J. Soc. Cosmetic Chemists, **17**, 581–594 (1966)

New Evaluation Techniques for Sunscreens

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Presented December 1, 1965, New York City

Synopsis—New techniques contributing to the laboratory evaluation of chemical, physical, and chemical/physical combination sunscreen products are presented. These enable determination of direct and total transmission, absorption, and reflection of ultraviolet and visible light through transparent or translucent thin films similar to those applied on the skin. The classical solution-dilution method using a Beckman Model DB Spectrophotometer and a new thin film technique using a xenon arc Monochromator and Cary Model 14 Spectrophotometer with integrating spheres were investigated. *In vitro* and *in vivo* measurements were made.

INTRODUCTION

Sunburn, photosensitivity reactions, aging, and carcinogenesis are some of the undesirable cutaneous responses to sunlight most commonly encountered by the dermatologist. Blum (1), Bachem (2), Everett (3), and Knox (4) have reviewed the physiological and the pathological cutaneous reactions to sunlight resulting from its broad action spectrum from the ultraviolet through the visible (290 to 740 mμ). In recent years, considerable activity has been evidenced in the cosmetic and pharmaceutical industry in developing products capable of efficiently screening out the harmful portion of the spectrum of light. This has resulted in the investigation of more effective and broad spec-

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trum physical and chemical sunscreens and their combinations in cosmetically elegant and functionally effective bases. Laboratory evaluation of such products, utilizing the classical methods, has failed to keep pace with the progress achieved in other areas in the field. Presented below are studies leading to a more adequate and meaningful evaluation of sunscreens and techniques devised to obtain representative, practical, and meaningful information regarding their physiological effectiveness.

Chemical and Physical Sunscreens

Chemical sunscreens are agents that protect the skin by absorbing and dissipating the energy of the damaging ultraviolet sunburn rays. Organic compounds which have absorbed radiation are either raised to a higher energy level or dissociated. The excited molecules may dissipate their absorbed energy by collision, fluorescence, or a reaction with other molecules at collision (5). *p*-Aminobenzoic acid and its derivatives have been the most widely employed suncreening agents.

Physical sunscreens (sunshades) are opaque, usually insoluble chemicals that produce their effect by reflecting and scattering light. Examples are titanium dioxide, talc, kaolin, zinc oxide, and bentonite. Occasionally these agents are used in combination with chemical sunscreens to complement the protection afforded by the latter. Even a cream vehicle will sometimes provide protection.

In this study the following experimental sunscreen products were studied:

A. *Chemical Sunscreen:*
  7.0% *p*-aminobenzoic acid in a clear viscous solution
B. *Physical Sunscreens:*
  (1) Opaque cream base
  (2) 10% talc in opaque cream base
C. *Chemical/Physical Sunscreen:*
  5% amyl *p*-dimethylaminobenzoate and 10% talc in opaque cream base

Solution-Dilution Method

The current laboratory method of evaluating sunscreens involves quantitatively dissolving the finished product containing the sunscreen in an ultraviolet-transparent solvent such as isopropanol, filtering the solution, preparing dilutions, and measuring transmission (6).

Figure 1 shows the transmission curves determined for the experimental sunscreen products by this solution-dilution method on a Beckman Model DB spectrophotometer.

Disadvantages of this method are: (a) Only the chemical sunscreen and isopropanol-soluble components in the product are extracted from the finished product; the insoluble components of the base are left behind. (b) Since the physical sunscreen is usually insoluble in the solvents used, it is not extracted and therefore cannot be evaluated. Thus, in case of a chemical/physical sunscreen combination, the total sunscreening capacity of the product cannot be determined. (c) The product is evaluated in a condition and concentration entirely different from those used on the skin. (d) The procedure is quantitative but involved and time consuming.

