Vol. 28 No. 12 December 1977

Journal of the Society of Cosmetic Chemists

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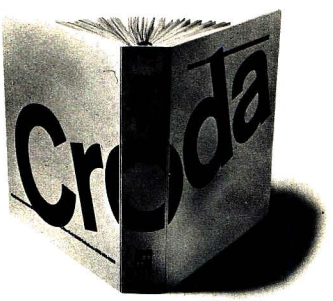

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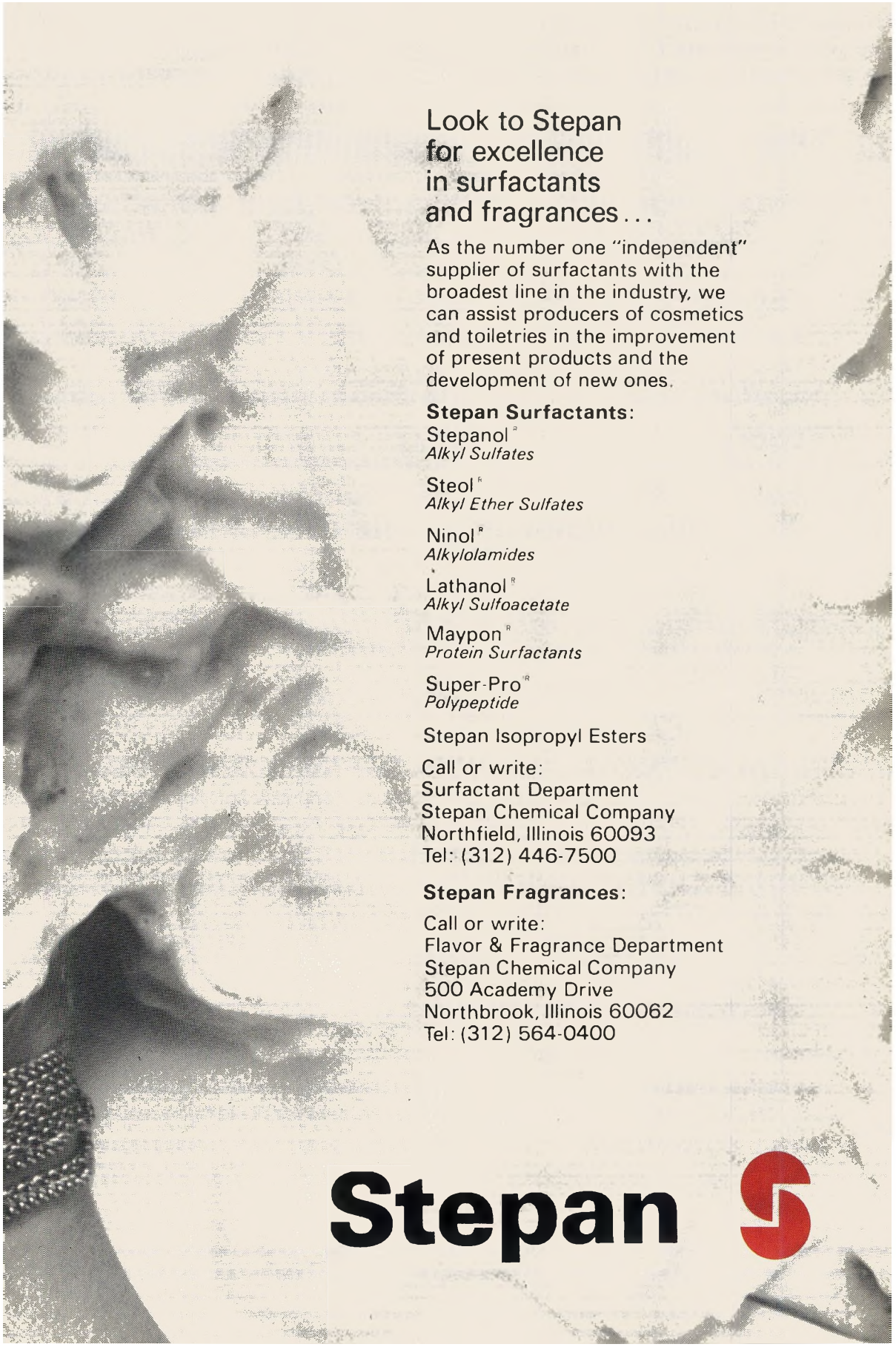

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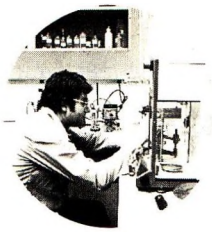

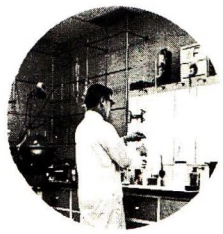

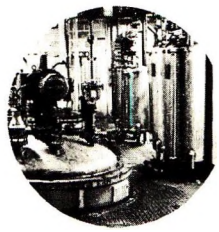

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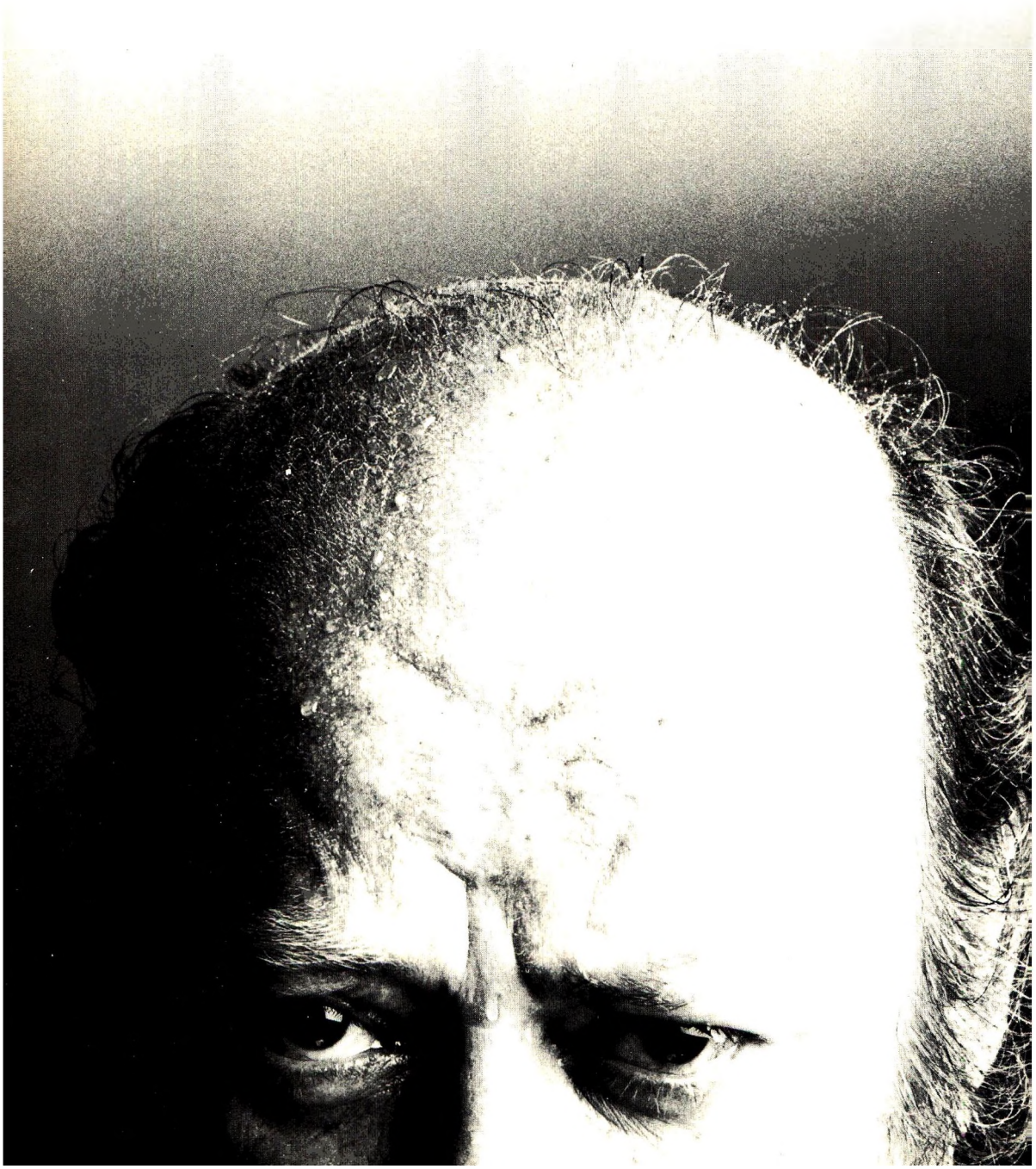

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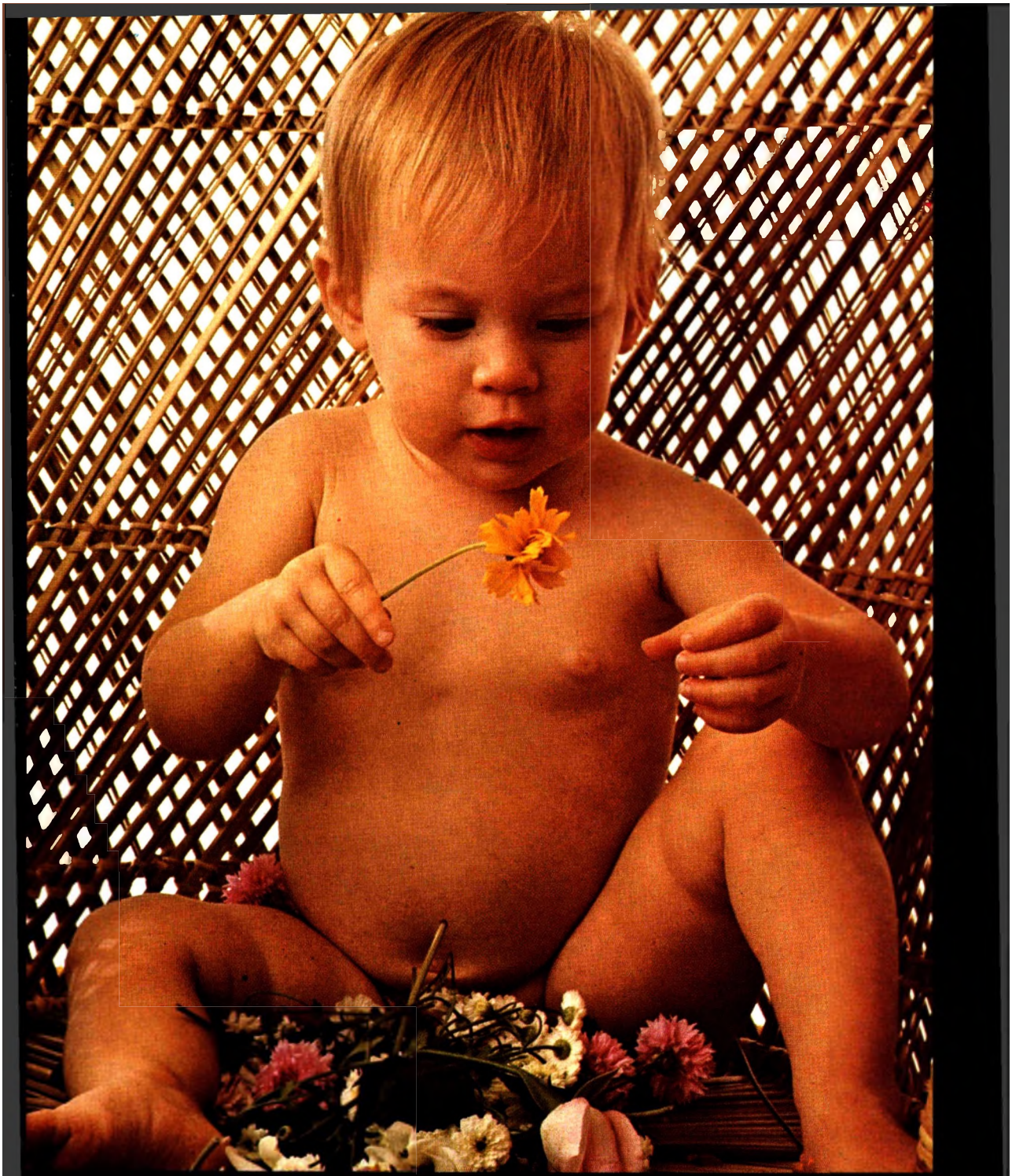

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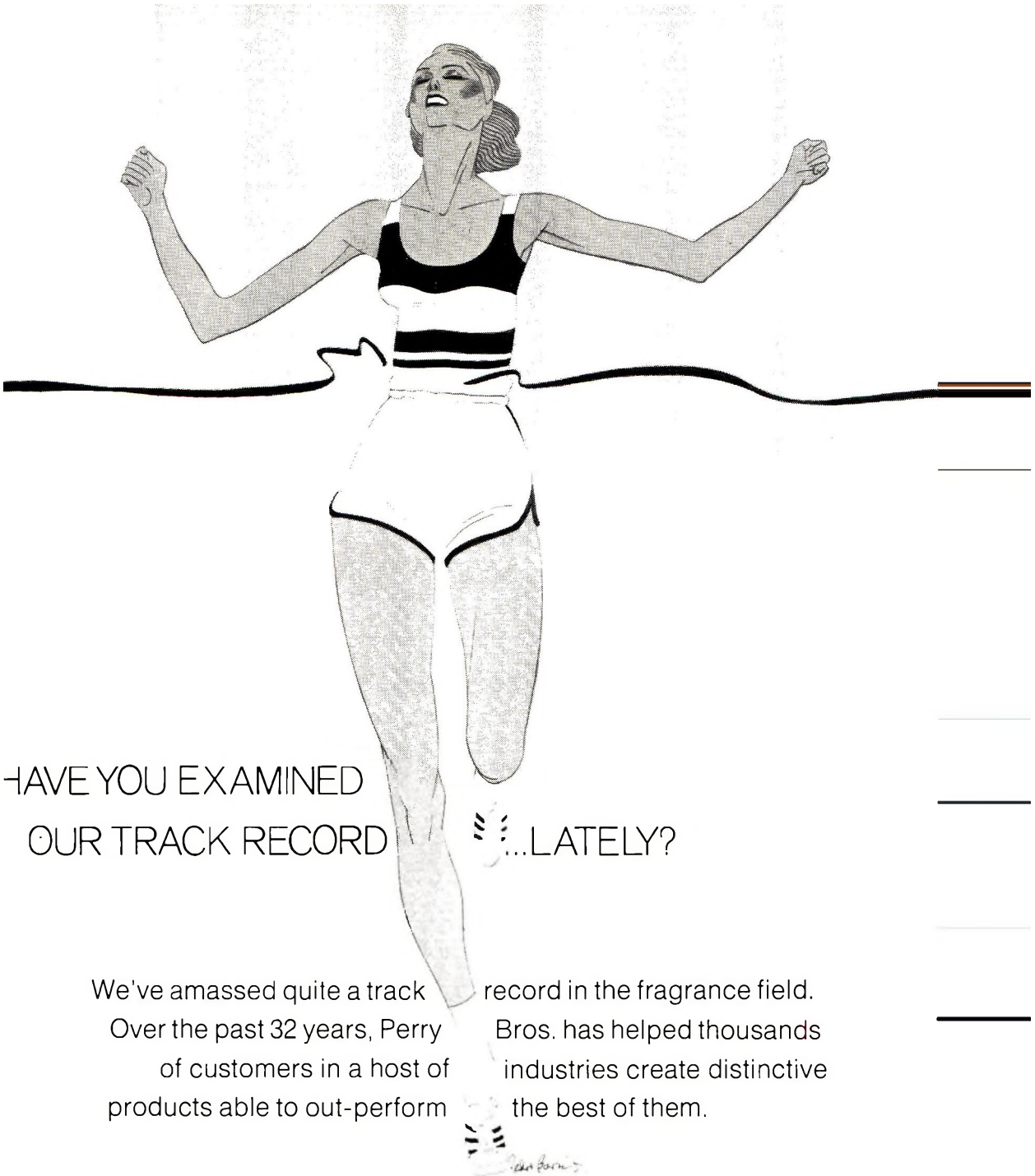

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SYNOPSES FOR CARD INDEXES

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

Effectiveness of reduction and oxidation in acid and alkaline permanent waving: J. G. Gumprecht, K. Patel and R. P. Bono. *Journal of the Society of Cosmetic Chemists* **28,** 717 (December 1977)

Synopsis—A study of the effect of permanent waving of hair keratin was undertaken to more clearly define the chemistry of the reduction and oxidation steps. The processing step was monitored by reacting the thiol groups with ethylene imine to show that 17 to 43 per cent of the cystine is reduced during typical optimal processing times. Hydrogen peroxide and sodium bromate show little differences in effectiveness as oxidizing agents, whereas air oxidation is not effective. Permanent degradation of 10 to 30 per cent of the cystine residues occurred with some cysteic acid being formed during the oxidation reaction. This permanent degradation is reflected in stress–strain properties. Minor amounts of lysinoalanine and lanthionine are produced as well. The major side reaction, however, is the formation of the mixed disulfide of cysteine and thioglycolate which serves as a diagnostic tool for permed hair. Utilization of a radioactive tag as well as amino acid analyses data have provided evidence that significant amounts of mixed disulfide in permed hair may not only be a result of direct reaction between cysteine and thioglycolate but may also be formed during acid hydrolysis after thiolation of lysine side chain amino groups. This latter synthesis route postulates the formation of a new type of crosslink in permed hair *via* disulfide formation between cysteine and thiolated lysine.

Development of water-in-oil emulsifiers and their application to cosmetic emulsions: J. P. McCarthy, S. J. Labruto, L. R. Mores and M. L. Schlossman. *Journal of the Society Cosmetic Chemists* **28,** 733 (December 1977)

Synopsis—The literature contains many references on the wide range of useful emulsifying agents for oil-in-water emulsions. On the contrary, little has appeared in the area of new emulsifiers for stabilizing water-in-oil systems. It is generally known that it is far more difficult to prepare stable w/o emulsions than those of the o/w type.

Polyglycerol esters having lipophilic surface activity derived chiefly from isostearic and oleic acids were prepared and characterized. A chemical description of these surfactants and other isostearic surfactants will be reviewed. Their physical and chemical properties are assessed as water-in-oil emulsifiers. Emulsifier efficiency is compared in the stabilization of simple w/o emulsions. Stability testing techniques are discussed.

Applications of these unique emulsifiers in preparing stable aesthetic functional w/o cosmetic emulsions are demonstrated.

Malodor formation in nitromethane stabilized trichloromonofluoromethane blends: S. Temple and R. G. Hirsch. *Journal of the Society of Cosmetic Chemists* **28,** 765 (December 1977)

Synopsis—Methyl isocyanide has been identified as the malodorous compound formed from nitromethane stabilized trichloromonofluoromethane (Propellant 11) in iron or steel containers. Oxygen, water, and iron are required for isocyanide formation. Water must be present as a separate phase, *i.e.*, above its saturation level in the blend. Methyl isocyanide forms from reaction of methylamine, the reduction product of nitromethane and the free radical intermediates obtained by reductive dechlorination of propellant 11. The presence of a separate water phase in propellant 11 may not be detected if dechlorination of the propellant and corrosion of the metal are occurring.

A quantitative human patch testing procedure for low level skin irritants: K. A. Smiles and M. E. Pollack. *Journal of the Society of Cosmetic Chemists* **28,** 755 (December 1977)

Synopsis—A week-long repeated insult occluded patch test methodology is presented along with an analysis procedure based on relative slopes of irritation development. This method allows for quantitative comparison of the primary irritation potential of low level irritants, culminating in statistical statements concerning the differences among samples. The use of the method to determine differences between commercial shaving creams, among various perfumes for a shaving cream formula, and among various roll-on antiperspirants is presented, as well as the identification and solution of an apparent irritancy problem associated with an anti-perspirant formula intended for pump spray delivery.

Irritancy potential of cosmetic ingredients: W. Kästner. *Journal of the Society of Cosmetic Chemists* **28,** 741 (December 1977)

Synopsis—The topical irritancy potential of fatty or fat-derived cosmetic ingredients on human skin in 24-hour patch tests was compared with that in various animal species. Different species exhibited widely varying reactivity under identical test conditions. The degree of irritation decreased in the order rabbit, guinea pig, hairless mouse, and man. These data support the conclusion that the rabbit or guinea pig are not useful for skin irritancy studies. Data on hairless mice are more likely to permit prediction of human responses. Skin tolerance to raw materials or finished preparations—as determined on animals—cannot be related directly to human experience unless it has been established that human skin reacts similar to animal skin. Consequently, topical studies of cosmetic components on human subjects should be included in the test program.

J. Soc. Cosmet. Chem., **28**, 717–732 (December 1977)

Effectiveness of reduction and oxidation in acid and alkaline permanent waving

JANET G. GUMPRECHT,* KANU PATEL, and ROBERT P. BONO, *Redken Laboratories, Inc., Biochemical Research Department, 14721 Califa Street, Van Nuys CA 91411.*

Received December 30, 1976. Presented December 6, 1976, SCC Seminar, New York, N.Y.

Synopsis

A study of the effect of PERMANENT WAVING of HAIR KERATIN was undertaken to more clearly define the chemistry of the reduction and oxidation steps. The processing step was monitored by reacting the thiol groups with ethylene imine to show that 17 to 43 per cent of the cystine is reduced during typical optimal processing times. Hydrogen peroxide and sodium bromate show little differences in effectiveness as oxidizing agents, whereas air oxidation is not effective. Permanent degradation of 10 to 30 per cent of the cystine residues occurred with some cysteic acid being formed during the oxidation reaction. This permanent degradation is reflected in stress–strain properties. Minor amounts of lysinoalanine and lanthionine are produced as well. The major side reaction, however, is the formation of the mixed disulfide of cysteine and thioglycolate which serves as a diagnostic tool for permed hair. Utilization of a radioactive tag as well as AMINO ACID ANALYSES data have provided evidence that significant amounts of mixed disulfide in permed hair may not only be a result of direct reaction between cysteine and thioglycolate but may also be formed during acid hydrolysis after thiolation of lysine side chain amino groups. This latter synthesis route postulates the formation of a new type of crosslink in permed hair *via* disulfide formation between cysteine and thiolated lysine.

INTRODUCTION

The amino acid composition of human virgin hair has been reported by several investigators (1–4), but there have been few reports of the chemical effects of permanent waving on human hair, particularly at the amino acid level. Zahn *et al.* (5) applied sulfur analytical techniques of wool research to hair. Robbins and Kelly (6) showed minor changes in the cystine and cysteic acid content using amino acid analysis, but they did not report side reactions other than the production of cysteic acid during oxidation.

Much discussion of the formation of the mixed disulfide of thioglycolate and cysteine in thioglycolate permanent waved hair has been published. Schöberl (7) suggested the

*Present address: UCLA Bone Research Labs, 1000 Veteran Avenue, Rehabilitation Building, Los Angeles, CA 90024.

