

Skin Lipid Structure and Barrier Function

By

**Linda D. Rhein, Ph.D.
Fairleigh Dickinson University
Teaneck, NJ
2005**

Outline

– General Background

- Structure and location of stratum corneum lipids & modification during differentiation
- Basis of macromolecular structure – free fatty acid & location of lipids within the structure

– Studies with the Model

- Effect of water and low relative humidity
- Effect of glycerine and other excipients

! Polymorphism of Lipid Structure

- Evidence of multiple phases
- Dependence on temperature, & on position within stratum corneum
- Altered polymorphism in disease states

! Concluding Remarks - Role of Polymorphism in Barrier Function

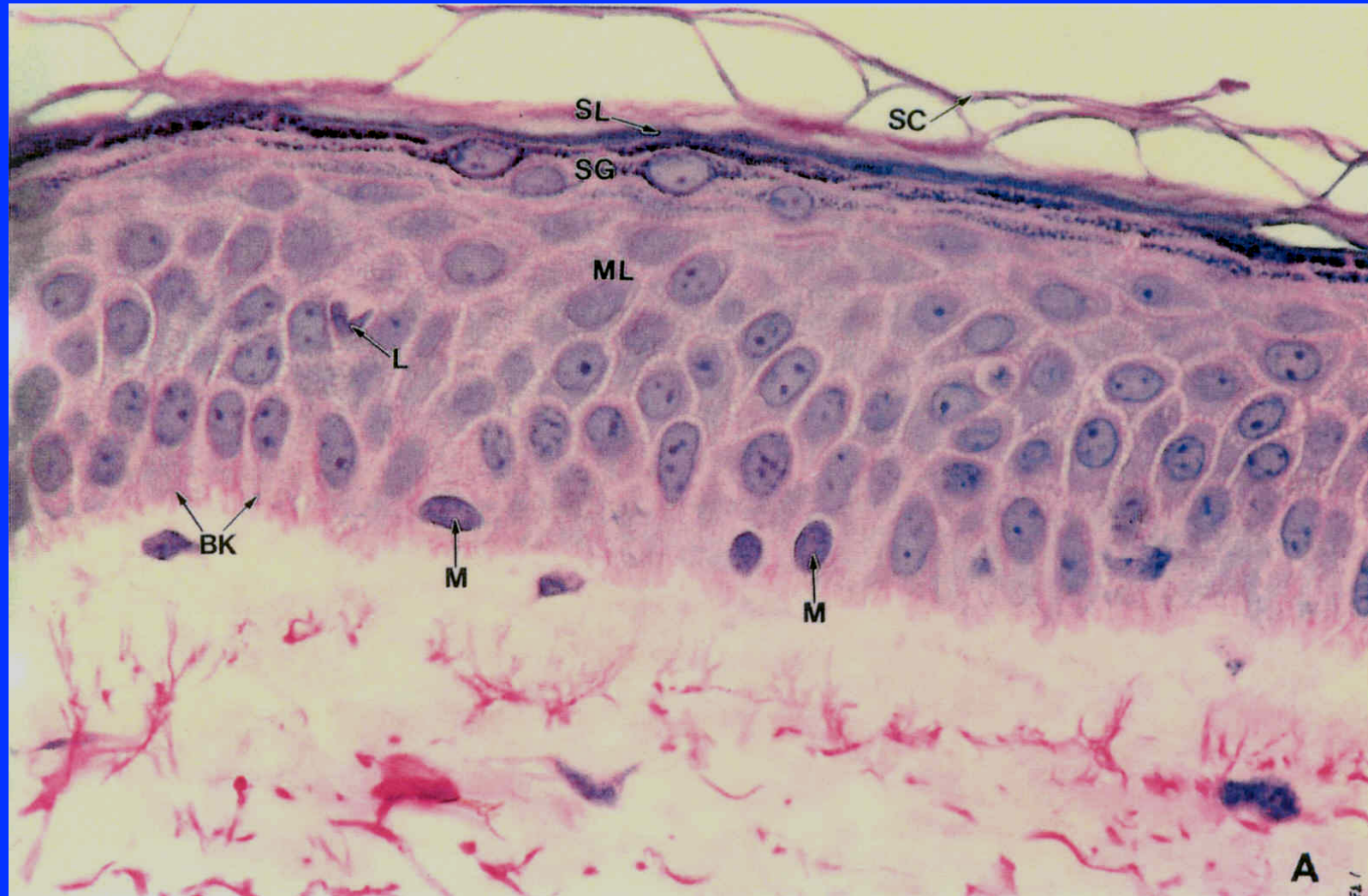
The Stratum Corneum is like a Brick Wall (Except that)

- **The bricks are flat disks of protein**
- **The bricks are joined by desmosomes**
- **The bricks overlap and may interlock**
- **The bricks are reinforced by microfibrils**
- **The mortar is partly attached to the bricks**
- **The mortar is lamellar and partly fluid**
- **OTHER THAN THAT THEY ARE JUST THE SAME!**

THE MORTAR

- Intercellular Lipids of the SC
- Multilamellar
- Ceramides, cholesterol and fatty acids
- Broad narrow broad pattern (6 & 13 nm)

Histological section of epidermis



The “Living” Stratum Corneum?

- SC is not alive in the classic sense
- No nuclei in corneocytes
- No ability to reproduce or utilize energy
- No cytoplasm
- Enzyme activities are degradative

EPIDERMAL LIPIDS

- Covalent-bound lipids
 - Attached to β -Sheet on the Cell Envelop that surrounds the dead corneocyte
 - Ceramides and fatty acids
- Intercellular
 - Multilamellar
 - Ceramides, cholesterol and fatty acids
 - Broad narrow broad pattern (6 and 13 nm)

LIPID PROCESSING IN THE EPIDERMIS

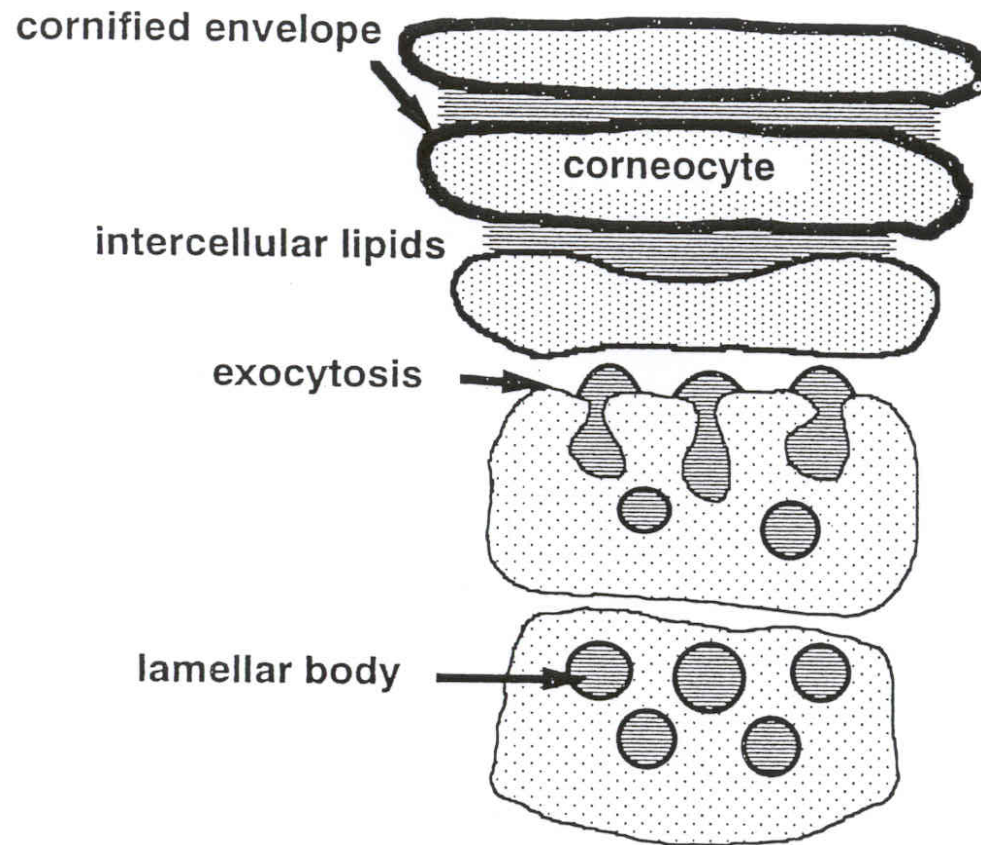


Figure Process of the epidermal lipid synthesis and formation of the intercellular lipid bilayer structure. The intercellular lipids are primarily generated from exocytosis of lipid-containing granules called lamellar bodies during the terminal differentiation of the epidermal keratinocytes.

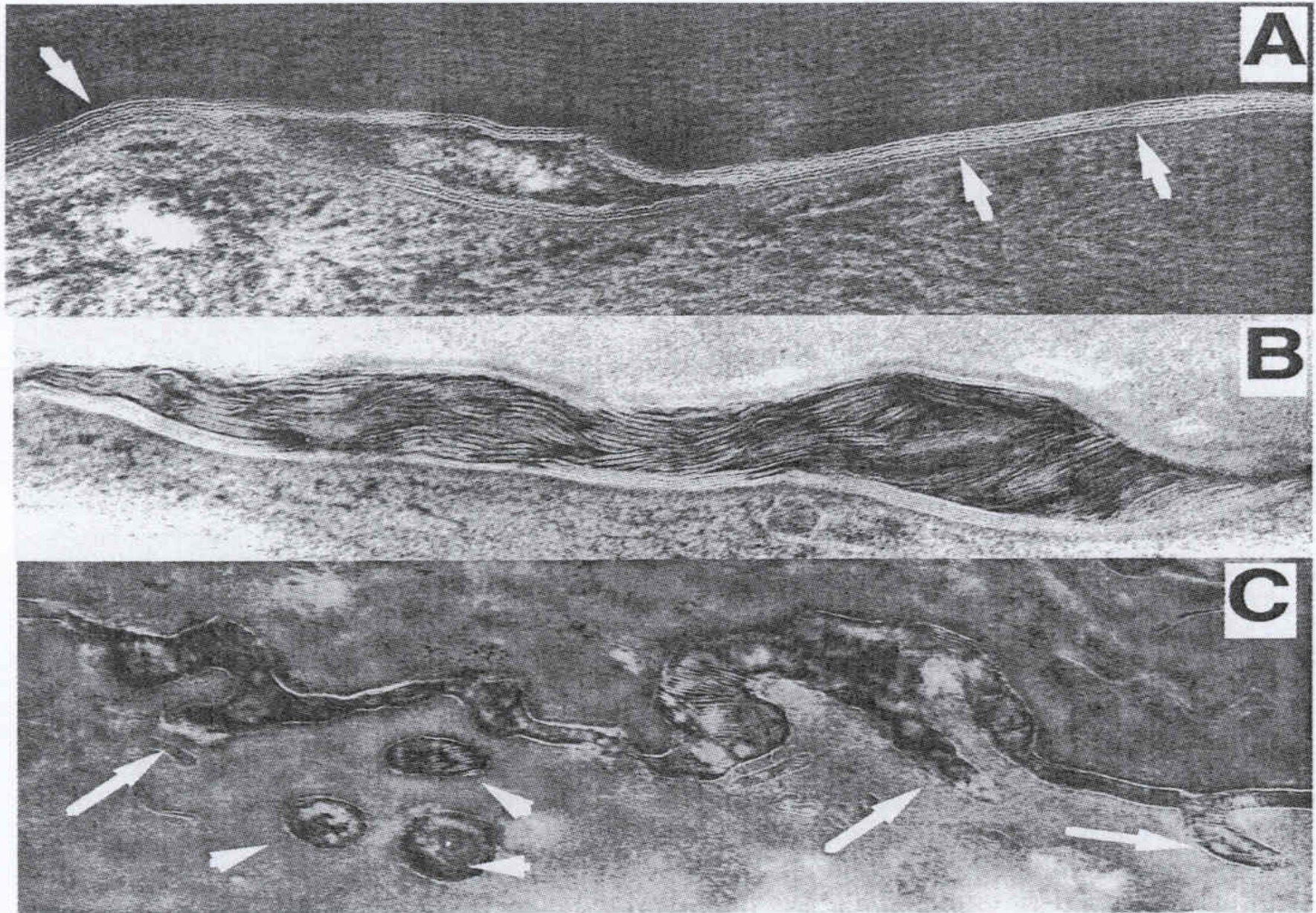
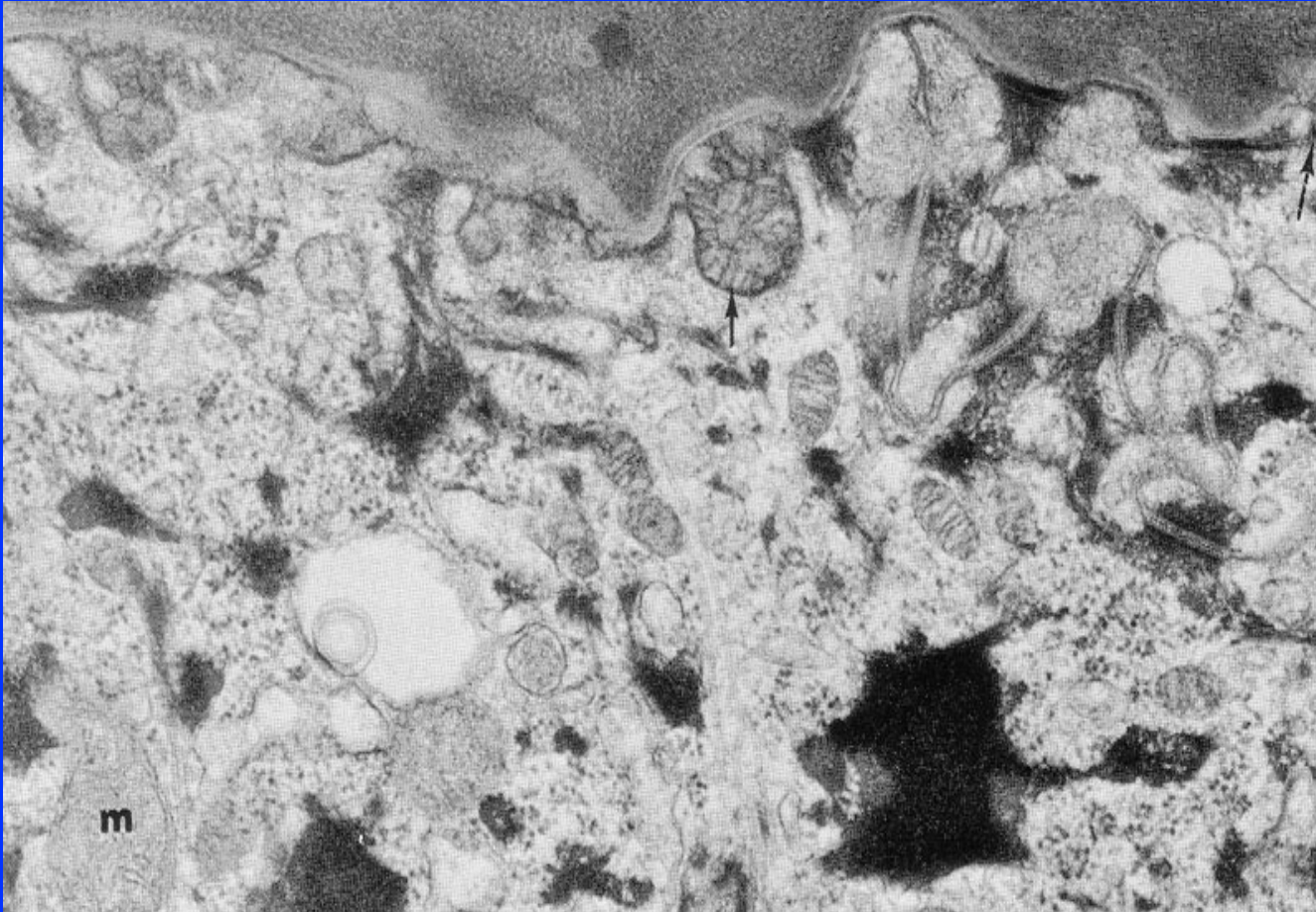


