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Contents

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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.

A new method for the quantitative determination of micro-organisms on human skin: E. M. STAAL and A. C. NOORDZIJ. *Journal of the Society of Cosmetic Chemists* **29** 607–615 (1978)

Synopsis—A new method is presented to quantify micro-organisms on the surface of human skin. With this method harvesting of micro-organisms from the skin is carried out with a Water-Pik® spray device, an instrument constructed for removal of food debris from the teeth. It will be shown that this method is operated very simple and gives reproducible figures about the microbial population of the human skin.

Short term effects of emollients and a bath oil on the stratum corneum: S. NICHOLLS, C. S. KING and R. MARKS. *Journal of the Society of Cosmetic Chemists* **29** 617–624 (1978)

Synopsis—Recently devised techniques were employed to detect and measure surface changes and structural alterations in the stratum corneum after single applications of both oil in water emollients and a dispersion type of bath oil. Flattening of the surface contours of the skin and 'plumping' of individual corneocytes were apparent on replicas of the skin taken from treated areas and suggested hydration. These effects were most apparent when replicas were examined by scanning electron microscopy and by surface contour tracing. Small changes observed in skin surface biopsies from treated areas may reflect alterations in the internal structure of the top layers of stratum corneum. An increase in skin furrow width was also detected in photographs of the surface of treated skin.

Water diffusion coefficients versus water activity in stratum corneum: a correlation and its implications: M. STOCKDALE. *Journal of the Society of Cosmetic Chemists* **29** 625–639 (1978)

Synopsis—Literature reports of rates of trans-epidermal water loss through skin exposed to differing external relative humidities have been used to generate water diffusion coefficients by means of Fick's law. These have been related, within a given series, to the diffusion coefficient at a standard average water activity to give relative water diffusion coefficients. These have been combined, together with literature values of water diffusion coefficients obtained by kinetic experiments on stratum corneum exposed to various humidities, to yield a correlation between the average water activity of stratum corneum and the relative water diffusion coefficient over the full range of water contents. The asymptotic relationship derived shows that the diffusion coefficient increases rapidly as the water activity approaches its maximum. The implications of this relationship in terms of water activity profiles of stratum corneum are discussed. A theoretical analysis of the subject is presented and water activity profiles of model systems are derived.

Design and evaluation of a water-resistant sunscreen preparation: RICHARD S. BERGER, JAMES A. MEZICK and CHRISTOPHER M. PAPA. *Journal of the Society of Cosmetic Chemists* **29** 641–649 (1978)

Synopsis—A water-resistant sunscreen preparation was formulated to function during swimming, exercising and sunbathing. The sunscreen agent, Octyl *p*-NN-dimethylamino benzoate (PABA) and an ammonium acrylate/acrylate ester polymer were combined in a cosmetically pleasing oil-in-water, fugitive amine, lotion vehicle. When applied to skin, a substantive film forms which does not interfere with transepidermal water loss or normal sweat gland function. Double blind clinical studies showed that this water-resistant sunscreen preparation provided protection from sunburn after 60 min swimming in fresh or salt water. This water-resistant sunscreen preparation was found to be safe and of low irritancy, allergenic, sting and stain potential.

The leaching of F.D. & C. Blue No 1 dye from its lake by electrolytes: N. A. ARMSTRONG, A. BIALKOWSKA and J. SMITH. *Journal of the Society of Cosmetic Chemists* **29** 651–655 (1978)

Synopsis—The effect of electrolyte solutions on the alumina lake of F.D. & C. Blue No 1 dye has been studied. Monovalent anions and cations cause a fairly rapid leaching of the dye for a period of about 1 h, followed by a much slower elution process. Multivalent cations cause a slight increase in leaching, but a more marked effect is obtained with salts of multivalent acids, where the dye is virtually completely removed from its substrate in 2–3 h. The results are explained by reference to the postulated structure of the alumina substrate of the lake, and the effects of pH on and ionic penetration into this structure.

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A new method for the quantitative determination of micro-organisms on human skin

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Received 26 January 1978

Synopsis

A new method is presented to quantify **micro-organisms** on the surface of human **skin**. With this method harvesting of micro-organisms from the skin is carried out with a Water-Pik® spray device, an instrument constructed for removal of food debris from the teeth. It will be shown that this method is operated very simply and gives reproducible figures about the **microbial population** of the human skin.

INTRODUCTION

On the surface of our skin in most areas a lot of micro-organisms do exist. Generally, these micro-organisms are completely harmless and will not interfere with the function of the skin.

The number of micro-organisms varies very greatly, as their existence is directly related to the quantity of nutritional substances and external factors such as humidity, heat and smoothness. Therefore big differences are found from place to place and from individual to individual.

In the survey of Williamson (1) about this subject differences of a thousand-fold are not rare. Kligman (2) gives a very detailed survey of the types of micro-organisms found on our skin. The factors hereby playing a part are further elucidated by Woodroffe (3). Extensive information about the skin population is also found in the book of Skinner and Carr (4), in which Marples extensively discusses these phenomena.

For the formulation of cosmetic products the study of the human skin population is very important. Several product groups are formulated to eliminate the harmless but inconvenient side effects of the micro-organisms on our skin. For example a deodorant product can be mentioned, which eliminates human malodour by killing skin micro-organisms with the help of a bactericidal agent.

As already mentioned before, micro-organisms will exist preferably on the most inaccessible areas of the skin. In these areas, for example the axillae, the conditions for bacterial growth are almost optimal and generally a huge number of micro-organisms is found in these places. Here especially, cosmetic products are used to combat the microbial side effects. For the determination of the efficacy of a cosmetic product in practice a method to quantify micro-organisms on the skin is necessary.

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Until now, all methods described are applied and are only suitable on a smooth skin surface, e.g. the skin of the underarm or forehead. Together with the abovementioned, the problem is raised that the known methods are not easily applicable for those areas of our body which are important for cosmetic treatment. Our experience was that in these areas, like the axilla, scalp and feet all methods failed for the quantification of the micro-organisms.

The known methods can roughly be divided into two basic techniques (5).

1 Excision technique

With the excision method (6) the upper layer of the epidermis is removed with a sharp knife. After that the micro-organisms are carefully separated from this part of the skin, collected and counted. The method is of course very accurate, but is not suitable to human subjects.

2 Direct sampling techniques

With these methods the micro-organisms are more or less effectively removed from the intact skin of the subjects. This can be done in the following ways.

(a) Dry sampling with a contact plate, tape stripping or velvet pad (7, 8, 3, 9, 10). Generally the yield of micro-organisms with this type of method is rather low, so the method is less suitable for a good quantitative investigation.

(b) Wet sampling with mechanical scrub or swab. With this method micro-organisms are separated from the skin by applying a detergent solution and rubbing on a restricted area of the skin. The yield is relatively high, but demands an accurate standardisation of liquid volume and applied rubbing force (11, 12, 13, 14, 15, 16, 17, 18).

(c) Wet ultrasonic sampling. This method was recently described by Stringer and Marples (15) and uses ultrasonic waves in a liquid phase to harvest micro-organisms from the skin. In fact, this method is a modified wet sampling technique with a well standardised quantity of applied energy. The advantage of this method is, therefore, its high accuracy, but the disadvantage is that the ultrasonic waves give a lot of discomfort to the subjects.

Until now, the most frequently used sampling method is the scrub method with a 'cup template' (13, 19, 20). Generally the washing liquid used with this method is a solution of the detergent Triton X 100. This solution is selected among sixteen other solutions of nonionic detergents after an extensive investigation by Williamson and Kligman (21).

Considering the advantages and disadvantages of the described methods an optimal sampling technique has to meet the following demands: (1) sampling must be easy to standardise; (2) the yield has to be reproducible; (3) the sampling may not produce discomfort to the subjects; (4) the method has to be suitable for hairy areas of the body; and (5) the method has to be suitable for vertical or upside down areas of the body.

Obviously none of the mentioned methods meets the demands of the ideal test method. This article describes a new method in which almost all mentioned advantages are collected.

For this we used a 'Water-Pik®' spray device. This instrument is a consumer product and is constructed to remove micro-organisms and food debris from the teeth with a fast pulsating, powerful water jet. Through these characteristics this instrument is, in fact, ideal for the separation and quantification of micro-organisms from the skin.

For application on the skin the Water-Pik was connected to a sampler with a silicone rubber sealing and a handle. The device has a volume of 1 ml and has a construction to permit application on every part of the body. The micro-organisms separated from the skin by the waterjet in the small sampler are collected through a closed system in a sterile bottle. This washing liquid is used afterwards to determine the number of viable micro-organisms. It appeared that the sealing of the sampler was so good that application on even hairy parts of the body could be done without water leakage.

MATERIALS AND METHODS

A Water-Pik® spray device is connected with a teflon tube to a sampler (*Fig. 1*). The outlet of the sampler is connected to a sterile collecting flask by means of a silicon rubber tube.

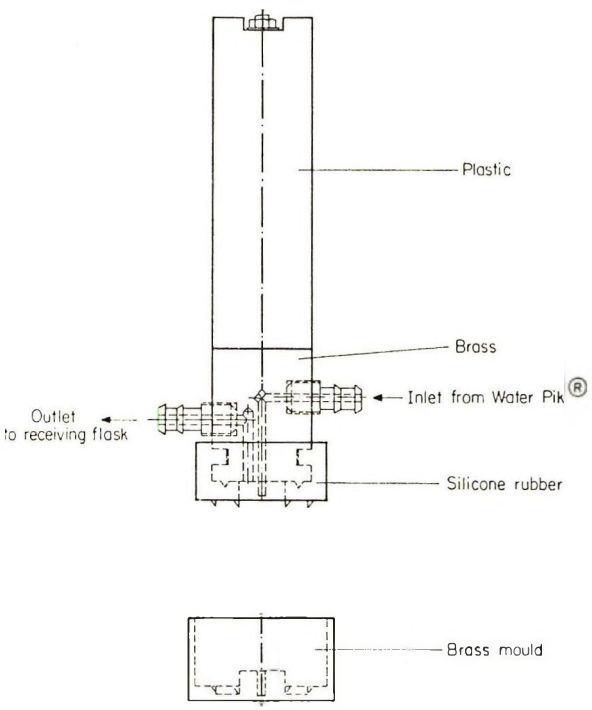

Figure 1. Samples attached to a Water-Pik spray device. Scale × ½.

The Water-Pik (model 49 EX) has a pulse frequency of 1200 pulses per minute and a discharge rate of 500 ml per minute. The sampler is built up by a brass body with a plastic handle. The body has a silicone rubber foot, so that a spray chamber is formed with a volume of about 1 ml. The rubber foot is prepared by mixing Sylastic 9161 RTV base* with Siliconoil F 128* with 1% of the catalyst N 9162* in a brass mould (*Fig. 1*).

For sterilisation the equipment was flushed with 750 ml of a 0·3% Chloramine T† solution or 750 ml 70% ethanol. To remove rests of chloramin or ethanol the apparatus is

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† (N-Chloro-*p*-toluene sulphonamido) sodium.

rinsed with approximately 1000 ml of sterile 0·1% Triton X 100* solution in 0·067 M phosphate buffer (pH 7·9). Sterile Triton X 100 is prepared by filtering the solution through a 0·22 μm membrane filter.

For operation the sampler is firmly pressed on the skin and the Water-Pik is started (range 5). The collected Triton X 100 solution is, if necessary, diluted with sterile 0·85% saline. The dilution grade depends on the expected number of micro-organisms. Samples from the forehead, axillae and the scalp normally give high counts and a dilution of a hundred thousand-fold is needed (12). Sampling of the scalp is demonstrated in *Fig. 2*.

Trypticase soy agar (BBL) with 0·5% glucose was used as a general medium for aerobic organisms; counts were made after 72 h incubation at 32°C. For anaerobic growth the trypticase soy agar medium according to J. A. Troller (22) is used; incubation was carried out during 6 days in a Brewer jar (23) at 37°C in an atmosphere of 90% nitrogen and 10% carbon dioxide (Gaspak BBL).

EXPERIMENTAL AND DISCUSSION

The investigation was started with the construction of the above mentioned sampler. The rubber foot of the headpiece has an opening with a diameter of 14 mm and thus an area of about 1·5 cm^2 is sprayed with this model.

A large number of orientating experiments were carried out in the axillae of twenty adult subjects. It was shown that fairly reproducible numbers of viable micro-organisms could be harvested, although these numbers were highly dependent on the quantity of the washing solution.

Generally a similar pattern was found between the obtained micro-organisms as a function of the wash volume. Figure 3 shows this pattern, averaged over twenty experiments. After washing the skin with 3 l of the washing liquid hardly any micro-organism could be found. The figures found for the density of the microbial population in the axillae agreed with figures from recent literature (13, 24, 21, 1).

Figure 3 shows that the number of micro-organisms in the first 100 ml is very high. The number of bacteria in the fraction from 100 to 1000 ml are not, however, negligible. We assumed that the micro-organisms in the first 100 ml originated from that part of the skin which was directly hit by the water jet from the spray nozzle. The micro-organisms in the 1000 ml fraction should possibly come from the surrounding area under the sprayhead and not from places deeper in the skin.

To confirm this assumption two plastic headpieces were constructed, with an opening of 2 and 5 mm respectively. In *Figs 4* and *5* the washing patterns of these sprayheads on the axillae skin are shown.

Comparison of the diagrams with the various sprayheads leads to the following conclusions. (a) If the exposed area of the skin is diminished by applying a smaller sprayhead, the quantity of washing fluid necessary for removal of all micro-organisms is also diminished. This means that the efficacy of a sprayhead increases when the opening decreases. (b) The efficacy diagram of the 2 mm sprayhead shows that with 100 ml washing liquid almost all available micro-organisms are harvested. With this sprayhead the complete exposed skin is directly hit by the water jet and the micro-organisms are loosened very fast from the skin.

In *Table I* the washing efficacies of the three sprayheads are given; for practical reasons only wash volumes of 50 ml and 100 ml are considered.

* Octylphenoxypolyethoxy ethanol, Rohm and Haas Co.

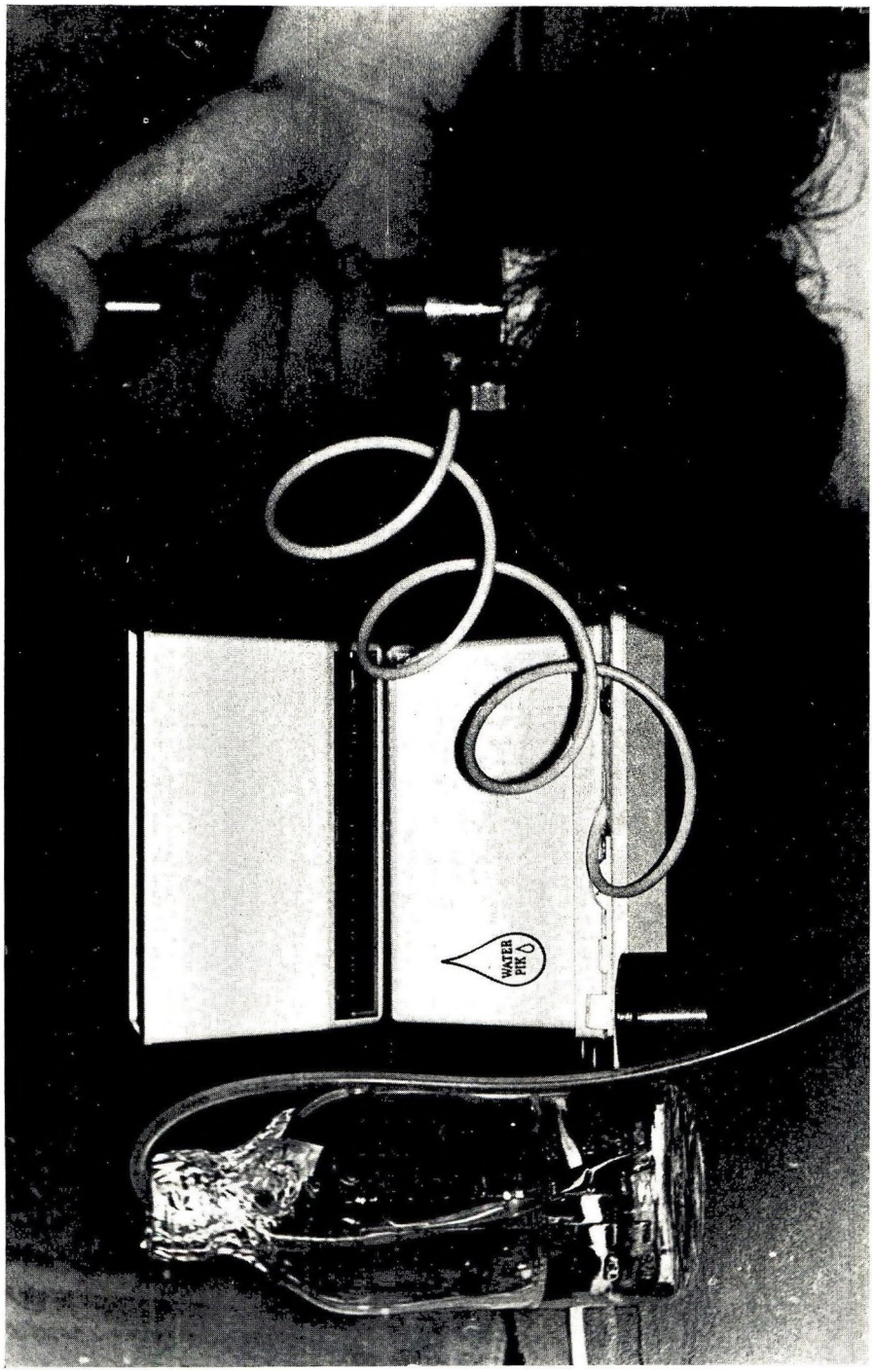

Figure 2. A new method for the quantitative determination of micro-organisms on human skin.

