*J. Soc. Cosmet. Chem.*, 44, 181–195 (July/August 1993)

# Investigations into biomechanisms of the moisturizing function of lanolin

E. W. CLARK and I. STEEL, *Westbrook Lanolin Company, Laisterdyke, Bradford, West Yorkshire BD4 8AU, England*

*Received January 12, 1993.*

**Synopsis**

A study of the lanolin/human stratum corneum system has been made, both *in vitro* and *in vivo*, to investigate where lanolin absorbed by skin lies within the tissues, and to determine possible mechanisms of the moisturizing action. Skin treated with lanolin rendered electron-opaque by lipophilic doping with either lead lanolate or lead oleate was examined by freeze-fracture and transmission electron microscopy of tissue specimens in order to identify the location and state of lanolin within the stratum corneum. Evidence was found that most of the electron-opaque material, presumed to be doped lanolin, occupied intercellular spaces down as far as the stratum granulosum, replacing natural lipid structures lost during sample preparation. In some cases applied material had penetrated into corneocytes and also appeared to have been taken up into the lipid strata of some trilaminar cell envelopes, some of which remained intact. Lanolin appeared to have a special affinity for regions of cell junction. Although this could possibly have been lead marker which had migrated, there appears to be no clear reason why the marker should have greater affinity for natural skin lipids than for lanolin. There was further evidence that lanolin within intercellular spaces had spontaneously emulsified epidermal water as a w/o emulsion with a droplet size of approximately 40 nm, thus providing a possible moisture reservoir.

## INTRODUCTION

The emollient or moisturizing action of lanolin on human skin is well established (1). Nothing is known of the biomechanisms involved, but considerable indirect evidence exists:

1. Microprofilometry and intracorneal cohesography of skin treated with lanolin or some lanolin derivatives demonstrated pronounced smoothing (2).
2. Reduction in TEWL of 30% to 32% after lanolin application has been reported (3,4).
3. It has been shown (2) that films of lanolin statically immersed in water become hydrated by spontaneous absorption of water to form a fine w/o emulsion and, conversely, that hydrous lanolin emulsions exposed to air lose water by migration through the substrate and evaporation from the surface; lanolin thus permits a two-way transport of water. The droplet size in spontaneously formed emulsions was measured at 50 nm to 6 μm, this dimension being significant in relation to observations described herein.

4. The penetration of topically applied lanolin down as far as the stratum lucidum has been demonstrated by tape stripping and chemical analysis (5).
5. Lanolin has some components in common with the natural intercellular lipids, such as free and esterified cholesterol and free and esterified fatty acids; some lanolic acids are hydroxy acids, and others are esterified with diols to form diesters with two long acyl chains, as in the case of ceramides.

This earlier work led to the hypothesis that the moisturizing effect of lanolin was a result of absorption into the stratum corneum, where it reduced TEWL by partial occlusion of the internal water pathways or other means, and possibly also of lanolin within the stratum corneum absorbing natural epidermal moisture and thus acting as a reservoir, releasing water if the moisture gradient across the stratum corneum should favor such loss. The present work explores this hypothesis in three separate stages.

## EXPERIMENTAL

### STAGE 1

This was an initial *in vitro* exploratory stage involving the application of lanolin to excised skin, which, after suitable fixing and sectioning, was examined by transmission electron microscopy. The lanolin was insufficiently electron-opaque, however, to be differentiated from other structures within the stratum corneum specimens. Therefore, this approach was discontinued and details are not reported here.

### STAGE 2: OUTLINE

This stage of the study was subdivided into two sections involving different methodologies. Section 1 consisted of treating the surface of human skin *in vivo* with anhydrous lanolin, and after a time removing the surface lanolin and examining it by freeze-fracture and scanning electron microscopy in order to study the microstructural characteristics.

Section 2 involved the doping of lanolin with lead to increase its electron opacity before application to human skin *in vitro* at a specific loading. Prepared specimens of treated skin sections were then examined by transmission electron microscopy.

The principle of lead doping to increase electron opacity had been previously used by Ghadially and co-workers (6) in studies of skin penetration by petrolatum. In those studies, doping was carried out by adding lead nitrate in the form of a physical suspension. Lead nitrate is soluble in water but insoluble in petrolatum and other lipids, and thus there is the possibility of migration of some of the lead salt out of the petrolatum into hydrous areas.

