The Discovery of Lysosomes

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Lysosomes ("lytic particles") act as the primary component of the intracellular digestive system in virtually all eukaryotic cells, both plant and animal. First recognized biochemically in rat liver, these organelles are membrane-bounded and contain a variety of digestive enzymes active at acid pH. Their existence and properties became evident during investigations concerning the latency of the enclosed enzymes. Initially defined by the presence of a single enzyme, acid phosphatase, which liberates inorganic phosphate from a number of monophosphoric esters, lysosomes are now known to contain at least 50 acid hydrolases, including various phosphatases, nucleases, glycosidases, proteases, peptidases, sulfatases, and lipases. Collectively, they are capable of hydrolyzing almost all classes of macromolecules according to the following scheme:

$$A - B + H_2O \rightarrow A - H + B - OH.$$

The breakdown products are usually available for metabolic reuse. Functionally, therefore, the lysosome appears to serve as a modern recycling plant (or refuse dump), scavenging and using whatever can be saved, and sometimes accumulating and sequestering indigestible residues as a final resort, sometimes for the life span of the cell.

Customarily, after introducing and characterizing a cellular organelle, one would then present a diagram or electron micrograph and describe its distinctive physical features, so that it would be easily recognized and remembered. In this respect, the lysosome is unique in that its size is variable (from very small to extraordinarily large), and its contents are typically heterogeneous and difficult to predict, because of dependency upon the recent "meal" and the amount of time elapsed since the ingestive event. This is somewhat analogous to the situation of a pathologist at autopsy, attempting to forecast the stomach content of a patient recently dead, in the absence of a reliable history. Indeed, it is this unparalleled aspect of polymorphism, even within the same cell, that makes the discovery of the lysosome different from that of other organelles, as the reader will appreciate in the story to be unfolded.

1949–1952: University of Louvain, Belgium

The trail of the discovery of lysosomes is not a difficult one to follow. "All we wanted was to know something about the localization of glucose-6-phosphatase, which we thought might

provide a possible clue to the mechanism of action or lack of action, of insulin on the liver cell"—so explained Christian de Duve upon acceptance of the Nobel Prize for Physiology or Medicine, December 12, 1974, a prize he shared with Albert Claude and George Palade (1). Although the facts of history do not change, the interpretation of history is always changing because the here-and-now reflects the current perspective of the observer. In sketching this brief history of lysosomes, some 25 years after their discovery in 1955, I can visualize the project as a modern-day grant proposal and progress report:

1949: Specific aim: to localize the enzyme glucose-6-phosphatase
Significance: to elucidate the mechanism of action of insulin on the liver.

1952: Progress Report: Unfortunately, no progress has been made on this problem; rather, we would like to report on ... "From Insulin to Latent Acid Phosphatase"....

The lysosome introduced itself in the Laboratory of Physiological Chemistry at the University of Louvain on December 16, 1949 as a crytic form of latent acid phosphatase. The new chairman, Christian de Duve, had just returned from a year of research in St. Louis with the Coris (Nobel laureates, 1947), the discoverers of hepatic hexose phosphatase and with Earl Sutherland, Jr. (Nobel laureate, 1971). He and his students, Jacques Berthet and Lucie Dupret, continued to work on enzymes involved with the metabolism of carbohydrates in rat liver and were able to characterize the hexose phosphatase as a specific glucose-6-phosphatase with a slightly acid pH optimum. In addition, they differentiated it clearly from the nonspecific acid phosphatase acting on glycerol-2-phosphate (β-glycerophosphate) and other phosphate esters upon which glucose-6-phosphatase is entirely inactive. These studies utilized extracts prepared "with typical disregard of cellular organization by vigorous dispersion of the tissue in a high-speed Waring blender in the presence of distilled water." When purification of the enzyme was next attempted, the investigators met an unexpected snag—once precipitated, the enzyme could not be redissolved (2).

At this point, a gentler technique—cell fractionation by differential centrifugation, which had recently been introduced by Albert Claude in 1946 (3)—was employed. Rat liver cells were ruptured with the use of the Potter-Elvehjem homogenizer as a grinding device and 0.25 M sucrose as medium, then further fractionated by several stages of centrifugation. After various procedural modification, the workers succeeded in localizing 95% of the enzyme activity in the microsomal frac-

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tion, thereby establishing the unique distribution of glucose-6-phosphatase in microsomes. (This accomplishment and subsequent experiments by other investigators concerned with the single focus of cytochrome oxidase in the mitochondrial fraction [4] led to the postulates of biochemical homogeneity and unique [sole] location of any enzyme, as discussed by de Duve in *The Harvey Lectures*, 1965 [5]. These two concepts served as working hypotheses in much of de Duve's later research.)

Among the enzymes assayed in the above study, however, was acid phosphatase, largely included for control purposes. To the surprise of the experimenters, acid phosphatase activity in the homogenate was only a 10% of what they had anticipated on the basis of previous assays of preparations subjected to the more drastic homogenizing action of a Waring blender. After 5 days, the same fractions (kept in the refrigerator) were again assayed; this time, the activity of the homogenate was of the right order of magnitude, with a distinct peak in the mitochondrial fraction (see Fig. 1). To quote de Duve: "...we could have rested satisfied with this result, dismissing the first series of assays as being due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome." (For a more detailed report, the reader is advised to peruse the charming and adventurous chapter called "The Lysosome in Retrospect" by de Duve [2].) Additional studies demonstrated that results of the first series of experiments were not due to a technical error, but that most of the enzyme content in the "fresh" preparations must have been present in masked form and become activated with storage. Only a few months of work were required to establish that the latency of acid phosphatase was attributable to a membranelike barrier limiting the accessibility of the enzyme to its substrate. "Thus, the lysosome had made itself known to us as a saclike structure surrounded by a membrane and containing acid phosphatase."

