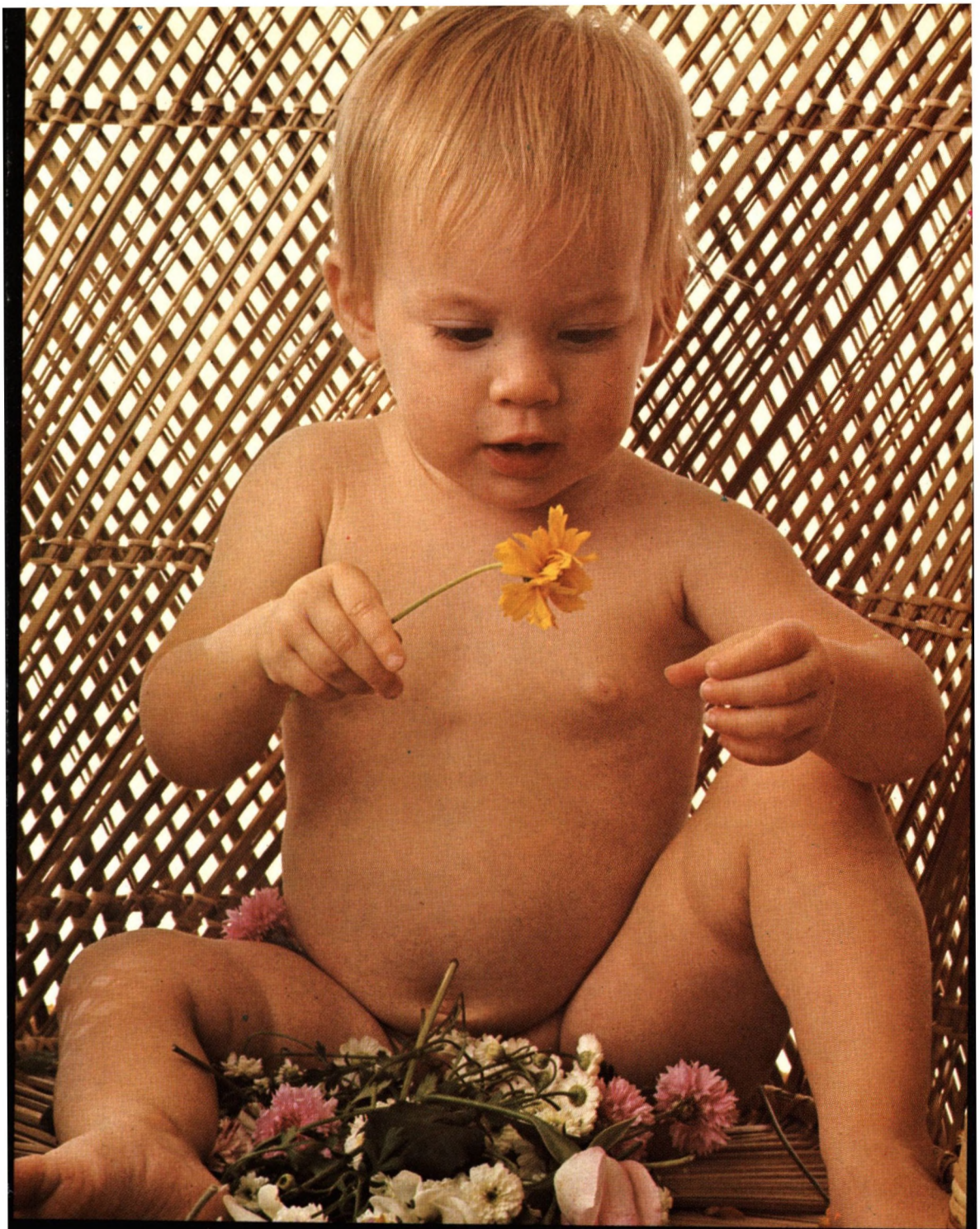

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In vivo skin friction measurements

A. F. EL-SHIMI *Past address: Lever Brothers Company, Edgewater, N.J. 07020. Present address: The Clorox Co., Pleasanton, CA 94566.*

Received March 8, 1976

Synopsis

In the area of SKIN CARE BENEFITS, consumers tend to rate SMOOTHNESS as an important attribute in their overall judgment. This paper describes a TECHNIQUE to measure the FRICTIONAL FORCE resulting from rotating a probe on the skin surface as a function of normal load and speed of rotation. A brief background review on FRICTION THEORY is presented. A number of factors were investigated. The highlights of our findings are as follows.

1. The use of a highly polished stainless steel disc or hemispherical probe produces "wrinkling" or "twisting" of the skin surface during rotation, especially at higher normal loads. The use of an intentionally roughened probe produces friction data which satisfy the simple laws of friction.
2. The force of friction is not a linear function of the normal load as suggested by Amonton's Law, $F = \mu L$, where F is the force of friction, L is the normal load and μ is a constant called COEFFICIENT OF FRICTION. A different expression, $F = KL^n$, was found to describe our results fairly well. K and n are constants. The deviation from Amonton's law is attributed to the elastic behavior of skin.
3. Dry skin produces low friction values. Much higher values are obtained on hydrated skin. A rationale for this behavior is proposed.
4. To produce immediate and significant changes in the friction properties of skin, sufficient quantities of beneficial agents have to be deposited on the surface. Talcum powder and silicone oil reduce the friction force. With silicone oils, fluid or hydrodynamic lubrication is involved.

INTRODUCTION

This paper describes a technique which assesses quantitatively the frictional properties of skin *in vivo* and the effects of product treatment on such properties. Cosmetic products, which are aimed at conferring smoothness to the skin, are thought to perform their function by depositing sufficient amounts of desirable ingredients leading to a perceptible change in the adhesion and friction properties of skin. The perception of such changes is usually subjective, and a fair assessment may not be possible because of the simultaneous interaction of other attributes. Obviously, a method to assess skin friction properties quantitatively not only offers a better way of generating basic information on the condition of untreated skin, but it could also provide valuable guidelines in the course of developing new products aimed at producing a desirable tactile feel.

The technique to be described in this paper features a cylindrical metal probe, which contacts the skin surface under a given normal load. The probe can rotate within a wide

range of speeds, and the resistance to the rotating motion can be measured directly via a torque measuring device. Specifically, we have set out to examine the applicability of the general laws of friction, derived mainly for metallic materials, to human skin as a substrate. A basic premise of modern friction theories relates to the distinction between the real area of contact between sliding materials and the geometrical area. The real area of contact is much smaller than the geometrical area due to surface roughness. This point has been examined in some detail using a rough and highly polished probe made from the same material. Also, the effect of lubricants (talcum powder and silicone oil) has been examined and an attempt was made to elucidate the lubrication mechanism of these materials.

THEORETICAL BACKGROUND

The coefficient of friction μ between two solids is defined as F/L, where F denotes the frictional force and L is the load or force normal to the surfaces. When μ is constant, $\mu = F/L$ is known as Amonton's law and expresses two important observations: (1) the friction force is proportional to the normal force; and (2) the friction force is independent of the apparent area of contact.

There is abundant evidence that even microscopically smooth surfaces are irregular on a molecular scale of distance. As a result of irregularities, two surfaces brought into contact will touch only in isolated regions. The true area of contact is then much less than the apparent area; it can be estimated, for example, from a measurement of the electrical conductivity between the two solids. It is also known that high local temperatures can develop during rubbing, as well as high local pressures, which can lead to plucking out of portions of the softer material by the harder one (1–4).

As the two surfaces are brought together, the pressure is large at the initial few points of contact, and deformation immediately occurs to allow more and more contact to develop. This plastic flow continues until there is a total area of contact such that the local pressure has fallen to a characteristic yield pressure P_m of the softer material. Thus, around each region of contact, there is a plastic zone, with further elastic deformation outside (2). The actual contact area is then determined by the yield pressure, so that

$$A = L/P_m \tag{1}$$

In a typical measurement of friction, a slider is pressed against a stationary block and the force F required to move the slider is measured. This force, in general, will consist of 2 terms. First, there is the force F required to shear the junctions at the points of actual contact. This is given by

$$F = AS_m \tag{2}$$

where S_m is the shear strength per unit area. The second term, F_1, is the force required to displace the softer material from the front of the harder one. With metals of different hardness, the harder one, if used as a slider, will plow a track in the softer, and F_1 is, therefore, a work term associated with this plowing action. In a general way, one expects F_1 to be proportional to the width of the slider, i.e.

$$F_1 = K A_1 \tag{3}$$

where A_1 denotes the width of the plowed track. Usually, the plowing term is important only for the case of a hard material rubbing against a soft one; if both are hard, the friction is due mostly to the shear term. As an approximation, then, A may be eliminated from equations (1) and (2) to give

$$F = L(S_m/P_m) \tag{4}$$

or

$$\mu = S_m/P_m = \text{constant} \tag{5}$$

This is Amonton's law as stated earlier. A point in connection with this law is that the two quantities, S_m and P_m, represent the resistance to plastic flow of the softer of the contacting materials to shear and compression, respectively.

The coefficient of friction may also depend on the relative velocity of the two surfaces. This will, for example, affect the local temperature, the extent of work hardening of metals, and the relative importance of the plowing and shearing terms. These facts work out such that the coefficient of friction tends to decrease with increasing sliding speeds (4, 5) contrary to Coulomb's law, which holds that μ should be independent of sliding velocity. At very low speeds, the effect is small.

A number of friction studies have been carried out on organic polymers in recent years (4–6). The detailed results show some serious complications, however. The coefficient of friction was shown to be dependent on the load as has been illustrated, for example, in the case of a copolymer of hexafluoroethylene and hexafluoropropylene (7), where it was suggested that the area of contact is determined more by elastic than by plastic deformation. The difference observed between the static and kinetic coefficients of friction (the force required to initiate sliding of the load gives μ_s, where s refers to static, and the force requires to sustain the motion gives μ_k, where k refers to kinetic) was attributed to the transfer of an oriented film of polymer to the steel rider used in the experiment during sliding and to low adhesion between this film and the polymer surface.

An important aspect of friction measurements in relation to cosmetic applications is the friction between lubricated surfaces. Two limiting conditions exist where lubrication is used. In the first case, the oil film (lubricant) is thick enough so that the surface regions are essentially independent of each other, and the coefficient of friction depends on the hydrodynamic properties, especially the viscosity, of the oil. Amonton's law is not involved in this situation, nor is the specific nature of the solid surfaces. As load is increased and relative speed is decreased, the film between the two surfaces becomes thinner and increasing contact occurs between the surface regions. The coefficient of friction rises from the very low values possible for fluid friction to some value that is usually less than that for unlubricated surfaces. This type of lubrication, i.e., where the nature of the surface region is important, is known as boundary lubrication and involves a strong physical adsorption of lubricant on the surface or even a surface chemical reaction leading to a very strong bond between the lubricant and the substrate. The general feature of friction between lubricated surfaces is usually represented by what is known as the Stribeck curve, which is a plot between the coefficient of friction and the so-called Sommerfeld number, $\eta V/P$. η is the viscosity of the lubricant film, V is the speed of sliding, and P is the normal load per unit area. This

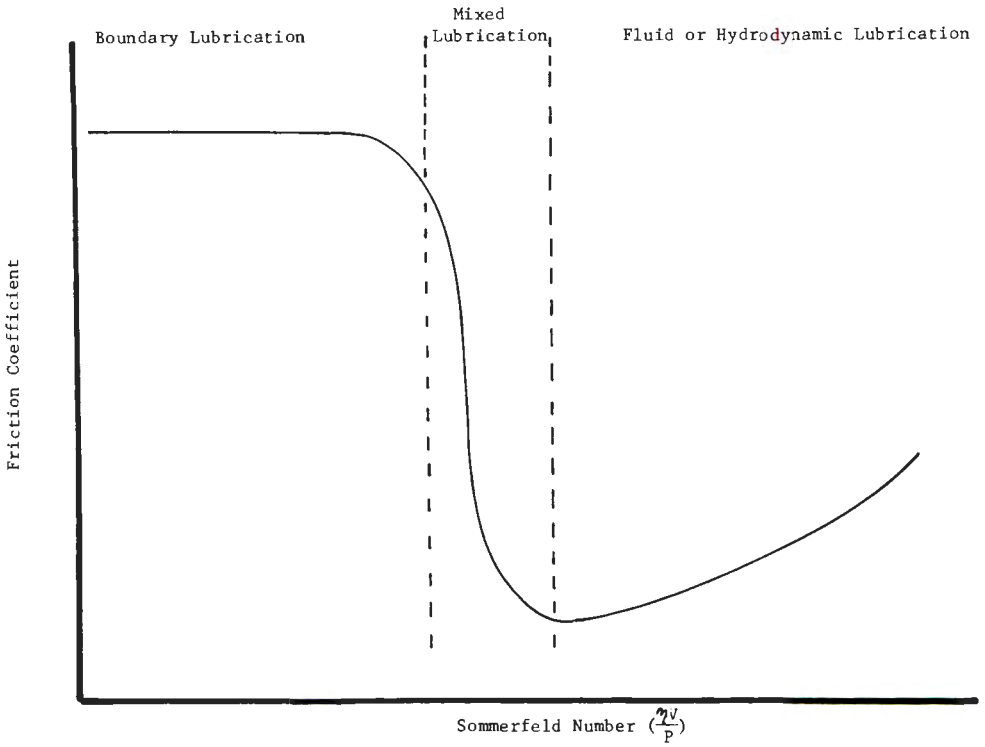

Figure 1. Stribeck curve showing the relationship between friction coefficient and the Sommerfeld number

relationship is represented schematically in Fig. 1. It has been shown that the value of μ in boundary lubrication depends greatly on the state of the adsorbed film and that, generally speaking, the film must be in a condensed state to give a low coefficient of friction. A number of models have been suggested to explain the mechanism of boundary lubrication (1, 4, 8, 9).

PREVIOUS FRICTION MEASUREMENTS ON SKIN

Few papers have been published on the frictional characteristics of skin. Naylor (10) measured the friction of a polyethylene ball rubbing against the skin. He found that the friction was higher when the skin was damp than when it was either very wet or dry. Appeldoorn and Barnett (11) have observed other distinctive frictional characteristics of skin as follow: (1) skin friction is "relatively high;" (2) a small amount of talcum powder greatly reduces skin friction. This is well known, but it is not a characteristic of the friction of other systems such as steel-against-steel, where talc increases friction; and (3) the skin friction is higher on a smooth surface than it is on a rough surface. This behavior is just the opposite to that normally encountered, but it can be verified by rubbing one's finger on a microscope slide. The friction is much greater on the clear glass (smooth) part than on the ground glass (rough) part.

Although Appeldoorn and Barnett have not conducted any *in vivo* work, they found that an *in vitro* model combining a rubber ball and a rotating stainless steel cylinder cor-

relates well with their observations on the behavior of skin friction. They concluded that the property of skin that gives it the unusual and characteristic behavior is not its roughness nor chemical composition, but its "flexibility." Like rubber, the skin can flex to conform to the shape of another surface. This gives it a relatively large area of contact and, therefore, a high coefficient of friction as compared to the relatively unflexible metal or plastic materials. These results have been confirmed by Prall (12) and by Comaish and Bottoms (13) on skin under *in vivo* conditions.

EXPERIMENTAL TECHNIQUE

Earlier experimental techniques for skin friction measurements have been reviewed by Prall (12). Traditionally, friction measurements involve the sliding of a probe over the skin and the force is determined as a function of load. Prall describes a friction dynamometer which features a constant-thrust friction head whereby a standard ground glass disc was pressed against the skin with a force of 200 g/cm^2. The friction head was attached to the shaft of an ac motor, which was energized by a variable transformer. In use, the friction head was presented to the skin, and the power to the motor gradually increased until the friction head just started to rotate.

In this work, we employ a modified Haake viscometer (RV-1)* to measure the friction behavior of skin *in vivo* with the help of a rotating stainless steel probe in contact with the arm (or any other part) surface. Preliminary experiments have shown the need for controlling the contact pressure between the skin surface and the probe. To this end, a special probe assembly was designed such that a constant load was maintained in contact with the skin surface in the course of the experiment. The assembly is depicted schematically in Fig. 2. The assembly features an adapter which fits tightly onto the shaft of the Haake measuring head. The part carrying the load and the probe is precisely machined so that it slides smoothly over the cylindrical adapter. The extent of vertical movement of the probe attachment is controlled by the size of the slit and a protruding knob on the adapter body. Loads can be added to the assembly by screwing on metal discs of known weight. The load is, thus, suspended and floats freely between the two ends of the slit. This design offers a convenient means to ensure a constant load contacting the skin. The panelist is only required to maintain the knob approximately in the middle of the slit during the experiment.

