Chapter 5

Role of Lysosomes in Cell Injury

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INTRODUCTION

Lysosomes are acidic cellular vacuoles that are heterogeneous in shape and size and function in degrading biological constituents derived from the intracellular and extracellular space (Lee and Marzella, 1992; Seglen and Bohley, 1992). In this chapter we will review the mechanisms that regulate the functions of lysosomes and discuss how alterations in these functions lead to cell pathology with special reference to acute and chronic cell injury.

For reviews on the physiology and pathology of biosynthesis, sorting, and processing of lysosomal enzymes and of transport of ions, amino acids, and other macromolecules to lysosomes, readers are referred to the reviews by Kornfeld (1990) and Thoene, (1992).

LYSOSOMAL DEGRADATION PATHWAYS

Autophagy and Heterophagy

The lysosomal pathway for degradation of cellular constituents is called autophagy. Autophagy is subdivided into *macro*autophagy, *micro*autophagy, and crinophagy. *Macro*autophagy is active in nonselective "bulk" degradation of organelles and is activated especially during nutrient deprivation (Mortimore et al., 1989; Seglen et al., 1991; Seglen and Bohley, 1992). *Micro*autophagy is an ongoing process for degrading cytosolic constituents in basal conditions (Marzella and Glaumann, 1987; Mortimore et al., 1988). Crinophagy participates in degradation of secretory proteins (Marzella and Glaumann, 1987).

Macroautophagic vacuoles are formed by membranes of the endoplasmic reticulum (ER) or Golgi apparatus (Marzella and Glaumann, 1987; Dunn, 1990; Yamamoto et al., 1990a,b; Ueno et al., 1991; Noda and Farquhar, 1992). The earliest structure identifiable by electron microscopy, the autophagosome, is typically bounded by two membranes which segregate intact organelles and cytosolic components (Figure 1). The autophagosome fuses with one or several preexisting lysosomes, which contain lysosomal hydrolases. At this stage this structure is called an autophagic vacuole and the degradation of the segregated cytoplasm is carried out. Undegradable substances are in some cases excreted to the extracellular space or more commonly remain stored intracellularly in residual bodies.

Microautophagic vacuoles are autophagic vacuoles forming within a lysosome. They arise by invaginations of surface membranes of lysosomes, leading to the for-

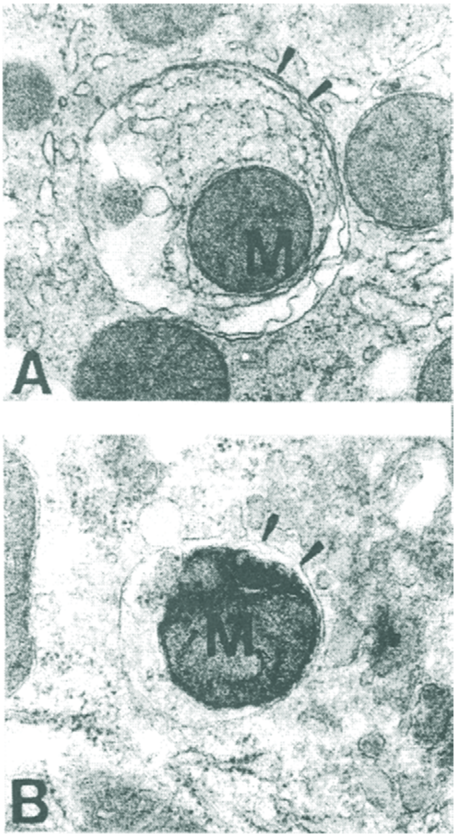

Figure 1. Ultrastructural appearance of autophagic vacuoles.**A.** The micrograph shows a newly formed autophagic vacuole from a cultured rat hepatocyte. The vacuole contains several intact cellular constituents, a mitochondrion (M), rough and smooth endoplasmic reticulum cisternae, and cytosol. At this stage, the autophagic vacuole is surrounded by two surface membrane (arrowheads). **B.** The micrograph shows an autophagic vacuole from a rat liver in a later stage of development. Only one surface membrane surrounds the vacuole (arrowheads) and the sequestered mitochondrion (M), and other constituents appear to be undergoing degradation. Magnifications: **A,** × 47,000; **B,** × 58,000.

mation of an intralysosomal vesicle. Cytosolic components, such as glycogen, ribosomes, or soluble proteins, seem to be taken up in this fashion (Marzella and Glaumann, 1987).

Crinophagic vacuoles are lysosomes containing secretory proteins undergoing intracellular degradation. Newly synthesized secretory proteins are either secreted constitutively or are packaged into secretory vacuoles for eventual extracellular discharge. In some instances, the secretory vacuoles fuse with the lysosomes and the secretory proteins they contain are degraded intracellularly.

The lysosomal pathway for uptake and degradation of materials from the extracellular space is called heterophagy and is particularly active in macrophages and other phagocytes (Figure 2 A,B). This pathway transports materials, such as microorganisms and cell fragments, from the cell surface to the lysosomes via phagosomes for degradation. Proteins, solutes, and other nutrients are delivered to endosomes via coated pits and vesicles. Internalized constituents, including mem-

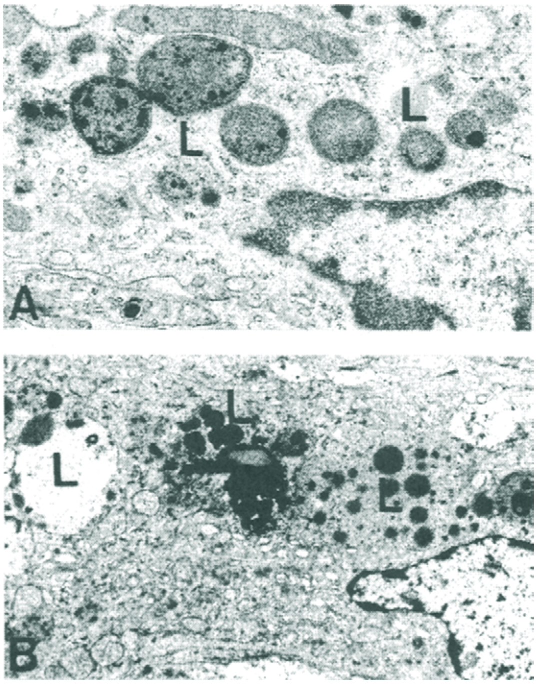

Figure 2. Ultrastructural appearance of heterophagic vacuoles. **A**. This micrograph shows a resting Kupffer cell from rat liver. Note the presence of numerous lysosomes (L). **B**. This micrograph shows a Kupffer cell from a human liver following shock. Note that the lysosomes (L) are full of amorphous materials and are markedly enlarged. These materials are probably derived from the phagocytosis of cellular debris present in the space of Disse or hepatocyte sinusoid. Magnifications: **A**, × 32,000; **B**, × 16,000.

brane, ligands, and receptors, are either recycled to the cell surface or shuttled to lysosomes via late endosomes for eventual degradation.

