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The Influence of Protein Vehicles on the Penetrability of Sunscreens

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Synopsis—The development of SUNSCREENS utilizing several established *in vitro* and *in vivo* products has been well documented in the literature; however, continuing publications in search of the optimal sunscreen indicates that the ideality in sunscreens has not yet been achieved. The objective of this paper is to outline a rational scientific approach for the evaluation of sunscreens using our investigations on PROTEIN VEHICLES as an example. Technology in *in vitro* and *in vivo* investigations are discussed.

In vitro investigations utilizing Kumler's "SUNSCREENS INDEX" provide a rapid means of assessing that AMINOBEZOIC ACID DERIVATIVES are choice agents, which absorb in the ERYTHEMOGENIC RANGE of 290 to 317 mμ in wavelength. Results in *in vivo* investigations in the rabbit-ultraviolet (uv) light-induced erythema demonstrated that PARA-AMINOBENZOIC ACID (PABA) was effective on both unrinsed and rinsed skin surfaces.

For the confirmation of the nonwashability of the selected aminobenzoic acid derivatives, analysis of tape stripped layers of epidermis was undertaken using the Bratton-Marshall method for primary amines for the quantitative analysis of sunscreen agents from tape stripped skin. PABA in an ethanolic protein vehicle appears to retard absorption into the tissue as well as to increase the amount of drug that is held at various tissue depths. This investigation confirmed the improved substantivity of PABA when it was combined with a protein vehicle.

Introduction

There are many detrimental effects of sunlight, which until recently, have received too little publicity. Because of the high incidence of skin cancer and other diseases precipitated by sunlight, dermatologists have become acutely aware of the harmful effects which the sun may have on the skin. The practicing physician is often questioned by patients about the effects of sun bath-

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ing, tanning, sunburning, sunscreening agents, palliatives, and simulative preparations on one's skin (1). The cosmetic sunburn and suntan preparations may be classified into the following three groups: (1) sunscreens, (2) palliatives, and (3) simulative preparations. Sunscreens represent the most important group of preparations designed to allow tanning with the minimum of sunburn. Palliatives are designed to alleviate the pain and irritation resulting from excessive exposure to sunlight, while simulative preparations are designed for those who wish to feign brown skin without the time required to obtain a suntan naturally (2).

The responses of normal skin to sunlight may be classified according to erythema and tanning (Fig. 1). Erythema is produced by a well-defined range of sunlight between 290 and 317 mμ in wavelength. The greatest effect occurs at 297 mμ, i.e., the middle range of the sunlight spectrum (Fig. 2). Severe burns may be produced by intensive or prolonged exposure to erythemogenic ultraviolet (uv) light. The process by which uv light causes a skin reaction to occur is probably due to the injured cells liberating leukotaxine and other substances, which induce inflammation. This type of sunburn may be prevented by a filter or a sunscreen which absorbs; glass, for example, filters out the erythemogenic rays of sunlight. The total effect produced by a given wavelength of light is a function of the intensity and duration of the exposure. The intensity of erythemogenic wavelengths of sunlight, in turn, depends on the latitude, amount of reflected light, water vapor, and dust particles, etc., as well as variables, which occur in the responsive individual's such as exposed areas or his complexion and, most important, the length of time (3) of exposure (Fig. 3).

Tanning on the other hand involves three basic changes. Two of the changes occur in melanin—a skin pigment—while the third is formation of new melanin. The melanin present in the skin is oxidized to a darker state following exposure of the skin to uv light. The greatest stimulus for this process results from wavelengths of about 300 to 420 mμ with a peak at about