In this part of our work, such a potential problem was avoided by doping lanolin with lead lanolate, which is the lead salt of the naturally occurring fatty acids of lanolin, at the level of 1.3% of lead as Pb. Lead lanolate has good solubility in lanolin but negligible solubility in water, and the possibility of migration into hydrous areas may be assumed to be negligible. There is at least the possibility of selective migration of lead salt into other lipids, but there seems to be no reason why these should have a greater affinity for lead salt than in the case of lanolin. Moreover, in a later section of

this work, a different lead salt was used (lead oleate). Despite the fundamental differences between oleate and lanolate salts, results appeared to be similar.

In all stages of our work, the biological treatment and electron microscopy of specimens was carried out by Dr. Ashley J. Wilson at the Centre for Cell and Tissue Research, University of York, England, in cooperation with and under the sponsorship of Westbrook Lanolin Company. Lanolin used in the work was anhydrous lanolin Eur. Pharm.; the lead lanolate was prepared in house by base exchange between aqueous solutions of sodium lanolate and lead nitrate.

STAGE 1, SECTION 1

*Methodology.* A 50-mm square of inner forearm of a male volunteer subject was lightly delineated and 50 mg of undoped lanolin was gently rubbed into the test area for one minute. After five minutes, small portions of lanolin were carefully scraped from the surface of the skin, avoiding any abrasion, and prepared for freeze-fracture studies. As controls, specimens of the original lanolin and of lanolin containing 25% w:w of water (as a mechanically prepared w/o emulsion) were similarly examined. Samples were put into a pair of hollow rivets held together by special forceps, the whole assembly being rapidly frozen by quenching in subcooled nitrogen at 69 K. Samples were fractured and replicated in a Leybold Heraeus Biotech 2005 freeze-fracture apparatus. Platinum carbon was evaporated at an angle of 45°, with carbon from above to strengthen the replicas. These were then cleaned of lanolin in trichloroethylene, followed by 5% sodium hypochlorite solution, and examined in a JEOL 1200 EX transmission electron microscope.

*Results.* Specimens of original anhydrous lanolin showed fracture surfaces composed of a mix of relatively amorphous solid lanolin and areas of crystalline lamellae thought to be fractions of lanolin that had been liquid at room temperature.

Freeze-fractured 25% hydrous lanolin showed fracture surfaces of similar general appearance but superimposed on it were spherical water droplets varying in diameter from 0.5 to 3 μm. Lanolin removed from skin after five minutes likewise showed spherical water droplets of 50 to 300 nm in diameter (Figure 1), presumably epidermal water which had passed from the skin surface into the lanolin layer where it formed a w/o emulsion. These droplets formed *in vivo* are of the same order of magnitude as the water spontaneously emulsified in lanolin *in vitro*, as previously reported (2).

STAGE 1, SECTION 2

*Methodology.* Full-thickness skin resulting from breast reduction operations was used, after removing subcutaneous fat down to the dermis. Prior to actual use, the skin samples were kept moist by saline-soaked swabs. From each piece of skin, three circular specimens of 20-mm diameter were punched, and two of these were clamped in holders with the dermis side of the skin held in contact with a wad of three discs of filter paper soaked in saline solution. The top plate of the holder ensured that a defined area of 1 sq cm was exposed to the atmosphere. About 4 mg of lanolin doped with lead lanolate was applied to each exposed area and spread evenly by means of a piece of surplus skin. The third disc of skin was also mounted in a holder but left untreated as a control. All three holders were placed in a 25-cm diameter desiccator fitted with a hair hygrometer. The

