PENETRATION OF ANIONIC SURFACTANTS INTO SKIN

II. Study of Mechanisms Which Impede the Penetration of Synthetic Anionic Surfactants into Skin*

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Data presented in the first paper of this series (1) led to the conclusion that sodium laurate,† but not sodium dodecyl sulfate (a synthetic anionic surfactant), could penetrate through the epidermis from dilute, unbuffered, mildly alkaline aqueous solutions into the dermis of excised human skin. Until more alkyl sulfates and the alkyl benzene sulfonates were studied, however, sufficient evidence was not available to justify the general conclusion that synthetic anionic surfactants as a class are unlikely to penetrate normal skin. Data are presented here to show that none of a series of synthetic anionic surfactants studied penetrates normal excised human skin in measurable amounts.

It has been stated (1) that the "ability of the proteins of the stratum corneum to bind the alkyl sulfates and alkyl benzene sulfonates" may reasonably be assumed to be "at least one mechanism whereby the skin is able to prevent the penetration of these compounds" into the epidermis and dermis of excised human skin from dilute, unbuffered, mildly alkaline aqueous solutions. Since publication of that statement, an effort has been made to determine the relative importance of this mechanism in impeding the penetration of surfactants through excised human skin. It was felt that if this mechanism were of major importance, penetration of surfactants might increase if protein-binding were prevented. The data to be reported here indicate that penetration does not increase when protein-binding has been reduced. It is unlikely, therefore, that protein-binding constitutes a major obstacle to penetration.

The series of observations to be reported here shows that synthetic surfactants do penetrate human skin after the stratum corneum conjunctum has been mechanically removed, or after the skin has been pretreated with lipid solvents. The rate-limiting barrier appears to be in the stratum corneum conjunctum, and can be altered by lipid solvents.

A. Penetration of Synthetic Anionic Surfactants Other Than Sodium Dodecyl Sulfate

In the study to be reported here, six pure sodium alkyl sulfates (octyl, decyl, dodecyl, tetradecyl, hexadecyl and octadecyl) and four pure sodium alkyl benzene sulfonates (p-decyl, p-dodecyl, p-(1-methyldodecyl) and p-(1-methylhexadecyl)) were used. Two compounds synthesized with radioactive sulfur were available: sodium dodecyl sulfate and an alkyl benzene sulfonate containing mixed molecular species (assumed average molecular weight, 346). Since only two compounds were radioactive, it was necessary to be able to determine quantitatively microamounts of these substances in the presence of large amounts of dermis by a biochemical or colorimetric method. For all of the alkyl benzene sulfonates and for the dodecyl, tetradecyl, hexadecyl and octadecyl sulfates, the method outlined in our previous paper (1) has been used. For the octyl and decyl sulfates, however, the methylene-blue method described by Jones (2) was more satisfactory; simple extraction with

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† Even though reference will be made throughout this paper to the "penetration of sodium laurate," the exact composition of the particle which penetrates from a solution of sodium laurate is not yet known. Perhaps it is an acid soap, or lauric acid, or the laurate ion. It is unlikely to be un-ionized sodium laurate.

water adequately removes these compounds from proteins.

The technic used for determining penetration into skin was that described in the first paper of this series (1). For the hexadecyl and octadecyl sulfates it was modified by substituting 60 per cent acetone for water as the vehicle. This change was made because the solubility of these two compounds in water is so low that 0.005 M aqueous solutions cannot be prepared.

After the skin had been in contact with about 5 ml of 0.005 M aqueous solution of each of the various alkyl sulfates or alkyl benzene sulfonates for between 18 and 20 hours (*i.e.*, overnight) at room temperature (20–23° C), not more than 15 mμmols per square centimeter of any one of these surfactants could be found in the dermis. This is less than 5 μg of surfactant. In evaluating results, it must be remembered that the accuracy of the colorimetric method used is open to question for amounts smaller than 5 μg. Therefore, when no more than this amount of surfactant is found in the dermis we shall speak of "no penetration" or "no penetration in detectable amounts."

When penetration of sodium dodecyl sulfate from water and from 60 per cent acetone was compared, no penetration of sodium dodecyl sulfate was observed from either vehicle. The hexadecyl and the octadecyl sulfates did not penetrate from 60 per cent acetone.