The lysosomal degradation pathways are shown schematically in Figure 3. This figure also illustrates the functional relationships between the endoplasmic reticulum, Golgi apparatus, and plasma membrane and the lysosomes.

Nonlysosomal degradation pathways are also present in cells. These pathways differ from the lysosomal pathways in subcellular localization, sensitivity to inhibitors, substrate specificity, pH optima, and physiological functions and are regulated independently. For a discussion of the regulation of nonlysosomal degradation pathways and their participation in cell pathology, see Lee and Marzella (1994).

Degradative Capacity of Lysosomal Enzymes

More than fifty lysosomal enzymes have been identified and have been shown to degrade nearly all biological molecules (proteins, lipids, carbohydrates, and nucleic acids) (de Duve, 1983). Most enzymes are "soluble" or loosely associated with the lysosomal membrane. A few enzymes, such as the membrane-associated form of acid phosphatase, are tightly bound and are considered integral membrane proteins (Himeno et al., 1989). Within the lysosomal matrix, the lysosomal enzymes may exist as aggregates. This property favors the retention of the lysosomal enzymes within the lysosomal matrix. It is not known whether individual lysosomes contain the full range of degradative enzymes or if variable numbers and types of hydrolases are present in each lysosomal organelle. The turnover rate of lysosomal enzymes is relatively rapid (approximately one to two days) (Burnside and Schneider, 1982). The half-life of individual lysosomal enzymes varies from 15 hrs to 3 days (Kominami et al., 1987).

Proteins are degraded to the level of constituent amino acids through sequential attack by a variety of lysosomal proteases (see below). The hydrolysis of lipids also takes place in the lysosomal compartment by the action of acid lipases to yield glycerols and fatty acids. Glycolipids, sphingomyelins, and phospholipids are all degraded in lysosomes. The degradation of glycogen can occur in lysosomes by acid α-glucosidases. The degradation of carbohydrate moieties in sugars, proteins, and lipids proceeds by the action of exoenzymes (e.g., glycosidases) giving monosaccharides. In the case of glycoproteins, the peptide backbone is degraded by various lysosomal proteases, followed by the breakdown of glycans. Nucleic acids are first hydrolyzed by an endonuclease, either acid deoxyribonuclease or ribonuclease, into oligonucleotides. Then the oligonucleotides are cleaved by an acid exonuclease to release 3'-phosphomononucleotides and subsequently nucleosides and inorganic phosphates. Breakdown products of lysosomal degradation (amino acids, fatty acids, glycerol, monoacylglycerols, cholesterol, and sugars) return to the cytosol by diffusion /permeation or are transported out of the lysosome by newly discovered specific carriers systems. At least 14 systems active in the transport of

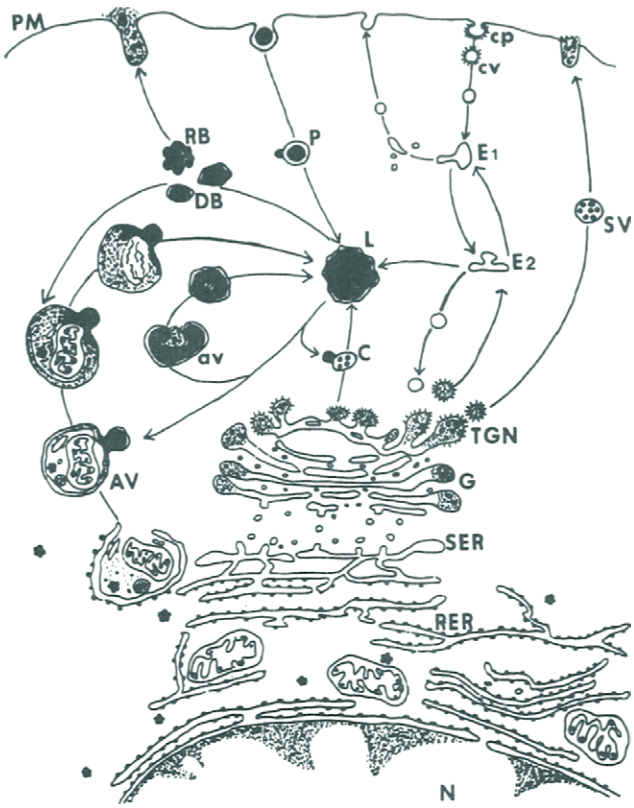

Figure 3. Overview of autophagy and heterophagy. Autophagy: cytoplasmic organelles are sequestered into macroautophagic vacuoles (AV). Proteins and other cytosolic constituents are taken up in microautophagic vacuoles (av). Secretory products diverted from the secretory pathway are taken up in crinophagic vacuoles (C). Macroautophagic and crinophagic vacuoles acquire digestive enzymes by fusing with lysosomes. As the vacuolar contents are degraded, the lysosomes (L) diminish in size, become electron-dense and are designated dense bodies (DB). Lysosomes containing undegradable residues such as lipofuscin, are designated residual bodies (RB), which may in some instances be extruded from cells. Heterophagy: extracellular constituents are carried via phagosomes (P) to the lysosomes for degradation. Proteins and solutes are transported to "early" endosomes (E_1) via coated pits (CP) and vesicles (CV) or pinosomes. Membranes and internalized constituents are recycled to the plasma membrane (PM) or are transported to the lysosomes via "late" endosomes (E_2). The transport of newly synthesized lysosomal enzymes to lysosomes follows the cytosol-rough endoplasmic reticulum (RER)-smooth endoplasmic reticulum (SER)-Golgi apparatus pathway taken by secretory proteins. In the trans-Golgi network (TGN), the lysosomal enzymes are sorted out from proteins destined for secretion and are transported to the lysosomes via late endosomes (E_2).

inorganic ions, amino acids, and other metabolites have been characterized. The specific transport proteins have not yet been identified (Thoene, 1992).

