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Lysosomes

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Dedicated to Jane and Lynn ... and all patients suffering of diseases with an impaired lysosomal function.

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PREFACE

The lysosome is the cell's main digestive compartment into which many types of macromolecules are delivered for degradation. However, during the last ten years it has become evident that lysosomes have more complex functions than simply being the "waste basket" of the cell. Lysosomes can be involved in various cellular processes such as cholesterol homeostasis (Chapter 9), autophagy (Chapters 14 and 15), repair of the plasma membrane (Chapter 13), bone remodelling (Chapter 12), defence against pathogens, cell death, and signaling. More than 50 acid hydrolases have been identified which are involved in the ordered lysosomal degradation of a variety of proteins, lipids, carbohydrates, and nucleic acids (Chapters 5, 6 and 11). These hydrolases are enclosed by a limiting membrane containing a set of highly glycosylated lysosomal membrane proteins (Chapter 4). Lysosomal enzymes are also components of cell type-specific compartments referred to as lysosome-related organelles and include melanosomes, lytic granules, MHC class II compartments, platelet-dense granules, and synaptic-like microvesicles. Functional deficiencies of several lysosomal proteins give rise to the lysosomal storage diseases (Chapters 6, 7, 8 and 10).

The biogenesis of new lysosomes or lysosome-related organelles requires a continuous delivery of newly synthesized components. The targeting of acid hydrolases depends on the presence of mannose-6-phosphate residues that in turn are recognized by specific receptors, mediating the intracellular transport to an endosomal/prelysosomal compartment (Chapters 2 and 3).

The more I work with the lysosomal compartment the more I realize that lysosomes are not simply a dead end in the endocytotic pathway. The majority of physiological processes involve at least a transient encounter with this cellular compartment. A more detailed understanding of the functions of lysosomes will shed light on the molecular basis of pathological conditions in which the degradation and transport processes of intracellular molecules are affected. This is reflected by the exponentially increasing volume of literature dealing with the biology of the lysosome. This book will only be able to pinpoint some of the essentials of what has been published recently. The goal of this book is to introduce the major features of lysosomes at a level which should be useful for both interested students as well as researchers and clinicians in need of a broad background.

When I was asked to edit a book aiming to provide information about lysosomal functions, I was fascinated by the chance to meet and talk with some of the leading experts in the field, but also a little apprehensive about whether I would be able to cover both the novel findings and the more established lysosomal research. I am now glad that I had this opportunity to interact closely with many colleagues who have been extremely generous with their time and efforts. I am especially happy that this book has turned out to be a blend of contributions from young colleagues who are pushing

the lysosome community forward with new concepts and ideas as well as contributions from more experienced and well recognized experts in various aspects of lysosomal function.

Once again, I would like to express my gratitude to all contributors and to the editor who helped me to realize this project. Last but not least I would like to thank Kurt von Figura, my teacher and mentor in "lysosomal matters", for his continuous support and interest in my research. Without his advice I would have missed many interesting aspects of this intriguing organelle.

Paul Saftig

CHAPTER 1

History and Morphology of the Lysosome

Renate Lüllmann-Rauch*

Abstract

The lysosome is the cell's main digestive compartment to which all sorts of macromolecules are delivered for degradation. The structure of the lysosome is variable and depends on the cell type and the actual conditions. In terms of function and cytochemistry, the lysosome is identified by the following criteria: acid pH, hydrolases with acid pH optimum, specific highly glycosylated membrane-associated proteins, and the absence of the mannose-6-phosphate receptor. The purposes of the present chapter are (a) to give a short overview on the morphology of the lysosome/endosome system for readers who are nonexperts in this field; and (b) to briefly trail the tracks and approaches which, during the first decades after the discovery of the lysosome, led to the present concept, with particular reference to morphology.

Introduction

Lysosomes are membrane-delimited organelles which occur in all mammalian cells except red blood cells. Lysosomes are defined by functional rather than structural properties. They contain a high proton concentration (pH $\leq$ 5) and more than 40 hydrolases with a pH optimum below 6. Their limiting membrane is endowed with specific integral proteins including a vacuolar-type H^+-ATPase, several highly glycosylated proteins and various types of transporters as reviewed in Chapters 4, 7, and 9 of this volume. Lysosomes are engaged in the degradation of macromolecules delivered from the cell's own cytoplasm (autophagy, Chapters 14 and 15 of this volume) as well as materials taken up from the extracellular space (endocytosis). Depending on the cell type and the functional state, lysosomes can considerably vary in structure. Therefore it is not surprising that the existence of lysosomes was first realized solely on the basis of biochemical results. The term *lysosome* was coined by DeDuve five decades ago.[1] Only thereafter, ultrastructural and enzyme-cytochemical studies of Novikoff and coworkers[2,3] uncovered the morphologic identity of lysosomes. Expanding research in this field gradually revealed the lysosomes as being part of the highly dynamic endosome/lysosome system (Fig. 1), a collection of several categories of vesicular organelles which to a certain extent exchange membrane constituents and contents thus having overlapping properties. An important feature of lysosomes which serves to discriminate them from endosomes and other related vesicles is the absence of mannose-6-phosphate receptors.

