ISSN 0037-9832

VOL 26 NO 1 JANUARY 1975

Journal of the Society of Cosmetic Chemists

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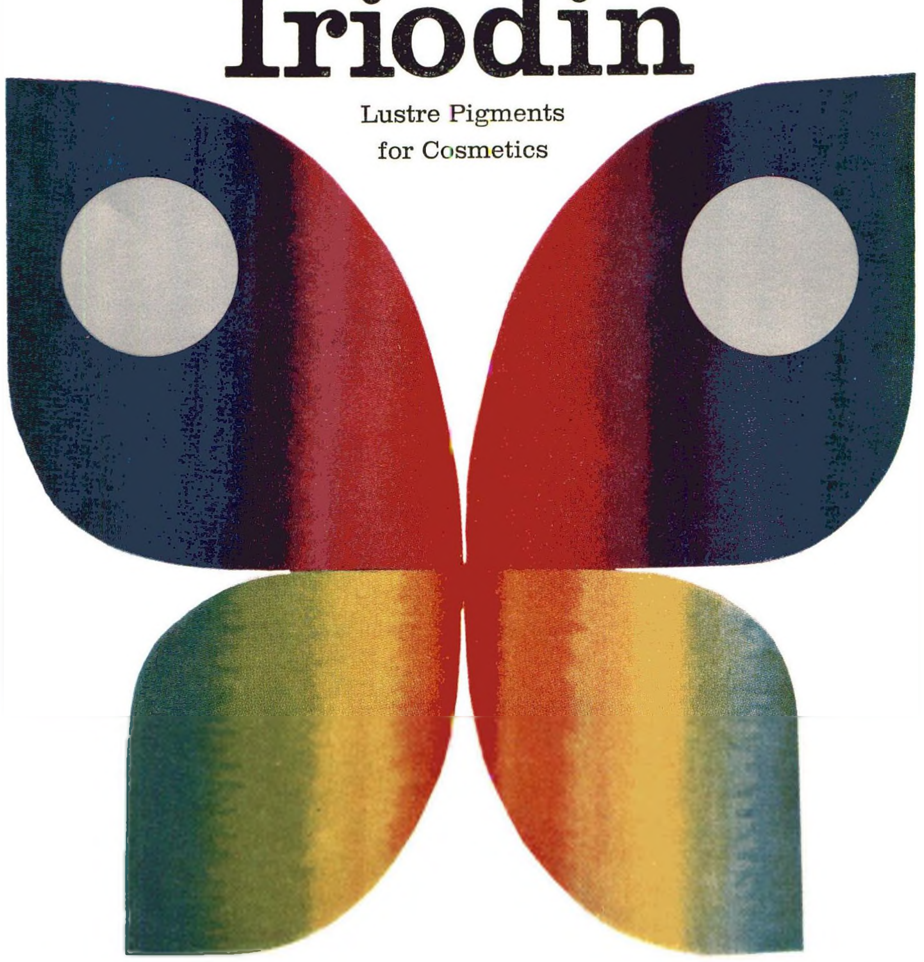

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal

The action and fate of sodium pyridinethione when applied topically to the rabbit: H. C. S. Howlett and N. J. Van Abbé. *Journal of the Society of Cosmetic Chemists* **26** 3–15 (1975)

Synopsis—Toxicological studies of sodium pyridinethione have warranted a study of absorption, distribution and excretion following topical therapy to rabbits.

The extent of percutaneous absorption of sulphur–35 labelled sodium pyridinethione has been related to the skin's structural integrity by radiometric assay for sulphur–35 analysis.

The distribution of the drug and metabolites for the major tissues and body fluids has been established, and the metabolic fate of the drug has been determined using a radiochromatographic technique.

Evaluation of antiperspirant preparations under normal conditions of use: M. W. Steed. *Journal of the Society of Cosmetic Chemists* **26** 17–28 (1975)

Synopsis—A method of assessing the effectiveness of antiperspirant agents under normal or near normal conditions of use has been developed. Silica gel moisture absorbing tins strapped to the body side of the axilla are used for sweat collection, and the change in ratio of sweat produced between axillae for a subject, when only one axilla is treated, is taken as a measure of antiperspirant effect. The advantage of the ratio method is that it eliminates the need for controlled conditions.

Using three aluminium chlorhydrate preparations, significant individual and group sweat reductions have been recorded.

It has also been observed that the individual response to these preparations varies considerably from one subject to another.

Factors which determine the skin irritation potential of soaps and detergents: Colin Prottey and Terry Ferguson. *Journal of the Society of Cosmetic Chemists* **26** 29–46 (1975)

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

The following papers have been accepted for publication in the *Journal*:

ORIGINAL SCIENTIFIC PAPERS

Exaggerated exposure in topical irritancy and sensitization testing
N. J. Van Abbé, F.P.S., D. Nicholas, Ph.D. and E. Boon, H.N.D.

Hair breakage: the scanning electron microscope as a diagnostic tool
A. C. Brown, B.Sc., Ph.D. and J. A. Swift, B.Sc., Ph.D.

Percutaneous absorption of Triclosan from toilet preparations
J. G. Black, B.S., Ph.D. and D. Howes, B.Sc.

Four methods for the characterization of dentifrices and other semisolids
M. Block

A parametric test to measure the cleaning power of toothpaste
W. B. Davies, B.Sc., A.I.M., D.A.E., M.Sc. and D. A. Rees, B.D.S.

The relationship between water-borne bacteria and shampoo spoilage
S. A. Malcolm, B. Pharm., Ph.D. and R. C. S. Woodroffe, M.I. Biol.

An appraisal of human head hair as forensic evidence
J. Porter, H.N.C. and C. Fouweather, B.Sc., Ph.D.

PRELIMINARY COMMUNICATION

Microbiological quality control—a case history
G. D. Breach

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 application, 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.

The percutaneous absorption of some anionic surfactants: D. Howes. *Journal of the Society of Cosmetic Chemists* **26** 47–63 (1975)

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 consumer-type applications and applications of longer duration, and the results have been compared with those from *in vivo* 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 from experiments with a series of sodium soaps of normal fatty acids, sodium lauryl sulphate, sodium lauroyl 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.

Implications of the enlarged European Economic Community on the quality and safety of cosmetics and toiletries: D. M. Gabriel. *Journal of the Society of Cosmetic Chemists* **26** 65–74 (1975)

Synopsis—Prior to the enlargement of the EEC in January 1973, when Britain, Denmark and Ireland joined, there was already some activity relating to the Cosmetic and Toiletry Industry. A proposal for a 'Directive on the Approximation of the Laws of Member States relating to Cosmetic Products', based on a negative, a restricted and a provisional list was being prepared. A Technical Study Group of Government experts had been formed to draw up the lists and a group of analysts assembled to recommend analytical methods to monitor the materials in the restricted and provisional lists. UK Government and Industrial representatives were included in these Groups and in addition a Microbiological group has been set up.

Several amendments to the draft Directive have been suggested and it is not likely to become law until 1975. There is a strong move by most member states towards a positive list but this could not be implemented for at least 5 years because of the immense difficulties which will have to be overcome.

In any case, the implications are that all companies manufacturing goods which come within the scope of the Directive will have to ensure that all their products offered for sale conform to the legislation. Details of the various lists and the proposed reference methods of analysis and control together with their implementation will be discussed.

Journal of the Society of Cosmetic Chemists

This edition is published for

THE SOCIETY OF COSMETIC CHEMISTS
OF GREAT BRITAIN

by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL
Hon. Editor: J. M. Blakeway
Chesebrough Pond's Ltd, Victoria Road, London NW10 6NA
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VOL. 26 JANUARY 1975 No. 1

GENERAL NOTICES

Publication dates: The 'Journal of the Society of Cosmetic Chemists' is published on the 5th of each month.
Five issues for the Society of Cosmetic Chemists of Great Britain
56 Kingsway London WC2B 6DX.
Seven issues by the Society of Cosmetic Chemists
50 East 41 Street, New York, N.Y. 10017, U.S.A.

Issue No	*Publication Date*	*Country of Origin*
1	January	Great Britain
2	February	U.S.A.
3	March	U.S.A.
4	April	Great Britain
5	May	U.S.A.
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Advertisements: All enquiries regarding advertisements in the British Editions of the Journal should be addressed to Blackwell Scientific Publications, Osney Mead, Oxford OX2 0EL.

Subscription: All members of the Society of Cosmetic Chemists of Great Britain receive one copy of each edition free. Further copies at non-member rates. Industriai and non-member subscribers: £30. Single issues: £2.75. If payments are made by bank transfer, all charges shall be at the remitter's expense.

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The action and fate of sodium pyridinethione when applied topically to the rabbit

H. C. S. HOWLETT* and N. J. VAN ABBE†

Presented on 28th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—TOXICOLOGICAL studies of SODIUM PYRIDINETHIONE have warranted a study of absorption, distribution and excretion following topical therapy to RABBITS.

The extent of percutaneous absorption of sulphur-35 labelled sodium pyridinethione has been related to the skin's structural integrity by RADIOMETRIC assay for sulphur-35 analysis.

The distribution of the drug and METABOLITES for the mjaor tissues and body fluids has been established, and the metabolic fate of the drug has been determined using a RADIO-CHROMATOGRAPHIC technique.

The safety evaluation of sodium pyridinethione needs to include the study of absorption, distribution and excretion following topical therapy. Using a radiometric assay for ^{35}S, the extent of percutaneous absorption of ^{35}S-labelled sodium pyridinethione has been examined in relation to the skin's structural integrity. Distribution of the drug and metabolites in the major tissues and body fluids has been established, and the metabolic fate of the drug determined using a radiochromatographic technique.

