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X-ray diffraction study of human stratum corneum

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Synopsis

A study of human thin skin stratum corneum was made using small-angle x-ray diffraction. This study elucidated how a new type of skin softener, 2-(alkoyloxy)-1-[(alkoyloxy)methyl]-ethyl-7-(4 heptyl-5,6-dicarboxy-2-cyclohexene-l-yl) heptanoate (G2), interacts with the stratum corneum. A broad diffraction peak from 50–80 Å, assigned to the lipid content of the stratum corneum, was characteristic of untreated normal stratum corneum. Washing of the specimen with a 0.1% soap solution removed the 50–80 Å peak, while treatment with G2 caused the occurrence of a diffuse diffraction peak from 30–45 Å for both unwashed and prewashed stratum corneum. Treatment with a triglyceride oil gave no change in the diffraction pattern. It appears obvious that G2 has a penetrative action on stratum corneum that is not present for unmodified triglycerides.

INTRODUCTION

Small-angle x-ray diffraction studies to determine the structure of human stratum corneum were first carried out by Swanbeck in the late 1950s (1,2). This landmark work provided a model that depicted the stratum corneum as being composed of 250 Å protein bundles surrounded by 80 Å thick lipid layers. Since this study, small-angle x-rays have been used by other investigators to examine the stratum corneum and the epidermal lipid it contains (3,4).

These studies have mainly used stratum corneum samples obtained from thick skin. However, as has been demonstrated (5), the barrier function and physical properties of thick skin stratum corneum are significantly different from those of thin skin stratum corneum. Since thin skin covers most of the body, especially those parts targeted by cosmetics, investigation of the properties of its stratum corneum are of obvious significance. In addition, it is reasonable that information gained from investigations on thick skin may not be totally applicable to the thin skin counterpart, since thin skin provides a much greater barrier function through its different structure.

Earlier diffraction studies concentrated on structural elucidation of the stratum corneum for both healthy skin and diagnosed dermatological conditions. However, there is no reported use of low-angle x-ray diffraction to study the effects of external treatments on the stratum corneum. Even the effect of washing upon the epidermal lipids has not been studied, despite the use of thorough washing with Ivory® soap by sebum donors two hours prior to collection (6). It was such experiments by Downing and co-workers

(6) that produced much of the current knowledge concerning the composition of human sebum.

Xerosis, the condition commonly referred to as dry skin, is a condition that is considered almost universal among the aged (7). We found the potential for a direct investigation of a cosmetic treatment on stratum corneum of great appeal and used small-angle x-ray diffraction techniques to investigate the effects of washing, treatment with G2, and ether extraction upon the stratum corneum of human thin skin samples.

EXPERIMENTAL

Fresh full thickness samples of human skin were obtained from radical mastectomy cases and prepared by a method described by Lampe *et al.* (8). The skin samples were from normal skin well away from the tumor site. After removal of the subcutaneous fat with a #22 blade, each sample was placed dermis side down on filter paper soaked with 0.5% trypsin in phosphate-buffered saline (PBS). The samples were then incubated while refrigerated for 24 hours prior to removal of the stratum corneum. Sheets of stratum corneum were then thoroughly rinsed with PBS and frozen on filter paper after being blotted dry. Immediately prior to x-ray analysis, the samples were thawed and vortexed in PBS to remove any granular cells or filter paper residue.

The samples, taken from the same skin specimen, were then prepared for low-angle x-ray measurements by one or more of the following techniques. "Washed" samples were vortexed in a 0.1% (by weight) Ivory® soap solution for ten minutes. Samples "treated with G2" were spread on filter paper soaked with purified G2 and allowed to remain in contact with G2 for one to thirteen hours. "Extracted" samples were vortexed in ethyl ether for 30 minutes. The prepared stratum corneum sheets were then rolled and placed in a 0.7-mm glass capillary tube and examined by small-angle x-ray diffraction. The untreated stratum corneum band was checked by multiple measurements of skin specimens from three different subjects, while the treated, washed, and extracted samples were determined by duplicate measurements from the same skin specimen. Only slight differences in intensity were encountered between spectra of the multiple runs.