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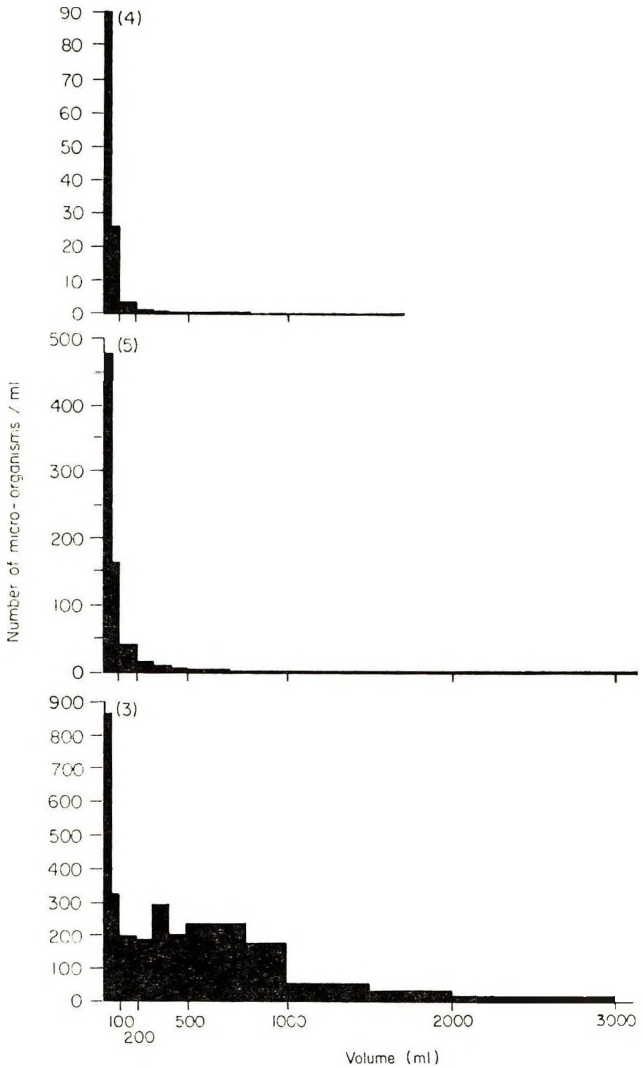

Figure 3. Recovery of aerobic bacteria from axilla over an area of 14ϕ (mm).
Figure 4. Recovery of aerobic bacteria from axilla over an area of 2ϕ (mm).
Figure 5. Recovery of aerobic bacteria from axilla over an area of 5ϕ (mm).

Table I. Observed washing efficiencies of different diameter sprayheads

Diameter of sprayhead (mm)	Percentage washing efficacy	
	50 ml	100 ml
2	69	89
5	58	78
14	18	25

As can be seen from the *Table I*, the micro-organisms, which are directly hit (2 mm sprayhead) are loosened very fast and almost quantitatively from the skin. The surrounding areas under the larger sprayheads release the micro-organisms at a slower rate that will depend on the distance between that area and the middle of the spraychamber.

The relationship between this relative efficacy and the distance towards the middle can be calculated from the above mentioned figures. These relative efficiency figures are shown in *Table II*.

Table II. Calculated efficiencies of micro-organism recoveries

Area		Relative percentage washing efficacy	
Inner diameter (mm)	Outer diameter (mm)	50 ml	100 ml
0	2	69	89
2	5	56	76
5	14	12·6	17·5

These data are shown graphically in *Fig. 6*. We believe the best interpretation of the diagram shows that with 100 ml wash fluid the relative efficiency right in the middle of the spraychamber is about 100%.

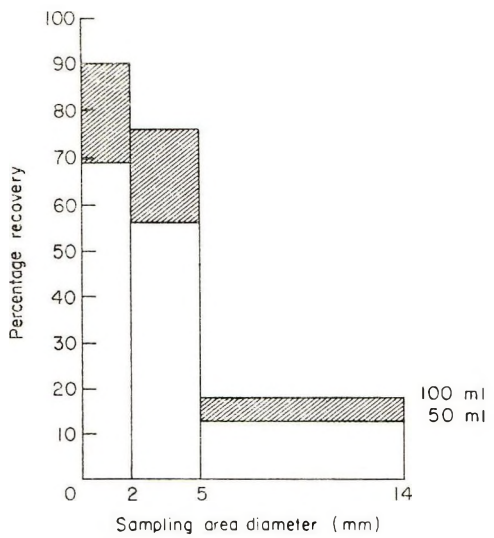

Figure 6. Percentage recovery of total aerobic bacteria from axilla.

In the tape stripping experiments of Kligman (2) and with the electron microscope studies by Montes and Willborn (25) it appeared that most of the bacteria are located on the upper layer of the epidermis. The high washing efficiency of the 2 mm sprayhead supports this statement.

Through its high efficacy the 2 mm sprayhead with a wash volume of 100 ml is an ideal combination for the quantification of the skin population.

In practice, however, we noticed that the sealing of the 2 mm headpiece was insufficient, especially on hairy areas of the skin. The use of a good rubber sealing was also impossible, due to the high flexibility in relation to the small opening diameter. For this reason all further experiments were carried out with our standard 14 mm sprayhead with a total efficacy of 25%.

As mentioned before the axillae and the scalp are parts of our body, which are interesting for cosmetic research. The study of the microbial population of the axilla is important through the application of a deodorant product and the microflora of the scalp can be used to study the effect of an antidandruff preparation. For this reason we tested the features of the Water-Pik method on these areas.

The microbial population of the axilla was determined by washing with 100 ml on five adjacent locations on each axilla. The complete quantity of washing fluid (500 ml) was collected and after homogenisation and dilution the number of aerobic micro-organisms was counted.

The Water-Pik method gave fairly reproducible numbers of micro-organisms, as can be seen from *Table III*.

Table III. Variation between individuals of the recovery of micro-organisms by a 14 mm head using the Water-Pik method

Subject	Totally recovered number of micro-organisms (14 mm head)	
1	L $1{\cdot}5 \times 10^6$	R $1{\cdot}3 \times 10^6$
2	$2{\cdot}2 \times 10^4$	$2{\cdot}5 \times 10^3$
3	$1{\cdot}3 \times 10^5$	$8{\cdot}8 \times 10^4$
4	$3{\cdot}9 \times 10^4$	$1{\cdot}0 \times 10^6$
5	$7{\cdot}7 \times 10^4$	$6{\cdot}9 \times 10^4$
6	$2{\cdot}5 \times 10^4$	$1{\cdot}7 \times 10^4$
7	$5{\cdot}5 \times 10^4$	$5{\cdot}0 \times 10^3$

In accordance with the results of other investigators (26, 1), a big individual variation is shown in the number of the micro-organisms. *Table III* shows a good relationship between the population of the left and the right axilla, regardless of the magnitude of these numbers.

For the sampling of the scalp also five times 100 ml with the 14 mm sprayhead was used. Here the total number of lipophylic micro-organisms was counted. In *Table IV* a survey is given of the scalp population of four subjects determined on several occasions. As it is hardly possible to sample twice on a scalp at the same time the reproducibility of our method on the scalp is difficult to give.

While sampling bacteria from scalp and axillae by the Water-Pik method scales were dragged along from the skin surface with the fluid stream (27). These scales did not interfere with the microbiological figures, as the microcolonies in the scales appeared to be easily dispersed in the Triton wash fluid. This finding is in accordance with the experiments of Williams (1) in which was demonstrated that samples wash fluid only contain disaggregated organisms.

In one of the long-lasting spray experiments on the axilla skin (*Fig. 3*), the micro-organisms in each fraction were identified. The types of micro-organisms agreed with

Table IV.

Time of sampling	Total lipophylic micro-organisms/cm² on the scalp			
	Subject 1	Subject 2	Subject 3	Subject 4
After 1 week	$7{\cdot}1 \times 10^5$	$2{\cdot}6 \times 10^6$	$5{\cdot}4 \times 10^5$	$6{\cdot}9 \times 10^5$
After 2 weeks	$1{\cdot}9 \times 10^5$	$1{\cdot}4 \times 10^6$	$5{\cdot}8 \times 10^5$	$4{\cdot}4 \times 10^5$
After 3 weeks	$4{\cdot}4 \times 10^5$	$1{\cdot}6 \times 10^6$	$1{\cdot}1 \times 10^5$	$2{\cdot}9 \times 10^5$
After 4 weeks	$3{\cdot}8 \times 10^5$	$1{\cdot}7 \times 10^6$	$1{\cdot}9 \times 10^5$	$9{\cdot}4 \times 10^5$
After 5 weeks	$4{\cdot}8 \times 10^5$	$3{\cdot}0 \times 10^6$	$1{\cdot}6 \times 10^5$	$7{\cdot}5 \times 10^5$

those given in the literature (28, 29). We also noticed that the ratio between aerobic and anaerobic micro-organisms did not change as a function of the quantity of washing fluid.

CONCLUSION

The Water-Pik method has been tested as a method to quantify the number of micro-organisms in the axilla and on the scalp. In these areas of the human skin, which are important for the study of the effectiveness of a deodorant or antidandruff preparation, the Water-Pik method appeared to be a suitable tool.

The Water-Pik method is easy to handle and gives little discomfort for the subjects. Not only for cosmetic research but also for medical or dermatological studies the described method can be recommended for the quantitative sampling of the skin population.

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Short term effects of emollients and a bath oil on the stratum corneum*

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Synopsis

Recently devised techniques were employed to detect and measure **surface changes** and structural alterations in the **stratum corneum** after single applications of both oil in water **emollients** and a dispersion type of **bath oil**. Flattening of the surface contours of the skin and 'plumping' of individual corneocytes were apparent on **replicas** of the skin taken from treated areas and suggested hydration. These effects were most apparent when replicas were examined by **scanning electron microscopy** and by **surface contour** tracing. Small changes observed in **skin surface biopsies** from treated areas may reflect alterations in the internal structure of the top layers of stratum corneum. An increase in skin furrow width was also detected in photographs of the surfaces of treated skin.

INTRODUCTION

Considerable symptomatic relief for patients with itching and scaling dermatoses is obtained by measures designed to increase hydration of the stratum corneum (SC). Apart from symptomatic relief this type of therapy may actually hasten recovery by the reduction of fissuring, excoriation and scaling. In this study, we have attempted to assess changes in the surface structure of skin after single treatments with either water containing a dispersion type of bath oil, or one of two oil in water emulsion emollients using techniques developed by our department.

MATERIALS AND METHODS

Materials used

Bath Oil A. Alpha Keri® (oil soluble fraction of lanolin, mineral oil and non-ionic emulsifiers), Westwood Pharmaceuticals. *Emollient B.* Keri lotion® (kerohydric® emollient, non-ionic emulsifiers), Westwood Pharmaceuticals. *Emollient C.* Oil of Ulay® (lipid particles in an aqueous phase), Garsalle Division of Richardson-Merrell, Ltd. The materials were supplied by the manufacturers and employed according to their instructions.

Skin surface biopsies (SSBs)

Samples of SC were taken onto glass microscope slides with a cyanoacrylate adhesive

* Based on a paper read at the European Society for Dermatological Research, Amsterdam, May 1977

0037–9832/78/1000–0617 $02.00

(Permabond Staident Ltd) using the skin surface biopsy technique to reveal the ruptured internal surface of the outer SC (1).

Skin surface replicas

Negative impressions of the skin surfaces were taken with standard Silflo impression material (J. & S. Davies, London). Positives of the skin surface were subsequently obtained from the 'negative' by covering them with DPX (R. A. Lamb, London) histological mounting medium (2). The negatives with DPX were then placed in a desiccator for 5 or 6 h or left overnight at room temperature before separating the 'positive' from the 'negative'.

Scanning Electron Microscopy (SEM)

Replicas and SSBs were mounted on stubs and coated with gold in a Polaron E5000, sputter coater, and examined in a Cambridge Stereoscan S2 scanning electron microscope at approximate magnifications of $\times 200$ and $\times 1000$.

Macrophotography

Photographs of the skin surface were taken using a 35 mm camera fitted with a 105 mm lens on a bellows attachment and an electric ring flash. Negatives were printed to give a final magnification of $\times 8$. Skin furrow widths were measured with an eye piece graticule ($\times 8$) by taking measurements in each of five standard areas in the photographs (*Fig. 1*), and averaging the values for the five areas.

Surface contour measurement of SSBs and replicas

SSBs and replicas were examined in an apparatus (3) designed for measuring surface contours (Surfometer, Planer Products, Ltd). The surfometer employs a stylus to traverse a test surface, the contours of which are traced on moving chart paper.

Skin surface biopsies (SSBs)

The areas under the curves of the surfometer tracings were measured with a planimeter and the mean peak height calculated for a representative 10 cm length of trace.

Skin surface replicas

Only very thin DPX positives were suitable for surface contour tracing. When separated from the negatives they were left to harden at room temperature. The DPX positives were then fixed to glass slides and the surface contours measured in our surfometer. The area described by the surface contour in 10 cm length of trace was measured with a planimeter. (For the Oil of Ulay study (C) tracings of 8·5 cm length of trace were used and the total length of contour was also measured using a curvimeter).

Ambient conditions

Laboratory personnel acted as volunteers for the experiments. They were instructed to avoid activities (such as promotion of sweating as a result of exercise) which may have masked any effect due to the treatment. Relative humidity (RH) and temperature (°C) were monitered in the laboratory areas. ($56 \pm 5\%$ RH $20{\cdot}5 \pm 0{\cdot}5$°C).

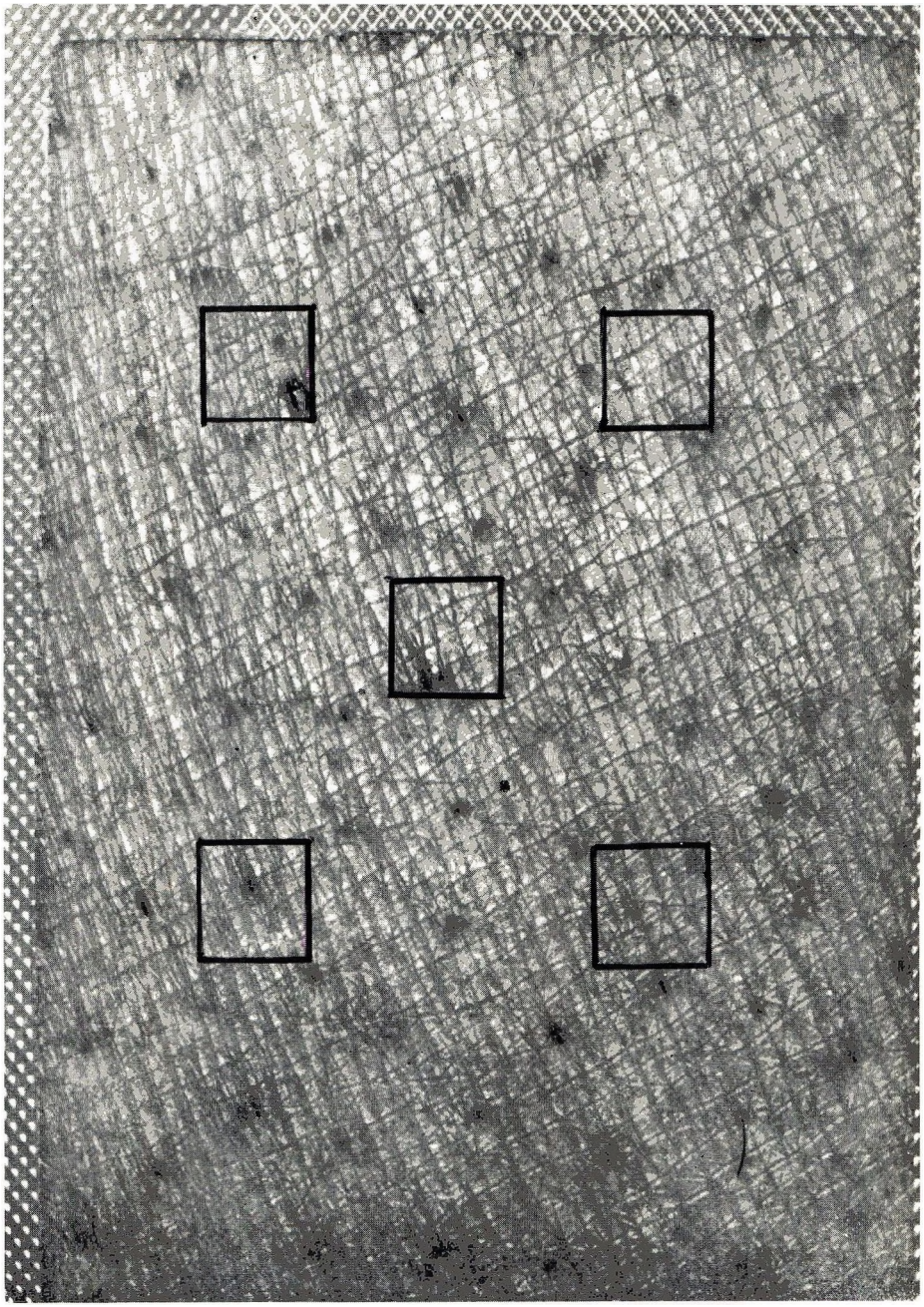

Figure 1. Macrophotography. Measurement of skin furrow width in five standard areas of the photograph.

