review

Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids

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Abstract This review article attempts to present an overview of the occurrence and function of lipid storage and secretory organelles: the lamellar bodies. Morphologically these organelles vary considerably in size (100 nm to 2400 nm); they are surrounded by a membrane and contain multilamellar lipid membranes. Lamellar bodies may also contain apolipoproteins and lytic enzymes and have an acidic pH, which confers on them a lysosomal character. Under normal physiological conditions, the main function of lamellar bodies is the supply of extracellular domains with specialized lipid components related to a specialized function. The lamellar bodies of the lung epithelium are best investigated in their functional and structural features and are the storage form of the lung surfactant. They provide a monomolecular lipid film of dipalmitoyl phosphatidylcholine (DPPC) on the surface of lung alveoli to lower surface tension necessary for optimal gas exchange and a hydrophobic protective lining against environmental influences. Additional cells of the respiratory system such as the mucosa of the human nose and the bronchia contain lamellar bodies. Lamellar bodies are also found in the gastrointestinal tract, in tongue papillae, oral epithelium, and mucosa cells of the stomach. The major phospholipid of lamellar bodies in mucosa cells of the stomach is DPPC, providing a hydrophobic protective lipid film against the tissue-damaging activities of gastric juice. The hydrophobic water-protective barrier of the skin, which consists mainly of neutral lipids, however, also originates from lamellar bodies secreted by epithelial cells. Lamellar bodies, mainly consisting of DPPC, also occur in mesodermal cell layers of sliding surfaces to provide the lubrication of joints, of the peritoneum, pericardium, and pleural mesothelium. In certain pathological conditions, such as atherosclerosis, Niemann-Pick disease, lecithin:cholesterol acyltransferase (LCAT) deficiency, cholestasis, degeneration of nerves and brain, and regeneration of nerves and wound healing, lipid-containing lamellar bodies have been observed in various cells, the function of which still remains to be elucidated. In early and late lesions of atherosclerotic plaques, lamellar bodies, consisting of unesterified cholesterol and phospholipids, are associated with the extracellular matrix of the intima. During regression of fatty streaks, lamellar bodies are seen intracellularly in macrophages and smooth muscle cells. Inherited metabolic disorders, such as Niemann-Pick disease type I and type II, result in the excessive accumulation of lamellar body-containing cells, for example in bone marrow, spleen, and lymphoid tissue. Type I is a deficiency in sphingomyelinase and type II is a defect in intracellular trafficking of lipoprotein-derived cholesterol. In both cases lamellar bodies that contain sphingomyelin and unesterified cholesterol accumulate. LCAT deficiency is characterized by a failure to esterify cholesterol in plasma. This leads to the formation of lamellar bodies containing unesterified cholesterol and phosphatidylcholine in spleen, bone marrow, histiocytes, and kidneys. In the plasma of these patients, lamellar vesicles called lipoprotein-X are also found, obviously resulting from the defect in normal processing of lipoproteins. During degenerative processes of nerves and brain tissue, formation of lamellar bodies may be due to defective lipid transport or autophagic activities of the cells. In regeneration of peripheral nerves and in wound healing, lamellar bodies in macrophages are probably intermediate lipid storage organelles. In summary, we conclude that lamellar bodies are lipid storage and secretory organelles, existing at least as four different types: *1*) those that are secreted, for example, by epithelial cells that serve a specialized role and function in the adjacent extracellular domains; *2*) intracellular lipid storage forms that may serve no specialized extracellular function; *3*) lamellar particles of extracellular origin derived from plasma lipids encountered in conditions such as LCAT deficiency or cholestasis; and *4*) pathologic accumulation of extracellular membrane. **—Schmitz, G., and G. Müller.** Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. *J. Lipid Res.* 1991. **32:** 1539–1570.

Supplementary key words lipoproteins • lipid droplets • multivesicular bodies • phospholipid lamellae • secretory vesicles • lung surfactant • keratinocytes • hepatocytes • bile canaliculi • cholesterol • phospholipids • atherosclerotic plaques • wound healing • lipidosis • macrophages • HDL • LCAT • apoE • lipoprotein-X • atherosclerosis • apolipoprotein

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; APF, anionic protein fraction; ARDS, Adult Respiratory Distress Syndrome; CDP, cytidine diphosphate; CTP, cytidine triphosphate; DPPC, dipalmitoyl phosphatidylcholine; EGF, epidermal growth factor; ER, endoplasmic reticulum; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; IL, Interleukin; IRDS, Infant Respiratory Distress Syndrome; LCAT, lecithin:cholesterol acyltransferase; LPC, lyso phosphatidylcholine; LDL, low density lipoproteins; LP-X, lipoprotein-X; MBP, myelin basic protein; MVBs, multivesicular bodies; PC, phosphatidylcholine; PDGF, platelet-derived growth factor; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; SPM, sphingomyelin; TGF, transforming growth factor; TNF, tumor necrosis factor; TGN, *trans* Golgi network; UC, unesterified cholesterol; VLDL, very low density lipoproteins; RXLI, recessive X-linked ichthyosis; NBD, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-6-aminocaproyl.

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I. LAMELLAR BODIES: DEFINITION AND DISTRIBUTION

Lamellar bodies are subcellular structures that have been detected by electron microscopy in various cell types under normal and pathological conditions. The best known and most intensively investigated lamellar bodies are produced by pneumocyte II cells in the lung alveoli to provide the lung surfactant system. In other tissues similar structures have been named lamellar granules, lamellar lysosomes, membrane-coating granules, Odland bodies, multilamellar lipid, secretory vesicles, and concentric membraneous structures, to mention only some. The purpose of this overview is to compare available biochemical data and putative biological functions of lamellar bodies of different cell types and to discuss whether they have a common biological role.

Screening the literature for the occurrence and function of lamellar bodies leads to the conclusion that lamellar bodies are specialized organelles determined to be a specifically regulated form for the storage and secretion of certain lipids with defined functions as demonstrated for the surfactant system of pneumocyte II cells of the lung, and for the waterproof "sealing layers" of epithelial cells of the skin, the hydrophobic lining of the gastric mucosa, and the lubrication of joints. Many other cell types of epithelial origin also produce lamellar bodies under normal conditions, but have not been intensively investigated. Lamellar bodies also occur in numerous pathological conditions where lipid metabolism is disturbed, e.g., in genetic abnormalities affecting lipid metabolism, secondary to other diseases, during wound healing, in degenerative processes in the brain or nervous system, and as a result of toxic drug effects. These findings suggest a more important and possibly a more general role for lamellar bodies than is widely accepted. Although we describe all kinds of multilamellated structures reported in the literature so far, we propose the following definition of lamellar bodies. Lamellar bodies are specialized lipid storage or secretory organelles that can be surrounded by a membrane and have a core composed of multilamellar membranes. They may contain lysosomal enzymes and apolipoproteins that are involved in the regulation of the functional and structural integrity of these lamellar bodies, and may be necessary for the specific role of these lamellar bodies in different tissues.

Our interest in lamellar bodies arose when we observed that, upon cholesterol-loading, macrophages form cholesterol- and phospholipid-containing lamellar bodies that may either originate from lysosomes or from cytoplasmic lipid droplets (1–3). This intracellular accumulation of lamellar bodies originating from cytoplasmic lipid droplets was previously observed when ACAT-inhibitors were added to macrophages (4).

On the basis of detailed electron microscopic studies and biochemical investigations performed in our laboratory with HDL-subclasses separated by preparative isotachophoresis, we propose the following model for the involvement of lamellar bodies and "slow" and "fast migrating" HDL-subclasses in cholesterol release from macrophages (see **Fig. 1**) (5–7). Two major routes exist by which macrophages can release excess cholesterol in addition to physicochemical exchange. Upon cholesterol loading, macrophages form cholesterol- and phospholipid-containing lamellar bodies that originate in lysosomes. These lysosomal lamellar bodies move towards the cell periphery, and the surrounding membrane fuses with the cell membrane to release their lipid content into the membrane or the extracellular medium. The formation of lysosome-derived lamellar bodies in cholesterol-loaded macrophages is promoted by dihydropyridine calcium antagonists and apoA-I/A-IV/LCAT-rich HDL particles that

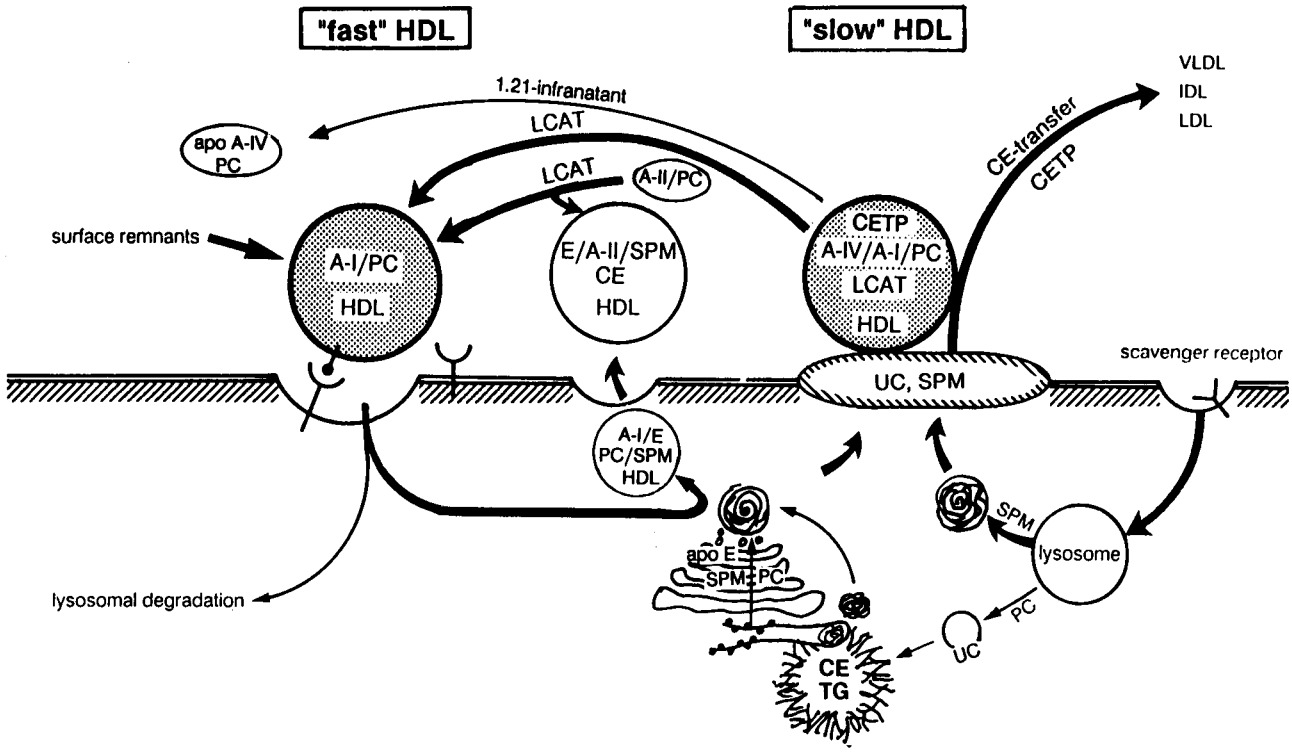

Fig. 1. Interaction of HDL subclasses with macrophages.

preferentially bind with a nonspecific component to the cell membrane and are obviously involved in their cellular release (5, 7). The second mechanism is assumed to be an HDL receptor-mediated cholesterol efflux where apoA-I-rich HDL particles bind with a specific component to a membrane-bound binding protein to take up cholesterol from lamellar bodies that are formed from cytoplasmic lipid droplets by involvement of ER and Golgi. Since these lamellar bodies are composed of cholesterol and phospholipids, we studied phospholipid metabolism in macrophages in detail (1) and demonstrated a strong correlation between cholesterol influx and efflux and the rate of phospholipid synthesis. The dihydropyridine calcium antagonist Nifedipine, which promotes the formation of lamellar bodies from the lysosomal route, down-regulates HDL binding and enhances sphingomyelin synthesis. In addition, an increase in the synthesis of apoE was observed, which may indicate that the formation and secretion of lamellar bodies is associated with ApoE (8).

In attempts to further understand the regulation of the intracellular movement or traffic of lamellar bodies, Tangier disease provides valuable new insights (7, 9–12). In Tangier disease, an inherited HDL deficiency syndrome intensively investigated in our laboratory, there is a cellular defect in the translocation of cellular cholesterol where HDL precursors are erroneously degraded in lysosomes, and the route of exchange of cholesterol from lamellar bodies originating from cytoplasmic lipid droplets in macrophages from Tangier disease patients is impaired. It is interesting to note here that several histochemical studies indicate (13–18) that in early atherosclerotic lesions a progressive accumulation of lipid particles rich in unesterified cholesterol appears extracellularly in the subendothelial space of the arterial wall. The extracellular lipid particles are distinct from cholesteryl ester-rich lipid droplets that accumulate in the cytoplasm of foam cells. Only recently these extracellular particles have been investigated for their chemical and structural properties (14–16). These particles consist of lamellar bodies which, from their lipid composition, were similar to lamellar bodies secreted by macrophages. Their peculiar feature is the unusually high ratio of unesterified cholesterol to phospholipid which is about 3:1 (see **Table 1**) (1). Many membranes, e.g., the normal plasma membrane, show only a ratio of about 1:3. It may be assumed that in addition to lipids these lamellar bodies are associated with

TABLE 1. Comparison of lamellar bodies (LB) secreted by MPM and LB found in human atherosclerotic lesions

	MPM (1,2)	Lesion (15)
Size	0.5–1.5 μm	0.07–0.3 μm
Density	1.21 g/ml	1.036 g/ml
Lipid composition		
UC	68%	59%
EC	8%	18%
PL	21%	23%
UC/PL	3.2:1	2.6:1

apolipoproteins and enzymes. However, further investigations are necessary.

