Effect of Polyoxyethylated Materials on the Interaction of Surfactants with Skin

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ABSTRACT

The effect of surfactants on skin has been investigated by means of two experimental techniques, viz., by permeability studies using isolated neonatal rat stratum corneum membranes, and by studies on the reduction of the electrophysiological potential across freshly excised frog skin membranes. Permeability studies indicate that typical cationic and nonionic surfactants are weak penetrants, unlike anionic surfactants, as exemplified by sodium lauryl sulfate (SLS), which readily penetrates and tends to destroy the integrity of stratum corneum membranes in a matter of hours. The addition of polyethylene glycols (PEG) or nonionic surfactants to solutions of SLS results in a considerable reduction in the last mentioned effects, the reduction tending to increase as the molecular weight and ethylene oxide content of the additive increase. By contrast with permeability, the electrophysiological measurements show that cationic surfactants can be extremely active: the typical surfactant, cetyltrimethyl ammonium bromide (CTAB), at a level of 0.5% in Ringer solution, destroys the potential across frog skin in minutes, and, indeed, is comparable in this respect to SLS. Nonionic surfactants are comparatively inactive, and their addition, like that of PEGs, to the ionic surfactant reduces the effect of the latter significantly. Preapplication of a solution of PEG to the membrane, rather than incorporation in the solution of the surfactant, affords better protection against the latter as judged by both permeability and potential criteria.

INTRODUCTION

The development of surfactants which are mild, i.e., nonaggressive towards humans, continues to be an important objective of the detergent and personal care industry. Likewise, agents which can be used as additives to reduce the irritancy of surfactants are continually sought. These factors underscore the need for reliable methods of testing for mildness of surfactant formations, i.e., outside of panel testing on human subjects.

In order for a surfactant to elicit a physiological response when applied to living mammalian skin, it is safe to assume that the agent must modify the outer layer of the skin by delipidization and interaction with the keratin, and/or penetrate through the epidermal layer into the dermis. The former process is expected to cause changes in the topography of the skin leading to rough "feel" (1); the latter is involved when there is an erythemal response attending entry of the aggressive agent into the living region of the skin, i.e., the region of living cells, capillaries and so on.

In this paper we present information on the use of two test methods evidently concerned with the latter of the above processes. The first test method involves a study of the rate of permeation of surfactants through stratum corneum membranes. In previous work (2) we have studied both the sorption of sodium lauryl sulfate by such membranes (and their consequent swelling) and its penetration through the membranes. This latter method is pursued in the current work and we will show, in confirmation of scattered references in the literature (3), that certain polyoxyethylated materials can significantly reduce the penetration. The second test method, viz., destruction of the electrophysiological potential across freshly excised frog skin (4) by surfactants, is used as a subsidiary technique to the first to confirm (or otherwise) the effect of the primary surfactant and that of the additive in the permeability test. Most of the work, with both methods, employed sodium lauryl sulfate—the "standard" surfactant for skin irritation testing. Differences observed between results obtained by the two methods are expected to allow inferences to be made concerning response mechanisms.

EXPERIMENTAL

Materials

Membranes. Neonatal rat stratum corneum membranes (NNRSC), from animals purchased from Marland Breeding Farms, West Milford, NJ, were separated by methods previously described (5). The source of the frog skin was the common American species, Rana Pipiens. The specimens, of body length 3-5", were obtained from NASCO Educational Materials, Ltd. and Mogul ED, Ltd., Wisconsin. They were stored in chlorine-free water at ca. 13 C and, just before use, were carefully washed, sacrificed, and the abdominal skin removed. The latter was washed, stored in Ringer solution and used within one hour of their dissection.

Surfactants. Sodium lauryl sulfate (SLS) was a pure (>99%) sample from B.D.H. Myristyldimethyl benzyl ammonium chloride (Barquat MS-100) was obtained from

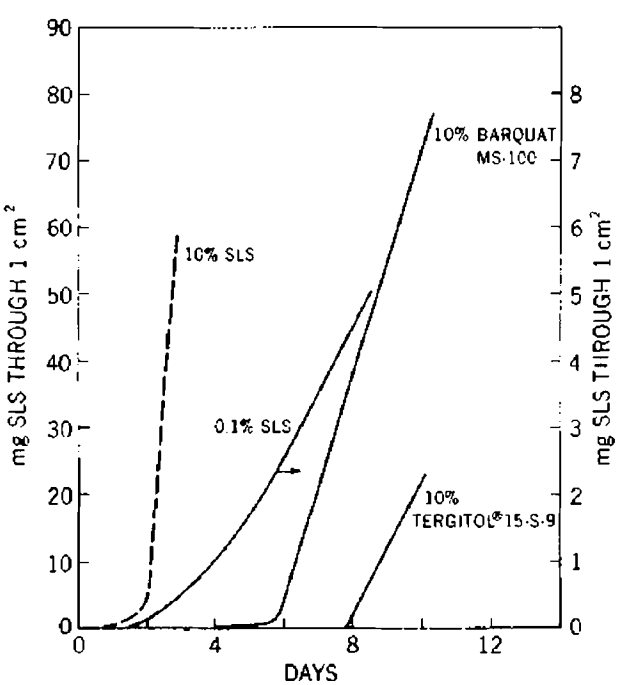

FIG. 1. Permeation of SLS, Barquat MS-100 and Tergitol® 15-S-9 through neonatal rat stratum corneum.