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PROCEDURES USED

Bath Oil A

The study was conducted on the skin of the lateral aspects of the lower legs of six volunteers. The area of skin (5 × 15 cm) on each leg was marked out in order that identically treated sites could be examined. Prior to immersion SSBs, surface replicas and macrophotographs were taken from the test sites. The lower leg was then immersed in water at 45°C for 20 min. To one of the vessels Bath Oil A was added to the water to give a final concentration of 0·02% (Manufacturer's recommendation). After 20 min the legs were removed from the baths, patted dry and the above assessments were repeated from adjacent sites 5 min after removal and at 60 min and 240 min.

Emollient B

The skin of the lateral aspects of the legs of twelve volunteers was examined, six with normal skin and six with 'socially' dry skin but without clinically significant skin disease. Assessments (as described for Bath Oil A) were performed on adjacent sites before treatment and after Emollient B had been applied, at 0 (immediately after application) 30, 60, 120 and 240 min. (Any excess oil remaining on the skin surface was wiped away.)

Emollient C

The study was conducted on the flexor aspect of the forearm of twelve normal volunteers. Assessments were performed before treatment and from adjacent sites after Emollient C had been applied after 60, 120, 180 and 300 min.

RESULTS

MACROPHOTOGRAPHY

Table I shows the effect of Bath Oil A on skin furrow width. *Table II* shows the effect of Emollient B. Macrophotography was not done for Emollient C.

Table I. Effect of Bath Oil A on skin furrow width

Post immersion time (min)	Oil leg width (mm ± SD)	Water leg width (mm ± SD)
Controls	1·48 ± 0·06	1·45 ± 0·18
5	1·51 ± 0·18	1·54 ± 0·22
60	1·58 ± 0·16	1·57 ± 0·13
240	1·65 ± 0·27	1·64 ± 0·23

It can be seen from *Tables I* and *II* that an overall increase in skin furrow width was detected subsequent to treatment by bath oil or emollient.

Table II. Effect of Emollient B on skin furrow width

Post application time (min)	'Dry' skin width (mm ± SD)	Normal skin width (mm ± SD)
Controls	1·41 ± 0·20	1·34 ± 0·19
0	1·51 ± 0·16	1·54 ± 0·16
30	1·80 ± 0·37*	1·54 ± 0·21
60	1·57 ± 0·11	1·45 ± 0·12
120	1·52 ± 0·16	1·37 ± 0·15
240	1·77 ± 0·40	1·43 ± 0·26

* Difference from control ($P = 0·05$)

SURFACE CONTOUR MEASUREMENT

SSBs

The effects of Bath Oil A and Emollient B on the surface contour of the internal ruptured face of SSBs are shown in *Tables III* and *IV*.

Table III. Effect of Bath Oil A. Area under surfometer tracing in 10 cm of trace from SSBs

Post immersion time (min)	Area (cm^2 ± SD)	Mean peak height ± SD
Controls	8·5 ± 2·2	21·6 ± 6·3
5	5·6 ± 1·5*	16·4 ± 4·5
60	8·2 ± 1·9	18·5 ± 1·8
240	6·9 ± 2·3	15·9 ± 5·0

* Significant difference from control $0·05 > P > 0·02$

Table IV. Effect of Emollient B. Area under surfometer tracing in 10 cm of trace from SSBs

Post application time (min)	Area (cm^2 ± SD)	Mean peak height ± SD
Controls	6·3 ± 1·5	13·0 ± 2·8
0	5·9 ± 1·8	11·2 ± 3·2
30	6·2 ± 2·2	11·8 ± 4·0
60	6·6 ± 1·4	12·2 ± 2·9
120	5·5 ± 1·7	11·4 ± 2·1
240	5·4 ± 1·5	10·9 ± 3·4

There were no significant differences between the control figures and the various post treatment time figures for Emollient B although a decrease in area and peak height measurement was apparent in SSBs from treated areas subjected to Bath Oil A. There were no significant differences for the effects of Emollient B on dry skinned individuals compared to normal individuals.

Skin surface replicas

Figure 2 shows a group of representative surfometer tracings from the replicas of a control area and areas treated with Emollient C. These tracings provide information about skin surface topography. We have attempted to quantitate the results by measuring the area (planimetrically) within the major contours in a known length of trace and determining the total length of contour by curvimetry. *Tables V–VII* summarise our findings.

Table V. Effect of Bath Oil A. Area under surfometer tracing in 10 cm length of trace from replicas

Post immersion time (min)	Mean area (cm^2 ± SD)	
	Oil leg	Water leg
Controls	14·3 ± 3·7	15·4 ± 5·7
5	10·9 ± 2·9	12·4 ± 2·9
60	8·4 ± 1·9*	9·3 ± 2·2†
240	8·9 ± 1·6*	10·1 ± 2·0

* Significant difference from control $P < 0.01$
† Significant difference from control $P < 0.05$

Table VI. Effect of Emollient B. Area under surfometer tracing in 10 cm of trace from replicas

Post application time (min)	Normal skin	Dry skin
Controls	9·2 ± 3·2	10·5 ± 2·0
0	7·9 ± 2·6	10·1 ± 3·7
30	7·8 ± 2·8	9·1 ± 2·4
60	7·4 ± 1·1	8·8 ± 3·2
120	6·7 ± 1·7	8·0 ± 2·4
240	6·9 ± 3·1	9·0 ± 1·8

Table VII. Effect of Emollient C. Length of contour of surfometer tracings from replicas

Post application time (min)	Length of contour (cm ± SD) in 8·5 cm of tracing
Controls	38·0 ± 6·3
60	34·4 ± 6·1
120	30·6 ± 7·09*
180	32·7 ± 6·4
300	33·3 ± 4·3

* Significant difference from controls ($P < 0.01$)

It can be seen from *Tables V* and *VI* that a decrease in replica area was apparent subsequent to treatment and that total contour length (*Table VII*) also appears to decrease after application of Emollient C.

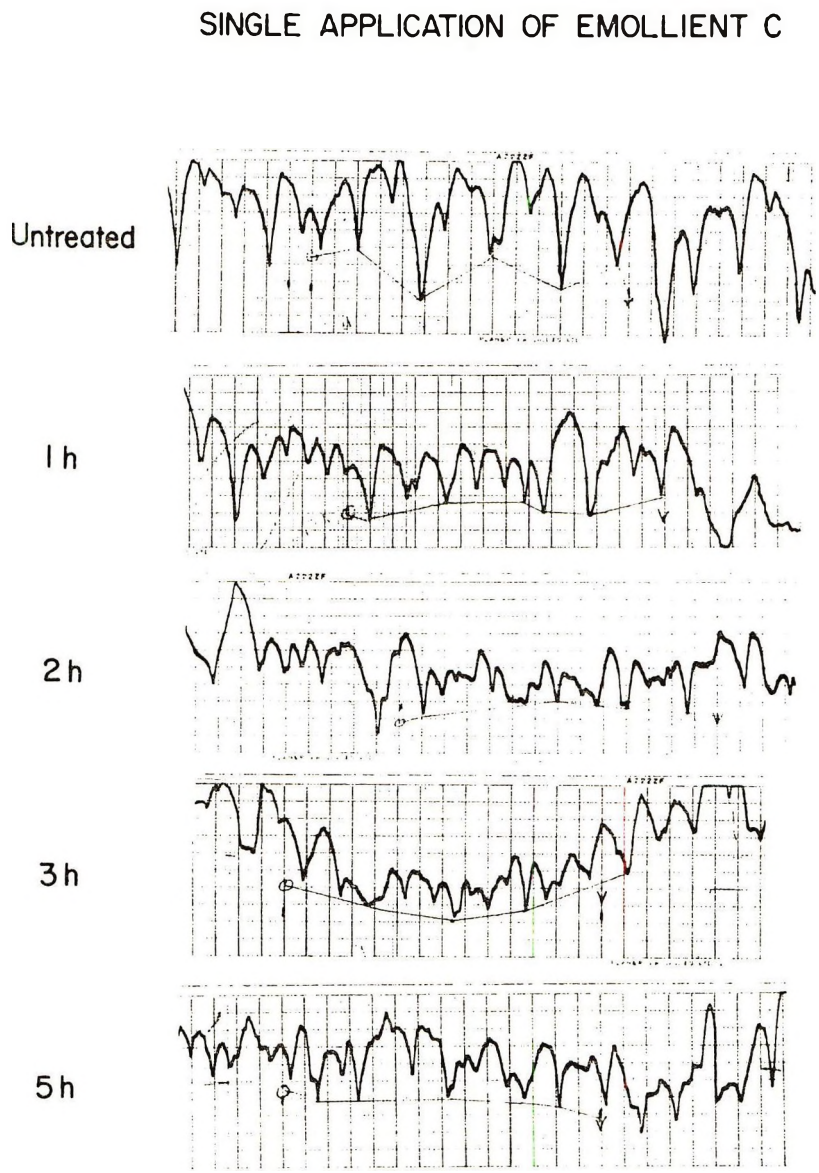

Figure 2. Surfometer tracings of skin surface replicas from adjacent forearm sites of a 23 year old male.

Scanning electron microscopy

Figures 3a and *b*, *4a* and *b* show examples of scanning electron micrographs of skin surface replicas from untreated forearms and forearms treated with a single application of Emollient C. Photographs taken at approximately × 200 revealed the general topography of the specimen. Surface features such as hairs, sweat orifices, lamellae of partially attached groups of corneocytes were revealed in some detail. Replicas taken from treated skin appeared to show a flattening of the major skin contours. This effect was still evident

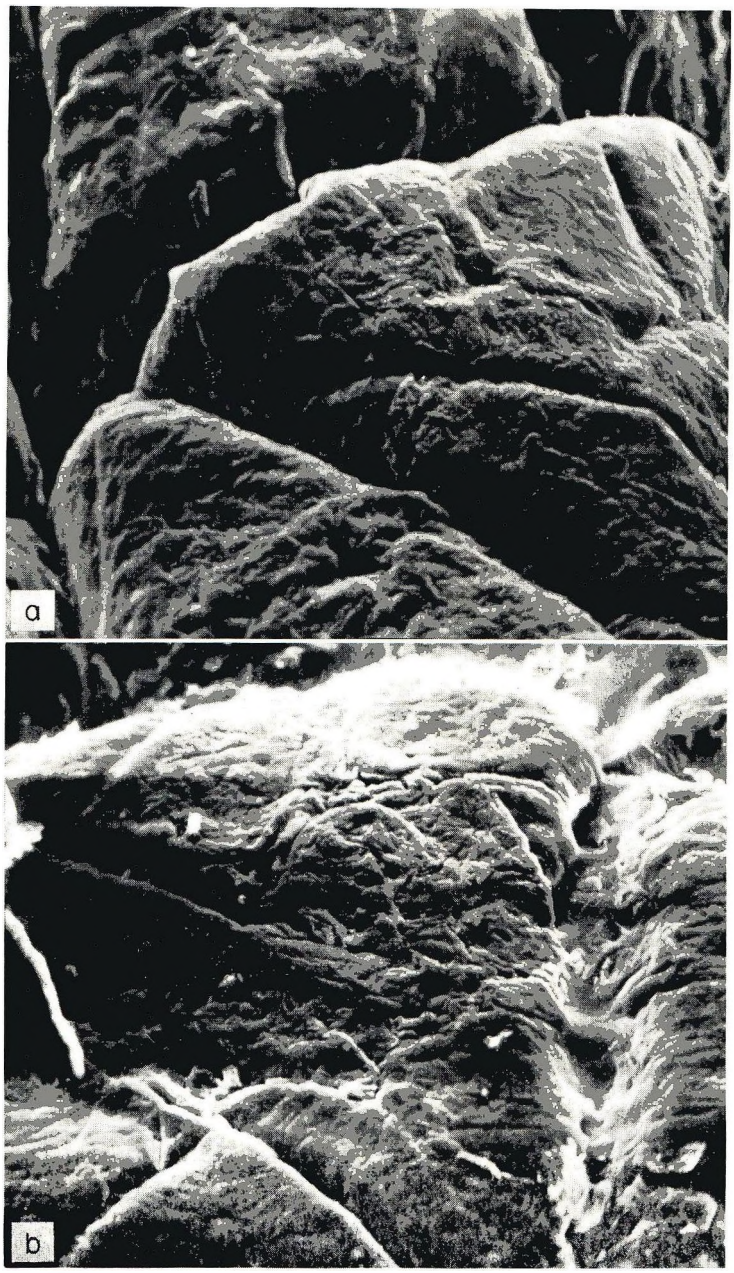

Figure 3 (a). SEM of a replica from untreated forearm of a 24 year old female (× 173). **(b)** SEM of a replica from an adjacent site 60 min after application of Emollient C. (× 170) showing considerable flattening of surface contour.

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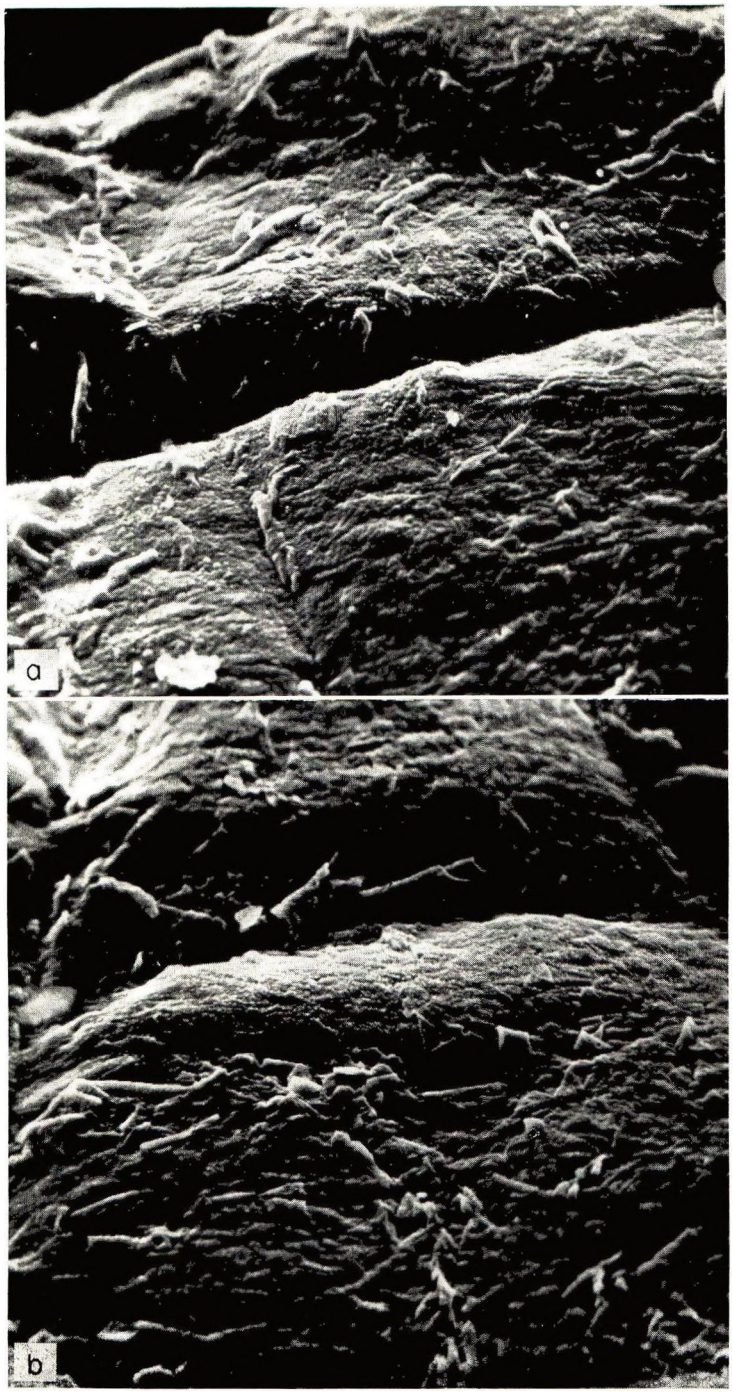

Figure 4(a). SEM of a replica from untreated forearm of a 49 year old male (× 188). **(b)** An adjacent site 300 min after application of Emollient C, again showing a flattening of contour.