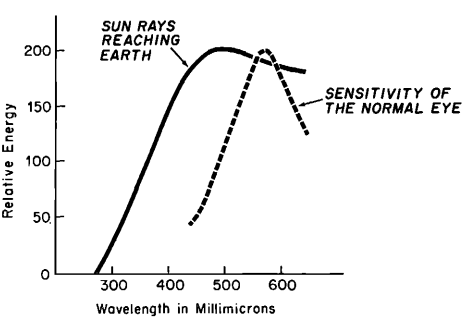

Figure 1. Spectrum of sunlight

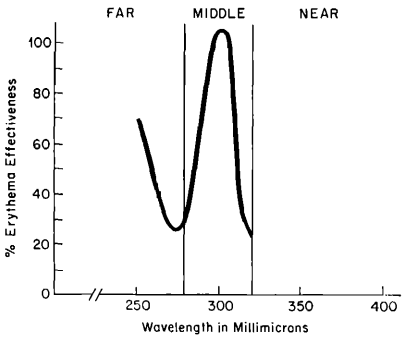

Figure 2. Erythema (middle uv) versus spectrum of sunlight

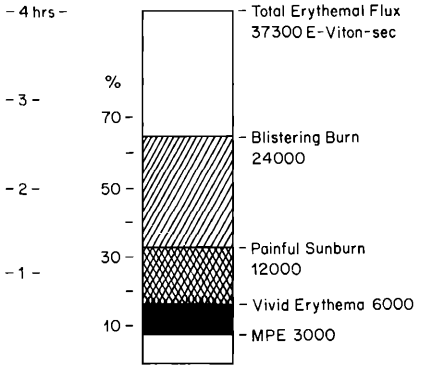

Figure 3. Skin response to sunlight

340 mμ (4). A second reaction of an upward dispersion of melanin is caused by uv light wavelengths, which measure between 280 and 310 mμ, with melanogenesis beginning about 2 days after exposure to uv light, but not achieving a peak pigment production until approximately 19 days after sun exposure (6).

Sunscreens should either scatter the incident light effectively or absorb the erythermal portion of the sun's radiant energy. The most important class of sunscreens are those which operate by absorbing the erythemal uv radiation. The properties of the ideal sunscreen should be as follows:

1. effective in absorbing erythemogenic radiation in a 280 to 315 nm range without breakdown;
2. should allow full transmission in the 300 to 400 nm range to permit maximum tanning effect;
3. should be nonvolatile, resistant to perspiration, resist washings—in other words, maintain a significant protection under conditions of normal usage;
4. should possess suitable solubility characteristics to allow the formulation of a suitable cosmetic vehicle;
5. should be nonodorous and sufficiently mild to be acceptable to the user and possess a cosmetic elegance; and
6. should be nontoxic, nonirritant, and nonsensitizing.

Draize (7) has stressed the need for nontoxicity and dermatological acceptability of sunscreens because these agents are unique as cosmetics since they involve multiple and extensive daily applications to large areas of the body surface and, in addition, may be applied to the skin already damaged by the sun or wind. Moreover, they are used on persons of all age groups in varying conditions of health. Ultimately, an eightfold margin of safety was prescribed by Draize involving acute and subacute dermal toxicity and potential sensitization tests (8).

Among the ideal characteristics listed previously for a sunscreen preparation, probably the most sought after attribute is nonwashability. Sunscreen preparations, by virtue of their use, are applied to the body preceding swimming or excessive exercise in which perspiration tends to wash off or dilute any material on the skin. Therefore, it is desirable, in order to avoid multiple applications, to find a sunscreen formulation, which in addition to protecting the user from erythemogenic rays of the sun and permitting tanning, can also be applied infrequently and which will remain on the skin even after continuous exposure to water via swimming, perspiration, or bathing.

The term used to describe the binding of a sunscreen agent to the skin is often referred to as "substantivity," a specific term referring to the capacity of an agent to adhere or combine with keratinized substrates. PABA is found to be absorbed by the intact epidermis, which results in partial chemical conjugation with the constituents of the horny layer and results finally in a substantive property of this specific sunscreen agent. Willis and Klingman (9) termed a compound truly substantive when the horny layer reservior was full (resulting in a substantive compound remaining fixed through physical or chemical conjugation.) Pathak *et al* (10) found that an ethanolic solution of PABA at pH 4.5 to 4.8 was substantive to the horny layer even after repeated washings with water. Others have not found this substantive property to be true under normal use conditions. The purpose of this presentation is to review how we approached the problem of evaluating sunscreen agents by developing the appropriate technology to allow us to determine significant performance improvements of some sample formulations and to investigate substantive properties on human skin. The sequence of test procedure we developed was as follows: (1) *in vitro* evaluation; (2) *in vivo* (animal) evaluation; and (3) *in vivo* (human) evaluation.

Experimental Evalaution

I. *In Vitro Evaluation*

Testing for the effectiveness of a sunscreening preparation begins with the uv-spectrophotometric evaluation to determine the absorption range and capacities. These may be expressed as E-vitrons (11), a sunscreen index (12), a K value (13), or any one of the *in vitro* terminologies that provide for spectrophotometric comparisons to be made of a sample sunscreen agent to a known standard (14).