Since the history of a scientific concept is appreciated particularly in the light of the present state of knowledge, the first part of this article briefly summarizes the basics of what is known today about the morphology of lysosomes including the morphological correlates of lysosome biogenesis. The second part provides a short historical review of the development of the lysosome concept during the first two to three decades following its original formulation.

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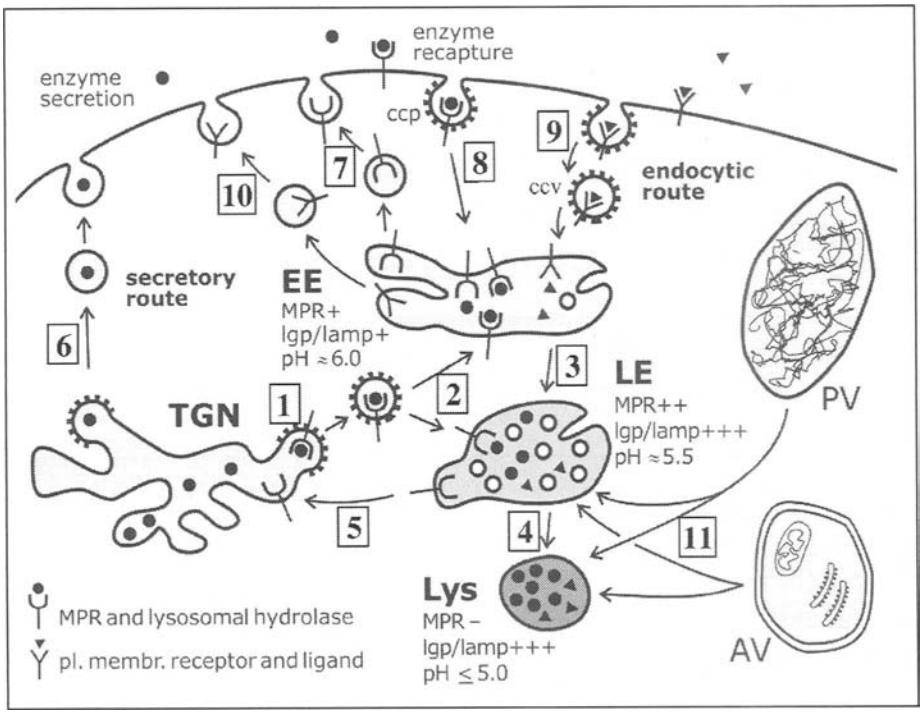

Figure 1. Diagrammatic summary of the endosome/lysosome system, with special reference to the biogenesis of lysosomes. After synthesis in the rER and modification in the Golgi apparatus (not shown) precursors of soluble lysosomal enzymes decorated with mannose-6-phosphate residues meet the mannose-6-phosphate receptor (MPR) in the trans Golgi network (TGN), are packaged (1) into clathrin-coated vesicles (ccv), and are transported (2) to late endosomes (LE) either directly or indirectly via early endosomes (EE) (3). The process of enzyme transfer (4) from the LE to the lysosome (Lys) is not fully elucidated yet; possibly LE matures to become Lys, or LE and Lys fuse to form a transient hybrid organelle (for further possibilities see text). The MPR is recycled (5) from the LE to the TGN, the lysosome is devoid of MPRs. A minor portion of the enzyme precursors gets into the secretory pathway (6) and is recaptured into clathrin-coated pits (ccp) by MPRs, which may be transferred (7) from the EE to the plasma membrane. Thus the enzyme precursors can reach the lysosome via the endocytic pathway (8) as do endocytic tracer molecules (9), whose receptors are recycled from the tubular extensions of the EE (10). Autophagic vacuoles (AV) and phagocytic vacuoles (PV) acquire lysosomal enzymes by fusion with lysosomes and/or LE (11) to become autolysosomes and phagolysosomes,[85] respectively. LE often resemble multivesicular bodies, i.e., they display invaginations of their membrane and internal vesicles budded off the invaginations (or representing cross sections of the invaginations). lgp/lamp, lysosomal membrane glycoproteins/lysosome-associated membrane proteins.

Morphology of Lysosomes

In terms of morphology, lysosomes are less clearly defined than other cell organelles such as mitochondria, peroxisomes or hormone storage granules. Yet, for normal cells of intact mammalian organisms lysosomes have so often been described in the literature, both ultrastructurally and cytochemically, that a combination of certain features may serve to identify lysosomes on the ultrastructural level. Lysosomes appear as cytoplasmic dense bodies, which are spheric, ovoid or occasinally tubular in shape, contain an amorphous osmiophilic dense matrix and are surrounded by a limiting membrane. Often lysosomes have a typical intracellular position, e.g., in hepatocytes near the bile canaliculus, in renal proximal tubule cells in the supranuclear cytoplasm, in cultured fibroblasts in the perinuclear cytoplasm. The size of lysosomes varies