* Pharmacology Department, School of Pharmacy, City of Leicester Polytechnic, Leicester.
† Beecham Products Ltd, Beecham House, Great West Road, Brentford, Middlesex.

The heavy metal salts of 2-pyridinethiol-1-oxide first synthesized by Shaw *et al.* (1) were first demonstrated to have antibacterial properties by Cox (2). Safety of the zinc salt was extensively studied by Brauer, Opdyke and Burnett (3) and by Opdyke *et al.* (4) and this compound is currently incorporated into several formulations for application to the scalp. Unlike the zinc salt, the sodium salt is readily water soluble, a property which, according to Davson and Danielli (5) can influence percutaneous absorption. The study reported here was intended:

(1) to establish the threshold dose levels for signs indicative of sodium pyridinethione toxicity in acute studies;
(2) to determine the extent of percutaneous absorption and ensuing toxicity of sodium pyridinethione applied to abraded and intact skin;
(3) to record the distribution and metabolic fate of sodium pyridinethione following dermal application.

Materials and methods

Materials

Sodium pyridinethione and pyridine-N-oxide-2-sulphonic acid were provided by Olin Research Centre, New Haven, Conn. Sodium pyridinethione labelled with ^{35}S was synthesized at Beecham Research Laboratories, Betchworth, Surrey, as a white powder, shown by tlc and ir examination to be 90% pure. All other chemicals and solvents were of analytical reagent grade and were used without further purification. Throughout the experimental work polyethylene apparatus was used to reduce drug losses from adsorption during recovery operations.

Preparation of animals

Acute toxicity studies

Two groups of five New Zealand white rabbits (1–1.5 kg) received an anaesthetic dose of sodium pentobarbitone 35 mg kg^{-1}, before i.v. infusion of sodium pyridinethione as a 4% w/v aqueous solution, at a constant rate of 20 mg (0.5 ml) per min into the cannulated jugular vein. The carotid artery was cannulated for blood pressure studies and the heart rate calculated from the electrocardiograph. One group of anaesthetized rabbits

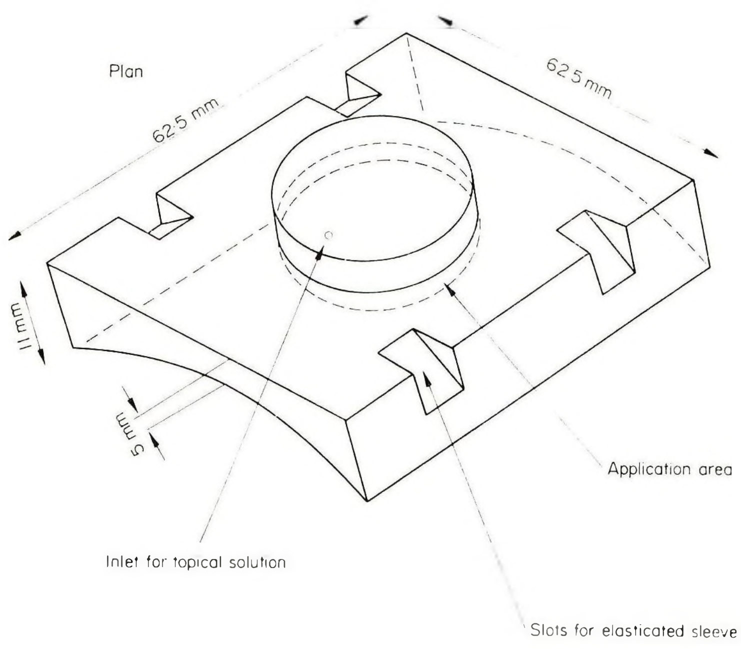
received artificial respiration whereas the other groups breathed spontaneously for the duration of the infusion. A control group of rabbits anaesthetized, but breathing spontaneously, was infused with a 1.8% w/v saline solution (equivalent in tonicity to 4.0% w/v sodium pyridinethione) at a rate of 0.5 ml min^{-1} for a period of time in excess of the time required to reach the lethal dose for any rabbit in either of the test groups. This experimental arrangement permitted the respiratory and cardiovascular systems to be monitored for the detection of functional changes at threshold dose levels.

Dermal application studies

All dermal applications were carried out on female New Zealand white rabbits weighing 1.6–2.5 kg. The rabbits were restrained in stocks to isolate the application site in the dorsal-lateral lumbar region. The application site was shaved 24 h prior to dermal administration and the aqueous topical application confined to an area of skin measuring 7.9 cm^2 using a moulded perspex occlusive device (*Fig. 1*). This device was secured to the skin by means of Stomaseal adhesive discs (Medical Products Division, 3M Company, St Paul, Minnesota) and Ostomy adhesive solution (Salt & Son Ltd, Birmingham). The entire device was harnessed to the animal by an elasticated sleeve positioned around the animal's trunk. An application volume of 4.2 ml was introduced into the device through the aperture designed to accommodate a size 12 hypodermic needle.

Abrasion of the application site was produced using a stripping technique employing cellophane tape (6). The net effect was to produce an application site which was erythematous without showing signs of capillary bleeding.

The dermal dose of ^{35}S-labelled sodium pyridinethione was 0.11 g kg^{-1} (specific activity 0.4 μCi mg^{-1}) in a constant volume of 4.2 ml. The topical application was left in contact with the skin for 4, 8, 12, 16, 20 and 24 h experiments, during which time blood samples were taken at 30 min intervals from the marginal ear vein for quantitative studies of drug serum levels. The animals were killed by cervical dislocation and exsanguinated. Percutaneous absorption of ^{35}S-labelled sodium pyridinethione from the site of application was quantified by a disappearance technique based on the method used by Parekh and co-workers (7). Tissues were removed, blotted and weighed in preparation for radiometric analysis.



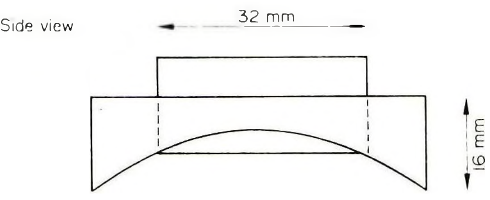


Figure 1. Occlusive device.

Radiometric methods

For distribution studies, 500 mg tissue samples or 0.5 ml of biological fluids were digested in 3 ml of 0.5 M solution of tissue solubilizer Protosol (N.E.N. Boston, Mass.) at 50° for 18 h. The ^{35}S radioactivity in the solubilized media was measured in plastic vials containing 14.5 ml of toluene-based scintillator containing 0.8% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis-(2,5-phenyloxazole) benzene (POPOP). The radiometric assay was

performed on a Tri-Carb 2000, model no. 3003 liquid scintillation spectrometer (Packard Instrument Co.) under conditions suitable for measuring ^{35}S. The channels ratio method of standardization employing ^{14}C as a secondary counting standard (8) was used to calculate the absolute disintegration rates for ^{35}S.

Excretion and metabolism studies

Urine and faeces were collected separately from rabbits, the former being collectively summated with urine withdrawn from the bladder at death. A radiometric assay of urine provided information for excretion rate studies, whereas urine subjected to solvent extraction and chromatographic techniques provided the means of establishing the metabolic fate of sodium pyridinethione following dermal administration.

One millilitre aliquots of urine were withdrawn from the bladder of rabbits exposed to sodium pyridinethione for 24 h and introduced directly onto a polyethylene column (1.5×100 cm) containing Sephadex G10 (Pharmacia Fine Chemicals Co.) and eluted with distilled water. This procedure was repeated for urine acidified to pH 4 with dilute hydrochloric acid and extracted with chloroform. Twenty-six fractions of 5 ml were collected automatically and assayed radiometrically. Fractions containing activity were summated and concentrated by freeze-drying; samples of the concentrated aqueous residue underwent paper chromatography (9) followed by radiochromatographic scanning. The Rf values were calculated. A similar analysis was performed for ^{35}S-labelled components in tissue extracts. Tissues were prepared as a protein-free aqueous filtrate by the Valov method. This filtrate was concentrated by freeze-drying and the concentrated tissue extract applied to the Sephadex column. The radioactive spots were eluted with water and examined in a Unicam SP 800 spectrophotometer.

Results and discussion

Acute toxicity

Rabbits received sodium pyridinethione by i.v. infusion 20 mg min^{-1} until a lethal dose was administered as described above. Results are given in *Table I*.

Table I. Lethal dose of sodium pyridinethione in rabbits with and without assisted respiration

Condition of rabbits	Group size	Mean lethal dose ($mg\ kg^{-1}$)
Anaesthetized and artificially ventilated	5	1608 ±230
Anaesthetized and breathing spontaneously	5	1041 ±196
Conscious	5	1159 ±228

The mean lethal dose for rabbits with assisted respiration was significantly greater than the other LD values ($P = 0.05$), i.e. assisted respiration aided the rabbit's tolerance for sodium pyridinethione.

