J. Soc. Cosmet. Chem., 43, 219–227 (July/August 1992)

Short-term penetration of lanolin into human stratum corneum

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

Received January 23, 1992.

Synopsis

Lanolin is readily absorbed by the human skin, and since it contains both free and esterified cholesterol and fatty acid esters, it has at least in part some similarities to the intercellular lipids in stratum corneum. It is widely used as an effective emollient.

As the first stage of a wider investigation into the mode of action of lanolin on the skin, the present study examines the extent of penetration of lanolin into the stratum corneum without attempting to find, at this stage, whether the substance is located intercellularly, in hair follicles, in sweat ducts, or elsewhere. It is already known not to lie merely in surface irregularities.

Anhydrous lanolin was applied at a loading of 2 mg cm^{-2} to an area of 2 × 1 cm of the flexor aspect of the inner forearm (*in vivo*).

The treated area of stratum corneum was then removed in layers by 30 successive tape strippings, and lanolin contents of the strippings were determined quantitatively by a spectrophotometric method based on the Liebermann-Burchardt reaction for steroids, developed to give a limit of determination in the region of 50 μg of lanolin and a sensitivity of 1.25 mg lanolin per unit absorbance on the linear portion of the calibration graph. The method is described in detail.

Total recovery of lanolin was between 98.8 and 103.1% of that applied, the major portion being found in the first 12 strippings but traces still being detectable in the deepest layers adjacent to the stratum lucidum. The profile of lanolin content in the stratum corneum, when graphed, gave a curve very similar to another one previously obtained in a pilot study, and also similar to a curve published by other workers which related corneocyte removal by tape stripping to a depth within the stratum corneum. There appeared to be no significant transport of lanolin through the stratum corneum into underlying layers.

Interference in the spectrophotometric method by the adhesive tape and other materials used was taken into account. The extent of interference by natural steroids in the stratum corneum was quantified by separate experiments and was found to be variable but very small.

INTRODUCTION

Intercellular lipids have been shown to influence the moisture-retaining capacity of human stratum corneum (1,2), and a bilayer arrangement of polar compounds such as cholesterol, glycerides, fatty acids, ceramides, and phospholipids has been demonstrated. A review was given by Ward and du Reau (3). Lanolin (the term implies the

anhydrous substance throughout this paper) is a naturally occurring lipid with polar surface-active properties, and it contains a high proportion of cholesterol and fatty acids, the majority of which are esterified. This similarity of lanolin to some components of the intercellular lipids suggests that lanolin molecules may have little difficulty in being incorporated in such bilayer structures.

Lanolin has long been recognized as an effective moisturizer for the skin, and this function has recently been demonstrated and quantified by Clark (4). It is readily absorbed by the stratum corneum, and about 2 to 3 mg cm^{-2} can be absorbed with no residual surface film. These attributes of the substance, together with the facts that it shows a very low incidence of specific allergy among the general population (5–9) and has a history of safe use extending back over centuries, has led to the widespread use of lanolin in cosmetics and pharmaceuticals intended for topical applications.

An investigation directed toward elucidating the mode of action of lanolin in the stratum corneum has now been started, and the present paper represents the first phase of the work. It is a quantitative study of the depth within the stratum corneum to which lanolin penetrates after application to the surface at a specific loading. The actual location of the absorbed substance (for example, whether it lies between corneocytes, in sweat ducts, or in hair follicles) is being investigated separately, but it has already been shown that lanolin does not merely lie in the natural folds and crevices in the skin's surface (1).

The basis of the present methodology was to apply lanolin at a specific loading to stratum corneum *in vivo,* after which the treated area of skin was removed a layer at a time by 30 successive tape strippings, sufficient to expose the stratum lucidum. The lanolin contents of the tape strippings were quantitatively determined by a sensitive spectrophometric method described later.

Several types of adhesive tape were evaluated, but most were impracticable because they caused severe interference in the colorimetric test method for lanolin. Scotch Magic Tape® was found to cause very little interference, and even this could be compensated for in the blanks used, and so this particular brand of tape was used in the work.

