PENETRATION OF ANIONIC SURFACTANTS (SURFACE ACTIVE AGENTS) INTO SKIN

I. Penetration of Sodium Laurate and Sodium Dodecyl Sulfate into Excised Human Skin*

IRVIN H. BLANK, Ph.D. and EDITH GOULD, B.S.

Soap has long been considered a cutaneous irritant. The synthetic detergents which were first incorporated into household cleansers have also been thought to be irritating to the skin, perhaps even more irritating than soap. Simple dryness of the skin can result if a substance penetrates into the stratum corneum only. Irritations more severe than simple dryness can occur only if a substance penetrates the major barrier of the skin and reaches the viable cells of the epidermis. It was decided, therefore, to study the penetration of various anionic surfactants (constituents of soaps and household cleansers) into the different layers of the skin and the factors which affect this penetration.

Sodium laurate, the salt of the twelve carbon fatty acid, is a constituent of most soaps. It is thought to be more irritating than the salts of the other fatty acids present in soaps (1). A mixture of the salts of various alkyl sulfates was one of the first synthetic detergents used in household cleansers. Today, alkyl benzene sulfonates are more commonly used. In this study, however, the sodium salt of dodecyl sulfate (a representative alkyl sulfate) has been compared with sodium laurate. Sodium dodecyl sulfate is thought to be more irritating than the sodium salts of other alkyl sulfates (2). Sodium laurate and sodium dodecyl sulfate were chosen for investigation because the size and shape of their molecules are similar (Fig. 1) and because each of these compounds can be obtained in relatively pure form and can be synthesized with a radioactive element.

This is the first of a series of papers about the penetration of anionic surfactants into skin. It presents data on the penetration from aqueous solutions of sodium laurate and sodium dodecyl sulfate into excised human skin.

METHODS

Penetration into Skin

Abdominal skin, obtained at autopsy, is used throughout this study. Non-hairy skin has been chosen whenever possible. Immediately after each piece of skin is cut to size, its electrical conductivity is measured (3). If there are no breaks in the skin barrier, the electrical conductivity at a potential of one volt is usually less than 5 microamperes immediately after the skin has been placed between the electrodes (16 mm. in diameter); if the barrier has been damaged, conductivity may be 100 microamperes or more. Those pieces of skin in which the conductivity is low are placed in the diffusion chambers previously described (4).

The aqueous preparations used in this study are 0.005 M solutions. At this concentration, the surfactant exists mostly in the molecular state; at higher concentrations micelles are present. Five milliliters of the surfactant solution are allowed to remain on the epidermal side of the skin in the diffusion chamber for 18 to 24 hours at room temperature (20–23° C). Drying of the dermis is prevented by placing a pledget of moist cotton in the lower portion of the chamber after the skin has been fastened in place, and by allowing the open end of the chamber to stand on a smooth surface, so that water cannot readily evaporate from the pledget. At the end of the exposure period, the surfactant solution is poured out of the chamber, the epidermal side of the skin gently washed six times with distilled water (total volume about 30 ml), the skin removed from the chamber and blotted between paper towels, and the electrical conductivity measured.

The next procedure is to remove the cornified epithelium by 10 to 15 successive strippings with

* From the Dermatological Research Laboratories of the Department of Dermatology, Harvard Medical School, at the Massachusetts General Hospital, Boston 14, Massachusetts.

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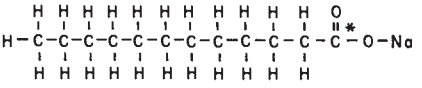
SODIUM LAURATE



SODIUM DODECYL SULFATE

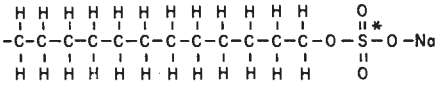


FIG. 1. Chemical structure of sodium laurate and sodium dodecyl sulfate. Asterisk indicates a radioactive element.

pressure-sensitive tape (Minnesota Mining and Manufacturing Company, #610, 1½ inches wide) and, when this has been done, to redetermine the electrical conductivity. When most of the stratum corneum and barrier have been removed, the conductivity is 500 μA or more.

A disc 16 mm in diameter is then cut with a cork borer from the area of skin which has been exposed to the surfactant solution. If this disc is held for 30 seconds between two pieces of dry metal which have previously been heated to 60° C, the epidermis may be easily separated from the dermis. To make certain that the portion of the dermis to be analyzed has not been contaminated by surfactant during the cutting procedure or by particles of epidermis which may adhere to the edge of the disc, a 10 mm disc is cut with a cork borer from the 16 mm disc. The epidermis from the 16 mm disc and the dermis from the 10 mm disc are then analyzed for surfactant.

