10. T. A. Smith and C. Sinclair, *Ann. Bot.* **31**, 103 (1967); A. Priebe, H. Klein, H.-J. Jäger, *J. Exp. Bot.* **29**, 1045 (1978).
11. D. Le Rudulier, G. Goas, *C. R. Acad. Sci.* **273**, 1108 (1971).
12. G. F. Munro, R. A. Miller, C. A. Bell, E. L. Verderber, *Biochim. Biophys. Acta* **411**, 263 (1975); J. W. Perry and T. Oka, *ibid.* **629**, 24 (1980); G. F. Munro, K. Hercules, J. Morgan, W. Sauerbier, *J. Biol. Chem.* **247**, 1272 (1972).
13. A. Speranza and N. Bagni, *Z. Pflanzenphysiol.* **81**, 226 (1977); M. J. Montague, T. A. Armstong, E. G. Jaworski, *Plant Physiol.* **63**, 341 (1979); Y. M. Heimer, Y. Mizrahi, U. Bachrach, *FEBS Lett.* **104**, 146 (1979); V. R. Villanueva, R. C. Adlakha, R. Calvayrac, *Phytochemistry* **19**, 787 (1980); R. Kaur-Sawhney, L.-M Shih, A. W. Galston, *Plant Physiol.* **69**, 411 (1982).
14. R. Kaur-Sawhney, L.-M. Shih, H. E. Flores, A. W. Galston, *Plant Physiol.* **69**, 405 (1982).
15. T. A. Smith, *Phytochemisty* **2**, 241 (1963); T. A. Smith, *ibid.* **18**, 1447 (1979).
16. M. R. Suresh, S. Ramakrishna, P. R. Adiga, *ibid.* **17**, 57 (1978).
17. A. Kallio, P. P. McCann, P. Bey, *Biochemistry* **20**, 3163 (1981).
18. B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. L. Casara, J. P. Vevert, *J. Am. Chem. Soc.* **100**, 2551 (1978).
19. A. Kallio and P. P. McCann, *Biochem. J.* **200**, 69 (1981).
20. R. Kaur-Sawhney, personal communication.
21. Y.-R. Dai and A. W. Galston, *Plant Physiol.* **67**, 266 (1981).
22. O. Heby, *Differentiation* **19**, 1 (1981).
23. M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
24. We thank P. P. McCann and W. L. Albrecht (Merrell Research Center, Cincinnati, Ohio) for providing the samples of DFMA and DFMO. We thank B. B. Stowe, M. H. M. Goldsmith, S. C. Maheshwari, R. Kaur-Sawhney, J. Gaynor, and N. Young for reviewing the manuscript. Study supported by grants from NASA and the Binational Agricultural Research and Development Foundation to A.W.G. and an American Cancer Society Summer Fellowship to H.E.F.

21 April 1982; revised 8 June 1982

Glycolipids in Mammalian Epidermis: Structure and Function in the Water Barrier

Abstract. *In the epidermis of terrestrial vertebrates, lipid lamellae between the horny cells are thought to form a barrier to water loss. The lipids are extruded from living cells after assembly in lamellar granules. This assembly might be promoted by recently identified 1-(3'-*O*-acyl)-β-D-glucosyl-*N*-(ω-hydroxyacyl)sphingosines, which have 30- and 32-carbon hydroxy acids as amides and linoleic acid esterified to glucose. Such a role for these molecules could explain the effects of essential fatty acid deficiency, in which the lamellar granules fail to assemble and the barrier to water diffusion is lost.*

The thin horny layer of terrestrial vertebrate skin forms an efficient barrier to water loss (*1*), which is greatly impaired by extraction with polar solvents (*2*). In mammals, birds, and reptiles, the intercellular spaces in the horny layer contain multiple membranous sheets (*3*). These appear to be derived from lamellar granules discharged from the uppermost living epidermal cells after the granules have accumulated during cell progression from the germinative basal layer toward the horny layer (*4*). In animals deprived of linoleic acid, the skin becomes scaly and more permeable to water (*5*), the lamellar granules appear empty, and the horny layer is deficient in intercellular membranes (*6*). A mechanism for this effect of essential fatty acid deficiency has not been explained.

