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Factors which determine the skin irritation potential of soaps and detergents

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Synopsis—Skin-surfactant interactions have been examined using specific laboratory tests with a series of pure SURFACTANTS. Effects of these compounds upon the STRATUM CORNEUM have been studied by means of KERATIN denaturation and the extraction of PROTEINS and AMINO ACIDS. It was found that strongly ANIONIC SURFACTANTS, such as sodium LAURYL SULPHATE, sodium LAURYL ETHER SULPHATE and sodium LAUROYL ISETHIONATE (*Igepon* A) had considerable activity, by virtue of their polar head groups, whereas sodium laurate and non-ionic ethoxylates had minimal effect upon the stratum corneum. The effect of lipophilic chain length of the surfactants was important in their overall activity, in particular, the lauryl moiety.

PERCUTANEOUS ABSORPTION of RADIOACTIVELY-LABELLED surfactants by guinea-pigs *in vivo* has been studied; sodium laurate and lauryl triethoxylate penetrated to a far greater extent than other compounds: lauryl hexaethoxylate, sodium lauroyl isethionate and sodium lauryl triethoxy sulphate, had lower penetrabilities and sodium lauryl sulphate and sodium LAURYL SULPHONATE were lower still. The effect of pure surfactants upon living cells was studied by means of measuring HISTAMINE release from rat peritoneal MAST CELLS *in vitro*. ALKYL SULPHATES, ALKYL ETHER SULPHATES and alkyl tri- and hexaethoxylates were potent mast cell lysins, whereas monoethoxylate and sodium laurate and sodium lauroyl isethionate were less effective. Chain-length studies showed that the capryl, lauryl- and myristyl moieties were the most potent lipophilic groups for releasing histamine.

Some of these surfactants were applied directly to the skin of RATS and the overall skin response determined by visual examination. Sodium laurate caused erythema after 24 h applica-

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tion, when other surfactants (sodium lauryl sulphate, ether sulphate, isethionate and non-ionic lauryl triethoxylate) had no effect. After 3 days of application, sodium lauryl sulphate had the greatest effect upon the skin in terms of dryness, scaling and cracking of the stratum corneum erythema and oedema. Correlation of these results are discussed.

INTRODUCTION

The skin irritation potential of soaps and detergents (hereafter termed surfactants) is an expression of the complex interactions occurring in the various regions of the skin following its exposure to solutions of these compounds. Often, in the skin of surfactant-treated laboratory animals (generally after exaggerated application) one may observe signs of primary cutaneous irritation, a response manifested by drying, scaling or even cracking of the stratum corneum, and oedema and erythema in the dermis. In this paper the complex response of skin has been defined in terms of the various types of skin-surfactant interactions which may occur and may contribute to the overall skin response. Specific laboratory test methods have been devised to study some of these interactions. In particular, the effects upon the stratum corneum have been examined by studying the way in which a series of highly pure model surfactants may denature keratin, a phenomenon which may contribute to the observed superficial skin roughness. Also, the extraction of soluble compounds from the stratum corneum by washing with surfactant solutions has been measured and related to the removal of natural moisturizer from the horny layer, an effect which may give rise to a lower water-binding capacity (1) and consequently a lower flexibility (2).

The rates of percutaneous absorption (skin penetration) of highly pure radioactively-labelled surfactants have been measured using guinea-pigs *in vivo*. Skin penetration of surfactants is a prerequisite of their causing a response in the living cells of the epidermis and underlying dermis (3). The interaction of surfactants with the living cells of the epidermis and dermis has been studied indirectly by measuring the ability of highly pure surfactants to release histamine from rat peritoneal mast cells *in vitro*. Histamine release from mast cells is an initial reaction in the development of erythema (4).

In each of these experimental methods on aspects of skin-surfactant interactions we have obtained evidence that the observed experimental result is related to the chemical structure of the surfactant used.

The overall response of rat skin *in vivo* to solutions of pure model surfactants has been assessed macroscopically, and the compounds have been ranked according to their observed irritation potential. It was found that notable differences between the various experimental tests existed which did not correlate with macroscopic results. Explanations for these differences are discussed.

