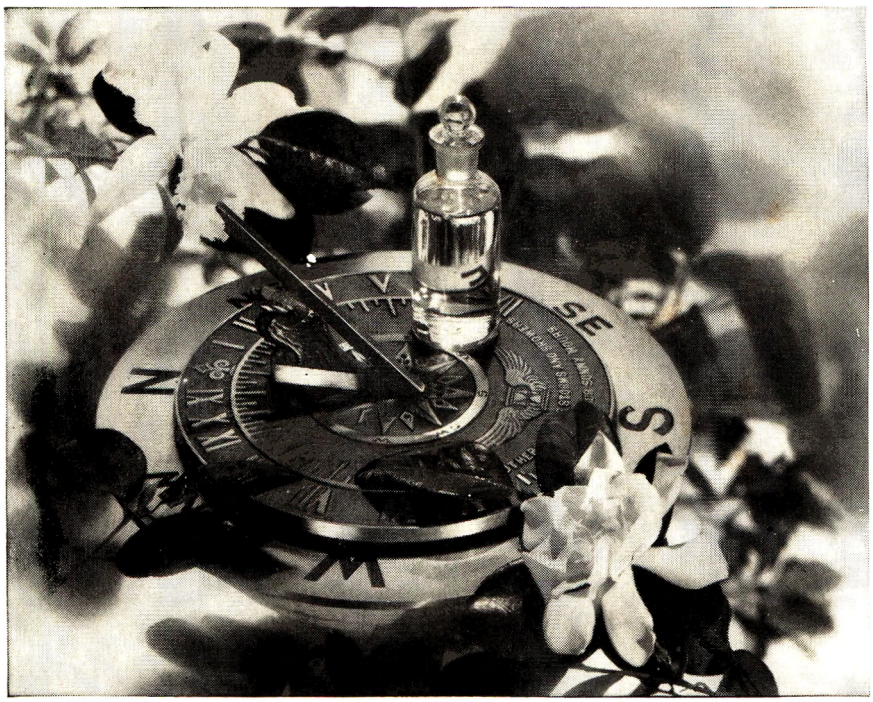
Vol XVI No 13 9 DECEMBER 1965

Journal of the Society of Cosmetic Chemists

Contents



Beauty of fragrance.....

made-to-measure for your success!

Beauty of fragrance is elusive . . . indefinable . . . yet vital to the success of a perfume or cosmetic!

It takes imagination to conceive a beautiful, original fragrance . . . skill and knowledge to give it exactly the right distinction and character.

Givaudan's imagination, skill and knowledge are reflected in many successful creations. They can provide you with matchless fragrances—made-to-measure for your success!

GIVAUDAN-DELAWANNA, INC.
321 West 44th Street, New York 36, N.Y.

GIVAUDAN

Journal of the Society of Cosmetic Chemists

VOLUME XVI • NUMBER 13

Published by The Society of Cosmetic Chemists, Inc.

Publication Office: 20th and Northampton Streets, Easton, Pa. 18043

Subscription: JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS is published six times per year, in February, March, May, August, September, and December. Yearly subscription price is $28.00 post-paid in North America and U. S. possessions and $29.30 in all other countries. The subscription rate to members of the Society is $8.00 and is included in the membership dues.

Missing Numbers: Because of uncertain and hazardous conditions, claims for missing numbers can be entertained only from subscribers in the country of origin of the particular Issue and must be made within 30 days from date of issue.

Change of Address: Members and subscribers are urged to give notice of change of address to the Editorial Assistant and the office of the Society.

Responsibility for Statements Published: The Society of Cosmetic Chemists, the Committee on Publications, and the Board of Directors assume no responsibility for statements or opinions advanced by contributors to this Journal.

Editors and Publishers: Abstracts or digest of articles not exceeding 400 words may be published, duly credited to the author and JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS. Reprinting or more extensive copying (whole pages or articles) are forbidden, except by special permission, in writing, from the Chairman of the Publication Committee.

Authors: When using Illustrations or quotations taken from copyrighted publications, authors must get written permission from the copyright holder to reproduce the same.

Manuscript: Manuscripts should be prepared in accordance with the "Directions to Authors," copies of which are available from Dr. Martin M. Rieger, 170 Tabor Road, Morris Plains, N.J. 07950

Second-class postage paid at Easton, Pennsylvania.

Send for your copy of our new catalogue GUIDE FOR THE PERFUMER *to-day*

Sole selling agent for the United Kingdom and Eire

P. T. Petley & Co. Ltd.

9 St. Cross Street, London, E.C.1

Telephone : HOLborn 4771

EST. 1871

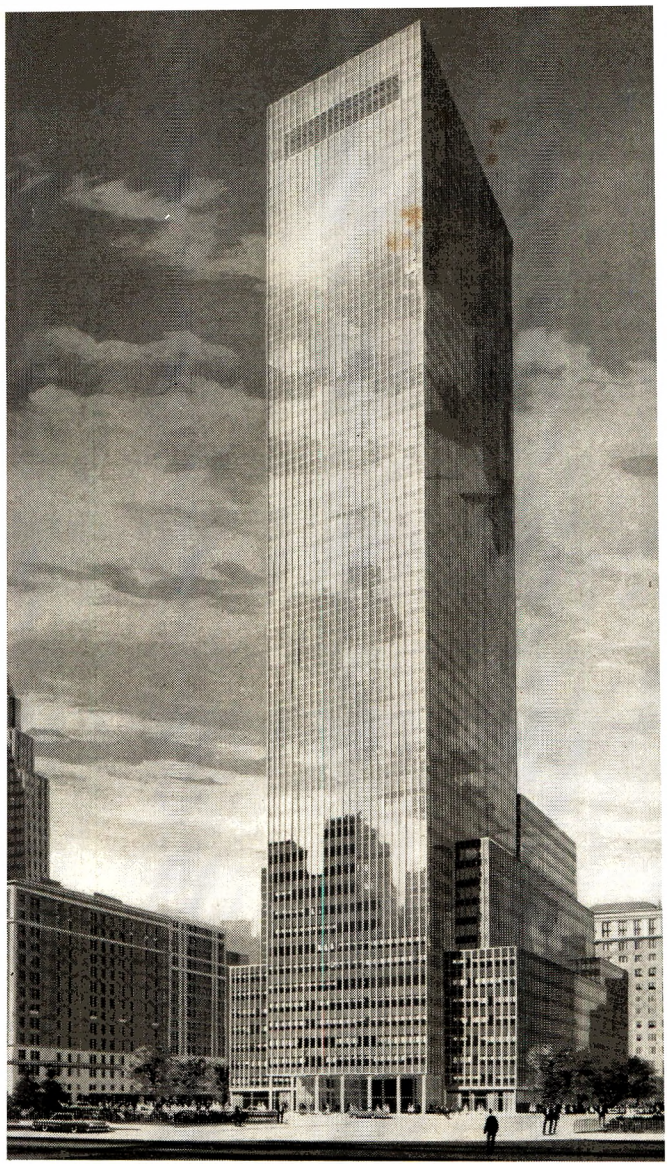
fritzsche

BROTHERS, INC.

76 NINTH AVE., NEW YORK, N.Y. 10011

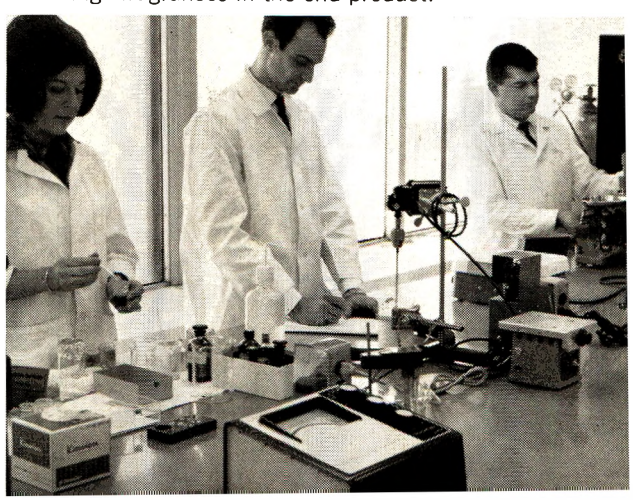
for creative perfumery

Firmenich now lives here



A Firmenich perfume technician implements creative perfume directions established by one of Firmenich's creative perfumers. More than 3000 components are available for developing the individualized fragrance.

The Cosmetic Application Laboratories test the stability of a Firmenich perfume in our client's cosmetic preparation, toiletry, aerosol, or soap. Accelerated, high and low temperature tests, are conducted. One of many steps in "custom-testing" fragrances in the end product.



THE NEW HEADQUARTERS FOR FIRMENICH INCORPORATED, UNITED STATES, IS NOW 277 PARK AVENUE, NEW YORK CITY.

These comprehensive facilities are dedicated to all Firmenich clients.

Now in midtown Manhattan, Firmenich creative and application laboratories are available to the fragrance industry's executive, marketing and technical staffs. Here every perfume marketing activity is performed—creation, application, physical end product testing and expert panel study to insure consumer acceptance.

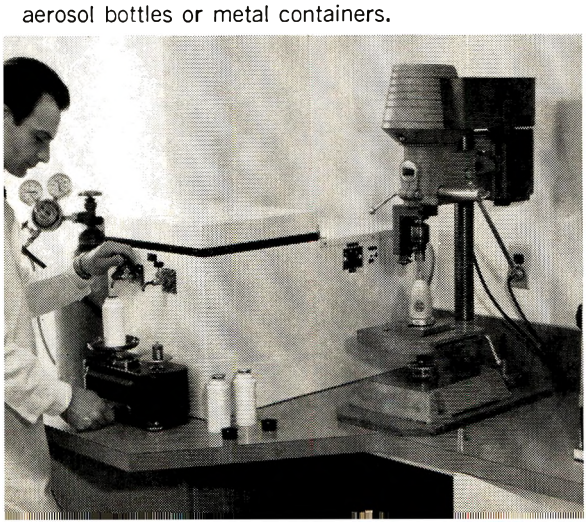
As the fragrance industry has changed over the years, so has Firmenich. Today's consumer marketing trends require the "tailor-making" of a perfume specifically for the customer's needs. It is at this that Firmenich excels.

You will find a visit to these new facilities most rewarding. In addition to the complete care devoted to technical and scientific efforts, you will experience an atmosphere conducive to true creativity.

FIRMENICH Incorporated · 277 Park Avenue · New York, N. Y. 10017 · Telephone: (212) 826-6060 · TWX 212-640-4446

Firmenich®

Aerosol filling equipment provides "pressure" and "cold-fill" methods. New perfume in aerosolized toiletries creations are tested for use in aerosol bottles or metal containers.



Firmenich fragrance experts meet in a special odor-free perfume panel room—often with delegates from a client company to carefully evaluate the aesthetic value and consumer appeal of new perfume blends.

Perfume creates an image...

An air of mystery or the sweetness of femininity...
each must possess the indestructible look of self-assurance.
You help to create the image she desires by offering
her D&O's enchanting fragrances.

Dodge & Olcott Inc.
SEVENTY-FIVE 9TH AVENUE • NEW YORK, N.Y. 10011

D&O
ESTABLISHED 1798

Your own development facilities are augmented by Atlas labs!

Here's an Atlas service that goes far beyond merely taking orders for Atlas products!

Our labs specialize in showing customers how to use polyols and surfactants to their own best advantage—your top source of polyol and surfactant information for the cosmetic industry.

Small-scale batch production of lotions, creams, toothpaste, and aerosol products facilitates study of polyol and surfactant effects on stability, skin-feel, physiological characteristics and many other properties.

ATLAS PRODUCTS FOR COSMETICS...

SORBO® Sorbitol Solution, USP, humectant & vehicle.

SURFACTANTS

ARLACEL® and **SPAN®** sorbitan fatty acid esters.
ARLACEL® monoglycerides.
BRIJ® polyoxyethylene fatty ethers.
MYRJ® polyoxyethylene stearates.
TWEEN® polyoxyethylene sorbitan fatty acid esters (polysorbates).
SOLUBLE LANOLIN DERVATIVES
SOLUBLE BEESWAX DERIVATIVES

YOU GET SOMETHING EXTRA WHEN YOU BUY FROM:

ATLAS

CHEMICAL INDUSTRIES, INC.
CHEMICALS DIVISION • WILMINGTON, DEL. 19899

The ability to "zero-in" on exacting specifications

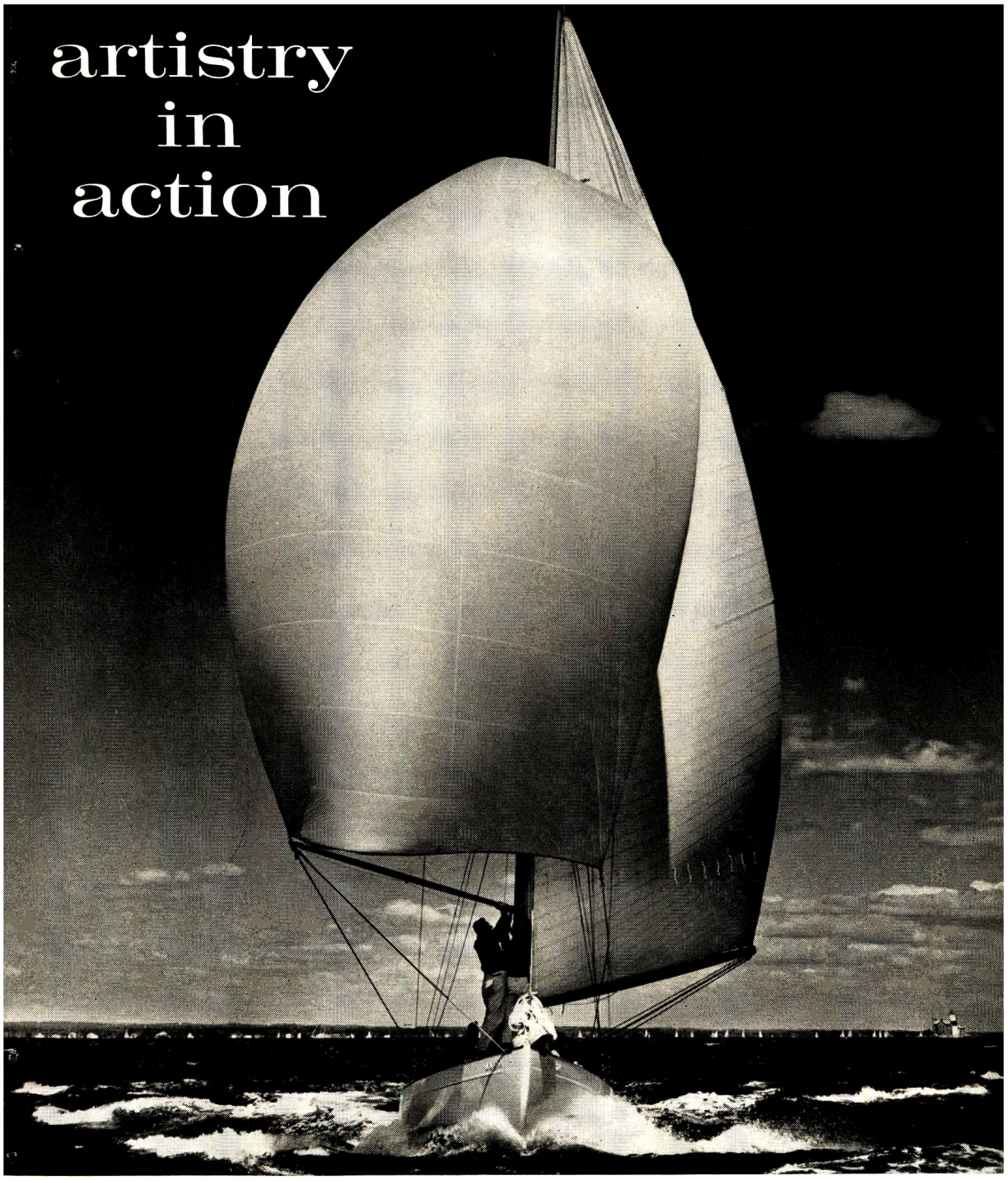
From time to time, customers ask us to modify one or more characteristics of our regular grades of White Mineral Oils or Petrolatums to meet their special requirements. Because of our long experience in the refining of high quality petroleum products, the flexibility of our refining processes, and our willingness to give conscientious attention to minute details, Penn-Drake is uniquely able to "zero-in" on such requests. The more exacting the requirements, the more valuable this capability becomes; it is a service that many of our customers find profitable.

When you use Penn-Drake White Mineral Oils, you will find that their high quality protects the quality of your finished products; their dependable uniformity smoothes production; our precise refining techniques assure strict adherence to your specifications.

If your requirements are becoming more exacting and you want white mineral oils and petrolatums—regular or special—that you can depend on, get in touch with Penn-Drake!

For complete information, tell us what you have in mind—in a letter, telegram, or if the need is urgent, telephone us collect. Pennsylvania Refining Company, Butler 29, Pa. Branches: Cleveland, Ohio; Edgewater, N. J.; Los Angeles, Calif.; Tokyo and Osaka, Japan

penn-drake®
WHITE MINERAL OILS
PETROLATUMS



This graceful racing sloop, skillfully manned by crewmen who are masters of the sailing arts, is an example of truly outstanding performance • At Fleuroma, the highly specialized skills and imaginative talents of world-renowned perfumers, combined with the finest technical and chemical facilities available, originate and create exciting fragrances that make your products unique . . . desirable . . . memorable.

