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Journal of the Society of Cosmetic Chemists

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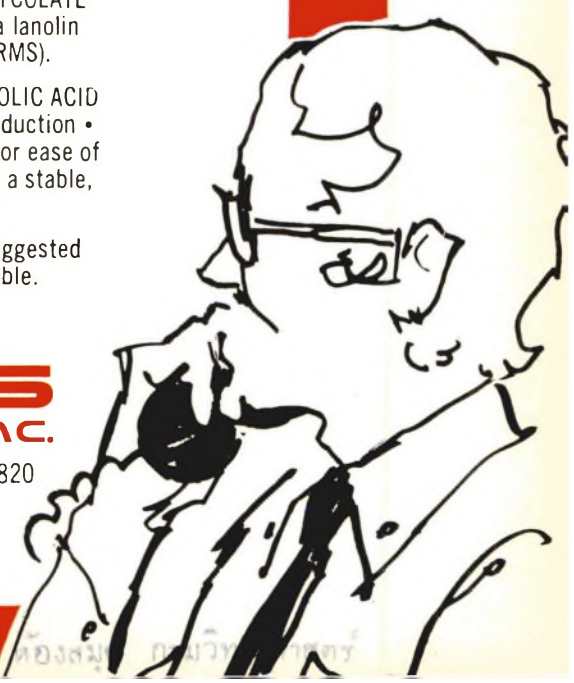

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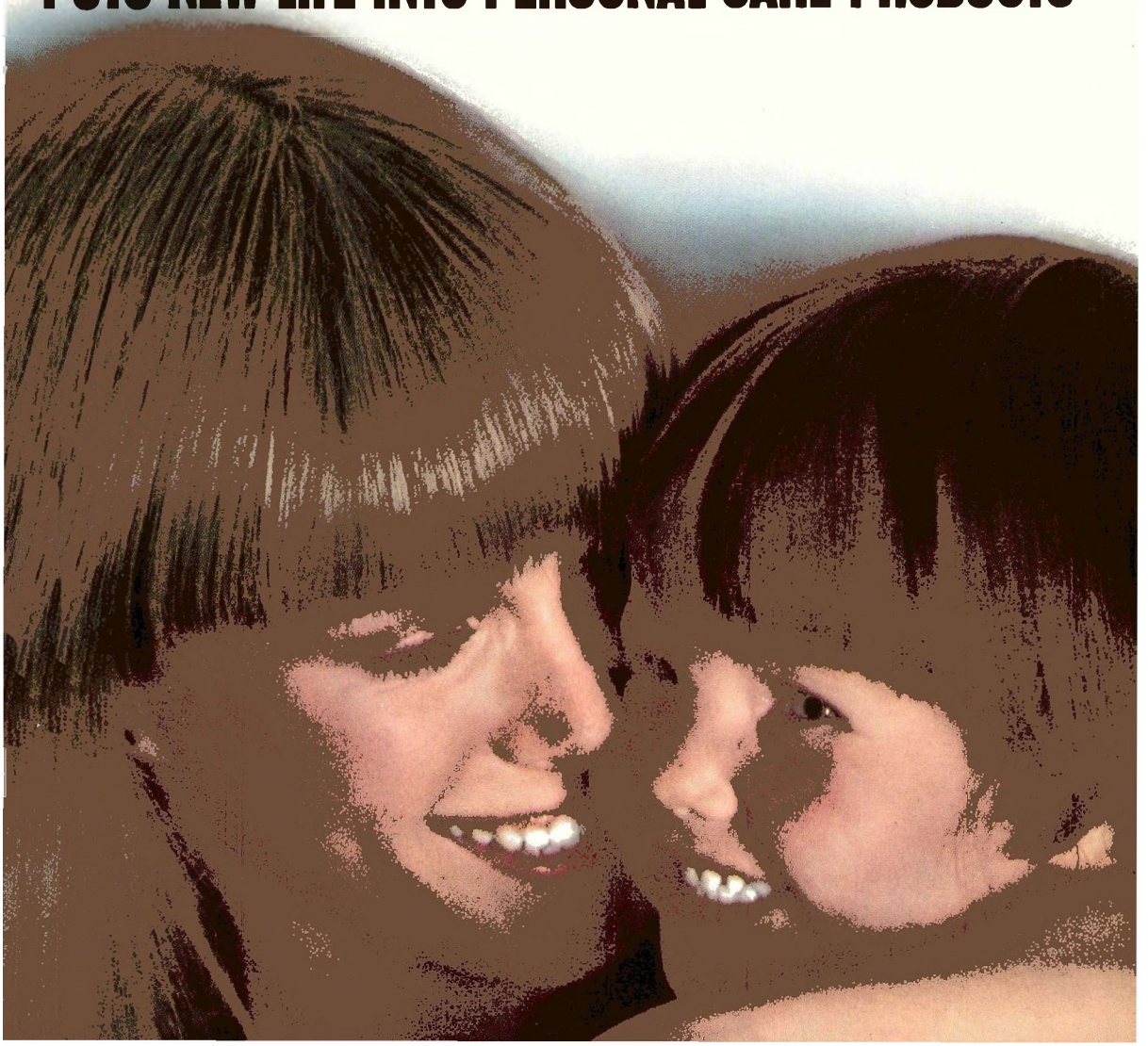

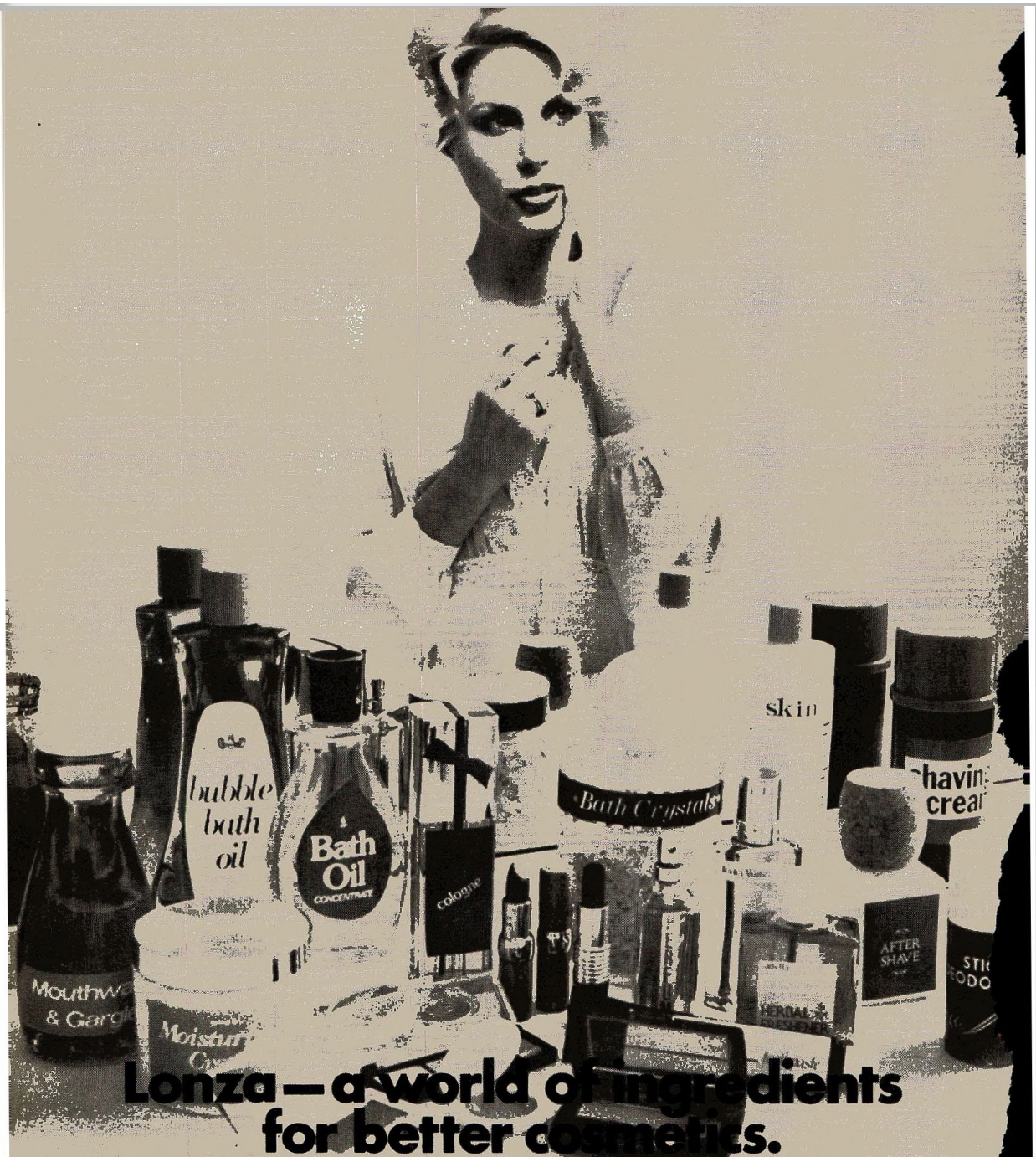

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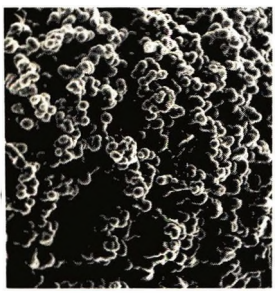

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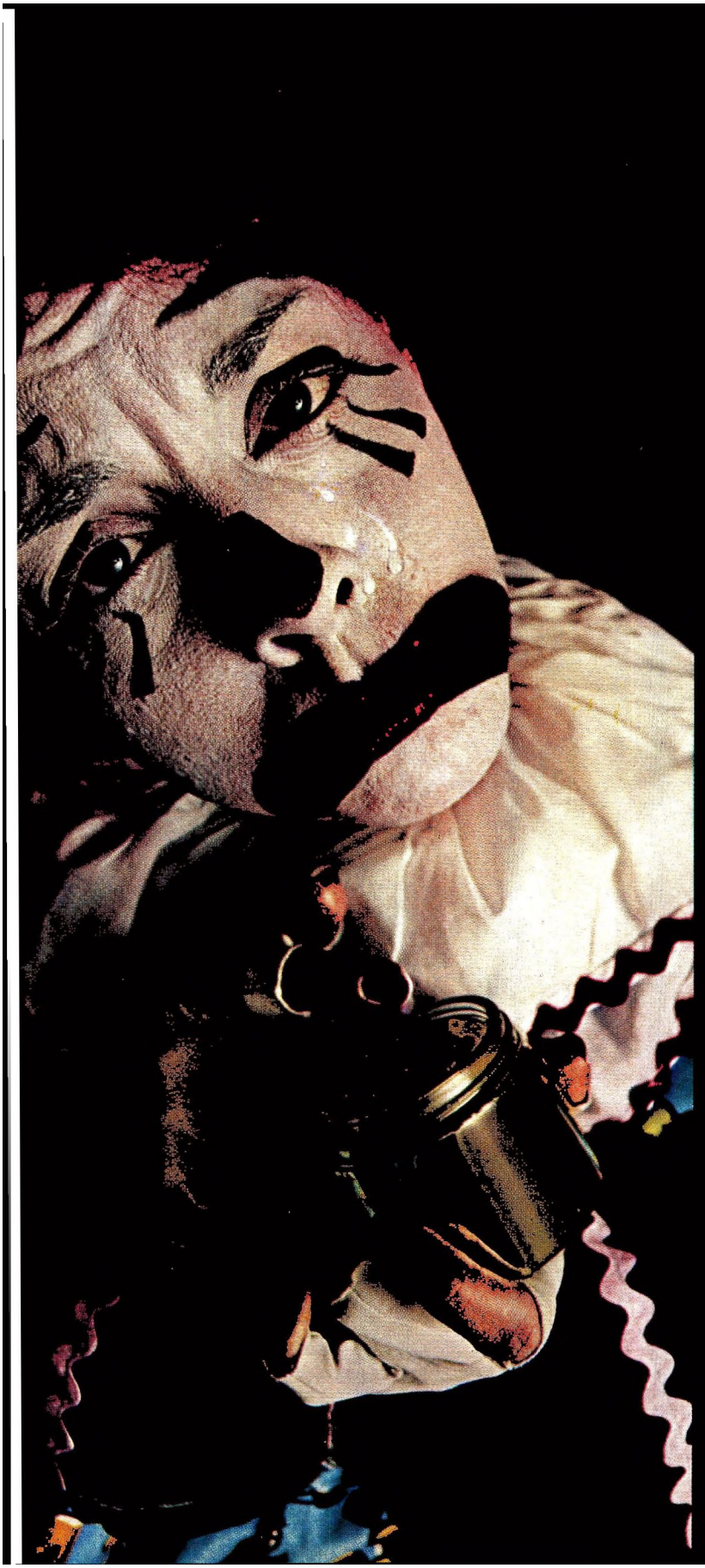

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SYNOPSES FOR CARD INDEXES

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

Laboratory methods for appraising the efficacy of sunscreens: K. H. Kaidbey and A. M. Kligman. *Journal of the Society of Cosmetic Chemists* **29,** 525 (September 1978)

Synopsis—Appraisal of a sunscreen entails measurements of an ensemble of capabilities, viz., determination of the sun protection factor, resistance to sweating, wash-off and abrasion, capacity to form a horny layer reservoir and protection against long ultraviolet rays (UV-A).

There is a good correlation between results obtained in sunlight and in the laboratory with the xenon solar simulator, but not with other "sunlamps."

Quantitative data are provided for a number of proprietory sunscreens ranging from chemical absorbers and physical blockers to polymeric film formers.

Use of microspectrophotometry in dermatological investigations: L. Grove, M. Lavker and M. Kligman. *Journal of the Society of Cosmetic Chemists* **29,** 537 (September 1978)

Synopsis—Quantitative estimation of dimensions of structure and amounts of material in skin at the light microscope level has, until now, required time-consuming and tedious methods that are often subject to observer error. We have overcome these problems by using a Vickers M86 scanning-integrating microspectrophotometer. This analytical light microscope detects the amount of light which can pass through a specimen and then electronically converts this value into units of absorbance and projected area. This approach is very versatile and is in fact applicable to any biological structure which can be identified at the light microscope level and in which an appropriate change in color intensity can be realized. The fundamental principles of visible light microspectrophotometry and its application to dermatological studies that objectively evaluate the pathophysiological status of skin are described.

Der einfluss erogener duftstoffe auf die visuelle wahrnehmung erotischer reize: W. Steiner, E. Hanisch and D. Schwarz. *Journal of the Society of Cosmetic Chemists* **29,** 545 (September 1978)

Synopsis—Pictures of multiple content were presented to twenty male subjects with the aid of a tachystoscope. As a rule, an erotic scene and a city view were projected simultaneously with one or the other dominant. The time of presentation ranged between 1/250 and 1/125 of a second. The presentation of pictures was conducted with and without simultaneous stimulation from presumably erogenous odors, for which perfumes available on the market were used. It was shown, with a high degree of significance, that the sex motives during odor presentation were more readily recognized even under adverse viewing conditions than the building scenes. Changes in the viewing time did not alter the influence of the odor on the results.

A new technique to assess sunscreen effectiveness: E. Pines. *Journal of the Society of Cosmetic Chemists* **29,** 559 (September 1978)

Synopsis—In this study photoacoustic spectroscopy (PAS), a new technique recently developed for the study of solid, semi-solid and biological samples, was used to obtain in situ ultraviolet absorption spectra from which the sunscreening effectiveness and the substantivity to skin of various formulated sunscreens were evaluated.

The uniqueness of PAS allows the measurement to be made directly on the sunscreen formulation applied to excised full-thickness, newborn rat skin. Thus the parameters which govern the spectral properties of the skin-sunscreen agent complex are maintained close to those of the "in use" situation.

Evaluation of a polymeric film-forming sunscreen preparation in tranquilized hairless mice: S. W. Scott, J. Suskevich and A. H. Cambell. *Journal of the Society of Cosmetic Chemists* **29,** 565 (September 1978)

Synopsis—A method is described for the evaluation of sunscreen preparations using hairless mice. The mice were treated with tranquilizers to prevent removal of the agents by grooming. Three preparations were evaluated: 5% p-aminobenzoic acid in absolute ethanol; 5% p-aminobenzoic acid in 55% ethanol with skin moisturizers, and 3.3% octyl dimethyl p-aminobenzoate in a vehicle that forms a polymeric coating on the skin. Each product was evaluated two ways: 1) exposure to fluorescent sun lamps and 2) immersion in water followed by exposure to the sun lamps. All three products provided comparable protection, provided the animals were not immersed in water. Only the product which formed a polymeric film provided suitable protection following immersion in water.

In vivo measurement of transepidermal water loss: B. Idson. *Journal of the Society of Cosmetic Chemists* **29,** 573 (September 1978)

Synopsis—An overview is presented of the background and principle methods for measuring transepidermal water loss (TWL) in vivo. Absolute values of TWL are a function of the particular technique and experimental conditions. TWL will vary with different skin sites and rise markedly if the skin barrier is removed or affected by pathologies. Early gravimetric methods lack sensitivity and require long testing periods as well as large areas of skin. The disadvantages have caused shifts to other techniques where absorption of water vapor is followed by a sensitive physical measurement. The majority of methods are based on determining the increase in moisture content of either a current of dried air or fixed humidity air conducted over the skin. Others have sought to avoid air flow, using changes in conductivity of inorganic crystals. Methods discussed include thermal conductance, electrohygrometry, infrared radiation, electrolysis of absorbed water vapor and calculation of vapor pressure gradient in the layer of air adjacent to the skin surface. The mechanism may be an additive effect of neural control of eccrine sweat gland activity and stratum corneum hydration.

The chemistry of nitrosamine formation, inhibition and destruction: M. L. Douglas, B. L. Kabakoff, G. A. Anderson and M. C. Cheng. *Journal of the Society of Cosmetic Chemists* **29,** 580 (September 1978)

Synopsis—*N*-Nitroso compounds are formed from the interaction of many types of organonitrogen compounds and nitrosating agents. Ease of nitrosation is determined by compound structure, nature of the medium and the presence of catalysts. The two categories, nitrosamines and nitrosamides, differ mainly in their chemical stability and mechanism of biological activity. Nitrosamines are more stable and difficult to destroy, but their formation can be inhibited by substances which react preferentially with the nitrosating agent. The carcinogenic activity of these compounds in laboratory animals varies widely from highly potent to innocuous.

J. Soc. Cosmet. Chem., **29**, 525–536 (September 1978)

Laboratory methods for appraising the efficacy of sunscreens

K. H. KAIDBEY and A. M. KLIGMAN *The Duhring Laboratory, Department of Dermatology, University of Pennsylvania, Suite 203, 3500 Market St., Philadelphia, PA 19104.*

Received February 10, 1978.

Synopsis

APPRAISAL of a SUNSCREEN entails measurements of an ensemble of CAPABILITIES, viz., determination of the sun protection factor, resistance to sweating, wash-off and abrasion, capacity to form a horny layer reservoir and protection against long ultraviolet rays (UV-A).

There is a good correlation between results obtained in sunlight and in the LABORATORY with the xenon solar simulator, but not with other "sunlamps."

Quantitative data are provided for a number of proprietory sunscreens ranging from chemical absorbers and physical blockers to polymeric film formers.

INTRODUCTION

Despite a plethora of commercial sunscreens, new ones are still being produced. This reflects the growing demand for more effective preparations. Not very long ago, Knox et al. (1) and Willis et al. (2) found that most marketed sunscreens provided only marginal protection. This is no longer true; a general upgrading has occurred. Moreover, modern formulations have tended to become more elegant and to possess greater powers against such stresses as sweating and swimming. Sunscreens after all are used under very exigent conditions in which environmental factors (high temperature, intense radiation, wind) and physical activity conspire to wipe out protection. Highly effective sunscreens must possess multiple capabilities.

Growing public awareness of the harmful effects of sunlight has also contributed to the expanding sunscreen market. The damaging effects of chronic sun exposure—premature aging and cancer—are becoming known to more and more people. Nonmelanoma skin cancer is the most prevalent of all human malignancies. Habitual use of effective sunscreens would substantially prevent these unhappy consequences of heedless exposure to the sun. Nonetheless, a rich tan is very highly prized. Sun worshipping is perhaps now the dominant religion in "advanced" countries. With increasing leisure, briefer clothing and longer life expectancy, there is even greater need for sunscreen which can protect the skin of all sorts of persons under all sorts of conditions be they

farmers, sailors, skiers, bathers, fishermen, workers, vacationers, retirees, playboys, etc. This is especially true for light-skinned persons who sunburn easily and tan poorly, notable examples of whom are persons of Celtic ancestry (Scotish-Irish-Welsh).

Laboratory assays are invaluable for appraising the efficacy of sunscreens prior to field trials. Unlike field studies, the conditions of exposure can be rigorously controlled. Sunlight is not only inconsistent from day to day but from hour to hour. In our experience, tests with sunlight give results which are far more variable than the solar simulator. We ourselves look to the laboratory for definitive study and resort to the outdoors for confirmation. Field studies are a final and necessary stage to show efficacy in real settings that impose rigors not present in the laboratory.

The methods (2) reported previously from this laboratory for assessing sunscreens are no longer adequate in view of the diversity of formulations and the demand for greater quantitative knowledge of their merits and limitations. This paper updates the methodology taking as examples some of the better known proprietory products. Needless to say, the sampling is small and arbitrary, though hopefully representative of the different types ranging from physical blockers to chemical absorbers of UV-B and UV-A.

MATERIALS AND METHODS

SUBJECTS

These were healthy Caucasian college students between the ages of 19 and 26 years. The untanned mid-back or flexor aspect of the forearm were used for testing. Informed consent was obtained. Panels of ten subjects were used for each test.

LIGHT SOURCE

This was a 150-W xenon solar-simulator. With the Schott WG-320, the emission in the sunburn range resembles the ultraviolet portion of mid-day summer sunlight in North temperate latitudes (3). With the Schott WG-345 filter, sunburning radiation is eliminated and the emission consists primarily of long ultraviolet radiation (UV-A) and some visible light. Intensity measurements at skin level were made with a calibrated Eppley thermopile (Eppley Laboratories, Newport, Rhode Island). The UV-B flux was 14.0 mW/cm^2 and UV-A 22.5 mW/cm^2.

THE MINIMAL ERYTHEMA DOSE

A series was given in which each exposure was 15 sec greater than the previous one. The minimal erythema dose (MED) was the least exposure which produced a uniform, mild erythema with a sharp border 24 hr later. The test agents were delivered to 2 cm squares of skin outlined by adhesive tape at a dose of 5 μl/cm^2, using either micropipettes (lotions and liquids) or 1 ml plastic tuberculin syringes (creams and ointments). The materials were spread evenly with thin glass rods.

THE SUN PROTECTION FACTOR (S.P.F.) IMMEDIATE PROTECTION

The S.P.F. is the ratio of the MED's on treated and untreated skin. Exposures were given 5 min after application. The sites were read 24 hr later.

RESISTANCE TO WASH-OFF

Treated *forearm* sites were covered by perforated plastic cups for 2 hr to enable diffusion into the horny layer. The forearm was then immersed in a tub of tap water at room temperature for 10 min, followed by air drying for 10 to 15 min. The challenge exposure was three MED's with reading of the degree of erythema on a 0 to 3 scale 24 hr later, as follows: 0, normal skin; 1+, minimal redness equivalent to one MED; 2+, more intense erythema; 3+, intense erythema and edema. This was found suitably discriminating and entailed considerably less effort than giving a series of exposures to determine the postimmersion S.P.F.

RESISTANCE TO SWEATING

The test agents were applied to the mid-back and the sites protected with perforated cups for 2 hr while the subjects remained inactive in an air-conditioned room. Sweating was then induced in an environmental chamber at 110°F and 65% R.H. for 30 min while the subjects sat upright. After leaving the chamber, the skin was air dried for 10 to 15 min and the sites challenged with three MED's.

CUMULATIVE EFFECT

This test measures the capacity of the material to form a horny layer reservoir. The agents were applied once daily each morning to the same forearm site for three consecutive days. The sites were not protected during this time; the subjects engaged in their usual activities. On the morning of the fourth day the arms were immersed in a tub of tap water at room temperature for 10 min, allowed to air dry and challenged with three MED's.

SCOTCH-TAPE STRIPPING

This test establishes whether the sunscreen is of the external type (on the surface) or internal (within the coherent portion of the stratum corneum). Practically speaking, this test corresponds to abrasion resistance and is preferred because of greater controllability. The test agents were applied as above to the same site once daily each morning for three consecutive days. On the fourth day the forearms were washed with soap and water and lightly dried with a towel. Each site was then stripped five times with scotch tape and challenged with three MED's.

DOSE-RESPONSE

S.P.F. values were determined immediately after application at three dosage levels: 2.5 μl/cm^2; 5.0 μl/cm^2 and 10.0 μl/cm^2.

PROTECTION AGAINST UV-A

This was assessed by determining the protective factor in skin pretreated with fluoranthrene, a polycyclic hydrocarbon photosensitizer. A solution of 20 μl of 0.5% fluoranthrene in 95% ethanol was applied to 2 cm squares of skin outlined by white adhesive tape, providing a dose of 5.0 μl/cm^2. After drying, the sites were covered with equal square patches of nonwoven cotton cloth (Webril, Curity) and fastened to the

skin by clear occlusive tape (Blenderm, 3M). Two hours later, the test sunscreens were applied at a dose of 5.0 μl/cm^2 and exposed to UV-A at 30-sec increments. The Minimum Phototoxic Dose (MPD) is the least exposure that produces erythema 24 hr later. The P.F. in this case is the ratio between the MPD in sunscreen treated and untreated skin.

COMPARISON OF LIGHT SOURCES

The S.P.F. values obtained by the solar simulator were compared to those from fluorescent sunlamp tubes. A bank of five Westinghouse FS20 fluorescent bulbs housed in an aluminum reflector was used at a distance of 15 cm. The UV-B flux (280 to 320 nm) at skin surface was 0.54 mW/cm^2.