At first, the particles containing acid phosphatase were believed to be mitochondria (6). This interpretation seemed reasonable because there were only three fractions—nuclear, mitochondrial, microsomal, and finally the nonsedimented portion, the supernate (see Fig. 1); the acid phosphatase activity clearly sedimented in the mitochondrial fraction. According to de Duve, progress was achieved in this area, again by chance, taking the inconvenient form of a breakdown in the high-speed attachment of the centrifuge. This caused Françoise Appelmans, who was then studying acid phosphatase latency on isolated "mitochondria" to prepare her mitochondrial fractions by a makeshift procedure using a less powerful ordinary table-

top centrifuge. She succeeded in sedimenting a sizable amount of particles in this way, but found to her great disappointment that her fractions were almost devoid of acid phosphatase activity. They did, however—as later experiments demonstrated—possess plenty of respiratory activity. Investigations prompted by these findings established that the old "mitochondrial fraction" could be subfractionated into a light and a heavy fraction, containing the particles with acid phosphatase and cytochrome oxidase, respectively (7). Eventually, the particles incorporating acid phosphatase were shown to comprise a distinct group, different from both the mitochondria and the microsomes, and designated "intermediate particles."

1952–1955: Extension to Other Acid Hydrolases: The Lysosome as a Biochemical Concept

In 1952, at the Second International Congress of Biochemistry in Paris, evidence that acid phosphatase belonged to a special type of cytoplasmic particle was presented. At this meeting, a young British biochemist, P. G. Walker, mentioned to de Duve that he had obtained data very similar to the Louvain group's findings on acid phosphatase, but on β-glucuronidase instead (8). With this statement in mind, the Belgian investigators tested a number of enzymes for presence in the key light (L) fraction and for latency. By 1955, five enzymes had been localized in the L fraction (Fig. 2) and proved to be
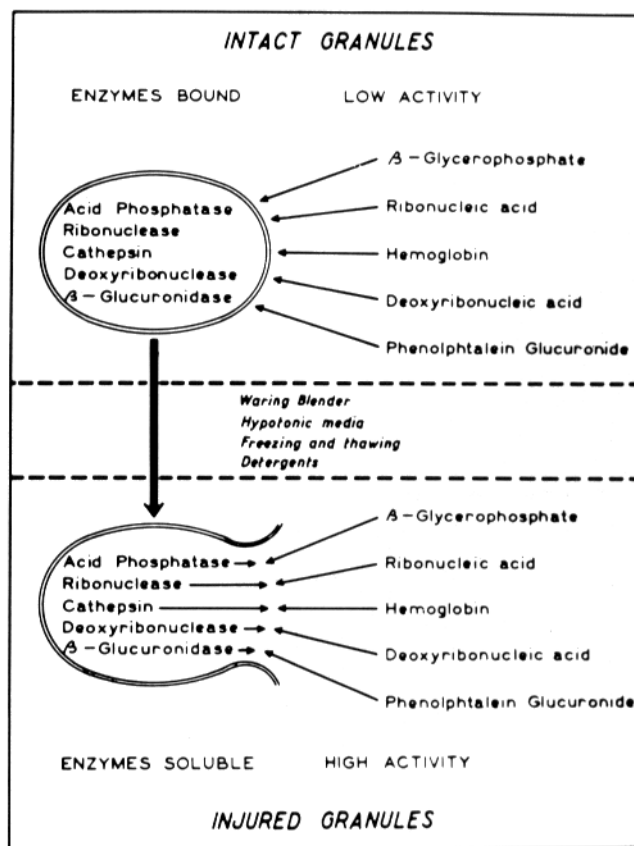

FIGURE 2 Biochemical model representative of rat liver lysosomes as first described by de Duve et al. in 1955. We now know that lysosomes contain at least 50 hydrolases (9), which can act on such diverse macromolecules as nucleic acids, proteins, glycoproteins, polysaccharides, and various lipids.

Fraction	Phosphatase acide en μ g P/20 min.	
	16/12/49	21/12/49
Homogénéisat	10	89
Noyaux	2	10
Mitochondries	7	46
Microsomes	6	10
Decantat	6	9

FIGURE 1 Acid-phosphatase activity in fresh and aged fractions separated from rat-liver homogenates. (Results of Berthet and de Duve [1951], copy of old slide [2].)

hydrolytic enzymes with an acid pH optimum (10). Moreover, all acted upon different sets of natural substrates. Such an apparent coincidence was considered biologically meaningful and interpreted to imply that the particles containing these enzymes fulfilled some sort of nonspecific lytic function. Hence the term "lysosomes," denoting lytic particles or bodies, was proposed (10). The lysosomes themselves were perceived as membrane-bounded granules enclosing five acid hydrolases in latent form (Fig. 2).

1955–1956: Morphological Identification of Rat Liver Lysosomes as the "Pericanaljcular Dense Bodies" of Rouiller