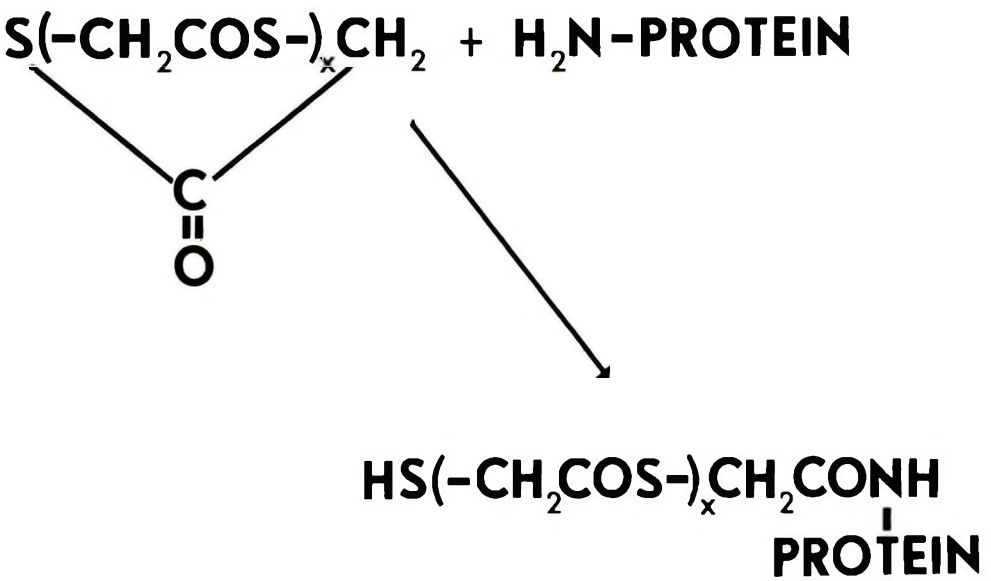

Figure 1.

presence of this mixed disulfide carboxymethylthiol-cysteine (CMT-CySH) in 1953, based on the increased sulfur content of treated hair. Zahn (5) concludes that there is essentially no CMT-CySH under normal waving conditions, but that it is an artifact of hydrolysis. Schulte and coworkers (8) have done some radioisotope studies with labeled thioglycolate with inconclusive results on the presence of the mixed disulfide in hair. Human and Springell (9) used S^{35} labeled mercaptoacetate to obtain evidence of the formation of the mixed disulfide in wool. Some of the bound label is probably a result of acylation of free amino groups by impurities in the thioglycolate as suggested by White (10) from experiments with thioglycolate reductions of ribonuclease. These impurities are formed by thiolesterification between the sulfhydryl and carboxyl groups of thioglycolic acid to produce the cyclic dimer, dithioglycolide, or chained polymers of x groups of thioglycolate. These polythioglycolides can result in thiolation of lysine side chains as is shown in Fig. 1. White was unable to show the presence of the mixed disulfide using amino acid analysis when redistilled thioglycolic acid was utilized. However, he did identify mixed disulfide when reagent thioglycolic acid was used.

Besides the possible side reaction of CMT-CySH formation in permanent waved hair, there are other suggested degradation products. Horn *et al.* (11) have shown that hair can undergo intramolecular rearrangements involving cystine in dilute alkali to yield lanthionine. β-elimination of cystine to yield dehydroalanine can also give rise to the reaction with ϵ-amino groups of lysine to produce lysinoalanine which has been identified in wool by Ziegler (12).

This research has been undertaken to reinvestigate the chemical changes in hair keratin during a permanent wave using improved hydrolysis procedures prior to amino acid analysis. Both the reductive and oxidative steps of permanent waving have been monitored and sensitive methods including radioisotope tagging have been investigated as aids in elucidating the permanent wave mechanism.

EXPERIMENTAL

HAIR SAMPLES

Salon treatments were performed on clients with virgin hair. Hair samples were individually cut from the nape not more than 3 in. long to minimize differences which occur down the hair shaft. The laboratory testing utilized one virgin hair swatch which was carefully cut so that all hairs were the same distance and length from the scalp. To verify that this hair swatch was representative of good competent keratin structure, the criteria of stress–strain, scanning electron microscopy (SEM), polarization microscopy, and amino acid analysis were used. In all cases, the hair shaft was in the average keratin range.

PERMING PROCEDURES

All salon permanent waves were performed in the standard manner. When hair swatches were utilized, procedures were used which simulated actual head waving. Thus the same amount of solution was applied to curlers, which were then placed in salon dryers at regulated temperatures for acid waves. A test curl was used to establish optimal perm time (designated as t) for all processing of different perms. Variations in time were related to this optimal time. In the same manner, variations in oxidation times are related to optimal times (t). The acid waves, which contained 8.4 per cent (wt/wt) ammonium thioglycolate, were formulated as usual with one exception: protein was left out. The alkaline wave was also devoid of protein and contained 4.2 per cent (wt/wt) ammonium thioglycolate at pH 9.4. Sodium bromate (12 per cent by weight) was the usual active ingredient in the reforming lotion except where variations in oxidizing incorporated the use of hydrogen peroxide (2.9 per cent by weight). When samples were removed prior to reoxidation, they were not rinsed, since this was found to cause reoxidation.

With the exception of one experiment, commercial ammonium thioglycolate was used to formulate wave solutions. In this experiment reagent grade thioglycolic acid (98 per cent) was vacuum distilled into 4 fractions under reduced pressure. Fraction I (105 to 110°C) was a clear liquid; Fraction II (100° to 125°C) was a slightly yellow liquid; Fraction III (130 to 134°C) was a yellow liquid, Fraction IV (residue from distillation) was a golden brown viscous liquid. Each fraction was water soluble except the residue which was soluble in 3M urea or in dioxane. UV spectra of Fractions I to III diluted 1/10,000 with water showed increasing amounts of thioester (White (10)). Reagent thioglycolic acid had an absorbance between those of Fractions II and III.

AMINO ACID ANALYSIS

The hair was washed consecutively with ether, ethanol, and distilled water and then air dried. Approximately 1 mg hair samples were weighed on a Mettler ME 22 electrobalance* to the nearest microgram. One complete strand of the swatch was used for each analysis. Hydrolysis of duplicate hair samples in double distilled constant boiling HCl was performed in sealed evacuated tubes at 110°C for 48 h. This was the time de-

*Mettler Instruments Corp., Princeton, NJ 08540.

termined for the best compromise between complete hydrolysis and optimal recovery of amino acids. Analysis was carried out on a Beckman 121M† amino acid analyzer according to the general procedure of Spackman *et al.*(13). Amino acid peaks were integrated with a systems AA integrator. In order to determine S-aminoethylcysteine (SAE-CySH), a 15-cm short column was used and SAE-CySH eluted between lysine and histidine.

STRESS-STRAIN TESTING

Stress-strain measurements were made by suspending a single hair fiber, 1.6 cm long, between a set of clamps. Force, applied to one end by a constant speed motor, elongates the fiber at a rate of 1.5 per cent/sec., while being monitored on the other end by a strain-gauge transducer.‡Stress versus strain graphs were plotted on an XY Recorder.* Ten hairs were tested and averaged.

CMT-CySH, LYSINOALANINE, AND LANTHIONINE IDENTIFICATION

The peak which elutes between serine and glutamic acid was shown to be different than methionine sulfone or homoserine by spiking a permanent wave hydrolysate containing this peak with known standards. To establish that it was the suspected mixed disulfide of thioglycolic acid and cysteine, a laboratory synthesis was carried out under conditions which should trap this intermediate. Equal molar amounts of thioglycolate and cystine were reacted for 30 min at pH 5.0, and then the solution was lyophilized and dissolved in 2 ml of pH 2.2 citrate buffer. This was run alone on the analyzer and also used as a spike. The CMT-CySH peak eluted coincidentally with the unknown permanent wave peak. The KF for alanine was used in the experiments to calculate CMT-CySH. Lysinoalanine and lanthionine were identified by comparison with standards.

RADIOACTIVE LABELING

Sample hair bundles were taken from scalps before permanent wave treatment, after reduction prior to rinsing and after permanent wave treatment. The amino acid composition was determined on the before and after samples. Reduced hair was immediately immersed into the reaction solution which had been flooded with dry nitrogen for 15 min. Carboxymethylation with 1-C^{14} iodoacetic acid in the presence of cold iodoacetic acid was then carried out according to the procedure of Hirs (14), except the time was increased to 2½ h to allow for sufficient penetration into the hair shaft. Afterward, the hair was carefully washed 7 times with distilled H_2O for a period of 15 min/wash with gentle stirring. A number of control experiments showed this washing procedure to be more than adequate for removal of unreacted iodoacetic acid trapped in the hair strands. Air dried C^{14} tagged hair samples were weighed accurately on a Mettler ME 22 electrobalance to approximately 4.000 mg and placed into glass scintillation vials. Distilled water (0.2 ml) was added to each vial and allowed to

†Spinco Division of Beckman Instruments, Inc., Palo Alto, CA 94340.
‡Stathan Instruments, Inc., Oxnard, CA 93030.
*Hewlett Packard, Inc., Palo Alto, CA 94303.

saturate the hair for 30 min. Then 1.0 ml of Soluene-350 tissue solubilizer was added to each vial which was then placed in a 55°C water bath for 1½ hours tightly closed. After cooling, 15.0 ml of Dimilume 30 scintillation fluid was added and the mixture was vortexed. Samples were placed in a Beckman LS-250 scintillation counter and counted one day after equilibration. Counting efficiencies were measured using 50 μl of C^{14} toluene as an internal standard. Counting efficiencies ranged from 80 to 88 per cent. From the DPM/mg protein, it was possible to calculate the moles of iodoacetic acid incorporated, and this was divided by the original half-cystine content to give the percentage of half-cystine molecules that were in the reduced state. Controls showed that the original cysteine was negligible. Unlabeled iodoacetic acid was used in parallel experiments and the S-carboxymethyl-cysteine (SCMC) content determined on the amino acid analyzer.

S-AMINOETHYLATED KERATEINE

Reduced hair (processed only) was alkylated immediately without rinsing, to prevent oxidation, by reacting with excess ethyleneimine under nitrogen atmosphere at room temperature according to the procedure of Cole (15), except that the reaction time was 2½ h. This was found as the optimal reaction time for alkylation before degradation of the hair fiber occurs. Amino acid analysis was done on samples before processing, after alkylation, and after the complete perm for comparison.

CHEMICALS

1-C^{14} iodoacetic acid and C^{14} toluene standard were purchased from New England Nuclear.* Urea was ultra-pure grade supplied by Schwarz/Mann†. Soluene-350 tissue solubilizer and Dimilume-30 were from Packard Instruments.‡. Lysinoalanine was a gift from Jim Vaughn, Durrum Instrument Corp.** Lanthionine and SCMC were purchased from Calbiochem.§ SAE-CySH was from Sigma.# All other chemicals were reagent grade.

RESULTS

Amino acid chromatograms of permanent wave hair hydrolysates always contained a peak between serine and glutamic acid not found in controls or in virgin hair (Fig. 2). This was identified as the mixed disulfide from thioglycolic acid and cysteine by spiking a perm hydrolysate with the synthesized mixed disulfide.

The amino acid chromatogram from the reaction of cystine with thioglycolate shows a complexity of products (see Fig. 3). Besides the mixed disulfide, cysteine, and cystine, there are several peaks (I, II, III) with high 440 nm absorption which elute between cysteic acid and aspartic acid. An interesting ninhydrin positive material is also present

*New England Nuclear, Boston, MA 02118.
†Schwarz/Mann, Orangeburg, NY 10962.
‡Packard Instruments, Downers Grove, IL 60515.
**Durrum Instruments Corp., Palo Alto, CA 94303.
§Calbiochem, La Jolla, CA 92037.
#Sigma Chemical Co., St. Louis, MO 63178.

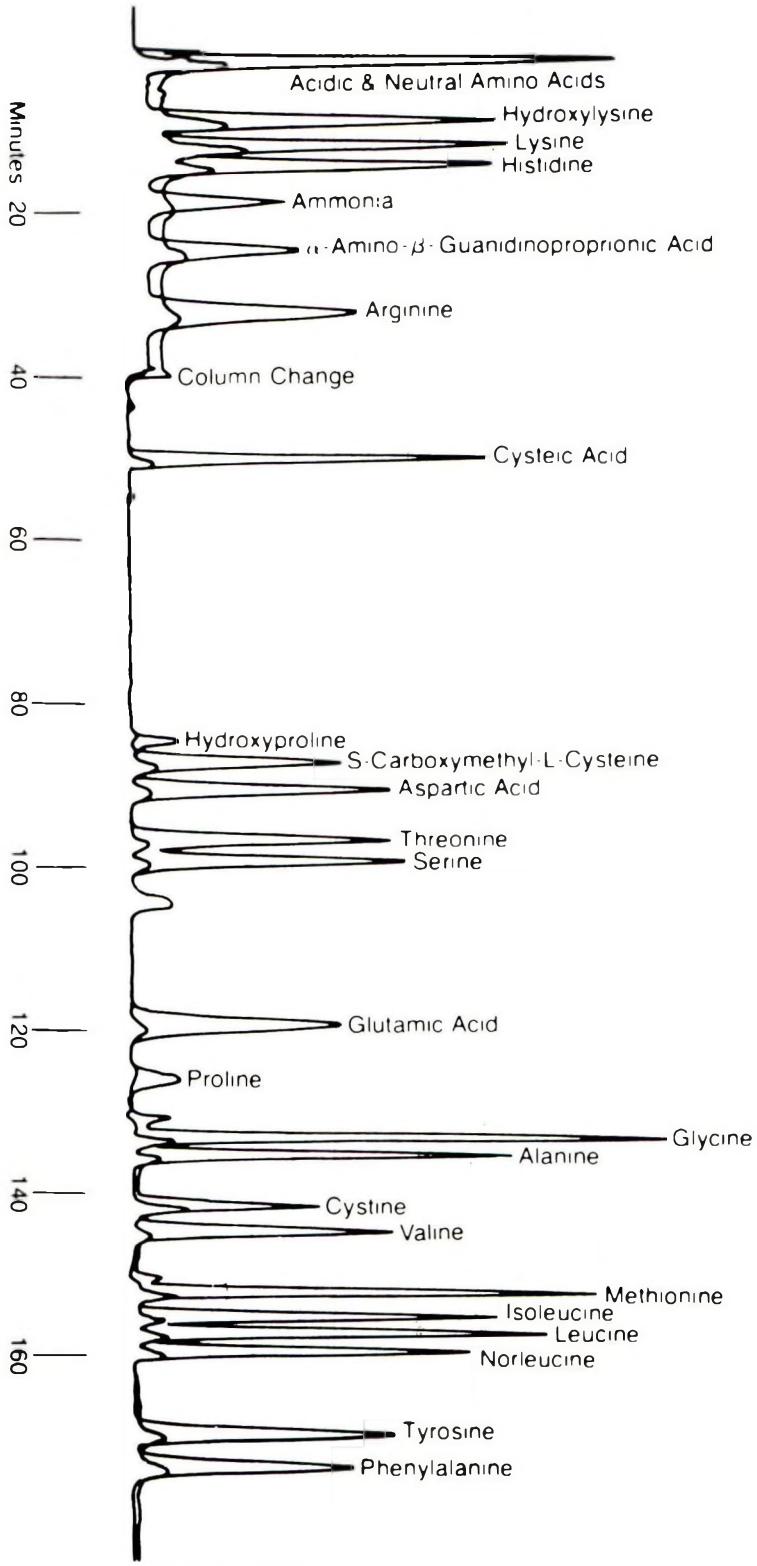

Figure 2. Chromatogram (tracing) of 2.5 nmol of Beckman amino acid calibration mixture with inclusion of peak found in hydrolysates of permanent waved hair.

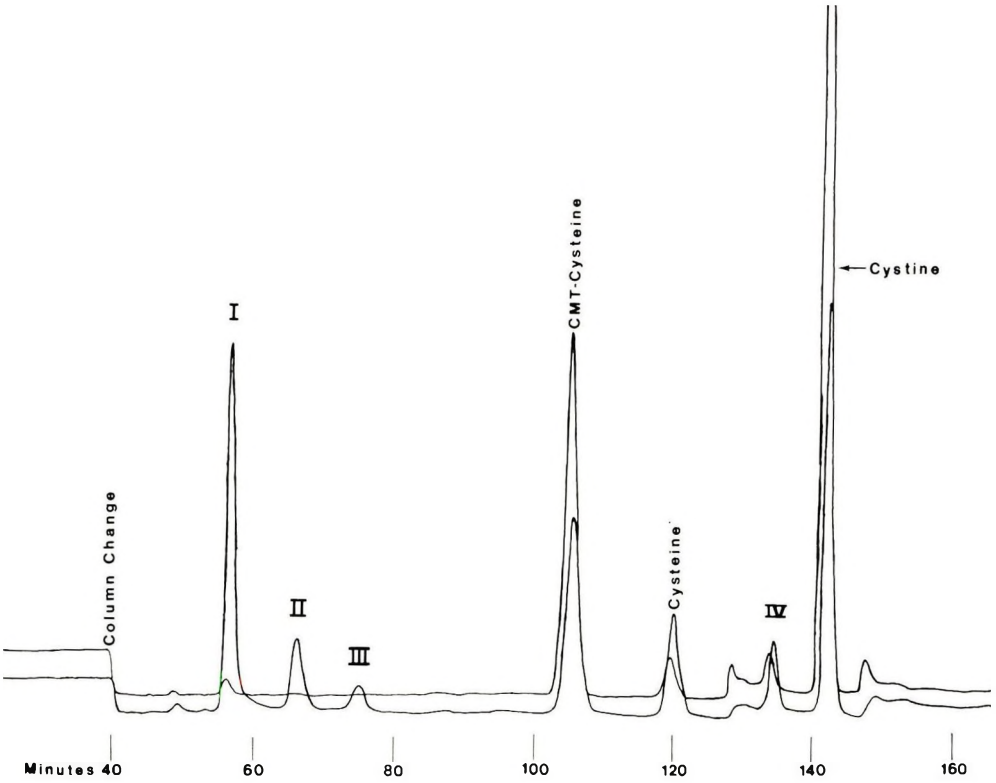

Figure 3. Chromatogram (tracing) of reaction products from cystine and thioglycolic acid incubation

Table I

	Acid Permanent Wave						
		(1/2)t—Process		t—Process		2t—Process	
Amino Acid	Control	Midpoint	Final	Midpoint	Final	Midpoint	Final
Lysine	2.4	2.2	2.2	2.2	2.3	2.2	2.2
Histidine	0.9	0.7	0.7	0.6	0.8	0.7	0.8
SAE-CySH	0	2.1	0	3.0	0	4.0	0
Arginine	6.6	6.6	6.2	6.8	6.2	6.3	6.6
Cysteic Acid	0.4	0.4	0.8	0.6	0.7	0.4	0.7
CMT-CySH	0	0.2	0.5	0.3	1.3	0.4	0.5
Aspartic acid	5.5	5.4	5.3	5.6	5.4	5.5	5.4
Threonine	7.9	7.7	7.6	8.0	7.6	7.6	7.6
Serine	12.2	12.6	12.3	11.7	13.0	12.5	12.2
Glutamic acid	12.3	12.2	12.4	12.9	12.4	12.5	12.4
Proline	8.3	8.4	8.7	8.5	8.2	8.6	8.6
Glycine	6.1	6.7	6.5	6.3	6.8	6.7	6.2
Alanine	4.7	4.9	4.7	4.6	4.8	4.9	4.6
Half-Cystine	15.5	13.0	14.7	10.5	13.6	9.8	13.8
Valine	5.1	5.2	5.1	5.5	4.8	5.4	5.5
Methionine	0.3	0.5	0.4	0.4	0.8	0.9	0.5
Isoleucine	2.3	2.2	2.2	2.6	1.9	2.4	2.6
Leucine	5.9	5.9	6.1	6.4	5.9	6.3	6.2
Tyrosine	2.1	2.0	2.0	2.1	2.0	1.9	2.0
Phenylalanine	1.6	1.5	1.5	1.5	1.5	1.5	1.5

Table II

Amino Acid	Alkaline Permanent Wave[a]						
	Control	(1/2)t—Process		t—Process		2t—Process	
		Midpoint[b]	Final	Midpoint	Final	Midpoint	Final
Lysine	2.4	2.3	2.2	2.3	2.2	2.3	2.3
Histidine	0.9	0.6	0.7	0.7	0.7	0.8	0.7
SAE-CySH	0	3.9	0	6.7	0	4.6	0
Arginine	6.6	6.3	6.7	7.0	6.0	6.4	6.6
Cysteic acid	0.4	0.4	0.9	0.5	0.8	0.4	0.7
CMT-CySH	0	0.5	1.6	0.6	1.0	0.5	1.9
Aspartic acid	5.5	5.3	5.5	5.4	5.7	5.4	5.4
Threonine	7.9	7.5	7.8	7.8	7.6	7.8	7.6
Serine	12.2	12.7	11.4	12.3	11.0	12.3	11.6
Glutamic acid	12.3	12.2	12.4	12.2	12.9	12.5	12.4
Proline	8.3	8.6	8.5	8.5	8.9	8.6	8.8
Glycine	6.1	6.8	6.3	6.6	6.8	6.5	6.5
Alanine	4.7	4.9	4.8	4.8	4.7	4.9	4.8
Half-cystine	15.5	10.4	11.9	6.8	10.8	9.5	12.6
Valine	5.1	4.8	4.9	5.0	5.8	4.9	5.2
Methionine	0.3	0.7	0.6	0.7	0.6	0.5	0.4
Isoleucine	2.3	2.2	2.5	2.3	3.0	2.2	2.4
Leucine	5.9	6.0	6.4	6.1	6.6	6.1	6.1
Tyrosine	2.1	2.0	2.1	2.1	2.1	2.0	2.0
Phenylalanine	1.6	1.6	1.7	1.6	1.7	1.6	1.6

[a]Amino Acids are expressed in moles/100 mol.
[b]Hair was treated with ethylene imine for 2 hrs after reduction step.

which elutes slightly after alanine but definitely before cystine (peak IV). The latter has a ratio of 440 to 570 nm similar to the mixture disulfide and may represent a mixed disulfide of cysteine and dithioglycolide.

The effect of processing time variations are shown in Tables I and II. The only amino acid that is significantly changed during processing in cystine. The decrease in this can be monitored accurately by covalently trapping the cysteine formed during the reduction step by reacting immediately with ethyleneimine to yield the stable derivative, SAE-cysteine. These values are shown in Table III for under, normal, and over processed waves.

