1 Pharmacology and the Skin
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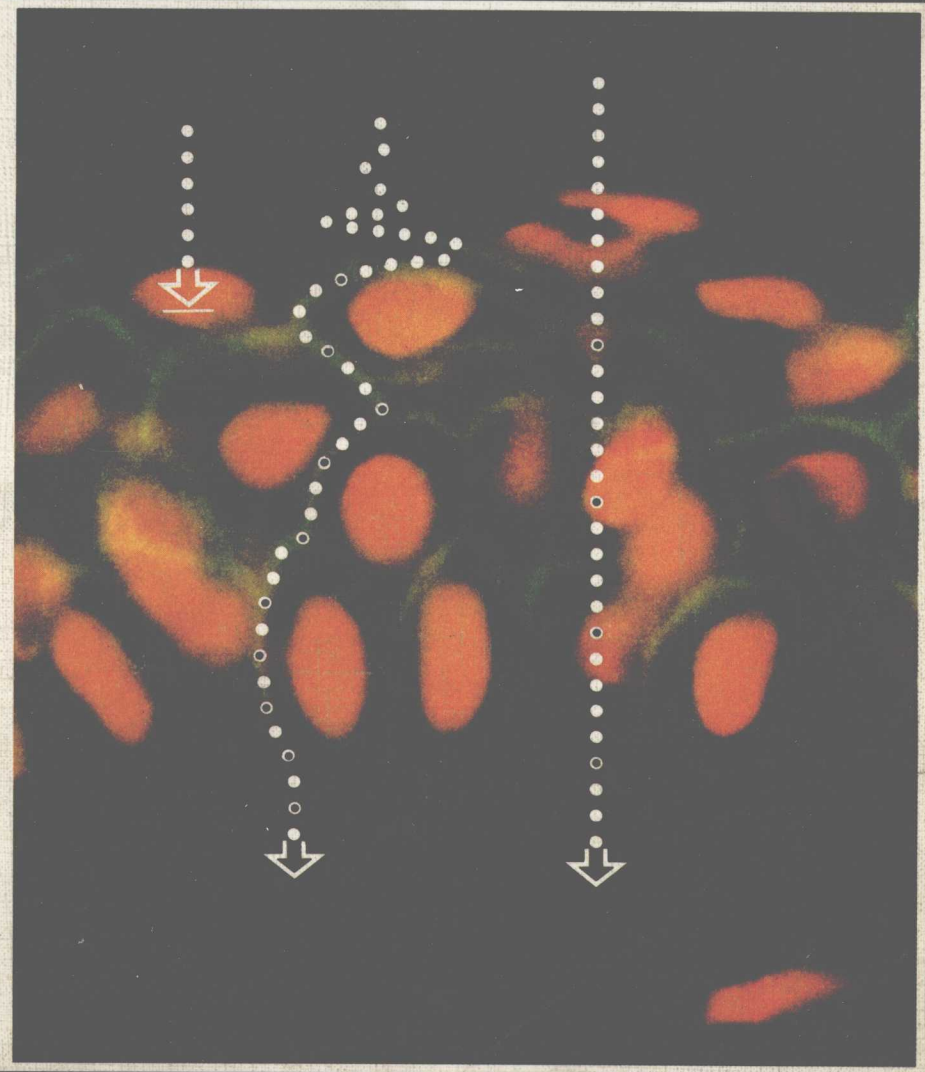

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Skin Pharmacokinetics

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The Stratum Corneum Two-Compartment Model and Its Functional Implications

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Evidence for the Two-Compartment Model