Not until 1955 did electron microscopy make its contribution to the identification of lysosomes. Independently of de Duve, a group of cell biologists headed by Alex Novikoff at the University of Vermont had been conducting experiments which involved systematic variations of the cell fractionation scheme in rat liver. They had examined closely a number of enzymes, including (in a remarkably prophetic manner) the use of markers for practically every distinct entity that has since been recognized in rat liver: 5'-nucleotidase (plasma membrane), succinate oxidase (mitochondria), acid phosphatase (lysosomes), urate oxidase (peroxisomes), and esterase (microsomes). Additionally, they had extensively studied the morphology of their fractions by phase-contrast microscopy (11). In 1955, during the Third International Congress of Biochemistry in Brussels, Novikoff visited de Duve's laboratory and was able to obtain the first electron micrographs of cell fractions containing partially purified lysosomes. These specimens were fixed in osmium, and, in addition to known particles (excessively sad-looking mitochondria), the pictures exhibited multitudes of characteristic bodies that had occasionally been observed in intact liver cells and had been termed "pericanalicular dense bodies" by Rouiller in 1954. Their function was unknown; the name signified only their preferential location in cells along the bile canaliculi and their electron density to the beam of the electron microscope (12). Identification of the lysosome activity with these dense bodies, a provisional association at the time, has since been confirmed by a diversity of techniques discussed later. (It happened that microbodies or peroxisomes were also present in such rat liver preparations [see Fig. 3a].) The next and extremely helpful step was the development of a reliable staining method for acid phosphatase reaction at light and electron microscope levels (Fig. 3b). The basic procedure, evolved by Gomori (14), is performed in two steps—the first yielding lead-phosphate, which can be seen by electron microscopy as dense, needlelike crystals (see Fig. 3b). The phosphate released by enzymatic hydrolysis from the substrate (β-glycerophosphate, grade I) at pH 5 is precipitated by the lead ions present in the incubation medium. In the second step, lead phosphate is transformed into lead sulfide by ammonium sulfide, a brown-black precipitate visible by light microscopy. Novikoff (15), Holt (16)and Barka and Anderson (17) effected significant improvements in extending this technique to the fine-structural level. Their work provided independent confirmation of the lysosomal nature of the dense bodies, and subsequently afforded considerable impetus to the study of the existence, origin, morphological features, and functional properties of lysosomes in a broad variety of biological tissues.

1958: Beginning of the Functional Concept

Although the thought that lysosomes might play a role in

intracellular digestion was mentioned in the Louvain group's first publication, it is fair to state that few people were ready to accept in 1955 what is now taken for granted, namely, that intracellular digestion is a general function common to virtually all animal and plant cells. The first definite clue to the function of lysosomes came from the work of Werner Straus, who deserves the credit for undertaking studies which would almost certainly have led to an independent discovery of lysosomes. Straus had obtained good evidence that the "droplets" of the proximal tubule of the kidney were a site of storage and breakdown of reabsorbed proteins. By 1954, he had succeeded in subfractionating these droplets and showed them to be rich in acid phosphatase and protease (18), and by 1956, he found other hydrolases similar to those described in liver lysosomes (19). This early work on the kidney provided the first clear link between lysosomal digestion and endocytotic uptake of extracellular materials. Together with a few other data obtained from organs as diverse as brain and spleen, as well as some lower organisms, de Duve presented the first schematic outline of the possible biological functions of lysosomes at a meeting organized by the Society of General Physiologists at Woods Hole, Massachusetts in June 1958 (20). It was postulated that the collection of acid hydrolases present in lysosomes could have but one function, that of acid hydrolysis. Furthermore, an attempt was made to link lysosomes with several natural processes. "These may comprise: digestion of foreign material, engulfed by pinocytosis, athrocytosis (old term for endocytosis) or phagocytosis; physiologic autolysis, as presumably occurs to some extent in all tissues, and particularly as part of the more specialized processes of involution, metamorphosis, holocrine secretion, etc.; pathological autolysis or necrosis." It should be mentioned that digestive and autolytic phenomena had been known for a long time, and their dependence on many of the enzymes found in lysosomes had been at least strongly suspected. However, no satisfactory explanation had been provided heretofore for their inhibition in the heathly cell. In developing the theory of intracellular acid digestion, considerable importance has always been attached to the structure-linked latency of the lysosomal hydrolases, which provided the first satisfactory explanation for the fact that autolysis is largely held in check in most cells, despite their content of highly active hydrolytic enzymes.

1953–1965: The Discovery of Peroxisomes—the Microbodies of Rouiller

We now know that the light fraction of rat liver contains two distinct populations of functional particles—lysosomes and peroxisomes (Fig. 3). The latter are membrane-bounded organelles containing enzymes which catalyze reactions involving hydrogen peroxide, and hence have been termed peroxisomes (21). Three of these catalyzing enzymes produce hydrogen peroxide (urate oxidase, D-amino acid oxidase, and α-hydroxyacid oxidase) and one (catalase) destroys it.