REGULATION OF LYSOSOMAL PROTEIN DEGRADATION

Enzyme Activity, Acidification

Several physiological conditions, such as growth or regeneration, can upregulate the activity of lysosomal enzymes by increasing their absolute concentration or their catalytic activity (de Groen et al., 1989; Rhodes et al., 1989). Changes in levels of naturally occurring enzyme inhibitors may also play a role (Barrett, 1987). Lysosomal acidification mediated by H^+-ATPase (pH close to 5) is essential to activate lysosomal enzymes. The proton gradient across the lysosomal membrane may also contribute to the transport of products of lysosomal hydrolysis to the cytosol (Ohkuma, 1987). Vacuolar acidification is critical for processing lysosomal proenzymes to mature hydrolases and for sorting them (Rothman et al., 1989).

The generation of a pH gradient in the lysosomes is almost exclusively due to an electrogenic proton pump driven by a H^+-ATPase in the lysosomal membrane (Schneider, 1987; Rodman et al., 1991). The H^+-ATPase is made up of eight or nine subunits. A 72-kDa subunit is the catalytic site for ATP hydrolysis, a 57 kDa subunit is a regulatory nucleotide-binding protein, and a 17-kDa subunit most likely participates in forming the proton channel (Klionsky et al., 1990).

Amino Acids and Hormones

Lysosomal protein degradation is regulated by amino acid levels in conjunction with the synergistic and additive effects of hormones. At normal plasma levels of amino acids, cellular proteolytic responses to hormones are commensurate with those caused by amino acid deprivation. The most important hormone to stimulate hepatic macroautophagy and lysosomal protein degradation is glucagon (Mortimore and Pösö, 1987; Mortimore et al., 1989). In skeletal muscle, increased catecholamine levels have been linked to stimulation of protein degradation (Nie et al., 1989). Epinephrine has also been shown to stimulate lysosomal protein degradation in the liver (Mortimore and Pösö, 1987). In muscle, workload is an important regulator of muscle mass.

Insulin and other growth-promoting factors inhibit lysosomal proteolysis (Ballard and Gunn, 1982). It has been found that glucocorticoids stimulate lysosomal protein degradation in hepatocytes (Hopgood et al., 1981). Estrogen can inhibit osteoclastic resorption activity by down-regulation of lysosomal gene expression (Oursler et al., 1993). The inhibitory influence of serum on protein degradation in cultured cells is also presumed to be mediated by growth factors and hormones in the serum. Serum deprivation causes only a transient increase in proteolysis. After 24 hours, the rates of protein degradation decline to levels equal to or even lower than controls. A diet deficient in protein reduces the lysosomal degradation of protein in muscle (Tawa et al., 1992).

Effects of Growth, Age, pH, and Calcium levels of Cells

The growth state of cells is important in the modulation of intracellular protein degradation (Ballard, 1987; Papadopoulos and Pfeifer, 1987). The stimulation of cytoplasmic growth seen during cellular proliferation (e.g., adrenocorticotropic hormone-stimulated proliferation of adrenal zona fasciculata) or hypertrophy (e.g., contralateral compensatory hypertrophy after unilateral nephrectomy) suppresses autophagic-lysosomal degradation (Müller et al., 1987; Jurilj and Pfeifer, 1990).

Cell age also influences intracellular protein degradation (Ballard, 1987; Dice, 1989). It has been proposed that decreased proteolysis may be responsible for the appreciable accumulation of posttranslationally altered proteins in senescent cells. The proliferative arrest in senescent cells may be due to a defect in certain proteolytic systems, and deficient degradation of oxidized proteins has been demonstrated in aging cells (Oliver et al., 1987).

A rise in intracellular pH causes a marked decrease in protein degradation and an increase in protein synthesis (Fuller et al., 1989). Increases in cytosolic Ca^{2+} levels also accelerate proteolysis in muscles by lysosomal and nonlysosomal pathways (Zeman et al., 1985). It has been proposed that a calcium transport system in the lysosomal membrane functions in regulating lysosomal protein degradation (Lemons and Thoene, 1991).

FUNCTIONS OF LYSOSOMAL ENZYMES

Degradation of Proteins

The degradation of proteins by lysosomes is accomplished by exoenzymes (exopeptidases) that cleave bonds only near the ends of molecular chains and by endoenzymes (endopeptidases or proteinases) that hydrolyze peptide bonds in the middle of molecular chains. Endopeptidases are subdivided into cysteine proteinases (e.g., cathepsin B, H, and L) and aspartic proteinases (e.g., cathepsin D and E) (Kirsche and Barret, 1987) based on the identity of the catalytic group at the active site. The degradation of proteins by lysosomal enzymes is essential for several cell function.

As a rule, lysosomal proteases, completely degrade the intracellular proteins segregated in the lysosomes and play an important role in the turnover of cellular proteins. The lysosomal proteases are also involved in changing cellular phenotype by degrading certain differentiation-related proteins (Teichert et al., 1989), and alterations in levels and/or activity of lysosomal enzymes occur during cell differentiation. A number of secretory proteins are degraded by lysosomal proteases (Willemer et al., 1990). Examples are hormones, such as insulin (Schnell et al., 1988), prolactin (Kuriakose, et al., 1989), parathormone (Pillai and Zull, 1986), corticotropin, melanotropin (Uchiyama et al., 1990), and catecholamines (Weiler et al., 1990). Partial proteolytic processing of certain proteins occurs in prelysosomal organelles (for a review, see Lee and Marzella, 1992). Examples of processing of secretory proteins by lysosomal proteases are the conversion of thyroglobulin to thyroxine (Rousset et al., 1989a,b; Rousset and Mornex, 1991), the conversion of prorenin to renin (Wang et al., 1991), and the conversion of procollagen to collagen (Helseth and Veis, 1984). In antigen-presenting cells, lysosomal proteases process endocytosed protein antigens and generate antigen-MHC II complex which are then translocated to the cell surface for presentation to T cells.