Chemical Analysis of Anionic Surfactants

Moore and Kolbeson (5) have described a colorimetric method for the determination of anionic surfactants in sewage and surface waters. It employs the formation of a methyl green-surfactant complex which is selectively extractable into benzene from an acidified aqueous solution. These authors observed that the presence of small amounts of protein in their solutions did not appear to interfere with the determinations. We, however, have been unable quantitatively to recover small amounts (3–50 μg) of surfactants from large amounts (100 mg dry weight) of protein (dermis). Apparently the protein-surfactant bond is stronger than the dye-surfactant bond.

It was necessary to find an extractant which would separate the surfactant from the protein-surfactant complex, and thus release it for subsequent combination with the dye. The extractant used (*abe*) is prepared as follows: 2 gm magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$) in 360 ml distilled water and 40 ml of 0.1 N sodium hydroxide are diluted to 1000 ml with reagent grade acetone. The flocculent precipitate of magnesium hydroxide which is formed need not be filtered, but the extractant should be agitated before use. Additional reagents used in the test are:

Buffer solution: To an aqueous solution containing 7.5 gm of glycine and 5.8 gm sodium chloride in 1000 ml, about 450 ml of 0.1 N hydrochloric acid are added until the pH is 2.5. A few drops of benzene are added and the solution is kept refrigerated.

Buffered water: To 1800 ml of distilled water, 200 ml of the buffer solution and about 55 ml of 0.1 N hydrochloric acid are added.

Methyl green solution: 0.5 gm certified methyl green is added to 100 ml of distilled water.

The epidermal or dermal specimen to be analyzed for surfactant is either frozen and ground in a Wiley Mill at −10° C or minced at room temperature with scissors, scalpel or razor blade. The specimen is held in 20 ml of the *abe* extracting solution, with occasional shaking, for 20–24 hours and then centrifuged for 15 minutes at about 2200 g. To a 10 ml aliquot of the resulting supernatant in a 125 ml separatory funnel, 10 ml of the buffer solution and 2 ml of the methyl green solution are added. Preferably, this aliquot should contain between 10 and 25 μg of surfactant; if much larger amounts are present, it is best to use a smaller quantity of supernatant and to bring the volume of the aliquot up to 10 ml with the *abe* extractant. Finally, 40 ml of benzene (reagent grade) are added to the funnel, and the entire mixture shaken for 1 minute. The aqueous layer, which separates out on standing, is carefully drawn off. Fifteen milliliters of the buffered water is added to the remaining benzene phase, and the funnel shaken for 20–30 seconds. If allowed to stand for 30–40 minutes after this second shaking, a clear benzene layer forms. The optical density of this layer at 615 mμ is determined with a spectrophotometer.

In each experimental series, a standard curve is set up for each surfactant. To relatively similar portions of the protein (dermis) are added known amounts of surfactant in volumes of water of not more than 4 ml. The surfactant-protein mixture is then extracted overnight with enough *abe* to bring the final fluid volume to 20 ml, and the supernatant submitted to the colorimetric procedure outlined above. The standard curve constructed from these data relates optical density to the amount of surfactant added. This standard curve parallels very well a curve constructed

from data obtained from colorimetric determinations of known amounts of surfactant in aqueous solution. For each specific amount of surfactant, the optical density is slightly higher when determined from the protein extract than from aqueous solution.

Analysis of Surfactants by Radioactivity Measurement

Since much of the sodium dodecyl sulfate used for this study contained S^{35}, it was possible to analyze the tissue extracts not only by the colorimetric procedure, but also by radioactivity measurement. One milliliter portions of the extracts are placed in nickel plated planchets (25 mm diameter), cautiously evaporated to dryness and the radioactivity measured by means of a Tracerlab #TGC-2 Geiger-Mueller tube and an Atomic Instrument Company scaler, Model #1081. The total amount of solids in 1 ml of the tissue extracts is small enough so that there is no significant absorption of radioactive particles.

Because sodium dodecyl sulfate may hydrolyze, splitting the sulfate group from the alkyl radical, determination of radioactivity alone does not necessarily indicate the presence of the entire alkyl sulfate molecule. Only after it is shown that the amount of alkyl sulfate as determined chemically is the same as that determined by radioactivity measurements, can counts of the radioactivity be accepted as an accurate measure of the alkyl sulfate present.