The purification of rat-liver peroxisomes was accomplished with good yield by Wattiaux and co-workers (22), taking advantage of their finding that a preliminary intravenous injection of Triton WR-1339 two days before sacrificing the animal caused a considerable decrease in the equilibrium density of lysosomes in a sucrose density gradient (Fig. 4). When these fractions were examined by electron microscopy, there was no doubt that the microbodies of Rouillier were indeed the particles biochemically characterized as peroxisomes (13, 23). Their morphology in intact rat liver is illustrated in Fig. 5.
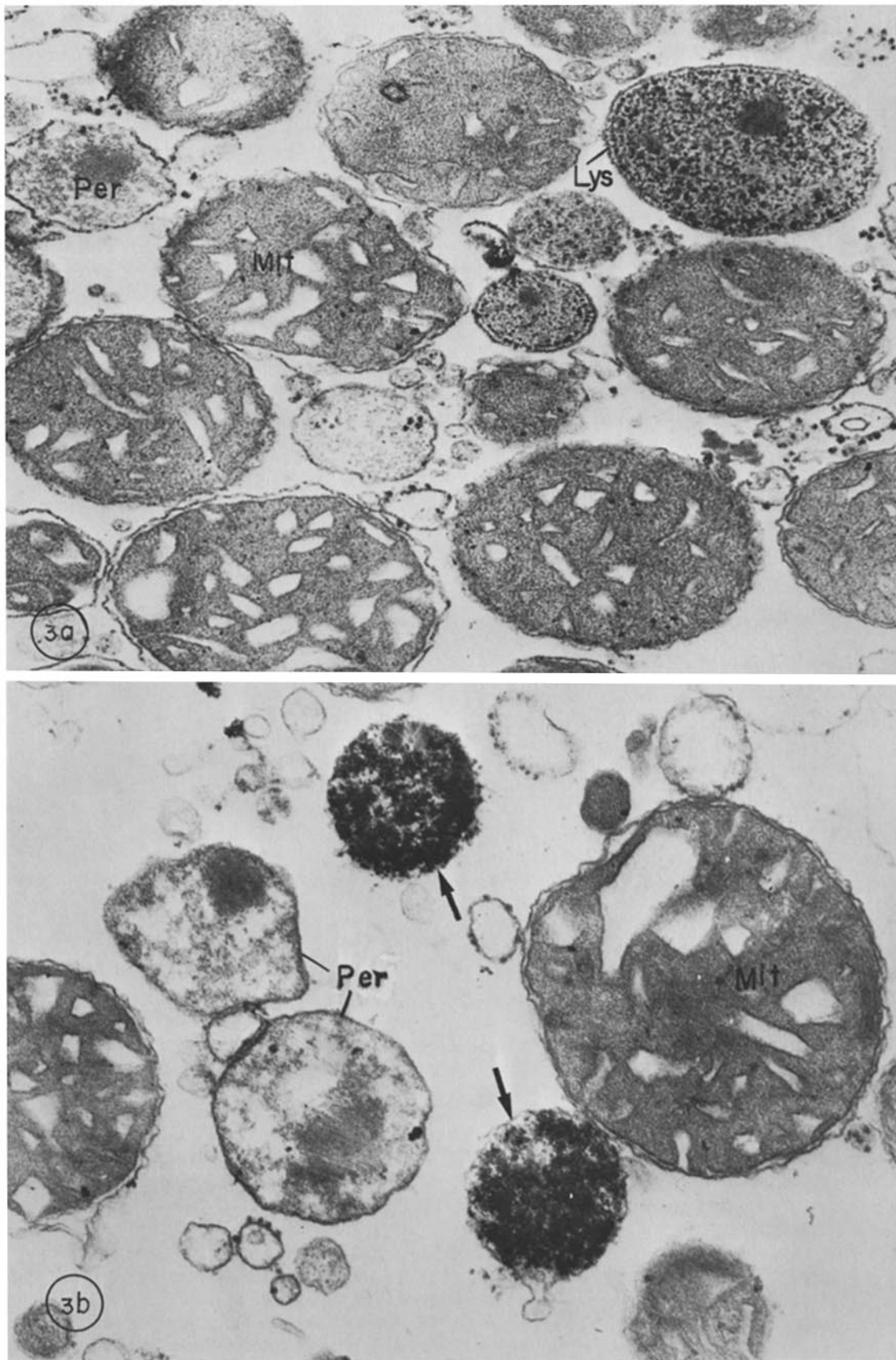
FIGURE 3   (a) Electron micrograph of the organelles present in the cell fraction, illustrating the distinctive morphology of dense bodies or lysosomes (Lys), microbodies or peroxisomes (Per), and mitochondria (Mit). This micrograph, however, is not the same as the original (see reference 12), because it was taken in 1967. Organelle morphology has now been much better preserved by glutaraldehyde fixation. × 58,000. (b) Same preparation as in a, but also incubated for acid phosphatase, which appears as a black precipitate in the dense bodies (arrows), but not in the peroxisomes (Per) or mitochondria (Mit). (From Baudhuin et al., 1967 [13].)
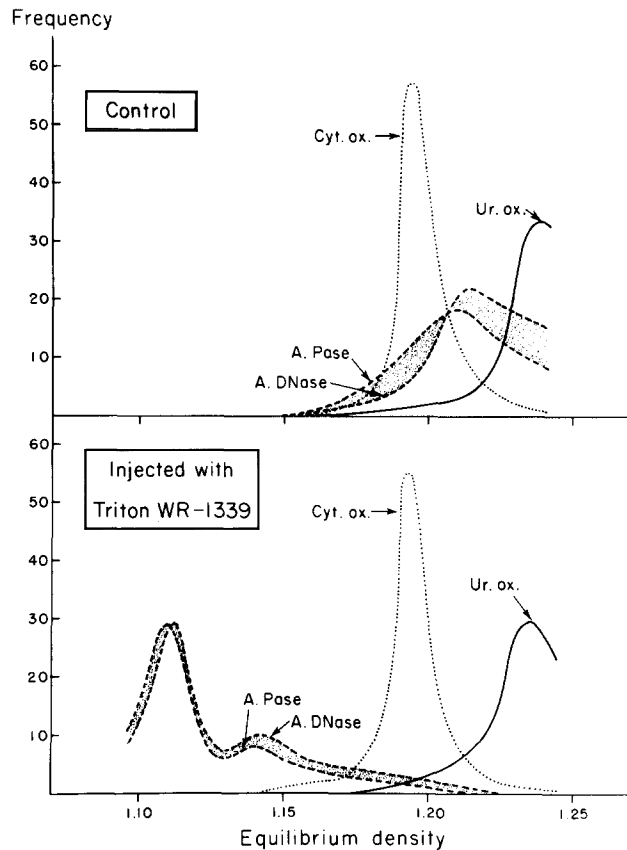
FIGURE 4 Effect of a previous injection of Triton WR-1339 on the equilibrium density of particulate enzymes. Density equilibration of mitochondrial fractions from rat liver in an aqueous sucrose gradient. Upper graph, control; lower graph, animal injected intravenously 4 days previously with 170 mg of Triton WR-1339. Note the selective shift of the lysosomal hydrolases. (Courtesy of Christian de Duve [5].)

1960–1966: The Lysosomal System

As more cells were studied and the ubiquitous distribution of lysosomes in mammalian cells was recognized, it became apparent that the lysosome is not actually a "body," but a part of a remarkably diverse and dynamic system. In addition to their polymorphism, lysosomes were discovered to be unique among other subcellular constituents by the variety of processes, both physiological and pathological, in which they participate. In fact, by 1963, when the Ciba Foundation Symposium on Lysosomes (24) was held, many pieces of the "functional puzzle" were beginning to fit into place. (A number of terms were introduced there that we now use quite frequently: for example, endocytosis, exocytosis, and primary lysosome.) Thereafter, the lysosome became popularized by publications in 1963 in the Scientific American (25); in 1964 in Federation Proceedings, organized by van Lancker, with contributions from Novikoff et al., Hirsch and Cohn, Swift and Hruban, and Weissmann, as well as de Duve (26); in 1965 in The Harvey Lectures series (5); and finally, in 1966 in an extensive review in the Annual Review of Physiology (27) entitled "Functions of Lysosomes." The various forms of lysosomes and related particles, together with the different types of interactions that may occur between them and with the plasma membrane, are presented in the diagram below, Fig. 6.