QUANTITATIVE DETERMINATION OF LANOLIN

A sensitive method of qualitative and quantitative analysis of skin lipids was described by Okamoto (10) but was considered unsuitable here, since it is based on thin layer chromatography. Lanolin, a very complex mixture, gives multiple spots by such a technique, making quantification difficult. Instead, a method was chosen based on the well-known Liebermann-Burchardt reaction, in which certain of the steroidal components of lanolin give a green color when dissolved in a mixture of chloroform and acetic anhydride treated with concentrated sulphuric acid. The resultant color is amenable to spectrophotometric determination (11). For present purposes the methodology was not fully developed statistically, but it is estimated to give a reliable limit of determination in the region of 50 μg of anhydrous lanolin and a limit of detection of about 10 μg. The method requires a calibration graph, the derivation of which is described in detail in Appendix 1.

The extent of any interference by steroids present in the natural intercellular lipids has been quantified and allowed for.

GENERAL METHODOLOGY

MATERIALS AND EQUIPMENT

- Scotch Magic® adhesive tape
- Glass fiber filter papers (Whatman GF/C, 11.0 cm)
- 55-mm glass funnels
- Glass rod, 120- × 5-mm flattened and flame-polished at one end
- 50-ml graduated stoppered glass cylinders
- Template, 20- × 10-mm rectangle cut in acetate sheet

PROCEDURE

The test subject was a 68-year-old male. A rectangular area 2 × 1 cm was marked out on the flexor surface of the inner forearm using a graphite pencil and the template. 4.2 mg of lanolin, weighed on a glass coverslip, were applied to this area and gently rubbed until absorbed, using the flame-polished glass rod. Ten minutes after application, tape stripping was commenced. Each stripping consisted of a 4-cm length of tape covering the test area, applied by thumb pressure for ten seconds and then removed by forceps. Successive strippings were collected in groups of three, each group being put into a 50-ml stoppered glass cylinder. To each cylinder 13.5 ml of chloroform were added, and after ten minutes the cylinder was shaken, causing the tape partially to disintegrate. 11.5 ml of acetic anhydride were then added, and the cylinder was reshaken, completing the disintegration of the tape and dissolution of any lanolin present. 0.5 ml of concentrated sulphuric acid was added, another shake given, and the mixture immediately filtered through a glass fiber filter paper and filled into an optical cell. Simultaneously a blank was prepared from 12 cm of virgin tape, treated in a manner similar to the actual test. As the green color in the test mixture developed, the absorbance at 458 nm and 25°C was continuously monitored on the spectrophotometer until a maximum had been reached and just passed. The maximum reading was taken as definitive.

The coverslip and glass rod used for lanolin application were washed with chloroform, and the residual lanolin thus recovered was determined by the same method as used for the tape strippings.

The mass balance is shown in Table I, and essential results plotted as a graph are shown in Figure 1. The cumulative recovery of lanolin from all sources was 4.33 mg out of 4.20 mg applied (103.1%). Even though this recovery was excellent, since it was slightly in excess of the weight applied, it was necessary to ascertain to what extent natural steroids in the stratum corneum of the test subject had contributed to the measured amounts of lanolin.

Accordingly, the whole experiment was repeated on the same subject, but this time without applying any lanolin. Faint positive reactions were found up to and including the fifth group of strippings, the results being shown in Table II and Figure 2. The

Table I
Recovery of Lanolin From Tape Strippings

Strippings	Lanolin found (mg)	
	Individual	Cumulative
Cleanings	1.03	1.03
1 to 3	2.47	3.50
4 to 6	0.43	3.93
7 to 9	0.15	4.08
10 to 12	0.07	4.15
13 to 15	0.06	4.21
16 to 18	0.05	4.26
19 to 21	0.02	4.28
22 to 24	0.01	4.29
25 to 27	0.01	4.30
28 to 30	0.03	4.33

cumulative recovery of "lanolin" was 0.18 mg (note: this is not a constant figure; see below), and after deducting this "blank" from the results of the original test with lanolin, the net recovery then becomes 4.15 mg out of 4.20 (98.8%). The weight of lanolin unaccounted for is only 50 μg, about the same as the limit of determination, and