Appreciation that the stratum corneum should be accorded more respect than a sheet of plastic wrap comes from a raft of observations made over the past few years [1–3] (table I). Although the importance of lipids for both barrier function [4] and the water-retentive properties [5] of the stratum corneum was well appreciated, Middleton [6] first noted that it was the organization of lipid into 'shells' that accounted for water retention. Soon thereafter, the existence of separate hydrophilic and hydrophobic pathways was suggested from physical-chemical observations [7]. Morphologic evidence for lipid-protein segregation came soon thereafter. First, freeze-fracture and thin section studies demonstrated lipid bilayers exclusively in the stratum corneum interstices, with the absence of lipid structures within the corneocyte cytosol [8–10]. Next, histochemical and cytochemical studies clearly displayed the process of lipid sequestration to membrane domains [10, 11]. Why these lipid domains had not been demonstrated previously, reflects the predominance of nonpolar lipids in these domains, and therefore the ease which they are extracted during routine microscopic processing [10]. The localization of lipids to intercellular domains can be further deduced from the ease with which certain lipid solvents disperse this tissue into cell suspension [11, 12], and the further ability of such solvent extracts to recombine with dispersed cells to produce a functionally competent tissue [12]. The first direct evidence for lipid localization to intercellular domains was provided by biochemical analysis of membrane couplets, which were prepared without loss of intercellular

Table I. Evidence for the stratum corneum two-compartment model

1 Physicochemical evidence for two pathways of transport of lipid and water-soluble molecules
2 Freeze-fracture morphology
3 Histochemistry and fluorescence staining of lipids in frozen sections
4 Dispersion by lipid solvents
5 Co-localization of lipid catabolic enzymes
6 Membrane isolation and characterization, including X-ray diffraction

lipid [13]. These preparations not only contained multiple bilayers, identical to intact stratum corneum, but they also were lipid-enriched, accounting for over 80% of all the lipid in the stratum corneum [13]. Moreover, they displayed both the same lipid distribution of whole stratum corneum [13], and duplicated the x-ray diffraction pattern previously ascribed to the 'shell' of lipids, previously thought to fill interfilamentous domains within the corneocyte cytosol [14]. Finally, one can assume that the localization of a variety of lipid catabolic enzymes (steroid sulfatase [15]; lipase [16]; sphingomyelinase [16]; phospholipases [16]) to stratum corneum intercellular domains represents co-localization of hydrolytic enzymes with their respective lipid substrates in stratum corneum membrane domains.

Formation of the Two-Compartment Model

It is now well appreciated that the two-compartment system is formed by the deposition of epidermal lamellar body contents in intercellular domains at the granular-cornified cell interface [8, 9]. Although secretion of this organelle has long been linked to both barrier formation and desquamation [1–3], its role in both of these functions was solidified with the isolation of these organelles [18, 19]. Subsequent characterization of the lipid and enzymatic content of lamellar bodies has provided a clearer picture of the molecular events associated with the formation of the stratum corneum intercellular compartment. Lamellar bodies are enriched in phospholipids [19, 20], free sterols [19], and glycosphingolipids [19], including certain distinctive acylglycosphingolipid species [21] that may be responssible for the disc-like bilayers that appear in lamellar bodies [22]. Although long suspected of being modified lysosomes due to cytochemical evidence of hydrolytic enzyme contents [1–3, 23], biochemical studies revealed a lim-

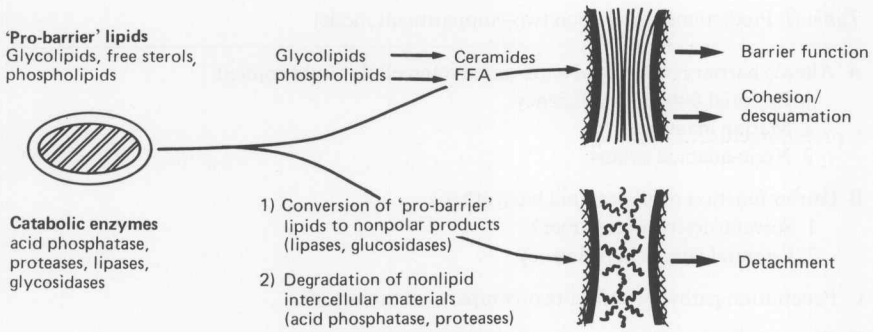

Fig. 1. Dual functions of the epidermal lamellar body. Summary of lipid biochemical and enzyme biochemical studies on isolated epidermal lamellar bodies [20]. Lamellar bodies contain free sterols and polar lipid precursors of stratum corneum lipids, and their lipid catabolic enzymes (lipases, glucosidases) presumably mediate this conversion after secretion. The other nonlipolytic enzymes (acid phosphatase, proteases) may participate in desquamation.