The measuring principle is as follows. The control console of the Haake RV-1 houses the operating controls, synchronous motor, electrical circuitry, and indicating meters. It drives the measuring head and the meter reading indicates only the torque induced by the frictional resistance to the rotating probe, and not the friction in the transmission. Torque is measured by the angular displacement of a creep-resistant torsion spring, mounted between two concentric conical shafts. The displacement angle is converted into an electrical signal by means of a high-precision potentiometer. The voltage output of the potentiometer is linear to the angular displacement of the spring. Thus, the torque exerted on the probe is proportional to the signal registered on the console meter.

*Haake Inc., Saddle Brook, N.J.

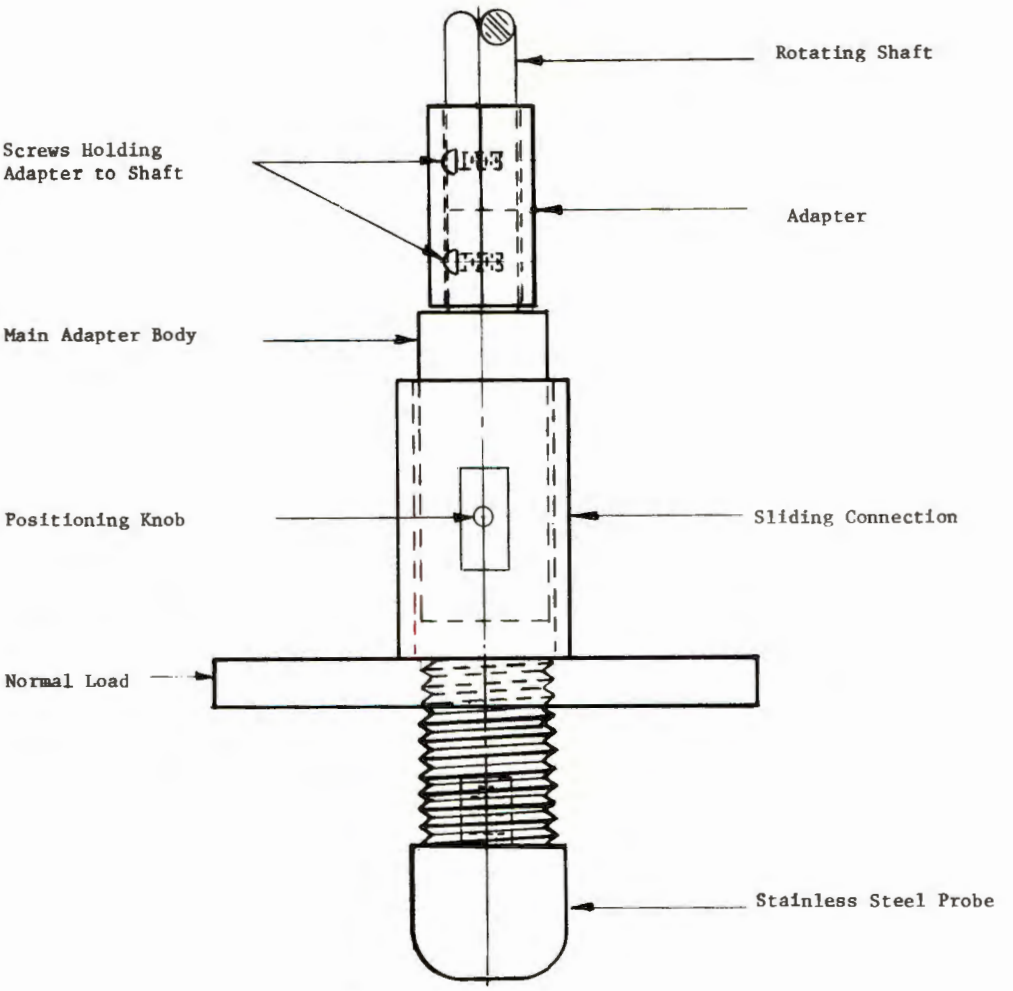

Figure 2. Friction probe assembly

We have calibrated the scale readings in terms of absolute force by determining the scale response to different weights attached to the probe with a thread. The measuring head was laid horizontally on a table such that the probe assembly protruded over the edge. Measurements were conducted at the lowest speed available (3.6 rpm) and care was taken that the thread windings did not pile up on the probe. The calibration curve obtained representing the force versus load was linear. Joy, Machin, and McGaw used a similar technique employing a modified Haake viscometer for measuring skin friction properties *in vivo* (14).

RESULTS AND DISCUSSION

A general view of the set-up is shown in Fig. 3. As will be discussed below, the state of skin hydration affects its friction properties, and, hence, it was necessary to conduct the measurements under controlled temperature and humidity (22°C and 55 per cent rela-

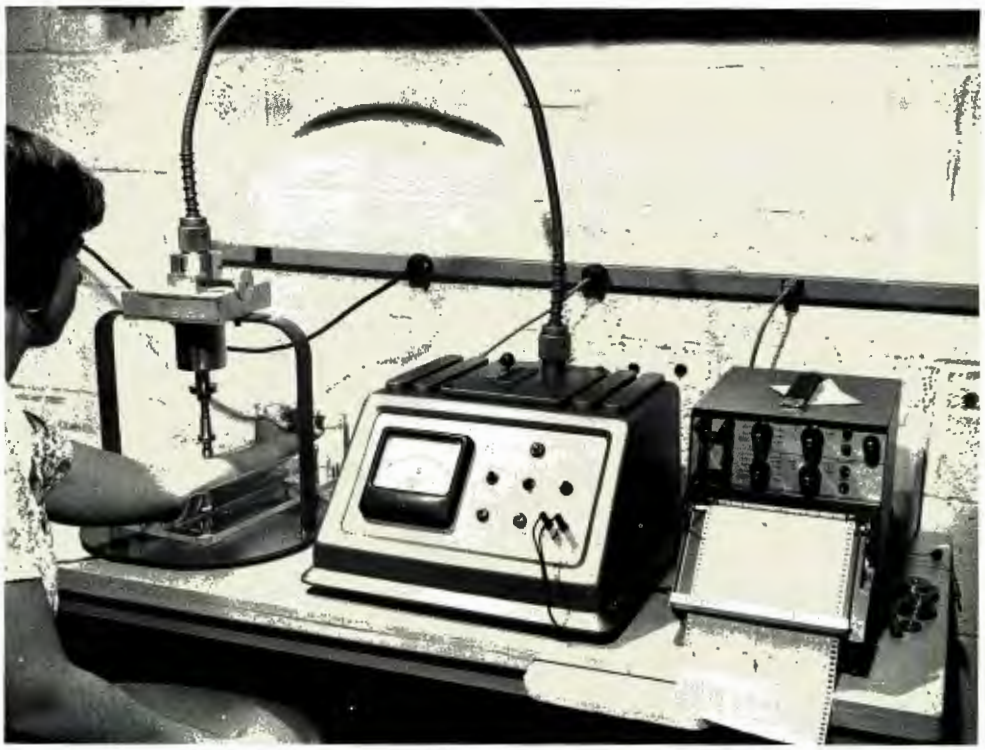

Figure 3. Friction measurement set-up

tive humidity). In general, measurements can be carried out on almost any part of the body, but for the sake of convenience, the test site used in this study was restricted to the volar forearm of about 20 female panelists ranging in age from 20 to 50 years. Normal loads of 0 to about 200 g were used. The friction force was determined 3 to 4 times at any given load to compute an average value. A number of factors were examined and these will be discussed separately.

EFFECT OF PROBE SHAPE

Initial attempts, using a disc-shaped highly polished stainless steel probe to measure the friction force, led to an interesting observation. It was found that in the course of the measurement, the friction increased with time accompanied by a certain degree of discomfort to the panelist, especially at higher loads. A closer examination of the skin in contact with the rotating disc revealed obvious "wrinkling," leading to what could be described as a "pinching-effect." This type of probe was discarded, and to alleviate this difficulty, we resorted to the use of a hemispherical probe (radius 0.6 cm). Two such probes were employed in this work; one probe was highly polished, and the other was intentionally roughened using emery paper. Both probes were of the same dimensions and made of stainless steel. The response of the two probes at a given load is shown schematically in Fig. 4, which demonstrates clearly the difference in behavior of the

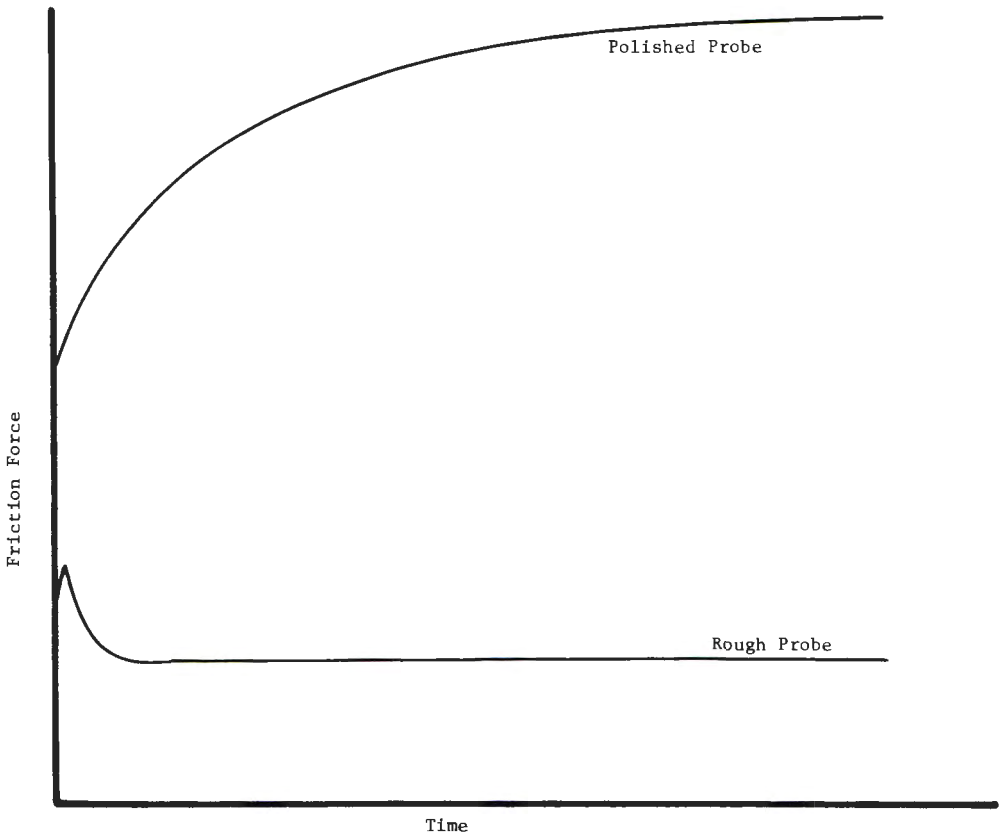

Figure 4. Schematic response of polished and rough probes during friction measurements on human skin *in vivo*

two probes. In the case of the rough probe, it can be seen that as the probe starts rotating, a maximum value for the force of friction is recorded which quickly tapers off to a constant value within a few seconds. The polished probe, on the other hand, produces a friction force—time profile which indicates an instaneous large response on the force axis, followed by a continual increase and a leveling off after a few minutes (3 to 4 min). Close inspection of the skin contacting the rotating probe revealed that while no "pinching-effect" was felt by a panelist, there was obvious wrinkling of the skin. The degree of wrinkling or twisting of the skin was found to be related to the load used in a given measurement. Thus, at low loads (*ca.*, 50 g) no wrinkling was observed with the polished probe, whereas, the disc-shaped probe produced wrinkling even at lower loads. Skin wrinkling was not observed with the rough probe over the whole range of loads used, and the results obtained with this type of probe were reproducible. Rather large fluctuations in the force values were observed with the polished probes, especially under high loads.

The formation of wrinkles is a complicating factor in skin friction measurement, since it is doubtful that the data obtained under such conditions relate to the inherent friction properties of the skin corneum. This point will be discussed further below. We

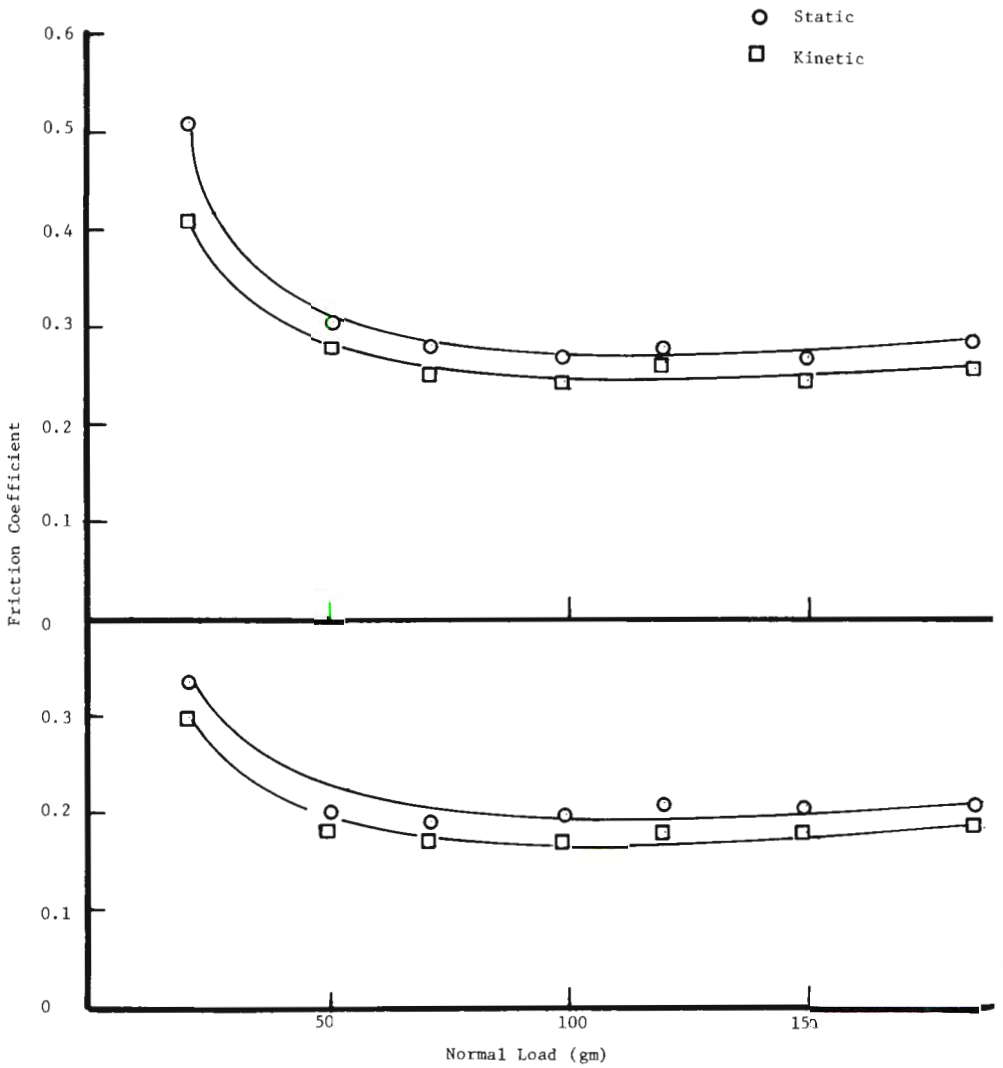

Figure 5. Static and kinetic friction coefficients as function of normal load

have, therefore, focused on the use of the rough probe, since the results obtained with this probe seem to conform in a much simpler way to the basic laws of friction.

The dependence of the coefficient of friction on load is shown in Fig. 5 for two panelists. The data are presented as the static (μ_s) and kinetic (μ_k) coefficient of friction. μ_s was determined from the maximum value of the friction force versus time curves and μ_k relates to the value of the force of friction after attainment of an equilibrium value (Fig. 4). It is clear from Fig. 5 that the friction force is not related linearly to the load, and, hence, Amonton's law is not obeyed.

All panelists examined showed the same general relationship as given in Fig. 5. These data confirm Comaish and Bottoms' findings that the friction coefficient for human skin *in vivo* increases as the load decreases (13).