Methods and results

Determination of sulphydryl groups (SH) liberated from human callus by treatment with surfactant solutions

If surfactants in contact with the stratum corneum can cause denaturation of the keratin, this may be assessed by measuring the increase in SH groups as the proteins unfold. The procedure of Harrold (5) was followed, with little modification, in which liberated SH was determined with 1-(4-*p*-chloromercuri - phenylazo) - naphthol - 2. Human callus (obtained from a chiropodist) was powdered and used with a series of pure surfactants all of which possessed the lauryl (12-carbon) lipophilic chain but with a variety of hydrophilic head groups. Two concentrations of each were studied (1 mM and 10 mM), the lower being at or below the determined critical micelle concentration of the surfactants (see *Tables VI* and *VII*). The results shown in *Table I* revealed that all of the anionic surfactants liberated more sulphydryl than water alone, but generally only at 10 mM. The three alcohol ethoxylate non-ionic surfactants did not denature the keratin. The results obtained were very similar to those reported by Harrold in that sodium lauryl sulphate, dodecyl benzene sulphonate, soap and non-ionics (in that order) possessed decreasing abilities to liberate sulphydryl. Also, as reported by Harrold, they had very little or no effect below the critical micelle concentration. From these data it is clear that the sulphate or sulphonate moiety of the surfactants imparts upon the molecules the ability to unfold keratin. In a second experiment we examined a series (C_{10}–C_{16}) of sodium alkyl carboxylates (soaps), alkyl isethionates and alkyl sulphates. In each instance considerably more SH was liberated from the keratin than by water. For all three groups of surfactants the C_{12} and C_{14} chain homologues showed maximum activity, but, nevertheless, it seems that the anionic head groups are the principle cause of keratin denaturation by surfactants.

Table I. Denaturation of human callus by surfactants

Surfactant	Concentration (mM)	SH liberated/g keratin (μmol)	% increase relative to water
Sodium laurate	1.0	1.071	0.0
	10.0	1.567	38.1
Sodium lauroyl isethionate	1.0	1.135	0.0
	10.0	2.049	84.5
Sodium lauryl sulphate	1.0	1.135	0.0
	10.0	2.03	78.9
Sodium lauryl sulphonate	1.0	1.407	24.0
	10.0	1.998	76.0
Sodium lauryl monoethoxy sulphate	1.0	1.135	0.0
	10.0	1.982	74.6
Sodium lauryl triethoxy sulphate	1.0	1.47	9.9
	10.0	1.583	39.5
1-(p-benzene sodium sulphonate)-dodecane	1.0	1.311	15.5
	10.0	1.583	39.5
6-(p-benzene sodium sulphonate)-dodecane	1.0	1.055	0.0
	10.0	1.839	62.0
Lauryl mono-ethoxylate	1.0	1.135	0.0
	10.0	1.135	0.0
Lauryl triethoxylate	1.0	0.96	0.0
	10.0	0.975	0.0
Lauryl hexa-ethoxylate	1.0	0.959	0.0
	10.0	0.975	0.0
Distilled water	—	1.135	—

Keratin denaturation was determined by measuring the release of sulphydryl groups (SH) after exposure to surfactants. The method of Harrold (5), using human plantar callus keratin was followed.

The extraction of materials from the stratum corneum by surfactant solutions

The technique described by Smeenk and Polano (6, 7) was followed using the Vermeer (8) washing simulator on guinea-pig dorsal skin *in vivo*. Each animal served as its own control, the left flank being washed with 20 ml distilled water and the right flank with 20 ml of the surfactant solution under test. The machine is illustrated in *Fig. 1*.

After washing, the wash liquors were analysed for soluble protein and amino acids and *Table II* shows the amounts extracted (expressed as mg

