Use of the Triethanolammonium Salts of Several Alkylsulfuric Acids as Dermatologic Vehicles

By JAMES C. KING* and WILLIAM J. SHEFFIELD

The triethanolammonium salts of dodecyl-, tetradecyl-, hexadecyl-, and octadecylsulfuric acids were prepared. Ointment bases were formulated from varying proportions of the salts, the corresponding alcohols, and propylene glycol. The release of salicylic acid, resorcinol, sulfadiazine, iodochlorhydroxyquin, and sulfur from these bases, polyethylene glycol ointment, and white petrolatum was studied by use of tissue digest procedures and microscopic examination, using guinea pigs as the test animals.

DURING THE past two decades, a great deal of effort has been made to devise dermatologics free of greasiness by preparing emulsions and by using water-soluble bases, as exemplified by polyethylene glycol ointment. However, both of these groups of mixtures possess certain inadequacies. Emulsion bases contain large amounts of water which may evaporate under storage conditions and/or might affect the stability of the incorporated medicament. Conversely, the addition of the therapeutic agent may influence the stability of the emulsion. The polyethylene glycol ointments are not completely satisfactory, cosmetically, and they are potential complex formers with some types of medicaments.

In this investigation, formulation was directed toward the preparation of a mixture miscible with both water and oil so that it might be removed easily by washing, while at the same time possessing a measure of compatibility with the oils of the skin. Toward this goal, a group of four fusible triethanolammonium salts of dodecyl-, tetradecyl-, hexadecyl-, and octadecylsulfuric acids was synthesized. By adjusting the proportions of these salts and their corresponding alcohols, it was possible to adjust the physical characteristics to meet any requirement as to consistency, appearance, and melting behavior.

An indication of the usefulness of these bases was obtained by comparing the release of medication from ointments prepared from representatives of the experimental base, white petrolatum, and polyethylene glycol ointment, into which sulfur, resorcinol, salicylic acid, iodochlorhydroxyquin, and sulfadiazine were incorporated. Drug availability from the bases was determined by the employment of direct tissue stain techniques and colorimetric assays of tissue digests.

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EXPERIMENTAL

Preparation of the Triethanolammonium Alkylsulfates.—The triethanolammonium salts of dodecyl-, tetradecyl-, hexadecyl-, and octadecylsulfuric acids were prepared by a modification of the procedure described by Maurer *et al.* (1). The crude alkyl sulfuric acid was dissolved in carbon tetrachloride and triethanolamine added slowly, with stirring, until the solution was neutralized. An equal volume of acetone was added and the mixture cooled to −20° and filtered through a sintered-glass suction funnel. Repeated crystallizations from boiling acetone produced yields in the order of 50% (Table I).

Preparation of the Base Mixtures.—Tests indicated that bases with satisfactory consistency and texture could be prepared from the triethanolammonium salts with the addition of the corresponding alcohols and a minimal proportion of propylene glycol.

The following formulas are representative of the ointment bases formulated for this investigation.

BASE A

	Gm.
Dodecyl alcohol	4.75
Tetradecyl alcohol	4.75
Hexadecyl alcohol	4.75
Octadecyl alcohol	4.75
Triethanolammonium dodecyl sulfate	19.00
Triethanolammonium tetradecyl sulfate	19.00
Triethanolammonium hexadecyl sulfate	19.00
Triethanolammonium octadecyl sulfate	19.00
Propylene glycol	4.80

BASE B

Dodecyl alcohol	6.40
Tetradecyl alcohol	3.20
Hexadecyl alcohol	3.20
Octadecyl alcohol	3.20
Triethanolammonium dodecyl sulfate	24.00
Triethanolammonium tetradecyl sulfate	32.00
Triethanolammonium hexadecyl sulfate	8.00
Triethanolammonium octadecyl sulfate	16.00
Propylene glycol	4.00

BASE C

Dodecyl alcohol	8.44
Tetradecyl alcohol	4.22
Hexadecyl alcohol	4.22
Octadecyl alcohol	4.22
Triethanolammonium dodecyl sulfate	42.20
Triethanolammonium tetradecyl sulfate	31.60
Propylene glycol	5.30