One can recall that Amonton's law expresses the relationship between the friction force and load for systems in which only plastic deformation occurs at the contact points. The nonlinearity of the force-load relation has been attributed to contributions of other mechanical components in the process of deformation such as elasticity. A simple equation describing the force-load relation under these conditions was proposed by Bowden and Tabor (2), and has the form

$$F = KL^n \tag{6}$$

where K and n are constants. The value of n ranges from 0.66 to unity. When n = 1, the above equation expresses Amonton's law. We have attempted to fit our data to the above equation using regression analysis with the help of a computer. Eleven cases were analyzed to evaluate the determination index (a measure of the goodness of fit) and the values of the regression coefficients at 95 per cent confidence limits. The results are given in Table I. A more detailed example of the computational analysis where the actual friction force values are compared with values calculated from equation (6) is presented in Table II.

Table I
Regression Analysis of Friction Data Fitted to the Equation $F = KL^n$ at 95 per cent Confidence Limit

Panelist Number	Index of Determination	Regression Coefficients	
		K	n
1	0.971	0.70	0.79
2	0.981	0.66	0.71
3	0.996	0.82	0.67
4	0.950	0.45	0.80
5	0.975	0.28	0.90
6	0.986	0.68	0.68
7	0.985	0.36	0.87
8	0.994	0.43	0.75
9	0.959	0.18	0.96
10	0.979	0.41	0.79
11	0.988	0.59	0.76

Table II
Example of Data Fitting According to the Equation $F = KL^n$ For Panelist Number 3 (Table I)

Actual Load (g)	Actual Friction Force (g)	Estimated Friction Force (g)	95 Per Cent Confidence Limits
20.9	6.25	6.21	5.77–6.68
50.5	11.25	11.18	10.74–11.65
71.4	14.38	14.10	13.63–14.58
98.9	16.56	17.52	16.93–18.13
119.8	19.38	19.92	19.18–20.68
149.4	23.75	23.08	22.09–24.11
187.7	27.50	26.88	25.53–28.30

In general, the equation seems to satisfy the experimental data reasonably well. A theoretical interpretation of the above equation has been suggested by Bowden and Tabor (2), based on the simple premise that if the shearing strength, S_m is constant

$$F = AS_m \tag{7}$$

then, the variation of the friction force with the load is due to the way in which A (the real area of contact) varies with the load, i.e.,

$$A = K_1 L^n \tag{8}$$

The differences in behavior of the polished and rough probes could be ascribed to the difference in the number of contact regions involved. With the polished probe, it would be expected that a much larger number of contact points would be established with the skin surface. Thus, upon rotating the polished probe on the skin substrate, the skin is "pulled" along, conceivably as a result of high adhesion. Continuous rotation should lead to the formation of wrinkles. The formed wrinkles will present an added resistance to the motion of the polished probe, which would be a function of the rate of "wrinkling." The measured value of the force under such conditions will not reflect the inherent friction properties of the substrate. The rough probe on the other hand, does not produce wrinkling because of the much smaller real contact area with the skin, so that the skin is not pulled along as the probe rotates, presumably because of the lack of adequate "grip."

Friction force measurements with the rough probe were highly reproducible at the lower loads showing variation of 2 per cent about the mean, but the variation increases to about 10 per cent at the higher end of the load range.

We have also examined the state of skin after contact with the rotating probe for 3 min for any possible plowing action or disruption in the surface. As mentioned earlier, the combination of a hard sliding probe on a softer substrate may lead to plowing. Scanning electron micrographs were taken of the same skin area before and after probe contact, using standard replicating procedures. Fig. 6 shows two such sites of a female panelist. There is no evidence of plowing or surface disruption as a result of contact of the rotating probe with the skin.

EFFECT OF SPEED OF ROTATION AND SITE-TO-SITE VARIATION

A study was made of the effect of the speed of rotation at a given skin site on the coefficient of friction under constant load. The speed ranged from 3.6 to 583 rpm. A number of measurements were also conducted to establish any variation in the value of the coefficient of friction along the volar forearm. Such measurements were usually carried out at a given speed of rotation and load. The general conclusions indicate that the effect of speed of rotation is negligible over the range examined, and that there is no site-to-site variation on the volar forearm if the measurements are restricted to the larger area close to the elbow.

EFFECT OF SKIN HYDRATION

After initial determination of the coefficient of friction at a given speed and load, a number of panelists were asked to rinse their arm with water and blot away any excess.

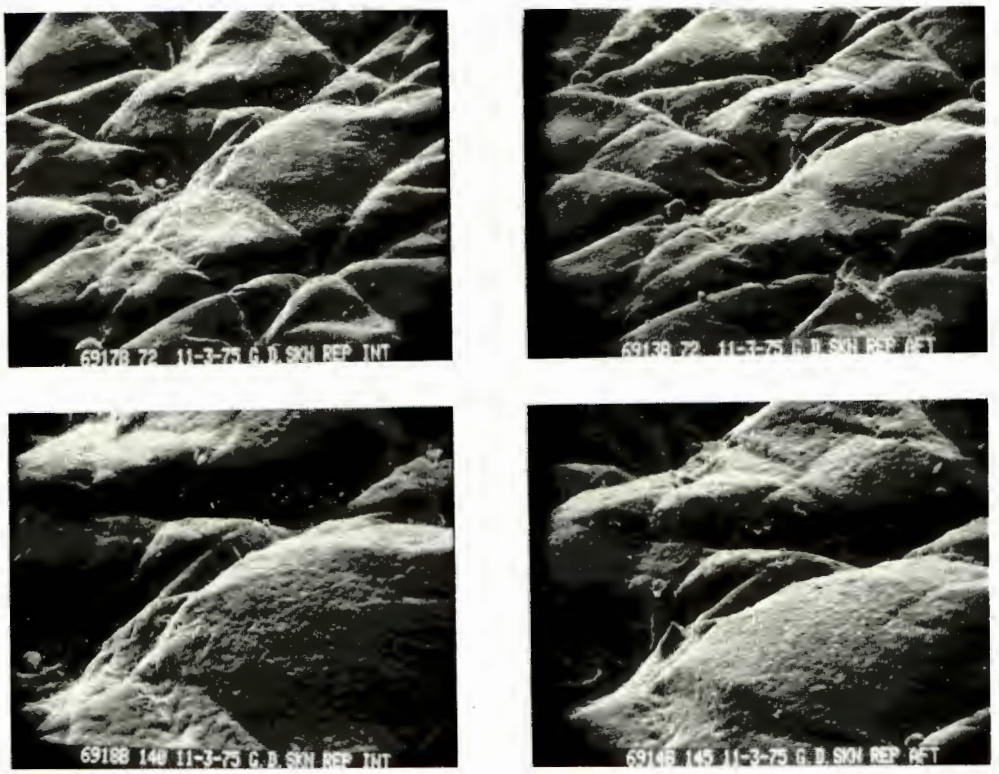

Figure 6. Photographs coded 6917 B and 6918 B show two different sites on skin surface *before* probe contact. Photographs coded 6913 B and 6914 B show the *after* effect

Measurements of the friction coefficient were then repeated immediately on the same test site. A 2–3 fold increase in the coefficient of friction was recorded on most panelists using the rough probe. It is interesting to note, however, that normal values of μ, obtained before rinsing, were again achieved after about 2 min. It is also worth noting that during the first measurement, immediately after rinsing and blotting, the rough probe exhibited a response similar to the polished probe, i.e., an increase of the force of friction with time. Subsequent measurements showed a normal response. The degree of wrinkling increased substantially when using the polished probe on the hydrated skin. These observations can be explained by assuming a substantial decrease in the compression modulus of skin due to presence of water, leading to an increase in the size and, perhaps, the number of the junction zones (hence, an increase in the real contact area) which would ultimately increase the coefficient of friction. The decrease in the value of μ, after about 2 min, to the original value, indicates a return of the mechanical properties to the normal values due to the fast evaporation of water from the skin surface. These findings support Appeldoorn and Barnett's observations concerning the effect of skin hydration on friction properties (11).

Table III
Comparison of Friction Values on "Normal" and "Dry" Skin
Load 98.9 g, Rotation Speed 2.03 cm/sec

	Friction Force (g)[a] Polished Probe	Friction Force (g)[b] Rough Probe
Normal Skin	62.5	16.0
Dry Skin	37	11.6

[a]Values after 20 sec.
[b]Values refer to kinetic friction force.

EFFECT OF SKIN DRYNESS ON FRICTION PROPERTIES

Because of the difficulty in inducing skin dryness under laboratory conditions, it was not possible to conduct an exhaustive study on the effect of dryness. We were able, however, to conduct some measurements on 1 panelist suffering from a severe case of skin dryness, which was obvious even to the untrained eye. The dry site was located on the dorsal forearm, and its extent was rather restricted. Friction measurements were made on a "normal" and the "dry" site using the polished and rough probes. The data are given in Table III. It can be seen that a substantially lower value of the force of friction is found on the dry site with both probes. A much larger decrease is observed with the polished probe, however.

EFFECT OF TALCUM POWDER

Before-and-after friction measurements were conducted using talcum powder as a solid lubricant on 7 panelists. The polished and rough probes were used at a speed of 32.4 rpm and 98.9 g normal load. As expected, some wrinkling was observed on the untreated skin with the polished probe, and the force readings were arbitrarily taken after 20 sec. A decrease averaging 50 per cent in the friction force was observed with the polished probe after application of talcum powder. No wrinkling of the skin or increase of the force with time was observed in the course of the measurement.

Little or no decrease in the friction force was registered with the rough probe after application of talcum powder. The amount of talcum powder applied to the skin was enough to cover the surface with a thick film, such that direct contact between the probe and the skin substrate was not possible. The effect of talcum powder can be attributed to its low S_m value and adhesion to the stainless steel surface; hence, a lower friction force would be expected. The fact that the same values for the friction force were obtained with the polished and rough probes after application of talcum powder, lends support to the notion that a film of talcum powder was transferred to the probe surface such that the measured values of the force reflect the property of the talcum/talcum system.

EFFECT OF SILICONE OIL

An investigation of the effect of silicone oil (polydimethyl siloxane) as a fluid lubricant, when applied as a thick film onto the skin surface, on the friction properties was

Table IV
Effect of Silicone Oil Viscosity and Probe Speed on the Friction Force. Load 98.9 g. Polished Probe

Speed (rpm)	Friction Force[a] (g) Untreated Skin	Viscosity of Silicone Oil (cks)		
		100	1,000	10,000
3.6	30.6	6.5	7.5	8.5
10.8	30.8	7.5	11.9	15.0
32.4	30.7	9.4	15.0	28.8
67.2	30.8	11.9	19.6	37.5

[a]Values at 20 sec.

conducted on 10 panelists. The effects of speed of rotation of the probe and of the viscosity of the silicone oil were examined in some detail to identify the mechanism of lubrication involved. The relevant data for 1 panelist have been compiled in Table IV. The results obtained indicate that the presence of silicone oil decreases substantially the friction force compared to untreated skin and that the mechanism involved is fluid or hydrodynamic lubrication, i.e., the friction force is dependent on the bulk properties (viscosity) of the lubricant. Both probes behave similarly in the presence of a fluid lubricant, both qualitatively and quantitatively.

A number of important points emerge from the above findings regarding *in vivo* friction measurements on skin as follows:

1. The effect of the surface condition of the probe. It has been shown that the type of finishing given to the probe surface has an important qualitative and quantitative effect on the results.
2. Low friction values for untreated skin do not necessarily mean a smooth skin condition. As has been shown in this work, obviously dry skin gave lower friction force values than seemingly normal skin. It is, therefore, necessary, before assigning any practical significance to the effect of product treatments on skin condition, to establish a meaningful correlation between instrumental measurements and what consumers perceive as an acceptable skin condition. This can be achieved by using a large panel and trained judges to help determine the range of the friction coefficients which describe the different skin conditions.
3. In order to bring about perceptible changes in the friction properties of skin through product application, it is obvious that a sufficient amount of some beneficial ingredient should be deposited on the surface. The simplest approach is to use the product directly as in the case of creams and lotions, for example. As suggested by this work, the properties of the residual film will probably have a direct bearing on consumer acceptability. Such products will probably exhibit hydrodynamic lubrication, and, hence, the viscosity of the applied film will be of considerable importance. Again, it will be necessary to define the optimum ranges of an acceptable friction coefficient under these conditions via panel testing. A very low value of the friction coefficient may be associated with "slippery feel" and too high a value will most likely be associated with "sticky feel."

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Screening of fragrance materials for allergenicity in the guinea pig
I. Comparison of four testing methods

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Synopsis

An OPEN EPICUTANEOUS TEST (OET) is proposed for the detection of SKIN IRRITATION and CONTACT HYPERSENSITIVITY induced in GUINEA PIGS. Thirty-two compounds described in the literature as being ALLERGENIC for man were tested in the guinea pig by the OET technique, and for the purpose of comparison, by three other techniques, namely the DRAIZE TEST (DT), the MAXIMIZATION TEST (MT) and a test with FREUND'S COMPLETE ADJUVANT (FCAT). In the OET, a high degree of correlation was found between the allergenicity of the tested compounds for the guinea pig and for man.

I. INTRODUCTION

A considerable amount of work has been done over many years developing procedures for detecting skin-irritating and/or sensitizing effects of chemicals on laboratory animals, in order to preselect compounds likely to be well tolerated by man. As in general toxicology and pharmacology, the results of such tests on animals cannot be fully valid for humans because of interspecies differences in the absorption, metabolism, and excretion of the compounds concerned. Furthermore, in the case of topical medication and of cosmetics, the conditions of exposure, such as concentration, frequency of application, and site of contact, can never be identical in human use and in animal experiments.

The problem of identifying contact allergens, in humans as well as in laboratory animals, was first approached critically and on a scientific basis by Kligman (1, 2, 3) and by Magnusson and Kligman (4, 5). These authors carried out comparative tests of numerous drugs, industrial contactants and cosmetics with well-known sensitizing properties, by various so-called predictive procedures commonly used on humans (6–21), and have shown conclusively that these procedures often fail to identify even known sensitizers. These procedures are, therefore, also likely to be inadequate for the recognition of the allergenicity of new synthetic compounds. By carefully analyzing all

*Deceased February 8, 1974.

the factors influencing contact sensitization in man and in the guinea pig, and by including all these factors in the same experiment, these authors developed a testing technique which they called the maximization test (MT). With this technique, they found a close correlation between clinical experience and experimental results. The authors conclude that by testing compounds on laboratory animals under extreme experimental conditions it should be possible to detect the whole sensitizing potentiality of a compound.

This statement should permit the assumption that the probability of a compound causing sensitization in humans could be estimated. The authors emphasize, however, that it is impossible, on the basis of animal experiments alone, to formulate fully reliable predictions valid for humans.

The purpose of our work, taking into account the knowledge acquired by Kligman and Magnusson, was to develop a testing procedure for guinea pigs convenient for testing new synthetic chemical compounds intended for use in fragrances and cosmetics. Special attention was paid to developing a test which would be simple to perform, would simulate the conditions in human use, would yield quantitative data, and would minimize the effects of subjective factors in evaluating the results. Like Kligman, we checked the reliability of our test by performing concurrently 3 other tests commonly used in this field. We tested a series of compounds with a well-known sensitizing capacity for man, in order to establish whether or not a correlation exists between clinical experience and the results of our animal experiments.

For the first step in these investigations, we chose 32 compounds described in the literature as being allergenic for man. These compounds were tested concurrently, on groups of 6 to 8 guinea pigs each, by the open epicutaneous test (OET), the Draize test (DT), the maximization test (MT) and an intradermal test with Freund's complete adjuvant (FCAT), all described below.

II. MATERIALS AND METHODS

CHEMICAL COMPOUNDS

Thirty-two compounds used in the perfume industry were tested. All these compounds were tested under code numbers, their nature and potential allergenicity being unknown to the experimenters prior to testing.

ANIMALS

The animals used were male and female outbred Himalayan white-spotted guinea pigs bred at the Institute of Biomedical Research, Füllinsdorf, Switzerland. The guinea pigs weighed 400 to 500 g. They were fed on pelleted feed supplemented with green vegetables, carrots and vitamin C in the drinking water, all available *ad libitum*.

TESTING METHODS

OET: All the compounds were tested undiluted as well as dissolved in acetone, ethanol, diethyl phthalate, etc., at concentrations of 30, 10, 3, 1, 0.3, 0.1, and 0.03 per cent (or less when necessary) in order to establish a dose-response curve making it possible to determine the minimal irritating and the maximal tolerated concentrations on an "all or

none" basis. This contrasts with another method still in use (11), where only one arbitrarily fixed concentration is used, and its activity is evaluated subjectively according to the intensity of the lesions observed.