ited array of hydrolases [16, 17, 20, 24, 25], but a striking absence of certain typical lysosomal enzymes, including β-glucuronidase, galactosidase, and arylsulfatases A and B [20, 25]. The lipid catabolic enzymes that have been demonstrated in lamellar bodies seem ideally suited to bring about the transformation of the polar lipid contents of lamellar bodies to the nonpolar species that eventuate in the stratum corneum (fig. 1). However, the regulation, timing, and location of these degradative events is still not certain. If all phospholipid and glycolipid species are completely absent from the stratum corneum, as claimed in some studies [26, 28], then degradation must occur very soon after secretion. Yet, others find some glycolipids and phospholipids in the lower stratum corneum [11, 17, 29], suggesting that lipid transformations in the intercellular spaces may be a more gradual process.

In addition to lipid catabolic enzymes, the lamellar body is also enriched in acid phosphatase [20, 24] and proteases [20]. Whether these enzymes participate in barrier formation is unclear at present, but it is more likely that they participate in desquamation, presumably by degrading extracellular glycoproteins, desmosomes, etc. (fig. 1). The recent demonstration of abnormal lipid contents of lamellar bodies in one disorder of cornification, suggests that not only the enzyme contents, but also disturbances in the bulk lipid content of these organelles could affect desquamation [30].

Table II. Predictions based upon two-compartment model

A Altered barrier is associated with varied intercellular lipid content
- 1 Essential fatty acid deficiency
- 2 Marine mammals
- 3 Xeric-adapted avians

B Barrier function regulates lipid biosynthesis
- 1 Solvent/detergent treatment
- 2 Essential fatty acid deficiency

C Penetration pathways reflect two-compartment structure

D Permeability is related to intercellular lipid content
- 1 Topographic variations
- 2 Solvent/detergent treatment
- 3 Marine mammals/avian models

E Desquamation is regulated by lipid content

Implications of the Two-Compartment Model

The two-compartment model has been amply tested in recent years. Of the predictions in table II, all have been established and exceptions have not been forthcoming. The importance of intercellular lipids for the barrier is underscored by the defective contents of lamellar bodies in essential fatty acid deficiency [31]. As a result of defective intercellular lipid, large water-soluble molecules traverse the stratum corneum interstices through once-impervious domains [31). That linoleic acid, presumably as an o-acyl ester within sphingolipids [32], is specifically corrective has been shown repeatedly. Moreover, prior conversion of linoleic acid to prostaglandins, with normalization of the high-turnover state, is not required [33–35].

The importance of intercellular lipids for barrier function is underscored by two recent, but separate bodies of work: Feingold et al. [36] have shown, first, that the epidermis is an important site of sterologenesis [36], and, second, that selective removal of intercellular, oil red O-stainable lipid from the stratum corneum with organic solvents provokes a compensatory burst in sterologenesis that returns to normal as the barrier abnormality corrects itself [37]. This dynamic approach has been extended to essential fatty acid deficient animals, who likewise demonstrate accelerated sterol synthesis in relation to barrier dysfunction [38]. The role of the barrier in regulating sterologenesis is underscored by localization of this burst in

synthesis solely to the epidermis, and specifically over those skin sites where barrier function is abrogated [37, 38]. Barrier function apparently regulates not only sterologenesis, but also fatty acid synthesis in the above models [39]. Hence, barrier requirements appear to regulate epidermal lipogenesis globally, rather than merely affecting specific epidermal lipid species.