II. LAMELLAR BODIES UNDER NORMAL PHYSIOLOGIC CONDITIONS

A. Surface coating and protection of the lung alveoli

1. The lung surfactant system

Lung surfactant is the most intensively investigated lamellar body system. Although it is not immediately obvious that lamellar bodies in other cells or tissues, e.g., in atherosclerotic lesions, have something in common with lung surfactant, a short survey of this field seems warranted, even though excellent review articles (19–23) are available. The secretory pathways for lipids as well as the intracellular traffic of lipids have, in general, remained a fairly esoteric branch of molecular cell biology when compared to the efforts directed towards the study of intracellular and extracellular protein transport and secretion. Insights into the most complicated assembly, secretion, and reutilization of lipid and protein components involved in the lung surfactant system could provide a guideline and model system for understanding the role of lamellar bodies in other cells just starting to attract attention.

The alveolar system of the lung is the largest surface of the body in contact with the environment and comprises ~120 m^2 in the phase of inspiration. It is composed of two types of epithelial cells, pneumocytes I and II (**Fig. 2**). Pneumocytes I cover 95% of the alveolar surface and are characterized by a flattened cell shape containing only a few organelles. The third type of cells present in the alveoli are macrophages, possibly involved in removal of surfactant, particles, and microorganisms invading from the air.

A specific morphological characteristic of lung pneumocyte II cells is the presence of lamellar bodies of 0.2–

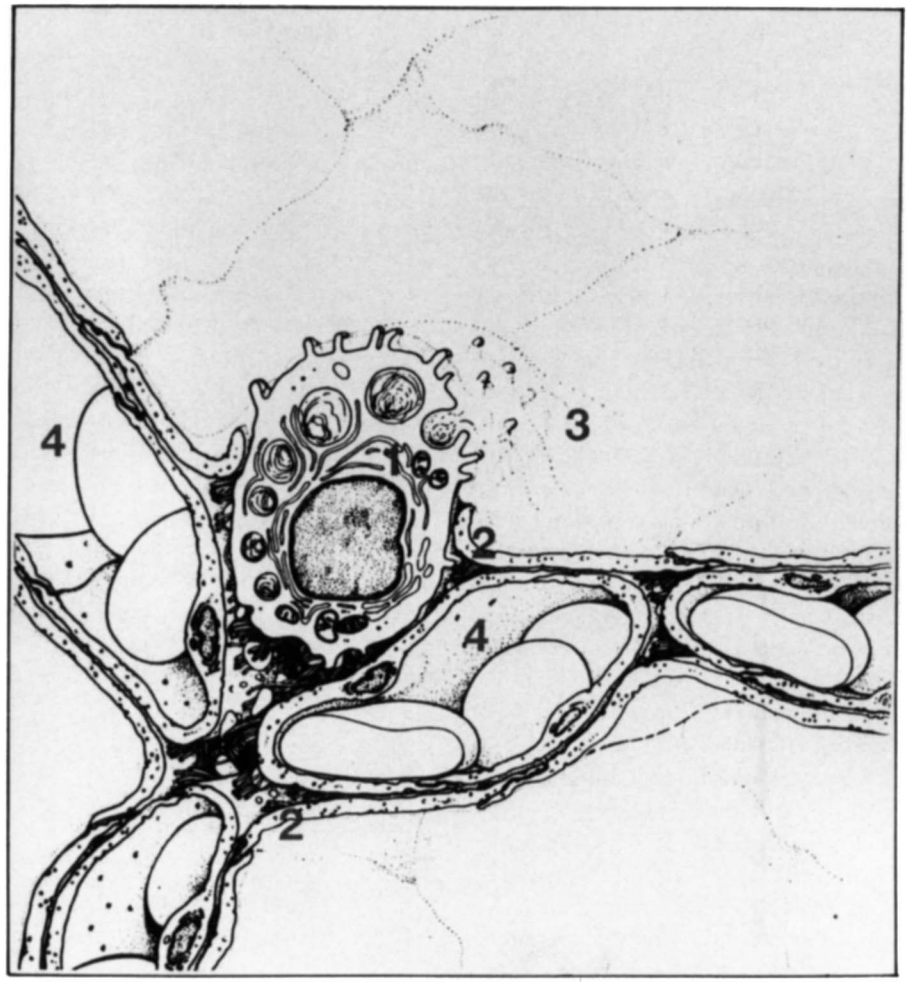

Fig. 2. Secretion of surfactant by pneumocyte II cells of lung alveoli. Pneumocyte II cell with lamellar bodies (1); pneumocyte I cell (2); surface area of lung alveoli with surfactant film (3); alveolar capillary (4). (With permission of K. Morgenroth (ed.) Das Surfactantsystem der Lunge. Walter de Gruyter Verlag, Berlin, 1986.)

2 μm diameter, which comprise 18–24% of the cytoplasm. These lamellar bodies are the storage form of lung surfactant which is composed of phospholipids and proteins that form a lipid monolayer at the alveolar air-liquid interface (21, 23, 24). Surfactant reduces surface tension along the alveolar epithelium and thereby maintains the integrity of the alveolus during respiration, thus allowing adequate gas exchange (25–27). In addition to its surface tension-reducing properties, surfactant may also aid in oxidant protection (28) and play a role in the defense mechanism of the lung against infection (29–32).

Surfactant is comprised of ~80% glycerophospholipid, ~10% cholesterol, and ~10% protein. The principal glycerophospholipid in surfactant dipalmitoyl phosphatidylcholine, is believed to be the major surface active component; the major apolipoprotein of pulmonary surfactant is a sialoglycoprotein SP-A (26–38 kDa) (20). It has been suggested that this major surfactant apolipoprotein, together with calcium, may play a role in the structural organization of tubular myelin. Tubular myelin (**Fig. 3**) is a lattice-like structure that represents the intermediate between the secreted lamellar body and the monomolecular surface film at the alveolar-air interface (33–35).

Evidence has been provided that surfactant is secreted at a fairly rapid rate: about 10–30% of the intraalveolar pool is replaced per h under resting conditions (36, 37). Images of lamellar bodies apparently undergoing exocytosis have been reported in electron microscopic studies (38, 39), which suggests that the limiting membrane that surrounds the lamellar body fuses with the plasma membrane of type II cells. Alveolar surfactant can be reinternalized into lamellar bodies and eventually resecreted (40–42). It has been estimated that between 25 and 95% of the alveolar surfactant enters this recycling pathway (**Fig. 4**).

2. *Pulmonary surfactant lipid components*

The lipid-protein composition of pulmonary surfactant from several mammalian species is very similar. **Table 2** shows the phospholipid composition of rabbit and human lung lavage surfactant. The major constituents are lipids, which make up 80–90% of its weight (19). Phosphatidylcholine (PC) and phosphatidylglycerol (PG) are the predominant phospholipids and make up 70–80% and 5–10%, respectively. About 70% of the phosphatidylcholine in surfactant is disaturated, with both fatty acids being palmitic acid. Small amounts of other phospholipids, triglycerides, free fatty acids, and cholesterol are also present. However, the only component in surfactant whose function is clearly understood is dipalmitoylphosphatidylcholine (DPPC), which is thought to be responsible for the lowering of surface tension and to provide alveolar stability because of its low surface compressibility. The choline residue of DPPC is polar and hydrophilic and associates with the liquid of the alveoli, while the palmitic acid residue is nonpolar and hydrophobic and points towards the air (43).

The enzymes required for the synthesis of surfactant phospholipids have been found in the microsomal and cytosolic fraction of type II cells (19, 44, 45). DPPC may be synthesized by at least two mechanisms: de novo synthesis from saturated diacylglycerols or by deacylation and reacylation of 1-saturated-2-unsaturated phosphatidylcholine involving phospholipase A_2 and acyltransferases (45–47). The rate-limiting enzyme in the synthetic pathway is CTP:cholinephosphate cytidylyltransferase. This enzyme is located both in particulate and soluble fractions and is associated with cytoskeletal actin (48) as has been found for several regulatory enzymes (49–53) and may be regulated by the state of microtubule and

Fig. 3. Electron micrographs of some of the structures found in surfactant. Left panel: lamellar bodies; center panel: vesicular structures; right panel: tubular myelin. The marker bar is 0.01 μm; magnification ×56,000. (By courtesy of M. C. Williams, University of California, San Francisco; ref. 34.)

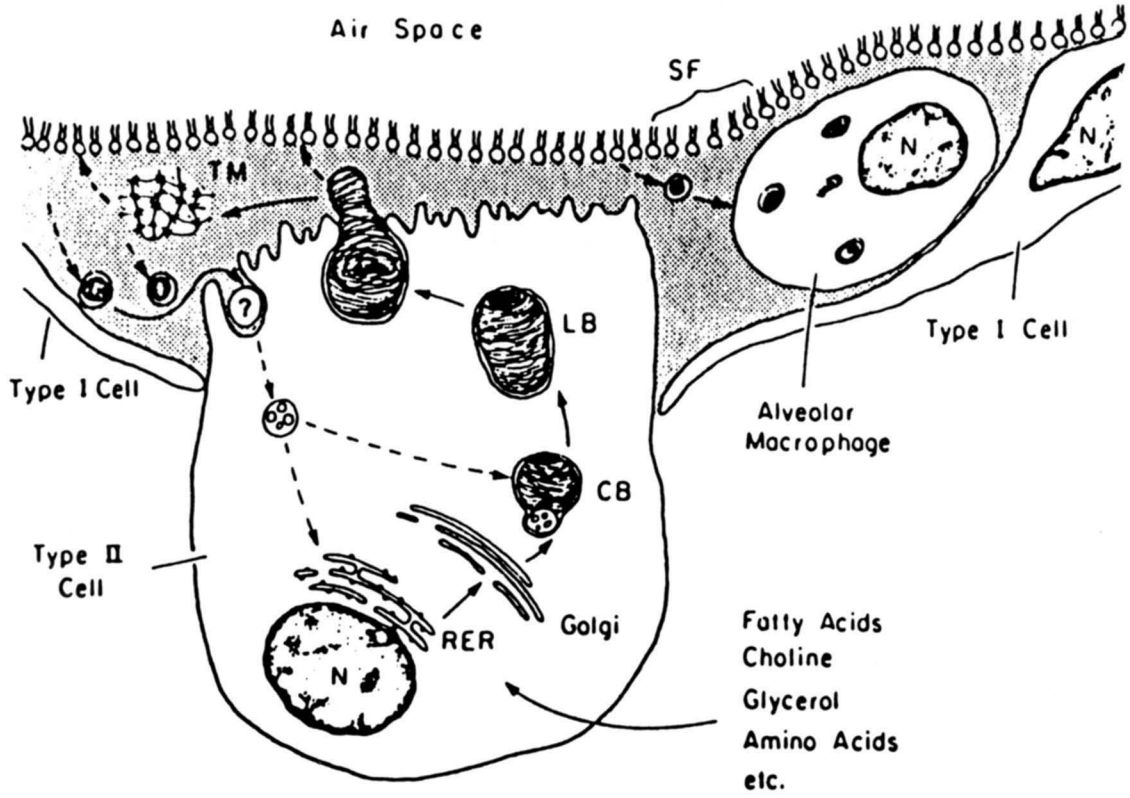

Fig. 4. A model of the metabolic pathways of lung surfactant. Nucleus, N; rough endoplasmic reticulum, RER; composite body, CB; lamellar body, LB; tubular myelin, TM; surface film, SF; vesicle, V. (By courtesy of J. R. Wright, University of California, San Francisco; ref. 20.)

microfilament assembly (48). Several studies have shown that cytidylyltransferase can be regulated by cAMP-dependent phosphorylation-dephosphorylation processes (54–56) as well as protein kinase C-dependent mechanisms (57, 58). These regulatory mechanisms are of special interest, since the secretion of lamellar bodies is also stimulated by agents that enhance cAMP formation and activate protein kinase C.

The site of phosphatidylglycerol (PG) synthesis is not well established. Phosphatidylglycerol and phosphatidylinositol (PI) are both synthesized from CDP diacylglycerol, and there is an inverse relationship between the amounts of PG and PI in lung surfactant (59–61). In most mammalian tissues, PG is synthesized in mitochondria and some studies have localized the enzymes in mitochondria as well as in the ER in type II cells (62, 63).

