THE PERCUTANEOUS ABSORPTION OF SOME ANIONIC SURFACTANTS*

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SYNOPSIS. The irritant action of a surfactant to skin may be related to the ability of that surfactant to penetrate the stratum corneum and act upon the underlying viable tissues.

The percutaneous absorption of some [^{14}C-] labelled anionic surfactants has been measured in vivo in rats, after both consumertype applications and applications of longer duration, and the results have been compared with those from in vitro studies using isolated rat skin and human epidermis.

The methodology for both the in vivo and in vitro studies will be outlined and results will be presented

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금년 런던에서 열렸던 IFSCC 8次 總會에서 發表 되었던 硏究論文 중 陰이온 界面活性劑의 皮膚吸收 및 이에 따른 皮膚刺戟을 動物實驗으로 硏究하여 가장 많은 關心을 일으켰던 本論文을 參考로 게재함.

from experiments with a series of sodium soaps of normal fatty acids, sodium lauryl sulphate, sodium lauryl isethionate and sodium dodecylbenzene sulphonate.

The in vivo techniques can also provide information as to the metabolic fate of topically applied surfactants under user type conditions. The usefulness of the in vitro techniques and their shortcomings will be discussed.

INTRODUCTION

Saponified fats of animal and plant origin are the traditional surfactants used in toilet soaps and in recent years these have been reinforced by synthetic surfactants of higher surface activity. The function of these surfactants is to solubilize and remove sebum, deposited soil and skin debris but the fate of topically applied surfactants is not fully documented especially the amounts which remain on the skin surface or penetrate the skin.

Techniques have been developed to study the percutaneous absorption of both therapeutic and toxic agents through skin but most of these are in vitro methods (1). Although good comparative data are often obtained using in vitro techniques the extrapolation of these data to the in vivo situation is difficult and Wahlberg (2) using guinea-pig skin indicated that there was little correlation between the two situations.

Maibach and his associates (3-5) have used radiotracer techniques for studies in vivo with a wide variety of compounds on both humans and experimental animals and has shown regional and interspecies

variations in percutaneous absorption. Sprott (6) measured the urinary recovery of ^{35}S after topical application of ^{35}S labelled n-alkyl sulphate to rat skin but no other data on surfactant penetration in vivo has been published.

This study reports the results from a series of experiments where the fate of in vivo of topically applied ^{14}C labelled surfactant solutions was compared with in vitro experiments using rat skin and human epidermis. The turnover of the ^{14}C labelled surfactants administered intraperitoneally and subcutaneously to rats is also reported.

MATERIALS AND METHODS

Surfactants

Decanoic acid ($C_{10:0}$), dodecanoic acid ($C_{12:0}$), tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$) and octadecanoic acid ($C_{18:0}$) were obtained from B.D.H. (Poole, Dorset) and were specially pure Biochemical grade. These were converted to their sodium salts by neutralization to pH 9.5 with sodium hydroxide. These acids were also obtained [1-^{14}C] labelled from the Radiochemical Centre (Amersham, Bucks). These [^{14}C] labelled acids were incorporated into a model soap system described below.

Sodium [1-^{14}C] dodecyl sulphate; 5.11 μCi/mg (^{14}C-SDS), sodium [1-^{14}C] dodecoyl isethionate; 1.7 μCi/mg (^{14}C-SDI) and sodium p-1-[1-^{14}C] dodecylbenzenesulphonate; 8.5 μCi/mg (^{14}C-DOBS) were synthesised in this laboratory and were shown to be chemically and radiochemically pure by thin layer chromatography and isotope dilution analysis.

Test solutions

The studies with the ^{14}C-labelled soaps were conducted from a model soap system in which all five soaps were soluble at 37°. This system was a 30 mMolar soap solution containing each of the five soaps at a concentration of 6 mM. Five such soap solutions were made each one containing a different [1-^{14}C] acid. The [1-^{14}C] decanoate soap solution was made in the following manner. The mass of the ^{14}C-labelled acid was determined from its specific activity (14.3 mCi/mM) and total ^{14}C activity in the sample (usually 250 μCi) i.e. 3.004 mg or 3.39 mg of sodium [1-^{14}C] decanoate. A total volume of 4.0 ml of test solution was made up by weighing 5.33 mg $C_{12:0}$, 6.00 mg $C_{14:0}$, 6.67 mg $C_{16:0}$ and 7.34 mg $C_{18:0}$ and (4.66-3.39) i.e. 1.27 mg $C_{10:0}$, soaps into a 'Duall' glass homogenizer (Kontes Glass Co. Ltd). The [1-^{14}C] $C_{10:0}$ acid was added using excess of diethyl ether which was removed in a stream of nitrogen and 4.0 ml of dilute sodium hydroxide solution (172 mg/l) was added. The resulting solution was homogenized and equilibrated for 24 h at 40° before adjusting the pH to 9.5 by addition of 0.01 N NaOH or HCl. The other [^{14}C] soap solutions were made up in a similar manner.