Before the induction procedure (see below), we determined the skin irritation caused by a single application. For this purpose, we applied 0.025 ml of each undiluted compound and of its progressively diluted solutions to an area measuring 2 cm^2 previously marked with a circular stamp on the clipped flank skin of 6 to 8 animals per group. In each case, the liquid tested was applied uniformly with a pipette. After evaporation of the solvent, the application site was left uncovered. The reactions were read after 24 h using an "all or none" criterion, i.e., the dose-response curve was established by end-point determination. The minimal irritating concentration was defined as the lowest concentration causing mild erythema in at least 25 per cent of the animals of the group concerned, and the maximal nonirritant concentration as the highest concentration causing no macroscopically discernible reactions in any of the animals of the group. The highest concentration of a compound used in this local application test was determined by its solubility and skin irritating capacity. Most of the substances used could be applied undiluted. However, high concentrations causing strong reactions, e.g., swelling, necrosis, or ulceration, were not used for evaluation because the end-point determination was considered a more sensitive index of activity.

The determination of the tolerance threshold on the guinea pig in the OET is mainly done for methodical reasons, in order to quantitatively realize an eventual sensitization, and besides it gives information on the concentration-dependent skin tolerance of substances. Carrying over these results onto man is possible under restriction.

Induction procedure: On day 0 we applied 0.1 ml of each undiluted compound and of its progressively diluted solutions to an area measuring 8 cm^2 on the clipped flank skin of 6 to 8 guinea pigs per concentration group, using 4 to 6 such groups for each compound. The applications were repeated daily for 21 days, always using the same skin site. The application site was left uncovered and the reactions were read 24 h after each application. The maximum nonirritant and the minimal irritating concentrations were determined by the same "all or none" criterion as described above. When necrotic or ulcerating reactions were provoked, the application site was changed.

Generally speaking, the degree of the topically irritant effect after one single or after repeated application is characterized by the intensity of the skin reaction and by the magnitude of the minimal irritating concentration. For evaluation of the irritations, we applied the following scale:

0 = no skin reaction	2 = redness plus swelling
0.5 = red spots	3 = redness, swelling, crusts
1 = confluent redness	4 = necrotic skin alterations

Challenge procedure: To determine whether or not allergic contact dermatitis was induced, all the groups of guinea pigs previously treated for 21 days as described above, as well as 6 to 8 untreated controls for each compound, were tested on days 21 and 35 on the contralateral flank with the same compound at the minimal irritating concentration and at some lower nonirritant concentrations. We used the minimal irritating

concentration of each compound in order to confirm the biological activity determined after the first application and to exclude false results. These tests were performed by applying with a pipette 0.025 ml of each concentration to skin areas measuring 2 cm^2, the reactions being read after 24, 48 and/or 72 h. This procedure enabled us to determine the minimal sensitizing concentration necessary to induce contact hypersensitivity and the minimal eliciting concentration necessary to cause a positive reaction. A concentration was considered allergenic when at least 2 out of the 8 animals of the concentration group concerned showed positive reactions with nonirritant concentrations used for challenge, based on practical experience.

The total dose of compound administered in the OET ranged from 2100 to 63 mg or less, depending on the concentrations used.

Solvents, like water, ethanol and acetone, even when applied repeatedly, yielded no macroscopically detectable alteration on the treated skin. When other bases were applied, like vaseline, diethyl phthalate or polyethylene glycol, additional controls for the vehicles were set in.

DT (11): A dose of 0.05 ml of a 0.1 per cent solution of the compound tested in isotonic saline was injected intradermally on day 0 and further doses of 0.1 ml each were injected on 9 alternate days (total dose = 0.95 mg). The treated animals and untreated controls were challenged intradermally with 0.05 ml of a 0.1 per cent solution on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.

MT (4, 5): On day 0 the animals were injected intradermally with 0.1 ml of a 5 per cent solution of the compound tested, with 0.1 ml of a 5 per cent emulsion of the same compound in Freund's complete adjuvant (FCA) and with 0.1 ml of FCA alone, each injection being given twice. In addition, 250 mg of the compound dissolved in petrolatum at a concentration of 25 per cent, which always causes mild to moderate skin irritation under occlusion, was applied on day 8 to a clipped skin area of the neck and was kept under occlusive bandage for 2 days (total dose 20 mg intradermally plus 250 mg epicutaneously). On day 21 an occlusive patch test with the compound at a subirritant concentration in petrolatum was applied to the flank for 24 h. The reactions were read 24 and 48 h after removing the patch.

FCAT: Doses of 0.05 ml of the undiluted compound mixed with the same volume of FCA were injected intradermally into the neck on days 0, 2, 4, 7, and 9 (total dose 250 mg). The control animals were similarly treated with 5 × 0.05 ml of FCA alone. All the animals were tested epicutaneously on days 21 and 35 as described in the section above.

III. RESULTS

The results obtained by testing the 32 compounds by the 4 procedures described above are presented in Table I. The compounds are subdivided into 4 groups according to their allergenicity in the 4 animal tests, namely: Group I, not sensitizing in any test; Group II, sensitizing in the OET and in one or more of the other tests; Group III, sensitizing exclusively in the OET; Group IV, not sensitizing in the OET but sensitizing in one or more of the other tests.

Table I
Skin Irritating and Sensitizing Properties of 32 Compounds in Guinea Pigs and in Humans

Compound	OET: Minimum Irritating Concn. in %		OET: Minimum Sensitizing Concn. in %	OET: Minimum Eliciting Concn. in %	Allergenicity in Guinea Pigs				Allergenicity in Humans	
	After 1 Application	After 21 Applications			OET	DT	MT	FCAT	Type	References
Group I										
Acetophenone	100	1			−	−	−	−	A	22
Benzophenone[a]	30	3			−	−	−	−	A	22
Diethyl phthalate	100	100			−	−	−	−	A	22
Dimethyl anthranilate	100	3			−	−	−	−	A	22
Dimethyl-benzyl carbinol	30	1			−	−	−	−	A	22
Hydroxycitronellal	30	10			−	−	−	−	A	22,50
Thymol[a]	3	3			−	−	−	−	B/E	24,29/22,23,30
Group II										
Benzyl alcohol	30	3	30	10	+	−	−	+	D	22,24,25,26,30,31,44,45
Benzyl cinnamate	3	3	3	0.3	+	+	+	+	D	22,24,25
Carvacrol	3	0.3	3	1	+	−	+	+	B/E	48/22,24
Cinnamic aldehyde	3	3	3	0.3	+	+	+	+	B/D	24,25,29,51/22,25,29,30
Citral	3	3	10	1	+	+	+	+	B/D	22,48,53/24,37
Citronellal	10	3	30	3	+	−	+	+	D	22,24,31,32,33,37,41
Cuminic aldehyde	30	3	3	3	+	+	+	+	A	22
Geraniol	30	3	10	3	+	−	+	+	D	22,24,25,29,31,32,33,37, 44,50
Heliotropin	30	3	30	1	+	−	+	−	D	22,25,30,32,37,40
Iso-eugenol	30	10	10	1	+	+	+	+	D	22,24,25,37
Limonene	3	3	100	1	+	−	+	+	D	24,25,30,42,43,44,48,52
Methyl cinnamate	30	3	30	3	+	+	+	+	D	22,24,25
Methyl heptine carbonate	10	0.3	3	0.3	+	−	+	+	B/C/E	22,29,34,36,50/22,35,1/22, 24,29,30,34,35,36,37,38
Methyl octine carbonate	10	3	10	3	+	−	+	+	C/E	35/22,24,35
Phenylacetaldehyde	0.3	0.3	0.3	0.03	+	+	+	+	D	22,25,27
Phenyl-ethyl salicylate	0.1	0.1	30	0.03	+	−	+	+	A	22
3-Phenyl-propionaldehyde	30	10	30	3	+	−	−	+	A	22
10-Undecenal	3	3	30	1	+	−	−	+	A	22
Group III										
Amyl salicylate	10	3	30	3	+	−	−	−	A	22
Benzyl salicylate	0.1	0.1	30	0.03	+	−	−	−	B/E	28/29,39,41,50
Bromostyrol	30	3	10	3	+	−	−	−	A	22
Methyl salicylate	3	3	30	1	+	−	−	−	B/E	23,30,37,49
Group IV										
Benzaldehyde	10	3			−	+	+	+	D	22,24,25,26,29,30,45
Cinnamic alcohol	10	3			−	−	+	+	D/E	25/32,44,50
Vanillin	30	3			−	−	+	+	D/B	22,24,25,29,30,44/22,48,52

[a]Benzophenone was not sensitizing at 60 per cent; thymol was not sensitizing at 10 per cent. Higher testing concentrations of these two compounds were not used because of systemic toxicity.

A compound shown as not sensitizing in the OET does not sensitize even when applied undiluted; otherwise, the minimal sensitizing concentration is given.

Data concerning the allergenicity of these 32 compounds for humans were collected from the literature and are summarized in the last 2 columns of Table I. Compounds considered as allergenic were classified into 5 types, as follows: Type A, compound simply described as "sensitizers," without any confirmatory data being presented; Type B, compounds with confirmed sensitizing capacity for man; Type C, compounds sensitizing experimental animals; Type D, compounds causing positive patch tests in eczematous patients hypersensitive to complex allergens, such as balsam of Peru and turpentine, which contain the incriminated compound or are known to cross-react with it; Type E, compounds causing positive patch tests in eczematous patients with no history of sensitization either to this particular compound or to the complex allergen known to contain it.

SKIN IRRITATION

The OET findings relating to skin irritation are summarized in Table II. It can be seen that, after one application, a few compounds caused mild erythema at a minimal concentration as low as 0.1 per cent, whereas a few others did so only when applied undiluted. In most cases, however, the minimal irritating concentration (after one application) was between 3 and 30 per cent.

After 21 applications, the minimal irritating concentration of most of the compounds tested was 3 per cent. Surprisingly, the minimal irritating concentration of 10 compounds was the same after 21 applications as after one application, whereas in the remaining 22 cases, it was lower after 21 applications, as expected. This may be explained by the accumulation of skin injuries after repeated applications or by the induction of contact hypersensitivity as demonstrated by subsequent contact testing. No relationship was found between the capacity to cause skin irritation and the capacity to induce contact hypersensitivity.

ALLERGENICITY AS DETERMINED BY THE OET

All the guinea pigs which had been treated for 21 days with the undiluted compounds or their various diluted solutions were challenged on the contralateral flank on day 21. By this procedure, the minimal concentration necessary for the induction of contact hypersensitivity and the minimal concentration for eliciting contact reactions at the time of challenge, which corresponds to the degree of hypersensitivity induced, could be determined and expressed in per cent. These data are presented in Table III. It can be seen that, out of the 32 compounds studied, 22 compounds (70 per cent) induced contact hypersensitivity at one or another of the applied dosages. It is also evident from Table III that there was no correlation between the minimal sensitizing concentration and the degree of hypersensitivity induced.

CORRELATION BETWEEN SKIN IRRITATION AND ALLERGENICITY

A scrutiny of the data presented in Tables IV and V shows that no relationship was found between skin irritation and the capacity to induce contact hypersensitivity, i.e.,

Table II
Minimal Skin Irritating Concentrations of 32 Compounds

Concentration %	100	30	10	3	1	0.3	0.1
Number of compounds causing irritation after one application	3	12	6	8		1	2
Number of compounds causing irritation after 21 applications	1		3	21	2	3	2

Table III
Correlation between the Minimal Sensitizing Concentration and the Degree of Contact Hypersensitivity

Minimal Sensitizing Concentration %	100	30	10	3	1	0.3
Number of sensitizing compounds	1	10	5	5		1
Minimal eliciting concentration %:						
10		1				
3		4	3	1		
1	1	3	2	1		
0.3				3		
0.1						
0.03		2				1

Table IV
Correlation between Skin Irritation and Allergenicity

Concentration %	100	30	10	3	1	0.3	0.1
Number of compounds causing sensitization after 21 applications	1	10	5	5		1	
Number of compounds causing skin irritation after 1 application		9	3	7		1	2

Table V
Correlation between the Minimal Sensitizing and the Minimal Irritating Concentrations

Minimal Sensitizing Concentration %	Number of Compounds	Minimal Irritating Concentration After 1 Application %	Number of Compounds
100	1	100	0
		30	0
		10	0
		3	1
30	10	30	4
		10	2
		3	2
		0.1	2
10	5	30	3
		10	1
		3	1
3	5	30	1
		10	1
		3	3
0.3	1	0.3	1

that the degree of hypersensitivity induced was independent of the irritating capacity of the compound concerned.

COMPARISON OF THE OET WITH 3 OTHER TESTS USED FOR DETECTING ALLERGENICITY OF FRAGRANCES FOR THE GUINEA PIG

In order to compare the sensitivity of the OET with that of the DT, the MT, and the FCAT as far as the detection of allergenicity is concerned, the 32 compounds tested were all tested concurrently by these 4 methods on separate groups of guinea pigs. It can be seen from Table VI that, by using all four tests, 25 of the 32 compounds were found to be allergenic in one or more of these tests.

Referring to the detailed data shown in Table I, it can further be seen that, of these 25 allergenic compounds, 22 were detected by the OET and 21 by the other tests. In other words, 4 allergenic compounds were detected exclusively by the OET and 3 others exclusively by one or more of the three intradermal tests. These results suggest that some compounds can only be recognized as allergenic when applied epicutaneously, whereas others only when injected intradermally. These differences may be due to the following: differences in the amount of compound administered in each test; the use of FCA which has well-known adjuvant properties, and/or; the nature of the solvent.

Thus, considering only the dosage, the differences are as follows. In the OET, 21 individual doses are used. Each individual dose may be 100, 30, 10 or 3 mg, or less, depending on the concentration used. In the DT, 0.1 mg is injected intradermally 10 times. In the MT, the total dose is once 20 mg intradermally plus once 250 mg epicutaneously. In the FCAT 50 mg is injected intradermally 5 times.

COMPARISON BETWEEN CLINICAL ALLERGENICITY AND THE FINDINGS ON THE GUINEA PIG

The correlation between the allergenicity of 32 "incriminated" compounds for humans and their allergenicity for the guinea pig can be derived from the data presented in Ta-

Table VI
Compounds Described as Allergenic for Man and Detected as Allergenic[a] for the Guinea Pig by the Four Tests Used

Group	Total Number of Compounds	Tests							
		OET		DT		MT		FCAT	
		+	−	+	−	+	−	+	−
Group II	18	18	0	7	11	15	3	17	1
Group III	4	4	0	0	4	0	4	0	4
Group IV	3	0	3	1	2	3	0	3	0
Subtotal	25	22	3	8	17	18	7	20	5
Group I	7	0	7	0	7	0	7	0	7
Subtotal	32	22	10	8	24	18	14	20	12
Total	32	32		32		32		32	

[a]The group code is the same as that in Table I (see Results Section of this paper for data). Group I represents compounds not sensitizing guinea pigs in any of the 4 tests used.

bles I and VI. Of these 32 compounds, 25 were found to be allergenic for the guinea pig in 1 or more of the 4 tests used. This includes 4 compounds found to be allergenic only in the OET and 3 compounds found to be allergenic only in 1 or more of the other 3 tests. The remaining 7 compounds were found to be nonallergenic in all 4 tests on guinea pigs. These 7 compounds are described in the literature (22) as sensitizing for humans, but without confirmatory data such as a positive history, a positive patch test or re-exposition. In view of our negative findings on the guinea pig, we consider that the reported allergenicity of these compounds for humans might perhaps reflect a possible cross-sensitizing capacity.

It can be seen from Table I that all the compounds with a well-established sensitizing capacity for man were found to be allergenic for the guinea pig in one or more of the 4 tests used. This could be expected for compounds of the "incrimination" Types B, C, D, and E. By contrast, the test results for compounds of Type A were divergent. These compounds induced hypersensitivity in guinea pigs when the incriminating data were accurate, and failed to do so when their reported allergenicity was based on an assumption or on an unconfirmed observation. The nonallergenicity of the first 6 compounds of Type A listed in Table I, as well as that of thymol, all of which failed to induce hypersensitivity in the guinea pig in any of the 4 tests used, may be considered fairly well-established, because they were negative in the OET even when tested undiluted (benzophenone and thymol could not be tested undiluted because of systemic toxicity, see Table I) and were also negative in the intradermal tests. On the other hand, 4 other compounds of Type A were shown to be weak sensitizers because they were positive in the OET at a concentration of 30 per cent.