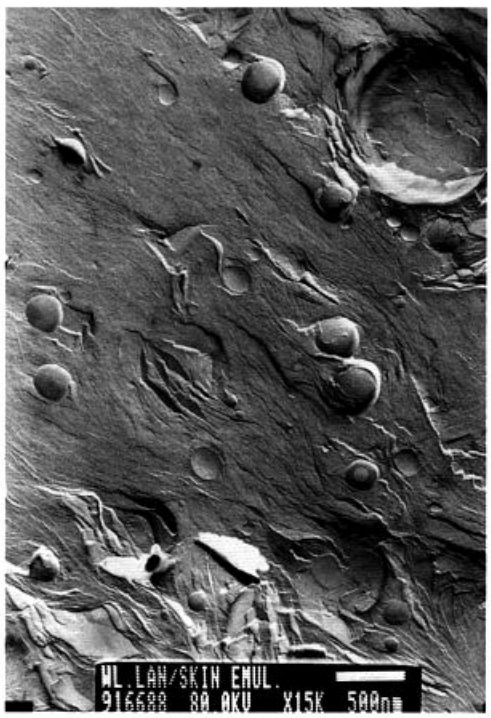


**Figure 1.** Freeze-fractured lanolin showing emulsified transepidermal water.

base of the desiccator held a saturated solution of calcium chloride dihydrate to provide a relative humidity of 38% within the chamber. The desiccator was kept in a constant-temperature room at 37°C. Treated skin was sampled after 16 hours. At the end of the treatment period, skin was removed from the holders, and small, roughly cubical pieces about 1.5 mm per side were cut for fixation by the standard operating procedure as follows:

| | |
|---|---|
| *Primary fixation:* | 3% Glutaraldehyde solution in phosphate buffer |
| *Buffer wash:* | 0.1 M Mixed phosphate buffer at pH 7.2 |
| *Secondary fixation:* | 1% Solution of osmium tetroxide in buffer |
| *Wash:* | Distilled water |
| *Dehydration:* | Ethanol series 50%, 70%, 90%, 100% (twice) |
| *Infiltration:* | Propylene oxide |
| | 50:50 Propylene oxide: resin |
| | 100% Resin at 37°C |
| | 100% Resin |
| *Embedding:* | 100% Resin in flat embedding molds |
| | Polymerized at 65°C |
| *Sectioning:* | By Reichert OMU2 ultramicrotome |
| *Staining:* | Some sections unstained, others stained in uranyl acetate and lead citrate solutions |
| *Observations and microscopy:* | JEOL 1200 EX transmission electron microscope |
| *Freeze-substitution medium:* | Methyl alcohol (100%) 100 ml |

Uranyl acetate 0.5 g
Osmium tetroxide 1.0 g
Glutaraldehyde stock solution (50%) 6.0 ml

*Results*. Figure 2 is a section of the outer layers of stratum corneum showing an excess of lanolin (grey) remaining on the surface (bottom of picture) after 16 hours, and also some lanolin (similarly grey) that has penetrated into intercellular spaces down to the third or fourth corneocyte. A dark line following the edge of some corneocytes suggests a particularly high concentration of lanolin (or at least lead marker) at those points, as though lanolin had become incorporated in the trilaminar outer membranes of corneocytes that in places had become detached from the cell. Most other intercellular lipids appear to have been lost during specimen processing. The surface lanolin is seen to contain some very electron-opaque particles, which are believed to be, in part, a residual impurity of unreacted lead nitrate in the doped lanolin. An examination of a thin film of this lanolin by electron microscopy showed similar small, very dense clumps. Figure 3 is of another skin section at greater magnification and shows even more clearly an accumulation of lanolin (and/or lead marker) in laminar structures.

The largest such feature is particularly interesting since is shows a distinct triple layer consisting of a central band about 200 Å thick sandwiched between two narrower (80 to 100 Å) bands that are more electron-opaque. This structure is in accordance with the findings of Swanbeck (9), who proposed the existence of fibrous protein layers surrounded by lipid bilayers, which, in our own case, have apparently been infiltrated by

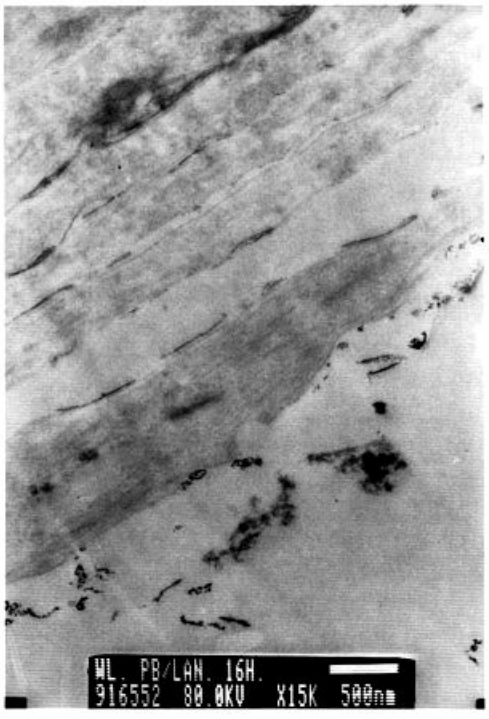


**Figure 2.** Lanolin on skin surface (bottom of picture) showing penetration into intercellular spaces and concentration at cell surfaces.