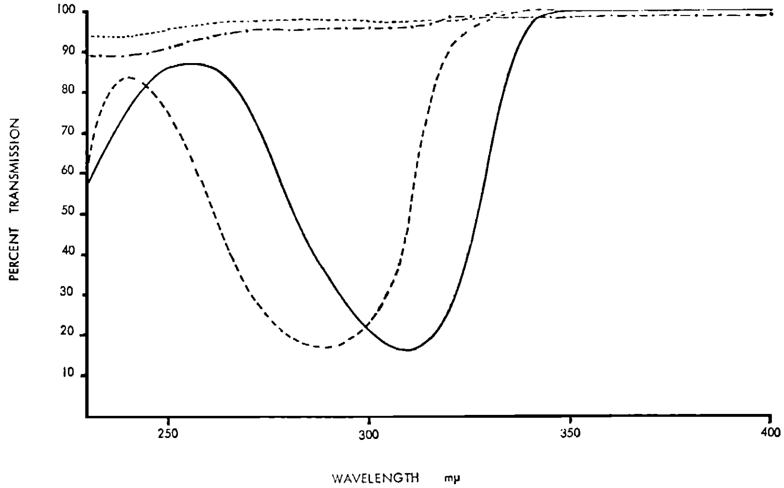

Figure 1. Ultraviolet transmission of sunscreen products using solution-dilution method and spectrophotometer. – – – 7% *p*-aminobenzoic acid in clear viscous solution (0.069672 g/l of isopropanol); —— 5% amyl-*p*-dimethylaminobenzoate + 10% talc in opaque cream base (0.12746 g/l of isopropanol); - · - · - 10% talc in opaque cream base (0.09929 g/l of isopropanol); and -·- opaque cream base (0.10914 g/l of isopropanol)

Thin Film Technique

This study, designed to avoid the disadvantages of the solution-dilution method, has resulted in the development of the Thin Film Technique. It consists essentially of sandwiching a thin film of the sunscreen product between two quartz slides separated by means of spacers of known thickness. The quartz slides* are ground and polished on

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both sides, measure 2.54 × 5.08 cm, and have a thickness of 1 mm with a tolerance of 0.000–0.001 mm. The spacers consist of undistorted 0.0254 ± 0.00254 mm thick No. 1100-0 alloy aluminum foil* cut into strips 3.2 mm wide × 25.4 mm long. The complete sunscreen product was used as such without any processing. A clean, quartz slide was placed on a hard, flat surface. Two strips of foil spacers were cut carefully to avoid distortion and placed on the slide at either end. A small amount of product was placed in the center of the slide. Another quartz slide was aligned and placed on this slide carefully, starting from the center and working away from it. Both ends of the slide were then firmly pressed simultaneously. A uniform film of the product showed in the center of the slide sandwich. While pressure was maintained, the slides were then taped† to one another at both ends above the foil locations. This thin film specimen was then ready for spectrophotometric, monochromatic, reflection, and other measurements. A blank was prepared in the same manner without the product. In the studies that follow, thin film specimens prepared as above were used.

Monochromator Studies

A high intensity xenon arc ultraviolet light monochromator system described by Sayre *et al.* (7) was used in conjunction with an RCA 935 photocell and specially constructed quartz cuvette/quartz slide holder for measurement of the transmission spectra of the sunscreen products. The technique utilized was to measure the output of the source from 250–400 mμ wavelength in 10 mμ increments, with blank quartz slides in the slide holder. Next, quartz slides with the thin film of the sunscreen product sandwiched between, as described above, replaced the blanks in the holder, and the light transmitted was measured by the photocell. The output of the source was again measured with the blank slides in the holder. Any significant change in the intensity of the source would thereby be detected; during the experiments the intensity, caused by wandering of the plasma in the arc, was found to vary up to 10%. The values of the light transmitted by the blank and sample are both expressed in volts; thus the ratio of sample to blank in volts gives the light transmitted by the sample. Transmission measurements were made for all the experimental sunscreen products. These are shown in Figs. 2 and 3 as "monochromator direct transmissions."