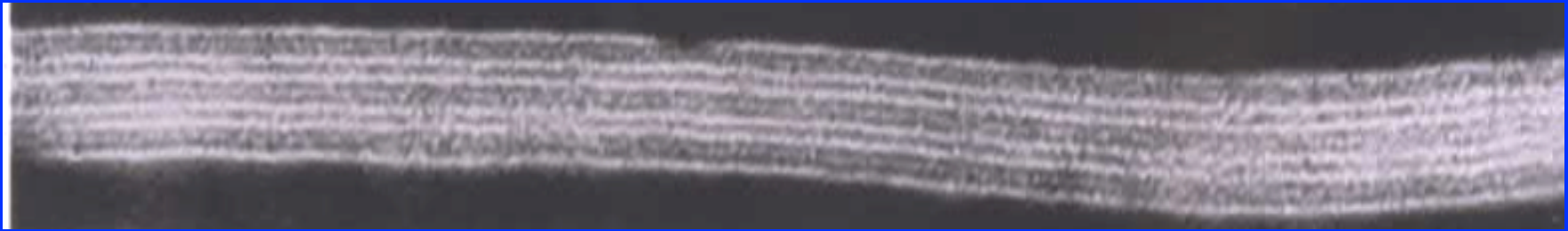
Figure 2 A: Electron micrograph of the intercellular lipid bilayer structure (arrows) in the human stratum corneum. B: Secreted lipids are stacked between the stratum corneum and the stratum granulosum. C: Lamellar bodies (arrowhead) and exocytosis of lamellar bodies (arrow) in the human stratum granulosum.

Lamellar Bodies Expel their contents into the intercellular space at the SG/SC interface

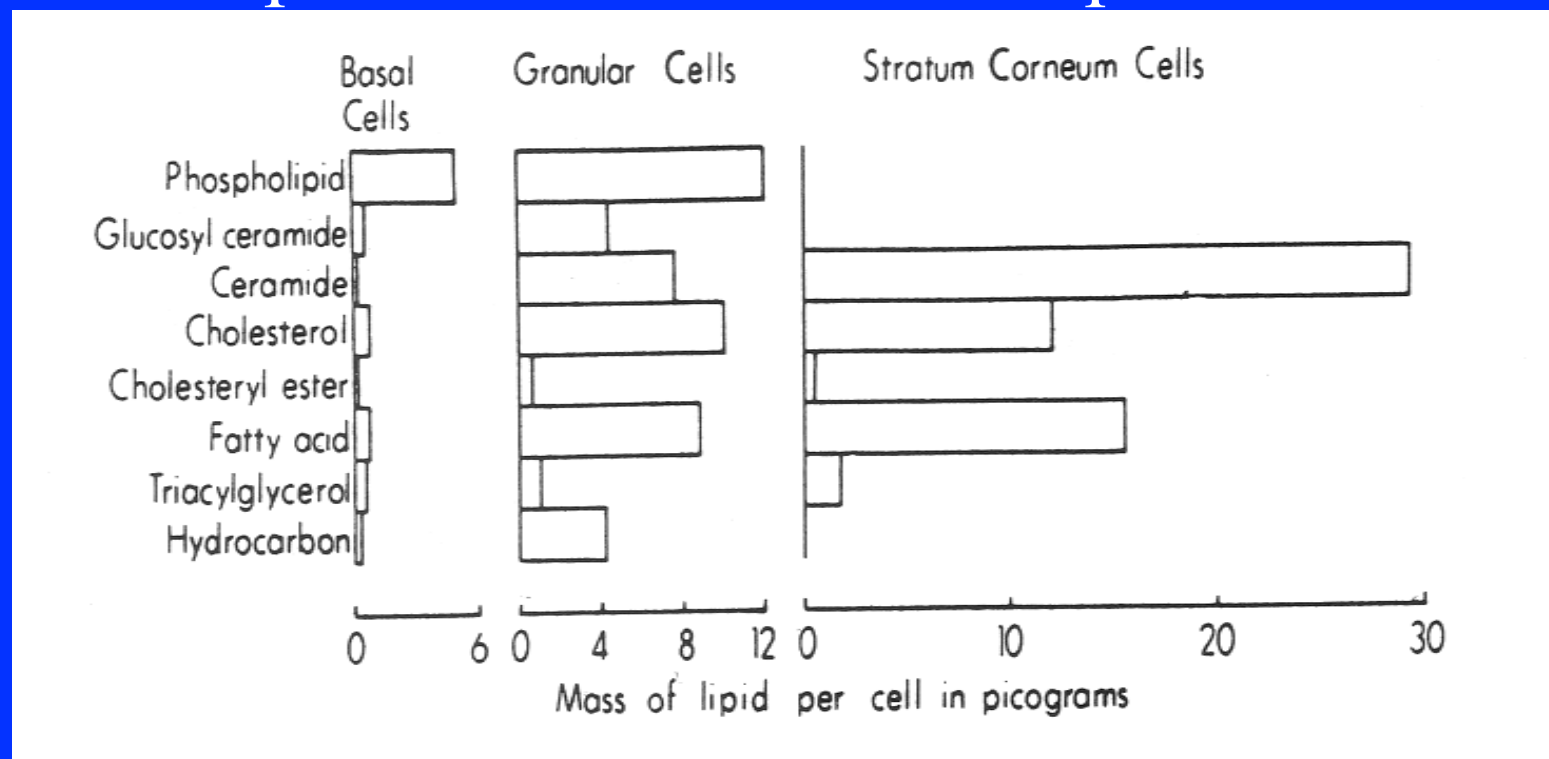


The Barrier Lipids of the Stratum Corneum

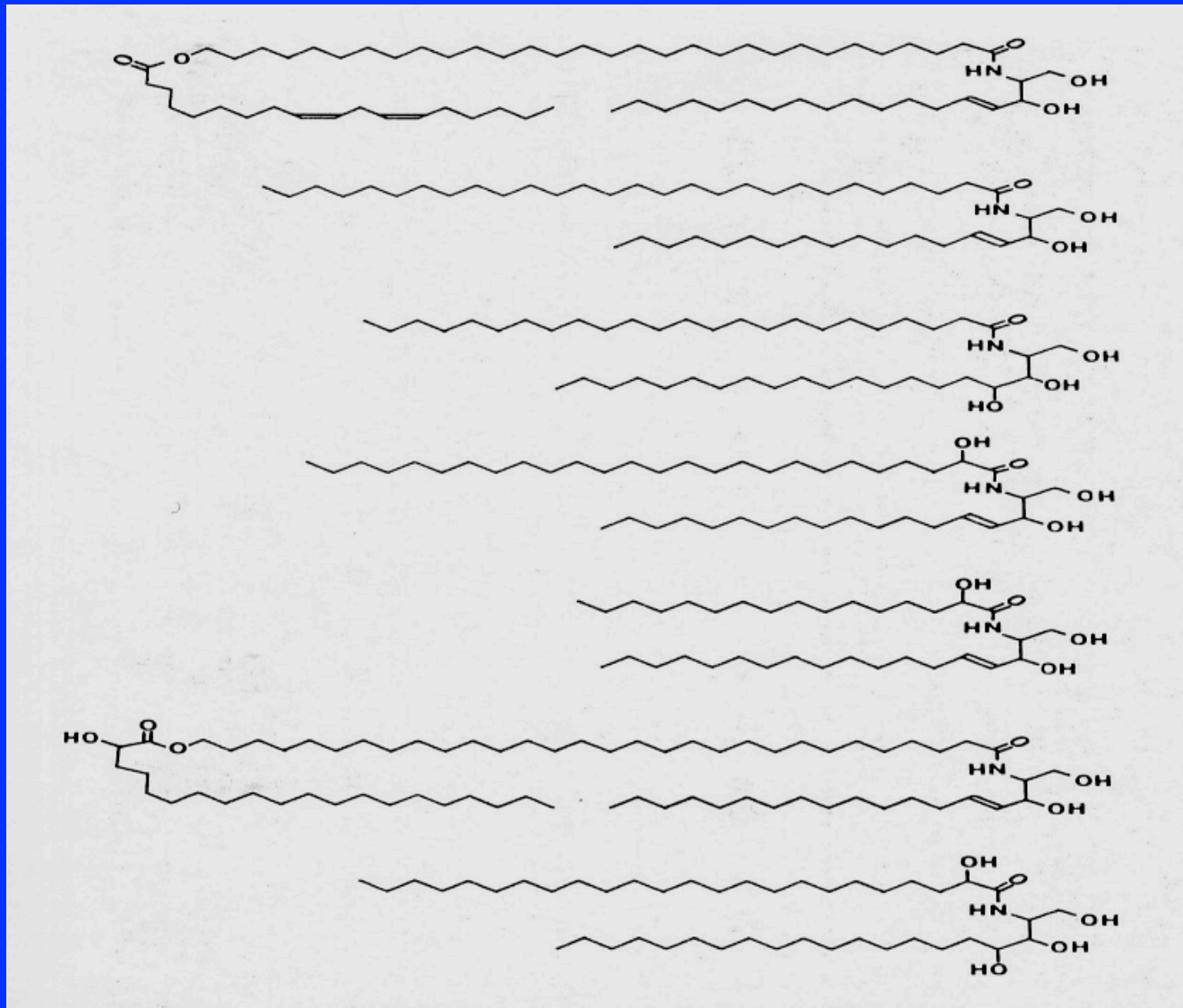
(courtesy of R. Warner)



