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Journal of the Society of Cosmetic Chemists

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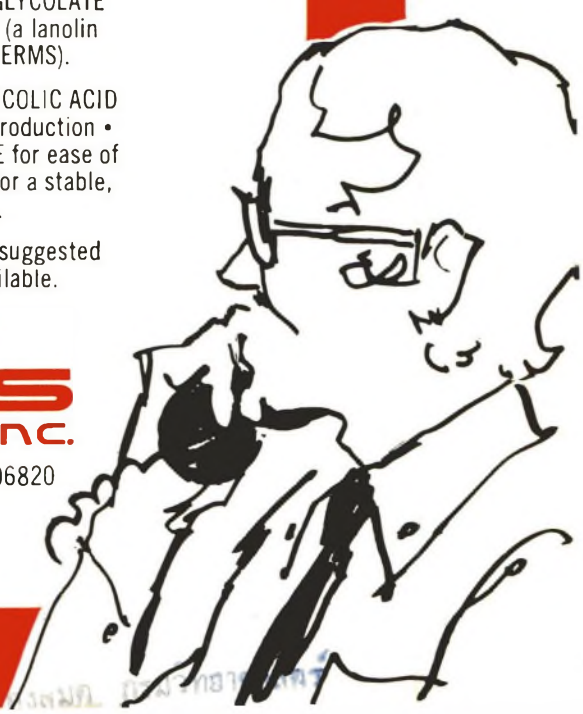

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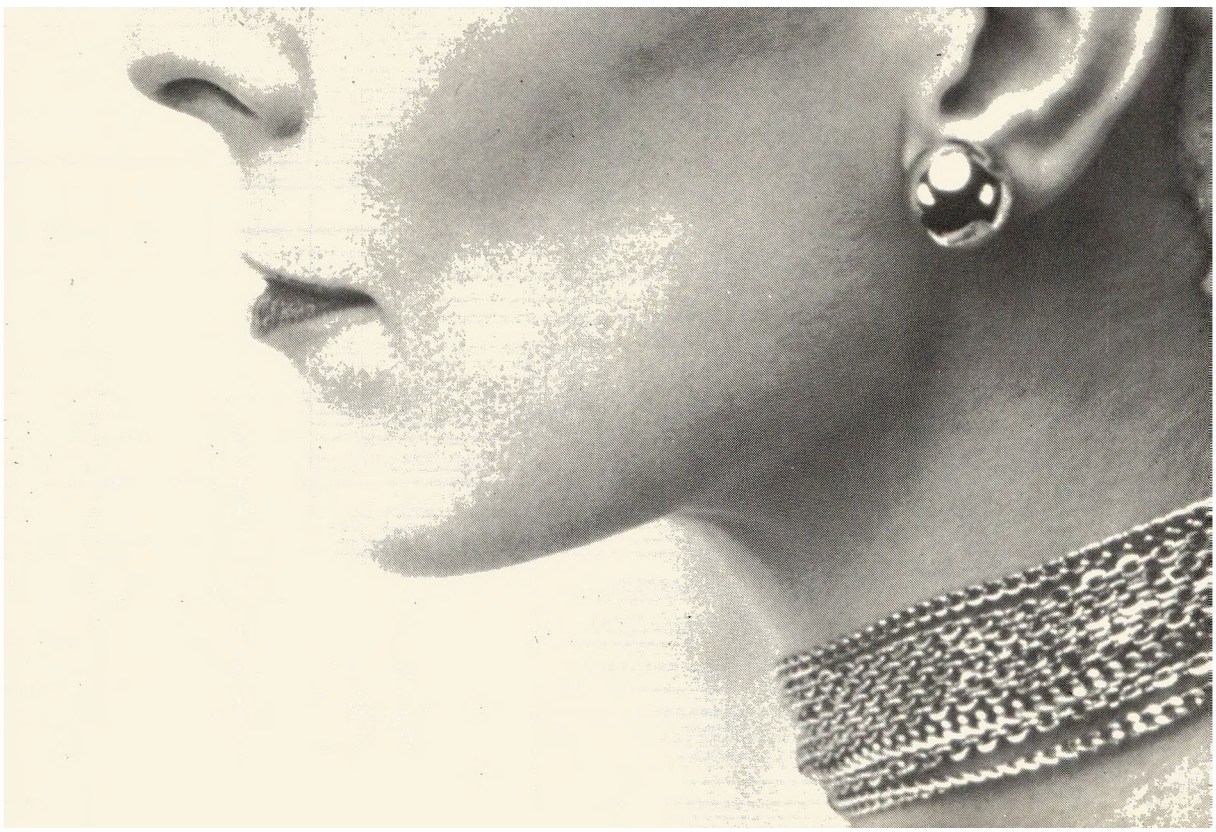

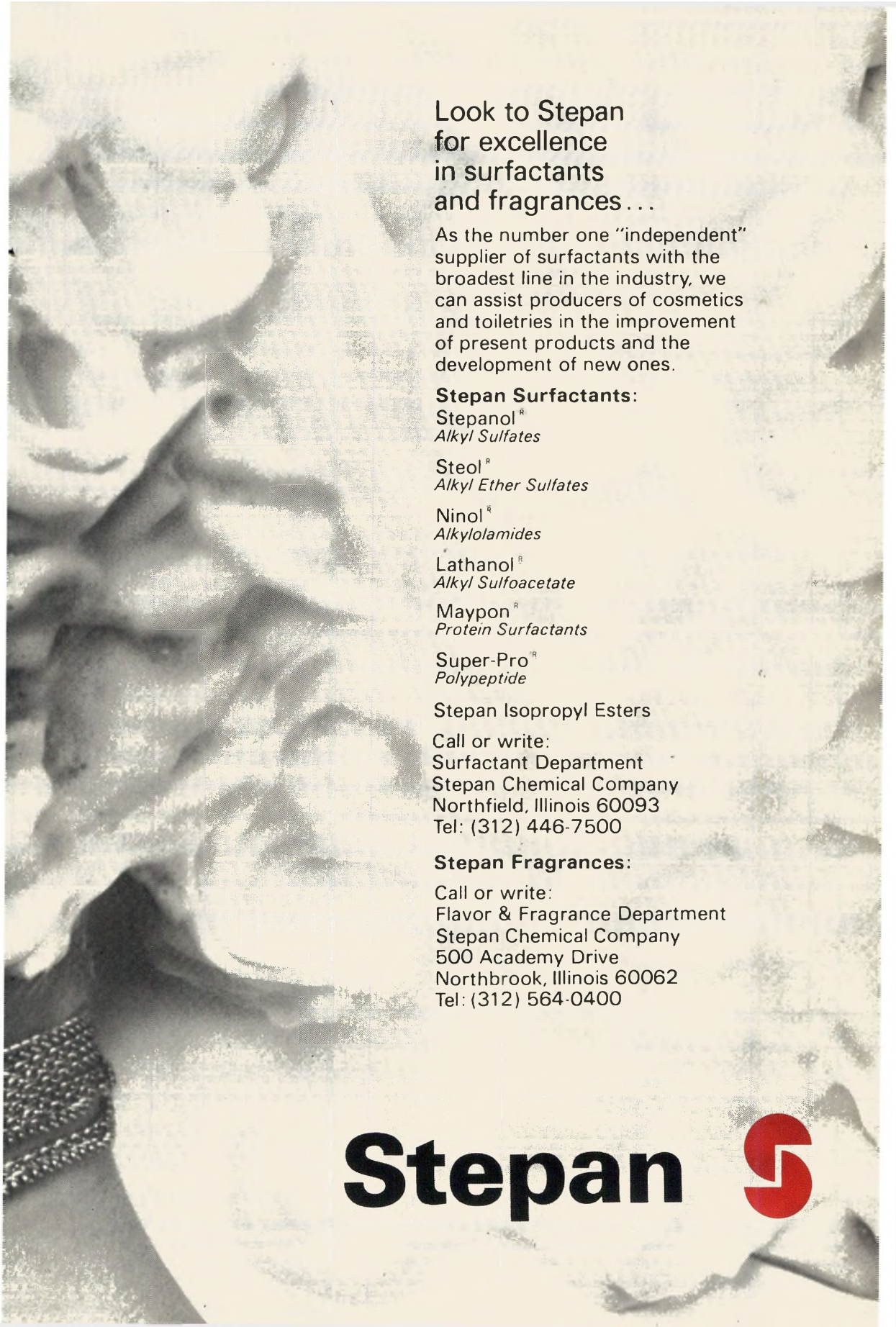

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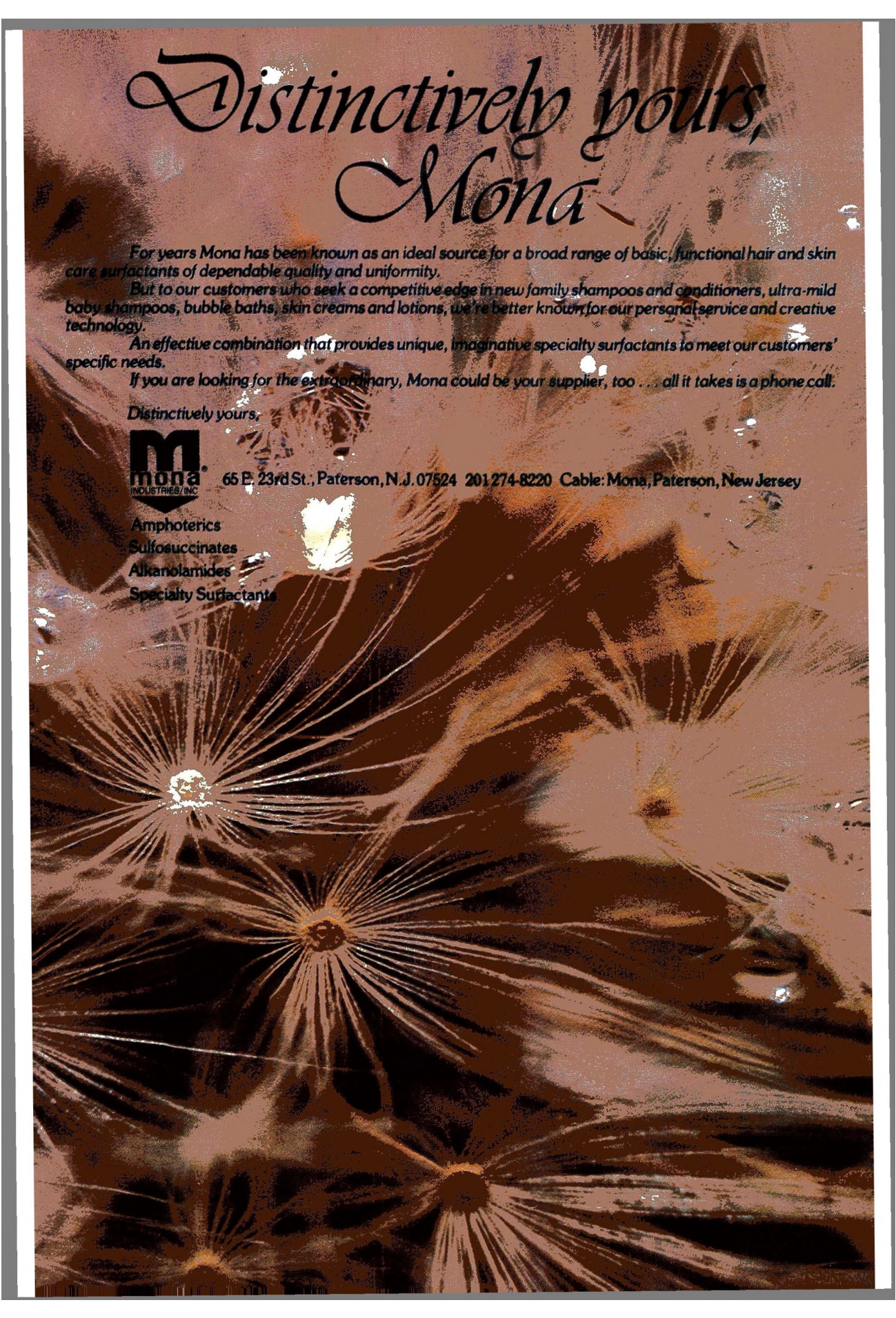
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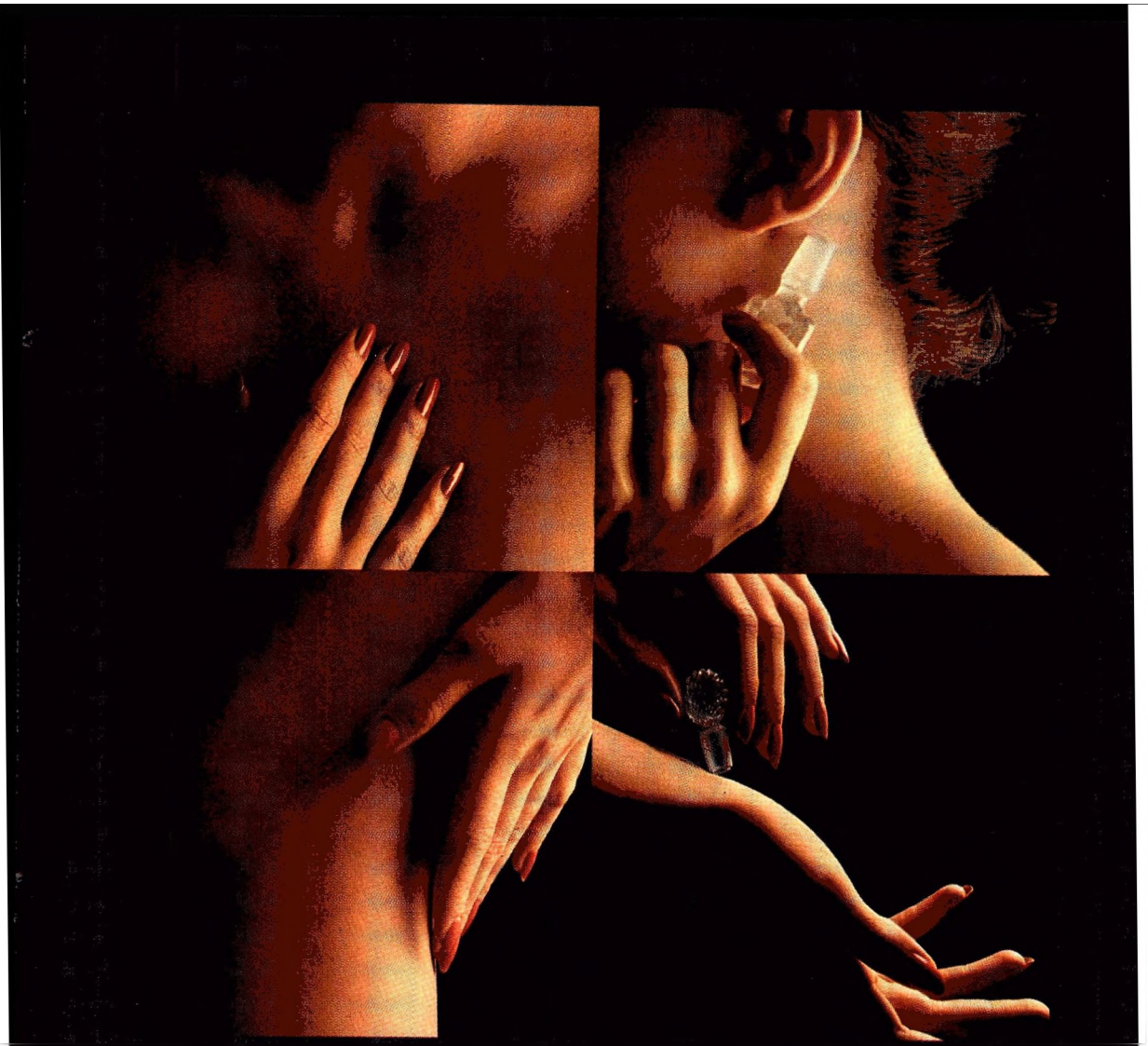

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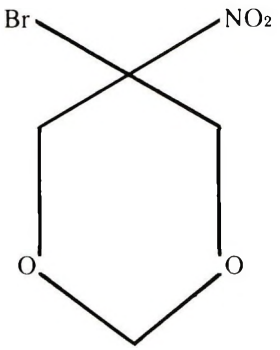

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The following synopses can be cut out and mounted on 3 × 5 index cards for reference, without mutilating the pages of the Journal.

Rheological properties of soap foam: I. apparatus for viscoelastic measurement on foam: H. Komatsu, H. Yamada and S. Fukushima. *Journal of the Society of Cosmetic Chemists* **29**, 237 (May 1978)

Synopsis—An apparatus was devised to measure the rheological properties of toilet soap foam. The principle of the measurement was based on the analysis of oscillation damped by foam.

The measurements by this apparatus were conducted at about 2.5 Hz (cycles/sec) on the soap foams obtained from a 5% (wt/wt) toilet soap aqueous solution at 40°C. The diameter of each bubble remained about 100μ while the specific volume varied widely with varying condition of the preparation.

The storage shear modulus increased from 500 to 850 dynes/cm^2, as the specific volume of foam increased from 10 to 25 cm^3/g. On the other hand, the dynamic viscosity was not much affected by the specific volume and was about 15 to 20 poise. The loss tangent, a parameter expressing energy dissipation, for the foams was calculated to be about 0.3.

The quantitative analysis of bergapten in perfumes: S. T. Zaynoun. *Journal of the Society of Cosmetic Chemists* **29**, 247 (May 1978)

Synopsis—The *Candida albicans* phototoxicity test of Daniels and the spectrophotometric method of Cieri were adapted for the quantitative analysis of bergapten (5-methoxypsoralen) and other photoactive psoralens in solutions such as perfumes. The *Candida albicans* test is simple and reproducible and has important advantages over the spectrophotometric method. Of a total of 108 perfumes investigated 57.4% contained bergapten in concentrations ranging from 0.00004 to 0.01080%. The significance of the presence of bergapten in perfumes and its relationship to skin hyperpigmentation is discussed.

Autoradiographic study on percutaneous absorption of several oils useful for cosmetics: M. Suzuki, K. Asaba, H. Komatsu and M. Mochizuka. *Journal of the Society of Cosmetic Chemists* **29**, 265 (May 1978)

Synopsis—Percutaneous absorption of five ^{14}C-labelled oils, n-octadecane, decanoxy decane, 2-hexyldecanoxy octane, isopropyl myristate and glyceryl tri-(oleate), generally used in cosmetics were studied from the point of view of their safety. In whole body autoradiography with hairless mice, there was no visible penetration into the skin and organs, whereas microautoradiography with guinea pigs showed local penetration. Isopropyl myristate penetrated to the greatest extent, whereas 2-hexyldecanoxy octane was hardly absorbed.

Percutaneous absorption of these two oils, therefore, was examined in Angora rabbits by microautoradiography simultaneously with skin irritation potential by histological method from the following aspects: 1. patterns of penetration and irritation according to application time and 2. fate within the skin and pattern of irritation after application. In addition, intradermal metabolic fate was also studied *in vitro*.

Noninvasive, rapid characterization of human skin chemistry in situ: R. E. Baier. *Journal of the Society of Cosmetic Chemists* **29**, 283 (May 1978)

Synopsis—The experimental yield of internal reflection spectroscopic and contact angle techniques applied to living human skin is demonstrated for natural, cosmetically treated and wounded epidermis. The critical surface tension, and chemical composition, of clean human skin surfaces is provided, along with spectral data bearing on the efficacy and quality of cosmetics, the depth profile of skin moisture, and the chemical nature of exudates from epidermal wounds.

Synthesen in der isocamphanreihe, IX. über den geruchseindruck von isocamphanderivaten: G. Buchbauer and M. Wiltschko. *Journal of the Society of Cosmetic Chemists* 29, 307 (May 1978)

Synopsis—A total of 55 isocamphane derivatives was studied in order to establish a correlation between the shape of the molecule and its odor as postulated by the theory of Amoore. The molecules were measured by use of the shadow matching method and compared with cineole, the camphoraceous odor standard. The agreement of the results of these measurements with other molecular parameters, e. g. boiling point, was also examined. Isocamphane derivatives with spherical shape exhibit a distinct camphoraceous odor, and this characteristic is reduced as the asymmetry of the isocamphane molecule increases.

Interaction of keratinous substrates with sodium lauryl sulfate: I. sorption: J. A. Faucher and E. D. Goddard. *Journal of the Society of Cosmetic Chemists* **29,** 323 (May 1978)

Synopsis—Use was made of radiotagged sodium lauryl sulfate (SLS) to determine its sorption by skin and hair. In the initial stages uptake is linear in square root of time, indicative of a diffusion process. The uptakes determined by radiotagged SLS were successfully correlated with data from a simple gravimetric method and showed that the latter procedure can be used satisfactorily under certain conditions when radiotagged compounds are not available. The influence of some additives on the sorption of SLS was studied. Salt increases the sorption, while nonionic surfactants (which are not themselves sorbed) substantially depress it. Finally, the relation of the sorbed SLS to water of hydration of keratin is examined. It is concluded that most, if not all, the sorbed material is bound to keratin, rather than existing in an "internal" solution.

Interaction of keratinous substrates with sodium lauryl sulfate: II. permeation through stratum corneum: J. A. Faucher and E. D. Goddard. *Journal of the Society of Cosmetic Chemists* **29,** 339 (May 1978)

Synopsis—Neonatal rat stratum corneum was used as a model membrane to investigate permeation through mammalian skin. Passage of materials through these membranes was determined by use of radiotagged compounds and by spectrophotometric analysis. The anionic surfactant sodium lauryl sulfate penetrates the stratum corneum even at low concentrations. The diffusion constant for this process is about 10^{-10} cm^2/sec, compared to 10^{-6} cm^2/sec for free diffusion in water. This surfactant is bound to the skin in large amounts, up to 50% by weight at high concentrations. Pretreatment of the membrane by a cationic cellulose polymer (which is itself strongly sorbed) greatly reduced the amount of surfactant which passed through the membrane.

J. Soc. Cosmet. Chem., 29, 237–246 (May 1978)

Rheological properties of soap foam: I. apparatus for viscoelastic measurement on foam

H. KOMATSU, H. YAMADA and S. FUKUSHIMA *Shiseido Laboratories, 1050 Nippa-cho, Kohoku-ku, Yokohama, Japan 223.*

Received March 31, 1977. Presented at 24th Meeting, Society of Rheology, October 1976, Maebashi, Gunma, Japan.

Synopsis

An apparatus was devised to measure the RHEOLOGICAL PROPERTIES of toilet SOAP FOAM. The principle of the measurement was based on the analysis of oscillation damped by foam.

The MEASUREMENTS by this APPARATUS were conducted at about 2.5 Hz (cycles/sec) on the soap foams obtained from a 5% (wt/wt) toilet soap aqueous solution at 40°C. The diameter of each bubble remained about 100μ while the specific volume varied widely with varying condition of the preparation.

The storage shear modulus increased from 500 to 850 dynes/cm^2, as the specific volume of foam increased from 10 to 25 cm^3/g. On the other hand, the dynamic viscosity was not much affected by the specific volume and was about 15 to 20 poise. The loss tangent, a parameter expressing energy dissipation, for the foams was calculated to be about 0.3.

INTRODUCTION

Foam is one of the dominant factors that determine the commercial value of such cosmetic products as soap, shampoo, shaving foam, cleansing foam, tooth paste, etc. For the purpose of offering those cosmetic products being favorably accepted by the consumer, it is necessary to evaluate various properties of foam such as foaminess, foaming capacity, foam stability and its mechanical properties. Among those properties, the mechanical property has been, to the best of our knowledge, rarely studied (1–4). The review in this area is given by Bikerman (5). The assessment of the mechanical properties of a products' foam has been mainly through sensory evaluation.

For this reason, we attempted in our present work to devise an apparatus to measure the rheological properties of foam. The principle of the measurement is based on the analysis of the oscillation of a coil spring damped by foam. This paper reports the design of the apparatus and the method of analysis, together with some experimental results obtained on toilet soap foam.

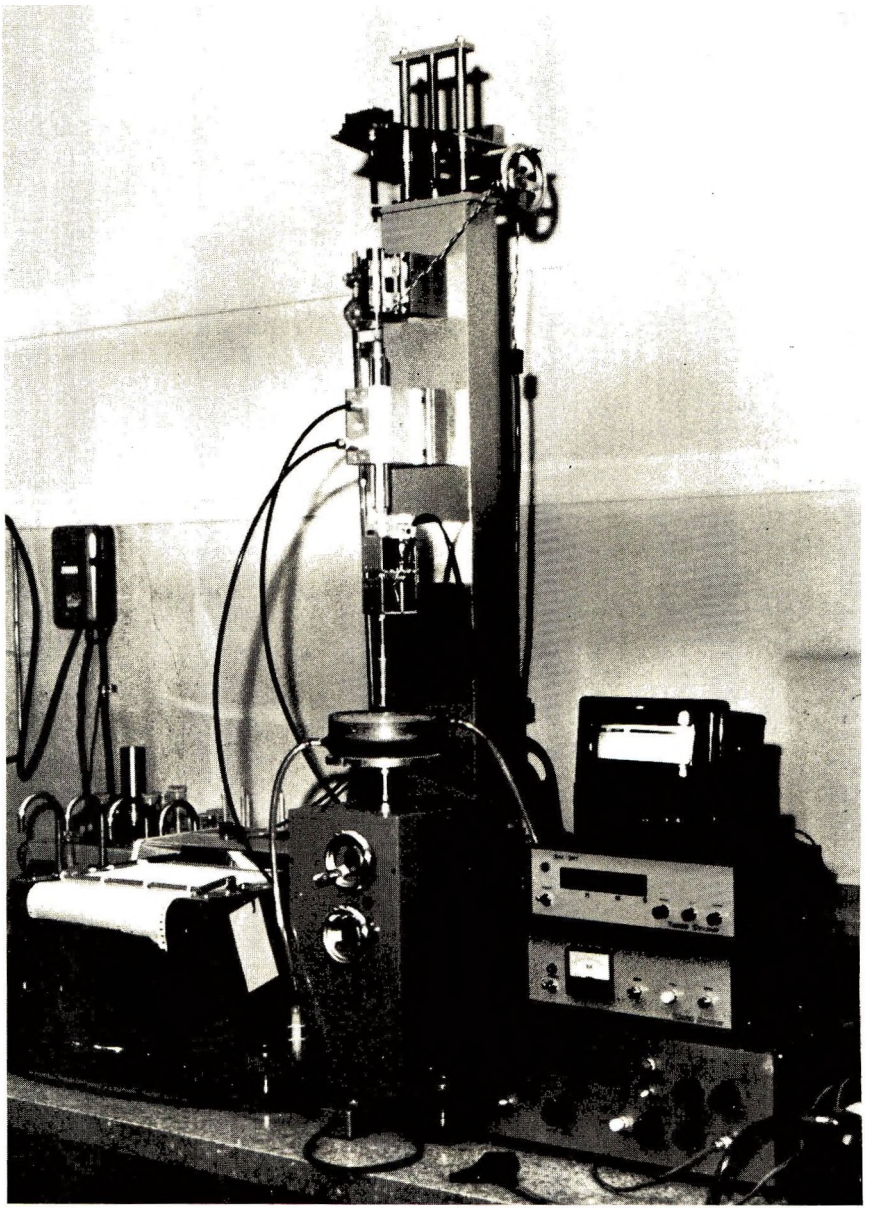

Figure 1. The photograph of the apparatus

DESCRIPTION

APPARATUS

A photograph of the apparatus is shown in Figure 1. Figure 2 shows a schematic drawing of its principle parts. The top end of a coil spring was fixed, and a differential transducer core, an air bearing core, a solenoid core, a weight vessel and a disk were suspended in that order to the lower end.

A foam sample placed in a measuring vessel was leveled up so as to come into contact

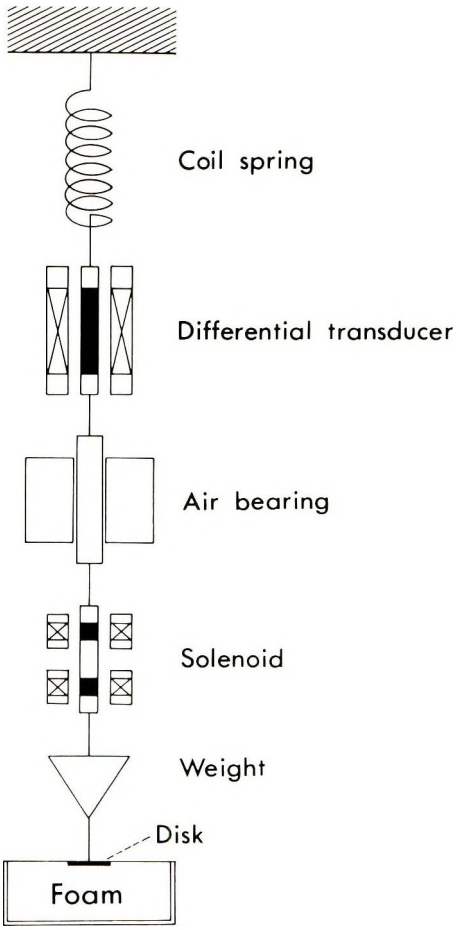

Figure 2. Schematic drawings of principal parts of the apparatus

with the disk (12 mm in diameter, 1 mm in thickness). A free oscillation of a given amplitude and frequency was applied to the sample with the aid of the solenoid. The oscillation damped by the sample was changed into an electrical signal. The signal taken from the differential transducer was passed to a transducer meter and, after amplification and filtration, was recorded by a recorder. At the same time, a period of the oscillation was measured by a digital period meter. The air bearing was used to prevent the eccentric motion of the oscillating system's axis.

An example of the oscillation damped by foam is shown in Figure 3 together with a control-free oscillation. The top pattern of the figure is without foam and the bottom pattern is with foam. The decrement of the oscillation was apparent with foam, however the oscillation was damped slightly even without foam. The decrement of the free oscillation, possibly caused by the viscosity of air, was taken into account when calculating the true decrement of the oscillation damped by foam.

THE DEVICE FOR SAMPLE PREPARATION

A commercial foam-generator (John Oster Manufacturing Co., Latherservice Machine®) was adapted by improving the following defects. First, we were unable to main-

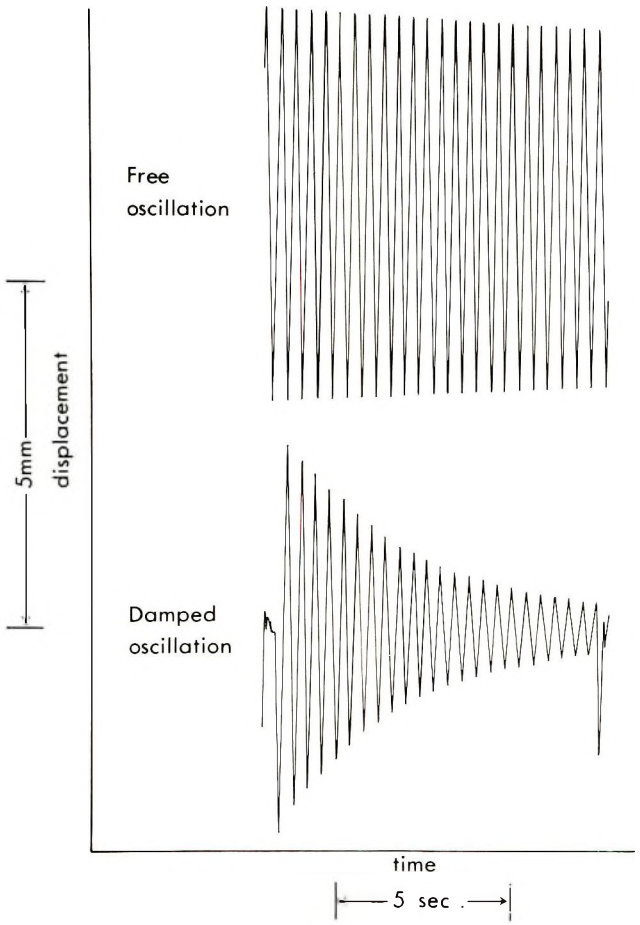

Figure 3. An example of the oscillation damped by foam, with control-free oscillation

tain the solution at a constant temperature; by the circulation of water at a constant temperature through a spiral glass tube inserted in the solution, we were able to solve this problem. Second, the machine motor's rotational frequency was hardly stabilized because of power insufficiency; the motor was replaced by a powerful one. Third, the flow rate of a solution to the foam-generating part of the machine was variable; using a tubing pump, a solution at a constant flow rate was injected.

A 5% (w/w) toilet soap aqueous solution was foamed by the use of the improved machine. Foam was taken into a measuring vessel that was then quickly transferred to the apparatus. The diameter of each bubble remained about 100μ whereas the specific volume of foams varied much with varying condition of the preparation. Water with a hardness of 70 ppm (as $CaCO_3$) was prepared for use in our present work by dissolving calcium chloride in deionized water.

THEORETICAL

A mechanical model for the measuring system was assumed, as shown in Figure 4. The equation of motion for the model is given by

$$M\ddot{x} + R\dot{x} + (K + k)x = 0 \qquad [1]$$

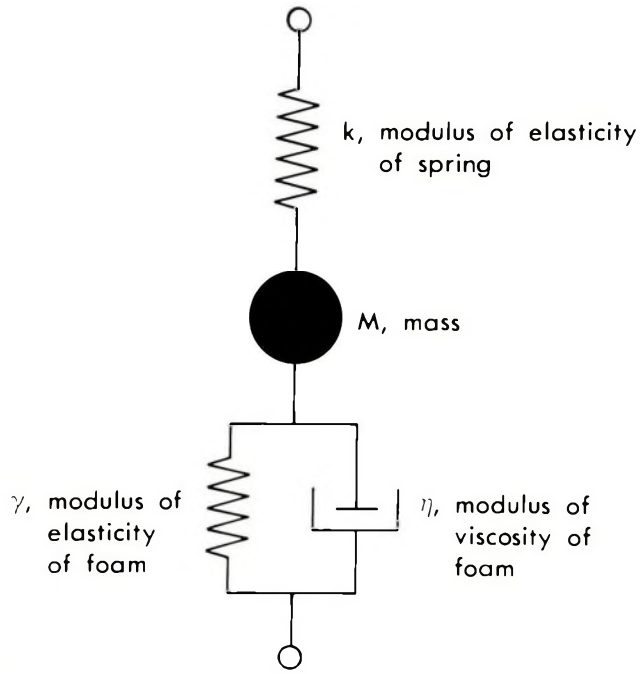

Figure 4. Mechanical model corresponding to the measurement by the apparatus

where M is the mass of mechanical system, k is the modulus of elasticity of the coil spring, K is the elastance of foam, R is its resistance and x is an axial displacement. There are three cases of the solution for eq [1].

$$\text{(a) } R/2M > \{(K + k)/M\}^{1/2}$$
$$x = Ae^{-\alpha_1 t} + Be^{-\alpha_2 t} \qquad [2]$$

$$\text{(b) } R/2M = \{(K + k)/M\}^{1/2}$$
$$x = e^{-Rt/2M}(Ct + D) \qquad [3]$$

$$\text{(c) } R/2M < \{(K + k)/M\}^{1/2}$$
$$x = Ee^{-Rt/2M} \sin\{t\sqrt{(K + k)/M - R^2/4M^2} + \varphi\} \qquad [4]$$

where α_1, α_2, A, B, C, D and E are positive constants, t is time and φ is phase difference.

A damped sinusoidal motion can be defined; both the period of the oscillation T_d and the logarithmic decrement δ_T are provided. T_d and δ_T are defined as eqs [5] and [6], respectively.

$$T_d = 2\pi/\{(K + k)/M - R^2/4M^2\} \qquad [5]$$

$$\delta_T = RT_d/2M \qquad [6]$$

From eqs [5] and [6], therefore, the elastance K and the resistance R of foam can be obtained experimentally. Provided the shape factor of the apparatus is represented by S, the modulus of elasticity γ and the modulus of viscosity η of foam can be written as

$$\begin{aligned}\gamma &= SK \\ &= SM\{4\pi^2(1/T_d^2 - 1/T^2) + \delta_T^2/T_d^2\} \\ &= Sk\{(1 + \delta_T^2/4\pi^2)T^2/T_d^2 - 1\}\end{aligned} \qquad [7]$$

$$\begin{aligned}\eta &= SR \\ &= SM\,(2\delta_T/T_d) \\ &= Sk\,(\delta_T/2\pi^2)\,(T^2/T_d)\end{aligned} \qquad [8]$$

where T is the period of free oscillation and is given by

$$T = 2\pi\sqrt{M/k} \qquad [9]$$

M and k were determined from eq [9] by measuring T at various weights. The shape factor S was calculated from eq [8] by using the experimental values obtained on a standard viscosity sample (Showa Petroleum Co., JS 2000).

We should point out that such an indirect determination of S involves the risk of allowing an error in measurement. The reason for having to adopt the indirect way to determine S can be summarized as follows. A large volume of foam sample was required for the measurement in order to satisfy the condition of the flow between parallel plates under which S is theoretically obtained, since foam is generally a coarse disperse system. On the other hand, if such a large volume of foam is used in measurement, it is unavoidable that the foam becomes more heterogeneous in film thickness because of drainage in the film. This problem is expected to be solved by devising an entirely new type foam-generator providing a homogeneous foam to a measuring vessel where the condition of the flow between parallel plates is taken into consideration; it is an important subject for further study.

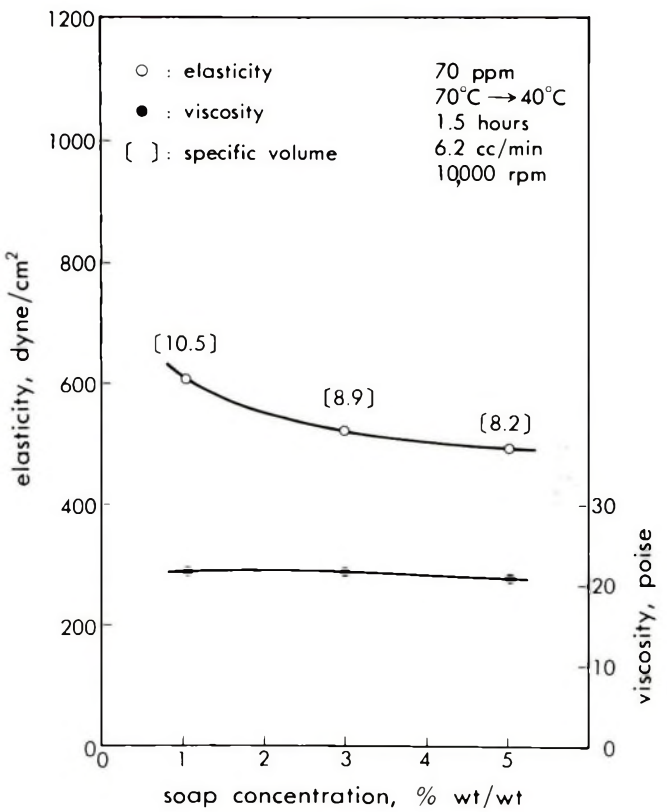

Figure 5. Dependence of the viscoelasticity of the foam on the toilet soap concentration. The specific volume of the foam sample is designated in brackets

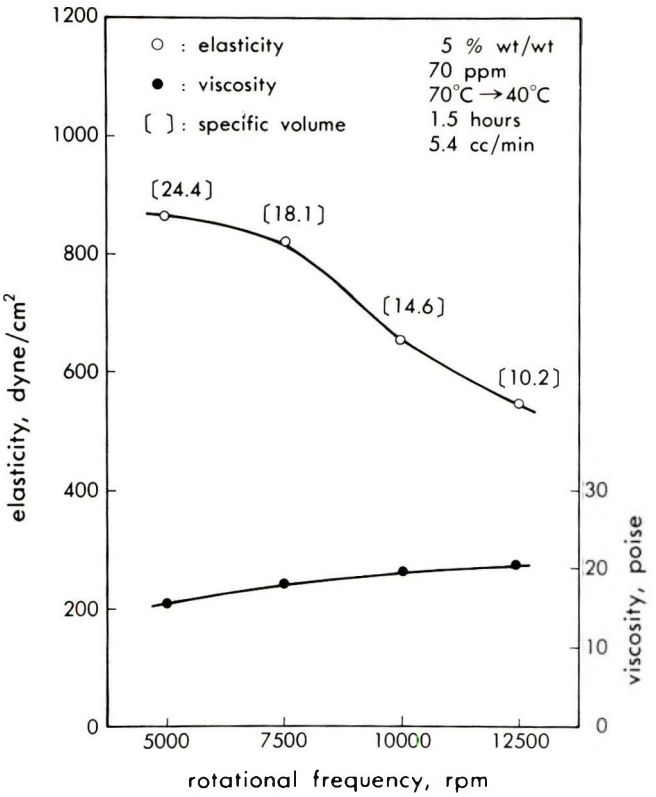

Figure 6. Effect of rotational frequency of the foam-generator on the viscoelasticity of the soap foam obtained from a 5% (wt/wt) toilet soap aqueous solution. The specific volume of the foam sample is designated in brackets

APPLICATION OF THE DEVELOPED SYSTEM TO THE MEASUREMENT ON THE FOAM FROM TOILET SOAP

A commercial toilet soap was used. A toilet soap aqueous solution was prepared by dissolving the toilet soap at 70°C in the artificial tap water described above. After dissolution, the solution was cooled to 40°C in a bath containing iced water. The solution was then allowed to stand for 1.5 hr at 40°C in the foam-generator before testing. Measurements were conducted at about 2.5 Hz on the foam at 40°C.

The dependence of the viscoelastic moduli of foam on soap concentration is shown in Figure 5. The foams were prepared under such condition that a flow rate of solution to the foam-generator is 6.2 cm^3/min and a rotational frequency is 10,000 rpm. A specific volume (cm^3/g) of a foam sample is designated in brackets. The elasticity decreased with increasing soap concentration, whereas the viscosity was not much affected by the soap concentration.

In Figures 6 and 7, the influence of a foaming condition on the viscoelastic moduli of the foam is shown. The soap concentration was 5% (wt/wt). The effect of a rotational frequency on the viscoelastic moduli of the foam is shown in Figure 6. The elasticity decreased from about 850 to 500 dynes/cm^2 as the rotational frequency increased, while the viscosity increased slightly. The effect of a flow rate of solution on the viscoelastic moduli of the foam is shown in Figure 7. The elasticity increased from about

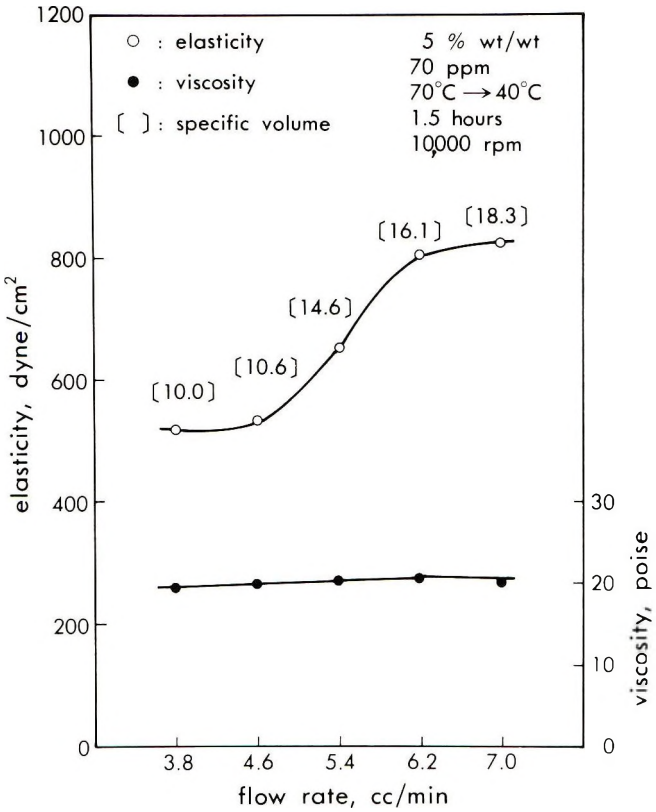

Figure 7. Effect of flow rate of the soap solution (to the foam-generator) on the viscoelasticity of the soap foam obtained from a 5% (wt/wt) toilet soap aqueous solution. The specific volume of the foam is designated in brackets

500 to 800 dynes/cm^2 with increasing flow rate, while the viscocity scarcely depended on the flow rate.