The reduction increases in relation to the time of processing in the acid waves done on swatches. In the optimally processed acid wave 19.4 per cent of the cystine is reduced to yield a good wave as shown by test curl pattern. The alkaline swatch shows faster and greater reduction with a t-reduction of 43.2 per cent of the cystine. Overprocessed hair with the alkaline wave contains less reduced cystine than the normal time. This may reflect some spontaneous reoxidation or possibly a pH change occurring with the longer time as the thioglycolate is used up. The figure of 43.2 per cent is high, but was reproducible. Other figures for typical reduction levels in the salon are shown and do vary between 17.3 to 43.2 per cent. Levels found in the lab correlated with those waved in the salon.

The chemical changes in the disulfide crosslinks are also reflected in the stress–strain data seen in Table IV. The hair had an average diameter of 59 μm and has normal values of 29.9 ± 5.4 S.D. grams force and percent elongation of 39.6 ± 1.2 S.D. before processing. When hairs were pulled at ½t without reoxidation, the grams force

Table III

Cystine Reduction

Sample	Control—Half Cystine[a]	Process Time	Alkylated Cysteine[a]	Per Cent Reduction
Acid wave (swatch)	15.5 per cent	(1/2)t	2.1[b]	13.6 per cent
Acid wave (swatch)	15.5 per cent	t	3.0[b]	19.4 per cent
Acid wave (swatch)	15.5 per cent	2t	4.0[b]	25.8 per cent
Alkaline wave (swatch)	15.5 per cent	(1/2)t	3.9[b]	25.2 per cent
Alkaline wave (swatch)	15.5 per cent	t	6.7[b]	43.2 per cent
Alkaline wave (swatch)	15.5 per cent	2t	4.6[b]	29.7 per cent
Acid wave (head)	15.0 per cent	t	3.9[b]	26.0 per cent
Acid wave (head)	16.1 per cent	t	4.4[c]	27.4 per cent
Acid wave (head)	15.7 per cent	t	5.7[c]	36.1 per cent
Alkaline wave (head)	15.0 per cent	t	2.6[b]	17.3 per cent

[a]Moles/100 mol amino acids.
[b]SAE-cysteine.
[c]CMT-cysteine.

Table IV

Stress-Strain Data—Acid Wave

	Average Force in Grams	Average Per Cent Elongation
Control	29.9 ± 5.4 S.D.	39.6 ± 1.2 S.D.
After processing (1/2t[a])	12.3 ± 2.1 S.D.	66.1 ± 7.9 S.D.
After oxidation (t)	18.3 ± 6.1 S.D.	48.7 ± 4.1 S.D.
After oxidation (2t)	16.4 ± 3.9 S.D.	47.2 ± 4.7 S.D.

[a]"t" refers to processing time.

Table V

Acid Permanent Wave

Amino Acid	Control	Hydrogen Peroxide (1/2)t	Hydrogen Peroxide t	Hydrogen Peroxide 2t	Sodium Bromate (1/2)t	Sodium Bromate t	Sodium Bromate 2t	Air 30′	Air 24 H
Lysine	2.4	2.5	2.3	2.4	2.4	2.3	2.5	2.5	2.5
Histidine	0.9	0.8	0.7	0.7	0.8	0.7	0.8	0.8	0.8
Arginine	6.6	6.6	6.1	6.2	6.6	8.2	7.0	6.6	5.8
Cysteic acid	0.4	0.5	0.4	0.6	0.6	0.7	0.9	0.4	
CMT-CySH		1.4	1.3	1.6	1.3	1.7	1.2	0.5	1.1
Aspartic acid	5.5	5.4	5.4	5.3	5.3	5.1	5.6	5.5	5.4
Threonine	7.9	7.6	7.7	7.4	7.4	7.2	7.4	7.2	7.4
Serine	12.2	11.7	12.1	12.0	11.4	11.8	10.1	10.5	11.9
Glutamic acid	12.3	12.5	12.6	12.3	12.2	11.8	12.8	12.8	12.6
Proline	8.3	7.9	8.2	8.3	8.1	7.5	8.5	9.8	9.2
Glycine	6.1	6.5	6.3	6.4	6.4	6.5	6.5	8.0	7.7
Alanine	4.7	4.6	4.7	4.7	4.7	5.0	4.9	5.0	4.9
Half-cystine	15.5	12.9	13.3	13.2	13.3	13.1	11.0	10.4	12.1
Valine	5.1	5.7	5.9	5.8	5.9	5.9	6.2	6.2	5.9
Methionine	0.3	0.7	0.5	0.5	0.6	0.5	0.6	0.6	0.5
Isoleucine	2.3	2.7	2.6	2.7	2.9	2.6	3.2	2.9	2.3
Leucine	5.9	6.3	6.4	6.2	6.3	6.0	6.7	6.6	6.1
Tyrosine	2.1	2.1	2.0	2.1	2.0	1.9	2.0	2.2	2.1
Phenylalanine	1.6	1.6	1.6	1.7	1.6	1.6	1.7	1.7	1.6

decreased over 50 per cent to 12.3, whereas the percent elongation increased over 50 per cent to 66.1. Normally processed hair regains 61 per cent of the original grams force and the percent elongation has increased 23 per cent. In over processed hair, the strength has decreased to 55 per cent grams force and the elongation is about the same, 19 per cent high.

Subsequent reoxidation, therefore, restores some of the original integrity of the fiber, but not all. Overprocessing of the hair (2t) results in more permanent damage.

Tables V and VI show the amino acid data when oxidation variations were studied. Again, only cystine is significantly changed. The oxidation is not strong enough to cause significant oxidation of tyrosine as is seen in bleached hair. The cysteic acid formed during oxidation is seen to increase slightly with longer oxidation times, and indicates damage results from overneutralization. SEM documentation in our lab is consistent with this observation. No consistent differences are seen with hydrogen peroxide or sodium bromate in either acid or alkaline waves. However, in both cases, the air oxidation is not effective. Even after 24 hr, there is still free cysteine in the hair. Evidence for this is obtained by noting the proline content. Using sodium citrate buffers, cysteine elutes under the proline peak. This peak is high in all of the air oxidation samples.

Table VII summarizes the cystine derivatives formed during permanent waving. Data on both swatches and salon treated hair involve permanent loss of cystine. This loss is accompanied by increases not only in cysteic acid during reoxidation, but also in the formation of CMT-CySH. There is some preliminary indication that CMT-CySH formation is greater under acidic conditions, but this remains to be established.

The final cystine content is seldom equal to the original value even taking into account

Table VI

Alkaline Permanent Wave								
		Hydrogen Peroxide			Sodium Bromate			Air
Amino Acid	Control	(1/2)t	t	2t	(1/2)t	t	2t	24 H
Lysine	2.4	2.2	2.3	2.3	2.3	2.4	2.3	2.3
Histidine	0.9	0.8	0.8	0.8	0.8	0.9	0.8	0.7
Arginine	6.6	6.5	7.5	6.4	6.4	6.4	6.7	6.7
Cysteic acid	0.4	0.9	0.9	1.4	0.7	0.6	0.8	0.5
CMT-CySH		+	+	0.1	+	0.3	+	+
Aspartic acid	5.5	5.5	5.5	5.2	5.4	5.3	5.2	5.6
Threonine	7.9	7.6	7.7	7.4	7.5	7.5	7.6	7.7
Serine	12.2	11.2	11.9	11.5	11.6	11.7	12.0	11.1
Glutamic acid	12.3	12.6	12.6	12.2	12.3	12.2	12.5	12.8
Proline	8.3	8.0	8.4	8.5	8.3	7.9	8.0	9.1
Glycine	6.1	6.0	6.3	6.3	6.2	6.3	6.6	7.0
Alanine	4.7	4.6	4.7	4.5	4.5	4.6	4.9	4.7
Half-cystine	15.5	14.1	12.4	14.6	15.0	15.4	14.6	12.1
Valine	5.1	5.6	5.8	5.6	5.5	5.6	5.8	5.6
Methionine	0.3	0.5	0.4	0.6	0.6	0.5	0.5	0.7
Isoleucine	2.3	2.8	2.7	2.8	2.7	2.8	2.4	2.8
Leucine	5.9	6.4	6.3	6.2	6.3	6.2	6.2	6.5
Tyrosine	2.1	2.1	2.1	2.1	2.1	2.0	2.0	2.1
Phenylalanine	1.6	1.7	1.6	1.6	1.8	1.6	1.5	1.7

Table VII
Cystine Derivatives Formed During Perming
(Moles/100 moles of Amino Acids)

Sample	Control		CMT-CYSH	Half-Cystine	Cysteic Acid
	Half-Cystine	Cysteic Acid	Final	Final	Final
Salon acid wave	16.1	trace	1.2	13.7	0.5
Swatch acid wave	15.5	0.4	1.7	13.1	0.7
Swatch alkaline wave	15.5	0.4	0.3	15.4	0.6
Swatch acid wave	15.5	0.4	1.3	13.6	0.7
Salon glycerol ester wave	15.5	0.4	4.4	11.8	0.9
Swatch acid wave	14.4	0.5	0.9	13.2	0.4
Salon acid wave	15.6	0.5	1.1	13.6	0.6
Salon acid wave	16.4	0.3	1.4	14.9	0.4
Salon acid wave	16.0	trace	1.2	13.3	0.5
Salon acid wave	15.5	0.4	2.3	13.8	1.1

the increase in cysteic acid content. The CMT-CySH formation varies considerably but plays a more significant role in the final analysis than the cysteic acid production.

A representative sampling of cystine recovery percentages varies between 70 and 90 per cent. There is no consistency of high recovery with certain perm types and, thus, these variations are results of other parameters.

Table VIII shows the data of CMT-CySH formation during the separate reductive and oxidative steps. In every perm experiment there is significant CMT-CySH formation during the reoxidation step.

Some experimentation was done to see if the CMT-CySH formation was related to the purity of the thioglycolate reagent. If impurities in the thioglycolic acid are in any way responsible for some CMT-CySH production, then distillation of the reagent should give fractions of different composition, which would show differences in waving characteristics when formulated into products. There was not enough of fraction I for perm formulation. Fraction II (the purest fraction which was tested) shows the lowest amount of CMT-CySH formed (58 per cent of that found using undistilled thioglycolic acid) and suggests that there is a relationship between the presence of polythioglycolides and CMT-CySH formation.

Chromatograms of lysine reacted at pH 9.0 with different distillation fractions of thioglycolic acid showed that lysine decreased most when reacted with the residue

Table VIII
S-Carboxymethylthio-Cysteine Formation
(Moles/100 moles of Amino Acids)

Sample	CMT-CySH Midpoint	CMT-CySH Final
Salon acid wave	0.3	0.6
Salon acid wave	0.3	1.2
Salon acid wave	0.1	1.8
Salon acid wave	0.8	2.7
Swatch acid wave	0.3	1.3
Swatch acid wave	0.4	1.0
Swatch acid wave	0.3	1.2
Salon alkaline wave	0.6	1.0
Swatch alkaline wave	0.6	1.0

Table IX
Radioisotope Alkylation With C^{14}-$ICH_2C^*O_2H$

Sample	Per Cent Cystine Reduction—Radioactive Tag	Per Cent Cystine Reduction—Cold Tag
1. Acid wave tag #1	40 per cent	36
Acid wave tag #2	41 per cent	35
Acid wave tag #3	22 per cent	20
Acid wave tag #4	44 per cent	30
Acid wave tag #5	44 per cent	29
2. Acid wave	45 per cent	N.D.
3. Acid wave	52 per cent	N.D.
4. Acid wave	25 per cent	N.D.
5. Alkaline wave	63 per cent	N.D.
6. Alkaline wave	30 per cent	N.D.
7. Alkaline wave	22 per cent	N.D.
8. Glycerol ester	37 per cent	N.D.
	70 per cent	N.D.

which, therefore, contains the largest amount of polythioglycolides. This indicates that thiolation of lysine is occurring. This decrease in lysine is accompanied by the appearance of a new peak in the chromatogram.

It was desired to devise a sensitive method for monitoring cystine reduction by using radioisotopes where several samples could be run and counted simultaneously without the long amino acid analyzer time involvement. C^{14}-$ICH_2C^*O_2H$ was used to alkylate the cysteine and numerous experiments utilizing this type of approach are summarized in Table IX. Samples 2 to 8 were monitored solely by scintillation counting.

Since these values seem quite high, an experiment (Sample 1) was carried out using both C^{14}-$ICH_2C^*O_2H$ and cold iodoacetic acid to monitor portions of the same swatch. This swatch was divided into 10 portions and 5 labeled with cold tag only and 5 with the inclusion of radioactive tag. In all cases, the values were higher with radioisotope counting versus direct monitoring of SCMC by amino acid analysis. $\bar{X}$ for SCMC alkylation is 30.0 per cent of the cystine, while $\bar{X}$ for radioactive carboxymethylation is 38.2 per cent. There was no evidence of reaction in iodoacetic acid with other amino acids as seen by the amino acid chromatographs and data.

All hydrolysates from permanent wave hair showed trace amounts of lanthionine and lysinoalanine. This was present in both acid and alkaline waves.

DISCUSSION

Amino acid analysis results indicate that cystine is the only amino acid in hair keratin to be changed significantly during permanent waving. Contrary to a simplistic mechanism involving reduction of cystine followed by subsequent reoxidation after wave formation, there is some permanent degradation (10 to 30 per cent in our studies) of cystine residues.

Both Robbins and Kelly (6) and Miyazawa *et al.* (16) report that cystine degradation is accompanied by a similar increase in cysteic acid. Although, some cysteic acid is produced in the oxidation step, the major side reaction is the formation of CMT-CySH. Zahn (5) has discussed the formation of the latter but suggests that it is an ar-

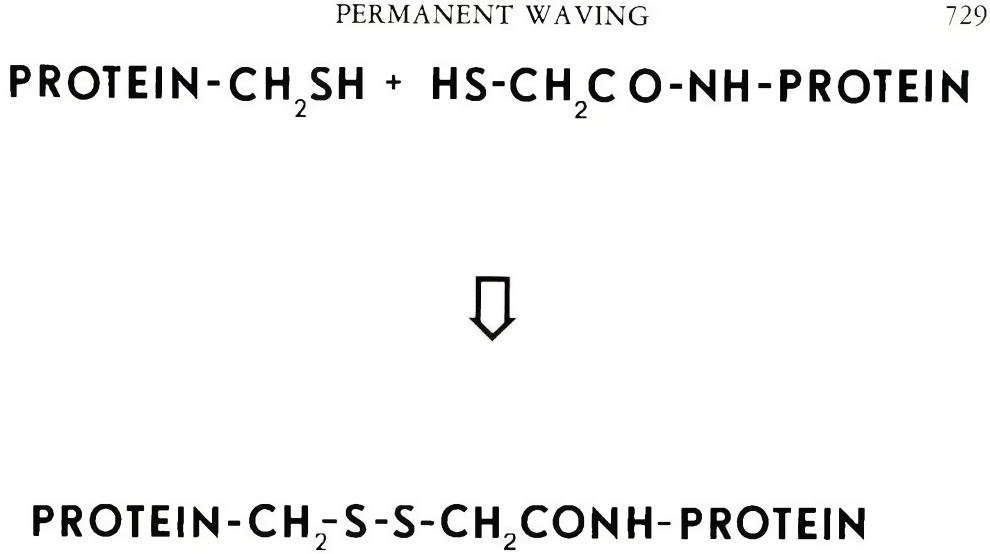

Figure 4.

tifact produced during hydrolysis in the presence of unremoved thioglycolate. Our lab has found insignificant production of mixed disulfide even during acid hydrolysis of hair in the presence of thioglycolate, so its formation appears to be directly related to the mechanism of perming.

The fact that CMT-CySH increases during the oxidative step could arise *via* two alternative routes. Excess thioglycolate present in the hair could react with cysteine in a direct reaction. An interesting mechanism for CMT-CySH formation involves the thiolation reaction proposed by White (10) (See Fig. 1). The introduction of this thiol group during processing could result in the formation of a new crosslink during oxidation (Fig. 4). If this does occur, one must speculate on whether this crosslink would be better than reformation of a disulfide bond. With the longer arm introduced by thiolation, there might be less strain in the hair fiber after oxidation. During acid hydrolysis, this crosslink would be cleaved at the amide bond (Fig. 5) to produce CMT-CySH. The interesting feature derived from White's research is that no mixed disulfide is formed with redistilled thioglycolic acid. This suggests that the thiolation mechanism is an important route for CMT-CySH formation.

There are several pieces of evidence that implicate the thiolation mechanism in permanent waving. Our distillation experiments showed less CMT-CySH formation when purer thioglycolic acid was used to formulate wave solutions. Reactions with lysine were greater with decreasing purity, suggesting that there are more polythioglycolides and/or dithioglycolides present to participate in the thiolation with lysine.

The radioisotope experiment can best be explained in terms of the thiolation mechanism. There is always higher incorporation of C^{14}- iodoacetic acid then accounted for by reaction of cold iodoacetic acid with cysteine as quantitatively determined by the amino acid analyzer. This greater amount of carboxymethylation could easily occur if thiolation introduces additional thiol groups which are available for reaction. The radioisotope labeling experiments do not provide an alternate route for determination of cystine reduction due to this reaction. However, they could provide information on the extent of thiolation when simultaneous experiments are carried out

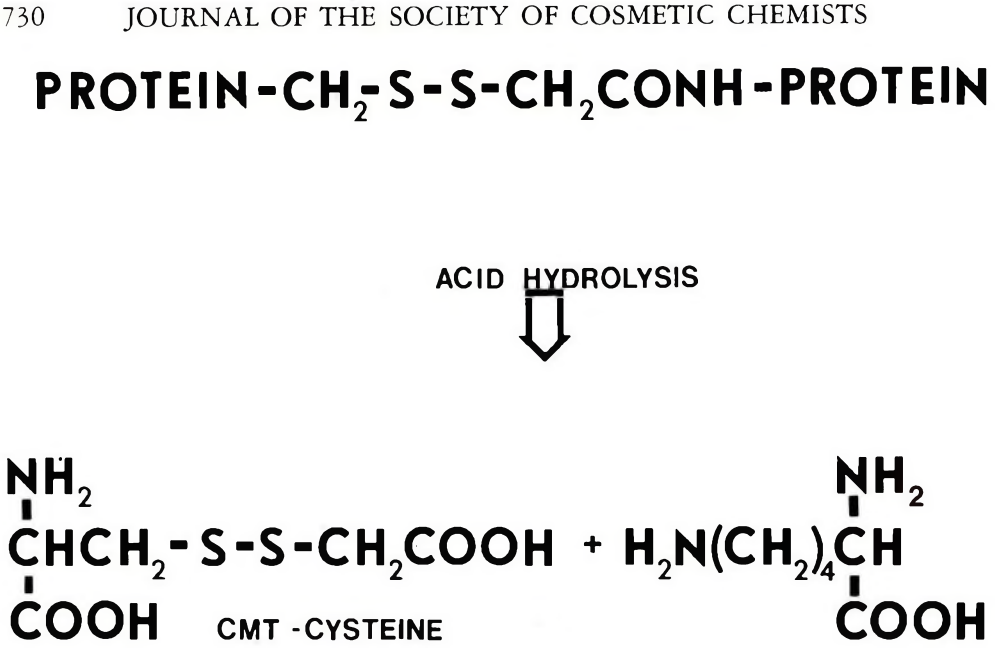

Figure 5.

to determine the specific labeling of cysteine with the amino acid analyzer. The excess labeling with the radioisotopes should reflect that specific to the thiolated lysine group. In the one experiment set up in this manner, the excess carboxymethylation amounted to 8.2 per cent. For this particular hair sample, this suggests that there are 27 per cent more thiol groups than accounted for by cystine reduction.

This suggests that oxidation produces more crosslinks in the hair due to thiolation of lysine side chains. These results are consistent with Schöberl's (7) report that there is a greater amount of sulfur present in permanent waved hair.

The CMT-CySH peak is a diagnostic peak for permanent waving, contrary to reports by Hiragama (17) that it is present in virgin hair. This has implications in the forensic science analysis of hair.

In addition to the side reactions that lead to cysteic acid and CMT-CySH formation, there are slight amounts of lanthionine and lysinoalanine formed *via* β-elimination involving cystine and serine with dehydroalanine as the intermediate (Asquith and Carthew (18)). These derivatives are found in both acid and alkaline waved hair fibers.

The range of cystine reduction necessary for good wave formation has been found to vary over a wide range from 13 to 43 per cent, in these experiments, of the original cystine content. This reduction is related to processing time, but is also indicative of the many parameters involved. These include type of hair, amount of stress during perming, age of perm reagent, operator skills, type of perm, as well as other parameters. Data from numerous experiments point out the difficulty in assigning the quantitative effect of individual parameters. Converting the thiol formed during reduction to a stable derivative using ethyleneimine or iodoacetic acid is a sensitive and accurate measure of cystine involvement in perming. Reports by Sanford and Humoller (19) and Inolex Corporation (20) on cystine reduction percentages by reacting with iodoacetic at 100°C in aqueous solution must be carefully scrutinized since these conditions could certainly cause cleavage of disulfide bonds.

Stress-strain data confirm that hair undergoes damage during permanent waving. The fact that reoxidation of thiol groups restores much of the loss of strength associated with disruption of disulfide bonds verifies the role of cystine crosslinks in the strength of the hair fiber. However, attempts to correlate quantitative cystine reduction with changes in stress-strain curves necessitates monitoring with amino acid analysis or some method with similar sensitivity and accuracy. This publication does not attempt to accomplish this, as the stress-strain data are included only as further evidence that permanent waved hair is damaged.

Both hydrogen peroxide and sodium bromate were shown to be effective in rebonding lotions. Air oxidation is not complete even after 24 h. There is cysteic acid formation during reoxidation of the thiol groups and increased neutralization times incur greater damage to the hair fiber as monitored by both SEM and amino acid analysis.

By looking at the reduction and oxidation steps individually, and by utilization of radioisotope methods as well, the mechanism of permanent waving has been reevaluated. In particular, the presence of significant amounts of CMT-CySH in permed hair may not only be a result of direct reaction between cysteine and thioglycolate but may also be formed during acid hydrolysis after thiolation of lysine amino groups and subsequent crosslink formation with cysteine during the neutralization step of perming. The mixed disulfide formation appears to be more significant in acid waving than alkaline waving from preliminary data. The implication of the introduction of a new type of crosslink during perming requires further investigation to assess whether its contribution is advantageous.

An important question in permanent waving involves ascertaining the actual areas in the hair fiber where these reactions are taking place. Using H^3-iodoacetic acid to tag the sites of thioglycolate reactions, we are using radioautography methods to show localization, and this will be presented in a subsequent paper.

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Development of water-in-oil emulsifiers and their application to cosmetic emulsions

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Synopsis

The literature contains many references on the wide range of useful emulsifying agents for OIL-IN-WATER EMULSIONS. On the contrary, little has appeared in the area of new emulsifiers for stabilizing WATER-IN-OIL SYSTEMS. It is generally known that it is far more difficult to prepare stable w/o emulsions than those of the o/w type.