TABLE I.—TRIETHANOLAMMONIUM ALKYLSULFATES

Triethanolammonium salt of:	M.p., °C. Reported	M.p., °C. Found	Anal.[a] % N Calcd.	% N Found	% S Calcd.	% S Found
Dodecylsulfuric acid	...	139–140	3.34	3.50	7.64	7.65
Tetradecylsulfuric acid	...	110–111	3.14	3.02	7.20	6.91
Hexadecylsulfuric acid	...	79.5–81	2.95	3.07	6.76	6.59
Octadecylsulfuric acid	86–86.5	86–86.5	2.79	3.00	6.38	6.22

[a] Microanalyses for nitrogen and sulfur were performed by Alfred Bernhardt, Mülheim, Germany.

The ingredients are melted together on a steam bath in a closed container. After mixing thoroughly, the mixture is heated to about 80° and subsequently milled until cool.

Incorporation of the Medicaments.—On the basis of a subjective evaluation of the ointment bases and in anticipation of a further lowering of the plastic range by the addition of medicament, base B was chosen as the base into which 6% salicylic acid U.S.P., 6% resorcinol U.S.P., and 10% medicinal grade colloidal sulfur were incorporated by simple levigation. Similarly, 6% sulfadiazine U.S.P. and 6% iodochlorhydroxyquin U.S.P. were incorporated into base A.

For purposes of comparison, a similar series of medicated ointments was prepared, using polyethylene glycol ointment U.S.P. and white petrolatum U.S.P. as the vehicles.

Stability.—Minimum stability studies were carried out to ascertain any gross changes in the ointments, prepared with the experimental bases, after storage at room temperature for varying lengths of time. All of the ointments were stored in opal ointment jars and opened frequently during the observation period. There appeared to be no consistency change or decomposition, as evidenced by lack of discoloration of the salicylic acid and sulfur ointments at the end of 8 months. This was also true for those containing sulfadiazine and iodochlorhydroxyquin, although the observation period was limited to 2 months. Characteristically, a slight brownish discoloration developed in the resorcinol ointments. However, by the end of a 6-month period, the intensity of discoloration was markedly less than in those resorcinol ointments based in polyethylene glycol ointment and petrolatum stored under similar conditions.

Evaluation of Drug Absorption

Preparation and Collection of Tissues.—Guinea pigs, weighing approximately 300 to 400 Gm., were chosen without regard to sex for use in this study. On the day prior to application of the ointments, the backs of the animals were clipped, under light ether anesthesia, with an Oster animal clipper and shaved with a Sunbeam electric shaver.

Animals prepared in the following manner were intended for the colorimetric assay of the skin for drug concentration. On the day following the clipping, again under light ether anesthesia, an area 5 × 5 cm. was marked off on the shaven back of each of four animals, and 1.5 Gm. of the ointment to be tested was spread over the area, with minimum friction and pressure. Without covering the inuncted site, the animals were returned to individual cages for 4 hr., following which the animals were sacrificed and the excess ointment removed thoroughly with clean, dry gauze sponges. The entire section of medicated skin was removed, stretched to original size and shape, and quickly frozen to −20°. Four skin samples from each of the four animals used for each drug in each base, a total of 16 samples, were assayed for drug concentration.

Samples were prepared in the following manner for photomicrographic procedures. The shaven back of the guinea pig was marked off into five areas. The four ointments of a given base (*i.e.*, sulfur, sulfadiazine, resorcinol, and salicylic acid in the experimental base, polyethylene glycol ointment, or white petrolatum) and an unmedicated control were applied, leaving sufficient margin to prevent intermixing. At the end of the 4-hr. absorption period, the animals were sacrificed and the entire treated integument removed for microscopic examination.

Microscopic Examination for Drug Absorption.—Samples of the fresh skin were mounted in 5% agar on a freezing microtome and 50-μ sections were taken. The samples were transferred rapidly to glass slides coated with a layer of the staining compound in a gelatinous medium to inhibit diffusion of the colored reaction products from the tissue absorption sites. Using a Tower 35-mm. reflex camera with microscope attachment, the specimens were examined immediately and photographed on high-speed Ektachrome Type B, Artificial Light, film (Kodak) at 1/2000 sec.