Further respiration studies on anaesthetized rabbits breathing spontaneously (*Fig. 2*) indicated a 20% increase in respiration rate during the

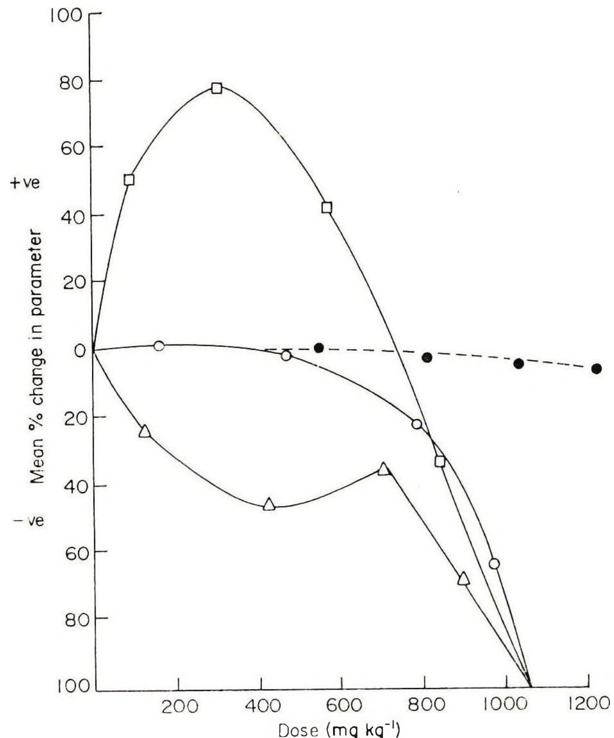


Figure 2. The mean effects upon respiration, blood pressure and heart rate of infusing sodium pyridinethione intravenously into the marginal ear vein of five anaesthetized rabbits. □, Respiration rate; ○, heart rate; △, blood pressure; ●, control.

infusion of 50 mg kg^{-1} sodium pyridinethione, which remained elevated until 700 mg kg^{-1} was infused. At this point respiration rate diminished dramatically as did the blood pressure and heart rate. The respiration rhythm was impaired at doses of 500 mg kg^{-1} and eventual collapse was climaxed by anoxic convulsions. It would appear from *Fig. 2* that infusion of sodium pyridinethione had a primary effect on blood pressure rather than heart rate, a hypotensive effect being recorded at the 50 mg kg^{-1} dose level. Results from the control group of rabbits indicated no gross changes in respiration, blood pressure or heart rate arising from the infusion, surgery or the anaesthetic during the time taken to infuse the lethal dose in the test group of rabbits. These studies showed that systemic levels of 50 mg kg^{-1} precipitated functional changes in the respiratory and cardiovascular systems of the anaesthetized rabbit.

Percutaneous absorption

Percutaneous absorption studies were carried out using ^{35}S-labelled sodium pyridinethione as described above. A dermal dose in excess of the threshold systemic toxic dose, namely 0.11 g kg^{-1}, was applied to intact and

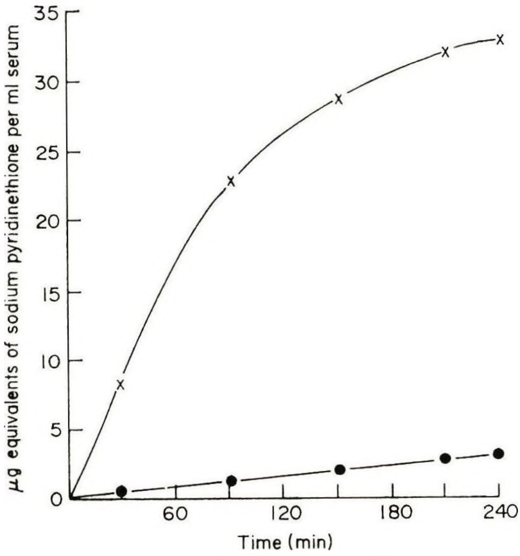


Figure 3. Serum levels of sodium pyridinethione for rabbits with (O) intact and (×) abraded skin.

abraded skin as an aqueous solution. During the 4 h exposure time, a profile of the blood serum levels was obtained by radiometric assay (*Fig. 3*). There was a twenty-five-fold increase in drug serum levels between abraded and intact skin after an exposure time of 2 h. These results emphasized the barrier function of skin and support well-documented evidence, e.g. Cronin and Stoughton (10), showing that abrasion facilitates penetration and percutaneous absorption. The extent of percutaneous absorption in the groups of rabbits with intact and abraded skin was quantified radiometrically by estimating the difference between the activity applied to the site and the total activity recovered from the application solution and excised skin (*Table II*).

Table II. Percutaneous absorption through abraded and intact skin

Condition of rabbit skin	No. of rabbits	Mean quantity absorbed (mg kg^{-1})	$\frac{\text{Quantity applied}}{\text{Quantity absorbed}}$ %
Intact	5	1.346 ± 0.795	1.20
Abraded	4	18.363 ± 4.012	16.42

Table II indicates a fourteen-fold increase in percutaneous absorption by applying sodium pyridinethione to broken skin, thereby greatly reducing the safety margin before toxic systemic levels are reached.

Distribution of labelled sodium pyridinethione

^{35}S-labelled sodium pyridinethione was administered to six groups of rabbits as a 0.11 g kg^{-1} aqueous solution for 4, 8, 12, 16, 20 and 24 h respectively for distribution studies as described above. *Table III* summarizes the results. Mean concentrations expressed as mg kg^{-1} were estimated for each group of five rabbits and this figure in *Table III* was taken as the representative figure for analysis. These results suggested that rapid urinary excretion of the ^{35}S-labelled parent compound and metabolites takes precedence over tissue concentration. A three-fold increase in concentrations was noted in the liver, kidney and lungs over 24 h but was not surprising because blood serum levels similarly increased with the rate of percutaneous absorption. The increased uptake rate of drug in the bile and small intestine, with no detectable levels in the faeces or colon, suggested the existence of a biliary

Table III. Distribution of ^{35}S-labelled material

Site	Exposure time (h)					
	4	8	12	16	20	24
Urine	0.49	1.33	2.23	3.88	5.67	6.94
Serum	0.057	0.11	0.14	0.16	0.15	0.16
Liver	0.10	0.15	0.19	0.25	0.22	0.32
Kidney	0.027	0.043	0.043	0.053	0.056	0.10
Lung	0.0052	0.0062	0.0070	0.0071	0.0067	0.014
Heart	0.0025	0.0028	0.0031	0.0046	0.0034	0.0037
Spleen	0.0006	0.0007	0.0006	0.0005	0.0005	0.0006
Brain	0.0014	0.0031	0.0044	0.0080	0.0089	0.0142
Bile	0.0011	0.0021	0.0026	0.0034	0.0041	0.0054
Skeletal muscle/G.	0.0004	0.0003	0.0008	0.0010	0.0009	0.0017
Pancreas/G.	0.0004	0.0006	0.0013	0.0016	0.0012	0.0017
Duodenum/G.	0.0009	0.0006	0.0039	0.0058	0.0059	0.0057
Small intestine/G.	0.0010	0.0009	0.0036	0.0039	0.0054	0.0056

enterohepatic shunt. No significant activity was located in the adrenal glands, gonads, skeletal tissue or adipose tissue.

Excretion studies

The excretion of ^{35}S-labelled material in these six groups of rabbits was mainly via the urine. *Fig. 4* shows that the excretion and absorption profiles after 12 h exposure to ^{35}S-labelled sodium pyridinethione were linear and parallel, implying that urinary excretion took precedence over tissue concentration and could be used as a parameter to quantify percutaneous absorption. *Fig. 5* revealed that drug serum levels reach a plateau about 12 h. During the initial 12 h exposure period, factors relating to skin penetration and tissue distribution would be expected to influence systemic drug levels, thus explaining the initial rise in serum levels.

Thirty rabbits received 0.11 g kg^{-1} of ^{35}S-labelled sodium pyridinethione dermally. Five rabbits were killed at 4 h intervals. Tissues were assayed for total activity. Total serum activity was calculated assuming the serum volume was 3% of the body-weight. The activity was converted to mg equivalents of drug from the specific activity and expressed as mg kg^{-1}. Mean values were calculated for each group of five rabbits and appear in *Table III*.

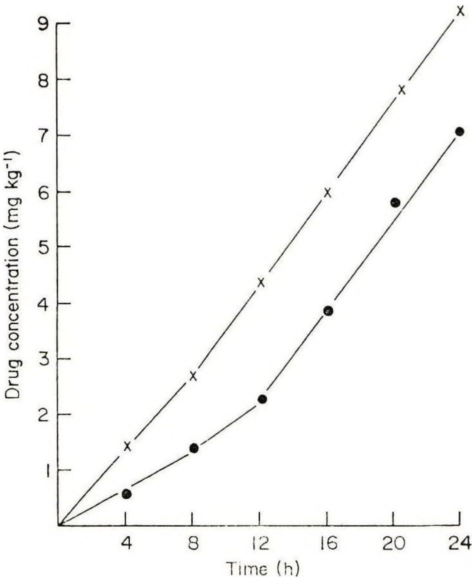


Figure 4. Absorption (×) and excretion (○) rates of sulphur-35 labelled sodium pyridinethione and metabolites over 24 h exposure time.

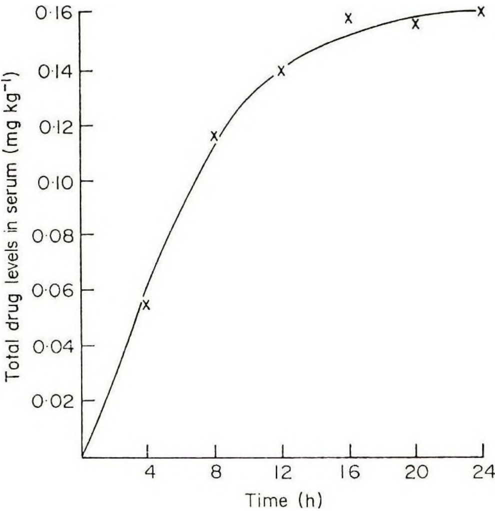


Figure 5. Serum levels of sulphur-35 labelled sodium pyridinethione and metabolites over 24 h exposure time.