Polyglycerol esters having lipophilic surface activity derived chiefly from isostearic and oleic acids were prepared and characterized. A chemical description of these surfactants and other isostearic surfactants will be reviewed. Their physical and chemical properties are assessed as water-in-oil emulsifiers. Emulsifier efficiency is compared in the stabilization of simple w/o emulsions. Stability testing techniques are discussed.

Applications of these unique emulsifiers in preparing stable aesthetic functional w/o cosmetic emulsions are demonstrated.

INTRODUCTION

Polyglycerol esters having lipophilic surface activity derived from isostearic and oleic acids were prepared. These esters, due to their chemical and physical properties, constitute a series of nonionic emulsifiers of the water-in-oil type. It is generally accepted by the cosmetic chemist that these emulsion types are the most difficult to stabilize. In this study, the following parameters were evaluated: (a) spreading coefficients and their relation to emulsion stability; (b) Nessler tube* separation studies and their relation to stability; and, lastly, (c) cosmetic emulsion applications utilizing these unique surface active agents.

CHEMICAL DESCRIPTIONS

Polyglycerol esters are simply prepared through the polymerization of glycerin followed by simple esterification with a fatty acid. The polymerization of glycerin can

*#45310, Kimble Glass Inc.

be controlled to give polyglycerols of varying average molecular weights and free hydroxyl groups. Increasing the extent of polymerization leads to very viscous liquids and eventually solids which are soluble in water and other polar solvents. They, like glycerol itself, are humectants.

Fatty acid esters of polyglycerols can be made to have water or oil solubility, or some of each, thus making them excellent emulsifiers. By varying the type and amount of fatty acid used, a wide range of products can be prepared with different performance properties.

In this study, efforts were concentrated on the esters of oleic and isostearic acids. These are liquid products having similar chemical and physical properties. The isostearates have the advantage of increased oxidative stability and lack of odor or taste when compared to the corresponding oleates.

The extent of esterification is governed by the ratio of fatty acid to polyglycerol and any number, up to the total, of hydroxyl groups present may be reacted. The one exception is a pure monoester, which is difficult to obtain since there are two equivalent primary hydroxyl groups at either end of the polyglycerol chain.

The preparation of these polyglycerols was carried out at atmospheric pressure and 260°C under an inert atmosphere of nitrogen. The amount of catalyst was generally 1 per cent by weight based on the glycerin charged. The amount of water of reaction, along with viscosity measurements and hydroxyl value, were measures of the extent of reaction. Any number of glycerin molecules may be polymerized, but at approximately ten, the polymer becomes quite viscous and is difficult to handle.

The esters of the polyglycerols were prepared by heating the desired acid and polymer at atmospheric pressure to 210 to 230°C under an atmosphere of nitrogen, without catalyst. The volume of the water of reaction and the acid value was used to monitor the progress of reaction. The fatty acids typically react with the primary hydroxyl groups initially before reacting with the secondary less active ones. The reaction at the internal groups is more or less on a random basis with steric hindrance probably being the controlling factor.

Table I lists typical specifications for these polyglycerol esters. The HLB values are calculated (1) values and can be rather precisely controlled by varying either the polyglycerol chain length and/or the degree of esterification.

EVALUATIONS

In evaluating these esters as w/o emulsifiers, some simple easily reproducible methods were chosen, since work in the area of w/o emulsions is not extensive and many theories that seem to apply to o/w emulsions do not fit the w/o situation.

EXPERIMENTAL

I. NESSLER TUBE SEPARATION STUDIES

These tests were designed to demonstrate the utility of the polyglycerol esters as emulsifiers. A rather simple emulsion, having the composition shown in Table II, was prepared by the following procedure: in a 250 ml beaker the emulsifier to be tested is

Table I
Typical Properties of Polygylcerol Esters

	Acid Value	SAP Value	Hydroxyl Value	HLB (calc)	Viscosity* (25°C)
Triglycerol diisostearate	4.0 maximum	140–160	205–235	4.7	900–1000 cst
Triglycerol triisostearate	2.9	168.6	86.7	2.6	500–600 cst
Hexaglycerol triisostearate	3.4	142.9	199.7	5.3	5500 cst
Hexaglycerol hexaisostearate	3.6	170.7	50.8	2.4	590 cst
Octaglycerol pentaisostearate	4.7	157.6	134.1	3.8	2100 cst

*Kinematic viscosity—ASTM D445.

Table II
Nessler Tube Test Emulsion

	Wt %
Mineral oil, 70 visc.	65.0
Emulsifier	10.0
Water	25.0

mixed with the mineral oil. The mixture was then agitated at 250 rpms with a Lightnin'® type of mixer* attached to a Powerstat® Variable Auto Transformer.† The water is then quickly added, and the emulsion formed is mixed for precisely 20 min. The emulsion is quickly transferred to a Nessler tube of 100 ml capacity (30 mm diameter, 20 cm high), placed on a rack, and observed for 3 weeks. Being a relatively unstable system, separation of a clear oily layer on the top of the emulsion occurs quite rapidly. The height of this separation is measured daily and recorded. After calculating a per cent separation based on emulsion heights, a graph is plotted; represented by Fig. 1.

Six emulsifiers were tested in this manner—Triglycerol Diisostearate, Octaglycerol Pentaisostearate, Octaglycerol Pentaoleate, Hexaglycerol Triisostearate, a 50/50 blend of Triglycerol Diisostearate-Glycerol Trioleate, and as a control—Sorbitan Sesquioleate. At least one supplier (2) advocates the use of Sorbitan Sesquioleate as an effective w/o emulsifier and its use as a control in this experiment seemed to provide a good basis on which to judge the performance of polyglycerol esters.

RESULTS:

The ideal HLB range for w/o emulsifiers falls in the range 3 to 6. As shown in Table I, the polyglycerol esters tested here all meet this requirement.

As shown in Fig. 1, all the polyglycerol esters, except the Octaglycerol Pentaoleate, had slower separation rates than the control. A comparison between the Octaglycerol Pentaisostearate and the Octaglycerol Pentaoleate demonstrates the superior effectiveness of isostearate ester versus the oleate ester. A more extensive study is currently underway to study this phenomenon of isostearate versus oleate esters. The branched

*Mixing Equipment Co. Inc., 138 Mount Read Blvd., Rochester, NY.
†Superior Electric Co., Bristol, CT.

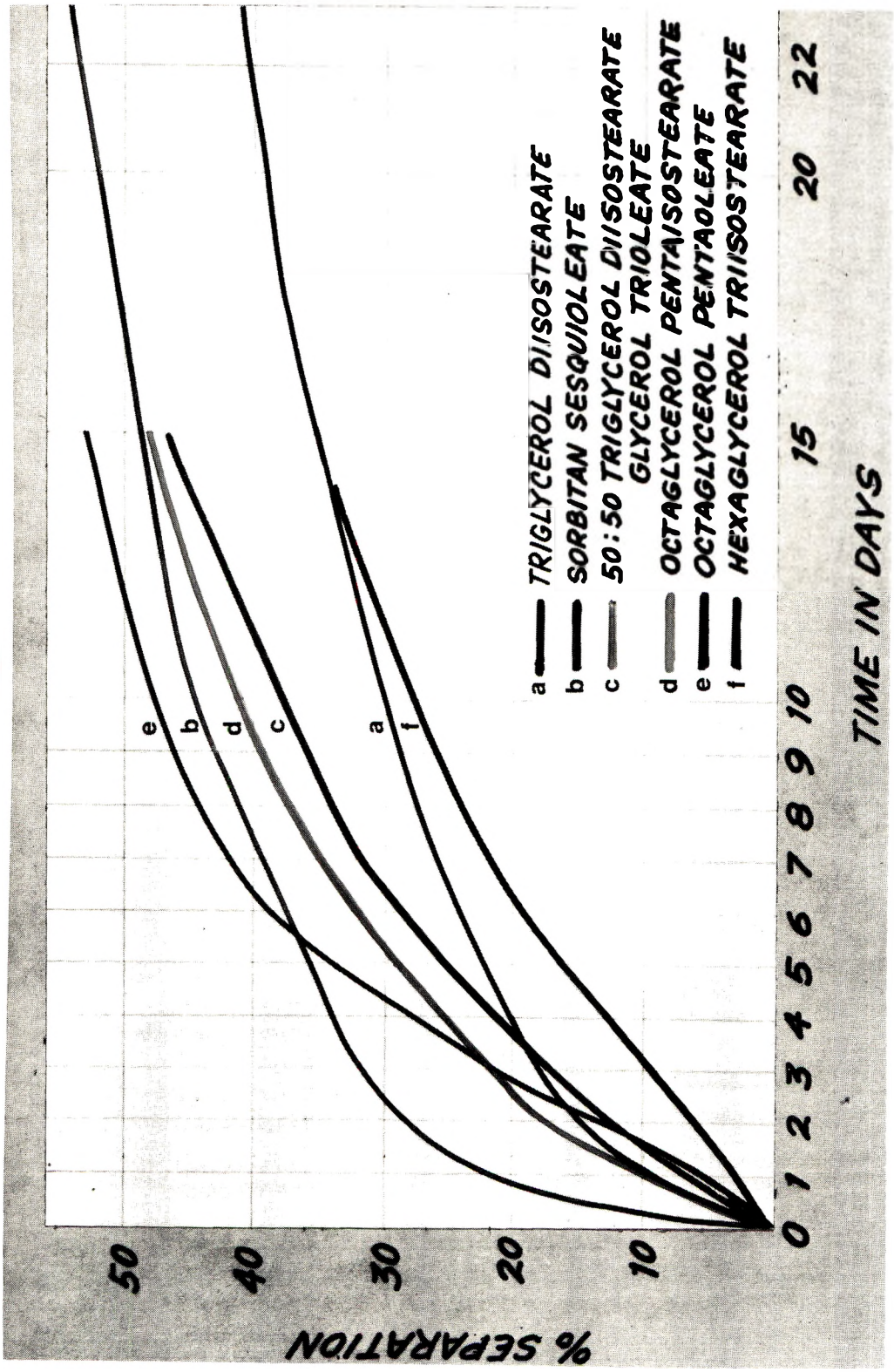

Figure 1. Per cent separation (Nessler Tube Study)

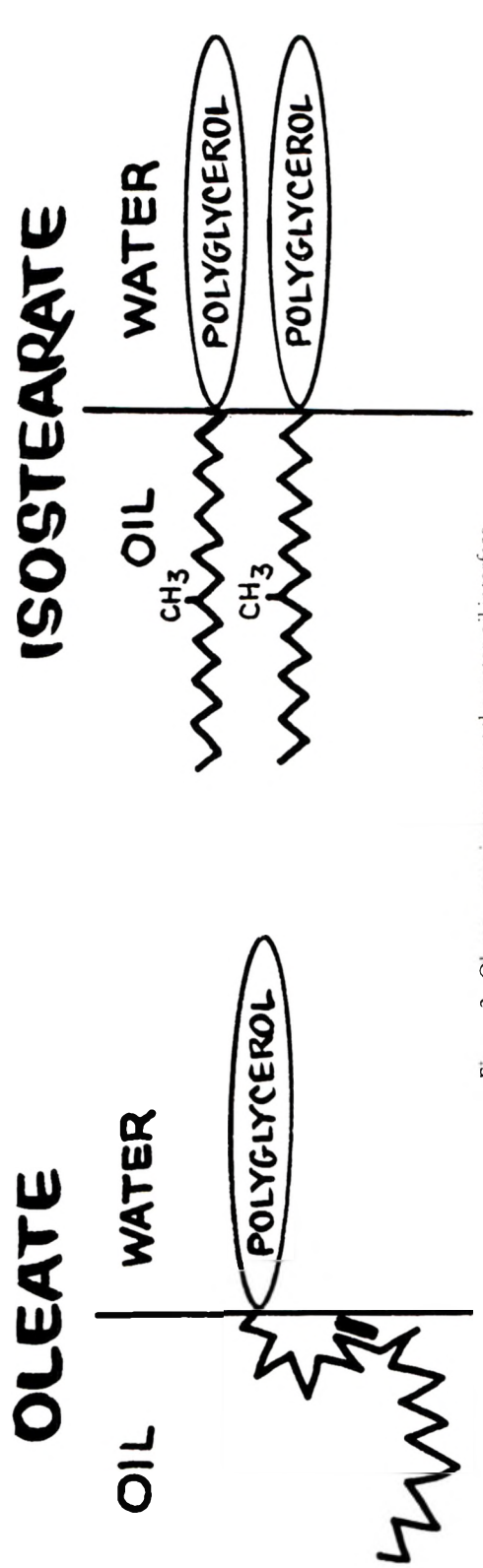

Figure 2. Oleate versus isostearate at the water-oil interface

Table III
Water-in-Oil Spreading Coefficients (S_2) (dynes/cm)
FOR SOME POLYGLYCEROL ESTERS IN VARIOUS OILS
$S_2 = \gamma$ soln oil $-$ (γ aq $+ \gamma$ interface)

	Mineral Oil	Isopropyl Palmitate	Castor Oil
Triglycerol diisostearate	−75.5	−73.2	−75.6
Hexaglycerol triisostearate	−75.4	−72.0	−72.0
Octaglycerol pentaisostearate	−75.7	−71.4	−70.0
Octaglycerol pentaoleate	−75.7	−73.2	−76.7

methyl groups on the iso-fatty acid seems to be more effective in bonding the water/oil interface than the double bond in an unsaturated acid (Fig. 2). One theory is that since the double bond contributes some polar nature to the hydrophobic chain, the chain is twisted back to the interface to satisfy the hydrophilic nature of the double bond. The branched saturated chain, on the other hand, has no hydrophilic site and is left free to penetrate into the oil, leaving room on the interface to be occupied by more emulsifier molecules and thus a more stable system.

II. SPREADING COEFFICIENTS

It has been proposed in the literature (3, 4) that spreading coefficients related directly to surface tension, and interfacial tension between 2 liquid phases, can be used quite accurately to predict emulsion stability. The theory is discussed in great detail by Becher (3) from which he derives the following 2 relationships for determining spreading coefficient (S) by the readily determinable quantities of surface tension (γ) and interfacial tensions.

$$S_1 = \gamma \text{ soln aq} - (\gamma \text{ oil} + \gamma \text{ interface})$$

$$S_2 = \gamma \text{ soln aq} - (\gamma \text{ aq} + \gamma \text{ interface})$$

where S_1 corresponds to the case of an oil droplet spreading on an aqueous solution of emulsifier (hence to an oil-in-water emulsion), and S_2 corresponds to the spreading of water on an oil-phase solution of emulsifier, and hence to a water-in-oil emulsion.

This work was concerned only with the values for S_2 relating to water-in-oil emulsions. Experimentally, these values are calculated simply from the readily determinable surface and interfacial tensions.

One per cent solutions of emulsifiers were prepared in mineral oil, isopropyl palmitate, and castor oil. The surface tensions were determined using a du Nuoy Ring Tensiometer.* The tensiometer was previously calibrated against benzene and distilled water and tensiometer performance was checked after each determination by repeating the measurement with distilled water. Results were always found to be reproducible. Interfacial tensions were determined with the same degree of precision, and the calculated values for S_2 yielded the results found in Table III.

*Cenco, Chicago, IL.

Table IV
W/O Cleansing Cream

	Wt. %
(A)	
Hydrogenated Vegetable Oil	25.0
Mineral Oil, 70 visc.	20.0
Beeswax	10.0
Lanolin	3.0
Emulsifier	5.0
Polysorbate 60	2.0
Propyl paraben	0.1
(B)	
Deionized water	34.0
Borax	0.7
Methyl paraben	0.2
	100.0

Procedure: Heat both phases to 75°C. Add (B) to (A) with stirring. Continue agitation until cooled to 35°C.

Becher states that the requirement for the stability of w/o emulsions is for the largest negative value of S_2. In the table, the values of -70 dyn/cm to -76 dyn/cm are sufficiently large enough to place these emulsifiers into the correct range for the formation of stable w/o emulsions.

III. COSMETIC EMULSIONS

A study was undertaken to investigate the auxiliary emulsifying properties of our emulsifiers in more complex cosmetically acceptable w/o systems.

The cleansing cream formula shown in Table IV was prepared for each of the auxiliary emulsifiers tested. Care was taken to insure that each cream was prepared identically. Samples of the creams were placed in a constant temperature oven at 49°C and in a freeze/thaw incubator (12 h. at 4°C/12 h. at 40°C). The samples were observed daily for signs of instability most notably water and/or oil layers forming as a result of instability for a period of a full month.

The results of the stability tests, summarized in Table V, indicate that most of the emulsions made with the polyglycerols exhibited better emulsion stability than the sorbitan ester controls.

Table V
Stabilities of W/O Cleansing Cream

Emulsifier	Freeze—Thaw	120°F
Octaglycerol pentaisostearate	Stable	Separation after 9 days
Octaglycerol pentaoleate	Slight Oil Separation	Separation after 7 days
Hexaglycerol triisostearate	Stable	Separation after 7 days
Trigylcerol diisostearate	Stable	Stable
Glycerol trioleate	Separation after 14 days	Separation after 7 days
Sorbitan monostearate	Separation after 1 day	Separation after 1 day
Sorbitan sesquioleate	Separation after 2 days	Separation after 2 days

SUMMARY

Various properties of polyglycerol esters of isostearic and oleic acid have been presented in this paper. Through spreading coefficient studies, we believe that their utility as w/o emulsifiers have been demonstrated in very simple and more complex w/o systems.

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Zur Speziesabhängigkeit der Hautverträglichkeit von Kosmetikgrundstoffen

WERNER KÄSTNER*

Synopsis — The topical irritancy potential of fatty or fat-derived cosmetic ingredients on human skin in 24-hour patch tests was compared with that in various animal species. Different species exhibited widely varying reactivity under identical test conditions. The degree of irritation decreased in the order rabbit, guinea pig, hairless mouse, and man. These data support the conclusion that the rabbit or guinea pig are not useful for skin irritancy studies. Data on hairless mice are more likely to permit prediction of human responses. Skin tolerance to raw materials or finished preparations — as determined on animals — cannot be relatet directly to human experience unless it has been established that human skin reacts similar to animal skin. Consequently, topical studies of cosmetic components on human subjects should be included in the test program.

1. Einleitung

Bei der Prüfung der Hautverträglichkeit von kosmetischen Grundstoffen und kosmetischen Präparaten kann man gelegentlich Unterschiede in der Hautverträglichkeit zwischen den üblichen Laboratoriumstieren Kaninchen und Meerschweinchen einerseits und der menschlichen Haut andererseits beobachten. Daß es hinsichtlich der Verträglichkeit tierartspezifische Unterschiede gibt, ist hinlänglich bekannt, daß es aber vorwiegend Fettkörper oder von Fetten abgeleitete Grundstoffe sind, die bei bestimmten Labortieren zu falschen Befunden führen, ist eine noch wenig beachtete Tatsache. So läßt sich erklären, daß die eine Untersuchungsstelle, die aus Erfahrung weiß, daß ein Produkt speziell nur bei Kaninchen oder Meerschweinchen Reaktionen verursachen wird und deshalb diese Tier-

* Aus den Toxikologischen Laboratorien der Henkel & Cie. GmbH, Düsseldorf.

arten für ihre Untersuchungen nicht heranzieht, ein Produkt als gut verträglich bezeichnet, während ein anderes Laboratorium wegen der bei diesen Spezies aufgetretenen Hautreaktionen Bedenken gegen ein Produkt anmeldet.

Solche Fälle haben uns veranlaßt, diesen Fragen nachzugehen und wir haben mit Grundstoffen vergleichende Prüfungen an drei Labortierarten und an Versuchspersonen durchgeführt. Zur Abrundung des Bildes haben wir auch aliphatische Carbonsäuren und Alkohole einschließlich der 2-Alkyl-alkanole in die Untersuchungen einbezogen, weil wir vermuten, bei diesen Stoffen am ehesten eine Gesetzmäßigkeit erkennen zu können.

In der Literatur finden sich einige Hinweise darüber, daß bei der Prüfung der Hautverträglichkeit Unterschiede in der Empfindlichkeit zwischen Versuchstieren und Menschen auftreten können. So weist Hopf (4) in den Empfehlungen für die Hautverträglichkeitsprüfungen von Körperpflegemitteln auf die besondere „Fettempfindlichkeit" der Meerschweinchen hin, die zu „falsch-positiven" Resultaten führen kann. Rieger und Battista (8) führen in ihrer Publikation über die Sicherheit von Kosmetika an, daß die Tierhaut als gänzlich verschieden von der des Menschen zu betrachten ist. Squalen führt z. B. bei Versuchstieren zu Haarausfall, ist jedoch normaler Bestandteil des menschlichen Sebum und wird weltweit in gut verträglichen Kosmetika eingesetzt. Durch die Applikation von Olivenöl können an der Rattenhaut Parakeratosen und Desquamation verursacht werden, trotzdem wird Olivenöl in Hautpflegemitteln seit biblischen Zeiten verwendet. Isopropylmyristat verursacht Reizungen auf der Haut (8). Roudabush et al. (9) kamen mit kosmetischen Fertigprodukten zu ähnlichen Ergebnissen. Sie fanden jedoch eine gute Übereinstimmung in der Empfindlichkeit zwischen der Haut von Kaninchen und Meerschweinchen. Mit teilweise ganz anders aufgebauten Stoffen (Formaldehyd u. a.) fanden Phillips et al. (7), daß mit der Patch-Test-Methode die Kaninchenhaut im allgemeinen leichter reizbar ist als die menschliche Haut unter gleichen Bedingungen. Davis et al. (2) kamen zu dem Schluß, daß das Kaninchen zur Prüfung der Hautverträglichkeit mehr aus historischen und praktischen als aus wissenschaftlich begründeten Überlegungen heraus verwendet wird. Es fiel den Autoren auf, daß ein auf dem Markt befindliches Antischuppenshampoo im Patch-Test bei Kaninchen schwere Reaktionen an der Haut verursachte, hingegen von Versuchspersonen fast reaktionslos vertragen wurde. Systematische Untersuchungen mit Kosmetikgrundstoffen wie Lanolin, Propylenglykol, Thioglykolat, Natrium-

laurylsulfat und kosmetischen Präparaten wie Cremeshampoo u. a. an verschiedenen Tierspezies (Maus, Meerschweinchen, Kaninchen, Schwein, Pavian und Mensch) zeigten die sehr unterschiedliche Empfindlichkeit der Tierspezies einerseits und zwischen diesen und dem Menschen andererseits auf. Die Kaninchenhaut reagierte bei fast allen untersuchten Produkten stärker als die menschliche Haut, die anderen Versuchstierarten zeigten teils stärkere, teils ähnliche oder geringere Reaktionen als die menschliche Haut. Die Ergebnisse machten deutlich, daß keine generelle Festlegung erfolgen kann, welche Tierspezies die am besten mit der menschlichen Haut übereinstimmenden Ergebnisse erbringen wird. Scholz (12) berichtete, daß besonders die Haut austrocknende Präparate wie glycerinhaltige Cremes und Salben an der Kaninchenhaut zu heftigen Reaktionen führen können, während sie von der menschlichen Haut gut vertragen werden. Die Ursachen hierfür wurden im differenten Wassergehalt der Haut als wesentlicher Unterschied zwischen Mensch und Tier gesehen. Besonders die durch die Schweißbildung mögliche Durchfeuchtung der menschlichen Haut hat keine Parallele in der tierischen Haut. Phillips et al. (7) sind hingegen der Ansicht, daß die leichtere Reizbarkeit der Kaninchenhaut im Vergleich zur menschlichen Haut in der unterschiedlichen Permeablität der Stoffe durch die Haut ihre Ursachen hat. Untersuchungen von Bartek et al. (1) haben gezeigt, daß die Haut von Kaninchen und Ratten deutlich durchlässiger für Stoffe recht unterschiedlicher Zusammensetzung als die Haut des Menschen ist.