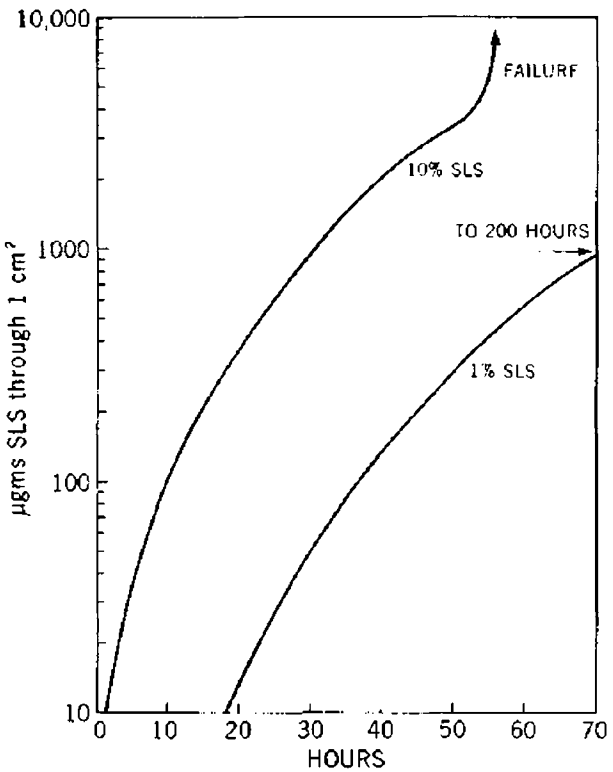

FIG. 2. Permeation of SLS through NNRSC membranes.

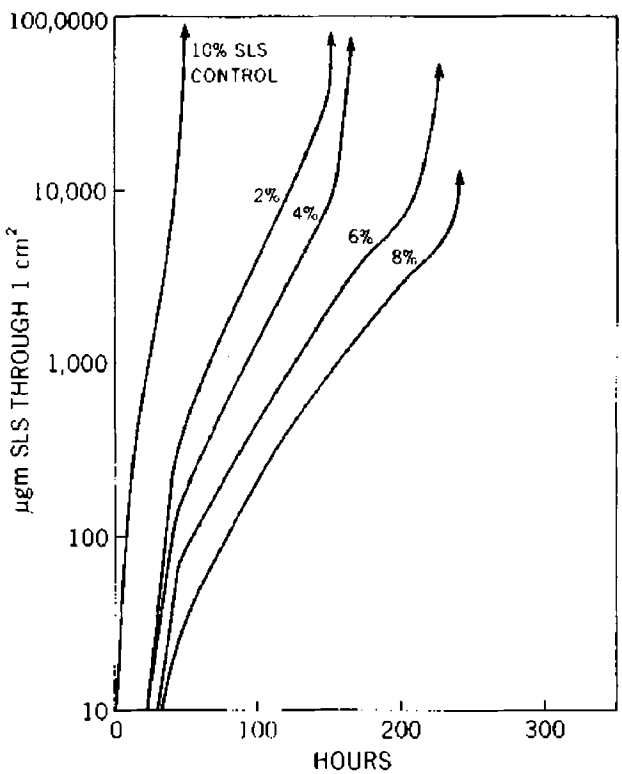

FIG. 3. Permeation curves for 10% SLS solutions with varying amounts of PEG 20,000; NNRSC membranes.

Lonza, Inc., and was used without purification. Cetyl trimethyl ammonium bromide (CTAB), used in the membrane potential measurements as the "standard" cationic surfactant, was a pure (>99%) sample from Fine Organics. The nonionic surfactants used were products of Union Carbide Corporation: Tergitol® 15-L-3, -L-9, and -L-20 are 3, 9, and 20 mole (av.) ethoxylates of C_{12-15} primary alcohol; Tergitol® 25-S-9 is the 9 mole (av.) ethoxylate of C_{11-15} random secondary alcohol. The Carbowax® polyethylene glycols (PEG) were also products of Union Carbide; their numerical designation refers to their approximate molecular weight.