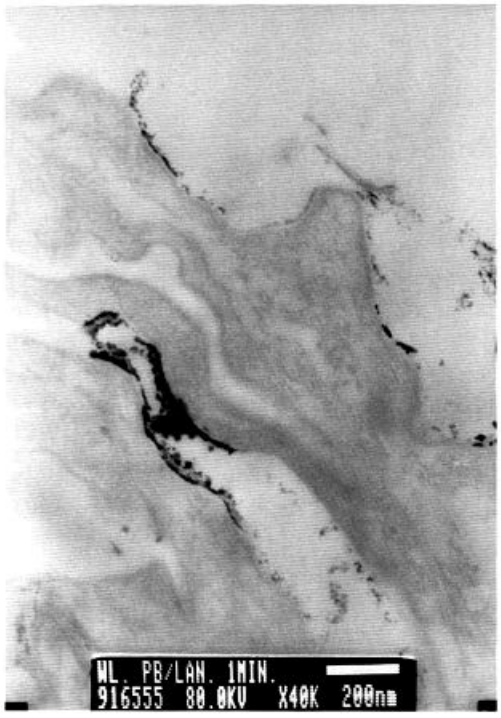


**Figure 3.** Concentration of lanolin/marker in trilaminar membrane of cell envelope.

lanolin and/or marker. The dimensions we see certainly agree well with Swanbeck's measurements. This trilaminar structure with a central protein band has been disputed, but lipid layers with a spacing of 50 to 80 Å were reported by Friberg *et al.* (10), who also showed that interaction of other lipids with the bilayers could affect the spacing. Other work reported by Ward and du Reau (11) also showed an alteration in spacing by the solubilization of oleyl alcohol into the bilayers and consequent increase in water capacity. It is possible in our case, therefore, that incorporation of applied substance into the lamellae has altered the spacing of lipid layers from that reported by Friberg *et al.* Trilaminar structures can also be detected in Figure 4, and here too, as in Figure 3, some of them have become detached from the cell surface. Trilaminar membranes have been previously reported by Odland and Holbrook (12) and Elias (13), and are now an accepted structural feature. In our case it appears that although most of the natural intercellular lipids have been lost during preparation of the specimens (except perhaps in Figure 10), the lamellae covalently bound to the corneocyte surfaces appear to have survived in places, although some have been detached from the cell surface. In an attempt to overcome this loss of lipids, further samples were prepared using a freeze-substitution technique that has been reported to fix and retain better, at low temperature, the natural lipids. This does not appear to have been so in our case, however, with most intercellular spaces still showing absence of natural lipids. Nevertheless, some interesting features are visible.

Figure 5 shows that lanolin (grey) has penetrated here into the intercellular spaces down as far as the stratum granulosum. Such stained material was not visible in the untreated

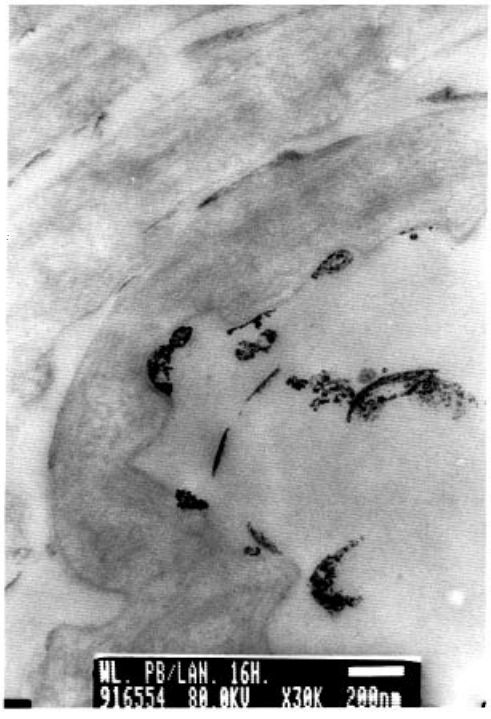


**Figure 4.** Lanolin/marker incorporated in membranes attached to, and detached from, the skin surface.

control (Figure 6), where the intercellular spaces appear white. There also appears to be a heavy concentration of electron-opaque material at a junction between the corneocytes (arrowed) in Figure 5 (perhaps a desmosome remnant), and more evidence of this is reported in Stage 3.