2. Versuchsmethodik

Als Versuchstiere wurden männliche ausgewachsene weiße New Zealand-Kaninchen, männliche Pirbrightwhite Meerschweinchen im Gewicht von ca. 300 g, männliche und weibliche ausgewachsene, haarlose Mäuse der Mutante hr/hr verwendet. Als freiwillige Versuchspersonen stellten sich weibliche und männliche Mitarbeiter unserer Abteilung zur Verfügung. Kaninchen und Meerschweinchen wurden 24 Stunden vor der Behandlung die Rücken geschoren. Jedem Kaninchen und jedem Meerschweinchen wurden vier Patch-Test-Pflaster mit verschiedenen Produkten nebeneinander auf den Rücken appliziert, wobei die Applikationsstellen randomisiert wurden. Jede haarlose Maus erhielt ein Patch-Test-Pflaster auf den Rücken appliziert. Um die Pflaster, die auf der Tierhaut nicht ausreichend gut hafteten, besser zu fixieren, wurden um den Körper der Kaninchen Ledermanschetten, die mit Zellstoff unterlegt waren, gelegt.

Die Pflaster der Meerschweinchen und haarlosen Mäuse wurden durch einen porösen Leukoplaststreifen (Porofix®) fixiert. Den Versuchspersonen wurden die Pflaster auf die Außenseite des Oberarms appliziert. Alle Pflaster blieben für 24 Stunden auf der Haut. Die Kaninchen wurden während dieser Zeit in Zwangskäfigen gehalten, die Meerschweinchen und haarlosen Mäuse einzeln in ihren gewohnten Käfigen. Jeder Grundstoff wurde an vier Versuchspersonen und an vier Versuchstieren der drei Versuchstierarten geprüft. Da es sich zum Teil um feste Stoffe handelte, war es notwendig, ein Verdünnungsmittel zu verwenden. Wasser kam dafür nicht infrage, da die verwendeten Stoffe meist nicht wassermischbar oder wasserlöslich sind. Olivenöl eignete sich dazu nicht, weil es von Kaninchen und Meerschweinchen nicht immer reaktionslos vertragen wurde. Wir entschieden uns für Vaseline weiß DAB 7, da diese Substanz unter den genannten Bedingungen nicht zu Reaktionen geführt hatte.

Die meisten der von uns geprüften Stoffe wurden als 50%ige Zubereitungen in Vaseline angewendet. Die in höheren Konzentrationen verwendeten Grundstoffe wie Silikonöl, Olivenöl, Vaseline, Wollfett und Glycerin wurden hingegen in unverdünnter Form auf die Haut appliziert. Geprüft wurden aliphatische gesättigte und ungesättigte Carbonsäuren und aliphatische gesättigte Alkohole der Kettenlänge C_4 bis C_{22} und die 2-Alkyl-alkanole der Kettenlänge C_{12} bis C_{28} sowie Alkanolamide, Fettsäureester, Fettalkohol-oxäthylate, Polyoxyäthylene, Stearinsäure und Stearate. Über die untersuchten Produkte gibt die Tabelle 1a und 1b Auskunft (Handelsname, chemische Bezeichnung, Kettenlänge und geprüfte Konzentration).

Tabelle 1a

Produkte, die in die Untersuchungen einbezogen wurden

Handelsname	Chemische Bezeichnung	Kettenlänge	Testkonzentration
	aliphatische Carbonsäuren (ges.)	$C_4 - C_{22}$	50 %
	Fettalkohole (gesättigt)	$C_4 - C_{22}$	50 %
	2-Alkyl-alkanole	$C_{12} - C_{28}$	50 %
	Ölsäure	C_{18}	50 %
	Rizinolsäure	C_{18}	50 %
HD-Ocenol K	Oleylalkohol	C_{18}	50 %
Comperlan LM	Laurinsäuremonoäthanolamid	C_{12}	50 %
Comperlan MM	Myristinsäuremonoäthanolamid	C_{14}	50 %
Comperlan 100	Kokosfettsäuremonoäthanolamid	$C_{12} - C_{18}$	50 %
Comperlan HS	Stearinsäuremonoäthanolamid	C_{18}	50 %
Comperlan LD	Laurinsäurediäthanolamid	C_{12}	50 %
Comperlan LMD	Laurinmyristinsäurediäthanolamid	$C_{12} + C_{14}$	50 %
Comperlan OD	Ölsäurediäthanolamid	C_{18}	50 %
Comperlan PD	Kokosfettsäurepolydiäthanolamid	$C_{12} - C_{18}$	50 %
Eumulgin B 1	Cetylstearylalkohol + 12 Mol ÄO		50 %
Eumulgin B 2	Cetylstearylalkohol + 20 Mol ÄO		50 %
Eumulgin B 3	Cetylstearalalkohol + 30 Mol ÄO		50 %
Eumulgin M 8	Oleylcetylalkohol + 7 – 8 Mol ÄO		50 %
Eumulgin O 5	Oleylcetylalkohol + 5 Mol ÄO		50 %
Cetiol A	Laurinsäurehexylester		50 %
Cetiol V	Ölsäuredecylester		50 %
	Isopropylmyristat		50 %
Cetiol HE	Polyol-Fettsäureester		50 %
Cutina GMS	Glycerinmonostearat DAB 7	$C_{16} + C_{18}$	50 %
Cutina MD	Fettsäurepartialglycerid	$C_{16} + C_{18}$	50 %
Myritol 318	Capryl-Caprinsäure-Triglycerid	$C_8 + C_{10}$	50 %
Eumulsan AGS	Äthylenglykolstearat	C_{18}	50 %
	Kaliumstearat	C_{18}	50 %
	Stearinsäure	C_{18}	50 %
Cutina LE	Gemisch aus Mono- u. Diglyceriden höher ges. Fettsäuren u. Cetylstearylsulfat	$C_{16} + C_{18}$	50 %
Cutina LM	Gemisch aus Fettalkoholen, Wachsen und Ölen		50 %

Tabelle 1b

Produkte, die in die Untersuchungen einbezogen wurden

Handelsname	Chemische Bezeichnung	Kettenlänge	Testkonzentration
Polydiol 400	Polyoxyäthylen	Mol-Gewicht 400	50 %
Polywachs 4000	Polyoxyäthylen	Mol-Gewicht 4000	50 %
Polywachs 6000	Polyoxyäthylen	Mol-Gewicht 6000	50 %
	Vaseline weiß DAB 7		100 %
	Wasserfreies Glycerin DAB 7		100 %
	Olivenöl DAB 7		100 %
	Silikonöl dünnflüssig		100 %
	Silikonöl dickflüssig		100 %
	Wollwachs DAB 7		100 %

24 Stunden nach dem Aufbringen der Pflaster auf die Haut wurden die Pflaster entfernt und die behandelten Hautstellen an Hand eines Schemas beurteilt. Eine weitere Beurteilung fand 24 und 48 Stunden nach der Pflasterabnahme statt. Es wurden die Hautschwellung und die Hautrötung in jeweils 3 Stufen (angedeutete, deutliche und starke Reaktion = 1, 2 bzw. 3 Punkte) beurteilt. Die Beurteilungsmerkmale wurden, um eine bessere Übersicht zu erzielen, in Punktwerte umgerechnet. Die Punkte der jeweils 4 behandelten Hautstellen wurden über alle drei Beurteilungszeitpunkte hinweg für jedes Präparat summiert. Diese Summe stellte das Kriterium für die Einteilung in 5 Reaktionsklassen dar. Das Beurteilungsschema ist in der Tabelle 2 wiedergegeben.

Tabelle 2

Beurteilungsschema für die Einteilung in die 5 Reaktionsklassen

Klasse	Punkte	hautreizende Eigenschaften des geprüften Stoffes
1	0 — 1	ruft praktisch keine Hautreaktionen hervor
2	2 — 5	ruft nur bei einigen Tieren der Gruppe angedeutete Reaktionen hervor, die rasch abklingen
3	6 — 10	ruft angedeutete oder leichte Reaktionen hervor, die rasch abklingen
4	11 — 20	ruft deutliche Reaktionen hervor
5	über 20	ruft starke Reaktionen hervor

3. Versuchsergebnisse

Die Reaktionen auf den 24stündigen Hautkontakt wurden in Form von Reaktionsklassen (Klasse 1-5) zusammengefaßt. Die Reaktionsklassen wurden als Säulendiagramme dargestellt (s. Abb. 1-4). Die jeweils erste Säule stellt die Reaktionen der Kaninchen, die zweite die der Meerschweinchen, die dritte die der haarlosen Mäuse und die vierte die der Versuchspersonen dar.

Ganz allgemein wurde festgestellt, daß nicht nur große Unterschiede in der Empfindlichkeit auf die verschiedenen Stoffe zwischen den verwendeten Tierarten bestanden, sondern daß es auch breite individuelle Streuungen gab. In unseren Versuchen zeigte sich, daß insbesondere in den Fällen, in denen die Stoffe nur geringe Reaktionen verursacht hatten, es auch einige Tiere gab, die überhaupt keine Reaktionen zeigten. Stärkere Reize wurden innerhalb einer Tierart übereinstimmender beantwortet als leichte Reize. Die individuellen Streuungen waren bei Kaninchen und Meerschweinchen deutlich größer als bei haarlosen

Mäusen und Versuchspersonen. Trotz dieser relativ großen Streuungen lassen sich Richtungen und Gesetzmäßigkeiten erkennen, wenn man sich die Versuchsergebnisse vergleichend ansieht.

3.1. gesättigte aliphatische Carbonsäuren (Abb. 1 a)

Die Kaninchen reagierten auf alle Carbonsäuren mit Ausnahme der Buttersäure mit den stärksten Reaktionen. Erst bei Kettenlängen von C_{20} und C_{22} waren die Reaktionen deutlich geringer. Bei den Meerschweinchen kam es von C_4 bis C_{12} zu einer Steigerung der Reaktionsstärke, ab C_{16} jedoch wieder zu einer Abnahme. Bei den haarlosen Mäusen war das Bild nicht so einheitlich, jedoch blieben ab C_{16} die Reaktionen praktisch aus. Überraschend waren die starken Reaktionen der haarlosen Mäuse auf die Buttersäure. Die Versuchspersonen reagierten nur auf die kurzkettigen Carbonsäuren (Buttersäure und Capronsäure wurde wegen ihres unangenehmen Geruchs beim Menschen nicht geprüft) empfindlich, ab Laurinsäure traten keine Reaktionen auf.

3.2 gesättigte aliphatische Alkohole (Abb. 1 b)

Während die kurzkettigen Alkohole bei Kaninchen keine Reaktion verursachten, führten die längerkettigen zu deutlichen Reaktionen, die ihr Maximum bei Tetradecanol (C_{14}) erreichten. Bei Meerschweinchen wurden Reaktionen nur bei den kurzkettigen Alkoholen bis C_{14} beobachtet. Bei haarlosen Mäusen und Menschen traten nur geringe Reaktionen bei den kurzkettigen Alkoholen auf.

3.3. 2-Alkyl-alkanole (Abb. 1 c)

Auf die 2 Alkyl-alkanole reagierten ebenfalls die Kanichen am empfindlichsten, während bei den Meerschweinchen nur leichte Reaktionen verursacht wurden. Von den haarlosen Mäusen und den Versuchspersonen wurden diese Substanzen reaktionslos vertragen.

3.4. ungesättigte aliphatische Carbonsäuren und Fettalkohole (Abb. 1 d und e)

Auch bei den ungesättigten Verbindungen zeigt sich die unterschiedliche Hautverträglichkeit der vier miteinander verglichenen Spezies. Am deutlichsten ist die artspezifische Reaktionsweise, in diesem Falle die besondere Empfindlichkeit der Kaninchen, beim Oleylalkohol zu erkennen.

3.5 Fettsäurealkanolamide (Abb. 2 a)

Die Monoäthanolamide riefen nur beim Kaninchen und Meerschweinchen leichte Reaktionen hervor, während sie bei haarlosen Mäusen und

Menschen keine Reaktionen verursachten. Die Diäthanolamide und das Polydiäthanolamid riefen bei Kaninchen starke, bei Meerschweinchen mittelgradige und bei haarlosen Mäusen und Versuchspersonen keine oder nur leichte Reaktionen hervor.

3.6. Fettalkoholoxäthylate (Abb. 2b)

Die Emulgatoren verursachten außer beim Menschen mittelgradige bis starke Reaktionen. Die stärksten Reaktionen traten jedoch bei Kaninchen auf, während bei haarlosen Mäusen und Meerschweinchen ähnliche Reaktionen beobachtet wurden.

3.7. Fettsäureester, Stearinsäure und -derivate sowie Gemische Abb. 3a-c)

Auch bei diesen Stoffen zeigt sich ein weitgehend übereinstimmendes Bild. Bei den Fettsäureestern reagierten die Kaninchen am empfindlichsten. Die Meerschweinchen zeigten nur beim Isopropylmyristat leichte Reaktionen, während sie bei den anderen geprüften Estern ebenso wie die haarlosen Mäuse und Versuchspersonen die Behandlung, ohne irgendeine Reaktion zu zeigen, vertrugen. Auch für die Gemische Cutina® LE und Cutina® LM trifft dies zu. Bei der Stearinsäure und Stearaten finden wir ebenso wie bei den Carbonsäuren eine typische Abstufung der Empfindlichkeit in der Reihenfolge Kaninchen, Meerschweinchen, haarlose Maus und Versuchspersonen.

3.8. Polyoxyäthylene (Abb. 4a)

Die Polyverbindungen sind wenig reizend. Sie riefen bei allen Versuchstieren keine wesentlichen Hautreizungen hervor. Meerschweinchen scheinen etwas empfindlicher zu sein als die anderen Tierarten und der Mensch.

3.9. Grundstoffe, die in unverdünnter Form geprüft wurden (Abb. 4b)

Die Abbildung 4b zeigt, daß Vaseline, Glycerin und Wollfett von allen Tierarten reaktionslos vertragen wurde, daß jedoch bei Olivenöl und Silikonöl mit leichten Reaktionen bei Kaninchen und Meerschweinchen gerechnet werden muß.

4. Besprechung der Versuchsergebnisse

Bei den meisten untersuchten Stoffen hat sich gezeigt, daß artspezifische Unterschiede in der Empfindlichkeit auf bestimmte Stoffe zwischen den verwendeten Versuchstierarten einerseits und diesen und dem Menschen andererseits bestehen. Wenn auch die Untersuchungen zu dem Ergebnis führten, daß nicht jedes Tier einer Art in gleicher Weise reagiert, eine Erscheinung, die von Phillips et al. (7) durch Wiederholungsversuche an Kaninchen demonstriert worden ist, so kann doch unter den hier gewählten Versuchsbedingungen ein bestimmter Trend herausgelesen werden, und es kann eine Abstufung der Empfindlichkeit auf die hier geprüften Grundstoffe zwischen den Arten vorgenommen werden. In den meisten Fällen reagierten die Kaninchen auf die applizierten Stoffe mit heftigen Reaktionen, eine Beobachtung, die schon mehrfach beschrieben worden ist (2, 7, 8, 9). Auch die Meerschweinchenhaut reagiert in allen Fällen viel stärker als die menschliche Haut auf die hier geprüften Stoffe. Es gibt jedoch auch Beispiele dafür, daß die Meerschweinchen deutlicher als die Kaninchen reagieren können (Silikonöl). Die von Hopf (4) erwähnte Fettempfindlichkeit trifft daher nicht nur für die Meerschweinchen, sondern im gleichen Maße auch für die Kaninchen zu, zumal auch Roudabush et al. (9) eine gute Übereinstimmung in der Empfindlichkeit der Haut von Kaninchen und Meerschweinchen festgestellt haben. Deutlich geringere Reaktionen im Vergleich von Kaninchen und Meerschweinchen wurden mit einigen Ausnahmen (Buttersäure) bei den haarlosen Mäusen beobachtet. Am wenigsten empfindlich auf fettähnliche Stoffe scheint der Mensch zu sein. Eine große Zahl von Stoffen rief beim Menschen überhaupt keine Reaktionen hervor. Schmid (11) beurteilt die Empfindlichkeit der Haut der weißen Maus als günstiger für den Vergleich mit dem Menschen als die Haut anderer Labortiere und ist der Ansicht, daß eine vorsichtige Übertragbarkeit der an der Maus erzielten Versuchsresultate auf den Menschen möglich ist. Er benutzte jedoch nicht die Patch-Test-Methode, sondern die wiederholte Applikation ohne Pflaster und als Kriterium für die Reaktionsstärke die Hautfaltendickenzunahme. Wir sind nach den hier beschriebenen Versuchsergebnissen zu der Überzeugung gekommen, daß sich haarlose Mäuse besser für solche Prüfungen eignen, da viel eher dem Menschen ähnliche Reaktionen an der Haut dieser Versuchstiere beobachtet wurden als bei Verwendung anderer Labortiere. Die Versuche haben nämlich gezeigt, daß die Unterschiede in den Hautreaktionen zwischen haarloser Maus und Versuchsperson auf die geprüften Stoffe meist nur geringgradig sind (meist eine, höchstens zwei Klassenunterschiede),

während die Unterschiede zwischen Kaninchen und Meerschweinchen einerseits und dem Menschen andererseits viel deutlicher waren (3-4 Klassen). Daß Versuchspersonen stärker als haarlose Mäuse reagieren, wurde nur in 2 Fällen (Octanol, Decanol) beobachtet, aber auch hier betrug der Unterschied nur eine Klasse.

Da Tierversuche notwendig sind, um eine erste Abschätzung der zu erwartenden Hautreaktionen zu erreichen, sollten entweder mehrere Versuchstierarten herangezogen werden oder es sollten nach Möglichkeit Tierarten benutzt werden, deren Hautempfindlichkeit mit derjenigen des Menschen so gut als möglich übereinstimmt. Allgemeine Regeln dafür, für welche Stoffgruppen welche Tierarten benutzt werden können, sind nicht leicht zu geben (2). Dazu sind die Angaben der Literatur zu widersprüchlich und wegen unterschiedlicher Versuchsmethoden auch zu wenig vergleichbar. Schaaf und Gross (10) fanden z. B. eine gute Übereinstimmung zwischen akanthogener Wirkung bei Meerschweinchen verschiedener Alkohole, Öle, Fette, Fettsäuren und Fettalkohole u. a. Stoffe, die als Salbengrundlage Verwendung finden, und den klinischen Erfahrungen hinsichtlich der Hautverträglichkeit beim Menschen. Levenstein (5) berichtet über eine gute Übereinstimmung der Hautverträglichkeit zwischen Kaninchen und Menschen bei der Prüfung von Shampoos u. a. Produkten im 24-Stunden-Patch-Test. Mehr als von übereinstimmenden Befunden wird von Differenzen berichtet und es wurde von verschiedenen Autoren davor gewarnt, die Ergebnisse von Tierversuchen über die Hautverträglichkeit bestimmter Stoffe kritiklos auf die Verhältnisse der menschlichen Haut zu übertragen. Wenn schon solche Stoffe, von denen bekannt ist oder vermutet wird, daß sie bei Kaninchen und Meerschweinchen Reaktionen verursachen können, an diesen Versuchstieren geprüft werden und zu positiven Ergebnissen führen, so ist zu fordern, daß diese Versuche am Menschen wiederholt werden, ehe ein Urteil über die Verträglichkeit oder Unverträglichkeit abgegeben wird. So schreiben Roudabush et al. (9), daß viele Kosmetika, die seit langem auf dem Markt sind, nicht zum Verkauf gekommen wären, wären mit diesen Produkten nur Tierversuche durchgeführt worden. Alle an Versuchstieren erzielten Versuchsergebnisse mit Stoffen oder Produkten, die für die Anwendung am Menschen bestimmt sind, müssen auf ihre Reproduzierbarkeit am Menschen überprüft werden. Für die Hautverträglichkeitsprüfungen sollten andere Tierarten als die bisher meistens verwendeten Kaninchen und Meerschweinchen herangezogen werden.

Über die Verwendung von haarlosen Mäusen zur Prüfung von Kosmetika bei einmaliger oder wiederholter cutaner Applikation ohne Pflasterverschluß liegen Erfahrungen vor (3). Unter diesen Versuchsbedingungen hat sich der Einsatz haarloser Mäuse bereits bewährt. Daß diese Versuchstiere auch für die Patch-Test- Methode verwendet werden können, zeigen die vorliegenden Untersuchungen. Die bessere Übereinstimmung der Befunde der Hautverträglichkeitsprüfungen zwischen Versuchspersonen und haarlosen Mäusen im Vergleich zu anderen behaarten Versuchstieren wird auf die Dicke des Stratum corneum der Haut zurückgeführt, welches an nicht behaarter Haut deutlich stärker als an der behaarten Haut ist (6). In vitro-Versuche von Stoughton (13) über die Permeabilitätsverhältnisse an der Haut von Menschen und haarlosen Mäusen haben eine gute Übereinstimmung zwischen beiden Spezies erbracht. Auch Bartek et al. (1) haben gezeigt, daß für die Bestimmung der dermalen Toxizität Kaninchen und Ratten wenig geeignet sind, weil der Durchgang der Stoffe durch die Haut bei diesen Tierarten wesentlich schneller verläuft als beim Menschen. Im Hinblick auf die Permeabilität ergab sich jedoch eine gute Übereinstimmung zwischen der Haut von Versuchspersonen und Miniaturschweinen, und es wird vorgeschlagen, zur Prüfung der dermalen Toxizität von Stoffen, die in Externa oder Kosmetika zur Anwendung kommen sollen, anstelle der bisher meist verwendeten Kaninchen besser Zwergschweine zu verwenden. Inwieweit sich diese Tierart für die Hautverträglichkeitsprüfungen eignet, muß noch näher untersucht werden.