Lysosomal proteases also degrade extracellular proteins intracellularly following endocytic uptake (Del Rosso et al., 1991) or extracellularly following the secretion of lysosomal enzymes (Ishii et al., 1991). By these mechanisms, lysosomal proteases participate in remodeling extracellular matrix and bone and in the degradation of plasma proteins, lipoproteins, and cells with finite life spans. Cathepsin B and L, for example, appear indispensable in bone resorption by degrading collagen in the bone matrix (Delaissé et al., 1991). Examples of extracellular proteins that are substrates for lysosomal enzymes are albumin (Baricos et al., 1987), the urokinase type of plasminogen activator (Buktenica et al., 1987; Jensen et al., 1990), low density lipoprotein (Brown and Goldstein, 1986), renin (Marks et al., 1991), and hemoglobin (Diment and Stahl, 1985).

In organs, such as the liver and kidney, lysosomal degradation of endocytosed or phagocytosed constituents assumes a specific physiological importance. For example, in the liver, the resident macrophages (Kupffer cells) clear from the portal circulation bacteria and endotoxin derived from the gastrointestinal tract (Ulevitch, 1991). In the renal tubular epithelial cells, the proteins filtered from glomeruli are rapidly reabsorbed via endocytosis. The internalized proteins begin to undergo degradation in the endosomes and are transported to lysosomes, where protein degradation is completed (Andersen et al., 1987, Haga, 1989). The endocytosis and degradation of filtered protein is greatly augmented when the glomerular filtration barrier is damaged (Haga, 1989). In these conditions, the number and volume of lysosomes increases markedly and acid hydrolases are greatly activated.

Degradation of Lipoproteins and Membrane Lipids

The degradation of phospholipids and neutral lipids segregated in lysosomes by uptake of cellular membranes and lipoprotein particles is accomplished by lysosomal acid lipases (Warner et al., 1981). A lysosomal lipase with broad substrate specificity is almost exclusively responsible for the lysosomal hydrolysis of cholesterol esters, triglycerols, and diacylglycerols. Lysosomes also contain phospholipase A (Löffler and Kunze, 1987; Bartolf and Franson, 1990). Several factors effectively mitigate against cell injury caused by the unregulated hydrolysis of membrane diacylphospholipids by this enzyme and prevent the loss of membrane integrity in normal cells. These factors include the lack of optimal pH in the cytosol, the presence of cations, such as Mg^{2+}, Ca^{2+}, Na^{+}, and K^{+}, and of intracellular and extracellular proteins, such as histone, albumin, fatty acid binding proteins, and immunoglobulins, which inhibit enzyme activity (Kunze et al., 1988). A lysosomal phospholipase C converts phospholipids into diglycerides (Matsuzawa and Hostetler, 1980). Lysosomal phospholipase C also plays a role in the degradation of membranes subjected to lipid peroxidation in injured cells.

LYSOSOMES IN ACUTE AND CHRONIC CELL INJURY

Intracellular Leakage of Lysosomal Enzymes

The lysosomal membrane provides a physical barrier separating the degradative activity of lysosomal hydrolases from cytoplasm. Impairment of lysosomal membrane integrity and release of hydrolases to the cytosol are severely detrimental to cellular physiological functions and integrity. The stability of the lysosomal membrane can be impaired by free radicals probably through lipid peroxidation reactions. It has been demonstrated that loss of lysosomal membrane integrity by lipid peroxidation occurs in photooxidation-induced cell injury (Olsson et al., 1989). Leakage of lysosomal enzymes also occurs after cell death and contributes to autolysis and necrosis.

Several naturally occurring enzyme inhibitors protect living cells against injury caused by the release of lysosomal enzymes (Barrett, 1987; Kirschke and Barrett, 1987). Examples are α_2-macroglobulin, α-cysteine proteinase inhibitor, and cystatins. The first two inhibitors are found in plasma whereas the latter is found in cells and body fluids (Kirschke and Barrett, 1987; Aoyagi, 1989). The cystatins are a group of low molecular weight inhibitors classified on the basis of the type of cell in which they are found. Cystatin A is present in epithelial cells and leukocytes. Cystatin B is in lymphocytes and monocytes. Cystatin C is in neuroendocrine cells. Finally cystatin S is in salivary glands.

Extracellular Release of Lysosomal Enzymes

Lysosomal enzymes released from neutrophils, macrophages, and other inflammmatory cells degrade critical extracellular proteins and may induce injury and loss of function in various organ systems (Kesava Reddy and Dhar, 1991). For example, increased activities of cathepsin B and L play a role in the degradation of cartilage collagens in arthritis (Maciewicz and Wotton, 1991). It has been proposed that one of the factors responsible for the development of smoking-related emphysema is an imbalance between proteases (e.g., elastase) and their inhibitors (α-1 antiprotease), resulting in the destruction of lung parenchyma and interstitium (Snider et al., 1991). Cysteine proteinases are the most likely source of the potent contact-dependent elastase activity of macrophages (Chapman et al., 1984). Cathepsin L has drawn considerable attention in this regard (Reilly et al., 1989).

Highly purified cysteine proteinases B and L are also able to degrade glomerular basement membrane (GBM) and isolated GBM constituents (Thomas and Davies, 1989; Baricos et al., 1991). In experimental models of glomerular disease, the administration of cysteine proteinase inhibitors decreases proteinuria (Baricos et al., 1991). Lysosomal aspartic and cysteine proteinases play a role in the degradation of filtered protein that is endocytosed by proximal tubular cells (Olbricht et al., 1987; Baricos and Shah, 1989).

Reumatoid arthritis is a systemic inflammatory process of unknown etiology in which the destruction of articular connective tissue occurs. Increased levels of cathepsin L and *ras* oncogene transcripts are detectable predominantly in synovial cells in the vicinity of sites of active joint destruction (Trabandt et al., 1990). Cathepsin L is a major *ras*-induced proteinase. *Ras*-induced proteinases have been implicated in the degradation of basement membrane that leads to the ingress and the pathognomonic accumulation of T cells in synovium in rheumatoid arthritis (Gay and Koopman, 1989; Ziff, 1989).

Free Radical Injury

Intracellular ferric iron is an essential mediator of membrane damage caused by free radicals and other reactive oxygen species. A cell pool of ferric iron is reduced by superoxide anions to ferrous iron at first. Ferrous iron in turn reduces H_2O_2 to hydroxyl free radicals. The degradation of iron-containing proteins by lysosomal proteases is an important source of free iron that is available for lipid peroxidation. Ferritin is segregated in the lysosomes by autophagy. The protein moiety is degraded, albeit slowly, by lysosomal cathepsins (Glaumann and Marzella, 1981) and iron is released due to the acidic pH (Sakaida et al., 1990; Hoffman et al., 1991). Modulation of autophagic protein degradation influences the size of the iron pool and the susceptibility of deferoxamine-treated hepatocytes to injury by *t*-butyl hydroperoxide (Sakaida et al., 1990).