Most of the experimental work has been done with a sample of sodium dodecyl sulfate which contained about 5 per cent impurities (sodium sulfate and water). Less than 0.02 per cent of the S^{35} was present in the form of sodium sulfate. Since the chemical determinations of sodium dodecyl sulfate in the extracts check well with determinations by radioactivity measurement, it can be said that the trace of radioactive sodium sulfate is not responsible for the radioactivity of the extracts. (Sodium sulfate produces no color in the test for the alkyl sulfates.)

Unfortunately, no simple chemical method is available for the determination of micro-amounts of sodium laurate in the presence of large amounts of proteins. We were obliged, therefore, to use radioactive sodium laurate, tagged with C^{14} in the 1-position, and to accept the radioactivity measurements as a quantitative measurement of the amount of sodium laurate present. In the extracts of the skin, one cannot say whether sodium laurate or lauric acid is being measured, or whether the laurate ion may have combined with some tissue constituent. It is unlikely, however, that the carbon in the first position will leave the laurate radical, and therefore radioactivity may be considered a measurement of the laurate radical. If known amounts of sodium laurate are added to samples of dermis and subsequently extracted with *abe* solution, they can be recovered quantitatively. It is possible that the sample of sodium laurate might contain traces of fatty acids of other chain lengths which would be radioactive. Our methods for estimating the amount of laurate in extractives would not distinguish between laurate and other fatty acids.

Autoradiography

After exposure to the surfactants, the pieces of skin to be used for autoradiography are frozen, mounted, and sectioned on a microtome in a cryostat (Linderstrom-Lang cabinet) at −10 to −15° C. The microtome is set at 8 μ. Sections are mounted on plain glass slides which are warmed momentarily to make the sections adhere to the glass. This slide is then placed adjacent to a radiosensitive slide (Type NTB2, Eastman Kodak Company) and the two slides clamped firmly together. The slides are held at 4° C for varying periods of time. After this exposure period, the slides are separated and the skin sections are stained with hemotoxylin-eosin. The radiosensitive slide is developed in full strength D-19 Kodak Developer for 2 minutes, washed and fixed with Kodak Acid Fixer for 3 minutes.

RESULTS

Penetration from Unbuffered, Aqueous Solutions

In an experiment in which the stratum corneum is removed by successive strippings, the amount and distribution of the penetrating substance in the stratum corneum can be determined by an analysis of the individual strippings (6). Since radioactive surfactants were used in this investigation of penetration, the amount of material in each strip could be estimated by a direct count of the strip. The amount of surfactant determined in this manner will be less than is actually in the strip, because the organic constituents from the stratum corneum absorb some of the low energy radiation from the radioactive elements (C^{14} and S^{35}). On any strip, the thicker the layer of cells from stratum corneum, the greater will be the error. It is not possible to remove the same amount of cornified epithelium on each strip,—some layers are thick, some are thin.

Table 1 shows the counts per minute per square centimeter of surface area and the equivalent mμM of surfactant, as calculated from the counts per minute, in fourteen strippings from

TABLE 1

Distribution of sodium laurate and sodium dodecyl sulfate in various portions of the skin after 20-hour contact with 5.0 ml. of 0.005 M solutions as determined from radioactivity measurements

Tissue	Stratum corneum strippng no.	Sodium laurate		Sodium dodecyl sulfate	
		Counts/min·cm²	mμM*	Counts/min·cm²	mμM*
	1	1778	77.3	5,372	36.5
	2	626	27.4	16,109	109.0
	3	245	10.8	1,136	8.0
	4	179	7.6	642	4.5
	5	168	7.6	840	5.5
	6	99	4.5	445	3.1
	7	127	5.4	619	4.2
	8	66	2.7	536	3.5
	9	45	1.8	533	3.5
	10	51	2.2	363	2.4
	11	100	4.5	742	5.2
	12	31	1.3	462	3.1
	13	47	2.2	170	1.0
	14	33	1.3	431	2.8
Stratum corneum (total in strippings)			*156.6*		*192.3*
Epidermis			*189.0*		*21.0(14.0)†*
Dermis			*221.0*		*<3.5(0)†*

* 1 mμM sodium laurate = 0.222 μg; 1 mμM sodium dodecyl sulfate = 0.288 μg.

† Figures in parentheses determined chemically.

each of two pieces of skin, sodium laurate having penetrated into one, and sodium dodecyl sulfate into the other. In this experiment, the surfactant solution was held on the surface of the skin for 20 hours at room temperature (20–23° C). The sodium dodecyl sulfate used in this experiment had higher specific activity than did the sodium laurate. When counted at infinite thinness with our equipment, 1 mμM of the sodium dodecyl sulfate gave 147 counts per minute and the sodium laurate 23 counts per minute (each corrected for background).