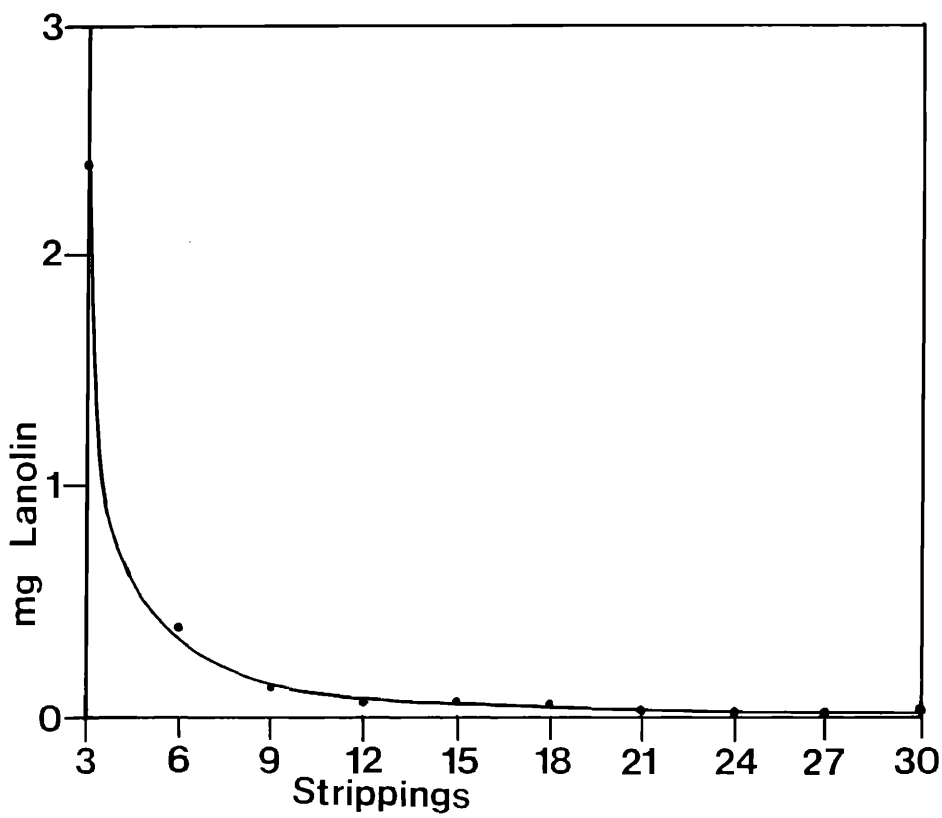

Figure 1. Lanolin removed by tape strippings.

Table II
Apparent Lanolin in Control Strippings

Strippings	Apparent lanolin found (mg)	
	Individual	Cumulative
1 to 3	0.09	0.09
4 to 6	0.05	0.14
7 to 9	0.02	0.17
10 to 12	0.02	0.18
13 to 15	Trace	0.18
16 to 18	Not detected	0.18
19 to 21	Not detected	0.18
22 to 24	Not detected	0.18
25 to 27	Not detected	0.18
28 to 30	Not detected	0.18

so no more than this, if any, appears to have penetrated the stratum corneum into deeper layers of the skin.

Further tape strippings were also made from two different individuals, and yet another set of strippings was made from the original test subject, in all cases without preapplication of lanolin. The number of strippings in each case was limited to six, comprising two groups of three, each group being analyzed for apparent lanolin content. The results are shown in Table III.

These figures demonstrate not only a large difference between individuals, but also that results from the same individual at different times can vary by a factor of more than three. In any case, interference with the test method used is relatively small.