3. *Surfactant apolipoproteins*

The pulmonary surfactant system consists of heterogeneous membraneous particles that include intracellular lamellar bodies, tightly packed myelin figures, sheets of parallel membranes, large highly ordered membrane

TABLE 2. Phospholipid composition of lung lavage surfactant: comparison with lavaged lung tissue

Phospholipid	Lung Lavage[a]		Lung Tissue[a]
	Rabbit	Human	Rabbit
Phosphatidylcholine	80.9	67.5	50.5
Saturated phosphatidylcholine	50.5	49.7	18.6
Unsaturated phosphatidylcholine	30.9	17.7	31.9
Phosphatidylglycerol	7.1	10.0	1.7
Phosphatidylethanolamine	3.2	5.3	21.2
Sphingomyelin	1.4	4.0	10.9
Phosphatidylserine	0.5	1.6	7.8
Phosphatidylinositol	3.2	3.6	4.6
Others	3.8	8.0	4.2
Saturated as % of total phosphatidylcholine	61.6	73.1	36.6

[a]Percent total phospholipid phosphorus. (With permission of S. A. Rooney, Yale University, New Haven, CT; ref. 19.)

aggregates named tubular myelin, and vesicular structures of many different sizes (64–70). Analysis of the composition of partially purified fractions suggests that each of the surfactant structures has a similar lipid composition, but a distinctive profile of associated proteins and apoproteins (71–73).

Transformation from one structure to another occurs in the alveolar space. In particular, the rate at which the different surfactant structures transform to phospholipid surface films and their clearance rates from the alveolar space varies greatly (73–75). These observations and reconstitution experiments suggest that the apoproteins associated with the surfactant lipids may regulate the structure, biophysical properties, and metabolism of the various lipoprotein complexes. Four particular proteins are generally considered to be specific surfactant apoproteins and are referred to as SP-A, SP-B, SP-C, and SP-D according to the nomenclature recently proposed by Possmayer (76).

Surfactant protein A. Pulmonary surfactant apoprotein A (SP-A) is the major protein component of the surfactant complex. It is polymorphic (26–38 kDa), which is a consequence of posttranslational modifications, such as variable glycosylation (77–80). Data from physicochemical studies demonstrate that the protein can interact with phospholipids and the extremely hydrophobic proteins SP-B and SP-C associated with the surfactant complex to promote the rapid formation of stable surface films of phospholipid (33, 81–84). SP-A also inhibits the secretion of PC and facilitates the uptake of phospholipids by type II cells (73, 85) thus regulating surfactant pool size by balancing secretion and uptake. These interactions of SP-A imply the involvement of a specific receptor for SP-A on the cell surface (85). The secreted form of SP-A is a large multimeric protein with at least 18 monomeric subunits. The overall organization of SP-A is similar to the organization of C1q (86–88). Both proteins have a collagen-like stem and a flower-like arrangement of the globular carboxy-terminal domain. The intracellular processing of SP-A still remains unclear, but there exists some evidence that correct folding and stabilization of the triple helical structure of SP-A and perhaps full assembly of the 18 subunits of the protein in the ER or Golgi is required before further intracellular processing (89–92). Since lamellar body contents are derived from both synthetic and endocytic pathways, it is not easy to establish that SP-A is secreted in association with lamellar bodies. However, SP-A and DPPC are released into the alveolar space with similar kinetics; therefore it is assumed that surfactant lipids and SP-A are secreted in a coordinated fashion (93).

Pulmonary surfactant proteins B, C and D. The secretion of apoprotein SP-B has been determined to be independent of the secretion of lamellar bodies, though mature SP-B was clearly identified in association with lamellar bodies (94, 95). Two cDNAs for SP-B have been characterized and the primary translation product is a proprotein of ~40 kDa (96). The untranslated regions of the SP-B gene contain consensus sequences for both glucocorticoid and cAMP regulatory elements (97). The mature form of SP-B has a molecular mass of 7–8 kDa, and contains seven cysteine residues that might be important in stabilizing its conformation. SP-B shows a periodic distribution of polar and nonpolar residues characteristic for amphiphilic proteins associated with membrane surfaces at the air/water interface (98).

SP-C is a hydrophobic protein with an unreduced molecular mass of 4–5 kDa (99–101). Although the exact function of SP-C is not clear yet, similar to SP-B, the addition of SP-C to a mixture of phospholipids facilitates the adsorption of the phospholipid to an air-liquid interface. The cDNA nucleotide sequence and amino acid sequence of SP-C have been reported (102, 103), and in the N-terminal part two palmitoyl groups are thioester-linked to two adjacent cysteine residues (104). This long-chain acylation of SP-C may constitute a means for association of the protein with membranes and could conceivably modulate the stability and biological activity of surfactant films.

An additional collagenous glycoprotein SP-D (43 kDa) has been found associated with surfactant, the function of which remains to be elucidated (105).

4. *Regulation of lung surfactant production and clearance*

There are several possible pathways by which the secreted surfactant may eventually be cleared from the alveoli including ingestion by alveolar macrophages, movement up to the airways, enzymatic degradation at the alveolar surface, and uptake by type II cells and other cells. Alveolar macrophages activated in vivo and in vitro secrete a number of soluble factors such as TGF-α, TGF-β, PDGF, IL1, TNF, and γ-interferon that may participate in the complex interactions involved in surfactant turnover, growth of pneumocyte II cells, inflammation, tissue repair, and fibrosis (106–110).

Agents that stimulate adenylate cyclase and increase cytosolic cyclic AMP levels such as β-adrenergic agonists, catecholamines, adenosine, or cholera toxin stimulate the secretion of surfactant by type II cells (111–113). The involvement of microfilaments may be associated with surfactant release (114–117). Surfactant secretion in vitro is also stimulated by activation of protein kinase C (118). A number of hormones, including glucocorticoids, prolactin, thyroid hormones, insulin, and estrogen, have been reported to enhance the synthesis of glycerophospholipids of lung surfactant (118, 119). Recent findings show that the growth modulator TGF-β inhibits, while EGF stimulates the synthesis of SP-A in fetal lung (120, 121).

B. Hydrophobic protective barrier of the skin

Lamellar bodies of the skin (see **Fig. 5**) were originally named Odland bodies, membrane-coating granules, or keratinosomes (122, 123). They are 0.2–0.3 μm in di-

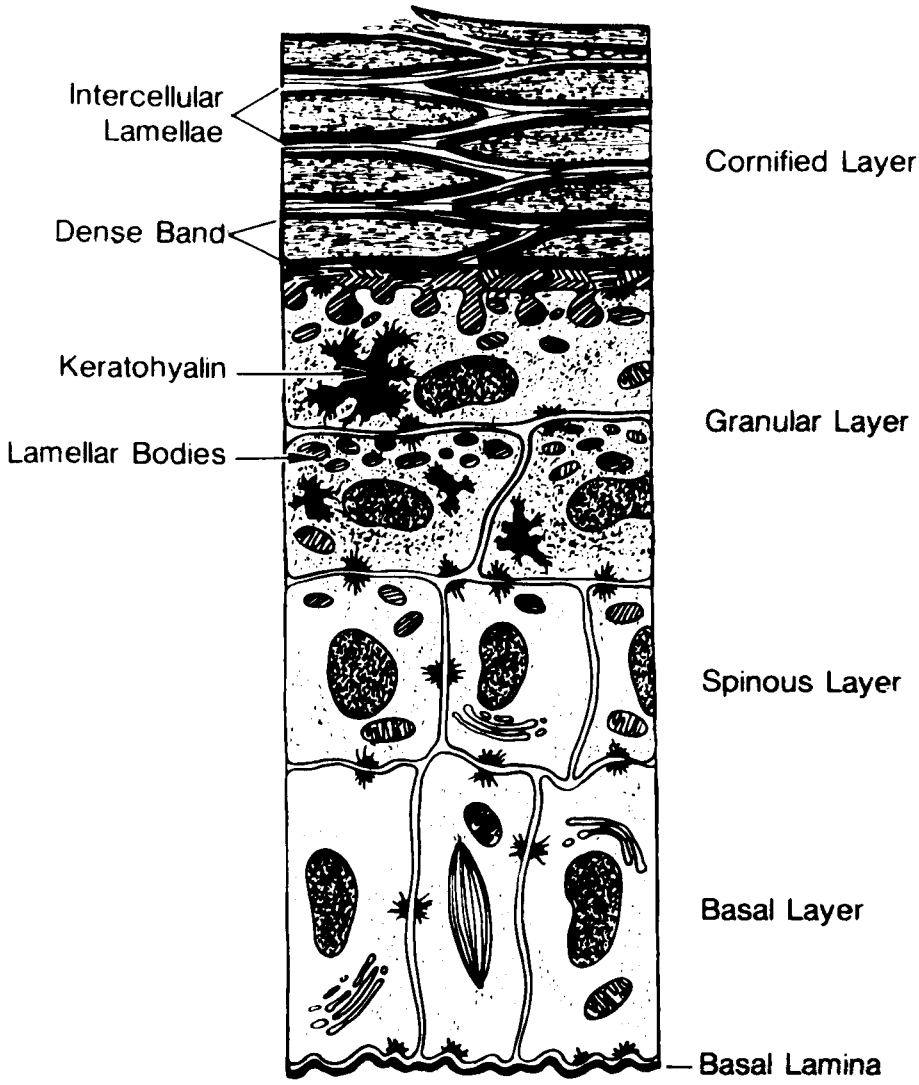

Fig. 5. Differentiation of epidermal cell layers and formation of lamellar bodies and intercellular hydrophobic barrier of the skin. (With permission of McGraw-Hill Inc., New York, F. B. Fitzpatrick et al., eds. 1987. Dermatology in General Medicine.)

ameter and are secretory organelles that are synthesized in the cornified layer of the epidermis. The role for this type of lamellar body is to provide special lipids to the intercellular space of the cornified layer (**Table 3**) which is responsible for barrier properties and desquamation of cornified cells (124, 125). Lamellar bodies have been isolated from the skin of neonatal mice and are enriched in phospholipids, sterols, and glycosphingolipids but not in other neutral lipids or ceramides (126). In addition, these lamellar bodies exhibit a lysosomal character and contain acid phosphatase, carboxypeptidase, cathepsin B, acid lipase, sphingomyelinase, and phospholipase A_2, but are strikingly depleted in all sulfatases and β-glucuronidase. Some of these enzymes could participate in the metabolism of lipid precursors of lamellar bodies to hydrophobic barrier constituents, while others may attack intercellular constituents, resulting in desquamation. However, the events that occur after secretion of the disc-shaped contents of these lamellar bodies into intercellular spaces are not yet elucidated. Elongation of discs begins to occur in the lower layers of the cornified layer, perhaps by end-to-end fusion. These processes are followed by subsequent formation of multilayered hydrophobic membrane bilayers that are found in the mid-to-outer cornified layer (127). These intercellular lipid domains are still in an active state of flux and several enzymes and even amphipathic glycoproteins may be involved. Several types of lipases localized in lamellar bodies have also been found in the intercellular domains of the cornified layer (128).

The co-localization of polar lipid precursors and nonpolar products with lipid catabolic enzymes, suggests that these enzymes mediate such a multi-stage sequence (see

TABLE 3. Variations in lipid composition during epidermal differentiation and cornification

	Layer		
	Basal/Spinous	Granular	Cornified
Polar lipids	44.5 ± 3.4	25.3 ± 2.6	4.9 ± 1.6
Cholesterol sulfate	2.6 ± 3.4	5.5 ± 1.3	1.5 ± 0.2
Neutral lipids:	51.0 ± 4.5	56.5 ± 2.8	77.7 ± 5.6
Free sterols	11.2 ± 1.7	11.5 ± 1.1	14.0 ± 1.1
Free fatty acids	7.0 ± 2.1	9.2 ± 1.5	19.3 ± 3.7
Triglycerides	12.4 ± 2.9	24.7 ± 4.0	25.2 ± 4.6
Sterol/wax esters[a]	5.3 ± 1.3	4.7 ± 0.7	5.4 ± 0.9
Squalene	4.9 ± 1.1	4.6 ± 1.0	4.8 ± 2.0
n-Alkanes	3.9 ± 0.3	3.8 ± 0.8	6.1 ± 2.6
Sphingolipids:	7.3 ± 1.0	11.7 ± 2.7	18.1 ± 2.8
Glucosylceramides	3.5 ± 0.3	5.8 ± 0.2	trace
Ceramides	3.8 ± 0.2	8.8 ± 0.2	18.1 ± 0.4

[a]Sterol/wax esters present in approximately equal quantities as determined by acid hydrolysis. (With permission of McGraw-Hill, Inc., New York. F. B. Fitzpatrick et al., eds. 1987. Dermatology in General Medicine.)

Fig. 6). The following model has been proposed (127). Initially, the phospholipase activity may generate lysophospholipids and fatty acids that could mediate the fusion of discs to form large structures. Ongoing phospholipase activity, in concert with other lipid catabolic enzymes, would then disperse residual phospholipid-maintained membrane bilayers into the broad membrane structures, highly enriched in nonpolar species. It could be shown by model membranes prepared from stratum corneum lipids with a composition of epidermal ceramides (55% by weight), cholesterol (25%), free fatty acids (15%), and cholesterol sulfate (5%) that multiple lamellae were formed without the presence of further proteins (129).