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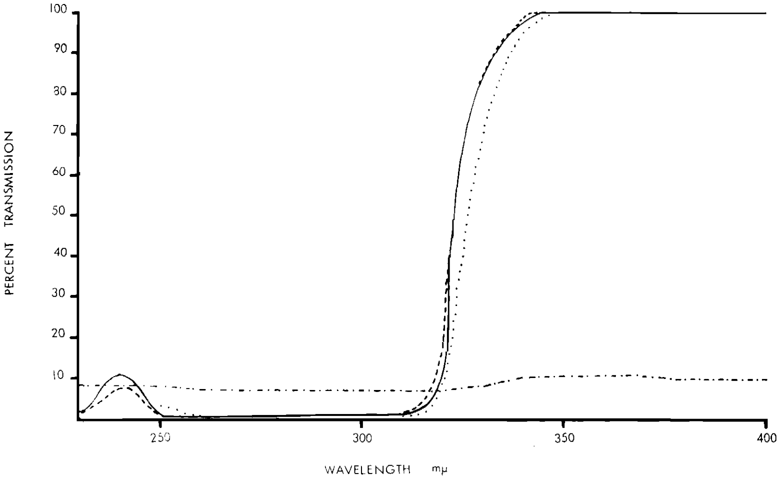

Figure 2. Ultraviolet transmission and reflection of 0.0254 mm thick film of 7% *p*-aminobenzoic acid in clear viscous solution. Using spectrophotometer with integrating spheres: —— total transmission, - - - direct transmission, and - · · - - percent reflection. Using monochromator: ... direct transmission

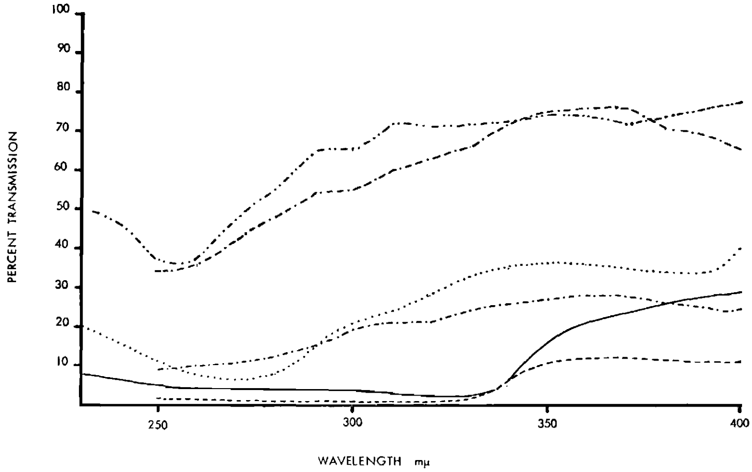

Figure 3. Comparison of two methods for determining transmission on 0.0254 mm thick films of three sunscreen products. Total transmission using spectrophotometer with integrating spheres; direct transmission using monochromator. 5% amyl-*p*-dimethylaminobenzoate + 10% talc in opaque cream base: —— total transmission and - - - direct transmission. 10% talc in opaque cream base: ... total transmission and - · · - - direct transmission. Opaque cream base: - · · - - · · · total transmission and — — · — — · — — direct transmission

Studies with Integrating Spheres

While the use of the monochromator and photocell indicated that a considerable portion of the light was scattered in a forward direction by samples containing both chemical and physical sunscreens, the total amount of this forward scattered light could not be evaluated due to the geometry of the equipment. Since the phototube subtends an angle of 26 degrees with the sample, only light within 26 degrees normal to the sample was measured.

Integrating spheres (Cell Space Reflectance Accessories*) were employed to adapt the Cary Model 14 Spectrophotometer for measurement of the total transmission of light (8). In addition to measurement of the total quantity of light scattered in a forward direction through the specimen, this instrumentation eliminated several errors in measurements due to the monochromator and photocell combination. First, this is a double beam instrument; second, its optics were designed for exact spectrophotometric measurements; third, its readout is automatically recorded; and fourth, its spectral range permits greater information to be obtained about each specimen. In addition to being able to measure the forward scattered light, the reflected or backward scattered light could also be measured so that the optical properties of each sunscreen were more fully determined.