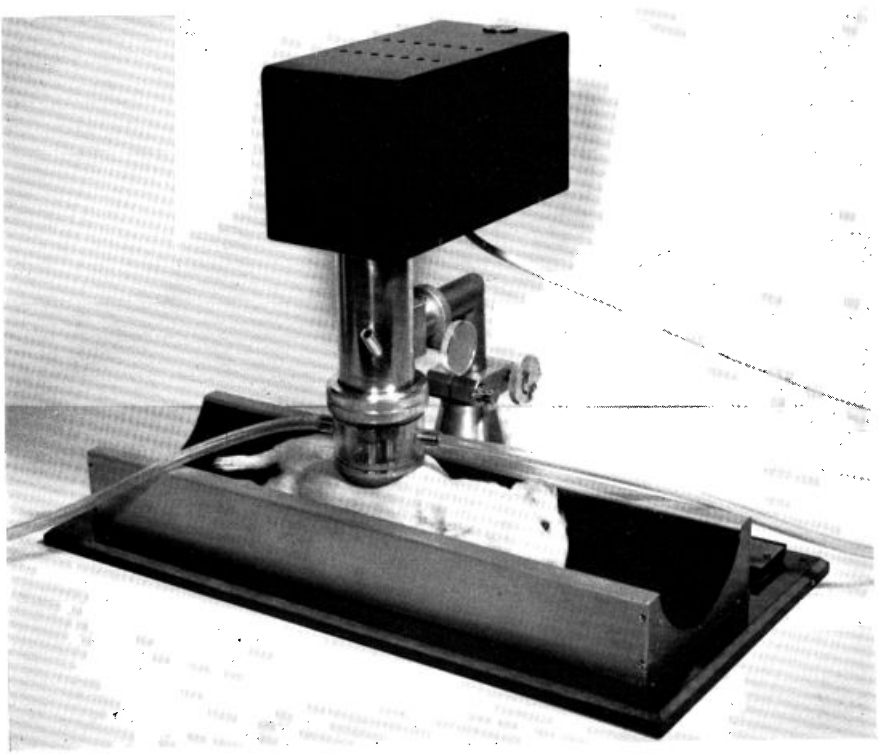

Figure 1. Use of Vermeer washing simulator on guinea-pig skin *in vivo*.

(*Facing p. 32*)

Table II. The extraction of amino acids and protein from the skin of guinea-pigs after washing with various surfactants of 12-carbon chain length

Animal no.	Flank washed	Washing solution (25 mM)	Protein extracted (as mg bovine serum albumin)	Amino acids extracted	
				(as mg phenyl-alanine)	% increase relative to water
21	Left	Water	0.0	13.1	75.0
	Right	Sodium laury sulphate	119.6	22.8	
31	Left	Water	0.0	13.2	8.0
	Right	Sodium lauroyl isethionate	52.4	14.2	
32	Left	Water	0.0	9.9	6.0
	Right	Sodium laurate	2.6	10.5	
33	Left	Water	0.0	13.5	0.0
	Right	Sodium lauryl triethoxy sulphate	22.6	13.2	
34	Left	Water	0.0	13.3	0.0
	Right	Sodium lauryl monoethoxy sulphate	75.08	13.2	
35	Left	Water	0.0	8.2	31.0
	Right	Lauryl mono-ethoxylate	0.0	10.8*	
36	Left	Water	0.0	6.4	134.0
	Right	Lauryl tri-ethoxylate	0.0	14.9*	

Washing performed in a washing simulator (see *Fig. 1*) designed by Vermeer *et al.* (8), for 5 min at 22° using 20 ml of solution: area of skin washed = 13.86 cm^2.

* Turbidity of these solutions make amino acid values doubtful.

bovine serum albumin for the proteins and mg phenylalanine for the amino acids) for various surfactants of 12-carbon chain length. Although water alone consistently removed no detectable soluble proteins from the skin, in the case of the four sulphated surfactants studied there were appreciable amounts eluted, by sodium lauryl sulphate in particular. The property of soluble protein extraction may be conferred by the anionic groups generally, as the two non-ionic compounds and sodium laurate (a weak anion) were without effect. Sodium lauryl sulphate was also the most efficient compound at eluting amino acids from the skin, no other surfactant

(with the possible exception of the lauryl mono- and triethoxylate) removed any more than that removed by water itself. Preliminary studies showed that only above the critical micelle concentration of the surfactants was there any increased extraction of amino acids and protein from the stratum corneum.

To investigate the effect of lipophilic chain length upon extraction an homologous series of sodium alkyl sulphates and sodium soaps were studied and the results are shown in *Table III.*

Table IIIa shows that all alkyl sulphates, especially those of chain length 11, 12 and 13, removed more amino acids and soluble proteins from the skin. Sodium lauryl sulphate was the most effective. *Table IIIb*, for the sodium soaps showed a similar trend, but here sodium myristrate removed far more soluble protein, and sodium laurate removed more amino acids from the skin than other members of the homologous series. The overall conclusions to be drawn from this study were that the ability to remove compounds from the skin, which probably contributes directly to the irritancy of a surfactant, is a function of both the nature of the polar head group and the length of the lipophilic chain, but the nature of the head group polarity of surfactants seems to be more important than length of the lipophilic chain in determining whether surfactants are able to extract materials from the stratum corneum.