Further evidence that excretion of the drug took place in preference to tissue concentration was gained when the ratio of urine, serum, liver and kidney levels were determined for the six groups of rabbits (*Table IV*).

Table IV. Ratio of ^{35}S-labelled material in urine, serum, liver, kidney

Location	Exposure time (h)					
	4	8	12	16	20	24
Urine	8.7	11.7	16.1	24.4	33.1	44.2
Serum	1.0	1.0	1.0	1.0	1.0	1.0
Liver	1.8	1.3	1.4	1.5	1.5	2.0
Kidney	0.5	0.4	0.3	0.3	0.4	0.6

The only amounts changing significantly with extended exposure were the urine levels.

Metabolic rate of sodium pyridinethione

In accordance with the method outlined, urine and tissues were subjected to a chromatographic and spectrophotometric analysis with the aim of identifying the metabolites following percutaneous absorption. *Table V* records the Rf values for reference compounds and components of urine and tissues with ^{35}S activity.

Table V shows that the urine and tissues examined contained pyridine-N-oxide 2 sulphonic acid; the urine and liver also had detectable levels of

Table V. Rf values for reference compounds and components of urine and tissues with ^{35}S activity

Reference compounds and materials examined	Solvent systems			
	Isobutanol 1: Propanol 4: Ammonia 2		N-Butanol 5: Acetic acid 1: Water 2	
Sodium pyridinethione	0.42		0.85	
Pyridine-N-oxide 2 sulphonic acid		0.25		0.18
Urine components	0.42	0.27	0.86	0.16
Tissue components: Liver	0.43	0.26	0.86	0.17
Kidney		0.26		0.16
Lung		0.25		0.16
Brain		0.24		0.16
Heart		0.26		0.17

sodium pyridinethione. Elution of the chromatographic paper spots with Rf value of 0.25 for spectrophotometric analysis, provided uv spectra with absorption peaks at wavelengths 217 and 265 mμm which was in agreement with the reference compound pyridine-N-oxide 2 sulphonic acid. Those spots with an Rf value of 0.42 absorbed at wavelengths 243 mμm, 282 mμm and 332 mμm in the uv spectrum confirming that the parent compound was excreted unchanged. Finally, the urine was assayed radiometrically before and after chloroform extraction to separate sodium pyridinethione from the aqueous soluble acid. Results from this separation indicated pyridine-N-oxide 2 sulphonic acid to be the major excretory product along with sodium pyridinethione in an 8:1 ratio.

These findings regarding the metabolic fate of sodium pyridinethione in the rabbit support the conclusions of Min *et al.* (11) following a dermal study on rats and monkeys. However, there was no evidence to support the findings of Kabacoff and his co-workers (12) whose studies proposed a conjugated mechanism with glucuronic acid as the major metabolic pathway for intravenously-administered sodium pyridinethione to rabbits.

Summary

Sodium pyridinethione was lethal to rabbits at 1 g kg^{-1} by i.v. infusion, the prime cause of death being respiratory failure. Respiratory and cardiovascular effects were evident at 50 mg kg^{-1}, a systemic dose level that was not approached by dermal application of 110 mg kg^{-1} to intact skin. From the findings on absorption, distribution and excretion there appeared to be no tissue concentration and the major proportion of material was excreted via the urine. The metabolic fate of sodium pyridinethione following percutaneous absorption in rabbits appears to be oxidation to pyridine-N-oxide-2-sulphonic acid.

Acknowledgments

The authors gratefully acknowledge the advice given by Dr Basil Northover in planning and carrying out these studies; thanks are also due to Olin Corporation, New Haven, Connecticut, and to Beecham Products, Brentford, England, for generous financial support.

(*Received: 6th June 1974*)

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J. Soc. Cosmet. Chem. **26** 17–28 (1975) © *1975 Society of Cosmetic Chemists of Great Britain*

Evaluation of antiperspirant preparations under normal conditions of use

M. W. STEED*

Presented on 13th November 1973 in Nottingham, at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—A method of assessing the effectiveness of ANTIPERSPIRANT agents under normal or near normal conditions of use has been developed. SILICA GEL moisture absorbing tins strapped to the body side of the AXILLA are used for SWEAT collection, and the change in ratio of sweat produced between axillae for a subject, when only one axilla is treated, is taken as a measure of antiperspirant effect. The advantage of the ratio method is that it eliminates the need for controlled conditions.

Using three ALUMINIUM CHLORHYDRATE preparations, significant individual and group sweat reductions have been recorded.

It has also been observed that the individual response to these preparations varies considerably from one subject to another.

INTRODUCTION

The primary function of an antiperspirant preparation is to reduce the rate of perspiration flow in the axilla. Progress in developing effective antiperspirants is, however, limited by the lack of a reliable *in vivo* method of assessing their action under normal conditions of use. There has been much literature published on the measurement of perspiration flow, but many of the methods have practical drawbacks. Basically there are three

* Reckitt & Colman Toiletry Division.

types of published method, visual or colorimetric, continuous humidity assessment and gravimetric.

The basis of the colorimetric method is to apply an anhydrous indicator to the skin and to observe the extent of colour formation, when sweat is produced. A developer may also be used to produce the colour, which is usually in the form of individual dots, each dot corresponding to a sweat gland. The disadvantage of this type of method is that it is not readily quantitative, and that it is only applicable to flat, accessable areas of the body such as the back or fore-arm. Many workers have studied the colorimetric approach and Daley (1) has produced a quantitative method that has given good agreement with other methods. It did, however, require a large area of skin such as the back, and depended on a numerical count of dots, not taking into account their size or intensity.

The continuous humidity approach is almost the opposite to the gravimetric in that it is readily suited to axillary sweating measurement, and is fully quantitative. It depends on directly measuring the humidity of the air in a cell held in the axilla, and sophisticated adaptions produced by workers such as James (2) are capable of giving a direct print out on an x–y recorder of the sweating ratio between axillae. The objection to the method is that the subject is severely restricted in movement, and adaptation to normal conditions of use is not really feasible.

Most attention in recent years has been directed at gravimetric methods of assessing perspiration flow, as they are direct or absolute and reasonably convenient. Much of the early work in this field was conducted by Fredell and co-workers (3, 4). Their method was to use pre-weighed absorbent pads held in place in the axilla without a harness for a fixed period of time. The increase in weight of the pad was a measure of degree of perspiration. The daily variation of weight of sweat produced by individuals was found to be high, but the ratio of sweat produced from one axilla to the other for an individual was reasonably constant. If after a control period only one axilla was treated, the change in ratio was a measure of antiperspirant effect. It was necessary with this sweat collection technique, however, that the subjects were static, and a hot room to induce perspiration was required. This method is the basis of the procedure used by Hill Top Research Inc., and typical results obtained using this procedure have been published by Martin (5). The great advantage of using the change in ratio as a measure of antiperspirant effectiveness is that it is independent of the absolute weight of sweat collected, which can vary considerably even under fairly controlled conditions.

To adapt the gravimetric method to be suitable for assessing antiperspirant preparations under normal conditions of use, Wooding *et al.* (6) used silica gel drying tins of the type used in analytical balances. These were strapped to the backs of subjects and had the advantages of being easy to handle, reusable and efficient.

From consideration of the different types of procedure described above, it was clear that the gravimetric method was the most adaptable for assessment of antiperspirant effect under normal conditions of use, if the ratio technique was employed. As to the method of sweat collection, it was found, after experimentation, that the moisture absorbing tins of Wooding *et al.* could be strapped to the body side of the axilla with waterproof tape, without greatly inconveniencing the tester, and that they gave more reliable and accurate results than the various pad and cup arrangements tried. The experimental method described in this paper is, therefore, a gravimetric one, whereby silica gel moisture absorbing tins are used to collect axillary perspiration and the change in ratio of sweat production between axillae for an individual, when only one axilla is treated, is a measure of antiperspirant effect.

Test procedure

The particular moisture absorbing tins selected were Silica Gel Air Dryers*, size No. 2, 60 mm × 9 mm (*Fig. 1*). These are perforated disc-shaped containers of silica gel, which are capable of absorbing 8–9 g of moisture. They are supplied in aluminium outer containers. The rate of loss of moisture by evaporation, from a used tin, is negligible for a tin in its container left on the bench for half an hour. Thus, during the time that the tin is removed from the axilla, placed in its container and weighed, there is negligible evaporation of sweat. The waterproof tape used is a microporous, plastic, self-adhesive plaster, *Elastoplast Airstrip*†, which was supplied in rolls 1½ inch wide. The tins and containers were numbered, and were used randomly during a test.

At approximately 09.00 every working morning, the tins were weighed in their containers to the nearest milligram. Subjects were asked to dry their axillae with tissue paper, and the tins were removed from their

* Silica Gel Limited, London.

† T. J. Smith and Nephew Ltd, Hull and Welwyn Garden City.

containers and placed in position on the body side of the axilla, as close to the apex of the axilla as possible without causing discomfort. They were completely covered and held in position by three horizontal strips of plaster (*Fig. 2*). Three hours later the tins were removed, placed in their respective containers and re-weighed. The tins were dried in the afternoon in a vacuum oven at 80–90°C for about 2 h, when they returned virtually to their original weight each time.