F L E U R O M A

43-23 Thirty-Seventh Avenue, Long Island City, New York / Chicago • London • Paris • Sao Paulo

A DIVISION OF UNIVERSAL OIL PRODUCTS COMPANY

A PROUD TRADITION OF SUPERIOR SERVICE TO THE PERFUMERY INDUSTRY

roure-dupont INC.

new york chicago hollywood

Sole agents in the United States and Canada for SOCIETE ANONYME DES ETABLISSEMENTS ROURE-BERTRAND FILS et JUSTIN DUPONT

Since 1904

◀ QUALITY

SERVICE ▼

EMULSYNTS

OF SPECIAL INTEREST:

EMULSYNT 505—O/W emulsifier for cosmetic creams with elegant "feel" over wide range pH. Forms stable permanent type hair dye creams.

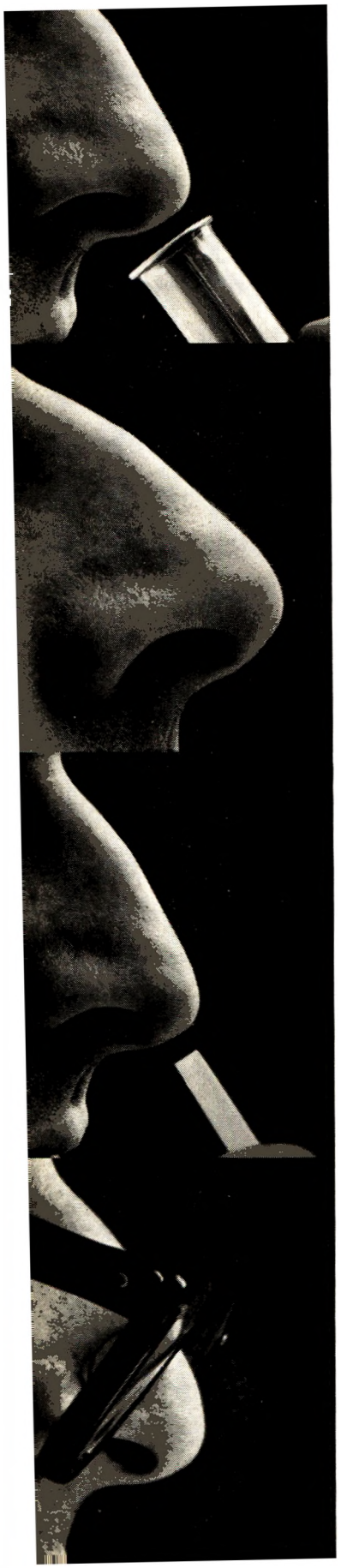
EMULSYNT 900—Highly efficient solvent and/or dispersant for dyes and pigments. Useful as an auxiliary emulsifier; imparts washability to anhydrous systems.

EMULSYNT 1055—W/O emulsifier for cosmetic and pharmaceutical lotions and creams. Excellent stabilizer when used as antagonistic emulsifier for O/W systems.

SINCE 1904
Van Dyk
BELLEVILLE, N. J.

VAN DYK & COMPANY, INC.

MAIN AND WILLIAM STREETS, BELLEVILLE, NEW JERSEY



with a strong inclination...

to create exciting aromas...

and you're looking for facts on synthetic aromatics, write us.

Send me product descriptions, classifications, and suggested formulations for Roche aromatics.

ROCHE®

NAME _____

TITLE _____

COMPANY _____

ADDRESS _____

_____

Aromatics Division,
Hoffmann-LaRoche Inc. Nutley, N. J.

INDEX TO ADVERTISERS

MOISTURIZERS

AMERCHOL® — sterol extracts. Amerchols such as L-101, CAB, C, H-9 and BL are a family of hypoallergenic lanolin derived products designed to provide a wide range of moisturizing and other valuable effects. Amerchol L-101, for example, is a superb emulsifier, emollient, stabilizer, and a powerful free sterol depressant of interfacial tension.

AMERLATE® P — isopropyl lanolate. Emollient ester of lanolin fatty acids. A particularly effective conditioner, lubricant and penetrant. Functions as a moisturizer by holding water to the skin in emulsified form. Melts at body temperature to form a nongreasy protective film.

SOLUBILIZERS

SOLULAN® — ethoxylated derivatives. Water soluble, yet emollient! Solubilizers of great general utility. Impart excellent plasticizing, lubricating, conditioning and pigment wetting qualities at low concentration.

PENETRANT

ACETULAN® — acetylated lanolin alcohols. Nonoily hydrophobic liquid emollient. Penetrates and lubricates, leaving a persistent velvety afterfeel that is truly remarkable.

EMOLLIENT

MODULAN® — acetylated lanolin.† Skin protective emollient with decided advantages over lanolin. Hypoallergenic, almost odorless, nontacky, oil soluble, and hydrophobic. Excellent for emulsions, soaps, baby oils, and brilliantines.

ENRICHERS

VISCOLAN® — dewaxed lanolin. Supplies all the natural benefits of lanolin in intensified, convenient liquid form. Oil soluble, low odor and color.

WAXOLAN® — lanolin wax fraction. Adds gloss and grooming effects. Stabilizes emulsions. Increases melting point, viscosity and consistency.

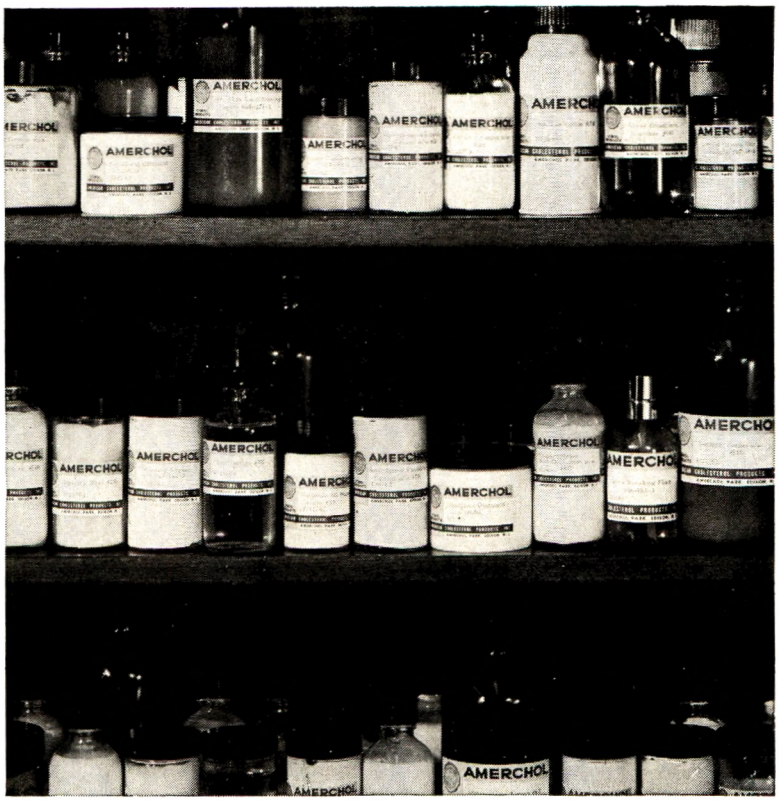
CHOLESTEROL USP — pure white and practically odorless. Suitable for the most exacting uses in pharmaceuticals and cosmetics.

UNSATURATES

POLYLAN® — essential polyunsaturate. Liquid wax ester. Combines the natural benefits of linoleic acid with the softening, protective, and conditioning properties of lanolin's most active components.

RICILAN® — lanolin ricinoleates. Provide valuable new skin oriented properties. Unusual combinations of selected lanolin alcohol and castor oil components designed especially for lipsticks.

†U.S. & foreign patents



ANSWERS
waiting for problems

Amerchol® lanolin derivatives have been developed for specific functional effects in formulations, and we have these shelves of finished, tested preparations which may be the answer to your formulation problem.

If the answer to your particular problem isn't here, we are prepared to put our extensive experience in formulating with Amerchol lanolin derivatives and other cosmetic raw materials to work for you. There is no cost or obligation for this confidential service.

Complete technical data, samples, and suggested formulas are available from our research laboratories.

Amerchol

AMERICAN CHOLESTEROL PRODUCTS, INC.

Amerchol Park • Edison, New Jersey

SYNOPSES FOR CARD INDEXES

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

An improved method for testing the safety of hair dye preparations: Steven Carson, Myron S. Weinberg and Richard Goldhamer. *Journal of the Society of Cosmetic Chemists* 16, 747 (1965)

Synopsis—The Draize procedure for subacute dermal studies in rabbits was compared with a modified procedure in which certain of the critical parameters were modified for the evaluation of oxidation-hair dyes. These included limitation of the duration of contact to one hour, clipping the hair to $\frac{1}{8}$ to $\frac{1}{4}$ inch instead of complete depilation, and thorough washing of the application sites after the one-hour contact. The results revealed that the modified method was equally sensitive with respect to the toxicological parameters examined. A further modification was employed in which the sites of application were excised and analyzed for hemoglobin content as an index of erythema. Addition of this procedure made it possible to detect effects within one hour of application. Significant differences were demonstrated between the irritation potential of 3 and 12% hydrogen peroxide.

Principles of consumer product testing: Jean F. Caul and Shirley A. Raymond. *Journal of the Society of Cosmetic Chemist* 16, 763 (1965)

Synopsis—After a review of the basic features of consumer panel testing, the requirements for a discriminating panel are described. The utility of this panel, especially in combination with a "Use Profile," is demonstrated with several examples. It appears to be entirely possible to relate consumer findings to laboratory measurements. Such data can guide reformulation or may be the go-ahead signal for marketing of the product.

The reaction of α-hydroxymethyl ketones with skin and amino acids: Karl Laden and R. Zielinski. *Journal of the Society of Cosmetic Chemists* **16,** 777 (1965)

Synopsis—The reaction of dihydroxyacetone and various α-hydroxymethyl ketones with callus, amino acids, and bovine serum albumin has been investigated. The reaction of callus and amino acids with α-hydroxymethyl ketones to produce colored products appears to be a general one. In addition, there is a suggestion that the reaction is enhanced if electron withdrawing groups are attached to the α-hydroxymethyl ketone. The failure of various analytical schemes to detect the presence of these ketones in callus suggests that although in some cases relatively intense color is produced very little material has actually reacted with the callus.

Identification of surface active agents as trimethyl silyl ether derivatives by gas chromatography: Robert Suffis, Thomas J. Sullivan and William S. Henderson. *Journal of the Society of Cosmetic Chemists* **16,** 783 (1965)

Synopsis—A method is presented for the analysis of some nonionic surface active agents by gas chromatography. The components of these agents are converted to their volatile trimethyl silyl ether derivatives prior to analysis by reaction with hexamethyldisilazane and trimethylchlorosilane. The volatile derivatives of the surface active agents may then be easily separated by gas chromatography. This procedure has been found to be applicable to a variety of glycol esters and sorbitan esters which are frequently utilized in cosmetic and toiletries formulations. In addition, the method could be utilized to provide information concerning the chemical properties of a surface-active agent. Rapid analysis for mono-ester and di-ester concentrations, free glycol, and fatty acid composition is possible through use of this technique.

CORRECTED SYNOPSIS

Approaches to a prophylaxis of skin aging: Margot Ippen and Hellmut Ippen. *Journal of the Society of Cosmetic Chemists* **16,** 305 (1965).

Synopsis—It is shown that smoking has a deleterious effect on skin condition and that this effect can be differentiated from that of damage by sunlight. Smoker's skin is identified as skin which suffers from loss of "turgor" and shows signs of flabbiness; in addition, the color of the smoker's skin is pale, with a grayish hue. Dermatological examination of 224 women up to now shows moderate correlation between their smoking habits and the appearance of their skin, as defined above. By contrast, smoking seems to have only a very minor effect on the skin of male smokers.

New York Chapter Award 1964

Shown from left to right are Mr. Herbert Edelstein, New York Chapter Awards Chairman, presenting the Chapter Award to Dr. Thomas F. McNamara, Miss Marianne L. Steinbach, and Mr. Benjamin S. Schwartz

During the September 9, 1965, meeting of the New York Chapter, the authors of the best paper presented before the New York Chapter of the Society of Cosmetic Chemists during the year 1964 were honored. Dr. T. McNamara, Miss M. Steinbach, and Mr. B. Schwartz of the Warner-Lambert Research Institute received certificates and a cash award. The award winning paper is entitled "Substantivity of Antimicrobials on Skin" and was published in the JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS (*16*, 499, 1965).

New York Chapter Honors Outgoing Chairman

Mr. Charles Fox, right, presents plaque to Mr. Henry Maso in recognition of his service as 1964 chairman of the Society of Cosmetic Chemists, New York Chapter, at the October 13, 1965 monthly meeting

New Members

Allured, Stanley E., 420 Papworth Ave., Wheaton, Ill. 60187
Barbuscio, Frank D., 80 Brush Hill Rd., Apt. 5, West Springfield, Mass. 01089
Bonduris, Angelo T., 57 Ross Ave., Metuchen, N. J. 08840
De Costa, Leander J., 137 Skyline Lake Dr., Wanaque, N. J. 07465
Delano, George M., 79 Giles Ave., Jersey City, N. J. 07306
Demestihas, Penelope, 1961 50th St., Brooklyn, N. Y. 11204
Diaz, Ramon B., 331 Hollywood Ave., Douglaston, N. Y. 11363
Fairchild, Charles M., 33 Mead Ave., Cos Cob, Conn. 06807
Gonzales, Dr. Israel P., 12 Burnett Dr., Chapeleroft, Wilmington, Del. 19803
Guimond, Ronald N., 43 Sunnybrook Lane, Clinton, Conn. 06413
Halasz, Dr. Alexander, Surrey Dr., Norwalk, Conn. 06851
Jackson, Maryanne, 208 Meadbrook Rd., Garden City, N. Y. 11530
Kaplan, Carl D., 3390 W. Hollywood Ave., Chicago, Ill. 60645
King, Michele M., 505 E. Ashman Ave., Midland, Mich. 48642
Laden, Dr. Karl, 12513 Montclair Dr., Silver Spring, Md. 20904
Levin, Norman A., 48 Stella Dr., Somerville, N. J. 08876
Lewandowski, Joseph J., 30 Skillman Ave. Jersey City, N. J. 07306
Libby, Henry, 630 Hilldale Ave., Berkeley, Calif. 94708
Matthews, Robert, 220 Eglinton Ave. E., Apt. 101, Toronto 12, Ontario, Canada
Murphy, Lawrence J., 13 Bell Court, East Brunswick, N. J. 08816
Natishan, John Jr., 106 Hillside Terrace W., Hackettstown, N. J. 07840
Panos, James J., 8627 N. 36th St., Milwaukee, Wis. 53209
Pollock, Carole L., 8849 Cedros Ave., Van Nuys, Calif. 91402
Polonsky, Thomas, 733 Gordon Terrace, Chicago, Ill. 60613
Roia, Frank C., Jr., 46 Budd Ave., Brockton, Mass. 02402
Saiewitz, Robert, 50 Hibernia Rd., White Meadow Lake, N. J.
St. Clair, James W., 728 Bellwood Ave., Bellwood, Ill. 60104
Sorrentino, Ralph P., 85–11 56th Ave., Flushing, N. Y. 11373
Stern, Arnold, 574 Green Lane, River Vale, N. J. 07675
Sushansky, Harold B., 3–53 Twenty-seventh St., Fair Lawn, N. J. 07411
Teitelbaum, Dr. Charles L., 225 W. Eleventh St., New York, N. Y. 10014

International Federation

of

Societies

of

Cosmetic Chemists

The Fourth Congress of the I. F. S. C. C. will take place in Paris in June, 1966.

The "Société Francaise de Cosmetologie" has created a scientific Committee consisting of:

Dr. Ir. Velon
Ir. Jean Morelle
Dr. Collin

This Committee wishes to receive conference papers before January 31, 1966.

Each paper can be typed in the original language of the speaker. The French Committee will take care of the translation in English, French and German.

General Secretary
Dr. P. A. M. E. van Velzen
Scheveningseweg 62
The Hague, The Netherlands

J. Soc. Cosmetic Chemists, 16, 747–761 (1965)

An Improved Method for Testing the Safety of Hair Dye Preparations

STEVEN CARSON, Ph.D., MYRON S. WEINBERG, Ph.D., and RICHARD GOLDHAMER, B.S.*

Presented November 4, 1964, New York City

Synopsis—The Draize procedure for subacute dermal studies in rabbits was compared with a modified procedure in which certain of the critical parameters were modified for the evaluation of oxidation hair dyes. These included limitation of the duration of contact to one hour, clipping the hair to ⅛ to ¼ in. instead of complete depilation, and thorough washing of the application sites after the one-hour contact. The results revealed that the modified method was equally sensitive with respect to the toxicological parameters examined. A further modification was employed in which the sites of application were excised and analyzed for hemoglobin content as an index of erythema. Addition of this procedure made it possible to detect effects within one hour of application. Significant differences were demonstrated between the irritation potential of 3 and 12% hydrogen peroxide.