The dead epidermal horny layer of mammals, birds, and reptiles contains ganglioside sulfates, ceramides, cholesterol, cholesteryl sulfate, and free fatty acids, but no phospholipids (*7, 8*). The living epidermal cells also contain these lipids, as well as phospholipids and several series of glucosylceramides (*8*). In mammalian epidermis, the major and least polar glucosylceramide was reported to have fatty acids esterified in the 3-position of glucose, and the amide-linked fatty acid was said to contain 35 carbon atoms and have two hydroxyl groups and two double bonds somewhere near the middle of the molecule (*9*). The esterified acids contained a high proportion of linoleic acid, ranging from 34 percent in neonatal mice to 56 percent in human skin and 77 percent in pig epidermis.

We have investigated the acylglucosylceramides from pig epidermis and found (*10*) amide-linked ω-hydroxy acids having mainly saturated and monounsaturated chains of 30 and 32 carbon atoms, respectively. Linoleic acid comprised 74 percent of the esterified acids. The dimensions of such molecules (Fig. 1) would allow the hydroxy acid chains to extend across both hydrocarbon palisades of a fluid bimolecular lipid membrane, with the hydroxyl group anchored in one polar surface and the glucose portion in the other surface. Furthermore, the fatty acids esterified to the glucose should be capable of extending into the hydrocarbon region of a second membrane, holding it in close apposition. This could constitute the mechanism by which multiple layers of intracellular membrane are assembled for inclusion in lamellar granules (Fig. 2). A deficiency of linoleic acid might result in synthesis of insufficient or inappropriate acylglucosylceramides, resulting in the observed nonassembly of lamellar granules during essential fatty acid deficiency (*6*).

Assembly of the lamellar granules appears to take place in the endoplasmic reticulum and Golgi regions (*11*). From the known compartmentalization of lipid synthesis (*12*), it can be inferred that the acylglucosylceramides are also produced in these regions. Thus, while the synthesis of 16-carbon fatty acids is accomplished by soluble enzymes in the cytoplasm, further extensions in chain length are mediated by enzymes bound in the endoplasmic reticulum. Such chain extension could continue, so that the growing chains progressed across the entire lipid region of the endoplasmic membrane until, at a length of 30 carbon atoms, the methyl group of the fatty chain would emerge into the endoplasmic space, there to be hydroxylated by a microsomal oxidase (*13*). Once thus anchored in the membrane, incorporation of the hydroxy acid into glucosylceramide would most likely occur in situ. Subsequent attachment of linoleic acid to the glucose portion could provide attraction for adjacent folds of membrane, resulting in the observed stacking of disk-shaped sections of membrane in cisternae (*11*).

Lipids with the molecular dimensions of the acylglucosylceramides should not be required for maintaining the extracellular sheets of lipid membranes once they are formed, since similar membrane structures exist in myelin and in liposomes prepared in vitro from myelin lipids or isolated phospholipids (*14*), all of which lack such molecules. We suggest that the function of the linoleic acid–containing structures is limited to aggregation of the disks of membrane that are stacked in the lamellar granules. These remain coherent, even though they are no longer enclosed in a bounding membrane, for some time after extrusion from the granular cells. Reorganization of the disks into intercellular sheets could depend on the observed disappearance of the acylglucosylceramides. Adequate polarity for the maintenance of the intercellular lamellae would then be provided by the ganglioside sulfates, which together with ceramides, cholesterol, cholesteryl sulfate, and free fatty acids,

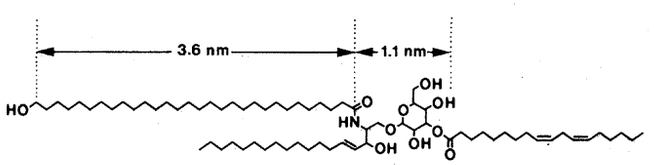

Fig. 1. Structure of the major acylglucosylceramide from pig epidermis, showing the dimensions of the molecule.

0036-8075/82/0924-1261$01.00/0

constitute the horny layer lipids (*7, 8*).

Lamellar granules are present in the epidermal cells of mammals, birds, and reptiles, but not of amphibians or fish (*15*). Measurements of the lamellar disks showed a uniform spacing of 9.7 nm in all of the terrestrial vertebrates (*15*). This spacing was said to indicate that the disks consist of not one, but two, bimolecular leaflets, as would be produced by flattening of a liposome. However, since the angle at which the disks were sectioned would have been random, the average angle would have been close to 45°, and the apparent spacing of 9.7 nm would indicate a true thickness of about 7 nm. From this must also be subtracted an unknown factor to account for swelling due to insertion of the heavy atoms used for visualization of the structures, leaving a spacing that would be more in accordance with a single leaflet. If, nevertheless, the flattened liposome model proves to be accurate, the acylglucosylceramide molecules might provide the impetus for apposition of the internal polar surfaces of a precursor liposome.