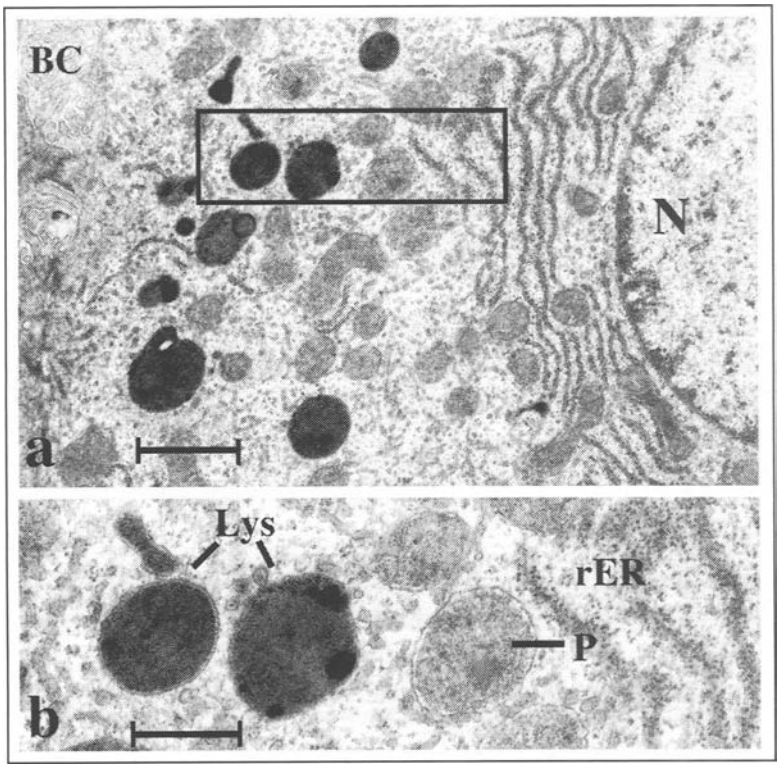

Figure 2. Lysosomes in a hepatocyte of a normal rat. a) The lysosomes appear as cytoplasmic dense bodies in close proximity to the bile canaliculus (BC). N, nucleus. b) Area marked in (a), shown at higher resolution. The limiting membrane and the amorphous dense matrix of the lysosomes (Lys) is seen. P, peroxisome. rER, rough endoplasmic reticulum. Bars represent 1 μm (a) and 0.5 μm (b).

between < 1 μm in many cell types as for example hepatocytes (Fig. 2) and neurons (Fig. 3) and several microns, e.g., in macrophages. On the light microscopic level (semithin epoxy resin sections), normal lysosomes—if visible at all—appear as intensely stained cytoplasmic bodies. Obviously, the diagnosis can only be tentative and requires verification by ultrastructural examination and/or enzyme-histochemical or immuno-histochemical demonstration of lysosomal constituents (enzymes or membrane proteins).

Different cell types show great quantitative differences regarding their equipment with lysosomes. In normal fibroblasts, hepatocytes or pituitary cells for example, the lysosomes usually contribute 0.5% or less to the cytoplasmic volume,[4,5] whereas in macrophages the fractional volume of lysosomes can be considerably larger.[6] The kidney may be taken as an example where the quantitative differences between cell types are immediately obvious (Fig. 4): The cells of the proximal tubules possess large numbers of lysosomes (corresponding to the high activity to endocytose and degrade proteins from the glomerular filtrate), whereas in the epithelia of the nephron segments downstream to the proximal tubules lysosomes are scarce.

The size and frequency of lysosomes can increase dramatically in any cell type, when the lysosomes accumulate nondegraded material. Such a condition can be induced by overloading the lysosomes with unphysiological substrates (e.g., saccharose,[7] polyvinylpyrrolidone, dextran), and by application of enzyme inhibitors (e.g., acarbose[8] inhibiting α-glucosidases or swainsonine[9,10] inhibiting mannosidases) or cationic amphiphilic drugs[11,12] and aminoglycoside

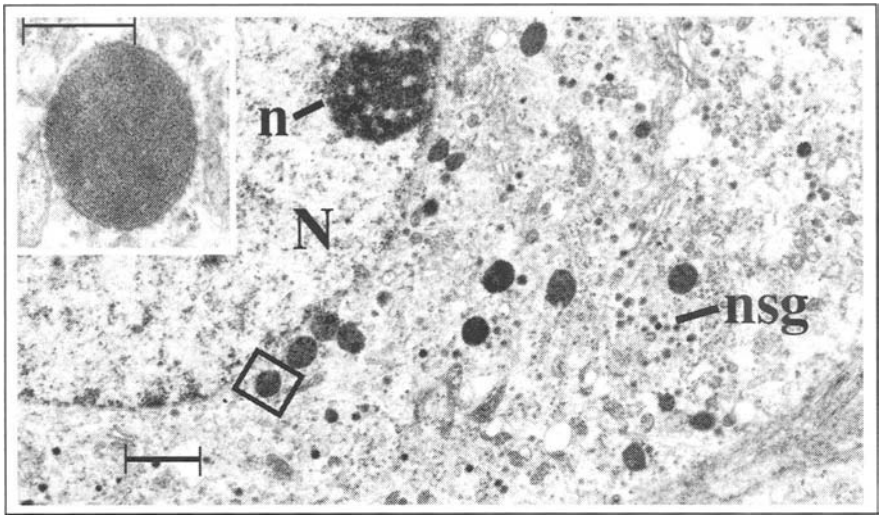

Figure 3. Lysosomes in a neurosecretory neuron from the hypothalamus of a normal rat. The lysosomes appear as dense cytoplasmic bodies. nsg, neurosecretory granules. N, nucleus. n, nucleolus. The *inset* shows one of the lysosomes at higher resolution. Bars represent 1 μm and 0.25 μm (inset), respectively.

