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Chronic Disabling Dermatoses

ANNUAL PROGRESS REPORT

by

Albert M. Kligman, M.D.

September 1968
(For the period 1 March 1967 to 30 June 1968)

U. S. Army Medical Research and Development Command

Washington, D. C. 20315

Annual Report to the Commission on Cutaneous Diseases
of the Armed Forces Epidemiological Board

Contract No. DA-49-193-MD-2137

University of Pennsylvania

Philadelphia, Pennsylvania 19104

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Annual Progress Report

Chronic Disabling Dermatoses

Period covered by the report: 1 March 1967 to 30 June 1968

Responsible investigator(s) and name of institution:

Albert M. Kligman, M. D., Ph. D.
University of Pennsylvania School of Medicine

Contract Number: DA-49-193-MD-2137

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Annual report to the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board

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ABSTRACT

1. Preparing Institution: University of Pennsylvania School of Medicine

2. Title of report: Chronic Disabling Dermatoses

3. Principal investigator(s):
   Albert M. Kligman, M. D., Ph. D.

4. Number of pages: 21
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   Annual Report to the Commission on Cutaneous Diseases of
   the Armed Forces Epidemiological Board

Summary

Our investigations have centered around two increasingly significant sources of chronic dermatitis, namely:

(1) bacterial infections
(2) photosensitivity reactions

In both areas we have been obliged to develop suitable experimental models and quantitative methods of assay. The ability to establish these disorders experimentally enables us to investigate the factors which contribute to oathogenesis as well as to assess the effectiveness of therapy.

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INTRODUCTION

Our investigations have centered around two increasingly significant sources of chronic dermatitis, namely, bacterial infections and photosensitivity reactions. In both areas we have been obliged to develop suitable experimental models and quantitative methods of assay. The ability to establish these disorders experimentally enables us to investigate the factors which contribute to pathogenesis as well as to assess the effectiveness of therapy.

I. Investigations into the Normal Bacterial Flora of Human Skin

Because of the realization that the normal and abnormal flora of the skin is not as simple as was previously believed[1] and that many strains previously regarded as non-pathogenic have been shown to produce or exacerbate disease under abnormal circumstances[2] it has become necessary to investigate and classify the organisms found on human skin in greater detail. Great impetus to this trend was given by the publications of Baird Parker[3] which established new criteria for classifying the cocci. Epidemiologic study will be greatly enhanced by this refinement in the taxonomy of these organisms; for example, it is now appreciated that Micrococcus type 3 is associated with coagulase negative coccal urinary infections.[4] We have applied the Baird Parker classification to 554 isolates of 339 strains of catalase positive cocci from normal human skin. Each isolate was chosen because of a difference in colonial morphology or pigmentation; for many strains multiple isolated colonies were subcultured. It was found that while colonies morphologically dissimilar or primary plates were often identical both morphologically and biochemically on subculture, the reverse was very rarely true.

The breakdown of the strains by site and by Baird Parker groups is shown in Table I.

TABLE I

Catalase positive cocci from normal human skin

	Scalp	Forehead	Axilla	Arm	Foot	All areas
Staphyloccus						
I	0	4	6	1	0	4
II	22	68	48	4	6	148
III	0	0	0	0	1	1
IV	2	2	0	1	0	1
V	2	4	11	0	3	20
VI	2	2	2	2	2	10
Micrococcus						
1	5	4	13	0	0	22
2	0	5	21	1	4	31
3	7	18	3	2	1	31
4	0	0	0	0	0	0
5	0	4	3	1	2	10
6	3	0	0	3	4	10
7	3	10	9	10	2	34
x	0	3	1	0	2	6
	46	124	117	25	27	339

It can be seen that Staphylococcus I (S. aureus) can be recovered from exposed areas as expected; S. II (S. epidermidis) is commonly recovered while other Staphylococcal groups are less regularly found. In the Micrococci types 1 and 2 are more often found in the axilla than elsewhere while M. 3 appears to be more common on the scalp and forehead. This suggests that mild pyodermas due to this type may be significantly more common than previously realized. The group labelled here M. x. does not fit the published classification. It is evident that different cocci inhabit different areas and because the interaction of the resident cocci with more pathogenic strains is now recognized as a protective factor in skin ecology, we intend to follow changes in the coccal flora in detail when systemic antibiotics are administered.

The diphtheroids, the other major component of the flora of the skin, are more difficult to classify. While C. acnes is implicated in endocarditis, septicemia and acne and C. minutissimum is associated with erythrasma[5], this group of organisms is basically of only low pathogenicity. However, their ecologic importance in preventing the establishment of virulent strains is being increasingly recognized. We have attempted to

characterize the common strains biochemically and have studied 85 isolates in detail. The significance of lipid dependence has been confirmed and the possibility of identification of ecologically significant groups attempted. Our preliminary findings indicate that at least two groups of lipophilic diphtheroids exist, that other diphtheroids may be lipophilic to a lesser degree, that T. C. 199 fluorescence is helpful and that lipid independent forms exist. Our approach has been based on the belief that some biochemical tests are valid only when growth supplements are present and that many of the carbohydrate based tests applicable to cocci are inadequate. A summary of our findings is listed in Table II.