Method

Details of the electrophysiological technique and principle (6) are given in the Appendix. The permeability test method which we have described before (2) employed a cell modelled after that of Loveday (7). The method involves a time study of the amount of surfactant which crosses a stratum corneum membrane separating the chambers of a two compartment cell. Surfactant solution was contained in the upper compartment and, intially, distilled water in the lower. Radiotagged material was purchased from Amersham-Searle (Des Plains, IL) in the form of small individual ampoules. Each ampoule contained 2.47 mg of SLS with an activity of 100 microcuries. The tag was present as the S-35 isotope. Solutions of desired concentration were made up of the nonradioactive powder, and one ampoule was added with stirring. For permeability experiments, a small sample (0.1 g) of the water in the lower part of the cell was removed by pipette and put in a one-ounce counting vial filled with the scintillant liquid, INSTAGEL. Radioactivity was determined by scintillation counting in a Packard 3255 Counter. Analysis of the other surfactants (see below) was carried out spectrophotometrically, Barquat MS-100 at 262 nM and Tergitol® 15-S-9 at 337 nM. Barquat MS-100 was in fact chosen for its ease of determi-

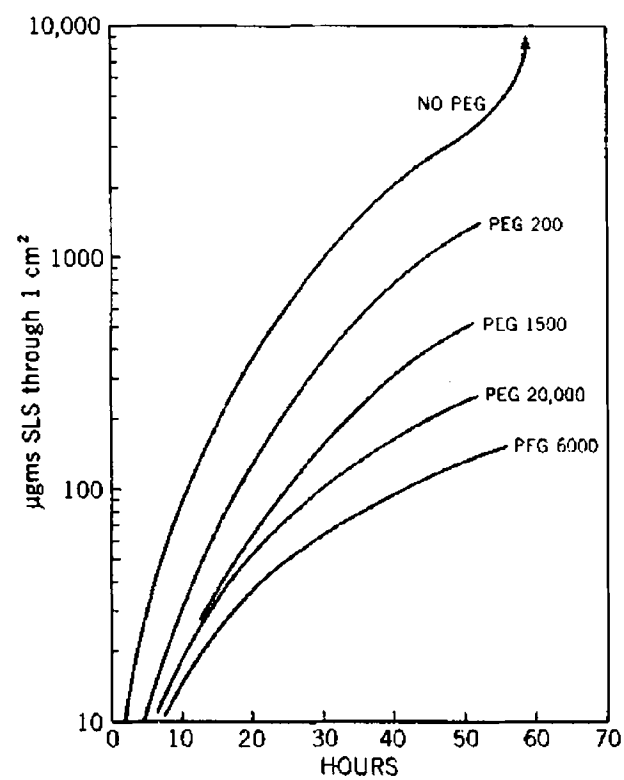

FIG. 4. Permeation curves for 10% SLS solutions containing 5% of various PEGs; NNRSC membranes.

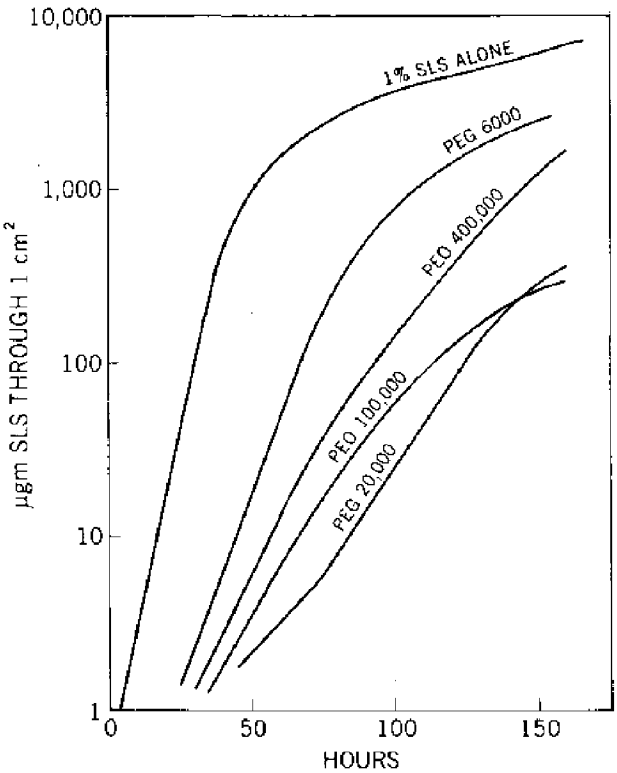

FIG. 5. Permeation curves for 1% SLS solutions containing 1% of various PEGs; NNRSC membranes.

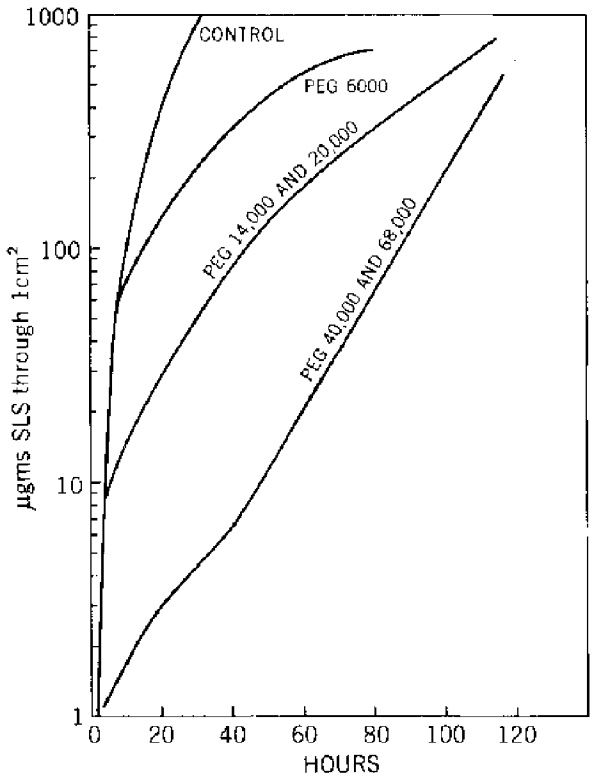

FIG. 6. Permeation of 10% SLS through NNRSC membranes pretreated with 50% solutions of various PEGs.

nation by this method. The pH of the (unbuffered) surfactant solutions ranged from 6 to 8.