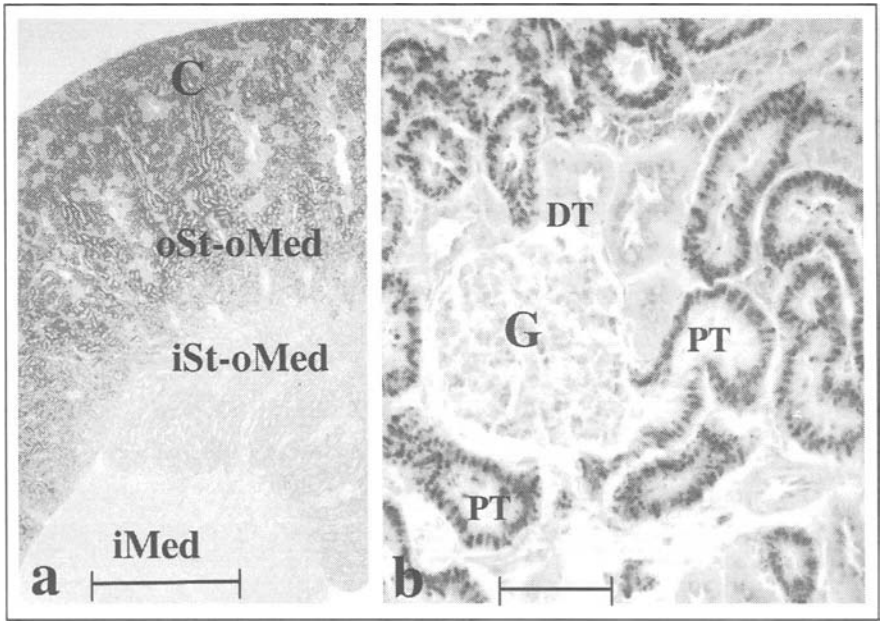

Figure 4. Kidney of a normal rat. Enzyme-histochemical demonstration of acid phosphatase.[86] The epithelium of the proximal tubules (PT) is rich of lysosomes, whereas in all downstream segments of the nephron (e.g., distal tubules, DT) and in glomerular cells (G) lysosomes are relatively scarce. Thus, intense staining is seen only in those regions which harbour PTs, i.e., cortex (C) and outer stripe of outer medulla (oSt-oMed). The inner stripe of outer medulla (iSt-oMed) and the inner medulla (iMed) are weakly stained. Bars represent 1 mm (a) and 50 μm (b).

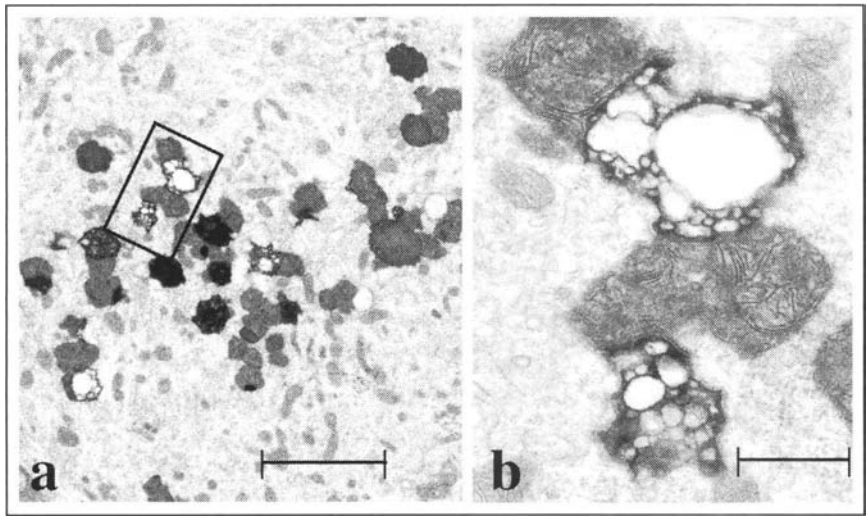

Figure 5. Lipofuscin granules in a dorsal root ganglion cell of an aged normal mouse (20 months). Several dense inclusions are seen in the cytoplasm, which are filled with polymorphous materials. Lipofuscin granules are generally regarded as telolysosomes, i.e., a subpopulation of lysosomes representing the terminal station for deposition of undigestible material which accumulates in long-lived cells. Bars represent 3 μm (a) and 0.5 μm (b).

antibiotics[13] which interfere with the intralysosomal digestion of certain substrates. Furthermore, an enormous augmentation of the lysosomal apparatus is typically seen in the inherited lysosomal storage diseases,[14-16] most of which are due to a genetically determined deficiency of a lysosomal enzyme (see Chapter 6 of this volume).

Contents of Lysosomes under Normal and Pathological Conditions

The structural heterogeneity of lysosomes is mainly a consequence of their function as digestive organelles. In macrophages of liver, lymphatic and hematopoietic tissues the lysosomes often are polymorphous and can contain remnants of undigestible or partly degraded materials. This is observed also in the retinal pigment epithelium[17] which constitutively engulfs and degrades fragments of the photoreceptor cell outer segments. Another example of lysosomes with polymorphous contents are lipofuscin particles, i.e., autofluorescent granules which are regarded as telolysosomes harbouring nondigestible ill-defined polymers of lipids complexed with proteins. Lipofuscin granules[18-20] typically accumulate in postmitotic and long-lived cells, for example neurons (Fig. 5), cardiomyocytes, hepatocytes or steroid-hormone producing cells.