The second group of experiments encompass comparative studies of lipid distribution and content in relation to barrier function in normal vs. xeric-adapted avians [40–42] and in nonfur-bearing marine mammals (cetaceans, manatees) [42, 43]. These studies can be summarized as follows: (a) intercellular lipid deposition is accelerated when avians are subjected to xeric stress, and their barrier function improves accordingly [40, 41], and (b) in marine mammals, intercellular stratum corneum lipid species remain more polar, and do not undergo the loss of polarity that characterizes terrestrial adaptation [42]. This difference presumably reflects the lesser barrier requirements of marine mammals. Moreover, the fatty acids on marine mammal lipids are shorter and more unsaturated than those on equivalent terrestrial species [43]. These results suggest that intercellular lipid location and composition in all terrestrial homeotherms subserve comparable functions, and are regulated by similar factors.

Based upon the two-compartment model, one also would predict that lipid-soluble agents, such as topical steroids, traverse intercellular domains. Although difficult to demonstrate morphologically, with the technique of in situ precipitation, lipid-soluble substances can be trapped at their penetration sites, and at least one such molecule has been localized to the intercellular pathway [44]. Moreover, after either solvent or detergent treatment, normally excluded, water-soluble agents gain entry to interstitial domains deep within the stratum corneum [12]. Moreover, site-specific variations in permeability have been shown to be related to differences in lipid content: neither the thickness nor the number of cell layers were contributory [45]. These latter studies provide rational explanations for two clinical problems: eczema, which occurs most readily on the palmar surfaces, has the lowest intrinsic lipid content, and hence is most vulnerable to further depletion from hot water, detergents, and solvents, while facial skin, which has the greatest lipid content, provides ready access to potent topical steroids – hence the all-too-common occurrence of atrophy after application of potent topical agents.

The two-compartment model also possesses vast implications for desquamation. That lamellar body-derived lipids regulate desquamation is supported by numerous studies linking abnormal desquamation to inher-

ited, lipid-metabolic disorders, and to drug-induced ichthyoses. Because the scope of this review is limited to the permeability barrier, the reader who desires further information about this subject is referred to several recent reviews [3, 46–47].

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Summary of Discussion

The following points were discussed with the participation of Drs. Bourke, Hadgraft, Gibson, Krueger, Pruniéras, Rougier, Busse, Schaefer and Ponec:

Intercellular lipid channels, which occupy more substantial volumes than generally assumed, are, at least in the mid and outer stratum corneum the main transport pathways involved in drug delivery. Whether this also holds true for the more compact lower layers remains to be proven by more refined techniques such as the use of soluble tracers and autoradiography.

Increased penetration of lipophilic drugs in the presence of DMSO or acetone may be caused by a transitory destruction of membranous structures.

There is no experimentally supported explanation for the abnormally rapid flux of some water-soluble substances such as glycerol. One explanation could be the existence of not only lipophilic, but also hydrophilic domains.

Experiments demonstrating the presence of catabolic enzymes in *all* layers of the stratum corneum have always been performed after *rehydration*. Whether these enzymes are thus active in the upper stratum corneum in vivo remains to be proven. Another problem is the availability of lipid substrates *in situ*.

Reports on the defective amount of lipids in atopic epidermis need further support from a comparative study of a statistically relevant number of normals and atopics (at least 100 each).

About 40% of epidermal linoleic acid is stored in the sphingolipids of the lamellar bodies. In animals receiving an essential fatty acid deficient diet, linoleic acid is replaced by oleic acid. This may explain the disappearance of disc structures in the lamellar bodies under EFA deficiency and their reappearance after oral linoleic acid intake. However, no correlation study has yet been done.

The heterogenicity of the stratum corneum impedes the transferability from the body site to the other of penetration data obtained by the application of Fick's law.

The flux of water molecules through the stratum corneum may be involved in the regulation of lipid biosynthesis. This possibility, however, needs further study.

Uwe Reichert