Introduction

Irritation is manifested by a tissue system in response to stimuli of either exogenous or endogenous origins. The characteristic reactions include rubescence (erythema), edema, inflammation, and possible impairment of the integrity of the associated vasculature, with necrosis and tissue degradation if the stimulus is sufficiently intense or prolonged.

In this report, two dermal test procedures employing rabbits have been compared for their utility in the evaluation of systemic safety and irritation potential of oxidation hair dyes. One of these methods, originally described by Draize (1), has been widely applied to the testing of cosmetics, topical pharmaceuticals, and industrial or agricultural chemi-

* Food and Drug Research Laboratories, Inc., Maurice Avenue at 58th Street, Maspeth, New York.

cals. A modified test procedure was used which more closely approximates use conditions. Oxidation hair dye formulations characteristically contain ammoniated bases which for use are mixed with an appropriate quantity and concentration of hydrogen peroxide. These dyes are used under specified conditions which include limited contact with the hair, followed by thorough shampooing and immediate rinsing. These conditions are employed both in the home and in the beauty salon.

In the Draize test prolonged (six-hour) contact is maintained with the test material by means of either a rubber dam or plastic sleeve wrapped around the trunk of the rabbits. The condition of the skin is

TABLE I
Evaluation of Skin Reactions

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm.)	3
Severe edema (raised more than 1 mm. and extending beyond area of exposure)	4
Total possible edema score	4

scored daily in accordance with Draize's grading system (Table I) for evaluating skin reaction, whereby emphasis is placed on the degree of edema, erythema, and eschar formation.

Under these test conditions, in which daily (5 days per week) applications to the abraded skin are made for 6 hours for 21 days, or to the intact skin for 90 days, even the most innocuous materials elicit adverse changes in the skin of rabbits. As a result of this treatment regimen the skin becomes dry and scaly, cracks, and thickens, with subsequent sloughing. Relatively large body areas become denuded, but regrowth of hair is generally observed in these areas. Since the test procedure precludes washing of the application area, it is intrinsically responsible for many of the physical changes observed. The effects due to any active components in the formulation are superimposed on these background reactions.

As a consequence, serious doubts have arisen concerning the validity of such exaggerated exposure conditions. The problem is compounded in the case of formulations which contain active ingredients where the dosages recommended are based on multiples of the human dosage. These are often scaled to 1, 3, and 10 times the human dose on a mg. per kg. body weight basis. The higher dosages often require volumes of test material considerably greater than can be applied to the trunk of the rabbit in single applications. When the total dose is placed under the plastic sleeve, the quantity of test material in direct contact with the skin is considerably less than if the same total quantity were applied as a thin layer covering the entire trunk. To avoid this problem of dosage, many workers administer the total dosage as a series of divided doses. These volumes are without doubt unrealistic in relation to the total body surface of the animal.

Recently, the Food and Drug Administration reviewed a protocol prepared by a group representing manufacturers of hair dyes. This group submitted a realistic procedure for evaluating oxidation hair dyes which bore a closer relation to the conditions of use. The results of several studies employing the original and modified procedures are covered by this report.

Characterization of the irritation response is one of the single most important criteria in these studies. Up to this time, only the Draize scoring procedure and subsequent histomorphological evaluation of tissue pathology have been applicable. The Draize system is an attempt to reduce subjective evaluations to numerical terms. Microscopic examination, a critical aspect of the total assessment, has serious limitations in terms of differentiating subtle differences in response.

A new procedure was employed which quantitates the inflammatory changes associated with irritation in terms of the fluids and cellular elements present at the application sites. It reflects the increased numbers of erythrocytes present due to the increased leakage through the capillaries and possible impaired integrity of these vessels. Frank hemorrhage is not required for definition. This procedure has revealed differences in the tissue fluids where no discrete visual evidence of erythrocytes had been noted.

Methods

These modified procedures were designed to approximate normal usage patterns. Adult albino rabbits weighing between 2 and 3 kg. were distributed into groups of 10 animals equally divided as to sex.

On the day prior to the initiation of applications, the dorsal hair was clipped to a length of 1/8 to 1/4 in. with an electric clipper, care being taken to avoid nicking or abrading the skin. One group served as the control, receiving all preliminary preparations without treatment. The *p*-phenylenediamine-resorcinol (P.P.D.-R.) type dye-peroxide mixture was prepared in accordance with label directions, all dilutions being discarded after a single use. Two graded concentrations of the mixture were applied within 20 minutes of preparation. The contact

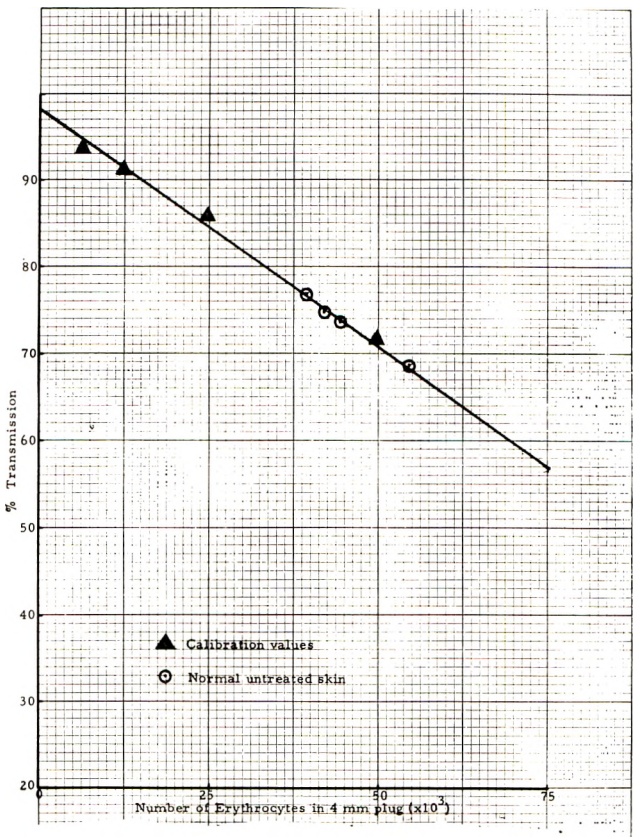


Figure 1. Standard values of rabbit blood cells-calibration curve for alkaline digestion procedure

time was fixed at 60 minutes. At the end of this exposure period, the dye mixture was rinsed off with lukewarm water. Four milliliters of a commercial shampoo intended for use after dye application was spread over the dyed area, worked into a rich lather, and then rinsed with lukewarm water until all traces of lather were gone. The rabbit was then

TABLE II

Primary Irritation Scores—Prolonged (6-hour) Daily Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose	Rabbit No.	Days 0	7	14	21
		Scores			
0	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1x	1	0	2	2	2
	2	0	2	2	2
	3	0	2	2	2
3x	1	0	2	2	2
	2	0	2	2	2
	3	0	2	2	2
10x	1	0	Died		
	2	0	2	2	2
	3	0	2	2	2

thoroughly towel-dried, and a warm air stream, from a commercial 300-watt heater equipped with a fan, was directed over the rabbits for approximately five minutes. This procedure was repeated after each daily application of the test material. The effectiveness of this two-stage drying was demonstrated by the significantly reduced incidence of upper respiratory disease during these long test periods. One-half the animals were sacrificed 24 hours, and the remainder 14 days, after the last application; no further treatment was given during these terminal periods.

Prior to starting treatment, a base line hemogram including hemoglobin concentration, hematocrit, platelet, and total and differential leukocyte counts was obtained on each animal. In addition, semi-quantitative determinations were made for specific gravity, pH, glucose, protein, and microscopic examination of the sediment of urine. These were repeated prior to termination after the last application. All animals that died and half the survivors, which were sacrificed after 20 applications, were autopsied. Nine tissues, lung, heart, liver, kidneys, spleen, pancreas, thyroid, gonads, and marrow were examined microscopically. The procedure was repeated on the survivors 14 days later, and target organs were examined.

Data obtained by this procedure were compared to a series in which the typical Draize procedure was followed. In this series, a commercially available oxidation hair dye similar to that used in the modified procedure was used. Groups of 10 rabbits (evenly divided as to

TABLE III

Primary Irritation Scores 1-Hour Contact with Graded Concentration H_2O_2

Rabbit No.	Application No.	H_2O_2, % 3	6	9	12
Males					
1	1	0	1	1	1
	2	0	0	0	2
	3	0	0	0	1
	4	1	1	2	1
	5	1	1	2	1
	6	H	H	H	H
2	1	1	1	1	1
	2	0	0	0	0
	3	0	0	0	0
	4	1	1	1	1
	5	H	H	H	H
	6	H	H	H	H
3	1	1	1	1	2
	2	0	1	1	1
	3	0	2	1	2
	4	2	2	2	2
	5	1	1	1	2
	6	1	1	1	1
4	1	1	2	2	2
	2	0	1	2	1
	3	1	1	1	1
	4	1	1	1	2
	5	H	H	H	H
	6	H	H	H	H
5	1	1	0	1	2
	2	0	1	1	2
	3	1	2	2	2
	4	1	1	2	2
	5	H	H	H	H
	6	H	H	H	H
6	1	1	0	2	2
	2	0	1	0	2
	3	1	2	2	2
	4	2	2	2	2
	5	1	1	1	2
	6	H	H	H	H
7	1	0	0	0	1
	2	1	1	1	1
	3	1	1	2	2
	4	1	1	1	1
	5	1	1	1	1
	6	H	H	H	H
8	1	1	1	2	1
	2	1	1	1	2
	3	0	2	1	2
	4	2	2	2	2
	5	1	1	1	1
	6	H	H	H	H

H = Indicates that hair regrowth was intense. No reading was made.

sex) weighing 2 to 3 kg. were prepared 24 hours prior to start of application. The entire dorsal surface was closely clipped by means of an electric clipper, removing as much hair as possible while avoiding nicking or abrading of the skin. The P.P.D.-R. dye-peroxide mixtures were prepared in accordance with the label instructions and applied five days a week for six hours to the body surface under plastic sleeves, following which the sleeves were removed. The excess was wiped off without shampooing. Skin scores were read daily. The physiological status of the rabbits was determined prior to and after the last applica-

TABLE IV
Body Weights 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

Dose Factor	No. of Rabbits	Days 0	Days 21	Net Gain
0	5M	2.3–2.6	2.4–3.0	0–1.1
	5F	2.5–3.7	2.7–3.9	0–0.9
1x	5M	2.4–2.7	2.8–3.0	0.3–0.6
	5F	2.4–2.9	2.7–3.3	0.5–0.7
2x	5M	2.2–2.8	2.5–3.0	0.3–1.1
	5F	2.3–2.9	2.7–3.5	0.3–0.7

TABLE V
Body Weights—Prolonged Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose[a]	Days 0	Days 21	Net Gain
0	1.9–2.1	2.3–2.6	0.2–0.7
1x	1.7–2.3	1.8–2.5	−0.5–0.6
3x	2.4–2.6	2.4–2.8	−0.2–0.4
10x	1.8–2.6	2.0–2.7[b]	0.1–0.2

[a] Three rabbits per treatment group (males).
[b] One animal died in this group.

tion. Blood and urine examinations were made as above described. The animals were examined daily, and unusual signs were recorded. They were sacrificed by over-barbitalization at the end of the 21st day after 15 test exposures or at 35 days, examined grossly at necropsy, and tissues and organs examined microscopically with particular emphasis being placed on the skin and adnexal areas.

From the observations made upon the differential pattern of erythema and injection of the vascular network of the dermal surface, it was ascertained that the gross scoring of the epidermal surface failed to reflect apparent differences between products or graded concentra-

TABLE VI

Hematological Findings (Rabbits) 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose	Sex	Hemoglobin, g./100 ml.		Hematocrit, %		Platelets, × 10^3		Leukocytes, × 10^3/mm.^{3}		Differential Count, %[a]									
										P		L		E		M		B	
		0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15
0	M	8.6–13.3	10.9–12.2	28–44	30–40	111–170	111–210	3.9–8.3	6.1–9.1	29–30	70–85	67–70	15–30	0	0	0–3	0	0–2	0
	F	10.6–12.2	11.2–12.2	33–38	35–41	90–180	190–240	4.6–9.1	5.9–8.2	24–28	69–82	72–75	18–31	0–1	0	0–1	0	0	0
1x	M	10.1–12.7	10.6–12.9	32–45	36–41	100–170	140–240	4.5–8.3	4.5–8.7	13–23	71–82	67–87	18–29	0	0	1–6	0	0–1	0
	F	9.6–11.2	10.9–13.2	33–40	35–43	111–161	100–216	5.9–9.6	5.0–10.9	20–30	77–82	70–80	18–23	0	0	0–2	0	0–2	0
2x	M	11.6–12.6	10.9–12.9	37–40	36–41	140–220	111–200	3.8–12.8	5.3–8.2	17–40	65–80	55–83	20–35	0	0	0–6	0	0–1	0–1
	F	9.9–11.7	10.3–12.2	30–38	35–39	117–161	120–210	6.2–9.7	4.2–6.9	16–22	78–83	76–83	17–22	0	0	0–2	0	0–2	0–1

[a] P = polymorphonuclear neutrophils; L = lymphocytes; E = eosinophils; M = monocytes; B = basophils.

TABLE VII

Hematological Findings—Prolonged Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose[a]	Hemoglobin, g./100 ml.		Hematocrit, %		Erythrocyte, × 10^6/mm.^{3}		Leukocyte, × 10^3/mm.^{3}		Differential Count, %[b]									
									P		L		E		M		B	
	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15
0	12.4–14.0	10.7–14.0	39–44	33–42	5.4–6.2	4.6–6.5	5.8–8.9	6.9–8.8	17–44	32–45	55–82	55–65	0–1	0	0	0	0	0
1x	12.1–12.7	10.3–13.1	39–47	33–43	4.6–5.7	4.8–5.8	6.3–9.3	6.4–11.7	15–52	13–61	48–85	39–86	0	0	0	0	0–4	0–1
3x	11.9–14.3	12.0–13.1	40–43	40–43	5.1–5.7	5.7–6.0	7.6–8.4	6.3–12.3	10–33	19–47	65–90	49–63	0	0	0–2	0	0–1	1–4
10x[c]	11.9–14.8	13.1–13.6	38–43	39–43	4.8–4.9	5.4–5.5	7.9–9.3	6.1–7.9	17–43	50–53	57–82	47–52	0	0	0	0	0–1	0–1

[a] Three rabbits per treatment group (males).

[b] P = polymorphonuclear neutrophils; L = lymphocytes; E = eosinophils; M = monocytes; B = basophils.

[c] One animal died in this group.

tions of a product. To differentiate these effects, a more definitive modification of the Ascheim (2) method was used. At autopsy, the skin was immediately stripped, and plugs 4 mm. in diameter were taken, using a carbide steel punch or a uterine biopsy forceps. This punch biopsy material was then placed in 1N NaOH for digestion overnight and examined for alkaline hematin content the next morning. The results could be expressed in terms of known erythrocyte counts (Fig. 1) or compared against standard curves for hemoglobin and expressed as mg. per 100 g. of skin. The latter alternative was preferred.

TABLE VIII

Incidence of Histopathological Findings[a] in Rabbits, 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

	None		P.P.D.-R. Dye + H_2O_2			
	Dose, ml./kg./day					
	0		2		4	
	5M	5F	5M	5F	5M	5F
Organ and Findings	Incidence					
Lungs						
Interstitial inflammation	2					2
Focal chronic inflammation	2	2				2
Focal acute congestion					1	
Focal thickening of pleura					1	
Kidneys						
Focal chronic inflammation						1
Testes						
Occasional abnormal germinal epithelial cell	1					
Brain						
Perivascular cuffing		1				
Skin—Treated						
Chronic inflammation in dermis, focal	1			1	1	
Epidermal thickening	1			1	1	1
Hyperkeratosis					1	1
Epidermal inflammation						1
Acute inflammation in dermis	1		1	1		
Abscess in keratotic layer	1		1	2	1	
Dermal fibrosis				1	1	2
Edema in dermis				1		
Atrophy of adnexal structures in dermis				1		
Acute and chronic ulceration in dermis						2

[a] Only positive findings are shown.

Four graded concentrations of aqueous hydrogen peroxide solutions of 3, 6, 9, and 12% (equivalent to 10, 20, 30, and 40 volumes per cent) were applied to groups of 10 rabbits each. These materials were applied

TABLE IX

Incidence and Severity of Histopathological Findings[a] in Rabbits[b] Prolonged (6-hour) Daily Contact with P.P.D.-R. Dye + H_2O_2

Organ and Findings	P.P.D.-R. Dye + H_2O_2 Dose, ml./kg./day			
	0	1	3	10
	Incidence			
Liver				
Focal acute necrosis				1
Focal lymphocytic accumulation	1			
Kidneys				
Focal chronic pyelonephritis				1
Chronic interstitial nephritis			1	
Marrow				
Congestion				1
Skin—Untreated		2^{2+}, 1^{3+}	1^{2+}	2^{2+}
Epidermal thickening		1^{2+}		
Skin—Treated				
Chronic inflammation in dermis	2^{1+}	1^{2+}, 2^{4+}	2^{2+}	
Epidermal thickening		1^{3+}, 1^{5+}	3^{4+}	
Hyperkeratosis	1^{1+}	2^{2+}, 1^{5+}	3^{3+}	
Acute inflammatory dermis			1^{3+}, 1^{4+}	
Abscess in keratis layer		1^{3+}, 1^{4+}	1^{3+}	
Dermal fibrosis		2^{4+}, 1^{5+}	2^{2+}	2^{5+}
Telangiectasia in dermis		1^{3+}, 1^{3+}, 1^{4+}		
Abscess in hair follicle		1^{4+}		
Atrophy of adnexal structures in dermis		1^{5+}	3^{5+}	
Foreign body material in dermis				1
Diffuse necrosis in epidermis				2
Early ulceration				1

[a] Only positive findings are shown.