Table IIIa. Effect of lipophilic chain length on ability of alkyl sulphate surfactants to extract material from the skin during washing

Alkyl sulphate (25 mM)	% increase in extraction relative to washing with water	
	Soluble protein	Total amino acids
Sodium nonyl sulphate (C9)	50.8	62.7
Sodium decyl sulphate (C10)	166.1	84.2
Sodium undecyl sulphate (C11)	119.5	100.4
Sodium dodecyl sulphate (C12) (sodium lauryl sulphate)	238.9	194.8
Sodium tridecyl sulphate (C13)	198·5	141·7
Sodium tetradecyl sulphate (C14)	163.9	110.3
Sodium pentadecyl sulphate (C15)	77.9	41.3

Experimental conditions resembled those described in *Table II*, except that total soluble protein in wash liquors was measured by the Folin-Ciocalteu method and total amino acids by a spectrocolorimetric assay, both of which are more sensitive than uv absorption as used in *Table II.*

Table IIIb. Effect of lipophilic chain length on ability of soaps to extract material from the skin during washing

Soap (25 mM)	% increase in extraction relative to washing with water	
	Soluble protein	Total amino acids
Sodium caprylate (C8)	125.7	0.0
Sodium caprate (C10)	196.8	43.3
Sodium laurate (C12)	186.7	234.9
Sodium myristate (C14)	792.8	147.5
Sodium palmitate (C16) ((insoluble at 22°)	147.6*	160.5*

Assay methods as in *Table IIIa.*

* Presence of insoluble soap make these readings doubtful.

Percutaneous absorption of [^{14}C] *labelled surfactants through guinea-pig skin* in vivo

As little is known of the rates of penetration of surfactants through the skin of live animals we studied a series of radioactively-labelled pure surfactants: these were applied to the dorsal skin of guinea-pigs and the amounts penetrating the skin barrier determined. The radioactive surfactants were all of 12-carbon chain length, labelled with ^{14}C at the α(1)-carbon position of the alkyl chain, the point of linkage to the head groups. These were either purchased from the Radiochemical Centre, Amersham, or synthesized in our laboratory by Mr C. T. James. The precise methods of synthesis and details of the penetration studies will be published elsewhere (9), and closely resemble the method described by Howes in the previous paper (11).

The fate of the eight cutaneously applied compounds during the 24 h following application is shown in *Table IV.* Attempts were made to account for all of the radioactivity applied to the animals and in most cases the percentages recovered are reasonable. In every case, by far the most radioactivity was accounted for in the skin rinsings, on the non-occlusive patches or bound to the skin of the animals at the original sites of application. With the exception of lauryl hexaethoxylate and lauryl alcohol there was much activity remaining in the skin. No attempts were made to determine if this activity was in the epidermis or dermis (i.e. whether it represented

Table IV. Distribution of radioactivity during 24 h following application of [^{14}C] labelled surfactants to skin of guinea-pigs

Surfactant applied to skin (μCi)	Recovered radioactivity (%)*									
	Exhaled CO_2	Urine	Faeces	Kidney	Liver	Carcass	Skin at site	Patch	Rinsings	Total
Sodium lauryl sulphate (16.3)	0.1	0.1	0.0	0.0	0.0	0.0	50.2	2.3	53.4	106.2
Sodium lauryl sulphonate (7.8)	0.0	0.1	0.05	0.0	0.0	0.0	38.3	2.1	66.5	107.1
Sodium lauryl triethoxy sulphate (9.8)	0.6	0.5	0.3	0.0	0.05	0.0	56.9	2.0	62.0	122.4
Sodium lauryl isethionate (17.9)	0.7	0.4	0.1	0.0	0.0	3.3	39.6	2.5	37.9	84.5
Lauryl triethoxylate (6.8)	0.2	17.9	1.9	0.05	0.1	0.0	29.2	0.3	40.7	90.3
Lauryl hexaethoxylate (7.4)	0.1	1.3	1.1	0.0	0.0	0.0	4.8	0.1	85.1	92.5
Lauryl alcohol (4.1)	1.7	0.1	0.1	0.0	0.0	0.0	4.7	0.8	27.9	35.3
Sodium laurate (12.2)	17.0	0.5	0.1	0.0	0.2	3.8	26.9	3.0	40.3	88.2