G2 was purified as described previously (9). Trypsin (98%) and dry phosphate-buffered saline (diagnostic) were obtained from Sigma Chemical. Absolute ethyl ether (Aldrich, Reagent) was used for extraction. All glassware was thoroughly cleaned and ultimately rinsed with ether to eliminate the possibility of external lipid contamination.

Small-angle x-ray diffraction spectra were collected for seven hours using a Kiessig low-angle camera from Richard Siefert. Ni-filtered Cu radiation was used and the reflections determined by a Tennelec position-sensitive detection system (Model PSD-1100).

RESULTS

Normal stratum corneum gave a characteristic broad diffraction peak of moderate intensity in the range of 50–80 Å (Figure 1). No other bands were distinguishable over the complete available range of 20–160 Å for collection times of twelve hours. This 50–80 Å band was removed both by washing and by extraction with ether as seen in Figure 2.

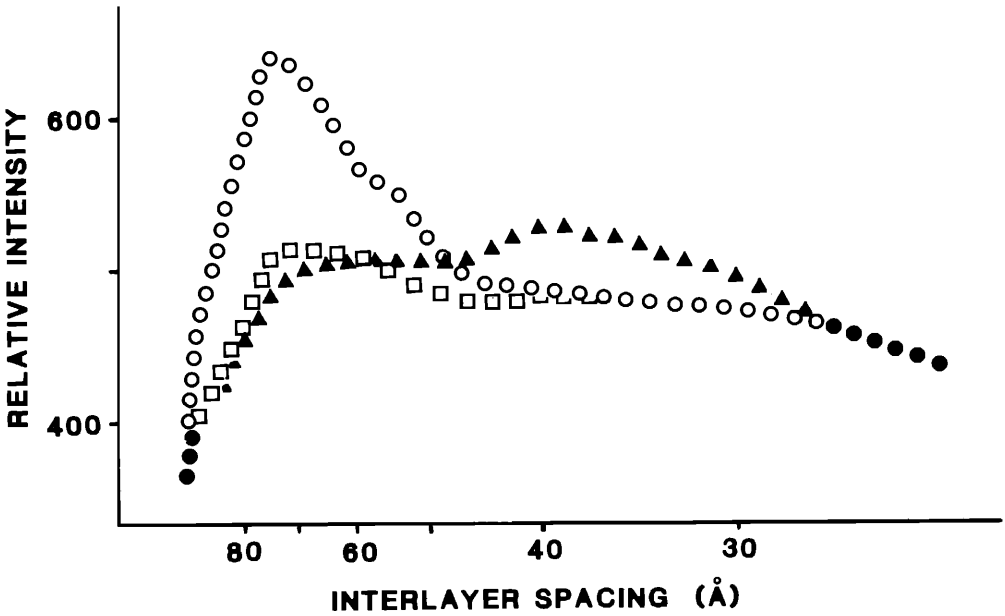

Figure 1. Effect of G2 on x-ray diffraction spectrum of washed skin. ○, untreated normal stratum corneum; □, washed normal stratum corneum; ▲, washed normal stratum corneum treated with G2 for one hour; ●, region of complete spectral overlap.

Treatment with G2 produced a weak diffraction from 30–45 Å. The intensity of this diffraction increased slightly as the treatment duration increased from one hour to thirteen hours (Figure 3), while the intensity of the 50–80 Å band significantly decreased for the same change in treatment duration. Treatment of the stratum corneum with soy oil for one hour produced a narrower, slightly shifted diffraction peak from 60–80 Å. This spectrum is compared to the normal stratum corneum pattern in Figure 4. Neat G2 produced no diffraction pattern.

When washed stratum corneum was treated with G2, the spectrum from 45 to 90 Å was indistinguishable from the pattern of washed stratum corneum (Figure 1), while the broad G2 peak from 30–45 Å was still present. By washing the stratum corneum after a one-hour treatment with G2, a spectrum indistinguishable from that of washed normal stratum corneum resulted for the entire 20–90 Å range.