Application of test materials was carried out by indivdual panelists at home. One arm of each subject was arbitrarily designated as the 'treatment arm' and the other as the 'untreated or control arm'. The ratio of the weight of sweat obtained from the treated arm to that obtained from the untreated arm, as explained above, was taken as a measure of the efficacy of the treatment and this had the advantage that such diverse factors, as air temperature, humidity and the activity of the subject, that affect the level of sweating, are automatically compensated for. In addition, the ratio is independent of the time for which the absorbing tins are in place, so any discrepancies in collecting time do not affect the results.

Mixed panels of approximately twelve subjects were used, and panel members were asked to shave the axillae a few days before the test. They were also requested to shave during the test when necessary. The panellists were asked to abstain from using an antiperspirant 2 weeks before the start of the test, and unperfumed soap was supplied for use during the test. The panellists were also supplied with a mild aerosol deodorant containing a very low level of cationic germicide, which they were allowed to use during control periods, if desired. The antiperspirant treatment was applied after washing in the morning and in the evening.

No attempt was made to control the quantity of application of the test product by the panellists, as:

(a) evaluation under normal conditions of usage was required;
(b) the surface area of one axilla to the next for different panellists varied very considerably;
(c) individual panellist accuracy in using aerosol products was very variable.

The experiment was divided into three phases; pre-treatment, during which neither arm received any treatment; treatment, during which the pre-designated treatment arm received the specific treatment; and finally a post-treatment phase, during which no treatment was given. The inclusion of a post-treatment phase, was to observe the carry over effect of any of the

Figure 1. Moisture absorbing tin (Silica Gel Ltd).

Figure 2. Moisture absorbing tin strapped to axilla.

(*Facing p. 20*)

treatments. (This effect was not quantitatively assessed owing to lack of post-treatment data.)

Test preparations

Although there are many potential antiperspirant agents available, aluminium chlorhydrate is the only one in widespread commercial use. It was decided, therefore, that at this stage only aluminium chlorhydrate would be evaluated, and its performance from different bases compared.

The following were the products tested.

Test 1

An aqueous solution containing by weight 25% aluminium chlorhydrate and 30% Industrial Methylated Spirit, which was thickened to an appropriate viscosity with hydroxyethyl cellulose. The product was dispensed from a bottle with a roll-ball applicator.

Test 2

An experimental aerosol preparation, where the concentrate was a W/O emulsion. The aqueous phase of the emulsion consisted of 50% aluminium chlorhydrate solution, and this was emulsified into light mineral oil. The phase ratio of the emulsion was 80 : 20 water/oil. The emulsion was dispersed in propellant 12/114 in an aerosol giving an overall concentration of 11.0% aluminium chlorhydrate and 5.5% mineral oil on can contents.

Unlike other aerosols the aluminium chlorhydrate in this product is deposited on the skin in aqueous solution.

Test 3

A leading brand of aerosol antiperspirant believed to contain 3.5% aluminium chlorhydrate powder, suspended in an emollient, and dispersed in aerosol propellant in conjunction with a silica suspending agent.

Statistical treatment of results

During the control period, ratios were calculated daily showing the relationship between the control arm output and the (untreated) treatment arm output. These ratios were then compared with the comparable daily ratios obtained when the designated arm was treated, in the treatment period. For each subject approximately 15 observations were obtained as control ratios and 10 as treatment ratios.

A standard (unpaired) *t*-test was used to compare the ratios (7). For each subject it was possible to state whether a statistically significant effect was observable at the conventional levels of significance (*P* 0.05, 0.01, 0.001).

For each subject a percentage inhibition was calculated, where

$$M = \frac{(P - T)}{P} \times 100$$

M = % inhibition;
P = mean pre-treatment ratio;
T = mean treatment ratio.

The calculations were conveniently carried out by using the logarithms of the perspiration outputs.

95% confidence intervals for M may be derived by the use of Fieller's Theorem (8). An acceptable approximation can also be obtained by applying the usual procedures for deriving confidence intervals after a *t*-test to the difference between the means of the logarithms of the two ratios.

Results

The tests were organized such that approximately 15 control readings (3 weeks) and ten treatment readings (2 weeks) were taken per person, and as far as was possible the same panellists were used on each test. The results (ratios) for each panellist were recorded on individual running charts, and a typical example of one is illustrated in *Fig. 3*.

The overall results for the three tests are given in *Tables I–III*. In these tables the individual panellists are referred to by code letters, A–Q, and pre-treatment and treatment ratio means, the significance of the change in

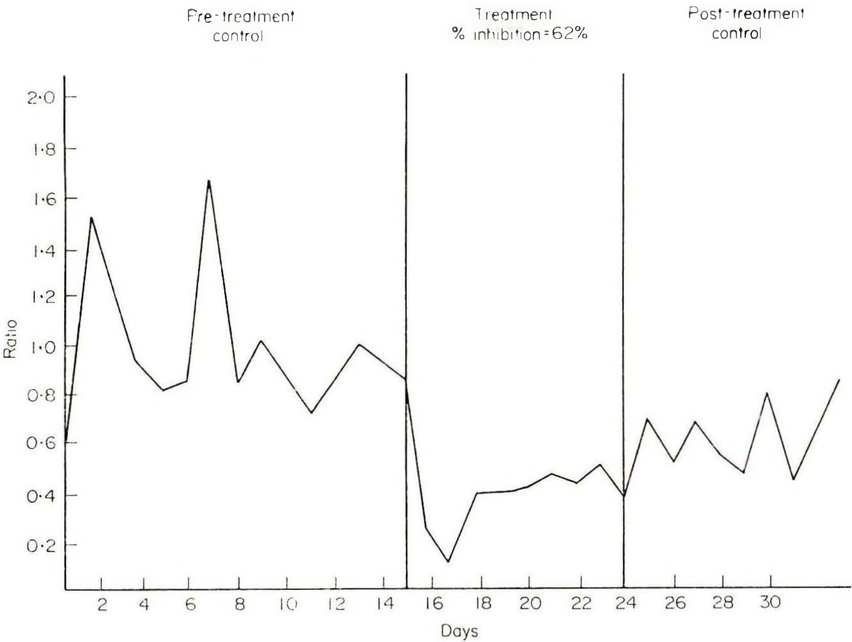

Figure 3. Subject A, Test 1.

ratio mean, the percentage inhibition in sweating caused by the treatment, and the 95% confidence of this figure are given. A combined, overall set of results is also given for each test.

The results, in general, confirm that aluminium chlorhydrate functions as an antiperspirant agent, but that the response from individuals is rather variable. Certain subjects such as panellists A, D and H show a consistently high degree of response, usually in the region of 40% or higher, whereas other panellists such as B, C and L, show only minimal response, often insignificant under the conditions of the test. This suggests that the overall results given for each test have only a very limited meaning, in that they predict an overall situation, but give very little indication of the likely response of an individual subject to a treatment. Such combined figures, therefore, should be used with caution. In this case, however, where the panels were basically similar and the pattern of response in each case was similar, it can be concluded that there was an order of response to the three preparations as follows:

Treatment II > Treatment I > Treatment III

Table I. Test 1

Subject	Sex	Ratio	Pre-treatment mean	Treatment mean	Change	% inhibition	95% confidence limits
A	M	R : L	0.97	0.37	× × ×	62	41 to 87
B	M	L : R	1.05	0.80	NS	23	−2 to 49
C	M	L : R	0.64	0.52	NS	18	−10 to 48
D	M	R : L	1.91	1.38	× ×	28	13 to 43
E	M	L : R	0.64	0.77	NS	−19	−108 to 69
F	F	R : L	1.56	1.79	NS	−14	−52 to 23
G	F	R : L	0.97	0.63	× × ×	35	20 to 52
H	F	L : R	1.16	0.61	× × ×	47	33 to 62
J	F	R : L	1.25	0.81	× × ×	35	26 to 45
K	F	L : R	1.03	0.66	× × ×	36	24 to 48
Overall			1.12	0.83	× × ×	26	13 to 39

× × × Significant reduction after treatment at 0.1% level.
× × Significant reduction after treatment at 1% level.
× Significant reduction after treatment at 5% level.
NS No significant difference.

Table II. Test 2

Subject	Sex	Ratio	Pre-treatment mean	Treatment mean	Change	% inhibition	95% confidence limits
A	M	R : L	1.16	0.66	× ×	44	15 to 75
B	M	L : R	1.18	0.93	NS	21	−6 to 51
C	M	L : R	0.88	0.65	NS	26	−15 to 73
D	M	R : L	1.81	0.97	× × ×	46	33 to 61
E	M	L : R	0.94	0.63	× × ×	34	20 to 49
F	F	R : L	1.74	0.67	× × ×	62	41 to 95
G	F	R : L	1.08	0.70	× × ×	35	17 to 55
H	F	L : R	1.16	0.64	× × ×	45	24 to 67
K	F	L : R	0.96	0.85	NS	11	−12 to 34
L	M	L : R	1.00	0.94	NS	6	−18 to 30
M	F	L : R	1.07	0.90	NS	16	−35 to 68
N	F	R : L	1.75	0.80	× × ×	54	29 to 82
Overall			1.22	0.78	× × ×	36	28 to 44

× × × Significant reduction after treatment at 0.1% level.
× × Significant reduction after treatment at 1% level.
× Significant reduction after treatment at 5% level.
NS No significant difference.