It can be seen that in the stratum corneum, a major portion of either the sodium laurate or sodium dodecyl sulfate is in the first two strippings. Some of each surfactant has reached the base of the stratum corneum, which is the site of the major barrier in the skin. Because the electrical conductivity of each piece of skin was high after the 14 strippings, we feel that the major portion of the barrier was removed by the stripping. We have reason to believe that the actual amount of surfactant in the stratum corneum was at least twice the amount shown in the figures; the absorption of radiation previously mentioned accounts for this discrepancy.

Table 1 also shows the amount of surfactant in the epidermis and dermis (1 cm² surface area). The figures obtained probably accurately represent the amounts of surfactant present in the tissues since the amount of solids in the planchets is so small that no appreciable adsorption of radioactive particles can occur. The figures (shown in parenthesis) for the amount of sodium dodecyl sulfate in the epidermis and dermis are the amounts as determined chemically.

The sodium laurate data show that there is appreciable radioactivity in the extracts from both the epidermis and dermis. Since we have not chemically analyzed the extracts for sodium laurate we cannot definitely say that it is sodium laurate which has penetrated the skin, but we believe that the radioactivity measured is present in the laurate radical. The pH of the sodium laurate solution as it was placed in contact with the skin was 10.3; at the end of the 20-hour exposure period, the pH was 7.6, a pH at which an appreciable amount of undissociated lauric acid may be present. A more detailed study of penetration of sodium laurate from buffer solutions over a wide pH range may answer this question.

It is seen that little or no sodium dodecyl sulfate reaches the dermis during a 20-hour exposure to a 0.005 M solution. Relatively little sodium dodecyl sulfate has reached the epidermis below the barrier. Because of the irregularity of the cutaneous surface, one cannot ever be entirely sure that all of the stratum corneum over a square centimeter of skin has been removed by the stripping technique. It is theoretically possible that most of the 13 to 21 mμM reported in the epidermis in this experiment is actually present in small remnants of stratum corneum which were not removed. If so, then even less than 21 mμM passed through the barrier.

It is to be remembered that the actual volume of a piece of dermis having a surface area of 1 cm² is much greater than the volume of the epidermis from a similar area and hence that the *concentration* of sodium laurate *in the dermis* is much lower than its *concentration in the epidermis*, even though, in the experiment discussed, the