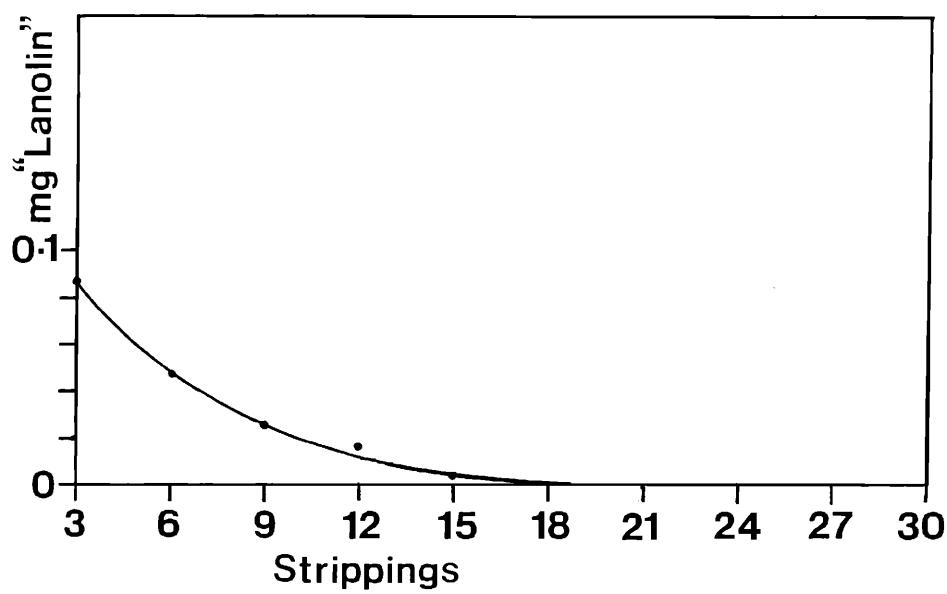

Figure 2. Apparent lanolin in untreated skin.

Table III
Apparent Lanolin in Control Strippings

	Apparent lanolin found (ng)		
Strippings	Male 68 years	Male 32 years	Male 36 years
1 to 3	36	Not measurable	11
4 to 6	15	Not measurable	Not measurable
Total	51	Not measurable	14

Note: results as low as these are of doubtful accuracy.

DISCUSSION

A sensitive method for determining lanolin in layers of human stratum corneum has been shown to give good results, taking into account interference from adhesive tape, filter paper, and naturally occurring components of the stratum corneum.

The reason for choosing a ten-minute waiting period for penetration to occur before stripping was twofold. First, corneocyte shedding from the stratum corneum is a continuous natural process and could be significant over a longer period, involving losses of lanolin with a resultant incomplete mass balance. The second reason is subjective, since when lanolin is used as an emollient, rapid penetration and effect is important to the user.

The mass balance obtained suggests that no significant penetration of lanolin through the stratum corneum takes place, at least within the time period covered by the investigation and with this particular test subject. Since about 96% of the lanolin was found in the first 40% of strippings, it seems unlikely that much, if any, penetration will occur. Whether there would be substantial inter-subject differences in penetration is not known at this stage, but it is hoped that the wider-ranging investigation as a whole may throw light on this point.

As a precursor to the experiment already described in detail, a pilot trial was made which followed the same broad technique, but with some differences:

1. The lanolin was applied to the test site through the open rectangle in the template that was temporarily taped to the arm.
2. The lanolin was applied by fingertip instead of by glass rod.
3. A higher loading of 5 mg cm^{-2} of lanolin was applied.

This was not a good method since some lanolin was forced underneath the template and thus could not be absorbed by the skin, although some was absorbed by the fingertip. Although unabsorbed lanolin was recovered from all locations by chloroform and determined, overall recovery was only 81.1%. Nevertheless, results were graphed and the general shape of the curve agreed closely with the one actually shown in Figure 1. Reproduction here was therefore considered unnecessary.