It was now evident that lysosomes, in combination with some

closely affiliated vacuolar structures devoid of hydrolases, formed an intracellular digestive system comparable (except for its discontinuity) to the digestive tracts of higher organisms; each separate component of the system was, to some extent, equivalent to a segment of the animal digestive tract. Moreover, it was further established that the material undergoing digestion in this system may be associated with heterophagy or with autophagy. In heterophagy, the material to be degraded is from outside the cell, whereas in autophagy, the material being degraded is of endogenous origin.

The word "lysosome" was chosen on the basis of the classification illustrated in Fig. 6. The choice can be defended, because lysosomes constitute the major functional constituents of the system, and also, usually the most numerous. Their identification, based essentially on the presence of acid hydrolases, is unambiguous. Within the lysosomal group, the primary lysosomes (also variously designated in the literature as pure, true, original, or virgin lysosomes) were distinguished as those containing enzymes which had never been engaged in a digestive event, whereas the secondary lysosomes represented sites of present or past digestive activity. The majority of secondary lysosomes are believed to have an acid pH, which activates their enzymes and allows them to function at optimal pH.

The most important components of the system that lack the acid hydrolase were the prelysosomes, with their contents of unattacked debris, generally destined for future digestion within lysosomes. At that time, the only well-known prelysosome belonged to the heterophagic line or phagocytic pathway: it was commonly called a phagosome (27). Postlysosomes, defined as degenerate telolysosomes that have lost their enzymes, were also included.

By the time of the comprehensive 1966 review (27), 330 references could be cited, indicating the vigorous investigative interest in lysosomes. Indeed, it is not easy to summarize the multiple and diverse contributions that have aided our understanding of the lysosomal system. Certainly, however, the following scientists have afforded significant new information.

(a) The contributions of Alex Novikoff and his co-workers should be mentioned first. In the late 1950s he had progressed from "once being a fledgling biochemist," using "grind-and-find" techniques, to becoming deeply submerged in microscopy and cytochemistry, where "seeing is believing" (28). It was Novikoff who assisted the lysosome, a biochemical entity, to its official entry into morphology and cell biology. Shortly after the Woods Hole meeting in 1958, Novikoff, who had accepted the invitation to write a chapter on mitochondria for The Cell edited by Jean Brachet and Alfred Mirsky, persuaded the editors to allow him to add a separate chapter on lysosomes (15). Largely stimulated by the work of Novikoff and his associates (29–33), over the years many investigators have sought to determine the formation and identification of primary (pure) lysosomes in many tissues and have pondered their relationship to the Golgi apparatus and endoplasmic reticulum (ER). He introduced the acronym GERL (34). "The specialized region of ER is referred to as GERL to suggest that it is ultimately related to the Golgi saccule (G), that it is a part of ER, and that it forms lysosomes (L)" (29, 32). It is valid to state that, along with the charismatic and articulate de Duve, the energetic and intuitive Novikoff continually brought the lysosomal system to the attention of a broad range of scientists.

(b) Hirsch, Cohn, and their colleagues at The Rockefeller Institute (now The Rockefeller University) clarified the manner in which lysosomes participate in digesting material engulfed
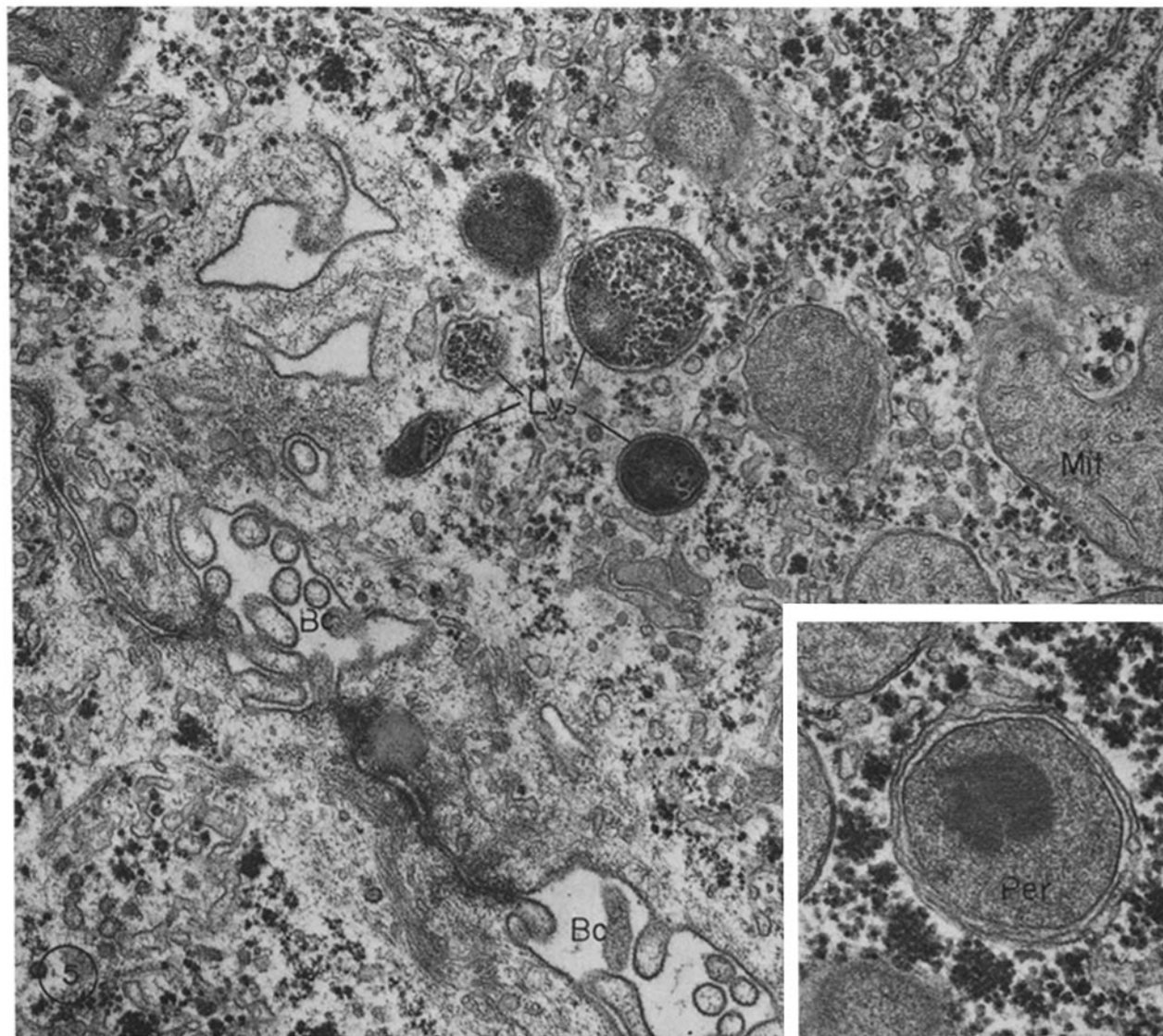
FIGURE 5 Electron micrograph of rat liver illustrating the morphology of the dense bodies (Lys) near the bile canaliculi (Bc). Inset illustrates the morphology of a peroxisome (Per) with a crystalloid in matrix. × 50,000. (Courtesy of Daniel S. Friend.)