In the formulation of sunscreen preparations, it is necessary to assess the efficiency both of the sunscreen and the prototype products. This was done by examining the absorption characteristics spectrophotometrically in terms of concentration, thickness of liquid through which the light passes, and wavelength. The absorption characteristics may be expressed as a percentage of the incident radiation absorbed or transmitted, or as the optical density with the

Table I

Sunscreen Index Published *(In Vitro)*

Sunscreen	Degree of Ray Filtration (in erythema range between 285 and 315 nm)
Ethyl-dimethyl-amino-benzoate	14.80
Ethyl-p-amino-benzoate	9.60
Isobutyl-p-amino-benzoate	9.20
p-amino-benzoic acid	7.40
Ethyl gallate	1.40
Lauryl gallate	0.85
Salicylic acid	4.30
Methyl salicylate	4.00
Salicylamide	3.90
Sodium Salicylate	2.40
Salicyl aldehyde	2.20
Dipropylene glycol salicylate	1.90
p-Amino-salicylate	1.90
Methyl umbelliferone	7.70
Umbelliferone acetic acid	6.00

latter being the logarithm of the ratio of intensities of the radiation before and after passage through the solution. This has the advantage of absorption characteristics being directly proportional to the concentration and the thickness of the material; hence, calculation is simple. The absorption characteristics of a chemical are frequently expressed as the molar absorbtivity, in order words, the calculated absorbance of a 1-cm layer of a molar solution or the absorbtivity calculated on the basis of the optical density of a 1-cm layer of a 1 per cent solution.

Kumler (1952) (12) uses this procedure as a simple and rapid method for the relative evaluation of sunscreens. He measured the absorbance of a 10-per cent solution in a 0.1-mm silica cell at 308 nm and converted the results to a sunscreen index, which corresponded to an absorbance of a 1 per cent solution in a 1-nm cell. Fifteen compounds are ranked in decreasing order of screening effectiveness (Table I) (12). In our laboratory, we have confirmed his findings from his evaluations of the sunscreen index of some of these products and, indeed, find this *in vitro* methodology to be effective. The method we used is described as follows. The solutions were measured with a Beckman* uv spectrophotometer using fused silica cells of 0.1-mm pathlength. The more effective compounds, which could not be adequately evaluated at

*Fullerton, CA.

this concentration and pathlength (because their densities were too high), were diluted tenfold using 95 per cent alcohol, which resulted in a 10 per cent solution, and brought the densities down to a value, where the relative absorbing power of the various compounds for the rays producing sunburn could be compared. The sunscreen index is a number obtained by dividing the absorbance of the solution at 3080 Å by the per cent of concentration that is found in the solution. The value of the sunscreen index then represents the density of 1 per cent solution of the compounds at a pathlength of 0.1-mm. The fact that isobutyl para-amino bezonate has a higher value than the propyl compound appears anomalous and may be due to the relative purity of the compounds.

The absorbance has been chosen to compare different compounds rather than to present transmission because sunburn depends on the amount of light absorbed by the skin in the sunburn range, and the amount of light that is screened out is given directly by the absorbance. On the other hand, the light screened out is a log function of the transmission; thus, the numbers obtained in the sunscreen index can be used to compare the effective sunscreening power of any compound with any other compound on a weight basis.

One will notice that PABA has a concentration per cent or sunscreen index of 7.4, and that there are other derivatives of benzoic acid (which we have already noted) with higher sunscreen index. However, one must keep in mind that this is an *in vitro* evaluation to give a relative comparison and, indeed, a final evaluation of the formulation must be made on intact skin before any conslusions can be reached. However, it was established that any material with the sunscreen index less than 6 would not be interesting for further evaluation. At best, these *in vitro* evaluations are qualitative in nature and provide an indication whether or not the sunscreen absorbs the burning rays. In order for a good sunscreen to be effective, a compound must possess high absorption properties at 308 nm while superimposing itself on the entire sunburn curve. However, one must be careful with these data, making certain that the sunscreen index does not exclude the effect the vehicle has on the absorption characteristics (15) as demonstrated by Van Ham *et al* (16, 17) (Fig. 4).