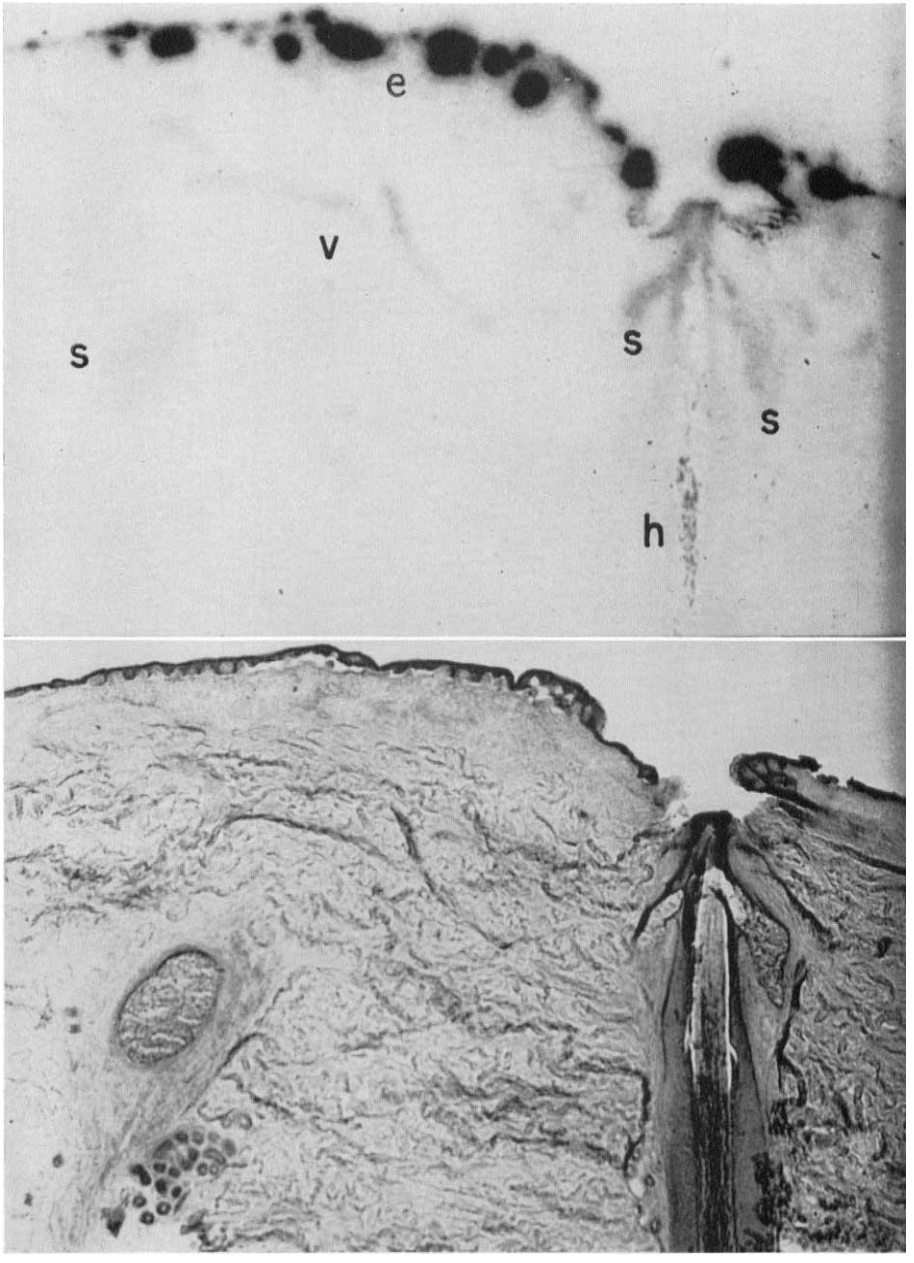


FIG. 2. Autoradiogram and histologic section (hematoxylin-eosin stain) from piece of skin into which radioactive sodium laurate has penetrated. Magnification: 40×. Shadows: *e*, epidermis; *s*, sebaceous glands; *v*, blood vessels; *h*, hair artifact.

amount per square centimeter is somewhat higher in the dermis.

Autoradiography

By autoradiography, the position of the radioactive chemical after penetration into the skin can be determined. For the autoradiogram shown in Figure 2, 5 ml of a 0.005 M aqueous solution of sodium laurate was held on a piece of excised abdominal skin for 22 hours at room temperature. The total radioactivity was 4.5 μc. The initial pH of the sodium laurate solution was 10.7. After 22 hours contact, the sodium laurate solution was discarded, the surface of the skin washed, the skin removed from the chamber, blotted with a paper towel, the stratum corneum removed by