On the other hand, a close relationship is probable between elasticity of foam and its specific volume, as is evident from Figures 5, 6 and 7.

The comparison of the viscoelastic moduli of the foam prepared by using deionized water with those of the foam prepared by using the aforementioned water is shown in Figure 8. The elasticity of the foam was much larger with the deionized water than that with the artificial tap water.

Figure 9 shows the influence of a soap composition on the viscoelastic moduli of the foam. The elasticity of the foam prepared from soap manufactured by mixing coconut oil with tallow in the ratio of 20 to 80 was larger than that of the foam from soap in which the ratio of coconut oil to tallow was 15 to 85.

CONCLUSION

The storage shear modulus increased from about 500 to 850 dynes/cm^2 as the specific volume of the foam increased from about 10 to 25 cm^3/g. On the other hand, the dy-

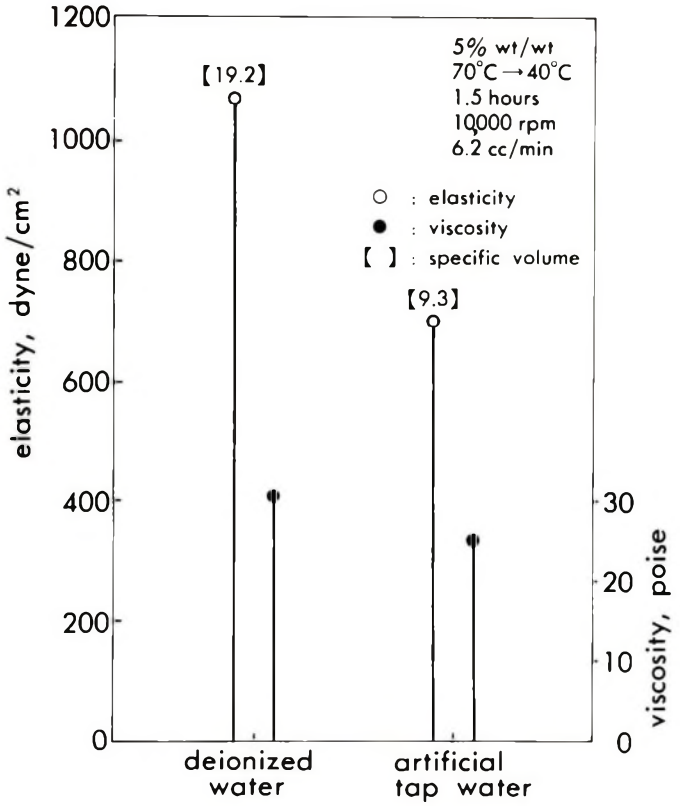

Figure 8. Influence of a hardness of water on the viscoelasticity of the foam from toilet soap. The hardness of the water was 70 ppm (as $CaCO_3$)

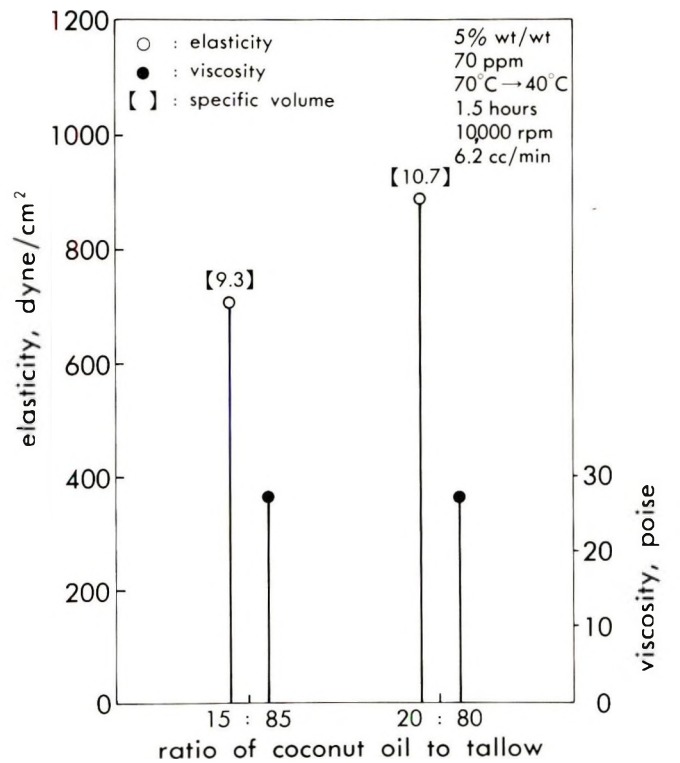

Figure 9. Influence of soap composition on the viscoelasticity of the foam from toilet soap

namic viscosity was not much affected by the specific volume and was approximately 15 to 20 poise. The loss tangent for the foams, a parameter expressing energy dissipation, was calculated to be about 0.3. The interrelation between the viscoelasticity of the foam and the specific volume suggests that the viscoelasticity of soap foam is dependent on the diameter of bubble and film thickness.

ACKNOWLEDGMENT

We would like to acknowledge the continuing guidance and encouragement of Drs. S. Ohta, Director, and T. Mitsui, Associate Director of Shiseido Laboratories, throughout the course of this work.

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The quantitative analysis of bergapten in perfumes

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Synopsis

The *Candida albicans* phototoxicity test of Daniels and the spectrophotometric method of Cieri were adapted for the QUANTITATIVE ANALYSIS of BERGAPTEN (5-methoxypsoralen) and other photoactive psoralens in solutions such as PERFUMES. The *Candida albicans* test is simple and reproducible and has important advantages over the spectrophotometric method. Of a total of 108 perfumes investigated 57.4% contained bergapten in concentrations ranging from 0.00004 to 0.01080%. The significance of the presence of bergapten in perfumes and its relationship to skin hyperpigmentation is discussed.

INTRODUCTION

In a previous study, Zaynoun, Johnson and Frain-Bell (1) have shown that a number of bergamot-containing perfumes produced erythema and subsequent hyperpigmentation following application to normal human skin and irradiation with long wave ultraviolet light (UVA). The bergamot response is due to a phototoxic reaction induced by bergapten or 5-methoxypsoralen (5-MOP), the only significant photoactive component of bergamot oil (2,3). Positive responses to perfumes and small concentrations of bergamot oil were reported in patients with Poikiloderma of Civatte and a possible relationship between the hyperpigmentation on the sides of face and neck and the use of perfumes containing significant quantities of bergamot oil was suggested (1).

As occurs with other psoralens, the response to bergapten is related to several factors, primarily the quantity applied and the dose of UVA delivered to the skin (4). Hence, it is considered important to determine the presence and concentration of 5-MOP in perfumes and advise against the use of those containing significant quantities of 5-MOP, particularly in individuals who have a tendency to develop a phototoxic response to small concentrations of the psoralen. In this paper, two methods for the quantitation of 5-MOP in perfumes are described and the 5-MOP content of 108 perfumes reported.

MATERIALS AND METHODS

Oil of bergamot (Coty), pure 5-MOP (Unilever Research Laboratories) and pure xanthotoxin or 8-methoxypsoralen (8-MOP) (Sigma Chemical Company) were ob-

tained. The 5-MOP content of this sample of bergamot oil was previously determined with the use of a spectrophotometric technique and was found to be 0.27 g/100 ml (2). Random samples of perfumes were supplied by local stores during the years 1974 to 1976.

Two methods for the quantitative analysis of 5-MOP in perfumes were used: the Candida albicans phototoxicity test (5) and the spectrophotometric test (6).

THE CANDIDA ALBICANS PHOTOTOXICITY TEST

The test consists of the application of test substances to Sabouraud's dextrose agar plates[1] (2.5 to 3 mm uniform agar thickness) streaked with *Candida albicans* and subsequent exposure to UVA; duplicate plates were kept in the dark as control. Phototoxic substances are then identified by the presence of a clear zone of killing of the yeast around the discs in the irradiated plate greater than that obtained in the control nonirradiated plate.

DOSE-RESPONSE RELATIONSHIP

It was first of all important to find out whether the test was useful for the quantitative assay of 5-MOP and other psoralens. An attempt was made, therefore, to derive dose-response curves which relate the concentration of the psoralen used to the diameter of the killing zone. Various concentrations of the bergamont oil, pure 5-MOP and pure 8-MOP in ethanol were prepared. Aliquots of 2 μl of each of these solutions were spotted on 20 × 20 cm aluminium-backed, thin-layer chromatography silica gel plates, thickness 0.25 mm (E. Merck). The plates were processed by the ascending method with hexane-ethyl acetate (75:25) to a distance of 8 cm from the origin, allowed to dry and viewed under ultraviolet light (Camag ultraviolet viewer, peak 366 nm). The fluorescent 5-MOP band in bergamot oil was identified by its yellowish–green fluorescence and its Rf, which was identical to that of standard 5-MOP. The 5-MOP fractions of bergamot oil spots were marked and the silica gel containing them was scraped onto clean paper and added to Sabouraud's dextrose agar plates freshly seeded with *C. albicans*; each spot occupying a circle 8 to 10 mm in diameter. Since the size of the killing zone varies with the thickness of the agar (5,7) and possibly with other factors as well, it was considered important when comparing the phototoxic effects of psoralens to apply similar quantities of each compound to the same agar plate. In these experiments, therefore, concentrations were chosen so that a similar range of quantities of 5-MOP derived by TLC from bergamot oil, pure 5-MOP and 8-MOP were applied to each agar plate (Table I). For example, 0.00025 to 0.001% solutions of pure 5-MOP and 8-MOP and the equivalent of 0.00034 to 0.00135% 5-MOP present in 0.125 to 0.5% bergamot oil (which contains 0.27% 5-MOP) were used in plate 1. Similarly, 0.0025 and 0.005% pure 5-MOP and 8-MOP and 0.0027 and 0.0075% 5-MOP in bergamot oil were applied to plate 2. Concentrations of 40% bergamot oil and 0.1% pure 5-MOP and 8-MOP were applied to two separate plates because of the large killing zones produced. One set of plates was kept in the dark and another set,

[1]Myocological peptone (Oxoid L 40) 10 g, Agar No. 1 (Oxoid L 11) 15 g, Dextrose 40 g/litre; pH approximately 5.6.

Table I
The Concentration of Bergamot Oil (Coty), 5-MOP and 8-MOP Applied to Each Sabouraud's Dextrose Agar Plate

	Bergamot Oil ml/100 ml	5-MOP g/100 ml	8-MOP g/100 ml
Plate No. 1	0.125	0.00025	0.00025
	0.25	0.0005	0.0005
	0.5	0.001	0.001
2	1	0.0025	0.0025
	2.5	0.005	0.005
3	5	0.01	0.01
4	10	0.025	0.025
5	20	0.05	0.05
6	40	0.1	—
7	—	—	0.1

with the plate covers on, was irradiated for two days with UVA from two fluorescent tubes (Philips Blacklight TW 20 W/08, wave-length range 300-355-400nm), placed 7 cm apart and 20 cm above the plates. The experiment was repeated six times and the diameter of the killing zone was measured, the minimum measurable zone being the diameter of the silica gel spot.

In comparing the efficacy of various photoactive psoralens, using this test, it was necessary to ensure that any difference in phototoxic effects based on the size of the killing zone is not due to a relative diffusion of the psoralens in the agar layer. A turbidimetric test using Sabouraud's liquid medium was devised.[2] In this test, 3 ml of the medium were delivered to 35 × 10 mm sterile tissue culture petri dishes (Flow Laboratories). The inoculom consisted of a suspension of *C. albicans* prepared by transferring 2 to 3 dabs from a two-day slant culture on a sterile cotton swab to 6 ml sterile water, to obtain an optical density of the suspension of approximately 1.2 at 600 nm. A volume of 0.1 ml was added to each of the petri dishes and a total of 20 dishes were prepared. Fresh solutions of 0.005% 5-MOP and 8-MOP in ethanol (w/v) were made and 0.1 ml of each added separately to each culture followed by gentle swirling to mix the contents. Five petri dishes containing 5-MOP and another five containing 8-MOP were exposed for 24 hr to UVA as previously described. Duplicates were covered with light-excluding material as controls. With the use of a glass rod, the contents of each culture plate were then mixed and any yeast growth on the walls of the petri dish brought into suspension. The optical density was then recorded at 600 nm.

THE 5-MOP CONTENT OF PERFUMES

As the presence of 5-MOP in perfumes is a result of the addition of 5-MOP containing bergamot oil, it was considered important in the assessment of 5-MOP concentration in perfumes to use bergamot oil of known concentration of 5-MOP as reference instead of pure 5-MOP. Aliquots of 5 μl of the perfumes and 5 μl of each 0.25, 0.5 and 1% bergamot oil Coty (contains 0.27 g/100 ml 5-MOP) in ethanol were

[2]Pancreatic digest of casein (Oxoid L 42) 5 g, Peptic digest of fresh meat (Oxoid L 49) 5 g, Dextrose 20 g per litre; pH approximately 5.7.

Table II
The Relationship of the Volume of Bergamot Oil (Coty) and Perfume Used in Each Agar Plate to the Concentration of Bergamot Oil Present in the Perfume

	Concentration of Bergamot Oil ml/100 ml	Volume of Bergamot Oil and Perfumes Applied (μl)
Plate No. 1	1	
	0.5	5
	0.25	
2	0.125	
	0.062	25
3	0.031	
	0.016	100

chromatogrammed as before. The silica gel containing the 5-MOP fractions was scraped from the TLC sheet and added in duplicates to agar plates innoculated with *C. albicans*. One plate was irradiated as before and the control was kept in the dark. The diameters of the killing zones were compared and the concentration of bergamot oil producing an inhibition zone equal to that of the perfume was recorded as the concentration of bergamot oil present in that perfume. When the zone of inhibition was greater than that of 1% bergamot oil, dilutions of the perfume in ethanol were prepared and the procedure repeated as above. On the other hand, when a zone smaller than that produced by 0.25% bergamot oil was obtained, 25 μl or more of the perfume and 0.016, 0.031, 0.062 and 0.125% bergamot oil were used. A rough guide to the amount and concentration of bergamot oil applied to each plate is shown in Table II. For a more precise measurement of bergamot oil concentrations in some perfumes, further dilutions of bergamot oil such as 0.75, 1.5 and 2.5% were also used.

The concentration of 5-MOP in each perfume was then calculated as follows:

$$\text{Concentration of 5-MOP (w/v)} = \text{Concentration of bergamot oil} \times \frac{0.27}{100}$$

RECOVERY EXPERIMENTS

The concentration of 5-MOP in Agua Brava aftershave and Paco Rabanne toilet water (for males), Caprice Cologne and Wind Song toilet water (for females) was determined as outlined above. A volume of 0.25 ml of 10% begamot oil (Coty) was mixed with 4.75 ml of each perfume, to give an additional concentration of 0.00135% and the 5-MOP content of the resultant solutions of perfumes was reassessed. In addition, the absorption spectrum of the bergapten TLC fraction of the prepared solutions was recorded and compared with that of the bergapten TLC fraction of bergamot oil.

THE IMPORTANCE OF TLC IN THE QUANTITATIVE ESTIMATIONS OF 5-MOP

In the original phototoxicity test (Daniels, 1965), the substances were applied to seeded agar plates as pure crystals, fresh plant materials or as dried concentrates in filter paper discs. The value of TLC in this study was therefore assessed by comparing the phototoxic effects of bergapten TLC fraction of perfumes with those of the whole

perfume applied to filter paper discs and also to silica gel. Yves Saint Laurent (YSL) toilet water (for males) was chosen as an example. Aliquots of 5 μl were chromatogrammed as before and a similar volume was added to 10 mm Whatman No. 1 filter disc and also to TLC plate. A total of ten agar plates were inoculated with *C. albicans* and to each plate a 10 mm disc, silica gel containing bergapten TLC fraction and silica gel containing the whole perfume (not chromatogrammed), was added. Five plates were exposed to UVA and the rest were kept in the dark. The killing zones were measured and the results subjected to statistical analysis. In addition to these experiments, all perfumes were tested for direct toxicity to the yeast by pipetting 25 μl of each perfume to 10 mm filter paper discs and dried under a stream of warm air from a hair dryer. The discs were added to seeded agar plates which were then grown in the dark. Any inhibition of the yeast growth around the discs was taken as a sign of toxicity. The toxic effects of each perfume were later compared with its phototoxic effects by growing one plate under UVA illumination and a duplicate in the dark.

THE SPECTROPHOTOMETRIC TEST

The technique used is a modification of the method described by Cieri (6) and later used for the estimation of the 5-MOP content of bergamot oil (2). In this technique, sixteen 25 μl aliquots of each perfume and a similar number of 25 μl aliquots of 1% bergamot oil in ethanol along with 25 μl of 0.05% pure 5-MOP in ethanol used as reference were chromatogrammed as previously described. The silica gel containing the 5-MOP fractions was scraped from the plates onto clean paper. All 5-MOP fractions of bergamot oil were transferred to a glass-stoppered tube and those of the perfume combined in another tube. 5-MOP was then extracted in 10 ml chloroform, centrifuged at 2000 rpm for 5 min and the absorption spectrum of the clear supernate obtained using an SP 8000 recording spectrophotometer (Unicam) with chloroform in the reference cell. The absorption at 310 nm was recorded and the concentration of bergamot oil and 5-MOP in each perfume was calculated as follows:

1. Percentage bergamot oil (v/v) = perfume absorption/bergamot oil absorption

where perfume absorption = absorption of supernate containing bergapten TLC fraction of perfume, and bergamot oil absorption = absorption of supernate containing bergapten TLC fraction of bergamot oil.

2. Percentage 5-MOP (w/v) = Percentage bergamot oil $\times \frac{0.27}{100}$

RESULTS

THE CANDIDA ALBICANS PHOTOTOXICITY TEST

TLC Patterns. On TLC, bergamot oil showed five main fluorescent bands (Figure 1). Band 1, 2 and 3 are known as bergamottin, 7-geranoxycoumarin and citropene respectively, while band 4, which has a yellowish–green fluorescence, is 5-MOP (2,6). The TLC pattern of perfumes containing bergamot oil was variable and for the sake of simplicity these perfumes were divided into two groups. Group A (*e.g.*, YSL toilet water) had five and sometimes six main fluorescent bands similar to those of bergamot oil

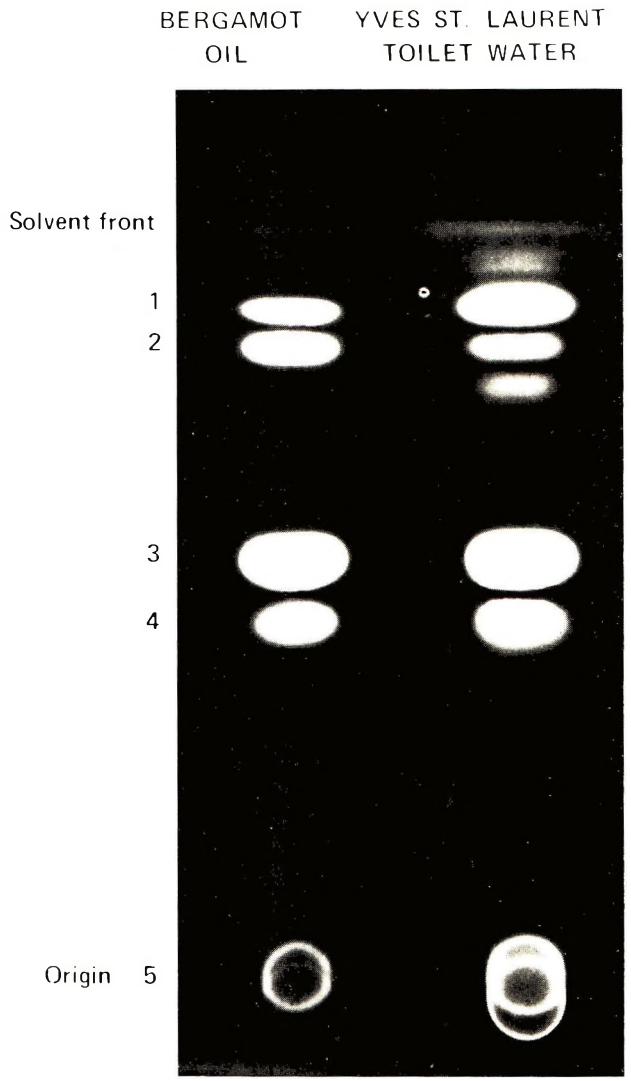

Figure 1. Thin layer chromatogram showing 1% bergamot oil in ethanol and Yves Saint Laurent toilet water as observed under UVA illumination. The perfume shows six main fractions, five of which are similar to those of bergamot oil. Band 4 is 5-MOP

(Figure 1). The bergapten TLC band of these perfumes was adequately separated from the adjacent TLC bands and had a yellowish–green fluorescence similar to that of the 5-MOP fraction of bergamot oil. However, the fluorescence of the 5-MOP band of this group of perfumes and of bergamot oil was not as bright and clear as that of pure 5-MOP. Perfumes belonging to Group B (*e.g.*, Brut aftershave), which formed the larger portion of the perfumes tested, had more than five to six main fractions and the 5-MOP band of most of these perfumes did not show a good separation from the adjacent bands and had a dull yellowish fluorescence indicating the presence of impurities. This was further confirmed by the absorption spectrum which was different from that of pure 5-MOP (Figure 2). Steam distillation, which was used in Cieri's method, was carried out to separate the steam-volatile portion of the perfume and

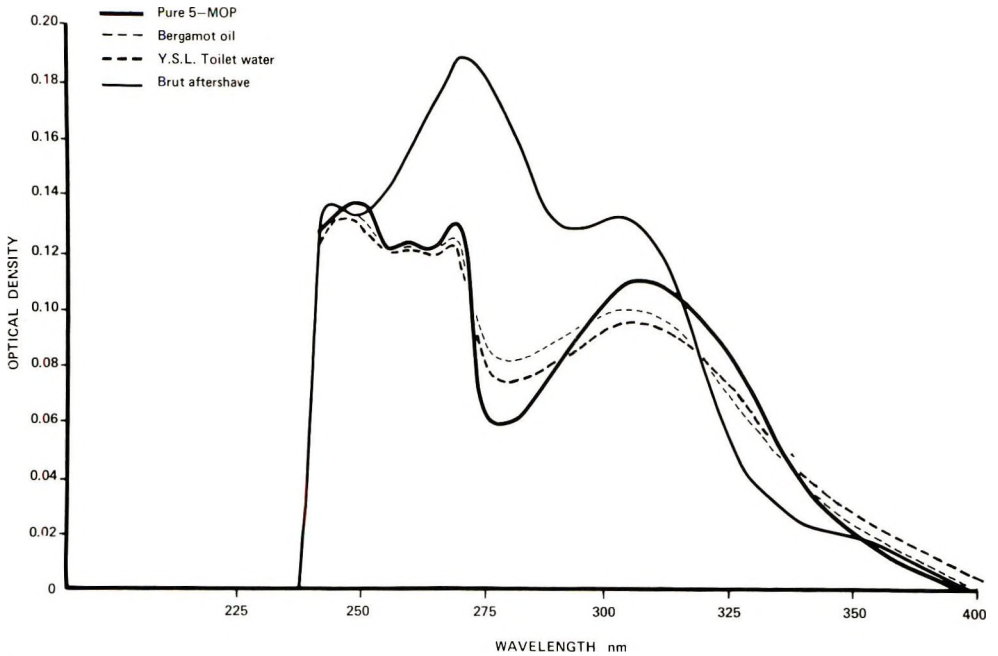

Figure 2. The ultraviolet absorption spectrum of the bergapten TLC fraction of bergamot oil, Yves Saint Laurent toilet water and Brut aftershave lotion compared with that of pure 5-MOP. The absorption spectra of bergamot oil and the toilet water are similar and resemble that of pure 5-MOP, whereas the absorption spectrum of Brut aftershave is different. The apparent lack of absorption characteristics below 240 nm is due to absorption of these wavelengths by chloroform itself. It should be noted that the absorption scale is from 0 to 0.20 rather than the usual 0 to 1.00

bergamot oil but did not significantly alter the absorption spectrum of the 5-MOP fraction.

Dose-Response Curves: Bergamot Oil. Using 2 μl aliquots and TLC, the minimal effective concentration of bergamot oil on the yeast was 0.25% (0.005 μl). Normal growth was obtained in all control plates. The dose-response curve showed a sharp rise with the smaller concentrations but tended to flatten out with higher concentrations (Figure 3). The results of 20 and 40% concentrations were not significantly different at 5% level when the Student's "t" was applied for comparing adjacent means. A significant difference, however, was obtained between the other adjacent means, the level of significance increasing when the means on the steeper part of the curve were compared. For example, the results of 10 and 20% concentrations were different at 5% level: 5 and 10% at 1%; 2.5 and 5% at 0.1% level.

Dose-Response Curves: 5-MOP. The dose-response curve of pure 5-MOP showed a pattern similar to that of bergamot oil (Figure 4). The concentrations of pure 5-MOP used for deriving the dose-response curve were 0.00025, 0.0005, 0.001, 0.0025, 0.005, 0.01, 0.025 and 0.1%. The concentrations of bergamot oil containing an equivalent amount of 5-MOP would therefore be 0.09, 0.19, 0.37, 0.93, 1.85, 3.71, 9.26, 18.52 and 37.04% (v/v) respectively. When the diameters of the killing zones produced by the various dilutions of pure 5-MOP shown in Figure 4 were plotted against the calculated concentrations of bergamot oil, a curve similar to that of bergamot oil was ob-

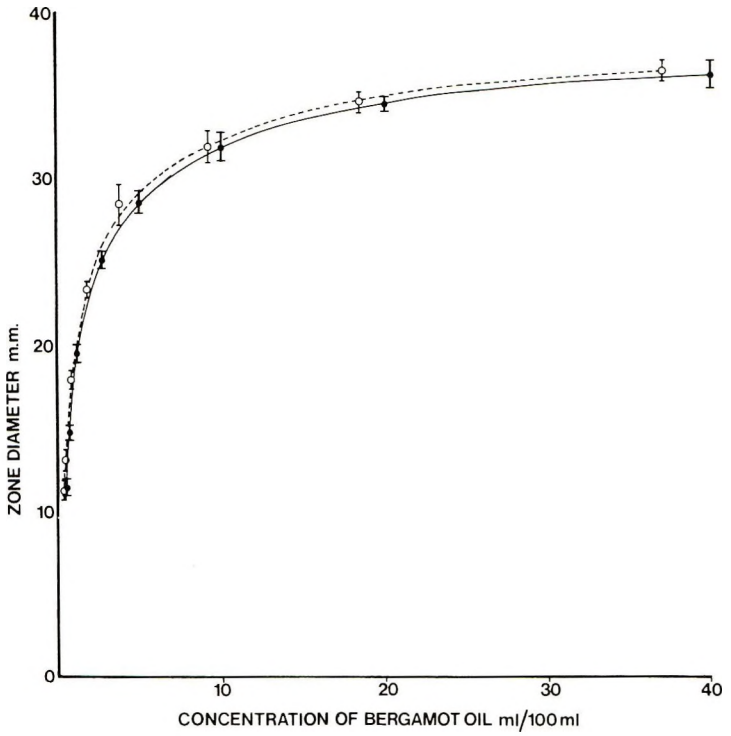

Figure 3. The effect of increasing concentrations of bergamot oil (Coty) on the diameter of the killing zone of the yeast, presented as the mean ± S.E.M. A sharp increase in the zone diameter is noted with the smaller concentrations, and a levelling-off of the response with the higher concentrations. The curve with the interrupted lines shows the relationship of the diameters of killing zones produced by known concentrations of pure 5-MOP (shown in Figure 4) to the concentrations of bergamot oil calcalulated from these concentrations, assuming the 5-MOP content of bergamot oil to be 0.27 g/100 ml

tained (Figure 3). The killing zones obtained with the calculated concentrations were, however, slightly larger than those produced by known concentrations of bergamot oil.

Dose-Response Curves: 8-MOP. The minimal effective concentration of 8-MOP to produce killing of the yeast was 0.0025%, concentrations of 0.00025, 0.0005 and 0.001% being ineffective. Higher concentrations were much less effective than similar concentrations of 5-MOP (Figure 4). Furthermore, the dose-response curve did not show a steep rise with the smaller concentrations as it did with 5-MOP. The results of the turbidimetric test are shown in Table III. Marked inhibition of yeast growth was

Table III

The Optical Density at 600 nm of Yeast Growth in Sabouraud's Liquid Medium 24 hr After Incubation with 0.1 ml of 0.005% Ethanolic Solutions of 5-MOP and 8-MOP. The Values in the Irradiated Petri Dishes Differ Significantly With a p Value of 0.001 Using Student's "t" Test

		Irradiated		Control	
		5-MOP	8-MOP	5-MOP	8-MOP
OPTICAL DENSITY	Mean	0.114	0.282	0.830	0.860
	S.E.M.	0.004	0.004	0.011	0.006

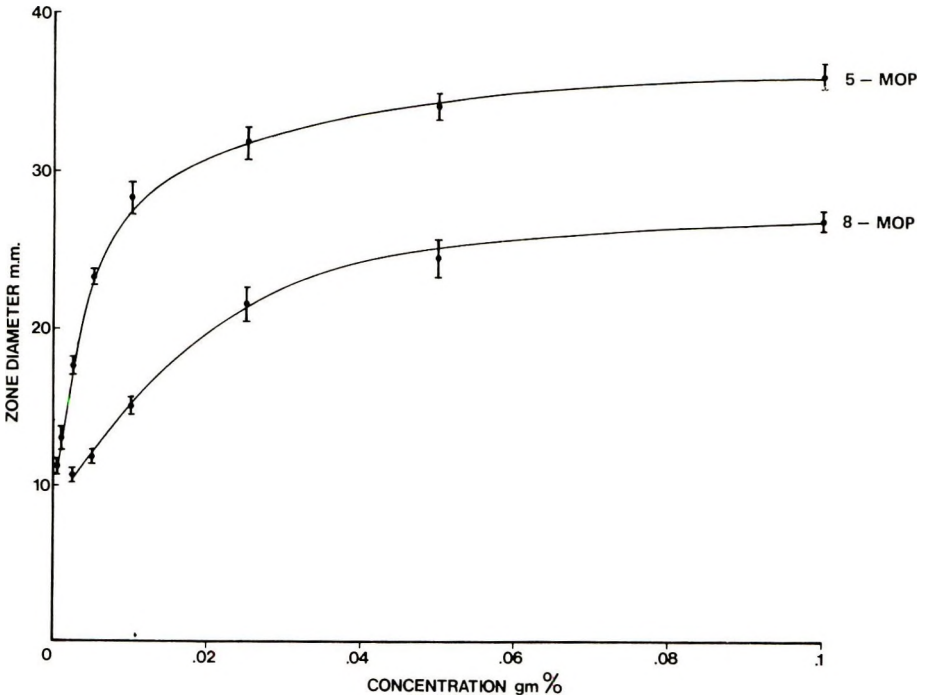

Figure 4. The effect of increasing concentrations of 5-MOP and 8-MOP on the diameter of the killing zones of the yeast (Mean ± S.E.M.) 5-MOP is noted to be more photoactive than 8-MOP and its dose-response curve is similar to that of bergamot oil

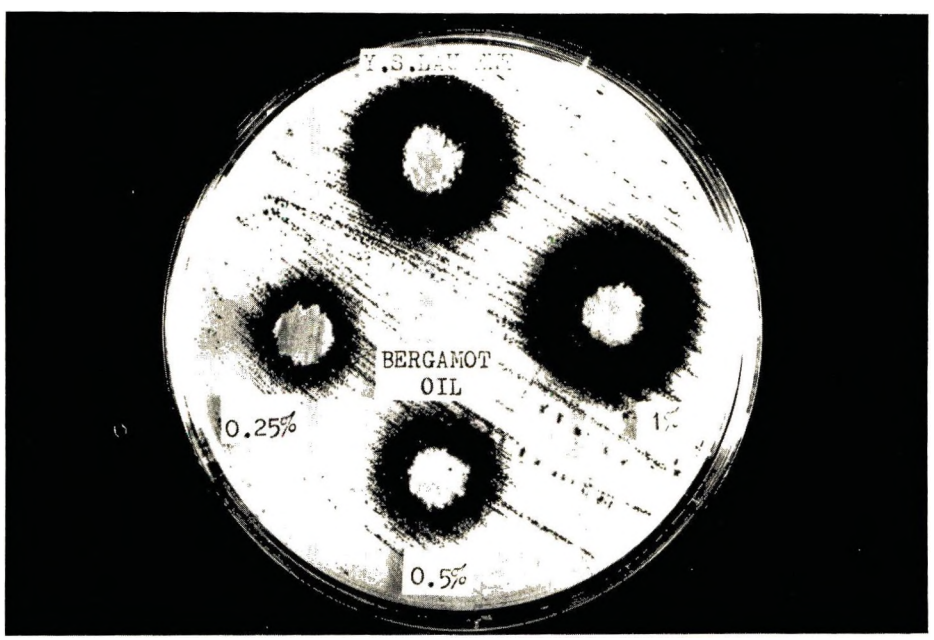

Figure 5. The results of the phototoxic effects of the bergapten TLC fraction of 0.25, 0.5 and 1% bergamot oil and Yves Saint Laurent toilet water

obtained in the irradiated cultures and the inhibition induced by 5-MOP was significantly greater than that of 8-MOP.

The Concentration of 5-MOP in Perfumes. The 5-MOP content of a total of 108 perfumes was determined. Taking YSL toilet water as an example, the killing zones obtained with 5 μl of 0.25, 0.5 and 1% bergamot oil and a similar volume of YSL toilet water are shown in Figure 5. It is noted that the clear zone obtained with YSL toilet water is slightly smaller than that of 1% bergamot oil. To determine, therefore, the exact concentration of bergamot oil in this toilet water the experiment was repeated using 5 μl of 0.5, 0.75 and 1% bergamot oil and the toilet water. The clear zone produced by the toilet water was greater than that of 0.75% and less than that obtained with 1% bergamot oil, indicating that YSL toilet water contains 0.75 to 1% bergamot oil. The concentration of 5-MOP would therefore be 0.00203 to 0.00270 g%. A similar approach was used in the determination of the concentration of bergamot oil in the remaining perfumes. The test was most accurate when quantities of bergamot oil smaller than 0.1 μl (0.27 μg 5-MOP) per TLC spot were used. Normal growth was obtained in all control plates. The 5-MOP content of a total 108 perfumes tested is shown in Table IV. The concentrations ranged from <0.00004 to 0.01080% (<0.016 to 4%

Table IV

The 5-MOP Content of Random Samples of Perfumes as Determined By the *Candida Albicans* Phototoxicity Test and the Estimated Bergamot Oil Equivalent Based Upon a 5-MOP Concentration of 0.27 g/100 ml Bergamot Oil (Coty)

Manufacturer	Perfume	Estimated Bergamot Equivalent Based Upon 0.27 g/100 ml 5-MOP (ml/100 ml)	Estimated 5-MOP Concentration (g/100 ml)
Avon	Charisma Cologne	0.062 to 0.125	0.00017 to 0.00034
	Deep Woods Aftershave	0	0
	Elegance Cologne	0	0
	Field Flowers Cologne	0	0
	FleurD'Avon Cologne	0	0
	Nearness Cologne	0	0
	New World Aftershave	0	0
	Oland Aftershave	0	0
	Promise of Heaven Cologne	0	0
	Roses Roses Cologne	0	0
	Tai Winds Aftershave	0	0
	Unforgetable Cologne	<0.016	<0.00004
	Windjammer Aftershave	0	0
Balenciaga	Ho Hang Toilet Water	<0.016	<0.00004
	Le Dix de Balenciaga	<0.016	<0.00004
	Quadrille Perfume	4	0.0108
Balmain	Miss Balmain Perfume	0.25 to 0.5	0.00068 to 0.00135
Boots	Boots 7 IF Cologne	0.5	0.00135
	Jade East Aftershave	0.25	0.00068
Caron	Bellodgia Perfume	0	0
Carven	Variation Perfume	0.25	0.00068
Chanel	Chanel Aftershave	0	0
	Chanel 5 Cologne	0.75	0.00203
	Chanel 5 Perfume	<0.016	<0.00004
	Chanel 19 Perfume	0.125 to 0.25	0.00034 to 0.00068

(Continued on Page 257.)

Table IV (Continued)

Manufacturer	Perfume	Estimated Bergamot Equivalent Based Upon 0.27 g/100 ml 5-MOP (ml/100 ml)	Estimated 5-MOP Concentration (g/100 ml)
Charles of the Ritz	Ritual Cologne	0.25	0.00068
	Ritz Perfume	0.25 to 0.5	0.00068 to 0.00135
Christian Dior	Diorescence Toilet Water	0.25 to 0.5	0.00068 to 0.00135
	Eau Sauvage Toilet Water	0.75 to 0.1	0.00203 to 0.00270
	Miss Dior Cologne	0.062 to 0.125	0.00017 to 0.00034
	Miss Dior Toilet Water	0.25	0.00068
Coty	Complice Cologne	0	0
	Imprevu Perfume	0.5	0.00135
	Masumi Parfum de Toilet	0.062 to 0.125	0.00017 to 0.00034
Cussons	Imperial Lather After Shave	0	0
Cyclax	Joie Vivre Cologne	0.125 to 0.25	0.00034 to 0.00068
	Vivaldi Cologne	0.5 to 0.75	0.00135 to 0.00203
D'Albert-Orlane	Casaque Cologne	0	0
Dana	Canoe Cologne	0.062 to 0.125	0.00017 to 0.00034
	Canoe Aftershave	0.062 to 0.125	0.00017 to 0.00034
	Canoe Toilet Water	0.062 to 0.125	0.00017 to 0.00034
	Tabu Toilet Water	0.125	0.00034
Dorothy Gray	Mon Secret Toilet Water	0	0
Elizabeth Arden	Blue Grass Toilet Water	0	0
Estee Lauder	Alliage Perfume	0.25 to 0.5	0.00068 to 0.00135
	Estee Perfume	0	0
	Youth Dew Perfume	0.031 to 0.062	0.00008 to 0.00017
Eyelure	Tabac Aftershave	0	0
Faberge'	Brut Aftershave	0.25	0.00068
	Brut 33 Splash-On Lotion	0.125 to 0.25	0.00034 to 0.00068
	Brut Toilet Water	0.5	0.00135
	Faberge' West After Shave	0.125 to 0.25	0.00034 to 0.00068
Le Gallion	Sortilege Parfum de Toilet	0	0
Germain Montel	Royal Secret Spray Perfume	1.5	0.00405
	Royal Secret Cologne	1	0.00270
Givenchy	Givenchy 3 Toilet Water	0	0
	Le De Givenchy Toilet Water	0.016 to 0.031	0.00004 to 0.00008
	Givenchy Gentleman	0	0
	L'Interdit Toilet Water	0.031 to 0.062	0.00008 to 0.00017
Guerlain	Mitsouko Toilet Water	1	0.00270
Guy Laroche	Drakkar Toilet Water	0.125 to 0.25	0.00034 to 0.00068
Helen Rubenstein	Apple Blossom Toilet Water	0	0
	Courant Toilet Water	0	0
	Heavensent Toilet Water	0.125	0.00034
Hermes	Caleche Parfum de Toilet	0.75	0.00203
	Caléche Perfume	1 to 1.5	0.00270 to 0.00405
Houbigant	Chantilly Cologne	0	0
	Quelques Fleurs perfume	0.031	0.00008
Lancome	Magie Perfume	0.016 to 0.031	0.00004 to 0.00008
Lanvin	Eau Arpége	0	0
Lenthric	Onyx Aftershave	0	0
Leonard	Fashion de Leonard Perfume	0.125 to 0.25	0.00034 to 0.00068
Max Factor	Citrus Music Aftershave	0	0
Molyneux	Fête Perfume	0	0
Nina Ricci	Farouche Perfume	0.125	0.00034
	L'Air du Temps Toilet Water	0.031 to 0.062	0.00008 to 0.00017

(Continued on Page 258.)

Table IV (Continued)

Manufacturer	Perfume	Estimated Bergamot Equivalent Based Upon 0.27 g/100 ml 5-MOP (ml/100 ml)	Estimated 5-MOP Concentration (g/100 ml)
Paco Rabanne	Calandre Perfume	0.5	0.00135
	Paco Rabanne Toilet Water	0.062	0.00017
Prince Matchabelli	Cachet Cologne	0	0
	Wing Song Toilet Water	0	0
Puig	Aqua Brava Aftershave	<0.016	<0.00004
	Puig Aftershave	<0.016	<0.00004
Revlon	Aquamarine Toilet Water	0	0
	Charlie Cologne	<0.016	<0.00004
	Intimate Toilet Water	<0.016	<0.00004
Rochas	Audace Perfume	2 to 2.5	0.00540 to 0.00675
	Eau de Rochas	1	0.00270
	Femme Cologne	0.125	0.00034
	Femme Toilet Water	0.125	0.00034
	Femme Perfume	1 to 1.5	0.00270 to 0.00405
	Madame Rochas Parfum de Toilet	0.031 to 0.062	0.00008 to 0.00017
	Monsieur Rochas Cologne	0.031 to 0.062	0.00008 to 0.00017
Shulton	Old Spice Aftershave	0.031	0.00008
Vanda	Si Jolie Cologne	<0.016	<0.00004
Vidal	Pino Sylvestre Cologne	0.75 to 1	0.00203 to 0.00270
Yardley	Bond Street Cologne	0.25 to 0.5	0.00068 to 0.00135
	Caprice Cologne	0	0
	Cougar Aftershave	<0.016	<0.00004
	Flair Cologne	0.125 to 0.25	0.00034 to 0.00068
	Khadine Cologne	0.062	0.00017
	Reverie Cologne	0.016	0.00004
	Sea Jade Cologne	0	0
	Shanida Cologne	0.125	0.00034
Yves Saint Laurent (Y.S.L.)	Rive Gauche Cologne	0.125	0.00034
	Y.S.L. After shave	0.75 to 1	0.00203 to 0.00270
	Y.S.L. Toilet Water (Males)	0.75 to 1	0.00203 to 0.00270
	Y.S.L. Toilet Water (Females)	0.062 to 0.125	0.00017 to 0.00034
	Y.S.L. Perfume	0.25 to 0.5	0.00068 to 0.00135

bergamot oil). It was impossible to predict the presence or absence of 5-MOP from the price, kind or manufacturer of the perfume.

Recovery experiments. As shown in Table IV, the concentration of 5-MOP in Paco Rabanne toilet water and Agua Brava aftershave, as determined by the *C. albicans* test, was 0.00017 and <0.00004% respectively, whereas Caprice cologne and Wind Song toilet water did not contain any 5-MOP. Following the addition of a known quantity of bergamot oil and using the same test, the concentration of 5-MOP in the resultant solutions was shown to be approximately 0.00135%. The recovery of bergamot oil from these perfumes was, therefore, virtually complete. Of the four resultant solutions, only Agua Brava aftershave had a TLC pattern similar to that of bergamot oil and Group A perfumes. The TLC pattern of Paco Rabanne toilet water, Caprice cologne and Wind Song toilet water was similar to that of Group B perfumes, which showed a poor separation of 5-MOP from the adjacent TLC bands. This was further confirmed by spectrophotometry where the absorption spectrum of the 5-MOP band of Agua Brava

Table V
A Comparison of the Phototoxic Activity of Yves Saint Laurent Toilet Water Using Bergapten TLC Fraction, Filter Paper Disc and Silica Gel to Which the Perfume Was Added

	Bergapten TLC Fraction	Whole Perfume On Filter Paper Disc	Whole Perfume On Silica Gel
Diameter of Killing Zone (mm)	26.8	25.2	23.6
S.E.M.	0.37	0.20	0.40

aftershave was similar to that of bergamot oil and different from that of three other perfumes.

The importance of TLC. The results of the phototoxic effects of YSL toilet water using the bergapten TLC fraction, 10 mm filter paper discs and silica gel containing the whole perfume, are shown in Table V. The killing zone was largest with the bergapten TLC fraction and smallest with the silica gel containing the whole perfume; the filter paper disc produced an intermediate result. The values obtained for the filter paper discs and silica gel differed significantly from the result of the bergapten TLC fraction at the 1 and 0.1% levels of probability respectively, when the Student's "t" test was used. A number of perfumes produced killing of the yeast in the nonirradiated plates when the whole perfume was applied to filter paper discs. The perfumes, which on TLC were shown to contain 5-MOP (Chanel 5, Quelques Fleurs and Fête perfumes, Charlie Cologne and Cougar aftershave), produced an inhibitory zone in the control plate smaller than that obtained in the irradiated plate. Others which did not show a definite 5-MOP fraction on TLC (Bellodgia, Youth Dew, Ritz and Estee Lauder perfumes and Aquamarine toilet water) produced an inhibition zone in the control plate equal to that obtained in the irradiated plate.