STAGE 3

*Outline and methodology.* Although this was basically a repeat of Stage 2, Section 2, freeze substitution was used throughout in the preparation of specimens, and there was a change in the lead-doping technique. Instead of using lead lanolate, doping was carried out with lead oleate, which has better lipid solubility and is easier to wash free from residual inorganic lead salts. Examination of thin films of the lead-oleate–doped lanolin by electron microscopy showed it to be quite homogeneous, with no dense particles. Tissue specimens were not stained, so that electron-opaque areas indicate the presence of lead oleate and presumably, therefore, of lanolin, unless selective migration of lead salt into other lipids has occurred, against expectation.

*Results.* Figure 7 confirms the penetration of lanolin deep into the stratum corneum. It also confirms findings reported in Stage 1, Section 2, of the existence of a structure with high lead and presumably lanolin content forming a layer on, or near, the surface of some corneocytes bounding intercellular spaces, more clearly seen in an enlargement of the area marked 'L' in Figure 8. Similar layers on or close to, and parallel with, corneocyte surfaces are very clear in Figure 9 and 10, where signs of a lamellar structure

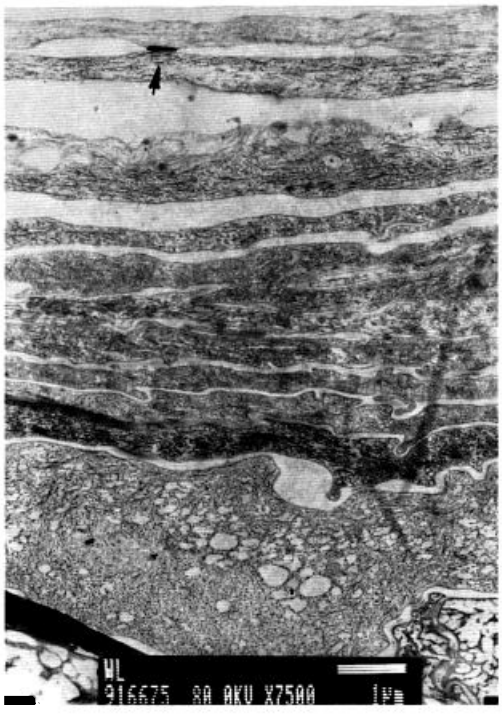

**Figure 5.** Concentration of lanolin/marker (arrowed) at cell junction, and lanolin visible in intercellular spaces down to the stratum granulosum.

can be seen. Also visible in Figure 10 is apparent evidence of water droplets emulsified in the lanolin.

The corneocytes themselves appear to be permeable to lanolin. Figures 11 and 12 show what seem to be concentrated accretions of lanolin (arrowed) within some corneocytes. The penetration of corneocytes by cholesterol was postulated by Garson *et al.* (14). Figure 12 also shows (arrows) concentrations of electron-opaque material at cell surfaces.

One of the objects of our work was to investigate the possibility of spontaneous emulsification of water in lanolin absorbed in stratum corneum, as is known to occur *in vitro*. Such an effect appears to be manifested not only in Figure 10 but also in Figure 13 (arrows), showing a mass of lanolin within an intercellular space, which is honeycombed with holes up to about 40 nm in diameter. These could be where emulsified water droplets had existed before the fixation of the specimen. Lead oleate without lanolin would not be expected to show this effect. The size of droplet observed is a close approximation to that of many of those shown in Figure 1, where epidermal water had migrated into a lanolin film on the skin surface, and strengthens the evidence pointing to spontaneous emulsion formation within the stratum corneum. If this does in fact occur, then lanolin would seem to provide a second mechanism by which essential moisture can be stored within the stratum corneum, in addition to the water held in the lipid bilayer structures.