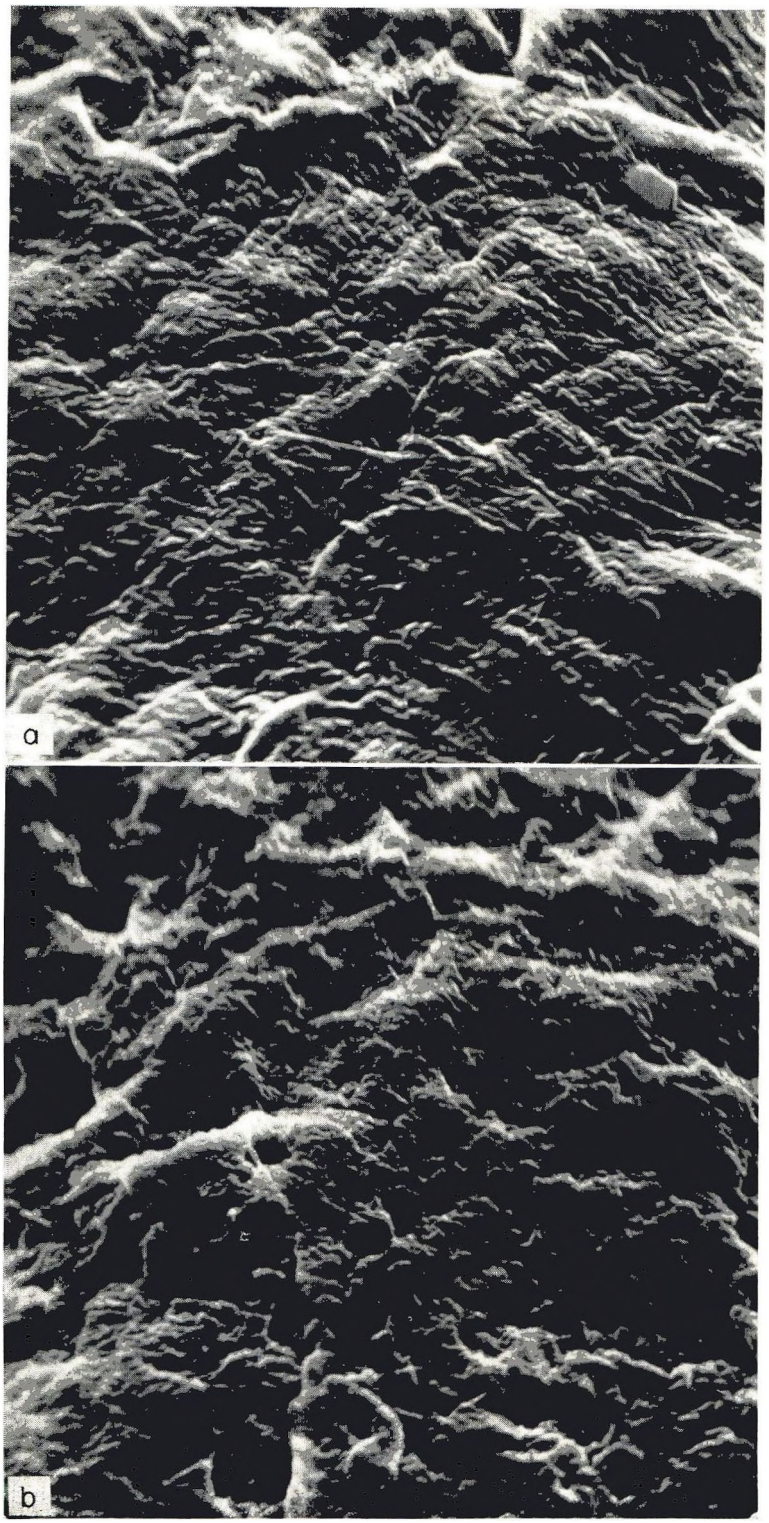

Figure 5 (a). SEM of a replica from the untreated leg of a 55 year old female (× 840). **(b)** An adjacent site 120 min after application of Emollient B, (× 894). Individual corneocytes appear more prominent. In some areas 'rolled' thickened edges of the corneocytes can be discerned.

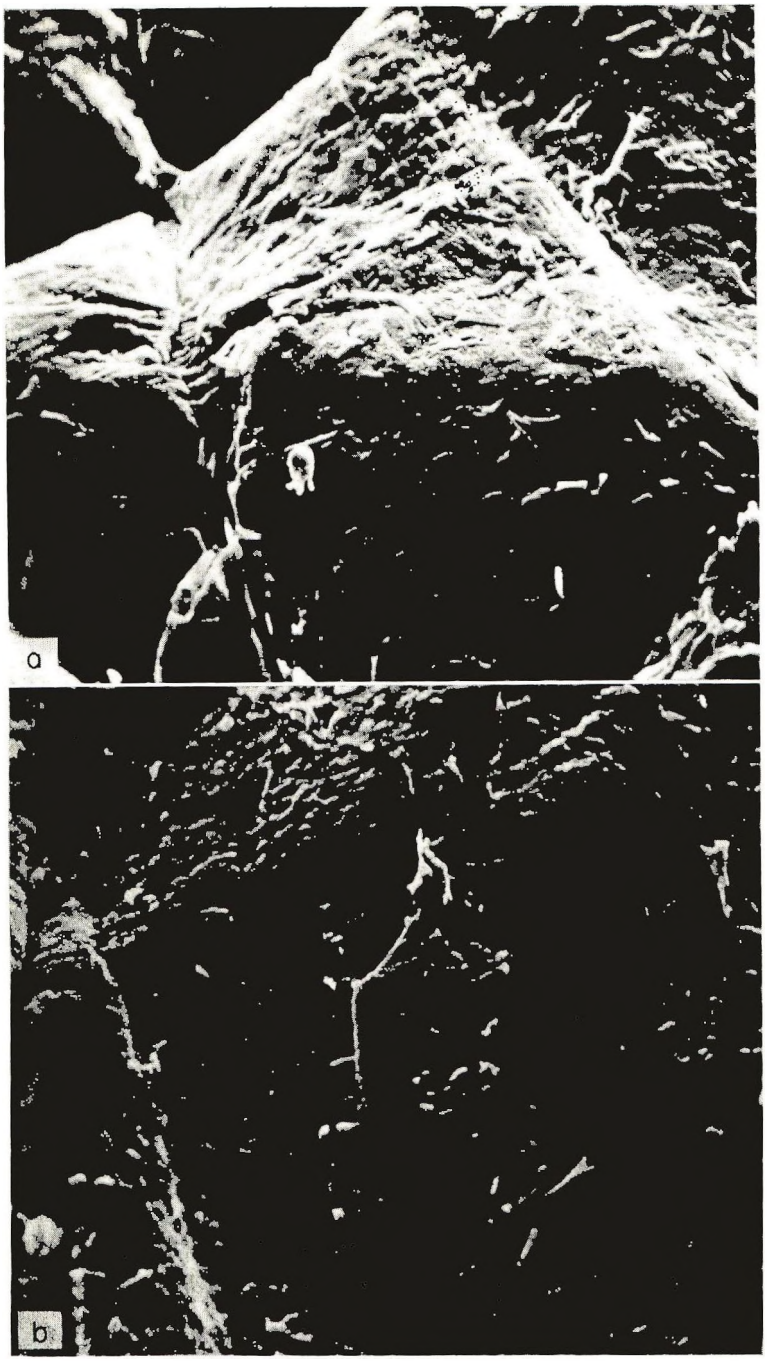

Figure 6 (a). Replicas from the leg of a 55 year old female with 'socially dry' skin (× 170). Fissuring is apparent between areas of apparently adherent corneocytes. **(b)** An area 240 min after treatment with Emollient B (× 168). Although a 'smoothing' of the surface furrows was detected, some fissuring of surface layers was still apparent.

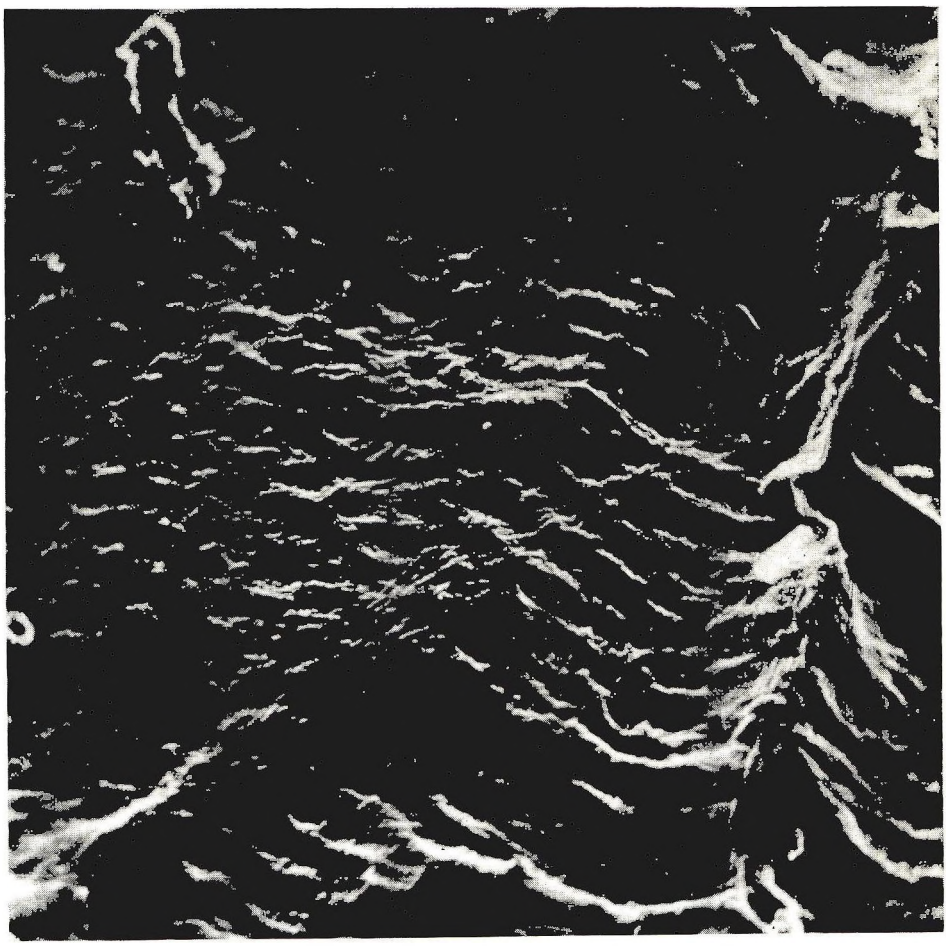

Figure 7. SEM of an SSB from untreated skin ($\times$ 200).

after 5 h but some 'recovery' was apparent 8 h subsequent to treatment. Higher magnifications (× 1000) of replica surfaces from areas treated with Emollient B indicated that a change in corneocyte morphology had taken place subsequent to treatment (*Fig. 5a* and *b*). The individual corneocytes often appeared thicker and the edges were more rounded and 'rolled' rather than angulated as in the controls. *Figure 6a* and *b* are scanning electron micrographs of replicas from both the untreated and the Emollient B treated legs of a woman with 'dry skin'. Although the emollient appears to have had a 'smoothing' effect on the surface furrows the uplifting of layers of surface corneocytes characteristic of dry skin (Nicholls, King and Marks, unpublished observations) were still apparent.

No obvious changes were observed in scanning electron micrographs of SSBs from treated sites. In some specimens, however, ridge patterns appeared less accentuated at low magnifications. *Figure 7* shows a typical ridge pattern of an SSB.

DISCUSSION

This study was designed to monitor the changes that take place at the skin surface and in the superficial parts of the horny layer following a single application of some of the commercially available emollients and bath oils. We have made no attempt at comparing the emollient preparations that we used although clearly comparative studies could easily be set up using the techniques that we describe.

The results obtained suggested that the two emollients (oil in water emulsions) and the bath oil were all quite effective in 'smoothing' the SC. SEM of replicas from legs soaked in water only showed no obvious changes from the control site, although a significant decrease in surface contour was detected at 60 min post immersion. Other workers have detected little change in replica appearance after soaking for 1 h in warm water alone, although a clinical change was detected (4). Surface contour measurements of the hardened DPX replicas in the surfometer provided us with quantitative information about skin surface topography before and after treatment and demonstrated a flattening of contour after use of the materials. Since replicas were taken from adjacent sites (repeated replicas on one site may alter or remove some surface characteristic) the heterogeneity in surface architecture was reflected in the surface contour tracings. Subsequent to treatment, however, the reduction in surface contour was usually greater than normal site variation in each individual. These observed changes seemed to correlate with the morphological appearance of the specimen when examined by SEM, i.e. a filling in of the topographical 'valleys'.

The small area and peak height changes demonstrable in SSB surfometer tracings suggested that some alteration in the internal structure of superficial corneum had also taken place subsequent to treatment although hardly any change could be demonstrated when the SSBs were inspected by SEM. Photography of skin sites has been used previously as a subjective means of evaluating emollients (5). A final magnification of × 8 was used in this study. The consistent (but non-significant) increase in surface furrow width on our macrophotographs is difficult to explain, but may be due to an optical effect from the decreasing furrow depth after application of emollient or bath oil. We do not believe that this method has a great deal to offer and believe that more accurate data can be obtained by contour measurements of replicas. Furthermore, we suggest that it is particularly helpful when assessing 'skin surface active materials' to use a combination

of replicas and SSBs for assessment. In this way it is possible to compare what is taking place at the surface with changes of some cell layers down in the SC.

We hope to employ the techniques described in this study to evaluate the effects of chronic application of topical preparations thought to have a hydrating action on the SC and to correlate these effects with changes in the pattern of epidermal growth and maturation.

ACKNOWLEDGMENTS

We are extremely grateful to Vick International Ltd, and Westwood Pharmaceuticals Inc for generous assistance towards these studies.

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Water diffusion coefficients versus water activity in stratum corneum: a correlation and its implications

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Synopsis

Literature reports of rates of **trans-epidermal water loss** through skin exposed to differing external relative humidities have been used to generate **water diffusion coefficients** by means of Fick's law. These have been related, within a given series, to the diffusion coefficient at a standard average water activity to give relative water diffusion coefficients. These have been combined, together with literature values of water diffusion coefficients obtained by kinetic experiments on stratum corneum exposed to various humidities, to yield a correlation between the average water activity of stratum corneum and the relative water diffusion coefficient over the full range of water contents. The asymptotic relationship derived shows that the diffusion coefficient increases rapidly as the water activity approaches its maximum. The implications of this relationship in terms of water activity profiles of stratum corneum are discussed. A theoretical analysis of the subject is presented and water activity profiles of model systems are derived.

INTRODUCTION

The importance of the water content of the stratum corneum in determining its observable properties is well documented (1–4). Simple diffusion theory shows that the water content will be lower in the outer layers than in the inner layers under normal ambient conditions. This variation with position will be non-linear, since the water diffusion coefficient will vary with water content (5, 6). A formula correlating the diffusion coefficient with the water content of the stratum corneum at that point would be useful and would enable a water content profile of stratum corneum under different external conditions to be derived.

Although there are several reports of variations in trans-epidermal water loss (TEWL) with external relative humidities, no single set of experiments has been sufficient to derive an empirical relationship between diffusion coefficient and water content. By combining the data from several literature reports, a correlation has been derived between stratum corneum water activity and relative diffusion coefficient.

DEFINITIONS

Fick's Law has been used extensively and successfully in relation to diffusion processes in stratum corneum (7). This is the basic equation involved in the theoretical treatment of this topic.

$$J = D_i \frac{dC_i}{dx} \qquad (1)$$

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where J = water transfer rate (TEWL) (mg cm^{-2} h^{-1})
D_i = diffusion constant at point i (cm^2 h^{-1})
C_i = water activity at point i (mg cm^{-3})
x = distance from the edge of the stratum corneum (cm)

Scheuplein and Blank (7) have shown that the water diffusion coefficient of the stratum corneum is the dominant factor in determining the permeability of whole skin and thus the great majority of the water activity differential across the skin will take place across the stratum corneum. For the purpose of this discussion, it will be assumed that any other water activity differential is negligible in comparison.

The definition of C_i needs further amplification. The concentration of water vapour in air in equilibrium with the water at point i is a useful concept, but since the diffusion process *in vivo* is generally non-isothermal, it is necessary to define C_1, in relation to a standard state. This is normally saturated vapour at the temperature of point i. The following definition of C_i will be used in this correlation,

$$C_i = \frac{4{\cdot}39 \times 10^{-2} \times \text{R.H.}_i}{100} \text{ (mg cm}^{-3}\text{)}$$

where R.H._i = relative humidity in air, at the temperature of point i, which would be in equilibrium with the water at point i. ($4{\cdot}39 \times 10^{-2}$ mg cm^{-3} is the saturated water vapour concentration at body temperature (8), i.e. C_{internal}).

Several investigators have reported values of TEWL at different external relative humidities, either *in vivo* (9–11), or *in vitro* (4, 12). Diffusion coefficients are obtainable from these reports. (This requires an assumption to be made about stratum corneum thickness, but since the values used finally are relative to a standard state, the accuracy of that assumption does not affect the correlation.)

These diffusion coefficients can then be plotted against the average water activity of the stratum corneum samples. In all cases the internal relative humidity was approximately 100%. If the initial assumption is made that the water activity profile is close to linearity, the average water activity is derived from the average of the internal and external humidities (a correction for the non-linearity of the water activity profile will be applied later).

$$C = \frac{4{\cdot}39 \times 10^{-2}(\text{R.H.}_{\text{internal}} + \text{R.H.}_{\text{external}})}{200} \text{ (mg cm}^{-3}\text{)}$$

In addition, El-Shimi and Princen (13) have determined diffusion coefficients at different humidities from kinetic sorption, desorption experiments. In these the relative humidity was the same on both sides of the stratum corneum samples, and thus

$$C_i = \frac{4{\cdot}39 \times 10^{-2} \times \text{R.H.}}{100} \text{ (mg cm}^{-3}\text{)}$$

The data from the above references were obtained using different sources of stratum corneum and under different experimental conditions. In order to attempt a correlation, it is necessary to convert the results to relative diffusion coefficients (relative to a standard water activity state). The reference state chosen for convenience was the common experimental conditions of $\text{R.H.}_{\text{internal}} = 100\%$, $\text{R.H.}_{\text{external}} = 0\%$ (i.e. $C = 0{\cdot}5 \times 4{\cdot}39 \times 10^{-2}$ mg cm^{-3}). The diffusion coefficient for this condition is denoted by D_0.

$$R = \text{relative diffusion coefficient} = \frac{D}{D_0}$$

where $D = \dfrac{J\,t}{\Delta C}$

$$\Delta C = C_{\text{internal}} - C_{\text{external}}$$

t = stratum corneum thickness (cm), assumed to be 15×10^{-4} cm, a typical value for human stratum corneum (14)

$C_{\text{internal}} = C_0 = 4{\cdot}39 \times 10^{-2}$ mg cm^{-3}

CORRELATION

The data used in the correlation is given in *Table I*. It was found that there was generally a linear relationship between D and $C_0/(C_0 - C)$. In those cases where the data does not include a value for D_0, this was obtained by extrapolation of D against $C_0/(C_0 - C)$ using linear regression. The extrapolated value of D_0 was then used to obtain values of R.