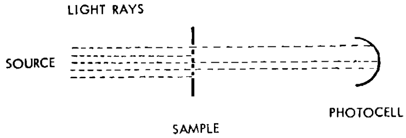

Figure 4. Direct transmission—spectrophotometer

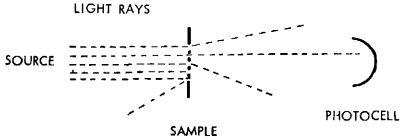

Figure 5. Direct transmission—spectrophotometer with sample that scatters and transmit light

All the thin film specimens of the sunscreen products, used for the monochromator, were also tested by this method. Three measurements were made upon each thin film specimen. The first was the ordinary or

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* Applied Physics Corp., Monrovia, Calif.

direct spectrophotometric transmission measurement. Figure 4 indicates the arrangement of the various components for this measurement. Only light passing straight through the thin film reaches the photocell and is evaluated as transmitted light. This light will be defined as direct transmitted light. Light scattered by the film as shown in Fig. 5 cannot be measured by this technique; moreover, measurements on substances that scatter light are likely to indicate erroneously that significantly less light is passing through the specimen.

The second measurement utilized the integrating spheres in conjunction with the spectrophotometer. An integrating sphere is essentially a hollow metal sphere, coated internally with barium sulfate, placed in the light beam of both the sample and reference compartment of the Cary spectrophotometer. The sample slide is placed directly in front of the integrating sphere. The light is reflected as it enters the sphere from a mirror to a standard reference surface in the sphere. The standard reference surface was prepared with magnesium oxide, having a reflectivity of essentially unity. Now the light is diffusely reflected about the interior of the sphere by its barium sulfate coated interior until it is reflected through the exit to the photocell. Differences between spheres and the greater amplification of light required are electronically compensated. The forward scattered light from the thin film is collected by the integrating sphere and evaluated by the photocell as the total transmission of the film. The arrangement of the components for these measurements is shown in Fig. 6.

By replacing the reference surface of the sample compartment sphere with the sample, the reflection or backward scattered light can be measured. Figure 7 illustrates the arrangement for these reflection measurements. All the light, no matter how it is reflected from the surface of the sample, is part of the flux in the sphere and is measured by the photocell.

Thus the optical properties of the thin film can be measured or calculated. Comparison of the first and second types of measurements (direct transmission and total transmission) indicated if the film behaves as just a chemical sunscreen (i.e., obeys the Lambert-Beer Law) or if it has both the chemical and physical sunscreen attributes. The forward scattering (FS) measurement indicates the percent total transmitted light. The backward scattering or percent reflection (BS) measurement determines the total light reflected by the specimen. The percent absorption (Abs.) of the sunscreen product can then be calculated: Abs. = 100 − BS − FS.

Measurements on Thin Film Specimens

Figure 3 illustrates the difference between total transmission and the monochromator direct transmission measurements on two physical sunscreen products and a chemical/physical sunscreen combination product. Figure 2 compares the results for the total transmission, direct transmission, and monochromator direct transmission for a product with only a chemical absorber. All three measurements readily agree in this instance. Hence only the total transmission of the thin film need be determined, regardless of the type of protection offered by the film, since the major protective value of each sunscreen is determined by its total transmission in the sunburn region of the spectrum. Little variation was obtained in total transmission in a thin film after drying. Obviously, if the film separates mechanically, physically, or chemically upon drying, its optical characteristics will change.

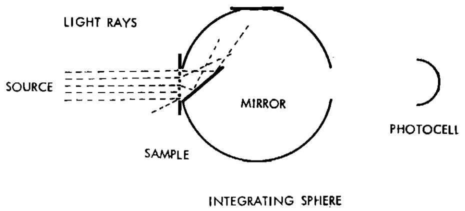

Figure 6. Diffuse scattering with integrating spheres

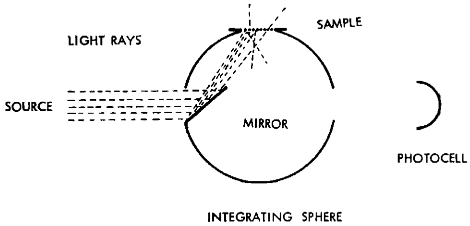

Figure 7. Diffuse reflectance with integrating spheres

In general, the total transmission values agree with the direct transmission values from the monochromator for each sample. Usually the monochromator values show less transmission than those from the integrating spheres total transmission values. This difference was anticipated since the monochromator with the photocell did not collect all available light from each sample. The most comparable values obtained were those in the region where the chemical absorber absorbed most efficiently, and the greatest divergence from the values occurred where the physical particles exerted the greatest effect in the total absorption of light (i.e., near 360–700 mμ and 230–270 mμ). The comparable runs without chemical absorbers and also without the physical sunscreen show even greater divergency between measurements, as shown in Fig. 3. Again this shows the loss of light due to scattering by physical particles rather than to normal absorption. The photocell-monochromator combination just could not detect as much of the scattered light as the integrating spheres.