RESULTS

IMMEDIATE PROTECTION

Fifteen proprietory sunscreens were evaluated. The S.P.F. values ranged from 3.8 to 13.5 (Table I). Nine of fifteen products had S.P.F.'s exceeding 8.0, indicative of

Table I
Sun Protection Factors[a]
(n = 10)

Sunscreen	Active Ingredients	Mean S.P.F. ± S.E.	Range
A	isoamyl-p-dimethyl aminobenzoate	3.8 ± 0.22	3–5
B	amyl dimethyl PABA	5.1 ± 0.35	4–7
C	homomenthyl salicylate 8.0%	5.3 ± 0.28	4–7
D	padimate and dioxybenzone	6.3 ± 0.47	5–9
E	titanium dioxide, 5.0% and menthyl anthranilate, 5.0%	6.6 ± 0.34	5–8
F	octyl dimethyl PABA 3.3%	7.3 ± 0.30	6–9
G	oxybenzone, 3.0% and dioxybenzone, 3.0%	8.8 ± 0.64	5–11
Zinc Oxide Ointment (U.S.P.)	zinc oxide	8.8 ± 0.67	7–11
H	cinoxate, 4.0% and menthyl anthranilate, 5.0%	9.0 ± 0.61	8–11
I	para aminobenzoic acid 5.0%	9.1 ± 0.62	7–11
J	sulisobenzone, 10.0%	9.2 ± 0.68	5–11
K	methoxy cinnamate	9.8 ± 0.93	8–15
Red Veterinary Petrolatum		12.6 ± 0.71	11–16
L	glyceryl PABA, 3.0% and octyl dimethyl PABA, 3.0%	13.1 ± 0.76	12–18
M	para aminobenzoic acid, 5.0%	13.5 ± 0.48	12–15

[a]In order of increasing efficacy.

Table II
Resistance to Wash Off

Sunscreen	Average Erythema[a] ± S.E.	Range
J	2.8 ± 0.16	0–3
Red Vet Petrolatum	2.2 ± 0.30	1–3
I	1.7 ± 0.12	1–2
Zinc Oxide Ointment	1.2 ± 0.27	0–3
H	1.2 ± 0.16	2–3
D	0.4 ± 0.20	0–1
C	0.4 ± 0.15	0–1
M	0.3 ± 0.25	0–2
F	0.1 ± 0.06	0–1

[a]Erythema 24 hr after three MED's:

0 = normal skin (complete protection)
1+ = minimal erythema
2+ = moderate erythema
3+ = intense erythema (little or no protection)

In order of increasing efficacy.

moderate-to-high protection. The highest values were obtained with formulations containing PABA and its esters. Nonetheless, the least effective (S.P.F.'s of 3.8 and 5.1) were also preparations containing PABA esters. S.P.F. values below 4.0 are indicative of poor protection. The benzophenones were intermediate. Zinc oxide ointment was quite effective as was red veterinary petrolatum.

RESISTANCE TO IMMERSION

Again, the test agents differed considerably (Table II). F provided virtually complete protection against three MED's in contrast to J and red veterinary petrolatum, which possessed but slight resistance to wash-off. It is noteworthy that four of the test agents performed very well with mean erythema values of 0.4 or less.

RESISTANCE TO SWEATING

Most of the agents withstood sweating well, with the exception of red veterinary petrolatum (Table III). The preparations containing PABA and its esters were the most effective.

Table III
Resistance to Sweating

Sunscreen	Average Erythema[a] ± S.E.	Range
Red Vet Petrolatum	2.3 ± 0.25	1–3
H	1.3 ± 0.45	0–3
J	1.1 ± 0.14	1–2
C	1.0 ± 0.21	0–2
Zinc Oxide Ointment	0.9 ± 0.29	0–2
D	0.7 ± 0.21	0–2
F	0.4 ± 0.14	0–1
L	0.3 ± 0.16	0–1
M	0.2 ± 0.14	0–1
I	0.2 ± 0.11	0–1

[a]Erythema 24 hr after three MED's.

Table IV
Cumulative Effects (Immersion)

Sunscreen	Average Erythema[a] ± S.E.	Range
H	2.8 ± 0.16	2–3
D	2.3 ± 0.42	1–3
J	2.2 ± 0.30	1–3
Zinc Oxide Ointment	1.8 ± 0.16	1–2
F	1.3 ± 0.36	0–3
L	1.0 ± 0.37	0–3
M	0.8 ± 0.30	0–2
I	0.8 ± 0.23	0–2

[a]Erythema 24 hr after three MED's.

CUMULATIVE EFFECT

Marked differences were observed among the test materials (Table IV). Those containing PABA and its esters showed high residual protection. Others, H in particular, demonstrated virtually no buildup.

SCOTCH-TAPE STRIPPING

Although removal of the uppermost superficial layers of the stratum corneum by stripping is a variable technique, there were striking differences. Protection from J, H and F was practically abolished (Table V). By contrast, I and L were highly effective.

DOSE-RESPONSE STUDIES

Protection was clearly dose-dependent (Figure). The S.P.F.'s increased with larger amounts of sunscreen, though not in any fixed way. With H increasing the dose from 2.5 to 5.0 μl/cm^2 practically doubled the S.P.F. At higher dosages (5.0 to 10.0 μl/cm^2), the increases in the S.P.F. for A and I were much less impressive than for H and zinc oxide ointment.

PROTECTION AGAINST UV-A

The test agents differed markedly. A PABA sunscreen (I) provided virtually no protection, while the benzophenone-containing agents were moderately effective, as were the physical blockers (Table VI). E, which contains 5% titanium dioxide, was more effective than zinc oxide ointment.

Table V
Cumulative Effects (Scotch-tape stripping)

Sunscreen	Mean Erythema Score ± S.E.	Range
F	2.63 ± 0.18	2–3
J	2.62 ± 0.18	2–3
H	2.50 ± 0.19	2–3
I	0.63 ± 0.26	0–2
L	0.50 ± 0.27	0–2

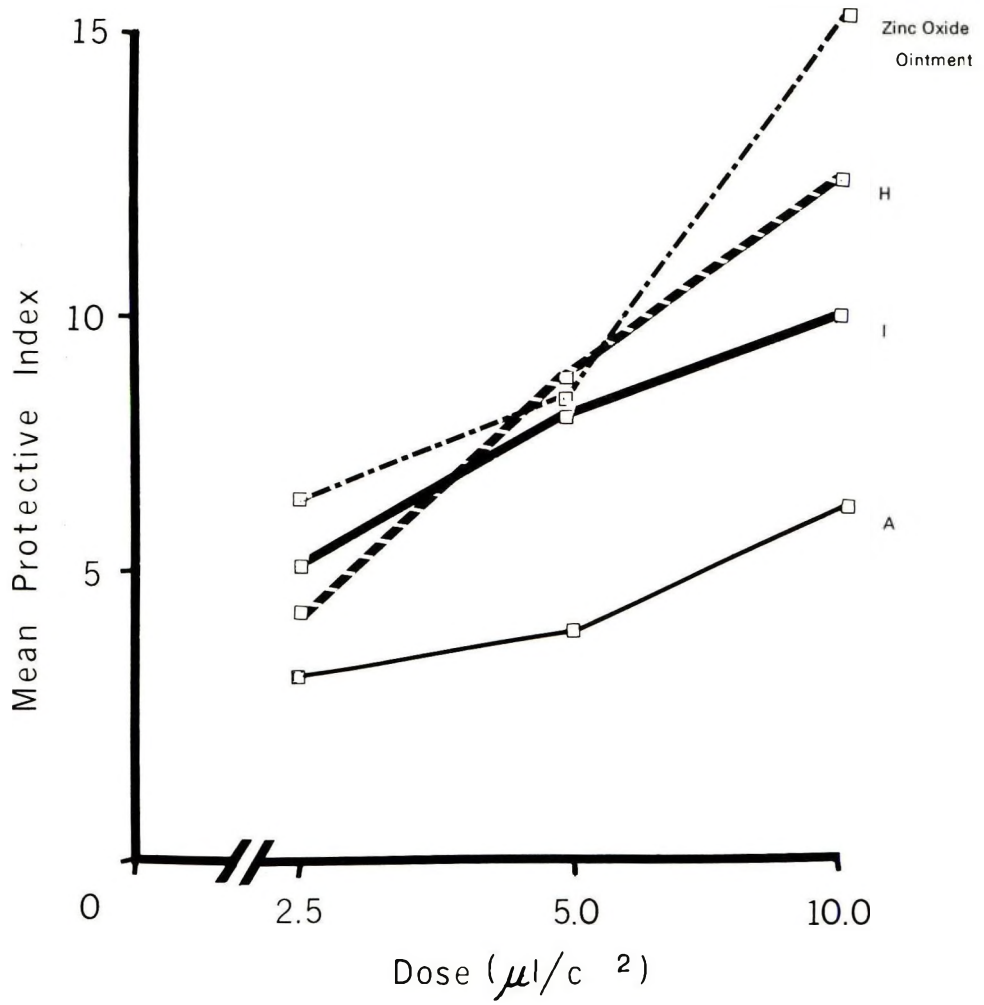

Figure 1. The influence of topical dose of sunscreen on the S.P.F.

Table VI
Protection Against UV-A Induced Phototoxicity to Fluoranthrene[a]
(n = 10)

Sunscreen	Mean P.F. ± S.E.	Range
I	1.2 ± 0.12	1.0–1.5
RVP	1.7 ± 0.17	1.0–2.0
H	2.0 ± 0.22	1.5–3.0
J	3.0 ± 0.62	1.7–5.0
Zinc Oxide Ointment	3.1 ± 0.55	2.0–5.0
E	4.4 ± 0.73	3.0–7.0
G	4.6 ± 0.83	2.5–8.0

[a]In order of increasing efficacy. P.F. values below 3.0 are indicative of poor protection.

Table VII
Solar Simulator Vs. Westinghouse FS-20 Sunlamps

Sunscreen ($5\mu l$ per cm^2)	Fluorescent FS-20 Sunlamp Tubes S.P.F. ± S.E.	Solar Simulator S.P.F. ± S.E.
J	13.4 ± 1.07	8.8 ± 0.68
A	7.2 ± 0.37	3.5 ± 0.22
I	62.0 ± 2.54	8.0 ± 0.42

COMPARISON OF UV-LIGHT SOURCES

In comparison to the solar simulator, the fluorescent sunlamps gave strikingly higher S.P.F. values for all the formulations (Table VII). The difference was most pronounced with I, where there was about an eightfold increase.

DISCUSSION

Recently, the Over-The-Counter (OTC) panel on topical analgesics has provided guidelines for the laboratory appraisal of sunscreens (4). These require the use of the solar simulator to determine the immediate S.P.F. of a specified UV-absorber at a dose of 2 mg/cm^2. This is intended to provide the consumer with an estimate of potency so that a choice can be made according to individual needs (skin type, geographic location, etc.) Valuable as this is, we regard the immediate S.P.F. as furnishing too limited information. Millions of persons have occupations or life styles which require the regular use of sunscreens under very diverse circumstances and different environmental stresses. Our data show that proprietory formulations vary markedly in their ability to withstand sweating, water wash-off and abrasion. Moreover, since we recommend daily use for persons at risk, it is important to assess other features of safety such as extent of percutaneous absorption as well as buildup in the horny layer reservoir. Often a sunscreen performs well in one test and poorly in another. Each formulation has a "personality"; a complex of features which may make it either exceptional or ordinary for particular uses and users. A comprehensive evaluation cannot be made without assessing these various properties. To develop appropriate methodology, we had to consider a number of factors of varying importance. Since these have not been adequately discussed in the literature, we shall briefly review our own thoughts and experiences.

Early on, the question arose whether S.P.F. values were related in any way to skin type. Fair-skinned, blue-eyed celts are far more susceptible to sunburn than darkly pigmented Mediterraneans. Would the S.P.F. for a given formulation be different in the two groups? We found that the mean S.P.F.'s were not different (unpublished observations). The S.P.F. is a ratio and is not influenced by the susceptibility of skin to sunburn. It is advantageous, however, to use fair-complexioned subjects since their MED's are lower; redness is more easily perceived and time is saved.

Another question is how do laboratory S.P.F.'s compare with those obtained with sunlight? The solar simulator mimics sunlight mainly in the sunburning UV-B range but not in other regions of the spectrum. UV intensity, for example, falls sharply above 360 nm. Sayre et al. found that the S.P.F. obtained with the solar simulator was higher than with sunlight (3.6 vs. 2.4) (5). Outdoor testing was done, however, at a tempera-

ture of 34°C and 85 to 90% relative humidity, conditions which favor sweating and dilution of the sunscreen. When laboratory testing was repeated after preheating the skin to 35°C, the S.P.F.'s were comparable. We have obtained excellent agreement for one well studied sunscreen (F). At a dose of 2 μl per cm^2, our laboratory S.P.F. was 4.1 ± 0.57 in ten subjects. In sunlight with a larger panel the S.P.F.'s at the same dose were 3.6 ± 0.4 before and 4.1 ± 1.96 after swimming (studies conducted by Paul Finkelstein, Ph.D., Johnson and Johnson Laboratories). This experience validates the solar simulator as a realistic instrument. Other pilot studies of our own show that the results secured with the solar simulator are applicable to the usage situation.

We found a wide range of individual S.P.F.'s for any single preparation; differences by as much as 50% being not uncommon. This variability is familiar to investigators who test sunscreens. Repeatability poses a real problem. Occasionally, tests conducted on the same person at different times will yield divergent results. It is well known that the MED may vary by as much as 50% in the same person from one day to another (6). The skin's surface does not resemble a glass slide. A variable amount of the test material is lost on the glass rod used to spread the test agents. This can have a large effect in view of the small amount applied. Even distribution of the test agents is difficult, especially with ointments and creams. We have long been aware that the variance is greater with ointments than with liquids. Delayed pigmentation may develop in dark complexioned persons, making it difficult to identify minimal erythema. This pigmentation is due to UV-A (7), which is transmitted freely by UV-B absorbers such as PABA and its esters.

As a check on undue variability it is advisable to include a standard formulation in every test. The OTC panel recommends a 4% ethanolic solution of PABA or, more recently, an 8% solution of Homomenthyl salicylate. These are convenient for UV-B absorbers. At 2 μl/cm^2, our S.P.F. for the 4% PABA standard is 4.6 ± 0.27 (unpublished observations). We customarily include a standard UV-B proprietary sunscreen, generally I, and when appropriate a UV-A absorber, generally J.

It was previously shown from this laboratory that hot quartz mercury lamps, which emit line spectra throughout the UV range, are woefully misleading for assessing sunscreens (2). The values were either falsely high or low depending on the absorption characteristics of the sunscreen. Our present findings show that this same handicap applies to fluorescent sunlamps. These emit a continuous spectrum that extends from about 280 to 360 nm, with about 55% of the energy below 320 nm. The inordinately elevated S.P.F. for I can be explained by the fact that the peak absorption of PABA parallels the maximal UV-B emission of the fluorescent sunlamp. We reemphasize that only the xenon solar-simulator provides realistic S.P.F.'s. Likewise, we would argue against the use of monochromatic radiation. Different wavelengths in the UV have different biologic effects and these may be augmentative rather than additive (8). Sunlight is of course polychromatic. It is inappropriate, therefore, to use monochromatic radiation.

It is clear that S.P.F.'s are significantly influenced by dose. The O.T.C. panel recommends a test dose of 2 mg/cm^2 (or 2 μl/cm^2 for liquids). This is entirely reasonable. We used 5 μl/cm^2 when we began our study and were constrained to continue with this dose for consistency. In several published studies, doses have ranged from 10 to 60 μl/cm (2, 9–11). Such amounts are greatly in excess of normal usage and yield artificially elevated S.P.F.'s. Schlagel et al. estimated the quantity of ointment and

cream necessary to sparingly cover the entire body when self-applied by a panel of volunteers (12). The amounts varied enormously ranging from 4.3 to 63.8 gm/m^2 with an average of 24.2 g/m^2 (or 2.4 mg/cm^2). There was no difference between the weights of ointment and cream applied. We conducted a similar, though more limited, study to compare a liquid, cream and ointment (unpublished observations). Five adult males applied petrolatum, cold cream U.S.P. and 25% aqueous propylene glycol to their entire bodies except the scalp. The sequence was first the solution, then the cream and finally the ointment at weekly intervals. The differences between the subjects using the same preparation were not great. Everyone, however, used considerably more of the solution (average 17.5 g/m^2 range 15 to 20 g) than the cream or ointment (average 9.1 range 6.5 to 11.5 g and 8.4 g/m^2 range 7.0 to 9.5 g, respectively). It is important to secure harder data concerning the amounts applied by the average consumer.

It is hardly surprising that the S.P.F.'s of commercial sunscreens differed so greatly, sometimes by as much as a factor of three. Clearly, consumers should be appraised of these differences so that persons at high risk can choose those which offer the greatest protection. In certain European countries the S.P.F. is printed on the label. This is very desirable provided the meaning of the value is adequately clarified. We find widespread misunderstanding in this regard even among specialists. A formulation with an S.P.F. of two will enable the user to stay out twice as long before developing a sunburn; in the average case, this might be 40 min. Suppose the sunscreen is then reapplied, will there be protection for another 40 min? Certainly not! During the first 40-min interval the subject would have received a certain subthreshold dose of UV. With reapplication, this threshold will be exceeded by the end of 2 hr and a sunburn will result. Complete protection cannot be afforded by reapplying the sunscreen every 40 min. The failure to develop redness does not signify that no radiation has reached the skin. Sunscreens are not perfect absorbers.

Among the UV-B absorbers some preparations were very good, some unimpressive. Previous studies have shown that PABA is superior to its ester derivatives (10, 13). Efficacy, however, depends on many factors such as concentration and vehicle design. An inappropriate base can vitiate efficacy, just as skillful formulation can greatly enhance activity.

Previous investigators have often reported greater S.P.F.'s than those given here. For the most part this is due to our having used lower dosages; but there are other reasons, too, such as the use of monochromatic radiation. Cripps and Hegedus (11) studied several preparations using monochromatic radiation at 305 nm. At a dose of 30 μl/cm^2, they found that an alcoholic solution of 5% PABA (I) was the most effective, with a S.P.F. of 17.6. Esters of PABA were less so and were comparable to benzophenones. However we obtained much better protection with red veterinary petrolatum (R.V.P.) for which they reported the astonishingly low S.P.F. of 3.2, compared to our 12.6. McCleod and Frainbell (14), also using monochromatic radiation at 305 nm and a dose of about 4.0 mg/cm^2, found that the S.P.F. for R.V.P. ranged between two and ten. With 5% PABA in 70% ethanol (2.5 μl/cm^2), their S.P.F. ranged from 8 to 17. Willis and Kligman, using a solar simulator, also reported an excessively high S.P.F., 23, for 5% ethanolic PABA solution, probably because of higher dosage (10).

The results of the sweating and water immersion tests were very informative. In general, physical blockers provided little protection while the chemical sunscreens

were much better owing to diffusion into the horny layer. The performance of F in the immersion test was outstanding, understandably so because it contains an acrylate polymer which forms an invisible film on the surface. It should be pointed out, however, that the film has little abrasion resistance: a few Scotch-tape strippings will remove it.

It is generally assumed that immersion and sweating are comparable tests, the former being merely more severe. We found important discrepancies, however. For example, postsweating protection with I was substantially higher than after water immersion, while the reverse was true, though to a lesser extent, for C suntan lotion. Also, the results with sweating are more variable, as expected.

The three-day cumulative assay clearly identified those sunscreens that have the capacity to form a reservoir in the skin. This was especially true for chemical sunscreens containing PABA and its esters. The resistance to water wash-off after only three daily applications was impressive. This finding has significant clinical implications and can be utilized to advantage by sunsensitive, fair-complexioned individuals.

As for protection against UV-A, both the benzophenone absorbers and physical blockers containing titanium dioxide offer moderate protection. More effective preparations are needed against these wavelengths as they can no longer by considered harmless. Not only is long ultraviolet radiation responsible for most photoallergic and phototoxic reactions, but UV-A is also carcinogenic to laboratory animals in large doses (15, 16). These rays penetrate to a far greater extent than UV-B (17, 18) and probably play a role in the induction of the deep dermal elastosis so typical of actinically damaged skin. UV-A also potentiates the harmful effects of UV-B (19). Unlike the latter, long UV wavelengths are present all year round, both in early morning and late afternoon (20). Protection against the dreadful, delayed effects of decades of mindless exposure to sunlight will require the development of better broad-spectrum sunscreens than are now available.

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Use of microspectrophotometry in dermatological investigations

GARY L. GROVE, ROBERT M. LAVKER and ALBERT M. KLIGMAN *Simon Greenberg Foundation, 3401 Market Street, Philadelphia, PA 19104.*

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Synopsis

Quantitative estimation of dimensions of structure and amounts of material in skin at the light microscope level has, until now, required time-consuming and tedious methods that are often subject to observer error. We have overcome these problems by using a Vickers M86 scanning-integrating microspectrophotometer. This analytical light microscope detects the amount of light which can pass through a specimen and then electronically converts this value into units of absorbance and projected area. This approach is very versatile and is in fact applicable to any biological structure which can be identified at the light microscope level and in which an appropriate change in color intensity can be realized. The fundamental principles of visible light MICROSPECTROPHOTOMETRY and its application to DERMATOLOGICAL STUDIES that objectively evaluate the pathophysiological status of skin are described.

INTRODUCTION

For many years, light microscopists have been obliged to rely on "eyeballing" to assess such items as acanthosis or atrophy of the epidermis, enlargement or shrinkage of sebaceous glands, and the amount of dermal ground substance in histochemically stained sections. Awareness of the crudeness of such estimates led to the development of more objective methods such as planimetry and stereographic grid analysis. Although these methods are certainly improvements, they also tend to be tedious, time-consuming and prone to human error. These problems led us to consider microspectrophotometry as an alternative method for analyzing dimensions of structure and amounts of material in the samples.

Until recently, this technique was considered to be of such an advanced nature that only specialized experts could dream of using it. The advent of commercially available instruments and improved histochemical methods have changed all this. Microspectrophotometry is currently at the heart of several exciting biomedical research projects and no doubt the next few years will see an increased number of instruments of this type being used in the routine situation for screening, diagnosis and other investigatory purposes.

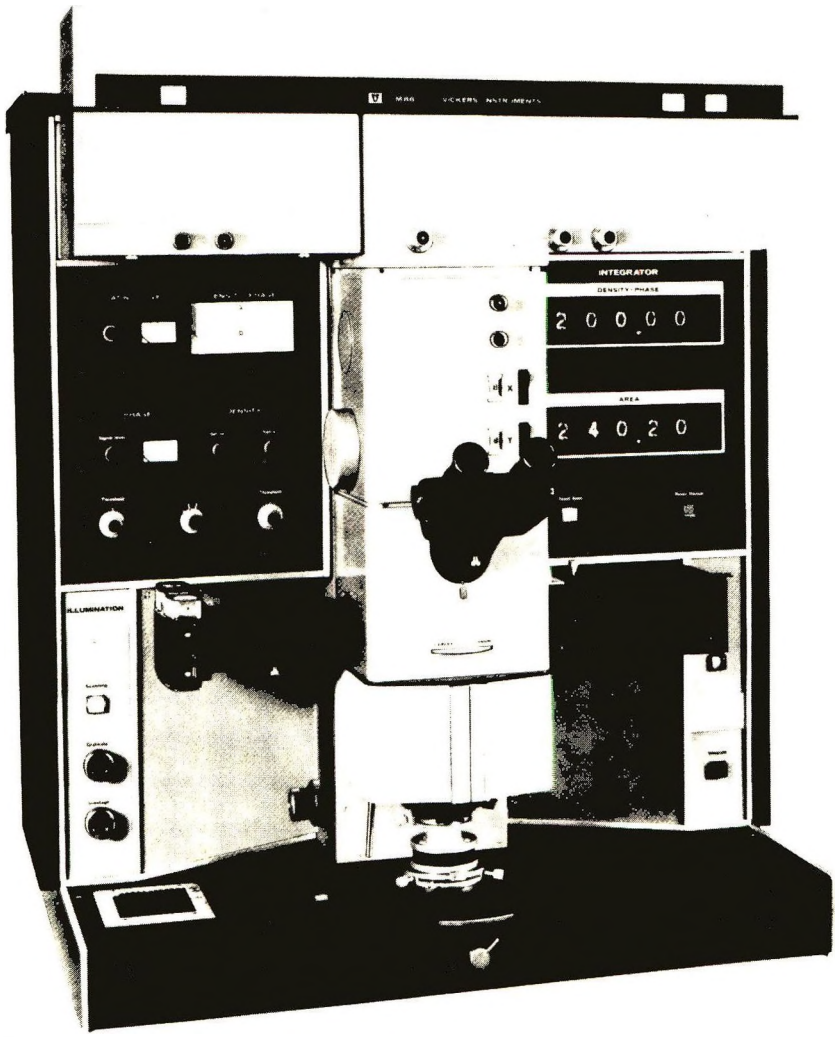

Figure 1. The Vickers M-85 scanning-integrating microspectrophotometer, courtesy of Mr. Robert Osgood, Vickers Instruments, Inc., Woburn, Massachusetts

With this in mind, we would like to describe the fundamental principles of microspectrophotometry and illustrate how a variety of parameters, which are useful in assessing the pathophysiological status of human skin, can thus be easily and rapidly measured. Special emphasis will be given to the Vickers M-85 scanning-integrating microspectrophotometer (Figure 1) that we routinely employ for our studies.