THE SPECTROPHOTOMETRIC TEST

The results of the determination of 5-MOP concentration in some of the perfumes tested using this technique are shown in Table VI.

It was impossible to perform accurate quantitative analysis of 5-MOP in Group B perfumes because the method was based on a comparison of the absorption of the 5-MOP TLC fraction of perfumes with that of bergamot oil and a significant contamination of this fraction was expected to yield higher results. This was indeed the case

Table VI
The Estimated 5-MOP Concentration in Perfumes Using the Spectrophotometric Test. Group A Represents Perfumes Which Have a Relatively Clear Bergapten TLC Fraction. In Group B Perfumes, the Bergapten TLC Fraction Shows Significant Impurities

Manufacturer	Perfume	Group	Estimated Bergamot Equivalent Based Upon 0.27 g/100 ml 5-MOP (ml/100 ml)	Estimated 5-MOP Concentration (g/100 ml)
Christian Dior	Eau Sauvage Toilet Water	A	0.90	0.00243
Faberge'	Brut Aftershave	B	2.70	0.00729
Rochas	Eau de Rochas	A	1.00	0.00270
Vidal	Pino Sylvestre Cologne	A	0.90	0.00243

with Brut aftershave, which was shown to contain 0.00729% 5-MOP using the spectrophotometric test and only 0.00068% with the *C. albicans* test. The results of Group A perfumes, on the other hand, were similar to those obtained with the *C. albicans* test in the samples tested. The bergapten TLC fraction of this group of perfumes showed only a slight impurity and the absorption spectrum was very close to that of 5-MOP fraction of bergamot oil used as reference. The spectrophotometric test was impractical for the assessment of Group A perfumes which contained very small concentrations of 5-MOP since a large number of 25 μl TLC spots would have been required for a reliable estimate to be made. For example, approximately 60 aliquots (25 μl) of Ritual Cologne and 500 aliquots of Intimate toilet water would have been required to obtain an adequate amount of 5-MOP in order to make a determination feasible. Attempts to concentrate these perfumes by chloroform extraction did not yield encouraging results and the use of a larger volume such as 100 μl TLC spots resulted in a poor separation of 5-MOP from the adjacent bands.

DISCUSSION

Fowlks, Griffith and Oginsky (8) described the photosensitization of bacteria by psoralens and related compounds using paper-disc diffusion method on agar plates. Filter paper discs were impregnated with the test compounds and arranged uniformly on freshly solidified agar layer in a Petri dish. Another agar layer with bacteria was poured over the discs, left to solidify and incubated at 37°C. Activity of the compound was determined by measuring the diameter of bacteria-free zones surrounding the discs.

Daniels' use of *C. albicans* (5) made this procedure much simpler, since the yeast has the advantage of growing rapidly at room temperature after direct inoculation onto the surface of agar plates. The need for incubation at 37°C and the addition of another layer of agar containing the microorganisms thus became unnecessary. Lethal photosensitization of the yeast was assumed to have occurred since many attempts to replate organisms from the clear zone had failed. Although the technique was designed primarily for screening purposes, preliminary studies of quantitative relationship between furocoumarins were performed and the possible use of the test in quantitative estimations of psoralens was suggested.

The spectrophotometric method was used by Cieri (6) for the determination of 5-MOP content of bergamot oil and some other essential oils. The procedure, however, was lengthy and tedious and a number of modifications were introduced which simplified the method, making it easier for ordinary day-to-day laboratory determinations (2). The test is based on a comparison of the ultraviolet absorption of bergapten TLC fraction of the perfume and a known concentration of bergamot oil. Any impurities present in the bergapten TLC band of the perfume would lead, therefore, to a greater ultraviolet absorption and a higher concentration. The bergapten TLC fraction of Group B perfumes showed a significant degree of contamination and the absorption spectrum was very different from that of pure 5-MOP. Hence, the spectrophotometric test could not be used for the determination of the 5-MOP content of these perfumes. Even the mild impurity of 5-MOP fraction of bergamot oil produced a slight increase in the concentration of 5-MOP in bergamot oil as pure 5-MOP was used as a reference. The small degree of contamination of the bergapten TLC fraction of Group A

perfumes did not affect the spectrophotometric determination since such an effect was cancelled by a similar degree of contamination of the 5-MOP band of the bergamot oil used as reference.

It is apparent from the results of the present experiments that the *C. albicans* phototoxicity test can indeed be useful for the determination of the concentration of photoactive psoralens in solutions such as perfumes. It is sensitive to 5-MOP, 8-MOP and bergamot oil in quantities of 0.01 μg, 0.05 μg and 0.005 μl respectively. A greater degree of accuracy was obtained when quantities smaller than 0.27 μg 5-MOP (0.1 μl bergamot oil) were applied to each test site on the agar plate. The volume of each perfume chosen for testing depended therefore on the concentration of 5-MOP in that perfume and had to be adjusted to give a final quantity in the range of 0.0135 to 0.27 μg (0.005 to 0.1 μl bergamot oil).

Using the yeast phototoxicity test, 5-MOP was found to be five times more photoactive than 8-MOP, although photopatch testing carried out on human skin showed that 8-MOP is slightly more active than 5-MOP (2). The difference was originally suspected to be due to relative diffusion of the psoralens in agar. Preliminary results, using a turbidmetric test in which diffusion of the psoralens does not affect the determination, support these findings however. The cause of the discrepency between the *in vitro* and *in vivo* tests is not clear, although factors which affect absorption and penetration of the chemical into the skin may play an important role. A discrepancy between the two systems was also noted with quinoline methanols (9), tetramethylthiurammonosulphide (10) and tribromosalicylanilide (11).

Although it is easier in the *C. albicans* test to avoid the use of the TLC procedure by applying the whole perfume on filter discs, TLC is considered an essential step in this test because it uncovers the nature of the photoactive component. In addition, TLC improves the sensitivity of the test since the diameter of the killing zone produced by the bergapten TLC fraction is significantly larger than that obtained with the filter paper disc containing the whole perfume. The larger killing zone was originally thought to be due to a better diffusion of 5-MOP from silica gel than from filter paper discs. However, the difference became more apparent when silica gel containing the whole perfume was compared with silica gel containing only the 5-MOP fraction, suggesting that the difference in zone size might have been due to incomplete release of 5-MOP from the other constituents of the perfume. Although no thorough investigations were performed to assess the effects of the impurities of the bergapten TLC fraction of Group B perfumes on the results of *C. albicans* test, it was considered unlikely that the impurities could greatly hinder the diffusion of 5-MOP. The preliminary results of the recovery experiments support this assumption. Although TLC offers a number of advantages, the disc technique is useful for screening purposes when an approximate estimation of the content of a known extract is required. In addition, it provides a good alternative where TLC cannot be adequately performed as with oily, greasy and semi-solid cosmetics. Direct toxicity to the yeast that has not been encountered with bergapten TLC should always be considered when the disc technique is used and should be assessed by means of duplicate nonirradiated culture plates.

The 5-MOP content of different samples of bergamot oil varies significantly and previous studies demonstrated the importance of determining the bergapten concentration prior to its use in photopatch testing (2). Similarly, it is important in the determination of the 5-MOP content of perfumes using the *C. albicans* test to use bergamot oil

with a known concentration of bergapten, since the test essentially compares the phototoxic effects of 5-MOP fraction of known dilutions of bergamot oil with 5-MOP fraction of bergamot oil present in perfumes. The results are therefore best expressed as 5-MOP content of perfumes but could well be reported as concentration of bergamot oil of known 5-MOP content.

Of 108 randomly selected perfumes, 62 (57.4%) contained bergapten in concentrations of 0.00004% or more. The significance of the presence of 5-MOP, even in small concentrations in perfumes, and its relationship to hyperpigmentation of the sides of the face and neck have already been demonstrated (1). Photopatch testing carried out on the skin of the midback showed a positive response, consisting of erythema and subsequent pigmentation, to a number of perfumes and also to bergamot oil (Coty) in concentrations of 0.25% (0.00068% 5-MOP) in some normal subjects and 0.125% (0.00034% 5-MOP) in some patients with Poikiloderma of Civatte (1). Individual variation in the degree of response to 5-MOP exists and the minimal effective concentration to produce a response is affected by many factors such as the kind of vehicle used, the site of phototesting, the hydration of the skin, the repeated applications and irradiations and the degree of natural or sun-induced pigmentation (4). Thus, a safe concentration for each individual cannot always be exactly defined and it is therefore recommended that perfumes free from 5-MOP or those which contain an extremely low concentration (*e.g.*, <0.00008%) be used by patients suffering from Civatte's Poikiloderma and subjects who on photopatch testing are shown to be sensitive to small concentrations of 5-MOP. It is hoped that the cosmetic industry will soon realize the noncosmetic effects of the presence of 5-MOP in natural bergamot oil and that, instead, 5-MOP–free or artificial bergamot oil will be used in perfumes.

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Autoradiographic study on percutaneous absorption of several oils useful for cosmetics

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Synopsis

PERCUTANEOUS ABSORPTION of five ^{14}C-labelled OILS, n-octadecane, decanoxy decane, 2-hexyldecanoxy octane, isopropyl myristate and glyceryl tri-(oleate), generally used in COSMETICS were studied from the point of view of their safety. In whole body AUTORADIOGRAPHY with hairless mice, there was no visible penetration into the skin and organs, whereas microautoradiography with guinea pigs showed local penetration. Isopropyl myristate penetrated to the greatest extent, whereas 2-hexyldecanoxy octane was hardly absorbed.

Percutaneous absorption of these two oils, therefore, was examined in Angora rabbits by microautoradiography simultaneously with skin irritation potential by histological method from the following aspects: 1. patterns of penetration and irritation according to application time and 2. fate within the skin and pattern of irritation after application. In addition, intradermal metabolic fate was also studied *in vito*.

INTRODUCTION

Skin irritations or eruptions caused by cosmetics are very serious problems from a social viewpoint. Skin penetration of irritants in cosmetics used topically is a prerequisite of their causing responses in the living cells of the epidermis and underlying dermis. Thus, cosmetic chemists have long been interested in percutaneous absorption as a very important phenomenon.

From the standpoint mentioned above, the authors have been studying percutaneous absorption of cosmetic constituents not only to examine their permeability, but also to evaluate its relationship to the occurrence of irritation and toxicity. The findings regarding these relations are expected to improve the quality of cosmetics in terms of their safety.

Though percutaneous absorption has been measured on some available materials used in small amounts in cosmetics, *e.g.*, germicides (1–4), hormones (5–7), vitamins (8,9) and surfactants (10–12), little data have been reported on the base materials of cosmetics. In this paper, percutaneous absorption of several oils used as bases in cosmetics were studied in hairless mice, guinea pigs and Angora rabbits.

Methods of measuring percutaneous absorption have been reviewed by Ainsworth (13), Barr (14), Tregear (15), Grasso and Lansdown (16) and Idson (17). In this study, the technique of microautoradiography was employed together with that of whole body autoradiography developed by Ullberg (18). Simultaneously, primary irritation potentials were observed histologically on the same substances.

In addition, intradermal metabolic fates of the oils were also examined radiochromatographically.

MATERIALS

Isopropyl myristate-^{14}C (^{14}C-IPM), decanoxy decane-^{14}C (^{14}C-DD) and 2-hexyldecanoxy octane-^{14}C (^{14}C-HDO) were synthesized from 1-^{14}C-myristic acid, 1-^{14}C-decyl bromide and 1-^{14}C-octanol respectively in our laboratory. The above starting materials, glyceryl tri-(oleate-1-^{14}C) (^{14}C-GTO) and 1-^{14}C-octadecane (^{14}C-OD) were obtained from Daiichi Pure Chemicals. These labelled compounds were chemically and radiochemically pure as checked by thin layer chromatography using several different solvent systems and scanning with a radiochromatogram scanner Aloka TLC-2D. Specific activities of these five oils were adjusted approximately to 0.2 μCi/mg suitable for this study.

An oil-containing hydrophilic ointment was prepared in the following formula: ^{14}C-labelled oil 5%, white petrolatum 30%, stearyl alcohol 15%, propylene glycol 12%, sodium lauryl sulfate 2% and distilled water 36%.

METHODS

WHOLE BODY AUTORADIOGRAPHY WITH HAIRLESS MICE

Male hairless mice (hr/hr strain) weighing 25 g (average) were used. Applied to the dorsal skin of animals under occlusion were 0.01 ml of the radioactive oils on 2.5 cm diameter Japanese papers backed with Lumirror® film (Toray Industries, Inc.) or 50 mg of the oil-containing hydrophilic ointments on 2.5 cm diameter filter papers (Toyo Roshi Co., Ltd.). The treated areas of skins were covered with 3M Co.'s Micropore surgical tape.

The mice were anesthesized with diethyl ether and immersed immediately in a dry ice-acetone bath (−78°C) at different intervals (1, 6, 24 and 48 hr). Subsequently, whole body autoradiography was carried out according to the Ullberg method (18). Forty μm sagittal sections adhering to Scotch® Tape No. 810 (Sumitomo-3M Co., Ltd.) were prepared with a Jung type microtome from Yamatokoki Co., Ltd., in a freezing room (−15°C). The sections were allowed to dry in that room, then brought into contact with Sakura X-ray film Industrial Type N (Konishiroku Photo Ind. Co., Ltd.), and exposed for 40 days. The film development was according to the usual procedure recommended by the manufacturer.

MICROAUTORADIOGRAPHY WITH GUINEA PIGS

Male guinea pigs (Hartley strain) weighing 340 g (average) were used. The hair on the dorsal region of animals was removed with a hair clipper and an electric shaver one day

prior to topical application. The radioactive oils (0.01 ml on 2.0 cm diameter Japanese papers backed with Lumirror® film) were applied to the clipped areas of skins. These treated sites were covered with Micropore® surgical tape.

The animals were sacrificed at 6 and 24 hr after application. The skins were excised and frozen immediately by immersion in hexane cooled with dry ice-acetone. Using a Jung type microtome, 20 μm frozen sections were cut and dried in a freezing room (−15°C).

After drying the sections were transferred onto the glass slides, and then covered with Fuji autoradiographic stripping films in the dark room. After 50 to 170 days of exposure at 5°C, the films were developed according to the usual procedure recommended by the manufacturer and the sections were stained in the usual manner with Harris' hematoxylin and mounted.

MICROAUTORADIOGRAPHY WITH ANGORA RABBITS

Male Angora rabbits weighing 2.5 kg were used. Two 5 × 6 cm areas were made on the dorsal region of an animal symmetrically with a median line by removing hair with the same manner mentioned above. Then, 0.02 ml of ^{14}C-IPM or ^{14}C-HDO on 3.0 cm diameter Japanese papers were applied to these sites for 2, 6 and 24 hr occlusively.

After the animals were sacrificed by air embolism, the treated skins were excised and divided into two pieces parallel to a median line. One was frozen in dry ice for microautoradiography, and the other was fixed in 10% formalin for histological observation. Microautoradiography was undertaken according to the usual method mentioned above.

HISTOLOGICAL STUDIES

For histological observation, the specimens were embedded in celloidin and paraffin. Serial sections cut at 5 μm mounted on slides were processed through xylol, alcohol to water. The slides were stained in the usual manner with hematoxylin and eosin, and mounted.

OBSERVATION OF FATE WITHIN SKIN

In the same manner described above, 0.02 ml of ^{14}C-IPM or ^{14}C-HDO were applied to the dorsal skins of Angora rabbits. After 24 hr the ^{14}C-compounds were removed and wiped from the skin surfaces with sanitary cotton. The treated sites were then protected with occlusive dressing during this experiment. At zero, one, three, six and ten days after the removal, the animals were sacrificed by air embolism and the treated skins were excised. Then microautoradiograms and histological specimens were prepared by the procedure described above.

METABOLIC FATE AFTER INTRADERMAL ADMINISTRATION

Angora rabbits, whose hairs had been removed with an electric clipper one day before injection, were injected intradermally in their dorsal skins with 0.05 ml of ^{14}C-IPM (0.14 μCi) or ^{14}C-HDO (0.18 μCi). Two rabbits were employed for each substance and six sites were used with each rabbit. At 0, 24 and 72 hr after injection, 1-cm-punch

specimens were taken from the injected sites of each animal. Two additional control specimens were obtained from each animal.

Metabolites were extracted from each specimen with 4 ml of chloroform in a vial for two days at −20°C in order to avoid decompositions of labelled compounds. After the extract was filtered with cotton wool, the filtrate was dried up quickly. Then the residue was redissolved in 1 ml of chloroform.

A 0.1 ml aliquot part of this solution was used for liquid scintillation counting with Aloka LSC-601, and the remainder was spotted on a silica gel plate. After developing, the plate was scanned with a radiochromatogram scanner Aloka TLC-2D in order to identify metabolites. After extraction, the specimen was digested and counted for radioactivity by the method of Petroff *et al.* (19).

RESULTS

WHOLE BODY AUTORADIOGRAPHY WITH HAIRLESS MICE

The distribution of ^{14}C-labelled IPM, DD, HDO, GTO and OD was assessed by whole body autoradiography with hairless mice sacrificed at 1, 6, 24 and 48 hr after topical application. Neither neat oils nor those in hydrophilic ointments penetrated into body organs. The oils were still localized on the applied regions 48 hr after application. The autoradiograms at 48 hr after application of oil-containing ointments are shown in Figure 1.

MICROAUTORADIOGRAPHY WITH GUINEA PIGS

The distribution of five ^{14}C-labelled oils was observed by microautoradiography in the skins of guinea pigs sacrificed at 6 and 24 hr after topical application. Each oil had a characteristic pattern of distribution in the skin depending on application time. The microautoradiograms of these oils are shown in Figure 2.

IPM. After 6 hr, the transfollicular penetration was observed, which resulted in the concentration of silver grains into the sebaceous glands. The silver grains are derived from and show the distribution of radioactive substance. The grains were distributed in the stratum spinosum. After 24 hr, the grains were distributed densely in the hair infundibula, the follicles, the stratum spinosum and particularly the sebaceous glands. Also, the dermis adjacent to them had a slight distribution of grains.

GTO. After 6 hr, the silver grains were distributed from the stratum corneum to the sebaceous glands, but not so marked as those in IPM. After 24 hr, however, the grains spread up to the hair bulges and concentrated considerably in the sebaceous glands. The grains were observed slightly in the dermis under the basal layer and around the hair follicles and the sebaceous glands.

OD. After 6 hr, the silver grains were distributed a little from the hair infundibula to the sebaceous glands. After 24 hr, those were concentrated in the sebaceous glands and spread to the dermis around them.

DD. After 6 hr, the silver grains did not appear in the skin. After 24 hr, the grains were observed slightly from the hair infundibula to the sebaceous glands. This substance was found to be absorbed at a slow rate.

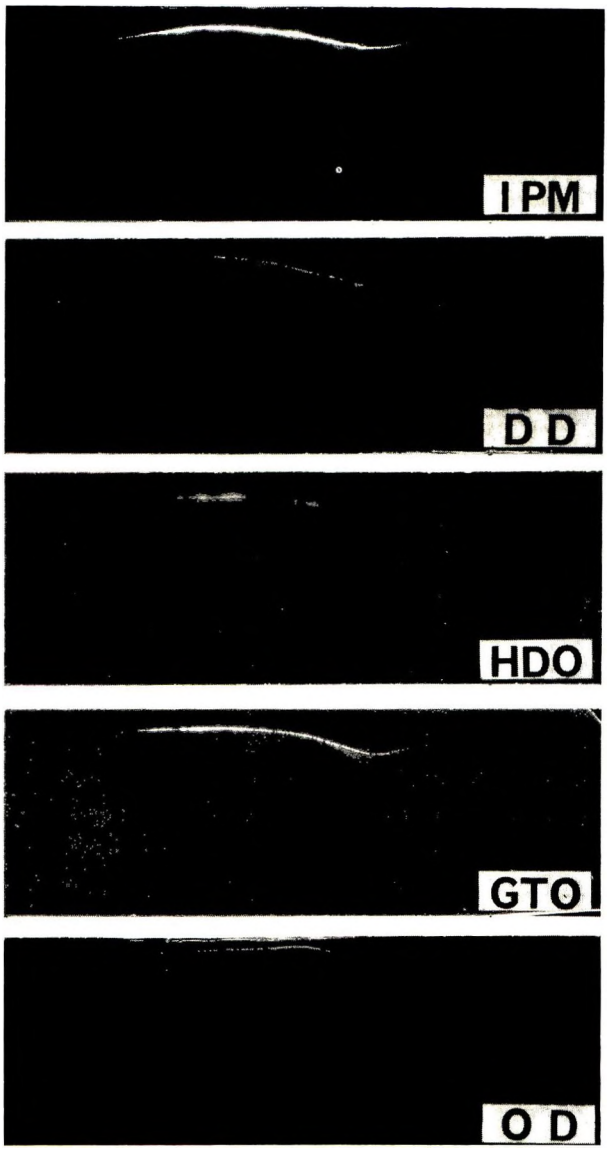

Figure 1. Whole body autoradiograms showing the distribution of radioactivity in hairless mice at 48 hr after topical application of ^{14}C-oil-containing hydrophilic ointments. No oils were penetrated into body organs. The radioactivities of oils were localized on the applied regions. The right of figure is head and the left is tail

HDO. The silver grains were not observed in the skin even after 24 hr. It was thought to be absorbed little or at an extremely slow rate.

From the results above, the absorbability of the oils was found reasonably in the following order from greatest to least: IPM, GTO, OD, DD and HDO.

Therefore, percutaneous absorption on IPM as the highest penetrant and HDO as the lowest was then examined in Angora rabbits by means of microautoradiography more deeply, simultaneously with skin irritation potential by histological method.

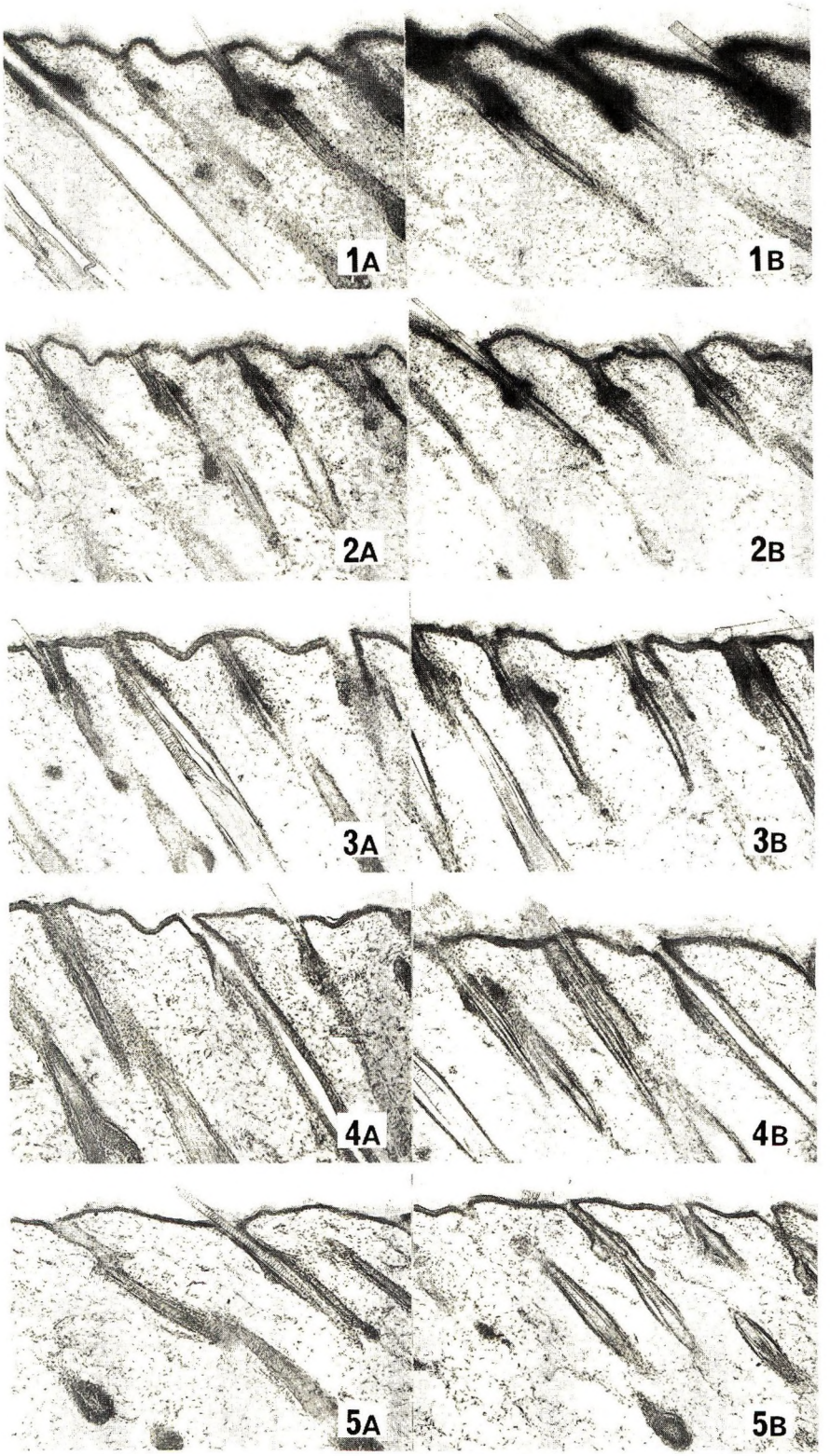

Figure 2. Microautoradiograms showing the distribution of radioactivity in the skin of guinea pigs after topical application of ^{14}C-labelled oils. 1) IPM, 2) GTO, 3) OD, 4) DD, 5) HDO and A) 6 hr, B) 24 hr (see text)

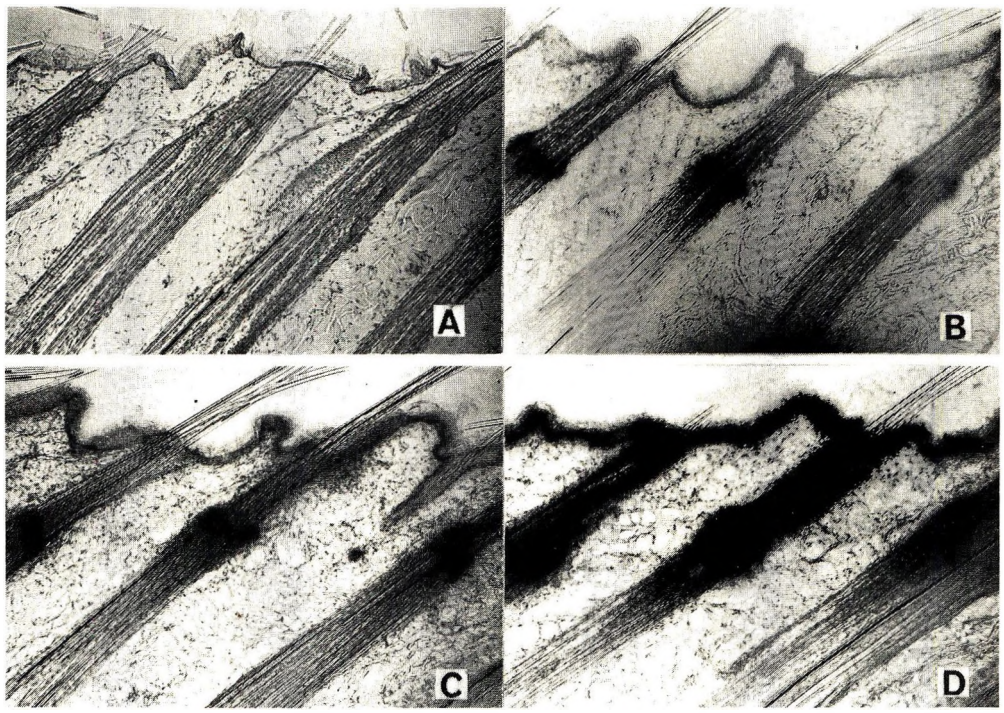

Figure 3. Microautoradiograms showing the distribution of radioactivity in the skin of Angora rabbits after topical application of ^{14}C-IPM. A) intact skin, B) 2 hr, C) 6 hr, D) 24 hr. Note the concentration in the sebaceous glands and epidermis

MICROAUTORADIOGRAPHY AND HISTOLOGY WITH ANGORA RABBITS

The distributions and skin irritation potentials of ^{14}C-labelled IPM and HDO were examined by microautoradiography and histological method in the skins of Angora rabbits sacrificed at 2, 6 and 24 hr after topical application.

IPM. 1. Microautoradiography: after 2 hr, the silver grains were already observed sparsely in the hair follicles and the epidermis, especially centered in the sebaceous glands. Then the grains became concentrated during the period of application. The dermis adjacent to the sebaceous glands and beneath the epidermis had an increasing distribution of grains with the time course (Figure 3).

2. Histology: histological changes due to IPM were not intense at 2 and 6 hr. The infiltration of polymorphonuclear leucocytes was observed in the hair follicles and the collagen fibers under the epidermis were seen to become fine. Also, the infiltration of small mononuclear and polymorphonuclear leucocytes into the upper layers of dermis was slightly observed.

After 24 hr, the epidermis was thickened to three or four cell layers. The epidermal cells became swollen and round. Vacuolation around nuclei was also observed. The keratohyalin granules disappeared in a wide area so that the stratum corneum was thinned. The sebaceous glands became larger. In the dermis, the collagen fibers became fine and marked vasodilatation was seen together with partial hemorrhage (Figure 4).

HDO. 1. Microautoradiography: after 2 hr, the silver grains were observed in the sebaceous glands and the adjacent dermis. Then the grains over the sebaceous glands and

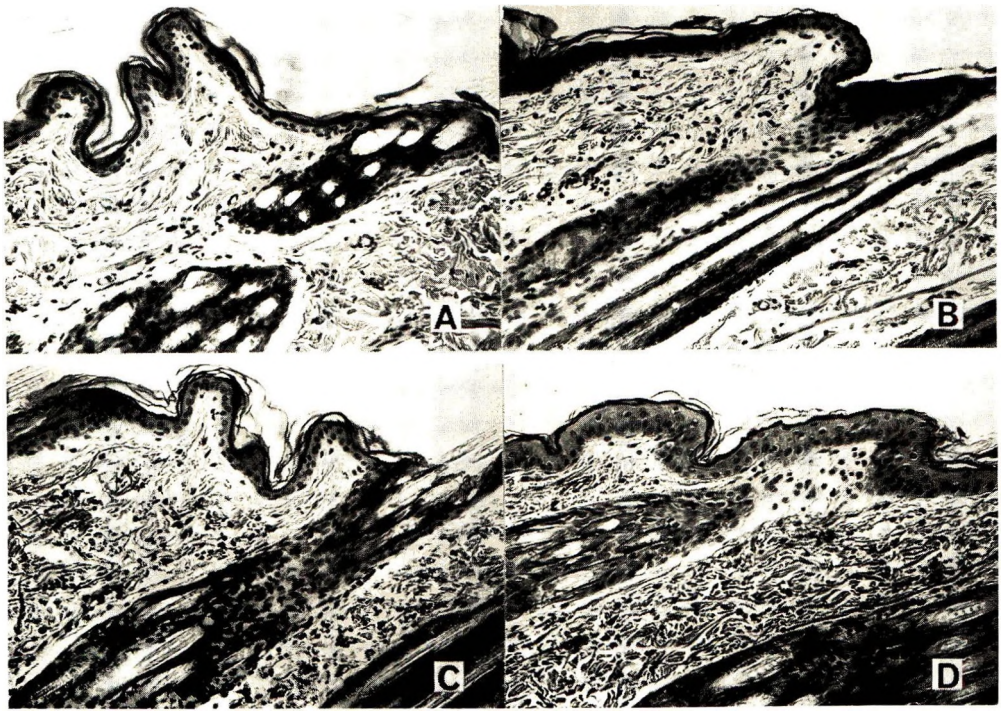

Figure 4. Light micrographs showing the histological feature in the skin of Angora rabbits after topical application of ^{14}C-IPM. A) intact skin, B) 2 hr, C) 6 hr, D) 24 hr. The changes of cells in the epidermis and collagen fibers in the dermis and the infiltration of polymorphonuclear leucocytes were observed

the hair bulges became concentrated during the period of application and, further, the distribution was spread to the deep regions of the hair follicles. Simultaneously, the surrounding dermis had an increasing distribution of grains. Also, the diffusion of grains from the epidermis down to the dermis appeared increased (Figure 5).

2. Histology: marked changes in the epidermis and the dermis were scarcely observed at 2 and 6 hr. The epidermis was composed of two or three layers and each cell became swollen and round. Weak edema was seen at 24 hr together with slightly increasing infiltration of small mononuclear leucocytes in the upper layers of the dermis. Vasodilatation or hemorrhage were not observed (Figure 6).

The results indicated that the irritation potential of HDO was extremely low.

OBSERVATION OF FATE WITHIN SKIN

The patterns of the distribution and the skin irritation potentials of ^{14}C-labelled IPM and HDO were observed by microautoradiography and histological method in the skins of Angora rabbits sacrificed at zero, one, three, six and ten days after 24 hr topical application.

IPM. 1. Microautoradiography: IPM was distributed into the skin both transeptidermally and transfollicularly immediately after 24 hr application. The density of grains in the epidermis and the hair follicles was maximal at one day after the finish of applica-

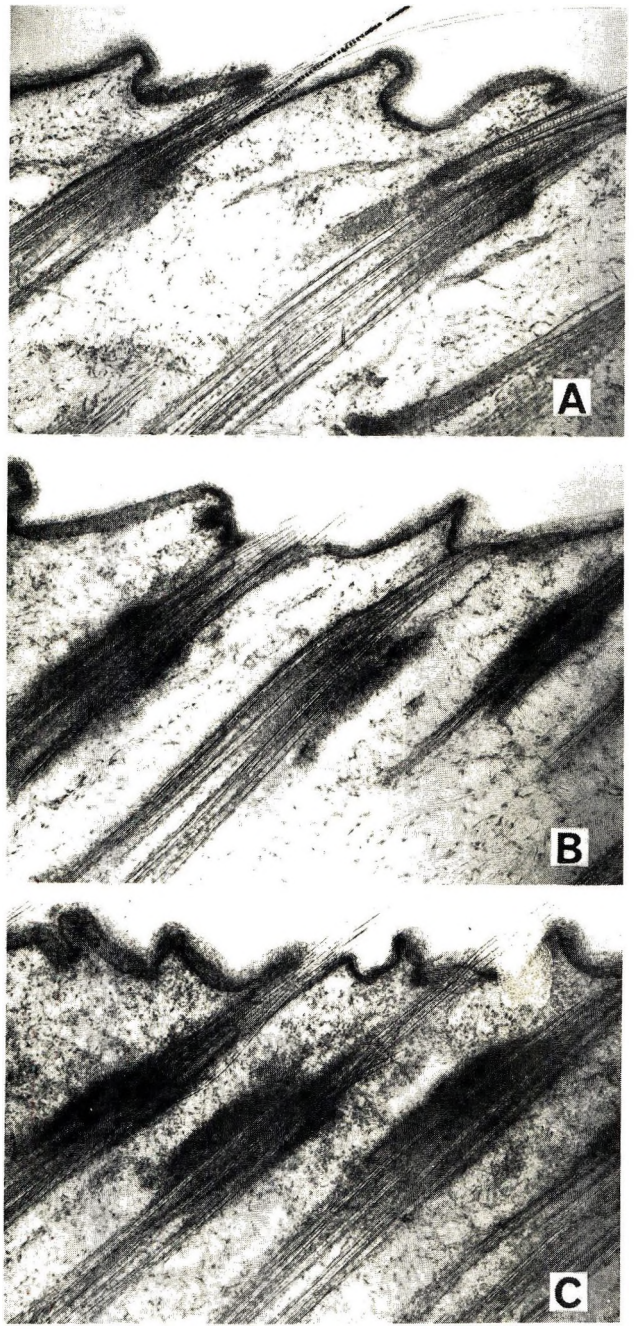

Figure 5. Microautoradiograms showing the distribution of radioactivity in the skin of Angora rabbits after topical application of ^{14}C-HDO. A) 2 hr, B) 6 hr, C) 24 hr. Note the concentration in the sebaceous glands and epidermis

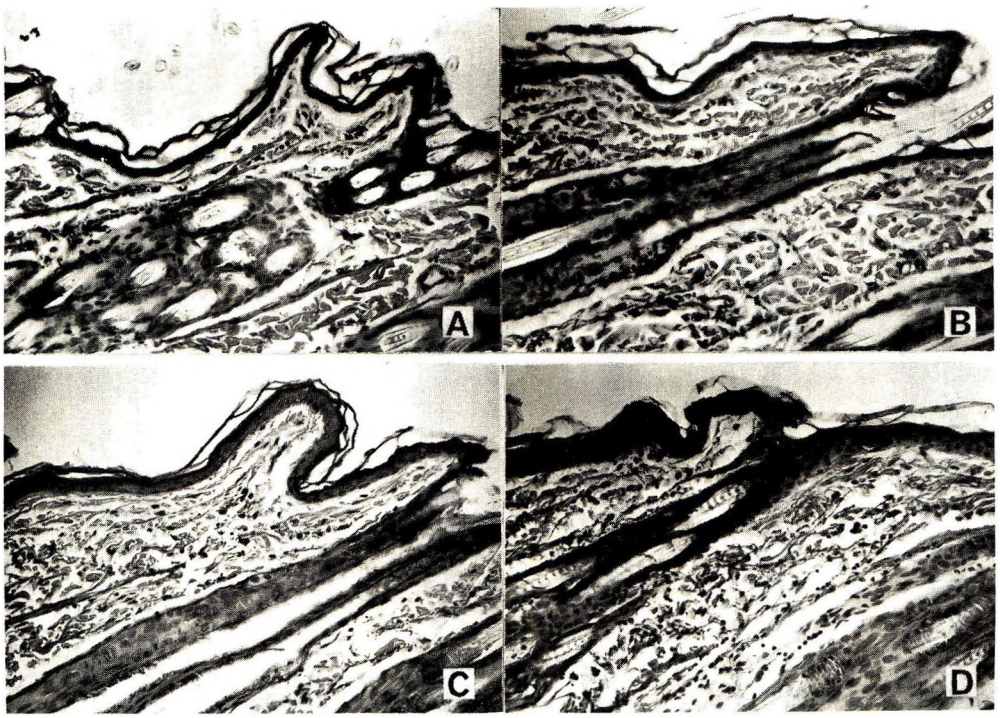

Figure 6. Light micrographs showing the histological feature in the skin of Angora rabbits after topical application of ^{14}C-HDO. A) intact skin, B) 2 hr, C) 6 hr, D) 24 hr. Acanthosis and edema were observed

tion. After that, the grains disappeared gradually from these sites. This finding was assumed due to the removal of the isotope from the skin by the turnover of the epidermis and the hair growth. After six days, the grains were not found in the sebaceous glands and after ten days, only the stratum corneum and a part of hair lumina had a distribution of grains. On the other hand, IPM in the dermis decreased abruptly from one to ten days, and so the grains disappeared completely from the dermis at ten days (Figure 7).

2. Histology: in the epidermis acanthosis increased from zero to three days and then decreased from six to ten days. After ten days, however, marked changes were still observed as compared with the intact skin. Namely, vacuolization and swelling of Malpighi layer cells remained and swelling of the follicular cells was also found. In the dermis, edema, vasodilatation and hemorrhage increased from zero to three days and remained high and severe from six to ten days (Figure 8).

The results showed that irritation reaction proceeded characteristically with the time course though IPM disappeared from the skin. Thus it was assumed that the revelation of irritation was influenced by the sensitivity and the repairability of a living body, not absolutely by the existence or remainder of an irritant.

HDO. 1. Microautoradiography: HDO was absorbed deeply along the hair follicles up to the hair matrixes, especially in the sebaceous glands, and in the upper layers of the dermis both transepidermally and transfollicularly after 24 hr application. Such a

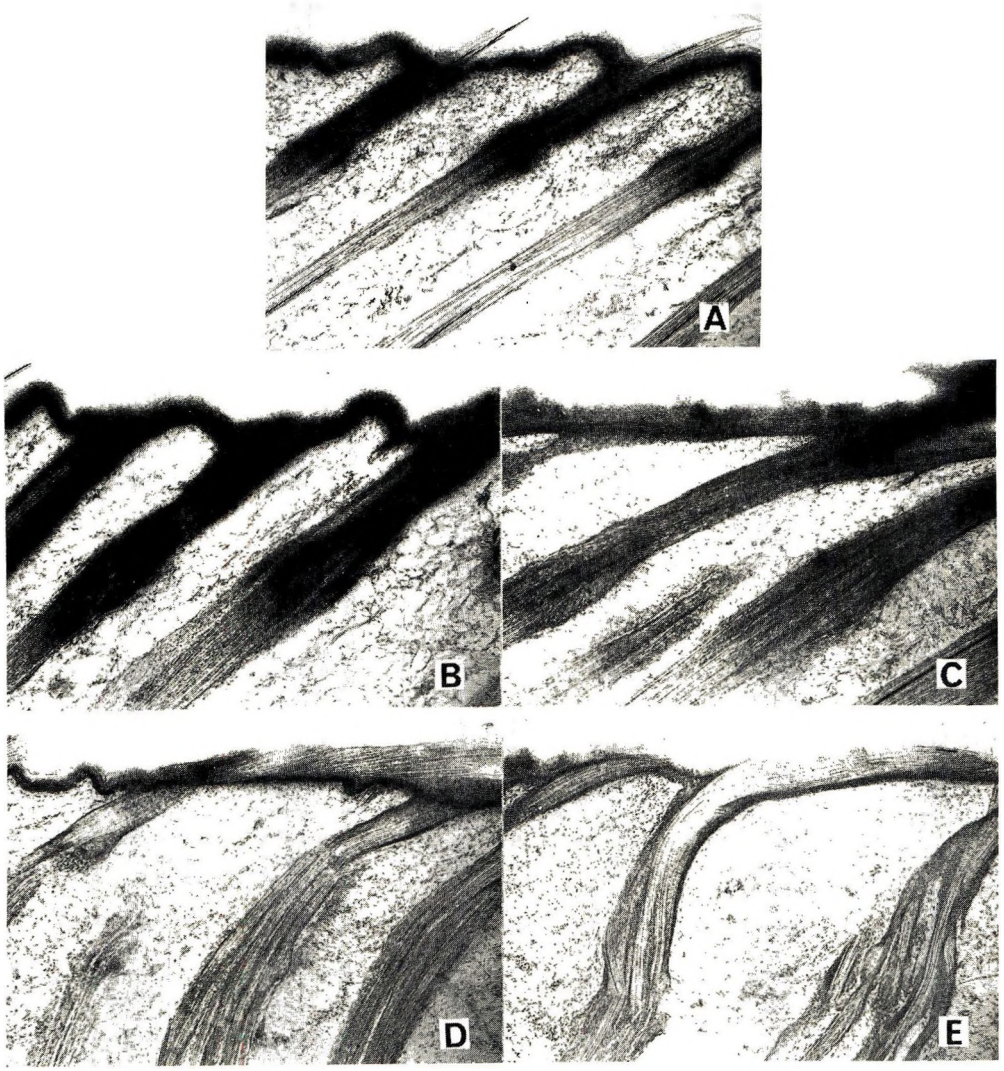

Figure 7. Microautoradiograms showing the distribution of radioactivity within the skin of Angora rabbits at various times after 24 hr topical application of ^{14}C-IPM. A) zero day, B) one day, C) three days, D) six days, E) ten days. Note the changes of concentration in the sebaceous glands and epidermis

tendency continued from one to three days after the finish of application. After six days, however, the grains remained only slightly in the sebaceous glands and became decreased in the dermis. After ten days, the density of grains in the sebaceous glands and the dermis was extremely diminished and almost unseen (Figure 9).