In a paper on the penetration of fatty-alcohol sulfates and sodium soaps of different chain lengths (C_8–C^{18}) into intact human skin, Szakall and Schulz (3) report that aqueous solutions of alkyl sulfates lose weight during contact with the the skin of the forearm. These authors interpret this loss in weight to be a result of penetration of the solution into the skin. Throughout most of their paper, they refer to "penetration of water into the skin;" only in their discussion do they mention penetration of the surface active agents themselves. The technics used by Szakall and Schulz do not determine whether the loss in weight of the applied solution is due to penetration of water alone or to penetration of both water and surfactant. From the data they have published, it is possible to calculate that, if it is the solution which penetrates, the penetration rate of sodium dodecyl sulfate is approximately 1 μg per square centimeter per minute. Since in our own experiments no detectable amount of sodium dodecyl sulfate reached the dermis after a similar solution of surfactant had been in contact with excised skin for 18–20 hours, either the loss in weight, as measured by Szakall and Schulz, if true penetration, must be due to penetration of water alone or sodium dodecyl sulfate must penetrate more rapidly into the intact skin of living man than into the excised skin of man.

Since none of the various synthetic anionic surfactants studied in our laboratory has been found to penetrate the barrier and reach the dermis of normal excised human skin in detectable amounts, we feel that it is important to learn how normal skin retards or prevents the penetration of these compounds.

B. Prevention of the Binding of Surfactants by Proteins of the Stratum Corneum

Many investigators have shown that the anion of anionic surfactants combines with the amino groups of various proteins. Many of the resulting compounds dissociate only slightly in water. One might argue, therefore, that if the proteins of the stratum corneum were to combine with anionic surfactants and hold them tightly, penetration through the stratum corneum and consequently into the dermis might be prevented or delayed. As a corollary, it might also be maintained that if such combination were prevented, penetration might take place.

We have attempted in several ways to prevent binding of surfactants by the proteins of the stratum corneum:

1) by increasing the alkalinity of the vehicle;
2) by using 60 per cent acetone solution containing an electrolyte with a divalent cation as a vehicle for the surfactant;
3) by pretreating the skin on the epidermal side with formaldehyde to block the amino groups;
4) by pretreating the skin on the epidermal side with acetic anhydride in order to acetylate the amino groups;
5) by saturating the amino groups with excess surfactant; and
6) by removing the stratum corneum by stripping with adhesive tape.

The results were as follows:

1) Raising the pH of the vehicle to 10 does not completely prevent dodecyl sulfate from combining with cutaneous proteins, although the amount which combines at this pH is much less than that

which combines when the pH is in the acid range. No detectable amount of dodecyl sulfate reaches the dermis from a buffered solution of pH 10.00 which has been placed on the epidermal surface.

2) When acetone and an electrolyte containing a divalent cation are present in the vehicle, less dodecyl sulfate combines with protein than when these substances are absent. In spite of this, however, dodecyl sulfate does not penetrate in detectable amounts to the dermis from 60 per cent acetone which contains magnesium sulfate at pH. 7.0.

3 & 4) After the epidermal surface of the skin has been pre-treated with formaldehyde or acetic anhydride, some of the amino groups on the protein will undoubtedly be blocked. In our experiments, skin was pre-treated by holding the epidermal surface in contact with either 4 per cent formaldehyde for 22 hours or acetic anhydride for 6 hours. The epidermal surface was then washed with water. No attempt was made to determine how completely the amino groups were blocked, but there was no evidence that the permeability to dodecyl sulfate of skin so treated was greater than that of untreated skin.

5) After attempting to saturate the amino groups by pre-treatment of the entire skin with an aqueous solution of sodium dodecyl sulfate, 0.005 M radioactive sodium dodecyl sulfate was placed on the epidermis of the pre-treated skin. No radioactivity was found in the dermis after overnight exposure of the epidermal surface to the radioactive dodecyl sulfate. When 0.05 M aqueous sodium dodecyl sulfate was in contact with the epidermal surface of untreated, normal skin overnight, no evidence of penetration could be obtained. In some experiments, dodecyl sulfate was found in the dermis after 3-day exposure to 0.005 M solutions, but in other experiments of this type no penetration was observed. Such exposure is severe and often causes complete separation of epidermis from dermis, and marked maceration of the stratum corneum. It seemed somewhat surprising that any skin was able to prevent penetration after being subjected to such severe "damage."