INSTRUMENTAL DESIGN

The principles of microspectrophotometry are similar to those of conventional spectrophotometry (Figure 2). Both instruments are comprised of three main units, viz., 1)

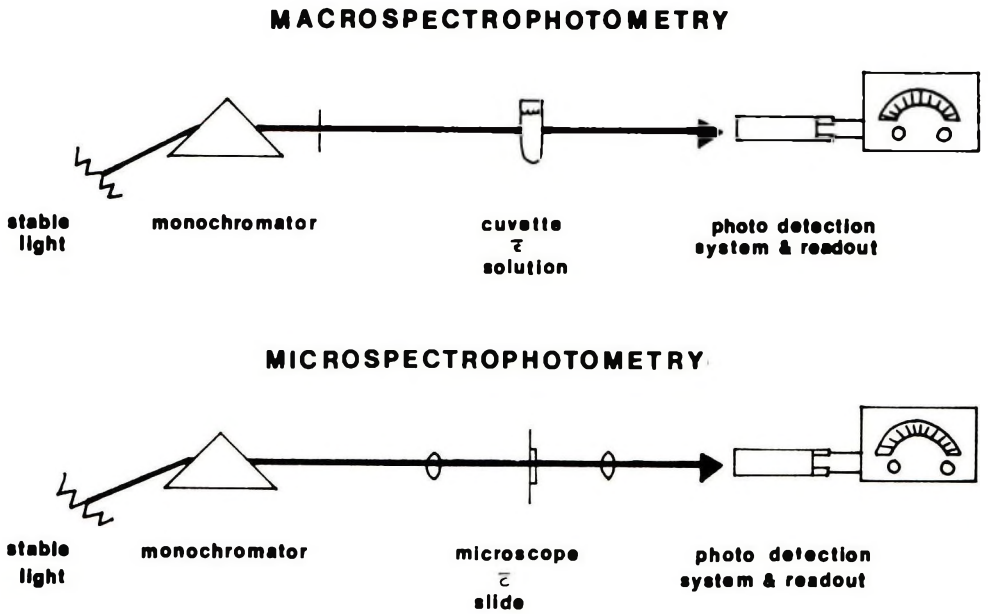

Figure 2. Comparison of microspectrophotometry with macrospectrophotometry, adapted from Chayen and Bitensky (3)

a stable source of monochromatic light, 2) a sample holder and 3) a photodetection system. The Beer-Lambert Law, which describes the exponential relationship between absorption of monochromatic light and the amount of absorbing material the light traverses, is the basis for the measurement in both. The differences arise from the nature of the material being measured. In conventional spectrophotometry, e.g., Lowry protein determinations, one measures how much light of a specific wavelength can pass through a cuvette containing a colored solution. In microspectrophotometry, the sample holder is replaced by the optical train of a microscope allowing measurements to be made on biological specimens. In contrast to the homogeneity of a colored solution, the majority of biological specimens are quite heterogeneous and subject to marked distributional errors. This basic problem can be illustrated by considering a square specimen (Figure 3) composed of four equal segments each of which has a different transmittance—an expression of how much light can pass through the specimen. The relative amount of absorbing material in the specimen can be calculated as the product of absorbance and area. Note that by determining an average transmittance for the entire specimen, an error of .054 units of 15% has been made. This is the "distributional error" and occurs whenever a single direct measurement of intensity is made on objects with regions of diverse transmittance. On the other hand, by calculating independently for each region and summing the results, one takes into account specimen heterogeneity and thus avoids the problem of distributional errors.

The technical aspects of the Vickers M-85 scanning and integrating microspectrophotometer have been described in detail elsewhere (2). In this instrument (Figure 4), the specimen is viewed by a conventional light microscope system and an adjustable photoelectric grating system is used to define the field to be measured. During operation this field is scanned in a raster fashion by a flying spotlight probe consisting of a small beam of light for which the material exhibits maximal absorption.

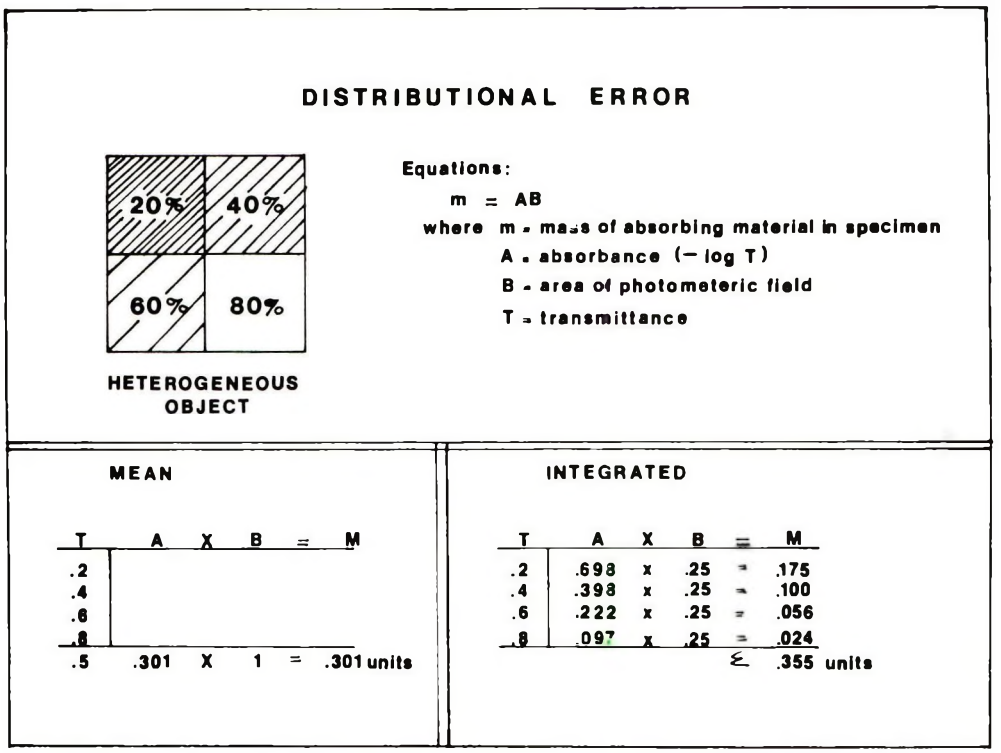

Figure 3. Distributional error in a model system

The specimen is divided into four equal areas of varying chromophore concentrations. The mean value was determined by measuring the transmittance of all areas simultaneously and the integrated value by measuring the transmittance of each area separately. The distributional error increases as specimen heterogeneity increases.

At each measuring point the light intensity of the object is transformed into absorbance by a specially designed analog convertor which makes use of the logarithmically decaying voltage of a discharging condensor to transform the signals from the photomultiplier into a train of 10 kHz pulses. Since this circuitry simulates the Beer-Lambert Law, the duration of each of these pulses is proportional to the absorbance at the point. The digitized value of each sample point within the electronically gated measuring field is stored in a computer. At the end of a scanning raster involving over 120,000 measurements of sample area, each small enough to be relatively free of distributional error, these signals are integrated to give a value proportional to the amount of absorbing material. Simultaneously a second digital meter gives a reading which is proportional to the area of the specimen which has an absorbance greater than any arbitrary chosen threshold value. By using standards of reference, the absorbance and area meters can be calibrated in absolute units of picograms and square microns, respectively.

APPLICATION USING ABSORBANCE MEASUREMENTS

Microspectrophotometry was originally designed to estimate the DNA content of an individual cell by measuring the absorbance of Feulgen-stained nuclei. This method has

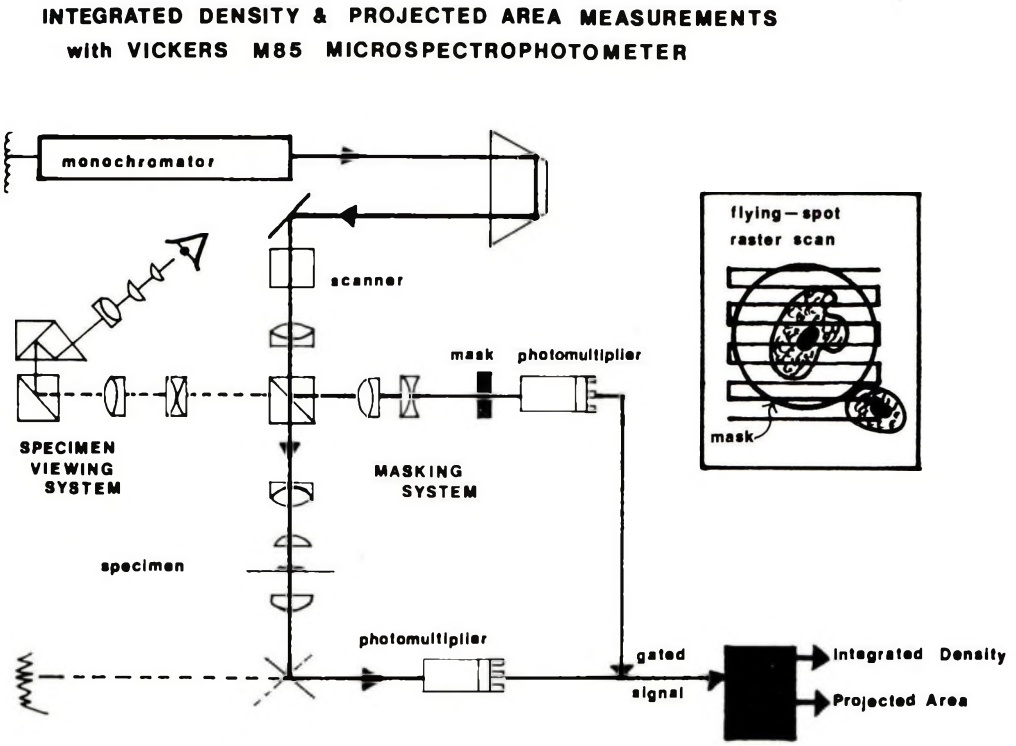

Figure 4. Schematic drawing showing relationship of the components of Vickers M-85 scanning-integrating microspectrophometer

been extremely fruitful in studies of the cell cycle (2). Cells in G_1 have a diploid or 2C DNA contents, cells in G_2, a 4C content, while cells in S have intermediate values. Thus the percentage of cells with DNA contents exceeding the diploid mode can be used to evaluate the degree of proliferative activity since it is these cells that are synthesizing DNA and preparing to divide. Psoriasis, a hyperproliferative skin disease (4–6), has been studied in this manner. As expected, there was a marked increase in the number of hyperdiploid nuclei in the lesional skin; of perhaps greater interest was the finding that proliferative activity was also elevated in the clinically normal-appearing skin as well. We are encouraged that this approach, which obviates the need for radioisotopes, may provide information that can be of diagnostic or prognostic value.

The Feulgen-DNA content distributions of tumor tissues often reveals subtle anomalies which aid in the detection of cancer. Recently two groups (78) have presented evidence which suggests that microspectrophotometry is useful in the cytodiagnosis of mycosis fungoides, a malignant skin reticulosis. Microspectrophotometric measurements were obtained in these studies from imprint specimens prepared by touching fresh biopsy material to a glass slide. Patients with clinically definite mycosis fungoides had abnormal Feulgen-DNA distributions with aneuploid and polyploid values. More importantly, even those patients in the premycotic stage who later went on to develop this disease could be prospectively identified on the basis of subtle but nonetheless real differences in their Feulgen-DNA content distributions. The final impact of being able to screen for mycosis fungoides in the early stages could be considerable and we are currently trying to further develop this cytodiagnostic tool.

Table I
Procedures Amenable to Microspectrophotometric Measurements

1) Histochemical & Cytochemical Staining Reactions:
   DNA (Feulgen, Gallocyanin-chromalium, methyl green)
   RNA (Azure B, Pyronin Y)
   Histones (Alkaline Fast Green, Eosin-Fast Green)
   Proteins (Naphthol Yellow S, Millon, Sakaguchi)
   Carbohydrates (PAS)
   Mucopolysaccharides (Alcian Blue, Mucicarmine, Colloidal iron)
2) Enzymatic Histochemistry:
   Lysosomal—Bitensky Fragility Test
   Mitochondrial—monoamine oxidase
   Pentose Shunt—glucose-6 phosphate dehydrogenase
3) Redox State:
   Prussian blue of Chevremont-Frederic
4) Natural Pigments:
   Cytochrome P-450
   Hemoglobin
5) Quantitative Autoradiography.

Although most absorbance applications have centered on Feulgen-DNA measurements, the current surge of interest in microspectrophotometric analysis probably stems from recent development in other histochemical methods. Although the details of these procedures are beyond the scope of this overview, a few examples that are amenable to microspectrophotometric measurements are listed in Table I. In general, these methods enable the detection of tissue chemical changes in the picogram (10^{-12} g) range with a routine accuracy of $\pm$ 2%.

The use of microspectrophotometry in conjunction with reliable histochemical methods offers several advantages over the conventional form of biochemical analyses ("grind and find"). First, it can be used to make many measurements on minimal amounts of sample tissue. Moreover, multiparameter analyses can often be achieved in the same specimen by using a combination of methods either simultaneously or sequentially. The most important advantage offered by this approach is that it allows the investigator to simply relate observed biochemical changes to the structure of the biological specimen being examined. Thus it is quite possible to measure such things as amount of mucopolysaccharides in the dermis, keratohylin content in the granular layer, the sudanophilia of lipids in sebaceous glands, lysosomal enzyme activity of the basal layer or sulfhydryl or disulfide groups of keratin in situ.

With the availability of such instrumentation one no longer needs to be content with making subjective appraisals of staining intensities. Instead it is now quite easy to quantify the precise amounts of specific material of a variety of dermatological specimens from normal or diseased skin.

APPLICATIONS USING AREA MEASUREMENTS

The Vickers M-85 scanning-integrating microspectrophotometer allows one to measure the projected area of the specimen. We have found this facility to be extremely useful in histogeometric analyses. For example, the need frequently arises to measure the mean epidermal thickness, a parameter which is markedly influenced by disease and experimental manipulations. In the conventional approach, the value

represents the average of 25 to 100 readings at random spots using eyepiece graticule (10). This is not only time consuming and tedious but is subject to considerable errors in specimens with prominent rete-ridge patterns. We are not the first to recognize this problem, as a method based on the Quantiment microdensitometer has been previously reported (11). The key word here is "microdensitometer," because this approach monitors only differences in grey levels—not color intensities. This presents problems when two or more colors are present in the same photometric field and requires that the sections be overstained with hematoxylen, which produces a dark blue color, enabling the Quantiment to detect these regions from fainter pink dermal components. With the Vickers M-86 microspectrophotometer these colors can be resolved and projected area measurements obtained for each colored component. Thus, by measuring the area occupied by the epidermis in a standard size field, one can obtain a global assessment of the dimensions of the viable epidermal compartment with little difficulty.

Much of our current research activity is concerned with developing noninvasive testing procedures for monitoring the physiological status of skin. One extremely promising area is exfoliative cytology, which analyzes cells shed from the body surfaces. Ken McGinley, in our laboratory, has devised a simple detergent-scrub method for quantitative sampling and cytomorphological visualization of the cells making up the desquamating portion of the horny layer (12). This approach has proved to be very valuable in our studies of psoriasis (13, 14), aging (15), dandruff (16, 17), contact dermatitis (18) and steriod atrophy (19). Many of these studies indicate that changes in corneocyte size permit a sensitive evaluation of altered skin physiology, especially epidermpoieses. Unfortunately, since these cells tend to be quite irregular in shape the techniques that have been employed to date to measure this parameter (axial filar micrometry and polar planimetry) are subject to considerable error. We can, however, rapidly and precisely measure changes in corneocyte size by using the projected area feature of Vickers microspectrophotometry.

CONCLUSION

The range of applications for both absorbance and projected area measurements covered by this brief survey hopefully has provided some idea of how valuable the microspectrophotometric approach can be. By measuring light absorbance characteristics we can analyze dimensions of structure and amounts of material in any biological structure that can be identified at the visible light microscopic level and in which an appropriate change in color intensity can be realized. The powerful combination of fast and accuarate geometric and absorbance measurements greatly expands the amount of information which can be obtained from dermatological specimens, viz., scrubs, biopsies, tissue slices, cultures, etc. In fact, the large number of measurements available and the rapidity with which they can be performed means that restraint must be exercised to avoid the unfortunate circumstance of being surrounded by reams of data which have little relevance to the question at hand.

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Der Einfluß erogener Duftstoffe auf die visuelle Wahrnehmung erotischer Reize

WALTER STEINER*, ERNSTFRIED HANISCH** und DIETER SCHWARZ***

Synopsis — Pictures of multiple content were presented to twenty male subjects with the aid of a tachystoscope. As a rule, an erotic scene and a city view were projected simultaneously with one or the other dominant. The time of presentation ranged between 1/250 and 1/125 of a second. The presentation of pictures was conducted with and without simultaneous stimulation from presumably erogenous odors, for which perfumes available on the market were used. It was shown, with a high degree of significance, that the sex motives during odor presentation were more readily recognized even under adverse viewing conditions than the building scenes. Changes in the viewing time did not alter the influence of the odor on the results.

A. Einleitung

Verhaltenskundliche, neurophysiologische und psychologische Untersuchungen lassen erkennen, daß der Geruchswahrnehmung eine wesentliche Steuerungsfunktion für das soziale Verhalten, insbesondere für das Sexualverhalten, zukommt. Dies wird dadurch unterstrichen, daß das limbische System, ein entwicklungsgeschichtlich in enger Beziehung mit dem Riechhirn stehender Gehirnteil, einen erheblichen Einfluß auf das Sozial- und Sexualverhalten hat.

Zahlreiche Tierversuche zeigen, daß das sexuelle Verhalten von einem gut funktionierenden Geruchsapparat und auch intakten, zentral-nervösen Strukturen zur Verarbeitung von Gerüchen abhängig ist. Schon für Insekten ist nachgewiesen, daß Geruchsstoffe in extrem niedriger Konzentration über weite Strecken hin die Auffindung des Sexualpartners gewährleistet. Bei Fischen werden die Identifikation der Spezies,

* drom, D-8021 Baierbrunn im Isartal.
** Dipl.-Psychologe an der Psychosomatischen Klinik in D-8911 Windach-Ammersee.
*** Chefarzt der Psychosomatischen Klinik in D-8911 Windach-Ammersee.

die Geschlechtszugehörigkeit und andere soziale Charakteristika wesentlich durch chemische Sinne vermittelt und die Bildung und Aufrechterhaltung stabiler Gruppen durch die Sinneswahrnehmungen reguliert. In zahlreichen Untersuchungen mit experimenteller Zerstörung des Bulbus olfactorius, einem Teil des Geruchsorganes, konnte nachgewiesen werden, daß die sexuelle Aktivität auch bei männlichen Tieren gehemmt wurde (1). Ähnliche Ergebnisse sind bei zahlreichen anderen Tierspezies erhoben worden, z. B. bei Ratten oder Goldhamstern.

Auch bei höher entwickelten Spezies konnten Hinweise auf entsprechende Beziehungen zwischen dem Riechsystem und sexuellen Reaktionen nachgewiesen werden. Zum Beispiel scheint der von der Östrogen-Androgenproduktion abhängige Vaginalgeruch bei Rhesusaffen die Attraktivität des Weibchens für männliche Affen zu steuern. Bei weiblichen Rhesusaffen wurden Pheromone aus der Vagina isoliert und eine Abhängigkeit des männlichen Verhaltens von der Anwesenheit dieser Duftstoffe nachgewiesen (2).

Vorwissenschaftliche Erfahrungen über die Wirkung von Duftstoffen auf den menschlichen Organismus lassen sich bis in vorgeschichtliche Zeiten nachweisen. Der objektive wissenschaftliche Nachweis derartiger Effekte beim Menschen bereitet jedoch, anders als bei vielen stammesgeschichtlich früheren Lebewesen, wegen seiner geringen Ausprägung und der großen individuellen Schwankungen erhebliche Schwierigkeiten. Selbst relativ einfache Differenzierungen wie die Geruchswirkung auf die Dimension Aktivierung/Dämpfung wurden bisher selten experimentell erfaßt (3). Die Abhängigkeit der sexuellen Reaktionen des Menschen von Geruchsreizen ist zwar eine alte Erfahrung, die in zahlreichen Darstellungen über aphrodisiakische Wirkungen erwähnt wird, sie ist bisher aber experimentell wenig belegt. Einer der Gründe dürfte sein, daß genetische Verhaltenssteuerungen beim Menschen und frühkindliche Prägungen stark von Lernfaktoren überlagert werden. Immerhin gibt es einige Beobachtungen, die die bisherigen vorwissenschaftlichen Erfahrungen bestätigen: Seit langem ist bekannt, daß die Empfindlichkeit des Geruchssinnes Schwankungen unterliegt (4), die vom Menstruationszyklus abhängen. Sehr interessante, den Ausschaltungsexperimenten bei Nagetieren analoge Beobachtungen wurden bei Menschen mit einer durch pathologische Prozesse bedingten Anosmie beobachtet, d. h. partielle Geruchsblindheit führt zu einer deutlichen Reduktion der sexuellen Reaktionen.

R. L. Henkin hat mit Recht darauf hingewiesen, daß dem Geruchssinn trotz seiner engen Beziehungen zu basalen, verhaltenssteuernden Hirnfunktionen bisher nur wenig Aufmerksamkeit geschenkt wurde. In

Fortführung früherer Arbeiten schien uns deshalb eine experimentelle Überprüfung psycho-sexueller Auswirkungen von Geruchsstoffen am Menschen erforderlich. Solche Untersuchungen tragen nicht nur zu einer besseren objektiven Differenzierung der bisher mehr intuitiv erfaßten Geruchswirkungen bei, sondern könnten auch das Verständnis der psycho-olfaktorischen Wirkungen und ihrer verhaltenssteuernden Effekte mit allen theoretischen und praktischen Implikationen fördern.

B. Angewandte Methode

Wahrnehmungsprozesse und Verhaltenstendenzen werden durch interne und externe Reizbedingungen gesteuert. Diese Tatsachen sind seit langem bekannt und sie bedeuten, daß der menschliche Organismus je nach Bedürfnislage die Leistungsfähigkeit der Sinnesorgane zu beeinflussen vermag. Ein Beispiel: Vielen Frauen ist die Erfahrung bekannt, daß sie im Zustand eigener Schwangerschaft auf der Straße schwangere Frauen eher sehen als sonst. Oder: einem hungernden Menschen fallen Objekte, die mit Nahrungsmitteln zusammenhängen, bereits unter Bedingungen auf, unter denen sie dem Gesättigten verborgen bleiben (5). Analog dazu stellten wir uns die Frage: Kann ein durch Parfum stimuliertes Individuum Reize sexuellen Inhaltes schneller erkennen als ohne Parfum?

Die meisten Untersucher, die sich mit der Abhängigkeit der Wahrnehmung von internen oder externen Reizbedingungen befassen, bedienen sich optischer Methoden, weil mit ihnen Prüfungsbedingungen besonders gut abzustufen und experimentell erfaßbar sind. Es lag deshalb nahe, auch für die von uns beabsichtigten Untersuchungen visuelle Reize zu verwenden.

Unser Experiment bestand aus der Darbietung von Diapositiven, auf welchen gleichzeitig eine städtebauliche Ansicht von München und eine erotische Szene zu sehen war. Diese Doppelbilder ergaben sich dadurch, daß wir ein Dia aus einem Andenkenladen und ein zweites mit einer sexuellen Szene übereinander legten. Wir verwendeten 8 solcher Bilder mit unterschiedlichen baulichen und erotischen Szenen, die wir so anordneten, daß beim 1. Bild eindeutig die Stadtansicht und beim 8. Bild eindeutig der sexuelle Inhalt dominierte. Die dazwischenliegenden Bilder wurden so variiert, daß von Bild zu Bild die Stadtansicht zugunsten des sexuellen Inhaltes abnahm. Der Sinn dieser Doppelbilder bestand darin, eine unklare Wahrnehmungssituation zu schaffen, wodurch die Versuchspersonen die Möglichkeit hatten, im Sinne einer Wahrnehmungsselektion den einen Inhalt zu bevorzugen und den anderen zu vernachlässigen.

Von den verschiedenen Möglichkeiten zur Abstufung visueller Reize wählten wir zwei aus:

1. Wie oben beschrieben: Die unterschiedlich dominante Einbettung sexueller Inhalte in einen neutralen Hintergrund.
2. Die Vorführung von Dias sexuellen Inhaltes mit wechselnden Darbietungszeiten. Im Rahmen sogenannter tachistoskopischer Experimente wurden die Bilder nur für Bruchteile von Sekunden gezeigt.

Durch die Versuchsanordnung sollten die folgenden Hypothesen überprüft werden.:

1. Die Wahrnehmung optisch sexueller Reize ist
   a) von der Dominanz des sexuellen Bildgegenstandes abhängig,
   b) von der Darbietungsdauer.
2. Unter gleichzeitigem Einfluß von Duftreizen kommt es zu einer Senkung der Wahrnehmungsschwelle für visuelle erotische Reize mit der Folge, daß:
   a) eine Wahrnehmung sexueller Stimuli bereits bei einer geringeren Dominanz eintritt,
   b) schon bei kürzeren Darbietungszeiten sexuelle Reize wahrgenommen werden.
3. Die Differenz zwischen der von der Duftwahrnehmung unbeeinflußten und der durch die Duftwahrnehmung geförderten Reaktion sollte im Ambivalenzbereich, in dem der städtebauliche Eindruck und der Sexeindruck ungefähr gleich stark ist, am stärksten ausgeprägt sein.

In dem folgenden Schaubild (Abb. 1) soll diese Anordnung schematisch veranschaulicht werden. In der oberen Reihe bedeutet die schwarze Fläche den Anteil des Sexreizes, die weiße Fläche den Anteil der Stadtansicht. Man sieht, wie sich die relative Dominanz der beiden Alternativen „Stadt" und „Frau" von Dia 1 bis Dia 8 allmählich verschiebt.

Um zu prüfen, ob der zu vermutende Einfluß der Duftdarbietung sich bei unterschiedlicher Projektionsdauer verschieden stark ausprägt, wurden 4 Diaserien mit vier verschiedenen Darbietungszeiten projiziert. Die Zeiten waren: $^1/_{250}$, $^1/_{200}$, $^1/_{170}$ und $^1/_{125}$ Sekunde. Wir nahmen an, daß bei erschwerter Wahrnehmung, d. h. bei sehr kurzen Darbietungszeiten, die Duftapplikation die Wahrnehmung in spezifischer Weise erleichtern würde, so daß bei den kürzeren Darbietungszeiten der Einfluß des Duftes

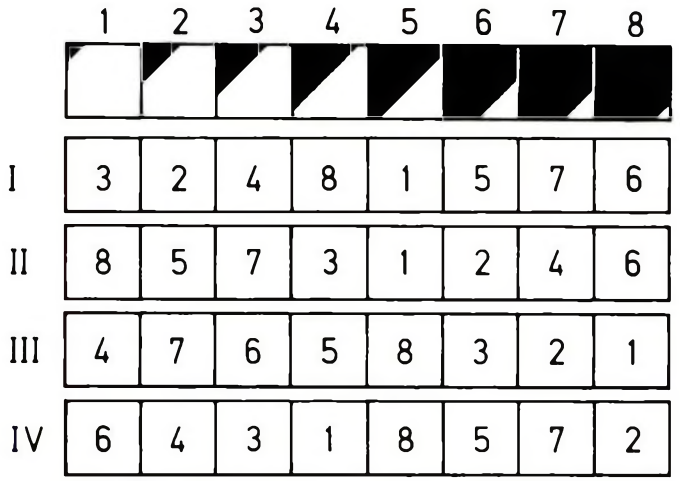

Abbildung 1

In dem oberen Schema bedeutet die schwarze Fläche den Anteil des Sexreizes, die weiße Fläche den Anteil der Stadtansicht in den vorgeführten Dia-Positiven. Die Zahlen geben die Dia-Nummer an. Wegen der vier Darbietungszeiten wurden vier Diaserien mit zufälliger Bildfolge zusammengestellt, die unter I, II, III und IV dargestellt sind.