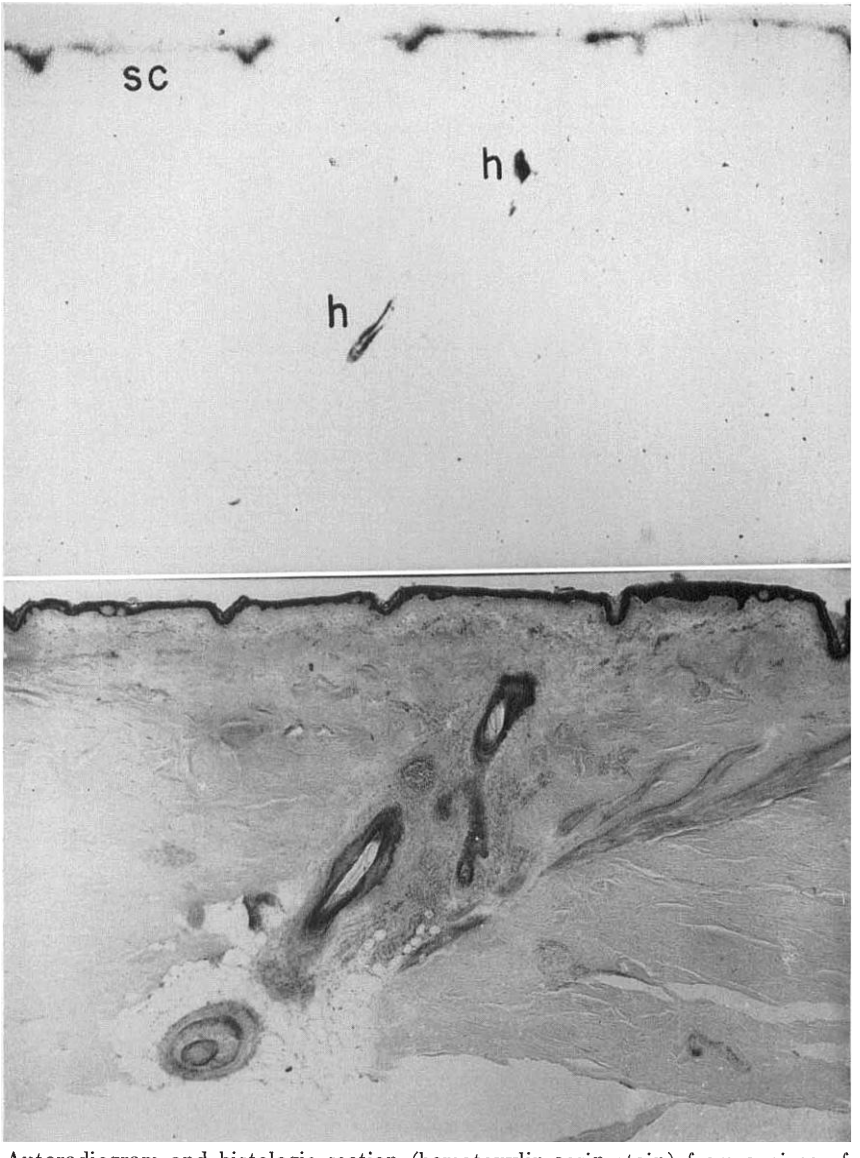


FIG. 3. Autoradiogram and histologic section (hematoxylin-eosin stain) from a piece of skin into which radioactive sodium dodecyl sulfate has penetrated. Magnification: 40×. Shadows: *sc*, stratum corneum; *h*, hair artifact.

10 strippings and the skin frozen for subsequent sectioning and autoradiography. For this autoradiogram the section was held in contact with the radiosensitive slide for 62 days before the radiosensitive slide was developed.

In Figure 2, very dense, discrete, circular shadows can be seen in the epidermis and upper dermis (e). These shadows can hardly have been produced from scattered radiation from sodium laurate in the stratum corneum, as most of the stratum corneum was removed by stripping. Why the shadows appear as patches of various size rather than as a continuous shadow is not known. Under higher magnification nothing unusual can be seen in the epidermis at the sites of the dense shadows. Less dense shadows are seen in the sebaceous glands (s) and still less dense in the sweat gland (difficult to reproduce photographically). Quite dense shadows are seen to correspond to the position of the blood vessels (v) in

the upper dermis. There is a generalized shadow throughout the dermis which cannot be seen at this magnification. The large-particle shadow, corresponding to the hair (h), is a stress artifact and is not caused by radioactivity, but rather by pressure of the hair against the emulsion.

With increased amounts of laurate in the sebaceous glands, sweat glands and sweat ducts (no ducts are seen in this autoradiogram), one might conclude that these were the avenues of penetration of the surfactant. We believe, however, that as yet this is not necessarily a justifiable conclusion. Certainly the blood vessels which show a dense shadow were not avenues of penetration. The route of penetration might have been directly through the epidermis and after diffusion throughout the dermis, the surfactant may have accumulated in those tissues for which it had the greatest affinity. This appears to us to be the most satisfactory explanation for the shadow in the blood vessel area. One cannot yet say, however, whether the surfactant reached the sebaceous gland through the hair follicle directly or through the epidermis, with subsequent diffusion through the dermis to the sebaceous gland.

Figure 3 shows an autoradiogram from a piece of excised abdominal skin which had been exposed to 5.0 ml of 0.005 M solution of sodium dodecyl sulfate for 20 hours at room temperature. The total radioactivity was 4.7 μc. The initial pH of the solution was 9.9.

At the end of the contact period, the surfactant solution was discarded and the skin handled in the same way as the skin in the sodium laurate autoradiography experiment, except that only three strippings were made and the section of the skin was held in contact with the radiosensitive slide for 25 days.

Figure 3 confirms the previous quantitative experiment which showed very little sodium dodecyl sulfate in the epidermis or dermis. Increased density in the autoradiogram is limited to a relatively continuous shadow in the stratum corneum (sc). No discrete, rounded patches of increased density are seen in the epidermis, as was observed for sodium laurate (Fig. 2). No shadows correspond to sebaceous gland, sweat gland or hair follicles, except two small stress artifacts (h). At higher magnification, little or no increased density is seen in the area of the dermis.

TABLE 2

Amount of surfactant which reaches the dermis after contact with 5.0 ml of 0.005 M solutions of different pH, as determined from radioactivity measurements

Sodium laurate			Sodium dodecyl sulfate		
Initial pH	Final pH	mμM in dermis	Initial pH	Final pH	mμM in dermis
9.3	9.2	2	9.3	9.2	9 (0)*
11.0	10.3	6	11.3	10.3	17 (<14)*
12.2	11.2	189	12.3	11.2	253 (267)*
12.7	12.1	482	12.5	11.8	320 (326)*

* Figures in parentheses determined chemically.