25 mM solutions of the [^{14}C] SDS and [^{14}C] SDI were used throughout the study. Two test solutions of the [^{14}C] DOBS were used, the first a 3 mM solution in 25% v/v Polyethylene Glycol 400 in water and a second a 3mM suspension in water prepared by homogenizing and equilibration in an all glass homogenizer as described for the soap solutions.

Analysis of ^{14}C

Liquid scintillation counting in a Packard Tri-Carb 4322 spectrometer was used to determine levels of ^{14}C. A channels ratio technique was used to determine the counting efficiency which was standardized using [1-^{14}C]-n-hexadecane (Radiochemical Centre, Amersham). All aqueous samples were counted in a Triton X-100: toluene liquid scintillator described by Patterson and Greene (7). The 50% aqueous ethanolamine samples from the $^{14}CO_2$ absorbers were counted in a dioxan:2-methoxy-ethanol-toluene scintillator described by Bruno & Christian (8). Freeze dired faecal samples and carcass homogenates were prepared for counting on a Packard Model 305 sample oxidizer.

In vitro penetration through rat skin

Female Colworth-Wistar rats (100-120 g) were clipped to expose dorsal skin 24 h before cervical dislocation. The skins were excised and mounted in 2.5 cm diameter penetration cells similar to those described by Ainsworth (9). 0.25 ml of the [^{14}C] surfactant solution was pipetted onto the epidermal surface of the skin and 10.0 ml of saline was added to the sampling compartment against the dermis. The cells were kept in a warm room at 37^{o} throughout the experiment and the saline was magnetically stirred continuously. The saline was monitored hourly for ^{14}C by removing 1.0 ml and replacing with fresh saline maintaining the volume of 10.0 ml in the sampling compartment. After 24 h the epidermal surface was washed with excess of distilled water which was monitored for ^{14}C by solubilizing 1 cm diameter autopsies in 'Soluene' (Packard Instruments Ltd) and counting as recommended by the manufacturers.

In vitro penetration through human epidermis

Female abdominal skin samples obtained at autopsy were frozen and stored at -70°. Samples of the skin were allowed to thaw out and were heated at 58° for 2 min and the epidermis removed in sheets. The epidermal samples were mounted in 1 cm diameter penetration cells similar to those described by Ainsworth (9). Saline containing 0.012% Penicillin and 0.01% Streptomycin was placed in contact with both surfaces of the sample and the cells were equilibrated at 37° for 24 h. The electrical resistance of the cells was measured and only cells with a resistance greater than 50000 Ω were used. The saline from the corneum surface was removed and 0.1 ml of the [^{14}C] surfactant solution was placed on the corneum. 1.0 ml aliquots of the saline in the sampling compartment (8.0 ml) were monitored for ^{14}C at 0.5, 1, 2, 3, 4, 6, 7, 8, 24 and 48 h, each time 1.0 ml of fresh saline was added to maintain the volume at 8.0 ml. At the end of the experiment the corneum was washed with excess of distilled water and the epidermal sample monitored for ^{14}C by solubilizing in 'Soluene'.

Animals and treatment

Female Colworth-Wistar rats weighing 100-120 g were used for all experiments.

Turnover of surfactants

The turnover of each ^{14}C-labelled surfactant was measured by injecting three animals intraperitoneally and three animals subcutaneously with 0.1 or 0.5 ml of surfactant solution. The animals were then placed in sealed metabolism cages where urine, faeces and expired

air were collected and monitored for ^{14}C. The metabolism cages consisted of airtight perspex cages mounted on polythene collection funnels which directed the excreta into 'Metabowl' urine/faeces separators (Jencons Ltd, Hemel Hempstead, Herts). Air was drawn through the cages at 1.5 l/min and bubbled through towers 30 cm deep and containing 240 ml of 50% aqueous ethanolamine. 1.0 ml aliquots of this solution were monitored for ^{14}C at regular time intervals. Each urinary sample was made up to 25 ml with cage rinsings and faecal samples were freeze dried. After 6 or 24 h the animals were killed by cervical dislocation. The carcasses of the animals were homogenized in an 'Atomix' blender (M.S.E. Ltd, Crawley, Sussex) and aliquots of the homogenate were freeze dried.