6) After adjacent pieces of skin had been stripped a varying number of times, their electrical conductivity was determined. The amount of surfactant which penetrates into the dermis of these pieces of skin following 21-hour exposure to 0.005 M aqueous dodecyl sulfate solution was determined. The pieces of skin were examined histologically at the end of the experiment. After 10 strippings, the stratum corneum conjunctum still appeared to be intact; only after 20 strippings was the entire stratum corneum removed.

TABLE 1

Effect of stripping with pressure-sensitive tape on the permeability of excised human skin to aqueous 0.005 M sodium dodecyl sulfate

Number of Strippings	Electrical Conductivity at 1 Volt (Microamperes)	mμmols of Sodium Dodecyl Sulfate per Square Centimeter of Dermis
0	1	0
5	2	0
10	15	0
20	800	71

Removal of the top portion alone of the stratum corneum does not seem to increase permeability; only when the stratum corneum conjunctum has been removed does penetration occur (Table 1). Combination of the surfactant with the upper layers of the stratum corneum does not seem to be a major factor in preventing penetration, because removal of these layers does not make penetration possible. The limiting barrier against the penetration of surfactants, like that against the penetration of water (4), appears to be in the stratum corneum conjunctum.

C. Pre-treatment of Skin with Lipid Solvents

Winsor and Burch (5) have been unable to show an increase in the permeability of excised human skin to water after the surface lipid film has been removed with lipid solvents. Szakall (6), measuring the absorption of water by intact skin, observed that after skin had been defatted for 3 minutes with ether, there was a temporary increase in the amount of water absorbed. Absorption appeared to return to normal approximately 2 hours after the defatting took place.

In our own experiments, removal of lipids from the cutaneous surface by washing with acetone, ethyl alcohol or an ethyl alcohol–ethyl ether mixture (1:9), or by short pre-treatment of the skin (*i.e.*, allowing these solvents to remain in contact with the epidermal surface for several hours) does not render the skin permeable to

TABLE 2

Effect of pre-treatment with lipid solvents on the permeability of excised human skin to anionic surfactants*

Solvent	mμmols of Surfactant per Square Centimeter of Dermis (Range of Values from Several Experiments)	
	Sodium dodecyl sulfate	Sodium laurate
Ethanol, 95%	0–150	90–740
Acetone, 100%	70–250	160–720
Ethanol–ethyl ether (1:9)	200–390	110–1580

* Complete immersion in approximately 500 ml of the solvent for 3 days, followed by 6 to 8-hour immersion in distilled water.

sodium dodecyl sulfate. The surface lipid film, therefore, does not appear to be the major barrier against penetration of anionic surfactants.

It was felt that more thorough removal of cutaneous lipids might alter the permeability of skin. To test the validity of this hypothesis, individual pieces of excised human abdominal skin, obtained at autopsy, were soaked in 500 ml of polar, lipid solvents at room temperature for 3 days with occasional agitation before their permeability was studied. Not only were the cutaneous lipids removed by this treatment, but also the skin became dehydrated, shrunken and less flexible. Following this immersion in solvents, the pieces of skin were put in several changes of distilled water for 6–8 hours and then blotted dry. Their electrical conductivity at this point had increased many fold. Table 2 shows the amount of penetration of sodium dodecyl sulfate and sodium laurate into the dermis of pieces of skin which had undergone this pre-treatment following 18 to 20-hour contact with 0.005 M aqueous solutions of these surfactants. The sodium laurate solution used was buffered to maintain a pH between 7.5 and 8.0,—a range within which as much as 100–200 mμmols may penetrate into the dermis of unextracted skin from a 0.005 M solution. It can be observed, however, that pre-treatment of skin with solvents increases its permeability to laurate. Little or no sodium dodecyl sulfate penetrates unextracted skin, but this compound does penetrate solvent-pre-treated skin. The greatest increase in permeability noted was in skin which had been pre-treated with the alcohol-water mixture.