It is also noted that the shape of the curve is remarkably similar to one obtained by King *et al.* (12) that related the number of corneocytes removed to the number of successive tape strippings. The majority of corneocytes were removed in the first 12 strippings. Some of the tape strippings made in the present work were examined by optical microscopy before their lanolin content was determined, and the number of corneocytes

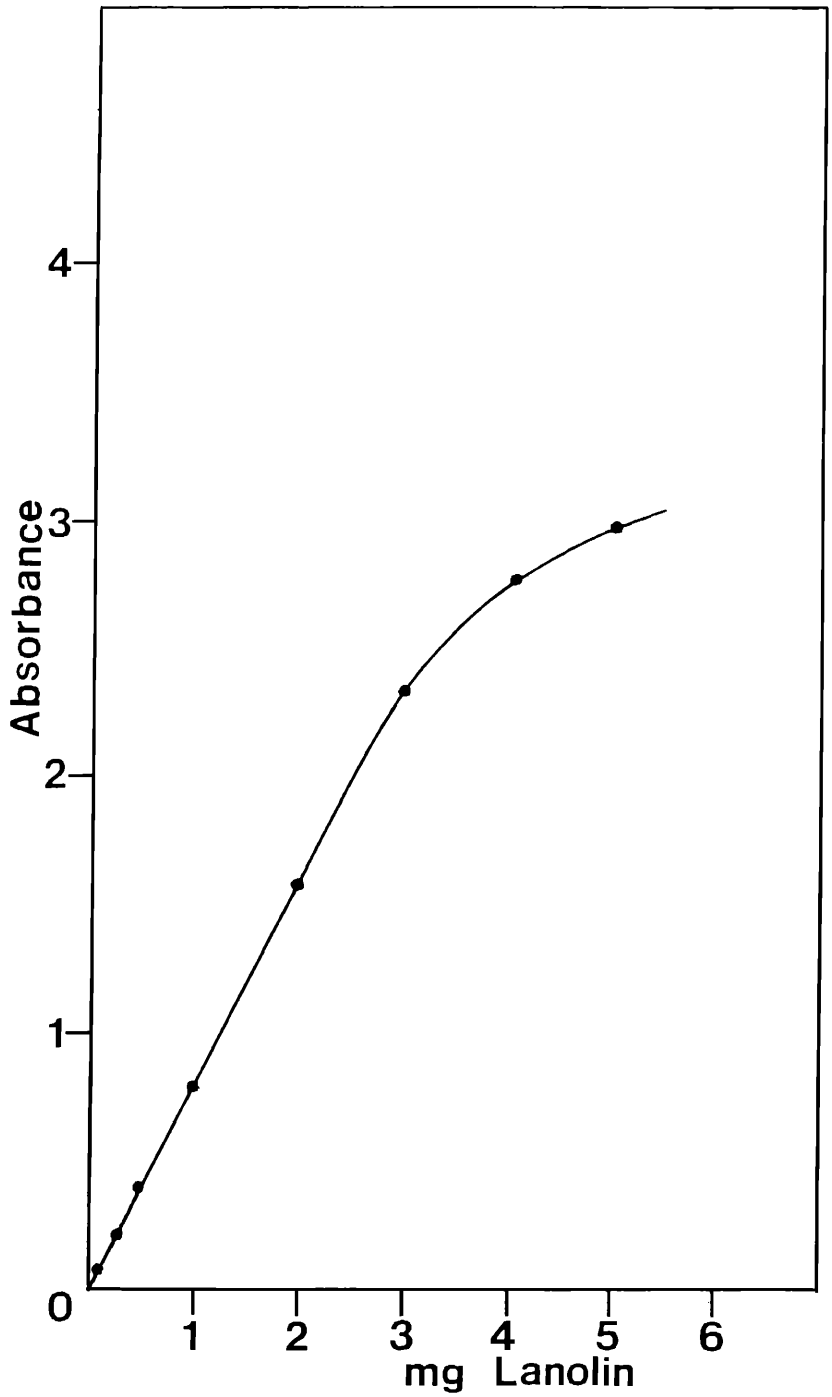

Figure 3. Calibration curve for lanolin determination.

removed visibly decreased as a function of depth, as would be expected from the other results.