The most dramatic accumulation of unusual contents is observed in inherited and induced lysosomal storage disorders. Thus, large amounts of intralysosomal glycogen particles are found in many cell types of individuals deficient in lysosomal acid α-glucosidase (glycogenosis type II, Pompe disease) (Fig. 6), and in experimental animals[8] treated with an inhibitor of this enzyme. In the majority of inherited lipidoses and drug-induced lipidosis, both of which are characterized by storage of polar lipids, the lysosomes are filled with multilamellated or paracrystalline materials reflecting the tendency of polar lipids to aggregate in highly regular arrays (Figs. 7, 9b). In a variety of lysosomal storage disorders characterized by accumulation of water-soluble substrates, for example mucopolysaccharidoses (storage of sulfated glycosaminoglycans) and mannosidoses (storage of low-molecular weight oligosaccharides containing mannose), the lysosomes in routine preparations appear as clear vacuoles (Figs. 8, 9c). This is

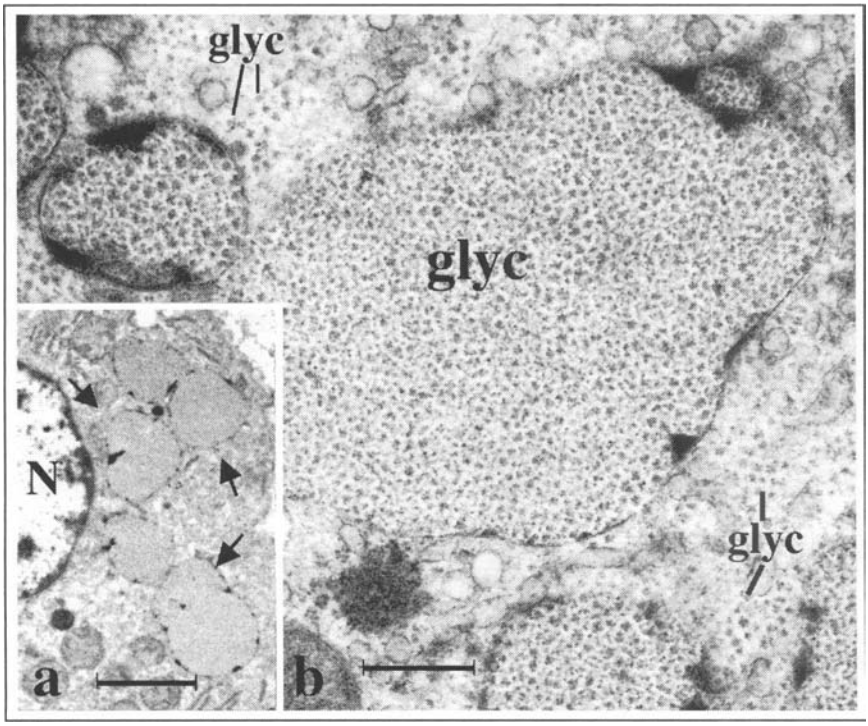

Figure 6. Lysosomal glycogen storage in a hepatocyte of a human fetus with Pompe disease (glycogenosis type II, deficiency for acid α-glucosidase). Glycogen rosettes (α particles) (glyc) are present in the cytoplasm (as observed also in normal hepatocytes) and in membrane-limited storage organelles (arrows in a). N, nucleus. Bars represent 2 μm (a) 0.5 μm (b).

due to the fact that the storage materials are not preserved by the usual fixation techniques and are therefore lost from the cells during tissue processing.

Biogenesis of Lysosomes

On the basis of the subcellular distribution of acid phosphatase as demostrated by enzyme cytochemistry, Novikoff and coworkers[21] proposed the concept of GERL (Golgi apparatus - endoplasmic reticulum - lysosome). It implied (a) that lysosomal enzymes, after biosynthesis in the rough endoplasmic reticulum (ER), are packaged into vesicles ("primary lysosomes") budding off from tubules which are continuous with the ER and intimately related to the Golgi apparatus, (b) that the vesicles are conveyed to preexisting lysosomes which have already been engaged in a digestive process ("secondary lysosomes"). This concept was later replaced by the concept of the *trans* Golgi network (TGN) as the common exit site for all products including lysosomal enzymes, secretory proteins and membrane proteins.[22,23] The most important modifications of the previous GERL concept were induced by data indicating that the intracellular transport of lysosomal enzymes is receptor-mediated and has to pass through a prelysosomal compartment (Fig. 1).