IV. DISCUSSION

The OET is a procedure proposed for testing on guinea pigs the skin irritating and allergenic capacities of chemical compounds intended for use in perfumes, cosmetics, and dermatics. In the OET, the compounds to be tested are applied undiluted and in a descending series of concentrations. By establishing a dose-response curve, the minimal irritating and minimal sensitizing concentrations of a compound can be determined quantitatively. The end-point reactions are read on an "all or none basis," thus largely excluding subjective bias in the evaluation of the results.

A total of 32 compounds described in the literature as allergenic for man were tested by the OET. For purposes of comparison they were also tested by the DT, the test with FCAT, and the MT described by Magnusson and Kligman (4, 5). The highest number of allergenic compounds were detected by the OET, somewhat fewer by the FCAT and the MT, and a few by the DT. However, certain compounds were detected exclusively by the OET and others exclusively by one or more of the intradermal tests (DT, MT and/or FCAT). The reliability of the OET, i.e., its predictive value for man, was investigated by us, as had been done by Magnusson and Kligman, testing on animals the sensitizing properties of fragrances known from clinical experience to be allergenic for man and some known to be innocuous. Of the compounds tested, all those with well-established allergenicity for man were detected by the OET (see Tables I and VI). On the other hand, compounds with an unconfirmed clinical allergenicity yielded divergent results in the animal tests, as expected.

Table VII
Allergenicity of Compounds Tested in Humans by the Maximization Test (47) and in Guinea Pigs by 4 Different Procedures

	Humans		Guinea Pigs				
	MT[a]		OET		DT[b]	MT[b]	FCAT[b]
Compounds	Test conc. %	Results	Test conc. %	Results	Results	Results	Results
Amyl-cinnamic aldehyde	3	–	100	–	–	–	–
Diethyl phthalate	5	–	100	–	–	–	–
Methyl-ionone	5	–	100	–	–	–	–
Ionone	4	–	100	–	–	–	–
Hydroxycitronellal	6	–	100	–	–	–	–
Vanillin	1	–	100	+	+	+	+
Cinnamic alcohol	2	–	100	–	–	+	+
Coumarin	4	–	10	+	–	–	–
			3	–			
Eugenol	4	–	10	+	+	+	+
			3	–			
Geraniol	3	–	10	+	–	+	+
			3	–			
Heliotropin	3	–	30	+	–	+	+
			10	–			
d-Limonene	4	–	100	+	–	+	+
			30	–			

[a]The occlusive eliciting concentration application was only at the user concentration × 2.
[b]DT, MT, FCAT: concentrations used see Section II of this paper.

Table VII shows a comparison of the results of our animal tests with those obtained by Greif (47) who used the MT on human subjects for testing several fragrance compounds "which had successfully weathered the test of time," i.e., the innocuousness of which for humans had been demonstrated by many years of practical use. This comparison confirms the high predictive reliability of the guinea pig OET for humans. Table VII shows that some compounds, all negative in the human MT, were not allergenic for the guinea pig in the OET even when tested in the undiluted form. Others did not sensitize guinea pigs at concentrations 2 to 100 times as high as the conventional concentrations used on humans. This shows that the guinea pig OET can be used to determine a quantitatively precise risk of sensitization for humans. Table VII also shows that the results of the intradermal tests on the guinea pig seem to be less predictive for man (see comments on Table VII) and that a better correlation was found between the human MT and the guinea pig OET.

In a later paper, the correlation between the results of Kligman's Human MT and the OET, especially, will be discussed.

V. SUMMARY

An OET is proposed for the detection of skin irritation and contact hypersensitivity induced in guinea pigs by various compounds intended for use in perfumes and cos-

metics. A dose-response curve was plotted for each compound tested, and the irritant and/or allergenic activity of each compound was established in terms of concentration in percent. The proposed procedure is simple, yields quantitative data, minimizes subjective factors when evaluating the results, and simulates the conditions in human use.

A total of 32 compounds described in the literature as being allergenic for man were tested by the OET technique and, for the purpose of comparison, by three intradermal techniques, namely, the DT, the MT and the FCAT. Most of the compounds described as being allergenic for man were found to be allergenic for the guinea pig in the OET and in 1 or more of the 3 intradermal tests. A few of the compounds tested, however, were found to be allergenic only in the OET, and a few others only in the DT, MT and/or FCAT. In the OET, a high degree of correlation was found between the allergenicity of the tested compounds for the guinea pig and their allergenicity for man.

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Das Bindegewebe der menschlichen Haut unter dem Einfluß von UV-Licht*

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Synopsis — The Influence of UV Light on Connective Tissue of Human Skin. — In separate UV-A and UV-B irradiation series on 30 test subjects, an increased biosynthesis of proteoglycans and of collagen was noted except that collagen biosynthesis interference effected not only histological and histochemical alterations but also modifications of the collagen backbone as demonstrated by immunofluorescence microscopy and by collagen atopy. On the basis of this study, skin changes caused by solar irradiation — which are later identified dermatologically as senile, i. e., actinic, elastoses — are interpreted as an expression of interference with proteoglycan, i. e., collagen metabolism of the skin.

Einführung

Wenn man sich heute mit den klinischen Aspekten der Einwirkung optischer Strahlung auf die Haut auseinandersetzt, so geschieht dieses aus mehreren Gründen:

1. Im Zeitalter des Massentourismus ist die Sonneneinwirkung im Winter (Skifahren) und im Sommer gleichermaßen gegeben.
2. Die Einwirkung des Lichtes auf das Bindegewebe der Haut wirkt sich klinisch erst nach Jahren bis Jahrzehnten aus.
3. Die Frage, welcher Anteil des ultravioletten Lichtes bei einem normalen Ablauf der Lichtreaktion zu Schädigungen des Bindegewebes führt, wird im Schrifttum unterschiedlich beantwortet.

Ob eine einmalige Schädigung der Fibroblasten und des Haut-Bindegewebes zum klinischen Bild der akuten UV-Strahlenreaktion beiträgt, ist offen. Der

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chronische Lichtschaden ist durch epidermale Veränderungen, wie Atrophie, Pigmentverschiebungen, Hyperkeratosen und teilweise auch Hautkrebs einerseits und durch bindegewebige Veränderungen, wie kollagene Degeneration (bei Zunahme des elastotischen Materials) und Verlust an Fibroblasten andererseits, gekennzeichnet. Insgesamt resultiert eine dünne, schlaffe, zur Fältelung bzw. Furchung neigende, alt erscheinende Haut (Landmannshaut, Cutis rhomboidalis nuchae), (1—9).

Im Gegensatz zu früherer Auffassung, daß die senilaktinischen Hautveränderungen eine bestimmte Art der Alterung darstellen, wird heute die Ansicht vertreten, daß diese durch jahrzehntelange chronische oder übermäßige Sonnen- bzw. UV-Bestrahlung zu erklären ist. Das Ausmaß dieser Hautveränderungen ist direkt proportional der Dauer und Intensität der Sonnenexposition (10).

Obwohl die genaue Entstehungsweise des histochemisch und histologisch nachweisbaren elasticaähnlichen Materials bisher noch nicht geklärt werden konnte, besteht aufgrund biochemischer, physikalischer und feinstruktureller Aussagen eine große Ähnlichkeit zwischen diesem und dem sog. elastischen Material (11—18). Neben einer degenerativen Umwandlung von Kollagen und/oder Elastin zu elastotischem Material wird auch ein aktiver Bildungsprozeß, der Ähnlichkeit mit der normalen Elastogenese hat, diskutiert (19 bis 21).

Elektronenmikroskopische, histologische und histochemische Befunde lassen sich wie folgt zusammenfassen:

Nach einmaliger Bestrahlung mit der 3—5fachen Erythemschwellendosis (UVB) findet man in den oberen bis mittleren Epidermisregionen nach anfänglichem inter- und intrazellulärem Ödem in einzelnen Zellen eine Kondensation des Zellplasmas und Kernpyknose (sunburn cells). In der Cutis ist außer einem Ödem keine Schädigung, insbesondere der kollagenen Fasern, zu erkennen. Nach einmaliger Einstrahlung hoher (erythematogener) UVA-Dosen sind nur eine geringgradige Schädigung der Epidermis, jedoch Ödem, Kapillarerweiterung, Endothelschwund sowie Kernpyknose in Fibroblasten und Endothelzellen festzustellen.

Nach chronischer UV-Strahleneinwirkung findet man eine Schrumpfung der Keratinozyten und ihrer Kerne, eine Erweiterung der interzellulären Räume und eine massive Zunahme an Melaningranula. In der Cutis sind die elastischen Fasern und kollagenen Mikrofibrillen degenerativ geändert (1—5, 22—28, 72—74).

In Kenntnis dieser bisher dem Schrifttum vorgelegten Befunde und im Hinblick auf die Erweiterung pathophysiologischer Vorstellungen im Binde-

gewebsbereich stellte sich für uns die Frage nach dem Ausmaß der Bindegewebsschädigung.

Dieses Bindegewebe zeichnet sich durch zahlreiche morphologische Erscheinungsformen aus (Knochen, Knorpel, Cornea, Blutgefäße, Sehnen, Haut). Sie alle bilden eine funktionelle Einheit, die auf das sog. Mesenchym zurückzuführen ist. Dieses ist die Bildungsstätte zellulärer Bestandteile (Fibroblasten, Fibrozyten, Makrophagen, Mastzellen) fibrillärer Elemente und der sog. Grundsubstanz. Dieser skizzierte unterschiedliche Aufbau des Bindegewebes ist offensichtlich nicht nur durch die Übernahme und Aufrechterhaltung differenzierter Funktionen zu erklären. So ist das Auftreten zahlreicher, wohl in ihrer Funktion gleichwertiger Zellen, z. B. als Ausdruck lokaler und möglicherweise situationsentsprechender Anpassungsvorgänge zu interpretieren. Dennoch steht eine Zelle im Mittelpunkt der Betrachtung: Der Fibroblast. Dieser stellt das stoffwechselaktivste Zentrum dar, das periphere Impulse aufnimmt, sie speichert und autonome Regulationsmechanismen in Tätigkeit setzt, um somit jeden Reiz mit einer Steigerung der Biosyntheseraten und Sekretion bindegewebiger Interzellularsubstanz zu beantworten, der über die Peripherie herangetragen wird (29—35). Zwei Bestandteile sind von grundsätzlicher Bedeutung:

1. Das Kollagen.
2. Der Gehalt an hochmolekulen Polysaccharid-Proteinkomplexen (PG), der in der menschlichen Haut weniger als 1 % des Feuchtgewichtes beträgt.
   - 2.1. Die Biosynthese dieser Proteoglycane in der Haut ist an 4 wesentliche Syntheseschritte gebunden.
   - 2.2. Die Bindung der Polypeptidketten (Akzeptorprotein).
   - 2.3. Die Erstellung der Bindegewebsregionen aus aktivierten Monosacchariden, die mittels spezifischer Transferasen an bestimmte Aminosäuren des Akzeptorproteins überführt werden,
   - 2.4. die Biosynthese der Polysaccharidketten aus aktiviertem Aminozucker und Hexuronsäuren mit Hilfe einer spezifischen Polymerase,
   - 2.5. die Sulfatierung der Polysaccharidketten mittels spezifischer Sulfotransferasen, die aktiviertes Sulfat auf Aminozucker oder Hexuronsäuren übertragen.

Verschiedene Typen dieser Polysaccharidproteinkomplexe sind in der Haut anzutreffen (Hyaluronsäure, Dermatansulfat, kleinere Anteile von Chondroitin-4- und Chondroitin-6-Sulfat sowie andere sulfatierte Proteoglycane).

Folgende Funktionen werden den Proteoglycanen zugeschrieben:

1. Fixierung von extrazellulärem Wasser,
2. Beeinflussung von Bau und Bildung kollagener Fibrillen (Wechselwirkung von Tropokollagen mit Proteoglycanen sulfatierter Glycosaminoglycane, sog. Matritzenfunktion),
3. Kationenaustauschereigenschaften,
4. Antigene Eigenschaften und Beziehung zur Blutgerinnung (36—40).

Neben diesen Proteoglycanen ist der Gehalt an Kollagen im Bindegewebe von Bedeutung, dessen Bildung sich aus mehreren Einzelschritten erklären läßt.

Erstellung aktivierter Aminosäuren;
Erstellung der Primärstruktur unter enzymatischer Steuerung, Hydroxylierung, Glykolysierung;
Spaltung, Fibrillenbildung und Ausbildung der Quervernetzung (dreidimensionales Faserflechtwerk), siehe Abb. I (41—45).

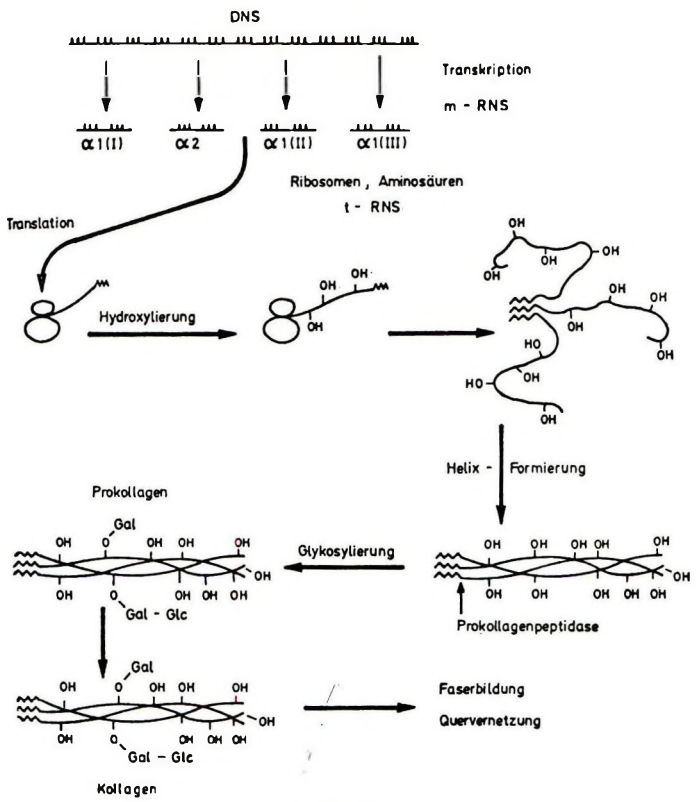

Abbildung 1

Die Biosynthese des Kollagen (nach Kühn 1974; Miller und Matukas 1974).

Methodik

Unsere molekularbiologischen Untersuchungen nach Ultravioletteinwirkungen sind wie folgt durchgeführt worden:

1. Als Strahlungsquelle für das gesamte ultraviolette Hg-Spektrum diente uns eine Kromayerlampe der Firma Hanau-Quarzlampengesellschaft.

   Eine nahezu monochromatische Strahlung der Wellenlänge λ (302 nm UVB) wurde durch Einschalten eines Interferenzlinienfilters der Firma Schott und Gen. Mainz, D max (maximale Durchlässigkeit) 13 % erreicht.

   Eine nahezu monochromatische Strahlung der Wellenlänge λ 366 nm (UVA) wurde unter Zuhilfenahme des Linienfilters E der Schottwerke Jena erzielt.

2. Zur Bestimmung der Erythemschwelle wurden bei jeder Versuchsperson in Abständen von etwa 1 cm Bestrahlungsfelder von 10 x 4 mm Größe an der Oberarmstreckseite gesetzt. Dabei hat jedes Bestrahlungsfeld gegenüber dem vorhergegangenen einen Dosiszuwachs von 40 %. Die Ablesung erfolgte nach 24 Stunden im Oberarmbereich.

3. Nach Ermittlung der Erythemschwellendosis wurde die Bestrahlung der Versuchsperson (n = 30) an der entsprechenden kontralateralen Oberarmseite durchgeführt. Die Berechnungszeiten für λ 302 nm (UVB) entsprechen dem 3—6fachen der Erythemschwellendosis, für λ 366 nm (UVA) dem 50fachen der UVB-Dosis. Die UVA-Dosis berechnet sich unter Berücksichtigung des Verhältnisses der Bestrahlungsstärken (λ 302/366 = 1 : 3) und der Filterdurchlässigkeiten (0,13 : 0,4).

24 Std. nach dieser Bestrahlung erfolgte die Exzision in Lokalanästhesie mit 0,1%iger Procainlösung. Das Exzidat wurde mit eiskaltem modifizierten Krebs-Ringer-Bikarbonatpuffer mit 11,6 M Glucose von pH 7,4 getränkt. Nach Entfernung des Fettgewebes, Anfertigung von Hautscheiben, ca. 0,5 mm dick, senkrecht zur Epidermis, mit einem Spezialmesser geschnitten, Feuchtgewichtsbestimmung.