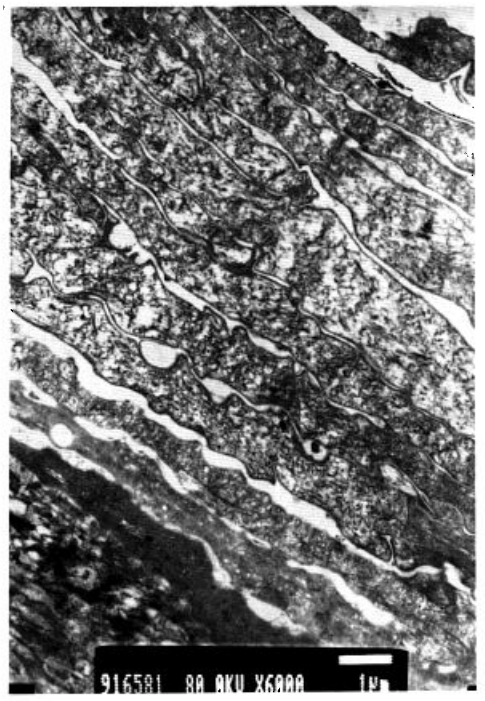

**Figure 6.** Section of control tissue showing no staining or electron-opaque material in intercellular spaces.


**Figure 7.** Concentration of lanolin/marker on cell surfaces bounding intercellular spaces L, M, and N.

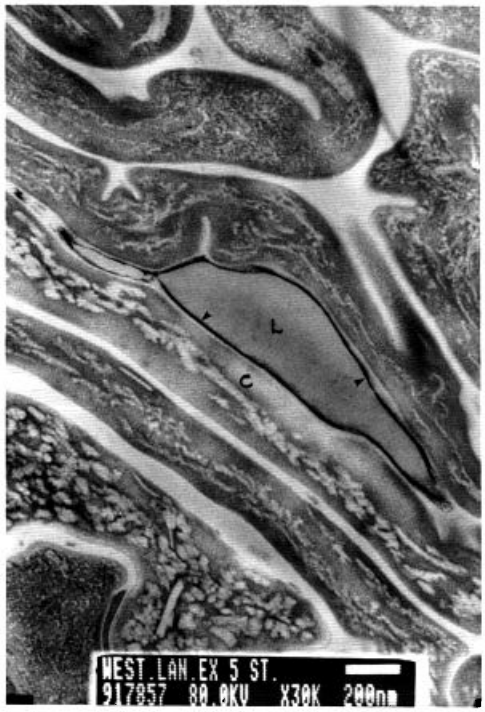


**Figure 8.** Enlargement of area L from Figure 11. (C indicates corneocyte about 11 layers from the surface.)


**Figure 9.** Lanolin/marker apparently concentrated in detached cell envelope membrane.

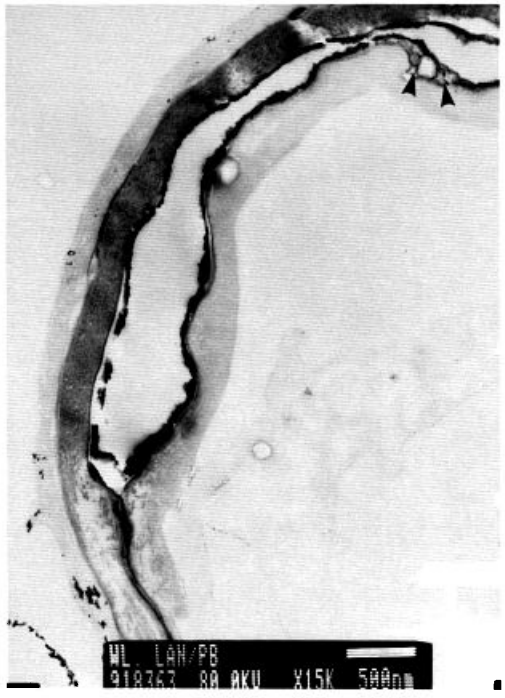


**Figure 10.** Lanolin/marker concentrated in trilaminar membrane, and possible emulsified water droplets in lanolin (arrowed).