Radioactive surfactants were applied in 0.6 ml of water (3 μmol) to an area of 22.5 cm² on the flanks of guinea-pigs. After 10 min of rubbing the treated areas were washed with water, then covered with non-occlusive patches for 24 h.

* Mean of four animals.

radioactivity which had actually penetrated the stratum corneum but still resided in the skin). Levels of radioactivity in the blood were also measured on samples obtained by cardiac puncture immediately before death of the animals and in no case was any discernible radioactivity found.

In order to calculate the rates of percutaneous absorption separate animals received similar doses of each labelled surfactant as were applied cutaneously, by intraperitoneal injection. The proportion of the known intraperitoneal dose excreted in a given time in urine, faeces and exhaled CO_2 was determined and used to calculate the amount of absorption through skin by dividing the excretion from the cutaneously treated animals by the excretion from the intraperitoneal treated animals, exactly as described by Howes in the previous paper (11).

Assuming that the sum of urinary, faecal and exhaled CO_2 radioactivity was related to *penetrated* surfactant, and knowing the similar values for the intraperitoneally administered experiments, the overall percentage of radioactivity applied which penetrated was calculated by dividing the former values by the latter. Thus, in *Table V* it is seen that, for example, about 25% of the lauryl triethoxylate applied to the skin penetrated during 24 h (20%÷81.9%), whereas for sodium lauryl sulphate the amount was less than 0.4% (0.3%÷84.9%). These values were standardized by expressing them as permeability constants (10), that is, the amount of surfactant penetrating per unit area per minute per unit concentration applied. Thus, sodium laurate and lauryl triethoxylate penetrate at relatively very high rates. The more strongly anionic sodium lauroyl isethionate penetrates at about one-tenth of this rate, whereas sodium lauryl sulphate penetrates at a rate approaching two orders of magnitude less than soap. Thus, the presence of strongly anionic head group in the surfactant molecules strongly impairs their ability to penetrate through the skin. Howes (11) has described the effect of different chain length upon penetrability of surfactants.

Histamine release from mast cells by surfactants

Histamine release from isolated mast cells *in vitro* has been studied extensively. For example, Frisk-Holmberg (12) demonstrated that certain lipophilic drugs effected its release, and Bloom and Haegermark (13) showed that the surfactant decylamine was an effective mast cell lysin. Here we have studied isolated rat peritoneal mast cells in contact with a variety of model surfactants. The methods used closely resemble those reported by

Table V. Rates of percutaneous absorption of pure surfactants

Surfactant	% intraperitoneal dose excreted in CO_2, urine, and faeces (X)	% cutaneously applied dose excreted in CO_2, urine and faeces (Y)	% penetration of cutaneously applied dose (Y/X)	Permeability constant (cm min^{-1})
Sodium lauryl sulphate	84.9	0.3	0.353	0.065×10^{-6}
Sodium lauryl sulphonate	73.2	0.15	0.205	0.038×10^{-6}
Sodium lauryl triethoxy sulphate	58.7	1.4	2.38	0.44×10^{-6}
Sodium lauryl isethionate	74.7	1.2	1.6	0.3×10^{-6}
Lauryl triethoxylate	81.9	20.0	24.4	4.52×10^{-6}
Lauryl hexaethoxylate	58.8	2.5	4.25	0.75×10^{-6}
Lauryl alcohol	67.0	1.85	2.76	0.51×10^{-6}
Sodium laurate	70.2	17.6	25.1	4.64×10^{-6}

For explanation of the calculation of permeability constants (10), see text.

many others for the study of specific histamine-releasing agents (14), and full details are to be published elsewhere (15).