Percutaneous absorption

The hair from animals' backs was removed with fine bladed clippers 24 h before topical application. Only animals with visibly undamaged skin were used in the topical studies and all animals were lightly anaesthetized with a cyclopropane:carbon dioxide:oxygen gas mixture during treatment.

Topical application of 0.1 or 0.5 ml of the [^{14}C] test solution was made from a microlitre syringe on to an area of skin (7.5 or 10 cm^2) previously marked out on the animal's back with a felt-tipped pen. The solution was lathered over the treatment area with a rounded glass rod for 1 min during application. After 15 min contact with the skin the animal was inverted over a 6" diameter funnel and the excess of soap solution was rinsed off with distilled water at 37^{o} from a wash bottle. After about 50 ml of water had been

used the treated area of skin was lightly drawn over the top of the funnel to squeeze excess of rinse water from the skin. This process was then repeated and the skin dried with paper tissues. The animals were then fitted with either restraining collars or non-occlusive protection patches and placed in the matabolism cages for collection of excreta as described above.

The restraining collar was a thin (0.25 mm) card disc, 10 cm diameter, in which was cut a central hole to fit around the animal's neck. The disc was opened by a single radial cut and placed around the animal's neck. The cut disc was then stapled up slightly overlapping the cut edges to form a shallow cone similar to a large ruff. This type of collar was successful in preventing small rats (up to 150 g) from grooming the treated area for up to 12 h after treatment.

The non-occlusive protective patch used in this study was similar to that described by Noakes and Sanderson (10). The treated area of skin was covered with a triple layer of surgical gauze approximately 1 cm larger in each direction to the treated area of skin. Over the surgical gauze a stainless steel gauze (100 mesh), approximately 0.5 cm smaller in each direction to the surgical gauze, was placed and 'Sleek' surgical strapping (Smith & Nephew Ltd, Welwyn Garden City, Herts), which had been punctured to give some 10 x 1 mm holes/ cm^2 over the treated area, was wrapped around the animal. This has been found to be effective in preventing grooming of the treated area of skin for 2 days and for some animals up to 4 days after treatment.

The effect of prewashing the skin on the penetration of the [^{14}C] soaps was examined by washing groups

of rats either once or three times with a non radioactive, 300 mM model soap solution (i.e. 60 mM solution of each of the five soaps studied). This soap solution (2 ml) was lightly lathered over the backs of rats for 1 min, left in contact for a total of 15 min, copiously rinsed with distilled water and dried with paper tissues. 2 h later the animals were treated with either 0.1 ml of the [^{14}C] soap solution over 7.5 cm^2 of skin or rewashed with the inactive soap solution twice, at 2 h intervals before treatment with the [^{14}C] soap solution as described above.

The topically-treated animals were treated similarly to the injected animals described above. Before homogenizing the carcasses however, the protective patch was removed and the treated area of skin was excised and frozen between glass places. Punch autopsies (1 cm diameter) from the frozen skin were monitored for ^{14}C by solubilizing in 'Soluene' and counting. Further samples of treated skin were sectioned histologically for autoradiographic analysis as described by Rutherford and Black (11).

RESULTS

Penetration in vitro of [^{14}C] surfactants through human epidermis and rat skin

A summary of the results from the experiments performed with isolated rat skin and human epidermis is presented in Table I.

The results show no measurable penetration of SDS, SDI, DOBS or the $C_{18:0}$ soap through rat skin up to 24 h after application, but 0.2 µg/cm^2 of the $C_{16:0}$ soap had penetrated at 24 h. Some 7.5 µg of the $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ soaps had penetrated per cm^2 at 24 h but the

TABLE I

In vitro penetration of surfactants through human epidermis and rat skin
surfactant penetrating μg/cm^2

Time after application (h)			Rat skin			Human epidermis			
			2	6	24	2	6	24	48
[^{14}C] surfactant		Conc. (mg/ml)							
Soaps*	$C_{10:0}$	1.2	0.2±0.1	1.1±0.2	8.6±3.4	0.2±0.1	1.3±0.6	16.8±5.1	-
	$C_{12:0}$	1.3	0.2±0.1	1.0±0.3	7.2±3.7	0.4±0.2	4.9±1.6	31.7±8.4	-
	$C_{14:0}$	1.5	0.2±0.1	1.0±0.2	6.9±3.0	0.1	0.6±0.2	9.6±3.6	-
	$C_{16:0}$	1.7	0.1	0.1	0.2±0.1	0.1	0.1	0.3±0.2	-
	$C_{18:0}$	1.8	0.1	0.1	0.1	0.1	0.1	0.1±0.1	-
SDS		7.3	0.1	0.1	0.1	0.1	0.1	3.9±3.6	87.2±24.1
SDI		9.8	0.1	0.1	0.1	0.4±0.3	3.0±1.7	8.4±3.4	30.1±13.6
DOBS		1.2	0.1	0.1	0.1	0.1	0.1	00.1	0.1

* The [^{14}C] soaps were applied as 6 mM solutions in a 30mM model soap solution.