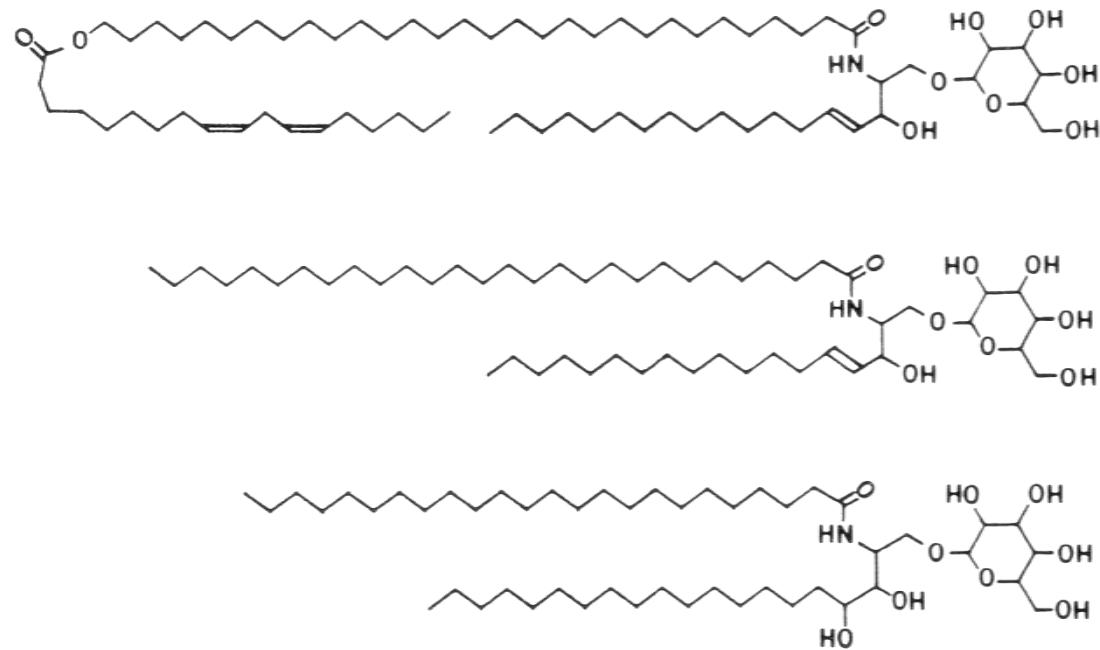
Lipid distributions in the epidermis



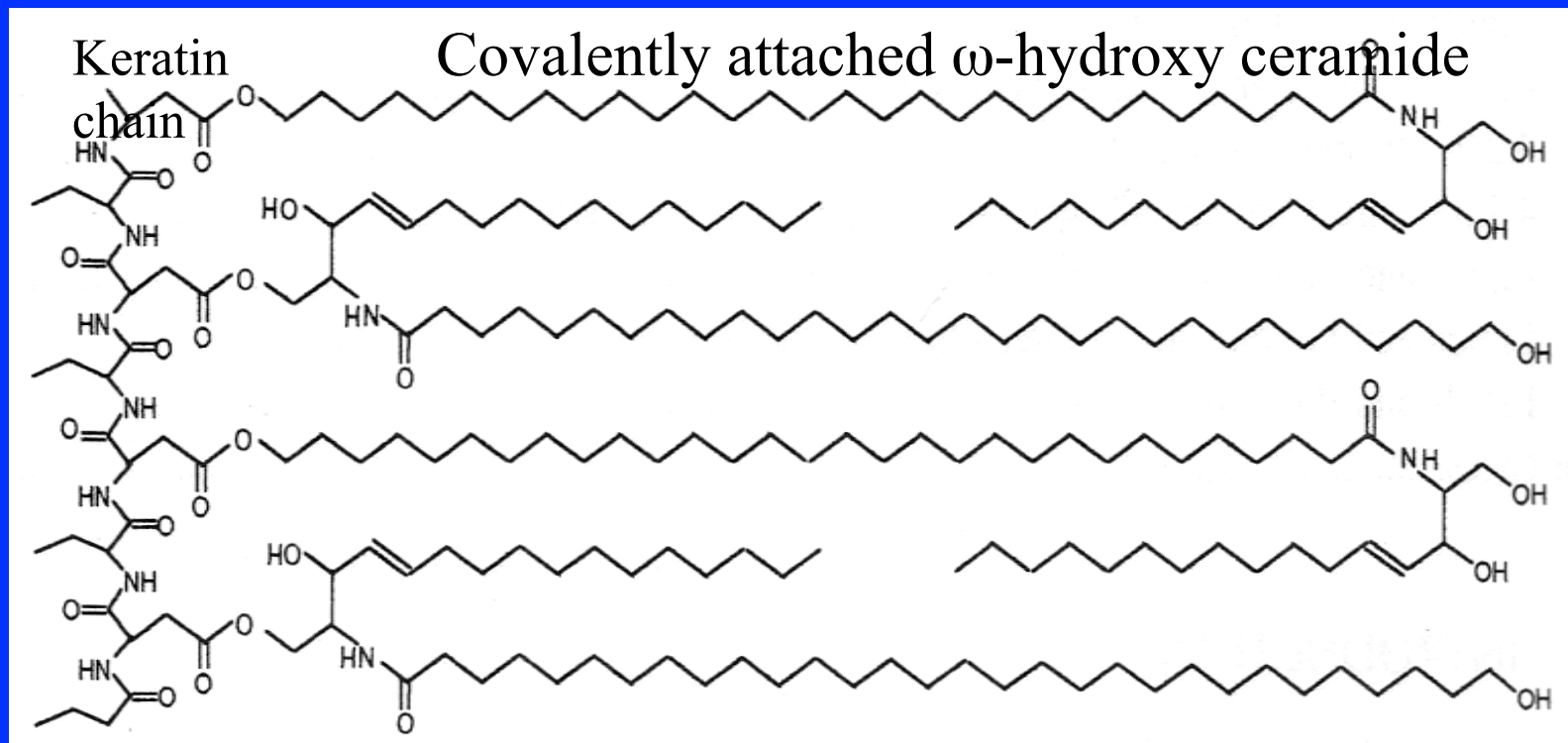
Ceramides



Glucosyl Ceramides

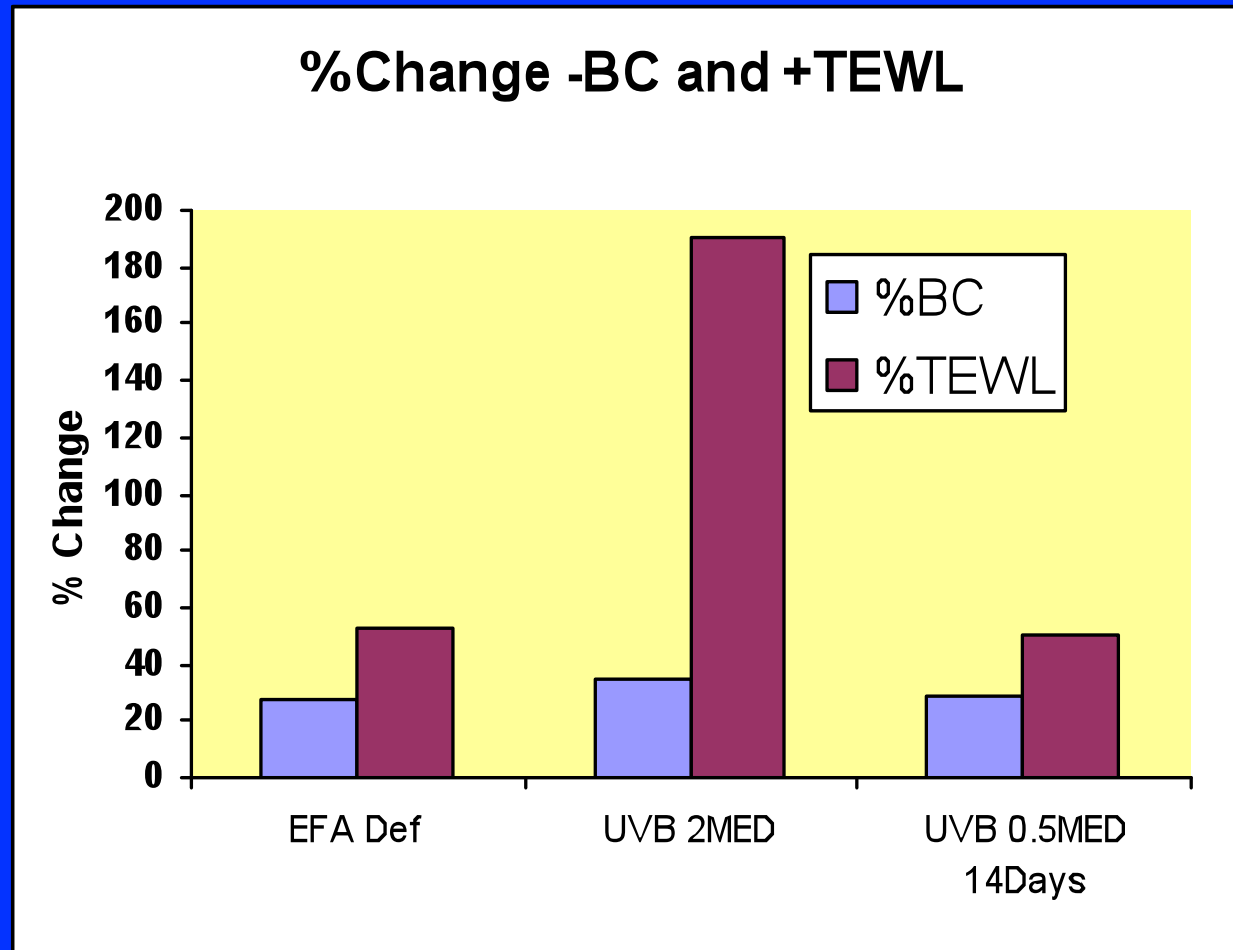


Covalently attached lipids of the cornified cell envelope



Note: Recent studies have shown that fatty acids are also attached to the SC cell envelope

Reducing Covalently bound ceramides increases TEWL



Data from Meguro et al Arch Dermatol Res(2000)292:463-468

Metabolic activity in the SC

- Conversion of phospholipids to fatty acids
- Glucosyl ceramides to ceramides
- Hydrolysis of desmosomes
- Cholesterol sulfate to cholesterol
- Filaggrin to NMF

Lipid Processing in the Epidermis

"Pro-Barrier" Lipids:

Glycolipids, Free Sterols,
Phospholipids



Conversion of "Pro-Barrier" Lipids to
Non-Polar Products (Lipases, Glucosidases)

Glycolipids

Ceramides

Phospholipids

FFA



Barrier Function

Catabolic Enzymes:

Acid Phosphatase,
Proteases, Lipases,
Glycosidases

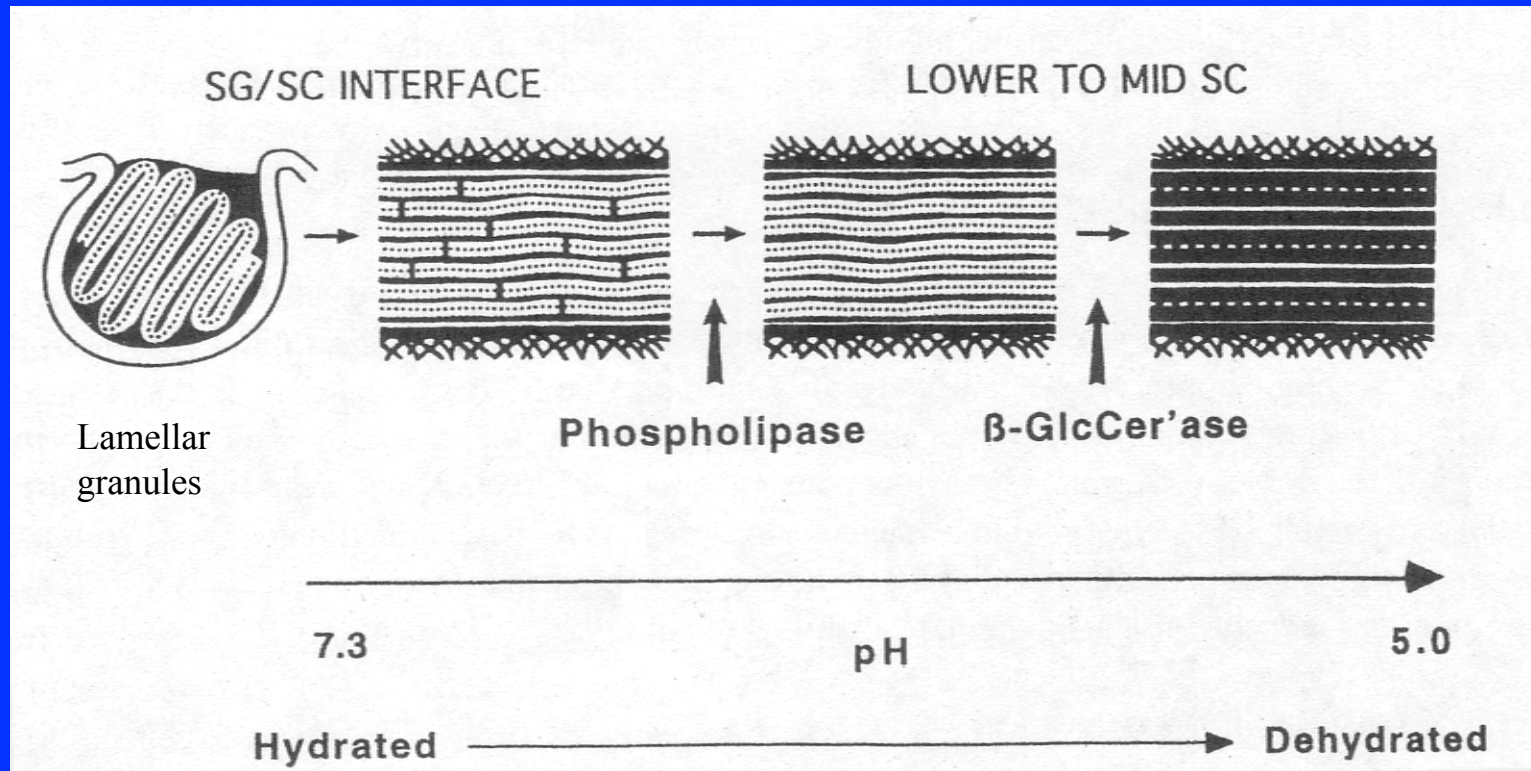
1) Release of Desmosomes into Intercellular
Space (Lipases)

2) Degradation of Non-Lipid Intercellular
Species (Acid Phosphatase, Proteases)

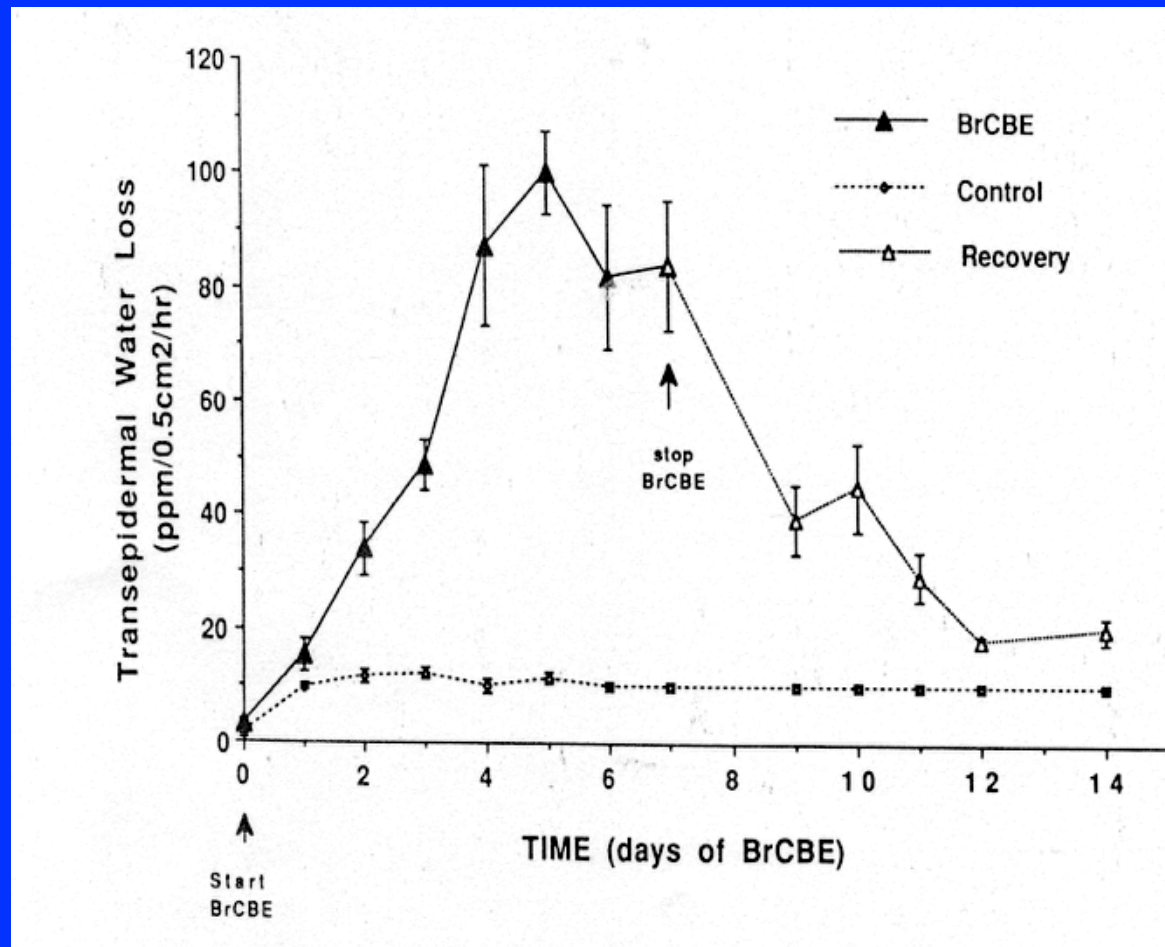


Desquamation

**Lipids are released in the intercellular space in the SG:
Extracellular processing is required for competent SC barrier
formation (Concept due to Elias)**



Inhibiting conversion of glucosyl ceramides to ceramides leads to barrier disruption

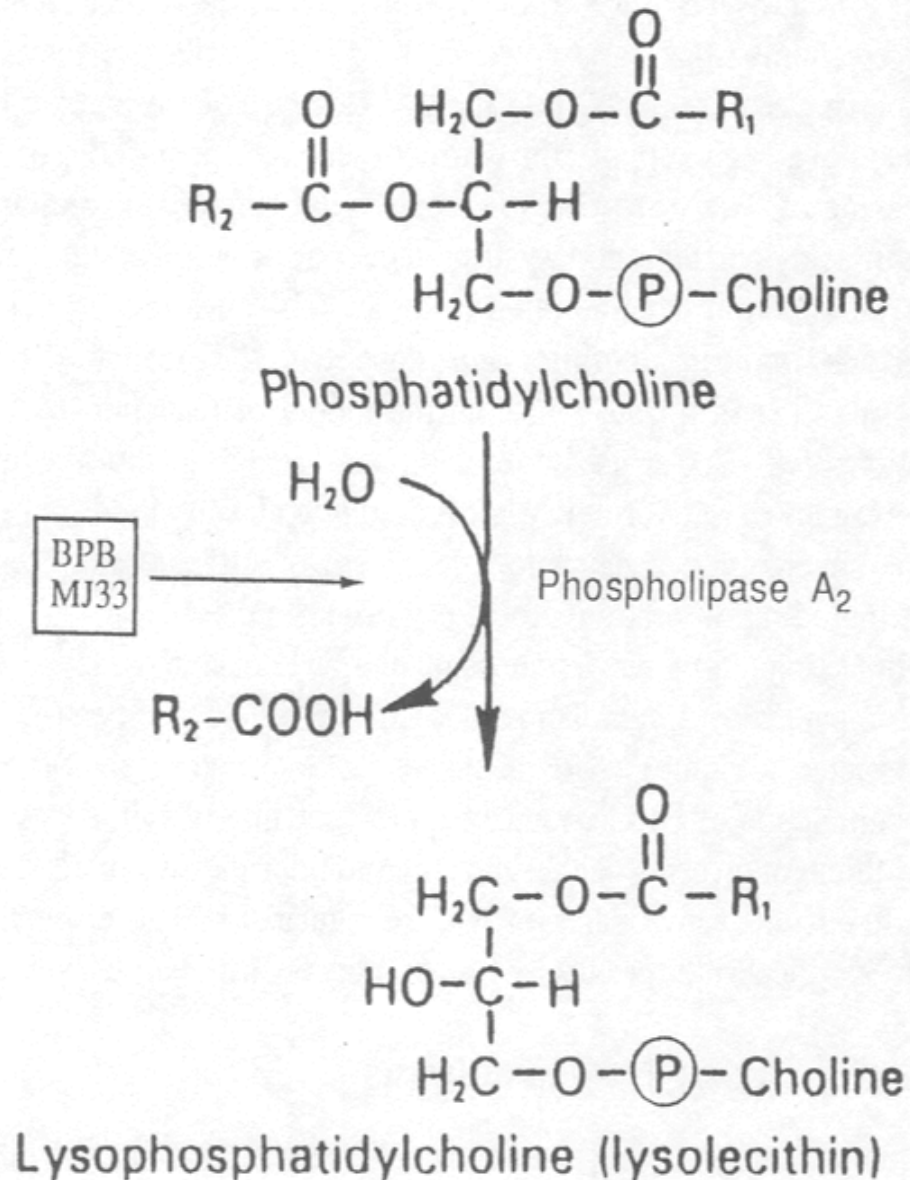


Holleran et al J Clin Invest. 911656-1664 (1993)

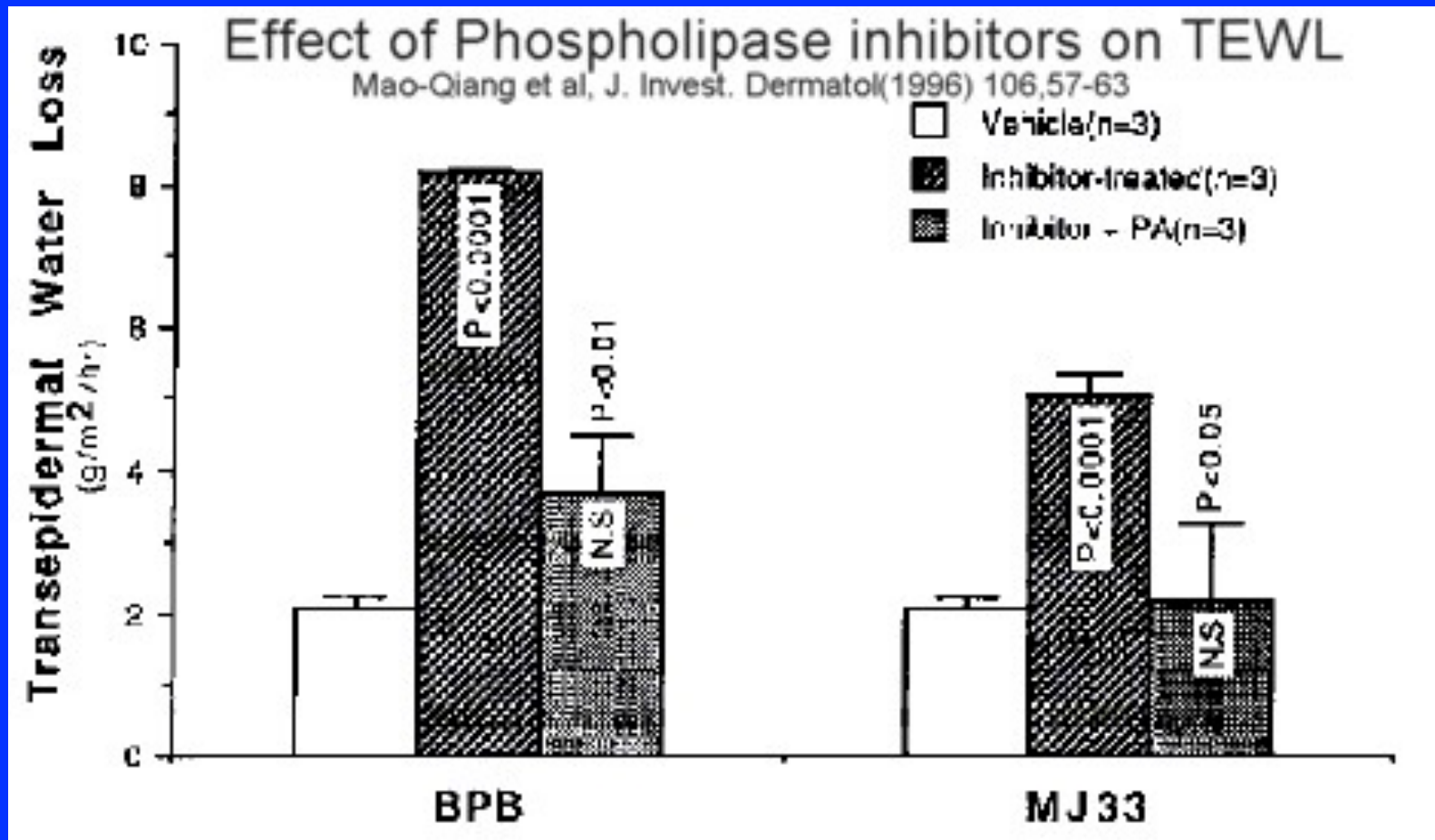
SC processing of phospholipids to fatty acids is necessary for barrier homeostasis.