Intracellular Activation of Zymogen Enzymes by Crinophagy

Intracellular degradation of secretory proteins by lysosomes can occur by crinophagy (Marzella and Glaumann, 1987). In the pancreatic acinar cell crinophagy may lead to intracellular activation of zymogen enzymes and to pancreatic injury (Resau et al., 1984). Serine proteases (e.g., trypsinogen) are probably activated inside the pancreatic parenchyma by lysosomal hydrolases (e.g., cathepsins), and autodigestion of pancreatic parenchyma occurs (Steer and Meldolesi, 1987; Willemer and Alder, 1991).

Cytotoxic Drugs

Many cytotoxic drugs induce alterations in lysosomes. It has been proposed that these alterations can further increase cell injury, although in many instances direct evidence of the mechanisms involved is lacking.

Aminoglycosides, such as gentamicin, are taken up by receptor-mediated endocytosis and accumulate in lysosomes of renal proximal tubular cells (Wedeen et al., 1983). Although the pathogenesis of the nephrotoxicity remains unknown, it has been proposed that changes in the physiological functions of lysosomes induced by aminoglycosides may alter cellular metabolism and ultimately cause cell death (Kaloyanides and Pastoriza, 1980). Gentamicin can reduce cathepsin B and L activities in renal tubular cells within 24h after inoculation by inhibiting enzyme activities and decreasing enzyme biosynthesis.

Another example of a nephrotoxic drug that induces marked alteration of lysosomes is the immunosuppressive drug cyclosporine (Palestine et al., 1986). This drug may decrease renal blood flow and induce toxic glomerulopathy, tubular atrophy, interstitial fibrosis, and arteriolopathy (Palestine et al., 1986). Kidneys of rats treated with toxic doses of cyclosporine contain numerous lysosomes, autophagic vacuoles, and myeloid bodies (Whiting et al., 1982). It is not clear if these alterations are simply the result of, or if they also contribute to cell injury.

Acidotropic Agents

The so-called acidotropic or lysosomotropic agents cause swelling of lysosomes by dissipating the H^+ gradient and inhibiting lysosomal protein degradation. These agents are weak bases, such as products of cellular metabolism (ammonia, NH_4Cl), or drugs such as chloroquine. They freely permeate into cells and subcellular organelles. Within acidic compartments, such as lysosomes, the bases are protonated and become trapped. The consumption of protons by the weak bases elevates the intralysosomal pH and decreases the catalytic activities of lysosomal enzymes (Krogstad and Schlesinger, 1987). The accumulation of protonated weak bases in the lysosomes is accompanied by an influx of water, leading to marked enlargement of lysosomes and cellular vacuolation (Kalina and Socher, 1991). In the case of inhibitors, such as the weak base chloroquine, lysosomal degradation is also impaired by direct inhibition of cathepsins and by inhibition of mannose-6-phosphate receptor (MPR) recycling, which causes enhanced secretion of lysosomal enzymes (Geuze et al., 1985; Brown et al., 1986). Quaternary ammonium compounds may also inhibit the activities of lysosomal enzymes by direct interaction with lysosomal proteases (Matsumoto et al., 1989).

Infections, Sepsis

Protein degradation in skeletal muscle is commonly augmented by fever and sepsis (see a review by Palmer, 1990). Sepsis markedly enhances (up to 50%) the degradation of muscle protein (Hasselgren et al., 1986) and alters the response of muscle protein turnover to the regulatory amino acid leucine.

The lysosomes of phagocytes are important in infections because they function as an antimicrobial defense system. The oxidative burst and oxygen-independent mechanisms, such as cationic and other nonenzymatic proteins, constitute the first line of defense against microorganisms. The acid proteases are a secondary defense mechanism and are responsible for degrading endocytosed pathogens (Yu and Marzella, 1990). The ability of some microbial pathogens to escape degradation by the lysosomes leads to disease. The mechanisms responsible for the failure of lysosomes to kill pathogens are (1) escape of microorganisms from endocytic compartments; (2) failure of fusion between phagosomes and lysosomes; (3) loss of capacity to degrade microorganisms; and (4) deficient lysosomal acidification.

Endocytosis of Microorganisms

Viruses use two routes to gain entry into cells. The first route of entry is nonspecific. Viruses penetrate the cell by direct fusion of the viral envelope with the cell surface membrane (Payne et al., 1990; Wittels and Spear, 1990). The second routeof entry is via receptor-mediated endocytosis (RME) (Pauza and Price, 1988). The acidification machinery is triggered immediately after the formation of early endosomes, and the H^+-ATPase is activated. At an acidic pH, the internalized viral envelope fuses with the endosomal membrane and releases the nucleocapsid into the cytoplasm before the endosome merges with a lysosome. Viruses thereby evade degradation by lysosomes (Pauza and Price, 1988). This event is observable in many viruses, such as orthomyxorabdoviruses, togaviruses, Semliki forest, vesicular stomatitis, influenza, and retroviruses (Yu and Marzella, 1990). The fusion between viral and endosomal membranes is pH-dependent and can be inhibited by weak bases.

Phagocytosis is a major route for the uptake of microorganisms (e.g., bacteria, fungi, or protozoans) in lysosomes. Some microorganisms, such as *Rickettsia* and *Trypanosomes*, can escape from phagosomes and survive within the cytoplasm (Weiss, 1982). In *Trypanosoma cruzi* infection, a protozoan-derived neuraminidase plays an important role in enhancing parasite access to the cytoplasm of host cells by removing terminal sialyl moieties on carbohydrate chains of lysosomal membrane glycoproteins (Fenton–Hall et al., 1992). In addition, the fusion of lysosomes with the parasitophorous vacuole seems to be required to facilitate the entry of *T. cruzi* into host cytoplasm (Tardieux et al., 1992).

Fusion between Phagosomes and Lysosomes

In cultured macrophages or monocytes lysosomes are unable to fuse with phagosomes containing certain types of pathogenetic microorganisms. These microorganisms include *Legionella pneumophila, Mycobacterium tuberculosis*, and *Leishmania braziliensis* (Lee and Marzella, 1994). Polyanions, such as those present in some microorganisms and the endogenously produced weak base ammonia, can block phagosome-lysosome fusion. Of interest, only viable microorganisms can suppress fusion between lysosomes and phagosomes. Microorganisms that are nonviable or inactivated intracellularly lose this ability (Krogstad and Schlesinger, 1987).