Table III. Test 3

Subject	Sex	Ratio	Pre-treatment mean	Treatment mean	Change	% inhibition	95% confidence limits
A	M	R : L	0.92	0.46	× × ×	50	34 to 68
B	M	L : R	1.13	0.87	× ×	23	9 to 38
D	M	R : L	1.65	1.00	× × ×	39	27 to 32
E	M	L : R	0.92	0.80	× ×	14	6 to 21
F	F	R : L	1.96	1.54	×	21	2 to 41
G	F	R : L	1.03	0.72	× × ×	30	15 to 46
H	F	L : R	1.13	0.84	× ×	26	11 to 42
K	F	L : R	0.92	0.75	×	18	2 to 35
L	M	L : R	0.93	1.14	NS	−23	−52 to 4
M	F	L : R	1.02	0.79	× ×	23	8 to 39
P	M	R : L	1.11	1.23	NS	−11	−34 to 10
Q	F	R : L	1.35	1.39	NS	−3	−24 to 18
Overall			1.17	0.96	× × ×	18	10 to 26

× × × Significant reduction after treatment at 0.1% level.
× × Significant reduction after treatment at 1% level.
× Significant reduction after treatment at 5% level.
NS No significant difference.

It is likely that this order is a dosage effect, and that the effect of the vehicle is minimal.

The actual sweating rates for panellists were, as anticipated, very variable, but it was noted that certain panellists, noteably E and F, sweated consistently at a much greater rate, an average of 20–30 times greater, than many of the others. In a normal 3 h sweat collection period up to 5 g of sweat could be collected from one axilla in the case of these subjects, whereas some of the other panellists rarely gave more than 0.2 g. There was no indication that initial rate of sweating influenced the response to aluminium chlorhydrate treatment. It is also of interest to note that, whereas the majority of panellists have a ratio approaching unity, certain individuals, notably panellists D and F, sweat also twice as much on one arm as on the other. As there was only one left-handed panellist, whose ratio was close to unity, the effect of right- or left-handedness of an individual sweating ratio could not be assessed.

Conclusions

A test method has been developed whereby it has been possible to assess the effectiveness of antiperspirant preparations under normal or near normal conditions of use. Three preparations containing aluminium chlorhydrate have been tested on panels of 11–12 subjects who used the preparations twice daily, and significant (1 in 1000) overall sweat reductions were obtained of a magnitude varying from 18 to 36%. These results agree with other workers (6, 9).

The most important single result appears to be that the response of individuals to the various aluminium chlorhydrate preparations is very varied. Some subjects responded significantly with a reduction in sweating as high as 60%, and in the region of 40% for all three preparations, whereas others consistently gave nil or marginal results. It is, therefore, misleading to quote overall panel sweat reduction figures obtained under normal conditions of use, unless they are qualified by giving the range of individual responses, which in this case was from insignificant responses, usually within ±20%, to very high significant responses in the region of 40–60%.

Acknowledgment

The author would like to thank the Statistical Services Unit of the

Reckitt and Colman Group Management Services Department for their contribution to the paper.

(*Received: 19th August 1973*)

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Factors which determine the skin irritation potential of soaps and detergents

COLIN PROTTEY and TERRY FERGUSON*

Presented on 28th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

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-

* Environmental Safety Division, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford.

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

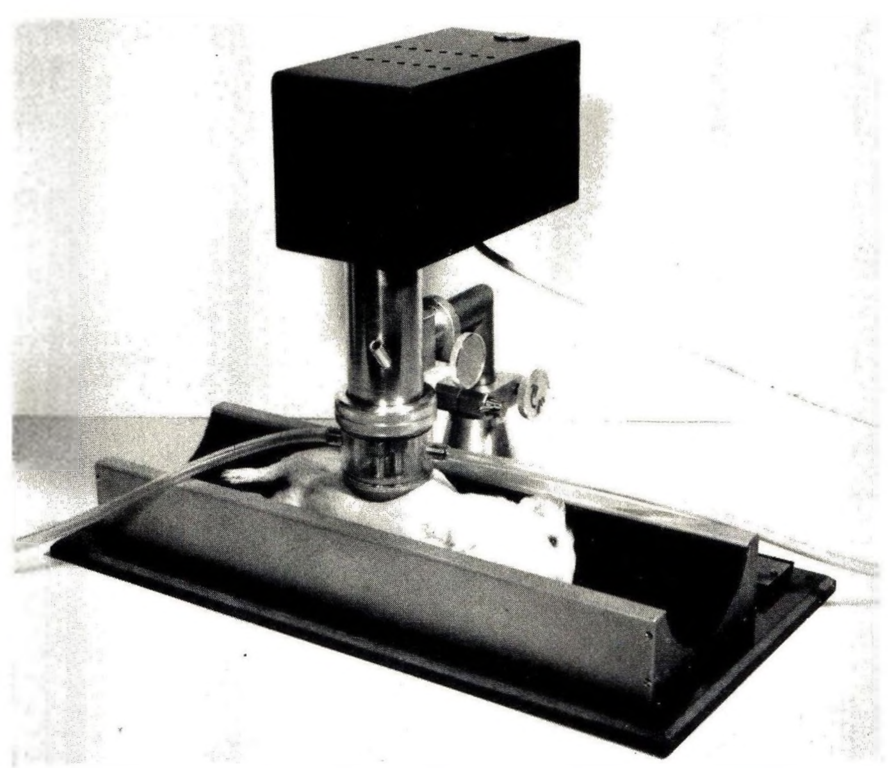

Figure 1. Use of Vermeer washing simulator on guinea-pig skin *in vivo*.

(*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)	Amino acids extracted % 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 cm².

* 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 cm^2 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 mono-ethoxy sulphate	0.05	0.15
Sodium lauryl tri-ethoxy 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 triethoxylate.

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 $\gg$ 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).

(*Received: 22nd May 1974*)

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The percutaneous absorption of some anionic surfactants

D. HOWES*

Presented on 28th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

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 consumer-type 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 from experiments with a series of sodium soaps of normal FATTY ACIDS, sodium LAURYL SULPHATE, sodium LAUROYL 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

* Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford.

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 have 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 *in vivo* fate 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^{-1} (^{14}C–SDS), sodium [1–^{14}C] dodecoyl isethionate; 1.7 μCi mg^{-1} (^{14}C–SDI) and sodium *p*-1-[1–^{14}C] dodecylbenzenesulphonate; 8.5 μCi mg^{-1} (^{14}C–DOBS) were synthesized 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 mM 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 3 mM 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 by Patterson and Green (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 and Christian (8). Freeze-dried 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° 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 and 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% Pencillin 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 50 000 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^{-1} 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-inch diameter funnel and the excess of test solution was rinsed off with distilled water at 37° 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 metabolism 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 10×1 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 plates. 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 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. 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 [^{14}C] 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±24.1 μg/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.

Table I. *In vitro* penetration of surfactants through human epidermis and rat skin (μg/cm²)

		Time after application (h)						
		Rat skin			Human epidermis			
[^{14}C] surfactant	Conc. (mg ml^{-1})	2	6	24	2	6	24	48
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 ± 0.2	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 ± 1.7	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	< 0.1	< 0.1

*The [^{14}C] soaps were applied as 6 mM solutions in a 30 mM 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 cm²) and 0.1 ml to the human epidermal samples (0.78 cm²).
All [^{14}C] surfactants were labelled in the 1-alkyl position with ^{14}C and were sodium salts.

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		% Applied dose			
		μCi	(mg)	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 excretion 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 $\mu g/cm^2$ 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.

The results show that from a single wash and rinse the order of penetrability of the soaps was $C_{12:0} > C_{10:0} \simeq C_{14:0} > C_{16:0} \simeq C_{18:0}$. 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

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 (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 ±SD.

* 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.

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] SDS 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 ± 0.09 µg 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 µg cm^{-1} but > 0.1 µg 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

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.

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 *in vitro* 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* 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.

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 μcm 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

* Permeability constant $= \dfrac{\mu g\ cm^{-2}\ m^{-1}\ \text{penetrating}}{\mu g/cm^{-3}\ \text{(concentration of applied solution)}}$.

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.

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 and Reller (14) showed a 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 μg 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 $C_{18:0}$ 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 10.0×10^3 dpm of ^{14}C respectively. For analysis of whole carcass a limit of accurate measurement of 1×10^4 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^{-1}] for which the limit of sensitivity was 0.3 μg cm^{-2}.

Penetration of the [^{14}C] soaps *in vivo* followed the same order as those obtained with excised human epidermis, i.e. $C_{12:0} > C_{10:0} > C_{14:0} > C_{16:0} \simeq C_{18:0}$. 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 ± 0.34 μg cm^{-2} for the $C_{12:0}$ to 0.7 ± 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 ± 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 frpm the topically treated animals (approximately 10 cpm above background) which indicated that small amounts (0.1–0.2 μg cm^{-2}) did penetrate *in vivo*. Subsequent experiments with [^{14}C] SDI with a specific activity of 17.6 μCi mg^{-1} have confirmed that small amounts (0.09 μg cm^{-2} from a 15 min application of a 10 mM solution) did penetrate from this type of application.

The penetration of the DOBS isomer was below our limits of detection (0.1 μg cm^{-2}) for all experiments. This is probably due to the very low solubility of this isomer ($\simeq$0.3 mM at 37°) which although present in commercial dodecylbenzene sulphonate, 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 μg cm^{-2}) and the least penetrating ($C_{18:0}$–0.07 μg 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.

Acknowledgments

I would like to thank Mr C. T. James for the synthesis of the ^{14}C labelled synthetic surfactants used in this study and Mrs A. Cordell for skilful technical assistance.