by phagocytic leukocytes. After establishing the lysosomal nature of the neutrophil granules, they demonstrated that these granules discharge their enzymes into the phagocytic vacuoles when the cells ingest bacterial and other particles (35, 36). Furthermore, in both neutrophils and macrophages, degradation of isotopically labeled bacteria occurred, as evidenced by the appearance of breakdown products of lipids, nucleic acids, proteins, and carbohydrates (37).

This work on amoeboid phagocytic leukocytes naturally reverted to a reanalysis (2, 26) of the discovery of phagocytosis by Elias Metchnikoff in 1883. During his exploration of intracellular digestion in lower animals and unicellular organisms, Metchnikoff recognized that the interiors of food vacuoles were acid, and assumed that they contained soluble enzymes called cytases. Although this vacuolar acidity is now a cornerstone of the lysosomal concept (26), the exact mechanism by which secondary lysosomes are acidified has still not been completely explained, but the participation of a proton pump appears likely (38, 39).

(c) In 1963, H. G. Hers (40) and his co-workers in Belgium were the first to identify a true, inborn, lysosomal storage disease. This was glycogen-storage disease, type II, wherein α-glycosidase, capable of degrading glycogen, is absent (Fig. 7a), and the liver contains large glycogen-filled vacuoles (Fig. 7b)—as would be expected if accumulation of the polysaccharide were due to lack of digestion within lysosomes. This condition and many others of similar etiology (a primary defect of one lysosomal hydrolase) have now been described. As a matter of fact, by 1973, Hers and van Hoof, editors of Lysosomes and Storage Diseases (41), could record at least 21 individual pathological entities—such as Gaucher's disease, with a defect in β-glucosidase, or Niemann-Pick disease, with missing sphingomyelinase. The list continues to grow (42). The clinical appearance of the primary defect in lysosomal protein results in intralysosomal accumulation of all complex molecules that require the missing enzyme for their degradation. Further research on these pathological conditions has now yielded valuable new data on the synthesis and transport of normal lysosomal enzymes and the presence of receptors (reviewed by Neufeld in reference 43), and will be discussed later.

(d) Marilyn G. Farquhar and her associates at the University of California, San Francisco described a unique type of
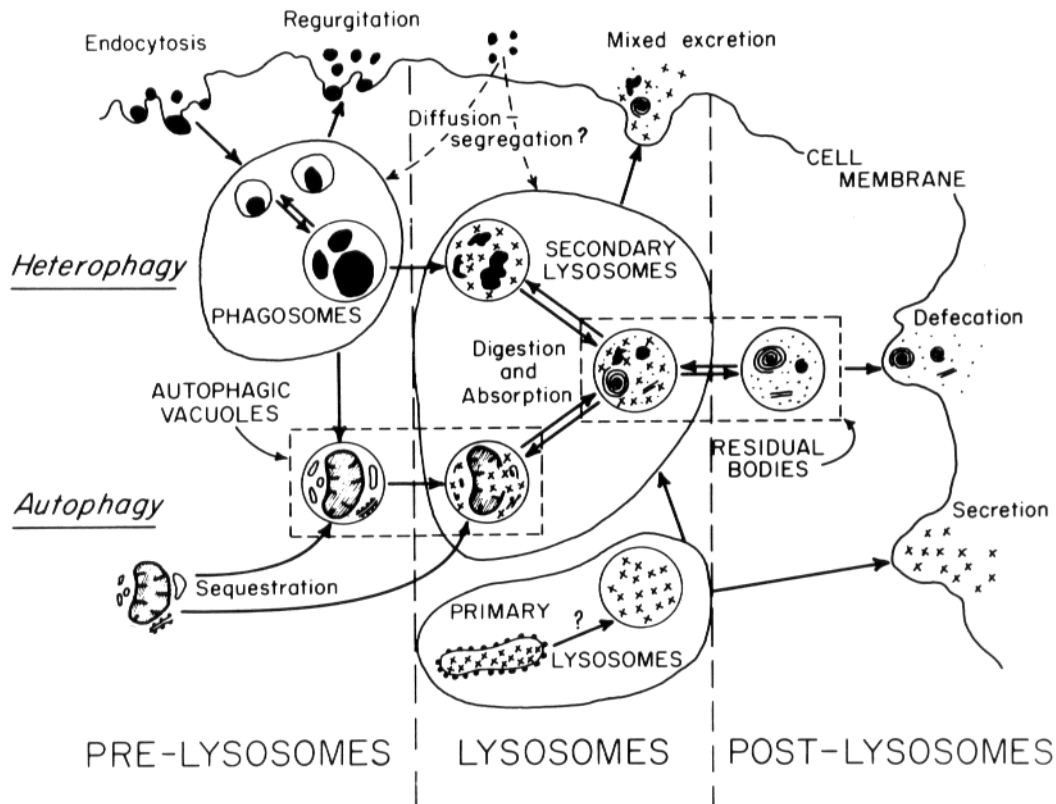
FIGURE 6 Synthetic diagram illustrating the various forms of lysosomes and related particles and the different types of interactions which may occur between them and with the cell membrane. Each cell-type is believed to be the site of one or more of the circuits shown, but not necessarily at all sites. Crosses symbolize acid hydrolases. (Reproduced from de Duve and Wattiaux, 1966 [27].)