It may be assumed, by analogy with the lung surfactant system, that apolipoproteins in addition to lipid catabolic enzymes may have regulatory functions in the secretion and transformation of the epidermal lipid system. Recently, a nonenzymatic amphiphilic protein of a molecular mass of 25 kDa has been found to be associated with epidermal lamellar bodies (130). In addition, keratinocytes secrete high levels of apoE and it has been hypothesized that apoE might also serve a role in the development and maintenance of the lamellated structures located in the intercellular spaces (131). This hypothesis deserves consideration, since apoE plays not only a role in reverse cholesterol transport of the organism, but possibly may be involved in local cholesterol transport between cells during myelin generation and turnover (132, 133). For a more detailed discussion of the role of apoE in local lipid transport see section III. I.

C. Hydrophobic protective lining of the stomach

The luminal surface of mammalian stomach is uniquely hydrophobic, having non-wettable properties similar to that of inert surfaces such as Teflon (134). These properties of the gastric mucosal barrier are considered to be the intrinsic mechanism by which the stomach protects itself against autodigestion, which could occur by the presence of the highly active gastric juice. It has been found that gastric ulcerogens (i.e., acidified aspirin, bile acids, ethanol, and hydrochloric acid) rapidly convert the stomach from a non-wettable to a wettable state (135). Interestingly, intragastric administration of a mixture of extrinsic phosphatidylcholine (DPPC), which is also the major phospholipid in lung surfactant, can significantly reduce gastric necrosis and bleeding in rats when administered before an ulcerogenic dose of acid. However, the luminal surface hydrophobicity is also dependent on a surface mucous gel layer (136). Mucin, the principal glycoprotein binds large amounts of lipids, which are also a protection against oxygen radicals (137).

It is well known that bananas exert a strong protective function on the stomach during ulcerous diseases. Electron microscopic investigations revealed that the banana

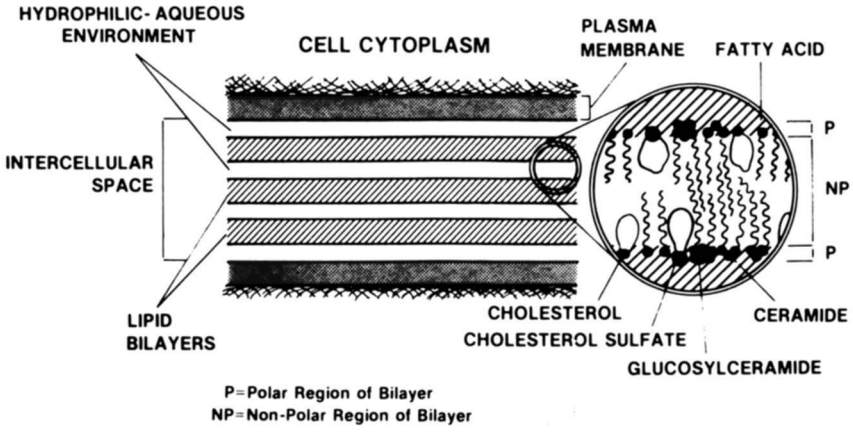

Fig. 6. Model of possible distribution of lipids within the intercellular spaces of the stratum corneum. (With permission of McGraw-Hill, Inc., New York. F. B. Fitzpatrick et al., eds. 1987. Dermatology in General Medicine.)

contains numerous lamellar bodies, the major constituent of which is DPPC (138), and that this surface-active phospholipid was responsible for the protective lining. Electron microscopic examination of mucous cells, especially the surface epithelial cells, revealed inclusion bodies consisting mainly of surface active phospholipids (139). These organelles were seen in the cytoplasm of these cells, some of which appeared to have a lamellar-like structure. Besides being observed intracellularly, intact lamellar-like structures and myelin-like coils were detected either directly associated with the plasmalemmal membrane of the surface of mucosa cells or already in the luminal space. Another interesting structure was observed, resembling the lipid surface film seen over the alveolar type II cells representing the monomolecular layer of pulmonary surfactant. Though further studies will be required to ascertain whether the observed diverse localizations of the inclusion bodies reflects the pathway of phospholipid biosynthesis, transport, and secretion by the gastric mucous cells, certain parallels to the lung surfactant system and to the protective lipid film of the skin are obvious. In view of the fact that lamellar bodies have also been detected in several other tissues of epithelial character such as kidney (140), oral epithelium (141), tongue papillae (142), organ of Corte (143), rabbit eustachian tube (144, 145), and the mucosa of the human nose (146), it may be proposed that lamellar bodies, consisting of a lipid pattern that is specialized for the functions of the organ, are organelles specialized for local lipid transport to the cell membrane or the extracellular space.

D. Sliding surfaces of joints and of serosal cells

Electron microscopic studies demonstrated lamellar bodies composed of PC on the articular surface and in synovial fluid. It has been proposed that synovial type A cells might be the producing cells. Oligolamellar phospholipid adsorbed to the articular surface contributes to joint lubrication (147). However, the apoprotein constituents and the mechanisms of release have not yet been studied in detail. Chronic inflammatory reactions of rheumatoid arthritis lead to changes of phospholipid content, accumulation of lipid peroxides, and lipid-laden macrophages in the synovial fluid (148, 149). In addition, concentrations in plasma HDL and vitamin E are considerably reduced (150, 151).

The lubricating function of phosphatidylcholine has also been found in sliding surfaces such as pericardium and pleural mesothelium (152, 153) and it remains to be seen whether lamellar bodies are also the source of PC in the epithelial layer of the serosa or the pleura. The mesothelial cell layer of the peritoneum has been characterized and shown to be carpeted with microvilli and motile cilia. Intracellularly, only ER and lamellar bodies are the predominant organelles. These lamellar bodies, which mainly consist of PC, are also secreted (154). A lubricating function for phospholipids has also been detected in lymph, thus facilitating lymph flow (155).

E. Lamellar phospholipid structures are major cholesterol carriers in bile

Since cholesterol is insoluble in water, certain cholesterol carriers are necessary for the solubilization (156). The general concept of cholesterol transport in bile that emerged from studies of model systems of the three biliary lipids cholesterol, bile salts, and phosphatidylcholine, led to the well-known triangular phase diagram (157). Its basic assumption was that cholesterol solubilization occurred mainly within mixed micelles and the solubility and precipitation depend on the relative proportions of the three lipids.

Several years ago, mixed micelles and phospholipid vesicles were shown to be important cholesterol carriers in human bile (158–161). Lamellar structures have been observed by some investigators using electron microscopy. They were variously interpreted as micellar aggregates (158), lipoprotein complexes (159), or artifacts (161). Recently, the three types of cholesterol carriers in bile were separated and characterized (162, 163). The typical phospholipid lamellae were seen by electron microscopy in great abundance in human gall bladder bile (**Fig. 7**). Small-angle X-ray scattering showed that sheets of lamellae and not multilamellar vesicles were present (164, 165). The main lipid components of the lamellated structures were cholesterol and phospholipids (63–91%) in a ratio of ~2:1, with only small amounts of bile salts (9–37 mol%) and proteins (0.06–0.3 wt%).

Fig. 8 shows the distribution of biliary lipids among the three separated lipid carrier species. The vesicular portions contained only 0–23% of the biliary cholesterol, the lamellar species 54–71%, and the micellar species 13–29% (162). Since phospholipid lamellae were shown to solubilize most of the biliary cholesterol compared to vesicles and micelles, it may be assumed that lamellar phospholipid aggregates are the main cholesterol carriers in bile. Reviewing the morphologic aspects of bile secretion from the liver reveals that the transport mechanisms are still unknown. Many substances are transported within the hepatocyte toward the pericanalicular zone in vesicles with an approximate diameter of 100 nm. No investigation has been reported as to whether vesicles containing cholesterol, bile acids, and phospholipids are formed inside the hepatocyte, at the canalicular membrane, or within the canalicular lumen. Addition of NBD-ceramide to isolated hepatocyte douplets leads to accumulation of NBD-sphingomyelin in the *trans* Golgi region only in the presence of taurocholate. This indicates that interaction of bile acids with phospholipids is important for the direction of these lipids to the secretory pole of the cells (166).

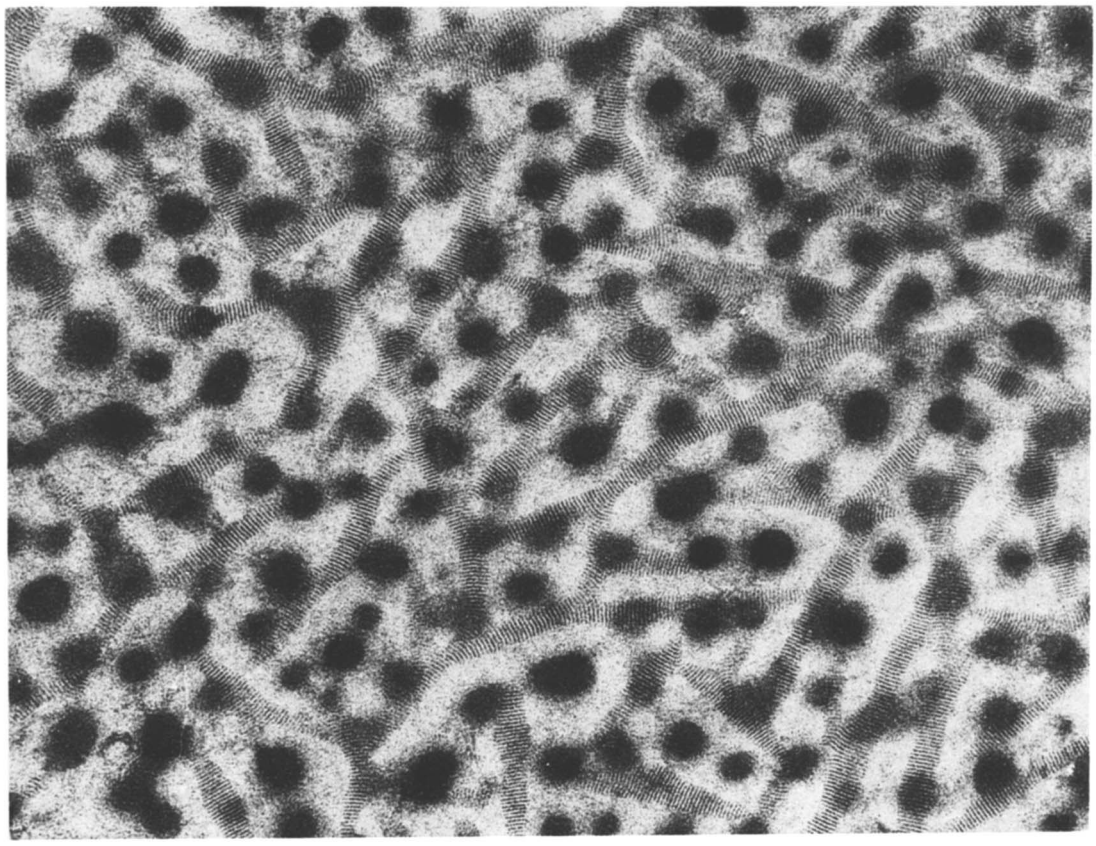

Fig. 7. Electron microscopy after negative staining of a fraction from the lamellar region of bile. Magnification ×243,000. (By courtesy of G. J. Sömjen, Tel Aviv University, ref. 162.)

Multivesicular bodies located in the bile canalicular pole of hepatocytes may also be intermediate organelles in the bile secretory pathway (167, 168). They contain endocytosed remnants of triglyceride-rich lipoproteins that are enveloped by additional bilayer vesicles. There is also evidence that in vivo reverse cholesterol transport from liver endothelial cells to parenchymal cells and bile is mediated by HDL (169). Radioactively labeled cholesterol from HDL, as well as apoA-I and anionic protein fraction (APF) associated with HDL, have been found in bile (170).

III. LAMELLAR BODIES IN DISEASE

In this chapter various diseases are described where the normal lamellar body production is either affected or where lamellar bodies occur due to a disturbance of lipid metabolism or lipid traffic. The various defects occurring at different sites may contribute to a deeper understanding of the importance of lamellar bodies.

A. Diseases affecting the lung surfactant system

Infant respiratory distress syndrome (IRDS) is the most common cause of neonatal morbidity and mortality. This disease of prematurely born infants is due to a primary failure of pneumocyte II cells to produce lamellar bodies (20). Obviously, the ability of these cells to produce lung surfactant is dependent on the stage of differentiation of the cells. It could be shown in isolated pneumocyte II cells that with progressing dedifferentiation of the cells in culture, the synthesis of lamellar bodies decreases. The lung is exposed to increased oxidative stress by inflammatory reactions of cells and by exposure to environmental pollution, such as ozone or smoke. Therefore, the lung has a huge potential for antioxidant protection, intracellularly by certain enzymes, extracellularly by the lung surfactant system (for review see 28).