TABLE II

Reactions of skin diphtheroids

	Lipo I	Lipo II	C xerosis	C minutissimum	Nonlipophilic I	Nonlipophilic II	Nonlipophilic III
Oxidase	(+)	(+)	-	+	d	d	d
Phosphatase	(+)	(+)	+	+	d	-	+
Urease	-	d	+	-	-	d	+
NO_3 Red.	-	-	-	d	-	-	d
V. P.	d	d	+	d	-	(+)	-
Fluor.	-	-	-	++	-	-	+
G.	(d)	d	+	+	d	d	-
L.	-	-	-	-	d	-	-
Maltose	-	-	+	d	-	-	-
Mannitol	-	-	-	-	-	-	-
Sucrose	(d)	-	-	d	d	-	-
Unsat.	+	+	+	+	+	+	+
Sat.	-	-	+	+	+	+	d
Growth on tween 20	-	-	-	d	+	-	-
Enhance by tween 80	++	++	+	-	-	+	-
Split tweens	+	-	-	+	-	d	d

( ) present on tween 80 only

II. Acne Studies

A. Gram Negative Folliculitis

We have delineated a new syndrome in patients attending an acne clinic.[6] The patients were older than the usual acne age group and had received many courses of broad spectrum antibiotics without effect. The lesions were histologically a folliculitis which, however, was not associated with comedones as in typical acne vulgaris. In some,

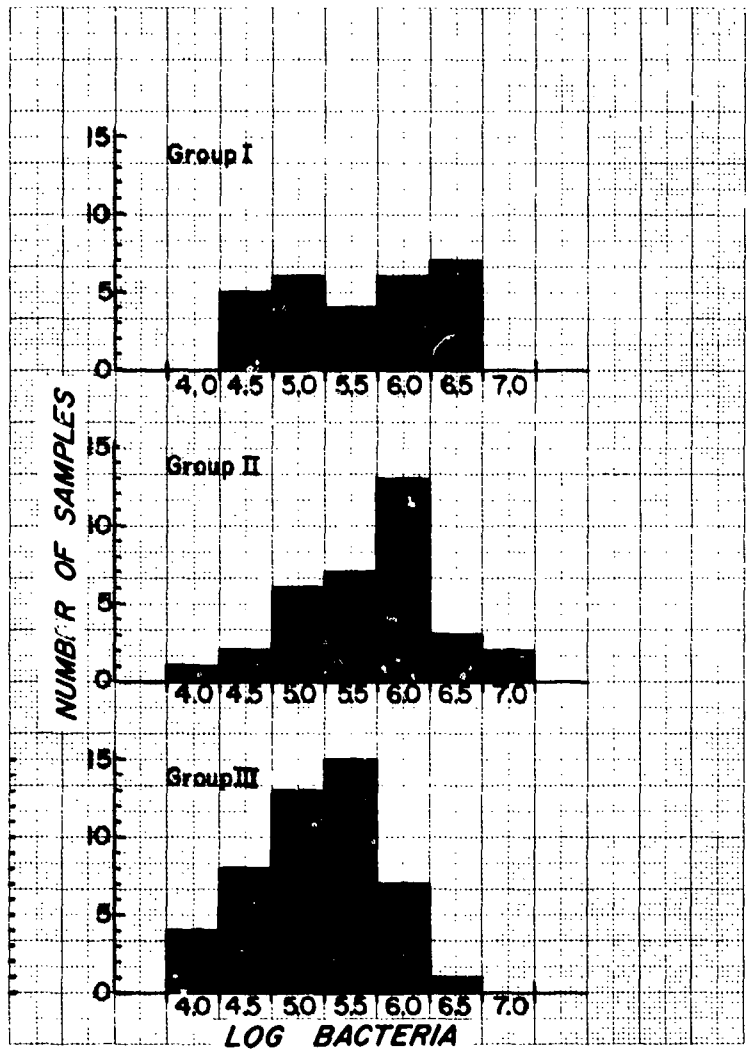
torpid, persistent nodules formed. The infecting organisms were gram-negative rods which could also be recovered from the nasal vestibule. Enterobacter, Proteus and Serratia marcescens were the usual ones. Conventional antibiotics are ineffective in this syndrome, hence, clinicians need to know how to differentiate this disorder from ordinary acne. A relationship with tropical acne is suggested.

B. The Effect of Antibiotics on the Nasal Flora

The enterobacteria and other gram negative rods are being increasingly implicated as pathogens, undoubtedly as a result of the wide spread use of antibiotics which suppress gram positive microflora. We studied the nasal flora in three groups of patients. 1) Acne patients receiving broad spectrum antibiotics; 2) acne patients not so treated over the last twelve months and 3) non-acne patients not receiving antibiotics. A total of 55 patients, 35 males and 20 females, were sampled bilaterally and samples studied quantitatively.

The total aerobic counts were distributed as shown in Figure 1. With the

Figure 1



30 Squares to the inch

method used the counts cluster between 10^5 and $10^{5.5}$ organisms. The untreated acne patients appear to carry a larger number of organisms than the control group while the distribution is flattened in the antibiotic treated group. The anaerobic C acnes did not outnumber the aerobic flora.