RESULTS

Skin Permeation

In Figure 1 plots of the permeation of SLS, Barquat MS-100 and Tergitol 15-S-9 are given. By comparison with the SLS, the cationic and nonionic surfactants show extremely limited permeability: no significant transport across the membrane was detected before 4 days. Accordingly, no further work by the permeability method was carried out on these surfactants as the primary penetrant.

In considering the effects produced by SLS on stratum corneum, it is important to note that this surfactant gradually attacks the skin membrane, increasing its permeability with time (2,8). The amount of attack is proportional to the concentration of the surfactant in solution. Because of this situation, a single measure of permeation (e.g., permeability constant) does not specify very well the state of the skin nor the efficacy of a material which reduces SLS penetration. Instead, it is instructive to consider a curve of the amount penetrated vs. time. Figure 2 shows the control data obtained for NNRSC exposed to both 1% and 10% SLS solutions. The logarithmic scale for the ordinate is useful, since the amount of SLS going through the membrane varies by several orders of magnitude as time progresses.

Most panel testing of surfactants utilizes rather concentrated aqueous solutions (~5% by weight) of these surfactants. A level of 10% concentration for the SLS solution was chosen for much of the present work on permeation. As can be seen in Figure 2, the skin fails catastrophically in about 2 days when exposed to this concentration if no protective additive in present. The addition of PEG to such solutions was found not only to decrease the absolute amount of SLS which penetrates, but also to greatly extend the lifetime of the membrane. For example, Figure 3 shows the effects of progressive increases in the amount of PEG 20,000 added. It can be seen that the presence of the latter at 2% concentration (20%, expressed by weight of the SLS) leads to a significant reduction of penetration and extension of useful membrane life. These effects increase progressively with PEG concentration.

Next, the effect of PEG molecular weight was investigated. A standard level of 5% in solution of various grade PEGs was added to 10% SLS solutions. The results in Figure 4 show that increasing PEG molecular weight, up to about PEG 6,000, brings about lower penetrations of SLS: a small reduction in efficacy is apparent on further increasing the PEG molecular weight to 20,000. At the 5% PEG level, it was not feasible to employ higher molecular weights, since the overall solution viscosity becomes too high. To circumvent this, the concentrations of both surfactant and PEG were lowered to 1%. Under these conditions, PEG 20,000 had a much stronger effect than PEG 6,000, but Figure 5 shows that further increases in PEG molecular weight led to somewhat reduced effects.

The addition of relatively large amounts of PEG to surfactant solutions is a somewhat inefficient way of reducing penetration. Hence, a method of preapplication was tried in order to maximize the effect. In this procedure, 0.1 ml of PEG solution was spread on the surface of the stratum corneum (area of 1.75 cm^2) and allowed to stand for 15–30 min. The amount of PEG left on the skin can be controlled by changing the concentration of the applied solution. Figure 6 shows data for solutions of 50% water/50% PEG by this method. Again, there is a general tendency for higher molecular weights to be more effective in reducing penetration. It was not feasible to go higher than PEG 68,000 because of viscosity problems at this concentration level. The long times and low permeation fi-

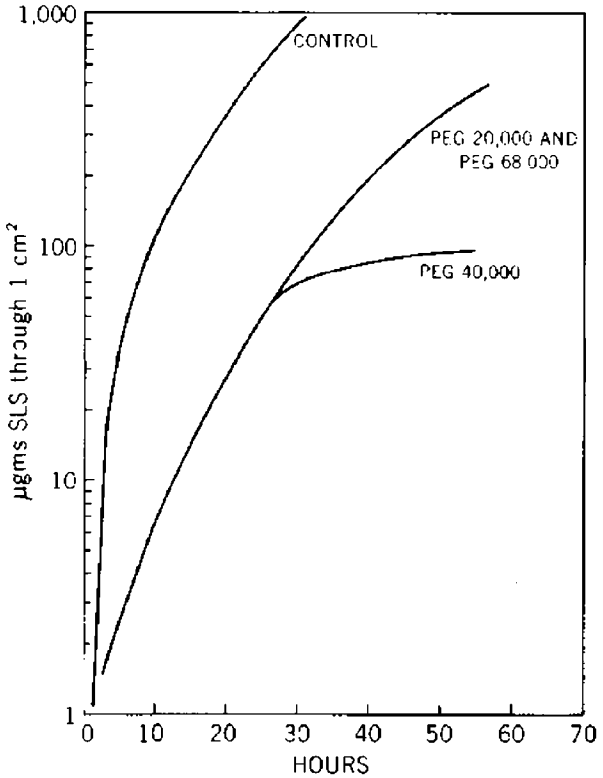

FIG. 7. Permeation curves for 10% SLS through NNRSC membranes pretreated with 20% solutions of various PEGs.