The importance of the structural and functional integrity of the lung surfactant system and its role in antioxidant protection is also obvious in adult respiratory distress syndrome (ARDS). In addition, it provides evidence for a physiological interdependence of plasma lipoprotein levels and lamellar body production. Current concepts of the mechanisms leading to this respiratory failure have been reviewed (171–174) and imply that endothelial injury of alveoli is possibly caused by different factors such as endotoxins (175) or the involvement of neutrophils, platelets, and monocyte-derived macrophages and their secretory products (173, 176). The initiating insult is followed by

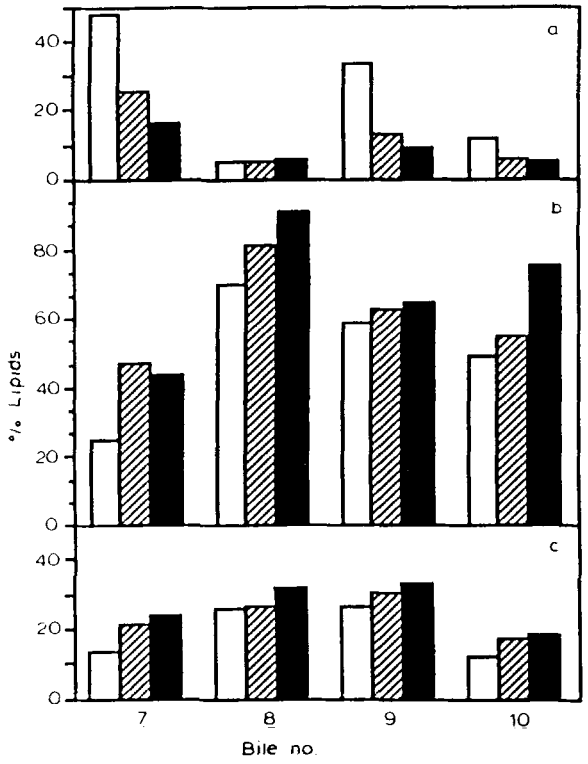

Fig. 8. Distribution of endogenous lipids of bile in the vesicular (a), lamellar (b), and micellar (c) regions; cholesterol (white); phospholipids (hatched); bile salts (black). (By courtesy of G. J. Sömjen, Tel Aviv University, ref. 162.)

permeability changes of endothelial cells, which lead to a leakage of plasma components such as fibrin monomers into the intraalveolar space and concomitant infiltration of cells (176–180).

Interestingly, fibrin monomers seem to directly interact with the phospholipid films; the mechanism, however, is unclear (181, 182). Changes in alveolar phospholipid composition result in decreased lipid fluidity (183, 184). As described above, ARDS is associated with increased oxidative stress caused by toxic oxygen radicals and it has been found that this is paralleled by abnormally low plasma concentrations of ascorbate and α-tocopherol (185) as well as increased levels of lipid peroxidation products (186).

There is also significant decrease in the plasma levels of total cholesterol and cholesteryl esters (187), concomitant with the appearance of discoidal particles in the HDL lipoprotein fraction, similar to that seen in familial LCAT deficiency indicating a decrease in LCAT activity (see chapter III. F.). Moreover, the plasma HDL-cholesterol levels in severely affected ARDS patients frequently fall below 5 mg/dl.

B. Psoriasis

Psoriasis is a common skin disorder in which the most important features are epidermal hyperproliferation and abnormal differentiation, associated with an inflammatory process. Psoriatic lesions have been used as a model in which to study benign cell hyperproliferation in relation to defects of intracellular messengers and incomplete differentiation (188). Psoriatic keratinocytes display a shortened S-phase of the cell cycle with an increased number of cells in the proliferative pool, which results in three layers of proliferating cells in the epidermis in contrast to one layer in normal skin (189). This leads to the formation of more cells containing lamellar bodies. The secretion of lamellar bodies, however, seems to be impeded: only few are found in the intercellular space, and most lamellar bodies remain intracellular in the cornified cells (123). Psoriasis could be an interesting model of the influence of intracellular messengers and differentiating agents on the production and secretion of lamellar bodies in relation to inflammation.

In psoriatic fibroblasts and red blood cells, the capacity of the regulatory subunits to bind cAMP seems to be defective (190). As a consequence fewer proteins are phosphorylated as compared to normal cells. A further defect in the intracellular signal transducing system as a primary or secondary event may be seen in elevated expression of the *ras* oncogene product *ras* p21 (191).

Several investigations (192–194) indicate a disturbance of lipid metabolism in these cells, which may be induced by the above-mentioned defects in the intracellular messenger systems. The membrane fluidity is much higher than in normal cells (194), and the free cholesterol content and the molar ratio of free cholesterol to phospholipid decrease significantly. Interestingly, hyperproliferative psoriatic cells, in contrast to normal keratinocytes, show abundant binding of low density lipoprotein (LDL) when LDL-gold is used as an ultrastructural marker (195), presumably reflecting different requirements for exogenous cholesterol.

C. Ichthyosis

Recessive X-linked ichthyosis (RXLI), an uncommon genetic disorder of cornification, is characterized by excessive scaling, due to prolonged retention of stratum corneum (196), and corneal opacity. RXLI is an excellent example of how lipids from lamellar bodies and interactions with enzymatic processes regulate the ordered desquamation of the skin. Patients with RXLI lack the enzyme steroid sulfatase (197) in skin and other tissues and accumulate cholesterol sulfate in serum, erythrocyte membranes, and the cornified layer (197–202).

In normal skin, cholesterol sulfate is stored and secreted by lamellar bodies. Steroid sulfatase is localized in the intercellular domains of the epidermis and continuous enzymatic degradation of cholesterol sulfate occurs in normal epidermis, thereby leading to a successive reduction in the cohesive forces of corneocytes, allowing a regulated desquamation. Important implications may be de-

rived from the comparison of normal differentiation of epidermis and ichthyosis.

Harlequin ichthyosis, another phenotype of ichthyosis, is characterized by abnormal expression of keratin and fillagrin and, in addition, lamellar bodies and intercellular lipids within the cornified layer are absent or abnormal (203). There is evidence that Harlequin ichthyosis belongs to a genetically heterogeneous group of disorders. It has been hypothesized that lamellar body production and secretion is related to the expression of differentiation markers such as structural proteins.

D. Fatty streaks and atherosclerotic plaques

There are numerous review articles covering the pathogenesis of atherosclerosis (204–209) and since the scope of this part of our article is limited to the role of lamellar bodies in atherosclerosis, only a short introductory overview is presented here.

The earliest lesion in the development of atherosclerosis is the fatty streak, which can be induced in animal models after 2 weeks of a high-fat, high-cholesterol diet. This stage is characterized by the accumulation of subendothelial macrophages that transform into foam cells. Cholesterol may also accumulate in smooth muscle cells that migrate from the media to the intima, thus increasing the severity of the lesions. Fatty streaks may easily regress by changes in diet and other factors, or they may be the precursors of fibrous plaques at older ages or upon progression of the lesion. Fibrous plaques are considered as the advanced stages of atherosclerosis and are characterized by an increased number of smooth muscle cells also transformed to foam cells and surrounded by connective tissue. The endothelial cells may still be intact or disrupted and the underlying tissue may be exposed to the circulation, resulting in platelet aggregation. The exact sequence of events leading to monocyte infiltration and foam cell formation and the conversion of fatty streaks into fibrous plaques is not yet clear.

Exposure of arterial walls to irritating stimuli such as high cholesterol levels may induce an inflammatory response in the arterial wall resulting in increased oxidative reactions of macrophages and endothelial cells. Recently, high levels of oxidatively modified LDL have been identified in atherosclerotic lesions in vivo (210–212). Oxidized LDL stimulate monocyte endothelial interactions (213) and may be ingested by macrophages via the scavenger receptor pathway. Massive intracellular and extracellular deposits of cholesterol, cholesteryl esters, and phospholipids are associated with plaques. Several investigations led to results suggesting a dynamic state of physical and metabolic interconversion of these extracellular lipid deposits during development of atherosclerosis (16, 18). During the first 2 weeks of a diet-induced hypercholesterolemia in rabbits, a continuous deposition of lamellar bodies in the vessel intima was observed. These lamellar bodies are rich in phospholipids and cholesterol and occur prior to monocyte infiltration. The data suggest that endothelial cells take up dietary cholesterol and transfer it to the intima by transcytosis (14). Increased synthesis and accumulation of phospholipids in the initial atherosclerotic lesions (214) have been considered to take part in the defense mechanism of the arterial wall against the sclerogenic effect of cholesterol. In early stages of atherosclerosis two types of extracellular lipid particles exist: lipid droplets enriched in esterified cholesterol and lamellar bodies enriched in unesterified cholesterol (15–18). Since these extracellular lipid droplets are 6 times smaller than those found in the cytoplasm of macrophages, it has been hypothesized that these lipid droplets originate from interaction of LDL with extracellular matrix components from the vessel wall such as elastin and proteoglycans (215–217). From the occurrence of "pits" and "blebs" in lipid droplets it was inferred that an extracellular hydrolysis of cholesteryl esters might take place (215). In spite of major differences in chemistry and structure, lipid droplets and lamellar bodies have certain features in common. They are similar in size (40–200 nm), have a molar ratio of UC/PL of 2.5:1, and sphingomyelin is the predominant phospholipid. The presence of substantial amounts of lysophosphatidylcholine (LPC, 7–9%) is also remarkable. Recently, it has been reported that LPC is a major chemotactic lipid component that may attract monocytes to infiltrate the arterial wall during early stages of atherogenesis (218). LPC also inhibits vascular relaxation in atherosclerotic vessels (219).

During regression of atherosclerotic lesions in a group of monkeys fed first with high- and then with low-cholesterol diets, accumulation and degradation of intracellular lipid droplet inclusions were accompanied by characteristic ultrastructural changes within the cell. During the early phase of regression, the accumulated lipid droplets start to shrink and are transformed into multilamellated secondary lysosomes and postlysosomes (208). The phase of late lesion regression is characterized by the absence of macrophages. Only smooth muscle cells with similar inclusions as macrophages and extracellular lamellar bodies and lipid droplets persist. The origin of lipid droplets and lamellar bodies remains to be elucidated, but our own results indicate that these lamellar bodies might resemble secretion products of cholesterol-loaded macrophages (1, 2). Secretion of lamellar bodies in macrophages in vitro is associated with increased secretion of apoE. A significant increase in mRNA expression of apoE has also been found in human atheroma (220).

E. Niemann-Pick disease

The Niemann-Pick group of diseases, excellently reviewed by Spence and Callaghan (221), are sphingomyelin-cholesterol lipidoses that are characterized by excessive foam cell formation and accumulation of lamellar bodies.

On the basis of etiology, these diseases may be classified in type I and type II forms, both of which are inherited by autosomal recessive mechanisms.

Type I. The metabolic defect in type I Niemann-Pick disease is a deficiency of lysosomal sphingomyelinase, which degrades SPM to ceramide and phosphocholine. This leads to excessive accumulation of SPM, which is stored in lamellar bodies within lysosomes. In type I disease a massive SPM accumulation has been found in liver and spleen tissue, and to a lesser extent in lymph nodes, kidney, lungs, and blood plasma. The molar ratio of SPM to cholesterol varies from organ to organ and between various phenotypes. In general, patients with type I disease tend to have higher ratios of SPM to cholesterol, for example 1.5–2.5 in liver compared to normal values of 0.2–0.3 (222). Increased levels of sphingomyelin always lead to an increase in unesterified cholesterol and vice versa, since SPM bilayers retain inserted cholesterol by forming tight complexes (223–228).

Apart from biophysical interaction, there are also several hypotheses about the interference between cholesterol and SPM metabolism. Incubating normal fibroblasts with cholesterol decreases SPM catabolism in intact cells and reduces sphingomyelinase activity (229). In macrophages loaded with acetyl LDL, an initial increase in SPM and PC concentration concomitant with an increase of free cholesterol was observed (1, 2). Taken together, these findings suggest a close relationship between SPM and cholesterol in vivo at a molecular and functional level in the membrane. It is not surprising, therefore, that mutations affecting the metabolism of either lipid could alter the metabolism of the other and cause clinical phenotypes with many similarities. The unusual sphingomyelin accumulation in type I cells certainly leads to the accumulation of unusual metabolites such as sphingosine phosphocholine, which may additionally lead to progressive cellular dysfunction by interference with protein kinase C and mitochondrial membranes (230–232). The possible interrelationship of sphingomyelin metabolism and the relevance to signal transduction pathways and formation of lamellar bodies is shown in **Fig. 9** and reviewed in ref. 233.