Abbildung 2

Dia 1 der acht für die Untersuchung verwendeten Dia-Positive. Man sieht eine städtebauliche Ansicht. Ein sexueller Inhalt ist nicht zu erkennen.

stärker zum Ausdruck kommen würde. Für unsere Versuche benötigten wir deshalb 4 Diaserien, wobei die Reihenfolge der Diadarbietungen zufällig variiert wurde. In den unteren vier Kästchenreihen von Abb. 1 sind die Reihenfolgen der Bilder in vier Zufallsserien abgebildet. Um einen Eindruck von dem von uns verwendeten Reizmaterial zu geben, seien zwei Dias aus der Serie der 8 Bilder ausgesucht:

Bei dem ersten Dia (Abb. 2) fällt offenkundig die nur städtebauliche Ansicht stark auf. Bei dem zweiten Dia (Abb. 3) dagegen ist der sexuelle Inhalt gut zu sehen.

Abbildung 3

Dia 6 der acht für die Untersuchung verwendeten Dia-Positive. Neben der städtebaulichen Ansicht ist deutlich der nackte Oberkörper einer Frau zu sehen.

Aus der Variation der genannten Reizbedingungen, also Bilder mit unterschiedlicher Dominanz der beiden Alternativen „Stadt" und „Frau", der verschiedenen Darbietungszeiten und der Bedingung mit bzw. ohne Duftapplikation, ergab sich der auf dem nächsten Bild dargestellte Versuchsverlauf (Abb. 4).

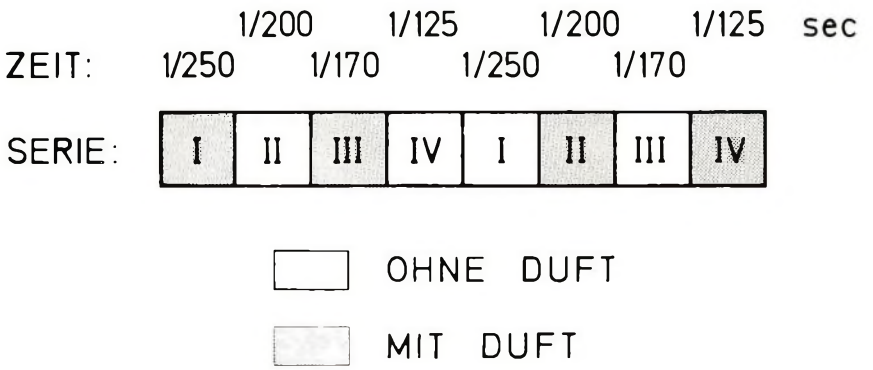

Abbildung 4

Schematische Darstellung, wie die in Abb. 1 aufgezeigten Diaserien nacheinander vorgeführt wurden. Grau bedeutet Diaserie mit Duft, weiß bedeutet Diaserie ohne Duft vorgeführt. Darüber stehen die Projektionszeiten in Sekunden.

Man erkennt, daß zunächst die Serie 1 mit der Darbietungszeit von $^1/_{250}$ Sekunde bei gleichzeitiger Wahrnehmung des Duftes, danach die Serie 2 bei $^1/_{200}$ Sekunde Darbietungszeit ohne Duft, schließlich die Serie 3 bei $^1/_{170}$ Sekunde Darbietungszeit wiederum mit Duft und dann die Serie 4 bei einer Darbietungszeit von $^1/_{125}$ Sekunde wiederum ohne Duft dargeboten wurde. Hier also liegt eine sukzessive Steigerung der Darbietungszeit vor, bei gleichzeitigem Wechsel der Diaserien und bei alternierender Duftapplikation. Die rechte Hälfte der Kästchenreihe stellt eine Wiederholung der linken Hälfte dar, wobei die bisher ohne Duft dargebotenen Serien bei jeweils derselben Darbietungszeit nun mit Duft appliziert wurden. Der Sinn dieser variierenden Abfolge lag selbstverständlich darin, Effekte, die durch die Wiederholung der Bildprojektionen zustande kommen könnten, auf die beiden Bedingungen mit und ohne Duftapplikation gleichmäßig zu verteilen und dadurch zu eliminieren.

Die Geruchsreize und ihre Darbietung

Die Versuchspersonen konnten zwischen drei der handelsüblichen Parfums auswählen: Das erste war ein nach Moschus, sehr animalisch riechendes Parfum, das zweite ein sehr blumiges, duftiges, und das dritte ein sehr schweres, süßes Parfum. Zu Beginn der Untersuchung hatten die Versuchspersonen sich für dasjenige Parfum zu entscheiden, dem ihrem subjektiven Empfinden nach am ehesten eine sexuell stimulierende Wirkung zukam. Zur Darbietung wurde mittels eines Atomizers eine relativ gleichbleibende Menge auf den Handrücken gesprüht und von dieser Stelle während der Bilddarbietung der Duft aufgenommen. Bei den Serien ohne Duft wurde ein Verströmen des aufgesprühten Duftes durch Überziehen eines Handschuhes verhindert. Den Versuchspersonen wurde gesagt, sie sähen entweder eine Frau oder eine Stadt. Dementsprechend waren drei Antworten möglich: Frau, Stadt oder ich sehe nichts.

Versuchspersonen und der Ort der Untersuchung

Getestet wurden 25 männliche Probanden im Alter zwischen 20 und 39 Jahren. Die Untersuchung wurde in der Psychosomatischen Klinik in Windach, Nähe Ammersee, durchgeführt.

C. Die Darstellung der Ergebnisse

Als erstes galt es zu prüfen, ob das von uns verwendete Bildmaterial tatsächlich nach dem oben dargestellten Schema bestimmte Antworten nahelegte. Aufschluß darüber gab die Summe aller „Stadt"- bzw. „Frau"-Antworten bei den acht Dias. Diese Summen sind in dem folgenden Kurvendiagramm (Abb. 5) dargestellt. Man sieht an der B-Kurve, daß bei Dia 1 praktisch alle Versuchspersonen die städtebauliche Ansicht wahrnahmen und keine einzige den darin versteckten Sexinhalt erkannte. Abgesehen von einigen Irregularitäten nahm dann die Anzahl der „Stadt"-Antworten, wie zu erwarten war, ab, während die Anzahl der „Frau"-Antworten (R-Kurve) zunahm. Der Schnittpunkt der beiden Kurven kennzeichnet jenen Bereich, wo eine Entscheidung zwischen „Stadt" und „Frau" als völlig arbiträr anzusehen ist. Bei den Dias 6 und 7 wurde also keine der beiden Alternativen favorisiert, so daß wir in diesem Bereich den stärksten Einfluß der Duftapplikation, sofern diese überhaupt einen Einfluß auf die Wahrnehmung hat, vermuten dürfen.

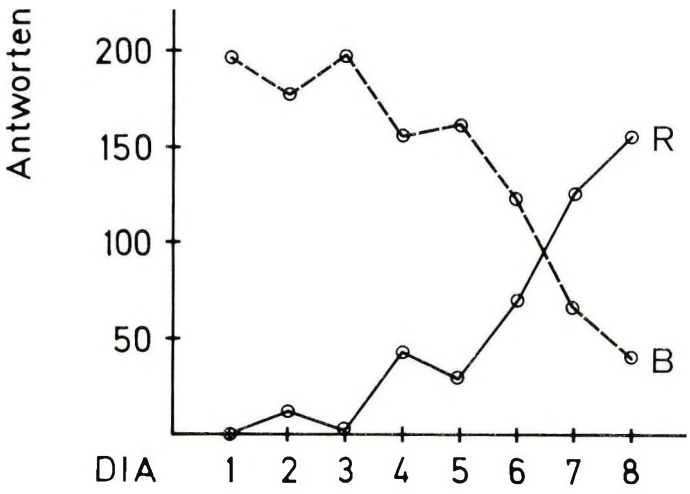

Abbildung 5

Auf der Abszisse sind die Dia-Positive 1 bis 8 aufgetragen. Die Ordinate zeigt die Summe aller „Frau"- bzw. „Stadt"-Antworten entsprechend der R- bzw. B-Kurve. Man erkennt dadurch die Wirkung der Dominanz des Stadtmotivs bzw. des Sexinhaltes der Dia-Positive im Sinne einer Wahrnehmungsselektion.

Im Säulendiagramm haben wir die Summen der „Frau"-Antworten (Abb. 6) bei den Dias 1 bis 8 eingezeichnet. Man sieht jeweils zwei übereinander gezeichnete Säulen, wobei die weiße Säule die „Frau"-Antworten,

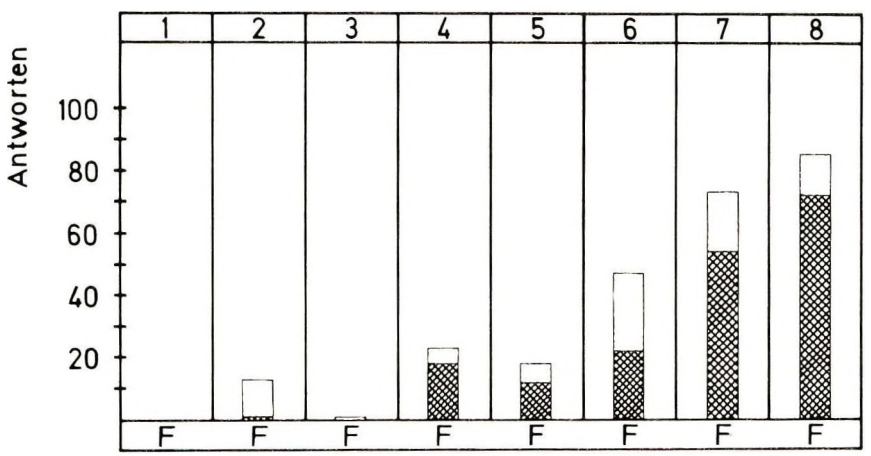

Abbildung 6

Die Höhe der weißen Säulen kennzeichnet die Summe aller „Frau"-Antworten mit Duft, die Höhe der schraffierten Säulen die Summe aller „Frau"-Antworten ohne Duft auf die in der oberen Zeile angegebenen Dia-Positive. „F" bedeutet „Frau"-Antwort.

die mit Dufteinwirkung, die schraffierte Säule die „Frau"-Antworten, die ohne Dufteinfluß abgegeben wurden, kennzeichnet. Die unterschiedliche Höhe der weißen Säule gegenüber der schraffierten Säule erweist somit den Einfluß des Duftes auf die Wahrnehmung sexueller Inhalte. Man erkennt, wie bereits bei der vorangegangenen Abbildung, daß die Tendenz, die Frauabbildung zu sehen, abgesehen von einigen Irregularitäten von Dia zu Dia zunimmt, wobei der Dufteinfluß bei den Dias 6 und 7 am stärksten ausgeprägt ist. Bei Dia 2 gab es einen nicht erwarteten Effekt, bei diesem haben die Versuchspersonen die Frau häufiger gesehen als die Experimentatoren erwarteten. Möglicherweise sind wir bei der Zusammenstellung der Bilder eigenen Verdrängungsmechanismen zum Opfer gefallen. Was bei Betrachtung des Diagramms der Augenschein bereits nahelegte, bestätigte eine statistische Analyse: Der Einfluß des Parfums auf die Wahrnehmung war bei den Dias 6, 7 und 8, sowie bei dem Dia 2 signifikant (Vierfeldertest nach McNemar).

Das nun folgende Bild (Abb. 7) faßt noch einmal das Ergebnis zusammen: Von Dia zu Dia wird die Stadtansicht seltener genannt und gleichzeitig nimmt die Tendenz, die Frau zu erkennen, deutlich zu, was durch die B- bzw. R-Linie dargestellt ist. Die zusätzlich eingetragenen Säulen sind ein Maß für den Einfluß des Duftes auf die Wahr-

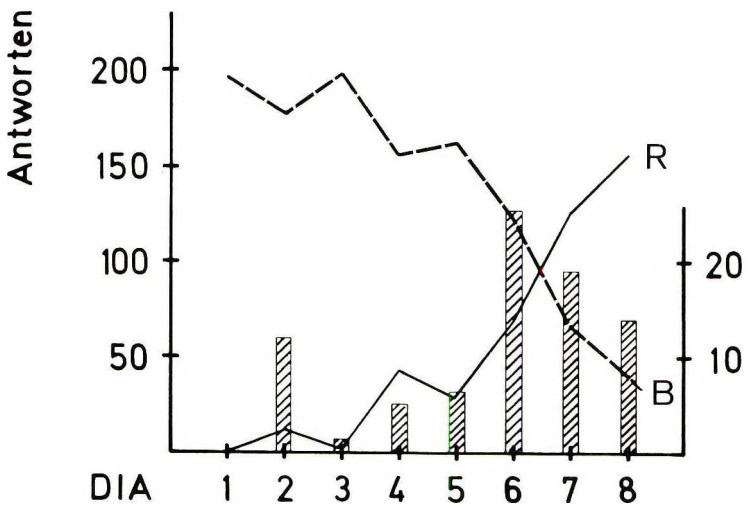

Abbildung 7

R- und B-Linien sind gleichbedeutend mit denen in Abb. 5. Zusätzlich wurde rechts als Ordinatenmaßstab die Differenz zwischen der Summe der „Frau"-Antworten mit Duft und der „Frau"-Antworten ohne Duft aufgetragen. Diese Differenz der einzelnen Dias ist durch die gestrichelten Säulen dargestellt.

nehmung des sexuellen Inhaltes. Ihre Höhe ergibt sich aus der Summe der „Frau"-Antworten unter Dufteinfluß, abzüglich der „Frau"-Antworten ohne Dufteinwirkung. Unsere Erwartung wurde damit bestätigt, daß der Effekt bei den Dias 6 und 7, also links und rechts vom Schnittpunkt der beiden Kurven, am stärksten zum Ausdruck kommt.

Unsere Hypothese, daß unter Duftbedingungen schon schwächere sexuelle Reize wahrgenommen werden, wird auch durch die Beobachtung bestätigt, daß die Zahl der „Frau"-Antworten bei Duftapplikation rascher ansteigt, als ohne Duft. So finden sich z. B. bei Dia 4 (Abb. 6) unter Dufteinwirkung bereits 23 „Frau"-Antworten, während diese Zahl ohne Dufteinwirkung erst bei Dia 6 erreicht wird.

Die bisherige Darstellung der Ergebnisse bezog sich auf die Summe der von allen Versuchspersonen gegebenen Antworten. Dabei blieb offen, welche Auswirkung der Duft bei den einzelnen Versuchspersonen auf deren Wahrnehmung hatte. Wir haben daher im folgenden Bild (Abb. 8) die individuellen Antwortsummen jeder einzelnen Versuchsperson aufgetragen. Die R-Kurve zeigt die Summe der „Frau"-Antworten unter Dufteinfluß, die B-Kurve dieselbe Antwortsumme ohne Dufteinfluß. Bei fast allen Versuchspersonen waren die „Frau"-Antworten unter

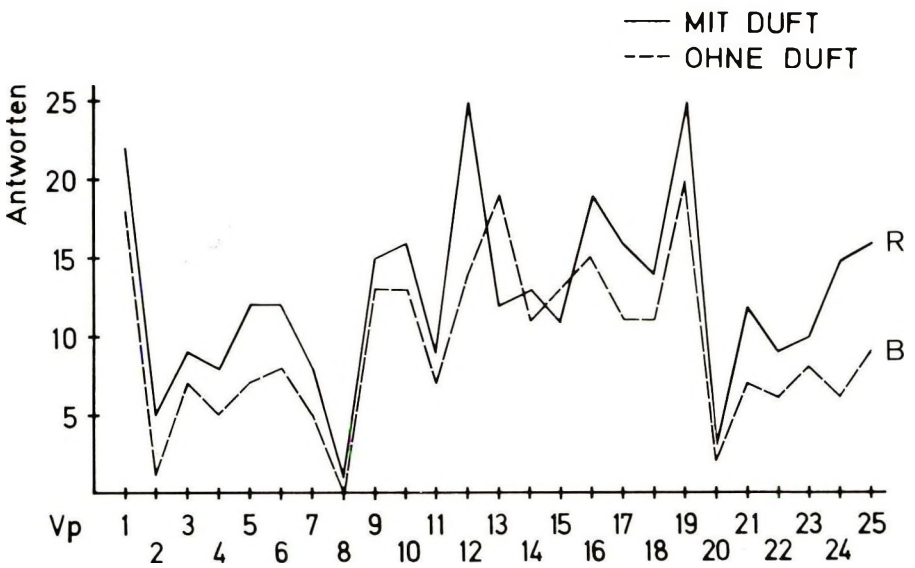

Abbildung 8

Die R-Linie bedeutet die Summe aller „Frau"-Atworten mit Duft, die jede einzelne Versuchsperson abgab. Die B-Linie gibt die „Frau"-Antworten ohne Duft wieder.

Parfumeinwirkung häufiger als die ohne Duft. Ausnahmen sind lediglich die Versuchspersonen 13 und 15. Außerdem erkennt man an dem unterschiedlichen Abstand der Kurven, daß manche Probanden durch erotische Düfte stärker zur Wahrnehmung sexueller Reize stimuliert werden als andere. Schließlich zeigt der enorm gezackte Verlauf der Kurve, wie unterschiedlich stark die Bereitschaft der untersuchten Personen war, unter den Versuchsbedingungen die Frau wahrzunehmen. Bemerkenswert ist, daß selbst jene Versuchsperson, die ohne Duft kein einziges Mal die Frau erkannte, sich mit Duft zu einer Sexantwort hinreißen ließ. Die hier dargestellten Unterschiede waren statistisch gesehen überaus signifikant. Die Zufallswahrscheinlichkeit beträgt 1 : 10.000.

Das letzte Schaubild (Abb. 9) bezieht sich auf den Aspekt unserer Hypothese, der einen möglichen Einfluß der verschiedenen Darbietungszeiten auf die Dufteinwirkung zum Gegenstand hatte.

Auf der Ordinate sind die Antworthäufigkeiten der „Frau"-Antworten, auf der Abszisse die vier verschiedenen Darbietungszeiten aufgetragen. Die untere B-Kurve gibt die „Frau"-Antworten ohne Duft, die obere R-Kurve die „Frau"-Antworten mit Duft wieder. Man sieht, daß sich hierbei unsere Erwartungen als nicht zutreffend erwiesen. Die Kurven verlaufen ungefähr parallel, was besagt, daß sich bei den von uns verwendeten Zeiten der Einfluß des Duftes auf die Wahrnehmung kaum verändert.

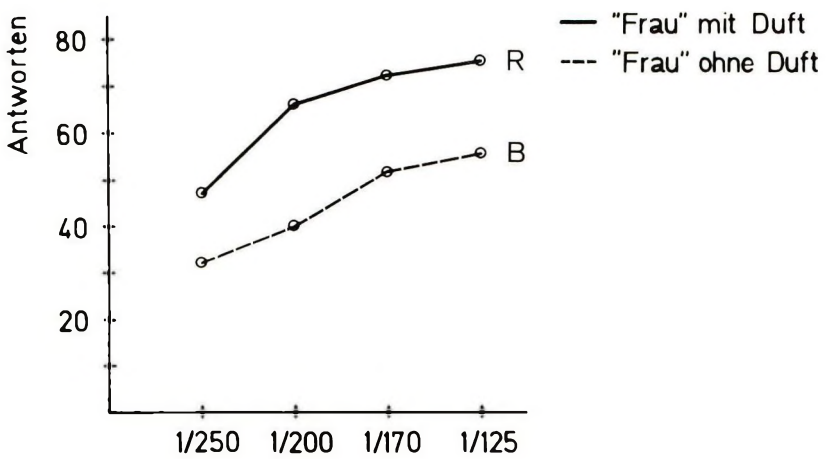

Abbildung 9

Die Ordinate gibt die Summe aller „Frau"-Antworten bei der betreffenden, auf der Abszisse angegebenen Darbietungszeit wieder. R-Linie: mit Duft, B-Linie: ohne Duft.

Diskussion der Ergebnisse und Ausblick

Vorwissenschaftliche Erfahrung über die Wirkung ätherischer Öle auf den menschlichen Organismus ist Jahrtausende alt. Der objektive Nachweis solcher Effekte beim Menschen bereitet jedoch — anders als bei vielen stammesgeschichtlich älteren Lebewesen — wegen seiner geringen Ausprägung und der großen individuellen Schwankungen erhebliche Schwierigkeiten. Ob ein Duft berauschend, belebend, beruhigend oder betörend wirkt, obliegt weitgehend subjektiver Erlebnisbeschreibung. Beim Versuch, die erogene Wirkung von bestimmten Düften aufzuzeigen, beschritten wir einen indirekten Weg: Eine der Psychologie seit langem bekannte Eigenschaft des Organismus besteht darin, daß seine jeweilige Bedürfnislage die Leistungsfähigkeit der entsprechenden Sinnesorgane beeinflußt. So nimmt ein hungernder Mensch jene Dinge, die mit Nahrungsmitteln zusammenhängen, bereits unter Bedingungen wahr, unter denen sie den Gesättigten verborgen bleiben. Ähnlich müßte — und diese Annahme galt es zu prüfen — ein durch sogenannte erogene Düfte stimuliertes Individuum Reize sexuellen Inhaltes schneller wahrnehmen als unter Vergleichsbedingungen.

Die von uns benutzte Untersuchungsmethode könnte sich unter bestimmten Voraussetzungen und entsprechenden Abwandlungen für die Beantwortung weiterer Fragen eignen, z. B.:

1. Gibt es Unterschiede zwischen den einzelnen Parfumtypen in ihrer Auswirkung so, daß die visuelle Wahrnehmung ganz bestimmter Sexreize begünstigt werden? Gibt es Düfte, die z. B. speziell bei schwarzhaarigen Frauen besonders wirken?
2. Kann man Assoziationen, die man bei dem Geruch eines Parfümöls empfindet, mit dieser Methode erhärten? Paßt zum Beispiel zu einem Parfümöl die Assoziation „grüne Wiese, Tau, Sommermorgen oder heißer Badestrand mit Palmen"?
3. Endlich könnte man die immer wieder auftauchende Frage stellen: gibt es noch Rudimente von menschlichen Pheromonen, also Stoffen, die in ganz bestimmter Weise sexuell wirksam sind?

Zusammenfassung

20 männliche Versuchspersonen, denen mittels eines sogenannten Projektionstachistoskops Bilder mehrdeutigen Inhaltes kurzzeitig dargeboten wurden, wurden getestet. Dargestellt war jeweils ineinander

eine erotische und eine städtebauliche Ansicht, wobei einmal die erotische Szene und dann wieder die städtebauliche Szene dominant waren. Die Darbietungszeiten lagen zwischen $^1/_{250}$ und $^1/_{125}$ Sekunde. Die Darbietung der Bilder erfolgte mit und ohne Stimulation von mutmaßlich erogenen Gerüchen. Als solche wurden auf dem Markt befindliche Parfums verwendet. Die Untersuchungen ergaben mit hoher Signifikanz, daß die Sexmotive während der Duftdarbietung gegenüber dem Stadtmotiv schon bei ungünstigerer Wahrnehmungssituation erkannt wurden. Ein Vergleich der Resultate bei verschiedenen Darbietungszeiten zeigten dagegen keinen Unterschied durch die Dufteinwirkung.

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A new technique to assess sunscreen effectiveness

E. PINES *Skin Biology Section, Exploratory Research Laboratories, Johnson & Johnson, New Brunswick, NJ 08902.*

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Synopsis

In this study photoacoustic spectroscopy (PAS), a NEW TECHNIQUE recently developed for the STUDY of solid, semi-solid and biological samples, was used to obtain in situ ultraviolet absorption spectra from which the SUNSCREENING EFFECTIVENESS and the substantivity to skin of various formulated sunscreens were evaluated.

The uniqueness of PAS allows the measurement to be made directly on the sunscreen formulation applied to excised full-thickness, newborn rat skin. Thus the parameters which govern the spectral properties of the skin-sunscreen agent complex are maintained close to those of the "in use" situation.

INTRODUCTION

One of the most effective means for studying the properties of matter nondestructively is to observe how matter interacts with photons by the use of conventional optical spectroscopy. At present, the two most common spectroscopic techniques are absorption and reflection spectroscopy. In biology, however, one must often deal with materials that in their intact, unmodified state cannot be readily studied by these conventional optical techniques—because of the sample's opacity, light scattering properties or surface characteristics.

In the spectroscopic investigation of skin or a skin-agent complex, some investigators (1–3) have attempted to minimize the above problems by treating the sample with fluids of matching refractive index. This is a cumbersome and not very effective approach. A more popular procedure is to solubilize the sample and then study the resultant optically clear solution. However, this approach likewise has its drawbacks: 1) the skin is chemically resistant to complete solubilization because of the strong cohesive nature of the keratinaceous stratum corneum matrix and 2) the question of whether the measured optical properties of the solution are exactly the same as those of the unsolubilized sample has been the subject of considerable investigation and remains unresolved.

Some investigators have tried by chemical means to extract selected constituents from the skin sample and subsequently study the extract solution. This procedure also can be very cumbersome and ineffective, particularly when the extraction procedure is in-

complete. In addition, in the solution environment the state of the constituent of interest may differ from its state in the intact membrane (solution as opposed to solid or film). Hence inappropriate or irrelevant spectral properties, such as line shape and intensity, may be observed.

An assessment of the sunscreening effectiveness and the substantivity to skin of various formulated sunscreens by use of conventional optical techniques is, therefore, often inappropriate for the following reasons: 1) skin itself is a highly effective light scatterer, especially in the ultraviolet region and 2) the parameters that govern the spectral properties of the skin-sunscreen complex are not the same as those of the diluted sunscreen formulation as determined by conventional techniques.