The composition of the nasal flora differed markedly when the treated group was compared with the two non-treatment groups. The incidence figures are shown in Table III. In the control group the flora was dominated (more than 50%) by diphtheroids

TABLE III

Incidence of Bacteria in Nasal Swabs

Bacterial group Aerobic	I	%	II	%	C	%	All Samples	%
Total samples	28		34		48		110	
S. aureus	4	14.3	12	35.3	25	52.1	41	37.3
Coag. neg. cocci	28	100	34	100	48	100	110	100
Streptococci	0	0	2	5.9	5	10.4	7	6.4
Lipophilic diph.	21	75.0	34	100	45	93.8	100	90.9
Other aerobic diph.	3	10.7	6	17.6	2	4.2	11	10.0
Enterobacteria	20	71.4	14	41.2	19	39.6	53	48.2
Pityrosporum	3	10.7	3	8.8	2	4.2	8	7.3
Other Yeasts	2	7.3	1	2.9	3	6.3	6	5.5
Anaerobic Samples	18		18		52		78	
C. acnes	15	83.3	13	72.2	49	94.2	67	85.9

in 38%, by coagulase negative cocci in 35% and by S. aureus in 17%. Enterobacteria were recovered from 40% of the samples. In the acne group not receiving antibiotics the flora was dominated by diphtheroids in 40%, by coagulase negative cocci in 47% and by S. aureus in 3%. Enterobacterial incidence was 41%. In the antibiotic-treated group, however, diphtheroid dominance was found in only 14%; S. aureus was never dominant and showed a lower incidence. Coagulase negative cocci dominated the flora in the other 86%. The incidence of enterobacteria rose to 71%.

These findings resemble the findings in the axilla under systemic treatment with dimethylchlortetracycline.[1] The effects of antibiotic administration are to depress the diphtheroid component of the flora and to select resistant coagulase negative cocci. While enterobacteria are also selected, overgrowth does not occur probably because of interactions with the cocci. The virtual elimination of S. aureus after treatment is unexpected and is probably due to the low incidence of resistant strains in this group of patients.

We have tentatively concluded that:

1. The nose is a likely source of secondary infection with enterobacteria particularly after antibiotic treatment.
2. The ecological determinant may be the density of lipophilic diphtheroids.
3. S. aureus and enterobacteria tend to be inversely related; carriage of S. aureus is associated with a low incidence of nasal gram negatives. The latter enter more readily when S. aureus is abolished.

C. Suppression of C. acnes in Sebaceous Follicles and Comedones

For an agent to affect the bacterial component of an acne comedone it is necessary for the agent to penetrate deeply into the follicular recess. If it is claimed that antibacterial substances are therapeutically effective, one might expect such drugs, whether topical or oral, to reduce the infra-follicular population of resident organisms, mainly C. acnes. By expressing the cheesy material from the follicles of the alae nasi by pressure, collecting the material using an empty gelatin capsule and after weighing, homogenizing the material in 5 ml. of Triton X-100 solution, it is possible to determine the number of viable C. acnes cells per milligram of such material. This is a crude yet reproducible method of sampling open sebaceous follicles in an area where anaerobic diphtheroids greatly outnumber the aerobic flora.

In preliminary studies we have sampled 22 subjects bilaterally 64 times. Individuals differ markedly in the amount of sebaceous material which can be collected but are reasonably consistent in one-week collections. The recovery of C. acnes from 51 untreated samples ranged from 820,000 to 58,000,000 with a geometric mean of 9,960,000 and a median value of 9,230,000 organisms per milligram.

The effect of a halogenated salicylanilid carbanalid mixture on the counts is shown in Table IV. The expected reduction in the aerobic flora was seen but the anaerobic counts are unaffected.

TABLE IV

Effect of topical antibacterials
Count per milligram

Subject		Pretreatment		Post treatment	
		Aerobic	Anaerobic	Aerobic	Anaerobic
A	L	20,300	4.7×10^6	2,170	7.83×10^6
	R	80,000	4.7×10^6	2,050	2.21×10^6
B	L	93,000	16.1×10^6	16,800	4.94×10^6
	R	51,700	6.0×10^6	2,180	5.42×10^6
C	L	28,800	13.2×10^6	200	3.45×10^6
	R	19,400	10.1×10^6	200	1.38×10^6

In subjects treated with 600 mg. demethylchlortetracycline daily for three weeks (Table V) there appears to be a real but small reduction in the number of C. acnes per milligram; the aerobic population has returned because of the development of resistance.

TABLE V

Sebaceous Material from ala nasi
Bacteria per milligram

		Pretreatment		3 weeks Declomycin	
		Aerobic	Anaerobic	Aerobic	Anaerobic
1	L	13,000	9.0×10^6	26,000	1.8×10^6
	R	52,000	14.4×10^6	14,000	2.8×10^6
2	L	9,400	8.8×10^6	11,500	14.0×10^6
	R	4,200	9.2×10^6	17,000	4.8×10^6
3	L	55,000	36.7×10^6	69,000	18.0×10^6
	R	155,000	42.0×10^6	91,000	16.0×10^6
4	L	16,000	4.0×10^6	103,000	1.2×10^6
	R	23,000	8.3×10^6	89,000	4.6×10^6
5			6.3×10^6		3.8×10^6
6			10.9×10^6		4.0×10^6
7			1.8×10^6		0.5×10^6

III. Antibacterial Agents

A. Substantivity

The problem of measuring substantivity, the persistence of an antibacterial effect after the end of a treatment period, is a requirement in comparing different antibacterials. Quantitative bacteriological studies using the axilla, a site normally supporting a high population, as the test site and following the recovery of the population after a course of treatment does permit this assessment. Figure 2 compares the recovery after intense washing with a non-poisonous soap to the recovery after six days usage of a hexachlorophene liquid soap. The prolonged reduction in numbers is evident.