Long pre-treatment with solvents, therefore, does render skin permeable to anionic surfactants. It seems unlikely that the observed penetration can be explained by a decrease in the combining power of the stratum corneum for the surfactants, as the result of pre-treatment with solvents. Solvent pre-treatment is known to remove lipids (free and/or combined), possibly from the major barrier in the stratum corneum conjunctum as well as from other parts of the skin. Whether increased permeability of the skin to surfactants results specifically from the removal of lipids or from some other structural or chemical change produced by the pre-treatment, has not yet been definitely established.

In a forthcoming paper, III of this series (7), we shall present data which show that penetration into skin from a sodium laurate solution is greater when the pH of the solution is 7.5 than when it is 9.5. Since the lipid solubility of sodium laurate increases as the pH decreases, one explanation of the greater permeability at the lower pH might be the ability of the laurate to utilize a lipid pathway through the skin. In the data presented here, however, an *increase in the permeability* of skin to sodium laurate accompanies *removal of the lipids* from the skin. Apparently, therefore, a lipid pathway does not seem to be essential to the penetration of sodium laurate at pH 7.5.

DISCUSSION

Although it is probably true that "the ability of the proteins in the stratum corneum to bind the alkyl sulfates and alkyl benzene sulfonates is at least one mechanism whereby skin is able to prevent the penetration of these compounds" (1), data presented here indicate that this is not one of the more important mechanisms involved. All attempts have failed to show that penetration occurs if binding of the surfactants is decreased. Only after removal of the entire stratum corneum, or after thorough pre-treatment of the skin with lipid solvents, has penetration been found to occur.

Most of the anionic surfactants which we have studied are strongly water-soluble and lipid-insoluble. Any lipid associated with the stratum corneum conjunctum might theoretically, therefore, cause difficulty in the establishment of a

continuous aqueous pathway through which water-soluble surfactants could diffuse. The natural channels through the stratum corneum conjunctum are probably very small. Accordingly, extraction of lipids should facilitate the establishment of a continuous aqueous pathway and at the same time enlarge the average size of channels through the stratum corneum conjunctum. Our experiments show that lipid extraction facilitates penetration of the surfactants.

The presence of lipids in the stratum corneum conjunctum need not necessarily render that layer completely hydrophobic, since the lipids themselves may be somewhat hydrophilic. If hydration of the stratum corneum conjunctum does occur, it will reduce the size of passageways which even under normal conditions are small. If particles attempting to pass through channels in the stratum corneum conjunctum carry an envelope of water, which surfactant ions can be expected to do, this envelope would increase the effective size of the particles and might prevent their penetration simply by making the effective size of the particles greater than the effective size of the channels in the skin (steric hindrance).

At the normal pH of the cutaneous surface, the net charge on protein fibers is electronegative. The anions of anionic surfactants also carry a negative charge. It may be that, because of electrostatic repulsion between the surfactant ions and the protein fibers, anionic surfactants cannot enter the relatively small passageways through skin even when the aqueous pathway is continuous.

We now suspect that at least four mechanisms may be involved in retarding or preventing penetration of synthetic anionic surfactants into and through the skin. These are:

1) Physical and chemical structures and reactions which make difficult the establishment of a continuous aqueous pathway through the skin
2) Steric hindrance
3) Electrostatic repulsion
4) Combination of surfactants with proteins in the stratum corneum.

The fourth mechanism does not now seem to us to be very important. The relative importance of the first three mechanisms has not yet been established, but on the basis of some evidence we now have, we suspect that electrostatic repulsion, like protein-binding, may be of minor significance. We know of no direct evidence either in support or refutation of the first two mechanisms. Whether these are mechanisms whereby the skin is able to prevent or retard the penetration of synthetic anionic surfactants, or whether there are other mechanisms not listed here which accomplish this, is not yet known.

SUMMARY

After 18- to 20-hour contact with the epidermis at room temperature, none of the many synthetic anionic surfactants studied has penetrated to the dermis of normal, excised, human skin in sufficient quantity to allow us to state definitely that penetration has occurred. Penetration into the dermis does occur when the entire stratum corneum has been removed, or when the skin has been thoroughly extracted with lipid solvents. All attempts to bring about penetration by preventing combination of the surfactant with cutaneous proteins have failed. The mechanisms whereby skin might retard or prevent the penetration of synthetic anionic surfactants are reviewed.

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