Separation of Lysosomal Enzymes from the Secretory Pathway

Most soluble lysosomal enzymes are synthesized as N-glycosylated precursors, the initial steps of biosynthesis are shared with the secretory proteins. The diversion of the lysosomal enzymes from the secretory pathway is dependent on the acquisition of the mannose-6-phosphate

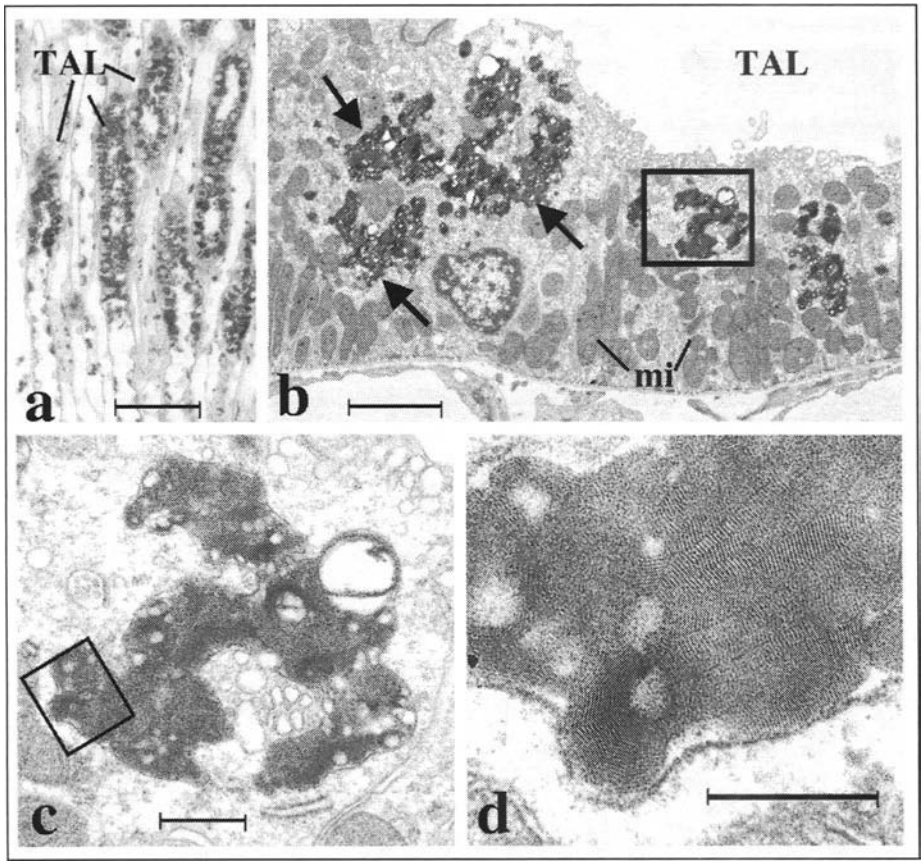

Figure 7. Lysosomal sulfatide storage in kidney of a mouse deficient in arylsulfatase A, representing an animal model for the metachromatic leukodystrophy (MLD) of humans.[87] a) Histochemical staining with alcian blue to detect sulfatide storage. All nephron segments shown in this photomicrograph are affected, particularly so the thick ascending limb (TAL) of Henle's loop, where normally the lysosomes are scarce. The micrograph shows the transition between inner medulla (bottom) and outer medulla. b) Ultrastructure of TAL epithelium. Bizzare-shaped inclusions are seen (arrows). mi, mitochondria. c) Lysosome marked in (b) shown at higher resolution. d) Portion from (c) shown at high resolution to show the limiting membrane and the fish-bone-like pattern, which is typical of intralysosomally accumulated sulfatides also in humans with MLD. Bars represent 50 μm (a), 4 μm (b), 1 μm (c), 0.5 μm (d).

(M6P) recognition marker; for review see reference 24. In the TGN the enzyme precursors meet transmembrane glycoproteins which recognize the M6P and bind the enzymes. Two distinct mannose-6-phosphate receptors (MPRs) have been characterized (for reviews see refs. 25, 26): The larger receptor is cation-independent (CI-MPR), whereas the smaller exhibits enhanced binding affinity in the presence of divalent cationes (cation-dependent, CD-MPR). The receptor-ligand complexes are sequestrated into clathrin-coated vesicles budding off from the TGN (see Chapters 2 and 3 of this volume). The vesicles are translocated and fuse with endosomes.

Endosomes

Endosomes can be roughly defined according to the temporal sequence in which they are reached by endocytic tracer molecules taken up from the extracellular space (Fig. 1); for reviews