Type II. Niemann-Pick disease type II is an autosomal-recessive neurovisceral lipid-storage disorder. The etiology of this disorder is not known and is not related to a primary deficiency of lysosomal sphingomyelinase, as are type I Niemann-Pick diseases. It was shown that LDL-cholesterol was abnormally sequestered in mutant fibroblasts, which resulted in the excessive accumulation of lamellar bodies composed mainly of unesterified cholesterol which represent secretory vesicles rather than lysosomes (234). This is underlined by the observation that abnormal storage of cholesterol occurs preferentially in the *trans* Golgi region. The defective regulation of cholesterol metabolism in type II cells appears to be specific for the lysosomal route of LDL-derived cholesterol (235). Although LDL is bound and internalized, LDL receptor activity is impaired. LDL does not stimulate cholesterol esterification or suppress cholesterol synthesis (236). Cholesterol derived from sources other than LDL are not subject to these defective pathways. Obviously, the movement of LDL-derived cholesterol from lysosomes to other cell membranes is impaired or delayed in type II cells (237–239).

Interestingly, imipramine, a drug known to induce lipidosis by excess accumulation of lamellar bodies, was shown to induce similar abnormalities in cellular processing of exogenous LDL-derived cholesterol as found in type II cells, even leading to excess accumulation of cholesterol in the Golgi (240). Processing of endogenously derived cholesterol was not affected. The mechanism of action of this drug may lead to the detection of a key regulatory function important for the processing of lysosomal cholesterol.

F. Familial LCAT deficiency, Fish Eye disease, and cholestasis

LCAT deficiency is characterized by failure of LCAT to esterify cholesterol in plasma. These patients show heterogeneous tissue and plasma lipoprotein abnormalities as reviewed by Norum, Gjone, and Glomset (241). The clinical symptoms include anemia, corneal opacities, and frequently proteinuria and early atherosclerosis. The formation of excess lamellar bodies in spleen, bone marrow, histiocytes, and kidney containing unesterified cholesterol and phosphatidylcholine is well established (242–244). Electron microscopy of the kidney revealed that arterioles and arteries have thickened intimas and subendothelial deposits of cholesterol and phospholipids, and the capillaries are filled with lamellar membranes. These accumulations are accompanied by fibrosis and hyalinization of arterial walls (245, 246). The general development of early arteriosclerosis with calcification in the aorta has been documented in some LCAT-deficiency patients before the age of 40 (247).

The plasma lipoprotein abnormalities involve all lipoprotein classes and affect shape, distribution, and concentration (241). Our interest is especially focused on the appearance of lipoprotein particles that are unusually shaped and of multilamellated structure in plasma. In LCAT deficiency VLDL appear to vary in size, phospholipid composition, and apolipoprotein content (248). The appearance of unusually large particles in the fractions of intermediate density lipoproteins IDL and LDL_1 (249) is very striking. LDL_2 also frequently includes large particles. Upon gel filtration through 2% agarose, three subfractions are obtained. The particles of 90 nm diameter have multilamellar structure and contain unesterified cholesterol and PC in the unusually high molar ratio of 2:1. The width of these lamellae is identical to cholesterol/PC bilayers formed in vitro (250, 251). A second subfraction of LDL_2 of size 30–80 nm has a disc-shaped

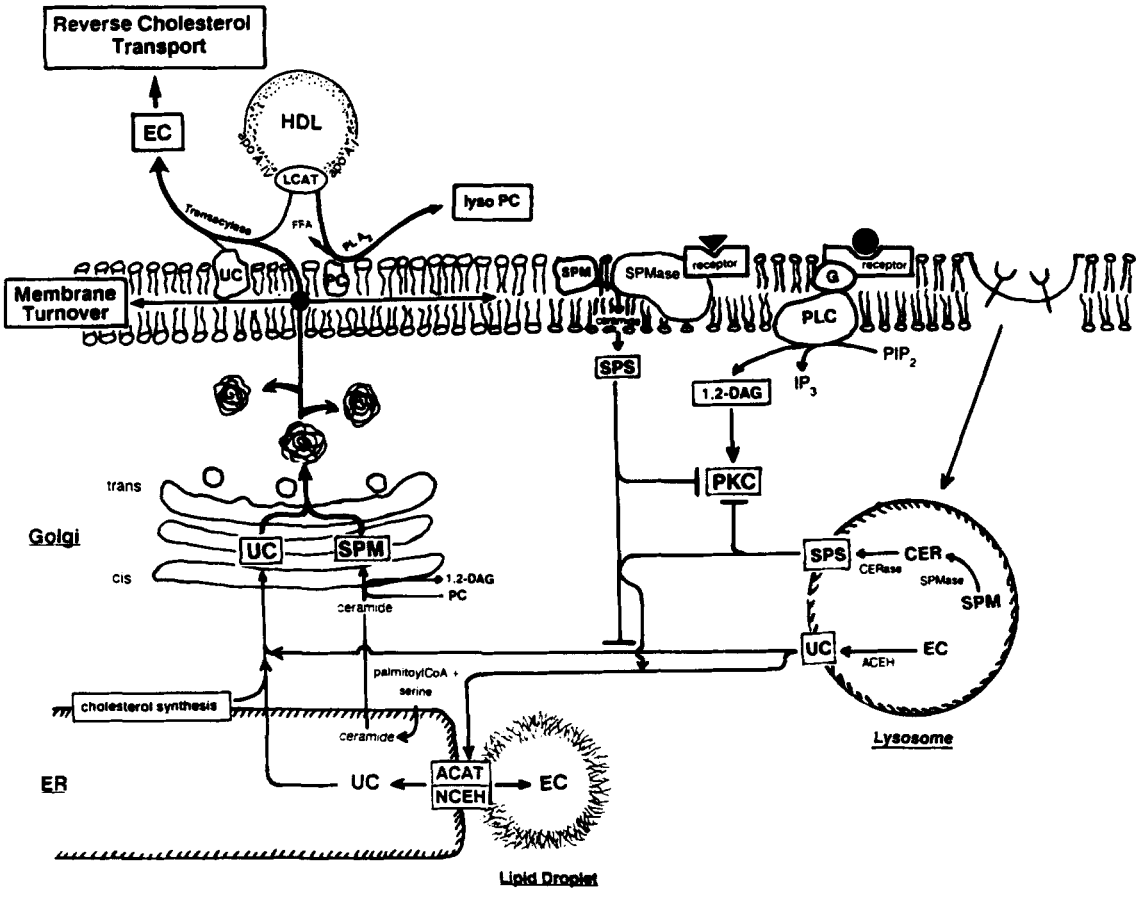

Fig. 9. Interrelationships among the steps of sphingomyelin metabolism, and their possible relevance to signal transduction pathways, which may interfere with the formation of lamellar bodies. Shown are the major intracellular compartments thought to participate in sphingomyelin metabolism and some reactions therein. Formation of the ceramide backbone begins in the endoplasmic reticulum with the condensation of serine and palmitoyl-CoA. Addition of the headgroup of sphingomyelin (SM) occurs in the Golgi apparatus and plasma membrane by transfer of phosphocholine from phosphatidylcholine (PC) to ceramide (cer), presumably releasing diacylglycerol (DG). Plasma membranes additionally contain sphingomyelinase (SMase) and ceramidase activities, and hydrolyze endogenous SM to sphingosine (SPS). Sphingosine is also released by lysosomal hydrolysis of SM and can be degraded to *trans*-2-hexadecenal plus ethanolamine phosphate in the cytosol, or perhaps moves to other locations in the cell. Some of these reactions appear to overlap with known lipid second messenger systems, viz., the receptor-mediated stimulation of phospholipase C (PLC) to hydrolyze phospholipids (PL) to diacylglycerols that activate protein kinase C. Recent studies have shown sphingosine to be a potent inhibitor of protein kinase C, and to affect other cell systems; however, it is unclear whether or not the plasma membrane sphingomyelinase is controlled by a receptor-coupled mechanism. (By courtesy of A. H. Merrill, Jr., changed version, ref. 233.)

appearance and often forms stacks (252). Similar particles (LP-X) have been found in cholestasis (253). Their apoC-I content is very high and the lipids are mainly unesterified cholesterol and phosphatidylcholine (254). HDL from these patients with LCAT deficiency include particles of disc-like shape and spherical particles that are unusually small in size (255–257). All patients have high concentrations of plasma unesterified cholesterol and phosphatidylcholine and low concentrations of plasma cholesteryl esters and lysolecithin, which may be due to the phospholipase A_2-inhibitory action of apoC-1. Cholesteryl esters contain abnormally high proportions of palmitic and oleic acids and an unusually low proportion of linoleic acid (258).

LCAT deficiency is a good example of how a primary metabolic shift, namely an increase in unesterified cholesterol, leads to many secondary, possibly compensatory, effects such as increased accumulation of tissue phospholipids that may function as a natural carrier of cholesterol.

Severe lens opacities consisting of membraneous deposits of excess unesterified cholesterol and phospholipids, besides other symptoms, are characteristic for Fish Eye disease (259–262). Plasma lipoprotein abnormalities in Fish Eye disease include increased amounts of triglycerides in whole plasma, VLDL, and LDL. HDL concentration is reduced by 90% and is mainly HDL_3 with a high concentration of unesterified cholesterol. Plasma fractions of patients with Fish Eye disease are able to esterify VLDL and LDL cholesterol but not HDL cholesterol. This leads to the conclusion that two different LCAT activities are present in plasma. In Fish Eye disease there is a lack of α-LCAT activity, while inheritable LCAT deficiency is devoid of α- and β-LCAT activity (263, 264).

In patients with cholestatic liver disease due to either biliary obstruction of certain infective diseases, a charac-

teristic elevation of unesterified cholesterol and phospholipids is due to the presence of an abnormal lipoprotein (LP-X), a lamellar body-like structure similar to that found in familial LCAT deficiency. During biliary obstruction bile passes from the bile duct to the hepatic lymphatics, from there to the thoracic lymphatic duct, and finally into the bloodstream. The lipid composition of LP-X isolated from serum shows great similarity to the lipids found in normal bile (265). In vitro, bile may be converted to LP-X by addition of serum albumin (251). LP-X in LCAT deficiency and biliary are of different origin and may differ in the composition of their apolipoproteins. However, the high ratio of UC/PL, in combination with certain surface and core proteins, seems to be a prerequisite for the formation of these lamellar bodies in plasma.

G. Lens and corneal opacities

Retinitis pigmentosa is a retinal dystrophy inherited as a dominant recessive or X-linked disorder, often accompanied by cataracts (266). The membranes of the photoreceptors exhibit an extremely high content of long chain polyunsaturated fatty acids and docosahexanoic acid (22:6), which are susceptible to oxidation by molecular oxygen (267).

As soon as replacement of lipid peroxidation products of these phospholipids is retarded, these polar substances enter the vitrious body and accumulate in the lens of the eye. The accumulation of these deleterious lipids together with other metabolic changes leads to formation of multilamellar lipid membranes in the lens, thus contributing to the development of cataracts (267, 268).

Corneal opacities with similar multilamellar lipid inclusions have been reported in LCAT deficiency, Fish Eye disease, and X-linked ichthyosis (263).

H. Degenerative processes of the nervous system

It is well known that during degenerative processes in peripheral nerves lamellar bodies may occur, as is reviewed in ref. 269. These lamellar bodies originate from lipids of intracellular organelles of the axon. Interestingly, with increasing numbers of lamellar bodies, concomitant disordered arrangement of tubuli has been observed. Certain degenerative diseases and increasing age are correlated with these pathologic changes. Long-term exposure to electromagnetic fields caused occurrence of lamellar bodies and strong cytoskeletal alterations (270).

Lamellar bodies have also been described in the granular layer of the cerebellum of people suffering from the unconventional slow-virus disease of Kuru, and lamellar bodies were demonstrated in mice and hamsters infected with scrapie (271) which belongs to the same group of diseases. Furthermore, degenerative processes of Purkinje cells and so-called "grumose degeneration" of the dentate nucleus are associated with lamellar body formation (272). Ultrastructural changes in the cerebral cortex of the cat after anoxia revealed hypertrophy of Golgi apparatus and formation of lamellar bodies (273). Lamellar bodies have also been proposed as markers of normal cholinergic neurons in ferret nucleus basalis (274).

I. Lamellar bodies in the regeneration of nerves

Regeneration of the peripheral nerve is a multistep process where axons, Schwann cells, and macrophages are involved especially in the turnover, intermediate storage, supply, and reorganization of lipids (275). First, degradation of myelin and an increase of accumulation of lipid droplets in surrounding macrophages that also secrete high amounts of apoE occurs (309, 310). With a retardation of 1 week as regeneration of multilamellated myelin progresses and lipid droplets in macrophages decrease, large lamellar bodies are formed intracellularly. After several weeks these lamellar bodies can also be detected in the extracellular matrix. The reason for the persistence of lamellar bodies in the extracellular matrix, however, still remains to be elucidated. Phagocytosis by other cells, exchange of lipids with plasma lipoproteins, and extracellular enzymatic degradation may be taken into consideration. The release of soluble proteins into the microenvironment implies that these proteins might play a major role in regeneration. ApoE undergoes a 250- to 350-fold increase in synthesis and constitutes 2–5% of the total extracellular protein (277). ApoE is also developmentally regulated in neonatal rat optic and sciatic nerves (278) and contributes to the availability of fatty acids, phospholipids, and cholesterol which are necessary for growing cells. A major intracellular apoprotein of Schwann cells is myelin basic protein (MBP), and four isoforms have been identified. MBP contributes to the induction and maintenance of the multilamellar structure of myelin (279–281). The lipid fraction of the membranes consists of phospholipids, sphingomyelin, cholesterol, and cerebrosides. MBP binds with high affinity to acidic phospholipids. Interestingly, T cell clones of patients with multiple sclerosis, an autoimmune, demyelinating disorder, react with MBP (282).