There was always a very low level of spontaneous histamine release from the mast cells on incubation (generally less than 10%) in the absence of surfactants. However, as the concentration of surfactant was increased, a level was reached when there was rapid release of the stored histamine (up to 85% of the total). Further increase in the concentration of surfactant did not release more. The relationship between a series of surfactants, all with a 12-carbon lipophilic chain, but with a variety of polar headgroups, and histamine-releasing potential is shown in *Table VI.* Sodium lauryl

Table VI. The ability of 12-carbon chain length surfactants of various polar head groups to release histamine from rat mast cells *in vitro*

Surfactant	Concentration (mM) at which histamine was released	Critical micelle concentration in buffer at 22° (mM)
Lauryl alcohol	No effect at 1 mM	Water insoluble
Lauryl monoethoxylate	0.2–0.5	0.1
Lauryl triethoxylate	0.03	0.03
Lauryl hexaethoxylate	0.02–0.05	0.035
Sodium lauryl sulphate	0.03	1.0
Sodium lauryl monoethoxy sulphate	0.05	0.15
Sodium lauryl triethoxy sulphate	0.05	0.2
Sodium laurate	0.4	10.0
Sodium lauroyl isethionate	0.15	1.2

sulphate and its mono- and triethoxy derivatives were the most potent anionic surfactants, and lauryl tri- and hexaethoxylates (non-ionic surfactants) were equally as effective at similar concentrations. However, sodium laurate and sodium lauroyl isethionate were far less potent by almost an order of magnitude. For the anionic surfactants the concentrations to cause mast cell degranulation (i.e. histamine release) were consistently below the critical micelle concentration (CMC).

The effect of chain lengths of different surfactants upon histamine release was studied and shown in *Table VII.* Of the anionic surfactants the series of alkyl soaps, alkoyl isethionates, alkyl sulphates and ether sulphates

Table VII. Effect of various alkyl chain length moieties of surfactants upon histamine releasing potential of surfactants

Surfactant	Concentration (mM) at which histamine was released	Critical micelle concentration (mM)
Sodium caprate	1.0	1.3
Sodium laurate	0.4	10.0
Sodium myristate	0.5	6.0
Sodium caproyl isethionate	0.75	6.5
Sodium lauroyl isethionate	0.15	1.2
Sodium myristoyl isethionate	approx. 0.5	not determined
Sodium capryl sulphate	0.5	9.0
Sodium lauryl sulphate	0.03	1.0
Sodium myristyl sulphate	0.025	2.5
Sodium capryl monoethoxy sulphate	0.5	1.0
Sodium lauryl monoethoxy sulphate	0.05	0.15
Sodium capryl triethoxy sulphate	0.2	1.5
Sodium lauryl triethoxy sulphate	0.05	0.2
Sodium myristyl triethoxy sulphate	0.02	0.06
Capryl monoethoxylate	0.2–0.5	1.0
Lauryl monoethoxylate	0.2–0.5	0.1
Myristyl monoethoxylate	no effect at 0.1, insol. above	0.013
Capryl triethoxylate	0.075	0.8
Lauryl triethoxylate	0.03	0.03
Myristyl triethoxylate	0.03	0.18

and of the non-ionics the alkyl mono- and triethoxylates, all revealed that 12 or 14 carbons in the lipophilic chain imparted the greatest lytic potential.

It is likely that the differences seen between members of homologous series of surfactants, or for those with various headgroups, are due to the intrinsic physical properties of the compounds in solution, such as polarity, hydrophilic-lyophilic balance (HLB), partition-coefficient between oil and water, detergency etc.

Observed irritancy of model surfactants to rat skin in vivo

Some of the pure surfactants used in the *in vitro* tests described above were also applied directly to the shaved dorsal skin of weanling rats as 0.25 M solutions (representing between 5 and 10% solutions by weight). Applications were twice daily for 3 consecutive days, similar to the method

previously described (16). After 1 day and 3 days of application the degree or irritation was assessed macroscopically in terms of erythema and oedema, scaling and cracking of the stratum corneum and drying of the stratum corneum superficially. The results are shown in *Table VIII.* When the effects of each surfactant were compared, after only 1 day's application, sodium laurate was by far the most irritant compound by virtue of the intense erythema and oedema which resulted. Indeed, none of the other surfactants showed differences from the water treatment. After 3 days, however, there was a much different picture. By this time sodium lauryl sulphate treatment was seen to have caused thickening of the epidermis with scaling and cracking of the stratum corneum. Sodium laurate also exhibited these changes but to a lesser degree and sodium lauryl triethoxy sulphate was the only other surfactant to cause erythema and oedema after 3 days, and then this was very slight. Both sodium lauroyl isethionate and (to a lesser degree) lauryl triethoxylate caused superficial dryness to the stratum corneum after 3 days, but there were no signs of accompanying inflammation (erythema and oedema).