DISCUSSION

The 50–80 Å band is due to lipid structures contained within the stratum corneum. This is based on similarities between the 50–80 Å thin skin band found in the present investigation and Swanbeck's 40–60 Å thick skin band (1). The removal of the band by ether extraction (Figure 2) supports the conclusion of the presence in both thin and thick skin of a lipid layer giving an interlayer spacing of the magnitude of 60 Å. The enhanced intensity at spacings in excess of 80 Å for the ether-extracted sample was not

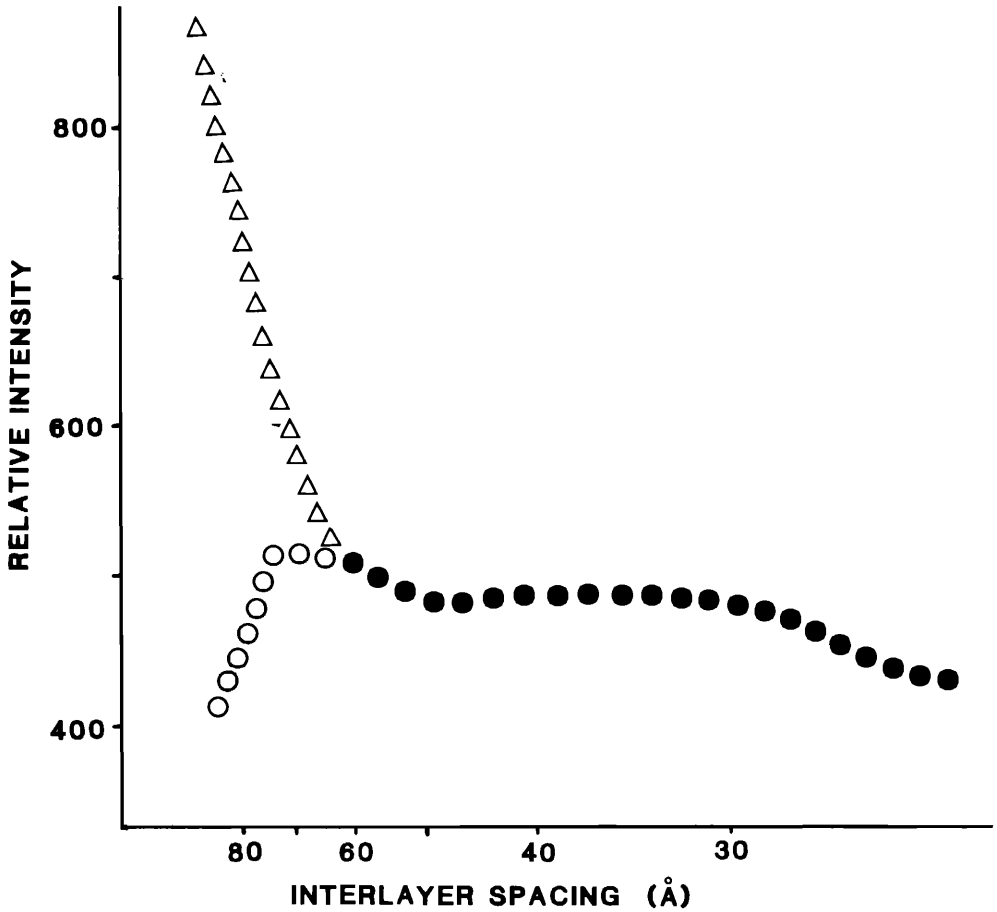

Figure 2. Comparison of x-ray diffraction spectra of washed and ether-extracted skin. △, normal stratum corneum extracted for 30 minutes with ether; ○, washed normal stratum corneum; ●, region of complete spectral overlap.

assigned with certainty. Tentatively, we interpret it as due to cavities formed in the structure by the extraction but are aware that other plausible explanations may exist.

With this interpretation of the prominent feature of normal stratum corneum, the results are readily interpreted. First, washing of the separated stratum corneum removed epidermal lipids. From Figure 2, it can be seen that washing does not have as dramatic an effect on the stratum corneum as does extraction; however, the effect on the 50–80 Å lipid region is virtually the same.