[b] Groups of 10 rabbits received each treatment.

once weekly for six weeks using a one-hour contact period. The skin was stripped at autopsy, a sample prepared for microscopic examination and another prepared for hemoglobin analysis.

In another series, groups of rabbits were placed on test to compare the results after 48-hours or two applications, each of one hour's duration. A third comparison was based on a single acute continuous 24-hour application of four commercial oxidation dyes purchased on the open market and of a vehicle common to each of the bases. These tests were terminated immediately after the period of application, and the sites were treated in the manner described.

Results

Primary irritation scores are shown in Table II for the daily six-hour contact. The maximum scores were 2 and indicative of slight ery-

thema. In the Draize system, effects scored as 2 or less are considered only mildly irritating, products eliciting scores of 2 to 5 are moderate irritants, and those with scores above 6 are considered to be severe irritants. There was no evidence of irritation in this modified procedure.

Primary irritation scores for the graded peroxide concentrations are shown in Table III. For this study, samples were applied on each animal in a randomized fashion so that in every instance each concentration used was placed adjacent to a different concentration.

Comparison of the data in Tables II and III indicates that the intensity of response to oxidative irritants of equal peroxide concentration is not a function of contact time. This may be due to the rapid decomposition of the hydrogen peroxide in contact with organic matter and air.

TABLE X
Incidence—Peroxide Studies, 1-Hour Contact Histopathological Findings in Skin[a]

Findings		Per cent H_2O_2			
		3	6	9	12
Chronic inflammation in dermis	1+	4	2	3	3
	2+		1		
	3+	1			
Epidermis thickening	1+	1			1
Epidermis inflammation	1+	1			
Hyperkeratosis	1+		1		1
Epidermis abscess	1+			1	
Dermal abscess	1+	1			
Dermal fibrosis	1+	3			
	2+		1		

[a] Two groups of 6 rabbits, each treated with each concentration.

Examination of a variety of physiological factors failed to demonstrate any evidence of systemic toxicity, following subacute dermal applications. The data shown in Tables IV and V indicate no adverse responses in body weights. No deleterious changes were seen (Tables VI and VII) in hemoglobin concentration, hematocrit, or total erythrocyte counts with either treatment groups. The variance in poly: lymphocyte ratio seen at all levels in the one-hour group (Table VI) indicates a complete reversal. This is commonly noted in rabbits and occurs spontaneously in nontreatment groups. Literature values for the ratio of these two leukocytes confirm the spontaneity of this shift. Critical examination of the differential slides revealed no change in the

incidence of juvenile or adult forms, indicating no treatment-related shift either to the left or right.

The histopathological assessments of the vital organs and of the skin of rabbits in which the two test methods were employed are shown in Tables VIII to X. The increase in number of skin lesions characterized by dermal fibrosis and ulceration in the 4 ml. per kg. group underscores the usefulness of the one-hour contact procedure for safety evaluation of this type of formulation. In general, these findings were observed in one or two rabbits in each group. Therefore, there were no differences between the treated and control groups or between the two treatment levels. The lack of significant findings in the viscera in both control and treated groups is indicative of the generally innocuous nature of the daily shampooing in this species.

TABLE XI
Hemoglobin Concentration in Skin Peroxide Studies, 1-Hour Contact

H_2O_2 Concentration, %				
Untreated	3	6	9	12
		mg./100 g.		
		Males		
60	230	550	520	330
20	180	230	640	840
10	270	20	220	350
60	230	210	20	330
30	270	270	250	710
10	240	240	470	290
20	230	290	350	290
60	460	340	310	360
		Females		
50	370	230	180	250
20	510	580	350	430
90	270	320	220	170
10	230	230	250	340
20	220	300	230	170
10	240	210	380	270
<10	200	200	200	270
20	220	500	320	400

The observations (Table IX) in both the abraded and intact skin treated for six hours each day appeared to have no adverse significance. Telangiectasia, atrophy of adnexal structures in the dermis, epidermal thickening, focal or diffuse chronic inflammation of dermis, hyperkeratosis, and occasional ulceration have been frequently seen in control

groups in both 21- and 90-day tests. The abrading process intensifies these changes. In these studies, epidermal thickening, inflammatory responses, and even hyperkeratosis were often noted near or adjacent to treatment zones. These changes which often appear as a consequence of the effects induced at the site of application may be seen 1 to 3 cm. away from a treated or abraded area.

The blood hemoglobin levels in the 3, 6, and 9% H_2O_2 groups did not differ from each other. The results of the hemoglobin determinations in the skin biopsies (Table XI) did reveal a significant difference ($p = 0.05$) between the groups receiving 3 and 12% H_2O_2. This is in contrast to the histopathological findings (Table X), where the incidence of these findings failed to indicate any differences related to the concentration of peroxide.

TABLE XII
Comparison of Hemoglobin Values in Rabbit Skin at 48 Hours

	P.P.D.-R. Dye + H_2O_2,		
	Depilated (no wash)	Depilated and Abraded (no wash)	Clipped ¼–⅛ in. (wash at 1 hour)
	mg./100 g.		
	1630	2830	1330
	2170	3550	860
	2350	3380	950
Means	2050	3250	1050

TABLE XIII
Erythrocyte Counts ($\times 10^3$) Estimated from Alkaline Hematin Analysis of Skin Plugs (Rabbit)

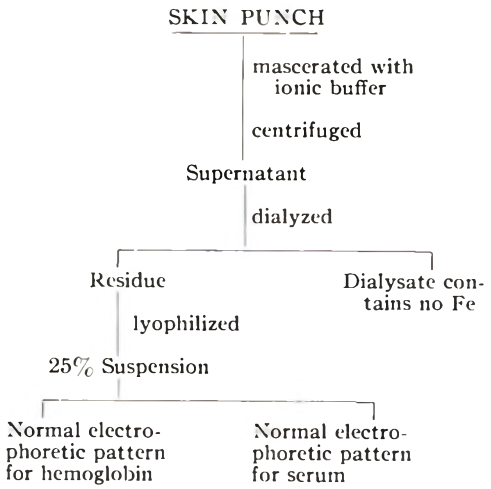
Dye sample →	1[a]	2	3	4	5
pH →	9.0	9.7	9.5	8.8	9.3
	67.5	95.0	83.0	86.5	85.0
	48.5	90.0	74.0	77.5	98.7
	40.5	83.0	85.0	81.3	91.3
	44.0	73.0		73.0	83.0
Means	51.0	85.2	81.0	79.6	89.6
Saline, pH	8.0		9.0	7.0	
	67.5		62.5	56.3	
	41.0		48.0	42.5	
	33.7		46.3	42.5	
Means	47.4		52.1	45.3	

[a] Product 1 = vehicle for products 2, 3, 4, and 5.

To ascertain effects at 48 hours, in rabbits treated by both the prolonged and the one-hour contact procedures, animals were sacrificed, the application sites examined, and skin plugs cut and prepared for hemoglobin analysis. The results are shown in Table XII. Marked differences in the values were observed between the untreated and treated skin. In each case, the treatment elicited increased quantities of hemoglobin-bearing red cells at the sites of application. The quantitative differences suggest that those animals treated by prolonged contact showed the more marked responses.

Data from the comparison of commercial oxidation dye preparations are shown in Table XIII. Product "1" was the vehicle for the various dyes. Products 2, 3, 4, and 5 were dye mixtures to be evaluated. The vehicle was included in order to assess the effects of the mechanics of the treatment. All, including product "1," were prepared with appropriate peroxide solution and applied for a one-hour period. The animals were sacrificed immediately thereafter and the skin subjected to the alkaline hematin test. For each product, replicate applications were made to a series of sites. Untreated controls and pH-adjusted saline were included. Data for these analyses are shown as red cell counts, read from a standard curve (Fig. 1). The values for products 2, 3, 4, and 5 are significantly higher than those for product "1," the vehicle, the pH-controlled saline, or the untreated skin (Table XI). These data generally correlated with the edema and erythema seen on exposure of the dermal surface of the skin.

To ascertain whether these alkaline hematin determinations are



indicative of the presence of blood *per se* or some foreign protein associated with injury, electrophoretic separations were made. The flow diagram for the procedure is shown in the scheme on page 760. No evidence of any unnatural or foreign protein was observed. The presence of hemoglobin protein was demonstrated by direct comparisons with an external standard.

Discussion and Summary

The modified procedure utilizing the one-hour application resulted in a marked diminution or absence of the characteristic cracking, thickening, sloughing, and necrosis so often seen with the original Draize procedure and which do not contribute to the safety evaluation. This was in large part due to the shampooing and drying of the rabbits after each daily exposure, thus avoiding drying of the residual test material. No significant differences were observed between the two methods being evaluated as toxicological tools. Not withstanding the similarity of findings with those of the Draize method, the one-hour procedure has distinct advantages with respect to utility and extrapolation to humans. Though both procedures yield comparable results, the one-hour contact more closely resembles use conditions with loss of sensitivity as a test method. Both procedures, though valuable in ascertaining irritation potential, failed to yield the means whereby more subtle differences between test preparations can be ascertained.

A revised method whereby the hemoglobin content of 4 mm. skin plugs taken from the application sites was determined by an alkaline hematin method permitted differentiation between graded concentrations of a hydrogen peroxide solution. Irritation and inflammation resulting from this contact is associated with the extravasation, through the capillaries, of red cells and their collection at these inflammatory sites. Electrophoretic identification of this material as hemoglobin was made. The value of the modified method as a means of differentiating potentially irritating materials is indicated by the comparison of commercial oxidation hair dyes and their common vehicle.

(Received May 20, 1965)

References

(1) Draize, J. H., *Appraisal of the Safety of Chemicals, Foods, Drugs and Cosmetics.* Association of Food Drug Officials of the United States, Editorial Office, Baltimore, Md. (1959), p. 46.

(2) E. Ascheim, *Am. J. Physiol.*, **206**, 327 (1964).

J. Soc. Cosmetic Chemists, 16, 763–776 (1965)

Principles of Consumer Product Testing

JEAN F. CAUL, Ph.D., and SHIRLEY A. RAYMOND, B.S.*

Presented May 4, 1965, New York City

Synopsis—After a review of the basic features of consumer panel testing, the requirements for a discriminating panel are described. The utility of this panel, especially in combination with a "Use Profile," is demonstrated with several examples. It appears to be entirely possible to relate consumer findings to laboratory measurements. Such data can guide reformulation or may be the go-ahead signal for marketing of the product.

Introduction

The chemists engaged in the development of new products and the statisticians responsible for the evaluation of these creations for the consumer market recognize the many difficulties in designing the perfect mathematical model for successful forecasting. These obstacles include considerations of cost and speed and of such concepts as statistical probability, quota sampling, Rorschach test, computer programming, decision-making, concept testing, and share of market. Another difficulty arises from the difference in emphasis between those trained in the physical sciences and those trained in mathematics, social psychology, and business. The chemist frequently emphasizes the product's properties; the mathematician stresses the exact and deducible relationships between quantities and operations; the social psychologist probes the explanations of group behavior; and the business-trained person is concerned with who exchanges money for merchandise.

* Arthur D. Little, Inc., Food & Flavor Section, Life Sciences Division, Cambridge, Mass. 02140.

Another difficulty which limits the correlation between product properties and consumer preference originates with marketing management. It is market-oriented rather than research-oriented and hence views the successful achievement of this correlation of various disciplines as virtually impossible.

Nevertheless, this laboratory's experience in the testing of product models and final formulations suggests the possibility of relating measurable product properties to consumer acceptance and rejection and thus sustains the hope that a product's specifications can be so drawn up in the laboratory as to make virtually certain its acceptance by consumers. The purpose of this paper, then, is to outline the use of consumer panels as product evaluation instruments. The first part of the discussion is concerned with the various kinds of consumer tests and discusses the do's and don'ts of consumer product testing; the last part explains methodology and the rationale underlying the suggested approach.

Tests Involving Consumers

Market Tests

Basically there are only two types of consumer tests: One in the marketplace where consumers exchange money for merchandise; the other, where they do not. Marketplace tests obtain information about the performance of the packaged, labeled, and priced product in actual sales situations, and such tests fall properly within the scope of sales development and market research people.

In all other consumer tests, that is, where consumers do not hand over their own money for products, information is obtained in a test situation about the consumers or the product or both. A test situation does not equate with a sales situation, but it eliminates some of the guesswork and, therefore, some of the risks in launching and merchandising a new product.

Consumer Testing

When information is obtained only about consumers, the approach should be called consumer testing. Like market testing, consumer testing or the testing of people falls within the scope of persons involved with sales and frequently needs to rely on the knowledge and training of social psychologists as well. It may involve hypothetical circumstances, as in evaluating a product concept when no product exists.

It may involve tricking consumers by having them evaluate the same product in three differently branded containers. It may aim to characterize consumers through depth interviews. Whatever the approach, consumers are under scrutiny, not the product.

Consumer Product Testing

Consumer product testing, on the other hand, should aim to test the product. At present there is some confusion of objectives about product testing because of two factions: the marketing people and the technical people. Marketing people have the task of taking the product, packaging it attractively, naming it, selecting a desirable label, getting it into distribution channels, developing enticing advertising copy, and pricing the finished product— all this by a target date that probably was set as soon as the green light was given to the technical staff to develop the product. Little wonder that they want to modify the product test with marketing-type questions. But all too often the marketing people appear not to remember that their best efforts will come to naught if the product is not right. They can attract first purchases, but they cannot bring about repeat buying unless the product they are trying to sell is in fact acceptable to consumers.

On the other side, the technical people have the responsibility of creating the product, and they necessarily have a different set of questions to be answered: Does the product meet its concept or design conceived for it? If not, how far along is it? What are its negative features? What are its positives? If it does meet the concept, is the concept acceptable? Is the product right?

In the authors' opinion, a consumer product test should test the product, and only if this primary objective will not be sacrificed may marketing-type questions be included in the test.

Components of Consumer Product Testing

In planning a consumer product test, one must consider four test components: 1) size and characteristics of the consumer panel; 2) number of different samples to be tested; 3) length of use of the product; and 4) method of obtaining information from the consumers. The key to selecting the best choice for each component is this: What must be learned about the product? In other words, this test is designed around the problem.

The Panel

Ordinarily, the selection of a panel of consumers is based on the premise that a consumer panel should be representative of the total population, and, therefore, statistical analysis can be applied to the findings. From such analyses, then, it should be possible to ascertain the probability or reliability of the findings.

There are two kinds of population samples to which statistics are justifiably applied: Random samples, that is, population samples selected without a definite pattern; and quota samples, in which the people who make up the test panel fit the characteristics of a predetermined pattern—such as proportionate representation of the various income and educational levels of a standard metropolitan area (SMA) as determined by the U. S. Bureau of the Census. Inasmuch as only persons who are willing to participate in a test are represented, no sampling is truly representative. Nevertheless, statistical analysis can be and is used successfully in making decisions by persons who take into consideration this and various other gaps in their methodology. Too often, however, statistical analysis is misapplied or interpreted incorrectly.

The larger the sample the greater the probability that the findings are not due to chance. But, as the sample size increases, so do the costs of the test. Therefore, depending on available funds and the degree of risk one is willing to take, a particular sample size is selected.

In addition to size and general representative qualities of the panel, specific characteristics of a panel could be deliberately selected according to the product's end use. For example, a hair conditioner for beauty shop use would be tested by a panel of beauty shop operators on their clients. Suppose that three popular hair conditioners are already on the market for beauty shop use. One could adjust the composition of the testing panel so that users of these first, second, and third place rank products would be proportionately represented. This subsampling technique would yield more information on the performance of the hair conditioner in comparison to its ultimate competition.

Different Samples to be Tested

Often technical people wish to obtain guidance from small consumer panels regarding the direction to be taken with an unfinished product. If this single product were given to the consumers, they would exaggerate its unfinished qualities and fail to see or evaluate its positive

features. Consumers, like people, emphasize the negatives. One solution is to give the consumer panel two products, both unfinished but having different negatives and positives.

Whenever two products are submitted to consumers, they will form the parameters of a comparison test. If one is a good product and the other only a fair product, the comparison will tend to emphasize the extremes; in this instance, the consumers would regard the good product as excellent and the fair one as poor. In any paired comparison both products should be of the same general quality.

It would be difficult to test two shampoos with the same consumer panel. An acceptable solution to this problem is to use two similar panels, one for each product. The panels should be alike in all pertinent variables: the same numbers of users and nonusers of cream rinses, for example; the same age distribution patterns; the same numbers of home permanent users; the same numbers of nonusers; and the like. When the returns are in, the balance of the two panels should be confirmed, and their findings can be compared.