The rat skin results are the mean from three pieces of skin ± SD.

The human epidermis results are the mean from four pieces of skin ± SD.

0.25 ml of test solution was applied to the rat skin (4.9 cm^2) and 0.1 ml to the human epidermal samples (0.78 cm^2).

All [^{14}C] surfactants were labelled in the 1-alkyl position with ^{14}C and were sodium salts.

results were not significantly different for the three soaps. For the three soaps which penetrated the skin there was a lag time of 1 h before any measurable penetration occurred but after this the rate of penetration steadily increased. At the end of the experiment, i.e. at 24 h after application between 60 and 70% of the applied [^{14}C] soaps and [^{14}C] SDI were rinsed from the skin and 30-40% was associated with the skin. The [^{14}C] SDS and DOBS were less easily rinsed from the skin as only 30% was recovered in the rinsings and 70% remained associated with the skin.

The results from the human epidermis experiments showed no measurable penetration of the [^{14}C] DOBS and no measurable penetration of the [^{14}C] SDS until 24 h after application when the rate of penetration was rapidly increasing so that at 48 h 87.2 $\pm$ 24.1 ug/cm^2 had penetrated. The [^{14}C] SDI showed a steadily increasing rate of penetration up to 48 h. The penetration of the [^{14}C] soaps from the model system showed different rates of penetration which ranked $C_{12:0} > C_{10:0} > C_{14:0} > C_{16:0} > C_{18:0}$. All of the surfactants which penetrated the epidermis showed increasing rates of penetration over the duration of the experiment which probably reflects the surfactant/stratum corneum interaction and the breakdown of the barrier properties. This effect was most marked for the SDS where no penetration was detected during the first 8 h of contact. It should be noted that all of the epidermal samples showed some degree of swelling after 48 h contact but this was most marked with the SDS treated samples.

The amount of [^{14}C] surfactant adsorbed to the epidermis was highest for the [^{14}C] SDS where some 75% of the applied ^{14}C was not removed by rinsing. For the

other surfactants 30-50% of the applied [^{14}C] was retained in the epidermis after rinsing.

Turnover of [^{14}C] surfactants in the rat

The rate and route of excretion of ^{14}C from intraperitoneally administered [^{14}C] surfactant solutions were the same as that from subcutaneously administered solutions. The recoveries are given in Table II.

TABLE II

Recoveries of ^{14}C from rats after injection with [^{14}C] surfactants

[^{14}C] surfactant*		Dose uCi	Dose (mg)	% Applied dose CO_2	Urine	Faeces	Carcass
Soaps	$C_{10:0}$	7.25	0.12	57±5	<0.1	<0.1	37±6
	$C_{12:0}$	10.49	0.13	65±7	<0.1	<0.1	30±7
	$C_{14:0}$	8.13	0.15	5±3	2.1±1.2	<0.1	85±9
	$C_{16:0}$	7.74	0.17	21±4	<0.1	<0.1	71±8
	$C_{18:0}$	8.59	0.18	38±9	<0.1	<0.1	56±16
SDS		18.60	3.64	1.5±0.4	77±4	2.6±0.7	15±3
SDI		8.29	4.90	80±7	2.7±0.2	1.7±0.5	12±5
DOBS		8.69	1.02	<0.1	78±4	1.5±0.6	22±5

Each result is the mean from six animals ± SD - three animals injected intraperitoneally and three animals subcutaneously. For the sodium soaps the collection time was 6 h after injection and for the other surfactants 24 h.

* All [^{14}C] surfactants were labelled with ^{14}C in the 1-alkyl position and were sodium salts.

These results showed that at 6 h after administration, the $C_{10:0}$ and $C_{12:0}$ soaps were readily metabolized and the main route of excretion was as $^{14}CO_2$. The $C_{14:0}$ soap was readily incorporated into the body and the ^{14}C turnover was slow. The $C_{16:0}$ and $C_{18:0}$ soaps showed some metabolism with subsequent $^{14}CO_2$ excretion but most of the ^{14}C was recovered in the carcass at 6 h. For both the [^{14}C] SDS and the [^{14}C] DOBS most of the administered ^{14}C was recovered in the urine at 24 h after dosing. The [^{14}C] SDI was metabolized and most of the dose was recovered as $^{14}CO_2$ at 24 h indicating the breakdown of the isethionate ester link. From the results the route of excretion of ^{14}C surfactant giving the most sensitive indication of percutaneously absorbed surfactant was indicated.