One cannot overemphasize the vehicle effects of a sunscreen preparation, particularly in an *in vitro* evaluation. In 1954 and 1955, Stambovsky (18, 19) published an extensive series of articles reviewing the factors considered important for a suntan preparation. In these articles he criticized the use of the spectrophotometer for the evaluation of sunscreen products on technical grounds (with which we and others (20) do not concur). The disparity in the results, which might exist between the spectrophotometric data and the results of skin testing, may be due to overlooking the effect of the vehicle components on the absorbent properties of the active compound. Properly designed spectrophotometric tests will always be more sensitive, rapid, and accurate guides for product evaluation than human testing. The latter can be

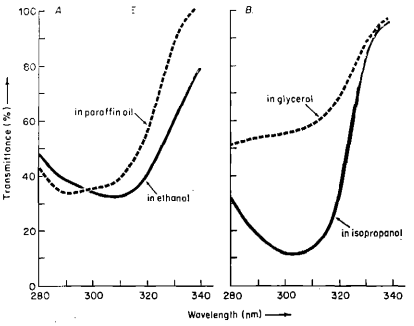

Figure 4. Influence of vehicles on sunscreens absorption characteristics: (A) oil soluble absorber; (B) water soluble absorber

reserved for the intensive study of the final formulation (21).

Having done the preliminary *in vitro* studies, we decided to study (PABA) for its effects in *in vivo* models. We chose an ethanolic vehicle and a protein vehicle for our *in vivo* effects. Proteins have been known to possess substantive properties on hair and skin. We sought to find if the substantivity of PABA could be increased by the addition of protein.

II. *In vivo Animal Evaluation*

The *in vivo* evaluation of laboratory animals under conditions of artificial lighting are wrought with many difficulties (22). The use of a uv-light apparatus has two major disadvantages: (1) the temperature differences, which can occur at the surface of the skin, and 2) the differences in uv emission between the mercury spectrum and the actual sunlight (Table II). For this reason, certain precautions must be taken to standardize the experimental conditions. The experimental procedure must be well controlled in terms of amounts applied, method of application, similarity of vehicle, and experimental design.

Methodology

Female rabbits, weighing from 1.7 to 1.9 kg, are selected with regard to overall appearance of their coat and skin. Twenty-four hours before use, they are carefully shaved in the abdominal region with clippers followed by a razor. The rabbits are tied down firmly in a supine position. Two strips of Blendrem tape* are placed in parallel, 5mm equidistant from the mid-line. The tape has 5 holes 1 cm in diameter punched out at 1-cm intervals. The

*3M Corp., Minneapolis, Minn.

Table II
Approximate Analysis of uv Content of Sunlight and uv Lamps

Light Source	Per Cent Wavelength		
	250-280 mμ	280-320 mμ	320+ mμ
Sunlight	None	40	60
Code quartz (mediquartz)	100	57	2
Fluorescent sun ray lamp (Westinghouse, 110 V)	41	57	2
R and S sun lamp Westinghouse 275 W, 110 V)	35	63	2
Hot quartz lamp, 100 V (Burdick)	63	35	2
Hot quartz lamp, 110 V (General Electric)	62	36	2
Xenon arc lamp (Osram SBO-501 W, Osram GMBH, Berlin)	12	28	60
Xenon arc lamp (450 W, Model 609, Rudolph Instruments Engineering Co., Little Falls, N.J.)	66	8	26

total length of the tape is approximately 12 cm. The 5 openings on each strip offer 10 sites for application, 6 for the test compound, and 4 for vehicle controls. The materials are applied to the center of the opening in a 95-per cent alcoholic solution in a volume of 0.1 ml and evaporated to dryness by an air stream. Any excess left at the end of this time is not apparent to the investigator. The sites are then exposed to uv light from a Kromayer #10 lamp* with a skin compressor lamp attachment, which is held in contact with the skin for 30 sec. The surface of the lamp is washed off with an alcohol swab and allowed to air dry for 5 sec before moving to the next site. All rays from the lamp are directed toward the opening by the use of a guarded colimator (see Fig. 5). After exposure of one site, the lamp is moved to the next site in a lateral back-and-forth fashion. The uv output is maintained constant at 2100 $\mu W/cm^2$ and standardized at a distance of 5 cm at 5 different distances from the skin surface. The inverse square law is applicable if the source is assumed to be 2 to 4 cm behind the quartz filter surface. The uv output is calibrated before the experiment and at the end of the exposure period to ensure that the ouput does not increase appreciably as the instrument warms up. Variation of output has never been greater than 2 per cent.