J. Lamellar bodies in wound healing

Injuries to tissue induce complex interactions of cellular and biochemical events; the topic is excellently reviewed in ref. 283. Platelets, polymorphonuclear leukocytes, lymphocytes, monocytes, fibroblasts, and endothelial cells migrate to the site of tissue damage and secrete soluble factors that regulate the formation of new tissue. Tissue repair can be divided into three phases: inflammation, proliferation, and remodeling. The function of inflammation is to eliminate injured tissue or pathogenic invading microorganisms. Platelets appear initially at the site of injury and are followed by neutrophils and finally by

macrophages and lymphocytes. Morphologically, the differentiation of monocytes into macrophages is accompanied by the development of secondary lysosomes, increased vacuolation, enhanced Golgi apparatus, and extensive formation of ER. Localized within the inflammatory site, these macrophages release oxygen intermediates and arachidonic acid products and undergo increased phagocytic and lysosomal activity. During these processes formation of lamellar bodies has been observed (284), suggesting a role in intermediate lipid storage and possible transfer during the regeneration process. At the time when the inflammatory stimulus is declining, migration and proliferation of connective tissue cells characterize the proliferation phase of wound healing. Factors secreted by macrophages, such as proteases, prostaglandins, and macrophage angiogenesis factor play a major role in the regulation of these processes.

K. Toxicological induction of lamellar bodies by drugs

At least 40 drugs with totally different pharmacological profiles such as antiarrhythmic, antidepressant, antimalarial, antibiotic activities, and inhibitors of cholesterol synthesis have been reported to induce the formation of concentric membraneous structures within cells (285–287) (**Table 4**). The common feature of these drugs is the cationic amphiphilic structure, and they are able to induce lipidosis in organisms. A hydrophobic portion of the molecule consisting of an aromatic or aliphatic ring structure is combined with a hydrophilic portion containing a primary or substituted nitrogen group that is charged at physiological pH.

The alveolar macrophage is particularly susceptible to the induction of lamellar bodies by drugs. However, other cells in the lung, liver, kidney, adrenal glands, and nerves have also been affected. The lipids of drug-induced lamellar bodies may originate from heterophagic or autophagic processes. During heterophagy, extracellular material enters the cell by phagocytosis or pinocytosis, as is the case in alveolar macrophages, which engorge large amounts of lung surfactant phospholipids.

In autophagy the membraneous material originates from intracellular sources such as organelles (287), which are degraded in lysosomes consisting of a double membrane. The origin of the limiting double membrane is the rough endoplasmic reticulum (RER), which surrounds the organelle to be degraded (288, 289).

The cationic amphiphilic drugs may interfere with the metabolism of phospholipids that accumulate by both the heterophagic or autophagic process in lysosomes which leads to the excessive deposition of these lamellar bodies. It has been demonstrated that interference of these drugs with lysosomal phospholipase A and C impairs the degradation of phospholipids (287, 290). Two possible mechanisms have been proposed: direct interaction with these enzymes or formation of an indigestible drug-lipid complex. In addition, it has been shown that these amphiphilic drugs selectively accumulate within lysosomes, thus increasing intralysosomal pH and inactivating enzymes of this organelle. However, numerous additional lysosomal and nonlysosomal functions may be disturbed by special amphiphilic drugs.

TABLE 4. Drugs that induce lamellar bodies

Class/Drug	
Anorectic	Antimalarial
Chlorphentermine	Chloroquine
Cloforex	Mepacrine
Fenfluramine	
	Antianginal
Antiarrhythmic	4,4'-Diethylaminoethoxy-hexestrol
Amiodarone	
Perhexiline	Antiestrogenic
	Tamoxifen
Antidepressant	
1-Chloramitriptyline	Antithrombotic
1-Chloro-10,11-dehydro-amitriptyline	RMI 10.393
Imipramine	
Iprindole	Neuroleptic
Maprotiline	Clozapine
Zimelidine	
Chlomipramine	Serotonin uptake inhibitor
	Fluoxetine
Antihistaminic	Citalopram
Chlorcyclizine	
Meclizine	Cholesterol synthesis inhibitor
Cyclizine	AY-9944
Norchlorcyclizine	Boxidine
Homochlorcyclizine	Triparanol
Hydroxyzine	
	Antibiotic
Antianxiety	Gentamicin
Ethyl fluclozepate	Erythromycin
Haloperidol	
	Interferon inducer
Secretolytic	Tilorone
Ambroxol	S3458-0
Bromhexine	
	Schistosomicidal
Tranquilizer	IA-3
AC-3579	

(With permission of M. J. Reasor, West Virginia University, Morgantown, WV; ref. 285.)

IV. FORMATION AND INTRACELLULAR MOVEMENT OF LAMELLAR BODIES

In several diseases lamellar bodies are formed in the lysosomal compartment of the cell, due to a defect in phospholipid-degrading enzymes, e.g., Niemann-Pick type I disease and phospholipidosis. Lamellar bodies of the lung and of the skin contain lytic enzymes and have an acidic pH, which confers a lysosomal character on them. It is of interest to follow the biogenesis of lysosomes

(reviewed in 291–298), so that predictions about lipid and protein trafficking and the intracellular movement of lamellar bodies can be made. Lysosomes may contain recently ingested material both from extracellular or intracellular sources. Receptor-mediated endocytosis is an important mechanism for the efficient uptake of extracellular substances (**Fig. 10**). During the process of internalization endosomes are formed, which contain a membrane-associated ATPase that concentrates protons and therefore acidified the lumen of the organelles (299). The contents of lysosomes may be destined for degradation, for intracellular storage, or for resecretion or fusion with the plasma membrane.

It was originally thought that the transfer of endosomal content to lysosomes takes place by fusion of the two organelles. The current view, however, is that a subclass of endosomes located near the Golgi apparatus undergoes conversion into a lysosome by acquiring the necessary complement of lysosomal hydrolases and proteins from clathrin-coated vesicles that originate from the *trans* Golgi region or *trans* Golgi network (298, 300).

The major function of the Golgi complex is to sort various cellular components and direct them to particular intracellular and extracellular destinations. Thus it is possible that membraneous material from the ER, mitochondria, or other cytoplasmic sites is directed via the Golgi to lamellar bodies. Lipids originating from different pathways, PC from ER, SPM from *cis*/medial Golgi, PS, PE from mitochondria, cholesterol from ER or endocytotic pathways, have to be assembled in the lamellar bodies. In the lung this may occur via small lamellar bodies (301, 302). Lamellar bodies of the skin and of the lung may contain at least nine different lipids and several proteins, and rather complicated and coordinated processes must be responsible for the assembly of lamellar bodies. Lipid molecules can move by at least three intracellular mechanisms: transport from one organelle to another by vesicle budding and fusion, by lateral diffusion between organelles connected by membrane bridges, and by transport of lipid monomers by specific binding proteins. Phospholipids may be transferred from the site of synthesis to the lamellar bodies by specific phospholipid

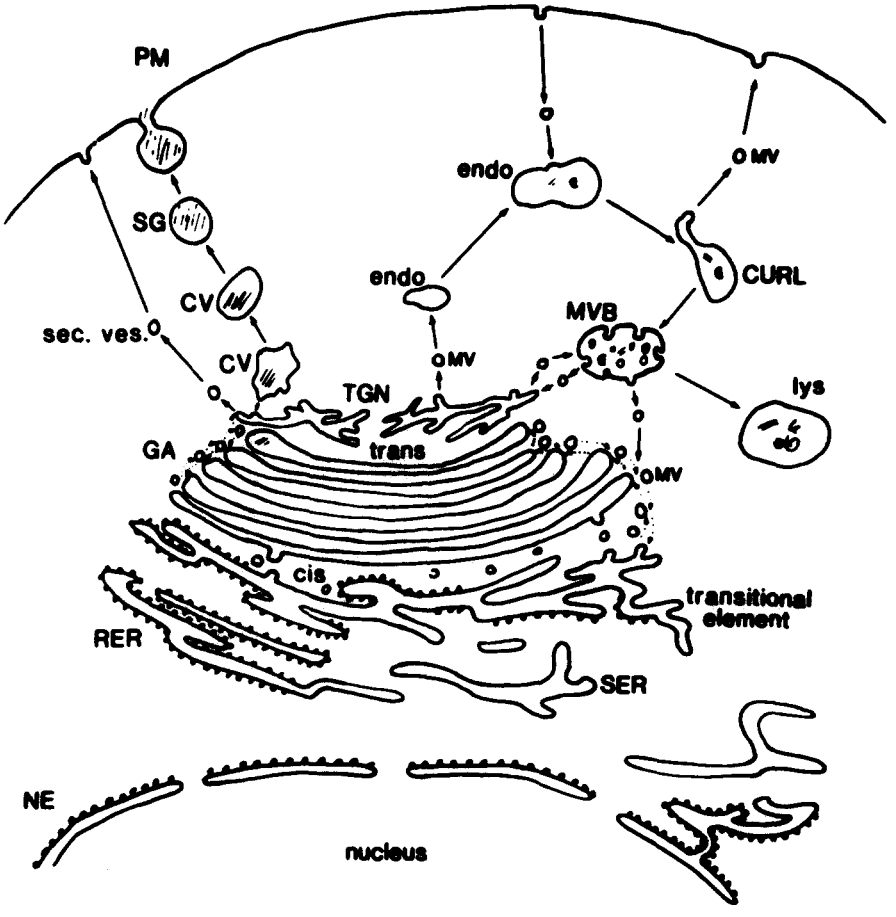

Fig. 10. Biosynthesis of lysosomes. The ligand-receptor complex is concentrated in clathrin-coated pits (CP) to form coated vesicles (CV) which fuse with endosomes (endo). By low pH the ligand is dissociated from its receptor and returned to the cell surface. Hydrolases are transported from the *trans* Golgi network (TGN) in coated vesicles to multivesicular bodies (MVBs) which are the precursor compartments of lysosomes. (By permission of McGraw-Hill Book Co., New York. C. R. Scriver *et al.*, eds. 1989. Metabolic Basis of Inherited Diseases.)

transfer proteins, which also have been demonstrated in the intact lung (303, 304) and in isolated type II cells (305, 306). At least three transfer proteins appear to exist in the lung: one specific for PG, another for PC, and a third that could transfer several phospholipids including PC, phosphatidylethanolamine (PE), PG, PI, and phosphatidylserine (PS) (304, 305). However, phospholipid transfer by these proteins to lamellar bodies has not been demonstrated experimentally. In addition, this transport rate would be too slow compared to the high secretion rate of lipids, which suggests dominance of vesicular transport. Two calcium-dependent phospholipid-binding proteins have been purified to homogeneity from rabbit lung cytosolic fraction which possess catalytic activities for aggregating small vesicles and for the fusion of small vesicles to the surfactant membrane (307). In lipid recycling studies, using fluorescent-labeled sphingomyelin and PC derivatives, it could be shown that the original fluorescent label inserted into the plasma membrane of fibroblasts was subsequently found in perinuclear endosomes to be returned to the cell surface membrane by translocational steps from *cis* to *trans* Golgi regions. This could indicate general pathways for intracellular lipid traffic (308, 309). In principle, lipids synthesized in the ER such as PC should move to the Golgi apparatus by vesicle transport (310). It has been shown that the intracellular site of SPM synthesis is the luminal site of *cis* and medial Golgi cisternae (311). There is some evidence for transport of lipids out of lysosomes to other organelles probably by nonvesicular processes. In particular, the movement of cholesterol out of lysosomes after endocytosis and degradation of LDL (312). Newly synthesized cholesterol is transported in vesicular form (313).

Translocation of proteins (apoproteins and enzymes) through the ER membrane may be accompanied by the acquisition of N-linked high mannose oligosaccharide chains and cleavage of the signal sequence similar to lysosomal peptides. Modifications that introduce the specific lysosomal marker, mannose-6-phosphate, which distinguishes these proteins from others, may lead to their specific lysosomal destination.

Proteins and lipids synthesized in the ER usually traverse the different stacks of the Golgi before they reach their intracellular or extracellular destinations, and there are indications that lipid and proteins are transported together in vesicular form (295). It may be assumed that lamellar bodies share the same pathways. Therefore, it is of interest to investigate mechanisms of the sorting processes by the Golgi where cellular constituents are directed to lysosomes, secretory granules, and to the plasma membrane (314–316). The *cis*, medial, and *trans* Golgi cisternae represent a series of subcompartments that sequentially carry out posttranslational modifications on newly synthesized proteins that traverse the organelle unidirectionally (294). It has been shown that acylation-deacylation reactions play a major role in permitting the transfer and recycling of vesicular carriers between Golgi cisternae, since a stimulation of transport by palmitoyl-CoA and an inhibition of transport by a nonhydrolyzable analogue of palmitoyl CoA was achieved in a cell-free system (317). These budding processes are complicated and at least one additional protein, a 76 kDa N-ethylmaleimide (NEM)-sensitive protein, was found to be essential (318, 319).