Absorption of [^{14}C] soaps through rat skin in vivo

The amount of the [1-^{14}C] labelled soaps penetrating through 7.5 cm^2 of treated skin was calculated from the levels of ^{14}C recovered in the expired CO_2, urine, faeces and in the carcass, after excision of the treated area of skin at 6 h after application. The terminal skin was examined by autoradiography which showed heavy deposition of ^{14}C on the stratum corneum, especially at the entrances of the hair follicles, and in the hair follicles for all soaps. Traces of ^{14}C were seen in the epidermis from all the soaps but only with the $C_{12:0}$ and $C_{14:0}$ soaps could detectable amounts of ^{14}C be seen in the upper regions of the dermis.

The amounts of [^{14}C] soap present in the skin at 6 h after application were between 2 and 5 ug/cm^2 of skin but there were no statistically significant differences between the five soaps even after the prewashing regime

with unlabelled soap solution.

The amounts of ^{14}C recovered in the expired CO_2, urine, faeces and carcass from rats washed for 15 min with the [^{14}C] soap solutions are summarized in Table III where the effect of prewashing the skin is compared with a single wash.

Table III

Penetration of the sodium salts of n-[1-^{14}C] fatty acids through rat skin *in vivo*

[1-^{14}C] Soap	Application* (µg)	Amount penetrating over 7.5 cm^2 of skin in µg		
		Number of prewashes with 300 mM≠ soap solutions		
		0	1	3
$C_{10:0}$	116	1.78±0.70	2.99±1.71	8.92±4.26
$C_{12:0}$	131	5.06±2.59	5.29±3.44	9.04±2.57
$C_{14:0}$	150	2.04±0.39	1.52±0.42	1.60±0.18
$C_{16:0}$	167	0.53±0.18	0.55±0.17	0.63±0.16
$C_{18:0}$	184	0.53±0.14	0.36±0.13	0.35±0.02

Results are the mean from three animals ± S D.

* 0.1 ml of model soap solution applied over 7.5 cm^2 of skin for 15 min.

Model soap solution was 6 mM of each of the five soaps used, i.e. 30 mM total soap concentration, and five solutions used each containing one of the [1-^{14}C] labelled soaps.

≠ 300 mM soap solution was a 60 mM solution of each of the five soaps used.

The results show that from a single wash and rinse the order of penetrability of the soaps was $C_{12:0}$ >

$C_{10:0} > C_{14:0} > C_{16:0} > C_{18:0}$. Approximately 10 times more $C_{12:0}$ penetrated than the $C_{16:0}$ soap. The results from the prewashed animals are only indications as to the actual amounts penetrating since inactive soap deposited on the skin would have diluted the [^{14}C] soap applied. The results, however, showed an increase in the penetration of $C_{10:0}$ and $C_{12:0}$ and no significant change for the other soaps. Thus since the true specific activity of the soaps penetrating must be lower than the test solution the amounts penetrating must be greater than the figures given in all cases.

Absorption of [^{14}C] SLS, [^{14}C] SDI and [^{14}C] DOBS through rat skin in vivo

[^{14}C] SDS and [^{14}C] SDI were applied (0.5 ml) as 25 mM aqueous solutions over 10 cm^2 of rat skin for 15 min. The [^{14}C] DOBS was applied (0.2 ml) as a 3 mM aqueous suspension over 7.5 cm^2 of skin for 15 min. The expired CO_2, urine, faeces and the carcasses of the animals, after excision of the treated skin, was monitored for ^{14}C at 24 h after treatment. The excised skin was monitored for ^{14}C and examined by autoradiography.

Autoradiography of the skins showed heavy deposition of all three of the surfactants on the skin surface and in the upper regions of the hair follicles. Only [^{14}C] SDS was seen in the lower regions of the hair follicles but some of the autoradiograms showed visible amounts in the dermis.

From all the tissue and excreta samples examined for ^{14}C, only the treated areas of skin and the urine from the [^{14}C] SLS treated animals contained quantifiable amounts; although ^{14}C was detected in the expired CO_2

from the [^{14}C] SDI treated animals the counts were less than twice background and were not quantifiable. The ^{14}C urinary level from the [^{14}C] SDS treated animals when corrected for a 77% recovery in the urine from injected SDS was equivalent to a penetration of 0.26 $\pm$ 0.09 $\mu g/cm^2$. The recoveries from these applications are given in Table IV.