In those experiments in which the skin is washed, areas which have received the sunscreen preparation or the circled areas receiving the test compounds are exposed to a flow of 50 ml of water for a period of 5 sec administered in 5 individual washings to approximate a rinse-wash condition. Twenty-four hours after the rabbits were exposed to the uv light, they were graded according to the scale shown in Table III.

*Engelhardt Hanovia Lamp Co., Slough, England.

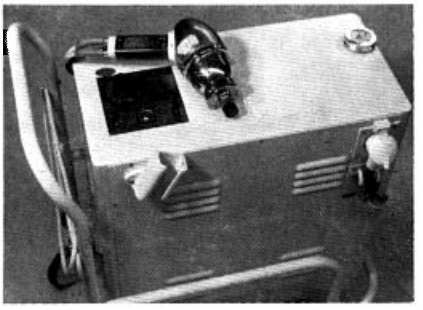

Figure 5. Kromayer model 10 lamp

The maximum erythemic score for each rabbit, comprising 10 sites, was 40. When scoring, the surrounding area was examined, since irritation due to shaving and/or the animal rubbing its abdomen against the floor of the cage will give rise to false results. If this irritation was evident the animal was discarded in its entirety. The experiment was conducted under blind conditions with the investigator and scorer being unaware of the treatment the animal received and all treatment sites being randomized by standard procedure.

The data obtained from this procedure can be applied to a regression analysis among individual compounds as well as to tests for parallelism between compounds (23). The index of precision (24) is low for this assay ($\lambda = 0.46$) which is indicative of the reproducibility of the dose-dependent response for sunscreen agents.

At this time, we were interested in evaluating if one could increase the substantivity of PABA by the additions of a protein adjuvant. Therefore, we selected several concentrations of PABA in ethanol and in a formulation in which a solubilized protein had been added to preserve its influence on a uv-induced dermal erythrema in rabbits. For this test, 5, 2, and 0.5 per cent

Table III
Scoring for the uv-induced Erythema in Rabbits

Score	Observation
0	No erythema
1	Faintly erythemic with less than 50 per cent of the area involved
2	Partially erythemic with more than 50 per cent of the area involved but less than 100 per cent
3	Completely erythemic with 100 per cent involvement with some edema
4	Edema with 100 per cent erythema and scaliness

Table IV
Influence of Sunscreen in the uv-induced Erythema in Rabbits

Concentration of Sunscreen Formulation	Per Cent Protected Sites[a]	
	Unrinsed	Rinsed
5 per cent PABA/ethanol	**86**	58
2 per cent PABA/ethanol	**54**	32
0.5 per cent PABA/ethanol	**18**	15
Vehicle	10	3.3
5 per cent PABA/protein	96	93
2 per cent PABA/protein	**85**	75
0.5 per cent PABA/protein	**75**	50
0.2 per cent PABA/protein	**35**	33
Vehicle	19	8.7

[a]Number of sites with score of 1 or less. All standard errors were less than 4 per cent.

PABA in ethanol was formulated using water as one vehicle and solubilized protein as another vehicle. The material was applied as discussed previously and the per cent protection obtained. The material was evaluated in unrinsed and rinsed conditions as described previously. PABA in ethanol has less effect (Table IV) when the skin was rinsed, but still maintained some activity due to the inherent binding of PABA to the keratin layer alone. This activity, however, in the rinsed condition was less than in the unrinsed condition.

When PABA was combined with a soluble protein vehicle, we noted no loss of efficacy in its ability to protect the uv-induced dermal erythrema in rabbits following rinsing. This gave us the first indication that possibly this material would offer an added advantage in a vehicle formulation.

Such *in vivo* results are a preliminary type of test, which can be used to screen out several simple parameters relative to the efficacy of the formulation, but one must keep in mind that the final formulation evaluation should occur on human skin under normal sun exposure conditions.

III. *In Vivo Human Evaluation*

Pathak *et al* (10) reported that 5 per cent PABA in 70 per cent ethanol was an effective sunscreen when it was used and evaluated in subjects exposed to intense, bright sun in the Arizona desert and, also, found the preparations to be effective even after swimming. Katz (25) repeated the evaluation of 5 per cent PABA in 70 per cent ethanol. In the latter study, the interval between ap-

plication and exposure, which was different from levels of the Pathik and Willis and Kligman (9) studies, may be of importance.