Recently there has been developed a new spectroscopic technique, photoacoustic spectroscopy (PAS) (4–6), which overcomes the drawbacks associated with opaque and light-scattering systems and permits spectroscopic investigations to be made in situ. For example, in this study measurements were made directly on the sunscreen formulation applied to excised, full-thickness skin. Thus the parameters which govern the spectral properties of the skin-sunscreen complex are maintained close to those of the "in use" situation.

In photoacoustic spectroscopy (5, 6), the sample to be studied is placed inside a sealed chamber, a photoacoustic cell. The cell contains a very sensitive microphone and is filled with a gas, such as air, at ambient temperature and pressure. The sample is irradiated with monochromatic light which is chopped at some acoustic frequency (50 to 5000 Hz). If the sample absorbs any of the incident radiation, some energy level in the sample is excited and this energy level must subsequently de-exite, usually by means of a nonradiative or heating mode of de-excitation. The periodic input of light thus results in a periodic heating of the sample and subsequent periodic heat flow from the sample to the surrounding gas. The gas at the sample-gas interface responds to this periodic heat flow with an oscillatory motion that produces a periodic pressure change in the sealed photoacoustic cell. The microphone in turn detects this pressure change as an acoustic signal which is then processed electronically and recorded. Typically, the sample is irradiated with less than 1 milliwatt/cm^2 of light, which results in only millidegree changes in the sample's temperature and in a periodic cell pressure change of less than 1 μ bar (10^{-6} atmospheres).

Since the strength of the acoustic signal in the photoacoustic cell is closely related to the amount of light absorbed by the sample, a plot of the acoustic signal vs. photon wavelength, that is a photoacoustic spectrum, bears a close resemblance to a true optical-absorption spectrum. Furthermore, since only absorbed light can produce an acoustic signal, scattered light, which presents such a serious problem in transmission spectroscopy, does not present an appreciable problem in photoacoustic spectroscopy.

The theory and mathematics of the photoacoustic effect have been published by Rosencwaig et al. (7). In general, the photoacoustic signal is a complicated function of thermal, optical and geometrical parameters which include thermal diffusivity, absorption coefficient, chopping frequency and sample thickness.

In this communication, we will consider the optical absorption coefficient, the only wavelength-dependent parameter associated with the photoacoustic effect and hence the one responsible for the observed line shape. The other parameters govern the overall magnitude and phase of the acoustic signal (6, 7). They can be experimentally varied so as to render optically opaque material photoacoustically transparent, as well

as to determine how far a periodic heat wave can travel in the sample before excessive heat dissipation occurs.

In the work presented here, PAS was used to obtain in situ ultraviolet photoacoustic spectra from which the sunscreening effectiveness and the substantivity of several sunscreen formulations applied directly to intact, excised newborn rat skin were evaluated.

MATERIALS AND EQUIPMENT

Neonatal rats were sacrificed 24 hr post partum. Samples of full-thickness skin 4 × 4 cm were excised and allowed to equilibrate at ambient conditions for 48 hr. The samples were cut into 1.5 × 0.5-cm sections to which the sunscreen formulations were applied. The formulated sunscreens used in this study are given in Table 1.

The dual-beam photoacoustic spectrometer was designed and built at our research facility. The light from a 1000 W Xe lamp was directed through a 0.25 m f/3.5 Ebert monochrometer equipped with two gratings, one blazed for the ultraviolet and the other for the visible region. The monochrometer was driven by a stepper motor equipped with TTL logic and controlled from an H-P 9825A calculator interfaced with an H-P 6940B multiprogrammer. The output from the monochrometer was mechanically chopped by a variable-speed chopper. The periodically chopped monochromatic light beam was split into two beams by a reflective beam splitter. The beams were directed into two photoacoustic cells (8), one containing the sample and the other containing carbon-black. The acoustic signal generated in each cell was detected by a high-sensitivity microphone whose output was processed by preamplifiers and magnitude- and phase-sensitive, lock-in amplifiers (one for each cell). The outputs from the lock-in amplifiers were directed to a ratio meter operating in the A/B mode, i.e., sample signal/carbon-black signal. The sample's signal was thereby normalized against the lamp power spectrum at all wavelengths to provide a normalized spectrum. The ratioed output was processed by the multiprogrammer and calculator to yield the photoacoustic spectrum which was plotted on a digital plotter.

METHOD

The actual "in use" situation was obtained by uniformly applying a fixed amount of the formulated product to the stratum corneum of the 1.5 × 0.5-cm sample of intact, excised, full-thickness neonatal rat skin. The sunscreening effectiveness and substantivity to the skin of the various sunscreen formulations were obtained as follows. After a 30-min postapplication drying time at ambient conditions, a photoacoustic spectrum over the 240 to 440-nm region was obtained. Immediately following the spectral measurement (53 min postapplication), the sample was soaked with constant stirring for 60 or

Table I
Sunscreen Formulations

Active Ingredients	A[a]	B[b]
PABA	5.0%	
Padimate-O[c]		3.3%

[a]Vehicle contains 55% alcohol.
[b]Vehicle contains an acrylic/acrylate copolymer film former.
[c]Octyl Dimethyl PABA.

30 min in a beaker containing one liter of water at room temperature. The sample was removed from the water, reequilibrated to ambient conditions (approximately 48 hr) and the postsoaking photoacoustic spectrum recorded. The previous was performed in triplicate. From these spectra, as will be shown, one can evaluate the sunscreening effectiveness and substantivity to skin of the various formulations.

RESULTS

Figure 1 shows the ultraviolet photoacoustic spectra of excised, full-thickness neonatal rat skin and the in situ pre- and postsoaking photoacoustic spectra of Formulations A and B.

The presoaking spectra are essentially the same. Both exhibit a broad line shape in the 270 to 340-nm region with a maximum at approximately 317 nm. Postsoaking, Formulation B retains its initial line shape, whereas Formulation A exhibits a vastly different line shape, one similar to that of the untreated, control, full-thickness skin.

As shown in Figure 1, the strength of the photoacoustic signal in the 270 to 340-nm region is associated with the sunscreen agent and the strength of the photoacoustic signal at wavelengths greater than 369 nm is associated with background signal. One can therefore, for the spectra shown in Figure 1, obtain a ratio of the PAS signal at 320 nm to its strength at 370 nm. This ratio can be thought of as a sunscreening effectiveness index. As can be seen from the spectra, the index decreases with the decreasing sunscreening effectiveness and substantivity for the UVB region (290 to 320 nm) protective sunscreen. The indices as determined from Figure 1 are: 8.7 and 2.3 for Formulation A pre- and postsoaking (30 min), respectively; 6.7 and 6.4 for Formulation B pre- and postsoaking (60 min), respectively; and 1.4 for the control, untreated, excised, full-thickness, neonatal rat skin.

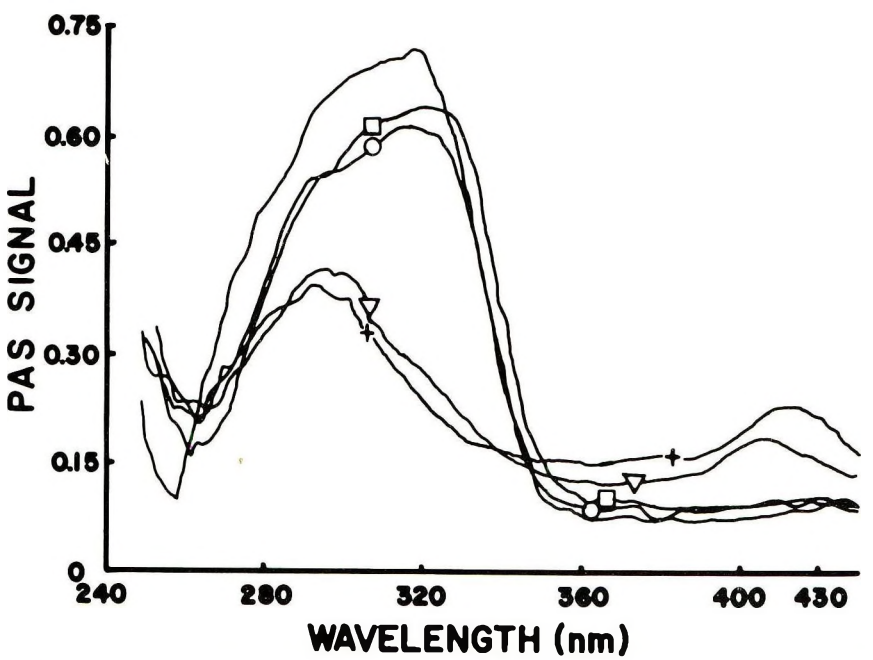

Figure 1. Photoacoustic spectra of newborn rat skin (+). Photoacoustic spectra of newborn rat skin with formulations applied: A pre-soak (−), post-soak (▽); B pre-soak (□), post-soak (○)

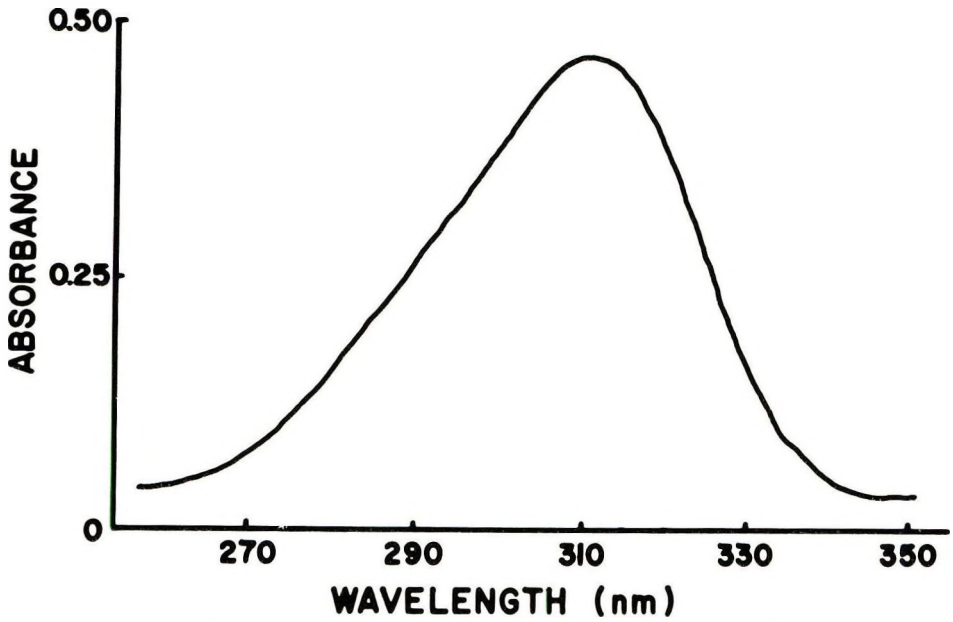

Figure 2. Optical absorption spectrum of a dilute alcohol solution of Padimate-O

The photoacoustic spectra of Formulation B (see Figure 1) exhibit a red shift and line broadening when compared to the optical absorption spectrum (see Figure 2) of a dilute alcoholic solution of Padimate-O. Moreover, as seen in Figure 1, both the red shift and line broadening are reduced with decreasing concentration of the retained sunscreen agent. These are common effects seen in the spectra of a compound as it undergoes changes in state, i.e., from solid to film to less concentrated film to dilute solution (6).

DISCUSSION

As mentioned earlier, the assessment of sunscreening effectiveness and substantivity would be most useful if it could be performed in a simple and direct fashion. Photoacoustic spectroscopy is an ideal method for achieving this goal since it allows for the spectral measurement to be made directly on skin; thus the parameters which govern the spectral properties of the skin-sunscreen complex are maintained close to those of the "in use" situation.

In addition, in actual practice, the measured absorbance values are frequently not directly proportional to the concentration of the solution. That is, so called "deviations" from the Beer-Lambert Law are quite common in analytical practice. Therefore conventional absorption data obtained on a dilute sunscreen product in solution, and extrapolated to higher concentration, are often inappropriate and misleading, particularly when the actual "in use" concentration lies in the nonlinear portion of the Beer-Lambert plot. As discussed above, the capabilities of photoacoustic spectroscopy enable one to study "in use" concentration, and the strength of the photoacoustic signal bears a close resemblance to the true absorbance. The spectra reported here are therefore more representative of the true sunscreening potentials since we obtain spectral information on undiluted samples in situ.

The observed red shift and line broadening as reported here indicate that in actual "in

use" situations the sunscreen provides protection over a broader wavelength region than that determined by dilute solution optical absorption investigation.

A broadened sun-protective region can be beneficial in designing a UVB-UVA (290 to 320, 320 to 400 nm) regions sunscreen product, where maximum blockage of both the burning rays (290 to 320 nm) and tanning rays (320 to 400 nm) is desired. However in designing a UVB region sunscreen product, where the desire is to block only the burning rays and allow for the tanning rays to penetrate the skin, the observed spectral shift and broadening may be detrimental because a substantial portion of the line shape may fall within the tanning region and thereby minimize the desired effect.

The photoacoustic spectral data shown in Figure 1 and the derived sunscreening effectiveness indices show that Formulation B is much more substantive to skin and is therefore a more effective postsoaking UVB region sunscreen when compared to Formulation A.

In a recent well controlled double-blind clinical study (9) the sunscreening effectiveness and substantivity of commercially available sunscreens similar to Formulations A and B were tested under controlled conditions of saltwater swimming at a beach. The result of this study showed that, postswimming, the commercially available product similar to Formulation B provided statistically significant better protection than the commercially available product similar to Formulation A.

CONCLUSION

The excellent agreement between the sunscreening effectiveness assessments as reported here by the use of photoacoustic spectroscopy and the clinical sunscreening beach study show that one can, in a rapid, simple and direct manner, use the new technique to evaluate undiluted sunscreen formulations in situ and under "in use" situations.

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Evaluation of a polymeric film-forming sunscreen preparation in tranquilized hairless mice

C. W. STOTT, J. SUSKEVICH and A. H. CAMPBELL *Johnson & Johnson Research Foundation, New Brunswick, NJ 08903.*

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Synopsis

A method is described for the EVALUATION of SUNSCREEN PREPARATIONS using HAIRLESS MICE. The mice were treated with TRANQUILIZERS to prevent removal of the agents by grooming. Three preparations were evaluated: 5% p-aminobenzoic acid in absolute ethanol; 5% p-aminobenzoic acid in 55% ethanol with skin moisturizers, and 3.3% octyl dimethyl p-aminobenzoate in a vehicle that forms a polymeric coating on the skin. Each product was evaluated two ways: 1) exposure to fluorescent sun lamps and 2) immersion in water followed by exposure to the sun lamps. All three products provided comparable protection, provided the animals were not immersed in water. Only the product which formed a POLYMERIC FILM provided suitable protection following immersion in water.

INTRODUCTION

Although the ultimate subject for sunscreen preparations is man, preliminary screening of such preparations in laboratory animals is desirable. Wolska et al. (1) have raised the question of the advisability of producing large areas of hyperpigmentation in human subjects in preliminary screening. Also the variation in the amount of pigmentation from subject to subject, and perhaps even between sites on the same subject, may tend to increase the difficulty of evaluation of the amount of protection afforded by different agents.

Physical methods (2, 3) in which the absorption characteristics of potential sunscreen agents are measured may be important in preliminary screening, such as the selection of compounds which absorb ultraviolet (UV) irradiation in the desired range and determination of the concentration of the agent in the product. The real test of efficacy, however, is the amount of protection afforded in vivo against biological damage during exposure to UV irradiation.

A number of laboratory animal species have been used in testing the effects of UV irradiation on normal and treated skin. Most of these, however, require the use of a depilatory or afford a very small area of relatively hair-free skin on which to conduct testing. We have found that the clipped abdominal skin of albino rabbits is markedly sensitive to many of these materials adding an additional impediment to evaluation of

protection. There are two hairless animal species available, the hairless mouse and the Mexican hairless dog. The former are relatively inexpensive and obtainable in large numbers. Also there is a total absence of pigment cells in the skin of these animals, which eliminates that variable.

Since erythema of mild degree is difficult to visualize in this species, it was felt that, instead of evaluating a preparation by determining the minimal erythema dose (MED) of skin treated with a product, a more realistic approach would be to compare the effects following exposures which produce severe damage in unprotected skin.

Many sunscreen products are ineffective because they are eliminated by perspiration or removed while swimming (4). Therefore, immersion in water following application of the test products is necessary in a realistic test of efficacy and substantivity.

We have also found that when hairless mice are painted with sunscreen preparations their natural grooming instinct results in the removal of some or all of the preparation. As an example, mice painted with 5% p-aminobenzoic acid, a proven effective sunscreen in man, 1 hr before exposure to the sunlamp had the same degree of injury as animals not treated with a sunscreen. We have arrived at dosages of tranquilizers which block the grooming response of these animals until after termination of the exposure to the sunlamp.

In this paper we present a method utilizing hairless mice which we feel is an effective and practical model for the evaluation of the efficacy of sunscreen preparations without, or following, immersion in water.

MATERIALS AND METHODS

Male hairless mice (JAX hr/hr, Jackson Laboratories, Bar Harbor, Maine), seven to ten weeks of age, were used in these experiments. They were housed in plastic cages, five per cage, on wood shavings and maintained on Purina® Laboratory Chow. The ultraviolet exposure was based on the method of Owens et al. (5). The mice were placed in groups of five in clean cages 7 in. wide × 12 in. long with 0.5-in. wire screen lids. No shavings were used in the cages and a few food pellets were placed on the floor to minimize climbing on the screen lids. Four cages were placed under a fluorescent light fixture with two Westinghouse FS40-T12 lamps. The lamps were 30 cm above the floors of the cages.

Three formulations containing sunscreens were tested. Product A was composed of 5% p-aminobenzoic acid in 95% ethanol. Product B contained 5% p-aminobenzioc acid, 55% alcohol and an undisclosed content listed as skin moisturizers. Product C contained 3.3% octyl dimethyl p-aminobenzoate and 8.6% ethanol in a vehicle which formed a polymeric film on the skin and is easily removed with soap and water. Products A and B contained 0.365 M p-aminobenzoic acid and Product C contained 0.140 M octyl dimethyl p-aminobenzoate.

Two test procedures were employed in this study: 1) UV exposure of mice immersed in water following sunscreen applicaiton and 2) UV exposure of mice not immersed in water following sunscreen application. In the nonimmersed study, all animals including controls were administered chlorpromazine hydrochloride, 9 mg/kg intraperitoneally. An additional control group was not treated with chlorpromazine hydrochloride in order to determine any possible phototoxic potential of the medication. A volume of

35 μl of the appropriate sunscreen was then applied over a 2 × 3.5-cm area on the backs of the mice (5 μl/cm²). One hour after application of the sunscreen, the animals were exposed to the UV source for 150 min. This was the equivalent of 17 × MED, determined by exposing groups of ten untreated animals/group and examining them for erythema at 24 hr.

For the immersion study, all animals were first administered 1.5 ml/kg intramuscularly of a 1:10 dilution in 0.15 *M* saline of Innovar®-Vet (Pitman-Moore, Inc.). The appropriate sunscreen was then applied as above. One hour after sunscreen application, all animals were immersed individually for 30 min in beakers containing enough water so that the animals could just stand on their hind legs. The water temperature was maintained at 37°C by the use of a waterbath. Upon removal from the waterbath they were administered chlorpromazine hydrochloride, 9 mg/kg intraperitoneally, and exposed to the UV source for 150 min. All animals were examined daily for four days. Written descriptions of the appearance of the animals were later translated into a numerical scoring system as follows: no visible damage = 0; erythema, dry scaley skin, but normal or nearly normal by 96 hr = 1; scattered whitish patches (edema?) with few scattered pinpoint erosions = 2; large area of whitish, thickened skin with numerous, tiny erosions = 3; large erosions 3 to 5 mm long = 4; and erosions >5 mm long = 5. The scores for each animal in the group were totaled and the total divided by the number of animals in the group to arrive at the "average burn score."

RESULTS

No differences in the various groups could be detected immediately following exposure to the sunlamps. All animals appeared erythematous. Differences could be distinguished at 24 hr but were much more obvious at 48 hr. In the animals that had received only slight damage, the treated areas appeared visibly normal within 96 hr. Animals that received little or no protection still had gross evidence of severe damage at 96 hr.

NONIMMERSION STUDY

The average burn scores are presented in Table I. No clear differences could be distinguished between the nondrug control group and the drug control group. These animals were generally erythematous with small pinpoint erosions and edema at 24 hr. At 48 hr there was marked edema with numerous small encrusted erosions. The sizes of the en-

Table I
Average Burn Scores of Hairless Mice Exposed to Ultraviolet Light

	Not Immersed		Immersed	
Treatment	N[a]	Score	N[a]	Score
Control	5	4.0	5	5.0
Drug Control	5	4.0	5	5.0
Product A	5	1.0	5	4.4
Product B	5	1.0	5	4.0
Product C	5	1.0	5	2.0

[a]Number of animals.

Figure 1. Hairless mice tranquilized with chlorpromazine and irradiated with UVL for 150 min—96 hr after irradiation

crusted erosions were in many cases larger at 72 hr and exfoliation was under way around the edges of the treated areas. At 96 hr much of the treated area was still covered with thickened, whitish dried skin, the encrusted lesions were very prominent and in areas where exfoliation had occurred there were deep pink to red spots (Figure 1).

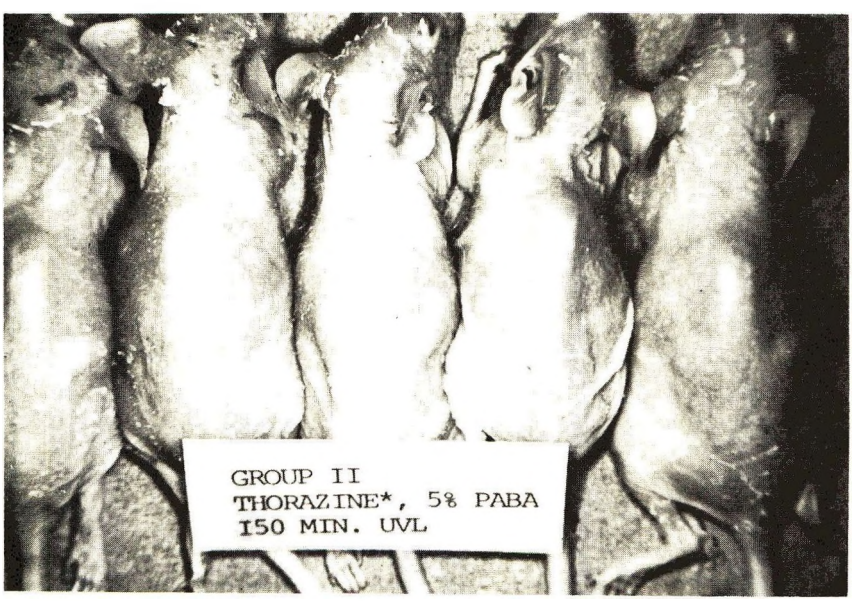

Figure 2. Hairless mice tranquilized with chlorpromazine, painted with Product A and exposed to UVL for 150 min—96 hr after irradiation

Figure 3. Hairless mice tranquilized with Innovar®-Vet, immersed in water 30 min, injected with chlorpromazine and irradiated with UVL for 150 min—96 hr after irradiation

The three groups treated with the sunscreen preparations were similar in appearance. Erythema was visible for 48 to 72 hr with some dry, scaley skin present at this time. By 96 hr the backs of these animals appeared to be normal or nearly normal (Figure 2).

WATER IMMERSION STUDY

The nondrug controls and the drug controls were similar to those in the nonimmersed groups, but the number and size of encrusted erosions were greater. Much of the center of the backs of these mice was still covered with whitish, thickened skin at 96 hr and, in areas where exfoliation had occurred, deep pink to red lesions were observed (Figure 3).

Of the five mice treated with Product A, four were similar to the controls, while the remaining one had fewer and smaller erosions (Figure 4). The group treated with Product B developed a few scattered erosions up to 4 mm in length and at 96 hr a large area of the back was still covered with the whitish, thickened skin (Figure 5).

In the group treated with Product C, the treated areas were erythematous with minor, spotty edema, dry scaley skin with some exfoliation at 48 hr. At 72 hr, most of the dry scaley skin was gone and the newly exposed skin appeared almost normal except for some very faintly erythematous spots. At 96 hr the treated areas of three mice appeared normal and the remaining two had a few spots of mild erythema (Figure 6).

DISCUSSION

This method provides an animal model for testing potential sunscreen agents and formulations for efficacy and substantivity. The use of tranquilizers ensures that the

Figure 4. Hairless mice tranquilized with Innovar®-Vet, painted with Product A, immersed in water 30 min, injected with chlorpromazine and irradiated with UVL for 150 min—96 hr after irradiation

agents tested are not removed by the grooming tendencies of the animals. Although we could not detect any obvious differences between drug-treated and nontreated animals in our study, the use of drug-treated control groups is necessary since chlorpromazine is a proven phototoxic agent whose activation wavelength lies within range of maximum output by the Westinghouse FS40-T12 fluorescent sunlamp.

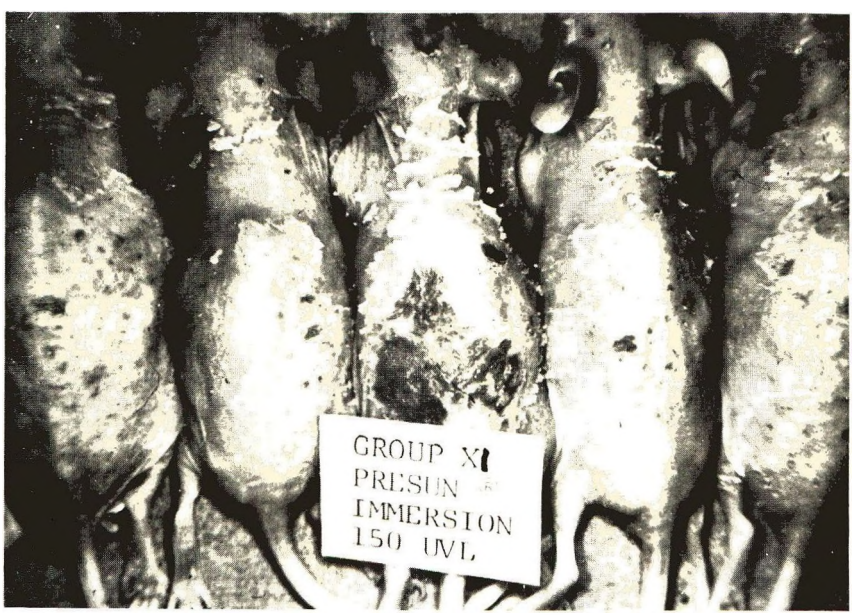

Figure 5. Hairless mice tranquilized with Innovar®-Vet, painted with Product B, immersed in water 30 min, injected with chlorpromazine and exposed to UVL for 150 min—96 hr after irradiation

Figure 6. Hairless mice tranquilized with Innovar®-Vet, painted with Product C, immersed in water 30 min, injected with chlorpromazine and exposed to UVL for 150 min—96 hr after irradiation

Since chlorpromazine depressed the mice to the point that they would sometimes drown during immersion, Innovar®-Vet was substituted. Following administration of this relatively mild neuroleptanalgesic agent, no deaths (from either drowning or medication) were recorded thereafter.