The results show that only small amounts of the applied surfactants penetrate the skin although considerable amounts are deposited on the skin. The level of ^{14}C in the expired CO_2 of the [^{14}C] SDI treated animals was very low and from these levels the amounts penetrating were shown to be <0.3 $\mu g/cm$ but >0.1 $\mu g/Cm^2$. No ^{14}C was detected in any of the excreta from the [^{14}C] DOBS treated animals.

DISCUSSION

Published data suggest that the penetration of anionic surfactants through skin is poor (12-14). These data are based upon measurements with excised human or animal skin. Using [^{14}C] soaps in this study confirms the findings of previous workers that the $C_{12:0}$ soap (sodium laurate) penetrates isolated human epidermis most readily of the soaps. The increasing rate of penetration of the surfactants during prolonged application was also confirmed. The penetration of SDI through human epidermis <u>in vitro</u> gave a penetration rate curve similar to that obtained with the soaps but SDS showed a long lag time (6 h) before any penetration occurred after which time the rate of penetration rapidly increased. From these data the permeability constants*

* Permeability constant = $\frac{\mu g/cm^2/min \text{ penetrating}}{\mu g/cm^3 \text{ (concentration of applied solution)}}$

TABLE IV

Recoveries from rats after a 15 min wash and rinse with [^{14}C] SDS, [^{14}C] SDI and [^{14}C] DOBS solutions

Surfactant	Application (µg)	Area of skin treated (Cm^2)	Rinsings (µg)	Skin levels (µg/cm^2)	Protective patch (µg)	Penetration (µg/cm^2)
[^{14}C] SDS	3640	10	1929±90	202±37	36±16	0.26±0.09
[^{14}C] SDI	4900	10	4297±353	75±18	5	<0.3
[^{14}C] DOBS	250	7.5	135±27	11±4	<2	<0.1

Results are expressed as µg recovered and are the Mean ± SD from six animals. The application of SDS and SDI were 0.5 ml of an aqueous solution and the DOBS was applied as 0.2 ml of an aqueous suspension. The contact time was 15 min for all the surfactants which were then rinsed off. The ^{14}C levels in the skin and protective patch were determined 24 h after application and the penetration results are based on levels of ^{14}C excreted in urine, faeces and expired CO_2 during the 24 h after application plus levels of ^{14}C in the carcass of the animals at 24 h.

for the penetration of the [^{14}C] surfactants through isolated human epidermis may be calculated and are presented in Table V. These results are comparable with those previously reported by Blank (12) and Bettley (13) but in addition show that application of a mixed micelle soap does not affect the penetration of the individual soaps.

Table V

Permeability constants (μcm min^{-1}) of some anionic surfactants through isolated human epidermis

Surfactant	Time of contact with surfactant solution (h)		
	6	24	48
Soaps $C_{10:0}$	5.4	18.6	-
$C_{12:0}$	18.2	25.0	-
$C_{14:0}$	1.6	9.4	-
$C_{16:0}$	0.1	0.2	-
$C_{18:0}$	0.1	0.1	-
SDI	0.7	0.9	1.3
SDS	0.1	1.8	35
DOBS	0.1	0.1	-

The sodium soaps were applied as a 6 mM solution in a model soap solution. The SDS and SDI were applied as 25 mM solutions and the DOBS as a 3 mM aqueous solution.

The results from the excised rat skin experiments showed penetration of the shorter chain length soaps, where the permeability constants were 2.5-3.9 μg cm min^{-1}

for the $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ soaps at 24 h after application, but the penetration of the other surfactants was not measurable. No autoradiographic studies on these skin samples were performed and little can be deduced from these results as to the distribution of the [^{14}C] surfactants in the skin. The observed rate of penetration will depend upon the time required for equilibration of the skin samples in the cell and the interaction between the skin and the surfactant. It is likely that some penetration occurred through the stratum corneum in most of the samples but, whereas in the *in vivo* state it would be removed in the peripheral blood supply in the *in vitro* state the dermis has to be traversed. Scala, McOsker & Reller (14) showed the dermal lag effect with tetrapropylene benzene sulphonate which took about 4 h to reach equilibrium. It is thus likely that the dermis may act as a barrier to penetrated surfactant and this probably accounts for much of the differences found between the penetration of these [^{14}C] surfactants through rat skin and human epidermis.

Extrapolation of these *in vitro* results to the use of these surfactants *in vivo* is difficult. From the rat skin data some deposition of surfactant on the skin surface could be predicted but the amounts of SDS, SDI, DOBS, $C_{18:0}$ and $C_{16:0}$ soaps penetrating from a 15 min wash and rinse would be very small. The $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ soaps had permeability constants of $\simeq$3 μ cm min^{-1} *in vitro* so that from a 15 min wash and rinse with a 6 mM solution a penetration of between 0.05 and 0.1 $\mu g/cm^2$ would be predicted.