Figure 2

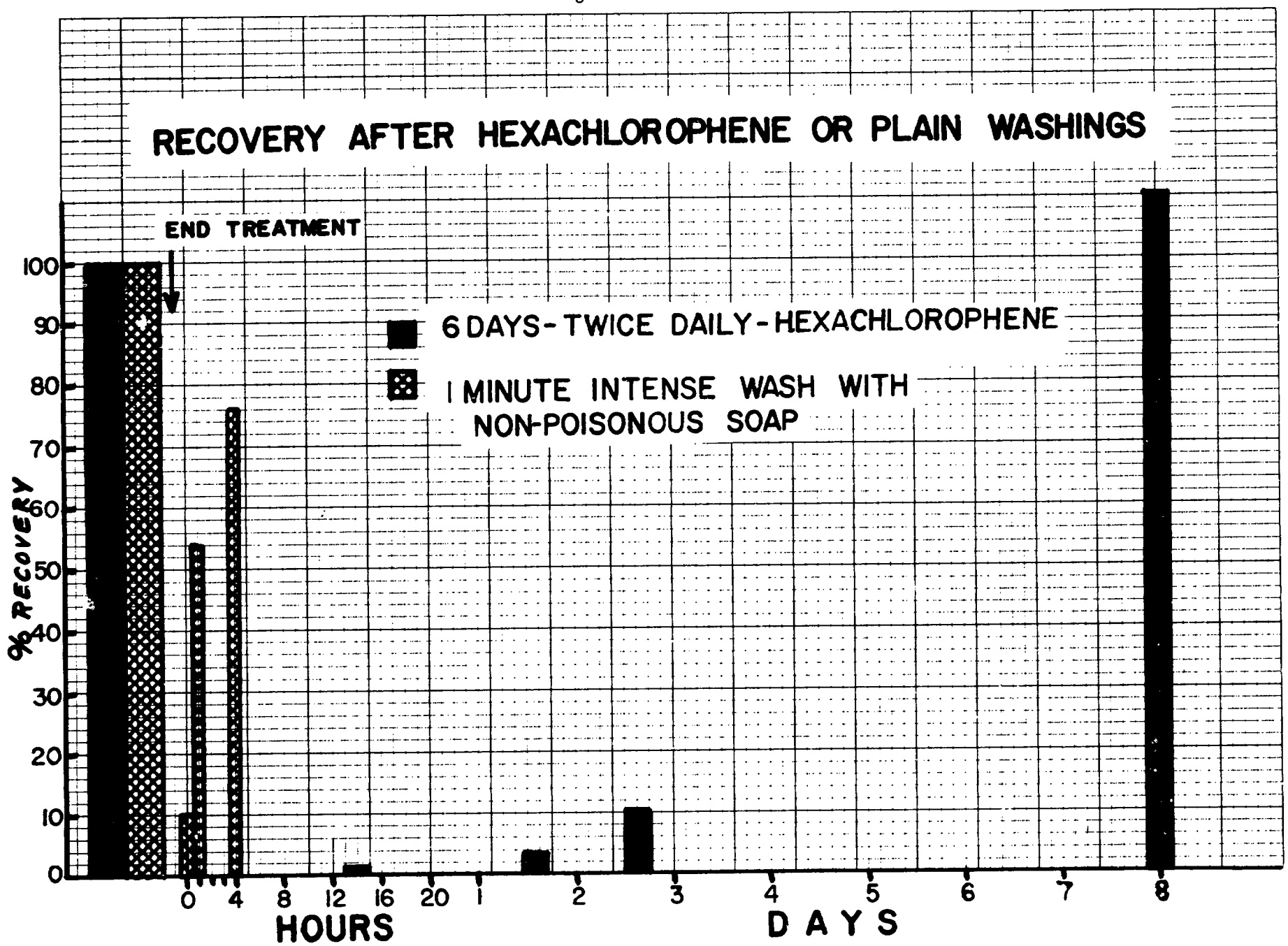
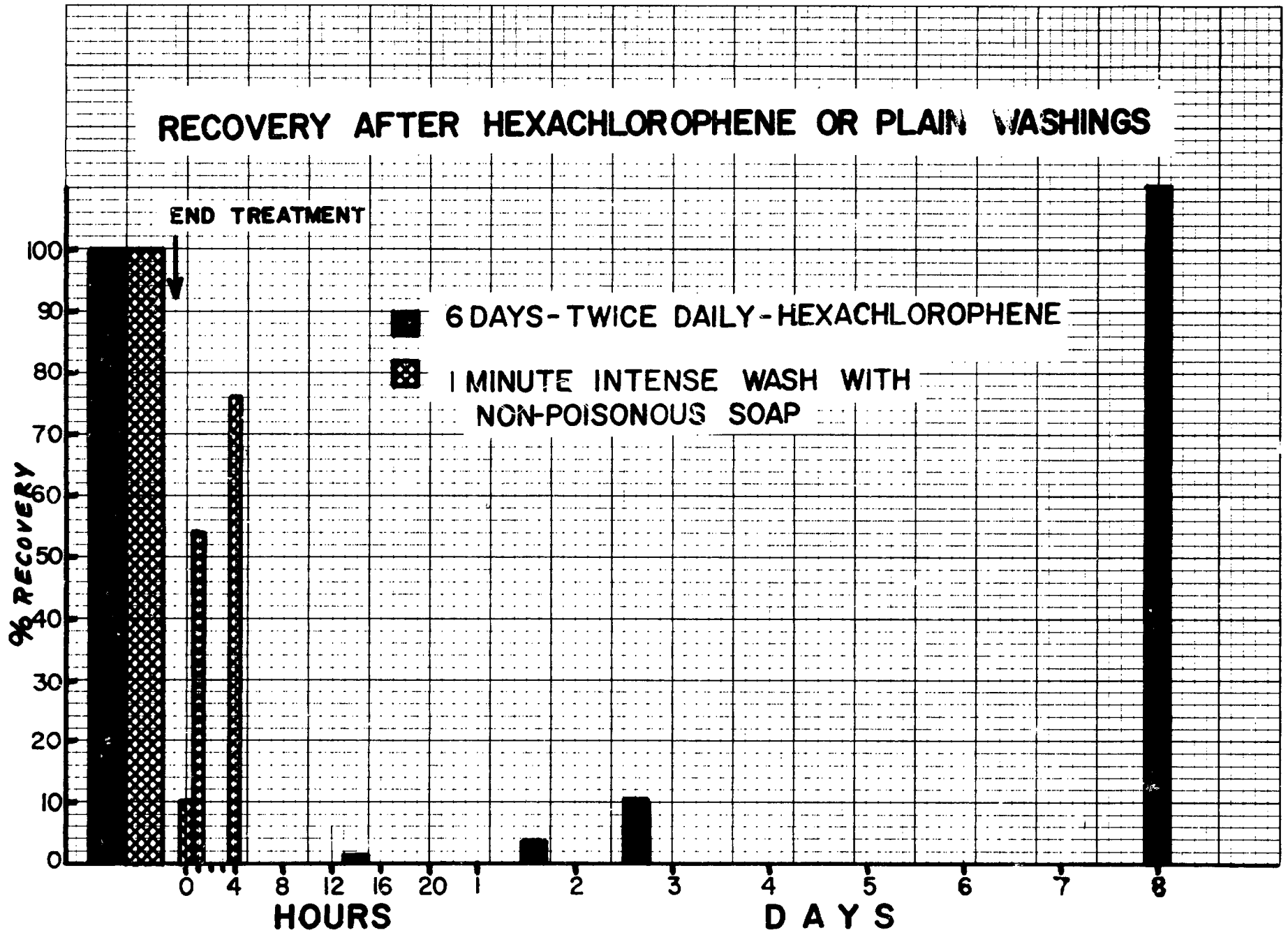