Overall, the irritancy of these surfactants could be ranked relative to water as follows: sodium lauryl sulphate $\gg$ sodium laurate $>$ sodium lauryl triethoxy sulphate $>$ sodium lauroyl isethionate $\geqslant$ lauryl trieth-oxylate.

With *in vivo* irritancy tests such as this, however, one must pay due regard to the significance of the various components of the overall irritation response. For example, moderate erythema may represent significantly greater irritation to the skin than a moderate drying of the stratum corneum. Also, the duration of application is important: changes such as cracking and thickening of the skin probably arise secondarily to inflammation, due to hyperproliferation of the epidermis. If applications had been for 1 day only, then sodium laurate would have been adjudged to be the most irritant of the surfactants studied, by virtue of the rapidly developing erythema, whereas when sufficient time was allowed for the complete irritation phenomenon to develop (3 days or longer) then the more drastic effects of sodium lauryl sulphate were evident.

Discussion

It has been suggested by Bettley (3) that irritancy to skin by surfactants is governed by their percutaneous penetrability and their toxicity to the

Table VIII. The irritancy of surfactants when applied to rat skin *in vivo*

Surfactant	Application time (days)	Subjective assessment of irritation*				
		Erythema	Oedema	Thickening of epidermis	Scaling and cracking of corneum	Superficial drying
Sodium lauryl sulphate	1	0	0	0	0	0
	3	++	++	+++	++	+
Sodium laurate	1	+++	++	0	0	0
	3	+	++	+	+	+
Sodium lauryl triethoxy sulphate	1	0	0	0	0	0
	3	+	+	0	0	0
Sodium lauroyl isethionate	1	0	0	0	0	+
	3	0	0	0	0	++
Lauryl triethoxylate	1	0	0	0	0	0
	3	0	0	0	0	0
Water	3	0	0	0	0	0

Each determination is the mean value of three animals.

* Degree of irritation was recorded as: 0, no discernible reaction; +, slight reaction; ++, moderate reaction; +++, strong reaction.

living cells of the skin, and so two of the laboratory methods we have employed deal specifically with percutaneous absorption and effect upon living cells (mast cells) in order to investigate this suggestion. In addition, we have included experiments designed to study the effect of surfactants upon the horny layer (namely, denaturation of keratin and extraction of corneum components), for, as the results in *Table VIII* show, the overall response of rat skin to exaggerated treatment with surfactant solutions is comprised of responses in the stratum corneum as well as in the living cells beneath. We attempted to use the data from the various experimental procedures (*Tables I–VII*) to predict the irritation potential of surfactants. For example, *Table VI* shows both lauryl triethoxylate and sodium lauryl sulphate to be equally potent as histamine-releasing agents upon mast cells, but as the former compound had a permeability constant of almost two orders of magnitude greater than the latter, we would have expected the nonionic triethoxylate to be far more irritant to the skin than the alkyl sulphate. *Table VIII* shows that this is not the case, however. Lauryl triethoxylate invoked no skin response after repeated cutaneous application, whereas after 3 days sodium lauryl sulphate had a pronounced effect, both in terms of denaturation of keratin and in extraction of proteins and amino acids. This would suggest that in defining an experimental approach to enable one to predict irritancy, one must consider other aspects of skin-surfactant interactions than merely penetration and effect upon the living skin cells.

The results in *Table VIII* show that the overall skin reponse to the five surfactants may be ranked in decreasing order of magnitude: sodium lauryl sulphate $\geqslant$ sodium laurate $>$ sodium lauryl triethoxy sulphate $>$ sodium lauroyl isethionate $\geqslant$ lauryl triethoxylate. However, when the various tables (*I–VII*) listing data from the experimental methods are examined, nowhere may one find a similar ranking of skin response to these surfactants, and, as such, must throw doubt upon the usefulness of these approaches for evaluating irritation potential of surfactants. We would suggest the following reasons for these differences.