MacKee *et al.* (2) employed *p*-dimethylaminobenzaldehyde in the histotopical identification of sulfonamides in tissue sections. However, to preclude the necessity of fixing the tissue before sectioning, and to avoid diffusion of the yellow color produced, it was possible to examine the sections directly by precoating the slides with an agar mixture into which the reagents were incorporated. The frozen section was placed directly on the gelatinous surface, and from 3 to 5 min. were allowed for the color to develop to its full intensity prior to photographing. A longer development period tended to obscure vivid delineation of the sites of drug distribution within the tissue.

p-DIMETHYLAMINOBENZALDEHYDE SLIDES

p-Dimethylaminobenzaldehyde	1 Gm.
Ethanol	95 ml.
Hydrochloric acid (37%)	5 ml.
Agar	5 Gm.
Water	100 ml.

Melt the agar in the water and add the *p*-dimethylaminobenzaldehyde, previously dissolved in the mixture of ethanol and hydrochloric acid. Spread the solution on glass slides with a glass rod and allow to gel completely.

Using silver nitrate slides, about 3 min. were required for full development of the black silver sulfide from samples inuncted with the sulfur ointments.

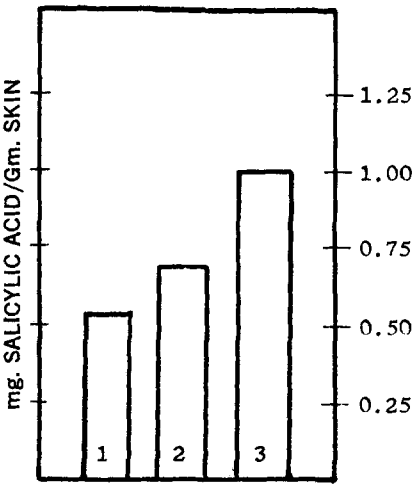

Fig. 1.—Comparison of absorption of salicylic acid from (*1*) petrolatum, (*2*) polyethylene glycol, and (*3*) experimental ointment base.

SILVER NITRATE SLIDES

Agar	2.5 Gm.
Silver nitrate	1 Gm.
Water to make	100 ml.

Melt the agar in 50 ml. of water, cool to about 50°, add the silver nitrate dissolved in the remainder of the water, and spread the melted mixture on glass slides with a glass rod. Protect the slides from light until ready for use.

For the detection of salicylic acid and resorcinol, ferric chloride in a glycerinated gelatin base was the most satisfactory.

FERRIC CHLORIDE SLIDES

Glycerin	50 Gm.
Water	40 ml.
Ferric chloride	5 Gm.
Gelatin[1]	5 Gm.

Mix the gelatin with the glycerin and heat on a steam bath until completely dissolved. Add the ferric chloride dissolved in the hot water, and spread the solution on glass slides with a glass rod. Allow to gel completely.

Determination of Salicylic Acid in Skin Samples.—Levels of salicylic acid in the skin of the test animals were determined according to the procedure devised by Brodie *et al.* (3) and modified by Plein and Plein (4).

The levels of salicylic acid in the skin, determined by the absorbance at 530 mμ on a Bausch & Lomb Spectronic 20 colorimeter, and expressed as milligrams of salicylic acid per gram of skin, are shown in Fig. 1.

Determination of Resorcinol in Skin Samples.—The findings of Etti (5) and Benedikt and Hazura (6) formed the basis for the colorimetric determination of resorcinol in the skins of the experimental animals.