Multiple product testing with the same panel can be done only if sufficient time elapses between the use of each product. The time lapse prevents carryover from one product to the next. Second, the time lapse must not exceed the user's ability to remember and compare the various products. Multiple product testing is one of the most misused techniques in food product testing for several reasons: the test designers seem to feel that by alternating the position of use of each product with succeeding consumers, they have canceled out product interactions and carryovers. Such is not the case. They also seem to feel that consumers remember each product's properties equally well. This is not the case either. Consumers do a paired comparison of the first two products, sort out the major differences, and then assess the succeeding products according to the major differences they remembered from the first two. The test designers frequently make another mistake by forcing consumers to respond to three or more products tasted at one session, headcounting these preference rankings and misapplying statistical analyses, coming up with probability figures that are accepted as true. The point here is that statistics should not be applied to data from improperly conducted tests. Fortunately, most cosmetic products require repetitive use before they can be truly evaluated by consumers, and multiple cosmetic product testing is therefore contraindicated.

With random panels of consumers who run the gamut in intelligence, there is always some danger of introducing unwanted biases induced by sample codes. A panel of intelligent consumers obviates the need to be deeply concerned about findings based on a like or dislike of the code used for an otherwise unidentified sample. But from common sense codes should be avoided that might take on meaning, e.g., the letter X, the letter-number code A-1, the butter-score numbers 88 or 93 for margarine, or G-11 for a soap. Most test designers avoid color codes and simple letter or number codes; they usually use two- or three-digit number-letter combinations.

Use Period

In food testing if only a simple "yes/no," "go/no-go" kind of result is needed, a product may be subjected to "one-shot" testing. This type of test is usually conducted in trafficked areas, such as a store, county fair, or bus terminal. This test resembles the man-in-the-street opinions poll. Persons who happen to pass by and who have the time and inclination are invited to participate. Often they are asked to choose between two samples on a preference basis. Considerable numbers of consumers can be reached by such a test method, but it should be remembered that the data relate only to first impressions.

Conceivably the aromas of two perfumes could be checked out in this way, if the bias inherent in such a consumer test population were recognized. But the actual properties of the perfumes and the consumer's changes in attitude could be learned only through a repetitive use test. For most products there is a first impression, a get-acquainted period, and a final impression. The final impression leads to final assessment, which in the marketplace would determine second purchase.

A use test, then, should be conducted for a long enough period to attain a final impression. This might be as short as three days for an after-shave lotion or as long as four weeks for a scalp conditioner. It is difficult to sustain a panelist's interest for more than two weeks unless re-stimulation is offered. Recently the authors were able to hold a home-use consumer panel of 200 persons together for three months by check postals, fresh samples, and friendly letters. The check postals really served a dual purpose. They activated the panelists and provided information on changes in attitude and frequency of product use so that one could be reasonably certain of obtaining final impressions at the conclusion of the test.

Methods of Obtaining Data

There are only two basic methods for obtaining responses from consumer panel members. The first is by questionnaire and the second by face-to-face or telephone interview. Even the interviews are commonly conducted by questionnaire. Usually these are structured questionnaires, whereby all interviewers use the same words, the same questions, the same procedure.

Interviews, understandably, are more expensive than questionnaires mailed to and from the consumer panel members. Interviews provide several advantages. First, they assure a response, whereas a mailed-in questionnaire can be totally ignored. Second, they assure early responses. A mailed-in questionnaire can be put aside until the respondent has forgotten details about the test product or until after the deadline for compiling and reporting results has passed. Third, more questions can be asked by an interviewer. Less patient consumers will not spend the time to fill out carefully a long and complicated questionnaire; a carelessly answered questionnaire is worthless.

In using the interview technique, considerable effort should be made to obtain reliable, honest interviewers and then to train them so as not to allow them to introduce a bias. In general, most testing agencies do not identify the client or the underlying reasons for testing the product. This eliminates another source of interviewer bias. A part of the interviewer training period is devoted to pre-testing the questionnaire, and if the interviewers are intelligent people, they may be able to point out ambiguous words, better sequences of questions, and suggest where structured probes could be inserted.

Questionnaires to be filled in by the consumer panel members also should be pre-tested. With experience the composer of questionnaires learns how to ask questions so that any literate person will understand them. But the danger of questionnaires lies in not knowing the meaning of terms, either those asked by the questionnaire or those used by the consumers. For instance, checklists in questionnaires have asked for preference on mildness. Does this refer to feeling sensations, or to flavor, or to level of flavoring? When a consumer says a toothpaste leaves his mouth clean, is he referring to cleansing ability or refreshment due to the flavoring? When he says there is too much carbonation in a beverage, does he mean just that or is he saying that the weak flavoring is subjugated by the carbonation?

Not knowing the meaning of terms results from not knowing the

product's properties. People who really know the product should participate in developing the questionnaire and in editing the responses before compilations are made. In fact, a great deal can be learned by reading through all answers from each respondent. This provides a perspective of the over-all issues before the specifics are examined, as well as an understanding of what each panelist is trying to say.

Requisites of Pilot Consumer Product Testing

The foregoing discussion of the component parts of consumer product testing was intended to orient the many facets of product testing from a technical person's point of view. The topography of consumer product testing is very similar to that of conducting a laboratory analysis. First, define the objective: What information is needed and with what precision? Second, select the measuring device and analytical procedure: What precautions and controls will be needed? Third, conduct the analysis and obtain the findings. Fourth, relate the findings to the objective.

This approach to product testing is always used in A.D.L.'s Food and Flavor Laboratory. During these studies several important principles have been developed which experience has shown are the most useful for product development problems. In the following discussion, it will become evident that the primary principle in applying consumer panels to product testing is to understand what is to be tested and why.

The Discriminating-Communicating Panel

Usually a random or quota sample is not used. Because of the desire to have accurate information that can, if necessary, be translated into technical terms, a panel of consumers is selected who have these particular qualifications: First, they are interested in testing the particular product. This means that they will probably complete the test, taking care to respond carefully to the questions and giving the product a fair trial. While aware that they are performing a special favor, the testers also feel they are influencing the design of products that they, as consumers, may someday see in the marketplace. Second, this consumer panel is intelligent. This means that they will follow our instructions, try to avoid confusing product identities or codes, and likely will be able to express themselves adequately. Their ability to communicate their observations is of paramount importance. Third, they observe or distinguish a product's properties accurately. This ability to discriminate has been demonstrated by pre-testing.

Then, as mentioned earlier, panelists will be selected according to specific characteristics. In testing a new instant coffee, care was taken to include families who used only instant coffee, families who used only brewed coffee, and those who used both. The "instant-only" users showed they had become accommodated to the flavor of the then insipid but not unpleasant instant coffees, while persons who were familiar with brewed coffee recognized the virtues of the flavor characteristics of the new product. This illustrates selection of panelists according to type of product they use.

Frequency of product use can also be a criterion of selection. In another test it was found that frequent users of the current product were strongly against a variant of the product, while occasional users were delighted with the variant. This could mean two products with an over-all increase in product use.

More attention is being paid to teen-age products, and the authors have a source of panelists, who have been found to be as discriminating as their parents and often more communicative.

Size of panels may be as small as 25 persons or families and as large as 100 persons or families. Since the size of the A.D.L. panels is not large, these tests are called pilot tests. The roster from which panelists are usually selected consists of the families of A.D.L. Cambridge employees who have expressed their desire to participate in product tests. Most of them have lived in the area more than five years. Naturally, then, the first question to face is: Is this a regional panel? The answer generally is "No."

If the type of product is used nationally—e.g., mouthwash—then its use properties can be evaluated by a discriminating-communicating panel anywhere. If the product is designed for a specific region in the U. S., this panel can indicate if the product has the qualities it is supposed to have. They may not particularly like the product, but they can isolate its elements.

The second question to face is: How do the results obtained from discriminating-communicating panels compare with those from a national and not necessarily totally discriminating panel? To probe this question, a paired comparison of two toothpastes was put through the A.D.L. consumer toothpaste panel and a national panel twice as large. The preference trends and reasons for preference were the same from both panels, but in analyzing the questionnaires, the information from the A.D.L. panel was found to be more definitive. This result was not unexpected.

But there is an even more important rationale to the use of discriminating panels. Because they do discriminate, they provide the basis for a rigorous test. If the product is acceptable to persons who can discriminate, its properties should also be acceptable to persons who cannot or do not discriminate.

Prerequisites

To plan a proper test—that is, to select the consumer panel, to decide how often the product should be used and for how long, to choose the method of obtaining responses from the panel, to develop instructions for the consumers, and to anticipate the terminology they might use—the designers of these tests charge themselves with two responsibilities: to be sure of the purpose of the test and to be sure of the test product's properties.

Every test is specifically designed around the product and the test objective. If the product is a model of a concept, the product development group may wish to know if it matches the concept and, if not, what modifications are needed. If it is to be a new product, is it in its present status acceptable as a whole; does it have more positive than negative features? If a variant of a currently marketed product-type, how do its attributes compare with those of the marketed product; is there a positive that could be exploited in advertising? If it is an improved version of an existing brand, do consumers see the difference and do they consider it an improvement; do they see it as a major or minor improvement?

Once the test objective is defined, extensive effort will be made to define the product. First, if such technical information is not already available, technical analyses or examinations of the product will be carried out. Since the authors' consumer product testing mainly concerns foods and other flavorful products, this technical examination, while including observations on pH, color, and viscosity, will mainly be a Flavor Profile. Flavor Profiles are produced by experienced panels, who work under controlled conditions and use standardized techniques for smelling and testing. The Flavor Profile is a tabular record of the product's sensory (aroma and flavor) properties.

Having completed the Flavor Profile, the panel members will work to produce a use profile. For a soft drink, they would drink (as opposed to taste) a bottleful of the beverage in much the same way that consumers will—for example, gulp it down, pour it over ice, let it warm up in the glass, and drink it from the bottle. The value of a use profile is

incalculable. It bridges the gap between the technical analysis and the consumer responses, so that the test operators will be able to relate consumers' descriptive terms to the use profile and finally to the technical or flavor analysis. To report consumer findings to the product developer, one must be able to speak in his technical terms.

In addition to use profiles, the panel will also produce abuse profiles. Such information enables one to characterize the inherent latitude of the product and also to anticipate untoward responses and to guard against them. If, for example, the directions for preparing a soup call for 10 minutes' simmering, the effects of under- and over-simmering should be known. Undercooking could cause the noodles to be tough and the flavor underdeveloped. Overcooking could concentrate the soup, making it strong and salty.

The Testing Situation

During this study period, the product elements that consumers are likely to observe are sorted out, and decisions are then made regarding the test method. As previously mentioned, if the product is not a finished one but is to be tested in order to obtain guidance for its future direction, one might decide to test it in comparison to another unfinished product. If it has a counterpart on the market, then its performance could be tested in comparison to the "blind" marketed product. And similarly, if it has been designed as an improved product, the assessment of its improvement features could be made through a comparison with the unchanged product. A new-concept product would of course be tested by itself; in fact, single-product testing may also be applied to any product if the test can be designed to meet its objectives.

The product's properties and its intended use will determine some of the instructions for the consumer panel as well as duration of the use period and, therefore, the supplies needed. A side-by-side comparison would be requested in a statistically designed difference test aimed at defining flavor attributes of puddings differing only in sweetener content. This technique provides for a minimum time lapse and thus a direct comparison, which is a stringent test. Stronger tasting products, such as mouthwashes, would not be amenable to such immediate comparisons. Instructions would therefore request alternate use of one mouthwash on one day and the other on the next day, both to be used according to the panelists' normal patterns. Alternating-day use gives a closer comparison than alternating-week use, for example.

Obtaining Responses

The test objective, the product's use properties, and its intended use will determine how to obtain information from the consumer panel. If, as in foods, eating quality is the primary product property to be tested, responses can be obtained by questionnaire. If, as in hard liquors, other unknown attributes may supersede flavor, face-to-face interviews are in order. Interviews are worth their higher costs for the advantages already cited, i.e., they are time saving and provide more definitive information.

The interviewers are personable technical people, who are made to be fully cognizant of the purpose of the test, the client, the product's Flavor Profile, and its use and abuse profiles. These technical people are experienced flavorists, members of the A.D.L. Food & Flavor staff who train in on the particular test. During their training period, they use the product as the consumers will and, under supervision by their peers, practice first among themselves and then with consumers. In their practice sessions they learn to establish rapport, how to conduct an unstructured interview on this product, and to train their memories so as to be able to write up the interview or fill in their data sheets away from the scene of the interview. In other words, they learn to have a conversation about the product, allowing the consumer to describe her own impressions without channeling her responses by checklist type questions. They rely mainly on open-end questions and are allowed to pursue whatever the consumer considers important, probing on the spot for clarity and definition of descriptive and vague terms.

Similar precepts guide the development of questionnaires to be filled in by the consumer panelists. The note transmitting the questionnaire tells the consumer in general terms why the test has been conducted. After asking about the use of the product, the questionnaire itself asks mainly open-end questions about the product. If the panelists have been stimulated to be communicative, they will give their appraisal, sort out the favorable features and those that, in their opinion, need to be improved, and indicate the relative importance of the features they have discussed. This result, however, is not left to chance. A pretest of the instructions, use period, and questionnaire is almost invariably conducted, using about five families.

Other things not left to chance concern the test samples themselves. Before they are placed for home use, they are sampled at random and checked out to make certain that they do represent the product to be

tested and that they do not vary significantly. After they have been placed, other samples are periodically checked in the laboratory so that any unanticipated changes occurring during the testing period will be known.

Interpretation of Consumer Responses

Since in most of this work the response sheets are a series of short essays, each one is read for meaning and perspective. The respondent, whether interviewer or consumer, assumes this will be done. For example, for Soup A the consumer may have written that its unfavorable feature was "not salty enough," and for Soup B, "good rich flavor." Since the Flavor Profiles showed that both soups had the same salt level, she is not saying she would like to taste salt. She is stating that Soup A's flavor needed something and to improve it she would have added salt. If her descriptions for each soup had been read separately, Soup A might have been tallied under "salt level low"; this would be inaccurate and misleading.

The value of use profiles for interpretation is illustrated by the following example. In a series of paired comparisons involving six balanced panels, the objective was to select the best flavored product of three. Each product had been tested against the other and also against a control. The six sets of response sheets were read separately, and it was found that Product B evoked a seemingly different reaction when tested against Product A than against the control. Against Product A it was called flat; against the control it was called pleasant tasting. Without the use profiles of the products, one could not have interpreted these findings. But they dovetailed nicely with the use profiles of A *vs.* B, A *vs.* control, and B *vs.* control, which showed each pairing had different flavor parameters. Product A's over-all flavor was stronger, more identifiable but less appropriate; the control's flavor had several negative components; Product B's flavor was weaker than A's, stronger than the control's, and more appropriate than either. In other words, the test situation was different with each pairing, and the use profiles had defined the differences.

A research study of an oral product is a final example of relating consumer findings to product properties. This study was aimed at defining the product's important flavor elements and the ranges wherein these elements could make positive and negative contributions to preference. First, the consumer panelists isolated the important elements. Then, in a series of paired comparison tests conducted over a two-year period, the

intensity of each flavor element was varied separately, each time relating the consumer findings back to the test products' Flavor Profiles. After only six tests the study furnished the flavoring formulators with a Profile-blueprint. This tabulation defined the flavor character notes, their upper and lower intensity limits, and their order of sequence. The formulator with his expert knowledge of flavoring materials could then create new models which he was able to evaluate at the bench.

Thus, it is possible to relate consumer findings to laboratory measurements in order to draw up a product's specifications in meaningful technical terms. If the basic principles of good testing are followed when discriminating consumers make up the test panel, a product that fails to pass a consumer product test can be purposefully modified; and a product that passes is ready to be turned over to the marketing people.

J. Soc. Cosmetic Chemists, 16, 777–782 (1965)

The Reaction of α-Hydroxymethyl Ketones with Skin and Amino Acids*

KARL LADEN, Ph.D.,** and R. ZIELINSKI, Ph.D.

Synopsis—The reaction of dihydroxyacetone and various α-hydroxymethyl ketones with callus, amino acids, and bovine serum albumin has been investigated. The reaction of callus and amino acids with α-hydroxymethyl ketones to produce colored products appears to be a general one. In addition, there is a suggestion that the reaction is enhanced if electron withdrawing groups are attached to the α-hydroxymethyl ketone. The failure of various analytical schemes to detect the presence of these ketones in callus suggests that although in some cases relatively intense color is produced very little material has actually reacted with the callus.

Introduction

Dihydroxyacetone (DHA) has been used in recent years in order to produce a simulated suntan on skin. This tanning effect has been attributed to the reaction of DHA with the amino acids in the skin and with the keratin itself (1–5). Other compounds have also been shown to be capable of producing a dark product when placed on skin or reacted with amino acids. Goldman *et al.* (1) have shown that glyoxal reacts in a manner similar to DHA. Wittgenstein and Berry (2) compared the reactions of solutions of DHA and solutions of fructose with various amino acids. It was found that, in some cases, the fructose also gave colored products. A similar type of "browning reaction" has been observed in the reaction of amino acids with sugars (6–11).

The purposes of this study were to determine if the reaction of α-hydroxymethyl ketones with skin is a general one and also to obtain some additional information concerning the nature of the reaction.