Table I. Water activity of stratum corneum versus water diffusion coefficients from literature sources

Source (Reference)	R.H.-external (%)	$C \times 10^2$ (mg cm^{-3})	$\frac{C_0}{C_0 - C}$	$D \times 10^3$ (cm^2 h^{-1})	R
	0	2·195	2·00	28·5	1·00
	29	2·83	2·81	31·2	1·09
9	55	3·40	4·43	43·7	1·53
	80	3·95	9·98	91·2	3·20
	88	4·13	16·88	125·0	4·39
	15	2·52	2·34	12·5	1·02
	31	2·88	2·91	13·4	1·09
4	51	3·31	4·07	16·05	1·31
	75	3·84	7·94	25·95	2·11
	89	4·15	18·20	43·5	3·55
	1·5	2·23	2·04	17·3	1·00
	26	2·77	2·69	20·25	1·17
	28	2·81	2·75	19·95	1·15
	28	2·81	2·75	20·4	1·18
	32	2·90	2·94	22·05	1·27
	33	2·92	2·99	19·95	1·15
	33	2·92	2·99	21·9	1·27
12	34	2·94	3·03	22·8	1·32
	45	3·18	3·64	23·55	1·36
	49	3·27	3·92	25·5	1·47
	53	3·36	4·26	29·85	1·73
	56	3·42	4·55	27·15	1·57
	59	3·49	4·88	32·55	1·88
	2·6	2·25	2·05	12·6	1·00
11	25·0	2·74	2·67	26·4	2·10
	49·0	3 27	3·92	31·5	2·50
	76·0	3·86	8·33	42·75	3·40

Continued on next page

Table I. (*continued*)

Source (Reference)	R.H.$_{external}$ (%)	$C \times 10^2$ (mg cm^{-3})	$\frac{C_0}{C_0 - C}$	$D \times 10^2$ (cm^2 h^{-1})	R
	20	2·63	2·50	58·5	1·25
	40	3·07	3·33	75·7	1·61
	80	3·95	10·00	222·1	4·77
	20	2·63	2·50	70·5	1·09
	40	3·07	3·33	96·2	1·49
	80	3·95	10·00	234·1	3·63
	20	2·63	2·50	47·8	1·10
	40	3·07	3·33	70·6	1·62
	80	3·95	10·00	179·4	4·14
10	20	2·63	2·50	50·4	1·13
	40	3·07	3·33	66·6	1·47
	80	3·95	10·00	158·9	3·53
	20	2·63	2·50	49·5	1·22
	40	3·07	3·33	67·2	1·67
	80	3·95	10·00	194·8	4·81
	20	2·63	2·50	45·7	1·11
	40	3·07	3·33	73·5	1·81
	80	3.95	10·00	198·2	4·89
	20	2·63	2·50	39·3	1·04
	40	3·07	3·33	69·5	1·84
	80	3·95	10·00	182·8	4·88
	20	2·63	2·50	40·1	1·04
	40	3·07	3·33	62·1	1·58
	80	3·95	10·00	146·9	3·77
				$D \times 10^{11}$ cm sec^{-1}	
	—	0·44	1·11	2·2	0·61
	—	0·88	1·25	2·5	0·69
	—	1·32	1·43	2·7	0·75
	—	1·76	1·67	3·3	0·92
13	—	2·20	2·00	3·6	1·00
(Guinea-pig)	—	2·63	2·50	4·8	1·33
	—	3.07	3·33	5·3	1·51
	—	3·51	5·00	5·0	1·42
	—	3·95	10·00	3·3	0·92
	—	0·44	1·11	5·6	0·63
	—	0·88	1·25	7·6	0·85
13	—	1·32	1·43	8·0	0·90
(Human)	—	1·76	1·67	8·6	0·97
	—	2·20	2·00	8·9	1·00
	—	2·63	2·50	10·4	1·17
	—	3·07	3·33	11·5	1·29
	—	3·51	5·00	11·0	1·24
	—	3·95	10·00	9·4	1·06

In the case of the data of Grice, Salter and Baker (11), D versus $C_0/(C_0 - C)$ was not linear, although the three higher humidity values are in line. The large standard deviation on the lowest humidity value (TEWL = 0·36 ± 0·4 mg cm^{-2} h^{-1}) may be relevant. Linear extrapolation on the other points gives a TEWL of 0·76 for this point. The second exception to the general linearity rule is the high relative humidity values

from the kinetic experiments of El-Shimi and Princen (13). Rieger and Deem (4) have shown that equilibrium times in steady state flux experiments at high humidities are long. Therefore it is not surprising that there are differences between steady state and kinetic experiments at these high humidities. The layers of skin with high water activities (the inner layers) will not be subject to large, or rapid variations in water content and the steady state results seem likely to be the most applicable. The El-Shimi and Princen data is useful, however, in extending the range of experimental points to lower water activity (C) values, where differences between steady state and kinetic experiments will be less important.

Excluding the data of Grice *et al.* and the two highest humidity points of El-Shimi and Princen, the following correlation is obtained by linear regression analysis:

$$R = 0{\cdot}29\left(\frac{C_0}{C_0 - C}\right) + 0{\cdot}50 \qquad (2)$$

$$n = 61;\ r = 0{\cdot}894.$$

Including the data of Grice *et al.* and all except the highest humidity points of El-Shimi and Princen, the correlation is

$$R = 0{\cdot}29\left(\frac{C_0}{C_0 - C}\right) + 0{\cdot}51 \qquad (3)$$

$$n = 67;\ r = 0{\cdot}885.$$

Correlations within a given set of data are better than the above. Most correspond well with linear relationships with some variation in the gradients.

WATER ACTIVITY PROFILES

In order to examine the effect of the above relationships on the water content profile of stratum corneum, it is necessary to assume that it is possible to extrapolate from the average values of water content and relative diffusion coefficient (C and R) to single point values (C_i and R_i). Weil and Princen (6) have illustrated one of the dangers of making such assumptions, in connection with the effect of surface barrier layers on TEWL. The data of El-Shimi and Princen used above, however, does not suffer from this disadvantage and the average gradient for their two sets of points is the same as that for the other sets of data. This suggests that the assumption will introduce less error than is inherent in the correlation itself.

Thus, from Equation 1,

$$\frac{dx}{dC_i} = \frac{D_i}{J} = \frac{R_i D_0}{J}$$

From Equation 2,

$$\frac{dx}{dC_i} = \frac{0{\cdot}29 D_0}{J}\left(\frac{C_0}{C_0 - C_i}\right) + \frac{0{\cdot}50 D_0}{J}$$

$$\frac{dx}{dC_i} = \frac{M}{C_0 - C_i} + N$$

where

$$M = \frac{0{\cdot}29 D_0 C_0}{J}$$

$$N = \frac{0{\cdot}50 D_0}{J}$$

For the case of $R.H._{external} = 0$, a normal value of J on human forearm skin is found to be 0·22 mg cm^{-2} h^{-1} (15) (equivalent to a value of $D_0 = 7{\cdot}52 \times 10^{-3}$ cm^2 h^{-1}, assuming $t = 15 \times 10^{-4}$ cm). Thus for these conditions:

$$M = 4{\cdot}35 \times 10^{-4}$$

$$N = 1{\cdot}71 \times 10^{-2}$$

$$x = \int \left(\frac{M}{C_0 - C_i} + N \right) dC_i$$

$$x = NC_i - M \ln(C_0 - C_i) + A \qquad (4)$$

where A = integration constant.

Applying the boundary condition $C_i = 0$; $x = 0$

$$A = -1{\cdot}36 \times 10^{-3}$$

Thus:

$$x = 1{\cdot}71 \times 10^{-2} C_i - 4{\cdot}35 \times 10^{-4} \ln (C_0 - C_i) - 1{\cdot}36 \times 10^{-3} \qquad (5)$$

When Equation 5 is plotted, the result is an asymptotic curve in which C_i approaches C_0 as x increases. C_i does not closely approach C_0, however, until x is much greater than the thickness of the stratum corneum (15×10^{-4} cm). At this stage no correction has been applied to the C values in *Table I* to account for the non-linearity of the water activity profile. This non-linearity will result in the average water activity (C) being higher than the simple numerical average of the inner and outer activities. The derivation of Equation 5 enables a short iterative process to be used to correct for this non-linearity, since Equation 5 represents the first approximation to the true water activity profile and indicates the nature of the deviation from linearity. Changes in the values of C used to derive Equation 5 will change the constants in the equation, but not the basic form.

In order to derive an approximate C value correction function, it is necessary to assume that the shape of the graph of Equation 5 is similar to the actual profile, except that the uncorrected constants result in a higher value of x when C_i closely approaches C_0 (which it must do at the inner edge of the stratum corneum) i.e. the graph is stretched in the x direction in comparison to the true profile.

A graph of Equation 5 shows that when C_i approaches C_0 to within 2·5% (at $x = x_{97{\cdot}5}$), the area under the curve (which is proportional to the average water activity, C) is 70% of the rectangular area bound by the lines $x = 0$; $x = x_{97{\cdot}5}$; $C_i = 0$; and $C_i = C_0$ (which is proportional to C_0). It can be shown that a similar result is obtained from the equivalent equation for the situation where $C_{external} = 0{\cdot}5\ C_0$ (i.e. that the area between the curve and the line $C_i = 0{\cdot}5\ C_0$ is approximately 70% of the area bound by the lines $x = 0$; $x = x_{97{\cdot}5}$; $C_i = 0{\cdot}5\ C_0$; and $C_i = C_0$).

The first approximate correction for the C values derived in this way is therefore as follows:

$$C = C_{external} + 0{\cdot}70\,(C_0 - C_{external})$$

$$\text{i.e. } C = 0{\cdot}7\ C_0 + 0{\cdot}3\ C_{external}$$

This function has been used to correct the C values of *Table I*. In fact, as will be shown below, this single iterative correction stage is sufficient to yield a relationship in which C_i closely approaches C_0 at $x = 15 \times 10^{-4}$ cm and one which would not be significantly improved by a second iterative stage.

In the case of the data of El-Shimi and Princen, no correction is necessary for the C values. The reference state for R determinations is, however, changed to $C = 0{\cdot}70\ C_0$ and thus the values of R need modifying. The corrected values of C and R are given in *Table II*.

Table II. Water activity versus diffusion coefficients, corrected data

Source (Reference)	R.H.$_{external}$ (%)	$C \times 10^2$ (mg cm^{-3})	$\frac{C_0}{C_0 - C}$	$D \times 10^3$ (cm^2 h^{-1})	R
	0	3·07	3·33	28·5	1·00
	29	3·46	4·72	31·2	1·09
9	55	3·80	7·44	43·7	1·53
	80	4·13	16·88	91·2	3·20
	88	4·23	27·44	125·0	4·39
	15	3·27	3·92	12·5	1·02
	31	3·48	4·82	13·4	1·09
4	51	3·75	6·86	16·05	1·31
	75	4·06	13·30	25·95	2·11
	89	4·25	31·36	43·5	3·55
	1·5	3·09	3·38	16·2	1·00
	26	3·42	4·53	20·25	1·17
	28	3·44	4·62	19·95	1·15
	28	3·44	4·62	20·4	1·18
	32	3·50	4·93	22·05	1·27
	33	3·51	4·99	19·95	1·15
	33	3·51	4·99	21·9	1·27
	34	3·52	5·05	22·8	1·32
12	45	3·67	6·10	23·55	1·36
	49	3·72	6·55	25·5	1·47
	53	3·77	7·08	29·85	1·73
	56	3·81	7·57	27·15	1·57
	59	3·85	8·13	32·55	1·88
	2·6	3·11	3·43	12·6	1·00
	25	3·40	4·43	26·4	2·10
11	49	3·72	6·55	31·5	2·50
	76	4·08	14·16	42·75	3·40
	20	3·34	4·18	58·5	1·25
	40	3·60	5·56	75·7	1·61
	80	4·13	16·88	222·1	4·77
	20	3·34	4·18	70·5	1·09
	40	3·60	5·56	96·2	1·49
	80	4·13	16·88	234·1	3·63
	20	3·34	4·18	47·8	1·10
10	40	3·60	5·56	70·6	1·62
	80	4·13	16·88	179·4	4·14
	20	3·34	4·18	50·4	1·13
	40	3·60	5·56	66·6	1·47
	80	4·13	16·88	158·9	3·53
	20	3·34	4·18	49·5	1·22
	40	3·60	5·56	67·2	1·67
	80	4·13	16·88	194·8	4·81
	20	3·34	4·18	45·7	1·11
	40	3·60	5·56	73·5	1·81

Continued on next page

Table II. (*continued*)

Source (Reference)	R.H.$_{external}$ (%)	$C \times 10^2$ (mg cm^{-3})	$\frac{C_0}{C_0 - C}$	$D \times 10^3$ (cm^2 h^{-1})	R
	80	4·13	16·88	198·2	4·89
	20	3·34	4·18	39·3	1·04
	40	3·60	5·56	69·5	1·84
10	80	4·13	16·88	182·8	4·88
	20	3·34	4·18	40·1	1·04
	40	3·60	5·56	62·1	1·58
	80	4·13	16·88	146·9	3·77
				$D \times 10^{11}$ cm sec^{-1}	
	—	0·44	1·11	2·2	0·37
	—	0·88	1·25	2·5	0·42
	—	1·32	1·43	2·7	0·45
	—	1·76	1·67	3·3	0·55
13	—	2·20	2·00	3·6	0·60
(Guinea-pig)	—	2·63	2·50	4·8	0·80
	—	3·07	3·33	5·3	0·90
	—	3·51	5·00	5·0	0·85
	—	3·95	10·00	3·3	0·55
	—	0·44	1·11	5·6	0·47
	—	0·88	1·25	7·6	0·64
	—	1·32	1·43	8·0	0·67
13	—	1·76	1·67	8·6	0·72
(Human)	—	2·20	2·00	8·9	0·75
	—	2·63	2·50	10·4	0·87
	—	3·07	3·33	11·5	0·96
	—	3·51	5·00	11·0	0·92
	—	3·95	10·00	9·4	0·79

Using the data in *Table II* (excluding the two highest humidity values of El-Shimi and Princen and the data of Grice *et al.*) the correlation becomes:

$$R = 0{\cdot}175\left(\frac{C_0}{C_0 - C}\right) + 0{\cdot}46 \qquad (6)$$

$$n = 61;\ r = 0{\cdot}902.$$

This correlation is illustrated in *Fig. 1* and Student's t test shows that the correlation could not have arisen by chance with a confidence of greater than 99·9%.

The water activity profile equation using Equation 6 and derived as before, for the case where R.H.$_{external} = 0\%$, is as follows:

$$x = 1{\cdot}54 \times 10^{-2} C_i - 2{\cdot}62 \times 10^{-4} \ln (C_0 - C_i) - 8{\cdot}23 \times 10^{-4} \qquad (7)$$

This profile is illustrated graphically in *Fig. 2*. In this case C approaches C_0 to within 3·4% at $x = 15 \times 10^{-4}$ cm. This is a considerable improvement on Equation 5.

For different relative humidities, a predicted value of D can be obtained from Equation 6, and hence a new value of J. By substituting this and the new boundary conditions into the corrected version of Equation 4, a new equation for the different condition can be derived.

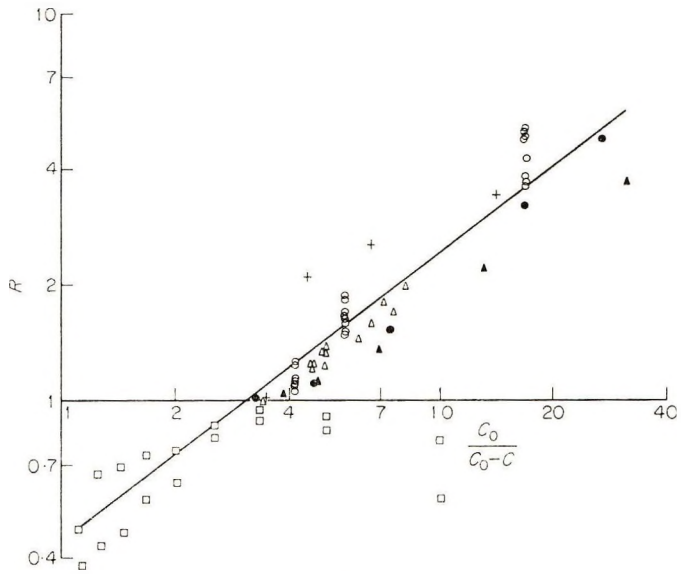

Figure 1. Plot of relative diffusion coefficient (R) versus $C_0/(C_0 - C)$. ○, data from ref. 10; △, data from ref. 12; +, data from ref. 11; □, data from ref. 13; ●, data from ref. 9; ▲, data from ref. 4. The data is plotted on a log–log scale for convenience.

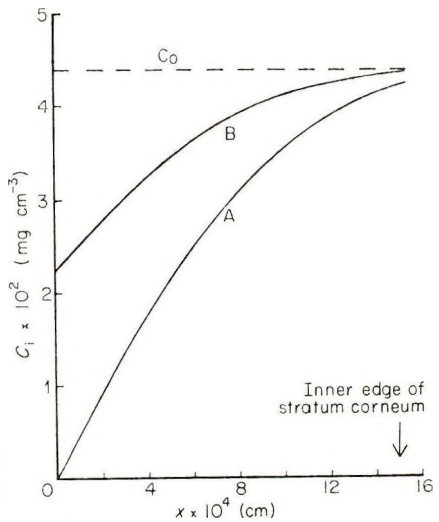

Figure 2. Water activity profile for normal skin. (A) R.H.$_{external}$ = 0%, Equation 7. (B) R.H.$_{external}$ = 50%, Equation 8.