Some difference in the shape of curves obtained by the different techniques is to be anticipated from the optical and mechanical inferiority of the monochromator as compared to the Cary spectrophotometer. Two important reasons are: (a) the intensity of the light source of the monochromator is unstable; it is not simultaneously in balance with a reference beam; (b) the monochromator optics are much poorer (wider slits, greater band width, etc.) since it was not designed for narrow-band spectrophotometric work.

Using films of different thicknesses, 0.0254, 0.0019, 0.0127, and 0.00635 mm ± 10%, with physical and chemical/physical sunscreen products, it was found that for transmission measurements the physical sunscreens do not obey the Lambert-Beer Law.

The reflective characteristics of the thin films were also examined to determine the degree to which reflection contributes to the protective ability of sunscreen products. In Fig. 8 the reflection spectra of films with physical and chemical/physical sunscreen products may be seen. Figure 2 shows the reflection spectrum of a film with a chemical sunscreen. The most striking feature of the reflection spectrum is that it appears to follow the total transmission spectrum. If the sample transmits well it also reflects well. If it absorbs well in some spectral region, or transmits little light, it does not reflect well. For example, the opaque cream base reflects uniformly at all wavelengths slightly more light than the *p*-aminobenzoic acid (clear viscous) solution. However, addition of 10% talc to the opaque cream base doubles its reflective capacity in the 300 mμ to 700 mμ region. Addition of 5% amyl *p*-dimethylaminobenzoate to the talc product did not increase the reflection very much. On the contrary, because of the absorbing capacity of the amyl-*p*-dimethylaminobenzoate in the sunburn range, the reflection of the talc product was reduced to the level of the opaque cream base in the 230 mμ to 350 mμ range; from 350 mμ to 700 mμ the reflection was equal to or slightly higher than that from the talc product.

Comparison of monochromator or integrating spheres measurements of thin films with the measurements of the solution-dilution method reveal interesting and important observations: The transmission characteristics are qualitatively similar for the chemical sunscreen product as determined by both methods (Figs. 1 and 2); however, these are entirely different for the physical and the chemical/physical sunscreen products, particularly in the longer wavelengths (Figs. 2 and 3). The physical sunscreen, being insoluble in solvents used in the solution-dilution method, obviously cannot affect the transmission spectrum as it does in the film specimens. This also is responsible for the lack of reflection in the solution-dilution method samples, although reflection is observed so well in the thin film specimens. Naturally, the lack of physical sunscreens in the extracted solution makes it impossible to determine the differences in transmission when one physical sunscreen is added to another.

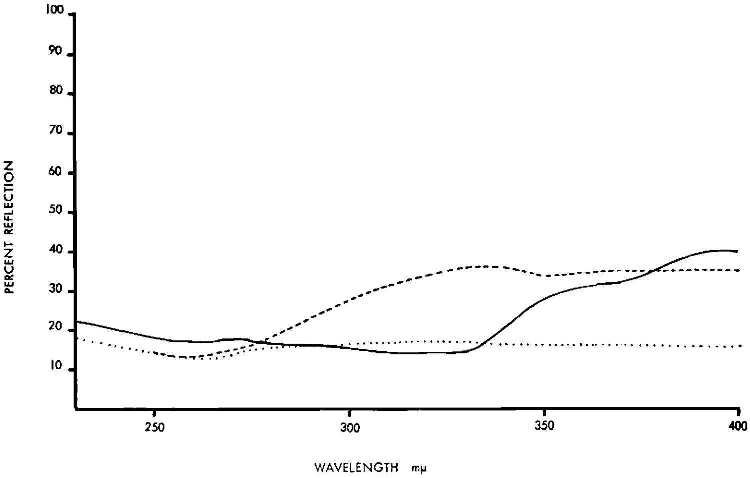

Figure 8. Percent reflection using spectrophotometer with integrating spheres of 0.0254 mm thick films of three sunscreen products. —— 5% amyl-*p*-dimethylaminobenzoate + 10% talc in opaque cream base; - - - 10% talc in opaque cream base; and . . . opaque cream base