(*Received: 1st April 1974*)

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J. Soc. Cosmet. Chem. **26** 65–74 (1975) © *1975 Society of Cosmetic Chemists of Great Britain*

Implications of the enlarged European Economic Community on the quality and safety of cosmetics and toiletries

D. M. GABRIEL*

Presented on 29th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—Prior to the enlargement of the EEC in January 1973, when Britain, Denmark and Ireland joined, there was already some activity relating to the Cosmetic and Toiletry Industry. A proposal for a 'Directive on the Approximation of the LAWS of Member States relating to Cosmetic Products', based on a negative, a restricted and a provisional list was being prepared. A Technical Study Group of Government experts had been formed to draw up the lists and a group of analysts assembled to recommend analytical methods to monitor the materials in the restricted and provisional lists. UK Government and Industrial representatives were included in these Groups and in addition a Microbiological group has been set up.

Several amendments to the draft Directive have been suggested and it is not likely to become law until 1975. There is a strong move by most member states towards a POSITIVE LIST but this could not be implemented for at least 5 years because of the immense difficulties which will have to be overcome.

In any case, the implications are that all companies manufacturing goods which come within the scope of the Directive will have to ensure that all their products offered for sale conform to the legislation. Details of the various lists and the proposed reference methods of analysis and control together with their implementation will be discussed.

In Britain, we have now become accustomed to the fact that we belong to the EEC but the implications as they relate to this industry are not always appreciated.

* Unilever Research, Isleworth Laboratory, 455 London Road, Isleworth, Middlesex TW7 5AB

At the beginning of 1973 when Britain, Denmark and Ireland joined the Community there was already under consideration a proposal for a Directive on the Approximation of the Laws of Member States relating to Cosmetic Products.

To simplify this title it will be referred to as the Cosmetics Directive. It is, however, of interest to note the exact wording of the full title which in effect means harmonization of existing legislation in the various states and not the creation of new laws.

Before entry, Britain had no specific legislation relating to cosmetics, but there were restrictions and recommendations, particularly in respect of hormones and certain therapeutic products, included in the 1968 Medicines Act and the 1972 Poisons Act.

None of the other members had legislative control either, but some had 'negative lists' of substances which were forbidden in all products offered for general sale.

The draft Directive had taken some years to prepare. The responsible Commissioner was Mr A. Spinelli of Italy, and the preparation of the Directive came under the Directorate General for Industrial, Technological and Scientific Affairs. In the early part of 1973, just after the enlargement of the EEC, responsibility was transferred to another Directorate General, namely The Internal Market and Harmonization of Legislation under the control of Mr F. O. Gundelach of Denmark.

At the end of 1972 the Draft Directive was submitted to the Council of Ministers, also to the Economic and Social Committee and the European Parliament for comment. Recommendations and resolutions from the latter two bodies have been received and are being considered with the main Directive by the Council of Ministers. It will then be rediscussed by Government experts and finally by the permanent representatives from the Member States. When the Directive is evenually agreed it will be binding but discretion is left to the National Authorities.

The contents of the draft Cosmetics Directive are appended in an abridged form. They include 15 articles embracing the scope of the proposal and the following Annexes.

Annex I—list of products regarded as cosmetics.

Annex II—a 'negative list' of 425 substances that must not be used.

Annex III—a 'restricted list' of substances.

Annex IV—a 'provisional list' of substances.

Annex V—a list of substances excluded from the Directive (only hormones and selenium disulphide are included at present).

I propose to highlight those parts which relate to Quality and Safety.

Article 2 states that 'Cosmetic products put on the market within the Community must not be capable of causing damage to human health when they are applied as directed'. The bland kind of statement with its complete lack of definition leaves many unanswered questions. For example: What are the criteria for damage to human health? . . . How should this be tested? . . . Should animals or human subjects be used? . . . How many should be tested? . . . What protocol should be used? . . . How should results be interpreted? . . . etc. etc. It will undoubtedly be the responsibility of the manufacturer to provide evidence that his product cannot cause damage to human health but no guidance or recommendations about the way this should be done are available. So for the time being the manufacturer continues to meet his own criteria regarding the safety of his product when used as directed.

Article 3 states that 'Member States shall take all the necessary measures to ensure that only cosmetic products which conform to the provisions of this Directive and its Annexes can be put on the market', and *Article 4* says that 'Member States shall prohibit the marketing of cosmetic products containing (a) the Substances in Annex II, (b) and (c) the substances in Annex III part 1 and 2 outside the limits and conditions fixed'. There is an EEC committee of analysts whose task is to recommend reference methods for checking whether products comply with the restrictions laid down in Annex III, but there is little indication that ways of detecting the possible presence of the long list of substances in Annex II will ever be considered. This must imply that the manufacturer is put on trust not to include any of these substances. It does not require much imagination to envisage the insuperable problems which could be created by an over-zealous Member State.

Then we come to the Provisional List (Annex IV). This is covered by *Article 5* which states that 'For a period of 3 years . . . Member States shall accept marketing of cosmetic products containing the substances in Annex IV parts 1 and 2'.

The same analytical committee is considering reference methods for products containing substances in this Annex. But what happens at the end of three years? This is covered in *Article 9* part 3: 'On the basis of the results of scientific and technical research the substance provisionally accepted shall at the end of three years be

—Finally included in Annex II or III
—or kept for a further period of 3 years in Annex IV
—or removed from any Annex to this Directive.'

So far there is no indication which criteria will be used to decide the fate of Annex IV substances. Is it the duty of the cosmetic manufacturer, the ingredient manufacturer or some other body to furnish evidence on which judgement can be made? Considerable costs could be incurred in checking a particular substance only to find that some other kind of information is needed.

Article 6 relates to labelling and advertising and this involves both Quality and Safety. Point 3 in this article states that special precautions of use must appear legibly on the container or, if this is impossible, on the outer pack and the enclosed leaflet.

In Annexes III and IV there are columns headed 'Conditions of use and warnings to be printed on the label'. Not all substances have a requirement of this type, but for those where it is necessary it can be quite wordy. For example, for diamino-benzenes, -toluenes and -phenols it is as follows: 'Can cause an allergic reaction (in persons sensitive to it). Requires a patch test (behind the ear or on the inside of the elbow) at least 24 hours before application. Add particulars of the method of making the patch test.'

So the manufacturer must make sure he has room to put this warning on the container or on the package and leaflet and that it is legible, which we must take to mean that the print size is large enough to be read without difficulty.

The fourth point of Article 6, although mainly concerned with advertising, is related to quality in that the manufacturer must not attribute characteristics to his products unless there is suitable scientific evidence.

In Britain we are already familiar with this requirement in the Trades Descriptions Act of 1968 and most manufacturers have already taken steps to ensure that they can back up their claims.

Article 8 is concerned with the supervision needed to ensure that cosmetic products conform to the provisions of the Directive. The responsibility lies with the Member States and so far there is no indication about how much effort will be devoted to this task or how it will vary from country to country.

I have already referred to *Article 9* when discussing Articles 4 and 5. The first paragraph of the first point requires procedures for the sampling and analysis of products containing substances in Annexes III and IV and the analytical committee are actively working towards this goal.

The second paragraph of the first point refers to bacteriological purity and a committee has recently been formed to decide on criteria and methods to ensure that products are acceptable in this respect.

Article 10 provides for the formation of a Committee of Adaptation. This committee will not be set up until the Directive is finally accepted but there is no doubt that it will be very necessary and will probably have a full programme of work. The methods recommended by the analytical committee will have taken into account most of the important factors and they will have been checked against a selection of relevant products on the market but it would be impossible to check every product from every country. This means that there could be some products which are not amenable to testing by the reference method. The onus is on the manufacturer to check whether his product gives the correct answer when tested according to the reference method. If it does not then he must bring this to the attention of the Committee of Adaptation using the mechanism outlined in Article 11.

Article 12 is of interest in that it allows for the possibility that a product could constitute a danger to human health even though it conforms to the Directive. Member States who find such products can forbid their sale in their territory for one year and they must inform the other Member States and the Commission, detailing the reasons. The Commission must start consultations within 6 weeks and eventually decide if the Directive must be revised.

Finally, *Article 14* states that when the Directive is finally agreed, all Member States must make the necessary provisions to ensure compliance within 18 months.

To summarize, the main points arising from this Directive are as follows.

(1) Products must not cause damage to human health.
(2) No product can be marketed if it contains any substance listed in Annex II.
(3) Products containing substances in Annex III must conform to the restrictions and conditions laid down.
(4) Products containing substances in Annex IV must also conform to the restrictions and conditions laid down, but at the end of 3 years their use will be reviewed in the light of any new safety data which is available.
(5) Products must be correctly labelled and claims must be justifiable.
(6) Reference methods will be published for the sampling and analysis of products containing substances in Annexes III and IV together with methods for checking bacteriological purity of all cosmetic products. Manufacturers must check that their products can be

analysed by these methods and if not they must bring the matter to the Committee of Adaptation.

(7) Any alterations or amendments to the Directive which individuals feel should be considered can be put forward according to the mechanism in Article 11.

(8) If a product, even though it conforms to the Directive, is found to be harmful, its sale can be forbidden.

(9) Within 18 months of acceptance of this Directive provisions will be introduced to ensure that all cosmetic products conform to the requirements of the Directive.

Present situation

Recommendations from the Economic and Social Committee and resolutions from the European Parliament are still being discussed by the Council of Ministers and the Directive is unlikely to be finalized this year (1974).

(*Received: 1st May 1974*)

Appendix

Proposal for a council directive on the approximation of the laws of the member states relating to cosmetic products

COM(72) 851 Final
Brussels 6th October 1972
Abridged version

Explanatory memorandum

(1) *General*

A comparative study of the laws, regulations, etc., in force in the Member States revealed divergences and the object of the Directive is to eliminate these divergences. The Commission formed a working party of experts and a technical study group, with provision for European representatives of Co-li-pa, to express their points of view on the technical problems.