The equation for R.H.$_{external}$ = 50% is as follows:

$$x = 1{\cdot}92 \times 10^{-2} C_i - 3{\cdot}21 \times 10^{-4} \ln (C_0 - C_i) - 1{\cdot}65 \times 10^{-3} \qquad (8)$$

This is also illustrated graphically in *Fig. 2* and again C_i closely approaches C_0 at $x = 15 \times 10^{-4}$ cm. The area under the curve in *Fig. 2A* is 65% of the area bounded by the lines $C_i = 0$; $C_i = C_0$; $x = 0$; and $x = 15 \times 10^{-4}$ cm. The area under the curve and above the line $C_i = 0{\cdot}5\ C_0$ in *Fig. 2B* is 72% of the area bounded by the lines $C_i = 0{\cdot}5\ C_0$; $C_i = C_0$; $x = 0$; and $x = 15 \times 10^{-4}$ cm. Thus, the use of a second iterative

stage in the correction of the water activity profile to account for its non-linearity is unlikely to lead to a significant improvement, and Equation 8 can be taken as the final equation.

SURFACE BARRIER LAYERS

Cooper and Van Duzee (5) suggested that the increase in diffusion coefficient with increasing water content could give rise to a situation where a surface barrier layer could lead to an increased TEWL. Weil and Princen (6) have challenged this on the basis that Cooper and Van Duzee assume an average value of the diffusion coefficient for whole stratum corneum may be used, an assumption which Weil and Princen consider invalid. The use of calculus illustrates that no increase in TEWL can result from a barrier layer application.

Using Equation 6, it is possible to derive an equation using average diffusion coefficients, which predicts up to a 10% increase in TEWL for the application of a weak surface barrier layer. When the more complete calculus approach is used in conjunction with Equation 6, a considerably different result is obtained.

$$J = \frac{1}{t}\int_{C_E}^{C_0} D(C_i)\,dC_i$$

$$J_B = \frac{1}{t}\int_{C_B}^{C_0} D(C_i)\,dC_i$$

where $C_E = C_{external}$

J_B = TEWL with a surface barrier layer applied

C_B = Water activity at the interface of the barrier layer and the skin surface.

$$J - J_B = \frac{D_0}{t}\int_{C_E}^{C_0}\left(\frac{0{\cdot}175\,C_0}{C_0 - C_i} + 0{\cdot}46\right)dC_i - \frac{D_0}{t}\int_{C_B}^{C_0}\left(\frac{0{\cdot}175\,C_0}{C_0 - C_i} + 0{\cdot}46\right)dC_i$$

$$J - J_B = \frac{D_0}{t}\{0{\cdot}46(C_B - C_E) - 0{\cdot}175\,C_0[\ln(C_0 - C_B) - \ln(C_0 - C_E)]\} \qquad (12)$$

For the case where $C_E = 0$:

$$J - J_B = \frac{0{\cdot}46\,C_B D_0}{t} - \frac{C_0 D_0}{t}\,0{\cdot}175\,[\ln(C_0 - C_B) - \ln C_0] \qquad (13)$$

In general:

$$C_B > C_E; \quad \ln(C_0 - C_B) < \ln(C_0 - C_E) < 0$$

Thus $J - J_B$ is positive and any surface barrier layer should lead to a reduction in TEWL. Any applied surface layer which leads to an increased TEWL probably interacts with the stratum corneum to reduce its barrier properties.

The only alternative explanation of an increased TEWL could be that the applied surface layer changes the nature of the skin surface so as to alter the size and effect of diffusion boundary layers in the air above it. The boundary layers will increase the actual value of C_E in comparison to the ambient water activity. The size of this effect will depend on the nature of the skin's surface, e.g. the presence of vellous hairs and loosely adhering skin cells. Where such surface structures are smoothed out by the surface layer, the boundary layers will be less important. It is possible to envisage a situation where

this effect could reverse the relative values of C_B and C_E, which would result in an increased TEWL.

It is possible to use Equation 13 to calculate C_B for an observed value of $J - J_B$. This should enable the calculation of an apparent diffusion coefficient for water through the barrier layer (D_B), providing the barrier layer thickness is known.

From Ficks' law:

$$\frac{D_B(C_B - C_E)}{t_B} = J_B = \frac{D(C_0 - C_B)}{t}$$

where t_B = barrier layer thickness (cm).

Where $C_E = 0$, this gives

$$D_B = \frac{Dt_B(C_0 - C_B)}{tC_B}$$

From Equation 6

$$D = D_0\left(\frac{0{\cdot}175C_0}{C_0 - C} + 0{\cdot}46\right)$$

$$C = 0{\cdot}7C_0 + 0{\cdot}3\,C_B$$

Thus

$$D = D_0\left(\frac{0{\cdot}58C_0}{C_0 - C_B} + 0{\cdot}54\right)$$

$$D_B = \frac{1{\cdot}04t_B D_0 C_0}{t\ C_B} - \frac{0{\cdot}46\,D_0 t_B}{t} \qquad (14)$$

Equations 13 and 14 enable the calculation of an apparent D_B from measured values of J, J_B and t_B.

DISCUSSION

In view of the variety of sources of information used in obtaining the correlation between water activity and relative diffusion coefficient (Equation 6), the degree of correlation obtainable is surprisingly good. This suggests that, although the value of the gradient and intercept may vary according to source of stratum corneum and experimental conditions, the general nature of the correlation, i.e. D is proportional to $C_0/(C_0 - C)$ must be basically correct. (The confidence limit is greater than 99·9%.)

This relationship is one in which the diffusion coefficient approaches infinity as the water activity approaches its maximum. This is reasonable when viewed in the light of known facts about the stratum corneum. As the humidity to which it is exposed approaches 100%, the stratum corneum increases its water content several fold. It has been shown that water is present in at least two states (16–19) and the relative proportion of unbound water will increase with the total water content (high humidity). It is reasonable to expect that when the water content of the stratum corneum increases considerably, the diffusion coefficient for water will tend to approach that for diffusion in pure water. The latter is several orders of magnitude greater than that for the stratum corneum at moderate hydration levels (7) and can be regarded as close to infinity in this context. Thus theory would predict a relationship similar to that of Equation 6.

The water activity profiles derived from Equation 6 (e.g. Equations 7 and 8 and *Fig. 2*) represent useful models, which can be used in conjunction with known water

activity/physical property relationships for stratum corneum (2–4, 20, 21), to extend our understanding of observed *in vivo* stratum corneum properties and appearances. In order to relate them to water content rather than water activity, it is necessary to combine them with sorption isotherms of water on stratum corneum (1, 2, 21, 22). These show a non-linear curve with rapid increases in water content at high humidities. *Figure 3* shows the results of combining the model activity profile of Equation 7 with the sorption isotherm of Wildnauer, Miller and Humphries (2). The 100% relative humidity point is taken to be the water content of the viable epidermis (7). In general, however, the curves of *Fig. 2* will be more useful, since the physical parameters, e.g. Young's modulus (20) are generally correlated with water activity, or ambient relative humidity, rather than actual water content.

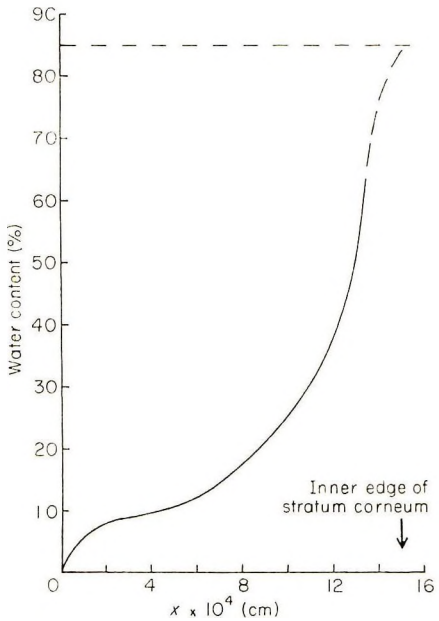

Figure 3. Water content profile for normal skin. Derived from Equation 7 and the water sorption isotherm for stratum corneum of Wildnauer *et al.* (2). $R.H._{external}$ = 0%.

In theory, it is possible to extend the treatment on water activity profiles derived from Equation 6, by considering models for abnormal stratum corneum conditions caused by either abnormal epidermal metabolism, e.g. psoriasis, or external conditions, e.g. regular exposure to detergents, etc. A simple model for the regular detergent exposure would be to assume increasing damage for the older, outer stratum corneum cells, due to longer total exposure. Mathematically this may be represented by a factor (F_i) applied to the diffusion constant, D_i, dependent on distance from the edge of the stratum corneum:

$$F_i = K^{(15-10^4 x)}$$

where K is a constant and skin thickness is assumed to be 15×10^{-4} cm.

For a threefold increase in TEWL, which is observable under these conditions (15), the constant K has a value of 1·24.

$$\frac{dx}{dC_i} = \frac{R_i F_i D_0}{J_D}$$

J_D = TEWL of damaged skin.

The above equation may be integrated, incorporating the functions for F_i and R. For the condition of external relative humidity = 0% and assuming again J (undamaged) = 0·22 mg cm^{-2} h^{-1}, and K = 1·24, the following equation results:

$$1{\cdot}84\times10^{-5}\,1{\cdot}24^{10^4x} = 5{\cdot}13\times10^{-3}\,C_i - 8{\cdot}73\times10^{-5}\ln(C_0 - C_i) - 2{\cdot}54\times10^{-4} \quad (9)$$

This equation is illustrated in *Fig. 4* and the resultant profile of water content, incorporating the water sorption isotherm, in *Fig. 5*.

The usefulness of models such as this must be assessed in relation to their ability to explain observed properties of skin. In the case of the above model, the area of skin concerned had a dry, flaky, opaque appearance and had a hard and rough texture. This

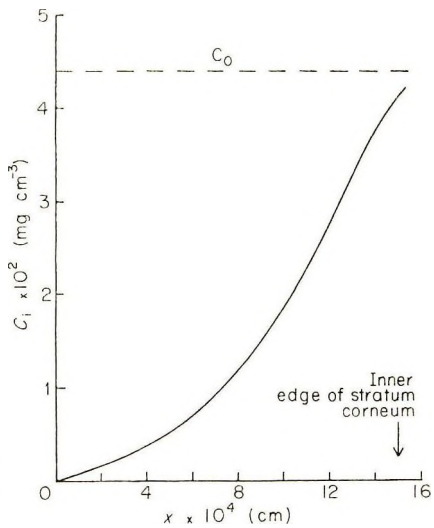

Figure 4. Model water activity profile for skin damaged by regular exposure to detergents derived from Equation 9. R.H.$_{external}$ = 0%.

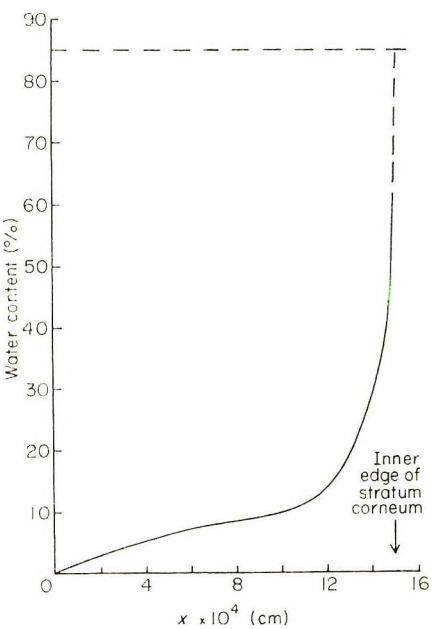

Figure 5. Model water content profile for detergent damaged skin derived from Equation 9 and the water sorption isotherm of Wildnauer *et al.* (2). R.H.$_{external}$ = 0%.

would be expected from the known relationships between water activity and visco-elastic properties (20) and light transmission (21).

The above example of a model system for damaged skin is not intended as an accurate description of the actual water activity profile. It does illustrate however that, in general, the outer layers will have suffered most from environmental insult, and that the true situation *in vivo* is liable to deviate from that depicted in *Figs 2* and *3*. The deviation will be qualitatively similar to that depicted in *Figs 4* and *5*.

The effect of a surface barrier layer will be to reduce the water activity difference across the stratum corneum, since some of the total water activity difference between ambient conditions and the viable epidermis will occur across the barrier layer. In terms of the effect on the water activity profile, this will be similar to raising the external relative humidity slightly. The size of the effect will depend on the effectiveness of the barrier. As can be seen from *Fig. 2*, this will have its greatest effect on the driest outer layers. This will be even more apparent in damaged skin where more layers will be relatively dry (*Figs 4* and *5*). These outer layers are those responsible for the 'dry' appearance of damaged skin. This explains the effectiveness of comparatively weak surface barrier layers in improving the appearance of dry, damaged skin.

It is worth noting that the principal effect of the other main class of moisturising ingredients, the hygroscopic humectants, will be on the water sorption isotherm and not on its water activity profile.

The data presented and analysed here does not represent an exhaustive, or totally complete analysis of the situations occurring in human skin *in vivo*. No account has been taken in the calculations of the relatively less important diffusion barriers of the dermis, the viable epidermis, or the surface air boundary layers. Nor have the complicating factors of expansion of the stratum corneum, or the temperature coefficient of the diffusion constant been incorporated. The effect of the former means that x in *Figs 2–5* should be regarded as being in units of skin layers rather than absolute distance. The effect of the temperature coefficient may be significant at low ambient temperatures, but less important at around 25°C.

The treatment, however, is sufficiently detailed to analyse important aspects of the water/stratum corneum system in a fairly detailed qualitative manner. Hopefully a more complete, integrated experimental programme and diffusion theory treatment would enable a more accurate quantitative analysis to be achieved.

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Design and evaluation of a water-resistant sunscreen preparation

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Synopsis

A **water resistant sunscreen** preparation was formulated to function during swimming, exercising and sunbathing. The sunscreen agent, **octyl** *p*-**N,N-dimethylamino benzoate** (PABA) and an **ammonium acrylate/acrylate ester polymer** were combined in a cosmetically pleasing oil-in-water, fugitive amine, lotion vehicle. When applied to skin, a substantive film forms which does not interfere with **transepidermal water loss** or normal sweat gland function. Double blind **clinical** studies showed that this water-resistant sunscreen preparation provided protection from sunburn after 60 min swimming in fresh or salt water. This water-resistant sunscreen preparation was found to be safe and of low irritancy, allergenic, sting and stain potential.

INTRODUCTION

Ultraviolet light (uvl) produces profound effects on human skin. This is especially true for the electromagnetic spectrum in the uv-B region of 290–320 nm. This radiation produces excitation of electrons which leads to chemical changes in DNA of epidermal cells and to the release of vasoactive substances, such as prostaglandins producing painful sunburn and cell destruction (1). This action spectrum is most likely responsible for the induction of skin cancers and ageing processes seen with prolonged solar exposure. With increased awareness of these sequelae and the popularity of out-door recreation, there is now a demand for effective products to protect individuals from these effects. In addition, there are a small number of individuals who require products to prevent sun-related exacerbation of underlying diseases, such as lupus erythematosus.

Most available sunscreen preparations are removed by active swimming because of the incorporation of a water soluble sunscreen agent or a water-washable emulsifying system. Our primary objective was to develop a sunscreen preparation for the mass market that would resist removal and provide effective uv-B protection during conditions of normal usage, such as swimming and exercising. Even if individuals are diligent about applying a sunscreen repeatedly, they may get burned while swimming, since water filters out very little uv-B. Therefore, the important feature of water-resistance is needed to protect people during and following swimming.

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Ideally, a sunscreen should have a proper uvl absorption and be non-sensitizing and non-stinging, so that it could be used on irritated or inflamed skin. The product should not stain clothing or skin and be cosmetically acceptable so that it can be used routinely on the face. Cosmetic acceptability includes being able to apply the product evenly to provide uniform protection.

Our approach was to combine a water-insoluble sunscreen agent with a water-resistant, skin adherent, film-forming polymer to prevent wash-off or rub-off of the sunscreen agent. Although the polymer had to remain on the skin during swimming and sweating, it had to be easily removed by soap and water, when desired. It was also important that this sunscreen preparation would not interfere with normal cutaneous functions, i.e. sweating.

METHODS

PHYSICAL CHEMICAL STUDIES

The water solubility of sunscreen agents (*p*-aminobenzoic acid (PABA), N,N-dimethyl PABA esters and benzophenones) was determined spectrophotometrically. Saturated solutions of each sunscreen agent were prepared by mixing and equilibrating the agent in water at 37°C for 24 h. Solutions were centrifuged and filtered through a 0·22 μ millipore filter. The concentration of each sunscreen remaining in solution was determined by absorbance using a Beckman® model 25 spectrophotometer.

Erythemal transmittance values of pure sunscreen agents were determined spectrophotometrically according to the method of Cumpelik (2).

POLYMER AND VEHICLE

An acrylate film-forming polymer was selected as the primary film-former for the sunscreen preparation. This polymer is skin-adherent and water-resistant. When dried on the skin, however, the film is easily removed with soap and water. An oil-in-water emulsion containing the acrylate polymer and octyl dimethyl PABA was prepared. Ammonium isostearate served as the primary emulsifier.

ANIMAL STUDIES

The hairless mouse model system (3–4) was used to evaluate the photoprotective effectiveness of the film-forming sunscreen preparation. For comparison, a commercial preparation containing 5% PABA in a hydroalcoholic lotion was included in the study. Test materials (5 mg/cm^2) were applied to the backs of the mice and allowed to dry for 1 h. One group of mice was exposed only to ultraviolet radiation and the second group was immersed in water for 30 min prior to uv exposure. The mice were irradiated for 150 min at a distance of 30 cm with a Westinghouse FS 40 sunlamp. Grading was done 120 h after irradiation.