Activity of Lysosomal Enzymes

Several microorganisms remain viable after reaching the lysosomes and a few even continue to replicate. Examples of these resistant pathogens are *Histoplasma capsulatum, Salmonella typhimurium*, and *Leishmania* spp. (Lee and Marzella, 1994). Mechanisms for the resistance to degradation of some of these microorganisms are inhibition of biosynthesis of lysosomal hydrolases (Chakraborty and Das, 1989), protection by carbohydrate moieties of surface glycoproteins, and release of excretory factors that inhibit lysosomal enzyme activity. It is noteworthy that the expression and secretion of cysteine proteinase aids some protozoa in degrading and invading host tissue. This mechanism accounts for the cytopathic effect of virulent trophozoites of *Entamoeba histolytica* (Keene et al., 1990).

Acidification of Phagosomes

Another mechanism that accounts for the survival of some microorganisms in lysosomal compartments of host cells is inhibition of lysosomal or phagosomal acidification (Sibley et al., 1985; Black et al., 1986). Microorganisms able to inhibit acidification include *Toxoplasma gondii, Legionella pneumophila, Nocardia asteroides*, and *Mycobacterium tuberculosis.*

Elevation of intralysosomal pH can arrest growth and proliferation of microorganisms (Krogstad and Schlesinger, 1987). A classic case is seen after the application of the antimalarial drug chloroquine or the weak base NH_4Cl to *Plasmodium falciparum.* These compounds raise the pH of the food vacuoles (lysosomes) of *Plasmodium falciparum* and suppress the degradation of hemoglobin that is indispensable for normal development and replication of the parasite (Krogstad and Schlesinger, 1987; Rosenthal et al., 1988).

Burn Injury

Burn injury increases protein degradation in skeletal muscle up to twofold by the second day after injury (Odessey, 1987). The induction of lysosomal enzyme synthesis may enhance proteolysis in burn injury (Odessey, 1987).

Myopathies and Denervation Injuries

In several pathological conditions, protein degradation is enhanced in skeletal muscle. Myofibrillar components are particularly affected, and pronounced muscle atrophy results. Several proteolytic systems appear to participate in the development of muscular atrophy (Fagan et al., 1987; Katunuma and Kominami, 1987; Driscoll and Goldberg, 1989). It has been proposed that both lysosomal cathepsins and Ca^{2+}-dependent proteases play a role in the enhanced protein degradation found in dystrophic (Turner et al., 1988) or injured (Furuno and Goldberg, 1986) muscle.

In the case of muscle atrophy induced by denervation, an increase in autophagic lysosomal proteolysis may be partially responsible for the atrophy because the activity of lysosomal proteases is augmented (Bird and Roisen, 1986). It is also proposed that Ca^{2+}-dependent neutral proteases (CANPs) mediate muscle atrophy in denervation and other conditions (Bond and Bulter, 1987; Hussain et al., 1987; Badalamente et al., 1989). At least three degradative pathways are active in denervated muscle. These are a non lysosomal pathway in basal conditions, a Ca^{2+}-dependent pathway activated during increased muscle tension (Baracos and Goldberg, 1986), and an autophagic-lysosomal pathway active during metabolic stress (Furuno et al., 1990).

Starvation and Stress

In the postabsorptive state, lysosomal protein degradation in the liver is essential to maintain amino acids and glucose levels in the bloodstream (Mortimore and Khurana, 1990). In stress responses, increased amounts of amino acids are made available through protein degradation to sustain metabolism and the synthesis of new proteins involved in cellular adaptive responses. During short-term starvation, hepatocytes are the most important endogenous source of amino acids. Beyond 48h of starvation, the degradation of nonrespiratory skeletal muscle is accelerated, and the amount of protein in muscle decreases (Mortimore and Pösö, 1987). Most if not all accelerated protein degradation occurs in the lysosomes.

Exercise of high intensity and long duration markedly intensifies protein degradation in the liver (Dohm et al., 1987) and in skeletal muscle (Parkhouse, 1988). Myofibrillary proteins are not affected. The degradation of these contractile proteins is actually diminished during exercise (Dohm et al., 1987; Kasperek and Snider, 1989). This has been explained by an elevation of intralysosomal pH through accumulation of ammonia after exercise and an increase in the permeability of lysosomal membranes (Tsuboi et al., 1993).

Accumulation of Iron and Other Metals in Lysosomes

Idiopathic hemochromatosis is a hereditary metabolic disease in which excessive iron accumulates within the parenchyma of many organs, particularly the liver. The intracellular iron is bound to apoferritin to form ferritin molecules, which are located in the cytosol. Ferritin also accumulates in lysosomes because of its relative resistance to degradation (Glaumann and Marzella, 1981). Although the pathogenesis of cell injury in idiopathic hemochromatosis has not been completely elucidated, it is postulated that the generation of free radicals and peroxidation of membrane lipid play an important role (Myers et al., 1991). Ultrastructural alterations of lysosomes occur in iron-overloaded cells in parallel with biochemical evidence of increased lysosomal fragility and leakage of acid hydrolases (LeSage et al., 1986). Lysosomes isolated from iron-overloaded livers appear enlarged and deformed and show an increase in membrane fragility, a decrease in membrane fluidity, and an rise in pH (Myers et al., 1991). Increased lysosomal membrane fragility is also observed in iron-loaded, cultured, cardiac myocytes (Link et al., 1993).

Untreated patients with hemochromatosis usually develop liver cirrhosis (Basset et al., 1986). Stål et al. (1990) found that in biopsies from livers with precirrhotic hemochromatosis, the volume density (V_d) of lysosomes increased in hepatocytes and Kupffer cells in parallel with increases in iron. The number of iron-laden lysosomes dramatically decreases and hepatic ultrastructure reverts to normal after therapeutic phlebotomies (Cleton et al., 1988; Stål et al., 1990).

The accumulation of other mineral elements in the lysosomes may also damage the lysosomal membrane and lead to cell injury. For example, lysosomal damage is closely correlated with the amount and duration of aluminum loading (Stein et al., 1987; Berry et al., 1988).