RECOVERY AFTER HEXACHLOROPHENE OR PLAIN WASHINGS
END TREATMENT
6 DAYS-TWICE DAILY-HEXACHLOROPHENE
1 MINUTE INTENSE WASH WITH NON-POISONOUS SOAP
% RECOVERY
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
1
2
3
4
5
6
7
8
HOURS
DAYS

Figure 2


RECOVERY AFTER HEXACHLOROPHENE OR PLAIN WASHINGS
END TREATMENT
6 DAYS - TWICE DAILY - HEXACHLOROPHENE
1 MINUTE INTENSE WASH WITH NON-POISONOUS SOAP
% RECOVERY
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
1
2
3
4
5
6
7
8
HOURS
DAYS

B. Penetration

We have developed an in vitro diffusion chamber technique using prepared whole skin for investigating the effects of vehicles on penetration of antibacterial agents. The test material is placed in the upper chamber which is separated from a saline containing lower chamber by a sheet of whole skin. After 24 hours incubation the skin is removed and the epidermis removed by dry heat at 60° for 2 minutes. Discs 6 mm. in diameter are cut from the dermis and placed on seeded bacterial plates. The zone sizes around the discs after incubation are measured. In preliminary studies we have found 70% alcohol to be superior to ethylene glycol monomethyl ether and propylene glycol, all of which are superior to water at pH 10 when used as vehicles for 1% hexachlorophene or tetrachlorsalicylanilide. Similarly, the penetration of antibiotics can be measured. In our early trials ampicillin, penicillin O and tetracycline penetrate to the dermis while chloramphenicol, streptomycin and penicillin G appear not to do so. Further studies are in progress.

IV. Drug Photosensitivity

The interaction between drugs and sunlight to create adverse reactions is no longer a clinical oddity. Within the last decade or so, an extraordinary variety and ever growing list of drugs have been shown to be capable of producing photoallergic and phototoxic reactions. The potentiality for photosensitization was scarcely foreseen and was usually appreciated only after extensive use. Some of the most frequently used drugs have turned out to be photosensitizers, including antibiotics, sedatives, tranquilizers, germicides and fungicides. The very prevalence of such reactions has stimulated the desire to know more about them and to find improved means of prevention and treatment.

We have particularly concerned ourselves with developing methods for identifying and rating the photosensitizing potentialities of new agents. Apart from such practical needs, we have begun an investigation of the basic mechanisms underlying phototoxic and photoallergic reactions.

A. Identification of Phototoxic Drugs by Human Assay

While initial screening for phototoxicity is feasible in hairless mice and guinea pigs, final evaluation should utilize the human subject because of species disparities. Weaker photosensitizers such as chlorothiazide and tolbutamide may be missed when such agents are parenterally administered to laboratory animals. We have designed standard, reliable procedures for detecting phototoxicity and estimating the clinical hazard.