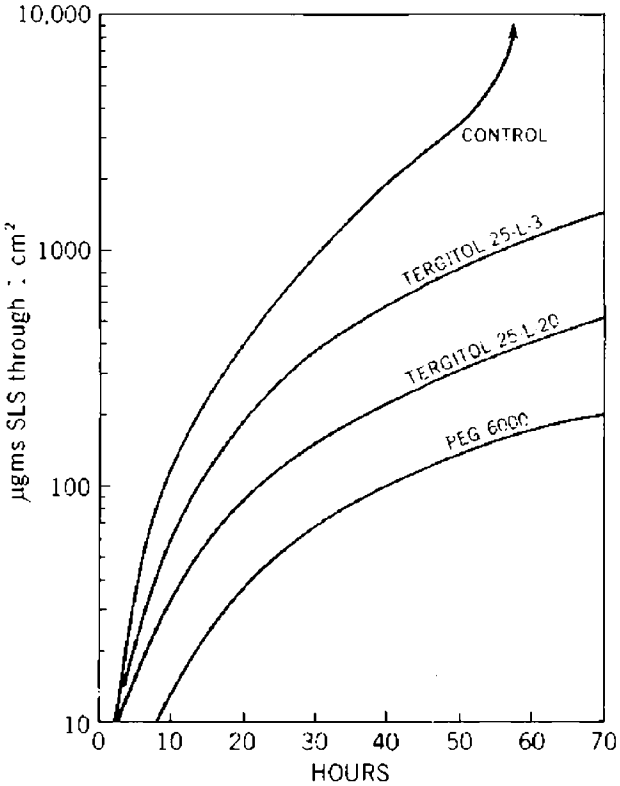

FIG. 8. Permeation curves for 10% SLS solutions containing 5% of various ethoxylated compounds; NNRSC membranes.

gures are testimony to the very effective action of PEG in the preapplication method.

When the concentration of the preapplied solution was lowered to 20%, an effect was still noticed, but it became difficult to distinguish any difference among the various molecular weight grades; see Figure 7. The amount of PEG applied in this case was ca. 10 mg per cm² of skin surface. However, it was still sufficient to reduce the SLS penetration 10 fold. Ethoxylated nonionic surfactants were also tested as additives to SLS in the permeability cell. Both preapplication and addition to solution were successful in reducing SLS penetration. Thus, Figure 8 shows the case of two of these materials added to 10% SLS solution. It is to be noted that the material with more ethylene oxide is more effective. Indeed, we have shown that many different types of ethoxylated materials, including block and random copolymers of ethylene oxide and propylene oxide, perform in this manner. Although the differences are sometimes slight, it is our experience that the most effective materials of all are the high molecular weight PEGs.

Frog Skin Membrane Potential

Results obtained by the electrophysiological potential technique (4,6) are given in Figure 9 and Figure 10 (surfactants at 0.5% concentration). The potentials are normalized to a starting value of 100% in order to compensate for skin-to-skin variation in the original potentials (see Appendix). While confirming the relative inactivity of the nonionic surfactant, this technique shows the quaternary ammonium surfactant to be highly active (more so than SLS) in destroying the poential across the frog membrane. The addition of as low a level as 0.005% CTAB completely destroyed the potential in ca. 60 min. It should be noted that, once the potential is destroyed in this way, repeated washing of the skin with fresh Ringer bathing solution will not restore it. In other words, the surfactants irreversibly impair the active metabolic sodium transport reactions which generate the potential. Comparison of the results of the two figures shows that an increase in concentration from 0.005% to 0.5% of both SLS and CTAB results in a considerable increase in decay rate of the potential. Our previous results indicate a rapid increase in the potential decay rate as the (ionic) surfactant concentration is increased through the critical micelle concentration (CMC); above the CMC the potentials can be reduced by 90% in less than 5 min (4). In the sulfate Ringer solution, the CMC values of SLS and CTAB are 0.04% and 0.015%, respectively (4). Because of the very rapid potential decay, further testing was limited to concentrations no higher than 0.5%.

Figures 10 and 11 show the effect of including PEG 6,000 at the level (1%) employed in the experiments referred to in Figure 5. As in the latter case, the presence of the polyglycol leads to a consistent reduction of the surfactant effect. The polyglycol alone is substantially without effect on the membrane potential. It is also evident that pretreating the skin membrane with a 1% PEG 6,000 solution has a slightly increased effect over that obtained when it is dissolved at this level in the SLS solution.

Finally, a test of the effect of adding a nonionic surfactant, Tergitol® 25-L-9, to SLS (both at 0.5%) confirms that the nonionic, like polyglycols, reduces the potential destroying action of the SLS. See Figure 12.