5. Zusammenfassung

Eine Reihe von fettähnlichen oder von Fetten abgeleiteten Stoffen, die z. T. in Kosmetika Verwendung finden, wurde auf ihre lokale Verträglichkeit im 24-Stunden-Patch-Test bei verschiedenen Versuchstierarten und bei Versuchspersonen vergleichend untersucht. Dabei wurden zwischen den Spezies unter gleichen Bedingungen sehr unterschiedlich starke Reaktionen festgestellt, wobei die Empfindlichkeit in der Reihenfolge Kaninchen, Meerschweinchen, haarlose Mäuse und Versuchspersonen abnahm. Aus diesen Befunden geht hervor, daß Kaninchen und Meerschweinchen für die Hautverträglichkeitsprüfung der hier untersuchten Stoffgruppen wenig geeignet sind und daß die an haarlosen Mäusen gewonnenen Ergebnisse eher einen Schluß auf die beim Menschen zu erwartende Verträglichkeit zulassen. Die im Tierversuch ermittelten Ergebnisse über die Hautverträglichkeit bestimmter Stoffe oder Zubereitungen, die bestimmte Stoffe enthalten, können demnach nicht auf

die Verhältnisse der menschlichen Haut übertragen werden, wenn nicht geprüft worden ist, ob die menschliche Haut in ähnlicher Weise reagiert. Die Prüfungen über die Hautverträglichkeit kosmetischer Grundstoffe sollten daher auch Untersuchungen an Versuchspersonen einschließen.

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Abbildung 1

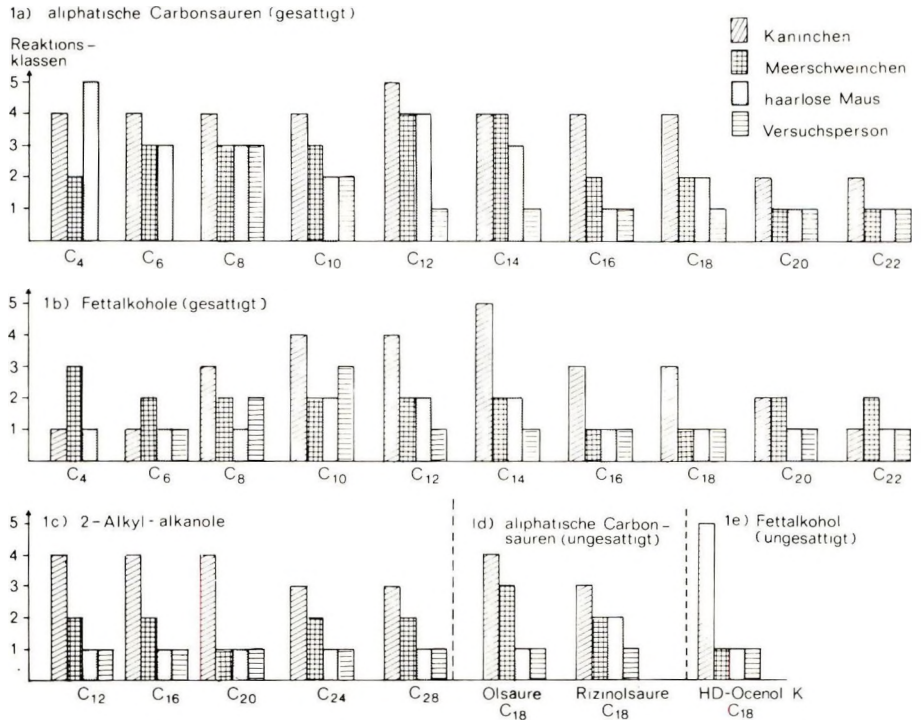
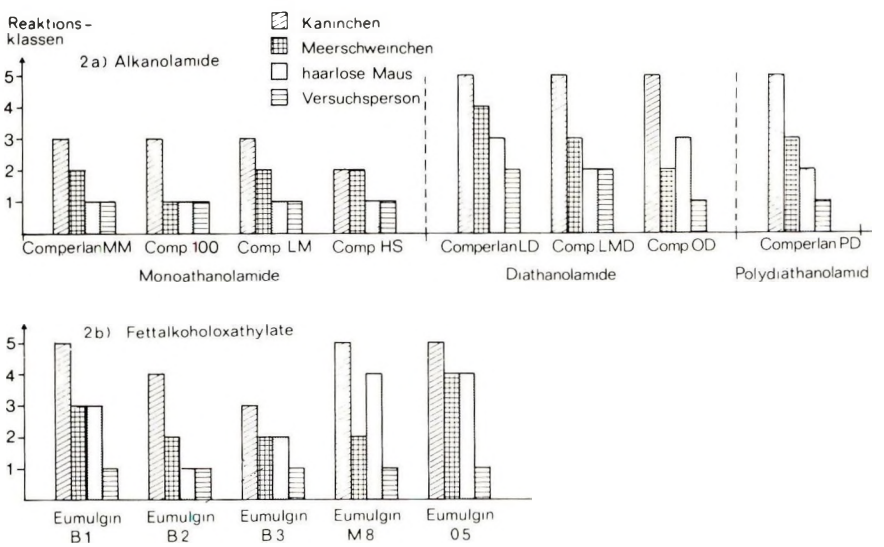


Abbildung 2



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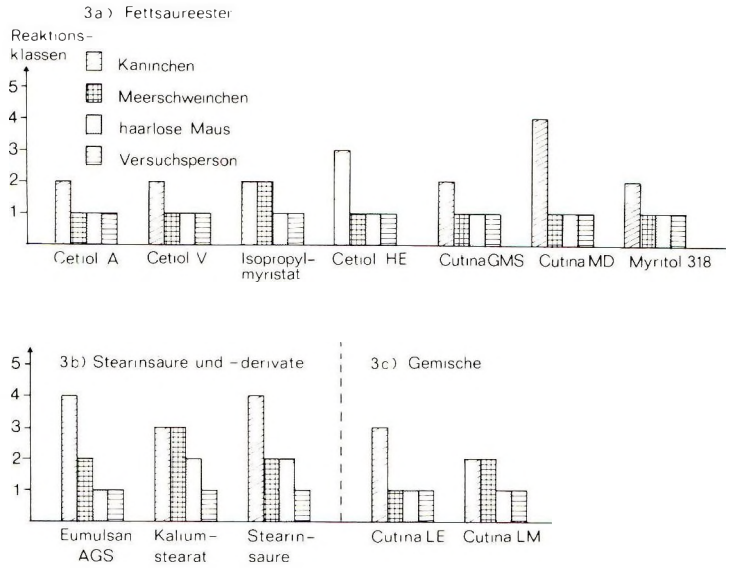

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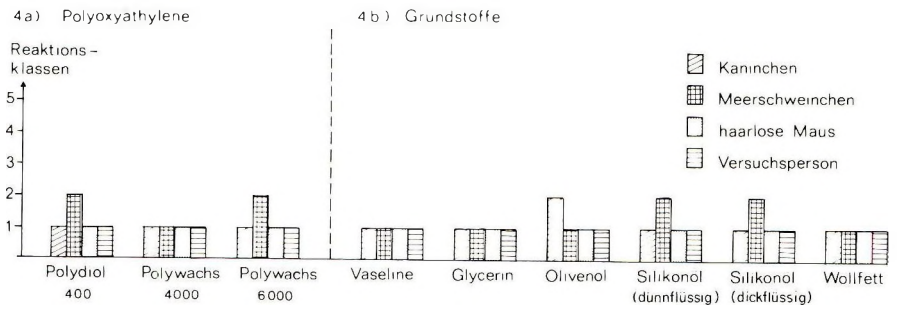

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A quantitative human patch testing procedure for low level skin irritants

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Synopsis

A week-long repeated INSULT OCCLUDED PATCH TEST METHODOLOGY is presented along with an analysis procedure based on relative slopes of IRRITATION DEVELOPMENT. This method allows for quantitative comparison of the primary irritation potential of low level irritants, culminating in statistical statements concerning the differences among samples. The use of the method to determine differences between commercial shaving creams, among various perfumes for a shaving cream formula, and among various roll-on antiperspirants is presented, as well as the identification and solution of an apparent irritancy problem associated with an anti-perspirant formula intended for pump spray delivery.

INTRODUCTION

Both the dermatologist and formulator have an interest in determining the inherent irritancy of various materials, medicaments, and cosmetics, which purposefully or otherwise come in contact with human skin. Animal testing, while often able to classify compounds into wide categories of irritancy, has not proved adequately predictable over the small range of irritancy associated with materials intended for human skin (1). Therefore, human testing is required.

Various human patch test procedures have been developed to quantify the primary irritation potential of a sample (2–11). All of these basically classify materials into categories of irritancy. If several samples fall within the same category, no statement can be made as to their differences. In order to make statements about relative irritancy on a quantitative basis, the IT_{50} test was proposed (12). Time until 50 per cent of the subjects had definitive irritation was measured. The method proved quite successful, but is hindered by the very long time required to get irritation with many materials of interest. The methodology and analysis procedure schema to follow was developed to assess the statistical significance of observed differences in the primary irritation potentials of similar products which produce low levels of irritation.

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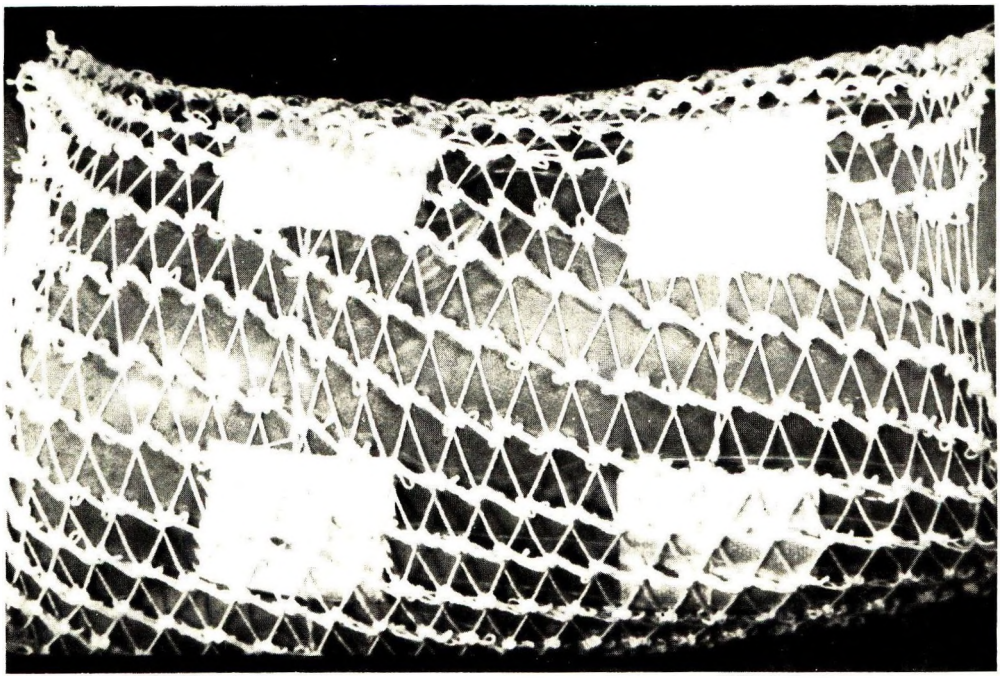

Figure 1 Occluded patches in place on a subject

METHODS

Eight or more subjects with normal skin acted as subjects for each test. The test method was a four-day repeated insult occluded patch test as developed by Finkelstein *et al.*(4) with several minor modifications. Four cotton flannel squares (2.54 cm on a side) were sewn onto a square piece of 0.08 mm thick poly-ethylene plastic film (11.2 cm on a side) with white cotton thread. The materials to be tested were applied to the cotton flannel patches on the plastic and were then applied to the inner aspect of the upper arms. The plastic patch combination was held in place with an elasticized web (Surgifix) (Fig. 1).

Subjects were selected who exhibited no evidence of irritation on the appropriate area of the arm, i.e., irritation scores (4) of zero. In the early afternoon of Day 1, treatments

Table I
Scores for Evaluation of Irritation

0.0	no reaction
0.5	trace of erythema or dryness
1.0	slight pink or scaliness
2.0	pink, 100 per cent of area, flakiness
3.0	dark pink, up to 50 per cent of area or wrinkly dry
4.0	red, up to 100 per cent or very dry
5.0	bright red, 50 per cent of area
6.0	deep or bright red, 100 per cent of area, cracky dry, slight edema
7.0	deep or bright red, 100 per cent of area, cracky dry, moderate edema
8.0	deep or bright red, 100 per cent of area, cracky dry, strong edema

were applied to the patches, secured in place with the elasticized web, and allowed to remain in place until approximately 9:00 A.M. the following morning. The treatment areas were then rinsed with water and patted dry with paper towels. Subjects were not scored immediately for irritation, but returned in the early afternoon. This permitted the generalized hydration and wrinkling of the skin to subside before evaluating the effect of treatments. Scoring was done using the criteria (4) in Table I, following which the treatments were reapplied to the same sites by the method previously described. This procedure was repeated for four consecutive days, except that treatments were not reapplied on the final day, Day 5. Treatments were discontinued on any site that exhibited an irritation score of six or greater during the test.

STATISTICAL DESIGN AND ANALYSIS

In the early stages of development an 8 × 8 Latin Square was used to assign treatments to subjects and sites. After some consideration, it was decided that a randomized blocks design was more appropriate. The Latin Square, unlike a randomized blocks design, permits separation of the sites and subjects effects from the treatments effect. The problem was that the sites effect thus isolated also contained the effect of the subjects x treatments interaction, which is the desired error term for the test of treatments when such an interaction exists. Indeed, in the early Latin Square designs the sites effect fluctuated so widely that the only plausible explanation, since any differences among sites would be expected to remain fairly constant, was a varying contribution of the subjects x treatments interaction. This interaction may be described as the extent to which different subjects respond differently to the same treatment, i.e., one subject is highly irritated by treatment A, but finds treatment B only mildly so; while another subject experiences the reverse situation. From a clinical viewpoint, this would not be a highly unusual event.

The randomized blocks design which was used in the tests described herein, randomizes the effect of sites in an unbiased manner and permits the testing of the treatments effect against the subjects x treatments interaction.

At the conclusion of any single irritation test, each subject had five recorded scores for each treatment: a score of zero representing the irritation level just before treatment and four scores representing the irritation response to treatment on four consecutive days. An analysis of the mean irritation score for the four treatment days is perhaps the most straightforward approach to comparing the irritancies of various treatments. Such a method is often illuminating and should be used in conjunction with information obtained from the method described below. A simple analysis of the mean irritation level may result in a loss of important information, however. For instance, consider the irritation scores of the following two hypothetical treatments:

	Irritation score for day #					
	1	2	3	4	5	Mean
Treatment A:	0.0	3.0	3.0	3.0	3.0	2.4
Treatment B:	0.0	1.0	2.0	3.0	6.0	2.4

Treatment A results in a moderate level of irritation occurring fairly rapidly, but does not seem to worsen with repeated applications. Treatment B begins with fairly low irritation levels, but repeated applications produce increasingly higher irritation scores.

The information contained in these scores is partially or totally lost by consideration of only the mean irritation score (means are the same for both treatments). Clearly, some method of assessing the rate of change in irritation would be useful. For instance, treatment B might be perfectly acceptable for a product intended for occasional use, whereas in chronic use situations it could prove much too irritating.

A technique described in detail by Mandel (13) can be used on data of this type. Briefly, this technique involves linearizing the changes in daily irritation scores, by expressing them as a function of the average irritation score for all treatments on each day. In other words, rather than plotting the irritation scores versus time which would undoubtedly produce a nonlinear plot, the x-variable is represented instead by the mean irritation score at each time over *all* of the treatments.

The five individual scores for each treatment on each subject are then regressed upon these overall mean irritation scores, and the slope of the line becomes the response used in the analysis. A slope of 1.0 represents an exactly "average" treatment; a slope greater than 1.0 represents a treatment more irritating than the average; and a slope less than 1.0 represents a treatment less irritating than the average.

RESULTS

This testing and analysis method has been used extensively over the past year within our laboratory, principally in evaluating prototype formulations and competitive products. The data to follow have been selected from several different experiments to demonstrate the utility of the test in different situations.

Tests indicated that mild shaving creams and soaps could be sensitively tested this way

Table II
Irritancy Scores of Two Shaving Creams (A & C) and a Mild Toilet Soap (B)—
All at 2.5 Per Cent Principal Irritant Level

Subject	1					2					3				
Day															
Treatment	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
A	0.0	2.0	2.0	2.0	3.0	0.0	0.5	0.5	1.0	2.0	0.0	0.0	1.0	2.0	0.5
B	0.0	0.0	1.0	2.0	2.0	0.0	0.0	1.0	2.0	2.0	0.0	0.0	0.5	3.0	3.0
C	0.0	1.0	2.0	2.0	2.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	1.0
Subject	4					5					6				
Day															
Treatment	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
A	0.0	0.0	2.0	2.0	2.0	0.0	0.0	0.0	0.0	1.0	0.0	0.5	1.0	2.0	2.0
B	0.0	1.0	1.0	3.0	2.0	0.0	0.0	0.5	1.0	1.0	0.0	0.5	1.0	2.0	2.0
C	0.0	0.0	0.0	0.5	1.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	1.0	0.0	1.0
Subject	7					8					9				
Day															
Treatment	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
A	0.0	0.0	2.0	2.0	1.0	0.0	0.0	1.0	2.0	2.0	0.0	0.0	1.0	1.0	2.0
B	0.0	2.0	2.0	2.0	2.0	0.0	0.0	1.0	1.0	2.0	0.0	0.5	2.0	3.0	3.0
C	0.0	1.0	0.5	0.0	0.0	0.0	0.5	1.0	2.0	3.0	0.0	0.0	1.0	2.0	2.0

Table III
Slopes of Irritation upon Average Daily Irritation Levels, by Product and Subject

Subject	Treatment A	B	C
1	1.283	1.379	1.155
2	0.945	1.379	0.017
3	0.804	2.037	0.383
4	1.410	1.414	0.529
5	0.383	0.689	0.192
6	1.251	1.251	0.287
7	1.027	0.900	−0.240
8	1.379	1.088	1.634
9	1.088	1.956	1.379

if the final concentration of soap or detergent was ~ 2.5 per cent. Using this constant concentration of active "irritant," two shaving creams and a commonly used toilet soap were compared. The raw data are shown in Table II and the resulting Mandel slopes are shown in Table III. For example, in Table III Subject Number 1 has a slope of 1.379 recorded for treatment B. This number is the result of a linear least squares regression of the irritation scores for Subject Number 1, treatment B,

Day 1	Day 2	Day 3	Day 4	Day 5
0.0	0.0	1.0	2.0	2.0

against the average of all 27 scores (nine subjects with three treatments each) for each day.

Day 1	Day 2	Day 3	Day 4	Day 5
0.000	0.389	0.963	1.482	1.667

Table IV shows the computational method used to obtain the "best fit" of the data from Subject Number 1, treatment B.

All other slopes were then calculated in the same manner, and the nine slopes cor-

Table IV
Sample Calculation on the Slope for Subject Number 1, Treatment B

	Average Irritation Score X	Individual Irritation Score Y	Deviations from Means $x = (X - \bar{X})$	$y = (Y - \bar{Y})$	Squares x^2	y^2	Products xy
Day 1	0.000	0.0	−0.900	−1.0	0.810	1.00	0.900
Day 2	0.389	0.0	−0.511	−1.0	0.261	1.00	0.511
Day 3	0.963	1.0	0.063	0.0	0.004	0.00	0.000
Day 4	1.482	2.0	0.582	1.0	0.339	1.00	0.582
Day 5	1.667	2.0	0.767	1.0	0.588	1.00	0.767
SUM	4.501	5.0	0.001[a]	0.0	2.002	4.00	2.760
MEAN	0.900	1.0					

$$\text{Slope} = \frac{\Sigma xy}{\Sigma x^2} = \frac{2.760}{2.002} = 1.379$$

[a]Rounding error produced non-zero sum.

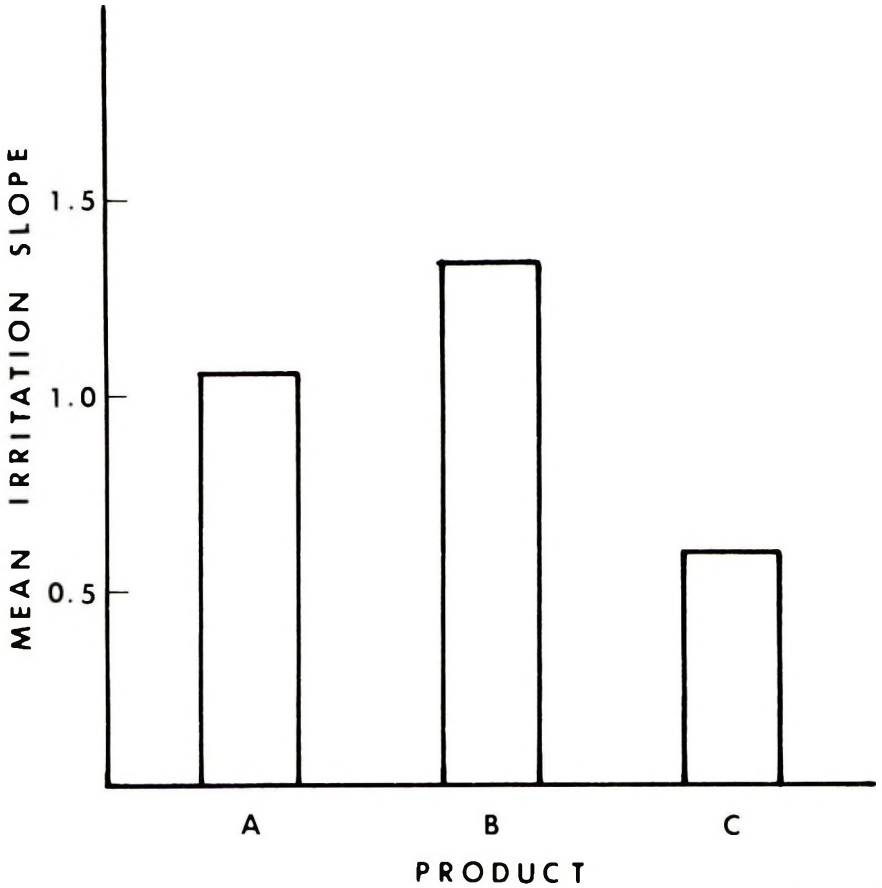

Figure 2 Relative mean irritation slopes of two shaving creams (A & C) and a mild toilet soap (B)

responding to a single treatment were averaged to obtain the treatment mean. Figure 2 shows the average irritation slope for each product, while Table V shows the analysis of variance. Shaving cream was substantially and significantly ($P < 0.01$) less irritating than the toilet soap, as well as possibly less irritating than the other ($P < 0.10$) shaving cream.

In a separate experiment, six perfumes were evaluated for irritancy in a standard foam shaving cream formula. All samples were diluted to a 2.5 per cent detergent level. Eight subjects were used, however, one failed to complete the test. Differences in mean irritation slopes were, nevertheless, apparent in the seven remaining subjects (Fig. 3), and Tukey's Test (honestly significant difference) indicated that the shaving cream with perfume A produced more irritation than that with perfume F ($P < 0.01$), and that the product with perfume D was perhaps more irritating than that with perfume F ($P \approx 0.1$).