We utilize window-glass filtered xenon-arc, solar-simulating radiation as a source of high intensity long ultra-violet light. We have corroborated the findings of previous workers that the major action spectrum lies in the near ultra-violet beyond 320 nm, extending slightly into the visible. Indeed, when radiation below 350 nm was eliminated by interposing the Schott WGI, 3mm filter, effectiveness was not diminished. When all ultra-violet light below 400 nm was filtered out, brisk reactions could still be elicited provided the exposure was increased by a factor of about 10.

We selected well known drugs for study: tetracycline congeners, phenothiazine congeners, sulfonamides, chlorothiazide, griseofulvin and tolbutamide. These were given orally, intradermally, and topically. The standard exposure contained long ultra-violet light which was about the equivalent of 1 hour summer, midday sunlight at 45° N. L.

Local administration, either by intradermal injection or topically, is especially appealing as a testing method since a small quantity of the drug may be adequate

and many tests can be accomplished on the same individual without encountering systemic toxicity. For intradermal injection, a suitable screening concentration was 0.1% in saline; insoluble agents could be conveniently handled by suspending them in a solution containing 0.1% Triton-X 100, a non-ionic surfactant. Once phototoxicity was demonstrated, the minimum phototoxic dose could be determined by simple titration, thus allowing an estimate of potency. The results with intradermal testing are shown in Table VI.

TABLE VI

Minimum Phototoxic Concentrations After Intradermal Injection

Drug	MPD (%)*
Demethylchlortetracycline	0.0016
Chlortetracycline	0.031
Tetracycline HCl	0.031
Methacycline HCl	non-phototoxic
Minocycline	non-phototoxic
Chlorothiazide	0.125
Chlorpromazine	0.0004
Promethazine	0.0008
Sulfisoxizole	0.125
Sulfanilamide	0.125
Griseofulvin	0.125
Tolbutamide	0.063

* The MPD is the least concentration giving a positive reaction in 3 of 4 subjects.

Several findings are of particular interest. The reactions often began immediately after irradiation and were usually at peak within 2-6 hours. This emphasized the necessity of making early readings instead of twenty-four hours. Moreover, irradiation immediately after injection instead of delaying 2 to 24 hours was not only optimal but absolutely imperative for some drugs such as chlorothiazide and sulfonamides. This could be an important source of false negative reactions.

Specificity of the test was verified by showing that drugs to which no suspicion of phototoxicity attaches do not yield reactions; viz., neomycin, penicillin, nickel salts, oleic acid, chloramphenicol, testosterone and others. To date, we have encountered neither false positive nor false negative reactions. It is interesting in this regard that whereas all the well known tetracyclines were found to be phototoxic, two new derivatives, methacycline and minocycline, were not. This demonstrates how a chemical can be tailored to exclude an undesirable property. This also demonstrates the

usefulness of the test as a screening procedure, for deliberate sunlight exposure of individuals taking these drugs is not attended by significant phototoxicity.

We were able to simplify the test still further by applying the test agents topically. The same sensitivity and specificity as for intradermal injection was demonstrated (Table VII). The test agents were suspended in petrolatum at 5-10% concentrations.

TABLE VII

Phototoxic Responses on Normal and Stripped Skin Treated with 10% Concentrations of the Drugs for 1 and 24 Hours Before Irradiation

Drug	No. Subj.	Positive Reactions			
		Normal Skin		Stripped Skin	
		1 hr.	24 hrs.	1 hr.	24 hrs.
demethylchlortetracycline	6	4	0	6	0
chlorpromazine	5	5	5	5	5
promethazine	4	4	4	4	4
sulfanilamide	4	4	4	4	4
griseofulvin	4	0	0	4	4
tolbutamide	4	0	0	4	4
chlorothiazide	4	3	4	4	4

With some drugs, griseofulvin and tolbutamide, it was necessary to scotch tape strip the skin first, evidently because of poor penetrability. Irradiating 30-60 minutes after application was optimal. Knowledge of such factors may be crucial; for example, irradiating a twenty-four hour application site may give a false negative response in the case of dimethylchlortetracycline.

Finally, we administered certain of these phototoxic drugs orally in order to appraise the hazard of actual clinical usage. We had become aware that the minimum phototoxic doses established intradermally did not adequately discriminate among drugs of different phototoxic potentiality. For example, tetracycline hydrochloride was almost as potent as demethylchlortetracycline, although clinical instances of phototoxicity to the former are decidedly uncommon. As it turned out, oral testing was sufficiently discriminating; we could achieve dose dependent reactions to oral dimethylchlortetracycline but could not show any phototoxicity even with 4 grams of tetracycline hydrochloride daily.

These experiences have led to well-defined guidelines in identifying phototoxic drugs and appraising the hazards attending their use.