Inhibition of PLA_2 by BPB or MJ33 led to barrier disruption mitigated by coapplication of palmitic acid.

Mao-Qiang et al J Lipid Res 36, 1925(1995)

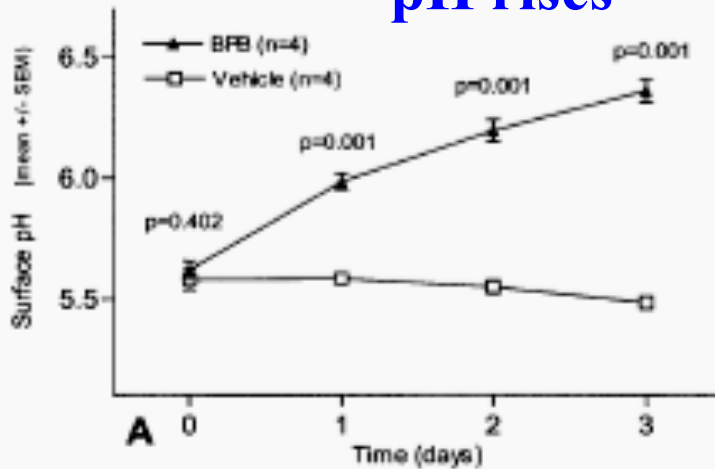


Inhibition of phospholipase increases TEWL: Effect is moderated by palmitic acid - PA

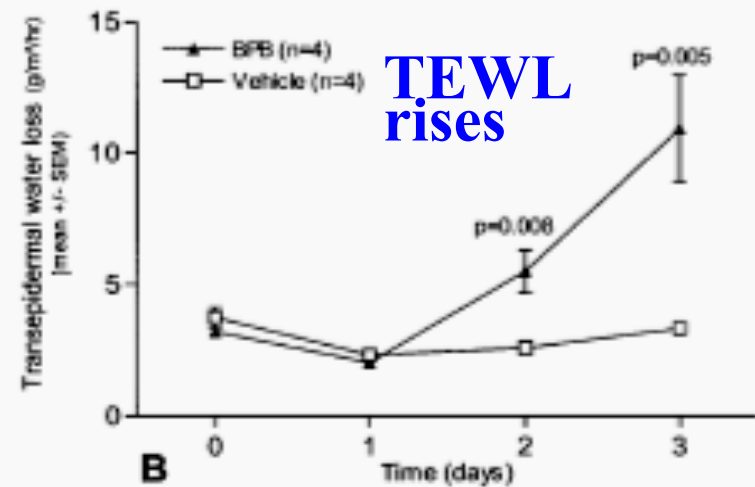


PLA₂ Inhibition also affects skin pH and cohesive properties

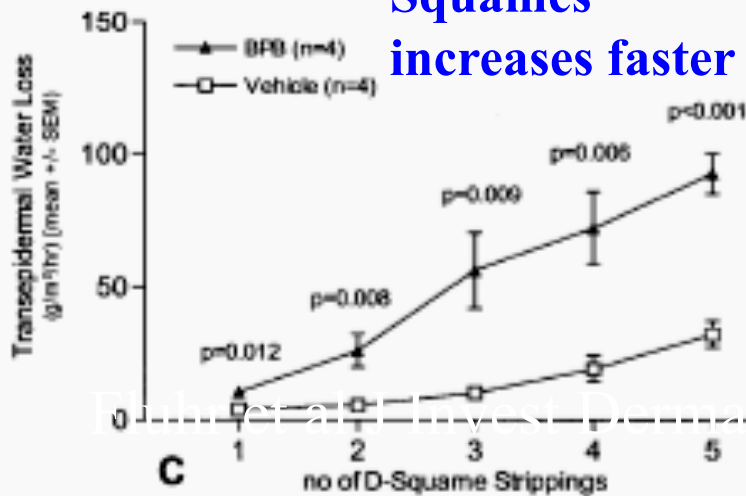
pH rises



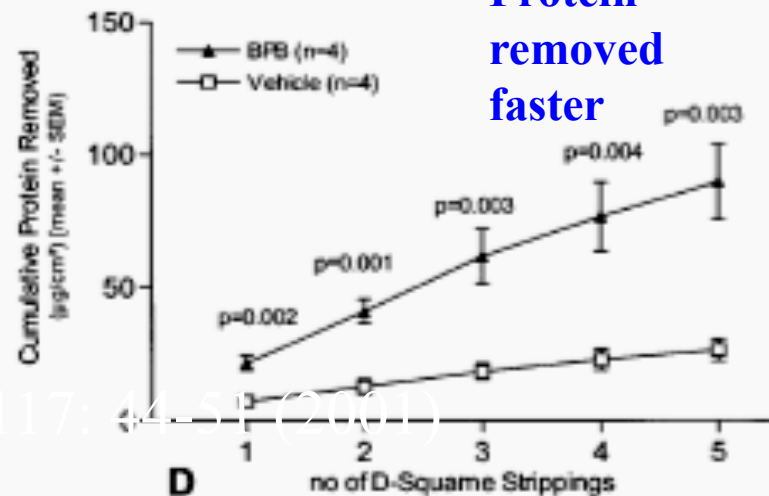
TEWL rises



Squames increases faster



Protein removed faster



Huber et al. Invest Dermatol 117: 44-5 (2001)

**STRATUM CORNEUM LIPID
MACROMOLECULAR
STRUCTURE**

FRIBERG MODEL

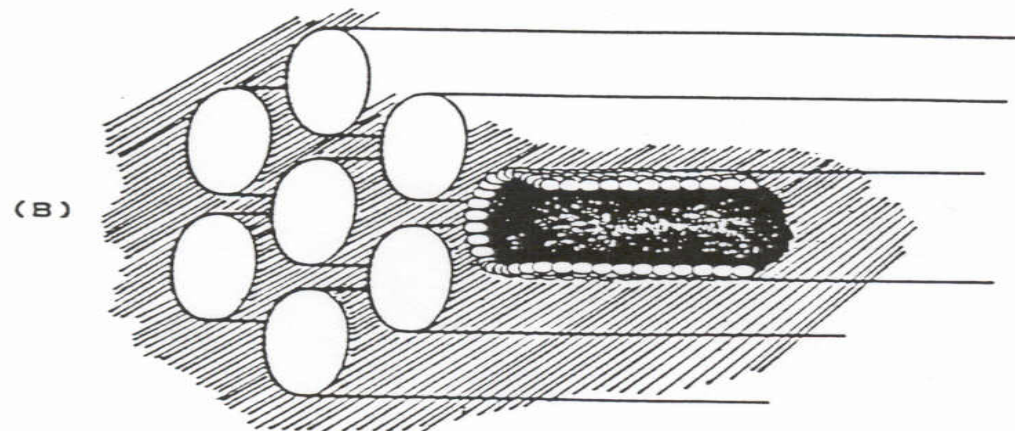
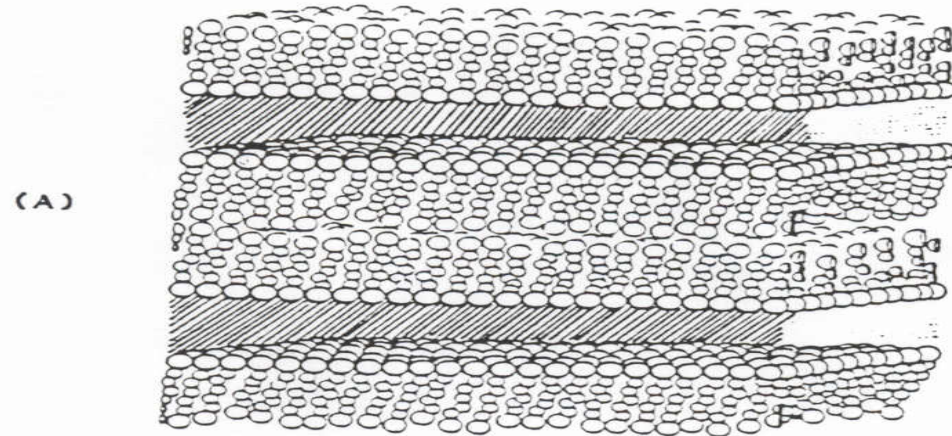
- | Friberg combined all epidermal and surface lipids in proportions found in skin plus 32% water
 - | Found a mixture of multiple phases
- | Adjusted mixture to pH 5

Key Findings

- | Liquid crystalline - could be lamellar or hexagonal structure
- | Results suggest fatty acid / soap is the basis of the layered structure

LIPID STRUCTURES IN STRATUM CORNEUM

A - lamellar and B - hexagonal gel phases

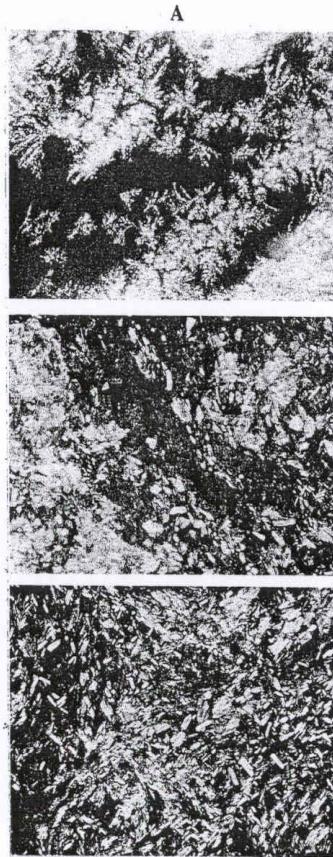


FRIBERG MODEL

- Rhein and Friberg studied the effect of low relative humidity and cosmetic ingredients on the model lipid using DSC
- Also the location of the various lipids in the model using small angle X-ray diffraction

EXPOSURE OF MODEL SKIN LIPID TO LOW RELATIVE HUMIDITY

Model
Skin
Lipid
Without
Lipid
Fluidizers

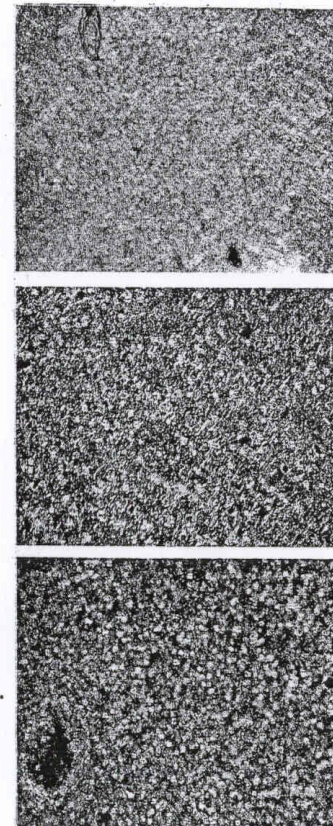


6 h

24

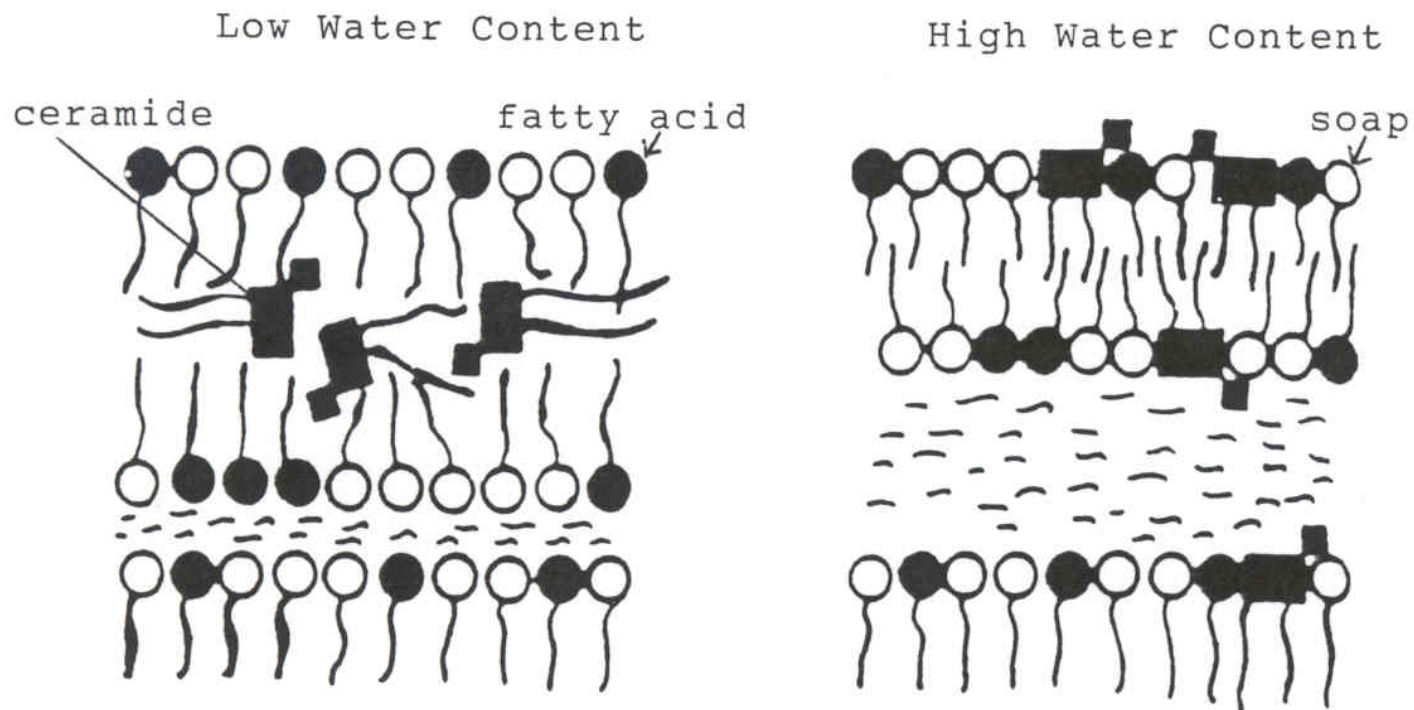
96 hr.