autophagy, and established the origin and identification of different forms of primary lysosomes. The significant findings of Smith and Farquhar (44) indicated that certain pituitary secretion granules may fuse with lysosomes under particular circumstances, and that this mechanism probably serves to dispose of excess secretory products when the stimulus for their discharge is lacking (Fig. 8). It should be emphasized that this is not a nondiscriminate process involving segregation of entire areas of cytoplasm, but rather a selective fusion process between the secretory granules and lysosomes. The process was designated as *crinophagy* by de Duve (2) to distinguish it from autophagy (45). Research on lysosomes in blood leukocytes by Bainton and Nichols (see review, reference 46) established that some leukocytes are unusual because they store lysosomal enzymes in morphologically distinct structures demonstrable as large storage granules (Fig. 9a). In most other cell types in which primary lysosomes have been identified, they take the form of small Golgi complex-derived vesicles, often coated (Fig. 9b), which transport hydrolytic enzymes from the Golgi complex to multivesicular bodies, some of which then become secondary lysosomes, as reported by Friend and Farquhar (47). It should be emphasized, however, that not all Golgi complex-derived vesicles are lysosomal in nature, nor are all small coated vesicles lysosomes.

After 1966, the development of lysosomal functions in physiological and pathological processes can be followed to the fullest extent in a series of books, *Lysosomes in Biology and Pathology*, edited by John T. Dingle and Honor B. Fell from the Strangeways Research Laboratory, Cambridge, England,

and beginning with the first number from 1969 and continuing through the sixth, published in 1979 (48). A recent, more concise survey by Eric Holtzman in 1976 (9) is also to be highly recommended. In addition, and perhaps most important, was the initiation of the Gordon Research Conferences on Lysosomes in 1967. The titles of the presentations alone indicate much of the chronological development of new data, as follows:

1967: "Biochemical and Structural Aspects of Self-Degradative Processes in Cells" (chaired by Christian de Duve).

1968: "Lysosomes and Host Defense" (chaired by Zanvil Cohn and Samuel Dales).

1969: "Lysosomes and Storage Diseases" (chaired by Alex Novikoff and H. G. Hers).

1970: "Autophagy" (chaired by James Hirsch and Michael Locke).

1972: "Immunity and Tissue Injury" (chaired by Gerald Weissmann and Stephen Malawista).

1974: "Lysosomotropic Agents" (chaired by de Duve).

1976: "Intracellular Turnover of Proteins and Eukaryotes and Prokaryotes" (chaired by Eric Holtzman and John Dingle).

1978: "The Origin of Lysosomal Enzymes" (chaired by Oscar Touster and Dorothy Bainton).