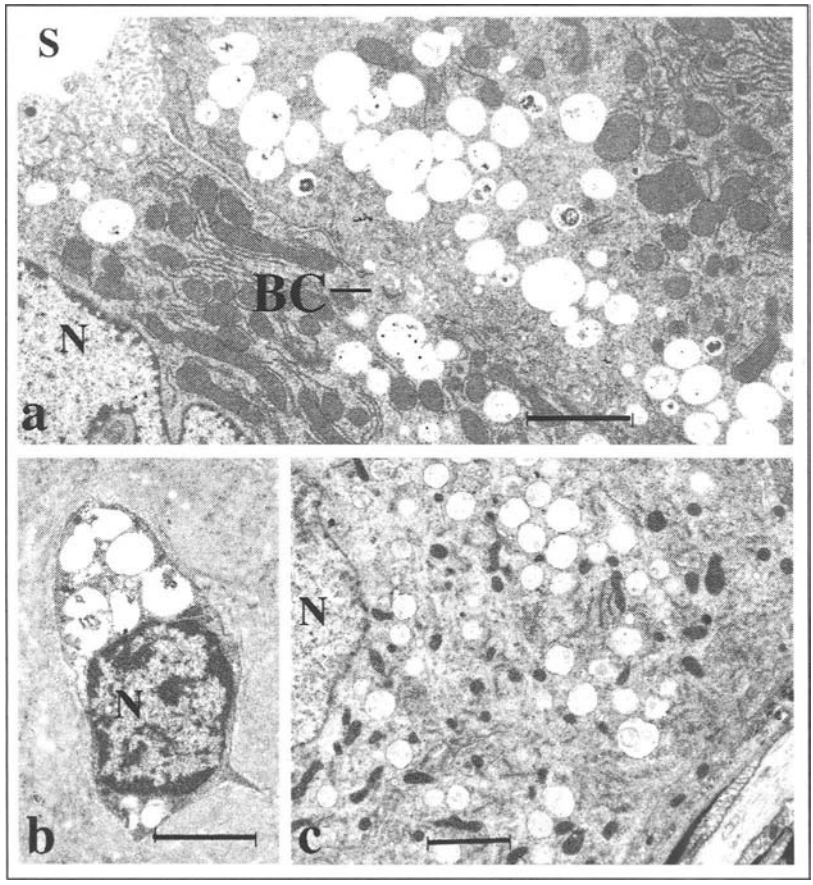

Figure 8. Lysosomes of mice deficient in α-mannosidase, which represent an animal model of the α-mannosidose of humans.[88] Hepatocyte (a), osteocyte (b), and vestibular ganglion cell (c). The lysosomes appear as empty vacuoles because the water-soluble storage material (mannose-containing oligosaccharides) are lost during tissue processing. BC, bile canaliculus. N, nucleus. S, sinusoid. Bars represent 3 μm (a) and 2 μm (b, c).

see references 27-29. After internalization by coated vesicles, the tracer appears in the early endosome (EE) within 1-5 min, in the late endosome (LE) within 10-15 min and accumulates in lysosomes after 30 min or later. EEs are less acidic (pH ≈6.0-6.2) than LEs (pH ≈5.5-6.0); for review see references 30, 31. EEs represents the compartment where the ligand-receptor complexes, if pH-sensitive, dissociate and from where the receptors are cycled back to the plasma membrane. The EE is often described to consist of two subcompartments (for reviews see refs. 32, 33): (a) the sorting endosome, i.e., electron-lucent vacuoles (diameter ≈0.5 μm) with tubular extensions; (b) the recycling endosome, i.e., tubules from where receptors are brought back to the plasma membrane and which are possibly formed by budding off from the sorting endosome. EEs can display a few internal vesicles which are formed presumably by budding off from invaginations of the limiting EE membrane. LEs are most probably generated by maturation of EEs. The internal vesicles (or membrane invaginations seen in cross section) increase in number as the EE matures to become an LE. Thus LEs often have the appearance of multivesicular bodies.