B

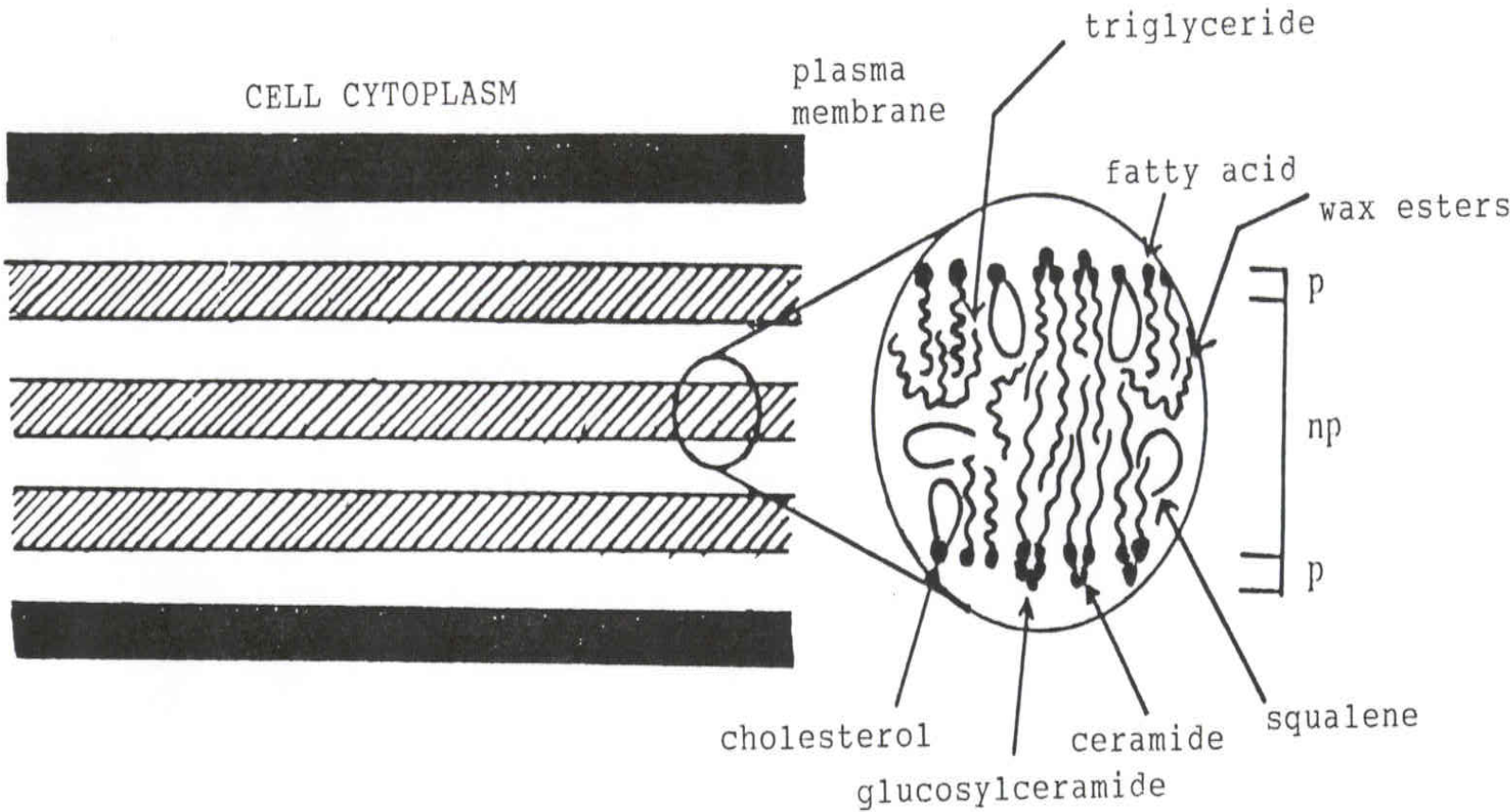


Model
Skin
Lipid
With
10% Lipid
Fluidizers

STRATUM CORNEUM LIPID STRUCTURE - EFFECT OF DEHYDRATION

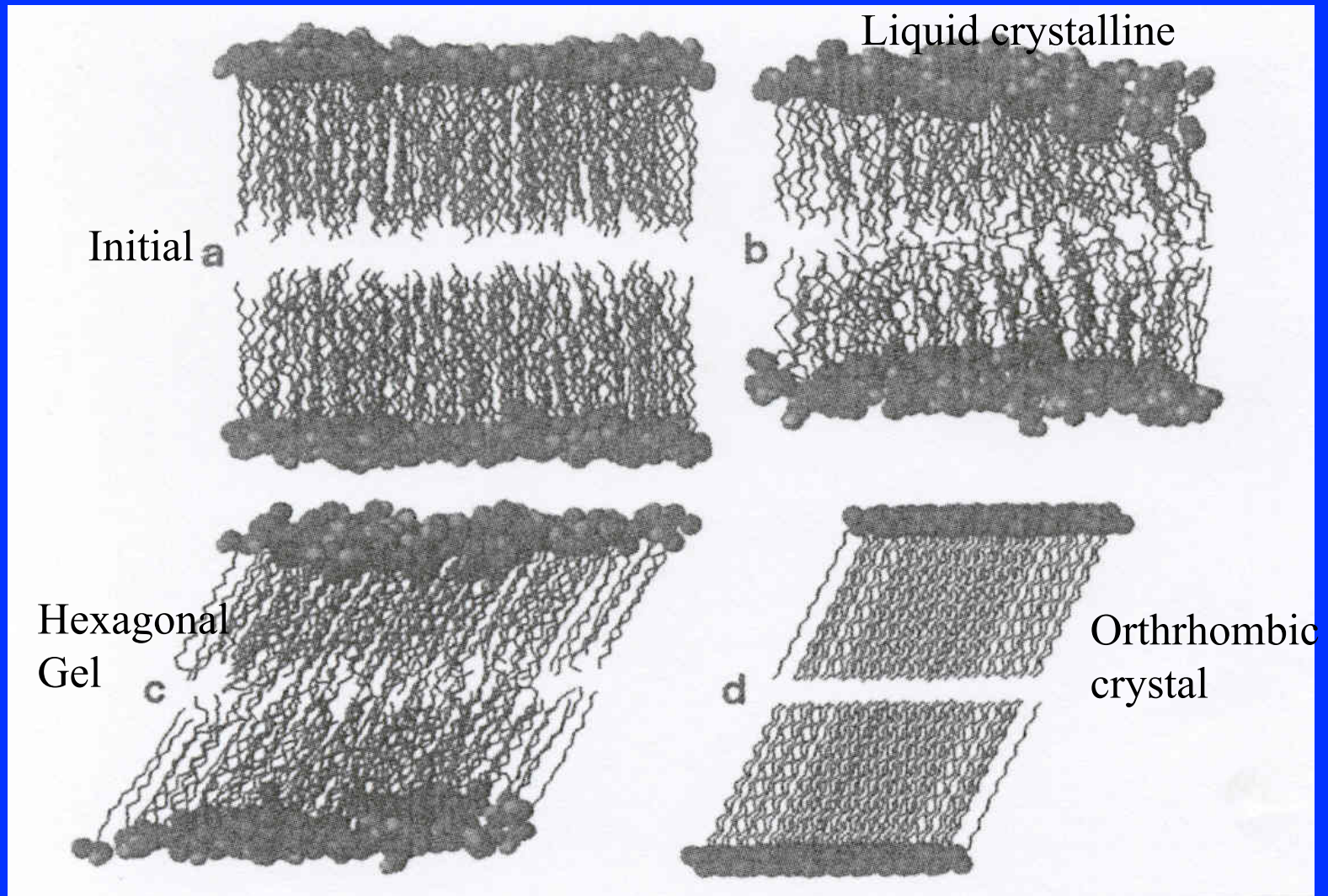


STRATUM CORNEUM LIPID STRUCTURE



**Evidence of Polymorphism
in Stratum Corneum
Lipid Structures**

STRATUM CORNEUM LIPID STRUCTURE

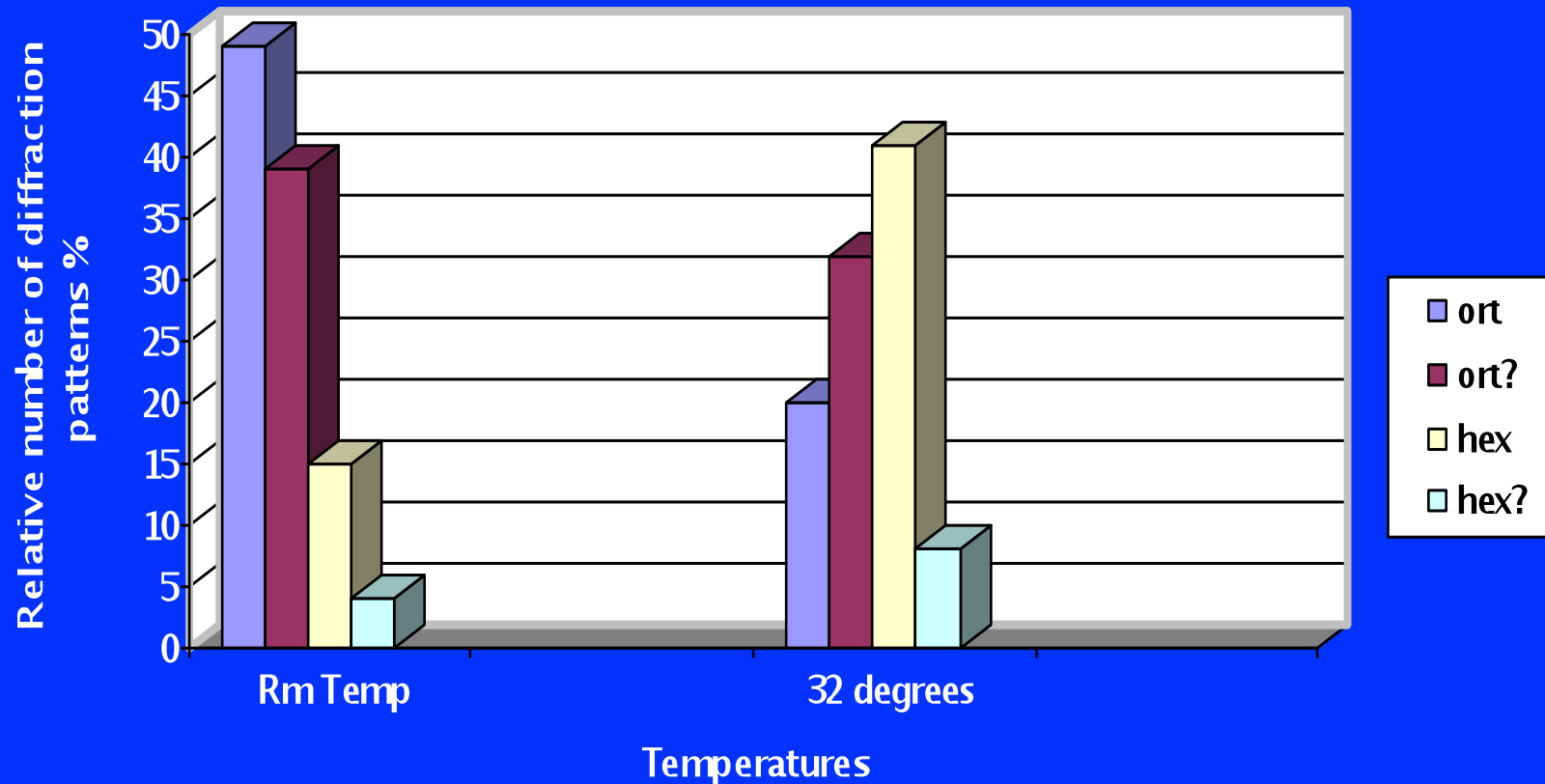


X-ray Diffraction Spacings in the 3rd & 5th Strips of Human Stratum Corneum

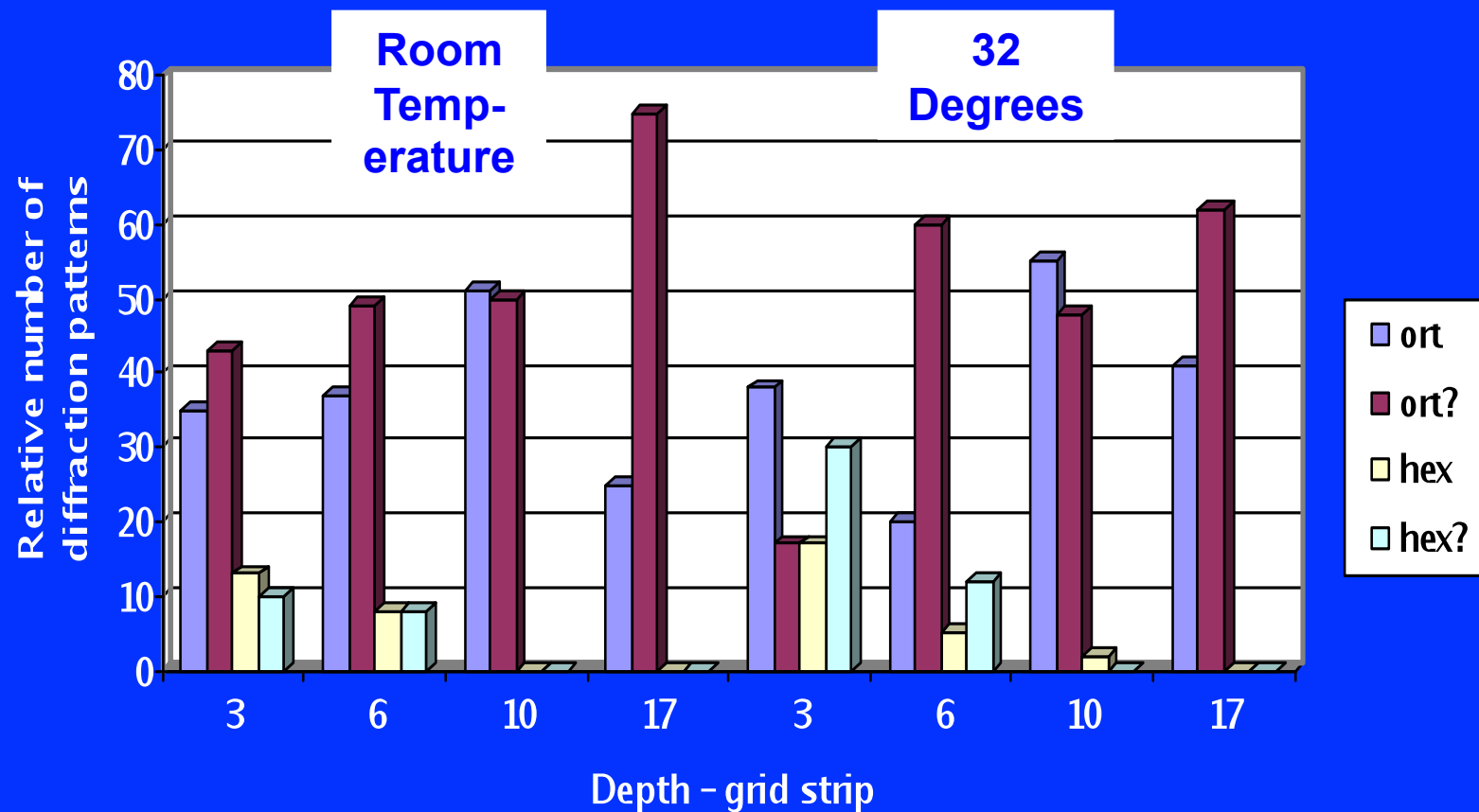
Spacings Found (nm)	Third Strip	Fifth Strip	Negative Control Lipid Depleted Stratum Corneum
0.48 – 0.50 nm (cholesterol)	present	present	absent
0.41 nm (hexagonal)	present	present	absent
0.37 / 0.41))* (orthorhombic)	present	present	absent
0.40 – 0.50 (keratin)	present	present	present

*For the 0.41 nm reflection hexagonal patterns cannot be ruled out

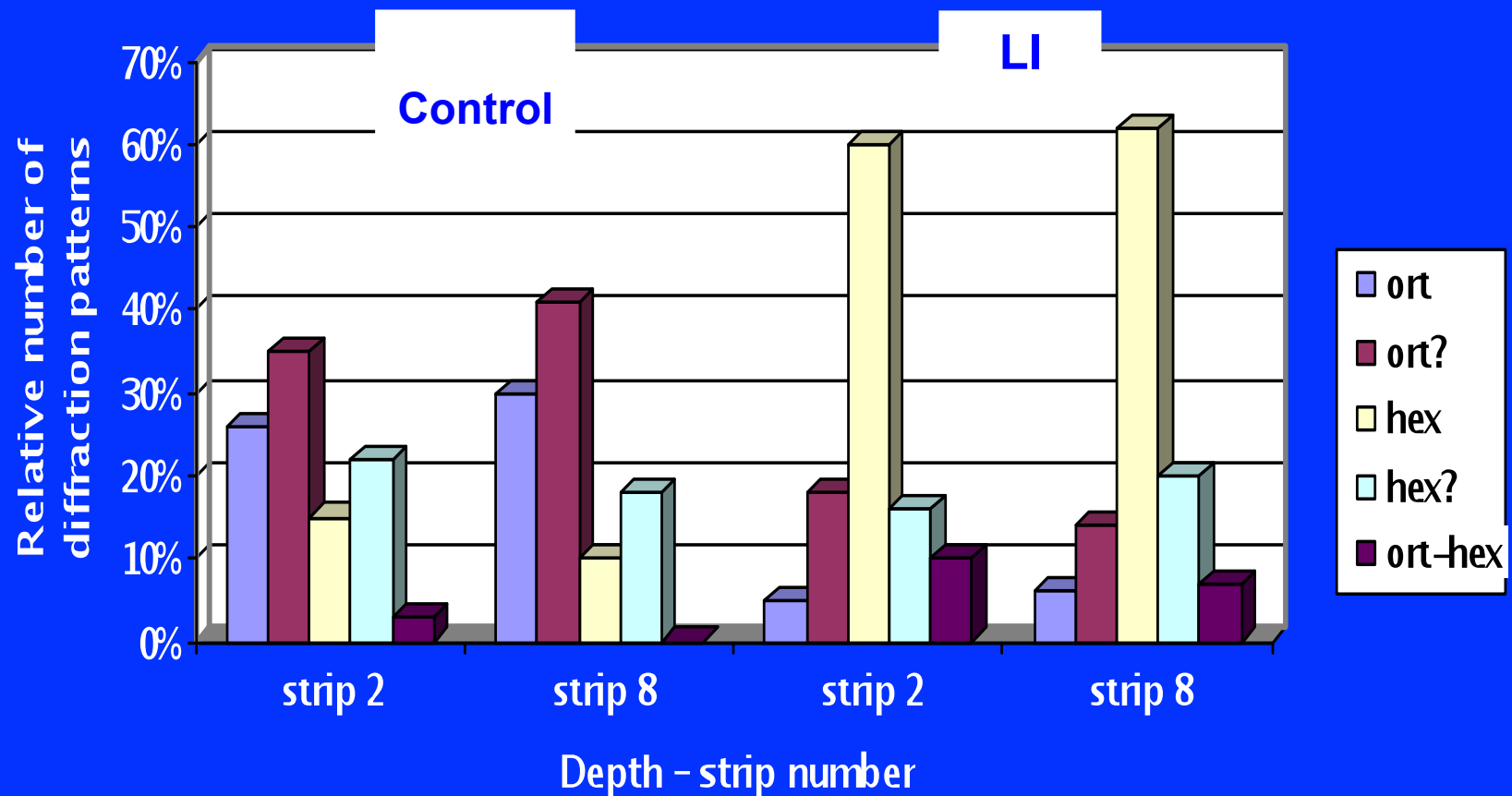
Distribution of Hexagonal and Orthorhombic Lattices at Different Temperatures in Human Stratum Corneum



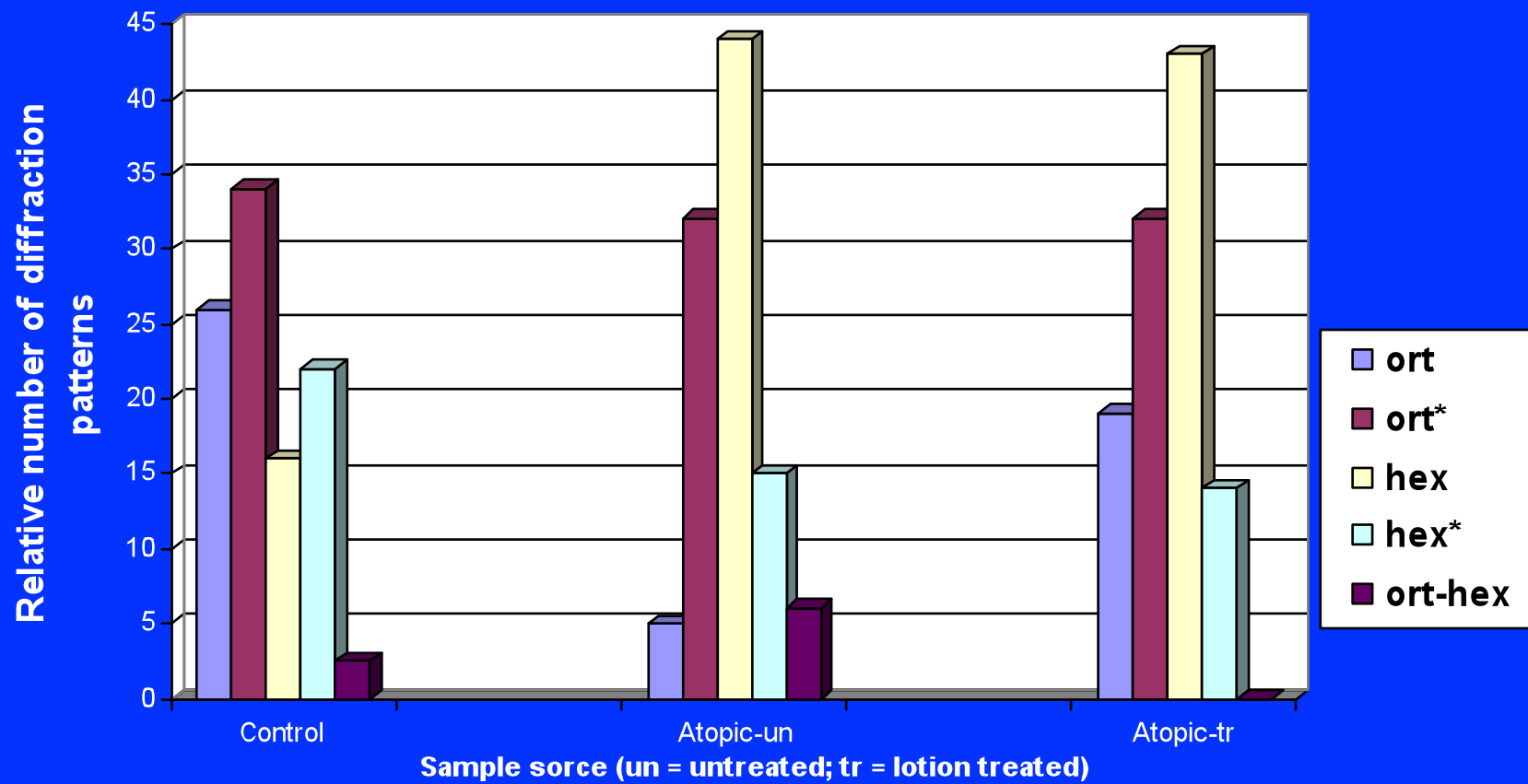
Distribution of Hexagonal and Orthorhombic Lattices in Relation to Depth in Human Stratum Corneum (see Pilgram)



Relative Distribution of Diffraction Patterns in Lamellar Ichthyosis (LI) Stratum Corneum



Relative Distribution of Diffraction Patterns from Controls, Atopic Dermatitis, and Treated Skin of Atopics



Conclusions from Polymorphism Studies

- ! Stratum corneum lipid exists as lamellar structures
- ! At room temperature mostly orthorhombic lattices are found in stratum corneum
- ! At physiological temperature, the lipid structure is both orthorhombic and enriched in hexagonal lattices, leaving it more fluid
- ! At higher temperatures the lipid alkyl chains are liquid
- ! The stratum corneum surface lipid is more enriched in hexagonal lattices compared to the lower layers
- ! The lipid structures in ichthyosis and atopic dermatitis stratum corneum are more enriched in hexagonal lattices
- ! Additives like glycerol disorder the lipid leaving it more fluid at physiologic temperature, slowing down dehydration at lower relative humidity SEEMS LIKE A DICHOTAMY???