From the human epidermis studies *in vitro* only small amounts of the $C_{10:0}$, $C_{12:0}$, $C_{14:0}$ soaps and the SDI would be likely to penetrate from a 15 min wash and

rinse *in vivo*. The low penetration rates of the $C_{16:0}$ and $C_{18:0}$ soaps and DOBS and the very long lag time before SDS penetrates suggests that little or none of these would penetrate from a 15 min wash and rinse *in vivo*.

The turnover of the [^{14}C] surfactants in the rat showed that there was no significant difference in the rate or route of excretion of ^{14}C given by intraperitoneal or subcutaneous administration. It was thus thought valid to assume that [^{14}C] surfactant penetrating the skin and entering the blood stream would be excreted at a similar rate. The turnover of the $C_{14:0}$, $C_{16:0}$ and $C_{17:0}$ soaps was slow but for the other [^{14}C] surfactants levels of ^{14}C in the excreta could be used as good indications of percutaneously absorbed material.

The *in vivo* techniques used in this study have been used for a variety of consumer type applications to experimental animals and have been shown to be reproducible for a number of compounds (15). The limit of detection for this type of assay is governed by the specific activity of the isotopically-labelled compound, the dilution in metabolic pools of the test animals and the overall counting efficiency for the isotope in these pools. For [^{14}C] labelled compounds, routine assays of 24 h collections of urine, faeces and expired CO_2 gave limits of accurate measurement of 2.0, 5.0 and 10.0 x 10^3 dpm of ^{14}C respectively. For analysis of whole carcass a limit of accurate measurement of 1 x 10^4 dpm is possible. These limits have been set by taking a count rate of twice background as the limit of sensitivity. Thus, in this *in vivo* study 0.1 μg of [^{14}C] surfactant penetrating per cm^2 of skin could be measured. The exception

is the [^{14}C] SDI which had the lowest specific activity [1.7 μCi/mg] for which the limit of sensitivity was 0.3 μg/cm^2.

Penetration of the [^{14}C] soaps *in vivo* followed the same orderas those obtained with excised human epidermis, i.e. $C_{12:0} > C_{10:0} > C_{14:0} > C_{16:0} \geq C_{18:0}$. The actual amounts of soap which penetrated from the 15 min wash and rinse applications to untreated skins with the 6 mM soap solutions ranged from 0.67 $\pm$ 0.34 μg/cm^2 for the $C_{12:0}$ to 0.7 $\pm$ 0.02 μg/cm^2 for the $C_{18:0}$. These amounts are considerably higher than those predicted from the *in vitro* study with excised rat skin. Prewashing the skin with 300 mM model soap solution--approximately 7.5% w/v solution which is similar to that found during consumer use, increased the permeability of the skin, especially for the $C_{10:0}$ and the $C_{12:0}$ soaps.

With regard to the synthetic detergents, the small amounts of SDS penetrating the skin (0.26 $\pm$ 0.09 μg/cm^2) from the application *in vivo* with a 25 mM solution, was not predictable from the *in vitro* studies. Blank and Gould (12) also found no measurable penetration *in vitro* of SDS which is inconsistent with the known irritancy of SDS to skin. Sprott (3) showed that SDS could penetrate rat skin but in that study, based on urinary ^{35}S levels after washing with [^{35}S] SDS some of the urinary ^{35}S could have been due to the animal ingesting [^{35}S] SDS deposited on the skin.

The SDI penetration *in vivo* was below our limits of accurate measurement in this study i.e. $<$ 0.3 μg/cm^2 penetrated from a 15 min wash and rinse. Small amounts of $^{14}CO_2$ were detected from the topically treated animals (approximately 10 cpm above background) which indicated

that small amounts (0.1-0.2 $\mu g/cm^2$) did penetrate *in vivo*. Subsequent experiments with [^{14}C] SDI with a specific activity of 17.6 $\mu Ci/mg$ have confirmed that small amounts (0.09 $\mu g/cm^2$ from a 15 min application of a 10 mM solution) do penetrate from this type of application.

The penetration of the DOBS isomer was below our limits of detection (0.1 $\mu g/cm^2$) for all experiments. This is probably due to the very low solubility of this isomer ($\triangleq$0.3 mM at 37°C) which although present in commercial dodecylbenzenesulphonate, is not typical of DOBS. The 3.0 mM suspension used in the topical studies at 37° was below the critical micellar concentration of this DOBS isomer.