The fatty chains in the horny layer lipids are predominantly saturated, with some monounsaturation. This should result in the formation of membranes in which the hydrocarbon regions are in the close-packed crystalline state rather than the fluid, liquid-crystalline state characteristic of biological membranes (*16*). In an electron diffraction study of isolated horny layer (*17*), the periodicities of 0.415, 4.25, 4.96, and 13.40 nm that were observed were abolished by solvent extraction. The first three would correspond, respectively, to the spacing between close-packed hydrocarbon chains, the thickness of the lipid regions, and the overall repeat distance between the polar regions of tightly packed membranes. The reported reflection patterns would be explained by multiple lipid membranes lying parallel to the surface between the horny cells, an arrangement that should serve effectively as a barrier to water permeation.

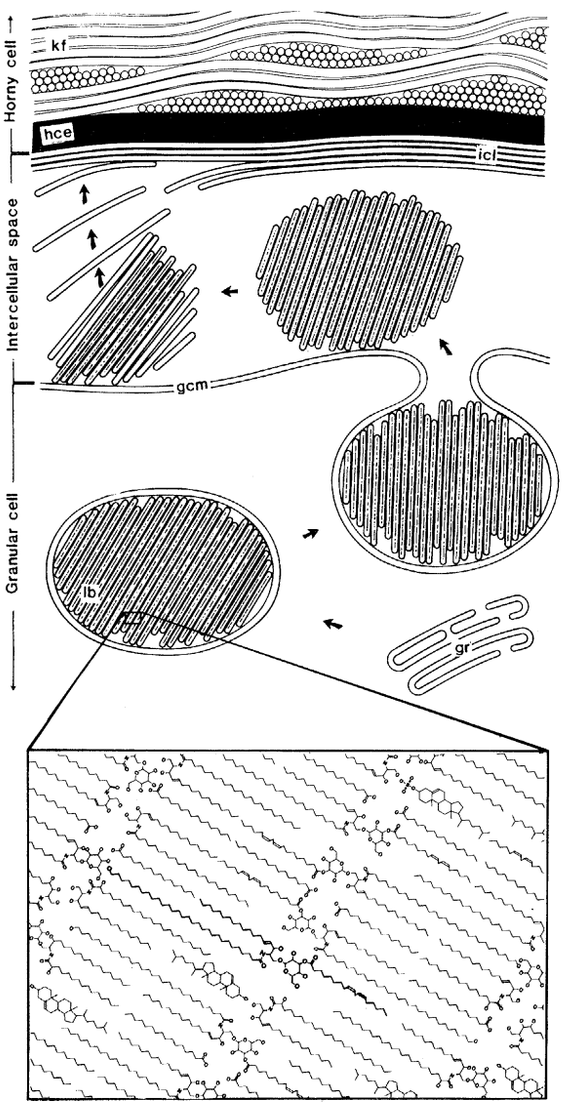

Fig. 2. A diagrammatic representation of events in the formation of the epidermal water barrier as seen in electron micrographs of thin sections stained with osmium (*3, 4*). The process includes assembly of a lamellar body (*lb*) from the Golgi region (*gr*) in an epidermal granular cell bounded by the granular cell membrane (*gcm*), subsequent expulsion of the granule into the intercellular space, and its rearrangement into the intercellular lamellae (*icl*) that lie parallel with the horny cell envelope (*hce*) and the keratin filaments (*kf*) of the horny cell. The expanded insert shows the proposed arrangement of the acylglucosylceramide molecules (bold) within multiple bimolecular lipid leaflets formed from lipids known to be present in the epidermal granular cells. For clarity, hydrogen atoms are omitted from the lipid structures. In essential fatty acid deficiency, the lamellar granules appear empty and the intercellular lamellae are largely absent (*6*), indicating that deficiency of a linoleic acid–containing molecule prevents assembly of the lamellar disks into granules.