Standardinkubation der Hautschnitte unter aeroben Bedingungen: (46).

Aufarbeitung der radioaktiv markierten Hautschnitte. Nach Inkubationsende, Isolierung von Sulfopolysaccharidpeptiden nach Inkubation von Radiosulfat ($^{35}SO_4$ trägerfrei der Firma Bucheler, 15 μCi, sowie 15 μCi D-Glucosamin-6 ^{3}H, spezifische Aktivität größer als 200 μCi sowie C_{14}-Prolin 15 μCi, spezifische Aktivität 100 μCi/mmol pro Inkubationsansatz, Volumen 3 ml).

Kollagenfraktionierung nach JACKSON, modifiziert nach LINDNER, Hydroxyprolinbestimmung nach JUVA und PROCKOP (47—49).

Ergebnisse

1. Proteoglycanstoffwechsel, *Tab. 1.*
2. Kollagenstoffwechsel, *Abb. 11.*
   Mittelwerte ($\bar{x}$) und Standardabweichung ($\pm s$)

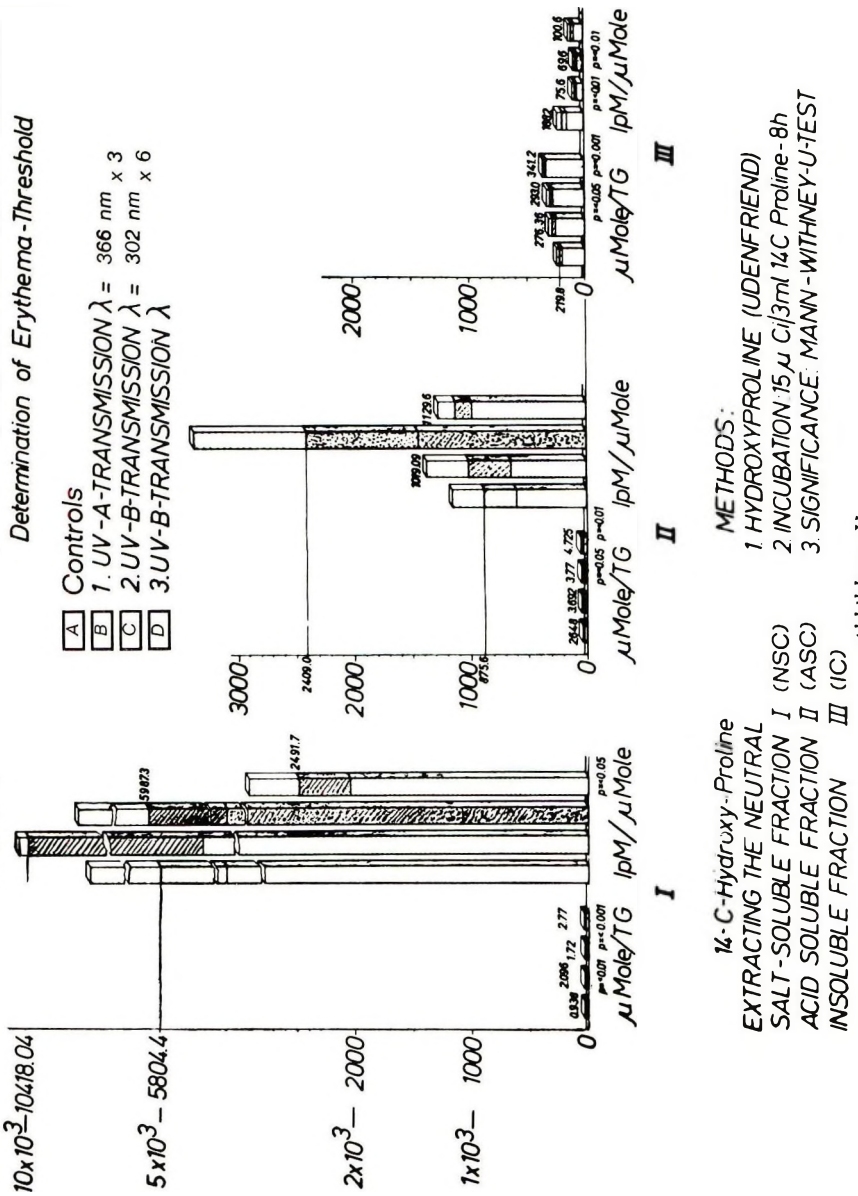

Abbildung 11

Tabelle 1

Der Einbau von $^{35}SO_4$- und ^{3}H-Glucosamin in die Sulfo-Polysaccharidpeptide der menschlichen Haut von gesunden und UV-bestrahlten Patienten (UVA, UVB).

Diagnose	Uronsäure µMol/g Trocken-gewicht	Sulfopolysaccharidfraktion					
		$^{35}SO_4$-Einbau					
		IpM/g Frisch-gewicht	Einbau-Kontrollen = 100 %	IpM/g Trocken-gewicht	Einbau-Kontrollen = 100 %	IpM/µMol Uronsäure	Einbau-Kontrollen = 100 %
Kontrollen n = 30	7,85 ± 1,49	911,76 ± 298,5		4919,6 ± 1603,9		494,2 ± 87,7	
UVA (×3) n = 30	9,40 ± 1,12	4912,0 ± 710,1 $p < 0,02$	538	20426,0 ± 4126,3 $p < 0,001$	415	3048,3 ± 356,0 $p < 0,001$	616
UVA (×6) n = 30	17,81 ± 1,91 $p < 0,01$	7100,0 ± 524,6 $p < 0,03$	778	92358,4 ± 24295 $p < 0,02$	1877	5378,2 ± 481,5 $p < 0,001$	1088
UVB (×6) n = 30	11,41 ± 1,30 $p < 0,05$	3889,4 ± 1253,6 $p < 0,02$	426	9623,4 ± 1256,3 $p < 0,01$	195	1784,0 ± 492,0 $p < 0,03$	360

(Fortsetzung)	^{3}H-Glucosamineinbau					
	IpM/g Frischgewicht	Einbau-Kontrollen = 100 %	IpM/G Trockengewicht	Einbau-Kontrollen = 100 %	IpM/µMol Uronsäure	Einbau-Kontrollen = 100 %
	2983,2 ± 535,8		18712,3 ± 3810,0		1745,0 ± 545,4	
	8221,5 ± 988,3 $p < 0,05$	275	68463,2 ± 10158,4 $p < 0,02$	365	9078 ± 1058,2 $p < 0,02$	520
	11305,2 ± 822 $p < 0,05$	378	120653,2 ± 27824 $p < 0,02$	644	14091 ± 2758,3 $p < 0,02$	807
	2336,6 ± 278,7	78	13703,4 ± 3304,9 $p < 0,03$	73	1613,1 ± 349,5	92

Mittelwerte mit Standardabweichung. Sicherheitswahrscheinlichkeitsbestimmung nach Mann-Whitney-U-Test. Weitere Einzelheiten s. Methodik.

Diskussion

Bei allen theoretischen Überlegungen zur Wirkung des Lichtes in der Haut spielt die Eindringtiefe des Lichtes (50—51) eine besondere Rolle. Wenn man hierzu die gegenwärtig vorherrschenden Meinungen zum Ablauf der Lichtreaktion an der Haut betrachtet, so kommt man zu folgender Feststellung:

1. Alle Prozesse gehen nicht auf eine einzige fotochemische Grundreaktion zurück.
2. Mehrere Reaktionsketten sind offensichtlich durch die gleichen oder verschiedenen Lichtqualitäten in verschiedenen Teilen der Haut in Gang gesetzt. So wird die Initialreaktion des Späterythems in Hornschicht und Stachelzellschicht dem UVB, die Sofort-Pigmentierungen in der Basalschicht und Stachelzellschicht und Fibroblastenschädigungen sowie Initialreaktionen des Früherythems an den äußeren Blutgefäßen der Cutis dem UVA zugeschrieben.

Betrachtet man unter diesen Gesichtspunkten unsere vorliegenden Befunde in Epithel und Corium, so ergibt sich folgendes Bild:

Unter den von uns gewählten, dem Sonnenlicht vergleichbaren UVA-UVB-Intensitäten findet sich eine signifikant verstärkte $^{35}SO_4$-Incorporation in die Haut (Bezugsgrößen IpM/g Feuchtgewicht/Trockengewicht) und in die sulfatierten GAG* (Bezugsgröße IpM/µMol Uronsäure) nach UVA mit erheblichen prozentualen Einbausteigerungsraten gegenüber Kontrollen. Ein ähnliches Verhalten dieser Parameter konnte nach der Bestrahlung mit UVB nachgewiesen werden. Ein Vergleich der prozentualen Einbauraten gegenüber UVA läßt eine Reduktion dieser Meßgrößen erkennen. Der erhöhte Bausteingehalt (µMol Uronsäure/Trockengewicht) kann als Ausdruck einer erhöhten Synthesekapazität bzw. auch als eine Störung in der Abbaugeschwindigkeit aufgefaßt werden. Diese Veränderungen wurden von NODA im Sinne einer Zunahme von Dermatansulfat interpretiert.

Da ^{3}H-Glucosamin als Baustein nicht nur in die polymeren Mucopolysaccharide der Haut, sondern auch in die nicht dialysierbaren Glykopeptide eingebaut wird — Glucosamin ist als Baustein beider Verbindungen anzusehen —, dürften auch die ermittelten Syntheseraten beiden Verbindungen zuzuordnen sein. Bei dieser Betrachtung zeigt sich deutlich, daß die ^{3}H-Glucosamin-Incorporationsraten gegenüber dem Radiosulfat 2- bzw. 3fach niedriger liegen, so daß die Möglichkeit zu diskutieren ist, daß Glykopeptide weniger an der Entstehung actinischer Dermatosen beteiligt sind.

* Glykosaminoglykuronane

Bei dem Kollagenstoffwechsel ergibt sich folgendes Bild:

1. Die bisher bei anderen Gewebsuntersuchungen beobachtete Korrelation zwischen GAG und Kollagenstoffwechsel ist hier nicht ohne weiteres erkennbar.
2. Der neutralsalzlösliche Kollagenpool zeigt nach UVA (Faktor 3*) einen deutlichen numerischen Anstieg, der auch im säurelöslichen Kollagenpool nachweisbar ist.
3. Bei einer Bestrahlung mit UVB (Faktor 3) ist ebenfalls im Bereich des löslichen Kollagenanteils (neutral- und säurelösliches Kollagen) eine Zunahme der Bezugsgrößen µMol HP/g Trockengewicht erkennbar. Nach Heraufsetzung des Bestrahlungsfaktors 6 ist im löslichen Kollagenpool eine Verminderung der Biosyntheserate erkennbar. Im unlöslichen Anteil dagegen fällt gegenüber Kontrollen eine deutliche Reduktion dieses Parameters auf.

Innerhalb der einzelnen Fraktionen ist eine Zunahme des Hydroxyprolingehaltes mMol HP/TG erkennbar. Die Werte lassen einen fast linearen Anstieg im Hinblick auf die gewählten Strahlenintensitäten gegenüber Kontrollen erkennen. Diese Aussage beansprucht unter Hinweis auf die Tatsache, daß in der menschlichen normalen Haut ein Kollagenmuster existiert, das dem Typ I, d. h. Nachweis zweier identischer α_1-I-Ketten und einer ähnlichen, nicht identischen α_2-II-Kette entspricht, unsere besondere Aufmerksamkeit. Immunfluoreszenzmikroskopische und biochemische Untersuchungen liefern gute Gründe dafür, hier nach UVB-Bestrahlung einen Kollagentyp anzunehmen, der dieser Regelmäßigkeit widerspricht und Ähnlichkeiten mit dem Nachweis sog. embryonalen Kollagens zeigt. Ähnliche Situationen im Gewebe der Haut konnten wir besonders bei den Hauttumoren (Melanom) in getrennten Untersuchungen nachweisen.

Die präsentierten biochemischen Daten sind den histochemischen und enzymhistochemischen Untersuchungen an die Seite zu stellen, die eine Schädigung der Bindegewebszelle zum Ausdruck bringen (52), die im Sinne einer Störung der sekretorischen Aktivität aufgefaßt wird. Proteoglycan einerseits und Kollagenbiosynthese andererseits zeigen nicht das sonst gewohnte synchrone Verhalten. Die ungewöhnlich hohen Incorporationsraten nach UVA und UVB deuten nicht nur auf einen erhöhten turn-over, sondern auch auf die Möglichkeit der Entwicklung von hybridisierten makromolekularen Strukturen hin (Übersulfatierung). Dieses mögliche Fehlverhalten nach UVB findet in dem Nachweis eines atypischen Kollagens in der Haut und einer

* dreifache Strahlendosis

Abnahme der Viskosität NSC gegen ASC ein entsprechendes biochemisches Korrelat.

Diesen eigenen Befunden sind Abnahme der Kettenlänge und Ruptur von Peptidbildungen nach ionisierenden Strahlenbehandlungen als Ausdruck einer Änderung der Proteinstruktur an die Seite zu stellen (53). Unter Hitzedenaturierung sind ähnliche Zustandsänderungen beobachtet worden, die sich primär in einer Aufspaltung innermolekularer Bindungen und Freisetzung wasseraffiner Gruppen ausdrücken. Der Zunahme der Wasserbindung (Anstieg der Proteoglycane) in der ersten Reaktionsphase folgt eine Abgabe gebundenen Wassers unter Bildung unlöslicher Aggregate (54, 55).

Während in der Haut die Bildung des Hydroxyprolins durch enzymatische Hydroxylierung von peptid- oder proteingebundenen Prolins als ausschließlich intracellulärer Vorgang beschrieben wird (56, 57, 58), wird in vitro eine Hydroxylierung unter Beteiligung von Wasserstoffperoxyd bzw. direkten Sauerstoff diskutiert (59, 60, 61). Dennoch könnte der in Relation zur UV-Lichtexposition zu beobachtende Anstieg des Hydroxyprolins in den einzelnen Fraktionen bei gleichzeitiger Abnahme der Biosynthese, vor allen Dingen nach UVB-Bestrahlung, im löslichen Kollagenbereich als Störung der Abbaugeschwindigkeit aufgefaßt werden (62, 63, 64). Ein ähnliches Verhalten ist auch in der Altershaut zu beobachten (65).

Zusammenfassung

In getrennten UVA- bzw. UVB-Bestrahlungsserien an 30 Probanden konnte eine Steigerung der Proteoglycanbiosynthese und eine Steigerung der Kollagenbiosynthese bzw. Reduktion der Kollagenbiosynthese (UVB) nachgewiesen werden. Diese sekretorische Störung führt nicht nur zu den histologischen und histochemischen Veränderungen, sondern auch zu immunfluoreszenzmikroskopisch nachweisbaren Veränderungen am Kollagengerüst und zum Nachweis einer Kollagenatypie. Damit können die unter Sonneneinstrahlung aufgezeigten und später dermatologisch wohl im Sinne einer senilaktinischen Elastose aufzufassenden Hautveränderungen als Ausdruck einer Störung im Proteoglycan- bzw. Kollagenstoffwechsel der Haut gedeutet werden.

*

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Society of Cosmetic Chemists
1976 Medal Award Presentation

Dr. Murray Berdick, Chesebrough-Pond's, Chairman

Dr. Robert P. Giovacchini, Gillette Research Institute, Awardee

The Society of Cosmetic Chemists awarded its highest honor for 1976 to Dr. Robert P. Giovacchini, Gillette Research Institute.

The Medal Award was presented to Dr. Giovacchini in recognition of significant contributions made over the years to industry and for his effectiveness in providing a liaison between the cosmetic and toiletries industry and the Food and Drug Administration over-the-counter Drug Panels.

The formal presentation was made at the December 6th, 1976 luncheon during the Society of Cosmetic Chemists' Annual Scientific Meeting by Mr. Joseph Kratochvil, 1976 SCC President. Dr. Murray Berdick, SCC Medal Award recipient for 1975, acted as Medal Award Chairman and Eulogist.

The Medal Award Dinner Dance, honoring Dr. Giovacchini, was held at the Americana Hotel.

Dr. Giovacchini is a member of the American Academy of Dermatology and Syphilology, Society of Toxicology, Sigma Xi, New York Academy of Science, Virginia Dermatological Society, and the American Academy of Clinical Toxicology.

He is the author of numerous scientific articles that have been published in many prestigious journals and symposia.