The test has demonstrated that commercially available products intended for daily use and in prolonged contact with the skin can vary considerably in their irritation potential.

In another test, three commonly used commercial roll-on antiperspirants were tested

Table V
Analysis of Variance of Mandel Slopes

Source	DF	SS	MS	F	P
Subjects	8	3.0988	0.3873	2.33	0.0710
Treatments	2	2.5911	1.2955	7.80	0.0044
Error	16	2.6579	0.1661		
Total	26	8.3478			

Treatment Means

A = 1.063
B = 1.379
C = 0.593

Tukey Test

HSD (0.10) = 0.4512
HSD (0.05) = 0.5368
HSD (0.01) = 0.7379

on eight subjects. It was found that Samples A and B were quite similar and moderately irritating, but that Sample C produced only slight irritation (Fig. 4). Tukey's Test indicated that Samples A and B were not significantly different ($P > 0.10$), while C was less irritating within the confines of this test methodology ($P < 0.05$).

Irritancy testing of another antiperspirant formula intended for pump spray delivery produced severe irritation when occluded repeatedly in this methodology. The formula contained approximately 70 per cent ethanol. Since the ethanol would be expected to evaporate in use, the patches were allowed to dry, then remoistened with water just prior to application to the subjects in order to more closely simulate use conditions in the axillary vault. Tested in this way, the formula produced less irritation and allowed for meaningful irritancy testing of other alcohol-containing antiperspirant formulae.

DISCUSSION

The presented material demonstrates that it is possible to clearly distinguish products and formulae of similar natures on the basis of their irritancy within the methodology of a repeated insult occluded patch test. Whether these results relate in any way to actual use experience has not been shown. The unique ability of this test is to show, with a degree of statistical confidence, that one product, formula, or compound is more irritating than another similar one.

Care must be exercised in using the analysis of variance that the underlying assumptions of homogeneous variances and normally and independently distributed errors, are reasonable. In situations where these criteria do not obtain, there are nonparametric procedures which can be applied to the randomized blocks design.

Samples with very low irritation potentials occasionally fall below the sensitivity of this test—they are essentially nonirritating. A scarifying technique similar to that recently proposed by Frosch and Kligman (14) may extend the range of this type of analysis, but this has yet to be tried.

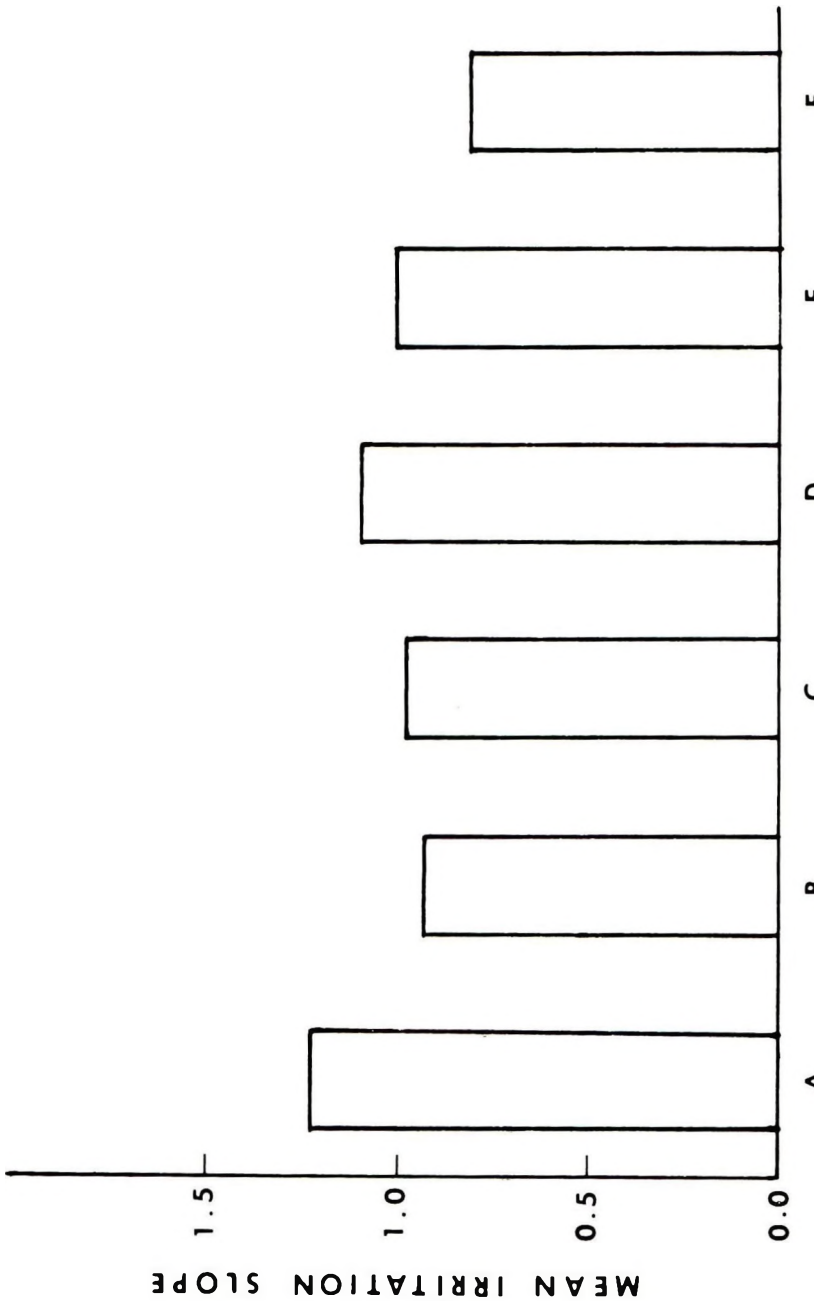

Figure 3 Relative mean irritation slopes of a standard shaving cream formula with six different perfumes

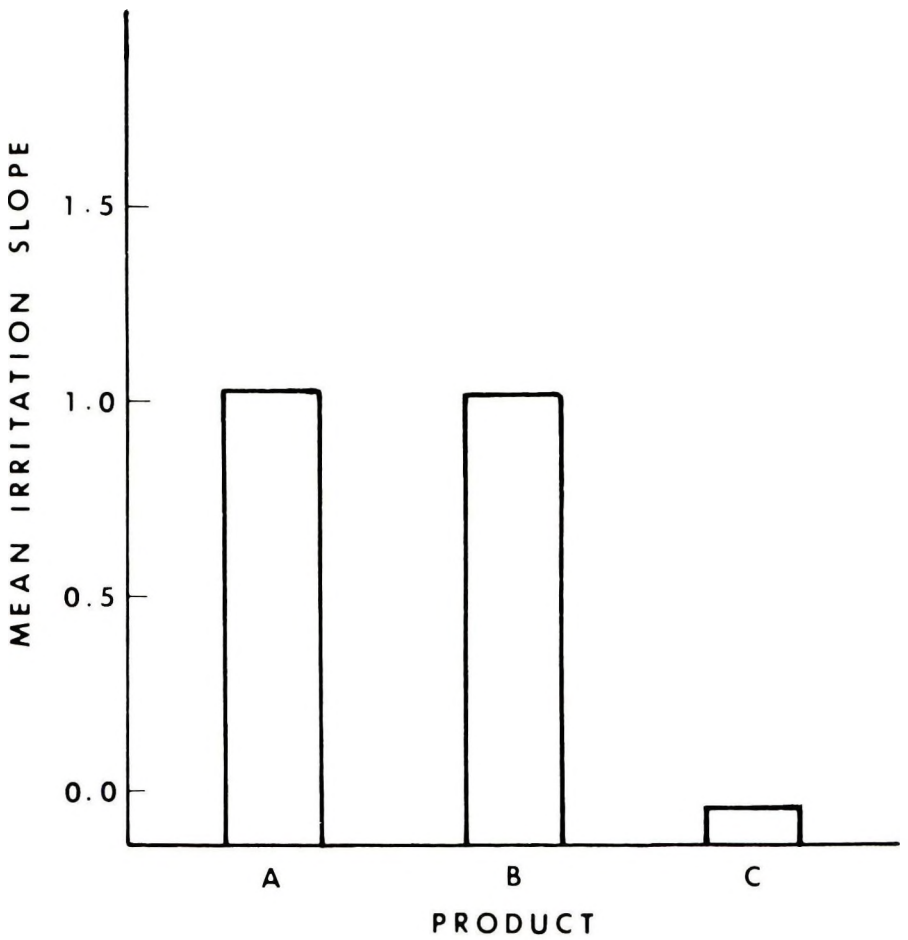

Figure 4 Relative mean irritation slopes of three roll-on antiperspirants

It is to be emphasized again that this method is not intended as a substitute for large-scale safety testing. The principal advantage of this test is that it can serve as a restraint to the formulator. When a potential product appears to be too irritating, multiple modifications to the formula may be made to attempt to reduce the irritation without incurring the costs associated with large-scale human testing at each step. Also, if the irritation averages of formulations are somewhat different, but it can be shown that the difference is within the range expected by random variation alone, avenues of investigation are not prematurely closed. When the formulator is satisfied that the product appears to have a low irritation potential, as well as satisfying any other criteria of interest, then large-scale testing can be more appropriately performed.

CONCLUSIONS

A repeated insult occluded patch test conducted on humans coupled with an analysis scheme based on relative slopes of irritation development can be useful in quantifying the differences in primary irritation potential of low level irritants. The utility of the

test has been demonstrated with shaving creams, roll-on antiperspirants, and an antiperspirant pump spray formulation.

ACKNOWLEDGMENT

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Malodor formation in nitromethane stabilized trichloromonofluoromethane blends

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Synopsis

Methyl isocyanide has been identified as the MALODOROUS compound formed from NITROMETHANE stabilized TRICHLOROMONOFLUOROMETHANE (Propellant 11) in iron or steel containers. Oxygen, water, and iron are required for isocyanide formation. Water must be present as a separate phase, i.e., above its saturation level in the blend. Methyl isocyanide forms from reaction of methylamine, the reduction product of nitromethane and the free radical intermediates obtained by reductive dechlorination of propellant 11. The presence of a separate water phase in propellant 11 may not be detected if dechlorination of the propellant and corrosion of the metal are occurring.

INTRODUCTION

Nitromethane, CH_3NO_2, has been successfully employed as a stabilizer in aerosol blends for over a decade (1). It is specifically effective in minimizing deterioration of alcohol based products formulated with propellant 11 blends, as well as preventing container corrosion. The reaction causing deterioration and corrosion in the above formulations has been shown to be free radical in nature, with reduction of propellant 11 to give HCl and acetaldehyde (2,3):

$$\text{I.}\quad CCl_3F + CH_3CH_2OH \rightarrow HCl + CH_3CHO + CHCl_2F$$

The HCl causes can corrosion and acetaldehyde reacts with the formulation ingredients to cause product deterioration. Nitromethane is believed to work as a free-radical reaction inhibitor, presumably by reacting with the initial radicals to produce a more stable, less reactive species. However, the actual mechanism is not certain, for several other inhibitors of such reactions are not as effective (as pointed out in (1–3)).

Several isolated instances of malodor formation from propellant 11/nitromethane blends have occurred in bulk storage facilities. The odor was described as garlic-like or pyridine-like by Marchio and Quick (4), who reported that a combination of propellant

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11/nitromethane/iron/H_2O is required for odor formation. They claim that propellant 11 serves only as an HCl source and that the odor is due solely to the reduction products of nitromethane, specifically formaldoxime:

$$\text{II.}\quad CH_3NO_2 + Fe + 3HCl \rightarrow CH_2 = NOH \cdot HCl + FeCl_2 + H_2O$$

its tautomer, nitrosomethane, and the various nitrogen compounds that may form by condensation of these compounds, e.g., pyridine and other foul smelling nitrogen heterocycles. Although water was correctly identified as a necessary reactant, the concentration of water necessary for odor formation in actual occurrences in the field was not specified.

The source of malodor has now been identified as methyl isocyanide, CH_3NC, a compound of extremely intense odor, i.e., having an extremely low odor threshold. The carbenic, or divalent carbon, has been shown to originate from propellant 11, and the rest of the molecule from methylamine by reduction of nitromethane

$$\text{III.}\quad CCl_3F \xrightarrow[Fe/H_2O/O_2]{} CCl_2F\cdot \text{ [or possibly } CClF\text{:]} + Cl\cdot$$

$$\text{IV.}\quad Cl\cdot + HOH \rightarrow HCl + [OH\cdot]$$

$$\text{V.}\quad CH_3NO_2 + 6HCl + 3Fe \rightarrow CH_3NH_2 + 3FeCl_2 + 2H_2O$$

$$\text{VI.}\quad CH_3NH_2 + CCl_2F \rightarrow [\text{"reactive complex"}] \rightarrow CH_3NC + HCl + HF$$

The processes by which radicals from propellant 11 react with methylamine have not been completely characterized. The presence of the isocyanide, however, is unequivocal. The actual concentration of CH_3NC, estimated from field studies is 10 to 20 ppb, while the estimated threshold limit for odor detectability is 0.1 ppb. These concentrations are far below the limit of conventional analytical tools. Complete characterization was provided by Plasma Chromotography (see the Methods section of this paper) as well as by odor identity of CH_3NC prepared by a tested literature procedure (see the Methods section of this paper).

As further proof of the presence of CH_3NC, its reduction product, dimethylamine, CH_3NHCH_3 (DMA), was also detected in contaminated storage facilities. DMA serves as a characterizing derivative of CH_3NC (5).

The "classical" method of isocyanide synthesis is the carbylamine reaction of haloforms, CHX_3 (X = halogen) with primary amines in the presence of caustic:

$$\text{VII.}\quad CHCl_3 \xrightarrow[KOH]{} CCl_2\text{:} + KCl + H_2O$$

$$\text{VIII.}\quad RNH_2 + CCl_2 \rightarrow [\text{reactive intermediate}] \rightarrow RNC + 2HCl$$

Since (1,2,3) clearly show that fluorocarbon 21, $CHCl_2F$, is formed during reduction of propellant 11 by ethanol formulations, we attempted to simulate conditions for malodor formation with FC-21 in place of P-11 and could not detect any isocyanide odor. Thus, FC-21 is not an intermediate in isocyanide formation.

Our interest in the cause of the malodor was initiated by an occurrence of intense malodor at the bulk storage facilities of an aerosol loader. Investigation of the system used for storing the nitromethane stabilized propellant showed a potentially active system for corrosion and oxidation-reduction reactions; several different metals (copper, iron, zinc). The lines, but not the tank, were badly corroded. There was indirect evidence of prior water contamination in the lines, although the propellant had not shown unduly high moisture concentrations; less than the saturation concentration.

Samples were taken from the walls of the corroded lines and three other lines at the site. One of the three lines was also corroded. Both corroded lines contained brown deposits that were shown by X-ray diffraction/fluorescence to be mixtures of crystalline oxides, $FeO/Fe_3O_4/Fe_2O_3$, containing traces of other metals used in construction.

The chloride analysis of deposits from both corroded lines was 12 per cent. In contrast, the deposits from the relatively uncorroded lines were amorphous, very hygroscopic, and had a chloride analysis of 28 to 30 per cent. This indicated that, under normal operation, some anhydrous $FeCl_2/FeCl_3$ may form on the walls of propellant lines. Once gross amounts of water are introduced, the chlorides are hydrolyzed, releasing their HCl, *via* the following sequence:

$$\text{IX.}\quad 2FeCl_2 + 3H_2O \rightarrow FeO\,(\text{hydrated}) + Fe(OH)_2 + 4HCl$$
$$\text{X.}\quad 4FeO \rightarrow Fe_3O_4 + Fe$$
$$\text{XI.}\quad 2Fe(OH)_2 + 1/2O_2 \rightarrow Fe_2O_3 + 2H_2O \quad (6)$$

The HCl released by this hydrolysis as well as from the reduction of propellant 11 is then removed by further corrosion processes at the metal surface.

It is relevant at this point, that during these hydrolytic processes at the metal surface, more H_2O is also being produced by reduction of nitromethane (Eq. V).

METHODS

A. IDENTIFICATION OF METHYL ISOCYANIDE; PLASMA CHROMATOGRAPHY

Plasma chromatography is a method developed for identification of trace materials in concentrations of $10^{-6}-10^{-10}$ v/v and below. The technique has been described in de-

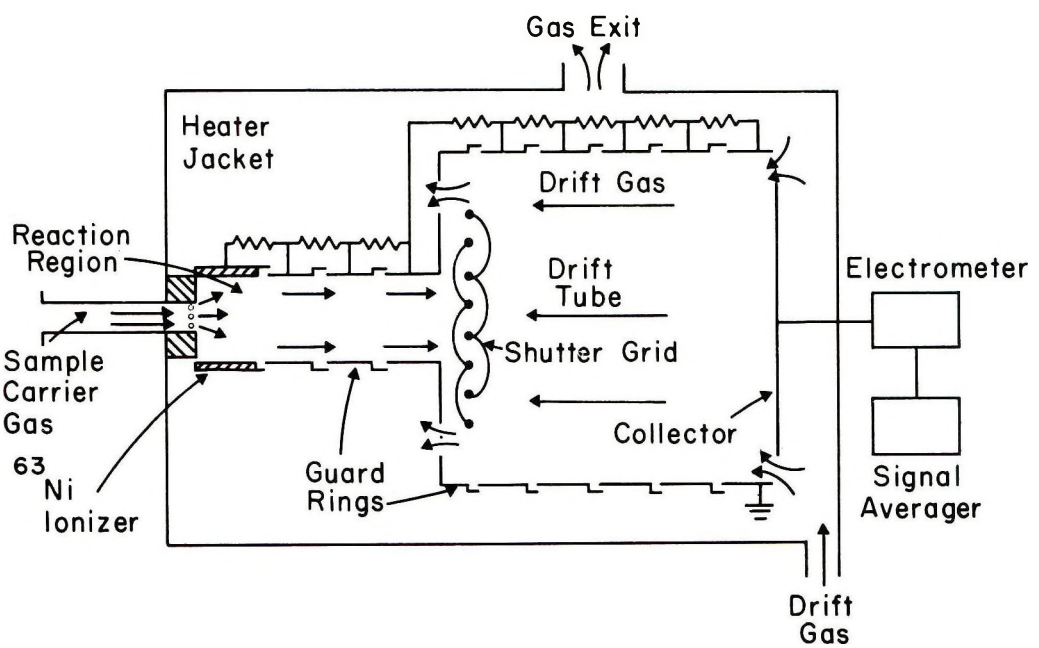

Figure 1. Plasma chromatograph (ion drift spectrometer).

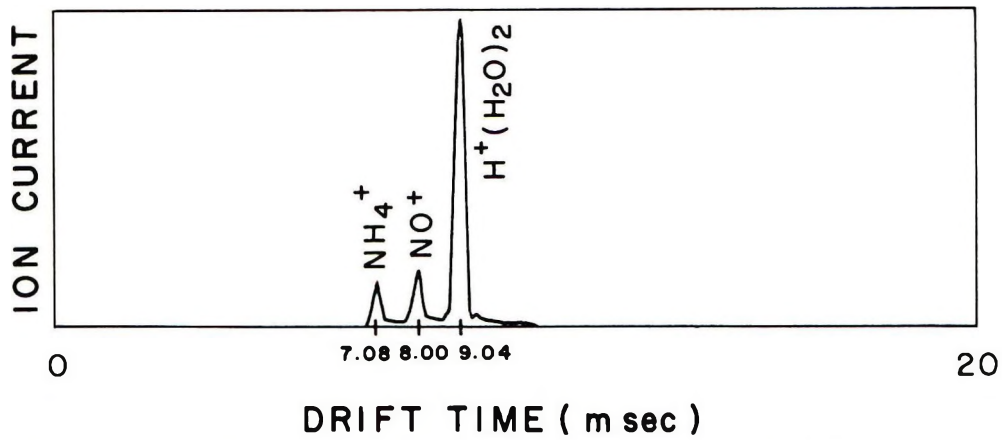

Figure 2. Plasmagram of N_2 carrier gas. Conditions: carrier gas (ultrapure) 100 cc/min; drift gas N_2 (ultrapure) 500 cc/min; temperature = 204°C; pressure = 760 mm Hg; scan time = 10.2 sec; gate width = 0.2 m sec; voltage = 3000 V/14 cm.

tail (7,8,9). Gas samples were obtained not only from contaminated propellant, but also from contaminated filters removed from the bulk storage facility. Vapor was sampled from filters enclosed in a plastic bag.

In this application of Plasma Chromatography, also called "Ion-mobility Spectrometry," sampling was accomplished with a gas tight syringe. The sample interacts with background ions produced by $^{63}Ni\,\beta$-rays.

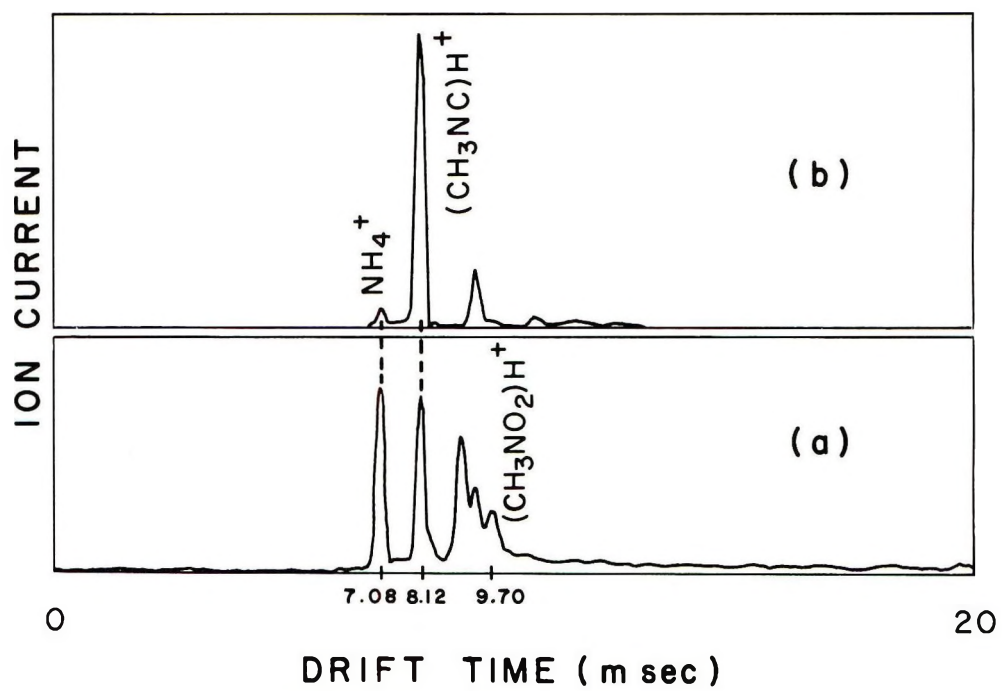

Figure 3. (a) Plasmagram of vapors from contaminated line filters; (b) plasmagram of pure CH_3NC.