* A contribution from The Toni Company, a Division of The Gillette Company, Chicago, Ill.

** Gillette Medical Research Institute, 6221 North Capitol St., N. E., Washington, D. C. 20011.

Experimental and Results

Initial experiments involved study of the reaction of several α-hydroxymethyl ketones with amino acids. The tests were run by placing two drops of a 1% solution of various amino acids on a piece of filter paper and, after drying, placing a drop of a 1% solution of the α-hydroxymethyl ketone on the spot where the amino acid solution had been placed. The filter paper was allowed to dry at room temperature and the color

TABLE I

The Reaction of Amino Acids with α-Hydroxymethyl Ketones on Filter Paper

	Proline	Norvaline	Threonine	Isoleucine	α-Alanine	Glycine
Dihydroxyacetone						
1 day	no color	lt. brown	v. lt. brown	lt. brown	lt. brown	brown
4 days	no color	dk. brown	dk. brown	dk. brown	dk. brown	brown
Phenacyl alcohol						
1 day	no color	lt. yellow	no color	no color	no color	no color
4 days	no color	yellow	yellow	yellow	yellow	yellow
p-Bromophenacyl alcohol						
1 day	no color	trace golden	no color	no color	no color	no color
4 days	no color	gold-yellow	gold-yellow	gold-yellow	gold-yellow	gold-yellow
Hydroxyacetone						
1 day	no color	lt. tan	no color	no color	no color	no color
4 days	no color	tan	tan	tan	tan	tan

Reactions performed as described in the text, using 1% solutions of amino acids and 1% solutions of α-hydroxymethyl ketones.

development observed. The results are presented in Table I. To show the effects of concentration of reagents similar trials were made with 10% concentrations rather than 1%. Some impressions as to the rate of reaction were also obtained by noting the time required for the first color to appear on the filter paper. Amino acids were selected so as to assess the role of structure of the amino acid on the reactivity. The results are presented in Table II.

In a second type of experiment 5 ml. of a 1% solution of the α-hydroxymethyl ketone in ethanol was added to 0.05 g. of each of the various amino acids, and the mixtures were allowed to stand overnight. The alcohol was evaporated on a steam bath, and the color produced was observed. While the end products were deeply colored, the color was not formed until the samples were heated. The results are presented in Table III.

TABLE II

The Reaction of Amino Acids with α-Hydroxymethyl Ketones on Filter Paper

Ketone Derivative	Time for Reaction with					
	Glycine	α-Alanine	β-Alanine	Aspartic Acid	Glutamic Acid	Arginine
p-Nitrophenacyl alcohol	hrs.	hrs.	min.	days	days	min.
Phenacyl alcohol	hrs.	hrs.	min.	days	days	min.
p-Bromphenacyl alcohol*	days	days	hrs.	...	...	hrs.
p-Dimethylaminophenacyl alcohol*	days	days	days	...	...	days
Dihydroxyacetone	hrs.	hrs.	min.	days	days	hrs.

Reactions performed as described in the text, using 1% solutions of amino acids and 10% solutions or saturated solutions (marked*) of α-hydroxymethyl ketones. Reaction times to first appearance of color are recorded as minutes = 1–15 min.; hrs. = 2–5 hrs.; days = 1½–2 days.

In order to investigate further this type of reaction with skin keratin, samples of 100 mg. of callus (ground to 60 mesh) were suspended in 15 ml. of a 5% solution of the α-hydroxymethyl ketone in ethanol. The samples were allowed to remain overnight at room temperature. The alcohol was then poured off and the callus washed three times with 15 ml. portions of alcohol and two times with 15 ml. portions of ether. It was then allowed to dry, and the color development was observed. The results are presented in Table IV. A second sample of ground callus was pretreated with formaldehyde before reacting with the α-hydroxymethyl ketones. The results are included in Table IV.

Since some of the hydroxymethyl ketones possess a slight color themselves (*p*-nitrophenacyl alcohol), it was of interest to ascertain that the color produced on the callus was not the result of simple adsorption. While many materials which impart color to keratin by simple adsorption can be effectively removed with an aqueous sodium chloride-acetone solution, the colors produced by the above materials were not affected by washing the callus with such a solution.

In the above experiments, color production was the only criterion used to detect reaction between callus and the test compounds. Several other methods were investigated to see whether the reaction could be detected.

1. Comparison of the I.R. spectra of samples of treated and untreated callus showed no differences in their spectra.

2. Comparison of the U.V. spectra of thin sheets of skin (carefully removed from the backs of sunburned subjects), before and after treat-

TABLE III

The Reaction of Amino Acids with α-Hydroxymethyl Ketones in Solution

	Alanine	Phenyl Alanine	Cystine
Dihydroxyacetone	brown liquid	brown liquid	yellow solid
Hydroxyacetone	brown liquid	brown liquid	yellow solid
Phenacyl alcohol	tan solid	yellow solid	white solid
p-Bromophenacyl alcohol	pink solid	gold solid	white solid

Reactions performed as described in the text, using 0.05 g. of amino acid and 1% solutions of the α-hydroxymethyl ketone in ethanol. Table indicates colors observed after ethanol evaporated on steam bath.

TABLE IV

Colors of Callus Samples Treated with α-Hydroxymethyl Ketones

	Untreated Callus	Callus Pretreated with Formaldehyde
Dihydroxyacetone	dark brown	no color
Hydroxyacetone	light tan	no color
Phenacyl alcohol	light yellow	no color
p-Bromophenacyl alcohol	yellow-gold	no color
p-Hydroxyphenacyl alcohol	v. lt. tan	no color
p-Nitrophenacyl alcohol	dk. yellow-brown	light yellow
p-Dimethylamino phenacyl alcohol	no color	...
3,5-Dichloro-4-hydroxy-phenacyl alcohol	light tan	...

Reactions performed as described in the text, using 100 mg. callus and 5% ethanolic solutions of the α-hydroxymethyl ketone.

ment with the α-hydroxymethyl ketones, indicated no detectable change in the spectra.

3. Comparison of the water-binding capacity of callus before and after treatment with the test materials indicated that no significant differences could be observed.

4. A measure of the extent of reaction could be obtained if the α-hydroxymethyl ketone had some functional group which could be easily determined. A callus sample that had been treated with *p*-bromophenacyl alcohol (and was deeply colored) was submitted to halogen analysis along with a control sample of callus. No differences were obtained between samples.

Samples of hair treated with *p*-bromophenacyl alcohol were also

analyzed for bromine. The amount of bromine in these samples was too low to be detected.

5. Bovine albumin is a water-soluble, ethanol-insoluble protein. Treatment of bovine albumin with absolute ethanol, if the times are not unduly prolonged, does not alter its water solubility. However, it was observed that, when a suspension of bovine albumin, absolute ethanol and either DHA, phenacyl alcohol, *p*-bromophenacyl alcohol, or *p*-nitrophenacyl alcohol was shaken overnight, the bovine albumin became slightly colored and was converted into a water-insoluble form. When *p*-dimethylaminophenacyl alcohol was used, the bovine albumin was not converted into a water-insoluble form, and no color change in the albumin was noted.

Discussion

The reaction of callus and amino acids with α-hydroxymethyl ketones to produce colored products appears to be a general one, and the rate of reaction appears to be related to the concentration of reagents. In addition, there is a suggestion that the reaction is enhanced (as judged by the intensity of the colors produced) if electron withdrawing groups are attached to the α-hydroxymethyl ketone. Thus, the order of decreasing color intensity of treated callus was *p*-nitrophenacyl alcohol, *p*-bromophenacyl alcohol, phenacyl alcohol and *p*-hydroxyphenacyl alcohol. In addition, it was noted that of the α-hydroxymethyl ketones reacted with bovine albumin only *p*-dimethyl amino phenacyl alcohol was without effect on its color and solubility. If the color formations were related to a Maillard type of reaction (8) (as suggested by the fact that removal of amino groups with formaldehyde blocked the reaction), such an activating effect would be predicted for electronegative groups.

Comparisons of the effects of amino acid structure on the rate of reaction are indicated in Table II. Thus, increasing the chain length by one carbon atom (glycine *vs.* alpha-alanine, aspartic acid *vs.* glutamic acid) has little effect on the rate. On the other hand, comparison of α- *vs.* β-alanine shows the latter to be substantially more reactive. Thus, compounds with more basic nitrogen groups appear to produce a color reaction much sooner. This is also seen in the case of arginine, which is a relatively rapid reactor, and in the cases of the dicarboxylic acids, which are relatively slow reactors.

The failure of the various analytical schemes to detect the presence of the reacted α-hydroxymethyl ketones on callus suggests that, although

in some cases relatively intense color was produced, very little material had actually reacted with the callus. It was observed, however, that in all cases where colored products were formed it was impossible to remove the color from the keratin by simple washing procedures.

(Received July 16, 1965)

References

(1) Goldman, L., *et al.*, *J. Invest. Dermatol.*, **35,** 161 (1960).
(2) Wittgenstein, E., and Berry, H., *Ibid.*, **36,** 283 (1961).
(3) *Idem*, *Science*, **132,** 894 (1960).
(4) Flesch, P., and Esoda, E. C. J., *Proc. Sci. Sect. Toilet Goods Assoc.*, **34,** 53 (1960).
(5) Maibach, H. I., and Kligman, A. M., *A.M.A. Arch. Dermatol.*, **82,** 505 (1960).
(6) Lento, H. G., *et al.*, *Food Research*, **25,** 750 (1960).
(7) *Idem*, *Food Research*, **25,** 757 (1960).
(8) Ellis, G. P., *Adv. Carbohydrate Chem.*, **14,** 63 (1959).
(9) Richards, E. L., *Biochem. J.*, **64,** 639 (1956).
(10) McWeeny, D. J., and Bruton, H. S., *Nature*, **196,** 40 (1962).
(11) Burton, H. S., *et al.*, *Ibid.*, **196,** 948 (1962).

J. Soc. Cosmetic Chemists, 16, 783–794 (1965)

Identification of Surface Active Agents As Trimethyl Silyl Ether Derivatives by Gas Chromatography

ROBERT SUFFIS, M.A., THOMAS J. SULLIVAN, B.S., and WILLIAM S. HENDERSON, B.S.*

Presented May 4, 1965, New York City

Synopsis—A method is presented for the analysis of some non-ionic surface active agents by gas chromatography. The components of these agents are converted to their volatile trimethyl silyl ether derivatives prior to analysis by reaction with hexamethyldisilazane and trimethylchlorosilane. The volatile derivatives of the surface active agents may then be easily separated by gas chromatography. This procedure has been found to be applicable to a variety of glycol esters and sorbitan esters which are frequently utilized in cosmetic and toiletries formulations. In addition, the method could be utilized to provide information concerning the chemical properties of a surface-active agent. Rapid analysis for mono-ester and di-ester concentrations, free glycol, and fatty acid composition is possible through use of this technique.

Introduction

The analysis of partial esters of polyhydric alcohols and other non-ionic surface active agents has been performed by chromatographic techniques. These methods have utilized silica gel columns and various solvent systems to effect these separations (1–3). In addition, there is considerable literature on the analysis of glycerides by paper (4, 5) and thin-layer chromatography (6–8). Research on these separations has also been performed utilizing countercurrent distribution (9) and liquid-liquid extraction (10). Most of the work in this field has been per-

* The Mennen Company, Morristown, N. J.

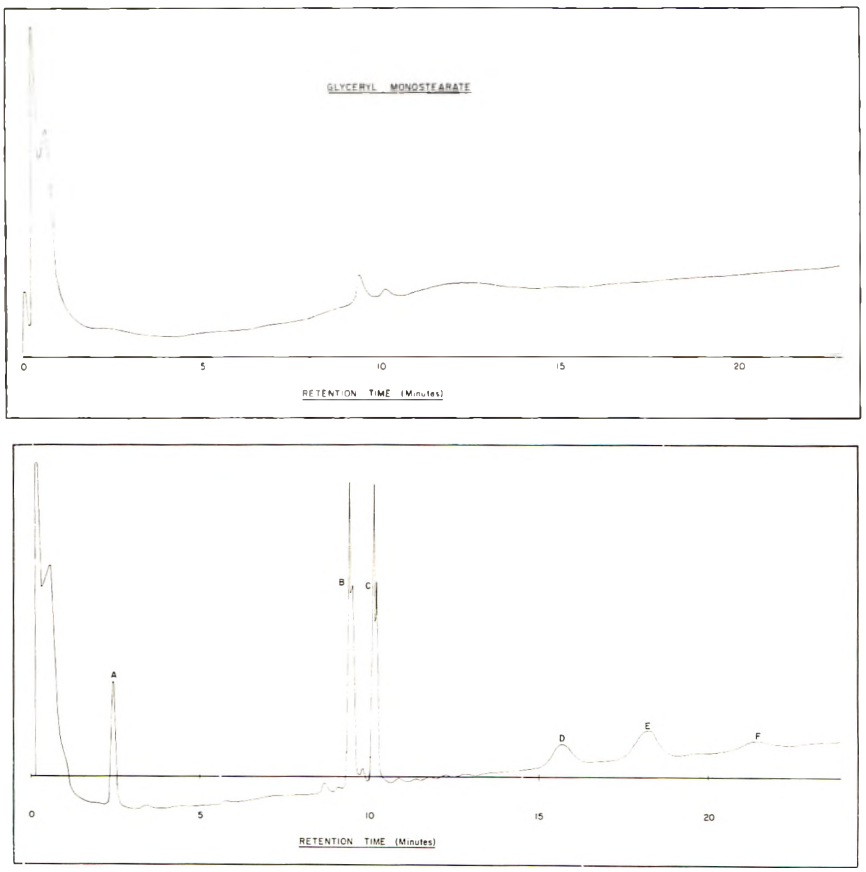


Figure 1. Top: Glyceryl monostearate in pyridine; Bottom: Glyceryl monostearate after trimethylsilylation

formed by lipid chemists. Therefore, most of the data cover fatty glycerides only. However, the same approaches should be possible for any glycol ester.

The above references describe techniques for the separation of the mono-ester, di-ester, and tri-ester components of the glycerides. These methods, applied to the identification of surface active agents, are lengthy, tedious, and usually not sufficiently specific for unequivocal identification.

There has been some research into the use of gas chromatography for the analysis of glycol esters. Triglycerides have been analyzed directly by high-temperature gas chromatography (11). However, the mono and diglycerides cannot be analyzed without conversion to a non-polar

derivative. The high boiling point and high polarity caused by the presence of one or more hydroxyl groups make it impossible to get good results by gas chromatography on the parent substance. Monoglycerides have been analyzed after conversion to allyl esters by a dehydration reaction (12). This method is applicable to glyceryl mono-esters only. Other work has been performed after converting the free hydroxyl groups

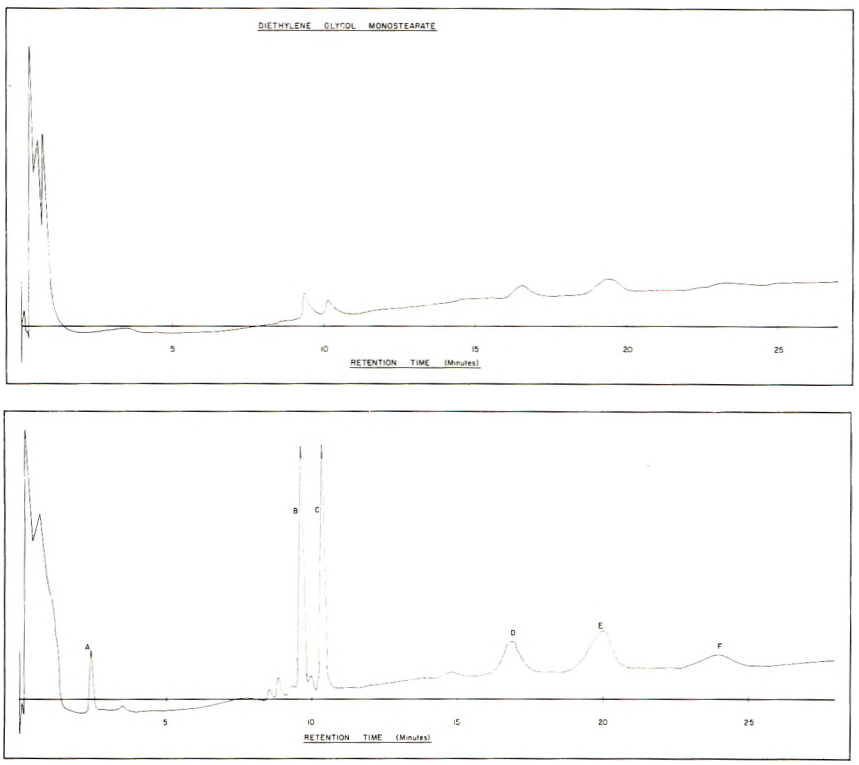


Figure 2. Top: Diethylene glycol monostearate in pyridine; Bottom: Diethylene glycol monostearate after trimethylsilylation

to acetates by reaction with acetyl chloride (13). These techniques have given some interesting results, but each one has some serious drawbacks. The conversion to allyl derivatives is very lengthy and applicable only to components with two adjacent hydroxyl groups. The acetate derivative preparation is also a lengthy procedure and gives rise to high boiling products.