The results gave essential information on the site of G2 in the stratum corneum. From the samples treated with G2 (Figure 3), it is evident that G2 interacts with and changes the structure of epidermal lipids. This is a relatively slow process continuing for several hours. Figure 3 reveals the trend of the change after one hour and also that the changes continue for a long time after that period. It is interesting to notice that the G2 dominated the spacings of the lipids. From earlier work on G2 (10), it was found that when G2 was added to a model skin-surface lipid mixture contained in a

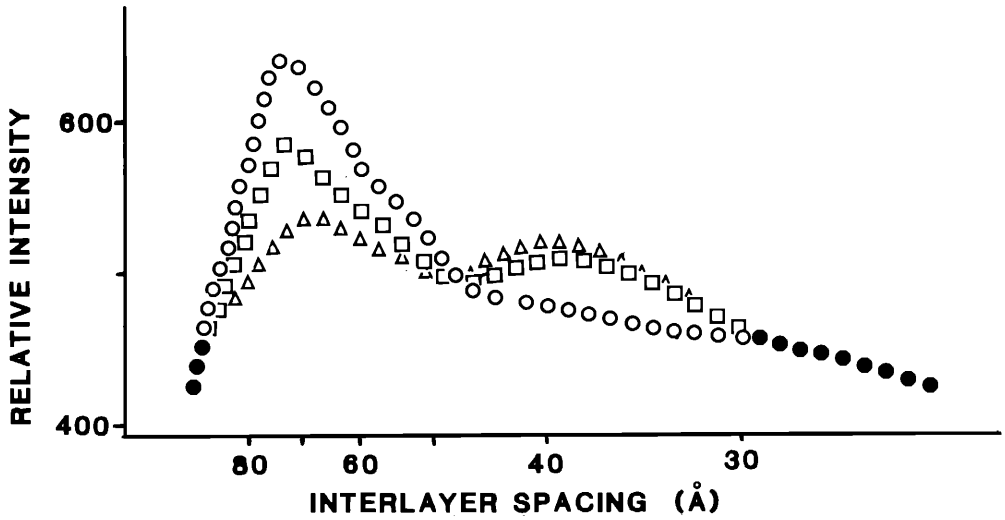

Figure 3. Effect of G2 with time on x-ray diffraction spectrum of skin. ○, untreated normal stratum corneum; □, normal stratum corneum treated with G2 for one hour; △, normal stratum corneum treated with G2 for 13 hours ●, regions of complete spectral overlap.

lamellar liquid crystal, the G2 dominated the interlayer spacing, giving a characteristic value of 45 Å. This effect was attributed to the ability of G2 to accomplish extremely good packing within the lamellar liquid crystal structure of a lipid mixture modeled exactly after the blend found in skin lipids (6,11,12).

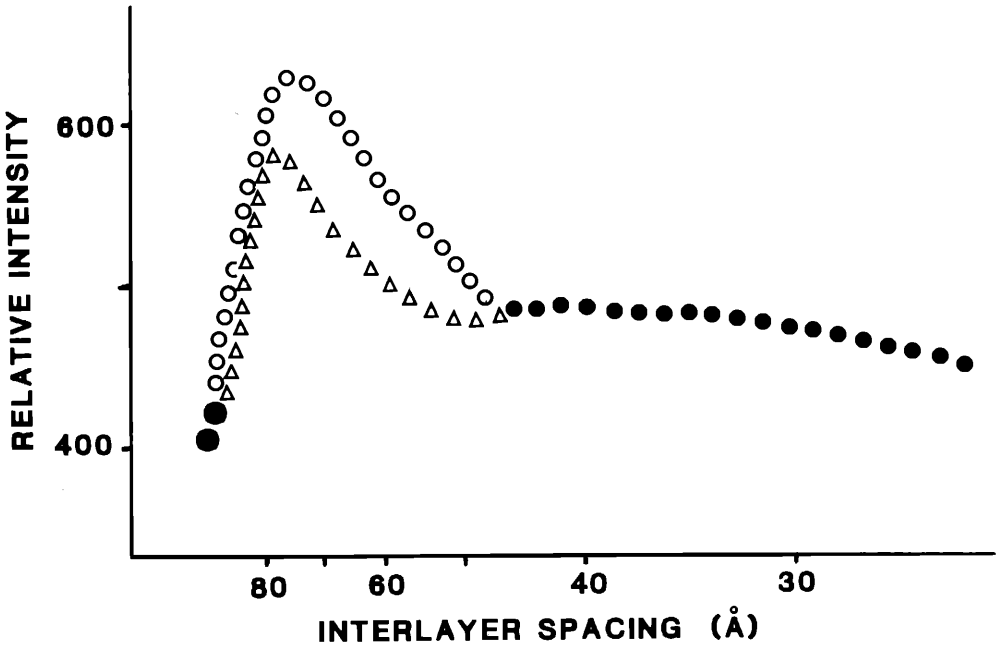

Figure 4. Effect of soybean oil on x-ray diffraction spectrum of skin. ○, normal stratum corneum; △, normal stratum corneum treated for one hour with soybean oil; ●, region of complete spectral overlap.