Mineral elements, such as aluminum, chromium, uranium, and cerium are reabsorbed by renal epithelial cells in the proximal convoluted tubules and are precipitated in lysosomes as insoluble phosphate salts by the action of acid phosphatase. Eventually, these phosphate salts are excreted in the urine. Pulmonary cells dispose of metal particles inhaled into the respiratory passages by precipitation within lysosomes. The accumulation of phosphate particles in the lysosomes of pneumocytes prevents the diffusion of these toxins into the interstitial capillaries. These metal salts and other inert inhaled particles are finally slowly cleared by pulmonary macrophages. Some metals can inhibit the activity of lysosomal enzymes. For example, Cu^{2+} inhibits lysosomal acid cholesterol ester hydrolase in the presence of hydroxylamine and ascorbic acid (Tanaka et al., 1988). Moreover, the breakdown of metalloprotein may be markedly decreased through inhibition of cathepsin B and/or L activity by protein-associated metal elements (Choudhuri et al., 1992).

Accumulation of Pigments in Lysosomes

Tissue necrosis, vitamin E deficiency, certain lysosomal storage diseases in the central nervous system, and ageing are associated with the accumulation of lipopigments, called ceroid and lipofuscin, in the lysosomes (Goebel and Busch, 1990;

Palmer et al., 1990). Ceroid is considered to be the undegraded remnant of material derived from heterophagocytosis and is characteristically seen in macrophages (Gedigk and Totovic, 1983). Lipofuscin, on the other hand, is thought to be made up of residues derived from autophagy. These polymerized lipid-protein complexes are resistant to hydrolysis and accumulate in the lysosomes.

Proteolytic decline and peroxidative stress may also contribute to the genesis of lipofuscin (Porta, 1991). In human senescent brains and in brains of patients with Alzheimer's disease, a defective or deficient degradation of a variety of proteins, such as amyloid precursor protein, by lysosomal enzymes plays an important role in the generation of β-amyloid deposits found, for example, within neuritic (senile) plaques (Cras et al., 1991; Tagawa et al., 1992).

Storage Diseases

Lysosomal storage diseases share the following characteristics: a complete or partial deficiency of lysosomal enzymes, an accumulation of undegraded materials within the lysosomes, and inheritance. Several genetic or induced abnormalities and deficiencies of lysosomal hydrolases or cofactors result in the accumulation of undegraded substrate in the lysosomes. The number and size of the lysosomes gradually increases in the affected cells, and the cells and organs become enlarged and dysfunctional (Patel, 1989).

Inherited

Several pathophysiological mechanisms induce these genetic storage disorders. The first mechanism is a lack of a protective glycoprotein that normally links certain lysosomal enzymes. This linkage reduces the susceptibility of the enzymes to proteolysis and is necessary for their activities (d'Azzo et al., 1989; Galjart et al., 1990). The second mechanism that induces storage disorders is a deficiency of nonspecific activator proteins (saponins A, B, C, or D) required for the lysosomal enzymatic hydrolysis of glycolipids (sphingolipids) (Li et al., 1988; O'Brien et al., 1988; Sandhoff et al., 1989). In their absence, despite normal lysosomal hydrolase activity, activator-deficient metachromatic leukodystrophy develops.

The third mechanism that induces inherited storage disorders is decreased levels or total deficiency of specific hydrolases caused by (i) decreased or defective biosynthesis due to genetic mutations resulting in amino acid substitution and/or deletion (e.g., Gaucher disease) (Galjaard and Reuser, 1984); (ii) incorrect sorting and targeting of hydrolases (e.g., I-cell disease) (Kornfeld and Mellman, 1989; Kornfeld, 1990); and (iii) defective synthesis of subunits which prevents the normal assembly or translocation of the enzymes (Lau and Neufeld, 1989; Paw et al., 1990). Finally, the fourth mechanism that induces inherited storage disorders is impaired carrier-mediated transport of degradation products from lysosomes (e.g., cystinosis; infantile free sialic acid storage disease, and vitamin B_{12} storage disorder) (Shih et al., 1989; Tietze et al., 1989; Mancini et al., 1991).

Acquired

Lysosomal storage diseases are inducible by cationic amphiphilic drugs. These compounds cause the formation of lamellar structures containing polar lipids in lysosomes. The mechanisms for the accumulation of lamellar structures include formation of undegradable drug-lipid complexes, raised lysosomal pH induced by the segregated drugs, and reversible inhibition of the activities of lysosomal phospholipases A and C (Reasor, 1989). Numerous reports have indicated that cell injury induced by these cationic drugs parallels the appearance of lamellar structures in cells.

Atherosclerosis

Lysosome participate in both physiological and pathological lipid metabolism. The stepwise buildup of free cholesterol in lysosomes of vascular smooth muscle cells and macrophages (Tangirala et al., 1993) has been proposed as one of the mechanisms responsible for atherosclerotic plaques.

The biosynthesis of cholesterol is modulated by receptor-mediated endocytosis and lysosomal degradation of low density lipoproteins (LDL) (Brown and Goldstein, 1986). In lysosomes, the protein/phospholipid coat of LDL is degraded and cholesteryl esters are hydrolyzed by lysosomal lipases freeing cholesterol (Brown and Goldstein, 1986). A rise in unesterified cholesterol derived from LDL or from endogenously synthesized cholesterol results in inhibiting cholesterol biosynthesis and activation of a cholesterol-esterification-catalyzing enzyme.

Perturbations in the endocytosis or degradation of LDL derived from the bloodstream or in the esterification of cellular cholesterol by microsomal ACAT can lead to the accumulation of lipids in cells (Tabas et al., 1987). In atherosclerosis, the arterial intima is infiltrated with pathognomonic, lipid-laden cells (so-called foam cells), derived from circulating monocytes or smooth muscle cells of the arterial media. In the early stages, a lipid of foam cells is predominantly localized in intracellular cytosolic inclusions. With the progression of the disease, intracellular lipid deposits become massive and the site of accumulation shifts to the lysosomes (Jerome and Lewis, 1985; Jerome et al., 1991).

LDLs modified by acetylation, oxidation, or conjugation with malondialdehyde are more effective in inducing the formation of lipid-laden (foam) cells. Unlike acetylated LDL, roughly only 50% of internalized oxidized LDL is ultimately degraded. This phenomenon has been ascribed to resistance of oxidized LDL to degradation by lysosomal cathepsins (Loughheed et al., 1991).