A secondary observation arising from the work was that test sites treated with lanolin prior to tape stripping healed significantly more quickly than untreated sites. This observation is being investigated further in a follow-up study.

REFERENCES

(1) G. Imokawa and M. Hattori, A possible function of structural lipids in the water-holding properties of the stratum corneum, *J. Invest. Dermatol.*, **84**, 282–284 (1985).
(2) G. Imokawa, S. Akasaki, A. Kawamata, S. Yano, and N. Takaishi, Water-retaining functions in the stratum corneum and its recovery properties by synthetic pseudoceramides, *J. Soc. Cosmet. Chem.*, **40**, 273–285 (1989).
(3) A. J. I. Ward and C. du Reau, Modelling the permeability properties of skin, *Cosmet. Toiletr.*, **105**, 53–59 (1990).
(4) E. W. Clark, New concepts of lanolin. In-Cosmetics Seminar, Birmingham NEC, England (March 1990).
(5) E. W. Clark, Estimation of the general incidence of specific lanolin allergy, *J. Soc. Cosmet. Chem.*, **26**, 323–335 (1975).
(6) E. W. Clark, E. Cronin, and D. S. Wilkinson, Lanolin with reduced sensitizing potential, *Contact Dermatitis*, **3**, 69–74 (1977).
(7) H. Ippen, Investigations on contact allergy to woolwax. 5th session of the International Contact Dermatitis Group, Barcelona (1980).
(8) E. W. Clark, A. Blondeel, E. Cronin, J. A. Oleffe, and D. S. Wilkinson, Lanolin of reduced sensitizing potential, *Contact Dermatitis*, **7**, 80–83 (1981).
(9) B. Edman and H. Moeller, Testing a purified lanolin preparation by a randomized procedure, *Contact Dermatitis*, **20**, 287–290 (1989).
(10) K. Okamoto, Studies on the quantity and chemical composition of surface lipids of human scalp and hair, *J. Dermatol.* (Tokyo), **7**, 85–99 (1985).
(11) E. V. Truter, *Wool Wax Chemistry and Technology* (Cleaver-Hume Press Limited, London, 1956), p. 185.
(12) C. S. King, S. P. Barton, S. Nicholls, and R. Marks, The change in properties of the stratum corneum as a function of depth, *Brit. J. Dermatol.*, **100**, 165–172 (1979).

Appendix I: DERIVATION OF A CALIBRATION CURVE FOR THE SPECTROPHOTOMETRIC DETERMINATION OF LANOLIN

MATERIALS AND EQUIPMENT

- Chloroform AR
- Acetic anhydride AR
- Sulphuric acid, concentrated AR
- Anhydrous lanolin BP (Westbrook)
- 50-ml graduated burette
- 50-ml graduated stoppered glass cylinders
- 1-ml graduated pipette
- Optical glass cells, 20-mm path length
- Spectrophotometer, Philips PU 8650

PROCEDURE

A standard solution of lanolin in chloroform was prepared with a concentration of 0.37 gl^{-1}. Eight different measured volumes of this standard solution were run from the

burette into a 50-ml graduated cylinder and made up to 13.5 ml (if necessary) with chloroform, then up to a total of 25.0 ml with acetic anhydride. To avoid buretting volumes less than 1 ml, the standard solution when appropriate was diluted 10-fold volumetrically with chloroform to a concentration of 0.037 gl^{-1}. To each prepared mixture of standard lanolin solution and acetic anhydride was added 0.5 ml of concentrated sulphuric acid by pipette, and the mixture was shaken and transferred to an optical cell. A blank was prepared similarly but using chloroform only, without lanolin, and used for comparison, the absorbance being measured at 458 nm and 25°C at frequent intervals. Absorbance reached a maximum after about 15 to 20 minutes, then began slowly to fall. The maximum reading was regarded as definitive. The total of eight readings obtained was plotted as a calibration curve (Figure 3), which followed the classical Beer-Lambert form. The sensitivity in the linear region of the graph is 1.25 mg lanolin per unit absorbance.

INDEX TO ADVERTISERS