The research described in this paper has made use of the reaction of hydroxyl groups contained in surface active agents with hexamethyl-

disilazane and trimethylchlorosilane. The products are volatile trimethyl silyl ether derivatives which are suitable for gas chromatography. The use of this reaction has received considerable attention in the sugar and carbohydrate field (14, 15). Compounds as high boiling as tetrasaccharides have been analyzed successfully. The reaction is simple and quantitative without any undesirable side reactions.

These derivatives are lower boiling than the corresponding acetates and give sharp, well defined peaks on the gas chromatograph. The use

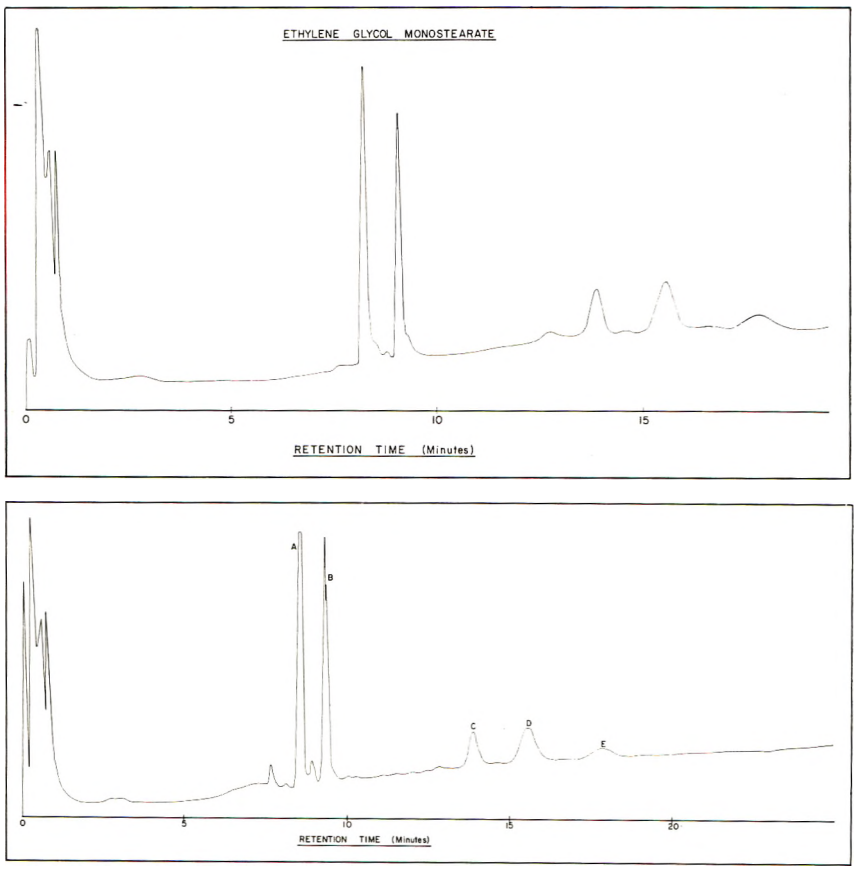


Figure 3. Top: Ethylene glycol monostearate in pyridine; Bottom: Ethylene glycol monostearate after trimethylsilylation

of this technique enables one to get a definitive chromatogram for each of the surface active agents investigated. A comparison of the chromatogram obtained from the initial pyridine solution with that of the reacted

product provides additional criteria for identification. This may be done easily by first analyzing a sample of the surface active agent in pyridine. Then the two reagents can be added and the chromatogram of the trimethylsilylated derivatives run next.

Experimental

Preparation of Derivatives

About 50–100 mg. of surface active agent is dissolved in 1 ml. of anhydrous pyridine (kept over KOH pellets) in a small plastic stoppered vial, and 0.2 ml. of hexamethyldisilazane and 0.1 ml. of trimethylchlorosilane are then added. The mixture is shaken vigorously for 30 seconds and then allowed to stand for 5 minutes. The solutions become cloudy, and a precipitate of ammonium chloride is formed. It is not necessary to remove this precipitate. The supernatant liquid may be run directly by gas chromatography (14).

Gas Chromatography

An F&M Model 810 Gas Chromatograph with thermal conductivity detector was used. All of the chromatograms were run using identical conditions. A 3 ft. by 0.125 in. o.d. column packed with 5% SE-52 silicone gum rubber on Anakrom A was employed. The helium flow rate was 20 ml./minute, and the column temperature was programmed from 100 to 300°C at a rate of 20°C/minute. One-microliter samples were injected into the gas chromatograph, using a Hamilton 10 microliter syringe.

Results and Discussion

Figure 1 shows a comprison of the chromatograms obtained from glyceryl monostearate before and after trimethylsilylation. The top chromatogram was obtained from a solution of glyceryl monostearate in pyridine. It is apparent that very little information can be obtained from this. The high boiling point and polar character of these components show very pronounced tailing for those peaks that have passed through the column. The bottom chromatogram shows the results obtained for the glyceryl monostearate derivatives after trimethylsilylation. The significant improvement is apparent.

All of the major peaks have been identified. Peak A is glycerin, and Peaks B and C are glyceryl mono-esters of palmitic and stearic acids, respectively. The small peak in front of peak B is due to glyceryl

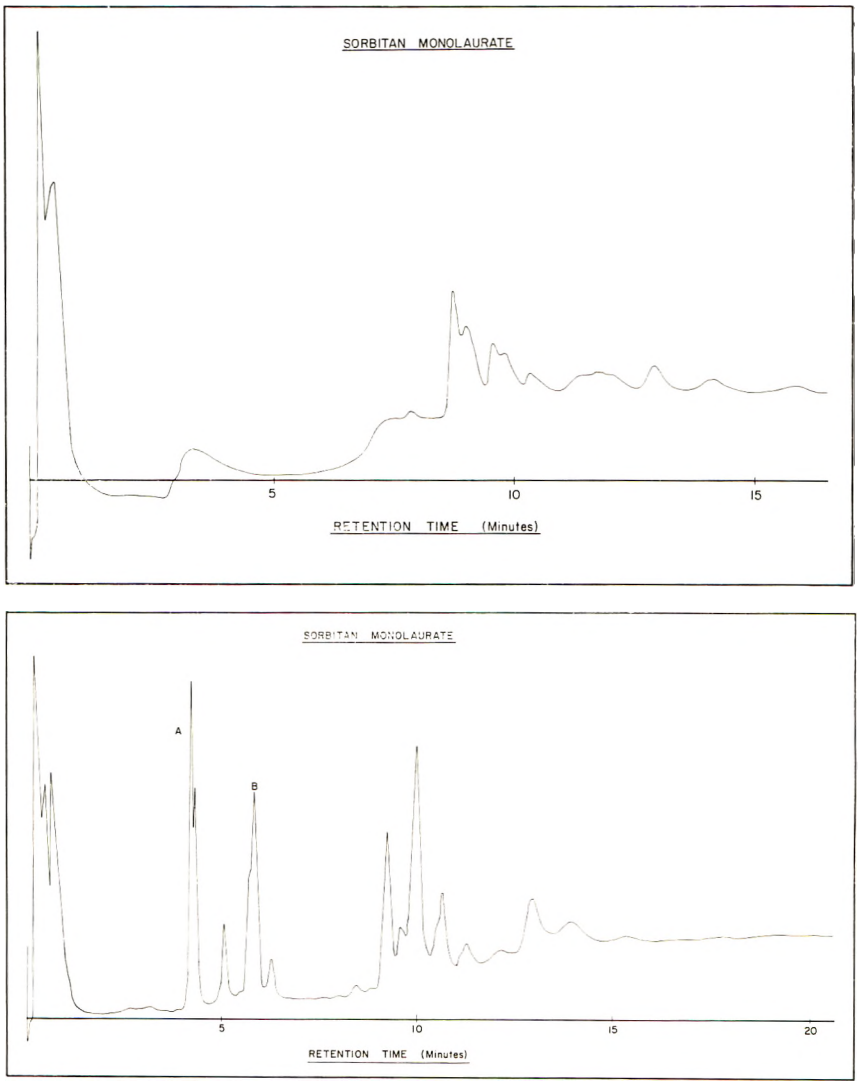


Figure 4. Top: Sorbitan monolaurate in pyridine; Bottom: Sorbitan monolaurate after trimethylsilylation

monomyristate. The composition of the monoesters is indicative of the fatty acid composition of the glyceryl ester. Peaks D, E, and F are di-ester peaks. Peak D is the dipalmitate; peak F is the distearate. The peak between these is due to the mixed ester of palmitic and stearic acids. These three peaks have been found to be in the expected ratio for the random combination of stearic and palmitic acids with glycerin. This

chromatogram together with the chromatogram of the glyceryl monostearate in pyridine provide for an absolute identification of this material.

Glyceryl esters of other fatty acids are also readily identifiable by this technique. It is simple to distinguish glyceryl monostearate prepared

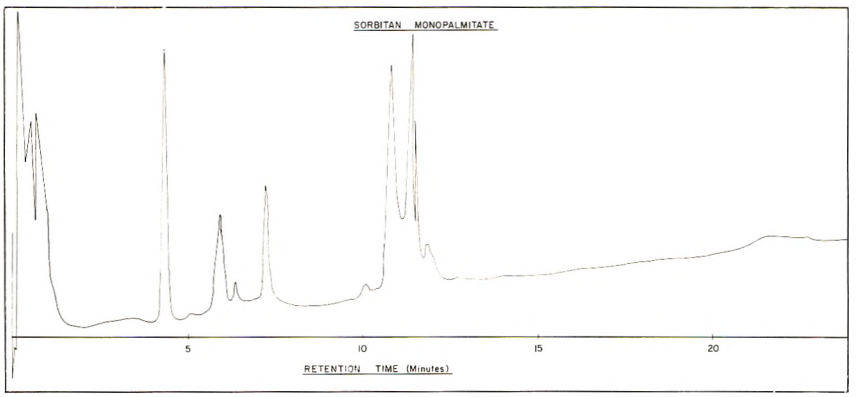


Figure 5. Sorbitan monopalmitate (Arlacel 40) after trimethylsilylation

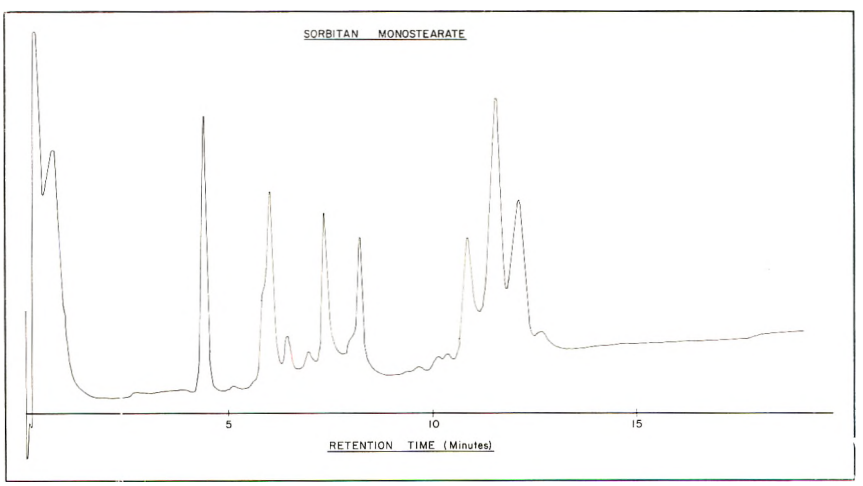


Figure 6. Sorbitan monostearate (Arlacel 60) after trimethylsilylation

from pressed stearic acid and that prepared from hydrogenated tallow fatty acids. Under the conditions of analysis utilized in this study there is no separation of the α- and β-monoglycerides. However, according to a recent publication (16) it is possible to separate these compounds using an ethylene glycol succinate column. This column has the disadvantage

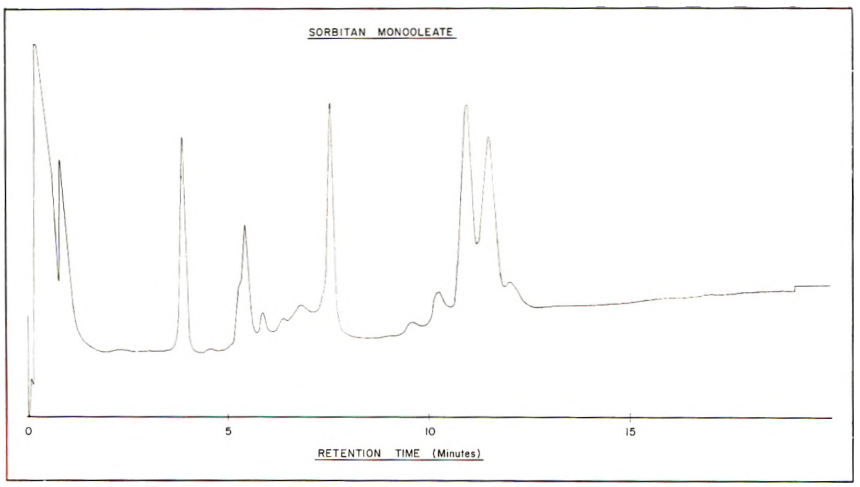


Figure 7. Sorbitan mono-oleate (Arlacel 80) after trimethylsilylation

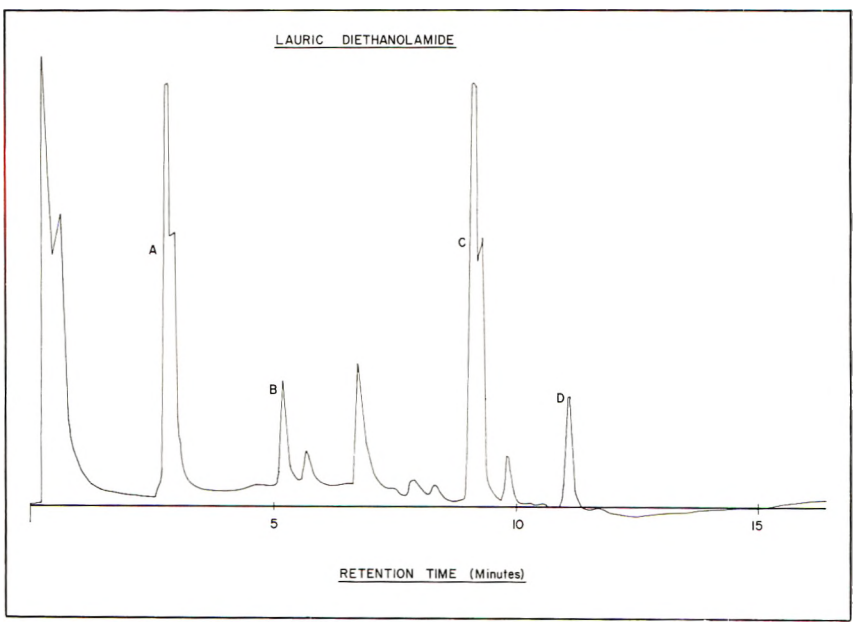


Figure 8. Lauric acid diethanolamide after trimethylsilylation

of comparatively low temperature stability, so it cannot be used for analysis of the di-ester components.

Figure 2 shows a comparison of the chromatograms of diethylene glycol monostearate before and after trimethylsilylation. One immediately notices the fact that peaks D, E, and F have identical retention

times in both chromatograms. These peaks are assigned to the di-esters present in diethylene glycol monostearate. These compounds have no available hydroxyl groups and, therefore, do not react with hexamethyldisilazane. The fact that these components are identical in the two chromatograms confirms the fact that this is a mixture of the di-esters derived from diethylene glycol. The mono-ester components, peaks B and C, which still contain a hydroxyl group do show a considerable change on trimethylsilylation. Peak A is due to free diethylene glycol.

Figure 3 shows a comparison of the chromatograms for ethylene glycol monostearate before and after reaction with hexamethyldisilazane. In this case, the di-ester peaks C, D, and E are identical in both chromatograms. Peaks A and B, due to the mono-ester, are also almost the same in both cases. This is due to the relatively non-polar characteristic of the mono-ester of ethylene glycol monostearate. However, there are changes that have occurred in the mono-ester peaks, before and after trimethylsilylation. The retention times of the two unreacted components are slightly longer than those of their counterparts in the bottom chromatogram. In addition, the reacted mono-esters show a considerably greater response for the same sample size. Even though the peaks have not undergone the considerable changes shown in the other examples, it is still apparent that some reaction has taken place. There is no free ethylene glycol peak, since the derivative of this component is so low boiling it is not resolved from the pyridine used as a solvent.

The three glycol stearates shown thus far can be easily distinguished by their chromatograms. Not only is there a difference between their response before and after trimethylsilylation, but in addition there are differences in the retention times of the various components which provide for absolute identification.