In their passage from *cis* to *trans* Golgi cisternae, all molecules are confronted with an environment of decreasing pH (320). The existence of proton pumps has been demonstrated in Golgi membranes. *Trans* cisternae and the *trans* Golgi network (TNG) are considerably more acidic than the *cis* Golgi cisternae. It has been suggested that the pH gradient may play a role in determining the unidirectional transport of molecules through the Golgi apparatus. Weak bases, such as chloroquine, traverse membraneous compartments and accumulate in acidic environments in their protonated forms, raising pH (321). These drugs appear to act at a late Golgi or post-Golgi stage, as well as in acidic lysosomal compartments. These agents completely block protein secretion as well as cholesterol secretion at the *trans* Golgi level (321). Since the movement of intracellular vesicles is closely related to interactions with cytoskeletal elements, these regulatory processes must also be disturbed directly or indirectly by the action of these drugs.

Only limited experimental data are available about involvement of cytoskeletal elements during the formation and secretion of lamellar bodies. Assuming a lysosomal origin of lamellar bodies as shown in Fig. 10, a directed translocation of vesicles and organelles must take place in a coordinated fashion. In general, it is believed that microtubules and actin filaments are involved (322, 323) and organelles or vesicles are cross-linked to the microtubules and microfilaments by force-generating molecules such as kinesin, dynein, or myosin (324, 325). Dynein has been shown to propel mitochondria along microtubules (326). Myosin I is responsible for the movement of membrane vesicles along actin filaments in intestinal microvilli (327), as an example of the in vitro models established recently.

A role for microfilaments in exocytosis has been hypothesized (328). The forces for the extrusion of secretory products are provided through the action of actin-myosin contractile systems. Intracellular movement of lysosomes depends on a functional integrity of both actin filaments and microtubules (329). In macrophages, decreasing cytoplasmic pH leads to movement of lysosomes to the periphery (330). The cytoskeletal mechanisms for

the formation, translocation, and exocytosis of lamellar bodies in different tissues, however, are still to be elucidated.

V. CONCLUSION

Numerous types of lamellar bodies have been detected in different cells and tissues, which may be classified according to their specific tissue or cellular function. In certain epithelial tissues lamellar bodies are secretory organelles for the directed transport of lipids to the extracellular or interstitial environment. The lipid and protein composition varies according to the special requirements of the organ, which is primarily the hydrophobic protection of the membrane of the epithelial cells. This is often associated with additional functions such as surface protection against oxidative stress and particles from the air; in the skin to form a protective water barrier and ordered exfoliation of corneocytes; in the stomach to form the protective lining against aggressive gastric juice; and in joints and pericardium to provide a lubricant phospholipid film. The ordered secretion and transformation of lamellar bodies into lamellar sheets and maintenance of the lipid layers imply the presence of certain apoproteins and enzymes. Intensive investigations of production and secretion of surfactant by lung has revealed a complex system of regulation and a similar complexity may be anticipated in other tissues. Remarkably, the cholesterol to phospholipid ratio in these systems is low in the lamellar bodies.

When we compare some morphological and biochemical data available for lamellar bodies (**Table 5, Table 6, Table 7**) the following conclusions may be drawn. The size, density, protein and lipid composition of lamellar bodies vary considerably. The size of lamellar bodies isolated from different tissues greatly varies from a minimum of 100 nm in stratum granulosum to 2400 nm in pneumocyte II cells. The same is true when comparing the different phospholipid contents. Lamellar bodies are specialized structures with a lipid and protein composition related to specialized functions. Within a given tissue associated with defined cell types they may be regarded as a tissue equivalent of plasma lipoproteins. From a functional aspect the main purpose of lipoproteins is the supply and turnover of certain lipids between organs within the organisms. The structural integrity, transformation, and the lipid exchange mechanisms are due to the associated apoproteins and enzymes. The main target for lamellar bodies is the supply of extracellular domains with specialized lipid components. These lipids are partially recycled within the same tissue or are lost to the outer environment, for example in the skin. As may be deduced from the complex sequence of events in the production and distribution of lung surfactant and the lamellar bodies of the skin, the structural integrity, transformation between different morphologic states, and changes in lipid composition are due to the presence of apoproteins and certain enzymes. Thus, lipoproteins and lamellar bodies of epithelial cells have certain features in common.

Tissue macrophages may deserve special attention in their ability to form and secrete lamellar bodies. In

TABLE 5. Composition of the lipoproteins in plasma of humans

					Composition						
							Percentages of Total Lipid				
Fraction	Source	Diameter	Density	S_f	Protein	Total Lipid	Triacylglycerol	Phospholipid	Cholesteryl Ester	Cholesterol (Free)	Free Fatty Acids
		nm	*g/ml*		%	%					
Chylomicrons	Intestine	100–1000	<0.96	>400	1–2	98–99	88	8	3	1	
VLDL	Liver and intestine	30–90	0.96–1.006	20–400	7–10	90–93	56	20	15	8	1
IDL	VLDL and chylomicrons	25–30	1.006–1.019	12–20	11	89	29	26	34	9	1
LDL	VLDL and chylomicrons	20–25	1.019–1.063	2–12	21	79	13	28	48	10	1
HDL_2	Liver and intestine	10–20	1.063–1.125		33	67	16	43	31	10	
HDL_3	VLDL? Chylomicrons?	7.5–10	1.125–1.210		57	43	13	46	29	6	6
Albumin-FFA	Adipose		>1.2810		99	1	0	0	0	0	100

VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; FFA, free fatty acids. VHDL (very high density lipoprotein) is a minor fraction occurring at density 1.21–1.25 g/ml. (With permission of R. K. Murray et al., eds. Harper's Biochemistry. 1988. Appleton & Lange, East Norwalk, CT.)

TABLE 6. Comparison of lamellar bodies from different tissue

Source	Ref.	Diameter	Density	Protein	Total Lipid	Carbohydrate
		μm	*g/ml*	%	%	%
Pneumocytes II, mice	71	0.5–2	1.062	8–10	90	2
Pneumocytes II, rats	69	1.5–2.4	1.053–1.059			
Epid. stratum granulosum	122, 126	0.1–0.2		14.3	72.5	
Mouse peritoneal macrophages	1	~0.5–1.5	1.21			
Foam cells coronary artery	15	0.25–0.8				
Cholesterol-fed rabbit aortas	15	0.07–0.3	1.036	<25		

several diseases such as atherosclerosis, arthritic rheumatism, peritonitis, and ARDS, lipid-laden macrophages occur. These diseases have certain features in common: the presence of lamellar bodies in the adjacent cell layers, chronic inflammation, increased oxidative stress, increased levels of lipid peroxides, changes in lipoprotein concentration of plasma and α-tocopherol or vitamin E, and later a development of fibrosis.

In atherosclerosis, macrophages as well as the extracellular matrix contain lamellar bodies of a high cholesterol to phospholipid ratio. The phospholipid cholesterol stacks in bile also have a high cholesterol to phospholipid ratio. Free cholesterol is not soluble and at a certain concentration would precipitate as deleterious crystals. Therefore multilamellar structures are phospholipid carriers for cholesterol. This might suggest that lamellar bodies of macrophages are specialized for the disposal of free cholesterol from the cells. Cholesterol cannot be degraded in macrophages and it is unlikely that these lamellar membranes with their unusually high cholesterol content form extracellular lipid sheets. It has been hypothesized that cholesterol-rich domains may be formed in the plasma membrane and these may be derived from lamellar bodies. These domains are destined for transfer of cholesterol to extracellular acceptors such as HDL.

Results obtained in our laboratory with macrophages in culture have shown that lamellar bodies with a high cholesterol to phospholipid content similar to those found in plaques may be secreted into the medium. Therefore these lamellar bodies have to be considered as organelles for storage and secretion of lipids under certain conditions of cholesterol overload. Several physiological reasons for the formation of these structures may be hypothesized. Lamellar bodies secreted into the bloodstream would probably disperse immediately, while in the extracellular matrix there may be exchange of lipids and possibly apolipoproteins with HDL. Our results with isotachophoretically isolated subclasses of HDL show that a slow migrating HDL species exists, which seems to be identical with pre-β_{1-3}-migrating HDL, that may interact nonspecifically with plasma membranes to exchange cholesterol, possibly by the LCAT reaction (332). The structure of lamellar

TABLE 7. Comparison of lipid composition of lamellar bodies (%)

Lipid Species	LB Epidermis	LB Macroph.	LB Rat Pneumocytes II
Phospholipids	44.5	20.8	97
Phosphatidylcholine	19.7	15.0	77
Lysolecithin/sphingomyelin	13.6	4.6	0.7
Phosphatidylethanolamine	8.8		
Phosphatidylserine/inos.	2.6	1.2	12
Phosphatidylglycerol			8
Neutral lipids	54.7	79.0	5
Free sterols	26.8	68.0	5
Sterol/wax esters	5.1	8.0	
Free fatty acid	8.0		
n-Alkanes	4.3		
Squalene	1.9		
Diglycerides	0.9		
Triglycerides		3.0	

bodies results in a larger surface area available for interaction with HDL particles. Adrenocortical cells are an example of such a system in which a large surface area of membrane facilitates the extracellular exchange of cholesterol by HDL particles (333). Since macrophages have only a limited intracellular space for storage, the extrusion of lamellar bodies to the extracellular matrix might accelerate these exchange processes. The lamellar bodies in the matrix may also be phagocytosed by other macrophages. This transfer of lamellar bodies from cholesterol-rich to cholesterol-poor cells may implicate these lamellar bodies as tissue lipoproteins.

Lamellar bodies found in macrophages and extracellular matrix during regeneration of nerves may have a similar function. It also seems that intermediate lipid storage and secretion and exchange of lipids may be the function of these lamellar bodies. From the sequence of events it may be inferred that the macrophages surrounding the Schwann cells accumulate cholesterol in cytoplasmic lipid droplets which are gradually transformed to lamellar bodies. It still remains to be investigated whether these lamellar bodies are secreted or remain intracellular for the purpose of cholesterol exchange with plasma lipoproteins, or whether transfer to Schwann cells occurs. The observation of a direct transfer of lamellar bodies from neurons to glial cells by endocytotic mechanisms (334) indicates that lamellar body secretion and uptake is a possible physiological event. Interestingly, in several cases of occurrence and secretion of lamellar bodies, an increased secretion of apoE has been observed, for example in keratinocytes, and atherosclerotic lesions, macrophages that secrete lamellar bodies, and in macrophages during nerve regeneration. It may be hypothesized that apoE has a very important regulatory role in these lipid transfer processes or might be directly involved in lipid transport or even associated with lamellar bodies. In addition, apoE might share some functional similarities to SP-A of lung surfactant, e.g., distribution of cellular lipids.

There is considerable evidence that SPM and cholesterol concentrations are closely correlated. It has been shown that these two lipids form tight complexes because of physical interaction and may interfere with metabolic pathways. Plasma membrane sphingomyelin appears to be one of the major determinants of the allocation of cell cholesterol (225). Removal of sphingomyelin leads to a dramatic redistribution of cholesterol within the cell and an up-regulation of ACAT activity (335). In Niemann-Pick type II only lysosomal-derived cholesterol is trapped in the *trans* Golgi, since SPM is synthesized in the early compartments of the Golgi apparatus. Thus, SPM concentration typically increases during hypercholesterolemia, in atherosclerotic lesions, and with increasing age. In Niemann-Pick type I disease the increase in lysosomal SPM is accompanied by accumulation of cholesterol. In Niemann-Pick type II disease, only lysosomal-derived cholesterol is trapped in the *trans* Golgi region, presumably because SPM is also synthesized in the early compartments of the Golgi. Cholesterol from biosynthetic pathways does not pass the Golgi and therefore is transported to the plasma membrane without disturbance.

There seems to be an inverse correlation between the amounts of SPM and PC in many membranes, which determines membrane fluidity. In polarized cells such as epithelial cells, intracellular lipid sorting to the apical and basolateral plasma membrane takes place in the *trans* Golgi network and is mediated by vesicular carriers (295). Membrane vesicles rich in SPM are destined for the apical membranes, and membrane vesicles rich in PC are destined for basolateral movement. Similarly, the synthesis and intracellular trafficking of lamellar bodies may also be influenced by their content of PC and SPM, with one fate for those enriched in PC, for example in epithelial cells, and another for those rich in SPM, for example in Niemann-Pick disease and atherosclerosis.

In some tissues, e.g., degenerating nerves, lamellar bodies are morphologically very obvious, but their significance remains to be elucidated. Since these lamellar bodies are often associated with cellular organelles such as ER, mitochondria, and Golgi, it may be hypothesized that either intracellular lipid transport of lipids originating from different organelles or autophagic lipid degradation is impaired. The associated disarrangement of microtubules at least indicates defective intracellular transport.

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