2. Histology: from zero to one day after 24 hr application, edematous changes were mainly observed in the dermis, while acanthosis became increased gradually but quite weakly in the epidermis. From three to six days, acanthosis and edema were found decreased; they almost disappeared at ten days. Vasodilatation first appeared at three

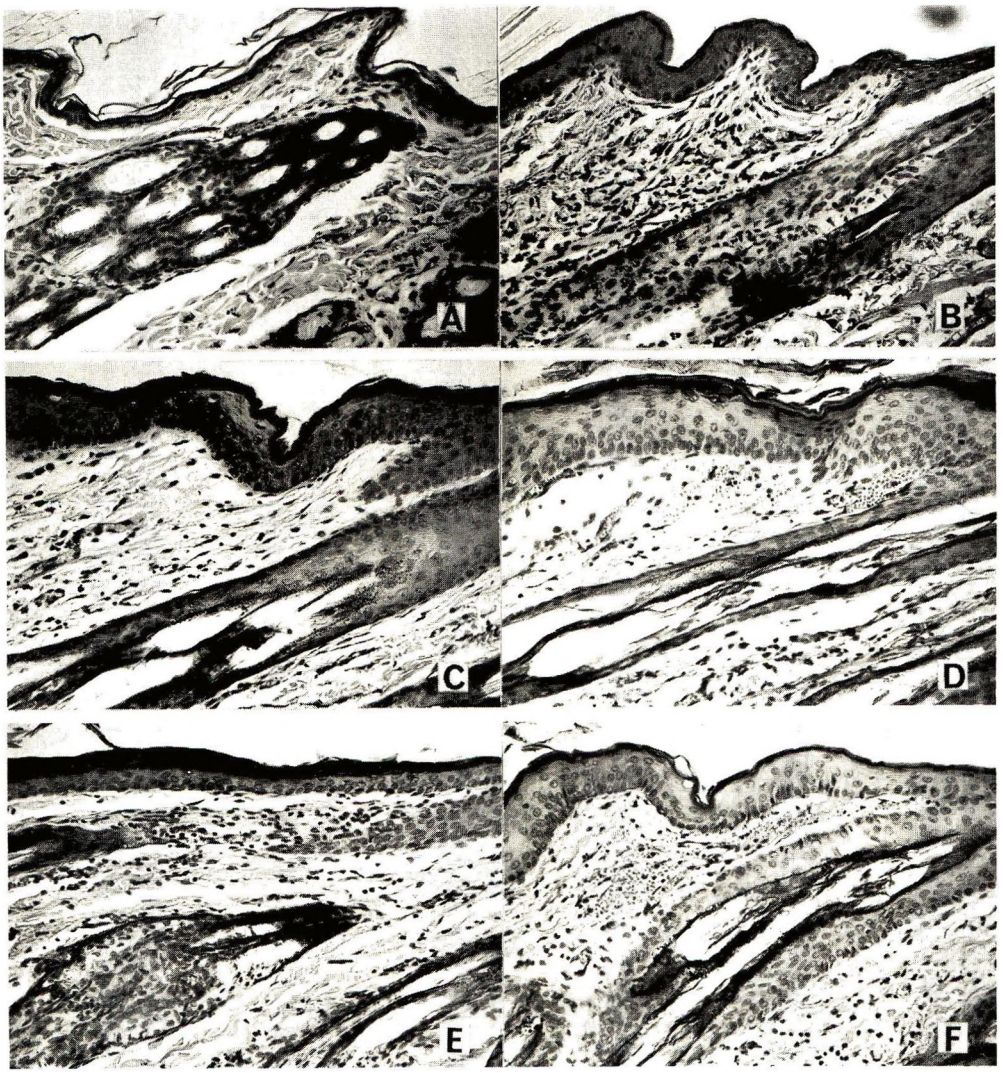

Figure 8. Light micrographs showing the histological feature within the skin of Angora rabbits at various times after 24 hr topical application of ^{14}C-IPM. A) intact skin, B) zero day, C) one day, D) three days, E) six days, F) ten days. The changes of acanthosis in the epidermis and edema, vasodilatation and hemorrhage in the dermis were observed

days and continued until ten days. The sebaceous glands were found swollen and large through the period of the experiment (Figure 10).

METABOLIC FATE AFTER INTRADERMAL ADMINISTRATION

The metabolites were extracted from the skin specimens in which ^{14}C-labelled IPM or HDO was injected. One tenth of the extracts for each specimen was taken for the liquid scintillation counting. Figure 11 shows the recovery percentage of labelled oils

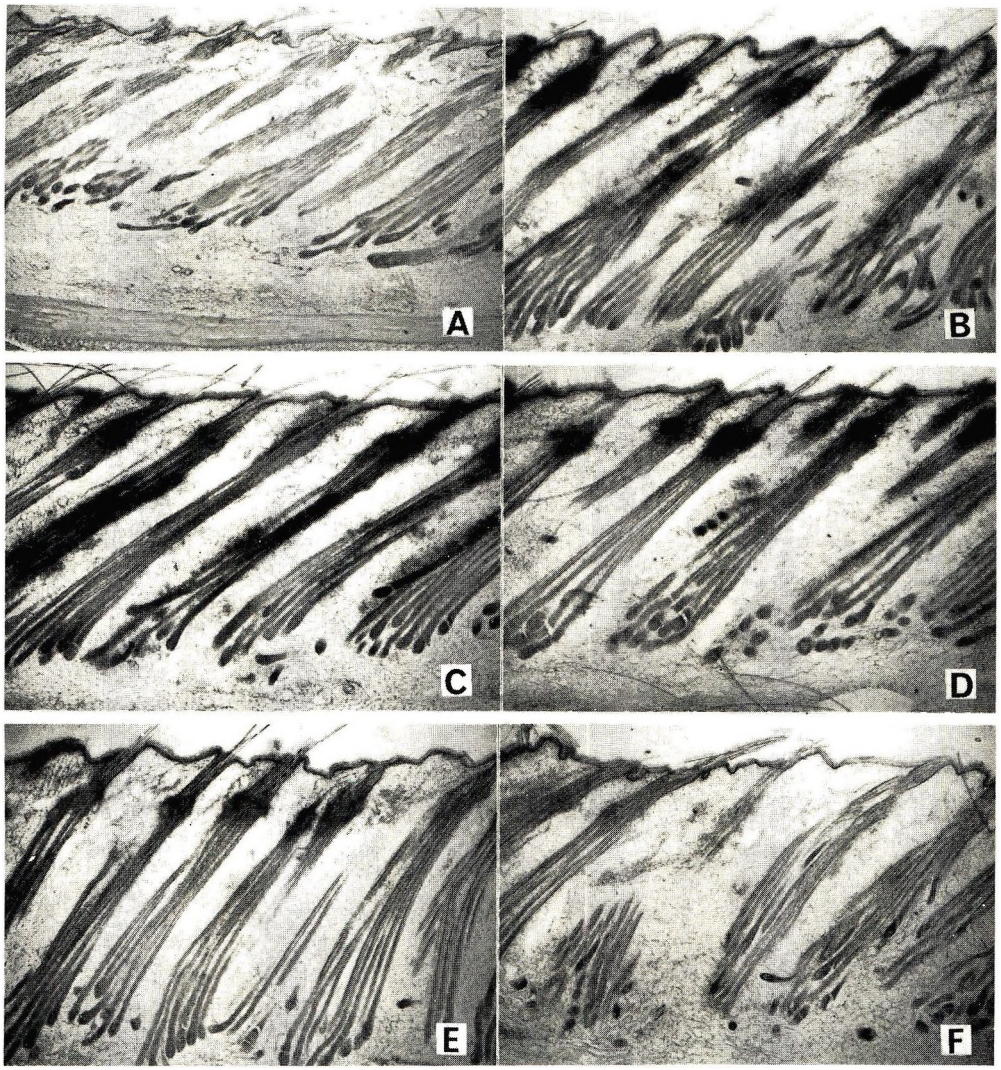

Figure 9. Microautoradiograms showing the distribution of radioactivity within the skin of Angora rabbits at various times after 24 hr topical application of ^{14}C-HDO. A) intact skin, B) zero day, C) one day, D) three days, E) six days, F) ten days. Note the changes of concentration in the sebaceous glands and dermis

plotted against time, which indicated that radioactivity remaining in the injected site was reduced to 43% at 24 hr and 20% at 72 hr for IPM, while HDO remained 42 to 43% at 24 and 72 hr.

Figure 12 shows the scanning curves of thin layer radiochromatograms of the extracts where no metabolites were found even after 72 hr for both oils. These results suggested that irritation was caused by the applied substances themselves and not by their metabolites in the case of IPM and HDO.

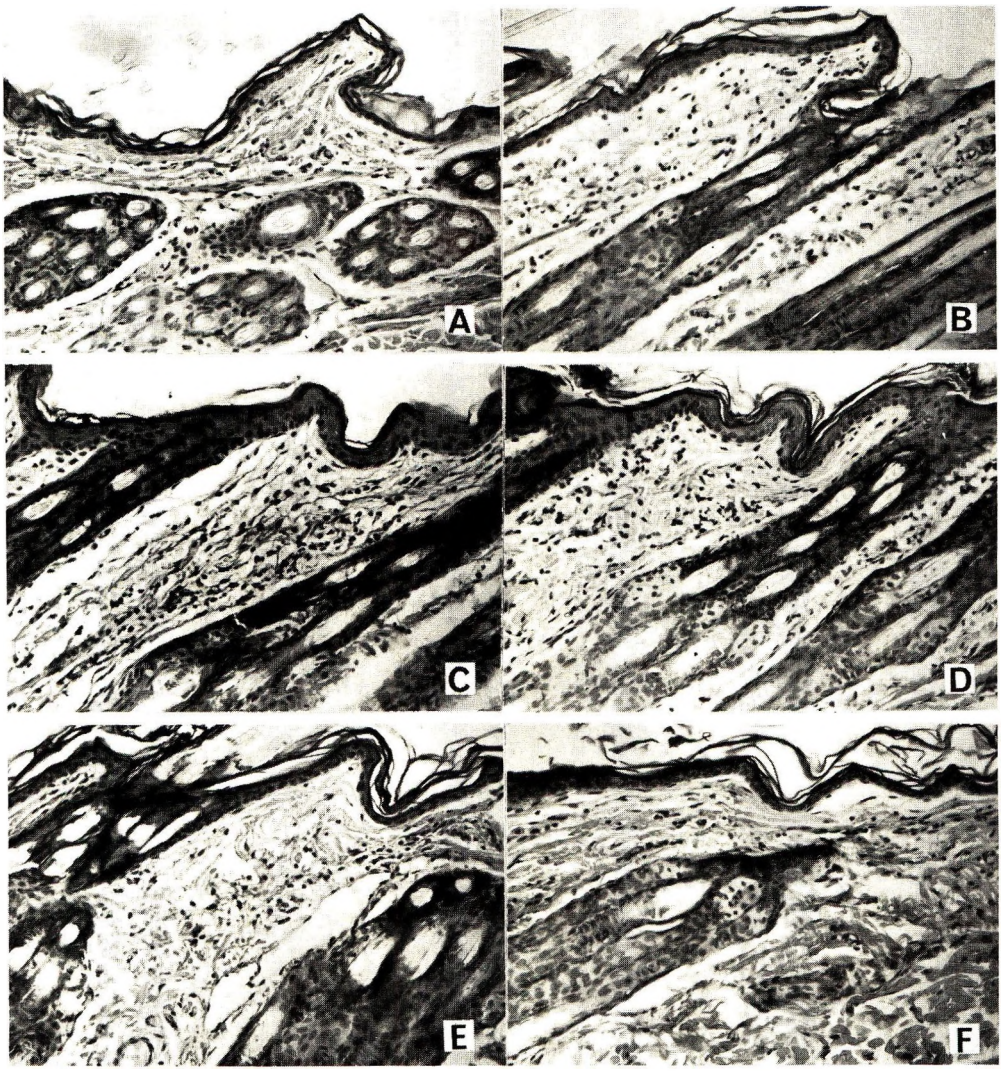

Figure 10. Light micrographs showing the histological feature within the skin of Angora rabbits at various times after 24 hr topical application of ^{14}C-HDO. A) intact skin, B) zero day, C) one day, D) three days, E) six days, F) ten days. The changes of acanthosis in the epidermis and edema and vasodilatation in the dermis were observed

DISCUSSION

Whole body autoradiography is a useful method since it can visualize the behavior of an applied material in the body and its possible metabolic routes then estimated. Figure 13 from our previous paper (20) showed autoradiograms of percutaneously absorbed ^{14}C-hexachlorophene where the isotope was accumulated in the liver and excreted through the gallbladder to the small intestine at 6 hr after topical application. Such a well absorbed material gives a clear autoradiographic feature. However it is not available for the materials of low percutaneous absorbability as shown in Figure 1. Besides,

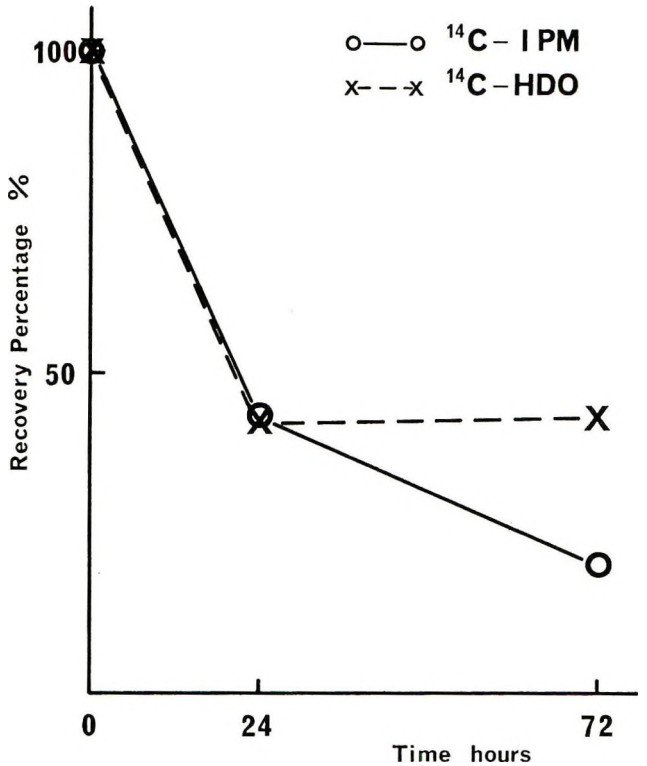

Figure 11. Recovery percentage of ^{14}C-labelled oils injected intradermally, plotted against time

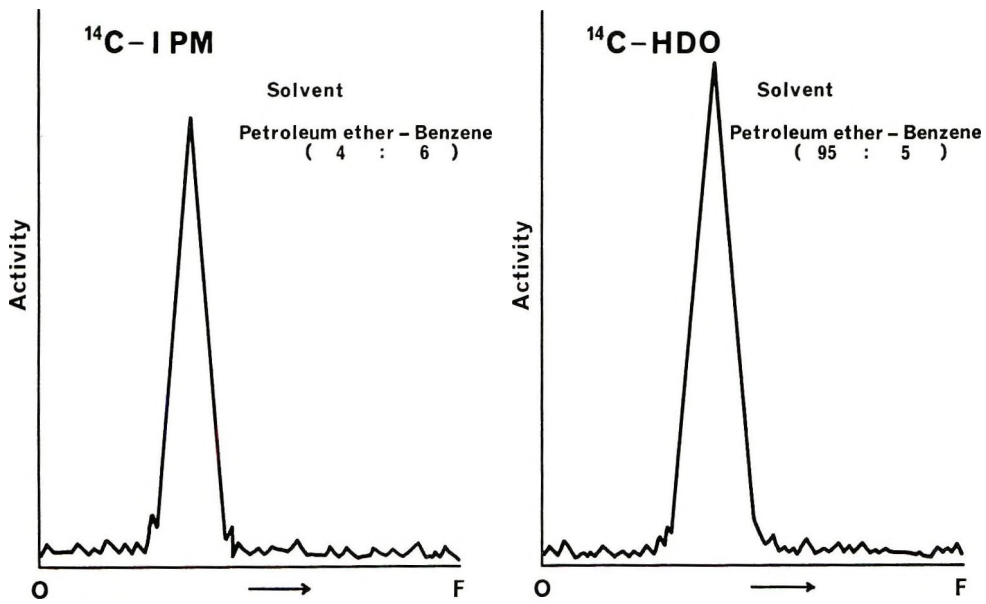

Figure 12. Scanning curves of thin layer radiochromatograms of extracts from skin specimens

Figure 13. Whole body autoradiograms showing the distribution of radioactivity in hairless mice after topical application of ^{14}C-hexachlorophene in hydrophilic ointment. A) 1 hr, B) 6 hr. After 6 hr, the radioactivity was distributed in the liver, gallbladder and small intestine

penetration routes of chemicals into the skin or their interactions with the skin cannot be examined. It is not possible to compare the absorbability of cosmetic oils of low permeability with each other.

From these viewpoints, microautoradiography was introduced to investigate and elucidate the percutaneous absorption of five oils with guinea pigs. The absorbability which could not be seen by whole body autoradiography was found to decrease in the following order by this method: IPM, GTO, OD, DD and HDO (Figure 2). The comparison of skin irritation potentials was attempted in this experiment by macroscopic observation of erythema. As shown in Table I, the skin irritation potentials were in agreement

Table I

Response of Guinea Pig Skin with Oil

substance	erythema 6 hr	erythema 24 hr
Isopropyl myristate	+	+ - ++
Decanoxy decane	±	+
n-Octadecane	±	±
Glyceryl tri-(oleate)	±	–
2-Hexyldecanoxy octane	–	–

Criteria
– no erythema
± slight erythema
+ moderate erythema
++ severe erythema

with the absorbabilities observed by microautoradiography. Therefore it is assumed that the penetration of a material is a necessary condition, though not sufficient in itself, for the occurrence of irritation.

More detailed investigations with Angora rabbits were made on the highest and the lowest absorbed oils to compare them and study the relationship between permeability and irritation. The relationship between accumulation or disappearance of the oils in the skin and occurrence or disappearance of irritation was also an interesting problem. Figures 3 to 6 showed that IPM revealed much more severe irritation than HDO did, though both of these oils penetrated into the skin. As shown in Figures 4 and 6, the irritation patterns of the two were rather different. Namely, IPM induced acanthosis, edematous degeneration of the collagen fibers and changes in the blood vessels, while HDO gave rise mainly to edema in the dermis. Figures 7 and 9 suggested that IPM had a good affinity for the epidermis and the hair follicles and showed a distinct boundary between the dense and sparse distribution of grains, while HDO had a continuous distribution from the epidermis or the sebaceous glands to the adjacent dermis. The grains of both oils increased a little after one day, but gradually disappeared from the skin during the experiment. IPM showed a higher skin irritation potential than HDO did also from the results of macroscopic observation of erythema and microscopic investigation of histology (Figures 8, 10). Since both oils are found to penetrate into the skin, the penetration of a material is not the sufficient condition for the occurrence of irritation. It is evident that the reaction potential of the penetrated material with the skin is the most important factor. The results in Figures 7 to 10 suggested that there existed a time lag from the arrival of the penetrated material at the living cells to the revelation of inflammation.

Difference in permeability between the animal species was also observed. The oils were more permeable into the skin of Angora rabbits than into that of guinea pigs. Bartek *et al.* showed in their comparison study of animal species (rat, rabbit, pig and man) that drugs penetrated most into rabbit skin and least into human skin, and that the permeability of the skin of miniature swine was the closest to that of human skin (21). IPM was found to penetrate into the skin of miniature swine by microautoradiography in our laboratory (22). According to these findings, this oil is thought to penetrate into human skin also, though IPM is known to generate no erythema on the skin of either miniature swine or man (23). Therefore observation of erythema alone is not sufficient for the safety of cosmetic chemicals.

Though the oils that penetrated into the sebaceous glands and the epidermis were seen to eject out of the skin, those in the dermis could not move out in this manner. Did they remain in the dermis or were they carried away by the blood circulation? Microautoradiography could not answer this problem clearly. Intradermal administration of the oils partly elucidated the problem showing that IPM became decreased gradually from the dermis; HDO tended to remain there, though both were not metabolized in the dermis (Figures 11, 12).

From the unpublished data of the authors, IPM was found to be distributed into almost all organs by means of whole body autoradiography when it was injected subcutaneously into mice. The extracts from the liver and kidney were determined as a fatty acid and a triglyceride. On the other hand, HDO was located in the subcutaneously applied site and not distributed into the body organs. These indicated

that HDO was localized in the skin and resistant to metabolization while IPM was easily distributed into the organs and metabolized.

When cosmetic bases are considered relative to their safety, their reactivity with or their localizing nature in the skin should be studied simultaneously with their macroscopic skin-irritation properties.

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Noninvasive, rapid characterization of human skin chemistry in situ

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Synopsis

The experimental yield of internal reflection spectroscopic and contact angle techniques applied to LIVING HUMAN SKIN is demonstrated for natural, cosmetically treated and wounded epidermis. The critical SURFACE tension and chemical composition of clean human skin surfaces is provided, along with spectral data bearing on the efficacy and quality of cosmetics, the depth profile of skin moisture, and the CHEMICAL NATURE of exudates from epidermal wounds.

INTRODUCTION

Professional groups, individual research chemists, consumer safety advocates and legislative bodies are increasingly calling attention to the potential hazards, and unverified claims for quality and efficacy, of cosmetic and therapeutic products applied to skin surfaces. Despite the pressures generated, even modern industrial research laboratories assessing the influence of various reagents on human skin continue to assume the end effects of frequent skin contact with certain chemical types without proof of the validity of such assumptions. For example, surfactant-containing liquids are assumed to damage human skin by excessive removal of skin lipids, deposition of the surface-active agents themselves (upon or immediately subjacent to the skin surface) and denaturation of the proteinaceous structures in the epidermal layers. These actions are supposed to lead to the abnormal features of human skin encompassed by clinical symptoms of roughness, scaliness, unsightly wrinkling, irritation and dermatitis.

Since many of the functional and aesthetic qualities of human skin are attributed to the skin moisture balance, a continuing goal of most cosmetic "moisturizing" preparations is to limit or prevent transepidermal moisture losses. Here, also, the *in situ* functions of the main ingredients of such preparations have not been convincingly demonstrated—merely assumed—to play a role in moisture retention, rather than simply lubricating and/or plasticizing the skin through ester imbibation. It is no longer necessary to accept without proof such assumptions in the development and certification of benefits for cosmetic or skin-healing products. Direct, noninvasive, relatively

inexpensive and widely applicable methods for assessing the fate and consequences of materials applied to human skin *in situ* are now available.

Some of the situations prompting wider application of these methods are the following. Concerns have been expressed by the American Medical Association that traditional vegetable and animal cosmetic products are safe but inefficient, and new products derived from synthetic compounds are capable of inducing dermatitis more readily (1). These concerns can be addressed by direct experimental techniques. Hazel Bishop has cautioned that, although necessarily safeguarding their existence by "the bread and butter techniques available to them," manufacturers of cosmetics who wish to share in the continued growth of the cosmetic industry must perform additional serious scientific research "which holds the key to the prestige—and the profits—which go with the dramatic new scientific find" (2). The type of research introduced here fits one of Bishop's categories "more likely to pay-off": creation of a chemical product to which one can validly apply exciting, yet unexploited claims, attracting potential customers. Even if the motives of professional concern and enhancement of profit were not operating, the mounting pressures from consumer research organizations, supplying well documented arguments to receptive committees of federal legislators, force the increased attention of cosmetic and skin treatment producers to unambiguous proofs of their product safety and efficacy.

In particular, the generally held belief that cosmetics stay on the skin, reflecting the impenetrable nature of the stratum corneum surface, has been convincingly challenged by the recitation of research results showing very high systemic absorption of topically applied chemicals; the protection of cosmetic preparations from the scientific and regulatory scrutiny that many other products have been subjected to will no longer be tolerated (3). Citing the routine exposure to skin-damaging strong soaps and detergents, one recent legislative brief also challenged the new generation of synthetic plastic resins (in skin-stripping agents and cosmetic "beauty masks") as further deteriorating already permeable skin (3), suggesting these products be banned by the Food & Drug Administration. Techniques for judging epidermal retention of such materials are described here. Natural polymeric extracts now incorporated in various cosmetic and wound-healing preparations, including proteoglycan preparations claimed to have both cosmetic and wound-healing properties (4), may also be more carefully studied *in situ*. The subject of epidermal wound healing has received more attention than has the subject of cosmetic improvement of human skin (5), but advances in the *in situ* evaluation of chemical influence on skin quality (6) and of measurement *in vivo* of the skin's moisture balance (7) suggest a growing willingness of the cosmetic industry to address the very real problems at hand.

The main purpose of this report is to expand the use and acceptance of two additional *in situ* methods for noninvasive characterization of skin chemistry (before and after the application of cosmetic preparations, or in various states of damage) by demonstrating the actual experimental yield of these methods in both cosmetic testing and wound-healing contexts.

METHODS AND MATERIALS

All of the measurements reported here were carried out in a constant temperature—constant humidity, clean room, free of dust and maintained at 21°C and 40% relative

humidity. Volunteer human subjects whose skin was to be characterized were, after their skin cleansing by a standardized technique described below, allowed at least 30 min equilibration with the clean room's atmospheric conditions prior to the initiation of any measurements on their skin. Contact angles with diagnostic fluids of known high purity and representing a range of surface tensions, molecular sizes and relative polar-to-dispersion-force interaction potentials, were measured and plotted according to the widely accepted techniques of Zisman (8). The resulting contact angle data plots yielded values of "critical surface tension" known to correlate well with the true outermost atomic constitution of organic surfaces, with their coefficients of friction, their qualities of adhesion and degrees of roughness (9). Similar studies of almost a decade ago, which were the pioneering efforts using contact angle techniques applied to cosmetically modified human skin, were mostly limited to measurements with droplets of water and acetone/water mixtures. These early studies showed striking relative differences in skin quality after various cosmetic treatments or cleaning procedures, but probably did not yield accurate data for the actual surface energetics of "clean" and modified skin surfaces (10).

Surface-specific infrared spectra of the natural, cosmetically treated, wounded and subsequently healed skin of volunteer human subjects were acquired by the internal reflection spectroscopic technique described by Harrick (11). A special mirror assembly, which was made to order for our purposes (12), allowed the mounting of internal reflection prisms in a horizontal plane rather than in the vertical plane common to all other internal reflection spectroscopic accessories. The necessary internal reflection prisms, constructed of the thallium bromide salt, KRS-5, or of pure germanium, were acquired from the same source (12). Infrared spectra were recorded on both Perkin-Elmer Models 457 and 700 spectrophotometers, but only traces produced on the latter instrument are included here to allow ready intercomparison among the many figures provided. The cosmetics that were spectrally characterized both before and after their application to human skin were of internationally distributed brands and were applied according to the written instructions provided by the suppliers. They are representative of products with similar generic functions widely distributed by the cosmetic industry. Epidermal skin profiling in depth, after the induction of mild epidermal wounds, was accomplished by the "Scotch tape stripping" technique commonly used in the study of epidermal wound healing (5). Prior to the initiation of any experimental series, the skin areas to be tested were gently washed with a standard liquid hand soap, thoroughly rinsed, towel dried and allowed to equilibrate with the clean room atmosphere.

Figure 1 illustrates the quite comfortable posture taken up by the volunteers during the recording of infrared spectroscopic signatures of their natural and treated skin zones. Contact angle measurements were obtained on the lateral regions of the same subjects' fingers, or the ventral surfaces of their forearms, supported—as in the spectroscopic device—on a horizontal stage of a contact angle goniometer. Figure 2 provides schematic drawings of the position of the horizontal prism mount in a recording spectrophotometer, together with sketches of the ventral surface of a subject's forearm upon the prism face during analysis, and an indication of the material transferred (insensible perspiration, cosmetic residues, cellular debris and/or serous exudates from wounded tissue) on the prism face after the approximately 10-min period of contact required for recording of the entire infrared spectral trace. Figure 3 illustrates,

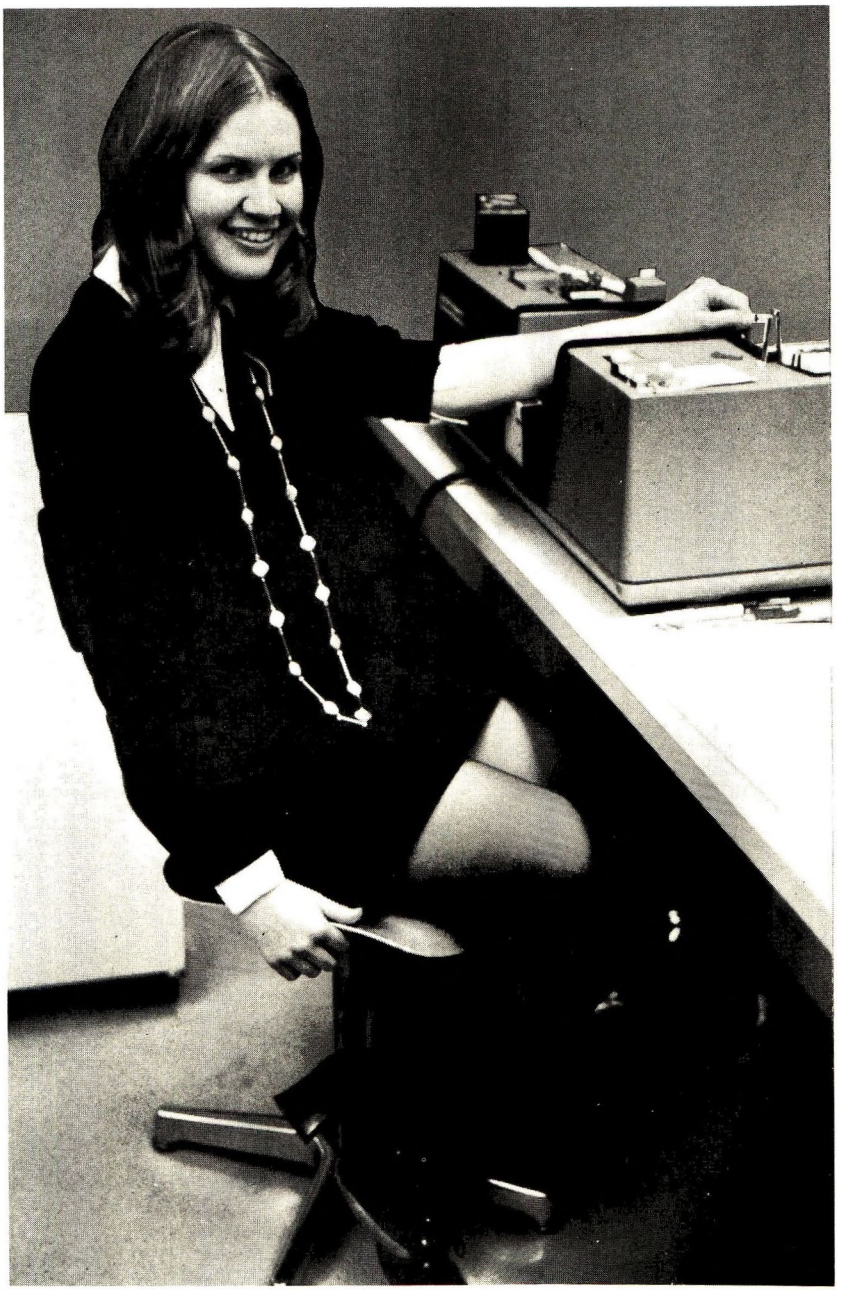

Figure 1. Illustration of the comfortable posture allowed for volunteer subjects during the rapid, noninvasive, analysis of their skin using a horizontal mount for multiple-attenuated-internal-reflection prisms. A similarly comfortable pose allows contact angle measurements on living skin which reveals the true outermost skin chemistry, before and after cosmetic application

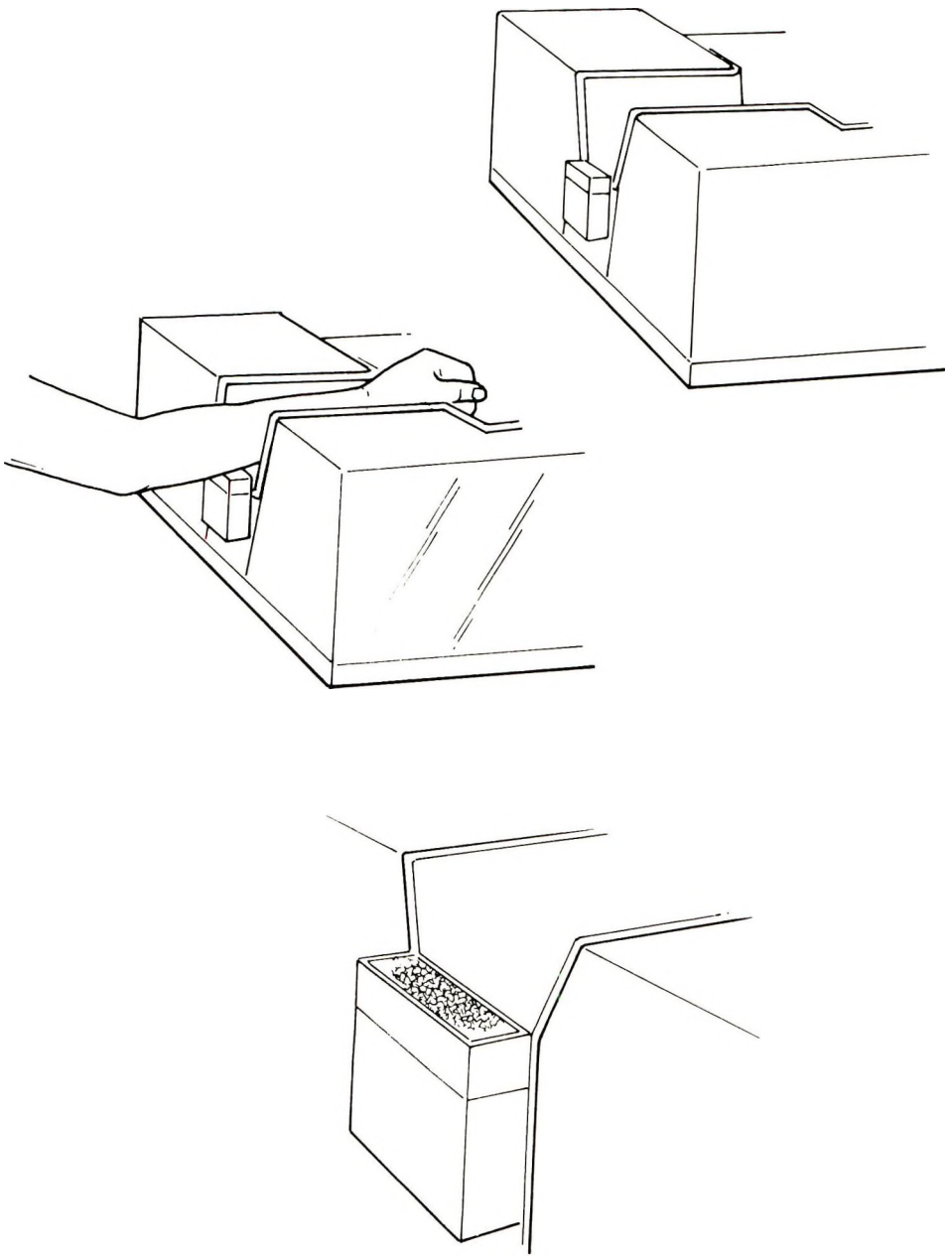

Figure 2. Schematic drawings of (top) the position of the horizontal prism mount protruding slightly from the sample space of a recording spectrophotometer, (center) of the forearm of a test subject in place for spectral analysis and (bottom) a close-up view of the residue usually left on the prism face for further analysis after the *in situ* skin chemistry has been ascertained

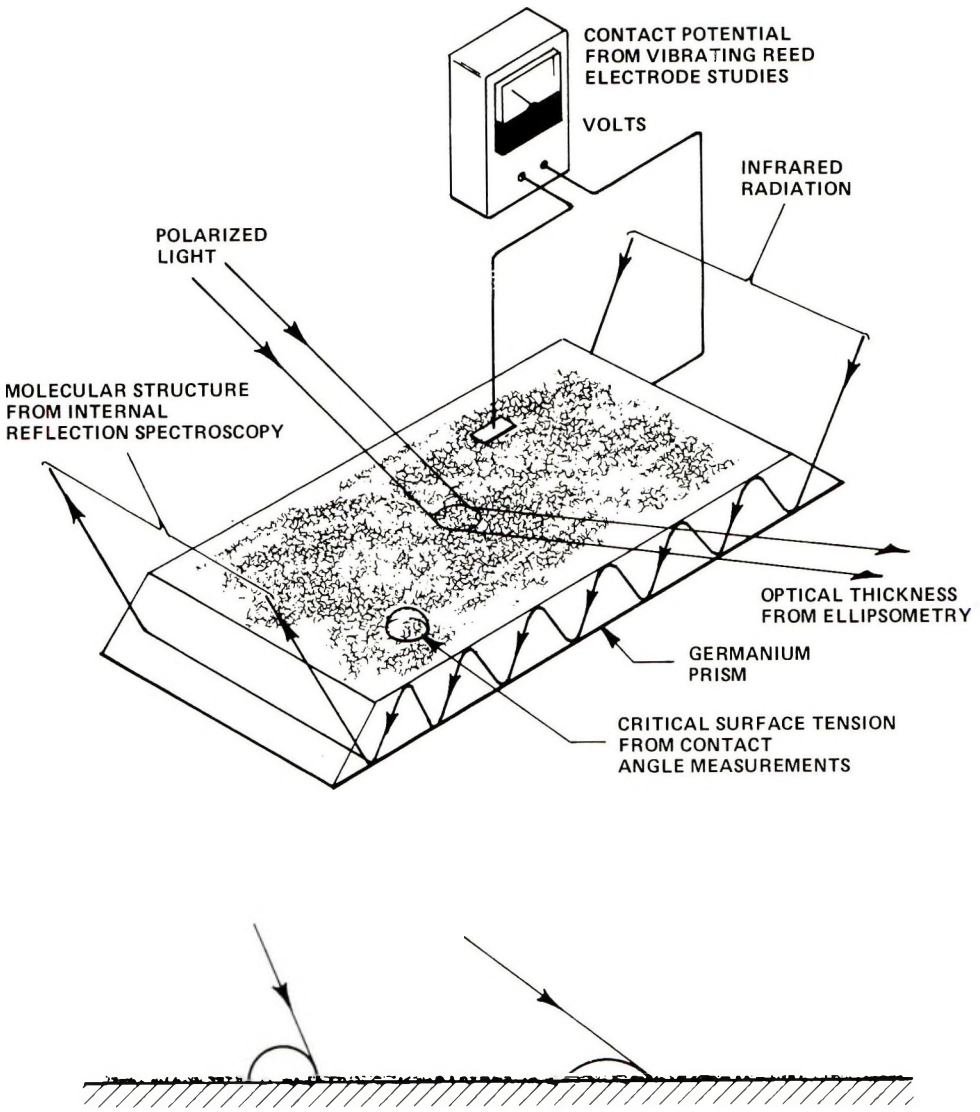

Figure 3. Schematic views of (top) the simultaneous application of four nondestructive analytical techniques which sensitively characterize skin residues or cosmetic films on germanium prisms and (bottom) varying contact angle profiles which can be observed on living skin, on skin deposits, or with varying cosmetic preparations to measure important features of wetting and spreading phenomena

schematically, nondestructive analytical techniques which can be utilized sequentially upon such a residue to characterize it by compositional criteria, organizational criteria (thickness and refractive index) and electrical character, in addition to the determination of the surface chemical composition and surface energetics by the simple measurement of contact angle profiles (of sessile liquid droplets on the residual films or on the skin surfaces themselves). Numerous applications of these simultaneous, nondestructive methods to biologically relevant surfaces have already been provided in a recent volume of the Advances in Chemistry series (13), including specific examples of

natural, cosmetically modified, old and freshly generated human skin *in situ*. Referring again to Figure 3, it should be noted that the internal reflection prism shown there is labelled as being constructed of germanium, an important requirement not only for the application of ellipsometry and contact potential measurements to the films in question but also assuring biological safety for the volunteer subjects especially when deliberate skin wounding was employed. Thallium bromide salts, such as employed in the more commonly used KRS-5 internal reflection prisms, can cause contact dermatitis and potential toxicity in such cases, while the biological acceptability of germanium seems much better (14).

RESULTS

WETTING AND SPREADING ON CLEAN HUMAN SKIN

Table 1 lists the variety of pure diagnostic liquids utilized in determination of the potential wettability and spreadability of cosmetic ingredients on clean human skin. These same liquids are useful in assessing the new surface condition of skin after treatment with specific ingredients in proofs of efficacy for cosmetic or medicinal preparations. In Figure 4, the average contact angle values obtained for these liquids on the surface of the skin of a male volunteer, after that skin had been cleansed with a liquid hand soap, well rinsed, towel-dried and equilibrated in clean room conditions, are plotted in the standard Zisman format (8, 9) to yield a critical surface-tension intercept near 38 dynes/cm. This is a typical value for uncontaminated, fibrous protein preparations and compares well with values already published for human skin, keratin, collagen and gelatin (15, 16). It might therefore be taken as a general case, for human skin treated by simple cleaning procedures as described here, that cosmetic or medicinal preparations having operational liquid/vapor surface tensions lower than 35 dynes/cm will wet and spread well upon those skin surfaces. Fluids with lower surface tensions will give excellent coverage and appearance, while cosmetic preparations with operational surface tensions greater than 35 to 38 dynes/cm will tend to bead up, retract, or leave interstitial voids on the same skin surfaces.

Table I
Wettability of Clean Human Skin, *In Situ*

Wetting Liquid and Surface Tension (γ_{LV}) (dynes/cm, 20°C)		Average Contact Angle (θ in degrees)
Water	72.8	65
Glycerol	63.4	66
Formamide	58.2	51
Thiodiglycol	54.0	54
Methylene Iodide	50.8	53
Sym-Tetrabromoethane	47.5	34
1-Bromonaphthalene	44.6	25
O-DiBromobenzene	42.0	18
1-Methylnaphthalene	38.7	24
Dicyclohexyl	33.0	0
n-Hexadecane	27.7	0

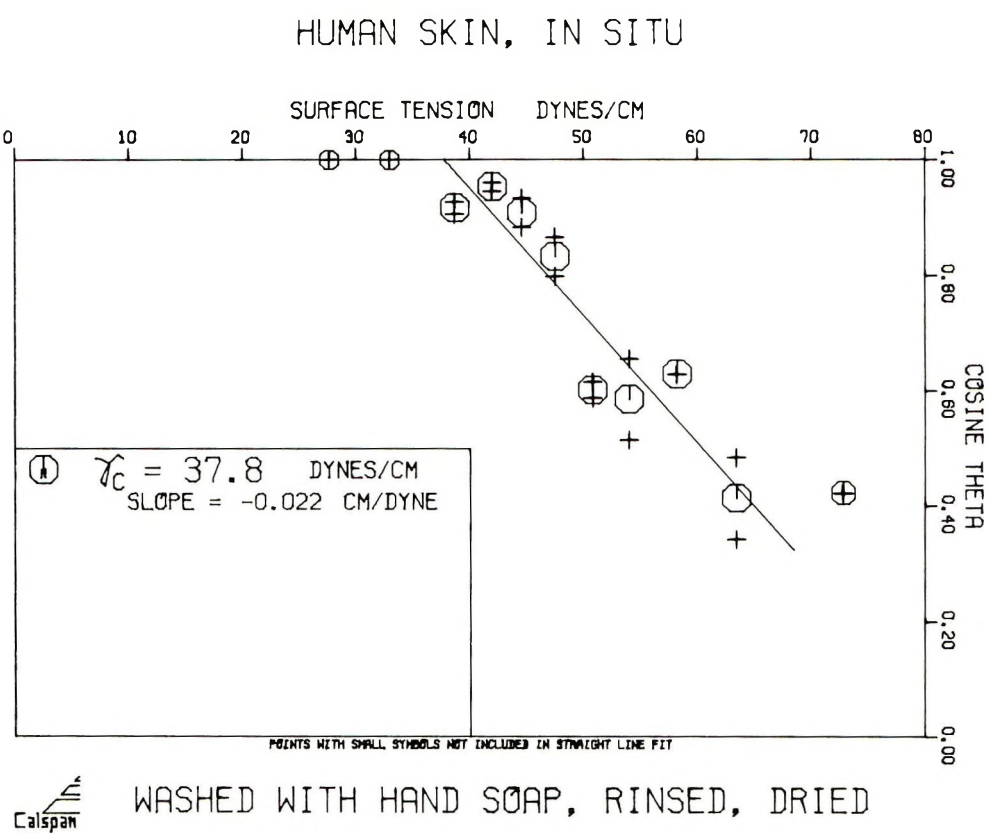

Figure 4. Contact angle data plot typifying the wettability and surface energy of human skin free of cosmetic preparations or greasy residues

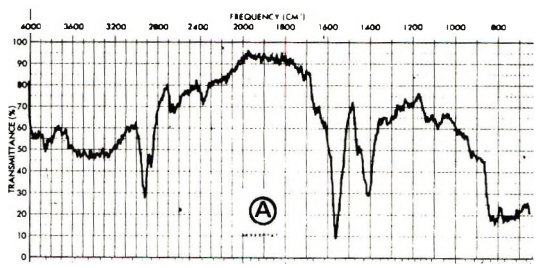

Figure 5. Infrared spectra illustrating the utility of internal reflection spectroscopy for the noninvasive evaluation of cosmetic preparations on human skin *in situ*. Compare with spectra of the cosmetics, alone, in Figure 6.