J. Soc. Cosmet. Chem., **28**, 79–82 (February 1977)

Solubility of cholesterol in isopropyl myristate

R. J. HARWOOD and E. M. COHEN, *Merck Sharp and Dohme Research Laboratories, West Point, PA 19486*

Received March 8, 1976

Synopsis

This report describes how the SOLUBILITY of CHOLESTEROL in ISOPROPYL MYRISTATE was determined by optical rotation. The procedure described is particularly attractive as compared to other analytical procedures used to determine cholesterol content quantitatively with respect to speed and simplicity of the measurement. The optical rotation procedure indicates that 5.26 per cent (w/w) cholesterol is soluble in isopropyl myristate.

INTRODUCTION

Cholesterol and its esters are common constituents of a number of pharmaceutical and cosmetic topical formulations (1–3). In the course of recent laboratory experiments, it was necessary to accurately determine the solubility of cholesterol in isopropyl myristate. This report describes how this was determined by optical rotation.

In addition, the procedure described herein would appear to be applicable as a general approach to the assay of optically active compounds in lipid and semisolid ingredients of the type generally used for cosmetics, toiletries, and topical pharmaceuticals.

EXPERIMENTAL

Qualitative determination of solubility of cholesterol in isopropyl myristate: Cholesterol* and isopropyl myristate† were heated to 95–100°C. The mixtures were stirred until solution was complete. The solutions were then cooled to room temperature (21°C) and observed for the presence or absence of crystals.

Quantitative determination of solubility of cholesterol in isopropyl myristate: Cholesterol and isopropyl myristate were mixed together and stored in covered beakers for at least 2 weeks. An aliquot of each solution (or an aliquot of the supernatant liquid) was withdrawn and diluted with an equivalent volume of anhydrous chloroform. Optical rotation measurements for the resulting solutions were made using the Perkin Elmer Model 141 Polarimeter‡ equipped with a 5 cc capacity, 1 decimeter cell.

RESULTS

Qualitative measurement: A series of solutions of cholesterol in isopropyl myristate were made in the concentrations as is shown in the chart that follows.

	Solution (per cent by weight)								
	A	B	C	D	E	F	G	H	I
Cholesterol	9.8	8	6	5.5	5.25	5	4.5	4	2
Isopropyl myristate	90.2	92	94	94.5	94.75	95	95.5	96	98

At t = 0, all solutions, except A and B, which were a mass of crystals, were placed at 5°C. Two and one-half hours later, solutions C, D, E, and F formed crystals and solutions G, H, and I were devoid of crystals. The mixtures were then placed at room temperature (21°C). Eighteen hours later, while at room temperature (21°C), there were no crystals in E, F, G, H, and I; C and D remained as a slurry of crystals; and A and B were almost a solid mass of crystals.

Quantitative measurement: A series of mixtures of cholesterol in isopropyl myristate was made. The system compositions ranged from 1.0 to 8.0 per cent by weight cholesterol. Table I indicates the observed rotations for all the samples made. A plot of the per cent composition versus observed rotation, at both 589 and 365 nm (as is shown in the chart that follows), over the concentration range of 1 through 5 per cent (by weight) cholesterol in isopropyl myristate was found to be linear. The rotations of the supernatants from solutions 6, 7, and 8 were used to calculate the cholesterol concentration in solution by use of the calibration curve obtained from the rotation data obtained for solutions 1 through 5 of Table I.

*U.S.P. grade supplied by Amerchol, Edison, N.J. 08817.
†Armak, Chicago, Ill. 60690.
‡Norwalk, Conn.

Per cent by weight of cholesterol in isopropyl myristate (starting mixture)	Per cent by weight of cholesterol soluble in isopropyl myristate (supernatant solution)	
	589 nm	365 nm
5.5	5.14	5.28
6.0	5.37	5.39
8.0	5.26	5.15
Average	5.26	5.27

This procedure would enable one to determine unknown cholesterol concentrations in isopropyl myristate accurately using the Perkin Elmer Model 141 Polarimeter (or equivalent) at least one order of magnitude below that reported for the lowest concentration shown in Table I. An 0.05 per cent solution of cholesterol in isopropyl myristate chloroform (1:1) has a rotation of −0.017° at 589 nm and −0.057° at 365 nm. Incidentally, at 365 nm, there is still sufficient readability of the rotation to go down to a 1 to 5 dilution.

Table I

Solution Number	Percent by Weight of Cholesterol in Isopropyl Myristate	Observed Rotation[a]	
		α589	α365
1	1.0	−0.163°	−0.562°
2	2.0	−0.366°	−1.217°
3	3.0	−0.540°	−1.760°
4	4.0	−0.695°	−2.382°
5	5.0	−0.868°	−2.920°
6	5.5[b]	−0.897°[c]	−3.103°[c]
7	6.0[b]	−0.938°[c]	−3.168°[c]
8	8.0[b]	−0.918°[c]	−3.028°[c]

[a]Rotation of cholesterol/isopropyl myristate solution diluted by an equivalent volume of chloroform. Readings were taken at 24°C.
[b]Crystals of cholesterol present.
[c]Supernatant solution assayed.

CONCLUSION

The estimation of cholesterol solubility in isopropyl myristate by the classical approach of visually ascertaining when the system composition can no longer support a homogeneous condition requires a large number of experiments in order to put upper and lower limits on solubility. The use of optical rotation yielded a precise solubility figure with a minimum of effort. In principal, one need only examine the supernatant of a single isopropyl myristate/cholesterol system for cholesterol content which contains solid in equilibrium with solution.

The simple optical rotation procedure indicates cholesterol is soluble in isopropyl myristate to the extent of 5.26 per cent (w/w). This technique conceivably can be

extended to include the determination of the solubility of cholesterol, cholesterol esters, and other optically active cholesterol congeners in isopropyl myristate and other optically inactive solvents. The optical rotation procedure described above is particularly attractive as compared to other analytical procedures (4) used to determine cholesterol content quantitatively with respect to speed and simplicity of the measurement.

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J. Soc. Cosmet. Chem., 28, 83–87 (February 1977)

Preservation of cosmetic lotions with imidazolidinyl urea* plus parabens

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THOMAS MATZIN and ARTHUR F. PETERSON *Wells Laboratories, Inc., Jersey City, N.J. 07306.*

Received February 27, 1976.

Synopsis

A model cosmetic LOTION containing 0.2 per cent METHYLPARABEN plus 0.1 per cent PROPYL PARABEN was previously shown to be unsatisfactorily preserved because it failed to kill *Pseudomonas (P. aeruginosa.* Addition of 0.3 per cent IMIDAZOLIDINYL UREA to the lotion gave an Imidazolidinyl Urea PARABEN PRESERVATIVE SYSTEM, which was effective against both an initial challenge of *P. aeruginosa* and two rechallenges.

INTRODUCTION

The evaluation of cosmetic lotion preservation using pure and mixed microbial culture challenges has been reported by the CTFA Microbial Preservation Subcommittee (1). In a study describing the results of challenging a "model" lotion with both pure and mixed cultures, the Subcommittee concluded that both types of challenge tests are valid, but that the information from pure culture tests can be "a valuable aid in reformulating borderline preservative systems."

The Subcommittee used two modifications of a model lotion (2) for evaluation, as shown in the following table taken from their report (1).

*Germall 115, registered trademark of Sutton Laboratories, Inc., Roselle, New Jersey 07203.

Ingredients	Lotion A (Unpreserved) Per cent w/w	Lotion B (Preserved) Per cent w/w
1. Lanolin Alcohol*	6.0	6.0
2. Acetylated Lanolin†	2.0	2.0
3. Stearic Acid XXX	2.0	2.0
4. Glyceryl Monostearate—Acid Stable	2.0	2.0
5. Sodium Lauryl Sulfate (100 per cent)	1.0	1.0
6. Magnesium Aluminum Silicate‡	0.5	0.5
7. Methylparaben	—	0.2
8. Propylparaben	—	0.1
9. Propylene Glycol	5.0	5.0
10. Water (deionized)	81.5	81.2

(The first four items in the above table constitute the oil phase and the remaining items, the water phase).

Lotion A, which had a pH of 5.6, was prepared without any preservative. Lotion B, which had the same pH, contained a preservative system of 0.2 per cent methylparaben plus 0.1 per cent propylparaben. Both lotions were challenge tested by the participating laboratories in the Subcommittee's study with pure cultures of the following organisms (1): *Escherichia (E.) coli,* ATCC 11229; *Pseudomonas (P.) aeruginosa,* ATCC 13388 (QMB 1468); *Staphylococcus (S.) aureus,* ATCC 6538; and *Candida (C.) albicans,* ATCC 10231.

When challenged with approximately 10^6 organisms/ml, Lotion A killed *S. aureus* within 7 days, but failed to kill the other 3 microorganisms. Lotion B, which contained both methylparaben and propylparaben, killed *E. coli, S. aureus,* and *C. albicans,* but failed to kill *P. aeruginosa,* which remained at high levels throughout the 28-day study period. The Subcommittee noted that "both test solutions were considered to be unsatisfactorily preserved, although it is clear that Lotion B was better preserved than Lotion A" (1).

METHODS

Since the parabens alone were not capable of adequately preserving this lotion, the authors decided to test the effect of adding Imidazolidinyl Urea at a level of 0.3 per cent to Lotion B, to give "Lotion B Modified." The pH of "Lotion B Modified" was 5.6, the same as that of Lotion B.

The detailed procedure for challenge testing with *P. aeruginosa* 13388 and evaluation by the quantitative pour plate count was described previously (1). All plate counts reported in Table I were carried out in triplicate. The efficacy and capacity of the preservative system was further tested by rechallenging the lotion two more times. One week after the initial challenge, sampling was done on the total sample. One-third of the sample was retained for further sampling, and the remaining two-thirds was

*Amerchol L-101, registered trademark of Amerchol, Edison, New Jersey 08817.
†Modulan, registered trademark of Amerchol, Edison, New Jersey 08817.
‡Veegum, registered trademark of R. T. Vanderbilt Co., Inc., New York, N. Y. 10017.

Table I

A

Result of Challenge of Lotion B with *P. aeruginosa* (Control Run)	
Time after Challenge	*Pseudomonas* Plate Count
0 days	5.6×10^5
1 day	6.8×10^5
2 days	5.4×10^6
7 days	7.1×10^6
14 days	8.4×10^6
21 days	8.2×10^6
28 days	6.6×10^6

B

Results of Challenge of "Lotion B Modified" (Lotion B plus Imidazolidinyl Urea) with *P. aeruginosa*	
Time after challenge	*Pseudomonas* Plate Count
0 day	4.0×10^5
1 day	1.3×10^2
2 days	0
7 days	0
14 days	0
21 days	0
28 days	0
Time after first rechallenge	
7 days	0
14 days	0
21 days	0
28 days	0
Time after second rechallenge	
7 days	0
14 days	0
21 days	0
28 days	0

challenged again with *P. aeruginosa*. This was sampled 1 week later and then subdivided into 2 portions. One portion was retained for further sampling, and the remaining portion was challenged again (a third time) with *P. aeruginosa*. All challenged samples were subcultured at 1, 2, 3, and 4 weeks. All were stored at room temperature.

RESULTS

Challenge testing confirmed that Lotion B failed to kill *P. aeruginosa*, which continued to grow vigorously over the 28-day test period in spite of the presence of paraben preservatives. In contrast, Lotion B Modified did kill *P. aeruginosa* within 2 days, and continued to show the absence of *P. aeruginosa* throughout the 28-day test period (see Fig. 1 and Table I). Counts of "0" in Table I are actually "<10 organisms/ml," and are, therefore, plotted as 10^1 in Fig. 1.)

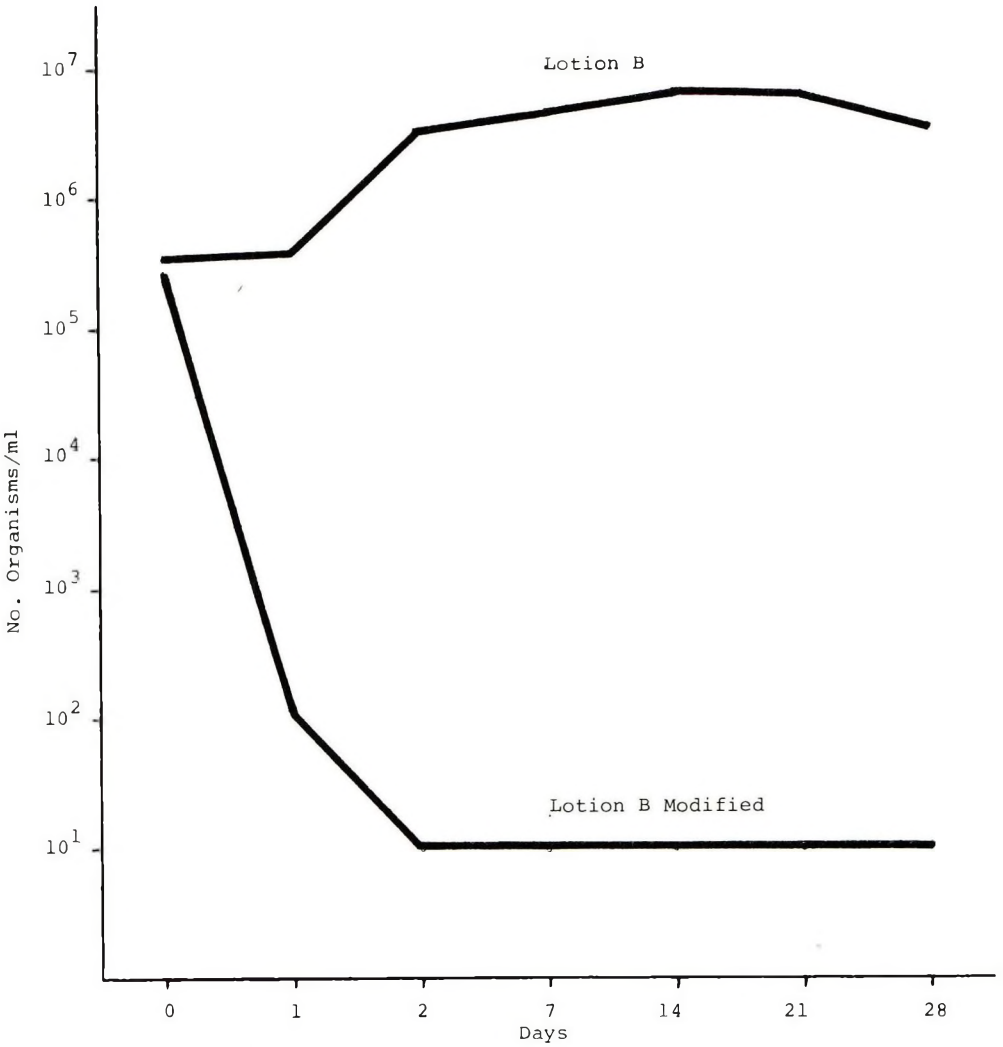

Figure 1. *P. aeruginosa* as challenge organism: test product Lotion B versus Lotion B modified

As can be seen from Table I, Lotion B Modified satisfactorily met the second challenge and the third challenge with *P. aeruginosa,* and no pseudomonads were detectable over the 28-day test period.

SUMMARY

Lotion B, containing 0.2 per cent methylparaben and 0.1 per cent propylparaben, was inadequately preserved. When 0.3 per cent Imidazolidinyl Urea was added to Lotion B, the Imidazolidinyl Urea-paraben preservative system was effective against both the initial challenge of *P. aeruginosa* and two rechallenges.

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(1) J. A. Rogers, G. Jacobs, E. M. Owen, D. C. Pease, Jr., A. F. Peterson, W. H. Riley, A. G. Schenkel, J. L. Smith, C. E. Stegmann, and J. A. Ramp, *CTFA Cosmet. J.*, 5, 2–7 (1973).

(2) *Amerchol Laboratory Handbook*, p. 56, column 1.

Society of Cosmetic Chemists
1975 Literature Award Presentation

Left to right: Dr. J. Alan Swift, Unilever Research, recipient; Dr. Hilda Feinberg, Revlon Research Center, Literature Award Chairman

The Society of Cosmetic Chemists Literature Award for 1975 was presented to Dr. J. Alan Swift, Unilever Research, Isleworth Laboratory, England on December 7th, 1976 at the Americana Hotel in New York City.

The presentation was made by Mr. Joseph Kratochvil, 1976 Society of Cosmetic Chemists President at the luncheon ceremonies. The Award consists of a scroll and an honorarium of $1,500.