Standards were prepared by heating known volumes of standard resorcinol solution (100 mg. resorcinol dissolved in exactly 100 ml. of water), sufficient water to bring that volume to 1 ml., with 1 ml. 6 *N* hydrochloric acid and 1 ml. of 0.5% vanillin in water in a 25-ml. volumetric flask on a steam bath for 30.0 min. The sample was cooled for 5 min. and 10 ml. of *n*-amyl alcohol added. The mixture was then shaken vigorously for 60 sec. and 2 ml. of a saturated solution of ammonium sulfate added before a final shaking of 30 sec. Finally, the entire mixture was transferred to a thick-walled centrifuge tube and centrifuged at 1500 r.p.m. for 5 min. The absorbance of the alcohol layer was determined on a Bausch & Lomb Spectronic 20 colorimeter at 455 mμ exactly 60 min. after the sample was placed on the steam bath. It was essential that the procedure proceed smoothly and that all variations in timing and temperature be avoided. A standard curve was constructed in the usual manner.

Skin samples were prepared in the same manner as for salicylic acid assays. One milliliter of 6 *N* hydrochloric acid and 1 ml. of freshly prepared 0.5% vanillin in water were added to the aliquot of tissue in a 25-ml. glass-stoppered flask which was heated on a steam bath for 30 min. At the end of the digestion period, the sample was shaken to break up the tissue, and after the 5-min. cooling period, 10 ml. of *n*-amyl alcohol was added. The procedure was completed as described above. Because there were substances in the skin which formed interference products, unmedicated control specimens were run simultaneously and all samples read against *n*-amyl alcohol blanks.

Recovery rates were determined by adding known quantities of resorcinol to 1-Gm. samples of previously unmedicated skin and processing those as described above. From the resulting recovery rate curve, it appeared that a rather constant absorbance was imparted to the alcoholic layer by the skin itself and that density was augmented uniformly by the presence of resorcinol.

Because of the interference products, the values reported were derived from the per cent transmittance calculated to milligrams of resorcinol represented, rather than absolute quantities of the drug. The color produced by the reaction of tissue components with the reagents in the procedure is

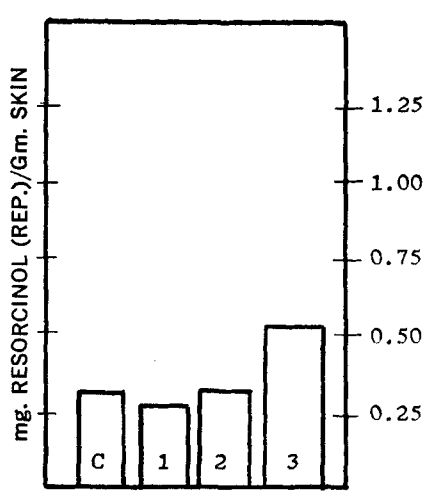

Fig. 2.—Comparison of absorption of resorcinol from (*1*) petrolatum, (*2*) polyethylene glycol, and (*3*) experimental ointment base. C = control level. (See text.)

[1] Marketed as Pharmagel A.

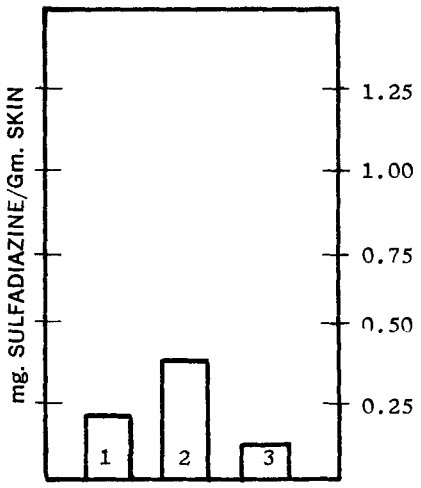

Fig. 3.—Comparison of absorption of sulfadiazine from (*1*) petrolatum, (*2*) polyethylene glycol, and (*3*) experimental ointment base.