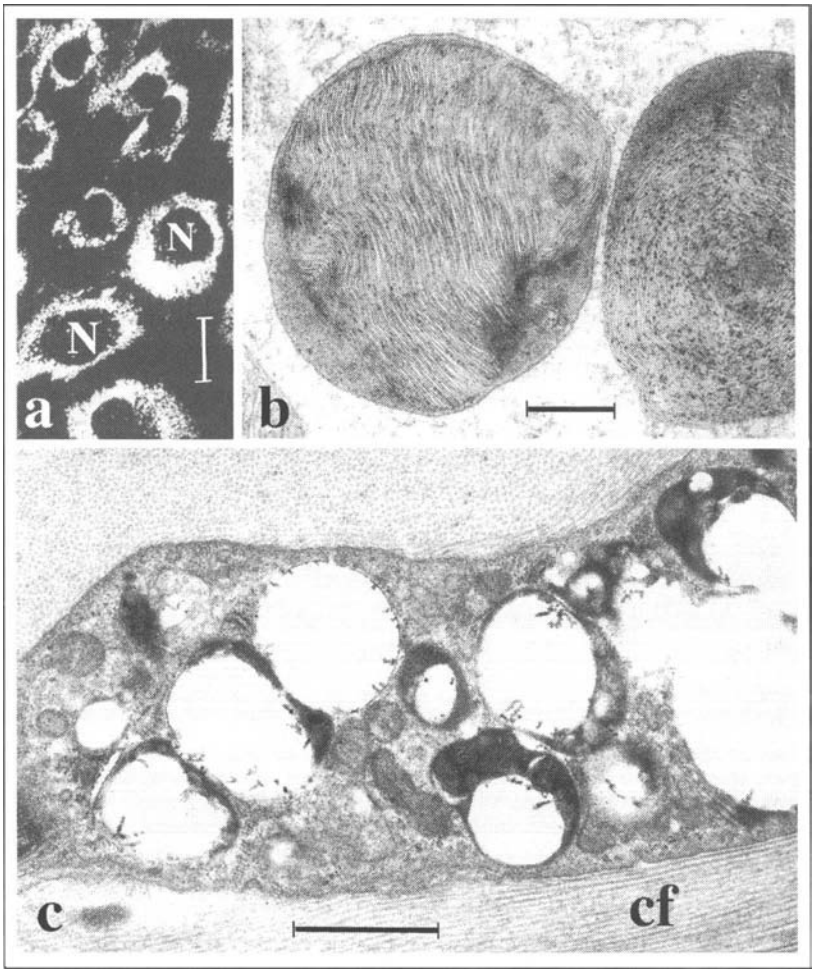

Figure 9. Induced lysosomal storage disorders as adverse drug effects. a) Cultured bovine corneal fibroblasts exposed to the antimalarial drug quinacrine (3 µM, 72 h).[49] Intralysosomal accumulation of the fluorescent drug. N, nucleus (unstained). b) Quinacrine-induced storage of polar lipids as indicated by the multilamellated material within lysosomes; choroid plexus epithelium[89] of rat after prolonged oral treatment with quinacrine, as an example of generalized drug-induced lipidosis.[11] c) Fibroblast from the cornea of a rat after prolonged oral treatment with the experimental anti-tumor drug tilorone,[90] as an example of generalized drug-induced mucopolysaccharidosis. The lysosomes appear as empty vacuoles because the water-soluble glycosaminoglycans, the storage of which has been demonstrated by histo- and biochemistry,[12] are lost during tissue processing. cf, collagen fibrils in longitudinal section and cross section (top). Bars represent 30 µm (a), 0.2 µm (b) and 1µm (c).

From the TGN to Lysosomes

It is not completely clear where the transport of lysosomal enzyme precursors merges with the endocytic pathway. The TGN-derived transport vesicles may deliver their cargo to the LEs directly or via the EEs. In any case, the LE is the main compartment where the pH-dependent MPR-enzyme binding dissociates. The receptors are retrieved into small vesicles, shuttled back to the TGN and become available for another round of enzyme tranfer (receptor recycling).

The last steps of enzyme maturation (dephosphorylation and trimming of the oligosaccharides and proteolytic processing) may be initiated in the LE and completed after arrival in the lysosomes. The way from the LE to the lysosome is not yet fully elucidated; the following possibilities have been proposed (for reviews see refs. 27, 34-36): (a) The LE itself or major portions of it may undergo further maturation to become lysosomes; (b) the lysosomal enzymes and lysosomal membrane proteins may be packaged into transport vesicles which fuse with preexisting lysosomes; (c) the LE and preexisting lysosomes may transiently fuse with each other ("kiss-and run")[37] to exchange contents, or (d) they may fuse to form transient hybrid organelles, followed by reformation of dense-core lysosomes. In any case, the lysosomes are, in contrast to the prelysosomal compartments, devoid of MPR. Under steady-state conditions, the MPRs are present in several populations of endosomes, in the Golgi apparatus, coated vesicles and on the cell surface, but consistently absent from lysosomes; for reviews see references 24, 27, 38.

Enzyme Secretion

The sorting mechanisms in the TGN are not fully efficient. Minor amounts of lysosomal enzyme precursors get into the secretory route, are released by exocytosis and recaptured by receptor-mediated endocytosis with the CI-MPR being responsible for the recapture. Cells of the monocyte/macrophage lineage additionally possess a mannose receptor which may be involved in the recapture of lysosomal enzymes.[39,40] The phenomenon of recapture is the basis for the "cross-correction" (see later) of genetically determined lysosomal storage and thus one of the mechanisms by which inherited lysosomal storage diseases are hoped to be treated efficiently (either by enzyme replacement or by transplantation of stem cells as founders of a competent cell population which serves as enzyme donor) (see Chapter 10 of this volume).

Alternative Sorting Mechanisms

In addition to the MPR-mediated trafficking of soluble lysosomal enzymes there may be additional or alternative mechanisms.This is concluded e.g., from findings on MPR knockout-mice[41] and from data on the human I-cell disease,[42] where the genetically determined lack of the M6P-recognition marker leads to secretion of the lysosomal enzymes from some but not from all cell types. Furtheremore, the MRP-dependent mechanism does not apply to membrane-associated lysosomal enzymes such as for example acid phosphatase, which is synthesized as a transmembrane protein and processed to a soluble enzyme only after arrival in the late endosome or lysosome. For a recent review on alternative mechanisms see reference 43.

Lysosomal Drug Accumulation and Drug-Induced Lysosomal Storage Disorders

A remarkable property of the lysosomes and associated acid compartments is the ability to accumulate weakly basic amines such as ammonia and chloroquine ("lysosomotropic" or "acidotropic" agents),[44] which is thought to be due to intralysosomal protonation of the bases and thus trapping within the acidic organelles. Using fluorescent lysosomotropic agents such as acridine orange or quinacrine, this principle can be used to visualize lysosomes in living cells (Fig. 9a). If such agents are applied at sufficiently high concentrations, they raise the pH in the acidic compartments,[45] interfere with the dissociation of the MPR and its ligand and thus with recycling of free MPRs. As a consequence, major proportions of the enzyme precursors get into the secretory route,[46,47] the cell is deprived of lysosomal enzymes, and all sorts of undigested macromolecules accumulate in the lysosomes. Apart from being useful tools for persuading cultured cells to secrete their lysosomal enzyme precursors, many of these weak bases, if possessing an aromatic ring system and thus being cationic amphiphilic in their charged form, can induce dramatic lysosomal storage of polar lipids and/or sulfated glycosaminoglycans in vivo and in vitro - notably at concentrations well below those enhancing enzyme secretion.[48,49] The intralysosomally accumulated drugs form fairly stable complexes