In conclusion, measurements with the thin film technique are more representative of conditions actually occurring on the skin since the product, when applied on the skin, will form a film, and the thickness of this film can be reproduced by this technique. In addition, the product is optically measured quickly and conveniently in its original form without being laboriously altered to the solution form, as in the solution-dilution method.

In Vitro Studies with Human Epidermis

Specimens of Caucasian human skin were surgically obtained at autopsy, and the intact epidermis was separated from the underlying tissues by the stretch technique (9). Histological examination of the epidermal skin revealed good, white, entire epidermis, with a total thickness of 40 μ, comprised of stratum corneum (16 μ thick), and stratum malphighian (24 μ thick). The epidermal section was then mounted on a quartz slide for total transmission and reflection measurements. The same measurements were repeated after the physical/chemical sunscreen was applied to the epidermis. The total transmission of the epidermal specimen was similar to that found by Everett *et al.* (10) using a similar technique of measurement. The application of the chemical/physical sunscreen to the epidermis considerably reduced the total transmission of both ultraviolet and visible light to less than 3% of the light penetrating the epidermis originally (Fig. 9). In the regions where the skin normally transmitted the greatest amount of light (320–700 mμ) the physical sunscreen component (talc) of the combination sunscreen product lowered the total transmission markedly. In the sunburn region (280–320 mμ) the total transmission was also markedly reduced because of the chemical sunscreen component.

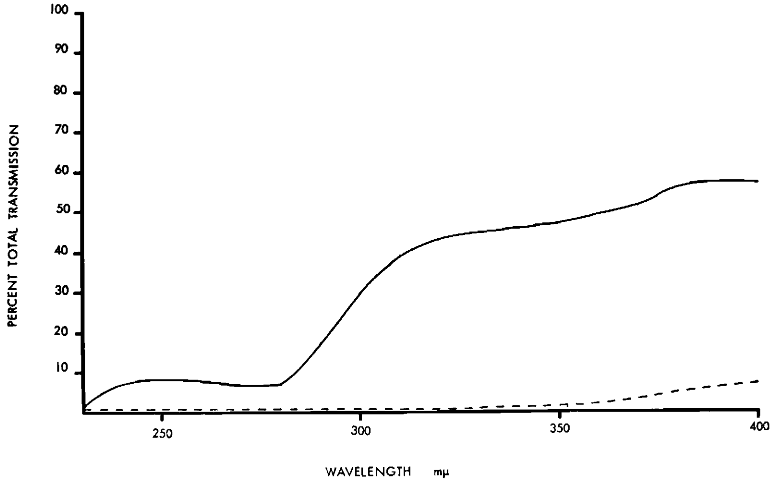

Figure 9. Total transmission of human epidermis (*in vitro*) using spectrophotometer with integrating spheres. —— human epidermis and - - - human epidermis with sunscreen product, 5% amyl-*p*-dimethylaminobenzoate + 10% talc in an opaque cream base, applied

A considerable quantity of the light in the sunburn and solar carcinogenic portion of the spectrum penetrates through normal epidermal layers to the dermis. The application of the sunscreen markedly reduces this penetration. The reduction in transmittance corresponds somewhat roughly to the observed erythemal protection obtained with this chemical/physical sunscreen product.

The initial reflection spectra obtained from these epidermal skin specimens (Fig. 10) were characteristic of those observed for other specimens by Everett *et al.* (10). The addition of a chemical/physical sunscreen product altered the reflection spectra in the sunburn region only a little. In the visible (370–700 mμ), the addition of the sunscreen markedly increased reflection to one and one-half times normal reflectance. In the region where the chemical sunscreen of the product as well as the skin absorbed the strongest (280–350 mμ) the skin and sunscreen combination reflected less light than was reflected by the skin initially. Throughout the shorter portion of the spectrum (230–280 mμ) the skin alone and skin with sunscreen reflected the same.