The first step is topical application to disclose whether the drug possesses phototoxic capabilities. The agent is formulated in 5 to 10% concentration in petrolatum and applied to the stripped skin of five subjects (white) for one hour, followed by irradiation with long U. V. As a source of high intensity long U. V., we have found the quartz-iodine lamp to be an effective substitute for the Xenon lamp. (Table VIII) It is considerably cheaper and less subject to technical troubles. The control is an unirradiated, drug treated, stripped site. If phototoxicity is demonstrated one can proceed directly to oral

TABLE 8

Oral Phototoxicity Testing With Different Light Sources

Drug	Daily Dose (mg.)	Xenon-Mylar Radiation (>310 nm)		Sunburn Radiation (290-310 nm)		Xenon-Solar Radiation (290->800 nm)		Black Light (320-400 nm)	
		No. subj.	Pos. Reactions	No. subj.	Pos. Reactions	No. subj.	Pos. Reactions	No. subj.	Pos. Reactions
Chlortetracycline	2,000	15	0	15	0	15	0	8	0
"	3,000	8	0	--	--	8	0	6	0
Tetracycline HCl	2,000	15	0	15	0	15	0	10	0
"	4,000	7	0	5	0	5	0	5	0
Demethylchlortetracycline	600	26	4	11	0	18	3	7	0
"	1,200	31	13	14	0	14	2	11	0
Chlorpromazine	400	8	2	8	0	8	0	8	0
"	800	11	9	10	0	11	4	8	0

testing. Intradermal testing will be superfluous in most instances though it may be used for confirmation.

The drug may then be given orally for a minimum of five days using a panel of ten white subjects. Both a normal and stripped site should be irradiated. If twice normal doses do not elicit reactions, it seems reasonable to assume that the phototoxic potentiality is low or absent.

B. Diagnosis of Photosensitization Reactions by the Scotch Tape Provocative Patch Test

We have previously shown that the sensitivity of the contact allergy patch test could be increased by methods which weakened or eliminated the horny layer barrier; viz., anionic surfactants and scotch tape stripping. This same device has also proved very useful in eliminating false negative photosensitivity reactions. Stripping the skin was found useful in identifying and rating phototoxic drugs, whether the test agent was given topically or orally (see above). For example, when dimethylchlortetracycline was given orally in twice normal dosage (1200 mg. daily), three of twelve subjects showed phototoxicity on normal skin, but ten of twelve were positive in stripped skin. Stripping enhances the penetration of light and increases the local concentration of the drug in the target tissue. With normal doses of 600 mg. daily, stripping is obligatory to disclose phototoxicity in a small panel of volunteers.

Provocative photopatch testing may also have some application in photocontact allergy. One example of enhanced sensitivity may be given. Investigators have found that patients photoallergic to salicylanilides generally do not cross react to hexachlorophene. In subjects strongly sensitized to tribromosalicylanide and bithionol, we found that only four of ten and two of ten reacted to hexachlorophene respectively on normal skin. Every subject gave a positive photopatch test when the agent was applied to stripped skin.

C. The Mechanism of the Persistent Light Reactor

We believe we have helped solve a peculiar problem which has greatly puzzled students of photoallergic contact dermatitis, namely the persistence of abnormal reactions to sunlight many months or even a year or more after all known contact has ceased. The usual cause of photosensitization in bacteriostatic substances incorporated in soaps. An extremely persistent photodermatitis can be almost ruinous to the afflicted person who must literally live in the shadows.

Although exotic theories have been proposed, we found the explanation extremely simple. The photoallergen simply remains in the skin for astonishingly long times. It is necessary to know that in strongly sensitized subjects, microgram amounts of the photosensitizing chemical and a few seconds light exposure are sufficient to excite a response.

We induced photocontact sensitization in prisoner volunteers to various salicylanilides, bithionol and hexachlorophene. In the first study, these agents were applied to sensitized subjects without exposure to light for three days. The site was then kept continuously light-sealed for periods up to ten weeks; at intervals, sites were uncovered and irradiated with window glass filtered Xenon arc radiation. Surprisingly, all subjects exhibited positive photopatch test reactions throughout and including the final ten week test period. Moreover, the intensity of the reaction did not greatly diminish during this time.

Next, we compared the duration of the dermatitis at photoallergic reaction sites which were either covered or continuously exposed after elicitation. The

subjects were studied in wintertime and had no exposure to direct sunlight; fluorescent bulbs constituted the lighting system in the prison where these studies were done. In covered sites the reaction generally subsided by 10-15 days. We found it extraordinary that the dermatitis remained clinically active for 26 weeks in three of twelve subjects. A dermatitis was evident in a majority up to ten weeks. This experience showed that diffuse, low energy winter room light was actually capable of maintaining a photodermatitis for many months in highly sensitized subjects. We subsequently showed that a photosensitization reaction could be elicited with less than one second of Xenon radiation.

A punch biopsy specimen of skin was removed from sites which had been active for ten to 24 weeks. The minced tissue was extracted with ethanol and examined in various ways for the presence of the photosensitizer. Firstly, the extracts fluoresced brightly under the Wood's Light; whereas, control tissue did not. By photofluorometry we found the excitation peaks to be identical with the authentic drugs that had been used to photosensitize. Absorbance curves of the extracts conformed closely to the authentic chemicals. Finally, the application of the extract to a photosensitized subject resulted in a positive photopatch test allergic reaction. Thus, the photosensitizer seems to reside in the tissue for as long as the reaction endures. Since epidermal turnover is complete in a month, we have concluded that the dermis must be the reservoir for the photosensitizer. These agents are notoriously substantive to skin. Evidently, small amounts can persist indefinitely.