In Vivo Testing

The model experiments above show that several poly ethoxylated materials decrease the penetration of SLS through the skin under in vitro conditions. We confirmed by in vivo tests that this correlates with reduced skin irritation. Two volunteers used daily preapplications

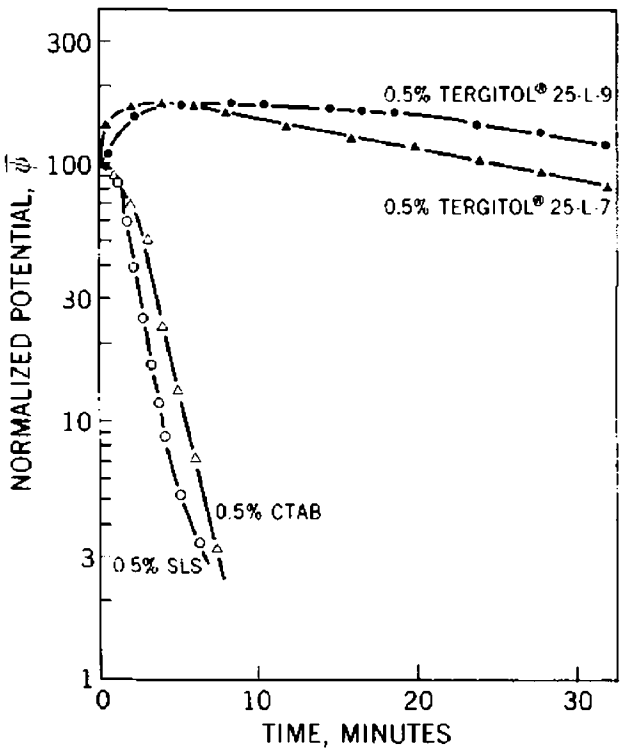

FIG. 9. Time course of potential (frog skin) in the presence of various surfactants.

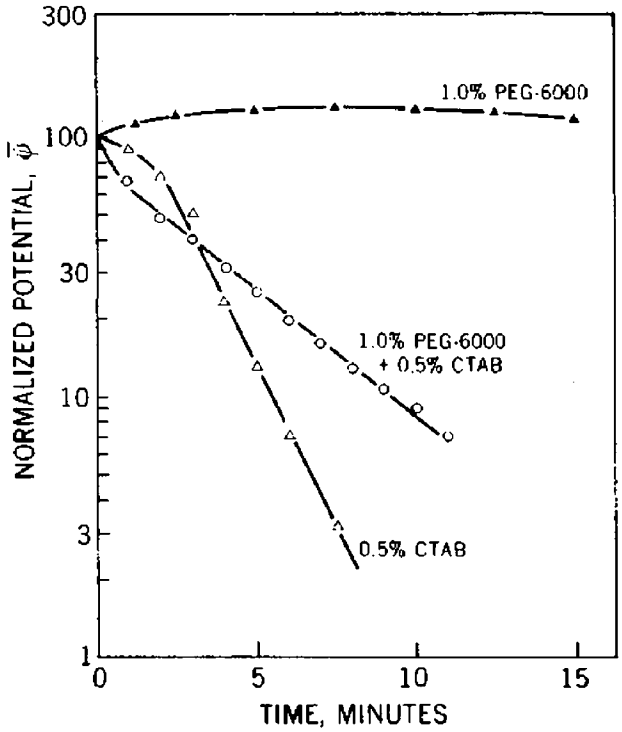

FIG. 10. Time course of (normalized) potential across excised frog skin in the presence of CTAB and PEG-6000.

of 50% PEG 20,000 on small patches of the forearm. After each preapplication gauze soaked in 5% SLS solution was allowed to contact the skin occlusively for 1 hr. These patches showed no reddening or irritation over a period of 3 days. However, control patches on the same volunteers that were not treated with PEG showed severe reddening and irritation in the same period. We point out that Garrett (3) successfully correlated the reduction of sorption of anionic surfactants by hide powder in the presence of ethoxylated nonionic surfactants with a reduction in in-vivo irritation.

DISCUSSION

It has been shown that sodium lauryl sulfate readily penetrates stratum corneum membranes, but that a representative cationic surfactant and a nonionic surfactant do not. In this respect this behavior parallels the known behavior of these surfactant types on skin: SLS is a recognized topical irritant and our in vivo tests have confirmed this; quaternary ammonium surfactants, on the other hand, are reported to be comparatively nonirritating when topically applied (9) as are nonionic surfactants. Evidently in order to elicit a physiological response, such as erythema or edema, when applied to the skin, a surfactant must be able to penetrate through the skin. The high level of intrinsic biological activity of cationic surfactants is well known, as evidenced by their potency as germicidal and lytic agents (9). The results obtained via the electrophysiological measurements are in full accord with the latter. They show that ionic surfactants, especially cationic surfactants, irreversibly impair the functioning of the frog skin cells, presumably by interfering with the active metabolic sodium transport reactions which generate the potential. The alternative explanation that ionic surfactants destroy the potential by altering the permeability of the membrane is inadequate since measurements showed an insignificant change in tritium transport across a membrane exposed to 0.5% SLS.