Ⓐ Residue of liquid hand soap used to establish "clean" skin conditions.

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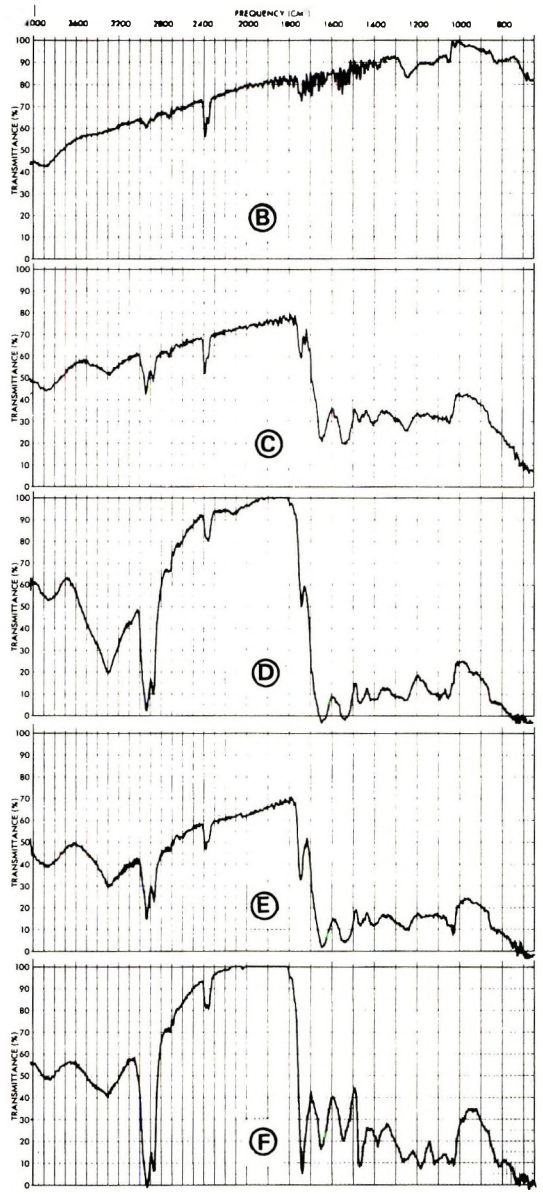

Ⓑ "Blank" prism baseline for an internal reflection plate constructed of the thallium bromide salt, KRS-5, 20 mm × 50 mm × 2 mm. Absorption bands shown are for trace hydrocarbon contaminant from prism polishing and from atmospheric CO_2 and H_2O unequally sampled between sample and reference beams of spectrophotometer.

Ⓒ "Clean" skin of forearm, female subject, resting upon face of "blank" prism of Ⓑ. Note absence of absorption bands which would indicate soap residues of Ⓐ.

Ⓓ Forearm skin of same subject, following use of cosmetic cleanser and skin toner rinse, according to instructions of supplier.

Ⓔ Forearm skin of same subject, after preparation as in Ⓓ, further treated with "skin mask" cosmetic preparation and "conditioner" preparation, again according to supplier's instructions.

Ⓕ Forearm skin of same subject, finally treated with "night cream" after "cleansing" and "conditioning" of Ⓓ and Ⓔ, following supplier's instructions.

EVALUATION OF COSMETIC EFFICACY AND QUALITY

Figure 5 presents a series of internal reflection, infrared spectra to illustrate the ease of applying this technique for noninvasive assessment of the results of cosmetics applied to human skin. The individual spectral traces in Figure 5 may be compared with the individual signatures of the cosmetics alone used in each treatment step characterized. At the top of the collection of traces in Figure 5, a spectrum of the residue left by the liquid hand soap used to establish the "clean" skin condition is provided, to demonstrate the band positions where soap residues on the skin might be detected in the infrared spectra. The second trace from the top of Figure 5 provides a "blank" absorption baseline for a typical internal reflection plate made from the translucent salt KRS-5 (a thallium bromide material), having external dimensions of 50 mm × 20 mm × 2 mm (thick). The only absorption bands seen in this "blank" baseline are for a trace hydrocarbon contaminant which becomes embedded in the prism surface during its optical polishing, and for the atmospheric carbon dioxide and water vapor of the space through which the analytical infrared beam travels. This beam is focused upon and collected from the bevelled prism edges by curved and planar mirrors in the internal reflection accessory that allows these spectra to be recorded. The third trace from the top of Figure 5 is a "clean" skin spectrum obtained by direct analysis of the lower forearm region of a female subject. The forearm was resting, under natural gravitational force, on the face of the prism whose "blank" baseline is given in the trace immediately above. The major infrared absorption bands shown are clearly those of the skin lipid and protein constituents. No evidence of the fatty-acid-soap absorption bands (as characterized in the top trace of Figure 5) is seen. Inspecting the spectral trace for clean human skin *in situ*, from left to right, one notes the following infrared absorption bands: at 3300 cm^{-1} for the N-H resonance; two sharp peaks centered on 2900 cm^{-1}, correlating with C-H groupings of most organic matter; a sharp absorption band between 2350 and 2400 cm^{-1}, for the atmospheric carbon dioxide also sampled by the infrared beam as it reflected from mirror to mirror in the internal reflection accessory; a sharp absorption band at about 1740 cm^{-1}, which is diagnostic for the presence of organic esters; the Amide I and II absorptions at 1640 and 1540 cm^{-1}, respectively, which are specific indicators of proteins; and, at frequencies lower than 1500 cm^{-1}, a group of confirming absorption bands on a generally climbing background, for the primary organic groupings already listed.

Just below the *in situ* characterization of the clean skin of our volunteer is another trace of that same skin area as spectrally characterized immediately following the use of a cosmetic cleansing agent and a skin toner rinse (according to the instructions of the cosmetic supplier). A significant increase in the proportion of hydrocarbon components, most likely from the mineral oil fraction of the skin cleanser preparation, is noted and should be compared with the top trace of the following Figure 6. Except for that evidence of imbibation of aliphatic organic matter, there is no further spectral evidence suggesting dominance of the skin epidermal layers (or masking of these layers) by any residual overcoat of either the cleansing agent or polyol skin toner. Rather, the exceptional increase in absorption strength for the N-H band, and for the Amide I and Amide II bands, of the skin's protein, together with a relatively constant proportion of fatty ester, as compared with untreated skin, illustrates more effective contact of the skin surface with the analyzing prism surface. This improved contact, allowing more efficient analysis of the skin layers and a greater effective "penetration

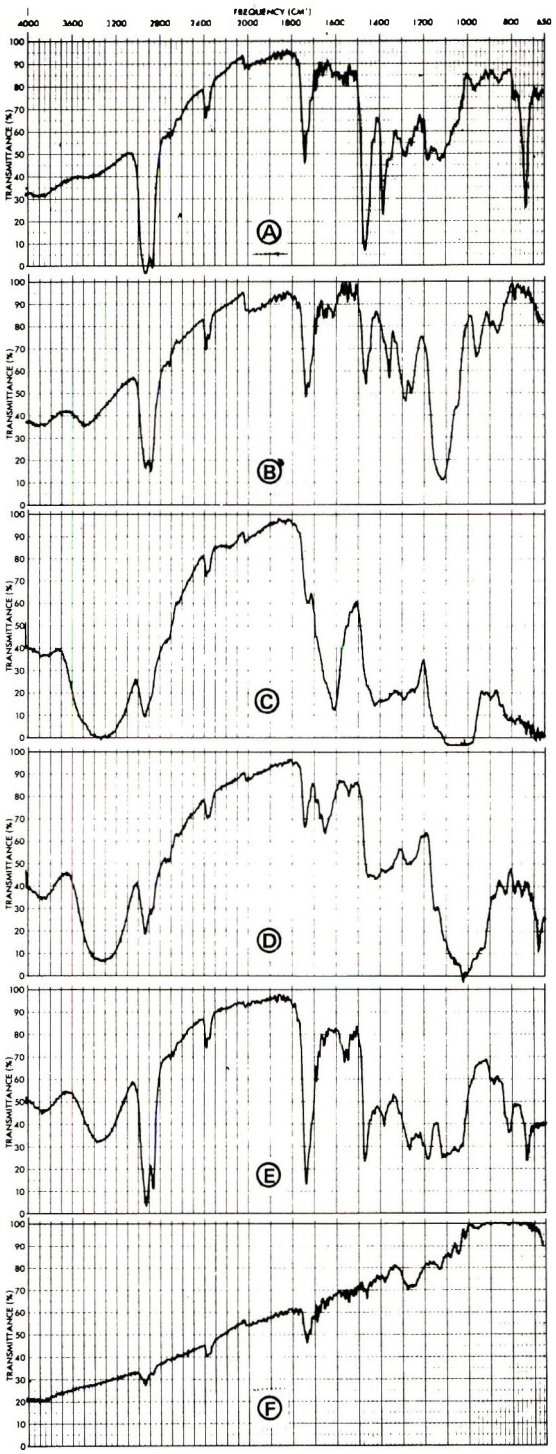

Figure 6. Internal reflection infrared spectra of cosmetic preparations as supplied. Compare with effects on human skin, *in situ*, as characterized in Figure 5. All samples applied directly to face of KRS-5 prism.
Ⓐ Skin "cleanser" Ⓑ Skin "toner" Ⓒ Skin "mask" Ⓓ Skin "conditioner" Ⓔ "Night cream" Ⓕ "Blank" prism baseline, after completion of a large series of both skin and cosmetic analyses. Note degradation of transmission, resulting from roughening of prism surface during multiple cleanings, as compared with starting baseline (see trace Ⓑ of Figure 5).

depth" of the electromagnetic sensing waves, is a consequence of use of the cosmetics. The cosmetics applied assisted in the removal of rough, loosely attached stratum corneum fragments and also improved the refractive index matching across the prism/skin interface by leaving the residual components of the formulations. It is significant to note, here, as will be confirmed in later figures, that no apparent change in the skin "moisture" level is demonstrated after this cosmetic treatment. The second lowest trace of Figure 5 characterizes once again the forearm skin of our volunteer, but now after further treatments with a "skin mask" cosmetic preparation and a skin "conditioner" (according to the suppliers' instructions for the intended use of these products). This infrared spectrum reveals, with the exception of some residual hydrocarbon and fatty-ester components imbibed into the epidermal layers, the return of the skin quality to, essentially, its pretreated state. This suggests that the polyol-containing "mask" and "conditioner" formulations did little besides remove excess hydrocarbon and (seemingly weakened) epidermal layers affected by the "cleanser" and "toner" formulations. Finally, the lowest infrared spectrum included in Figure 5 characterizes the forearm skin of our subject as treated with a "night cream" after its "cleansing" and "conditioning" according to the cosmetic supplier's instructions. As the spectra collected in Figure 6 show, "moisturizing" cream has the highest fatty-ester content of any of the cosmetics used in this series. It is, in fact, these fatty esters which are taken up into the skin to soften, lubricate, or plasticize it. The only indication of a truly increased "moisture" content (that is, additional water retention) of the skin is the slight skewing of the N-H absorption band in the lowest trace of Figure 5. This band, normally symmetrical about 3300 cm^{-1}, is shifted towards the 3400-cm^{-1} absorption maximum which characterizes the O-H resonance of retained hydroxyl or water groups. The continued observation of intense absorption bands in the Amide I and Amide II region of the spectrum illustrates the "night cream" to have been taken up into the epidermal layers rather than to be lying in a thin film above them. In the latter case, even a film thickness of a few microns would significantly damp the analytical sensitivity to proteinaceous layers beneath them.

Some judgment of the actual influence of a number of cosmetic products on human skin *in situ*, examined without distress to the volunteer subject and in a rapid, noninvasive manner, is thus easily accomplished by the internal reflection, infrared spectroscopic technique. The second important feature of the method, beyond its ability to evaluate skin before and after its modification, is the method's suitability (without modification) for the direct analysis of the cosmetic preparations themselves. The technique does not require their dilution, extraction, or complex processing in any manner beyond that experienced in their normal application to the skin. Figure 6 collects internal reflection, infrared spectra of neat cosmetic preparations (as supplied and applied directly to human skin with the outcome characterized in Figure 5). All of the samples were applied directly to the face of the same KRS-5 prism used in recording the skin spectra. The top trace of Figure 6 characterizes the skin "cleanser" formulation. Scanning from left to right across this trace, it is seen that the major infrared bands are for hydrocarbon components at 2950 and 2850 cm^{-1}; fatty esters, at 1740 cm^{-1}; confirming absorptions for hydrocarbons at 1470 and 1380 cm^{-1}; and confirming absorptions for the ester components at 1280, 1180 and 1130 cm^{-1}. A final confirming absorption band for the long chain, aliphatic hydrocarbons present in this formulation appears at 740 cm^{-1}. On the basis of numerous similar analyses, primarily of pure known compounds and their mixtures, the top spectrum in Figure 6 would be compati-

ble with the following typical formulation for skin cleansing products: mineral oil, 50%; beeswax, 10%; lanolin, 4%; and fatty acid esters used as emulsifiers, 1%. The approximately 35% fraction of water in the original formulation is not revealed in this infrared spectrum since the sample was allowed to dry "naturally" on the prism face, as it would upon the skin, prior to the analysis. Spectral traces recorded immediately after applying cosmetics to the prisms do show intense absorption bands for water, but these quickly disappear. The second trace from the top of Figure 6 characterizes the skin "toner" preparation. It contains, in addition to the mineral oil and fatty-ester components common to many cosmetic formulas, a polymeric ingredient of highly polar character as indicated by the strong absorption band in the region between 1100 and 1200 cm^{-1}. Similarly, the third and fourth traces from the top of Figure 6 present internal reflection infrared spectra of the skin "mask" and "conditioner" products, respectively, actually used by our volunteer subject. Noting the strong absorption bands in the regions between 3000 cm^{-1} and 3600 cm^{-1}, and between 1000 cm^{-1} and 1200 cm^{-1}, both indicative of the O-H bond containing groups of polyols, it can be readily seen that these products contain highly hydroxylated components. The next-to-last spectrum of Figure 6 characterizes the "night cream" preparation which was used, showing a band sequence and relative intensity ratio which indicates this typical composition for such products: stearates and palmitates, 3%; mineral oil, 3%; fatty alcohols and other hydroxylated materials (such as poly propylene glycol), about 5%; and both animal and vegetable fatty esters, about 6%. The remaining quantity, water, had dried from the preparation before its analysis. The lowermost trace of Figure 6 presents once again a "blank" baseline spectrum for a KRS-5 internal reflection prism. This prism was used throughout the skin analysis series and also a series of direct characterizations of different cosmetics, including those represented in both Figures 6 and 7. It is easily noted, comparing this final trace of Figure 6 with the "blank" baseline for the same prism at the top of Figure 5, that there has been some degradation in the transmission of infrared energy through the prism. This is especially apparent at the higher frequencies which are more subject to scattering losses caused by degradation of the optical quality of the prism's surface. Soft KRS-5 salt prisms are more easily damaged during multiple, between-sample cleaning operations (using cotton swabs and acetone to remove deposits on the prism face) and more susceptible to "etching" by water than are the germanium prisms preferred for this work.

Figure 7 collects additional infrared spectra of typical cosmetic products having the following general purposes: facial washing cream, hand cream, deep cleanser, suntan cream, moisture cream and a perfumed ointment. The facial washing cream is characterized by an internal reflection spectrum compatible with a formulation containing approximately 50% mineral oil, 15% beeswax, 5% emulsifiers and 30% water (which evaporated prior to recording this trace). The hand cream spectrum presented in Figure 7 would be compatible with a formulation having this typical composition: stearic acid, 15%; sorbitol and fatty alcohols, 10 to 12%; and triethanolamine, 2%; the remainder (nearly 72% water) dried from the preparation prior to this analysis. The "deep" cleansing product provides an infrared spectrum consistent with the following proportion of ingredients: isopropylpalmitate, 2%; saturated fatty acids (such as stearic acid), 2%; unsaturated fatty acids (such as oleic acid); polyols (such as sorbitol), 2%; triethanolamine, 1%; and the remaining 80+% of the product water evaporated from the specimen prior to its analysis. The most complex of the infrared spectra collected in Figure 7 is for the suntan cream, where the numerous sharp absorption bands reflect

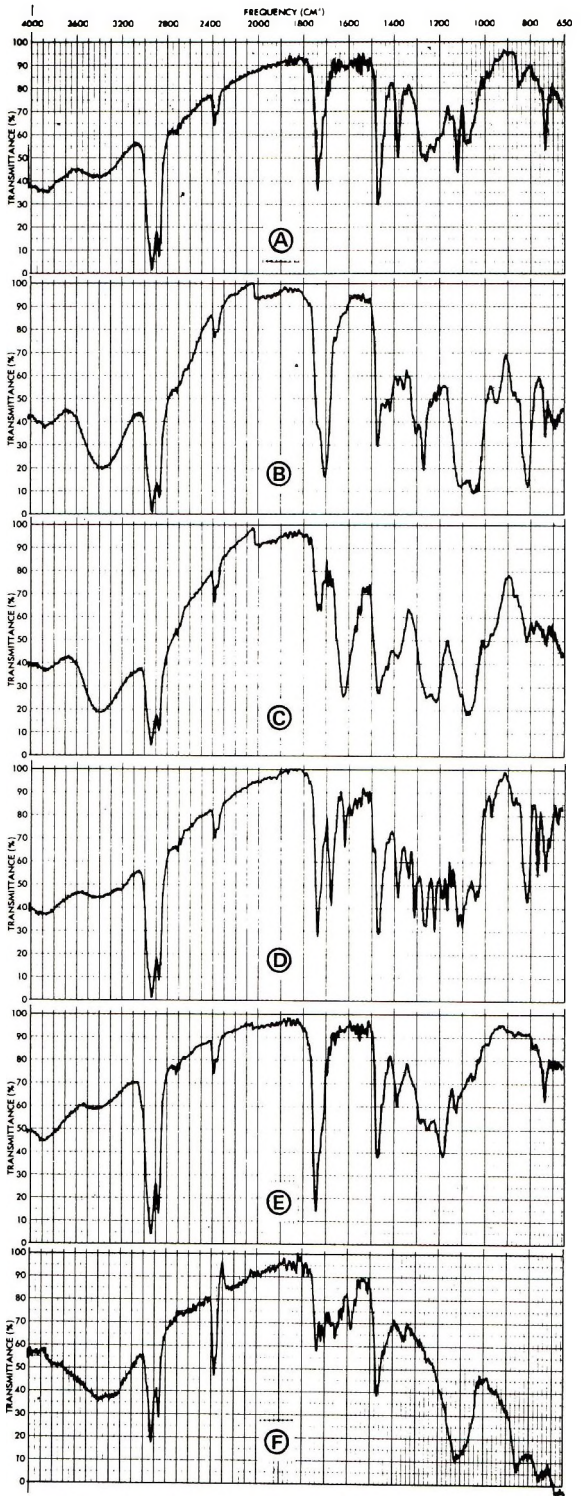

Figure 7. Additional infrared spectra of typical cosmetic products, applied directly to the face of a KRS-5 internal reflection plate.

Ⓐ Facial washing cream Ⓑ Hand cream Ⓒ "Deep" cleanser Ⓓ Suntan cream Ⓔ Moisture cream Ⓕ Perfumed ointment.

the presence of a large quantity (about 5%) of an active sun screening agent such as isobutylparaamino benzoate, along with about 10% fatty esters. The vehicle for this preparation, about evenly split between alcohol and water, quickly evaporated away. The moisture cream spectrum reveals a formulation of fatty ester materials in an aliphatic hydrocarbon base. This spectrum was closely matched by a test formulation containing some 25% of hydrogenated vegetable oil, 20% mineral oil, 10% beeswax, 3% lanolin, and emulsified with surface-active agents (present to about 7% of the original formulation) in 35% water. The lowermost trace of Figure 7 characterizes a commercial, perfumed ointment base containing what is spectrally identified as a polymeric ingredient similar in many ways to polyethylene oxide, a highly hydrophilic film-forming material.

ASSESSMENT OF SKIN CHEMICAL AND MOISTURE LEVELS

Figure 8 presents another series of internal reflection, infrared spectra of human skin *in situ,* to demonstrate the utility of the method for rapid and noninvasive characterization of intact and damaged epidermal layers, together with the exudates and/or debris from wounded skin surfaces. At the top of Figure 8, the bowl-shaped but otherwise relatively featureless trace is for the "clean" baseline of a 50 mm × 20 mm × 2 mm germanium prism. This prism was mounted in the same horizontal attachment used for the characterization of cosmetics described in the foregoing section. The only change made for skin-profiling work has been to the more biologically safe and moisture resistant germanium internal reflection element. The second trace from the top of Figure 8 provides the infrared spectrum of the forearm skin of a male volunteer, after that skin area had been cleaned in our standard manner with hand soap (see the top trace of Figure 5) followed by rinsing, towel drying, and equilibrating the skin with the constant conditions of a clean room (72°F, 40% relative humidity). Immediately below that spectral characterization of human skin *in situ* is a rerun of the spectral characteristics of the germanium prism after lifting the arm from contact with the prism. This trace characterizes only the residue on the prism face, deposited during the approximately 10 min of skin contact which it experienced. The interpretation of the small infrared absorptions shown on this trace is that they are hydrocarbon, ester and glycoproteinaceous residues from the dried insensible perspiration accumulated on the prism face during the skin analysis, along with some shed epidermal cells that were revealed to be present by microscopic inspection. The fourth spectrum from the top of Figure 8 characterizes, again, the forearm skin of our male volunteer, but this time after ten "Scotch tape strippings" of the epidermal layers. It is immediately clear that this process caused the exposure of more highly hydrated (subsurface) cellular zones than had characterized the external skin surface in equilibrium with the room's relative humidity. Note particularly the large general increase in absorption in the region from 3000 to 3600 cm^{-1}, and the equally large increase in intensity (and lack of definition, characteristic of hydrated proteins) in the Amide I and II bands. The next trace (5th from the top in Figure 8) illustrates the character of the residue left by this mildly damaged skin wound during its period of contact. Although slightly greater in quantity than that left by the undamaged skin, the qualitative characteristics of the residue are similar. Little evidence is shown for there having been sufficient damage to the epidermis by ten skin-strippings to change its permeability to serous components of the

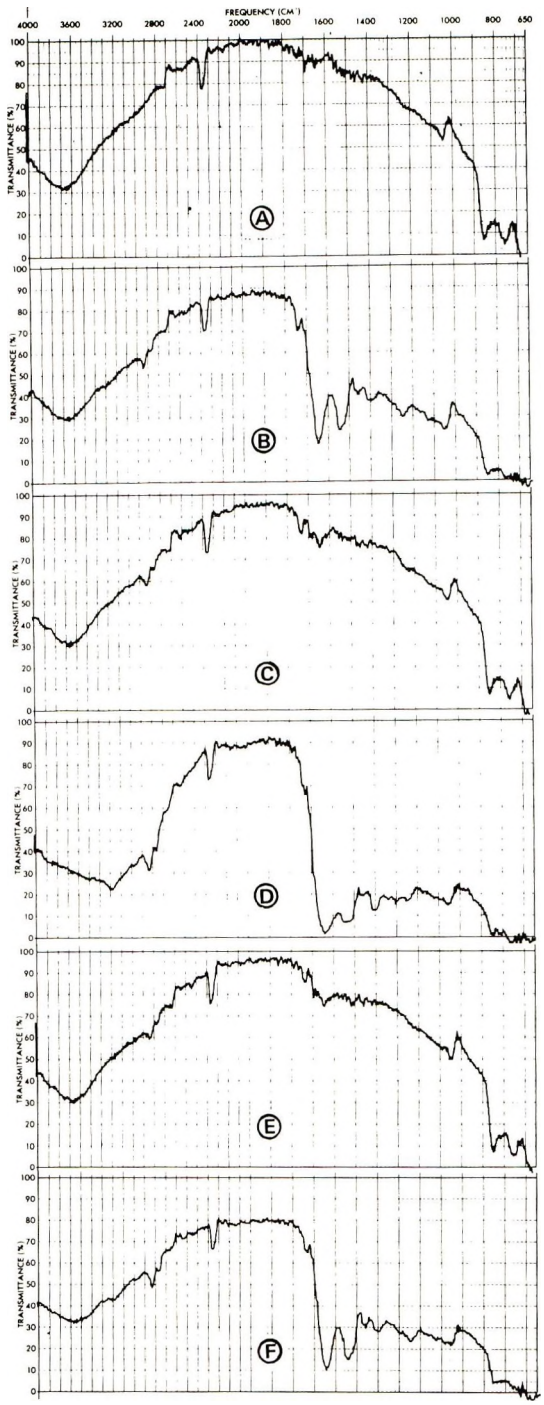

Figure 8. Internal reflection infrared spectra of human skin *in situ*, demonstrating the rapid, noninvasive characterization of both natural and damaged epidermis and the exudates therefrom.

Ⓐ "Clean" Baseline for 50 mm × 20 mm × 2 mm germanium prism mounted in horizontal mirror attachment, prior to applying subject's forearm. Ⓑ Forearm skin of male volunteer, after cleansing with liquid hand soap (see trace A of Figure 5), thorough rinsing, towel drying, and equilibrium with constant conditions of "clean room," 72°F, 40% R.H. Ⓒ Residue left on prism after skin analysis of Ⓑ. Ⓓ Forearm skin of male volunteer after 10 "Scotch-tape strippings" of epidermis. Ⓔ Residue on prism following contact analysis of "stripped" skin, *in situ*. Ⓕ Re-hornified skin of Ⓓ, after overnight aging and morning shower.

subjacent tissues. The final infrared trace of Figure 8 characterizes, once again, the equilibrated skin chemistry of our subject, dried and rehornified for approximately 16 hr following the group of ten Scotch tape strippings.

Figure 9 presents spectra collected in another investigation of the changes in skin chemistry and moisture level with depth through the epidermal layers, as those layers were sequentially removed by successive "strippings" with Scotch tape. The uppermost trace of Figure 9 is the internal reflection, infrared spectrum following 20 Scotch tape strippings of the epidermal layers of human skin. This infrared characterization is not significantly different from that shown in Figure 8 after only ten skin strippings. However, as the second trace from the top of Figure 9 shows, following 30 skin-stripping operations the epidermal layers under analysis were significantly more hydrated. This is illustrated by the growing infrared absorption band in the region between 3000 and 3600 cm^{-1}. No further significant changes in composition, for example in the lipid to protein ratio, were revealed. The next spectrum shows the consequences of 40 Scotch tape strippings of a forearm skin area, it now becoming clear that the moisture level of these deeper tissues is considerably greater than that nearer the skin surface. The O-H absorption band between 3000 and 3600 cm^{-1} becomes a major band of the spectrum. The fourth trace from the top of Figure 9 characterizes the skin chemistry after 50 Scotch tape applications had stripped away most of the drier epidermal layers, and the fifth trace presents the infrared characterization of the physically wet "glistening" wound produced by 60 such Scotch tape strippings of the epidermis. The final trace of Figure 9 shows the return of the skin chemistry to its equilibrium, normally hydrated state, after the wounded skin zone had healed for six days. Note the reproducibility of both the positions and relative intensities of the infrared absorption bands characterizing the "natural" state of a given subject's skin, as is represented in the three independently obtained traces for that skin included in Figures 8 and 9.

EXUDATES AND HEALING OF EPIDERMAL WOUNDS

Figure 10 collects internal reflection infrared spectra of the serous exudates collecting at the surface of human skin, *in situ*, after its wounding to successively deeper layers. The top trace characterizes the exudates of skin "stripped" 20 times with Scotch tape. It is comparable to the spectrum for insensible perspiration and moderate cellular debris found for natural or mildly wounded skin as already characterized in Figure 9. The second trace from the top of Figure 10 characterizes the exudates produced after 30 Scotch tape strippings of the epidermal layers. The next trace (third from the top of Figure 10) characterizes the matter extruded from human skin that had been wounded by 40 applications to and sudden removals of Scotch tape from the same skin area. Note, in this 40-layer-deep wound, the beginning of the collection of a wound fluid. The composition of the dissolved components of this fluid, as characterized by infrared absorption bands in the Amide I and Amide II area, is seen to be dominated by proteinaceous substances. The proteins were probably provided by hydrostatic pressure-induced leakage of plasma components through the damaged tissue bed. The infrared traces for human skin wounded to depths of 50 and 60 layers by Scotch tape stripping show increasing amounts of wound fluid collected on the face of germanium prisms during identical contact times, attesting to the increasing compromise of permeability barriers as the skin layers were stripped away. The final trace of Figure 10 shows that, with only a few days of healing, this "glistening" epidermal wound exuded

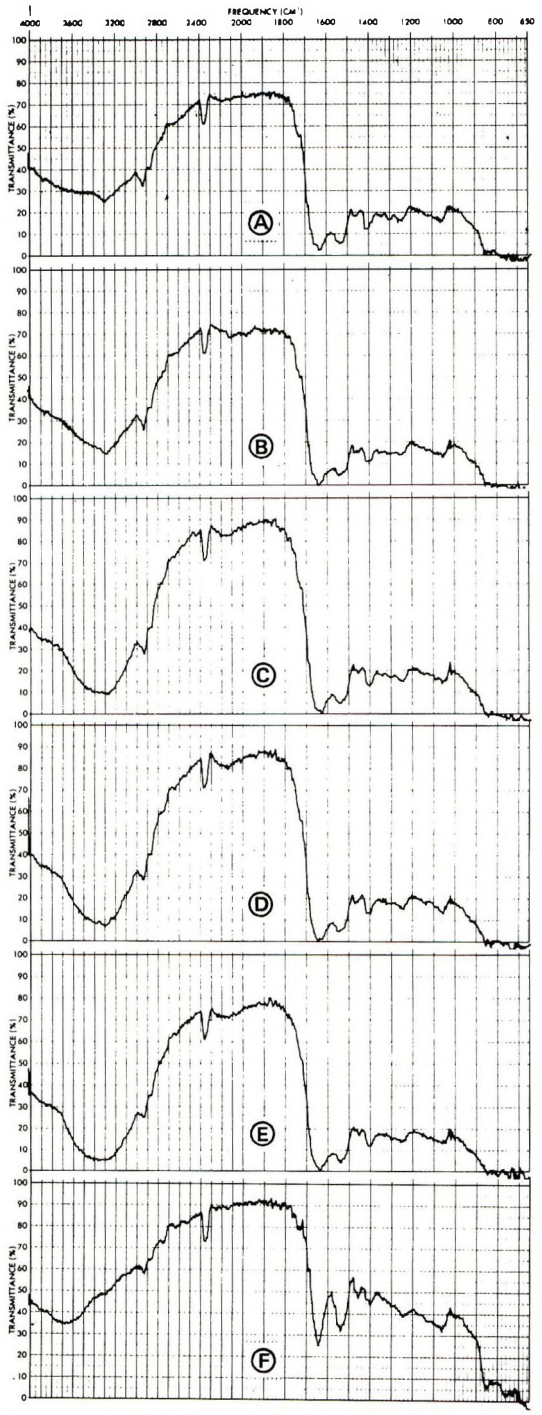

Figure 9. Internal reflection infrared spectra profiling human skin *in situ*, as epidermal layers were successfully stripped away. Ⓐ Forearm skin of male volunteer, following 2 groups of 10 "Scotch tape strippings" of epidermal layers. Compare with less damaged skin of same subject in Figure 8. Ⓑ After third group of 10 skin strippings. Ⓒ After fourth group of 10 skin strippings. Note that N-H absorption band is almost obscured by increasing O-H band intensity. Ⓓ After fifth group of 10 skin strippings. Ⓔ After sixth group of 10 skin strippings, producing a "glistening" wound of dimensions 80 mm × 60 mm. Ⓕ Healed skin, 6 days following wound production by 60 "scotch tape strippings."

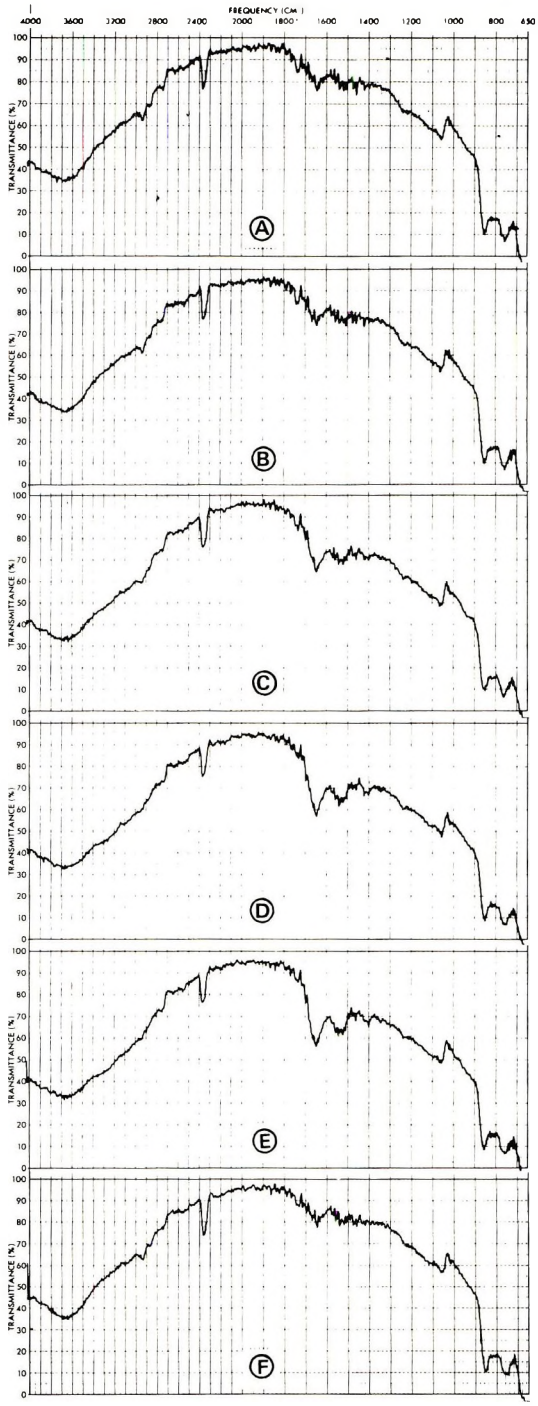

Figure 10. Internal reflection infrared spectra of exudates from skin wounded to successively deeper layers. Ⓐ Exudate upon contact with skin "stripped" 20 times with Scotch tape. Compare with skin spectrum, *in situ*, in Figure 9. Ⓑ Exudate after 30 strippings of epidermal layers. Ⓒ Exudate after 40 strippings. Ⓓ Exudate after 50 skin strippings. Note the absence of lipid absorption bands from this spectrum of purely proteinaceous matter. Ⓔ Exudate from "glistening" forearm wound produced by 60 skin strippings. Ⓕ Exudate from wounded skin area after 6 days of healing.

Table II
Wettability of Proteinaceous Film Formed by Serous Exudate From "Glistening" Wound of Forearm Skin

Wetting Liquid and Surface Tension (γ_{LV}) (dynes/cm, 20°C)		Average Contact Angle (θ in degrees)
Water	72.8	42
Thiodiglycol	54.0	53
Methylene Iodide	50.8	45
1-Bromonaphthalene	44.6	35
1-Methylnaphthalene	38.7	24
Dicyclohexyl	33.0	14
n-Hexadecane	27.7	7
n-Decane	23.9	0

only insensible perspiration, containing fatty-ester components not seen at all in the serous wound fluids.

In the healing of epidermal wounds, it is primarily the dried films formed by serous exudates (wound fluids) that cover the propagating cells. These cells must move freely as they seek to close the breach of the environmental seal. It becomes important,

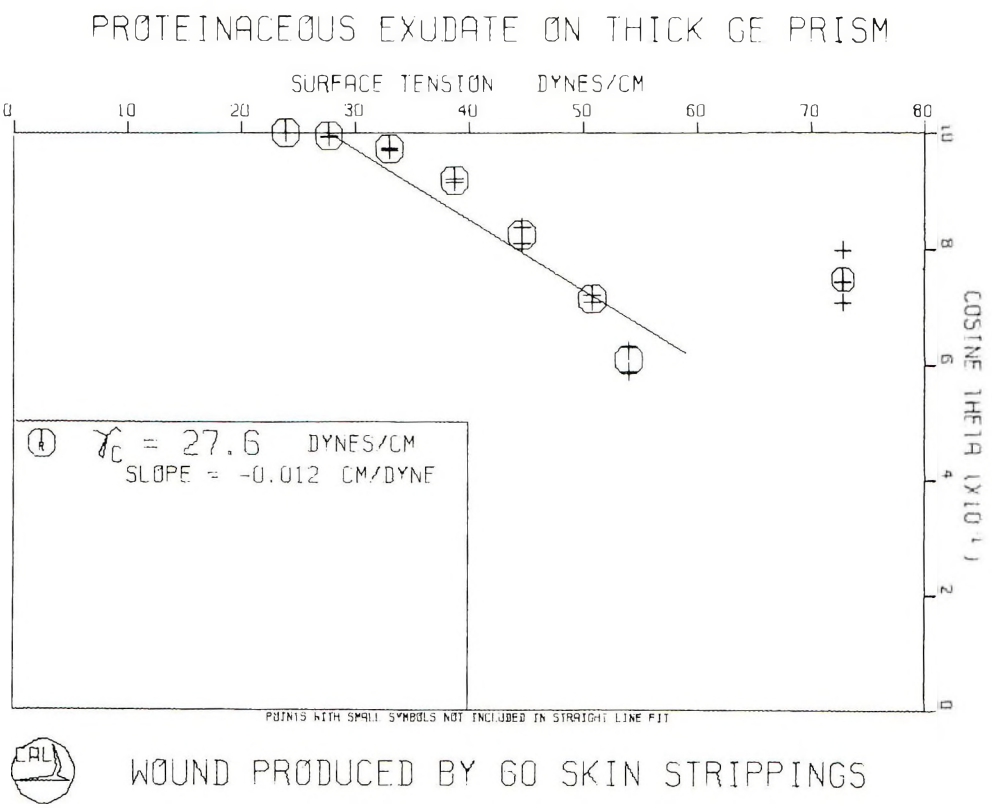

Figure 11. Contact angle data plot characterizing the wettability and adhesive quality of the dried, serous exudate from a "glistening" skin wound.

therefore, to assess the relative adhesive qualities of such wound fluid films. These qualities must include compatibility with the subjacent cells, supporting their tendency to move smoothly beneath it rather than becoming adherent and resistant to such movement. Table 2 provides contact angle data that characterizes the wettability of a typical proteinaceous film formed by the serous exudate from a "glistening" wound of the forearm skin, the wound having already been characterized in Figure 9, and the exudate characterized in Figure 10 by spectroscopic criteria. Figure 11 plots these contact angle data to yield a critical surface tension value in the mid 20's dynes/cm, a value often found to characterize "biocompatible" natural and synthetic surfaces and argued to have the minimum adhesive potential for living cells (17, 18). The history of the collection and examination of wound fluids goes back over 20 years (19, 20), but the collection of adequate quantities for analysis of the proteins, glycoproteins and lipids present in such fluids has required the use of experimental animals and surgically

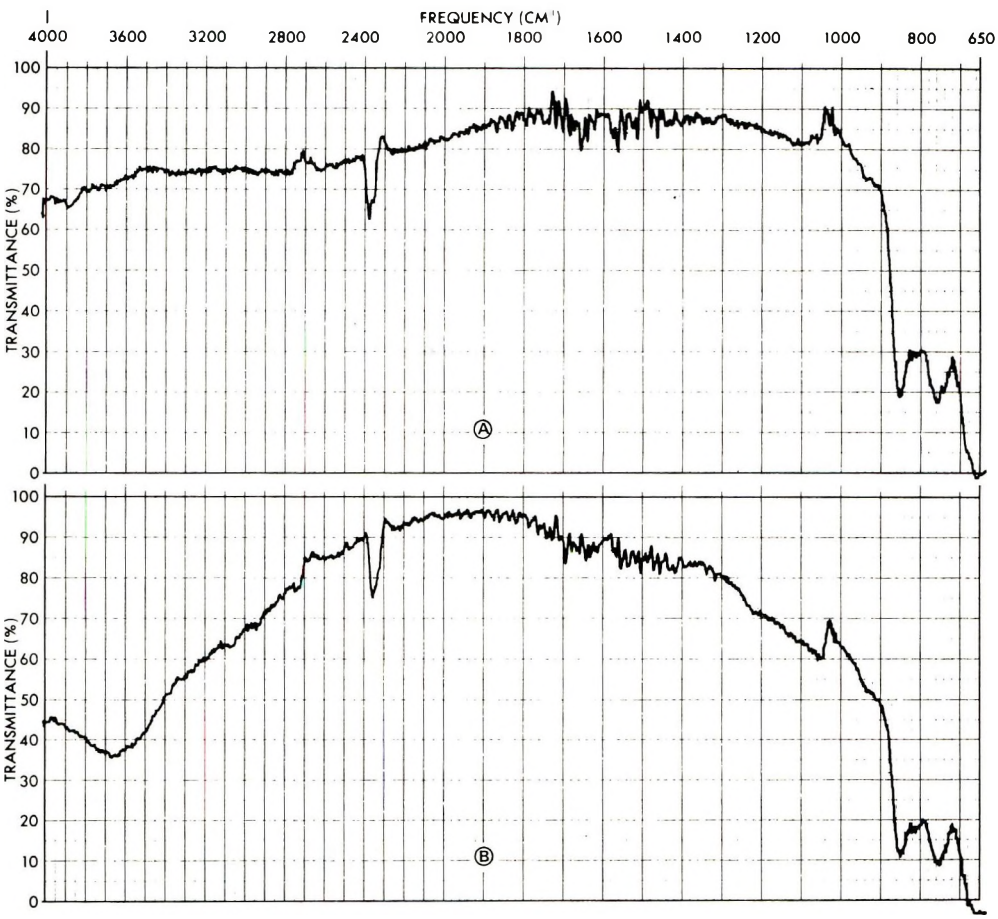

Figure 12. Demonstration of the different baselines obtained when an internal reflection prism is mounted in different mirror devices for spectroscopic examination.

Ⓐ Germanium prism (50 mm × 20 mm × 2 mm) baseline obtained in vertical mirror mounting unit. Note flatter response over most of the spectral range.

Ⓑ Same prism baseline obtained in horizontal mirror unit, which allows more convenient and even-pressured skin or liquid cosmetic contact.

implanted devices. The invasive techniques for placement and removal of collecting receptacles have serious drawbacks when the applicability of the results to mild skin wounds, producing tiny amounts of exudates, are to be considered. The internal reflection, infrared spectroscopic method presented here, supplemented by contact angle measurements of surface properties, and further supplemented by measurements of ellipsometric and electronic parameters for these exudates (13, 31), should prove more useful because of the noninvasive character, nondestructive analysis and exceptional sensitivity to thin films of this method.

DISCUSSION

There is an important departure in the internal reflection, infrared method described here from the normal practice of this technique in other analytical fields. This is the utilization of internal reflection elements in a horizontal position to allow comfortable, gravitationally aided rather than hindered, skin analysis. Horizontal prisms also allow the fascile an· .ysis of fluids, creams and/or medicinal preparations, which would drain from vertic surfaces. This departure from standard methods is not without some sacrifice. Figure 12 demonstrates, for example, the different baselines obtained for the same internal reflection prism mounted in a standard vertical mirror unit *vs.* the horizontal mirror unit preferred for cosmetic and wound-healing studies. The "baseline" quality for the simpler vertical mounting unit is considerably flatter and spectroscopically more desirable than that for the horizontal device. The extreme, concave down, skewing of the baseline for the horizontal device is a disadvantage that must be accepted in order to avoid more serious difficulties experienced when a volunteer is asked to hold a skin surface, with constant pressure, against the vertical face of an internal reflection prism or when drainage causes redistribution of a cosmetic preparation on the prism face during analysis.