SKIN SAFETY

To test for irritation potential of the film-forming sunscreen preparation on damaged skin, adhesive tape stripped wounds (1·27 × 2·54 cm) were made on the backs of human volunteer subjects. Commercial sunscreen preparations containing 5% PABA (lotion); 3% glyceryl PABA plus 3% amyl dimethyl PABA (lotion); 2·5% amyl dimethyl PABA

(alcoholic vehicle); and 10% sulisobenzone (lotion) were included in the study. Each test material was applied to gauze (0·5 ml), placed on the wound, occluded for 18 h and evaluated on a 0–4 scale 2 h after removal of the gauze. Standardized predictive tests, i.e. Draize, maximisation, cumulative irritation, phototoxicity, photoallergy and subtotal inunction tests were conducted on the film-forming sunscreen preparation. Also, known acrylate sensitive volunteers were tested for allergic sensitivity by a 48 h occlusive patch test.

TRANSEPIDERMAL WATER LOSS

A 5·08 × 5·08 cm area was outlined on the backs of six volunteer subjects. Transepidermal water loss readings were measured with an air flow hygrometer on these normal skin sites. The polymeric film-forming sunscreen product was applied (0·2 ml) to the outlined site and allowed to dry for 30 min before measuring transepidermal water loss.

ECCRINE SWEAT STUDY

To determine whether the acrylate film would interfere with sweating and/or produce sweat retention problems, the polymeric film-forming sunscreen preparation was applied daily (2·5 mg/cm²) to 10·16 × 10·16 cm sites on human volunteers under chronic use conditions (12 h per day for 7 days) and misuse conditions (continuous use for 4 days). At the end of both experiments, the films were removed with soap and water and the areas allowed to dry at least 2 h before sweat prints were made. Sweat prints were obtained with the silastic impression technique of Harris (5). A Saran® wrap occluded site served as a positive control.

CLINICAL STUDIES

Double blind clinical investigations included the following.

1. Two fresh water swimming pool studies. Weather conditions varied from a hot, dry climate in Phoenix, Arizona (100°F, 5% relative humidity) to hot, humid conditions in Bradenton, Florida (90°F, high humidity).

2. One salt water ocean study conducted in Holmes Beach, Florida, with hot and humid weather conditions, (85°F, 68% relative humidity) in winds of 20 mph and rough seas.

The film-forming sunscreen preparation containing 3·3% octyl dimethyl PABA and commercial products containing the following sunscreen agents were evaluated in these studies: 5·0% PABA (lotion); 3·0% amyl dimethyl PABA plus 3·0% octyl dimethyl PABA (lotion); and 10% sulisobenzone (lotion).

A single application of product was applied to the backs of each subject (2·5 mg/cm²) at Phoenix and Holmes Beach and (5·0 mg/cm²) at Bradenton. Product sites were randomised on each subject. Sun exposure was between 10:00 a.m. and 2:00 p.m. with 60 min swimming before 12:00 noon. Erythema was graded on a scale of 0–3 (0, none; 1, mild; 2, moderate; 3, severe), 6 h after the study.

FABRIC STAIN STUDY

A multifibre fabric obtained from Testfabrics, Incorporated, and approved by the

American Association of Textile Chemists and Colorists (AATCC) was used. This multifibre fabric consisted of 1·5 cm strips of wool, orlon 75, dacron 54, nylon 6·6, bleached cotton and acetate. Fabrics were impregnated with 0·05 ml of the following sunscreen preparations: 3·3% octyl dimethyl PABA plus film-former (lotion); 3·0% amyl dimethyl PABA plus 3·0% glyceryl PABA (lotion); and 5·0% PABA (lotion). A glassine paper was applied over the fabric and a 2 kg weight placed on a glass plate over the paper was allowed to sit undisturbed for 60 s. Each fabric was exposed to direct sunlight for 4 h and 8 h later, washed with All® detergent in a 'normal' wash of 12 min in a washing machine.

RESULTS AND DISCUSSION

SUNSCREEN AGENT

The sunscreen agent, octyl dimethyl PABA, was selected for the film-forming preparation because it fulfils many important features of a desired sunscreen agent. The presence of the octyl group and the two methyl groups (*Fig. 1*) make this derivative less reactive, less water soluble and more photostable than the parent compound, PABA. The water insolubility of octyl dimethyl PABA (*Table I*) promotes its water-resistance.

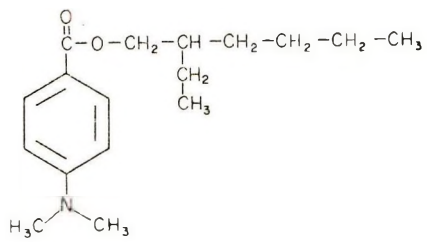

Figure 1. Structure of octyl dimethyl PABA.

Table I. Water solubility of pure sunscreen agents

Sunscreen agent	Solubility (%)
Octyl dimethyl PABA	0·0001
Homosalate	0·0003
Amyl dimethyl PABA	0·0005
Oxybenzone	0·0013
PABA	0·80
Glyceryl PABA	18·0
Sulisobenzone	40·0

Figure 2 shows that octyl dimethyl PABA absorbs energy in the sunburning range, having an absorption maximum at 312 nm, and transmits energy in the tanning range (320–400 nm), thus allowing the user to obtain a gradual tan. At 3% concentration, octyl dimethyl PABA transmits less than 1% erythemal energy (*Fig. 3*).

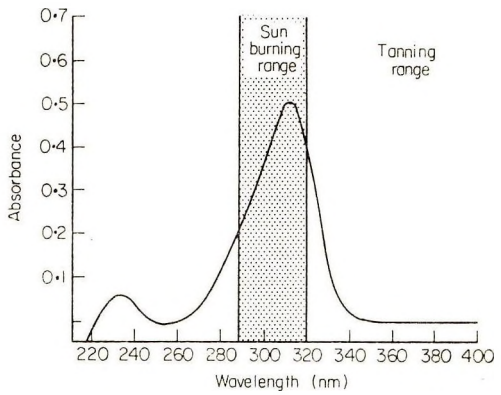

Figure 2. Ultraviolet absorption spectrum of octyl dimethyl PABA.

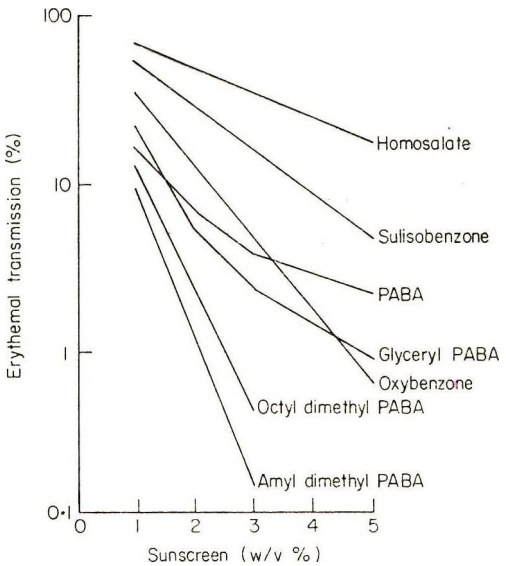

Figure 3. Erythemal transmission values of pure sunscreen agents.

Since staining of fabric is a problem with PABA compounds, a complete line of fabrics was impregnated with the film-forming sunscreen product and exposed to sunlight for 4 h. After washing, only a slight discolouration on cotton was observed with the film-forming sunscreen product, whereas, other sunscreen preparations containing PABA or PABA derivatives produced a strong discolouration on more fabrics (*Table II*).

Table II. Stain study

	Fabric					
Product	Wool	Orlon	Dacron	Nylon	Cotton	Acetate
A	−	−	−	−	+	−
B	+	+	+	+	+	+
C	−	−	−	+	−	+

A, 3·3% octyl dimethyl PABA plus film-former (lotion); B, 3·0% amyl dimethyl PABA plus 3·0% glyceryl PABA (lotion); C, 5·0% PABA (hydroalcoholic lotion).

POLYMERIC FILM AND VEHICLE

An acrylate film-forming polymer was selected which could be solubilised by forming the ammoniated salt in an anionic soap emulsion. When the sunscreen preparation dries on the skin, the ammonia evaporates, fixing the insoluble polymer to the skin. Due to this 'fugitive amine' transformation, the film is insoluble in water and resistant to rub-off. It is easily removed, however, by washing with soap and water (*Fig. 4*).

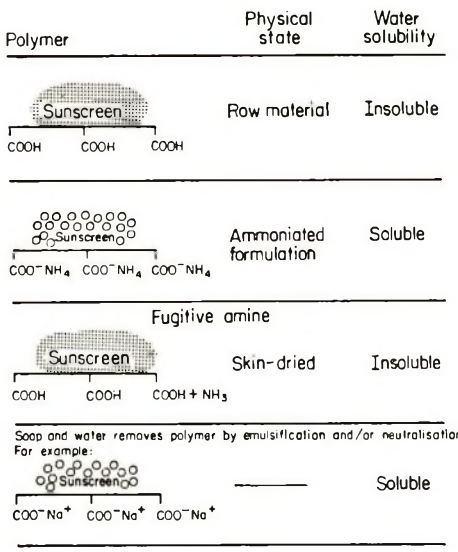

Figure 4. Film-former action of polymer (fugitive amine).

Scanning electron microscopic examination of the skin following the application of this formulation shows that the film has multiple small holes (*Fig. 5*). These minute holes account for the findings that the film is non-occlusive and does not interfere with transepidermal water loss through the skin or human eccrine sweat function (*Tables III* and *IV*).

Table III. Transepidermal water loss (TWL)

Area	No treatment	Film-forming sunscreen product*
TWL (Average of six subjects) (mg h^{-1} cm^2)	0·40	0·35

* 3·3% octyl dimethyl PABA

ANIMAL STUDIES

Using a hairless mouse model, the amount of uvl and water immersion were carefully controlled. With no water immersion and uvl exposure, both the film-forming preparation containing 3·3% octyl dimethyl PABA and a commercial preparation containing 5% PABA protected the mice from severe skin damage, whereas, the untreated sites were severely damaged. In the group immersed in water for 30 min, followed by uvl exposure, only the film-forming preparation provided significant protection (*Table V*).

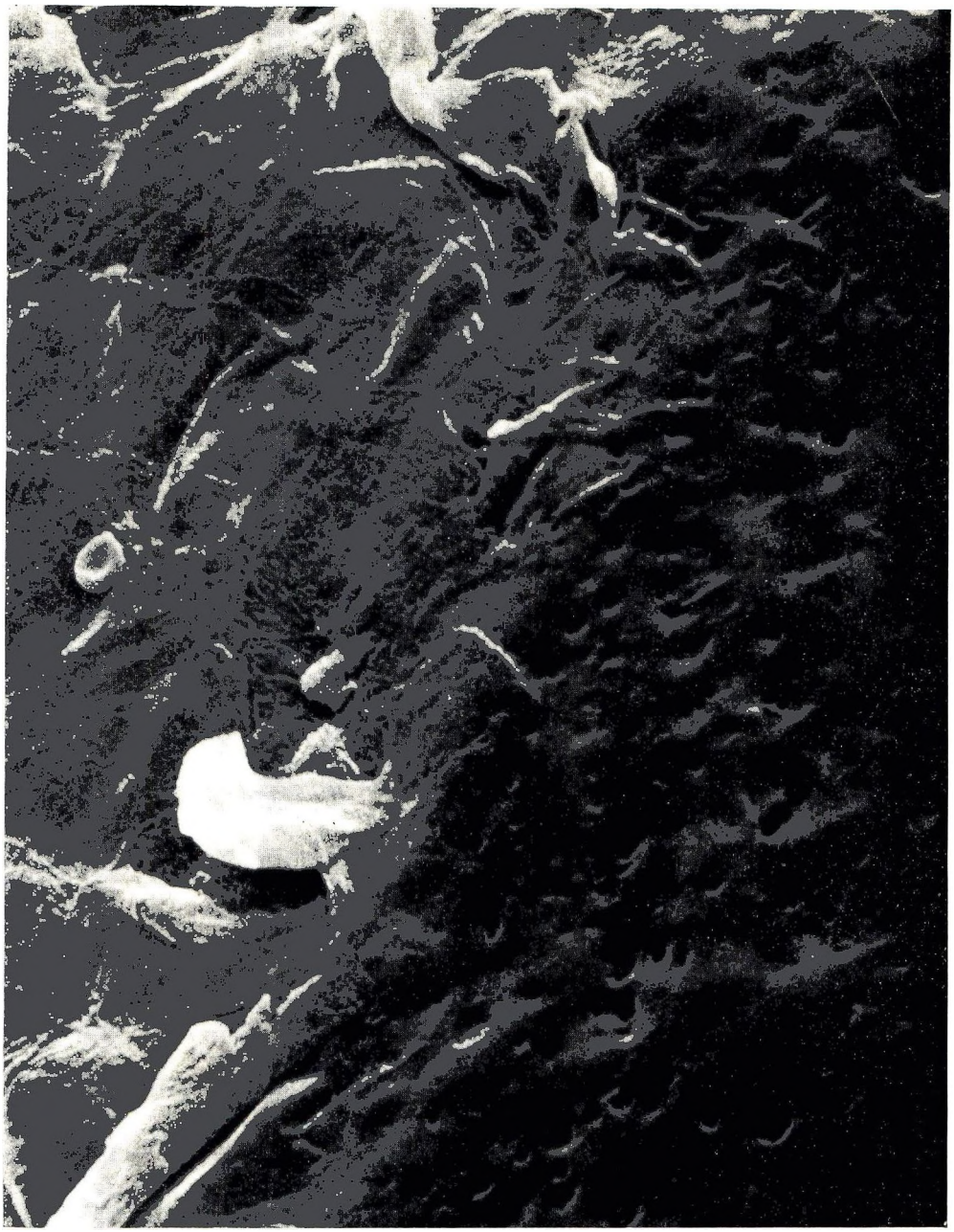

Figure 5. SEM of polymeric film on human skin.

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Table IV. Human sweat study

Sweat gland activity after 7-day use for 12 h per day			Sweat gland activity after 4 days continuous use			
	Active sweat glands/cm²			Active sweat glands/cm²		
Subject	Sunscreen treated	Untreated control	Subject	Sunscreen treated	Untreated control	Saran occluded
1	144	149	7	106	98	78
2	119	122	8	81	80	50
3	99	96	9	85	81	30
4	103	103	10	105	91	17
5	86	80	11	77	82	23
6	47	56	12	89	96	23

No significant difference between sunscreen treated and untreated control by one-way analysis of variance. Saran occluded and untreated control are significantly different by one-way analysis of variance.

Table V. Hairless mouse study

	Average grade		
Condition	A	B	C
150 min uvl	0·7	2·3	6
30 min water immersion + 150 min uvl	1·6	5·6	7

A, 3·3% octyl dimethyl PABA plus film-former (lotion); B, 5·0% PABA (hydroalcoholic lotion); C, untreated: 0–3 mild; 3–5, moderate; 6–7, severe.

SAFETY TESTS

Safety tests including Draize (204 subjects), maximization (twenty-eight subjects), cumulative irritation (twenty-seven subjects), phototoxicity (ten subjects), and photoallergy (twenty-five subjects) established that the film-forming sunscreen product had little or no allergy or irritancy potential. Only slight irritation was noted on stripped skin in human volunteers with the film-forming formulation, whereas, other commercial preparations produced moderate to severe irritation (*Table VI*).

Table VI. Human stripped skin irritation

Sunscreen products	Mean irritation score	Level of irritation
A	0·3	Slight
B	1·4	Slight to moderate
C	2·7	Moderate to severe
D	1·2	Slight to moderate
E	3·1	Moderate to severe
Untreated control	0·0	None

A, 3·3% octyl dimethyl PABA plus film-former (lotion); B, 3·0% amyl dimethyl PABA plus 3·0% glyceryl PABA (lotion); C, 5·0% PABA (hydroalcoholic lotion); D, 10·0% sulisobenzone (lotion); E, 2·5% amyl dimethyl PABA (hydroalcoholic lotion).

The film-forming product did not elicit a sensitivity response to 48 h occlusive tests in four subjects known to be allergic to acrylates. Clinical use tests confirmed that octyl dimethyl PABA did not produce facial irritation with sweating and swimming, a problem seen with amyl dimethyl PABA (6–8). Because it is anticipated that patients may use the product repeatedly, a subtotal inunction (abuse) test was conducted on twenty-one subjects. In this test, the product was applied to the arms, legs and back for 28 consecutive days. Complete physical exams and laboratory evaluations were carried out at biweekly intervals. No local or systemic toxicity was noted.

CLINICAL EFFICACY

Double blind studies in Arizona (forty-seven subjects) and Florida (sixty-two subjects) compared the efficacy of the film-forming product to competitive products. Although no significant differences were observed in ordinary sunbathing or exercising, the film-forming sunscreen provided significantly better protection from sunburn than the other commercial products after 60 min swimming in two fresh water swimming pool tests and one salt water test (*Table VII*).

Table VII. Clinical sunscreen studies for sunbathing plus swimming (1 h) erythema

Study	No of Subjects	A	B	C	D	E
Phoenix	23	1·7	2·7	2·8	2·9	2·9
Bradenton	12	1·4	2·6	2·9	3·0	3·0
Holmes Beach	25	1·9	2·5	2·8	*	3·0

A, 3·3% octyl dimethyl PABA plus film-former (lotion); B, 3·0% amyl dimethyl PABA plus 3·0% glyceryl PABA (lotion); C, 5·0% PABA (hydroalcoholic lotion); D, 10·0% sulisobenzone (lotion); E, placebo (no sunscreen). * Not tested. Erythema was graded on a scale of 0–3 (0, none; 1, mild; 2, moderate; 3, severe).