We conclude that, from the point of view model test methods for skin irritation, the permeability and potential methods are to some extent complementary. The former will give an indication as to whether a surfactant is likely to be able to cause irritation, i.e., to enter the sensitive sublayers of skin. The latter is expected to indicate the damage a surfactant can cause once it has penetrated, and may be more relevant to behavior on impaired (chapped, cracked, erythemic or swollen) skin. It should also be considered as a possible model test for irritation of eyes and mucous membranes.

The application of PEGs as anti-irritants is a logical extension of the interesting inquiry begun in the 1960s by H.E. Garrett (3). He showed, for example, that the addition of certain ethoxylated nonionic surfactants to sodium lauryl sulfate decreased the rate of sorption of the latter by the model substrate, hide powder. Also, he successfully correlated this phenomenon with in vivo reduction of irritation. Practical use of Garrett's findings has been made in the hair care industry: the addition of nonionics to anionic shampoos in order to reduce eye irritation is becoming a common practice (10).

The reduction of surfactant penetration and of membrane potential destruction brought about by PEGs and nonionic surfactans can be considered to be part of the general phenomenon of "anti-irritation." Although this behavior has been observed for several chemical substances, there is little understanding of the action(s) involved. A recent review by Goldemberg and Safrin (11) discusses possible mechanisms, which are brought under three headings.

1. Prevention of intimate physical contact of irritant and skin (occlusion).
2. Complexation.
3. Blocking of otherwise reactive sites on the skin.

These three categories are considered in turn:

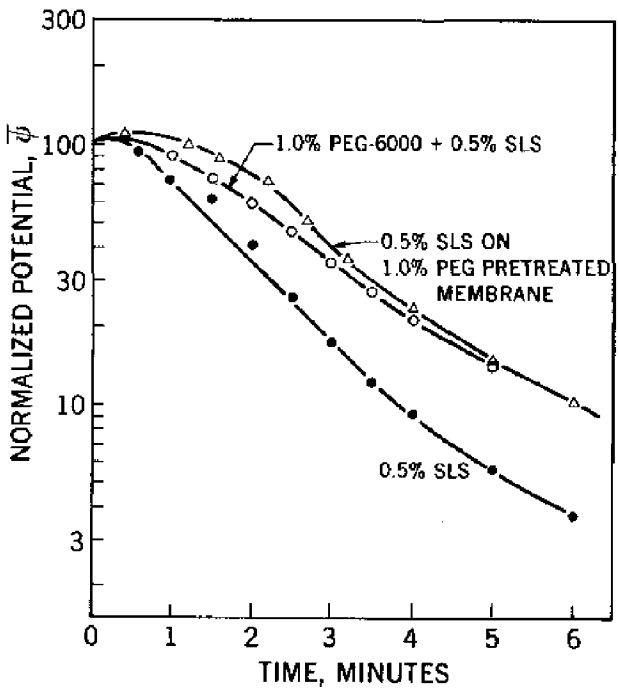

FIG. 11. Time course of potential (frog skin) in the presence of SLS and PEG-6000.

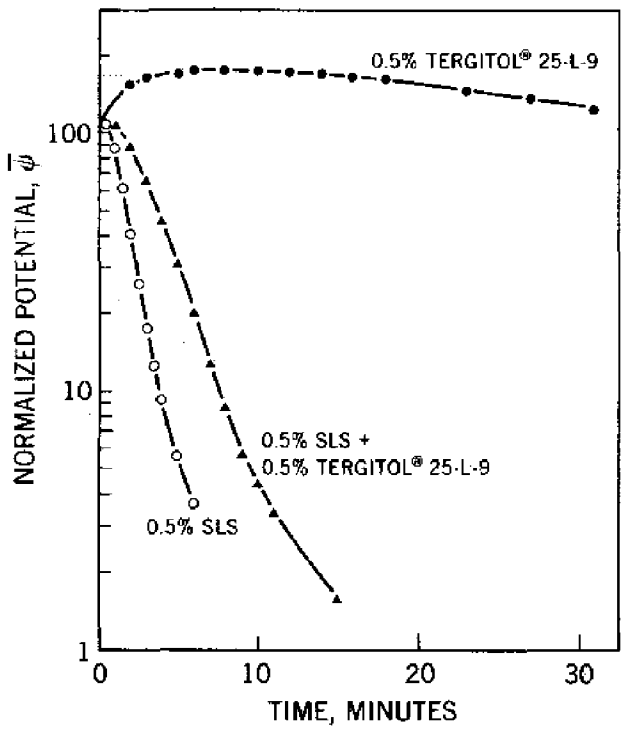

FIG. 12. Effect of Tergitol® 25-L-9 in reducing the decay of potential (frog skin) caused by SLS.

1. Prevention of contact or occlusion does not seem to fit the observed facts very well. Neither PEGs nor nonionic surfactants can be regarded as effective film formers on the skin. Also, these materials are effective anti-irritants when merely dissolved in solution, and under these conditions occlusive films are unlikely to form.