The first indication that a solubilized protein concentrate was applicable for topical preparation was reported by Swartz in a preliminary finding (26). He was investigating the application of a solubilized protein concentrate in teenagers with acne vulgarus of varying severity, and he initially reported a residual beneficial effect, which was noted for some time. Collagen, which is a normal constituent of the keratin layer, has been alluded to as having an adjuvant effect when included in a vehicle formulation for many preparations (27, 28). However, no well-defined basis for this activity has been documented.

To confirm our findings in animals, we decided to evaluate the influence of the protein derivative on the penetrability of PABA. If the protein does enhance the penetration or retard the removal of a sunscreen agent, particularly after washing or bathing, one should find greater concentration of the sunscreen agent in the presence of a protein in a vehicle. We undertook a feasibility study among in-house volunteers for the evaluation *in situ* of the penetration or localization of PABA. The vehicle in the sunscreen preparations was ethanol in water or a protein derivative utilized to determine whether the compounds exhibit favorable adjuvant effects. Appropriate control solutions were also included. Studies reported here were confined to: (1) the analytical determination of the amount of drug eluted from the scotch-tape stripped skin defined as a function of skin depth, and (2) the effect of protein adjuvant on tissue penetration. The materials utilized and the methodology followed in this study are outlined as follows.

A. *Methods*

1. *Standard Curve for PABA:* A 1 mg/ml stock solution of PABA was prepared in 0.1 *N* sodium hydroxide. The working standard consisted of a dilution of stock in 0.1 *N* sodium hydroxide to a concentration of 0.01 mg/ml.

The standard curve was prepared as follows: 0.010, 0.025, 0.050, and 0.1 ml of the working standard were pipetted into individual 10-ml graduated centrifuge tubes to give final concentrations of 0.1, 0.2, 5, 0.5, and 1.0 μg/ml. 0.5 ml of a 2 *N* hydrochloric acid and 1 ml of a 0.1 per cent sodium nitrate were added to each tube, mixed well, and allowed to stand 3 min. After the diazotization procedure, 1 mg of ammonium sulfamate was added, mixed well and allowed to stand for 10 min to destroy excess nitrite. Color was developed by the addition of 1 ml of 0.1 per cent *N*-(1-napthyl)-ethylene-diamide-dihydrochloride in distilled water, mixed well, and allowed to stand for 30 min. The red color was read against the reagent blank at 540 nm in a spectrophotometer.

2. *Sunscreen Preparations and Treatment Sites:* The sunscreen preparations used in this study are in two different vehicles—a protein vehicle compound with an appropriate ethanol and a water reference vehicle (Table V).

Table V
Recoveries of PABA from Blenderm Tape

PABA (μg/ml)	Per Cent Recovery			Mean[a]	Standard Deviation[b]	Standard Error[c]
10	104	102	99	**102**	± 2.5	1.4
5	96	92	97	**95**	± 2.6	1.5
2.5	103	116	85	101.3	±15.5	8.9
1	81	118	94	**97.6**	±18	10.8

[a]Mean: 96 107 93.7.
[b]Standard deviation: ±10.6 ±12.2 ±6.1.
[c]Standard error: ±5.3 ±6.1 ±3.1.

3. *Application of Sunscreen and Skin Stripping Procedures:* Ten subjects were utilized for these studies over a two-day period. The lower back area was utilized for application of the coded formulation preceded by a preliminary swabbing with an alcohol sponge. When the area was dry, two sites on either side and parallel to the spinal column, measuring 2½ x 15 cm, were marked with indelible ink using a 10-point spotting procedure. One-tenth ml of 5 per cent solution of PABA in the various formulations was delivered to the site with a 1-ml disposable syringe in a randomized manner. Following application, the material was spread by inunction utilizing a glass rod with 20 uniform forward and backward strokes.