We obtained an insight into another puzzling feature of these patients, namely, their heightened sensitivity to sunburn as manifested by low MED's. We used the fluorescent FS-20 Sunlamp to determine MED's in photosensitized subjects using bacteriostatic soaps to wash their forearms. It did appear that the MED was considerably lower than on the opposite forearm washed with plain soap. However, closer study showed this to be specious; the histologic picture was not that of sunburn but of photoallergic contact dermatitis which at threshold levels cannot be clinically distinguished from sunburn. By spectral analysis, we found that the FS-20 Sunlamp has two strong line emissions at 365 and 405 in addition to continuous energy in the erythemic range. Thus, long U. V. photoactivating rays are present in such lamps, a fact which seems generally not to be recognized, not even by the manufacturers. Because the photosensitizer is universally distributed over the skin surface by the very act of washing, phototesting anywhere with light containing unsuspected long U. V. will elicit photoallergic reactions which will be misinterpreted as sunburn.

V. The Mechanism of Photoallergic Contact Dermatitis

As a result of an extensive experimental analysis we believe we now have a satisfactory explanation for the mode of action of photocontact sensitizers; the phenomenon turns out to be less mysterious than supposed.

Investigators agree that photocontact sensitization resembles contact allergy clinically and histologically. Our studies lead to the conclusion that the two are in fact indistinguishable. The sole role of light is to transform the original sensitizer into a more contact allergen; this derived product can fully reproduce the reaction in the absence of light.

Using solar-simulating radiation from the Xenon arc lamp we photosensitized volunteers to the bacteriostatic chemicals which have proved to be photosensitizers when incorporated in soap and toiletries. These included: tetrachlorosalicylanilide (TCSA), tribromosalicylanilide (TBS), bithionol, hexachlorophene and trichlorocarbanilide (TCC).

A. Histologic and Clinical Study

Reactions were elicited by photopatch testing sensitized subjects with Xenon radiation filtered through window glass to eliminate erythematogenic rays. Biopsies were examined at 24, 48 and 72 hours. The histologic patterning of both the mild and severe reaction was entirely typical of contact allergy: intra-epidermal vesicles, lymphocytic exocytosis and peri-venular cuffing with mononuclear cells. The evolution of the response beginning with round cell infiltration was identical with contact allergy. Similarly, clinical observations of the eczematous reactions revealed no feature which was different from ordinary contact allergy.

B. Patch Testing with Irradiated Chemicals

According to our hypothesis, light acts solely on the chemical and not on the skin. The phototransformation should just as readily take place *in vitro*. Alcoholic solutions of the photosensitizers were irradiated in quartz curettes with solar simulating radiation. Closed patch testing with these solutions uniformly induced typical reactions in the absence of light; the unirradiated control solution had no effect whatever.

C. Patch Testing with Authentic Photodecomposition Products

Coxon et al.[7] have identified the products which formed when halogenated salicylanilides were irradiated. Halogens on the salicyl ring were found to be successively replaced by hydrogen atoms through free radical formation. Thus, tribromosalicylanilide was first transformed to dibromosalicylanilide; this was then converted to monobromosalicylanide. The same sequence obtains for TCSA, chloride atoms being successively lost in this case.

We obtained these photodecomposition products and performed closed patch tests (no light) in TCSA and TBS sensitized subjects. Typical reactions were regularly elicited. We felt it to be significant that the reaction was increasingly more severe with each additional loss of a halogen. This seemed to mean that progressive photodecomposition created increasingly more potent allergens.

D. Contact Sensitizing Capabilities of Photodecomposition Products

The last idea was tested by evaluating the contact sensitizing powers of the photodecomposition products using the maximization procedure developed in our laboratories. The results conformed to expectation: each further loss of a halogen atom was associated with a step-up in contact allergenicity. Thus, DBS sensitized more subjects than TBS and MBS sensitized more subjects than DBS.

E. Photopatch Reactions in Contact Sensitized Subjects

If the photodecomposition product can evoke a reaction without light, the reverse must also be true. In a contact sensitized subject, photopatch testing with the parent compound should elicit a response. This turned out to be so. In DBS contact sensitivity for example, photopatch tests with TBS gave equal reactions. The same relationship held when MBS sensitized subjects were photopatch tested with DBS, the parent compound. In MBS contact sensitivity irradiation did not intensify the reaction. This is instructive as well as a theoretical necessity since MBS is photostable and no photodecomposition product can arise from it. Light does not eliminate halogen atoms from the aniline ring of the salicylanilides.

In summary, our view is that photosensitizors are intrinsic contact allergens. Light creates products which are more potent sensitizers; that is to say, the photodecomposition products are haptens with more binding power for skin proteins. Moreover, there is

a good correlation between photocontact and contact sensitizing potentialities. Potent photosensitizors, such as TCSA, are powerful contact allergens. Conversely, hexachlorophene, a weak contact allergen, is a weak photosensitizer.

The maximization procedure permits us to identify and assess the potency of contact allergens. It is now possible to establish whether such substances are also capable of acting as photosensitizors.

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13. ABSTRACT

Our investigations have centered around two increasingly significant sources of chronic dermatitis, namely:

(1) bacterial infections
(2) photosensitivity reactions

In both areas we have been o. iged to develop suitable experimental models and quantitative methods of as ay. The ability to establish these disorders experimentally enables us t. investigate the factors which contribute to oathogenesis as well as to assess the effectiveness of therapy.

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