All three sunscreen formulations appeared to provide equivalent protection from ultraviolet radiation when the mice were not immersed in water following application of the sunscreen. In the groups which were immersed, those animals treated with Products A or B were not protected due to the elution of the sunscreen agents from the skin. Although the burn scores for the immersed group treated with Product C indicate some loss of protection when compared with the nonimmersed group, the comparison of this group with the other two groups treated with Product A or B and immersed is dramatic. Product C still provided significant protection following immersion in water for 0.5 hr.

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EDITOR'S NOTE: In the paper entitled "The quantitative analysis of bergapten in perfumes," written by Shukrallah T. Zaynoun, M.D., and published in the May 1978 issue of the *Journal of the Society of Cosmetic Chemists* (Vol. 29, No. 5), part of the author's address was inadvertently deleted. The bulk of Dr. Zaynoun's research was done while he was with the Department of Dermatology, University of Dundee, Dundee, Scotland, U.K. We apologize for this omission.

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In vivo measurement of transepidermal water loss

B. IDSON *Hoffmann-La Roche, Nutley, NJ 07110.*

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Synopsis

An overview is presented of the background and principle methods for MEASURING TRANSEPIDERMAL WATER LOSS (TWL) IN VIVO. Absolute values of TWL are a function of the particular technique and experimental conditions. TWL will vary with different skin sites and rise markedly if the skin barrier is removed or affected by pathologies. Early gravimetric methods lack sensitivity and require long testing periods as well as large areas of skin. The disadvantages have caused shifts to other techniques where absorption of water vapor is followed by a sensitive physical measurement. The majority of methods are based on determining the increase in moisture content of either a current of dried air or fixed humidity air conducted over the skin. Others have sought to avoid air flow, using changes in conductivity of inorganic crystals. Methods discussed include thermal conductance, electrohygrometry, infrared radiation, electrolysis of absorbed water vapor and calculation of vapor pressure gradient in the layer of air adjacent to the skin surface. The mechanism may be an additive effect of neural control of eccrine sweat gland activity and stratum corneum hydration.

INTRODUCTION

Water exerts a major role in all well-being but particularly in skin health to maintain its desirable soft, flexible mechanical properties (1). The lack of adequate water in the upper layer of the skin, the stratum corneum, results in dry and chapped skin (2–6). Dermatologic and cosmetic interest has focused on techniques that generate information on the state and quantity of water in the stratum corneum, the mobility of the water and the influence exerted by components of the stratum corneum on the diffusion characteristics of the water (1).

Water is lost through skin in two ways, eccrine sweating and transepidermal diffusion. Under severe thermal stress as much as 2 l/hr may be lost as sweat. By contrast, diffusional or transepidermal water loss (TWL) is a steady passive process in which water vapor diffuses from the highly hydrated underlying tissues through the avascular stratum corneum, dissolves in it and diffuses to the exterior surface where it evaporates. Emphasis is placed on the stratum corneum since this biologically inert membrane—due to its dense, fibrous, lipoprotein matrix—represents the principle physical barrier to the penetration of molecules through the integument (7). The magnitude of TWL has been widely used as a measure of the effectiveness of this barrier in dermatological disease states (8–11). In pathologies such as psoriasis, ichthyoses and

eczema there is impaired barrier function with increased TWL. Normal intact skin has an average TWL of 0.31 mg cm^{-2} hr^{-1}, rising about ten-fold in psoriasis and eczemas. If the barrier is removed, e.g., by Scotch tape stripping, the TWL rises to 15 to 45 mg; a 50-to 150-fold increase over intact skin. No biological membrane of comparable thickness offers such resistance (12–14).

Water loss through the skin is dependent on environmental factors, of which the most important are the ambient temperature and humidity. Comparison of results of water loss can only be valid if readings are made when these two factors are constant. Like all membrane-diffusion processes, TWL has a characteristic activation energy and therefore its magnitude is temperature dependent (9, 15, 16). Decreasing skin temperature is accompanied by a decreasing TWL. A 5°C fall in skin temperature lowered the TWL by about 45%. The fall appeared to be related to skin temperature and not directly to the reduction in body temperature. A rise of skin temperature of 7 to 8°C doubled the TWL rate (9). A number of "in vitro" and "in vivo" techniques have been described to measure the transepidermal water diffusion from selected areas of the skin. Isolated skin has been studied in vitro in diffusion chambers (17–19). This paper, however, will only be concerned with "in vivo" methodology on human subjects. Early techniques of measuring TWL, prior to 1965, have been critically reviewed by Bettley and Grice (8) and Baker and Kligman (20).

Analysis of the extensive literature data indicates that the absolute values of TWL largely depend on the technique and experimental conditions used in its measurement (21). However it is evident that with any given technique the values of the TWL consistently vary topographically from skin site to skin site (11, 17, 22). Considerable regional variation was noted in certain areas, even after the readings had been corrected for varying horny layer thickness and expressed as diffusion constants. Compared with that of the back, the diffusion constant is four times greater on the forehead, nine times greater on the back of the hand and 100 times greater through the palm (20).

Technical difficulties have been encountered with all methods. Basically this is because all measurements of TWL must, of necessity, be made under artificial conditions; variations in these conditions might be expected to alter the water loss. Broadly, the methods can be classified as "ventilated" and "unventilated": ventilated—in which a continuous flow of gas or air passes through a capsule attached to the skin and the change in the humidity of the gas is measured by a sensing element in the outflow; "unventilated"—in which a container is used with its open end placed on the skin surface, the water vapor given off alters the relative humidity within the chamber and this rate of change is a measure of the rate of insensible water loss (11). Any unventilated method is much less satisfactory if the water loss is considerable since water droplets may develop on the skin and fail to evaporate completely.

TECHNIQUES

Until recently, in vivo determinations have depended upon gravimetric estimation of the water taken up by a hygroscopic medium enclosed in a chamber placed over the skin or removed from a stream of dried air passed through a skin chamber. Pioneering studies (23) involved passing dry oxygen over a small brass chamber attached to the abdomen and collecting the water vapor in the effluent air in freezing coils. In variant

gravimetric methods (8, 24), a chamber containing a bag of calcium chloride was fastened over the skin. The change in weight of the hygroscopic salt indicated the amount of water transpired per unit of time. In an interesting cosmetic study, Powers and Fox (25) strapped small tared dessicators containing silica gel to the arms of subjects and reweighed them after 2 hr. If there was a decrease in weight, the material was a good occludant. While crude, the method yielded the first quantitative proof of the superiority of petrolatum as an occlusive agent.

While simple to perform, gravimetric methods are crude and inaccurate. Results may only be obtained at the price of considerable effort and care (20). A main disadvantage is the lack of valuable rate data. Long periods are necessary for testing, with lack of sensitivity in assessing minimal day-to-day differences. The large areas of skin needed has limited use in dermatology where the interest lies in local deviations of small areas of the skin. Actually, the earlier methods of weighing absorbed water vapor have been gradually abandoned when limited areas are involved in investigation. In addition, the tests can be compromised by eccrine sweating, which cannot be discounted, particularly when long periods of testing are used. Since eccrine sweating is so much greater than transepidermal diffusional loss, most subsequent investigators have sought to inhibit the former by use of anticholinergic drugs (9, 20) and keeping the ambient temperature low. However, excessive emotional sweating usually appears as transient rapid increases in water loss which are easily distinguished from baseline TWL (21).

The disadvantages of the gravimetric method have caused shifts to other techniques where absorption of water vapor is followed by another more sensitive physical measurement, such as the electrical conductance of a chemical sensor cell or electrolysis of the absorbed water.

The majority of current methods are based on the increase in moisture content of a current of dried air conducted over the skin. Some investigators consider it a disadvantage that the skin is exposed to dry air instead of the normal environmental humid air (26). Since the permeability of the skin depends on the water content of the stratum corneum, the water content of the horny layer of the skin alters when the water content of the atmosphere changes. Thus many investigators prefer to study the water vapor loss of the skin when exposed to air of a fixed humidity, which can be obtained by bubbling the air through a saturated sodium chloride solution before it reaches the skin (27). Other investigators even want to avoid a flow of air along the skin surface and record the increasing humidity inside a cup placed upon the skin (28). Ideally, the skin should be investigated under unaltered atmosphere conditions so that the skin does not need time to acclimatize to a changed environment. Some methods approach this ideal, e.g., where environmental humid air is conducted over the skin and hygrometers are mounted in the air both before and after it has passed the skin (20, 21, 29). As will be discussed, only large areas of skin have been used and the sensitivity of the hygrometer is critical.

Investigators such as Thiele and Schutter (28) have sought to avoid air flow entirely. They consider that the streams of a carrier gas, used to transfer moisture from the skin to the measuring vessel, create abnormal water vapor gradients. They developed a sensitive method based on the change of conductivity at the surface of a temperature-controlled halite (rock salt) crystal, resulting from the adsorption of minute amounts of water evaporating under normal conditions from the skin surface. Without this temperature control, the temperature of the salt crystal would be adversely affected by

heat radiation from the skin surface. The temperature of the crystal is kept constant by means of a cooling system connected with a circulation themostat. The sensing element is a thermal conductivity cell comprising two compartments. The air passes through the first compartment before it reaches the skin. After the air has been humidified by passing over the skin, the air is led through the second compartment of the cell. A thermistor is mounted in each of the two compartments which are then incorporated into the arms of a Wheatstone bridge circuit. Any difference in the composition of the air between the two compartments causes an imbalance in the bridge, which is recorded directly (29).

The measurement of the thermal conductivity of the air allows the measurement of the insensible perspiration of 1 cm^2 of forearm skin (30). It is possible to quantitatively detect 0.1 to 30 $\mu g/cm^2/min$ evaporating from the skin. Simultaneously, the water vapor pressure at the skin surface can be recorded. From this vapor pressure can be calculated the relative humidity of the skin surface which is a measure of the moisture content of the outermost skin layers.

Quattrone and Laden (31) have adapted the thermal conductivity method to use a carrier gas, in a method which they call transpirometry. The investigators employ an apparatus wherein a stream of dry nitrogen, passing in a flow-through chamber on the skin, and a stream of identical pressure flowing independently of the skin are compared for their thermal conductivity in a gas chromatograph. Two of these systems, each equipped with integrators, allow for simultaneous measurement of the rate of moisture loss at two separate sites (i.e., a control and a test). In the actual method, for each unit, streams of dry nitrogen are split into two equal components—one passing directly into the chromatography unit, while the other streams into a flow-through probe on the skin before entering this thermal conductivity analyzer. The difference in the conductance between the split streams is measured and a signal from each chromatograph is sent to a dual pen recorder. The latter is equipped with two repeating potentiometers, allowing for integration of each signal. Standard curves are obtained for each system before use each day by application of known quantities of water to filter paper sealed within each chamber.

The previous static-conductance method can be replaced with a dynamic electrohygrometer technique (20, 21, 29) wherein ambient humidity air is swept through a skin chamber over a plate coated with a sensor chemical whose electrical conductivity is a function of the ambient humidity. Sulzberger and Herrmann (32) were the first to attempt to monitor humidity changes by means of electrohygrometry. They passed air through a skin chamber and over a plate coated with lithium sulfate. A group of inorganic sensor salts, of which lithium bromide is the most commonly used, has subsequently been refined and made commercially available. It should be noted that the "humidity sensor" devices are limited by the fact that they operate within an enclosed area. Therefore the measurements have to be accomplished very quickly in order to avoid saturation of the air contained within the chamber. Electrohygrometry has lowered the skin conditioning time, as opposed to gravimetric methods.

In electrohygrometric TWL measurements, a current of air is either predried by passing through a freezing mixture (8) or is passed across a pre-sensor to record the humidity of the inflowing air (11) and is then conducted into a sampling chamber. The chambers have extended from 15 cm^2 area of skin (21) to 60 cm^2 (8). The apparatus usually incorporates a humidity transducer which provides continuous monitoring and a thermistor for measuring skin temperature (8, 9). As previously discussed, the

presence of sweat is either inhibited by inactivation of sweat glands by use of anticholinergic drugs (20) or ascertained by the galvanic conductivity of skin (9). If the sweating is not inhibited, attempts are made to hold skin temperature below 34°C, which is the lower threshold of sweating. Low air-flow rates are appropriate for diffusion measurements; in intact skin between 50 and 300 ml/min is the usual range, but the flow must be adjusted to the rate of water loss. Too small a flow will allow the humidity to build up in the system, or, in sweat studies, may fail to vaporize the droplets as they form. Too large a flow may result in uneven mixing since the relative humidity (RH) and temperature (T) of the air is monitored prior to and following its passage over the skin surface, the difference in relative humidity (Δ RH) represents the water vapor picked up at the skin surface (21). Each measurement requires approximately 15 to 20 min. Most TWL measurements are performed with a Sage electric hygrometer, Model 154 (Sage Instruments, White Plains, New York) using lithium bromide sensing elements.

The TWL may be calculated according to the formula (21):

$$TWL = \frac{\Delta RH}{100} \times D \times AF \times \frac{1}{A} \tag{1}$$

where:

TWL is the transepidermal water loss in mg cm^{-2} hr^{-1}
ΔRH is the difference between the incoming and effluent relative humidities
D is the weight of water per liter of saturated steam at the temperature of the air passing over the skin in mg l^{-1}
AF is the volumetric air flow rate in 1 hr^{-1}
A is the area of skin in cm^2

the density of saturated steam (D) at different temperatures is obtained from tables.

In a variant hygrometer method, TWL was determined by Berube et al. (33), using compressed air as a carrier gas. The flow of the gas was split into two streams to flow over the left and right arm of subjects. Each stream then flowed over a Sage hygrometer where the moisture is swept from the surface of the skin into the gas stream. The stream passes through the sensing chamber Dew Point hygrometer (Cambridge Systems, Newton, Massachusetts) where the amount of moisture present is measured utilizing the dew point principle.

The concentration of water vapor in a gas stream can also be measured by its absorption of i.r. radiation (15). TWL can be measured on areas from 20 cm^2 (34) to as low as 1 to 4 cm^2 (35). The principle is that infrared radiation passes through two conduction tubes and then into the i.r. detector. In practice, dry gas is passed over the skin surface and the moistened gas is then passed through the analyzer. When the gas stream containing water vapor is passed through the conduction tube, while the other is flushed with dry gas, some radiation is removed by the wet gas stream. This produces an imbalance in the radiation absorption between the two sides of the detector which is a measure of the TWL. The measurement is made when water loss becomes constant at a particular flow of dry nitrogen gas over the skin. This is called the equilibrium state because loss of water from the skin surface is exactly matched by water diffusing up from the epidermis. Measured in this way, it was found that the rate of TWL was modified by the rate of flow of dry gas; increases in the flow of dry gas produces cor-

responding increases in the skin water loss and these two variables show a linear relationship.

Electrolysis of absorbed water vapor is among the most sensitive methods for determining TWL. Spruit (36, 37) adapted the MEECO (Model W, Manufacturers Engineering and Equipment Corporation, Warrington, PA) industrial electrolytic water analyzer to measure water vapor on only 1 mm^2 of forearm skin with the same accruacy as had previously been determined on 20 mm^2. In the TWL analysis, a current of dry nitrogen is passed through a cup placed on the skin. This is conducted through a tube containing two platinum wires separated by a thin layer of phosphorus pentoxide that will absorb the water vapor. An electric potential is established between the platinum wires which splits the water into oxygen and hydrogen. The electric current resulting from the electrolysis is registered by a recorder.

The measurement of the water vapor loss of the skin may be completed in 5 to 15 min. The reading of the instrument will then be constant and will remain so for several hours. This may seem surprising because one would expect the skin to be dried by nitrogen and the swelling and water solubility to decrease in the course of the measuring procedure. A probable explanation is that the diffusion constant increases at the same rate as the solubility decreases (15).

The MEECO values can only be compared to the previously discussed salt crystal values, when the MEECO values are converted. With the MEECO, an absolutely dry atmosphere is used as a reference. In the case of the Salt Crystal Meter, the moisture transport is induced by a vapor-pressure drop from the skin to the ambient atmosphere.

A recent study of TWL in newborn infants (38) has developed a method based on calculation of the vapor pressure gradient in the layer of air immediately adjacent to the skin surface. If this gradient is known, the amount of water evaporated per unit time and area can be calculated from the equation

$$\frac{1}{A} \cdot \frac{dm}{dt} = -D' \frac{\delta p}{\delta x} \qquad (2)$$

where

$\frac{1}{A}\frac{dm}{dt}$ is the amount of water evaporated per unit time and area (g/m^2 hr), expressed as evaporation rate (ER)

D' is a constant (0.670×10^{-3} g/mhrPa)

$\frac{\delta p}{\delta x}$ is the vapor pressure gradient of the water vapor (Pa/m).

This equation is valid in the immediate vicinity of the evaporative surface, i.e., in the zone of diffusion, which is about 10 mm wide. At a constant rate of evaporation the vapor pressure in the diffusion zone decreases linearly with the distance from the skin surface.

The vapor pressure gradient is therefore proportional to the difference in vapor pressure at two separate points located on a line perpendicular to the evaporative surface. The vapor pressure at the two measurement points is calculated as the product of the relative humidity and the saturated vapor pressure, the latter being a function of

the temperature alone. According to eq 2, the water evaporation per unit time and area (ER) can then be calculated as a constant multiplied by the measured difference in vapor pressure between the two measurement points.

MECHANISM

The physiological mechanism and relative contributions of chemical composition, membrane structrue and topographical skin area in TWL is relatively ill-defined. Wildnauer and Kennedy (21) suggest that the phenomena of TWL is not simply a physical process following physicochemical laws, but that some physiological processes can participate in the mechanism and therefore influence the magnitude of TWL. They cite evidence (39–41) which strongly suggests that this physiological involvement may be the neural control of the eccrine sweat gland. The resultant of this interaction of eccrine gland activity with stratum corneum properties that influence TWL can be interpreted as a hydration effect on membrane permeability.

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The chemistry of nitrosamine formation, inhibition and destruction

M. L. DOUGLASS *Colgate-Palmolive Company, 909 River Rd., Piscataway, NJ 08854;* B. L. KABACOFF *Revlon Research Center, 945 Zeraga Ave., Bronx, NY 10473;* G. A. ANDERSON *The Dow Chemical Company, 1710 Building, Midland, MI 48640, and* M. C. CHENG, *The Procter and Gamble Company, Ivorydale Technical Center, 5299 Spring Grove Ave., Cincinnati, OH 45217.*

Received June 29, 1978. Authors represent the Nitrosamine Task Force of the Cosmetic, Toiletry and Fragrance Association, 1133 15th St., N.W., Washington, D.C. 20005.

Synopsis

N-Nitroso compounds are formed from the interaction of many types of organo-nitrogen compounds and nitrosating agents. Ease of nitrosation is determined by compound structure, nature of the medium and the presence of catalysts. The two categories, nitrosamines and nitrosamides, differ mainly in their CHEMICAL stability and mechanism of biological activity. NITROSAMINES are more stable and difficult to DESTROY, but their FORMATION can be INHIBITED by substances which react preferentially with the nitrosating agent. The carcinogenic activity of these compounds in laboratory animals varies widely from highly potent to innocuous.

I. INTRODUCTION

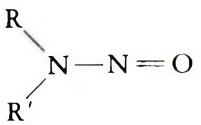
Advances in analytical techniques allow modern industrial society to detect trace amounts of undesirable substances in its physical environment. There has been legitimate concern that we are creating conditions that have serious adverse effects on human health. Recently, minute levels of nitrosamines have been found in some consumer products, including cosmetics (1). While not attempting to judge whether these substances at parts-per-billion levels have a significant physiological effect, we are presenting a review of nitrosamine chemistry to aid workers in the cosmetic and allied industries in their research on the subject.

II. TYPES OF *N*-NITROSO COMPOUNDS

N-Nitroso compounds are formed by the interaction of a nitrogen-containing organic compound—such as an amine, amide, urea, guanidine, urethane or cyanamide—and a nitrosating agent, such as a nitrogen oxide.

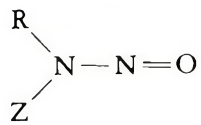
These compounds can be divided into two categories—nitrosamines and nitrosamides—which differ in their chemical stability, the mechanism of their carcinogenicity and their mutagenicity (2–4).

N-Nitrosamines



R,R′ = alkyl or aryl

N-Nitrosamides



R = alkyl or aryl

Z = $R'C(=O)-$ nitrosamide

$R'_2NC(=O)-$ nitrosourea

$R'_2NC(=NH)-$ nitrosoguanidine

$R'OC(=O)-$ nitrosourethane

$NC-$ nitrosocyanamide

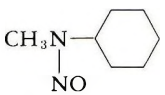
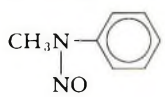
$R'SO_2-$ nitrososulfonamide

The nitrosamines are very stable once they are formed. They require chemical modification in an enzyme-catalysed reaction before they exhibit carcinogenic and mutagenic activity (2, 3, 5). By comparison the nitrosamides can be hydrolysed, especially in neutral and alkaline solution. They exhibit carcinogenic and mutagenic activities without modification and malignant tumors are produced at the site of their application (2–5).

III. CARCINOGENICITY OF *N*-NITROSO COMPOUNDS

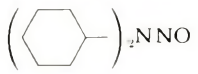
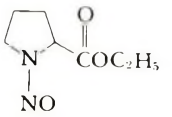
The first report that nitrosamines cause cancer in laboratory animals was that rats fed low levels (50 ppm) of dimethylnitrosamine in their diet developed liver cancer (6, 7). Since then more than 120 nitrosamines and nitrosamides have been examined for

Table I
Varying Carcinogenic Activity of Nitrosamines

Classification by Range of log (l/D_{50})[a]	Structure	D_{50}[a]	log (l/D_{50})
Highly Potent, >3.0			
	$(CH_3CH_2)_2NNO$	0.00063	3.2
	$CH_3NCH_2CH_2Cl$ NO	0.00061	3.2
	$CH_3NCH_2C_6H_5$ NO	0.00080	3.1
Potent, 2.1 to 3.0			
		0.0010	3.0
	$CH_3NCH_2CH_2NCH_3$ NO NO	0.0039	2.4
	$(CH_3)_2NNO$	0.0054	2.3
	$(n\text{-}C_3H_7)_2NNO$	0.0088	2.1
Intermediate Activity, 1.1 to 2.0			
	$(NCCH_2)_2NNO$	0.012	1.9
		0.012	1.9
		0.011	1.9
	$CH_3CH_2NCH_2CH_2OH$ NO	0.016	1.8
	$(n\text{-}C_4H_9)_2NNO$	0.025	1.6
		0.025	1.6
	$n\text{-}C_4H_9N(CH_2)_4OH$ NO	0.031	1.5
		0.039	1.4
Minimal Activity, ≤1.0			
	$(i\text{-}C_3H_7)_2NNO$	0.11	1.0
	$(CH_3COCH_2CH_2)_2NNO$	0.18	0.7
	$(n\text{-}C_5H_{11})_2NNO$	0.26	0.6
	$CH_3NCH_2CO_2H$ NO	0.24	0.6
	$(HOCH_2CH_2)_2NNO$	0.89	0.05

continued on p 584

Table I (continued)
Varying Carcinogenic Activity of Nitrosamines

Classification by Range of log (1/D_{50})[a]	Structure	D_{50}[a]	log (1/D_{50})
No Detectable Activity			
	$(C_6H_5)_2NNO$	—	—
	$(C_6H_5CH_2)_2NNO$	—	—
		—	—
		—	—
	$(HO_2CCH_2)_2NNO$ (12)	—	—

[a] D_{50} = mean total carcinogenic dose, expressed in mol/kg body wt, for production of tumors in 50% of the animals.

carcinogenic activity in animals. Comprehensive reviews of the results have been published (2, 4, 5).

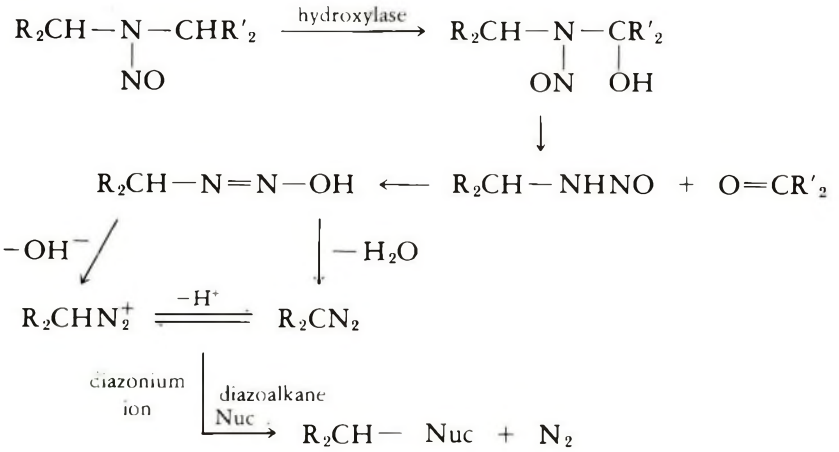
Although there is no direct evidence that *N*-nitroso compounds cause cancer in man, their carcinogenicity has been demonstrated in many other animal species including mice, rats, hamsters, fish, rabbits, guinea pigs, dogs and monkeys (4, 5, 9).