Following application, the subject was supplied with a loose fitting cotton shirt and was allowed to return to work. One-half hour after application of the sunscreen, the sites were washed for 2 min with water delivered at a rate of 2 l./min at a temperature of 37 to 39°C using a Cal-pump, Model #1200.* Following the wash period, the area was air dried and the subject assumed a prone position in preparation of the stripping procedure. The stripping procedure was accomplished by 2 persons working simultaneously on either side of the spinal column. Blenderm tape strips measuring 2½ x 15 cm were applied to the areas and removed in a standardized manner, each strip removing one layer of cells. This procedure was repeated 25 times on each site. Following stripping, a thin layer of vaseline was applied to the site and the person was requested to report for examination of the test site 2 days later. Tissue stripping procedures produce a mild erythema comparable to a mild sunburn.

4. *Extraction procedure of the Skin Strip Tape:* Blenderm tape strips, 2½ x 15 cm, were placed over an equal area of medicated skin. After removal of the tape, the centermost portion measuring 2.5 x 7 cm was cut away and

*Cal Corporation, San Francisco, Calif.

placed in a 1.5 x 12.5 cm test tube containing 5 ml of 0.1 *N* sodium hydroxide for elution of PABA. Each sample was eluted on a Vortex* mixer for 1 min at the fastest mixing rate. Recoveries indicated that a 1-min elution was sufficient to remove as much as 10 μg of drug. PABA was assayed by the Bratton-Marshall method (29).

5. *Procedure for Recovery of PABA from Blenderm tape:* Various amounts of PABA in 0.1 *N* sodium hydroxide were added to 2½ x 7 cm strips of Blenderm tape for a calibrated syringe. The solvent was allowed to evaporate in a hood and the strips extracted as mentioned previously. Skin blanks obtained on 20 strips gave an average absorbance of 0.007 in the Bratton-Marshall test. All subsequent absorbency reading were blank corrected.

B. *Results and Discussion*

Standard curves for PABA are given in Fig. 6, whereas the recovery of drug from tape are given in Table V. Overall recovery of PABA by the elution of the tapes with 0.1 *N* sodium hydroxide was 99 per cent. In earlier studies on PABA, the recoveries, which occurred during the use of an alcoholic vehicle, were low due to the dissolution of the tape adhesive, which after evaporation, trapped drug in the glue-adhesive matrix. Recoveries of PABA were considerably improved by the use of concentrated alcoholic solutions, which were diluted with chloroform. Small volumes of chloroform applied to the tapes evaporated rapidly, thereby preventing the drug from dissolving into the tape adhesive. Recoveries under these conditions were quantitative.

The statistical analysis of PABA in the presence and absence of protein is given in Table VI. The data represented in Fig. 7 are the average amounts of the drug found in 5 subjects per group distributed over 50 skin strips per group. As indicated in Fig. 7 and Table VI, strips 4 and 5 with 5 degrees of freedom show significance at the 95 per cent level. The significance at such high levels for small sample numbers lends credence to the enhanced substantivity and/or penetration effect of the protein. From these data, the probability exists that if considerably more samples were obtained and the techniques of assay, application, and removal of stripped skin were better standardized, the variance would be reduced considerably, which in effect could show significant differences over many more stripped areas than were found here. Our animal studies with uv-induced erythema in rabbits shows that PABA sunscreens prepared with 2 per cent protein gel on rinsed sites gave enhanced substantivity over similar formulations lacking the protein vehicle. Although these studies are preliminary, the protein curve shown in Fig. 7 would seem to indicate that protein produces a retardation of penetration possibly by adsorption to certain acidic molecular sized fragments. The implica-

*Scientific Products, New York, N.Y.