How do the Different Lattices Dictate Barrier Function?

- From our own studies, low relative humidity appears to crystallize the lipid, probably promoting enrichment with orthorhombic lattices
- These conditions also lead to dry, xerotic skin
- This results from increased water loss and hence dehydration of the lipid structure
- Inclusion of glycerol, a well known moisturizer, blocks or slows down the formation of crystalline lipid at low relative humidity
- Glycerol greatly facilitates healing and moisturizing of xerotic skin
- This suggests that the crystalline nature of the orthorhombic lattice is insufficient for optimal barrier function if this is the primary action of glycerol.

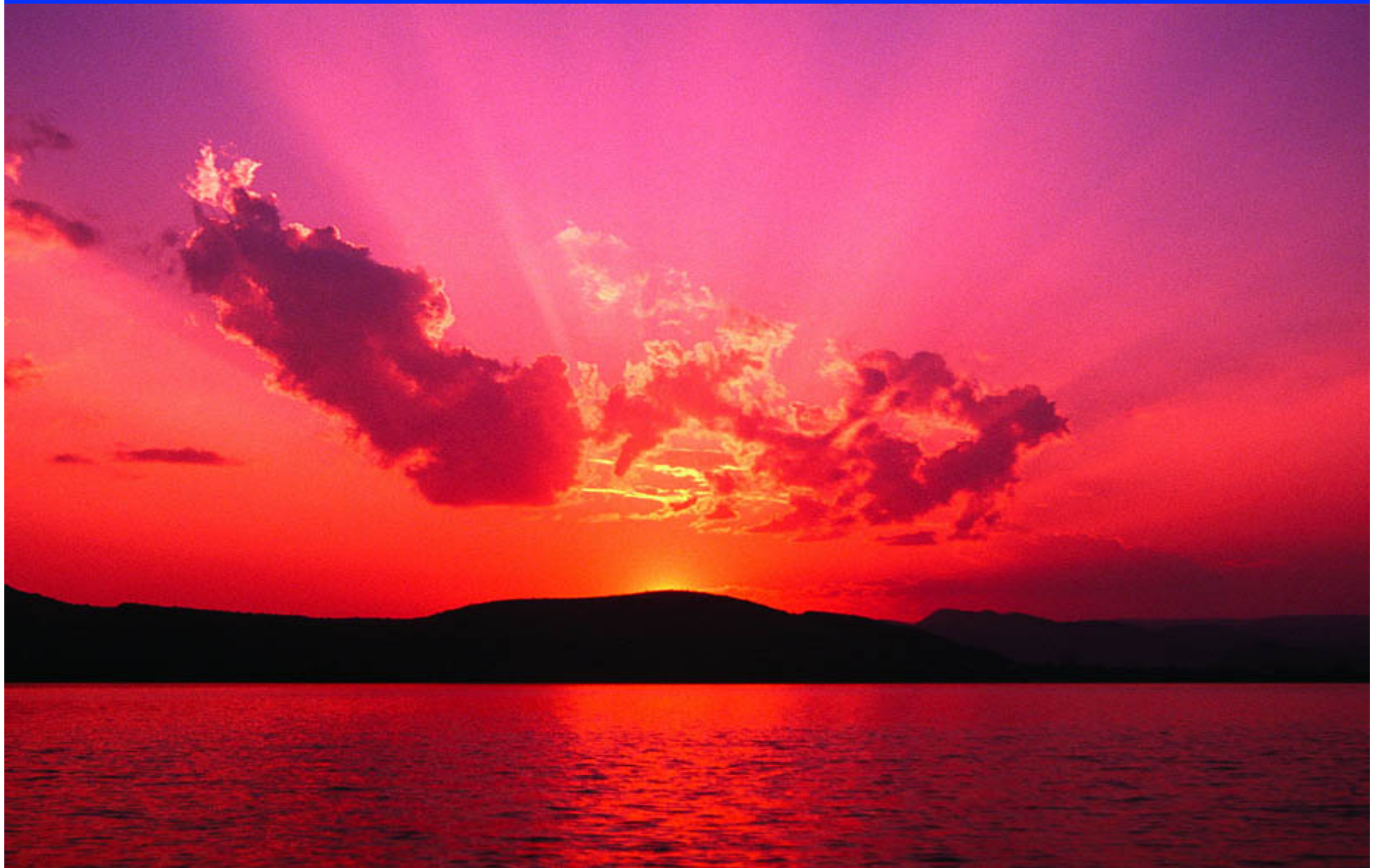
On the Other Hand...

- Normal healthy skin is relatively more enriched in crystalline orthorhombic lattices than skin of atopics and ichthyotics
- Treatment of atopic skin with a regular lotion seemed to promote enrichment with crystalline orthorhombic lattices
- However at physiologic and higher temperatures the diffraction patterns attributed to orthorhombic lattices decrease and hexagonal lattices increase especially at the skin surface
- These results suggest that a balance exists in stratum corneum between crystalline orthorhombic lattices, hexagonal liquid crystal structures and other transient phases.
- Disturbances of this balance can be caused by many factors including temperature, relative humidity, inflammatory dermatoses, harsh cleansing, and penetration enhancers.

Proposed Relationship between Barrier Function, Lipid Lattices, and Interactions with Actives

- Multiple lamellar lipid lattices exist and are in balance
- There is an optimal balance for normal healthy skin
- A disordered and fluid lattice leads to excessive penetration of hydrophobic substances and lacks rigidity
- A crystalline orthorhombic lattice is probably too rigid and lacks flexibility and may actually become leaky
- Barrier altering agents / conditions can act in a variety of possible ways with the lipid, ie by shifting the balance to more fluid or more rigid phase, phase separation, etc
- This effects penetration and action of the agent within the skin
- Future research needs to focus on defining these mechanisms as they relate to improved barrier health

THANK-YOU



Descriptions of the Interactions

- **Phase Transitions** from orthorhombic to hexagonal lattice leads to decreased packing density and increased mobility of hydrocarbon chains
- **Grain Boundries** are sites where fluctuations in packing density occur due to mismatches between separate crystals; exists between phase separated lipid domains or different orientations of the same crystals
- **Phase Separation** can be caused by insertion of an ingredient leading to coexistence of different phases such as a fluid phase along with others
- **Fluidization** can occur by intercalation of exogenous ingredients that reduce the van der Waals or hydrogen bonding interactions increasing mobility of the hydrocarbon chains

Do all these interactions occur in stratum corneum and what is the impact of each type on barrier function?

High – Angle Spacing (Å) Observed in Stratum Corneum as a Function of Temperature

Temperature			Interpretation
25 °C	45 °C	75 °C	
9.4s	9.4s	9.4s	Both of the sharp lines, 9.4 and 4.6 originate from the protein in the corneocyte envelope
4.6s	4.6s	4.6s	
4.6b	4.6b	4.6b	
4.16s	Absent	Absent	Both 4.16 and 3.75 Å spacings are due to the crystalline alkyl chains organized as an ortho-rhombic perpendicular subcell. There may be a distribution of alkyl chains in the gel state because the 4.16 line is wide
Absent	4.12s	Absent	The 4.12 Å spacing is due to gel – state alkyl chains organized as hexagonal subcell-transition from crystalline state at 25 °C (the 4.16 and 3.75 Å bands)
3.75s	Absent	Absent	Both 4.16 and 3.75 Å spacings are due to the crystalline alkyl chains organized as an orthorhombic perpendicular subcell.

s = sharp, b = broad referring to the width of the reflection.

Assimilated from Hou, et al (43)

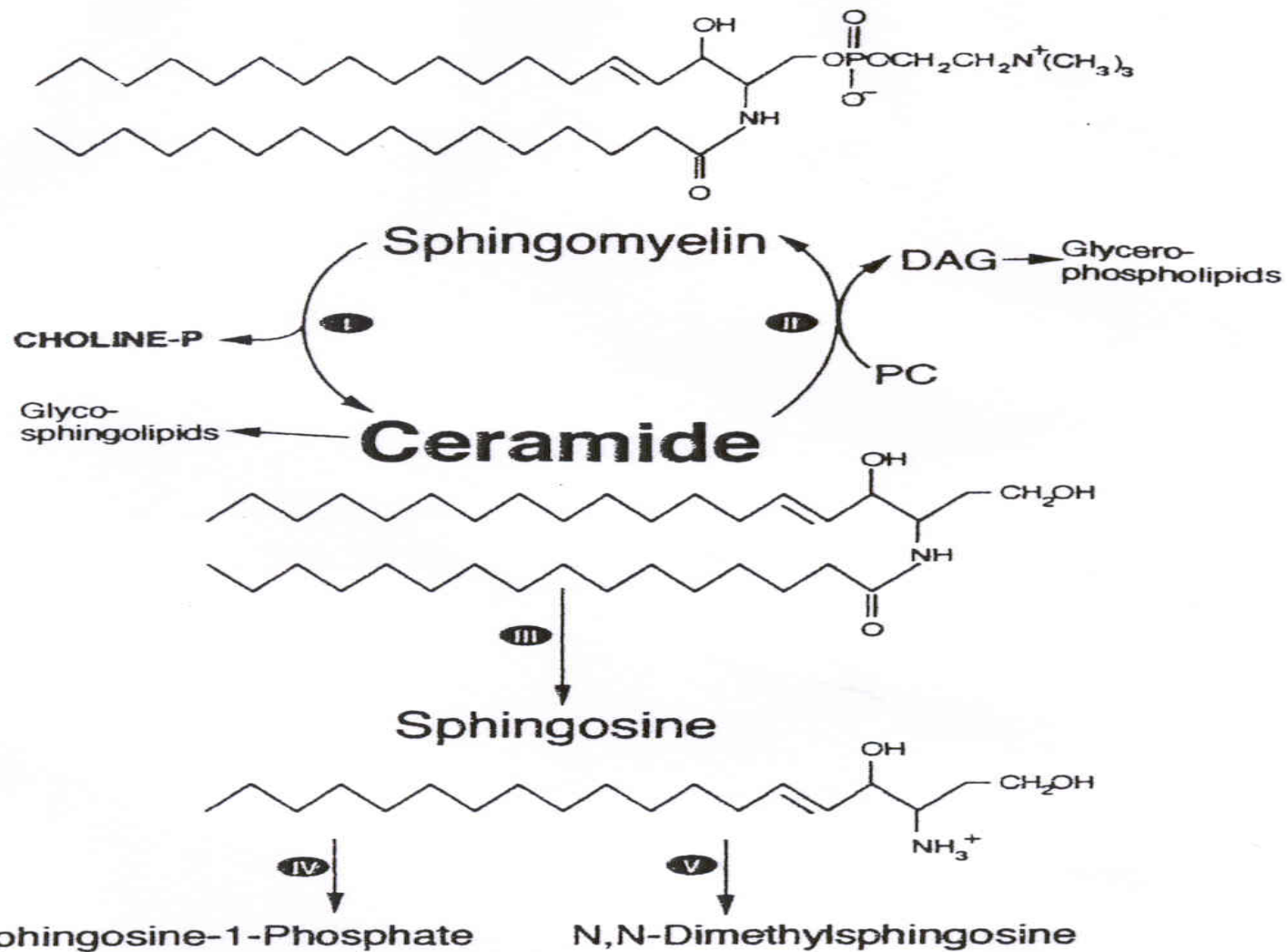


Fig. Ceramide metabolism. The central role of ceramide in sphingolipid metabolism. The enzymes involved in ceramide metabolism are: I, SMase; II, phosphatidylcholine:ceramide choline phosphotransferase; III, ceramidase; IV, sphingosine-1-kinase; V, transmethylase.

SLS Induces IL-1 and TNF α