Nevertheless, there are numerous circumstances in which the simpler setup and superior baseline of the more standard internal reflection, spectroscopic devices may be accommodated to the needs of cosmetic or dermatologic analyses. As an example, the rapid analysis of wound fluid components collected in the crevice between shear-separated epidermal layers of friction blisters may be accomplished by first evaporating the fluid to dryness on a horizontal prism face and then turning the prism 90° for mounting in a standard vertical unit. Figure 13 presents an internal reflection infrared spectrum of the essentially pure proteinaceous components present in the fluid beneath such a friction blister on human skin and compares it with the spectral baseline for the clean prism in a vertical mounting device. Although friction blisters are very common causes of civilian and military disabilities, they have received very little scientific attention (22). Perhaps the availability of the simple analytical technique described here will allow their more careful examination and the development of improved wound dressings for their more rapid healing.

Finally it must be noted that as cosmetic and medicinal preparations begin to incorporate more natural products, and especially products of proteinaceous origin (23), the differentiation among natural skin, damaged skin and the proteinaceous ingredients of various cosmetics, salves, or ointments will become a great deal more difficult than has been the evaluation of cosmetic or therapeutic agents of predominantly hydrocarbon composition. In the former cases, it will be necessary to resort to refinements in the

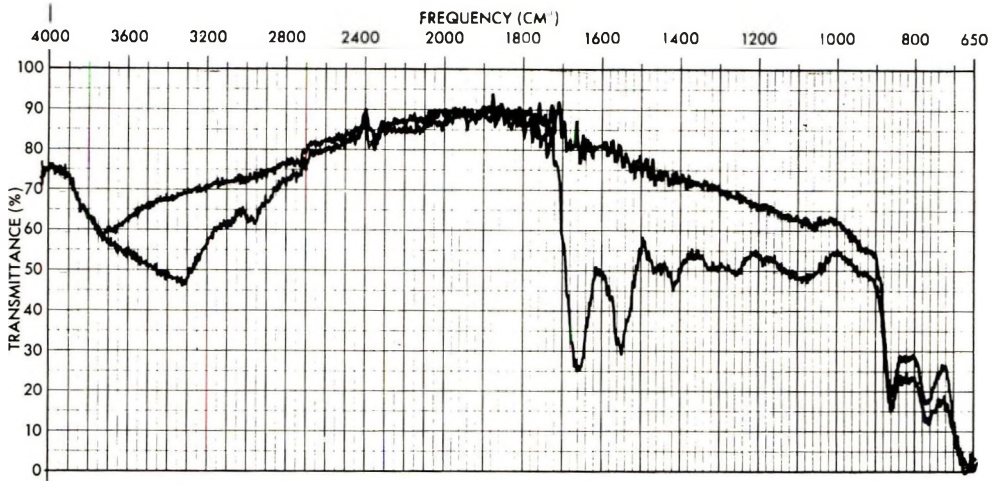

Figure 13. Internal reflection infrared spectra of clean germanium prism and of same prism coated with film formed by fluid from beneath a friction blister on human skin.

internal reflection spectral analyses, including attention to the specific locations and polarizations of the protein absorption bands, and to specific features of contact angle data plots, which have been described elsewhere (24, 25).

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SYNTHESEN IN DER ISOCAMPHANREIHE, IX.*

Über den Geruchseindruck von Isocamphanderivaten

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Synopsis — A total of 55 isocamphane derivatives was studied in order to establish a correlation between the shape of the molecule and its odor as postulated by the theory of Amoore. The molecules were measured by use of the shadow matching method and compared with cineole, the camphoraceous odor standard. The agreement of the results of these measurements with other molecular parameters, e. g. boiling point, was also examined. Isocamphane derivatives with spherical shape exhibit a distinct camphoraceous odor, and this charcteristic is reduced as the asymmetry of the isocamphane molecule increases.

Nach J. E. Amoore (1) ist die Gestalt bzw. die Raumerfüllung eines Moleküls der wesentliche Faktor für die Entstehung der verschiedenen Geruchseindrücke. Wenn nun einige Moleküle einen gleichen oder ähnlichen Geruch hervorrufen, so müssen sie nach dieser Theorie daher auch gleiche oder ähnliche Gestalt haben und von anderen Molekülen mit anderen Gerüchen in der Gestalt mehr oder minder deutlich verschieden sein. Es erschien daher interessant, eine größere Anzahl von Isocamphanverbindungen, die entweder als Riechstoffe selbst oder als Zwischenprodukte bei der Synthese von Riechstoffen von potentieller

* 8. Mitteilung, G. Buchbauer und E. Klissenbauer, Mh. Chem., im Druck.

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Der Firma Dragoco, Wien-Liesing, danken wir für wohlwollende Unterstützung.

Bedeutung sind, mittels des Schattenkorrelationstestes von Amoore miteinander zu vergleichen und auf diese Weise innerhalb einer Verbindungsklasse die Abhängigkeit des Geruchseindruckes von der räumlichen Gestalt zu überprüfen.

Es soll gezeigt werden, inwieweit die stereochemische Theorie von Amoore den Geruchseindruck der in Tabelle 1 angeführten Isocamphanderivate zu erklären vermag. Demzufolge sollen die kleineren und vor allem kugeliger geformten Moleküle dieser Reihe den camphrigen Geruchseindruck hervorrufen und es sollte mit zunehmender Größe und asymetrischer Gestalt der Moleküle dieser Geruchseindruck abnehmen.

Material und Methodik

Zur Untersuchung kamen die in Tabelle 1 angeführten Isocamphanverbindungen und einige Norisocamphane (Nr. 1, 5-7, 30, 42), die aus Gründen der Vollständigkeit in diese Arbeit einbezogen wurden. Die Substanzen wurden teils von uns synthetisiert (Nr. 5, 7-9, 12, 15-17, 19, 20, 23-25, 27-30, 34, 36, 37, 38, 39, 40, 42, 44, 45, 47-50, 53), teils von E. Klissenbauer (2) (Nr. 32, 33, 35, 43, 46, 51, 54-56) zur Verfügung gestellt. Die Substanzen Nr. 1-4, 6, 10, 11, 13, 14, 18, 21, 22, 26, 31, 41, 52, sind in der Literatur entnommen.

Zum Bau der Molekülmodelle wurden Stuart-Briegleb'sche Kalottenmodelle der Firma Leybold-Heraeus, Köln, verwendet. Die Untersuchung erfolgte nach der von Amoore angegebenen „shadow-matching-method" (1). Die Molekülmodelle wurden aus drei verschiedenen, aufeinander senkrecht stehenden Richtungen fotografiert. Hierzu wurde eine Kodakkamera, Retina II F, mit folgenden Einstellungen verwendet: Blende 8, Belichtungszeit $^1/_{500}$ sec. Nach einigen Probeaufnahmen zeigte sich, daß ein schwarz-weiß-Film mit DIN 21 für diese Arbeit am besten geeignet war. In Anlehnung an diesen Schattenkorrelationstest wurde zunächst versucht, die Molekülmodelle so zu fotografieren, daß die Kamera 1 m vom Objekt entfernt war. Das Molekülmodell lag auf einer Milchglasplatte, unter welcher ein Blitzlicht angebracht war. Es wurden auch Aufnahmen gemacht, bei welchen das Blitzlicht unmittelbar neben der Kamera angebracht war und schließlich wurde auch noch ausprobiert, ob bessere Resultate durch die Arbeit in der Dunkelkammer oder bei Tageslicht erzielt werden konnten. In Abänderung der "shadow-matching-method" wurden sämtliche Molekülmodelle direkt und nicht ihr Schatten fotografiert. Durch eine besondere Montage des Blitzlichtes unmittelbar neben der

Kamera war aber gewährleistet, daß der Umriß des Bildes genau dem Schatten des fotografierten Objektes entsprach. Durch die Wahl dieser Arbeitsmethode war es möglich, mit einfacheren Mitteln ein optimales Ergebnis zu erzielen. Um die Molekülmodelle exakt fotografieren zu können, war es außerdem nötig, die Objekte mittels kleiner Styroporstückchen zu stützen. Moleküle mit einem längeren aliphatischen Rest wurden zum Fotografieren in der sterisch am wenigsten behinderten Form angeordnet. Auch wurde darauf geachtet, daß sich die einzelnen Wasserstoffatome in gestaffelter Stellung zueinander befanden. Die Annahme, daß dies die wahrscheinlichste Molekülform ist, läßt die obzitierte Anordnung berechtigt erscheinen.

Die Filme wurden entwickelt und die Aufnahmen so vergrößert, daß ein mitfotografierter, 1,5 cm langer Meßstreifen wieder dieser Größe entsprach; nach Angaben der Firma Leybold-Heraeus entspricht der Abstand von 1,5 cm bei den Kalottenmodellen einem Å. Die Molekülbilder wurden ausgeschnitten und dann der Schwerpunkt dieser Bilder auf der Figur ermittelt. Vom Schwerpunkt ausgehend wurden von 0° bis 360° alle 10 Grade Linien gezogen, welche zur Vermessung gegenüber der Standardsubstanz dienten. Als Vergleichsmodell diente Cineol für den camphrigen und 3-Methyl-1-phenylpentan-3-ol für den blumigen Geruch. Die Vermessung erfolgte durch Vergleich der Bilder des betreffenden Moleküls und der Standardsubstanzen. Die Bilder wurden so Schwerpunkt auf Schwerpunkt gelegt, daß die 10°-Linien deckungsgleich waren und die Unterschiede — ob länger oder kürzer — mit einem Lineal abgemessen werden konnten. Anschließend wurde die Summe der metrischen Werte in Å-Werte umgerechnet und als Absolutwerte in die Tabelle 3 eingesetzt. Diese Summe der $\triangle$-Werte für jede der drei Raumrichtungen ergibt die Ähnlichkeit mit dem Standardmolekül. $\overline{\triangle}$ ist das arithmetische Mittel aus allen gemessenen molekularen Radialdifferenzen, also der mittlere Unterschied des Molekülradius und gibt somit die Differenz in der Molekülform an. Die Verwendung des Begriffes similarity (1) hat sich für die vorliegende Aufgabe nicht bewährt.

Weiter werden in der Tabelle 2 das Molekulargewicht, das Volumen des Moleküls und die Kondensationspunkte der einzelnen Verbindungen angegeben. Das Volumen des Moleküls (= Raumerfüllung) wurde in folgender Weise bestimmt: Die einzelnen Moleküle wurden in ein mit Wasser gefülltes kalibriertes Gefäß gegeben und die Flüssigkeitsverdrängung abgelesen. Die Kondensationspunkte wurden ebenfalls in dieser Arbeit berücksichtigt, da nach Mazziotti (3) innerhalb einer Stoffklasse die Geruchsqualität in Beziehung zum Siedepunkt stehen soll. Der Siedepunkt

stellt ein Maß der intermolekularen Bindung einer Substanz dar, so wären diese Bindekräfte mitverantwortlich für die Geruchsqualität. Die Kondensationspunkte wurden nach der Methode von Reckhard (4) in °Kelvin umgerechnet.

Die statistischen Berechnungen wurden nach den in Lit. (5) angegebenen Formeln durchgeführt und für jede Korrelation die Ausgleichsgerade berechnet. Bezüglich des Molekulargewichtes und des Volumens des Moleküls wurden alle 55 Verbindungen gegenüber dem Cineol verglichen, bei den Siedepunkten wurden 52 Substanzen berücksichtigt, da einige Verbindungen nur durch den Schmelzpunkt charakterisiert sind.

Tabelle 1

Formelübersicht

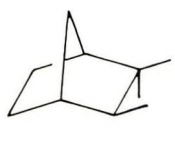
Nummer	Chemische Bezeichnung	Literatur
1	2,2-Dimethyl-bicyclo[2.2.1]heptan (Camphenilan)	6
2	1,2,2-Trimethyl-bicyclo[2.2.1.]-heptan (Isocamphan)	7
3	Camphen	8
4	2,2,3-Trimethyl-bicyclo[2.2.1.]-hept-5-en (endo-Isocamphen)	9
5	3,3-Dimethyl-bicyclo[2.2.1.]heptan-2-on (Camphenilon)	10
6	2-Amino-3,3-dimethyl-bicyclo[2.2.1.1-heptan (Camphenilylamin)	11
7	2-Hydroxy-3,3-dimethyl-bicyclo-[2.2.1]heptan (Camphenilol)	10
8	2-Cyano-3,3-dimethyl-bicyclo[2.2.1.]-heptan (Isocamphenilansäurenitril)	12
9	2-Formyl-3-3-dimethyl-bicyclo[2.2.1]-heptan (Isocamphenilanaldehyd)	12
10	2,3-Dimethyl-bicyclo[2.2.1]hept-5-en-2-yl-methanol	9

Nummer	Chemische Bezeichnung	Literatur
11	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-methylamin (Isocamphenilanylamin)	13
12	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-methanol (Isocamphenilanol)	12
13	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]-octan (Cineol)	14
14	2-Hydroxy-2,2,3-trimethyl-bicyclo-[2.2.1]heptan (Camphenhydrat)	15
15	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-5-en-2-yl)-ethanon	16
16	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-5-en-2yl)-ethanol	17
17	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-ethanon	16
18	2-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yliden)-ethanol (Camphenilydenethanol)	18
19	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-ethanol	17
20	3,3-Dimethyl-bicyclo[2.2.1]heptan-2-carbonsäure (Isocamphenilansäure)	12
21	1-Phenyl-3-methyl-pentan-3-ol	14
22	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yliden)-propan-2-on (ω-Acetyl-camphen)	19
23	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-propanon	17
24	1-(3,3-Dimethyl-5,6-epoxy-bicyclo-[2.2.1]-hept-2-yl)-ethanon	20
25	3-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-propionaldehyd	21
26	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-N,N-dimethyl-methylamin (Isocamphenilanyldimethylamin)	8
27	2-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-propan-2-ol	17
28	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)propanol	17
29	3,3-Dimethyl-bicyclo[2.2.1]heptan-2-carbonsäuremethylester (Isocamphenilansäuremethylester)	12

Nummer	Chemische Bezeichnung	Literatur
30	2-Acetoxy-dimethyl-bicyclo[2.2.1]-heptan (Camphenilylacetat)	9
31	3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl-nitromethan (10-Nitro-isocamphan)	22
32	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-but-2-enon	2
33	4-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-but-3-en-2-on	2
34	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-butanon	17
35	3-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-acrylsäure	2
36	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-carbonsäuredimethylamid (Isocamphenilansäuredimethylamid)	12
37	5-(3,3-Dimethyl-2-hydroxy-bicyclo-[2.2.1]hept-2-yl)-2-methyl-pent-2-en	23
38	2-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-butan-2-ol	17
39	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-butanol	17
40	3-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-propionsäure	21
41	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-N,N-dimethyl-methylamin-N-oxid (Isocamphenilanyldimethylamin-N-oxid)	8
42	2-Brom-3,3-dimethyl-bicyclo[2.2.1]-heptan	10
43	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-penta-2,4-dienon	2
44	3-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-2-methyl-pent-1,4-dien	21
45	5-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yliden)-2-methyl-pent-2-en (Isosantalen)	23
46	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-pent-2-enon	2
47	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-pentanon	17
48	1(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-pentanol	17

Nummer	Chemische Bezeichnung	Literatur
49	3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl-methylbromid (Isocamphenilanylbromid)	21
50	5-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yliden)-2-methyl-pent-2-enol (iso-Santalol)	23
51	1-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-4-methyl-pent-2-enon	2
52	5-(3-Methyliden-2-methyl-bicyclo-[2.2.1]hept-(2-yl)-2-methyl-but-2-enol (β-Santalol)	24
53	5-(3,3-Dimethyl-bicyclo[2.2.1]-hept-2-yl)-2-methyl-pent-2-enol (δ-Santalol)	21
54	1-(3,3-Dimethyl-bicyclo[2.2.1]hept-2-yl)-4-methyl-pentanon	2
55	3-(2'-Furyl)-1-(3,3-dimethyl-bicyclo-[2.2.1]hept-2-yl)-prop-2-enon	2
56	3-Cyclohexyl-1-(3,3-dimethyl-bicyclo-[2.2.1]hept-2-yl)-propanon	2


1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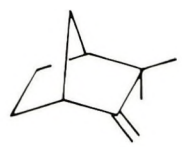
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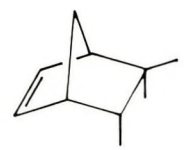
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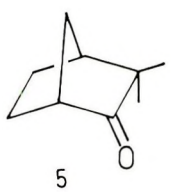

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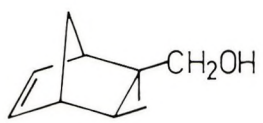

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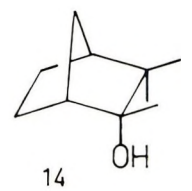

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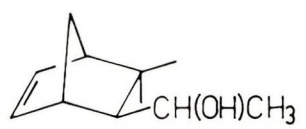

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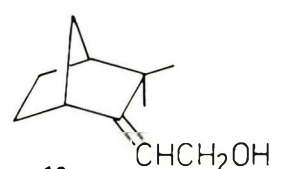

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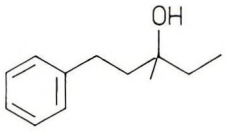

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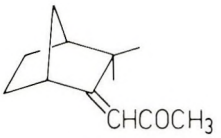

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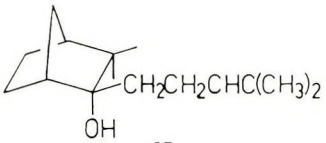

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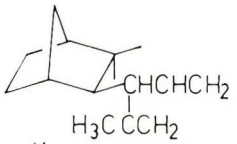

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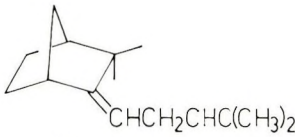

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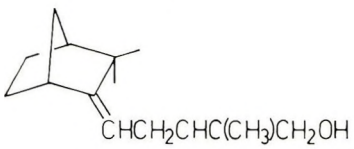

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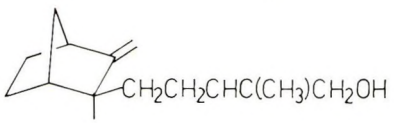


52

6 R= NH_2	23 R= COC_2H_5	33 R=$CHCHCOCH_3$
7 R=OH	25 R= CH_2CH_2CHO	34 R= COC_3H_7
8 R=CN	26 R= $CH_2N(CH_3)_2$	35 R=CHCHCOOH
9 R=CHO	27 R= $C(CH_3)_2OH$	36 R= $CON(CH_3)_2$
11 R= CH_2NH_2	28 R= $CH(OH)C_2H_5$	38 R= $C(OH)\langle^{CH_3}_{C_2H_5}$
12 R= CH_2OH	29 R= $COOCH_3$	39 R= $CH(OH)C_3H_7$
17 R= $COCH_3$	30 R= $OCOCH_3$	40 R= CH_2CH_2COOH
19 R= $CH(OH)CH_3$	31 R= CH_2NO_2	41 R= $CH_2\overset{\downarrow}{N}(CH_3)_2$ O
20 R=COOH	32 R= $COCHCHCH_3$	42 R= Br

43 R = $COCHCHCHCH_2$

46 R = $COCHCHC_2H_5$

47 R = COC_4H_9

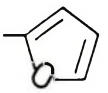
48 R = $CH(OH)C_4H_9$

49 R = CH_2Br

51 R = $COCHCHCH(CH_3)_2$

53 R = $CH_2CH_2CHC(CH_3)CH_2OH$

54 R = $COCH_2CH_2CH(CH_3)_2$

55 R = COCHCH- 

56 R = $COCH_2CH_2C_6H_{11}$

Tabelle 2

Physikalische Konstanten

Nummer	Molekular-gewicht	Siedepunkt in K	Volumen des Molekülmodells in ml
1	124	416	311,0
2	126	435	374,5
3	134	431	325,5
4	136	424	325,5
5	138	465	304,0
6	139	458	342,0
7	140	361	333,0
8	149	520	360,0
9	152	490	356,5
10	152	— (Schmp. 375)	374,5
11	153	491	378,5
12	154	501	369,5
13	154	449	376,0
14	154	478	369,5
15	164	483	371,0
16	166	497	384,0
17	166	494	393,0
18	166	507	384,0
19	168	497	406,0
20	168	545	378,5
21	178	515	452,0
22	180	385	407,5
23	180	506	429,5
24	180	— (Schm. 316)	371,0
25	180	607	429,5
26	181	483	451,5

Nummer	Molekular-gewicht	Siedepunkt in K	Volumen des Molekülmodells in ml
27	182	513	442,5
28	182	509	440,5
29	182	491	415,0
30	182	569	415,0
31	183	— (Schm. 198)	397,0
32	192	633	460,0
33	192	551	460,0
34	194	519	466,0
35	194	615	445,5
36	195	553	460,5
37	195	579	546,0
38	196	553	479,0
39	196	522	479,5
40	196	705	451,5
41	197	543	468,5
42	203	409	370,0
43	204	625	479,5
44	204	517	518,0
45	204	585	502,0
46	206	649	496,5
47	208	537	502,5
48	210	543	515,5
49	217	593	397,0
50	220	607	524,0
51	220	667	533,0
52	220	595	563,5
53	222	651	546,0
54	222	648	539,0
55	244	695	555,5
56	262	701	626,0

Tabelle 3

$\overline{\Delta}$ in Absolutbeträgen

Nummer	$\overline{\Delta}$	Nummer	$\overline{\Delta}$	Nummer	$\overline{\Delta}$	Nummer	$\overline{\Delta}$
1	579	16	773	30	691	44	1122
2	506	17	776	31	553	45	1269
3	539	18	517	32	940	46	936
4	539	19	751	33	1142	47	1229
5	625	20	834	34	1036	48	1111
6	497	21	1253	35	993	49	927
7	676	22	683	36	1016	50	1362
8	819	23	845	37	1133	51	1318
9	695	24	902	38	1025	52	1243
10	543	25	781	39	915	53	1123
11	682	26	821	40	988	54	1277
12	669	27	926	41	743	55	1537
14	515	28	853	42	748	56	1545
15	747	29	843	43	1009		

Tabelle 4

In der Literatur beschriebene Geruchseindrücke

Nummer	Geruchseindruck	Nummer	Geruchseindruck
1	camphrig	30	camphrig/blumig
2	camphrig	31	camphrig
3	camphrig	32	blumig
4	camphrig	33	blumig
5	camphrig	34	blumig
6	camphrig mit Fischgeruch	35	schwach blumig
7	camphrig	36	schwach camphrig
8	camphrig	37	blumig
9	camphrig	38	camphrig
10	camphrig	39	blumig
11	camphrig	40	blumig/süßlich
12	camphrig	41	camphrig
13	camphrig	42	camphrig/stechend
14	camphrig	43	blumig
15	camphrig	44	zedernholzartig
16	camphrig	45	schwach santalolartig
17	camphrig	46	blumig
18	camphrig	47	blumig
19	camphrig	48	blumig
20	schwach camphrig	49	camphrig/stechend
21	blumig	50	sandholzähnlich
22	blumig	51	blumig
23	blumig	52	Sandholzgeruch
24	camphrig	53	schwach holzartig
25	blumig	54	blumig
26	camphrig mit Fischgeruch	55	blumig
27	camphrig	56	blumig
28	blumig		
29	camphrig		

Ergebnisse

In Abbildung 1 ist auf der Ordinate das Volumen der Moleküle in ml und auf der Abszisse $\overline{\Delta}$ in Å aufgetragen. Werte für das Cineol konnten in den Abbildungen nicht angegeben werden, da diese Verbindung als Vergleichssubstanz diente. $\overline{\Delta}$ gibt die Abweichung in Absolutbeträgen gegenüber dem Cineol an. Bei der Angabe von Plus- und Minuswerten hätte Cineol in die Abbildungen aufgenommen werden können. Auf der Abbildung ist deutlich ein Bereich der camphrigen Gerüche, ein Bereich der blumigen Gerüche sowie ein Mischbereich zu erkennen. Die Korrelationskoeffizienten sind ebenfalls aus den einzelnen Abbildungen ersichtlich.

In Abbildung 2 sind auf der Ordinate die Siedepunkte in °Kelvin und auf der Abszisse $\overline{\Delta}$ in Å aufgetragen. Hier ist die Streuung im Vergleich zur Abbildung 1 größer, jedoch ist auch hier ein Bereich der camphrigen und ein solcher der blumigen Gerüche und ein Mischbereich zu sehen.

In Abbildung 3 ist das Molekulargewicht in g auf der Ordinate und $\overline{\Delta}$ in Å auf der Abszisse aufgetragen. Auch hier sind wieder die 3 Bereiche der camphrigen, blumigen und Mischgerüche — (dieser ist jedoch im Vergleich zu den anderen Abbildungen kleiner) —, zu erkennen.

Die in den Abbildungen 1 bis 3 eingekreiste Substanz ist der für die blumigen Gerüche gewählte Standard.

In Abbildung 4 sind Mittelwerte ($\overline{\times}$) und Standardabweichung (s) für die camphrigen Gerüche angegeben. Es wurden 31 Verbindungen dieser Geruchsklasse ohne die Standardsubstanz Cineol vermessen. Für den blumigen Geruch — es wurden 24 Verbindungen inklusive der Standardsubstanz vermessen — sind $\overline{\times}$ (bei 1123 Å) und s (± 205) nicht extra hervorgehoben. Man sieht sehr deutlich, daß der Großteil der vermessenen camphrigen Verbindungen im Bereich der Standardabweichung liegt.

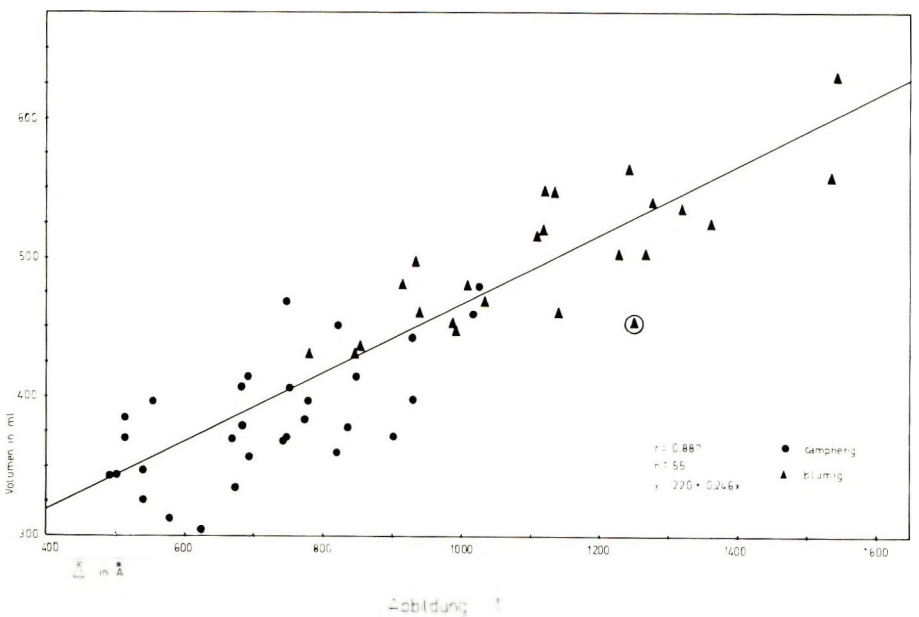


Abbildung 3

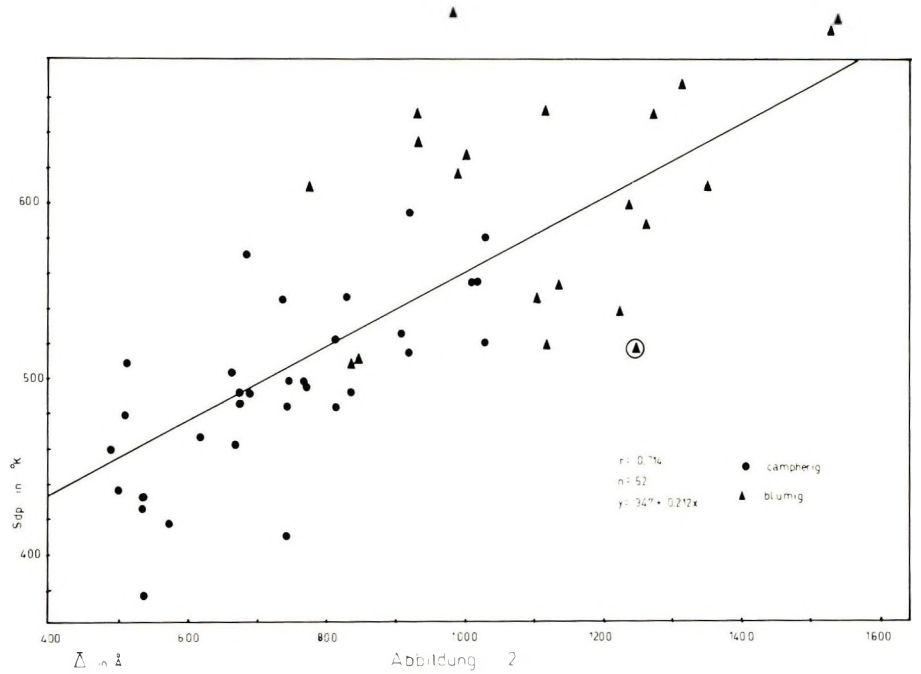


Abbildung 2

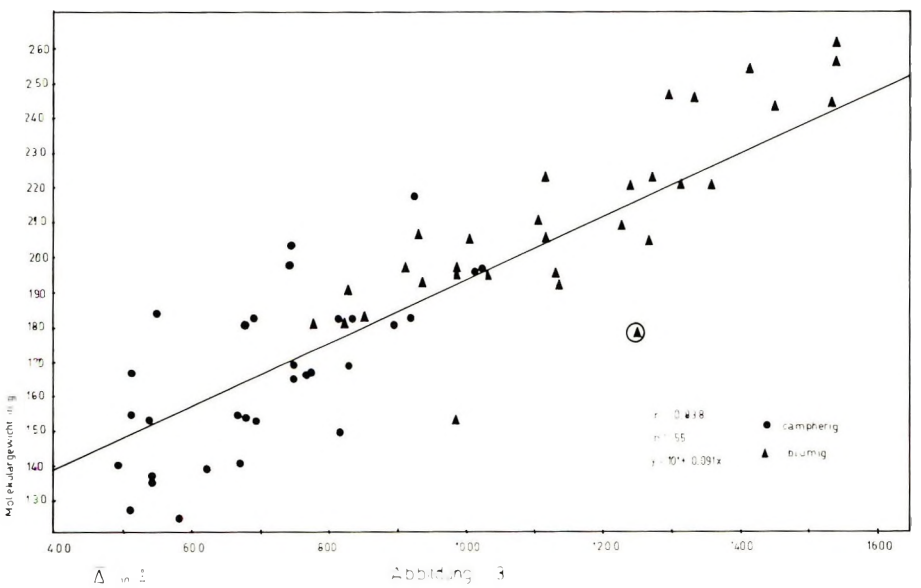


Abbildung 3

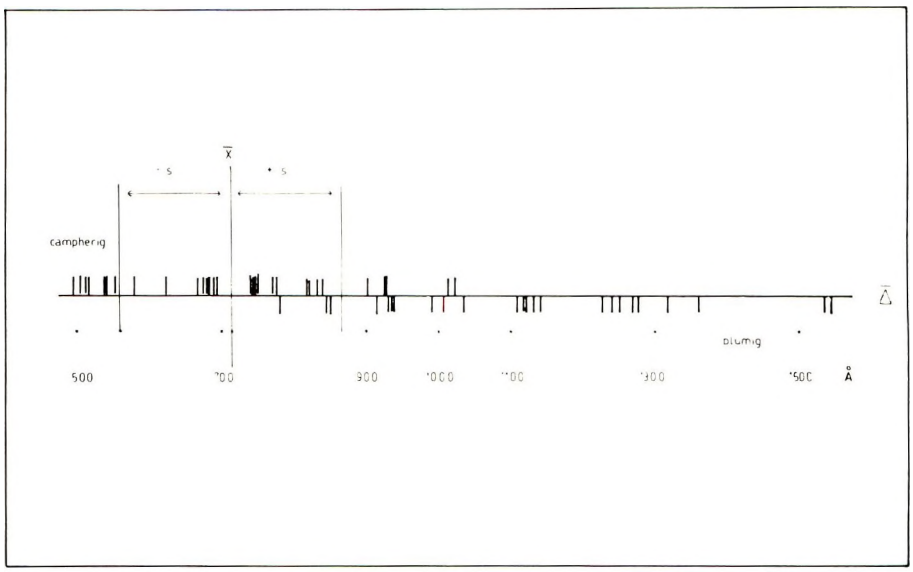


Abbildung 4

Dikussion der Ergebnisse

Mit dieser Studie sollte gezeigt werden, daß eine Beziehung zwischen chemischer Konstitution und Geruch besteht. Der in Abbildung 1 angegebene Korrelationskoeffizient (0,887) zeigt die Übereinstimmung, die durch die Bestimmung der Raumerfüllung der in Tabelle 1 angeführten Isocamphanverbindungen auf zwei voneinander unabhängigen Wegen (Volumsmessung und Vermessen der Unterschiede der Schattenbilder im Schattenkorrelationstest) erzielt wurde. Darüber hinaus ist dieser Korrelationskoeffizient eine Bestätigung für die Genauigkeit und Reproduzierbarkeit der Messungen und somit auch für die Tauglichkeit des Schattenkorrelationstestes. Es besteht, bedingt durch die gute Übereinstimmung der Ergebnisse, die Möglichkeit, bei zukünftigen Untersuchungen in dieser Richtung den etwas aufwendigen Schattenkorrelationstest durch die einfachere Volumsmessung ersetzen zu können.

Ein größerer Aussagewert bezüglich der Amoore'schen Theorie ist durch die Abbildung 2 erkennbar. Die Siedepunkte sollen nach Mazziotti (3) innerhalb einer Stoffklasse in Beziehung zur Geruchsqualität stehen. Abbildung 2 gibt einen deutlichen Beweis für diese Beziehung, was auch durch den Korrelationskoeffizienten zum Ausdruck

gebracht wird. Die Irrtumswahrscheinlichkeit, daß dieses Ergebnis zufällig ist, beträgt weit unter 1%. Etwas überraschend ist die gute Korrelation von $\bar{\Delta}$ mit dem Molekulargewicht. Denn a priori ist es nicht vorauszusehen, daß mit steigendem Molekulargewicht sich nicht auch der Geruchseindruck ändert. Tatsächlich geben die in Abbildung 3 angeführten Ergebnisse der Messungen einen weiteren Beweis dafür, daß für den „Grundgeruch" (Primärgeruch) hauptsächlich die Molekülgestalt und somit die Raumerfüllung verantwortlich ist.

Eine Erklärung für die weite Streuung der als blumig riechenden Verbindungen kann übrigens darin gefunden werden, daß unter dem Begriff „blumiger Geruch" auch holzige, fruchtige und andere ähnliche Geruchsnoten mit einbezogen werden. Die Vielfalt der unter „blumig-fruchtig" einzuordnenden Gerüche ist ja bekannt.

Yamasaki und Mitarbeiter (25) zeigten in einer Studie, daß die camphrig riechenden Substanzen in einem Diagramm auf einer verhältnismäßig kleinen Fläche, die man mit einer Ellipse umschreiben könnte, beisammen liegen, während die blumigen Gerüche, die an die Verbindungen mit camphrigen Geruch in der Richtung der geringeren Flüchtigkeit anschließen, eine weite Streuung aufweisen. Das Gebiet der blumigen Gerüche ist nach diesen Angaben in sich nicht geschlossen, sondern umschließt auch unter anderem die minzig und faulig-fäkalartig riechenden Substanzen.

Jedenfalls ist — wie vor allem aus Abbildung 4 ersichtlich ist — der über $\bar{\Delta} = 900$ liegende Bereich nicht den camphrigen Gerüchen zuzuordnen.

Die Theorie von Amoore stimmt mit dem Ergebnis der Geruchsprüfung bezüglich Grundgeruch der untersuchten Isocamphanverbindungen überein. Dies gilt jedoch nicht für Geruchsnuancen, denn es ist unwahrscheinlich, daß kleine Änderungen des Moleküls eine wesentlich andere Raumerfüllung zeigen, womit ein anderer Primärgeruch hervorgerufen werden könnte. Diese Geruchnuancen werden sicherlich durch Sekundäreffekte, die auch im Bereich des Rezeptors liegen, und weiteren molekularen Parametern hervorgerufen.

Die in der Einleitung gegebene Problemstellung, derzufolge die kleineren, kugelig geformten Moleküle dem camphrigen Geruchstyp zuzuordnen sind, während die mit längerer Seitenkette ausgestatteten Verbindungen einen anderen Geruchseindruck hervorrufen sollten, wurde durch die vorliegende Arbeit voll bestätigt. Es kann also als Resumee die Gesetzmäßigkeit gezogen werden, daß innerhalb der Isocamphanreihe mit abnehmender Kugelform des Moleküls auch der camphrige Geruchs-

eindruck verblaßt. Dies müßte auch für endo-konfigurierte Substanzen am C_2-Atom zutreffen. Die Übereinstimmung der Ergebnisse mit der Amoore'schen Theorie ist bei der Verbindungsklasse der Isocamphane gegeben.

Zusammenfassung

An Hand von verschiedenen Isocamphanverbindungen wurde gezeigt, inwieweit die stereochemische Theorie von Amoore zur Erklärung des Geruchseindruckes angewendet werden kann. Mit Hilfe des Schattenkorrelationstestes wurden die Moleküle gegenüber der Standardsubstanz 1,8-Cineol vermessen. Die Übereinstimmung der Ergebnisse mit anderen Molekülparametern wurde geprüft. Die kugelig geformten Moleküle dieser Reihe rufen einen camphrigen Geruchseindruck hervor und es nimmt dieser mit zunehmender Asymmetrie der Moleküle ab.

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Interaction of keratinous substrates with sodium lauryl sulfate: I. sorption

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Synopsis

Use was made of radiotagged SODIUM LAURYL SULFATE (SLS) to determine its sorption by skin and hair. In the initial stages uptake is linear in square root of time, indicative of a diffusion process. The uptakes determined by radiotagged SLS were successfully correlated with data from a simple gravimetric method and showed that the latter procedure can be used satisfactorily under certain conditions when radiotagged compounds are not available. The influence of some additives on the SORPTION of SLS was studied. Salt increases the sorption, while nonionic SURFACTANTS (which are not themselves sorbed) substantially depress it. Finally, the relation of the sorbed SLS to water of hydration of KERATIN is examined. It is concluded that most, if not all, the sorbed material is bound to keratin, rather than existing in an "internal" solution.

INTRODUCTION

Surfactants and soaps are known to be irritating to the skin and under extreme conditions can have adverse effects on hair. Scientific study of the action of these materials is hampered by a lack of data on their uptake by various keratin substrates. The availability of sodium lauryl sulfate (SLS) in radiotagged form makes it relatively easy to study the kinetics of sorption of this model surfactant over a wide range of concentrations and times, and to explore the various effects of additives.

There are few data in the literature concerning the sorption of surfactants by human hair. Two studies (1,2) have dealt with long-chain quaternary ammonium halides (typical cationic surfactants), but both were conducted at concentrations well under 1%. A brief radiotracer study was made of sodium acyl sarcosinates (3) in which the concentration was as high as 5%. However, nothing has appeared dealing with nonionics, amphoterics or more common anionics such as sodium lauryl sulfate and its ethoxylates; nor have concentrations in the range of 10% been investigated, corresponding to the actual strength of shampoos.

The situation is similar for the substrate skin. There is a large, medically oriented literature on the percutaneous absorption of surfactants–particularly anionics like SLS, but the uptake of surfactant by the skin is not usually considered in these works. Two not-

able exceptions are Harrold and Pethica (4) and Blank and Gould (5). However, both of these papers describe only long times (18 to 24 hr) and low concentrations. Another related study deserves to be cited, namely the work of Garrett (6) on the sorption of surfactants by hide powder. Only low concentrations were employed, but the fundamental $\sqrt{t}$ dependence was shown at short times.

It is believed that the present study is the first to examine short times and high surfactant concentrations, corresponding in some degree to normal-use conditions.

EXPERIMENTAL

Undamaged and bleached hair samples were obtained from DeMeo Bros., New York City, and were used as received.

The stratum corneum from neonatal rats was used as a model for human skin. The details of preparation of these membranes have been given in a previous publication (7). This stratum corneum has well developed barrier properties, at least in respect to the transmission of water vapor, as has been shown by Singer and coworkers (8) and confirmed by our own determinations (7).

Sodium lauryl sulfate was obtained as a pure white crystalline powder from BDH Chemicals, Ltd., Poole, England. Tagged material was purchased from Amersham/Searle Corp., Arlington Hts., Illinois, in the form of small, individual ampoules. Each ampoule contained 2.47 mg of SLS with an activity of 110 microcuries. The "tag" is present as the S-35 isotope and is thus in the anion of the surfactant:

Standapol ES-2 (Henkel Co.)—sodium lauryl ether sulfate with 2 mol of ethylene oxide.

Standapol ES-3 (Henkel Co.)—sodium lauryl ether sulfate with 3 mol of ethylene oxide.

Standapol 130E (Henkel Co.)—sodium lauryl ether sulfate with 12 mol of ethylene oxide.

Tergitol 15-S-9 (Union Carbide Corp.)—the 9 mol ethoxylate of secondary C_{13} to C_{15} alcohol.

Solutions of desired concentration were made up of the nonradioactive powder and one ampoule of tagged material was added with stirring. Hair samples of about 100 mg each were placed in 20 ml of the solution for times which varied from a few minutes to 8 hr. They were then removed and rinsed twice for a few seconds to remove entrained solution. For stratum corneum the sample size was approximately 2 mg and the solution was 10 ml. In either case the exposed substrate was dissolved with Unisol (Isolab Inc.) and Unisol-Complement was added. The resulting clear solution was counted by the scintillation method on a Packard 3255 Tri-Carb Spectrometer to determine the amount of SLS sorbed. Triplicate experiments were run and averaged for each experimental point on the figures.

A GRAVIMETRIC PROCEDURE

The availability of accurate sorption data from the radiotracer experiments with SLS described below furnished a benchmark from which a simpler gravimetric technique

has been worked out for materials not available in tracer form. In studies of hair care products such as proteins, polymers and conditioners it is often of interest to determine the amount of material which is sorbed by the hair. The high cost of human hair and its difficulty in handling dictate that laboratory samples be of small size, that is of the order of 100 to 200 mg in weight. If the sorbed quantity is in the range 1 to 10% (often the case), then the incremental weight is 1 to 20 mg, an amount that is easily detected with an analytical balance. For best results the hair should always be weighed directly, not in a container. A suitable configuration is achieved by winding the 100 mg strand around one's finger and stuffing it into a 1 oz vial. Enough distilled water is added to wet the hair and the vial is left open overnight in a 50°C oven. By the next day, after drying, a well shaped curl has formed which retains its configuration and can easily be removed by forceps and placed on the balance pan.

Hair is very hygroscopic, a fact which creates a problem in gravimetric work. Thoroughly dried hair will gain several percent of its weight in moisture when it is exposed to the atmosphere for only a few minutes. It is thus extremely important to make sure that the sample being weighed is always at the same reference state relative to water vapor. For example, this could be a "bone dry" condition such as is achieved by drying over P_2O_5 or at high temperatures. However, drying by desiccants is time-consuming, while high-temperature drying tends to alter the hair protein irreversibly. On the other hand, simple equilibration for some time at ambient atmosphere is quite unsatisfactory, owing to changes of relative humidity. As a compromise, oven drying at 50°C was adopted. This is low enough so that no damage seems to occur to the hair. The amount of time required to reach equilibrium water content is of the order of 8 to 12 hr. Thus samples can conveniently be left overnight in the oven before weighing. (A 10 min period in a desiccator to cool the sample is advisable between the oven and balance.) The reproducibility achieved in this way has generally been very satisfactory. For example, two samples of bleached hair were conditioned in a 50°C oven for a day. Their weights were recorded as 104.8 and 123.4 mg. The samples were then placed in distilled water for three days, removed and dried in the oven overnight. The following day their weights were, respectively, 105.0 and 123.6 mg.