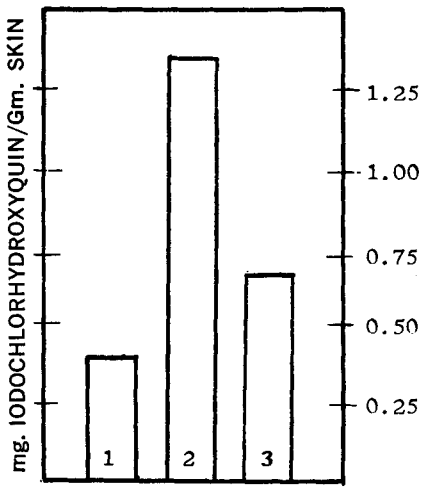

Fig. 4.—Comparison of absorption of iodochlorhydroxyquin from (*1*) petrolatum, (*2*) polyethylene glycol and (*3*) experimental ointment base.

responsible for the apparent resorcinol content in control samples, as shown in Fig. 2 which serve as the basis of comparison of medicament release. It must be pointed out that it was not the intent of this investigation to determine absolute drug content in the tissue, but rather only relative concentrations attained from the bases employed and to attribute the apparent differences, if any, to the vehicle.

Determination of Sulfadiazine in Skin Samples.—Levels of sulfadiazine in the skin of the test animals were determined according to the procedure devised by Bratton and Marshall (7) and modified by Strakosch and Clark (8).

The absorbance was determined at 545 mμ on a Bausch & Lomb Spectronic 20 colorimeter and compared with the standard curve. The results are shown in Fig. 3.

Determination of Iodochlorhydroxyquin in Skin Samples.—Relative quantities of iodochlorhydroxyquin absorbed by the skin samples, as reflected by total iodine content, were determined by the method presented by Zak and Boyle (9) for the determination of organic bound iodine.

Procedure.—To a 25-ml. volumetric flask containing a weighed sample of skin, add 100 ml. of chloric acid and evaporate at the low temperature of a hot plate to fumes of perchloric acid. The flasks must be watched closely during the early part of the digestion to avoid loss by boiling over. Dilute the digested sample to volume. Transfer a 2-ml. aliquot to a clean 25-ml. volumetric flask, add 1 ml. of 1% potassium iodide, and again dilute to volume.

The absorbance was determined at 353 mμ on a Beckman DB spectrophotometer and compared with a standard curve, prepared in the usual manner, using a freshly prepared solution of 100 mg. of iodochlorhydroxyquin in 100 ml. of chloroform.

The comparison of absorption of iodochlorhydroxyquin by the skin from the ointment bases is indicated in Fig. 4.

Evaluation of the Results.—Results of the tissue digest analyses on the skin samples treated with each medicament–base combination were analyzed statistically by use of the Student *t* test and analysis of variance, following the experimental pattern of Little (10).

DISCUSSION AND CONCLUSIONS

It has been shown in this study that quite effective dermatologic vehicles can be prepared using the triethanolammonium salts of certain alkylsulfuric acids and the corresponding alcohols as the major components of the base. The more plastic of the bases might be useful for the incorporation of large amounts of insoluble powders. Thus, it could be possible to achieve whatever plasticity might be desired for the finished product.

These vehicles leave no perceptible residue when rubbed onto the skin, and they are completely washable in water. In patch tests, no evidence of irritation could be detected on the glabrous forearm surfaces of 30 volunteers on whom the bases were allowed to remain for 24 hr.

When the freshly microtomed frozen tissue section, containing salicylic acid, was placed on a ferric chloride gelatin slide and examined immediately, the color intensity was least in those skins treated with the medicament in the petrolatum base. Especially, as seen in the case of salicylic acid, the drug was confined chiefly to the epidermis. When the drug had been incorporated into the polyethylene glycol base, salicylic acid could be observed down to the level of the hair roots. The routes of penetration appeared to be both transepidermal and transfollicular. An accumulation of salicylic acid was clearly apparent in the epidermis of the integuments treated with that drug in the experimental base. In gross aspect, those sections assumed a violet color throughout the entire corium.

As seen in microscopic examination, sulfur incorporated into the experimental base produced a striking effect on the epidermis, while little or no action was exhibited from the other bases.

The yellow produced by the interaction of sulfadiazine and *p*-dimethylaminobenzaldehyde was observed in the hair follicles and epidermis of those tissue sections to which that drug had been applied.

When polyethylene glycol ointment had been used as the vehicle, the color was not only more intense but also presented additional distribution in the corium.