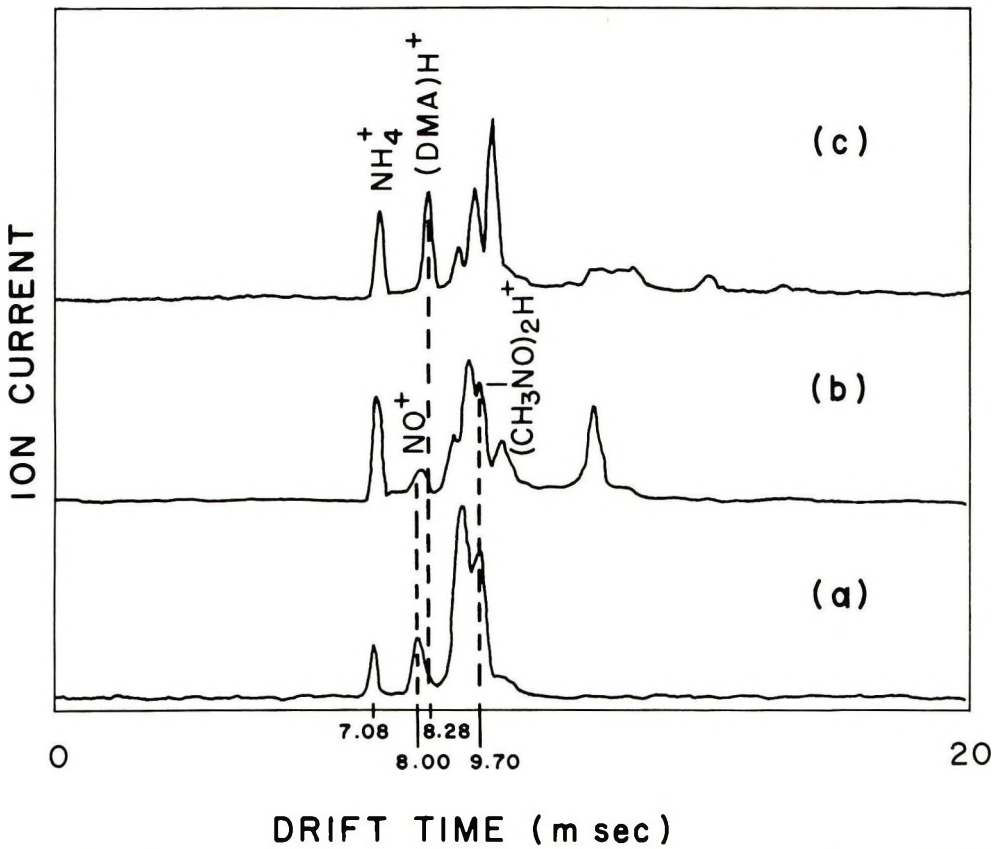

Figure 4. (a) Plasmagram of 60/40 propellant 12/11 containing *ca.* 0.12 per cent CH_3NO_2; (b) plasmagram of contaminated 60/40 propellant 12/11 containing CH_3NO_2; (c) plasmagram of DMA.

The resultant ions are propelled by an electric field in a counter flowing stream of nitrogen (Fig. 1). The characterization of the contaminants is then made on the basis of drift time and the approximate mass of aggregates of the type $[M(H_2O)]H^+$. This method is 3 to 4 orders of magnitude more sensitive than conventional Mass Spectrometry and is conducted at normal atmospheric pressures.

For the present study, methylisocyanide was prepared by a standard procedure (10) from freshly distilled N-methyl formamide and p-toluenesulfonylchloride in quinoline.

Vapor samples from contaminated propellant and filters were obtained using gas tight microliter syringes. It was necessary to enclose the filters in plastic bags in order to obtain a sufficient amount of sample. Figure 2 shows the plasmagram of the background in clean N_2 carrier gas at 204°C. Figure 3(a) shows a plasmagram of vapors from the contaminated filter and Fig. 3(b) a plasmagram from a control sample of pure CH_3NC. Both plasmagrams have a pronounced peak at 8.12 μsec. drift time, which is characteristic of CH_3NC.

A standard mixture of 60/40 propellant 12/11 containing CH_3NO_2 stabilizer gave the plasmagram shown in Fig. 4(a). The peak at 9.70 μsec. was shown to be due to CH_3NO_2. Comparison of this with the plasmagram of a contaminated sample of propellant (Fig. 4(b)) showed an additional peak at 8.28 μsec., which was identified as

dimethylamine (DMA) by comparison with a sample of pure DMA (Fig. 4(c)). DMA is a known reduction product of CH_3NC (11).

B. LABORATORY STUDIES ON CH_3NC FORMATION (SEE TABLE I FOR DETAILS)

All laboratory studies were run in 3-oz capacity Fisher-Porter Aerosol* compatibility tubes, type 110-007, fitted with a manifold fitted with an Ashcroft Maxisafe gauge† (30 psig vac to 100 psig pressure) and two Whitey‡ needle valves. Materials that were liquid or solid under ambient conditions were loaded directly into the tube. For materials that were gases under ambient conditions, the tube was placed on the manifold, cooled in a dry ice propellant 11 mixture, and evacuated (oil pump). A cylinder of the desired propellant was connected to the manifold and propellant condensed into the tube.

Purging air from the tubes was generally accomplished by heating the tubes in a water bath until an internal pressure of 60 psig was reached and then venting off small amounts of vapor by opening the Whitey valve.

Most runs were made with the tube contents agitated by a finned magnetic stirrer and heated in a water bath using a Thermo-Stir hot plate.#

The reaction studies were conducted at two different laboratory sites. Runs 1 to 8 were run in a laboratory where lack of night supervision required shut down of heating every evening and weekend. Furthermore, these reactions were run during the winter. Gauge pressures with P-11 run were often less than zero psig on workday mornings and absolute certainty of anaerobic conditions could not be assumed. All other runs were made where continuous heating was possible throughout the run and positive pressure was assured. Isocyanide formation could only be detected when air was deliberately admitted to the tubes (Runs 9 to 17). Run 16 was made without purging air so the moisture content may have been slightly greater than the 15 ppm specification of P-11/CH_3NO_2 blends, but considerably less than the saturation point of 110 ppm in H_2O in P-11 (at 25°C). Run 17 was made with FC-21 which contained 65 ppm P-11. Besides running this experiment in the presence of air, a larger amount of water was added because FC-21 has a greater H_2O saturation concentration than P-11.

In addition to the above, duplicate sets of runs were made with Propellant 11 containing *ca.* 0.3 per cent CH_3NO_2 in pipe bombs fashioned from both corroded and uncorroded pipe from Propellant 11 service. Runs were made both in the presence and absence of air for one month in ovens held at 60°C. No isocyanide odor was detected.

MATERIALS

Propellant 11 and methylene chloride were commercial materials and better than 99.8 per cent pure. Fluorocarbon 21 (50 to 500 ppm P-11) was obtained as a byproduct of Propellant 22 manufacture and purified by distillation. It was 99.9 per cent pure by gas liquid chromatography (GLC). Nitromethane** was of 95 per cent minimum purity, 99 per cent minimum nitroalkane. All other reagents were commercial ma-

*Fisher-Porter Co., "Lab-Crest" Division, Warminster, PA.
†Ashcroft Division of Dresser-Industries, Stratford, CT.
‡Whitey Co., Cleveland, OH.
#Penninsular Manufacturing Co., Gainesville, Fl 32602.
**Commercial Solvents Corp., 245 Park Ave., NYC 10017.

Table I
Summary of Experiments on Generation of Methyl Isocyanide

Run Number	Organic Substrate	H_2O (ml)	Metals(s)	Other	Time (hours)	Remarks
1.	P-11 50 ml, CH_3NO_2 0.15 ml	0.5	Galvanized Iron (Note 1)	$FeCl_3$ 50 mg	696	Strong stench, typical CH_3NC
2.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g/Cu powder, 0.2 g (Note 2)	None	892	Strong stench, typical CH_3NC
3.	FC-21 50 ml, CH_3NO_2	0.8	Galvanized Iron	None	648	No odor change, extensive corrosion, milky liquid
4.	FC-21 50 ml, CH_3NO_2 0.2 ml	0.8	Fe Wire, Cu Wire, (Note 3)	None	648	Slight odor change; unidentifiable. Brown particulate matter, brown liquid
5.	P-11 50 ml	0.004	Galvanized Iron	CH_3NH_2 HCl 0.25 g	48	Very strong CH_3NC stench, corrosion milky liquid
6.	P-11 50 ml	0.004	Fe powder, 1 g Cu powder, 0.2 g	CH_3NH_2 HCl 0.25 g	48	Definite CH_3NC odor, turbid liquid
7.	FC-21 50 ml	0.004	Fe powder, 1 g Cu powder, 0.2 g	CH_3NH_2	96	No odor, green liquid after 2 h
8.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g	Fe_3O_4 (Note 4)	500	No odor change
9.	P-11 50 ml, 0.15 ml CH_3NO_2	0.08	Fe powder, 1 g	$FeCl_2 \cdot 4H_2O$, 0.05 g air purged	396	No odor change
10.	P-11 50 ml, 0.18 ml CH_3NO_2	0.08	Fe powder, 1 g	$FeCl_2 \cdot 4H_2O$, 0.05 g air admitted (Note 5)	64	Strong CH_3NC odor
11.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g	$FeSO_4 \cdot 7H_2O$ 0.05 g air purged	400	No odor change
12.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g	$FeSO_4 \cdot 7H_2O$ 0.05 g air admitted	216	Strong CH_3NC odor
13.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g	$FeCl_3$ 0.05 g air purged	500	No odor change
14.	P-11 50 ml, CH_3NO_2 0.15 ml	0.08	Fe powder, 1 g	$FeCl_3$ 0.05 g air admitted	600	No odor change
15.	P-11 50 ml, containing ca. 0.05 wt. % CH_3NH_2	None	None	None	150	Amine odor

(Continued on page 772.)

Table I (*Continued*)

16.	P-11 50 ml, CH_3NO_2 0.15 ml	None	Fe powder, 1 g	$FeCl_2$ anhydrous 0.05 g (Note 6)	>500	No odor change
17.	FC-21 20 ml	0.08	Fe powder, 1 g	$FeCl_2$ $4H_2O$ 0.025 g air admitted (Note 7)	216	Very faint odor (questionable)

1. Galvanized iron strips were deliberately scratched to expose the underlying iron.
2. Iron powder, 200 mesh (J. T. Baker). Copper, electrolytic, dust (Fisher).
3. Iron and copper wire were twisted together in a coil to maintain bimetallic contact.
4. Fe_3O_4, "magnetite," commercial material, not necessarily identical to the hydrated Fe_3O_4 in corrosion deposits.
5. Air was admitted to the system by cooling the tubes until gauge pressure was zero psig and quickly opening the valve momentarily and then closing it tightly.
6. $FeCl_2$ anhydrous was obtained from Research Organic/Inorganic Chemical Corp.
7. The FC-21 contained 65 ppm P-11.

terials. All runs were made with the tubes immersed in water baths held at approximately 60°C.

DISCUSSION

Previous literature on Propellant 11 dechlorination, as cited in the references, demonstrates that the reaction is a homolytic reduction of Cl. The usual products formed in substantial amounts are HCl and FC-21. Nitromethane has been shown to be an excellent inhibitor of this reaction, although the mechanism of its inhibitory action is not clear.

Essentially, all of the uses of nitromethane inhibited systems have been in aerosol formulations with homogeneous liquid phases, e.g., ethanol formulations. Robey (12) has shown that peroxide initiated reaction between Propellant 11 and ethanol was markedly increased by added H_2O when the phase stability of the system was decreased, i.e., when a water-rich phase could separate. Robey also demonstrated the need for oxygen initiation and postulated alkylhydroperoxides as the true initiators.

The present study demonstrates the need for oxygen and a separate water phase in the formation of methyl isocyanide from nitromethane/propellant 11 systems (cf. Table I, Runs 9, 10, and 16). Since HCl is produced in this reaction, the well-known reduction of nitrocompounds to the corresponding amines by the iron/H+ system can readily occur. Any resultant amine will be formed as its salt, which will be soluble in the aqueous phase. The reduction of nitro compounds will, of course, produce more H_2O.

The source of the carbenic, or divalent carbon in the CH_3NC appears to be the Propellant 11 and not FC-21 since FC-21 did not produce CH_3NC under any of the conditions where propellant 11 formed isocyanide. It is more than likely that the FC-21 and isocyanide are derived from a common unstable intermediate, be it radical or carbene.

The conclusions above explained the field observations in all respects but one; moisture analyses on malodorous samples taken from the contaminated storage facilities were not unduly indicative of a separate water phase. This apparent contradiction was resolved by the work of C. C. Seastrom (13) and T. N. Jones, III (14), who

demonstrated that HCl formation will lower the H_2O saturation level of Propellant 11, leading to the formation of a separate phase of aqueous HCl that will tend to wet out on the surface of the vessel. We have carried this one step further, demonstrating that corrosion products and processes will also remove H_2O from propellant 11. Examples of this are shown in Table II.

Table II
Effect of Corrosion Products Upon H_2O Analyses in Propellant 11 Containing 0.3 per cent CH_3NO_2[a] [b]

Initial H_2O ppm	76, 76
$FeCl_3$ added	8, 4
Fe_3O_4 added	7
Mixture of Fe/Cu powder added	15

[a] Approximately 4 days elapsed between treatment and analysis.
[b] Analyses by Karl-Fischer method.

Thus, once corrosion processes begin, any water contamination may go unobserved.

The presence of several metals in the system with different positions in the electromotive series, is a more active electrolytic system and, therefore, is more active in promoting oxidation-reduction reactions (Table I, Runs 1-6) whether or not the second metal is above or below iron in the electromotive series.

The exact role of oxygen in initiating the reaction is not yet certain. The unusually high activity of the ferrous ion/iron system in accelerating the reaction (Table I, Run 10) is also uncertain. It is known, however, that oxygen may be cathodically reduced to hydrogen peroxide and that ferrous ion/H_2O_2 is a common initiator of free radical reactions.

The reaction $Fe^{++} + H_2O_2 \rightarrow OH^\circ + OH^-$ is unlikely to be the source of 'Cl' abstraction, since hydroxyl radicals have a low affinity for halogen in polyhalocarbons. On the other hand, the hydrogen of H_2O would also be an unlikely source of reduction until, or unless, acid were present in the system. In the propellant 11/ethanol system, the hydrogen source is the CH_2 group, not the OH, as shown by (2) and (3).

A possible explanation for the lower activity of $FeSO_4$ compared to $FeCl_2$ is the known lower corrosivity of sulfate ion to ferrometals compared to halide ions (Table I, cf. Runs 10 and 12).

Still more puzzling is the role of oxygen and ferrous iron in the presence of the large excess of metallic iron. Ferric iron is inactive (Table I, Run 14) or possibly inhibitory in this system, yet $Fe^{+++} + Fe^\circ \rightarrow Fe^{++}$. Ferrous iron is readily oxidized to ferric ion by oxygen.

Known radical promoting systems such as Fe(II)/Fe(III) or Fe(II) + H_2O_2 were not run because they would not deal with the reality of a system containing excess iron, oxygen and ferrous ion. Clearly, there is room for further study.

CONCLUSIONS

Methyl isocyanide is the cause of malodor in nitromethane stabilized propellant 11 undergoing reduction in iron storage containers. A separate water phase must be present initially for the reaction to proceed, i.e., the H_2O concentration must exceed the satu-

ration level in the propellant. Once dechlorination of the propellant is initiated, any water is forced out of the organic phase, along with the acid formed. The resultant aqueous acid wets out on the metal to cause corrosion and reduce nitromethane to methylamine. The methylamine then scavenges the reactive intermediates from the reduction of the propellant to form, ultimately, the methyl isocyanide and further reduction products, such as dimethylamine. Oxygen is necessary for the reaction to proceed. The reaction will proceed more readily if galvanic systems of dissimilar metals are present.

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BOOK REVIEWS

CTFA COSMETIC INGREDIENT DICTIONARY, 2nd Ed., Edited by Norman F. Estrin, Ph.D. The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C. 1977, IX + 513 pages. Price $45.

This long awaited, greatly expanded revision of the CTFA Dictionary, first published in 1973, can without hesitation be described as a monumental accomplishment. It required prolonged and tireless efforts on the part of a great many individuals, drawn from the various scientific and technical disciplines which make up the cosmetic industry, to produce this sizable, well organized and clearly printed lexicon of necessary information.

The book is dedicated to the world's consumers, perhaps because the various consumer movements were instrumental in pointing out the need for clarification of names, definitions, structures, composition, etc. However, the Dictionary is of immense value to the cosmetic chemist, to the cosmetic industry and to various government agencies concerned with cosmetics. Although the first four chapters are brief introductory ones, they are important because they establish the ground rules for standardizing the procedures which are employed in chapters V through IX pertaining to nomenclature, abbreviations, Register and CRMCS numbers, information sources, chemical and trade names.

Chapter V is an extensive monograph section (350 pages) consisting of brief descriptions of cosmetic raw materials. Chemical configurations are presented for most well-defined substances and information sources are listed—very useful when a detailed follow-up is necessary.

Chapter VI consists of a listing (109 pages) of chemical terms, trademarks, generic names and common names cross-indexed to CTFA Adopted Names. This is a valuable aid to the formulating chemist who is looking for alternate sources of supply although, as the editor points out, CTFA Adopted Names do not reflect all the components present in a product. The importance of this section to those concerned with product labeling cannot be overemphasized.

Chapter VII is an alphabetical index listing names, addresses and telephone numbers of suppliers of all the trade name ingredients included in the Dictionary.

Chapter VIII is concerned with indexing the numerical listing of Chemical Abstract Services (CAS) Registry Numbers to Recognized Disclosure Numbers for CTFA Adopted Names.

Chapter IX performs a similar indexing function; i.e., indexing Raw Material Composition Statement Numbers (CRMCS Numbers) to trade named materials included in the Dictionary.

This reviewer feels safe in predicting that this revised CTFA Dictionary will be used regularly by all persons in the cosmetic industry who have a need to refer to such respected reference manuals as the U.S. Pharmacopeia and the Merck Index. The book is well-written, in concise, crisp English which will bear translation into many foreign languages without undue difficulties. It undoubtedly will enjoy worldwide distribution and use, although periodic supplements will have to be issued to keep the Dictionary up-to-date.—LESTER I. CONRAD—Amerchol

ENCYCLOPEDIA OF CHEMICAL PROCESSING AND DESIGN, Vol. 1, A/ACR. Edited by John J. McKetta. Marcel Dekker, Inc., New York, 1976, xiii + 496 pages. Price $95.00 per individual volume; $1500.00 for the set of 20 volumes.

This is the first volume of an encyclopedia of current chemical technology, with emphasis on practical design and the economics of operation. Twenty volumes are planned (two per year) to cover the entire field of chemical processing and design.

The intent is to serve the needs of chemical engineers, designers, managers, and technical people in the chemical industry who are involved in day-to-day work in the plant. Technologically important processes, materials, practices, products, and standards are described by competent authors for, not only the chemical industry, but also such related dustries as plastics, petroleum, and rubber. Generally, the style is readable and informative.

Articles in the first volume include: Abrasives, Coated Cutting Tools; Absorption; Absorption, Falling Film; Acetaldehyde; Acetal Resins; Acetate and Triacetate Fibers; Acetic Acid: Acetic Acid Derivatives; Acetic Anhydride; Acetic Anhydride Design Problem; Acetone Design Problem; Acetylene and Derivatives; Acrolein and Derivatives; Acrylic Acid and Derivatives; Acrylic Emulsions; and Acrylonitrile-Butadiene Rubbers.

Comprehensive surveys of the fundamental chemistry, as well as industrially-important reactions, are presented in such articles as those on acetaldehyde, acetic acid, acetylene, and acrolein. The authors have included helpful bibliographies, flowsheets, tables, graphs and other illustrations; mathematical and chemical equations are presented where necessary, but not in such profusion as to be burdensome. Paper and printing are of good quality, with only rare misspellings. A detailed analytical index to the entire encyclopedia will appear in the final volume.

This reference work will be a useful acquisition for libraries.—ALFRED WEISSLER—Consultant, Chevy Chase, Maryland.

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

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

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

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

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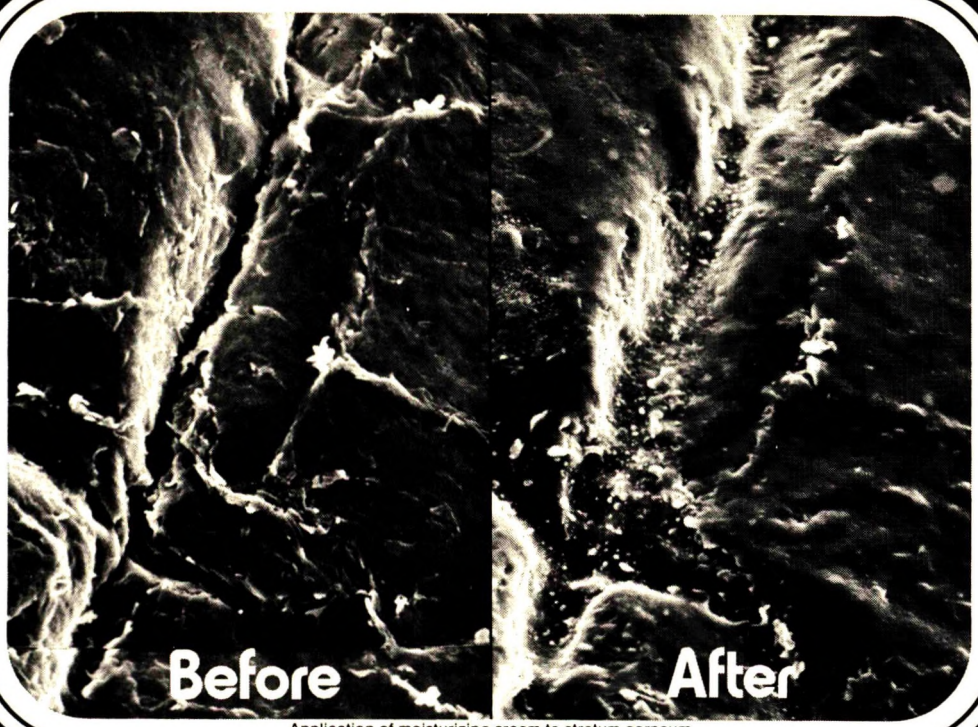

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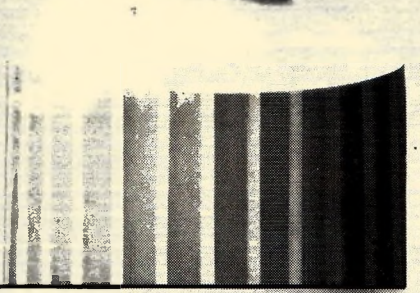

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