For accuracy, the hair samples should be dried and weighed several times, both initially and after sorption, until constant values are obtained. In actual practice it was usually found that the weight after the first drying does not change appreciably upon further drying. It is always advisable to run several "controls" for each sorption experiment; these are hair samples which are exposed to distilled water for the same time as the duration of the sorption. Normally the controls will return to their initial weight; but in some cases systematic (*i.e.*, more than one sample) deviations of 0.5 to 1 mg can occur. This is possibly related to large changes of ambient laboratory humidity. Such deviations should be taken into account for the sorption samples.

Some discussion about rinsing procedure is in order here. To a certain extent the method followed must be adapted to the material which is sorbed. Thus, for a cationic polymer, which is very tenaciously sorbed, the hair should be thoroughly rinsed several times. The polymer sorbed will not come off easily even with vigorous washings; and more important, the viscous polymer solution which is easily entrapped in the hair must be removed or erratic results will be recorded. At the other end of the spectrum are substances like salts (see below for results on NaBr). In this case sorption is very weak and thorough rinsing completely removes the salt; a different procedure must be

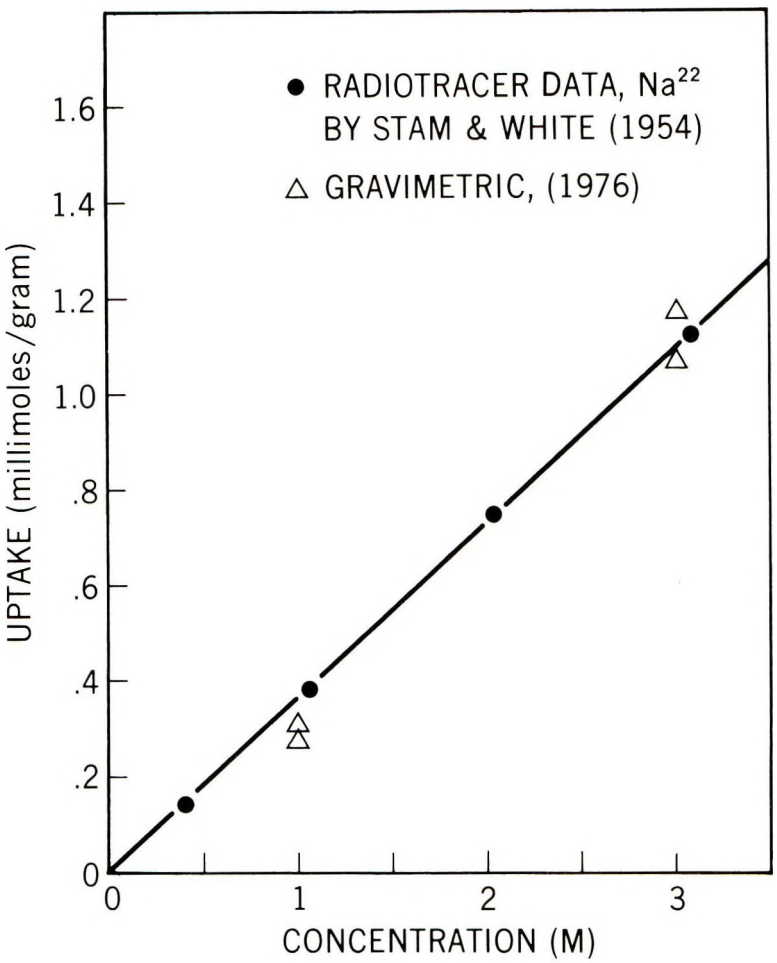

Figure 1. Sorption of sodium bromide by blonde hair

followed, which consists in simply patting dry the hair fibers with tissue. This effectively removes entrapped liquid. The weight of a tress treated in this way is surprisingly reproducible and corresponds to a kind of "fully hydrated" state. The anionic surfactant sodium lauryl sulfate (also treated below) corresponds to an intermediate case. The surfactant is fairly tightly bound by the hair substrate and is located internally as well as on the surface. However, upon very thorough washing (15 to 30 min) a third or more of it will be desorbed. Hence a compromise protocol is advisable, such as two or three successive short rinses in distilled water to remove adhering liquid and foam.

With suitable care the gravimetric procedure outlined above has yielded results which are quite close to those obtained by radiotracers, as shown in the following cases:

Sodium bromide, NaBr. Stam and White (9) have reported on the uptake of NaBr by undamaged blonde hair from aqueous solution, using a Na^{22} tag. Their results for several concentrations are given in Figure 1 and show a linear relation between sorption and concentration. Our gravimetric data (also with undamaged blonde hair) done in duplicate at two concentrations are plotted in the same figure. The agreement is surprisingly good, considering that the hair samples are completely different. Note that

this is a relatively favorable case owing to the large weight of sodium bromide; 1 millimole/g corresponds to about 10% by weight.

Sodium lauryl sulfate (SLS). In this instance, the uptake of SLS from 10% solution was measured on a single series (three samples for each time period). The solution was tagged with the compound described in the experimental section. First, weighing was done according to the gravimetric method above, yielding the open triangles of Figure 2. The samples were then dissolved and counted for radioactivity content following the procedure of the experimental section. This gave the solid circles of Figure 2. The agreement of the two methods is excellent indeed.

Thus, for relatively favorable cases (bleached hair as substrate and large uptakes), sorption of some materials by hair can be determined gravimetrically with reasonable accuracy. (The authors have not found it practical to adapt a similar procedure to stratum corneum as substrate.)

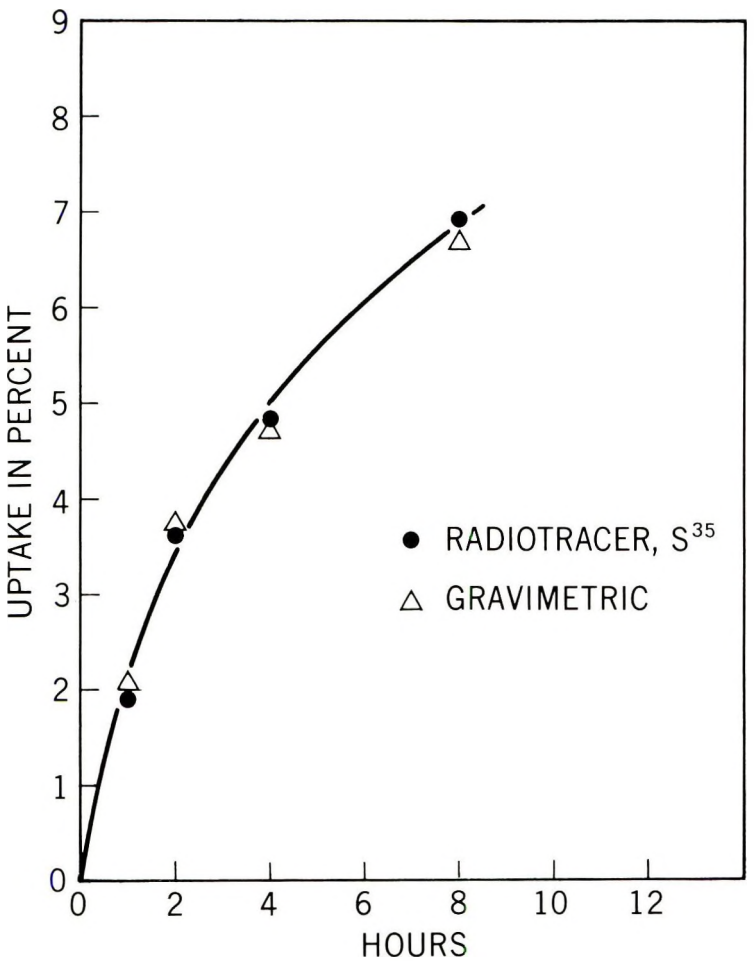

Figure 2. Sorption of 10% sodium lauryl sulfate by bleached hair; gravimetric and radiotracer determinations were made on the same samples of hair

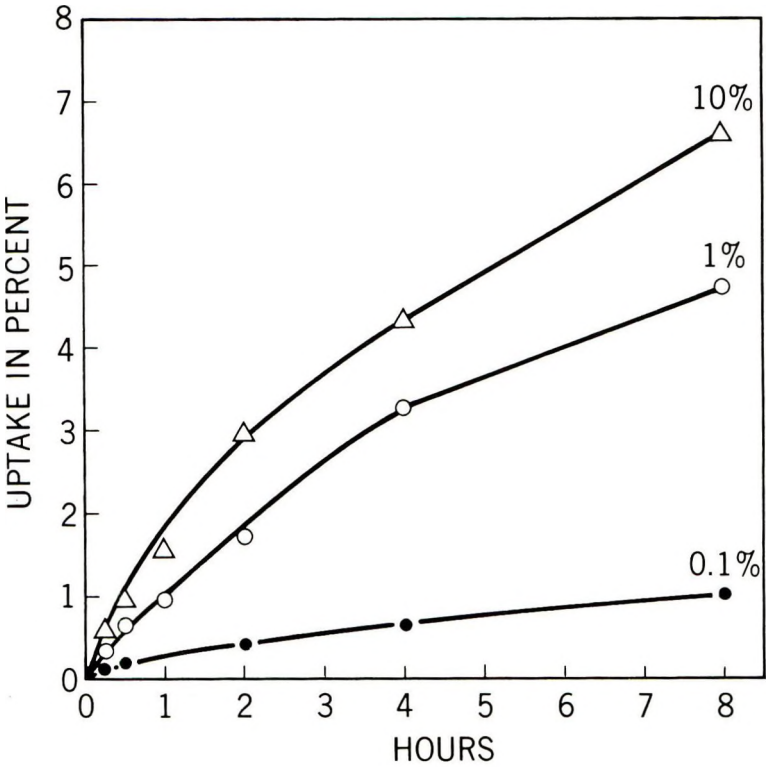

Figure 3. Sorption of sodium lauryl sulfate by bleached hair

RESULTS AND DISCUSSION

SODIUM LAURYL SULFATE

Typical sorption curves at various concentrations are shown in Figure 3 for bleached hair and in Figure 4 for stratum corneum. Similar curves were obtained for undamaged hair and the uptakes in that case were approximately an order of magnitude less than found for bleached hair. For all these substrates the course of sorption follows closely a linear dependence on the square root of time, consistent with a diffusion process. In Figure 5 the data are plotted in this manner for both bleached and undamaged hair. A linear dependence is observed except for the first 15 to 30 min, where a sort of "lag time" is observed. Analogous behavior has been noted before in the dyeing of wool, a physically similar type of process (10,11). The initial lag is characteristic of the presence of a surface barrier, which in this case is postulated to be the so-called epicuticle (12). The data for stratum corneum (Figure 6) also shows good linearity in $\sqrt{t}$ but there is no evidence of a surface barrier.

The slopes of the uptake – $\sqrt{t}$ lines can be regarded as a measure of the rate of sorption and it is evident that these rates continually increase with concentration. Thus Figure 7 shows the uptakes at 1 hr (which are proportional to the slopes) plotted against the total surfactant concentration for stratum corneum and bleached hair. An interesting feature occurs in both cases: the rate function closely approximates two straight lines which intersect at the critical micelle concentration, CMC, *i.e.*, the point

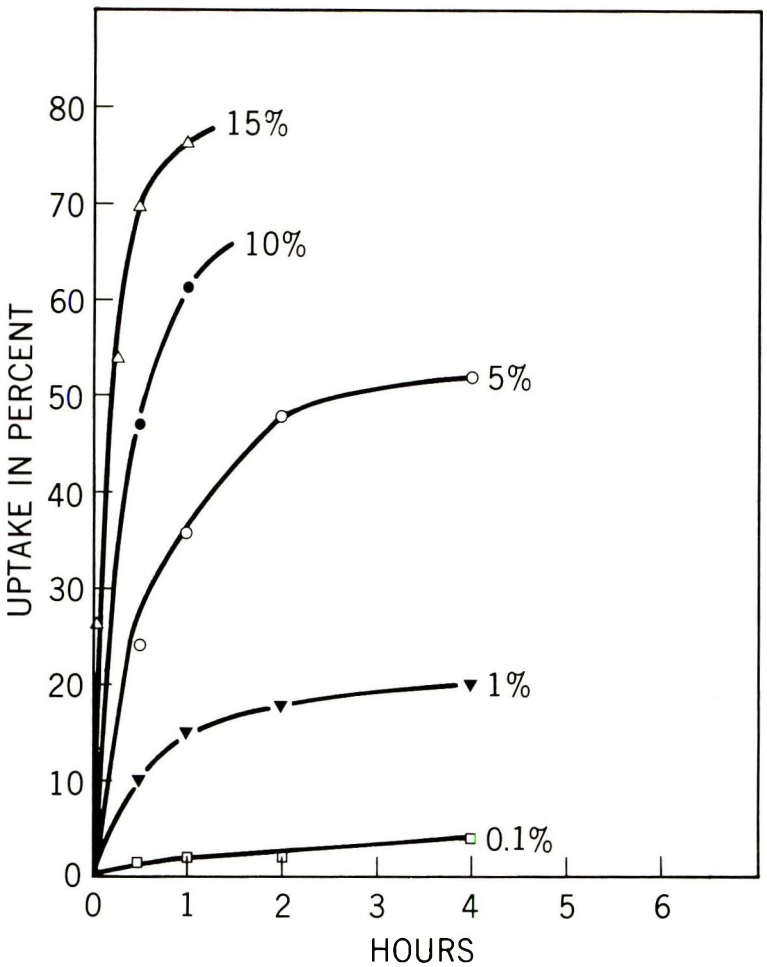

Figure 4. Sorption of sodium lauryl sulfate by stratum corneum

where micelles begin to form. (For SLS this concentration is 0.24%.) Undamaged hair also shows this phenomenon, but the rates are considerably lower than for bleached hair.

It is not surprising that the CMC is important in terms of sorption rate. The diffusion mechanism of sorption strongly suggests that it is the monomer species which enters the substrate. Above the CMC most of the added surfactant is known to exist in the solution as micelles, *i.e.*, roughly spherical aggregates of 50 to 100 surfactant molecules. These entities do not seem to penetrate the keratin, probably because of the combination of size and negative charge. Little is known about the precise monomer concentration above the CMC, although it is often stated that it remains constant. There is evidence, however, that it may increase slowly. For example, osmotic pressure data (13) show a pattern of two intersecting lines similar to Figure 7, but the measurements were not extended very far above the CMC. Mysels (14) in some ingenious dialysis experiments has shown that the rate of passage of SLS through a membrane impermeable to micelles continues to increase above the CMC and he cites this as evidence for increas-

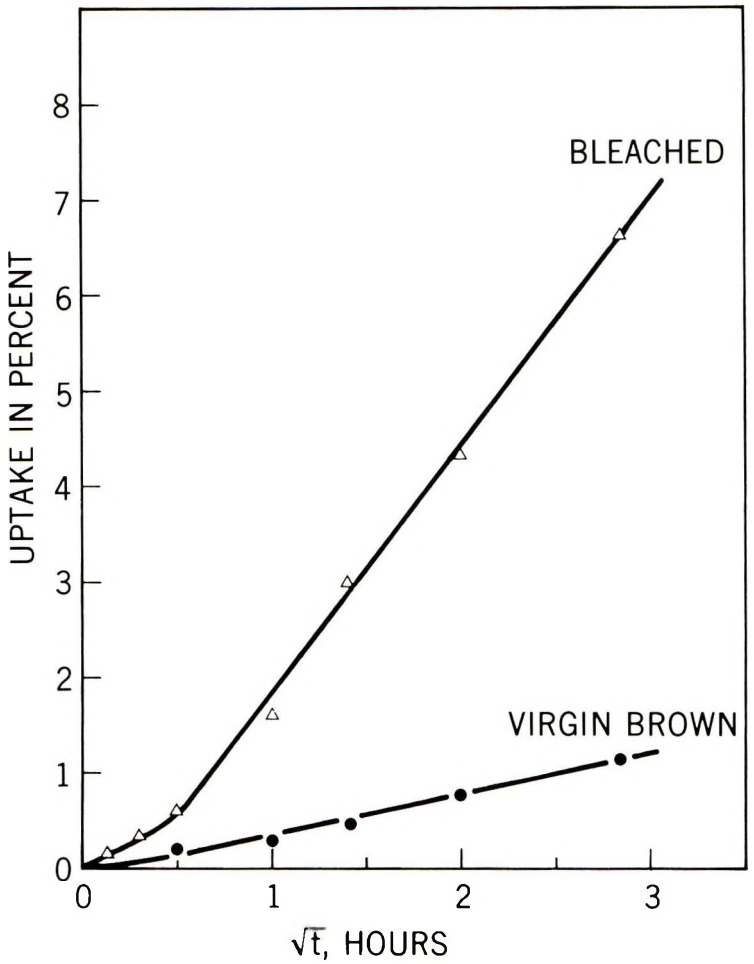

Figure 5. Sorption of 10% sodium lauryl sulfate by bleached and virgin brown hair

ing activity of the monomer in this region. Unfortunately it is very difficult to make direct measurements that are unambiguously related to the SLS monomer concentration in the region well above the CMC. Thus, while it is not clear that monomer concentration in fact does increase there, the sorption data shown here are consistent with such an interpretation and they show the same kind of phenomenon found in the dialysis experiments of Mysels.

THE DIFFUSION PROCESS

From the uptake *vs.* $\sqrt{t}$ curves a rough estimate can be made of the diffusion constant of SLS in the keratinous medium, either hair or skin. For hair, the formulation commonly employed is that which represents diffusion into an infinitely long cylinder at short times (9):

$$\frac{Q(t)}{Q(\infty)} = \frac{4}{r}\sqrt{\frac{Dt}{\pi}}$$

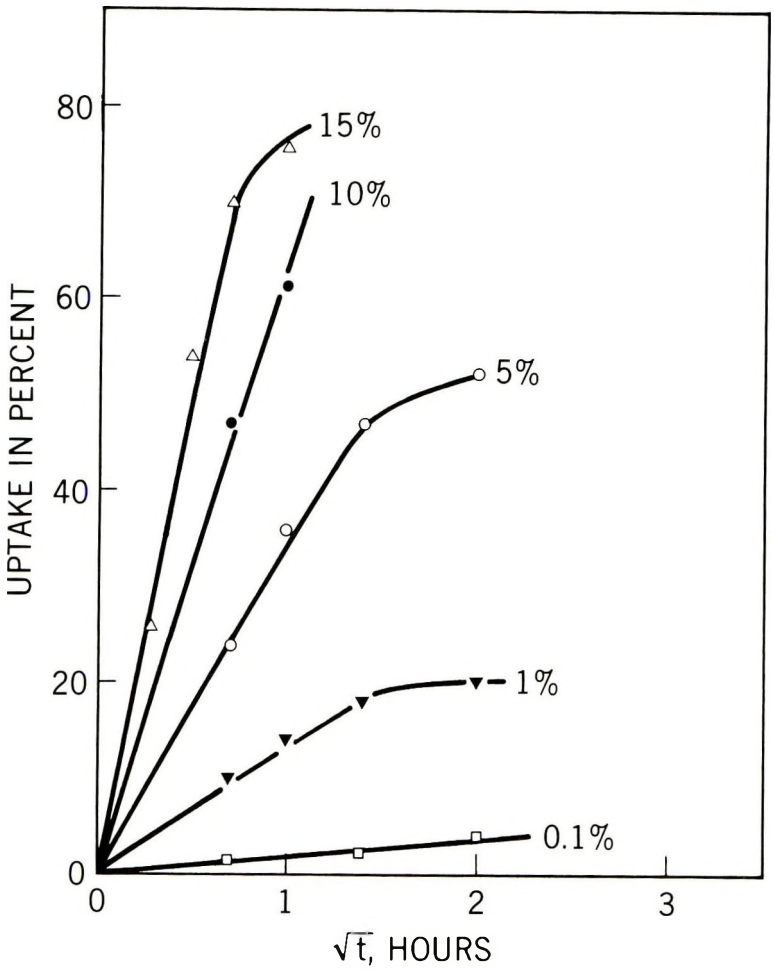

Figure 6. Data of Figure 4 *vs.* $\sqrt{t}$

where r is the radius of the hair fiber, D is the diffusion constant and Q (∞) is the "equilibrium" uptake, *i.e.*, at very long times. If r is taken as 25×10^{-4} cm and a rough estimate is made for Q (∞) by measuring uptake after several days, one obtains for bleached hair D = 1 to 3×10^{-11} cm²/sec and for undamaged hair D = 1 to 2×10^{-12} cm²/sec. Within the uncertainty of estimation of values for Q (∞) the magnitudes of the diffusion constant D were found to vary only slightly for the concentration range between 0.1 and 10%. They compare well with values reported by Griffith (15), 1×10^{-11} cm²/sec, and by Chen (16), 4×10^{-11} cm²/sec, in both cases for wool, a closely related substrate. These authors made use of the formula cited above.

In the case of skin, we prefer to use the well known formula of A. V. Hill (see reference 7 for a derivation):

$$Q(t) = 2C_0\sqrt{\frac{Dt}{\pi}}$$

where Q is the uptake in g/cm², C_0 is the external concentration in g/cm³, t is the

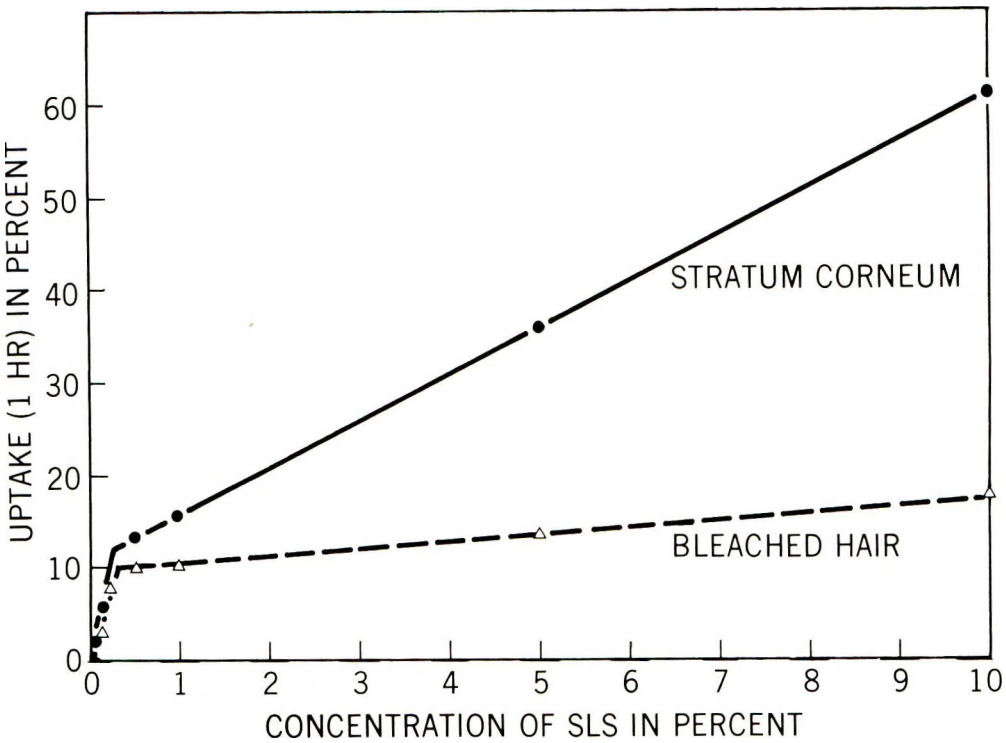

Figure 7. Concentration dependence of sorption rate: uptake of sodium lauryl sulfate by stratum corneum and bleached hair

time in seconds and D is the diffusion constant. The diffusing species is assumed to be the SLS monomer; hence C_0 corresponds to the total solution concentration only in the range below the CMC (0.24%). Using data at 0.1 and 0.2% one obtains by this formula $D = 3$ to 7×10^{-9} cm²/sec, about three orders of magnitude higher than in undamaged hair but still considerably lower than for SLS in water.

SORPTION OF SODIUM LAURYL ETHER SULFATES

Having measured the sorption of 10% SLS by a simple weighing procedure, it was of interest for comparison to determine the uptakes of closely related surfactants: Standapol ES-2, ES-3 and 130-E. These are, respectively, the 2, 3 and 12 mol ethoxylates of SLS and they represent a chemical series which increases in ethylene oxide content. Radiotagged samples of these materials are not available, so the gravimetric procedure described above was used. In Figure 8 their uptakes *vs.* time are plotted and compared to SLS. Bleached hair was the substrate. There is clearly a reduction in sorption with increasing number of ether groups in the surfactant molecule—a reduction which also persists on a molar plot. There are several possible explanations for this effect. The simplest is that the molecules increase in size in this series and hence have more difficulty getting into the hair structure. Also very convincing is the fact that the CMC decreases markedly for these compounds as the ethylene oxide content increases (17). Thus the monomer concentration available for diffusion will be a decreasing func-

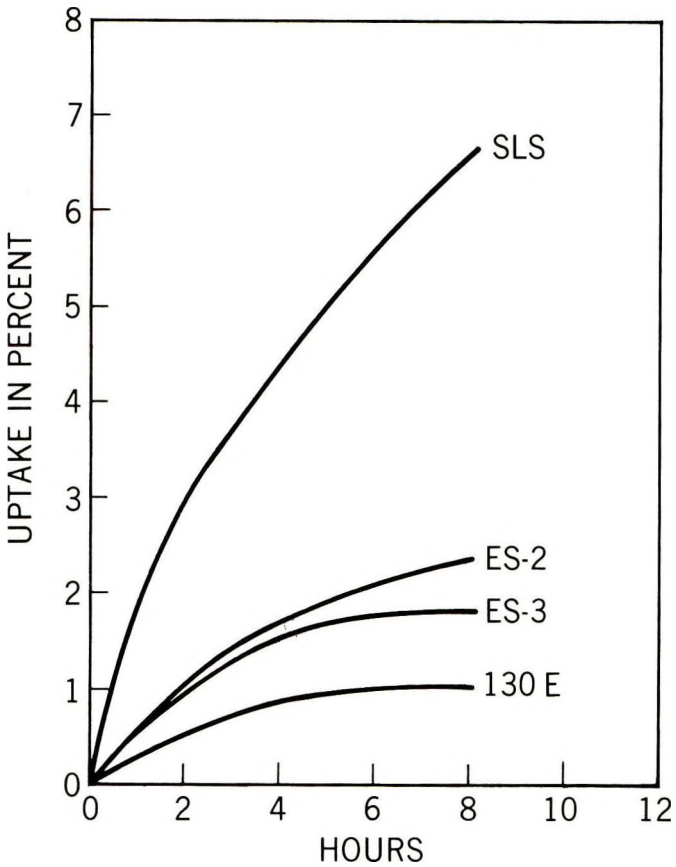

Figure 8. Sorption of sodium lauryl ether sulfates by bleached hair from 10% solution

tion also. This decreased sorption correlates well with the well known milder properties of highly ethoxylated anionic surfactants compared to SLS.

THE EFFECT OF ADDITIVES

A feature of SLS sorption is that it is strongly influenced by the addition of certain other compounds. For example, sodium chloride generally causes an increase in sorption. This effect is well known from work on wool (15,16). It is even more pronounced with stratum corneum as the substrate, as the data in Figure 9 show. Harrold and Pethica (4) found the same phenomenon with finely divided epidermal keratin. Salt decreases the CMC of SLS, so the monomer concentration will be lowered in its presence. It seems, therefore, that the salt must act on the substrate in a way that makes it more available to the surfactant or by a nonspecific electrical screening effect.

On the other hand, the addition of a nonionic surfactant such as Tergitol 15-S-9 considerably decreases the sorption of SLS, both for hair and skin. This is not due to competition between the two surfactants for sites in the keratin, because the nonionic material is hardly sorbed at all by itself. Instead it is known that mixed micelles of the two surfactants are formed. For a very similar system Schick and Manning (18) have

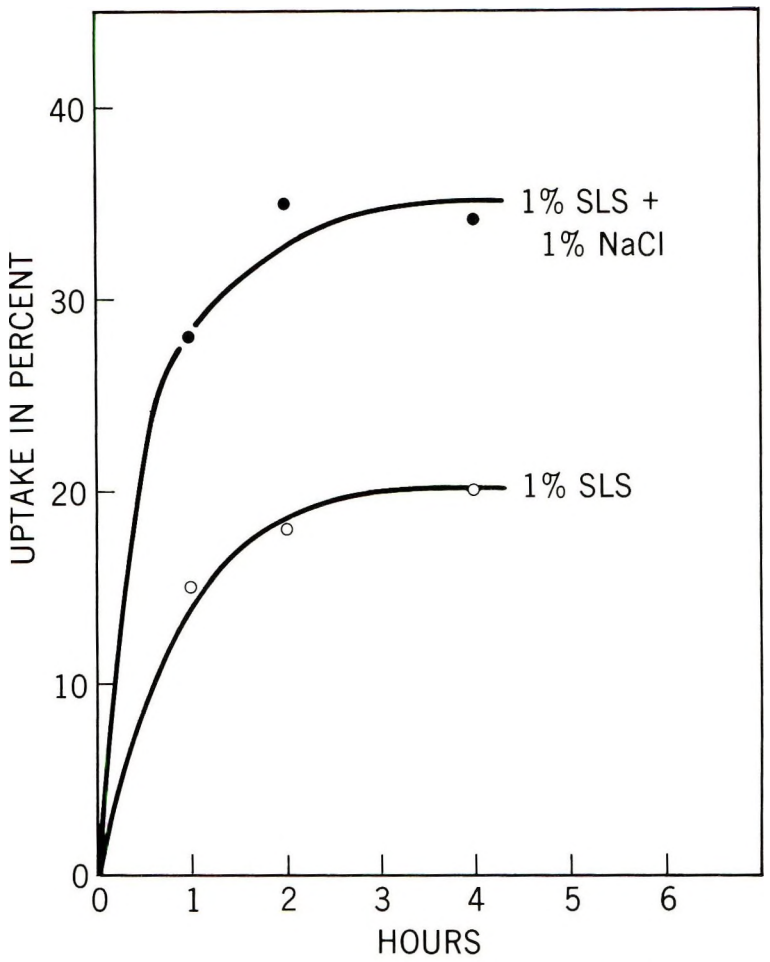

Figure 9. Effect of salt on the sorption of sodium lauryl sulfate

shown that even small additions of a nonionic surfactant have a large effect in lowering the CMC of sodium lauryl sulfate. This brings about a lowering of the SLS monomer concentration and, hence, lower sorption. Figure 10 demonstrates the effect in a striking way. This furnishes a physico-chemical explanation of the findings of Finkstein (19) who showed that a reduction of irritation of anionic shampoos occurs on the addition of nonionic surfactants in spite of the fact that the total surfactant concentration increased. In this case, lower irritation is attributed to decreased sorption of the anionic surfactant by proteins of the skin and cornea.

RELATION OF SORBED SURFACTANT TO WATER OF HYDRATION

Both hair and stratum corneum absorb water when placed in solution. It is therefore conceivable that some, if not all, of the sorbed surfactant may be present as a solute in this "internal" solution, rather than being truly bound to the keratin. The analytical method employed here does not distinguish these cases. It is not easy to decide this point conclusively, but the available evidence indicates that the surfactant is bound to the substrate.

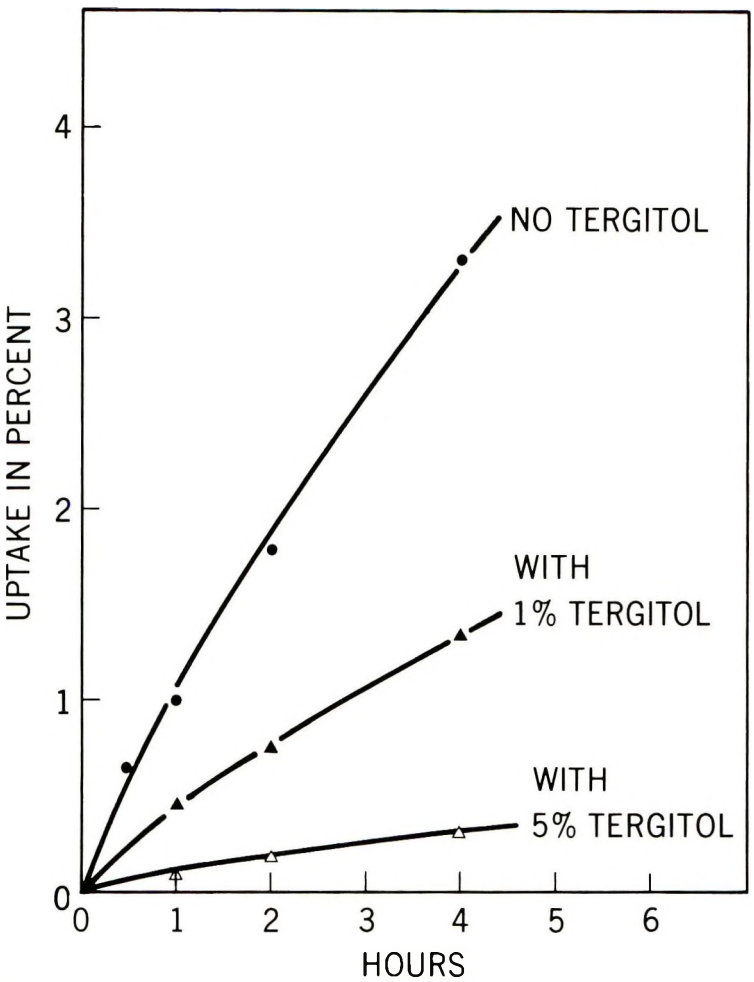

Figure 10. Effect of a nonionic surfactant on the sorption of sodium lauryl sulfate by bleached hair

For undamaged hair the water of hydration amounts to about 35% by weight of the dry substrate; this water is absorbed in less than 1 min. However, the SLS sorption, as shown in Figure 1, goes on for many hours. Furthermore, at low solution concentration of SLS the ultimate amount of surfactant taken up by hair can amount to ten times as much as would be calculated solely from the "external" solution. A study has been made by NMR of the mobility of water in hydrated hair (20). In this work it was found that such water is quite immobile and tightly bound to the keratin. It seems unlikely that SLS can exist as a normal solute in such an environment.

The case of stratum corneum is somewhat different in that this substrate absorbs as much as 1000% of its own dry weight over a period of many hours when immersed in aqueous solution. A detailed study of this water (21) shows, however, that much of it is quite restricted in mobility and probably located in the interior of the keratin cells. Again, it seems unlikely that this water of hydration can behave like the bulk "external" solution; in particular, the existence of ordinary micelles therein is improbable because of exclusion effects. Figure 11 shows two curves which compare the actual measured

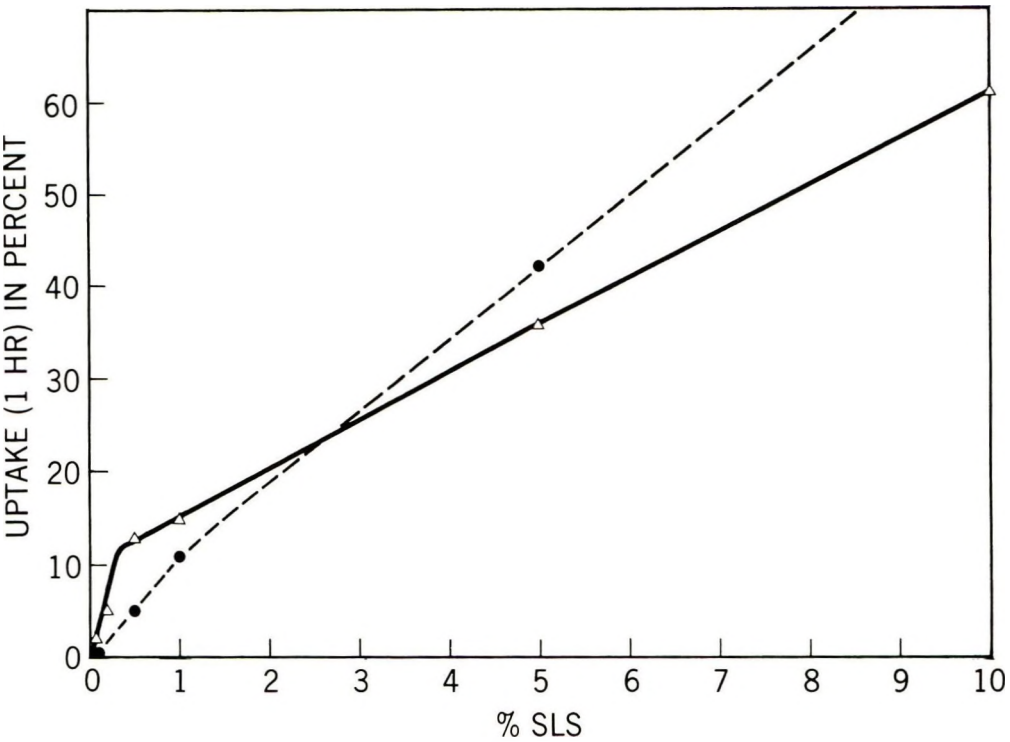

Figure 11. One hour uptakes of sodium lauryl sulfate in stratum corneum: solid line, as measured experimentally; dotted line, calculated by assuming that water of hydration has the same composition as the external solution

uptakes of SLS at 1 hr with the calculated uptakes at each concentration assuming that the water of hydration or "internal" solution has the same concentration as the "external" solution. The latter curve was calculated from swelling data obtained at a 1 hr exposure for a number of concentrations of SLS. It will be noted that the uptakes at low concentrations (below the CMC) are much greater than the calculated uptakes. But at high concentrations (above 5%) the calculated uptakes are larger than the measured ones. This lack of agreement clearly shows that the internal solution does not have the same concentration as the external one. It does not exclude the possibility, however, of some SLS monomer existing in free solution inside the stratum corneum. The evidence above suggests that this possible state is unlikely to amount to more than a small fraction of the measured uptake. In this connection it may be recalled that collagen and protein in general can bind large amounts of SLS. Nelson (22) has shown that as much as 1.1 to 2.2 g of SLS/g of protein can be bound under the most favorable conditions. Thus the inference that all of the SLS uptake reported here is bound to the keratin is not unreasonable. More light could be shed on this point by a detailed NMR study of the state of the lauryl sulfate anion in hydrated stratum corneum and hair.

CONCLUSIONS

It has been shown that the uptake of anionic surfactants by hair and stratum corneum membranes is appreciable. With sodium lauryl sulfate, SLS, the uptake increases markedly with concentration even above the critical micelle concentration, and it also

increases in the presence of added salt but decreases in the presence of added nonionic surfactant. Lauryl ether sulfates are sorbed to a lesser extent than SLS and their uptake decreases with ethylene oxide content.

By comparison of sorption data obtained by radiometric and gravimetric techniques, it has been demonstrated that a simple weighing technique can be employed for measuring the uptake of surfactants and simple salts, in view of their relatively high sorption values.

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Interaction of keratinous substrates with sodium lauryl sulfate: II. permeation through stratum corneum

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Synopsis

Neonatal rat STRATUM CORNEUM was used as a model membrane to investigate PERMEATION through mammalian skin. Passage of materials through these membranes was determined by use of radio-tagged compounds and by spectrophotometric analysis. The anionic surfactant SODIUM LAURYL SULFATE penetrates the stratum corneum even at low concentrations. The diffusion constant for this process is about 10^{-10} cm^2/sec, compared to 10^{-6} cm^2/sec for free diffusion in water. This SURFACTANT is bound to the skin in large amounts, up to 50% by weight at high concentrations. Pretreatment of the membrane by a cationic cellulose polymer (which is itself strongly sorbed) greatly reduced the amount of surfactant which passed through the membrane.

INTRODUCTION

Considerable study has been made in the past of the effect of surfactants on the permeability of mammalian skin. For example, Bettley and Donoghue (1) showed that soap not only penetrates the skin barrier, but also makes the barrier more permeable to water and other solutes. A number of studies thereafter emphasized the increased permeation of water or salts (2–6). Relatively little, however, has appeared on the permeation of surfactants themselves through skin. Perhaps the first quantitative study was by Blank and Gould in 1959 (7) showing that anionic surfactants in low concentration penetrated human epidermis with difficulty. Blank's subsequent work on a cationic surfactant indicated virtually no penetration at all (8). With somewhat greater concentrations, Scala *et al.* (9) and Howes (10) demonstrated more permeation by various anionic surfactants. All of these previous studies were quite limited in terms of the range of concentrations studied and the time scale investigated; yet both factors have been specifically recognized to be of great importance in the permeation process (5). The authors' interest in the permeation of surfactants through skin arose in connection with a clinical study of antiirritation effects of a cationic polymer (11). A detailed study of both sorption and permeation of the anionic surfactant sodium lauryl sulfate has been made with the aim of clarifying the mechanism of action of these observed protective effects.

EXPERIMENTAL

Stratum corneum of neonatal rats was used as a model for human skin. Live young rats one or two days old were obtained from Marland Breeding Farms, West Milford, New Jersey. The animals were sacrificed by being placed in an atmosphere of CO_2 for several hours; after death the whole skin was removed by a surgical scalpel. The skin was placed in a desiccator jar and exposed to ammonia vapor for 1 to 3 hr. Following this, the skins were put in water and the epidermal layer was gently separated from the dermis. The epidermis so obtained was floated on the surface of a pan of water. After an hour the membrane was removed by bringing up a metal screen under it. The membrane was placed top down on a wet paper towel and the screen removed. At this point the Malpighian layer could be gently scraped off, leaving the desired stratum corneum on the towel. The paper and stratum corneum were placed again in water until separation occurred. The stratum corneum layer was recovered by a small Teflon screen and air-dried. Isolation of the stratum corneum follows a method outlined for us by E. J. Singer and E. Boisits of Lever Brothers Co., Edgewater, New Jersey. A typical piece of stratum corneum was about 25 μm thick and 5 $\times$ 6 cm in area. It weighed about 20 mg, corresponding to a density of 0.7.

The permeability cell used was modeled after a description by Loveday (12), and is shown schematically in Figure 1. The temperature of the experiments was that of the laboratory, 23°C $\pm$ 1°C.

The surfactants used in this study were:

Tergitol 15-S-9 (Union Carbide Corp.), the 9 mol ethoxylate of a secondary C_{11} to C_{15} alcohol.

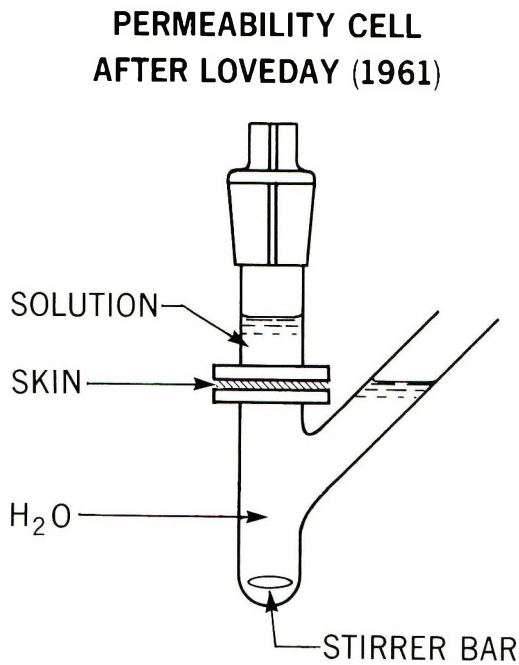

Figure 1. Schematic drawing of permeability cell

Barquat MS-100 (Lonza, Inc.), myristyldimethylbenzyl ammonium chloride.

Sodium lauryl sulfate (SLS) was obtained as a pure white crystalline powder from BDH Chemicals.

Analyses of the first two materials were made spectrophotometrically, by absorption at 337 nm for Tergitol and at 262 nm for Barquat. In these cases, the small amount of protein leached from the stratum corneum does not interfere with absorption peaks of the surfactants.