Although exhaustive attempts were made to demonstrate the presence of iodochlorhydroxyquin visually, no satisfactory method could be found.

The levels of resorcinol attained in the skin from the experimental base were highly significant ($p < 0.001$), while the concentrations reached from petrolatum and the polyethylene glycol base were not significantly different from control samples ($0.2 > p > 0.1$ and $p > 0.9$, respectively). Similarly, the levels of salicylate in the skin from the experimental base were very significantly higher than those from the petrolatum base ($p < 0.001$). The significance of the difference between tissue levels of salicylate from the polyethylene glycol base and petrolatum, although less marked, was nevertheless still significant ($0.01 > p > 0.001$). The difference in tissue levels of sulfadiazine attained from petrolatum and the experimental base would not appear to be significant ($0.05 > p > 0.02$), although the concentration reached from the polyethylene glycol was very significantly higher ($p < 0.001$). Likewise, the absorption of iodochlorhydroxyquin from the petrolatum and experimental ointments by the skin were found to be not significantly different ($0.05 > p > 0.02$), whereas a significantly higher level was attained from the polyethylene glycol base ($p < 0.001$).

In conclusion, it appears that dermatologic vehicles based upon the experimental formulas, such as those presented here, present a promising innovation in the formulation of topical medication.

REFERENCES

(1) Maurer, E. W., Stirton, A. J., and Weil, J. K., *J. Am. Oil Chemists Soc.*, **27**, 34(1960).
(2) MacKee, G. M., *et al.*, *J. Lab. Clin. Med.*, **28**, 1642 (1943).
(3) Brodie, B. B., Udenfriend, S., and Coburn, A. F., *J. Pharmacol. Exptl. Therap.*, **80**, 114(1944).
(4) Plein, J. B., and Plein, E. M., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 705(1957).
(5) Etti, C., *Monatsh. Chem.*, **3**, 643(1882).
(6) Benedikt, R., and Hazura, K., *ibid.*, **5**, 168(1884).
(7) Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, **128**, 537(1939).
(8) Strakosch, E. A., and Clark, W. G., *Am. J. Med. Sci.*, **205**, 518(1943).
(9) Zak, B., and Boyle, A. J., *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 260(1952).
(10) Little, J. M., "An Introduction to the Experimental Method," Burgess Publishing Co., Minneapolis, Minn., 1961.

Cholinergic Anionic Receptors I

Steric Requirements for Quaternary Ammonium Inhibitors of Acetylcholinesterase

By JAMES C. KELLETT, Jr., and CHARLES W. HITE*

As an initial effort to discern some of the fine features of cholinergic anionic receptors, a study was made on inhibitors of acetylcholinesterase which used inhibitors that are relatively free from conformational variation. The inhibitors used were quaternized 1-azabicyclo(2.2.2)octanes. The bicyclic parent amine was prepared by several conventional procedures. The salts were evaluated as competitive inhibitors of acetylcholinesterase by a titrimetric procedure. The affinity of these compounds for the anionic site of this enzyme was compared to the affinity of a series of related salts subject to conformational variation. These comparisons suggest that there are stereochemical requirements for the anionic site of this cholinergic receptor more specific than heretofore suspected.

THE AUTHORS are engaged in a study of cholinergic receptors with the aim of further defining the chemical requirements of receptor substances activated by acetylcholine (ACh).

The active site of acetylcholinesterase (AChE) has been the object of a great many investigations. [See Koelle (1) and Krupka (2) for leading reviews.]

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It is almost universally accepted that the active site of AChE consists of an anionic site (which is responsible for the first contact between the enzyme and ACh) and an esteratic site which, in turn, consists of a serine hydroxyl, an acid site, and a basic site. These last three functional groups act in an integrated fashion to effect hydrolysis of the substrate. Little or no information is available about the details of the anionic site. All expressions for describing the enzyme's activity involve the formation of a Michaelis complex. It is generally accepted that at least a significant portion of the binding in the AChE–ACh complex is an electrostatic attraction for the cationic head of ACh and an anionic site of the enzyme; this point has been