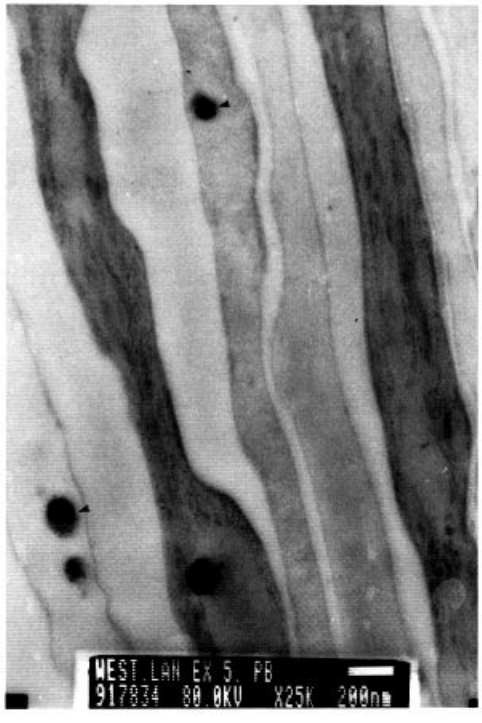


**Figure 11.** Accretions (arrowed) of lanolin/marker within corneocytes.


**Figure 12.** Lanolin penetration into intercellular spaces and apparent concentration at cell surfaces.

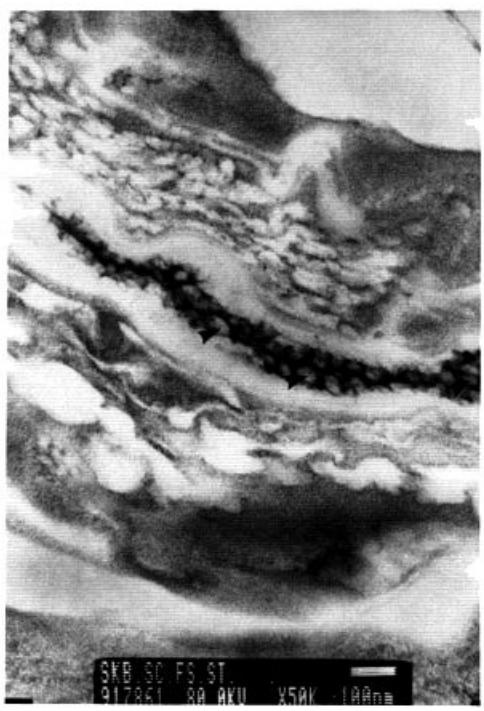


**Figure 13.** Evidence of emulsified water droplets in lanolin in intercellular spaces (arrowed).

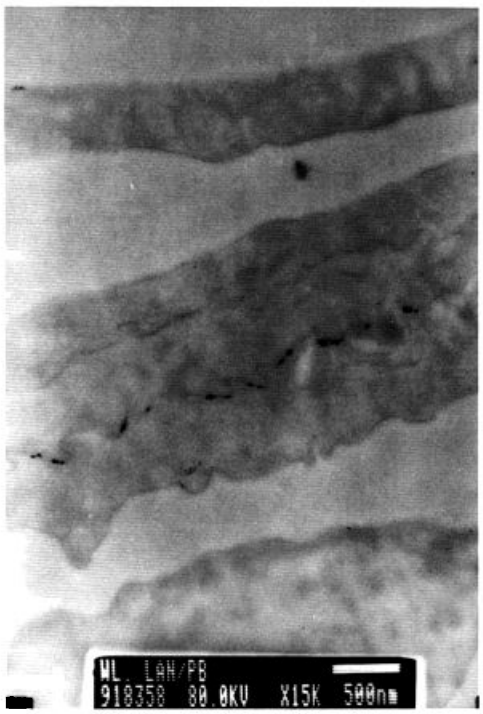


**Figure 14.** Lanolin/marker concentrated at long area of cell contact.

A feature of Figure 14 is a long line of contact between two corneocytes where lanolin or marker (arrows) has concentrated, presumably in the lipid layer on the surface of corneocytes. Evidence of this was seen earlier in Figures 2 and 12.

Figure 15 is also interesting, showing distinctly a number of short trilaminar structures, with a spacing of 25 to 50 nm, which have taken up a high concentration of lanolin or lead salt. These apparently lie within corneocytes about three layers down from the surface of the skin. This spacing seems too great for intercellular lipid/water bilayers, but it is closer to that of cell envelope material, although how fragments of envelope could penetrate to the interior of the corneocytes is not clear. It may perhaps be evidence of a lipid layer surrounding a short protein fibril.

## CONCLUSIONS

Evidence has been found that is in agreement with the postulated mechanisms by which lanolin could act as an emollient and moisturizer:

1. Lanolin applied to the stratum corneum penetrates throughout its full depth, down to the stratum granulosum.
2. Lanolin located in intercellular spaces appears to form spontaneously a w/o emulsion with some of the epidermal water.
3. Lanolin (or lead marker) can penetrate to the interior of corneocytes.
4. Lanolin and/or lead marker appears to have the power to become incorporated in the