Penetration from Buffered Solutions of High pH

Independent of the initial pH, weak, aqueous solutions of the surfactants studied usually approach a pH of 6 to 8 after overnight contact with the skin, because the skin itself is a good buffer. The pH of the solutions can be held more nearly constant if the surfactants are dissolved in buffer solutions.

In an attempt to evaluate the effect of high pH on penetration, 0.005 M sodium laurate and sodium dodecyl sulfate solutions were prepared in glycine-sodium chloride-sodium hydroxide buffers. The pH of these solutions initially ranged from 9.3 to 12.7, and decreased somewhat following contact with the skin, but not so much as in the case of the unbuffered solutions of surfactants. The rate of change of pH was not studied.

The data shown in Table 2 are from two experiments. The sodium laurate solutions were held on the skin for 21 hours and the sodium dodecyl sulfate solutions for 18 hours. When the solutions were removed, those skins whose epidermis had been in contact with the more highly alkaline solutions of either surfactant (pH 11.0 or higher) were swollen, and the epidermis was visibly damaged. The electrical conductivity of these pieces of skin exceeded 500 μA. The epidermis was often loosened from the dermis; routine stripping techniques were not possible. Determinations of surfactant in the epidermis were not reliable; only the surfactant found in the dermis is shown in Table 2. There is good agreement between the amounts of sodium dodecyl sulfate as determined chemically and as determined by radioactivity measurements.

In this experiment, relatively little sodium laurate or sodium dodecyl sulfate has reached the dermis from buffered solutions in which the final pH did not exceed 10.5. When the pH is 11.0 and higher, large amounts of both surfactants reach the dermis, probably after the protective barrier has been damaged. There is an apparent discrepancy between the penetration of sodium laurate from an unbuffered solution whose final pH was 7.6 (Table 1) and the limited penetration from the buffered solution with a final pH of 10.3 (Table 2). Penetration from buffered solutions of low pH and unbuffered solutions maintained at a high pH have not yet been studied.

DISCUSSION

The data presented in this paper are taken from single representative experiments. The conclusion should not be drawn that these data show the average amount of surfactant which may penetrate into the skin and reach the various layers. As might be expected, the amount of surfactant which penetrates varies from experiment to experiment. The variation is large and no average figure would be significant unless a large number of experiments were run.

Even though there may be appreciable variation from subject to subject, it is possible to compare the penetration of two different surfactants into adjacent pieces of skin from the same subject, as has been done in the experiments reported in this paper. Many experiments of this type have confirmed the data reported here, which show that sodium laurate seems to be able to penetrate from unbuffered aqueous solution, but that little or no sodium dodecyl sulfate reaches the dermis from a similar solution of this salt. From the data of comparable experiments (to be reported later), it can be seen that the sodium salts of alkyl sulfates other than dodecyl sulfate appear not to penetrate either from solutions of a pure chemical compound or from a solution of mixtures of alkyl sulfates of various chain lengths. The alkyl benzene surfactants, which represent another class of synthetic surfactants, have also failed to penetrate under experimental conditions similar to those described for the study of the alkyl sulfates. We have not studied the salt of any fatty acid other than lauric.

The ability of proteins to combine with the anions of synthetic detergents has been reviewed in detail by Putnam (7); more recently Stüpel and Szakall (8) have discussed the pertinent data on the subject as they apply to the action of soaps and detergents on the skin. It seems reasonable to assume that the ability of the proteins of the stratum corneum to bind the alkyl sulfates and alkyl benzene sulfonates is at least one mechanism whereby the skin is able to prevent the penetration of these compounds.

A mechanism which is thought to be involved in penetration is the solubility of the penetrating substance in cutaneous lipides. At the pH of the cutaneous surface, some of the sodium laurate is converted to lauric acid (9). Lauric acid is only slightly ionized and might be expected to be soluble in cutaneous lipides, and it may, therefore, be the un-ionized lauric acid which penetrates into the skin from aqueous solutions of sodium laurate. Dodecyl sulfate, on the other hand, functions as a strong acid and is more highly dissociated.

It still remains to be shown that the data obtained for penetration into excised human skin can be applied to problems of penetration into the intact skin of living humans. If such data do apply, then the data reported in this paper, which show that sodium laurate can penetrate into the epidermis and dermis, help explain why this substance can irritate the skin. As yet there are no data to show either the concentration at which sodium laurate or lauric acid damages epidermal cells, or the concentration of these salts within the epidermis which results from penetration from outside the barrier. To explain how sodium dodecyl sulfate can irritate normal skin is difficult since so little of this substance appears to be able to penetrate any part of the skin. If irritation does result from contact with this chemical, very low concentrations must be able to damage epidermal cells, or very small breaks in the barrier must allow higher concentrations to reach the epidermal cells.