It can be readily seen that through the use of calibration standards it should be possible to analyze these glycol stearates quantitatively for free glycol, mono-esters, and di-ester concentration. Presently used methods for glycol and mono-ester are rather lengthy wet chemical procedures. There is no direct quantitative method for di-ester content commonly in use. The di-ester content could be an important factor in the performance of a surface active agent, such as glyceryl monostearate

Other glycol esters that have been studied by this procedure include diethylene glycol mono-oleate, propylene glycol monostearate, diethylene glycol monolaurate, diglycerol monostearate, triglycerol monostearate, and decaglycerol monostearate. In each case the surface

TABLE I

Relative Retention Times of Glycolesters and Sorbitan Esters (Glyceryl Monopalmitate = 1.000)

	Glycerol Mono-Stearate	Ethylene Glycol Monostearate	Diethylene Glycol Monostearate	Diethylene Glycol Monooleate	Propylene Glycol Monostearate	Diethylene Glycol Monolaurate	Decaglycerol Mono-Stearate	Diglycerol Mono-Stearate	Triglycerol Mono-Stearate
Free Glycol									
A	0.257		0.259	0.259		0.217	0.247	0.264	0.247
B							0.452	0.622	0.446
C							0.531		0.608
D							0.613		
Monoester									
A	1.000	0.898	1.026	0.866	0.804	0.833	0.774	0.860	0.769
B	1.077	0.983	1.104	1.098	0.901	0.920	0.849	1.013	0.854
C					0.983	1.004	0.987	1.095	1.011
D						1.075	1.036	1.188	1.091
E								1.253	1.188
F									1.258
Diester									
A	1.659	1.466	1.794	1.759	1.339	1.222	1.183	1.348	1.344
B	1.928	1.650	2.123	2.053	1.458	1.295	1.328	1.454	1.462
C	2.269	1.903	2.540	2.487	1.643	1.387	1.587	1.688	1.753
D					1.858	1.518		1.962	1.989
E						1.716		2.344	2.389
F								3.022	3.064
G									3.914

active agent could be readily identified by its chromatogram, which was distinguishable from any other gylcol ester.

Several sorbitan esters have been investigated by this technique. Figure 4 shows a comparison of the chromatograms for sorbitan monolaurate (Arlacel 20) before and after trimethylsilylation. The Arlacel 20 is broken down into a large number of components. Peaks A and B represent the main components of free sorbitan. Peak A is probably the hexide or five-membered ring dehydration product of sorbitol. Peak B has been assigned as the hexitan or six-membered ring dehydration product. This pattern is repeated with some minor modifications in each of the sorbitan ester combinations.

In the example shown, the fatty acid component is lauric acid. No attempts have been made thus far to make any definite assignments to these peaks. For the purpose of this investigation it is significant to note the differences among the sorbitan esters and use these data as a method of identification. The differences between Arlacels 20, 40, 60, and 80 are apparent after comparison of Figs. 5, 6, and 7 with Fig. 4.

It is also apparent that this gas chromatographic method could be an important tool in the elucidation of the composition of these surface-active agents. Table I gives a listing of the relative retention times of the major peaks of each of the glycol esters and sorbitan esters run by this technique. In each case there is no problem in identification of the surface active agent from its definitive chromatogram.

The trimethylsilylation reaction has also been applied to several other cosmetic raw materials. Figure 8 shows a chromatogram of lauric acid diethanolamide after trimethylsilylation. Peak A in this chromatogram is due to free diethanolamine, peak B to free lauric acid, and peak C (the main peak) to the amide. Peak D has been identified as the amine ester. The minor peaks have not been identified. Further investigation of this raw material may provide for identification of the amide ester that is usually present in this raw material. Possibly other more selective columns might perform an improved resolution job. The use of this technique for analysis of ethanolamines is another important application. The diethanolamine impurity gives an excellent peak (Fig. 8). Equally sharp, well resolved peaks are given by monoethanolamine and triethanolamine.

Summary

The technique of trimethylsilylation followed by gas chromatography appears to be applicable to the identification of some non-ionic surface-

active agents. It has been found to be particularly useful for glycol esters and sorbitan esters. Some of the possibilities of extension of this work to quantitative analysis of the components of these surface-active agents has been mentioned. In addition, fatty amides and ethanolamines have been indicated as areas of research that would merit further interest.

(Received July 16, 1965)

References

(1) Hofmann, A. F., *J. Lipid Res.*, **3**, 391 (1962).
(2) Hamilton, J. G. and Holman, T. T., *J. Am. Chem. Soc.*, **76**, 4107 (1954).
(3) Huebner, V. R., *J. Am. Chem. Soc.*, **35**, 325 (1958).
(4) Holasek, A., and Fried, J., *Mikrochim Acta*, **1957**, 469.
(5) Dieckert, J. W., and Reiser, R., *J. Am. Oil Chem. Soc.*, **33**, 123 (1956).
(6) Privett, O. S., and Blank, M. L., *Ibid.*, **39**, 520 (1962).
(7) Rybecka, S. M., *Chem. Ind.* (London) **1962**, 308.
(8) Vioque, E., and Holman, R. T., *J. Am. Oil Chem. Soc.*, **39**, 63 (1962).
(9) Perry, E. S., and Brokow, G. Y., *Ibid.*, **32**, 652 (1955).
(10) Monick, J. A., and Treybal, R. E., *Ibid.*, **33**, 193 (1956).
(11) Fryer, F. H., Ormand, W. L., and Crump, G. B., *Ibid.*, **37**, 589 (1960).
(12) McInnes, A. G., Tattrie, N. H., and Kater, M., *Ibid.*, **37**, 7 (1960).
(13) Huebner, V. R., *Ibid.*, **36**, 262 (1959).
(14) Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W., *J. Am. Chem. Soc.*, **85**, 2497 (1963).
(15) Bentley, R., Sweeley, C. C., Makita, M., and Wells, W. W., *Biochem. Biophys. Research Commun.*, **11**, 14 (1963).
(16) Wood, R. D., Raju, P. K., and Reiser, R., *J. Am. Chem. Soc.*, **42**, 161 (1965).

ERRATUM

Due to language difficulties an unavoidable error occurred in the synopsis of "Approaches to a Prophylaxis of Skin Aging," by M. and H. Ippen, *J. Soc. Cosmetic Chemists*, **16**, 305-8 (1965). The corrected synopsis should read as follows:

Synopsis—It is shown that smoking has a deleterious effect on skin condition and that this effect can be differentiated from that of damage by sunlight. Smoker's skin is identified as skin which suffers from loss of "turgor" and shows signs of flabbiness; in addition, the color of the smoker's skin is pale, with a grayish hue. Dermatological examination of 224 women up to now show moderate correlation between their smoking habits and the appearance of their skin, as defined above. By contrast, smoking seems to have only a very minor effect on the skin of male smokers.

For the convenience of the readers, the corrected synopsis is repeated on page xxxvii for use in card indexes.

Book Reviews

ÜBER DIE WIRKUNGSWEISE INDIFFERENTER SALBEN UND EMULSIONSSYSTEME AN DER HAUT IN ABHÄNGIGKEIT VON IHRER ZUSAMMENSETZUNG (Action of Plain Ointments and Emulsion Systems on Skin as a Function of Their Composition), edited by H. Tronnier. Editio Cantor Kg., Aulendorf, 1964. 178 pages, indexed. Price 25 DM, paper bound.

This booklet is Volume V of the Berufsdermatosen monographs. In it Tronnier describes the performance of 36 different ointment and lotion bases and discusses their effect on normal or healthy skin. Tronnier uses eleven tests to evaluate these formulations: adhesion to the skin; transfer from the skin; effect of bases on the dyeing and washability of the skin; immersion test; penetration through the bases (from the outside to the skin and from inside to the surrounding); effect of temperature of the skin; melting of the bases at skin temperature; gloss on the skin; friction on the skin; change of resonance frequency of the skin (moisturizing action); and effect of the bases on alkali-neutralization by the skin.

The cosmetic chemist and dermatologist will find much of interest in this booklet. Although one need not necessarily agree with all of Tronnier's conclusions nor approve his test procedures, the ideas are provocative and merit careful review. It is evident that the discussion of plain bases is only part of the problem since the bases do not include the "active" component of the cosmetic or dermatological formulation. Once the "actives" are included in the base, questions of drug transfer, penetration, and inactivation become important. Nevertheless, a few of the comments by Tronnier are particularly noteworthy. He observed little or no influence by the pH of the bases, whether they be o/w or w/o emulsions. The addition of silicones, in the hands of Tronnier, has failed to increase the barrier properties of petrolatum or of standard o/w emulsions.

In summary, this volume describes a considerable amount of original experimental work. The experimental data and the background are supported by numerous (over 300) references to the original literature. As a result, this booklet is worthwhile reading for all cosmetic chemists.—M. M. RIEGER—Warner-Lambert Research Institute.

THE PROTEINS—COMPOSITION, STRUCTURE AND FUNCTION, VOL. III, edited by Hans Neurath. 2nd Edition, Academic Press, New York and London, 1965. 585 pages, illustrated and indexed. Price $21.

This, the third volume of this massive compendium, upholds the tradition of excellence established by Volumes I and II. The subjects covered in this volume are rather diverse, although there is some overlap of information.

Two chapters are concerned with general characteristics of proteins. The first of these, Fractionation of Proteins by Sober and co-authors, deals with methods of purification and separation of proteins and is primarily descriptive. The second chapter, by Weber and Teale, deals with the interaction of proteins with radiation. This chapter is interesting reading; it should be of particular value to cosmetic chemists who are concerned with the interaction of proteins with u.v. radiation. However, as is so often the case in books containing contributions by various authors, the lack of cross references in this chapter is painfully apparent. Certainly, brief references to the appropriate material on spectral data and X-ray structure in Volumes I and II would have been appropriate.

The remaining four chapters of this volume are concerned with a detailed examination of the structure and function of a variety of proteins, i.e., those of viruses, plasma, antigens and antibodies, and blood coagulation. These chapters are, of course, of primary interest to students or experts in these particular fields. Suffice it to say that the novice will need much effort to master the diversity of information included in these four chapters. As a result, this reviewer feels that the inclusion of detailed discussions of specialized proteins in this general treatise might be questioned. Again a lack of cross references is noted, especially between the chapter on antigens and antibodies and that on plasma proteins.

Careful study of this book should be rewarding and informative for most readers. On the other hand, the detailed and comprehensive treatment of specialized topics in several chapters appears to be the main value of this particular volume.—M. M. RIEGER—Warner-Lambert Research Institute.

AUTHOR INDEX TO VOLUME XVI

SUBJECT INDEX TO VOLUME XVI

BOOK REVIEW INDEX TO VOLUME XVI

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION (*Act of October 23, 1962; Section 4369. Title 39 United States Code*)

1. Date of Filing October 1, 1965.
2. Title of Publication THE JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS.
3. Frequency of Issue Six times per year.
4. Location of Known Office of Publication (*Street, city, county, state, zip code*) Twentieth and Northampton Streets, Easton, Pennsylvania 18043.
5. Location of the Headquarters or General Business Offices of the Publishers (*Not printers*) 2 East Sixty-third Street, New York, New York 10021.
6. Names and Addresses of Publisher, Editor and Managing Editor.
   Publisher (*Name and address*) The Society of Cosmetic Chemists, Inc., 2 East Sixty-third Street, New York, New York 10021.
   Editor (*Name and address*) Dr. Martin M. Rieger, 170 Tabor Road, Morris Plains, New Jersey 07950.
   Managing Editor (*Name and address*) None
7. Owner (*If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given.*) Name Society of Cosmetic Chemists, Inc. Address 2 East Sixty-third Street, New York, New York 10021
8. Known Bondholders, Mortgagees, and Other Security Holders Owning or Holding 1 Percent or more of Total Amount of Bonds, Mortgages or Other Securities (*If there are none, so state*) None
9. Paragraphs 7 and 8 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting, also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner. Names and addresses of individuals who are stockholders of a corporation which itself is a stockholder or holder of bonds, mortgages or other securities of the publishing corporation have been included in paragraphs 7 and 8 when the interests of such individuals are equivalent to 1 percent or more of the total amount of the stock or securities of the publishing corporation.
10. This Item Must Be Completed for All Publications Except Those Which Do Not Carry Advertising Other Than the Publisher's Own and Which Are Named in Sections 132.231, 132.232 and 132.233 Postal Manual (*Sections* 4355*a*, 4355*b*, *and* 4356 *of Title* 39, United States Code)

		Average No. Copies Each Issue During Preceding 12 Months	Single Issue Nearest to Filing Date
A.	Total No. Copies Printed (*Net Press Run*)	2400	September
B.	Paid Circulation		
	1. Sales Through Dealers and Carriers, Street Vendors and Counter Sales	None	
	2. Mail Subscriptions	2109	
C.	Total Paid Circulation	2109	
D.	Free Distribution (*including samples*) By Mail, Carrier or Other Means	67	
E.	Total Distribution (*Sum of C and D*)	2176	
F.	Office Use, Left-Over, Unaccounted, Spoiled After Printing	224	
G.	Total (*Sum of E & F—should equal net press run shown in A*)	2400	

I certify that the statements made by me above are correct and complete.

(*Signature of editor, publisher, business manager or owner*)

George J. King
(Signature of business manager)

they said it couldn't be done... but

WE'VE DONE IT!

Now, for the first time, thanks to the efforts of our Research Laboratories, we offer you practical, long-lasting and completely effective masking agents for:

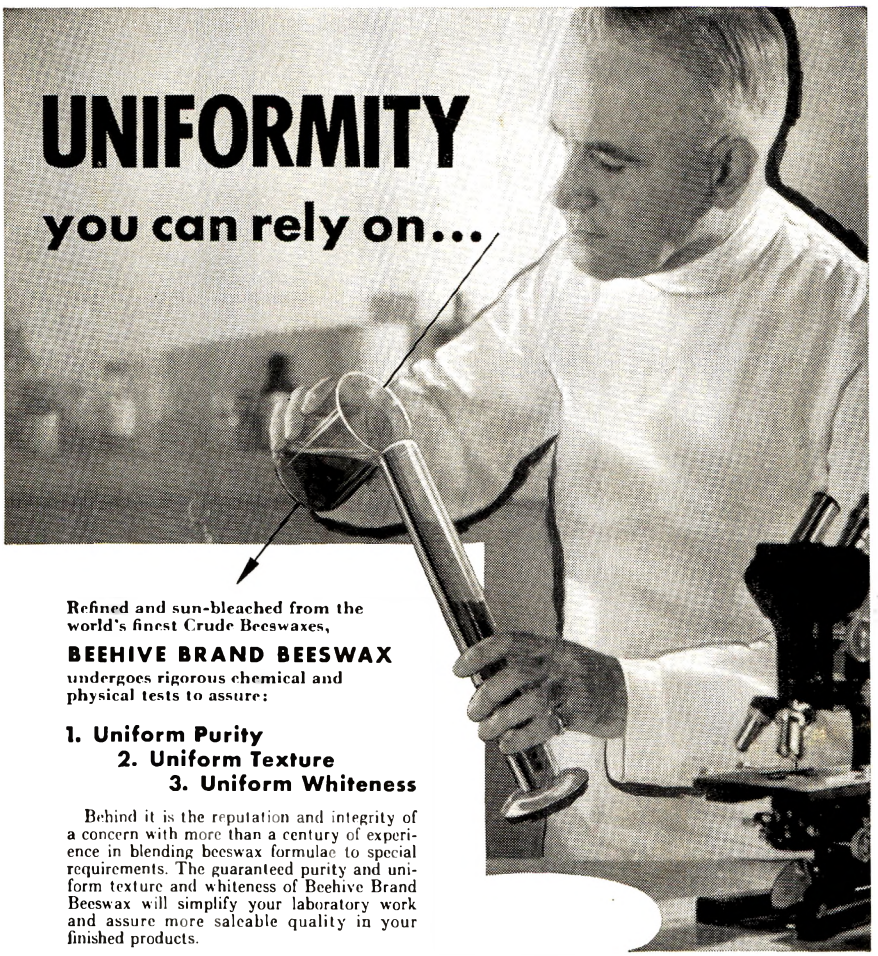
ISOPROPYL ALCOHOL
THIOGLYCOLIC ACID · AMMONIA

Not only do our new products effectively odor-mask the chemical odors but they also add their own pleasant fragrance to your products.

We admit this is almost too good to believe –so we invite you to send for free samples for your own tests! Write us *today* (on your company letterhead, please). Specify whether you want samples for Isopropyl Alcohol, Thioglycolic Acid, or Ammonia.

P. ROBERTET, Inc. 37 West 65th Street, New York, N.Y. 10023 • Tel: 873-6400

LOCAL MANUFACTURING FACILITIES: New York City • Mexico City • São Paulo • Buenos Aires • Keciborlu • Grasse • Reus



Refined and sun-bleached from the world's finest Crude Beeswaxes,

BEEHIVE BRAND BEESWAX

undergoes rigorous chemical and physical tests to assure:

1. Uniform Purity
2. Uniform Texture
3. Uniform Whiteness

Behind it is the reputation and integrity of a concern with more than a century of experience in blending beeswax formulae to special requirements. The guaranteed purity and uniform texture and whiteness of Beehive Brand Beeswax will simplify your laboratory work and assure more saleable quality in your finished products.

WILL & BAUMER Candle Co., Inc., Dept. JSC, Syracuse N. Y.

BEEHIVE BRAND *Beeswax*

Free **Consultation Service**

- The experimental data and practical manufacturing experience of more than 100 years' specialization in beeswax and beeswax compounds are at your service without cost or obligation.

Write us about your beeswax problems.

NEW YORK 10
300 Park Ave. So.

BOSTON 9
71 Broad St.

CHICAGO 6
162 N. Franklin St.

LOS ANGELES 15
952-4 S. Flower St.