Dr. Swift is credited with some 45 published papers concerned mainly with the structure and chemistry of human hair and with the application of the electron microscope in toiletries research. He has presented papers at three of the last 4 Congresses of the IFSCC and, with his colleague Dr. Brown, was awarded the prize for the most meritorious paper read at the 8th IFSCC Congress held in London in 1974.

Book Reviews

THE USE OF FRAGRANCE IN CONSUMER PRODUCTS, by J. Stephan Jellinek. John Wiley & Sons, New York, 1975, 213 pages, Indexed. Price $16.75.

In his preface the author states ". . . this is the first book written expressly for nonperfumers who in their work need to know something about the use of fragrance in products." To this end, the author has succeeded fairly well.

In general, the text reads well and the points the author attempts to make can, in most instances, be readily appreciated. Unfortunately, from time to time, the author attempts to discuss a topic in such broad generalities that the reader will want to reread a section in order to ferret out its meaning.

For the development chemist, the highlights of this book are Chapters 1, 2, and 7. Chapter 1: "The Role of Fragrance," does a good job of explaining how fragrance can and is used in a consumer product to make the difference between success and failure. The principals taught in this chapter should be most useful to the development chemist in formulating a product that conveys those all important messages over and above product performance. Chapter 2: "Fragrance Selection: Marketing Considerations," similarly does a good job of explaining and illustrating how a product may be tested and the "witchcraft" that seems to go into the market research evaluation of the chemists creation. Chapter 7: "Some Properties of Fragrance," is concerned with the uses and chemical characteristics of fragrances in finished products. The more advanced development chemist would also profit from Chapter 4: "Relations with Fragrance Suppliers." This chapter deals extensively with the problems of the fragrance houses, as if they only had problems, but does give one insight into how to get the most from the suppliers you deal with. The chapter on technical considerations is too shallow to be of more value than to illustrate that instability can and will be a problem. Chapters 6 (Organizational Aspects of Fragrance Decisions) and 5 (Monitoring Fragrance Quality) appear to be out of place in this text. Their subject matter seems to be directed at individuals who would need to be expert in fragrances and managing their use in consumer products. The fledgling nonperfumer could find them of general interest but little more.—CARL B. FELGER—Gillette Research Labs.

PERFUMERY TECHNOLOGY ART: SCIENCE: INDUSTRY, by Marcel Billot and F. V. Wells. Ellis Horwood Limited, Chichester, Susses, 1975. 343 pages. Price $41.00.

Because 25 years have passed since a new comprehensive perfumery text had been offered, the present contribution was awaited with high anticipation. The anticipation was fueled by the authors' preface; its first sentence mirroring my sentiment exactly.

The first of 15 chapters entitled "The Study and Practice of Perfumery" cover an array of current topics, many of which post date previous textbooks.

For example, the discussion of topics such as: Safety on Skin, The Use of Modern Instruments, and The International Dimensions of Perfumery, are recent developments.

Unfortunately, the remaining 14 chapters deal largely with traditional topics handled just as they might have been 30 years ago, with almost nothing to show with regard to what recent developments have impinged on perfumery.

The historical review in Chapter 2 is a most comprehensive treatment of this early period, but, unfortunately it fails to delineate between the extensive use of name dropping which is characteristic of early perfumery and the more face value and matter of fact characteristics of this field in its recent 20 years.

The perfumer's raw materials are organized in chapters III through VI, using several devices to catalogue and cross-reference the large number of odorant substances.

The old order of rose, jasmin, orange flower, muguet, green, burnt, salicylate and so on, seems more contrived these days then it used to, being neither an odor classification system which it resembles, nor a convenient system for storing and retriving information. In the same sense, this reader wonders why the book's alphabetically cross-indexing of synthetic odorants carries the long forgotten arrangement, among English speaking perfumers, of being translated from a French list without being realphabetized. This "short list" of synthetics includes only two or three materials that are newer than twenty years old.

Chapter VII is devoted to the discussion of odor classification systems and is well annotated listing 32 references.

Chapter VIII, "Creating a Perfume" is unique in its comprehensiveness.

A careful reading of this chapter will reward any perfumer with many helpful hints, even if he or she must disagree with some of the views that are expressed.

Chapters IX, X and to some degree, XI deal with formulary. As one would expect, the formulas are mostly "golden age." Except for some specialties that are a little newer than 1931, these formulas recall those of Felix Cola in their construction. It is, of course, understandable that no perfumer can publish currently commercially valuable formulas. A word of caution to the trainee perfumer: some materials recited as possibly sensitizing in Chapter I are employed without comment in some of these formulas.

Of the remaining chapters, Chapter XIII is singled out for comment; entitled "Perfumes for Many Purposes," this chapter deals with the greatest part of the productivity of the world's professional perfumers, the compositions that scent functional consumer products. This chapter contains the most up-to-date information in the book—save perhaps Chapter I.

The book is highly recommended as required reading to all student or trainee perfumers, even if mostly for them to gain a detailed picture of the antecedents of their chosen profession. All perfumers should add this book to their far too small technical library.—HARRY C. SAUNDERS—Shaw Mudge & Co.

SYNOPSES FOR CARD INDEXES

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

In vivo skin friction measurements: A. F. EL-Shimi. *Journal of the Society of Cosmetic Chemists* **28,** 37 (February 1977)

Synopsis—In the area of skin care benefits, consumers tend to rate smothness as an important attribute in their overall judgment. This paper describes a technique to measure the frictional force resulting from rotating a probe in the skin surface as a function of normal loan and speed of rotation. A brief background review on friction theory is presented. A number of factors were investigated. The highlights of our findings are as follows.

1. The use of a highly polished stainless steel disc or hemispherical probe produces "wrinkling" or "twisting" of the skin surface during rotation, especially at higher normal loads. The use of an intentionally roughened probe produces friction data which satisfy the simple laws of friction.
2. The force of friction is not a linear function of the normal load as suggested by Amonton's Law, $F = \mu L$, where F is the force of friction, L is the normal load and μ is a constant called coefficient of friction. A different expression, $F = KL^n$, was found to describe our results fairly well. K and n are constants. The deviation from Amonton's law is attributed to the elastic behavior of skin.
3. Dry skin produces low friction values. Much higher values are obtained on hydrated skin. A rationale for this behavior is proposed.
4. To produce immediate and significant changes in the friction properties of skin, sufficient quantities of beneficial agents have to be deposited on the surface. Talcum powder and silicone oil reduce the friction with force. With silicone oils, fluid or hydrodynamic lubrication is involved.

Screening of fragrance material for allergenicity in the guinea pig 1. Comparison of four testing methods: G. Klecak, H. Geleick, and J. R. Frey. *Journal of the Society of Cosmetic Chemists* **28,** 53 (February 1977)

Synopsis—An open epicutaneous test (OET) is proposed for the direction of skin irritation and contact hypersensitivity induced in guinea pigs. Thirty-two compounds described in the literature as being allergenic for man were tested in the guinea pig by the OET technique, and for the purpose of comparison, by three other techniques, namely the draize test (DT), the maximization test (MT) and a test with Freund's complete adjuvant (FCAT). In the OET, a high degree of correlation was found between the allergencity of the tested compounds for the guinea pig and for degree of correlation was found between the allergenicity of the tested compounds for the guinea pig and for man.

The influence of UV light on connective tissue of human skin: H. W. Kreysel, W. Stermann, A. Wiskemann, and J. Kimmig. *Journal of the Society of Cosmetic Chemists* **28,** 65 (February 1977)

Synopsis—In separate UV-A and UV-B irradiation series on 30 test subjects, an increased biosynthesis of proteoglycans and of collagen was noted except that collagen biosynthesis interference effected not only histological and histochemical alterations but also modifications of the collagen backbone as demonstrated by immunofluorescence microscopy and by collagen atopy. On the basis of this study, skin changes caused by solar irradiation—which are later identified dermatologically as senile, i.e., actinic, elastoses—are interpreted as an expression of interference with proteoglycan, i.e., collagen metabolism of the skin.

Solubility of cholesterol in isopropyl myristate: R. J. Harwood and E. M. Cohen. *Journal of the Society of Cosmetic Chemists* **28,** 79 (February 1977)

Synopsis—This report describes how the solubility of cholesterol in isopropyl myristate was determined by optical rotation. The procedure described is particularly attractive as compared to other analytical procedures used to determine cholesterol content quantitatively with respect to speed and simplicity of the measurements. The optical rotation procedure indicates that 5.26 per cent (w/w) cholesterol is soluble in isopropyl myristate.

Preservation of cosmetic lotions with imidazolidinyl urea plus parabens: William E. Rosen, Philip A. Berke, Thomas Matzin, and Arthur F. Peterson. *Journal of the Society of Cosmetic Chemists* **28,** 83 (February 1977)

Synopsis—A model cosmetic lotion containing 0.2 per cent methylparaben plus 0.1 per cent propylparaben was previously shown to be unsatisfactorily preserved because it failed to kill *Pseudomonas (P.) aeruginosa*. Addition of 0.3 per cent imidazolidinyl urea to the lotion gave an Imidazolidinyl Urea-paraben preservative system, which was effective against both an initial challenge of *P. aeruginosa* and two rechallenges.

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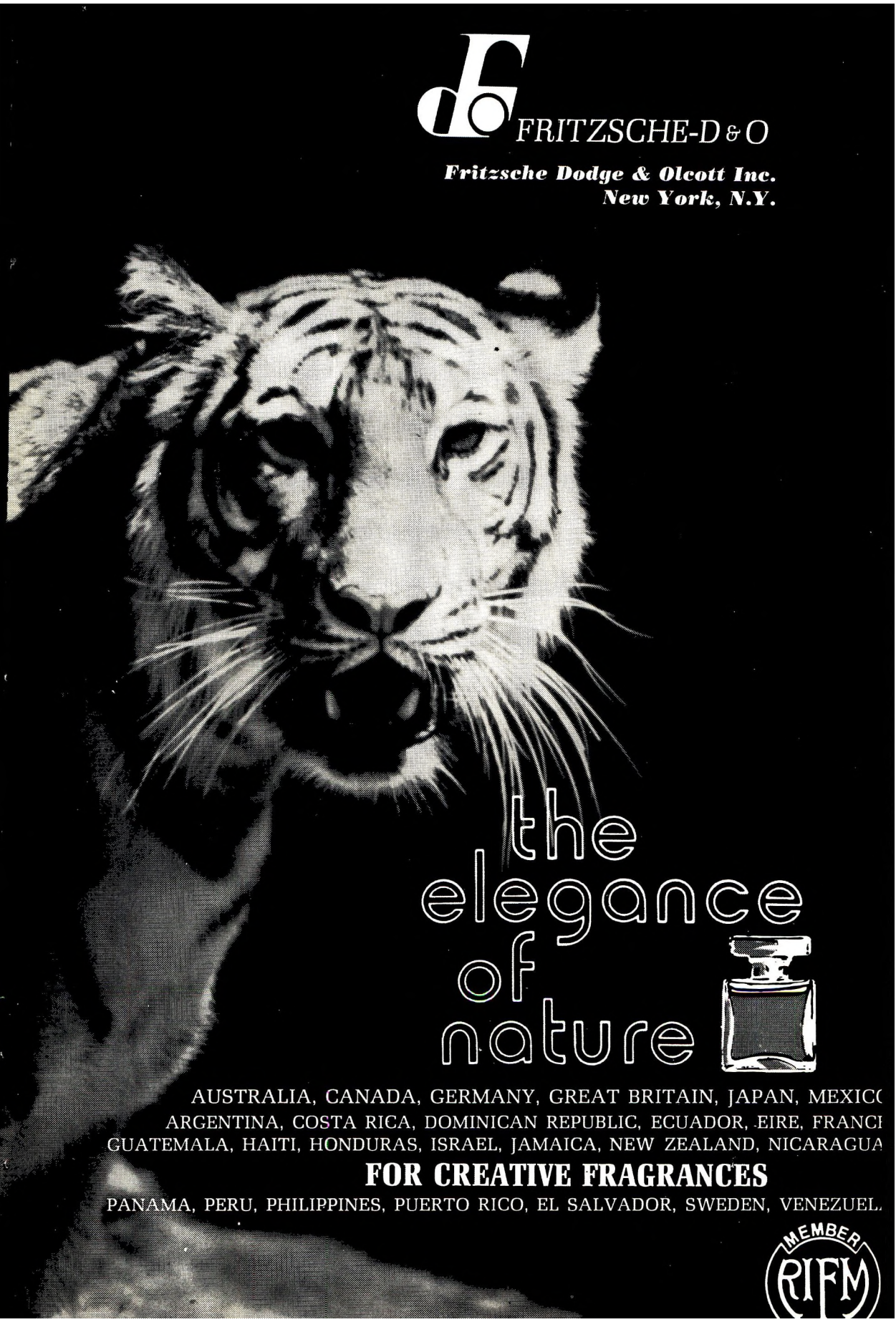
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At Amerchol, we put out fires. That's why the scientists who formulated this sunburn preparation called us when they had a problem with heat stability and staining.

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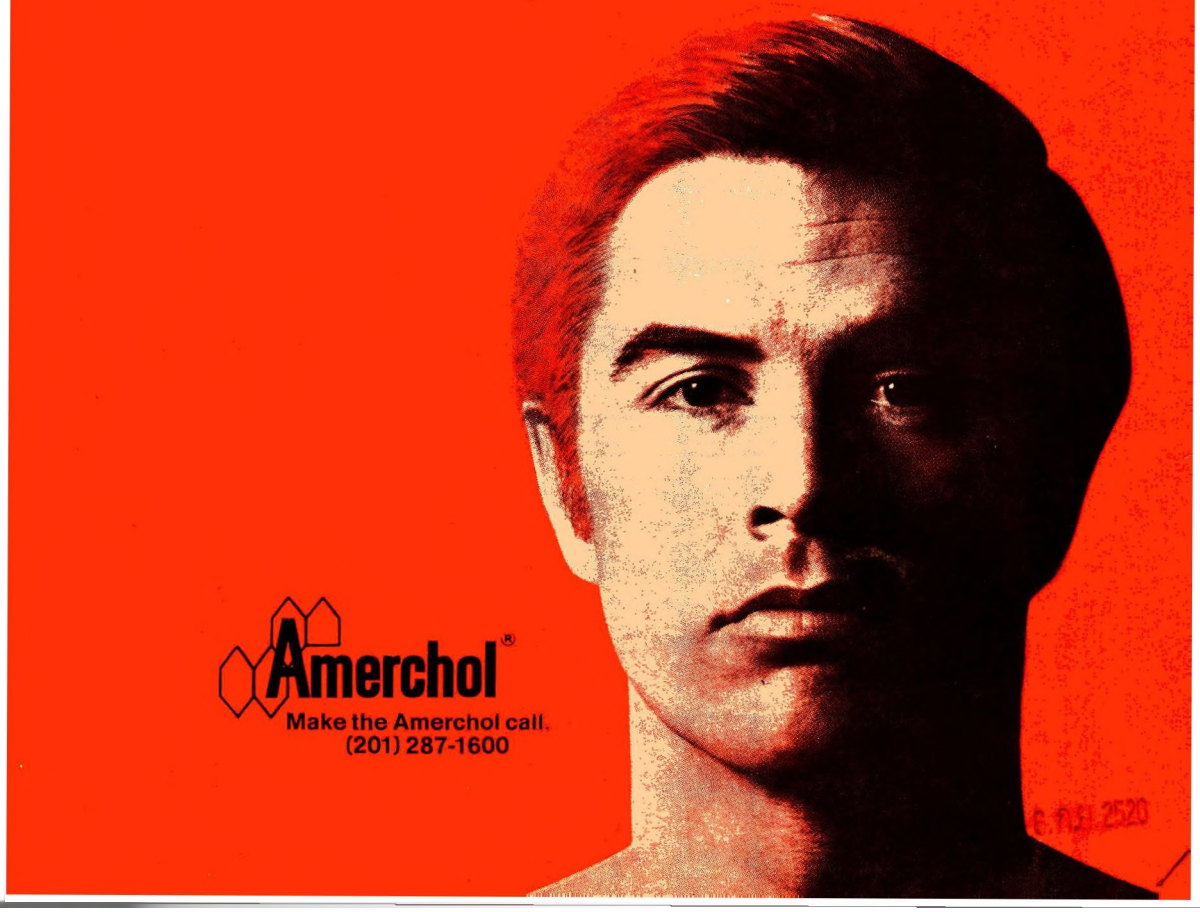

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