Reverse transport of cholesterol from lysosomes to plasma membrane is known to take place. This efflux is constitutive. High density lipoprotein particles (HDL) remove the free cholesterol from the plasma membrane of cells. A negative correlation exists between plasma HDL levels and atherosclerosis indicating the crucial role of cholesterol efflux from lysosomes in the pathogenesis of this disease.

LYSOSOMAL DEGRADATION IN TUMORIGENESIS AND TUMOR METASTASIS

Lysosomal Protein Degradation and Acidification in Cancer Cells

Normal cells respond to a variety of stressful stimuli, such as nutritional deprivation by increasing protein degradation. It has been hypothesized that cancer cells may be resistant to stimuli that accelerate protein degradation and may also down-regulate basal proteolysis. These changes could enhance the survival and growth of the cancer cells particularly in conditions of nutrient stress (Lee et al., 1989 and 1992). It is generally accepted that cancer cells manifest lower basal protein degradation and decreased lysosomal enzyme activities, compared with normal cells (Schwarze and Seglen, 1985).

The viability of normal hepatocytes incubated in nutrient-free media increases to the same level as that of transformed hepatocytes when an autophagic inhibitor, 3-methyladenine, is added to culture media (Schwarze and Seglen, 1985). These observations support the postulation that the capacity to down-regulate autophagic protein degradation increases the resistance to injury and enhances the growth of cancer cells.

Role of Lysosomal Proteases in Tumor Invasion and Metastasis

Unlike normal parenchymal cells (Hohman and Bowers, 1984), cancer cells secrete a variety of proteases that degrade extracellular matrix and facilitate local invasion and metastasis of tumors (Boyer and Tannock, 1993). These proteases include urokinase and tissue type plasminogen activators (Rifkin et al., 1989; Hollas et al., 1991; Oka et al., 1991), collagenases (Nakajima et al., 1987), trypsin (Koivunen et al., 1991), metalloprotease (gelatinase) (Matrisian, 1990; Chen et al., 1991), glycosidase (Nakajima et al., 1984), stromelysin (Matrisian, 1990), and the lysosomal cysteine or aspartate proteases (Nathalie et al., 1990), B (Watanabe et al., 1987), D (Capony et al., 1989; Rochefort et al., 1990), H (Tsushima et al., 1991), and L (Dong et al., 1989).

The balance between the levels of lysosomal and nonlysosomal proteases and levels of their inhibitors, altered synthesis and translocation of cathepsin B, and in particular an enhanced secretion of enzyme are critical determinants of tumor growth and invasion. Sloane et al. (1990) have proposed that malignant tumor cells are capable of establishing an acidic extracellular microenvironment, in which a variety of lysosomal proteases (e.g., cathepsin B) and glycosidases (e.g., β-hexosaminidase) function optimally. By this mechanism, the destruction of basement membrane and connective tissues matrices may thus be intensified.

Cathepsin B

Secretion of the cysteine proteinase cathepsin B by malignant and benign tumors has drawn a great deal of attention because secretion of cathepsin correlates with the metastatic potential of tumors (Keren and LeGrue, 1988). Cathepsin B activity is regulated by an intracellular cysteine proteinase inhibitor (CPI), also known as cystatin. In many tumors the regulation of protease activity by cysteine proteinase inhibitors is lessened because of decreases in the levels of CPI or in the affinity of CPI for cysteine proteases (Lah et al., 1989).

Cathepsin L

Secretion of procathepsin L, a lysosomal cysteine protease precursor, is markedly up-regulated by tumor promotors (Gal et al., 1985), growth factors (e.g., epidermal growth factor [EGF]; fibroblast growth factor [FGF]; platelet-derived growth factor [PDGF]) (Frick et al., 1985; Chiang and Nilsen-Hamilton, 1986; Dong et al., 1989), and viral transformation (Hiwasa et al., 1991). The level of expression of cathepsin L in H-*ras*-transformed murine fibroblasts is closely associated with their metastatic potential of the cells (Denhardt et al., 1987).

Cathepsin D

Several lines of evidence have indicated the importance of up-regulated biosynthesis and increased secretion of cathepsin D in enhanceing tumor proliferation, invasion, and metastasis (Rochefort et al., 1990). For example, the forms of cathepsin D secreted by cancer cells may have autocrine mitogenic potential (Vignon et al., 1986; Garcia et al., 1990). Also critical in tumorigenesis is the role of procathepsin D in degrading or activating specific substrates. Indeed, this secreted enzyme has been shown to be able to degrade basement membranes, impair growth factor receptors, modulate antigen processing, activate cathepsin B and other proteases, and activate transforming growth factor-β (TGF β) (Briozzo et al., 1988; Pagano et al., 1989; Rochefort et al., 1990).

Direct evidence of the association between intensified metastatic competency and overexpression of cathepsin D gene has been provided. Clinical investigations have indicated that the level of cathepsin D in primary breast cancer is correlated with recurrence and metastases and may be the best indicator of prognosis, independent of other parameters (Thorpe et al., 1989; Tandon et al., 1990).

SUMMARY

Lysosomes are acidic intracellular vacuoles of heterogeneous shape, size, and content. Lysosomes contain hydrolytic enzymes that degrade proteins, lipids, carbohydrates, and nucleic acids derived from intracellular (through autophagy) and extracellular (through heterophagy) sources. Lysosomal degradation regulates several physiological cell functions. These include turnover of cellular organelles and extracellular constituents; amino acid and glucose homeostasis; processing of proteins; lipid metabolism; cell growth, differentiation, and involution; host defenses against microorganisms and other pathogens; and removal of necrotic and foreign material from the circulation and from tissues.

Lysosomal degradation also plays an important role in the pathophysiology of acute and chronic cell injury, inflammation and repair, and tumor growth and metastasis. The participation of the lysosomes in the specific types of cell injury we have discussed is due to altered regulation of one or more of the following processes: turnover of cellular organelles by autophagic degradation; levels and activities of lysosomal hydrolases; levels of intracellular and extracellular lysosomal hydrolase inhibitors; transport of degradation products from the lysosomal matrix to the cytosol; permeability of the lysosomal membrane to hydrolases; lysosomal vacuolar acidification; transport of degradable substrates and of pathogens to the lysosomes; transport and processing of secretory proteins and lysosomal hydrolases during biogenesis; traffic and fusion of lysosomal vacuoles and vesicles; secretion of lysosomal hydrolases; and accumulation of metals, particularly iron, acidotropic agents, and undegraded and/or undegradable materials in lysosomes.

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