CONCLUSIONS

Controlled laboratory and clinical studies, show that a sunscreen preparation containing 3·3% octyl dimethyl PABA and an acrylate film-forming polymer is highly water-resistant and provides sunburn protection while permitting gradual tanning during swimming, sunbathing and exercising. It has little potential for irritation or adverse side effects and it does not unduly irritate inflamed skin or interfere with normal water loss from the skin. It remains steadfast during swimming but is easily removed with soap and water. This cosmetically pleasing product can easily be applied evenly to all body areas due to the oil in the water vehicle. It is invisible on the skin when dry and does not strain most clothing. Thus, it is well suited for daily use as well as for people who enjoy swimming and water sports.

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The leaching of F.D. & C. Blue No 1 dye from its lake by electrolytes

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Synopsis

The effect of **electrolyte** solutions on the alumina lake of **F.D. & C. Blue No 1** dye has been studied. Monovalent anions and cations cause a fairly rapid **leaching** of the dye for a period of about 1 h, followed by a much slower **elution** process. Multivalent cations cause a slight increase in leaching, but a more marked effect is obtained with salts of multivalent acids, where the dye is virtually completely removed from its substrate in 2–3 h. The results are explained by reference to the postulated structure of the alumina substrate of the lake, and the effects of pH on and ionic penetration into this structure.

INTRODUCTION

Though the use of water soluble dyes is common in cosmetics and pharmaceuticals, any process which involves the use of a moving solvent front may give rise to uneven colour distribution as the dye travels with the solvent. Thus, on drying for example, dye will be transferred progressively to the surface from which evaporation is taking place, resulting in an excess of dye at that point, whilst leaving a deficiency elsewhere [Jaffe and Lippmann (1) Armstrong and March (2)]. The use of the lake of the dye, in which the dye is adsorbed on to an insoluble substrate, is often recommended to avoid this problem, and was used with some success by Armstrong and March in an attempt to produce uniformly coloured tablets containing F.D. & C. Blue No 1 dye. It was noted, however, that under certain conditions, for example when the tablets contained calcium phosphate, no reduction in dye migration took place. Goodhart, Everhard and Dickcius (3) found a similar effect using F.D. & C. Red No 3 dye lake in the presence of calcium sulphate, implying that ionic species presence in the system may remove the dye from its insoluble substrate. Accordingly, the role of electrolytes in displacing F.D. & C. Blue No 1 dye from its substrate has been investigated.

EXPERIMENTAL

MATERIALS

F.D. & C. Blue No 1 dye Batch X7663 D. F. Anstead Ltd, F.D. & C. Blue No 1 lake Batch X8432, D. F. Anstead Ltd, pure dye content, 13%. Both substances were used without further purification. All other reagents were of Analar or laboratory reagent quality, and solutions were prepared from water which had been distilled from glass subsequent to deionisation.

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ELUTION STUDIES

F.D. & C. Blue No 1 lake (50 mg) was placed in a glass centrifuge tube. The electrolyte solution (20 ml) was added, the pH measured (Phillips model PW 9418 pH meter), the tube closed with waterproof film and agitated in a horizontal position at 27°C. After the requisite time had elapsed, the tube and contents were centrifuged (3300 rpm) for 15 min, the pH of the supernatant measured, and the latter then diluted appropriately with Teorell and Stenhagen pH 5·0 buffer. The concentration of dye was then determined at 628 nm (Cecil model CE272 spectrophotometer). All experiments were triplicated and blank experiments using the lake suspended in water were carried out in each case. The amount of dye leached from the lake is expressed as a percentage of the total dye present.

RESULTS AND DISCUSSION

The elution data of F.D. & C. Blue No 1 dye from its lake in the presence of molar concentrations of the chloride and bromide salts of sodium and potassium are shown in *Fig. 1*. Water causes very little elution, the dye concentration reaching a plateau level of about 0·3% in approximately 6 h. It is likely that this represents free dye unadsorbed on the alumina substrate, rather than dye eluted from the substrate.

In the case of the electrolytes, a fairly rapid increase in free dye concentration is obtained over the first period of about 1 h, followed by a more gradual increase. Throughout this period, the potassium salts are more effective eluents than the corresponding sodium salts, whilst the chloride is more effective than the corresponding

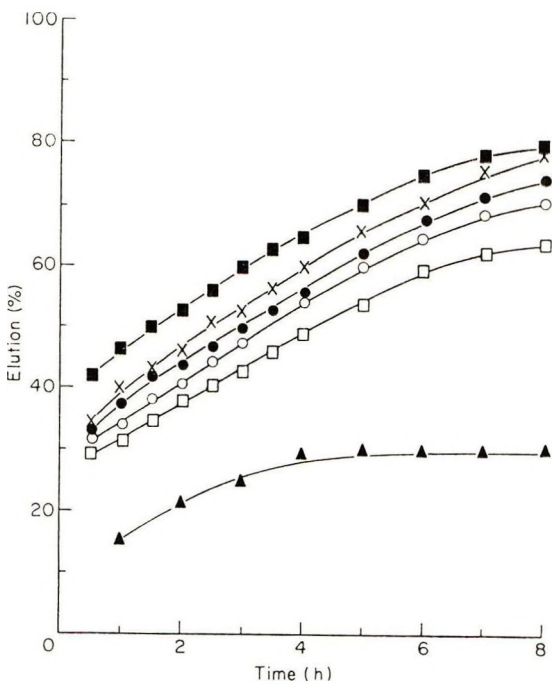

Figure 1. The elution of F.D. & C. Blue No 1 dye from its lake by molar solutions of salts of monovalent acids. ×, potassium chloride; ●, sodium chloride; ○, potassium bromide; □, sodium bromide; ■, calcium chloride (0·5 M); ▲, water (× 100).

bromide. Even so, the most effective eluent in this series, potassium chloride, removes only about 40% of the dye from its substrate after one hour and about 80% after 8 h. These effects are concentration dependent, 0·01 M and 0·1 M potassium chloride solutions causing about 2 and 25% elution respectively over a period of 8 h.

Increasing the valency of the cation has little effect on elution. Calcium chloride shows an increased degree of elution compared to potassium and sodium chloride, but if a correction is applied for the two chloride ions contributed by each molecule of calcium chloride, the elution of 0·5 M calcium chloride is only slightly greater than that achieved by 1·0 M sodium chloride.

In order to study more closely the eluting effects of polyvalent anions, elution studies were carried out using 0·5 M solutions of sodium sulphate, selenate, thiosulphate, and succinate. In all cases, elution occurred much more rapidly, about 70–80% being achieved after 0·5 h and elution being virtually complete in most cases within 4 h (*Fig. 2*). Elution caused by magnesium sulphate is slightly quicker than that achieved by sodium sulphate confirming that while increasing the cation valency increases elution, it is not a major influence.

As part of a comprehensive survey of the properties of alumina, Mutch (4) studied the eluant effect of a number of sodium salts on acidic dyes adsorbed on alumina. His findings contradict those of the present study, since he reported that in general salts of monovalent acids were ineffective as eluents, while those of multivalent acids caused

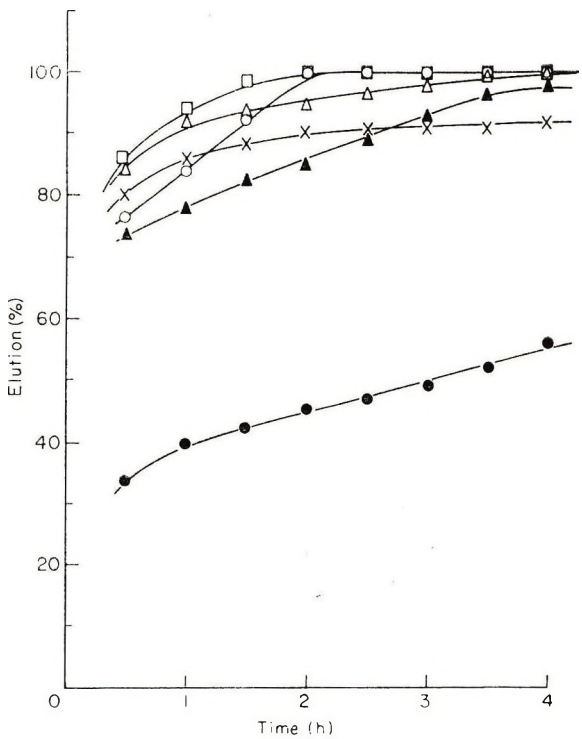

Figure 2. The eluting effect of 0·5 M solutions of salts of dibasic acids on the lake of F.D. & C. Blue No 1 dye (1·0 M sodium chloride is included for comparative purposes). □, sodium selenate; △, sodium thiosulphate; ×, sodium succinate; ○, magnesium sulphate; ▲, sodium sulphate; ●, sodium chloride.

elution. Exceptions were found, however. Sulphate and thiosulphate, both effective eluents in the present study were reported as being ineffective, as were chloride and bromide. No mechanism was suggested by Mutch to support either his findings or their exceptions.

Structures for hydrated aluminium hydroxides have been postulated by Thomas and co-workers. Individual alumina units are joined by hydroxide ions to give two- or three-dimensional polymeric structures, the process being termed 'olation'. Thomas and Whitehead (5) noted that on storage of alumina suspensions, changes in solubility occurred, and the liquid phase became more acidic, this being attributed to a hydrolytic process (*Fig. 3a*). The addition of neutral electrolytes, however, caused an increase in pH, the magnitude of the change being dependent on the salt used. For example, Thomas and Tai (6) studied the effect of seven potassium salts, and found that the magnitude of increase in pH was in decreasing order, oxalate, sulphate, acetate, iodide, bromide, chloride, nitrate. This was explained in terms of anion penetration, the anion displacing a hydroxo (H_2O) or ol (OH) group, the latter causing the increase in pH (*Fig. 3b*). It was found that in general, salts of multivalent acids raised pH to a much greater extent than the salts of corresponding monovalent acids (7).

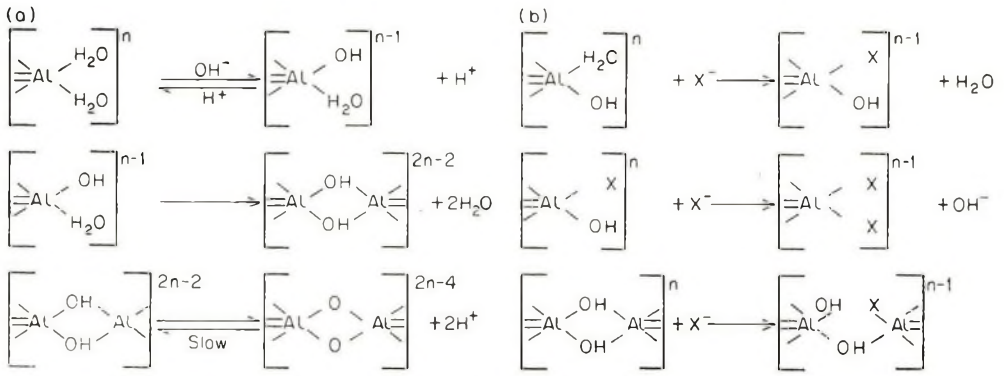

Figure 3. Proposed mechanisms for (a) hydrolysis and (b) anion penetration in hydrated alumina structures [derived from Thomas and Whitehead (5)].

It is probable that a similar mechanism can explain the elution of F.D. & C. Blue No 1 dye from its lake. The degree of elution depends firstly on the valency of the anion, and within any valency group, the order of elution is comparable to that described by Thomas and Tai. This is confirmed by a study of the pH changes in the supernatant during the elution process where a high degree of elution is associated with an increase in supernatant pH.

For example, water which shows negligible elution is associated with a fall in pH of 1·4 after 4 h, whilst almost all electrolytes which cause elution are associated with a rise in supernatant *p*H of between 0·5 and 1·5 units. The only exception to this is sodium succinate where a fall of 0·9 units is obtained. This is probably explained by the high starting pH (8·90) and the buffering capacity of the electrolyte itself.

These elution effects are not associated with the disruption of the alumina lattice structure itself, which is stable between pH 3 and pH 9 (8). If electrolytes are used whose solutions give pH values outside this range, however, then elution will occur which is due to dissolution of the alumina substrate. Thus sodium phosphate and aluminium chloride

(the pH values of 0·5 M solutions of which are 12·2 and 3·1 respectively) are both associated with the rapid appearance of a large amount of the dye in the supernatant, but this effect will be acheived, at least in part, by disruption of the alumina itself. This mechanism may also be involved with sodium succinate (pH of a 0·5 M solution = 8·9).

Between pH values of 3 and 9, however, the pH value itself is of minor importance. *Table I* shows the degree of elution at pH 4, 6 and 8, these pH values being achieved by either buffering with Teorell and Stenhagen citrate-borate-phosphate buffer, or by the addition of small amounts of N/10 hydrochloric acid or sodium hydroxide (i.e. an unbuffered system). The buffered suspensions show a much higher degree of elution at any given pH. This indicates that rather than pH *per se*, it is the presence of the buffer electrolytes used to achieve that pH which is the primary eluting factor. (Teorell and Stenhagen's buffer at pH 4 for example, contains approximately 0·06 M of sodium as borate, citrate and phosphate, all of which are stated by Mutch to be effective eluting electrolytes). pH changes, also shown in *Table I*, are those expected if the anionic penetration mechanism proposed by Thomas and Tai is appropriate in this case.

Table I. Variation with pH of the elution of F.D. & C. Blue No 1 dye from its lake after 24 h, using (a) buffered and (b) unbuffered systems

Original pH	Buffered system		Unbuffered system	
	Elution (%)	pH change	Elution (%)	pH change
4·0	98·4	+1·35	4·4	+0·9
6·0	96·2	+1·00	7·9	−0·85
8·0	94·0	+0·50	9·3	−2·55
Water pH 7·0 (control)	—	—	1·08	−1·65

The significance of this data in formulation is considerable. Small amounts of electrolyte impurities present in the dye itself or other associated solids may cause elution, particularly when the quantity of water present is also small, since in that case, reasonably concentrated electrolyte solutions may readily be achieved. Also the concentration of electrolytes in human perspiration (0·03 M, 0·02 M, and 0·07 M with respect to chloride, phosphate and sulphate), may well cause a significant degree of dye elution.

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Publications Prize 1977

17–19 April 1978, Eastbourne

Figure 1.
Mr K. V. Curry, President of the Society (right), presenting the 1977 Publications Prize to Mr Francisco Serra, Past President of the IFSCC, who received it on behalf of the winning Spanish authors, Messrs J. Garcia Dominguez, J. C. Parra, R. Infante, Carlos M. Pelejero, Francisco Balaguen and T. Sastue for their paper, 'A new approach to the theory and permeability of surfactants on keratinic proteins: the specific behaviour of certain hydrophobic chains'.

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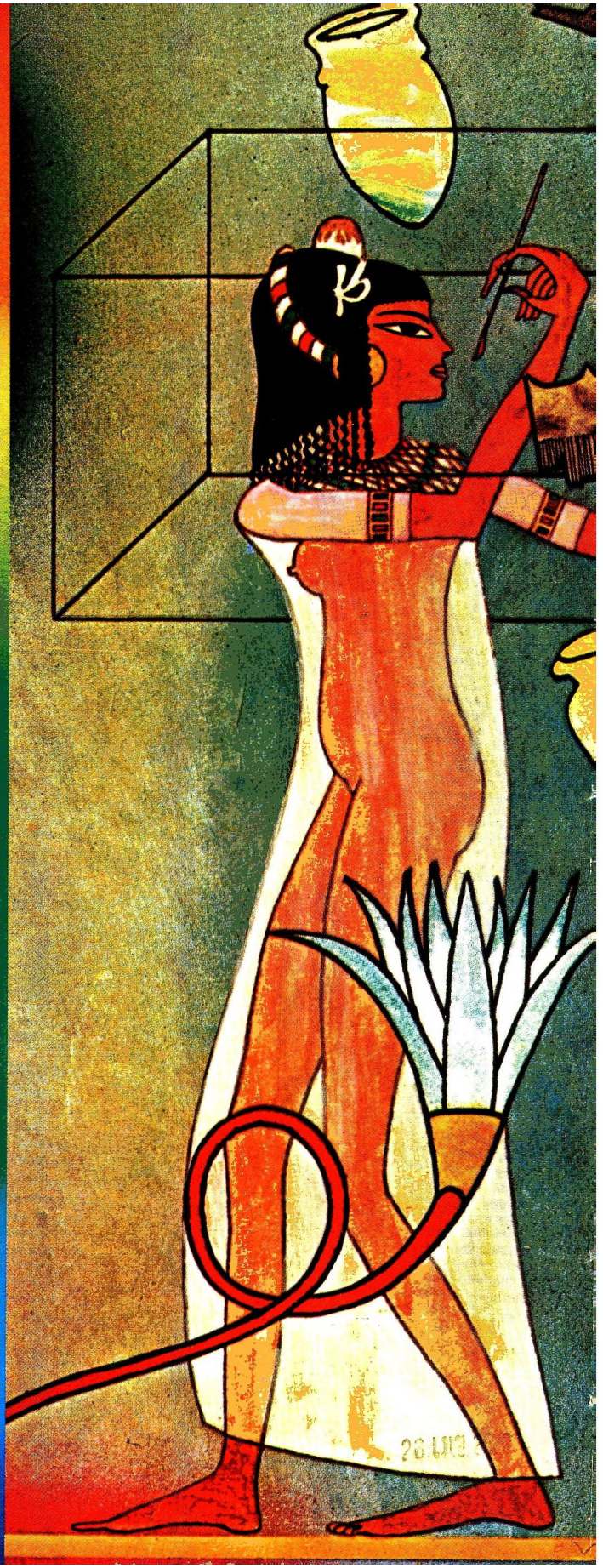

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