(2) *Harmonization solution*

The Directive is based on harmonization and the result will be the substitution of Community provisions for the national laws in force.

(3) *Notes on the proposal*

These cover the scope of the Directive as exemplified by the 15 Articles and 5 Annexes. The system chosen is based on negative listing: con-consequently all substances not specifically prohibited are accepted, so any cosmetic product is accepted providing it does not contain any of the prohibited substances in Annex II and meets the restrictions laid down for any substance in Annex III and IV.

(4) *Acceding countries*

Technical contacts with the experts of acceding countries have been established.

(5) *Consultation of the European Parliament and the Economic and Social Committee*

The opinion of these bodies is necessary to comply with the provisions of Article 100 of the EEC Treaty of Rome. Application of the Directive will require amendments of the laws of all Member States.

The Directive

Article 1 (Definition of cosmetic)

'Cosmetic product' means any preparation intended to be placed in contact with superficial parts of the body or with teeth and mouth with a view principally for perfuming, cleaning, protecting, keeping in good condition, changing appearance or correcting body odours. Annex I lists cosmetic products included in the Directive. Annex V lists substances excluded from the Directive.

Article 2 (Products must not cause human damage)

Cosmetic products must not be capable of causing damage to human health when they are applied as directed.

Article 3 (To be marketed—the product must conform)

Member States shall take the necessary steps to ensure that only products conforming to the Directive and its Annexes are put on the market.

Article 4 (Prohibitions Annex II; Restrictions Annex III)

Member States shall prohibit the marketing of cosmetic products containing:

(a) the substances listed in Annex II;
(b) the substances listed in Annex III (part 1) outside the limits and conditions fixed;
(c) colourants other than those in Annex III (part 2) if the products are intended to be used near the eyes, on the lips or in the mouth.

Article 5 (Provisional Restrictions—Annex IV)

For a period of 3 years Member States shall accept marketing of cosmetic products containing

(a) substances listed in Annex IV part 1;
(b) colourants listed in Annex IV part 2 if these products are intended to be used near the eyes, on the lips or in the mouth.

Article 6 (Packaging and labelling requirements)

(1) The container or outside package must bear the name and address of the manufacturer/packer/importer/distributor having his registered place of business within the Community.

(2) The container or outside package shall state the net content at time of packing in legal metric units and expiry date for products which do not have an unlimited shelf life.

(3) Special precautions of use shall appear legibly on the container, or if impossible on the outside package and an enclosed leaflet.

(4) The use of names, trademarks, images or other signs suggesting a characteristic which the products do not possess is prohibited. A recommended amendment to this section includes prohibiting claims for products where there is no, or insufficient, scientific evidence.

Article 7 (Freedom of marketing to Member States)

Member States must not prohibit or hamper the marketing of products which conform to the Directive but they can demand that the particulars required in Article 6 are expressed in their national language(s).

Article 8 (Supervision)

Member States shall take the necessary measures to supervise that cosmetic products conform to the Directive.

Article 9 (Sampling, analysis, bacteriological criteria, amendments, fate of Annex IV substances)

(1) Procedures for sampling and analytical methods necessary for supervision are determined in accordance with Article 11. Similarly, criteria for bacteriological purity.

(2) Amendments necessary for the adaptation of Annexes II and III shall be adopted in accordance with the same procedure.

(3) On the basis of results of scientific and technical research the substances and colourants provisionally accepted in Annex IV shall at the end of the 3-year period provided for in Article 5 be:

—finally included in Annex II or III;
—or kept for 3 more years in Annex IV;
—or be removed from any Annex to this Directive.

Article 10 (Committee for Adaptation)

A Committee of Adaptation shall be established, composed of representatives from Member States and chaired by a representative of the Commission.

Article 11 (Mechanism for raising matters)

(1) In cases where reference is made to the procedure the matter is brought before the Committee of Adaptation by the chairman, either on his own initiative or at the request of a representative of a Member State.

(2) The chairman shall submit a draft of the measures to be taken. The Committee of Adaptation shall give its opinion within the time fixed by the chairman and decide by a majority of 12 votes according to the weighting laid down in Article 148 of the Treaty. The chairman shall not take part in the vote.

(3) (a) When the Committee of Adaptation is in accord the Commission shall lay down the proposed measures.

(b) If not, or in the absence of an opinion from this Committee, the Commission shall forward to the Council a proposal concerning the measures to be taken and the Council shall act by a qualified majority.

(c) If after 3 months the Council has not acted, the measures proposed shall be adopted by the Commission.

Article 12 (Procedure when conforming products are found to be harmful)

(1) If a Member State ascertains that a cosmetic product presents a danger to human health, although it conforms to the Directive, that State,

in accordance with the procedure in Article 11, can provisionally restrict or prohibit the sale on its territory. At the same time it must communicate to the other Member States and the Commission the measures envisaged and the reason for the decision. (A likely amendment limits the time to 1 year.)

(2) If after 30 days no measure has been laid down by the Commission or the Council, the Member State may take the measures envisaged until such time as a decision is made according to Article 11. (A likely amendment requires the Commission to start consultations within 6 weeks and if the Directive has to be revised this must be completed within 1 year.)

Article 13 (Possible restrictions by Member States)

Precise reasons shall be stated for any individual measure taken to restrict or ban the marketing of cosmetic products. These shall be communicated to the interested party together with particulars of remedies available under the legislation in force in the Member States and the time limit for the proceedings.

Article 14 (Date for compliance)

(1) Within 18 months of notification the Member States shall introduce the provisions necessary to comply with the Directive and shall inform the Commission forthwith.

(2) Member States shall ensure that the text of such provisions of national law as they adopt in the field governed by this Directive is communicated to the Commission.

Article 15

The Directive is addressed to the Member States.

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The Journal of the British Allergy Society

Edited by J. Pepys

Volume 4, Number 4, December 1974

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British Journal of

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Edited by R. H. Champion

Volume 91, No. 6. December 1974

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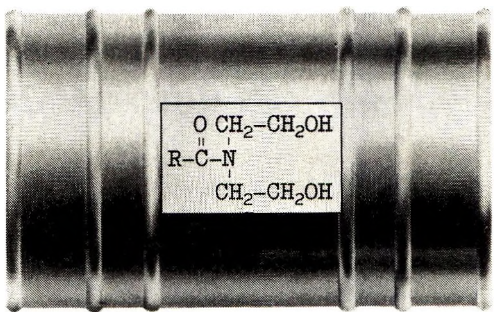

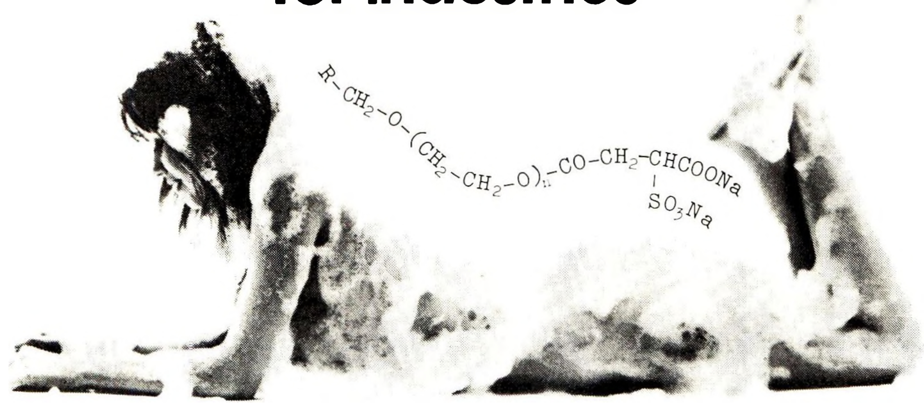

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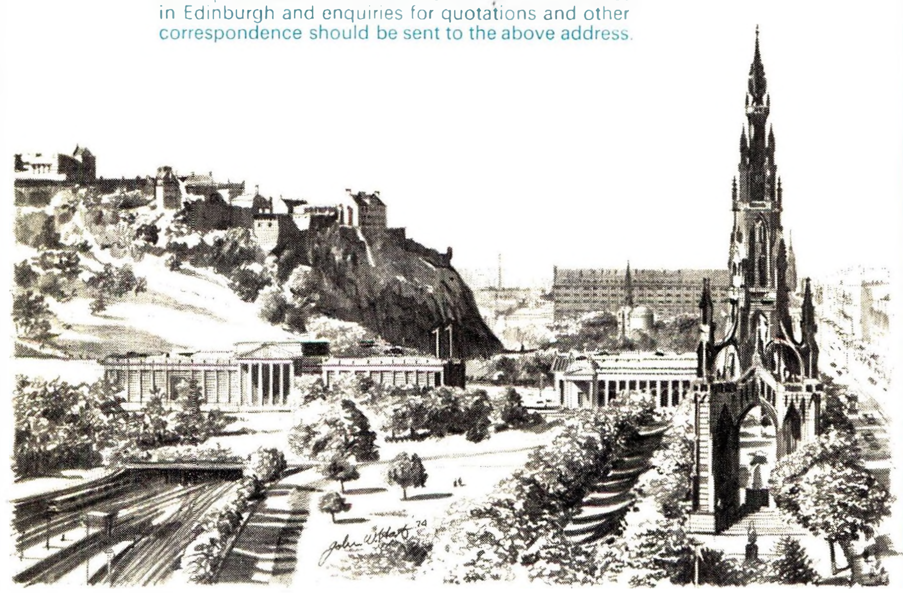

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