About 80% of the *N*-nitroso compounds tested are carcinogenic to some degree. Their potency varies widely, from compounds where a single dose is sufficient to induce tumors to those where large doses given repeatedly produce no malignancy (2, 5). To illustrate the range of activity representative nitrosamines are classified in Table I according to carcinogenic potency (10). The carcinogenic dose is expressed in the way suggested by Wishnok et al. (11) so that larger numbers indicate higher carcinogenicity.

Wishnok and coworkers (11) recently demonstrated that the carcinogenic potency of many nitrosamines correlates quantitatively with a combination of their hexane-water partition coefficients and the electronic inductive effects of substituents on the α-carbon. Earlier Wishnok and Archer (13) showed that carcinogenicity is inversely related to the number of carbon atoms of acyclic dialkyl nitrosamines. Lijinsky (14) found that the reverse is true for cyclic nitrosamines, where the larger molecules are more potent, and that there are major changes in target organs with a change in ring size.

The frequently proposed mechanism of action (2, 3, 15) shown below accounts for the enzymatic activation required by nitrosamines, but not nitrosamides, and indicates that only nitrosamines containing an α-hydrogen are carcinogenic.

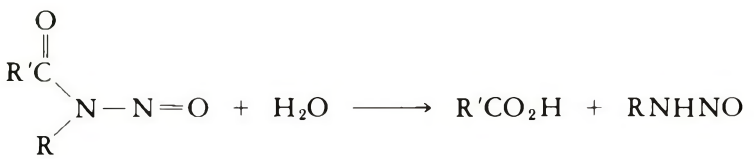
The requirement for activation of nitrosamines is defined as an enzyme-catalysed hydroxylation of an α-carbon. This step is supported by correlations of the degree of carcinogenicity with α-carbon substituents (11) and by recent work showing that preformed α-acetoxy nitrosamines are direct acting carcinogens not requiring enzymatic modification for activity (16).



The hydroxyalkyl group is eliminated as an aldehyde or ketone leaving an unstable primary nitrosamine. The latter tautomerizes to a diazonium hydroxide.

Alkylation of nucleophilic sites (Nuc) in DNA, RNA and proteins by *N*-nitroso carcinogens has been demonstrated (5), but the evidence conflicts as to whether a diazonium ion or the diazoalkane is the alkylating agent (2, 4, 5, 17). In nucleic acids the principle site of alkylation is at N(7) of guanine. Alkylation of nucleic acid oxygens has also been demonstrated (18).

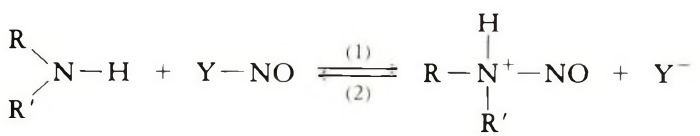
Nitrosamides do not require metabolic activation because they can be hydrolysed in vivo to an unstable primary nitrosamine (2, 3), the proposed precursor of the alkylating agent.



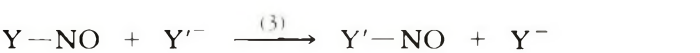
IV. CHEMISTRY OF *N*-NITROSO COMPOUNDS

A. INTRODUCTION

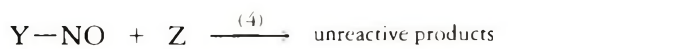
Much of the chemistry of *N*-nitroso compounds in aqueous solution can be summarized by the following scheme.



(1)
(2)



(3)



(4)

Nitrosation of secondary amines and amides is described by eq 1. The effectiveness of the nitrosating agent Y—NO depends on the nature of Y. *Catalysis of nitrosation* by Y′ species results from its prior reaction with Y—NO (eq 3), which produces the more active nitrosating agent Y′—NO. When Y is a secondary amine function, eq 1 describes *transnitrosation* as it is defined in this paper.

Inhibition of nitrosation occurs by reaction of inhibitor Z with nitrosating agent Y—NO in the irreversible eq 4, which is much faster than 1 and produces unreactive products. *Destruction* of *N*-nitroso compounds by denitrosation is described by eq 2. Addition of Z, in this case called a trap or scavenger, is necessary to prevent via 4 the reversal of denitrosation, eq 1.

Details of these reactions and the chemistry of *N*-nitroso compounds not included in this scheme are described below.

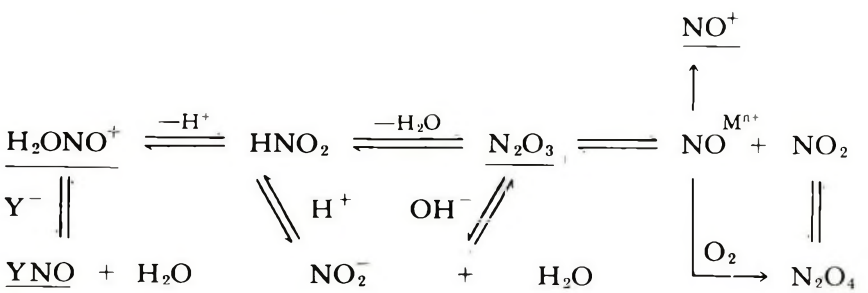
B. FORMATION

1. Nitrosating Agents

a. Inorganic Species. Several nitrogen oxide species are nitrosating agents, but nitrous acid (HONO) and the nitrite ion (ONO^-) are themselves inactive (19). Known inorganic nitrosating species are:

Substance	Medium
N_2O_3	gas (20, 21)
	water (19, 22–27)
	organic solvent (2)
NO_2/N_2O_4	water (25–27)
	organic solvent (28, 29)
	gas (21, 30)
YNO	water (19, 22, 23, 31–37)
H_2ONO^+	water (19, 22, 38–40)
NO	plus O_2 (25, 27, 36)
	anaerobic, M^{n+} (19, 27)

The interrelationship between active nitrosating agents (underlined) and inactive species is summarized below. For simplicity, the equations are not balanced.



In moderately acidic aqueous nitrite solutions the nitrosating agent is nitrous anhydride, N_2O_3 (19, 22–24), formed from nitrous acid, $pK_a = 3.138$ at 25° (41, 42), after protonation of nitrite ion according to eqs 5 and 6.

$$H^+ + ONO^- \rightleftharpoons HONO \qquad (5)$$

$$2HONO \rightleftharpoons ONNO_2 + H_2O \qquad (6)$$

At lower pH, more rapid nitrosation by the nitrous acidium ion (19, 22, 38–40) becomes important, especially for weakly basic aryl amines and amides.

$$HONO + H^+ \rightleftharpoons H_2ONO^+ \qquad (7)$$

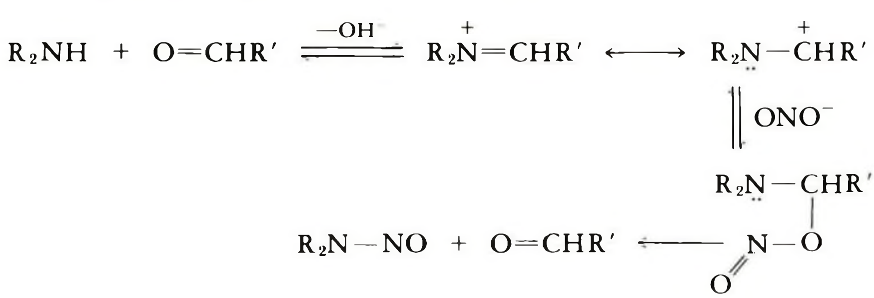
Certain anions, Y^-, catalyse the reaction in water by forming nitrosating species Y—NO which are more reactive than N_2O_3.

$$HONO + Y^- + H^+ \rightleftharpoons Y{-}NO + H_2O \qquad (8)$$

Of the anionic catalysts studied thiocyanate has the greatest effect (23, 31–37). Halide ions are also catalytic in the order SCN^-, $I^- >> Br^- > Cl^-$ (19, 22, 23, 31, 33, 35). The equilibrium concentration of YNO (eq 8) mainly determines the order of the catalytic effect, rather than the actual reactivities of YNO (36). As the pH is lowered below 2, rapid nitrosation by Y—NO dominates over that by N_2O_3, lowering the pH at which the nitrosation rate is maximum compared to the uncatalysed reaction (23, 32–34, 37). Perchlorate and sulfate ions are not catalytic (22, 31, 33). Hydrogen phosphate and carboxylate anions may catalyse nitrosation (31), but only weakly (19, 33).

Substances capable of forming micelles exert a catalytic effect on the nitrosation of amines in acid solution. The rate of nitrosation of dihexylamine at pH 3.5 increases 800-fold in the presence of decyltrimethylammonium bromide micelles (43). Other cationic and nonionic substances at levels higher than their critical micelle concentrations are also catalytic (43, 44). The magnitude of the catalytic effect is smaller for secondary amines with alkyl chain lengths shorter than C_6. Some nitrosation rate enhancements observed in the presence of microorganisms have been explained as due to an analogous hydrophobic interaction between amine and a cellular constituent (45).

In aqueous solution at pH > pK_a of HNO_2 the rate of nitrosation drops rapidly with increasing pH, because the concentrations of active nitrosating species generated in situ decrease. No nitrosation by aqueous nitrite has been observed above pH 7.5. When formaldehyde (equimolar with amine) is added to neutral or basic solutions, nitrite can nitrosate secondary amines, but at a slower rate than in acid solutions (46, 47). Nitrosamine yields vary with steric accessibility of the nitrogen atom. Chloral (46, 47), pyridoxal and various benzaldehydes (48) are also catalytic, but less so than formaldehyde. Acetone and acetaldehyde are inactive. The proposed mechanism (eq 9) involves nucleophilic attack by nitrite on an iminium ion intermediate following by collapse of the adduct releasing the carbonyl catalyst.



(9)

In organic solvents NOCl, N_2O_3, N_2O_4 and $NOBF_4$ have been used as preparative nitrosation reagents (23, 25). Use of N_2O_3 (2) or N_2O_4 (28) in carbon tetrachloride or acetic acid gives high yields of nitrosamides. With secondary amines N_2O_4 in methylene chloride reacts cleanly either as a nitrosating agent at 0° or as a nitrating agent at −80° (29). Since NO_2 is in equilibrium with N_2O_4 (49), statements in the literature on effects of either substance must be critically viewed.

Nitrosation of amides occurs faster in two-phase systems composed of an organic solvent and aqueous HNO_2 solution at pH 1 or in methylene chloride extracts of 2 *M* aqueous HNO_2 than in water alone at pH 1 (50). Nitrosation of amines dissolved in methylene chloride in contact with solid sodium nitrite occurs by a reaction which involves the solvent (51).

Recent studies have shown that secondary amines react with N_2O_3 and N_2O_4 gases dissolved in aqueous alkaline solutions (pH 6–14) at a rate greater than in acidified nitrite (25–27). Although both nitrogen oxides might be expected to undergo rapid hydrolysis at pH > 5 to yield unreactive NO_2^- and NO_3^-, amines of widely different reactivity compete effectively with water and OH^- for dissolved N_2O_3 and N_2O_4.

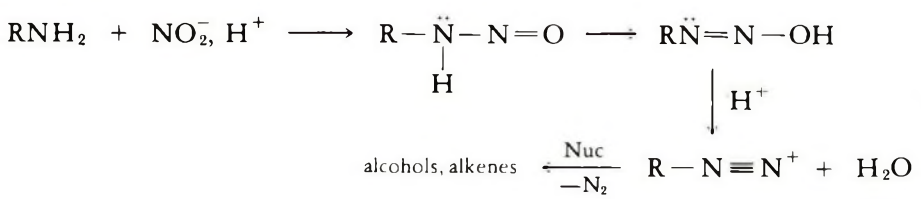
Nitric oxide (NO) alone is inactive but is oxidized by oxygen to NO_2 and thus to the reactive nitrosating agents N_2O_3 and N_2O_4 (25, 27, 36). Rapid nitrosation by NO under anaerobic conditions occurs in the presence of iodine or Ag(I), Cu(I), Cu(II), Zn(II), Fe(III) or Co(II) salts (19, 27).

b. Organic Species. *N*-Nitrosamines themselves act as nitrosating agents. Aromatic nitrosamines, such as nitrosodiphenylamine, transnitrosate secondary amines under neutral conditions in organic solvents (52, 53) probably by a free radical mechanism (53). The process is more rapid in acidic aqueous solution and occurs by a heterolytic mechanism (53, 54). The slower transnitrosation between aliphatic secondary amines requires more extreme conditions or catalysis by nucleophilic agents, such as thiocyanate and halide ions (23, 55, 56).

Nitrosation of morpholine by aromatic and aliphatic *C*-nitro compounds in tetrahydrofuran at 70°C has recently been reported (57). Further work is required to ascertain whether nitrosation occurs by direct reaction of amine with the C-nitro function or is caused by agents derived from inorganic nitrite present as a synthetic contaminant or decomposition product. Primary and secondary nitroalkanes decompose to nitrite in dilute alkaline solutions (58).

2. Nitrogen Compounds

a. Primary Amines. The well-known deamination of primary aliphatic amines with nitrite in cold aqueous acid yields a variety of products (22). The rapid reaction proceeds through unstable primary nitrosamine and diazonium ion intermediates. The latter reacts with nucleophiles present to form substitution, elimination and rearrangement products.

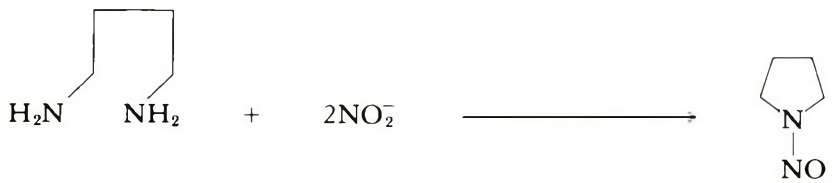


(10)

Secondary amines and subsequent nitrosamines formed by reaction of the diazonium ion with the primary amine starting material (eq 11) have been isolated (22). This reaction occurs in low yield because the amine is largely protonated and unreactive under the strongly acidic and low temperature conditions commonly used.

$$R-N\equiv N^{+} + RNH_2 \xrightarrow{-N_2} R_2NH \xrightarrow{NO^{-}_2,H^{-}} R_2NNO \qquad (11)$$

Diamines with a second primary amine function appropriately located for intramolecular reaction with the diazonium ion form secondary nitrosamines at high temperatures or long reaction times as illustrated by the following examples (59).



HCl salt	moist	160°C, 2 hr	22% yield
0.025 *M*	0.050 *M*	in citrate-phosphate buffer, pH 3.4	
		100°C, 1 hr	1.6%
		22°C, 6 days	0.39%

Under similar conditions *n*-butylamine, a monoamine, gave much lower yields of *N*-nitrosodibutylamine.

Higher levels of stable α-alkoxynitrosamines are produced from the reaction of primary amines with aldehydes in the presence of alcohols and nitrite under mildly acidic conditions (60, 61).

$$RNH_2 + CH_2O + R'OH + NO_2^{-}, H^{+} \longrightarrow R-\underset{\underset{NO}{|}}{N}-CH_2OR' \qquad (12)$$

However, mixtures of primary amines and aldehydes without alcohol do not react with nitrite at pH 3 (62).

b. Secondary Amines. Nitrosamines formed directly from secondary amines are stable. In moderately acidic aqueous nitrite solutions N_2O_3, formed from two molecules of HNO_2 (eq 6) is the nitrosating agent. The rate-determining step in the reaction is electrophilic attack by N_2O_3 on the free electron pair of the unprotonated amine (eq 13). Rate equation 14 describes the kinetics.

$$R_2NH + N_2O_3 \rightarrow R_2NNO + HNO_2 \qquad (13)$$

$$\text{rate} = k[R_2NH][HNO_2]^2 \qquad (14)$$

Thus, two factors determine the effect of pH on the rate of nitrosation:

(i) extent of conversion of NO_2^{-} to HNO_2 and thus to N_2O_3 (favored by lower pH)

(ii) concentration of unprotonated amine (favored by higher pH).

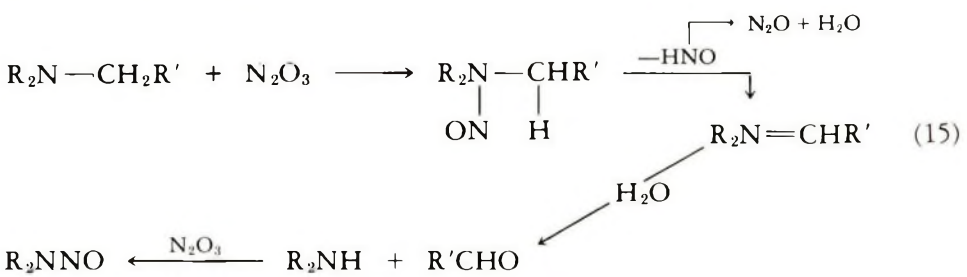
As predicted by the rate equation (33) for simple basic amines ($pK_a > 5$), the nitrosation rate in water is maximum at $pH_{max} = 3$ to 3.4 (23, 24, 40, 63, 64) near the pK_a of HNO_2. For a given amine as the pH increases above pH_{max} the rate decreases, because the concentration of HNO_2 decreases. As the pH decreases below pH_{max} the rate decreases, because the concentration of unprotonated amine decreases. At a given pH the rate of nitrosation increases as the basicity of the amine decreases, because of the higher relative concentration of unprotonated amine present. Thus, the following order of reactivity is found at pH 3.0:

Amine			
pK_a	11.2	8.7	5.57
Relative rate (23, 33)	1	930	180,000

Nitrosation of secondary amino acids (65, 66) occurs at an optimum $pH_{max} = 2.25$ to 2.5 (34, 40). The reaction follows rate eq 14, but the pH-rate profile is changed by the fact that two amine species react—$RNHCHR'CO_2^-$ and $RNHCHR'CO_2H$.

c. Tertiary Amines and Related Compounds. Tertiary amines have generally been regarded as inert to nitrosation, even though their conversion to secondary nitrosamines was reported over 100 years ago (67). Because dealkylation is required, the reaction occurs to a significant extent only at elevated temperatures in weakly acidic media (68–70). At 25°C and pH 3.4 nitrosation of tertiary amines is about 10,000 times slower than that of related secondary amines (23).

The following mechanism has been proposed (23, 68, 69) for nitrosative dealkylation and nitrosamine formation:



(15)

With mixed tertiary alkyl aryl amines ring C-nitrosation also occurs (19).

Two related compounds—the nitrogen acetal hexamethylenetetramine (71) and the drug antipyrine which has an eneamine structure (23, 72)—undergo *N*-nitrosation much more rapidly and extensively than normal tertiary amines. In both compounds at least one of the three *N*-substituents is in a higher oxidation state than in typical tertiary amines and nitrosation undoubtedly occurs by a different mechanism (70).

d. Quaternary Amines and Amine Oxides. Quaternary ammonium compounds apparently react slowly with nitrite in acidic media. The initial dealkylation required accounts for their lower activity compared to tertiary amines and may not involve the nitrosating agent (73). The relative reactivity of secondary, tertiary and quaternary

amines is indicated by the following data gathered for reaction of a ratio of 5 mol $NaNO_2$/mol amine at 78°C and pH 5.6 for 4 hr (73).

Amine	% Yield of $(CH_3)_2NNO$
$(CH_3)_2NH$	9.6
$(CH_3)_3N$	0.9
$(CH_3)_4N^+$	0.6
$(CH_3)_2NCH_2CH_2OH$	1.6
$(CH_3)_3N^+CH_2CH_2OH$	0.0002

Several naturally occurring quaternary ammonium compounds were found to be much less reactive than the tetramethylammonium ion (73). The tribenzylmethylammonium ion is reported to be unreactive under similar conditions (68). No nitrosation of 10^{-3} *M* hexadecyltrimethylammonium bromide by a 20-fold excess of nitrite at 25°C and pH 3.5 was observed after 40 min (44).

Tertiary amine oxides in the presence of excess nitrite at pH 1 to 3 and temperature 25 to 75°C are converted to secondary nitrosamines to a greater extent than are tertiary amines (74). However, at 90 to 100°C and pH 4 to 5 both classes show similar reactivity (68, 70, 74). Two mechanisms that account for the change in relative reactivity with conditions have been proposed (70, 74).

e. Secondary and Tertiary Amides. For secondary amides, as with amines, the nitrosation condition most widely investigated has been nitrite in aqueous acid.

N-Alkyl ureas and carbamates are rapidly nitrosated at pH 1 to 2. The nitrous acidium ion is the main nitrosating agent for these and other amides (eq 16) and the reaction rate follows eq 17 (23, 39, 40).

$$ZNHR + H_2ONO^+ \rightarrow ZN(NO)R + H_2O + H^+ \quad (16)$$

$$\text{rate} = k[ZNHR][HNO_2][H^+] \quad (17)$$

The reaction rate increases about ten times for each 1-unit drop in pH from 3 to 1 and does not show a pH maximum. At pH > 2.5 nitrosation by N_2O_3 contributes (39).

In acidic aqueous media nitrogen substrates decrease in propensity toward nitrosation in the order 2-imidazolidone > acyclic *N*-alkylurea > *N*-arylurea > *N*-alkylcarbamate > less basic dialkyl and secondary aromatic amines ($pK_a < 9$) and tertiary eneamines > more basic dialkyl amines > *N*-alkylamides, *N*-acylureas, *N*-alkylguanidines and tertiary amines (23).

High yields of nitrosamides are obtained from reactions of amides with N_2O_3 (2) or N_2O_4 (28) in organic solvents. However, *N*-methylacetamide in aqueous solution at pH 13 does not react with added N_2O_4, conditions under which secondary amines are rapidly nitrosated (27). Apparently the weakly basic amide is too unreactive to compete with hydrolysis of the nitrosating agent.

Nitrosation of tertiary amides in acidic aqueous solutions of nitrite at high concentrations and temperatures produce either nitrosamides or nitrosamines (23, 70, 75). Nitrosation of trialkylureas gives the corresponding nitrosoureas. Dialkylnitrosamines are the major product from dialkyl- or trialkylthioureas, 1,1-dialkylureas, 1,1-dialkyl-3-phenylureas and tetraalkylureas.

C. INHIBITION OF NITROSATION

Studies of nitrosamine inhibition have consisted of the use of substances which compete with the amine for nitrosating species. The reduction potentials of various nitrogen oxides (76) listed below can aid in selecting appropriate oxidizing and reducing agents for destruction of nitrite.

In acid solution:

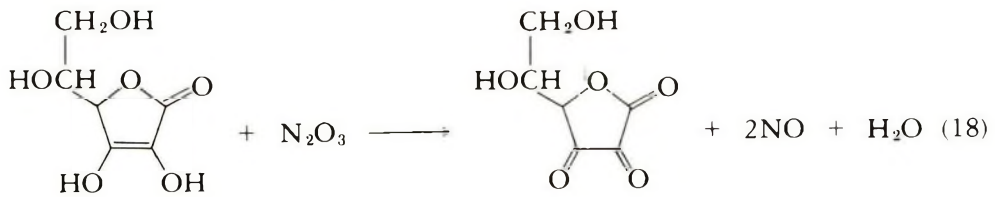
	E° (volts)
$HNO_2 + H_2O \rightleftharpoons NO_3^- + 3H^+ + 2e^-$	−0.94
$NO + H_2O \rightleftharpoons HNO_2 + H^+ + e^-$	−0.99
$N_2O + 3H_2O \rightleftharpoons 2HNO_2 + 4H^+ + 4e^-$	−1.29

In basic solution:

$NO_2^- + 2OH^- \rightleftharpoons NO_3^- + H_2O + 2e^-$	−0.01
$NO + 2OH^- \rightleftharpoons NO_2^- + H_2O + e^-$	+0.46
$N_2O + 6OH^- \rightleftharpoons 2NO_2^- + 3H_2O + 4e^-$	−0.15

1. Inhibition by Ascorbic Acid

Ascorbic acid inhibits nitrosamine formation by rapid reduction of the nitrosating agent (77). Since the product NO can be air-oxidized to



Ascorbic acid

Dehydroascorbic acid

the nitrosating agent N_2O_4, excess ascorbic acid must be added to inhibit nitrosation in systems exposed to air.

Literature reports describing ascorbic acid inhibition of nitrosamine formation in amine-nitrite systems are summarized in Table II. Under in vitro conditions ascorbic acid inhibited nitrosamine formation. It inhibited the toxic and carcinogenic effects attributable to in vivo nitrosamine formation with two exceptions. In one case adenoma induction by *N*-nitrosomorpholine and mononitrosopiperazine increased with added ascorbic acid (78). In another it inhibited in vivo synthesis of *N*-nitrosomorpholine in rats and consequent liver tumors, but enhanced forestomach papillomas and carcinoma (79).