PHILIP W. WERTZ
DONALD T. DOWNING
Marshall Dermatology Research Laboratories, Department of Dermatology, University of Iowa College of Medicine, Iowa City 52242

References and Notes

1. R. J. Scheuplein, *J. Invest. Dermatol.* **55**, 31 (1976); N. F. Hadley, *Am. Sci.* **68**, 546 (1980); P. F. A. Maderson, A. H. Zucker, S. I. Roth, *J. Exp. Zool.* **204**, 11 (1978).
2. A. G. Matoltsy, A. M. Downes, T. M. Sweeney, *J. Invest. Dermatol.* **50**, 19 (1968); T. M. Sweeney and D. T. Downing, *ibid.* **55**, 135 (1970).
3. A. S. Breathnack, T. Goodman, C. Stolinski, M. Gross, *J. Anat.* **114**, 65 (1973); P. M. Elias and D. S. Friend, *J. Cell Biol.* **65**, 180 (1975); R. M. Lavker, *J. Ultrastruct. Res.* **55**, 79 (1976); A. G. Matoltsy and J. A. Bednarz, *ibid.* **53**, 128 (1975); J. V. Frei and H. Sheldon, *J. Biophys. Biochem. Cytol.* **11**, 719 (1961); A. I. Farbman, *J. Cell Biol.* **21**, 491 (1964); L. Frithiof and J. Wersall, *J. Ultrastruct. Res.* **12**, 371 (1965); A. G. Matoltsy and P. F. Parakkal, *J. Cell Biol.* **24**, 297 (1965); I. R. Martinez and A. Peters, *Am. J. Anat.* **130**, 93 (1971); A. G. Matoltsy, *J. Ultrastruct. Res.* **29**, 438 (1969).
4. A. G. Matoltsy, *J. Invest. Dermatol.* **67**, 20 (1976).
5. R. T. Holman, in *Progress in the Chemistry of Fats and Other Lipids*, R. T. Holman, Ed. (Pergamon, Oxford, 1968), vol. 9, pp. 275–348; C. Prottey, *Br. J. Dermatol.* **94**, 579 (1976).
6. P. M. Elias and B. E. Brown, *Lab. Invest.* **39**, 574 (1978).
7. E. Leikola, E. Nieminen, A.-M. Teppo, *J. Lipid Res.* **10**, 440 (1969); *ibid.* **11**, 306 (1970).
8. G. M. Gray and H. J. Yardley, *ibid.* **16**, 441 (1975); H. J. Yardley and R. Summerly, *Pharmacol. Ther.* **13**, 357 (1981); C. S. Birkby, P. W. Wertz, D. T. Downing, *Comp. Biochem. Physiol.*, in press.
9. G. M. Gray, R. J. White, J. R. Majer, *Biochim. Biophys. Acta* **528**, 127 (1978).
10. The full thickness epidermis was separated from fresh pig skin by heat treatment and the lipids were extracted with a mixture of chloroform and methanol (2:1). The least polar of the glucosylceramide fractions was isolated by thin layer chromatography and subjected to mild alkaline hydrolysis to remove the esterified acids. The amide-linked fatty acids were freed by hydrolysis with 1*N* HCl in aqueous methanol, and their structures were determined by chromatographic, chemical, and spectroscopic means in comparison with similar acids isolated from carnauba wax [D. T. Downing, Z. H. Kranz, K. E. Murray, *Aust. J. Chem.* **14**, 619 (1961)]. The methyl esters of the esterified acids were fractionated on $AgNO_3$, and the positions of unsaturation in the C_{18} dienoic acid were determined by the method of D. T. Downing and R. S. Greene [*Lipids* **3**, 96 (1967)]. Details of the isolation and structure determination of the acylglucosylceramides will be published.
11. K. Wolff and E. C. Wolff-Schreiner, *J. Invest. Dermatol.* **67**, 39 (1976).
12. L. M. G. Van Golde and S. Van den Bergh, in *Lipid Metabolism in Mammals*, F. Snyder, Ed. (Plenum, New York, 1977), vol. 1, pp. 1–33; M. Gan-Elepano, E. Aeberhard, J. F. Mead, *Lipids* **16**, 790 (1981).
13. A. J. Fulco, *Annu. Rev. Biochem.* **43**, 215 (1974).
14. L. L. M. van Deenan, in *Progress in the Chemistry of Fats and Other Lipids*, R. T. Holman, Ed. (Pergamon, New York, 1965), vol. 8, pp. 1–127; W. Stoeckenius, in *The Interpretation of Ultrastructure*, R. J. C. Harris, Ed. (Academic Press, New York, 1962), vol. 1, p. 349.
15. L. Landmann, *J. Ultrastruct. Res.* **72**, 245 (1980).
16. S. J. Singer and G. L. Nicolson, *Science* **175**, 720 (1972).
17. L. A. Goldsmith and H. P. Baden, *Nature (London)* **225**, 1052 (1970).

18. Supported in part by grants from the U.S. Public Health Service (AM 22083) and by Richardson-Vicks, Inc., Wilton, Conn.

21 June 1982