Radiotagged material was purchased from Amersham-Searle (Des Plaines, IL) in the form of small individual ampoules. Each ampoule contained 2.47 mg of SLS with an activity of 100 microcuries. The tag was present as the S-35 isotope and is thus in the anionic part of the surfactant. Solutions of desired concentration were made up of the nonradioactive powder and one ampoule was added with stirring. For permeability experiments a small sample (0.1 g) of the water in the lower part of the cell was removed by pipette and put in a 1-oz counting vial filled with the scintillant liquid, Instagel. Radioactivity was determined by scintillation counting in a Packard 3255 Counter. For sorption experiments skin samples of about 2 mg each were placed in 20 ml of tagged solution for various set times, removed and rinsed twice for a few seconds with distilled water to remove entrained solution. The skin was then dissolved in UNISOL and a scintillant cocktail added. Radioactivity was again determined in the Packard Counter.

Polymer JR is a quaternary nitrogen-containing cellulose ether (13). The JR-400 grade was used for the experiments reported here. Its approximate molecular weight is 400,000.

NOTE ON CALCULATIONS

For biological membranes such as skin, whose thickness is difficult to measure, it is convenient to work with the apparent permeability defined by (14)

$$Q/A = P\,\Delta c\,t, \qquad [1]$$

where Q is the amount of solute which penetrates through area A in time t; P is the apparent permeability constant and Δc is the difference in concentration between the two sides of the membrane. For this work Δc is taken to be simply the initial concentration of the upper solution, since the concentration in the stirred water is always close to zero. An idealized Q *vs.* time curve is shown in Figure 2. There is an initial slow growth of Q leading to a straight line portion, the extrapolation of which back to the time axis gives the "lag" time, T_0. From this time a diffusion constant, D, can be calculated by (14)

$$D = h^2/6T_0 \qquad [2]$$

where h is the thickness of the swollen membrane. The plot illustrates how both P and D can be derived from permeability data. For purposes of calculation h has been assumed here to be 50 μ, which is about twice the dry thickness of the stratum corneum. (It should be appreciated that individual membranes vary in thickness and that the swollen thickness changes slowly with time.)

In the ideal case, the apparent permeability is related to the diffusion constant, D, by the equation:

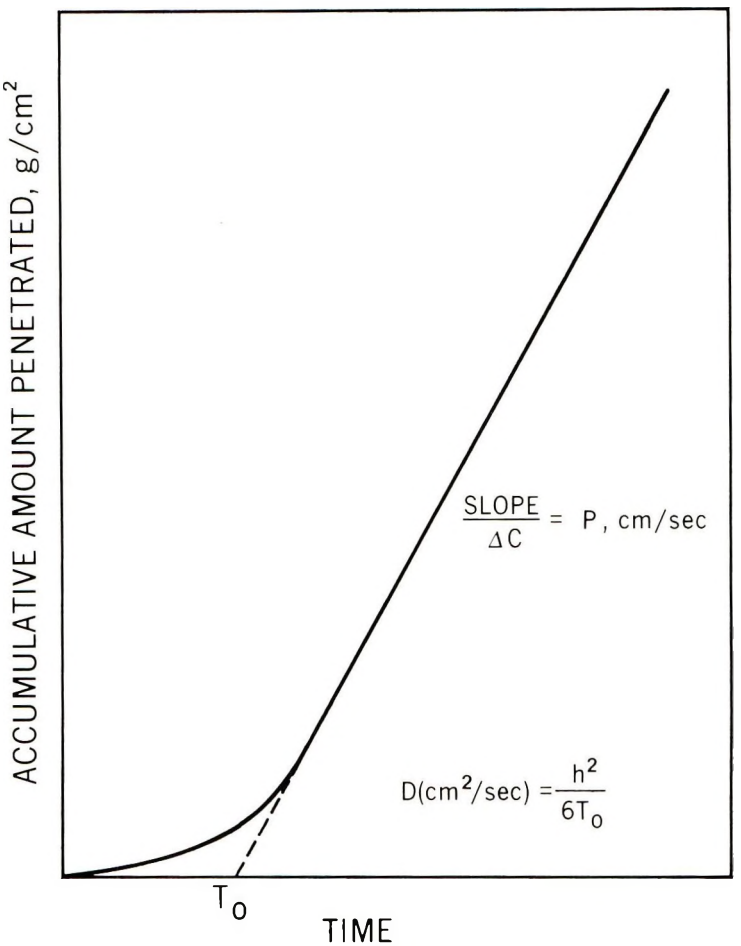

Figure 2. Idealized permeation curve

$$P = (KD)/h \qquad [3]$$

where K is a unitless partition coefficient, being the ratio at equilibrium of the concentration of solute in the membrane to the concentration of solute in solution (15). This allows determination of K, or of P by two different methods if K is known.

RESULTS AND DISCUSSION

In view of the fact that each permeation run uses a different individual skin, some general comments on reproducibility are appropriate. Generally speaking, precision was found to be good. For example, Figure 3 shows three separate stratum corneum samples treated for permeability to 10% SLS, a very aggressive environment. Better results could be obtained at lower concentration: Figure 4 contains data for two different runs at 0.5% SLS. However, it was not always possible to obtain such good agreement, particularly at the higher concentrations where the membranes are substantially dissolved with time. Occasional skins showed immediate passage of

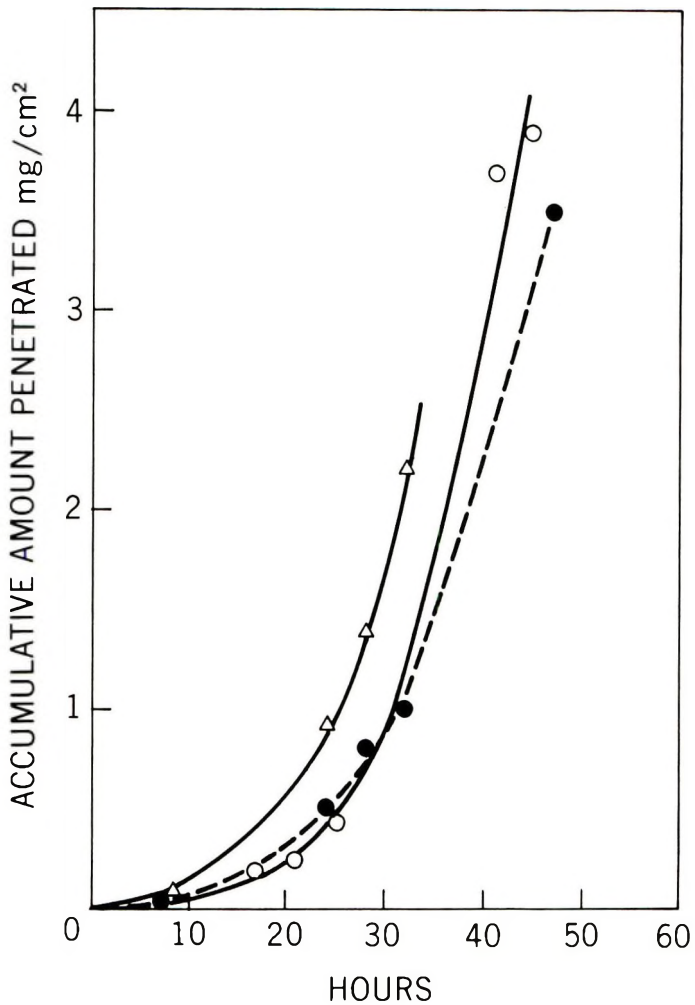

Figure 3. Three separate runs at 10% sodium lauryl sulfate

surfactant, presumably because of a pinhole or quick breakdown. Such membranes were discarded and the data not used.

BARRIER PROPERTIES

Extensive studies of moisture vapor transmission have shown that the barrier function is well developed at birth for young rats (16) and we have confirmed these findings (17). In this sense the stratum corneum of such animals can be regarded as a reasonable model for human skin. An examination was also made of the permeability of liquid water (tagged by tritium oxide) through these membranes. The permeability was found to be 5.5×10^{-7} cm/sec. This is in satisfactory agreement with the value of 2.8×10^{-7} cm/sec determined for human stratum corneum by Scheuplein (15).

It should be noted that this relatively low value of permeability means that there is rather slow, back diffusion of water from the lower reservoir of the permeability cell

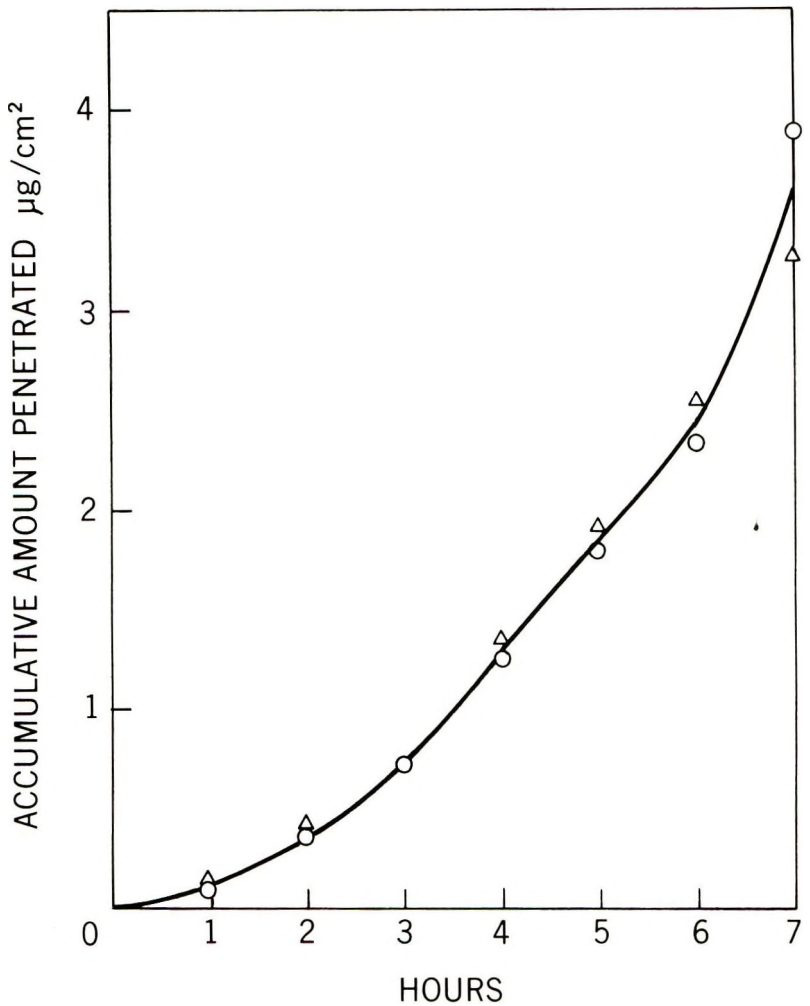

Figure 4. Two different runs at 0.5% sodium lauryl sulfate

into the concentrated surfactant solution above the membrane (osmotic effect). This flow can be calculated to be something on the order of 1 to 2 mg/hr/cm^2, and is a negligible factor for the time and concentrations described below.

PERMEATION OF SURFACTANTS

Seemingly contradictory opinions have appeared in the literature concerning the permeability of mammalian stratum corneum to surfactants and ionic species in general. The viewpoints of Blank (7,8), that both cationic and anionic surfactants penetrate human epidermis with great difficulty or not at all, and of Scala (9), that permeability of human skin continually increases with exposure to these surfactant types, were reconciled by Scheuplein (5) who showed the great effect of concentration. The data for sodium lauryl sulfate given below confirm his interpretation.

As a preliminary it may be noted that considerable barrier strength does exist in the stratum corneum against nonionic and cationic surfactants. Thus the cationic, myristyldimethylbenzyl ammonium chloride (Barquat MS-100) applied at the level of 10% to neonatal rat stratum corneum requires about six days for penetration. Figure 5 shows the permeability curve, which resembles the schematic of Figure 2. Using eq 1 above, one can calculate from the slope an apparent permeability, P, of 2.2×10^{-6} cm/sec, and from the lag time of six days $D = 0.8 \times 10^{-11}$ cm^2/sec. The permeability value is unreasonably high since it is even greater than that for water (see above). If one now uses these values of P and D in eq 3 to calculate the partition coefficient K, a value of 1380 is obtained. For the surfactant concentration of about 0.1 g/cm^3 this implies a concentration of 138 g/cm^3 of Barquat MS-100 in the swollen skin, a physical impossibility. It is plausible that sometime between three and six days the membrane has been structurally altered to permit such a high permeation. Since this change may well be non-uniform throughout the membrane, there is little point in the calculation of P or D in such a case. The same phenomenon was found for the nonionic Tergitol 15-S-9 at

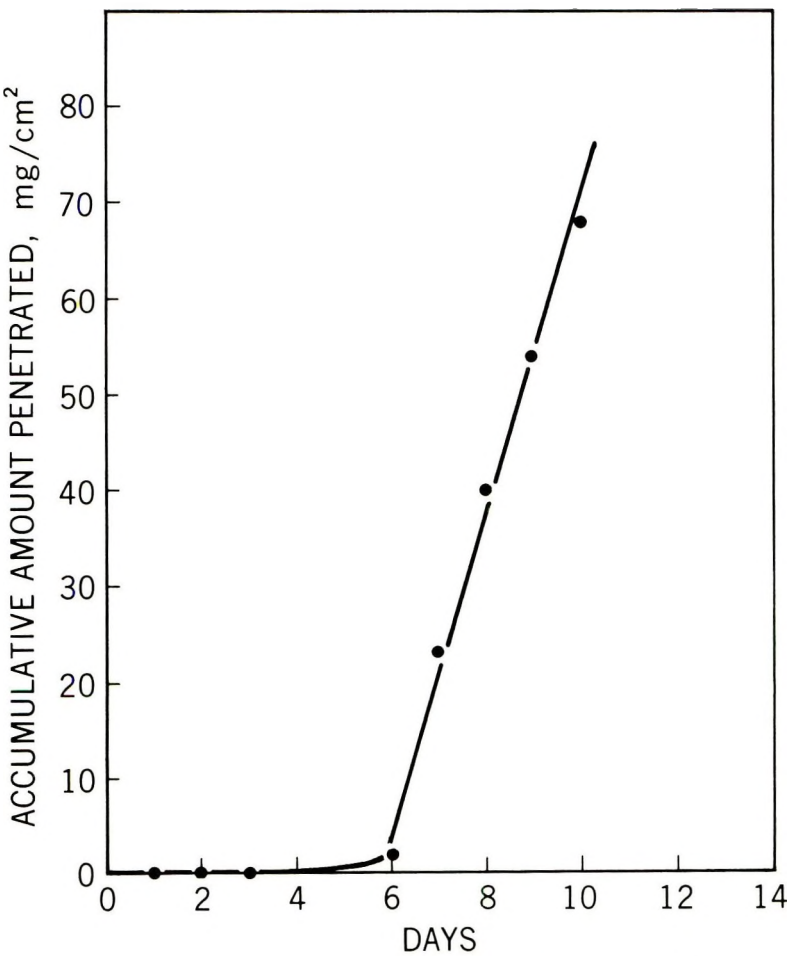

Figure 5. Permeability of Barquat MS-100 at 10% concentration

10% and also for a number of simple electrolytes such as NaCl, RbCl and $CaCl_2$, all at 1 to 2 M. In each case the lag or "breakthrough" times for these materials ranged from two to six days, which indicates that the stratum corneum provides a fairly good barrier even against concentrated salts. This is also confirmed by previous studies (18).

SODIUM LAURYL SULFATE

However, the stratum corneum is not such an effective barrier against the anionic surfactant sodium lauryl sulfate. At the higher concentrations a steady attack on the membrane appears to take place as evidenced by permeation curves with continually increasing slope; see for example the data of Figure 3 and Figure 4. But at the very lowest concentrations the permeability curves begin to resemble the idealized curve of Figure 2, especially for the initial stages. Furthermore, approximately the same lag time is obtained for different concentrations, so that an attempt can be made to calculate P and D. Figure 6 shows data for runs at 0.01 and 0.001% SLS which give a P of 1.7 to 2.3×10^{-8} cm/sec and a D of 1.0×10^{-10} cm^2/sec. The only study in the literature with which a meaningful comparison can be made seems to be the work of Howes (10); he

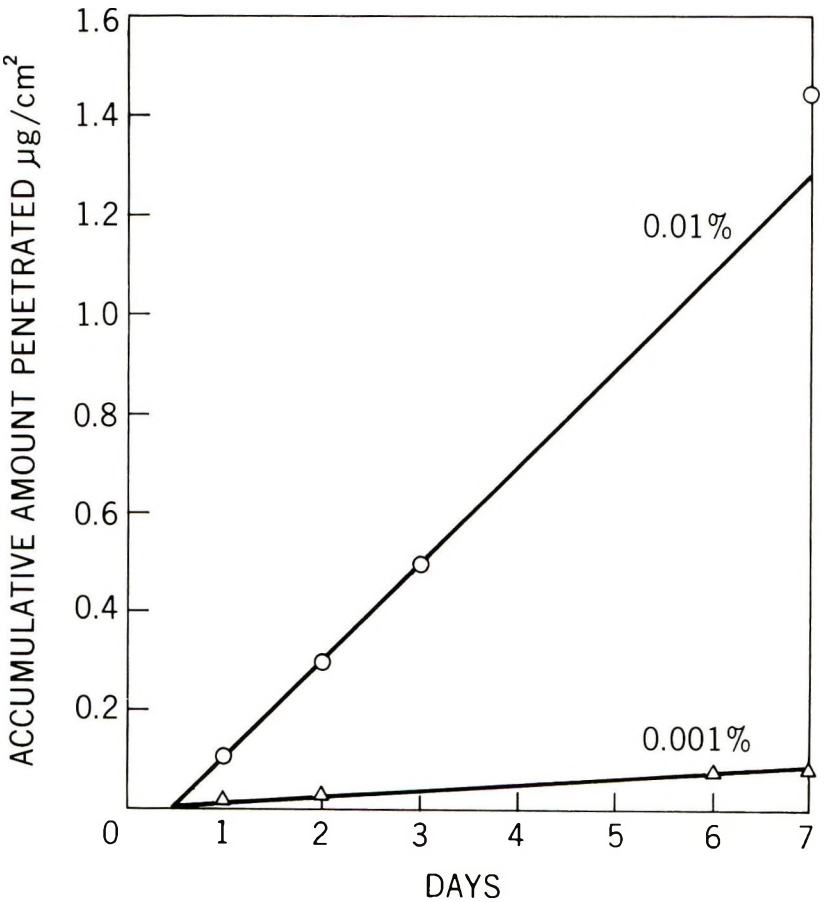

Figure 6. Permeability of 0.01 and 0.001% sodium lauryl sulfate

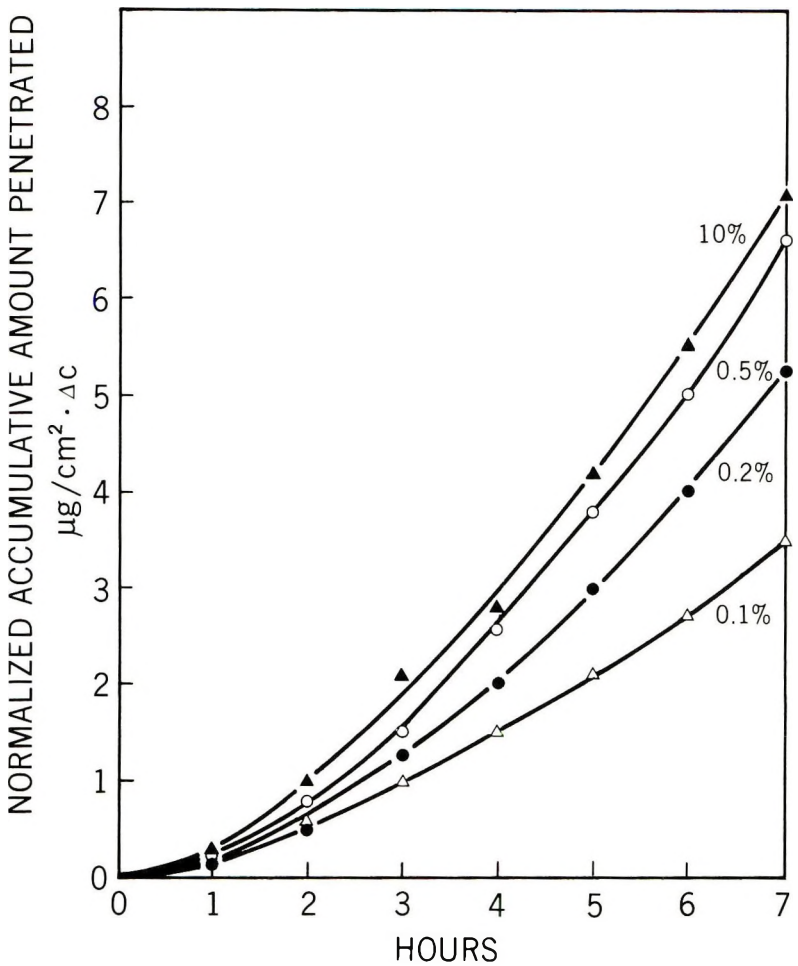

Figure 7. Normalized permeability curves for sodium lauryl sulfate permitting data at different concentrations to be shown on the same plot. Data at 10% were divided by 10, data at 0.1% divided by 0.1, etc.

obtained permeability constants for SLS through human epidermis of 0.17 to 3.0 × 10^{-8} cm/sec, the lower value being at 6 hr of contact and the higher at 24 hr. His concentration was about 0.7% SLS, which is somewhat higher than those in Figure 6.

As the concentration increases, still greater changes occur in the membranes. This can be conveniently shown by a series of "normalized" permeation curves in Figure 7. In this plot the flux has been divided by the solution concentration. Thus the slopes of the various curves are directly proportional to permeability. There is a continual increase in slope with concentration, although the effect is not large for the first 8 hr. Figure 8 shows the same type of behavior over a wide range of concentration and time. This is perhaps the most impressive example from this study of the vigorous attack by SLS on skin.

The data of Figures 7 and 8 raise an interesting question about the mechanism of penetration of SLS through the skin. Above the critical micelle concentration (CMC),

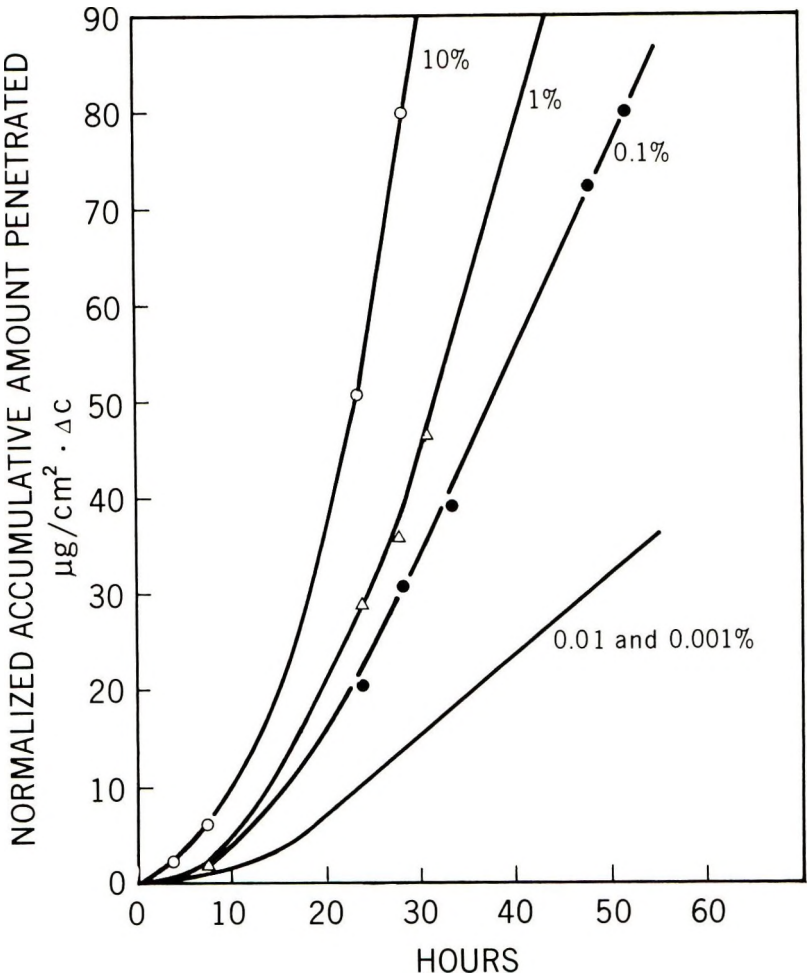

Figure 8. Normalized permeability curves at longer times. Calculation procedure is the same as in Figure 7

which is about 0.24% for SLS, the surfactant molecules in solution are thought to exist mainly in the form of micelles, *i.e.*, approximately spherical aggregates of 50 to 100 molecules. These entities presumably are too large to diffuse as such into or through the stratum corneum, and it is the monomer molecules of SLS which are the actual diffusing species; yet their concentration in solution is generally held to be nearly constant above the CMC. On the face of it then, the flux through the membrane might be expected to level off above the CMC. From Figures 7 and 8 it is clear that this is not the case. Undoubtedly alteration of the membrane by surfactant is responsible for some of the increased flux. However, a rather similar phenomenon was found by Mysels (19) in dialysis experiments of SLS through cellulose membranes, where no degradation occurred.

EFFECT OF POLYMER JR

Clinical studies with volunteers by Professor A. M. Kligman (11) have shown that preapplication of the cationic cellulose derivative, Polymer JR (13), is effective in

reducing skin irritation caused by sodium lauryl sulfate. It seems likely that sorption or permeation (or both) of the surfactant must be influenced by the presence of the polymer. It is true that the polymer does interact with SLS in solution (20). However the amount placed on the skin in the clinical studies is too little to explain the observed phenomena either by interaction or by film formation.

Experiments on the effect of Polymer JR upon sorption of SLS were carried out in two ways: (1) with the polymer present in the SLS solution and (2) by application to the stratum corneum, *i.e.*, soaking for 1 hr in a 1% solution. Neither had any effect on the sorption of SLS. Thus Figure 9 shows data for 0.1% SLS with pretreatment. Other concentrations of SLS gave the same results.

However, permeation experiments did show a pronounced effect, one that was concentration-dependent. Figure 10 shows data for preapplication of Polymer JR (1 hr

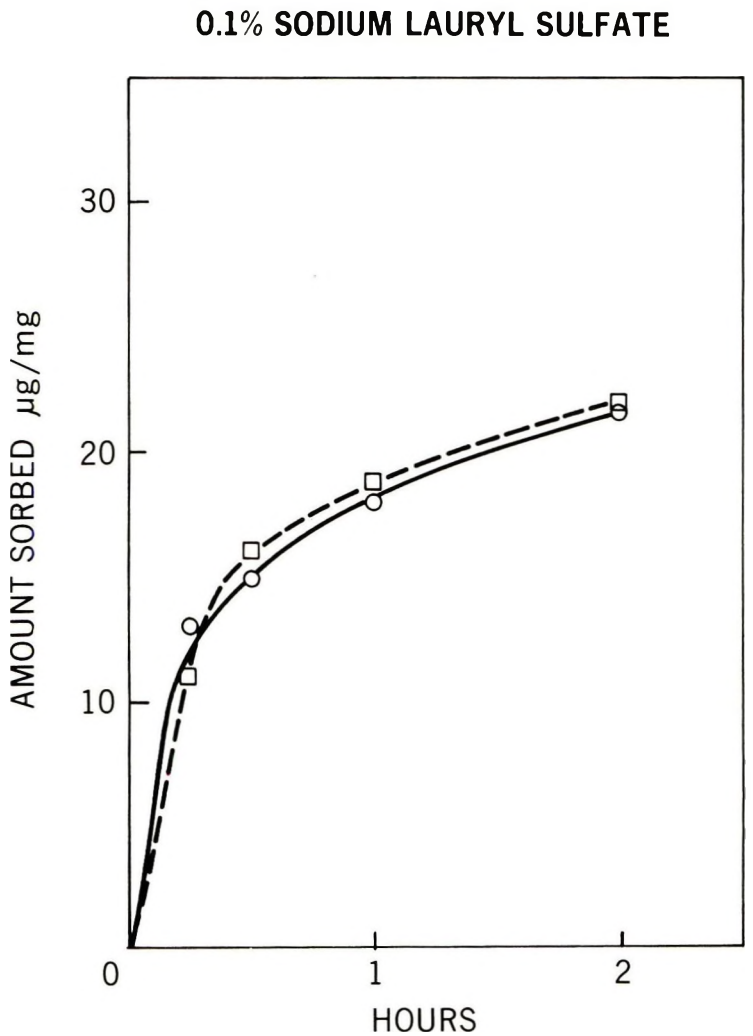

Figure 9. Effect of Polymer JR on sorption of sodium lauryl sulfate. Solid line: untreated. Dashed line: pretreatment for 1 hr with 1% Polymer JR

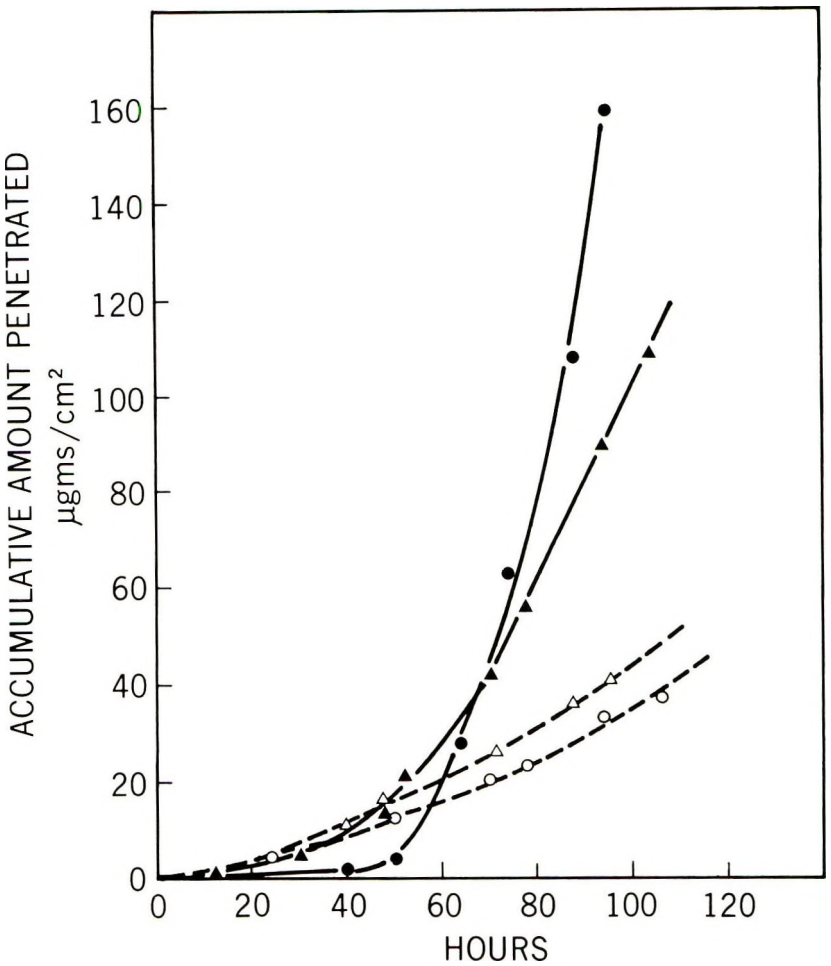

Figure 10. Effect of pretreatment by Polymer JR on permeability of 0.1% sodium lauryl sulfate. Solid line: untreated. Dashed line: pretreated for 1 hr with 1% Polymer JR

of 1% solution) and permeation of 0.1% SLS; a time of some 60 to 70 hr is necessary before the permeability of the untreated membrane begins to rise sharply above that of the treated skin. This effect is reproducible as the repeated curves show. Even more striking are the results of Figure 11 at 10% SLS. Here significant differences are already evident in less than 10 hr. An exact correspondence cannot readily be made to the conditions and times of the clinical testing cited above (11); but it is clear that these latter experiments involving prolonged occlusion and lasting for several days must represent many hours of effective contact with concentrated surfactant.

These experiments of sorption and permeation begin to suggest a plausible mechanism for the action of the polymer on the stratum corneum. The polymer does not act primarily as a barrier to penetration of the surfactant since it has no influence on sorption. However, it does help to maintain the physical integrity of the membrane. That is, it seems to slow down the changes in structure which cause the greatly increased permeation as time goes on (Figures 7 and 8). The exact means by which the polymer strengthens the stratum corneum is not known at this time; but it has been established

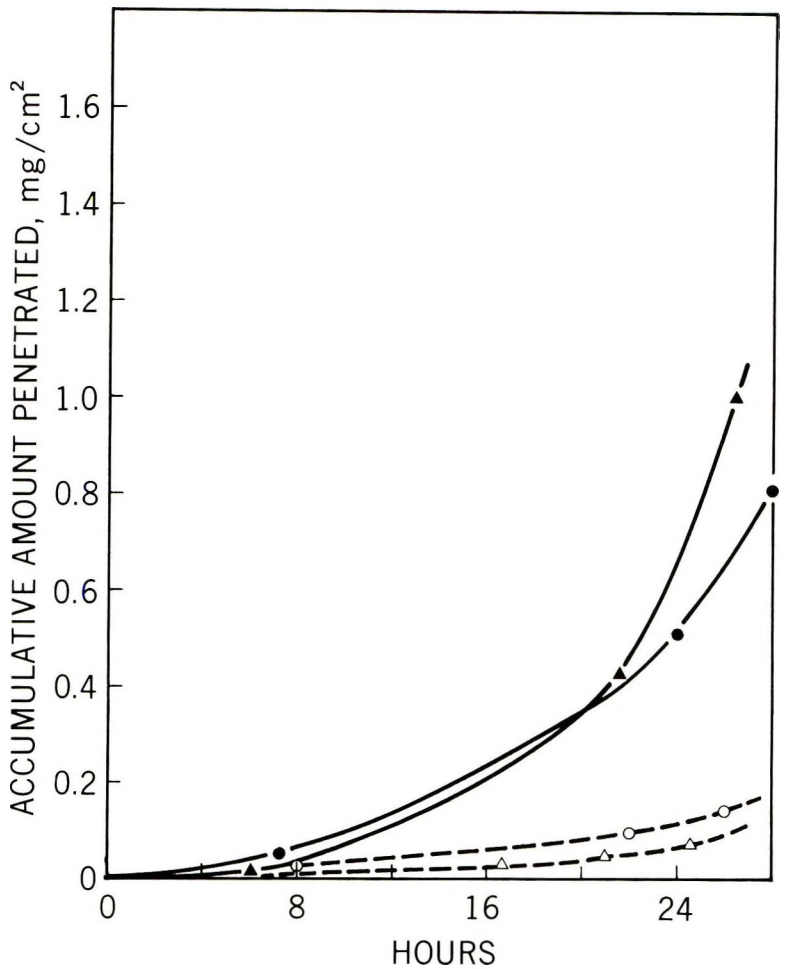

Figure 11. Effect of pretreatment by Polymer JR on permeability of 10% sodium lauryl sulfate. Solid lines: untreated. Dashed lines: pretreated for 1 hr with 1% Polymer JR

that Polymer JR is itself highly substantive to stratum corneum (17). Its action may therefore be analogous to the glue-like property of cationic starches which are used to hold cellulose fibrils together in the paper-making process (21).

CONCLUSION

Diffusion studies have shown that penetration of (radiotagged) sodium lauryl sulfate, SLS, through neonatal rat stratum corneum is relatively rapid and increases with concentration even above the critical micelle concentration. Penetration by a cationic surfactant and a nonionic surfactant was also found but required a period of several days rather than hours as was observed for SLS. It is postulated that the high sorption and diffusion obtained with SLS are due in part to structural changes in the membrane brought about by this surfactant. These changes are mitigated by the preapplication of a cationic polymer which was observed to reduce markedly the diffusion of SDS through the membrane.

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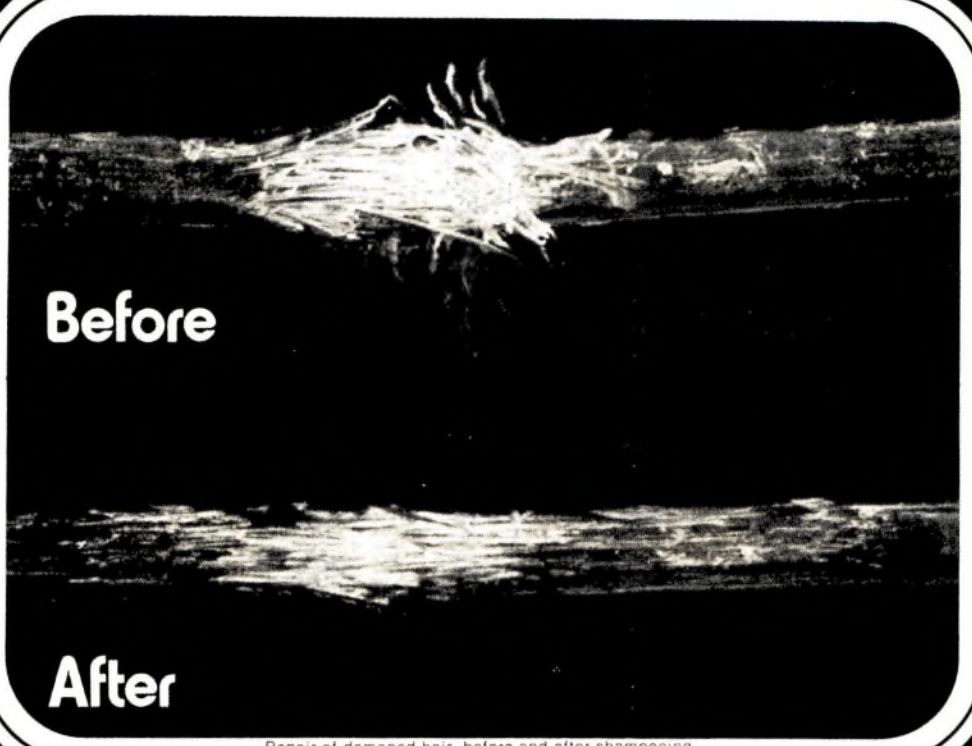

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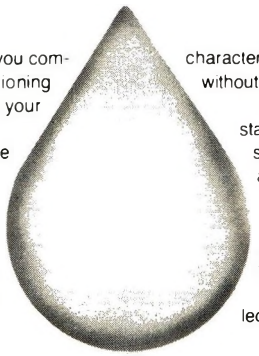

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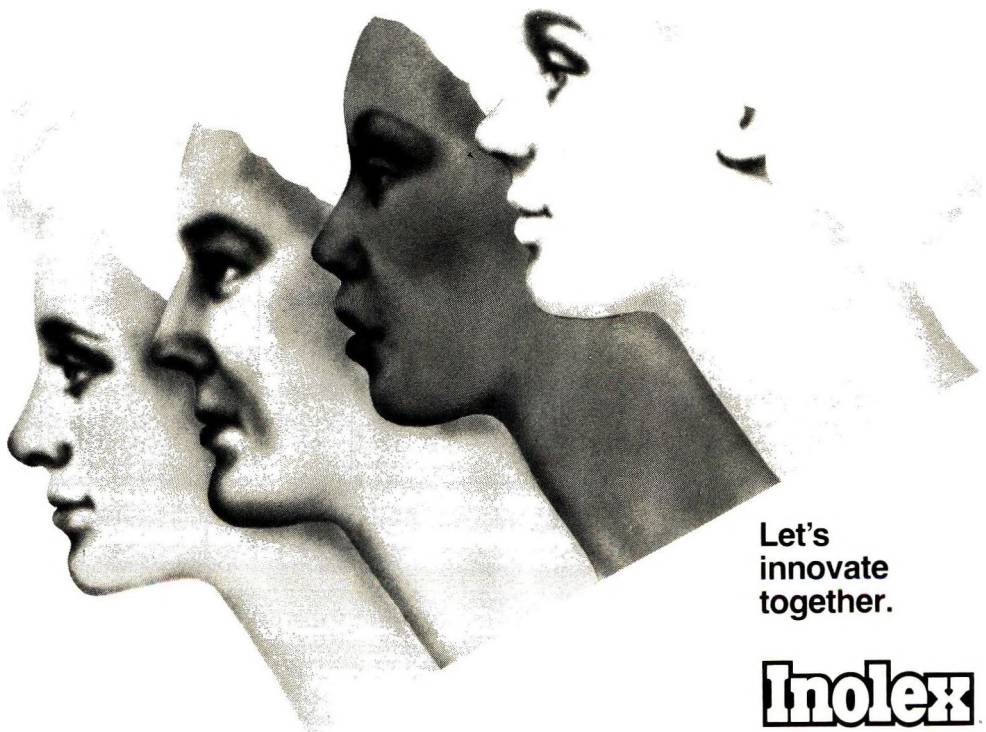

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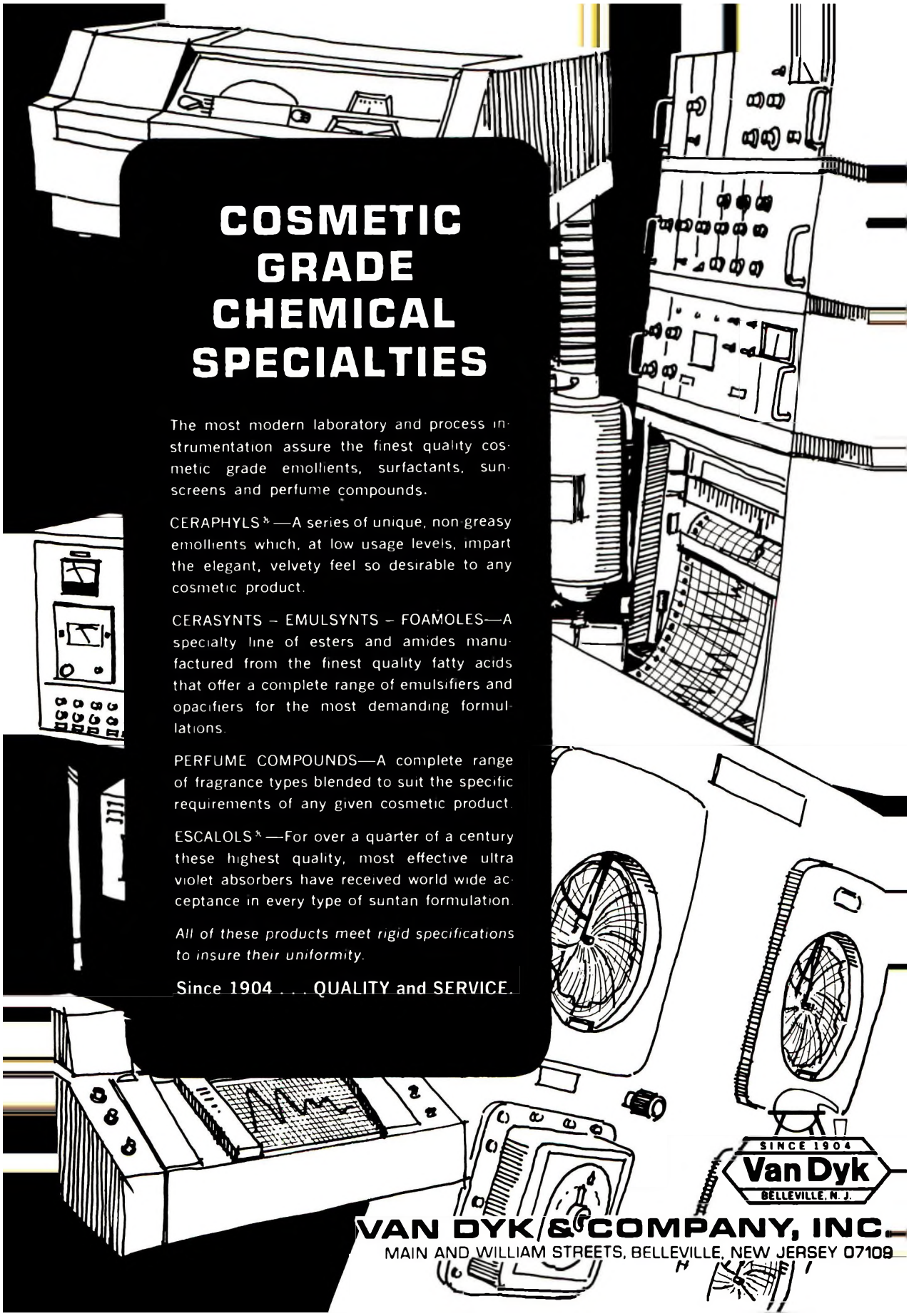

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