Firstly, the conditions necessary for the full response to develop (*Table VIII*) were repeated application twice daily, for 3 consecutive days, and not merely a single application. Using the Vermeer washing simulator we have found that if the washing procedure was repeated daily for several days on guinea-pigs, surfactants such as sodium lauryl sulphate continued to extract more components than, say, sodium laurate, and so cumulative action of surfactants would affect the skin's ability to replace quickly and

fully natural moisturizer etc. removed during this washing. Equally, the fact that we have found some surfactants denature keratin or modify the stratum corneum suggests that the skin may be sufficiently altered after one application to behave quite differently during subsequent applications. Although sodium lauryl sulphate has a very low rate of penetration (permeability constant of 0.065×10^{-6} cm min^{-1}) it is probable that if the animals had been previously washed with this surfactant prior to application of the radioactive compound, sufficient changes in the stratum corneum may have occurred to allow greater amounts to be absorbed. This has certainly been shown to be true for sodium lauroyl isethionate. *Table V* gives a permeability constant of 0.3×10^{-6} cm min^{-1}. When additional guinea-pigs were washed three times with this surfactant on the day prior to the penetration study we found that the average value of the permeability coefficient was 0.92×10^{-6} cm min^{-1}. Thus, frequency of treatment has a direct bearing on observed penetrability. This would suggest that laboratory methods designed to examine individual aspects of the skin's response to surfactants should be designed to resemble normal methods of application of these compounds.

Secondly, in the present study we have examined by no means all of the salient parameters of skin-surfactant interaction. Middleton (1) showed that the amount of lipid extracted from stratum corneum was dependent upon the type of surfactant used, and its removal affected the water-binding capacity and the flexibility of the skin. We have not as yet studied lipid removal by model surfactants. It may well be that this aspect is more important than, say, removal of proteins and amino acids from the stratum corneum. Indeed, the stability of the skin's lipid mantle during washing and its subsequent rate of recovery may be a rate-limiting factor. Equally, we have no precise data on substantivity. If a seemingly non-penetrating surfactant actually tightly binds to the stratum corneum this may in practice leave a large cutaneous pool which gradually penetrates during many hours after a single exposure, and so overall irritation potential would be greater than first thought. Also, we have not considered partition coefficients of the surfactants used: it is possible that solubility in the components of the skin (lipids, aqueous phase) is important to overall irritancy.

Each of the four approaches dealing with individual aspects of overall skin-surfactant interactions we have described, indicate that the chemical structure of surfactants is very important in determining its effect upon the skin. Head-group polarity determines whether a surfactant can denature protein, extract compounds from the stratum corneum and penetrate to the living cells. Also, the length of the lipophilic chain imparts properties of

extraction, penetrability and cell-lytic ability. The concentration of surfactant used is most important, below the critical micelle concentration, when the surfactant behaves as an ideal solution, denaturation and extraction of the corneum is not so important. This suggests that adequate knowledge of the physical chemistry and solution thermodynamics of surfactants is vital to an understanding of how surfactants may invoke a skin response.

The studies reported here suggest that no one experimental procedure can adequately replace that in which surfactants are directly applied to the backs of animals, and the skin's response is assessed by the naked eye. If one wishes to compile a mathematical equation for skin irritancy, this must necessarily be a complex function, and more than just an expression of penetration and cell toxicity. One must also include expressions governing the binding of surfactants to skin, the modification of the stratum corneum which allows greater penetration, the polarity of the surfactants involved, etc. Thus, when one attempts to employ laboratory methods dealing with various parameters of the skin's response to surfactants, one must clearly define the questions being asked by such tests. For example, knowledge of the effect of surfactants upon living cells would have no value in evaluating whether a surfactant formulation had a drying effect upon the stratum corneum, whereas studies on extraction ability would. On the other hand, substantivity and knowledge of penetrability through the skin would be important in deducing whether a compound was able to cause erythema.

The data described above do not permit us to state which type of laboratory test would be the most adequate to give an indication of whether a surfactant is potentially irritant to the skin, rather, we have shown that there are many parameters of the response to be considered, each playing a specific part in the overall phenomenon of skin-surfactant interactions. Similar conclusions have been drawn by Brown (17).

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