2. Complexation seems at first like a reasonably good explanation for the anti-irritation and permeability reduction phenomena. It is known, for example, that there is a solution interaction between PEG and SLS (12,13). And, in fact, a minimum molecular weight of ca. 6,000 is necessary to observe such interactions. However, it is not at all clear that such complex formation will be very effective in reducing penetration of the surfactant. To test this, Visking cellulose casing was put in the permeability cell instead of rat stratum corneum. Numerous experiments showed that neither addition of PEG to SLS solution nor pretreatment of the casing by PEG solution had any appreciable effect on passage of SLS. These experiments appear to rule out complexation and indicate in a general way that there is some specific effect between the PEG and the stratum corneum. On the other hand, with added nonionic surfactants, reduction of transport of SLS by mixed micelle formation (i.e., nonspecific complexation) can be anticipated since nonionic surfactants have a low critical micelle concentration (CMC) and thus would lower the monomer concentration of SLS (14). However, the larger effect seen with the higher ethoxylated (higher CMC) surfactant in Figure 8 suggests that ethoxylate content, per se, may be more important than the mixed micelle effect.

3. As regards the "blocking" mechanism, comments at this point can only be speculative. However, the data are consistent with a mechanism involving sorption of polyoxyethylated (PEO) materials, either themselves or as complexes with SLS, onto sites of the keratin which normally attract SLS and which are thereby involved in the transport of SLS across the keratin membrane (15). On the basis of such a mechanism, if PEO material in solution is effective, then preapplied PEO should be much more effective, as was indeed observed. Preliminary results we have obtained indicate a finite uptake of PEGs by hide powder, but more data are required before rigorous conclusions can be made.

APPENDIX

The Frog Skin Membrane Potential Technique

Principle of the Method. When a freshly excised, "still living" frog abdominal skin is bathed in aerated Ringer solution, a steady state potential is developed (6) under open circuit conditions. The magnitude of this potential, with the innerside of the skin electrically positive in relation to the outside, can be as high as 100 to 150 mv (6,15). The origin of such potentials is thought to be metabolic sodium and potassium transport reactions associated with active sodium transport or the "sodium pump" process (17–19) by the skin cells. This causes spontaneous transport of sodium ions from the outside to the inside of the skin, even against an electrochemical gradient. Such active sodium transport is accompanied by passive transport of other anions and cations along their electrochemical gradient. The magnitude of passive ion transport is directly proportional to the permeability of the membrane to the specified ions. The overall generated potential, Ψ, under open circuit conditions can be represented as $\Psi = J_R/\lambda$, where J_R is the ionic flux caused by the sodium pump mechanism and λ is the membrane permeability.

The existence of such potentials is vital to the functioning of animal systems (20) and is associated with a number of coupled transport processes (18), control of cell volume (17), and so on. Introduction of surfactant molecules, which interfere with metabolic reactions responsible for the sodium pump, can destroy the generated potential. In the cases of skin, the rate of potential destruction is likely to be one indication of the agressiveness of the surfactant towards the skin cells.

Apparatus. The apparatus used for the electrometric

measurement is similar to that developed by Ussing (4,16). In brief, the cell consists of two conical compartments, each of 20 ml capacity, separated and sealed from each other by the membrane under investigation. These compartments are filled with "sulfate" Ringer solution of pH 8 (111.2 mM Na_2SO_4, 2.0 mM K_2SO_4, 1.0 mM $CaSO_4$, and 2.6 mM $NaHCO_3$) to an appropriate level. Stirring and simultaneous aeration are achieved by a rising stream of bubbles from purified air supplied to each compartment.

The electrical potential measuring device consists of two calomel sleeve junction reference electrodes which are coupled to each of the half cell compartments by agar bridges. These reference electrodes in turn are connected to a Keithley 610A electrometer coupled to a chart recorder (7132A, Hewlitt-Packard).

The experiments were conducted by first carefully mounting the membrane in between the two half cell compartments. This was followed by addition of 25 ml sulfate Ringer solution to each half cell and aeration of these cell compartments until a steady state potential was recorded. Next, an aliquot of surfactant stock solution (generally 1 5 ml) was added to the outer compartment to obtain the required surfactant concentration. To avoid any hydrostatic disturbances across the membrane, the surfactant solution addition was accompanied by the simultaneous addition of an equal volume of sulfate Ringer solution to the inner compartment. All the surfactant stock solutions were prepared in the sulfate Ringer solution. Sulfate Ringer solution was preferred over the conventional chloride Ringer solution as the membrane is less permeable to sulfate ions than to chloride ions, and hence a higher skin potential which is stable and less sensitive to hydrostatic pressure gradients is generated.

Actual starting potentials varied appreciably from skin to skin, and use of normalized potential, in which the starting value is set at 100%, was found to facilitate comparison of surfactant effects and to yield good reproductibility for a particular treatment. In the absence of added surfactant, the skin potential maintained its value to within 10% even after 8 hr.

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