Thus the *in vivo* studies show that all of these [^{14}C] surfactants penetrate rat skin with the exception of the [^{14}C] DOBS, the solubility of which was very low. From the *in vivo* penetration data presented, it can be seen that there is an order of magnitude difference between the most penetrating of the soaps ($C_{12:0}$ - 0.6 $\mu g/cm^2$) and the least penetrating ($C_{18:0}$ - 0.07 $\mu g/cm^2$) when applied as 6 mM solutions. The penetration of the synthetic surfactants from 25 mM solutions showed that some 0.25 µg of SDS and 0.15 µg of SDI penetrated per cm^2 of skin. Thus, provided a linear relationship between the amount penetrating and concentration of these surfactants in the applied solution exists, then the $C_{12:0}$ soap is about ten times as penetrating as SDS or SDI which penetrate at similar rates to the $C_{18:0}$ soap.

Autoradiography of the treated skins from the 15 min wash and rinse applications showed deposition of surfactant on the skin surface and in the hair follicles especially at their entrances. This deposition suggests

that penetration occurs both transepidermally and via the hair follicles which have been regarded as the main source of penetration for applications of short duration (16,17). The presence of ^{14}C in the epidermis and upper dermis at 6 h after application of the $C_{10:0}$ and $C_{12:0}$ soaps shows the penetration of these soaps but gives no indication when they penetrated. Penetration may have occurred only during the 15 min washing time but penetration may also have taken place from the labelled soap deposited on the skin surface. The fact that the rate at which $^{14}CO_2$ was recovered from the animals washed with $C_{12:0}$ soap was slightly slower than from animals injected with $C_{12:0}$ soap may be a reflection of the route of administration but is probably due to the fact that penetration occurs from the [^{14}C] soap deposited on the skin.

This experiment is the first of a series on the percutaneous absorption of surfactants and shows that although the in vitro systems give some useful data on relative penetrations they do not completely reflect the in vivo situation and extrapolation from in vitro to the user conditions is difficult. The in vivo experiments in this study have not examined the relationships between penetration and the concentration of the applied solution, duration of contact or number of applications. The 15 min applications in this study were an exaggerated 'consumer type' application but a range of contact times from 1 to 20 min with four different concentrations of surfactant are being examined and the effects of multiple application of test solution. It is felt that this type of in vivo study gives data which can be related to human use of all types of products coming into contact with skin.

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RESUME

L'action irritante d'un surfactant sur la peau peut être liée a l'aptitude de ce surfactant à pénétrer la couche cornée et d'agir sur les tissus vivants sous-jacentes.

L'absorption percutanée de certains surfactants [^{14}C-] dit anioniques a été mesuree in vivo sur des rats, après des applications de courte durée et longue durée, et les résultats ont été comparés avec ceux des études in vitro utilisant de la peau de rat et de l'épiderme humaine.

Les méthodes utilisées dans études in vivo et in vitro seront décrites ainsi que les résultats obtenus après action de savons. Na d'acides gras ordinaires, de lauryle sulfate de sodi de lauryle isothianate de sodium et de dodécilebenzéne sulfonate de sodium.

Les techniques in vivo peuvent aussi fournir des renseignements sur le destin métabolique de surfactants appliqués topiquement dans des conditions normales d'emploi. L'intérêt de ces techniques in vitro et leurs limites sera discuté.

ZUSAMMENFASSUNG

Die Reizwirkung eines Schaumerzeugers auf die Haut mag auf die Fähigkeit des Schaumerzeugers zurückzuführen sein, zum Stratum corneum durchzudringen und auf die darunterliegenden lebensfähigen Gewebe einzuwirken.

Die perkutane Adsorption einiger [^{14}C-] indizierter anionischer Schaumerzeuger ist in vivo an Ratten gemessen worden, sowohl nach einer Gebrauchsüblichen Auftragung als auch nach langfristigeren Anwendungen. Anschliessend wurden die Ergebnisse mit denen von Versuchen in vitro an isolierter Rattenhaut und

Menschenepidermis verglichen.

Es wird ein Überblich über die Methodik der Versuche in vivo und in vitro gegeben, und es werden die Ergebnisse von Experimenten mit einer Reihe von Natriumseifen aus normalen Fettsäuren, Natriumlaurylsulfat, Natriumlauryl-Isäthionat und Natrium-Laurylbenzol-Sulfonat dargestellt.

Die Methodix der Versuche in vivo kann ausserdem Auskunft darüber erteilen, was mit under gebrauchsüblichen Bedingungen lokal aufgetragenen Schaumerzeugern in Bezug auf den Stoffwechsel geschieht. Die Brauchbarkeit der in vitro Techniken und deren Mängel werden besprochen.