SUMMARY

From weak, unbuffered, mildly alkaline aqueous solutions, sodium laurate seems to be able to penetrate into the epidermis and dermis of excised human skin. From similar solutions, sodium dodecyl sulfate, on the other hand, appears to penetrate only in very small quantities below the barrier of normal undamaged skin and for the most part to be retained in the stratum

corneum. When in quite alkaline, buffered solutions (pH > 10.5) both of these surfactants penetrate into the dermis. At this alkalinity the natural barrier is damaged.

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DISCUSSION

Dr. Peter Flesch (Philadelphia, Penna.): Some years ago we did some work on the percutaneous absorption of agaric acid which may be considered a surface active agent with a 16 C atom lipophilic and a hydrophilic citric acid grouping. In spite of all efforts we were unable to force this substance through the epidermis.

In a work with detergents it is important to distinguish between percutaneous penetration and combination with epidermal proteins which may cause considerable damage to the barrier. In other words, the detergent may enhance the penetration of water and other compounds, without penetrating itself into the dermis. This view is also in keeping with Szakall's recent work in which he showed that the barrier function is effectively destroyed by certain detergents. Therefore the damage from detergents may be an indirect effect and may be due to removal of essential water-soluble components from the epidermis by the aqueous medium.

Dr. Stephen Rothman (Chicago, Ill.): Sodium laurate is alkaline and sodium lauryl sulfate is neutral. I wonder if the difference in absorption may have something to do with this difference in the initial pH. Also, it was not quite clear to me as to whether after scraping off the horny layer there was a clear-cut increase in absorption. Theoretically, one would not expect such increase unless the barrier is damaged.

Dr. Richard B. Stoughton, (Cleveland, Ohio): It is difficult to determine whether the material measured by Dr. Blank is in the corium, or in the hair follicles, or areas apart from the corium. I want to ask Dr. Blank how he gets around this problem, because we are interested in such experiments ourselves.

Dr. Irvin H. Blank (in closing): I agree entirely with Dr. Flesch's underlying thesis that it is important in any study of percutaneous absorption to inquire whether or not the penetrating substance can injure the skin's normal defenses against penetration. If it is capable of injuring the major barrier or any other part of the skin's defense mechanism, then any penetration which is observed may have occurred subsequent to the injury and the fact that penetration has been observed should not be construed as indicating that the normal skin is permeable to the substance. We believe that the penetration we observed for surfactants at high pH occurred subsequent to barrier damage. We are not yet willing to say that the damage resulted from the removal of water-soluble components of the skin or from combination of the surfactants with epidermal proteins. We are not yet sure by what mechanism the surfactant was able to damage the skin.

In answer to Dr. Rothman's first question, it is correct that an aqueous solution of pure sodium laurate is more alkaline than an aqueous solution of the sodium salt of dodecyl sulfate. When we compared the penetration from aqueous solutions of these two compounds we added small amounts of alkali to the sodium dodecyl sulfate solution so that the two solutions had the same pH at the beginning of the experiments. Since

these particular solutions are unbuffered, their pH decreases from about 10 to about 7.5 when they are in contact with skin overnight. We are now studying the rate of change of pH. As Dr. Rothman indicated, penetration from a sodium laurate solution does not necessarily mean that sodium laurate itself has penetrated. Actually, we have followed only the radioactive carbon atom, but we believe that during penetration this carbon atom remains with the laurate radical. When the pH of a sodium laurate solution drops, the amount of non-ionized lauric acid in the solution increases and under these conditions it may be molecules of lauric acid which penetrate. This increase in the amount of lauric acid can be prevented by using alkaline-buffered solutions.

I believe that Dr. Rothman's second question and Dr. Stoughton's question can be answered together. Dr. Stoughton correctly points out that in the separation of epidermis and dermis, which we made, epidermal appendages often remain in the dermis. He asks if the surfactant which we stated was in the dermis was in the true dermis or in the epidermal appendages remaining in the dermis as we analyzed it. I believe that some of the surfactant was in each area. I would be more concerned about this if I were presenting data in support of penetration into the dermis. Remember that for sodium dodecyl sulfate (other than strongly alkaline solutions) I am emphasizing the negative results of our work,—very little surfactant is found in that portion of the skin we have called dermis. We are sure that for sodium laurate, penetration into the true dermis has occurred. This can be seen well in the autoradiograms. Not much shadow is seen in the hair follicles, but a considerable increase in density of the silver granules can be seen to correspond to the area of the true dermis. This is more apparent when the autoradiograms are viewed under higher magnification than could be used in preparing the slides for projection.