Table II
Inhibition of In Vitro and In Vivo Nitrosamine Formation by Ascorbic Acid in the Presence of Nitrite

Amine (or Amide)	System Investigated	Effect of Ascorbic Acid	Reference
Aminopyrine	Hepatic necrosis, Mice	2 *M* excess of ascorbate prevented necrosis. Equimolar ascorbate gave incomplete protection.	80

continued on p 593

Table II (continued)

Amine (or Amide)	System Investigated	Effect of Ascorbic Acid	Reference
Aminopyrine	Hepatotoxicity, Rats	Inhibits elevation of GPT, Ala. aminotransferase, nitrosodimethylamine serum levels.	81, 82
Aminopyrine	Carcinogenesis, Rats	Greater incidence of cancer in rats in absence of ascorbic acid.	83
Chlordiazepoxide	In vitro	Inhibits nitrosamine formation.	84
Chlordiazepoxide	Toxicity, Rats	Ascorbate protected against increases in liver and spleen wt.; decrease in adrenal wt., increases in GPT, LDH.	85
Dimethylamine	Acute tox., Rats	Hepatic necrosis inhibited. GOT, GPT elevation inhibited.	86
Dimethylamine	In vitro	Low conc. of ascorbate *enhanced* nitrosamine formation. High concentrations inhibited such formation. Formation and inhibition are a function of pH.	87
Ethylurea	Pregnant rats	Prevents carcinoma in offspring.	88
Morpholine	In vitro	Amount of ascorbate required depends on the presence or absence of O_2.	89
Morpholine	In vitro	Inhibits nitrosamine formation.	90
Morpholine	Nitrosomorpholine formation and tumorgenesis in rats	Inhibits nitrosomorpholine formation and liver tumors. *Enhanced* forestomach papillomas and carcinoma.	79
Piperazine	In vitro, human gastric juice	Inhibits nitrosamine formation.	91
Proline	Fried, nitrite-cured bacon	Formation of nitrosopyrrolidine from proline is inhibited.	92
Dimethylamine Pyrrolidine Proline	Model food systems	Inhibits nitrosamine formation.	93
Dimethylamine, Pyrrolidine, Piperidine	Meat-curing mixtures	Inhibits nitrosamine formation.	94
Morpholine Piperazine Methylurea	Adenoma, lung, in mice	Ascorbate decreased adenoma frequency in some cases. Increases adenoma frequency when given with the nitrosamines.	78
Morpholine, Oxytetracycline, Piperazine, N-Methylaniline, Methylurea, Dimethylamine	In vitro	Inhibits nitrosamine and nitrosamide formation.	95
Piperazine, Aminophenazone	In vitro, human gastric juice	Inhibits nitrosamine formation.	96
—	—	Review	97

Table III
Effect of Phenols on Nitrosamine Formation In Vitro and In Vivo

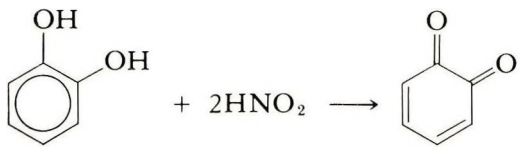
Phenol	Amine	System	Effects of the Phenol	Reference
Phenol	—	In vitro	Phenol reacts with nitrite 10^4 X as rapidly as dimethylamine.	98
Gallic Acid	Morpholine	Adenoma induction, Mice	Adenoma strongly inhibited.	78
Gallic Acid	Diethylamine	In vitro	Inhibited or catalysed depending on pH and rel. conc. of reactants.	99
Gallic Acid	Diethylamine	In vitro	Catalyses nitrosamine formation (see text).	100
Gallic Acid	Piperazine Aminophenazone	In vitro, human gastric juice	Inhibited nitrosamine formation.	96
Propyl Gallate	Proline	Oil/water system	Inhibited nitrosamine formation.	93
Propyl Gallate	Proline	Fried, nitrite-treated bacon	Inhibited nitrosopyrrolidine formation.	92
Propyl Gallate	Dimethylamine	Hepatotoxicity, Rats	Inhibited liver pathol. GOT, GPT and ornithine carbamoyl transferase.	101
Tannic Acid	Dimethylamine	In vitro	Inhibited nitrosamine formation.	93
Tannin	Piperazine, Aminopherazone	In vitro, human gastric juice	Inhibited nitrosamine formation.	96
α-Tocopherol	Dimethylamine	In vitro	Inhibited nitrosamine formation.	93
α-Tocopherol	Dimethylamine	Cigarettes	Inhibited dimethylnitrosamine formation.	102, 103
	Proline	Bacon	Inhibited nitrosopyrrolidine formation.	
	Aminopyrine	Hepatotoxicity, Rats	Inhibited hepatotoxicity.	
t-Butyl-hydroquinone	Pyrrolidine	In vitro, Oil/Water	Inhibited nitrosamine formation.	93
t-Butyl-hydroquinone	Dimethylamine	Hepatotox., Rats	Inhibited hepatox. GOT, GPT, ornithine carbamoyl transferase.	101
2,6-Di-*t*-butyl-*p*-cresol (BHT)	Dimethylamine	Hepatotox., Rats	Relatively ineffective.	101
Butylated Hydroxyanisole (BHA)	Dimethylamine	Hepatotox., Rats	Relatively ineffective.	101
4-Methyl-catechol	Piperidine	In vitro	Catalysed nitrosamine formation.	104
Chlorogenic Acid	Piperidine	In vitro	Catalysed nitrosamine formation.	104
Vanillin	Dimethylamine	In vitro	Inhibited nitrosamine formation.	93
Hydroquinone	Dimethylamine	In vitro	Inhibited nitrosamine formation.	93
Thymol	Dimethylamine	In vitro	Inhibited nitrosamine formation.	93

2. Effect of Phenols on Nitrosamine Formation

In Table III are summarized literature reports of the effect of phenols on the formation of nitrosamines in amine—nitrite systems. In most cases phenols inhibited nitrosamine formation, but sometimes their presence intensified nitrosamine production.

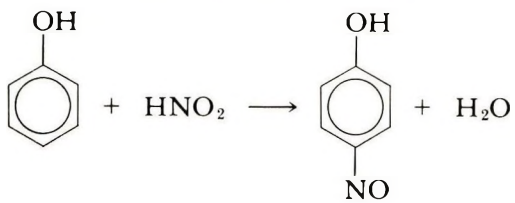
In systems containing nitrite, phenols and secondary amines several reactions compete:
—formation of quinones (eq 19)
—formation of *C*-nitrosophenols (eq 20)
—direct formation of *N*-nitrosamines
—phenol-catalysed formation of *N*-nitrosamines
—aerobic oxidation of *C*-nitrosophenols to noncatalytic nitrophenols (105).

Inhibition of nitrosamine formation by phenols occurs by reduction of nitrite to unreactive nitric oxide (104)



(19)

or by removal of nitrite via *C*-nitrosation (98):



(20)

Under some conditions phenols can catalyse nitrosamine formation. In the presence of excess nitrite 4-methylcatechol catalyses the nitrosation of dimethylamine and piperidine (104) and both *p*-cresol and *p*-nitroso-*o*-cresol catalyse the nitrosation of pyrrolidine (105).

Walker, Pignatelli and Castegnaro (100) investigated the effects of 0–65 m*M* gallic acid on the formation of nitrosodiethylamine from 75 m*M* nitrite and 500 m*M* diethylamine. Figure 1 and Table IV are adapted from their data obtained at pH 4.2 where maximum nitrosamine formation occured. In the absence of gallic acid 0.39 m*M* nitrosamine was formed. At the lowest level of gallic acid added, 12.5 m*M*, nitrosamine formation increased nine-fold. However, further increases in gallic acid concentration *decreased* nitrosamine formation linearly. Extrapolation of the linear relationship (Figure 1) indicates that addition of 144 m*M* gallic acid would result in complete inhibition of nitrosamine formation. This is equivalent to approximately 2 mol of gallic acid per mol of nitrite.

This result is consistent with that obtained by Davies and coworkers (105) who found that the rate of nitrosation of pyrrolidine by nitrite *increased* linearly with the concentration of *p*-nitroso-*o*-cresol. They demonstrated that the nitrosating species responsible for catalysis is an adduct of nitrite and a tautomer of the nitrosophenol. A similar mechanism probably operates with gallic acid where a large excess of nitrite would lead to catalysis by *C*-nitrosogallic acid.

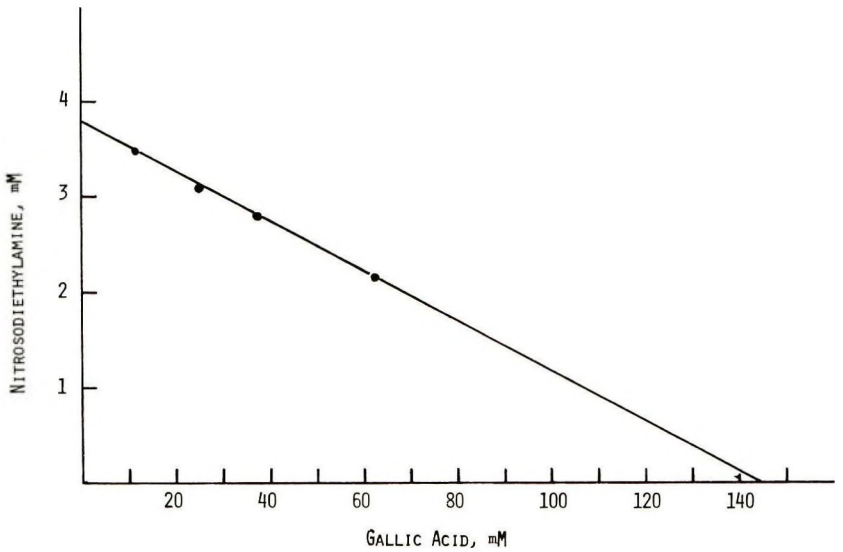


Figure 1. Effect of gallic acid on *N*-nitrosodiethylamine synthesis

Thus, whether a phenol inhibits or catalyses nitrosamine formation largely depends on the relative concentration of nitrite and phenol. Excess nitrite *C*-nitrosates the phenol and subsequently forms the catalytic species. A large excess of phenol removes nitrite so that it is unavailable for reaction with amine, either directly or catalytically. No catalysis should occur with phenols such as α-tocopherol, which are not *C*-nitrosated because the ring is fully substituted.

Table IV
Effect of Gallic Acid Concentration on Nitrosodiethylamine (NDEA) Synthesis from 75 m*M* Nitrite and 500 m*M* Diethylamine at pH 4.2

	m*M* NDEA	
m*M* Gallic Acid (g)	Found (N)	Calc. (N^a)
62.5	2.15	2.15
37.5	2.81	2.80
25.0	3.10	3.13
12.5	3.48	3.46
0.0	0.39	—

$N^a = -0.0263\ g + 3.79$, the least squares line of best fit.

3. *Inhibition by Sulfur Compounds*

Bisulfite reduces nitrite in two steps (106)—first to nitric oxide (eq 21) and then to nitrous oxide (eq 22). Sulfamate reduces nitrite to molecular nitrogen (107) (eq 23). These substances inhibit nitrosamine formation (Table V).

$$SO_2 + 2HNO_2 \rightarrow 2NO + H_2SO_4 \quad (21)$$

$$SO_2 + 2NO + H_2O \rightarrow N_2O + H_2SO_4 \quad (22)$$

$$NaNO_2 + H_2NSO_3H \rightarrow NaHSO_4 + N_2 + H_2O \quad (23)$$

Table V
In Vitro Inhibition of Nitrosamine Synthesis by Sulfur Compounds

Sulfur Compound	Amine	System	Effect of Sulfur Compound	Reference
Sodium Bisulfite	Dimethylamine	Model food systems	Inhibits nitrosamine formation.	93
Ammonium Sulfamate	Dimethylamine, morpholine, piperazine	In vitro	Inhibits nitrosamine formation.	93
Sulfamic Acid	Piperazine Aminophenazone	Human gastric juice, in vitro	Inhibits nitrosamine formation.	108
Cysteine	Dimethylamine	In vitro	Inhibits nitrosamine formation.	93
Cysteine	Piperazine Aminophenazone	Human gastric juice, in vitro	Inhibits nitrosamine formation.	108
Glutathione	Dimethylamine	In vitro	Inhibits nitrosamine formation.	93
Glutathione	Piperazine	Human gastric juice, in vivo	Inhibits nitrosamine formation.	91
Methionine	Dimethylamine	In vitro	Inhibits nitrosamine formation.	93

The thiols cysteine and glutathione also inhibit nitrosamine formation. The thioether methionine is less effective. It is postulated that nitrite oxidizes methionine to the sulfoxide or sulfone and is in turn reduced to nitric oxide (93).

Thiols react with nitrite to form *S*-nitroso compounds (109). In the absence of nitrite preformed nitrosocysteine reacts with *N*-methylaniline, morpholine and pyrrolidine to form *N*-nitrosamines (105). In contrast catalysis of nitrosation by *p*-nitroso-*o*-cresol does not occur in the absence of nitrite. One would suggest transnitrosation of secondary amines by nitrosothiols, except that molecular oxygen appears to be necessary (105).

4. Miscellaneous Inhibitors

The ammonium ion reacts with nitrite to form molecular nitrogen (107) by the following sequence:

$$\begin{aligned} &NH_4^+ \rightleftharpoons NH_3 + H^+ \\ &2H^+ + NO_2^- \rightleftharpoons H_2ONO^+ \\ &H_2ONO^+ + NH_3 \rightarrow H_2O + NH_3NO^+ \rightarrow N_2 + H^+ + H_2O \end{aligned} \tag{24}$$

Hydroxylamine reduces nitrite to nitrous oxide (107).

$$NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O \tag{25}$$

Vitamin A reacts with nitrite in acid solution but not under neutral conditions (108). Presumably oxidation of the vitamin involves its double bonds.

Table VI summarizes literature indicating that urea, caffeine and ethanol are relatively ineffective inhibitors, but reduced nicotinamide adenine dinucleotide (NAD) is effective.

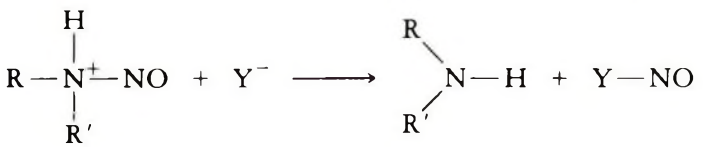
In alkaline solution even weak oxidants such as O_2 convert nitrite to nitrate (110).

Table VI
Miscellaneous Nitrosamine Inhibitors

Inhibitor	Amine	System	Effect of Inhibitor	Reference
Urea	Dimethylamine	In vitro	Relatively ineffective in inhibiting.	93
Urea	Piperazine Morpholine	In vitro	Inhibitory effect decreases with time.	95
Reduced NAD	Dimethylamine Pyrrolidine Piperidine	In vitro	Inhibits nitrosamine formation.	93
Caffeine	Morpholine	Lung adenoma, Mice	Moderately inhibited.	78
Ethanol	Chlordiazepoxide	In vitro	Slight inhibitory effect.	99

D. DESTRUCTION OF N-NITROSO COMPOUNDS

N-Nitrosamines are stable compounds and are difficult to destroy once they are formed. They are stable in neutral and strong alkaline solutions in the absence of light (2, 5). Denitrosation (eq 2) occurs slowly in acid solution (1 to 5 *M*) and is catalysed by nucleophiles in the order of effectiveness $Y = I^- > SC(NH_2)_2 > SCN^- > Br^- > Cl^-$ (36, 111). To prevent reversal of the reaction a substance, which reacts irreversibly with YNO (eq 4) and more rapidly than amine, must be added. Relative efficiency of various nitrite traps in 5 *M* H_2SO_4 was found to be hydrazoic acid and hydrazine > sulfamic acid > aniline > hydroxylamine > urea (112). Ease of denitrosation varies in the order R,R′ = aryl > R = aryl,R′ = alkyl > R,R′ = alkyl (53, 113).



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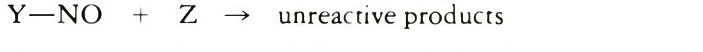

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Quantitative denitrosation of nitrosamines can also be achieved at room temperature using a solution of HBr (5 to 10%) in glacial acetic acid if water is excluded. Analysis of the nitrite released provides a measure of the original nitrosamine concentration (114).

When exposed to ultraviolet light nitrosamines decompose either to aldehydes, nitrogen and nitrous oxide or quantitatively to amine and nitrous acid depending on the wavelength used. The reaction is fastest in acid and faster in neutral than basic solutions. Apparatus and conditions for the photochemical destruction of nitrosamines in solution in the presence of a HNO_2 scavenger have been described (115, 116).

Nitrosamines can be reduced by zinc in acetic acid, sodium amalgam, tin in hydrochloric acid, lithium aluminum hydride and catalytic hydrogenation (4, 117). A reduction procedure for destruction of nitrosamines in alkaline solution with aluminum has been published (118). The corresponding hydrazines are usually formed (eq 26), but other products can be produced depending on the reducing agent and experimental conditions (119). Many hydrazines are carcinogenic, but about 100 times less so than the corresponding nitrosamines (2).

$$R_2NNO \xrightarrow{\text{reduction}} R_2NHNH_2 \qquad (26)$$

N-Nitrosamides are hydrolytically unstable. In aqueous acid they decompose by both denitrosation and deamination pathways (120, 121). Hydrogen bromide in carbon tetrachloride has been used for synthetic conversion of nitrosamide to amide (28).

At alkaline pH nitrosamides decompose to diazoalkanes (eq 27) (2, 4, 17, 23, 122).

$$\begin{array}{ccccccc} RCH_2N(NO)-Z & + & H_2O & \xrightarrow{OH^-} & RCH_2NHNO & + & ZOH \\ & & & & \downarrow & & \\ RCH{=}N{=}N & + & H_2O & \longleftarrow & RCH_2N{=}NOH & & \end{array} \qquad (27)$$

The rate of decomposition increases with increasing pH and varies with amide structure (2). At pH 9 the order of stability was found to be nitrosourea < nitrosamide < nitrosourethane < nitrososulfonamide < nitrosoguanidine (2).

In the solid state *N*-nitrosamides sometimes decompose explosively (2). Nitrosourea samples should be frozen, not merely refrigerated (123). Nitrososulfonamides are stable only if kept cool and dry (124).

V. PRACTICAL CONSIDERATIONS

The basic problem in minimizing nitrosamine formation is prevention of the reaction between nitrosating species and amines. The nitrosating species are ubiquitous in the environment. Roughly 50 ppb of nitrous oxide and nitrogen dioxide are present in the atmosphere of our cities (125). In soils, streams and rivers, organisms of the genus nitrosomonas oxidize ammonia to nitrite (126). Some foods have a high nitrate content. These can be reduced in vivo after ingestion of the food. Nitrites are added to some foods to prevent growth of botulinus organisms. Nitrites are also widely used as metal corrosion inhibitors.

Removal of nitrosating species from our environment is a sociological task not amenable to immediate solution. In certain cases, steps can be taken to minimize such contamination. Already industry is moving to replace nitrite as a corrosion inhibitor in some applications and reduce its use as an additive in meat.

A more likely general approach to preventing the reaction of nitrosating species and amines is the inclusion of appropriate scavengers into raw materials and finished products. For example, in the production of organic raw materials, where a nitration step occurs in the synthesis, a small amount of SO_2 can be added before solvent removal in the final step to destroy any traces of nitrite. The excess SO_2 would be eliminated by the drying process. Alternatively, a nontoxic nitrite scavenger, such as ascorbic acid, can be incorporated into the raw material or finished product.

Scavengers which reduce nitrosating species can be classified into those which convert nitrite to NO and those which reduce it further. Most inhibitors described here reduce nitrite to NO. In the presence of molecular oxygen NO is readily oxidized to N_2O_4 which is a good nitrosating agent. Thus, a sufficient excess of these inhibitors should be incorporated to scavenge oxidized NO. Sulfamates and sulfites reduce the nitrites to

N_2 and N_2O, respectively, which are not reoxidized by molecular oxygen. These inhibitors are not as innocuous as some of the weaker reducing agents, however.

Cosmetics are frequently in the form of emulsions. Mirvish has shown that lipids readily extract nitrosating species from water. Under these conditions, nitrosation reactions are very fast. Since amines are also more soluble in the oil phase of emulsions, it is appropriate to incorporate oil soluble inhibitors, such as ascorbyl palmitate and α-tocopherol, into such products for maximum inhibition of nitrosamine formation.

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Solvent	$K \times 10^5$
Gas Phase	382
CCl_4	8.05
$CHCl_3$	5.53
C_6H_6	2.23

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Editor's Note:

With the acceptance of this review article, the *Journal of the Society of Cosmetic Chemists* inaugurates the review section of the Journal. We are especially appreciative and indebted to the Cosmetic, Toiletry and Fragrance Association for granting the authors permission to publish this article in our Journal. Review articles are solicited by special invitation from the Editor and Editorial Committee and are not subject to review by the Editorial Committee. The Journal intends to continue publication of review articles of this nature.

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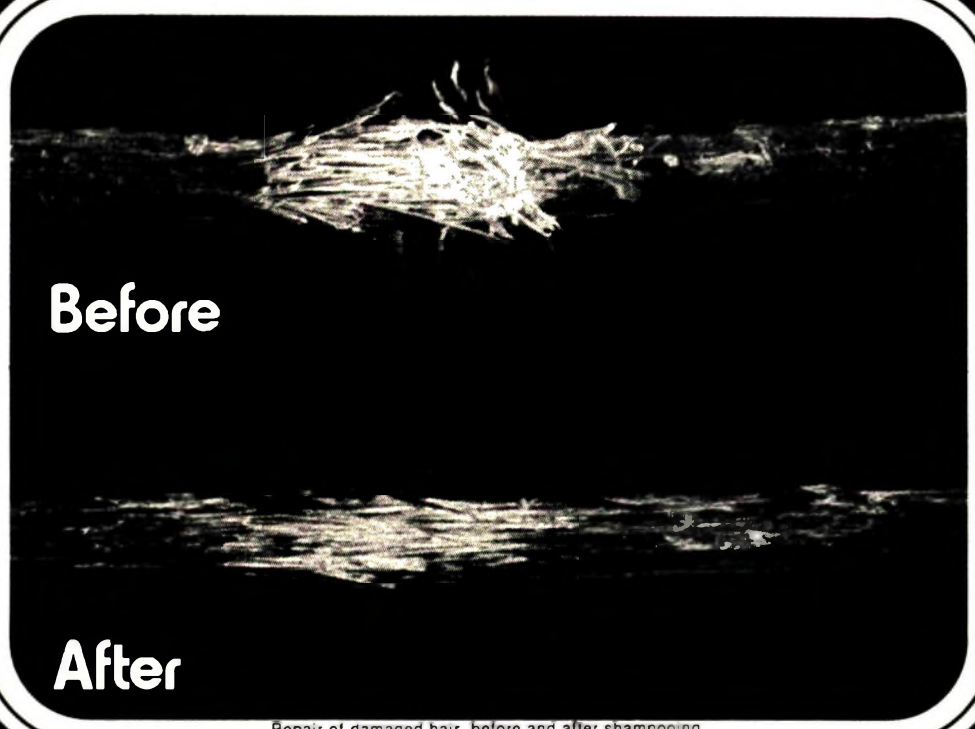

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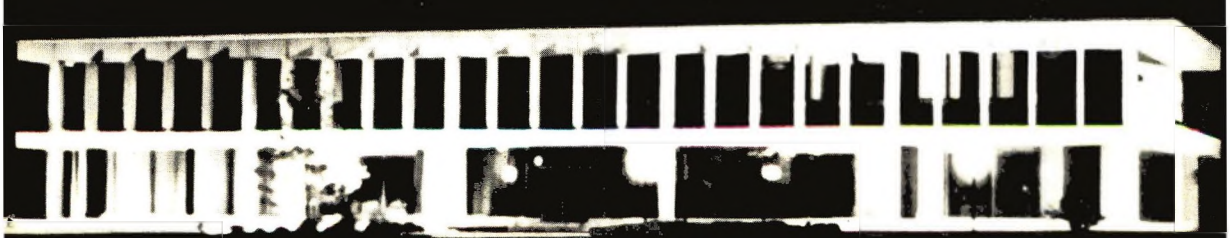

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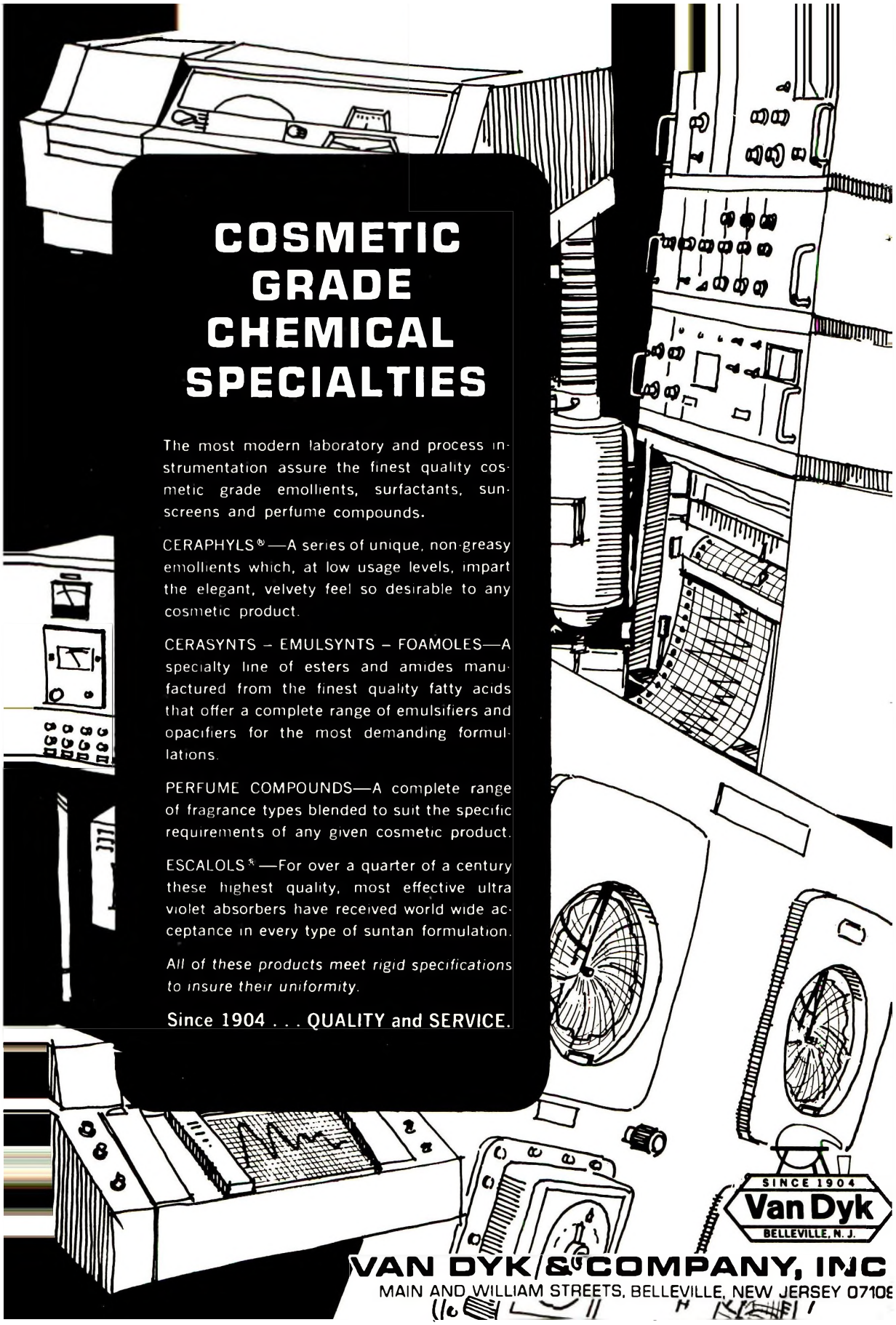

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