It is essential to realize the difference between the influence on skin lipids by a fatty oil (a liquid triglyceride such as soybean oil) and by G2. Stratum corneum treated with soybean oil (Figure 4) showed little difference in lipid interlayer spacing, while treatment with G2 caused a new band to appear at 30–45 Å. Hence, the comparison of soybean oil-treated stratum corneum with G2-treated stratum corneum demonstrates that the 30–45 Å band is neither the result of G2's triglyceride structure nor trace impurities remaining in the purified G2.

From the studies of G2 treatment on washed stratum corneum, we find that G2 can interact with the stratum corneum even after the bulk of the lipid has been removed by washing. This is concluded after comparison of the 30–45 Å band for G2-treated stratum corneum with the 30–45 Å band of G2-treated stratum corneum after washing (Figure 1). It is also shown that most of the lipid structural change caused by G2 is removed with the lipids during washing, since the spectrum for stratum corneum washed after treatment was identical to washed untreated stratum corneum.

SUMMARY

From the small-angle x-ray diffraction study of human thin skin stratum corneum, it is proposed that the broad diffraction band from 50–80 Å is due to epidermal lipids. Whereas washing removes epidermal lipids, treatment with G2 changes the packing structure of epidermal lipids. It is proposed that this interaction is the primary source of the skin-softening properties of G2. This interaction can occur even after removal of epidermal lipids by washing; however, when the stratum corneum is washed after treatment with G2, the characteristic x-ray reflection caused by addition of G2 was removed.

REFERENCES

(1) G. Swanbeck, Macromolecular organization of epidermal keratin, *Acta Dermato-Venerelogica,* 39, 1–37 (1959).
(2) G. Swanbeck and Nils Thyresson, A study of the state of aggregation of the lipids in normal and psoriatic horny layer, *Acta Dermato-Venerelogica,* 42, 445–457 (1962).
(3) L. A. Goldsmith and H. P. Baden, Uniquely oriented epidermal lipid, *Nature,* 225, 1052–1053 (1970).
(4) G. L. Wilkes, An-Lac Nguyen, and R. Wildnaver, Structure-property relations of human and neonatal rat stratum corneum. I. Thermal stability of the crystalline lipid structure as studied by x-ray diffraction and differential thermal analysis, *Biochimica et Bio-Physica Acta,* 304, 267–275 (1973).
(5) A. M. Kligman, "The Biology of the Stratum Corneum," in *The Epidermis,* W. Montagna and W. C. Lobitz, Jr., Eds. (Academic Press, New York, 1964), pp 387–433.
(6) D. T. Downing, J. S. Strauss, and P. E. Pochi, Variability in the chemical composition of human skin surface lipids, *J. Invest. Dermatol.,* 53, 322 (1969).
(7) B. A. Gilchrest, *Skin and Aging Processes* (CRC Press, Inc., Boca Raton, Florida, 1984), p 39.
(8) M. A. Lampe, M. L. Williams, and P. M. Elias, Human epidermal lipids: Characterization and modulations during differentiation, *J. Lipids Res.,* 24, 131–140 (1983).
(9) S. E. Friberg and D. W. Osborne, A lamellar liquid crystal as an in situ surface balance. II. The conformation of a glyceridacid, *Colloids and Surfaces* (in press).
(10) D. W. Osborne, Ph.D. dissertation (under preparation).
(11) E. Haahti, Major lipid constituents of human skin surface with special reference to gas-chromatographic methods., *Scand. J. Clin. Lab. Invest.,* 13 (Suppl. 59), 1–108 (1961).
(12) H. J. O'Neil, L. L. Gershbein, and R. G. Scholz, Identification of pristance in human sebum and related lipid sources, *Biochem. Biophysical Res. Comm.,* 35, 946–952 (1969).