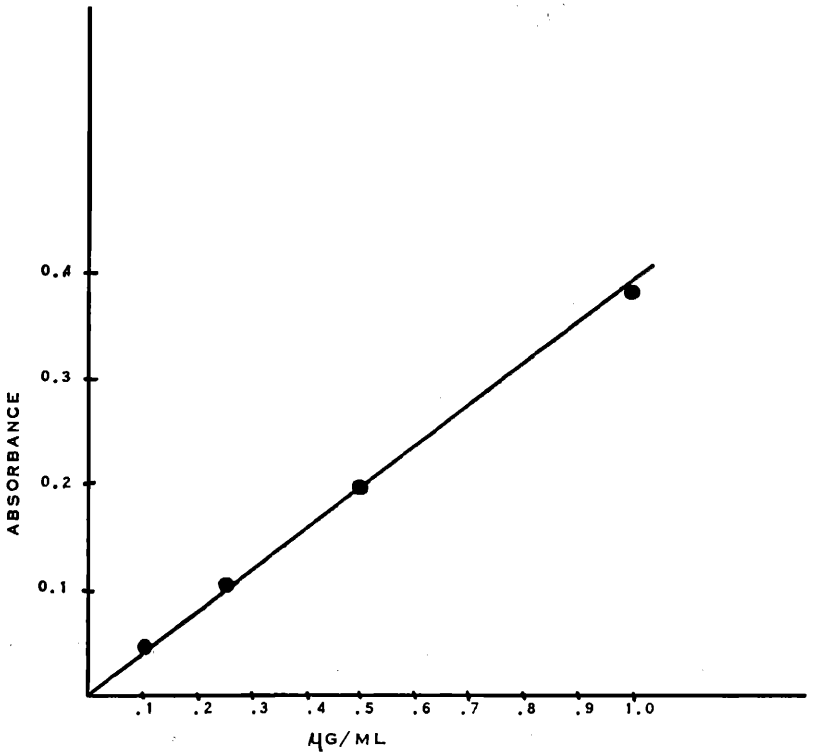

Figure 6. Concentration of PABA. Bratton-Marshall reaction 540 nm

Table VI
Statistical Comparison of Individual Skin Strips of PABA Sunscreen With and Without Protein: Paired T-Tests

	Strip Number[a]					Cumulative	
Values	1	2	3	4	5	[1 + 2]	[3 + 4 + 5]
t =	−0.86	−1.05	1.11	3.56	3.09	−0.95	2.35
p =	–	–	–	.05	.05	–	.10

[a]All strips are for 4 differentials and are comparisons of PABA in alcohol and United State's PABA in alcohol with protein adjuvant.

tion is that absorption was maximum and analytical analysis of the strips represents the strata of drug deposited, which remains in their respective skin tissue, and which would offer considerable advantages over nonprotein containing formulations from a protective standpoint.

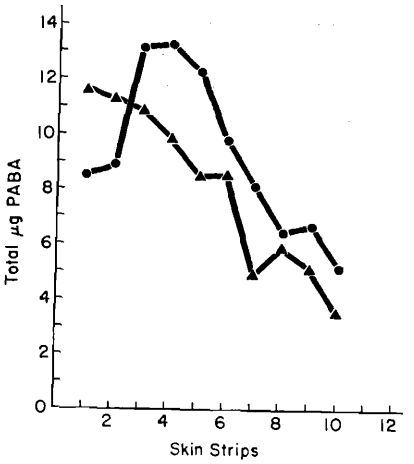

Figure 7. Comparison of PABA sunscreen in alcohol vehicle, with and without protein adjuvant: (●) with protein; (▶) without protein. Statistics: T–test for strips 4, 5, with protein: (4) $T = 3.56$, $p < .05$; (5) $T = 3.09$, $p < .05$. Note: significance at such high levels for small sample numbers lends credence to efficacy of addition of protein to vehicle

VI. *Discussion and Conclusion*

As can be seen from the data presented, the addition of a protein material to PABA, a known effective sunscreen agent, appears to improve the penetration and adsorption. We have yet to evaluate whether, indeed, this compound in combination with the protein vehicle does produce a greater effectiveness as a sunscreen agent under standard clinical usage conditions.

We have presented an *in vitro* and *in vivo* laboratory approach of a typical sunscreen program in which one first selects the compound to be studied, as well as possible vehicle desired, by doing spectrophotometric adsorption studies *in vitro*. These latter studies are very rapid and can be useful in selecting the agent of choice. Second, we proceeded with some formulations applied to an animal model in which we evaluated, using an artificial light condition, the effectiveness of the compound to protect the uv-induced dermal erythema, both under unrinsed and rinsed conditions. Third, we evaluated a penetrability/substantivity study, based on earlier data of animal studies, which indicated that PABA may be substantive to skin. We investigated this by using a tape-stripping technique incorporating the Bratton-Marshall method for primary amines with a spectrophotometric method for the quantitative analysis of the sunscreen agents from Blenderm tape strip skin. Our findings supported the adjuvant effect of protein initially seen in our rabbit study and substantially confirmed in our clinical situation. Under the conditions of the experiments, protein appears to have properties, which allow significantly larger

quantities of drugs to be retained near the epidermal surface. The test methodology presented represents a typical screening method for sunscreen evaluation and, hopefully, will lead to better agents which will meet the criteria of an ideal sunscreen formulation.

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