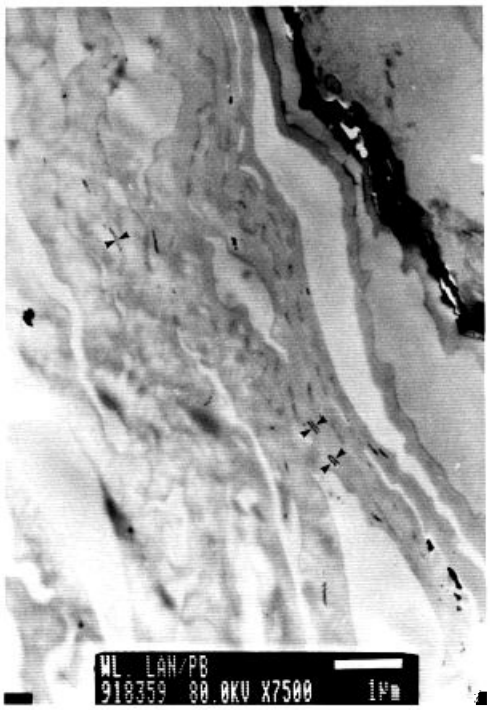


**Figure 15.** Small, apparently intracellular, laminar structures that contain lanolin/marker in their outer layers.

intercellular lipid bilayers, at least (apparently) in trilaminar structures forming the outer envelope of corneocytes.

This work suggests that lanolin achieves its moisturizing action by absorption deep into the stratum corneum. Once there, whether its action is partial occlusion of natural water pathways, or due to being taken up into natural lipid bilayers, or due to providing an additional water reservoir, is not yet entirely clear. There is, however, some evidence suggesting that lanolin may penetrate corneocytes and become incorporated into laminar structures such as cell envelope material. Evidence suggesting the spontaneous emulsification of epidermal water by intercellular lanolin has been seen.

## ACKNOWLEDGMENT

The assistance of Dr. Ashley J. Wilson of CCTR, University of York, Heslington, England, in interpreting the photomicrographs is gratefully acknowledged.

## REFERENCES

(1) E. W. Clark, A brief history of lanolin, *Pharm. Historian,* **10,** 5–6 (1980).
(2) E. W. Clark, New concepts of lanolin. Presentation at In-Cosmetics Seminar, NEC, Birmingham, England, March, 1990.
(3) D. H. Powers and C. Fox, A study of the effect of cosmetic ingredients, creams and lotions on the rate of moisture loss from the skin, *Proc. Sci. Sec. Toilet Goods Assoc.,* **28,** 21–26 (1957).

(4) D. Spruit, Interference of some substances with water vapour loss of human skin, *Amer. Perfum. Cosmet.*, **86**, 27–32, (1971).
(5) E. W. Clark, Short-term penetration of lanolin into human stratum corneum, *J. Soc. Cosmet. Chem.*, **43**, 219–217 (1992).
(6) H. E. Jass and P. M. Elias, The living stratum corneum, *Cosmet. Toiletr.*, **106**, 47–53 (1991).
(7) M. A. Lampe, M. L. Williams, and P. M. Elias, Human epidermal lipids: Characterisation and modulation during differentiation, *J. Lipid Res.*, **24**, 131–140 (1983).
(8) R. Ghadially, L. Halkier-Sorenson, and P. M. Elias, Effects of petrolatum on stratum corneum and function, *J. Am. Acad. Dermatol.*, **26**, 387–396 (1992).
(9) G. Swanbeck, Macromolecular organisation of epidermal keratin, *Acta. Derm. Venereol. (Stockh.)*, **39**, Suppl. 43 (1959).
(10) S. E. Friberg, D. W. Osborne, and T. L. Tonbridge, X-ray diffraction study of human stratum corneum, *J. Soc. Cosmet. Chem.*, **36**, 349–354 (1985).
(11) A. J. I. Ward and C. du Reau, Modeling the permeability properties of skin, *Cosmet. Toiletr.*, **105**, 53–59 (1990).
(12) G. P. Odland and K. Holbrook, The lamellar granules of epidermis, *Curr. Probl. Dermatol.*, **9**, 29–49 (1981).
(13) P. M. Elias, Lipids and the epidermal permeability barrier, *Arch. Dermatol. Res.*, **270**, 95–117 (1981).
(14) J. C. Garson, J. Doucet, G. Tsoucaris, and J. L. Leveque, Study of lipid and non-lipid structure in human stratum corneum by X-ray diffraction, *J. Soc. Cosmet. Chem.*, **41**, 347–358 (1990).