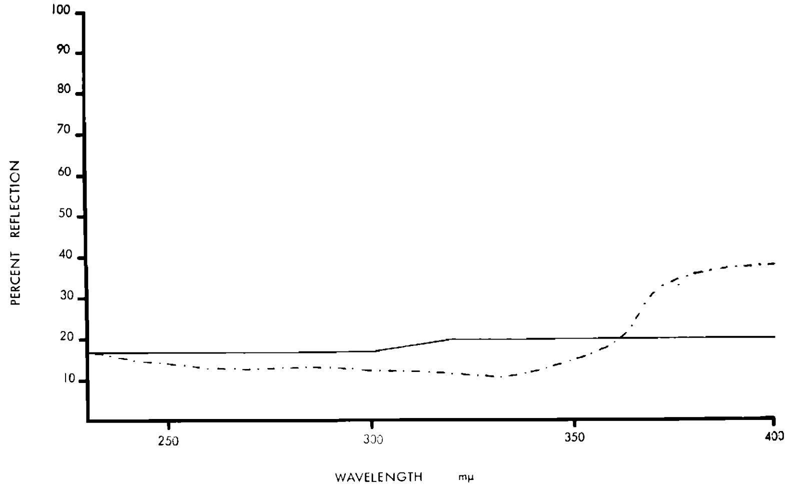

Figure 10. Percent reflection of human epidermis (*in vitro*) using spectrophotometer with integrating spheres. —— human epidermis and - · - · - human epidermis with sunscreen product, 5% amyl-*p*-dimethylaminobenzoate + 10% talc in an opaque cream base, applied

In Vivo Reflection Studies with Human Skin

Based on the *in vitro* studies, *in vivo* studies were attempted, using the dorsal surface skin of subject's hand in place of the epidermis specimen. Three *in vivo* studies indicate very little difference between the reflection of the skin *in vivo* with or without the protection of a sunscreen. This appears to be true in the visible as well as in the ultraviolet range. The slight differences between the reflectance spectra could be as easily attributed to differences in pressure or positioning of the subject's hand over the opening to the integrating sphere. The fact that in one experiment the skin with the sunscreen actually reflected less light than the skin without sunscreen, while in the other two experiments the reverse of this occurred, would tend to support the contention that positioning, pressure, and trauma from applying the sunscreen may be more influential in changing the reflection spectra than the actual physical additive. Further studies are in progress to obtain information as to how changes in the circulation and the trauma of application of the sunscreen affect the reflection of the human skin and how to modify the equipment to eliminate these drawbacks.

SUMMARY

Development of sun-protective preparations must continue to provide products giving a broad range of protection against the undesirable cutaneous responses to sunlight, such as sunburn, photosensitivity reactions, accelerated aging, and carcinogenesis. Most of the products available provide protection against sunburn and promote increased pigmentation. One of the drawbacks is a lack of a suitable laboratory method that will yield realistic information about the protective ability of the sunscreen product without the need for prolonged and strong exposure tests in humans to sunlight and other light sources with similar spectra. Use of the thin-film technique presented in this paper with a xenon arc monochromator has afforded such information. Modifying this method by using integrating spheres with a Cary spectrophotometer Model 14, in place of the monochromator, has made it possible to measure the total transmission or forward scattering and reflection or back scattering in addition to the direct transmission usually measured. The thin film technique makes it possible to determine rapidly these measurements on the sunscreen films simulating those applied on the skin without altering in any way the sunscreen product, as is commonly done with the classic solution-dilution method. The new method has, in addition, made it possible to evaluate not only the chemical sunscreens but also the physical sunscreens and chemical/physical sunscreen combinations. This has not been possible with previously published standard laboratory methods. The versatility of the thin film technique

makes it possible to measure the protective ability of sunscreen products on excised human epidermis as well as conduct similar measurements on human skin *in situ.*

(Received February 16, 1966)

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