1980: "The Role of Lysosomes in the Uptake, Storage, and Recycling of Membranes and Membrane-Bound Molecules" (chaired by Dorothy Bainton and Samuel Silverstein).
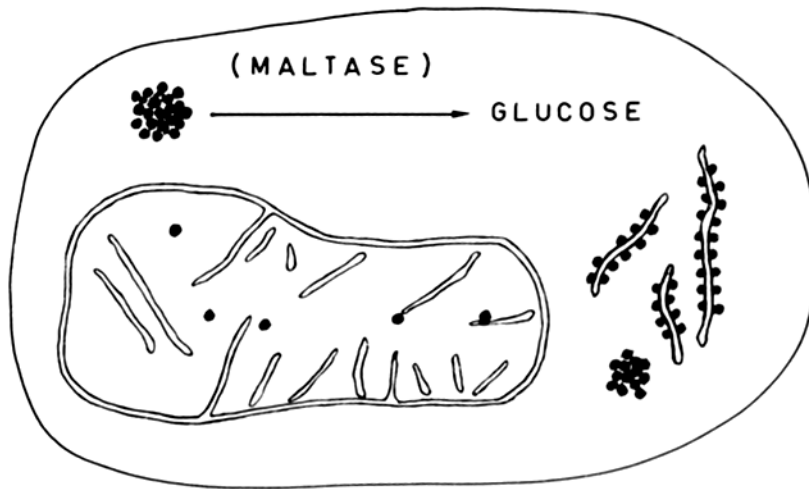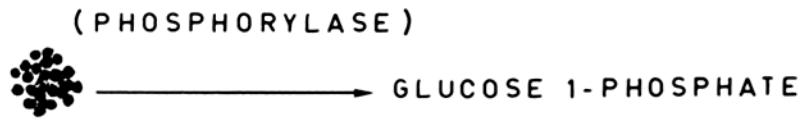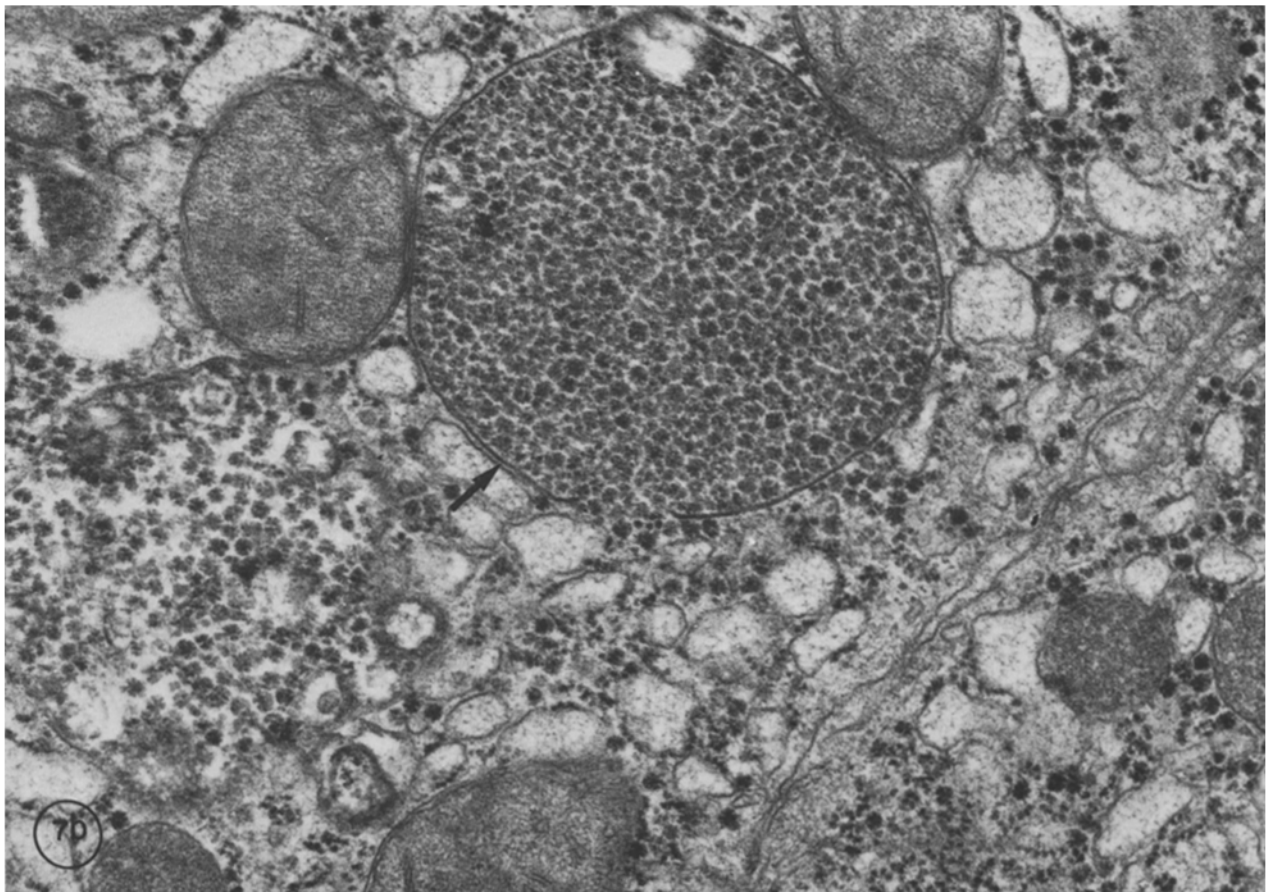
FIGURE 7   (a) Schematic representation of tne two pathways of glycogen degradation within cells. The upper one is cytoplasmic; the lower one is within the lysosome. (b) Part of a liver parenchymal cell from a patient with glycogen storage disease, type II. One vacuole, a lysosome, is filled with α-particles of glycogen (arrow). (Courtesy of Hers and van Hoof [41].)
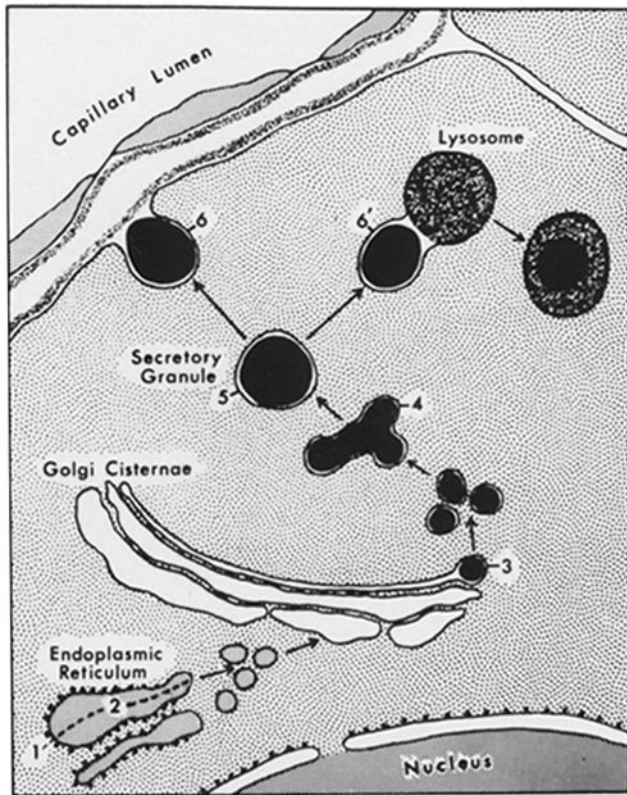
FIGURE 8 Diagram of the events of crinophagy as studied in mammotrophic cells of the rat anterior pituitary gland. Mammotrophic hormone is believed to be synthesized and transported through the cells as outlined in steps 1–6. If the secretory activities of the cells are suddenly discontinued, as takes place when the pups are separated from the lactating rats, the cells dispose of the excess stored hormone by fusion of the granules with lysosomes (6'). (Courtesy of Smith and Farquhar [44].)
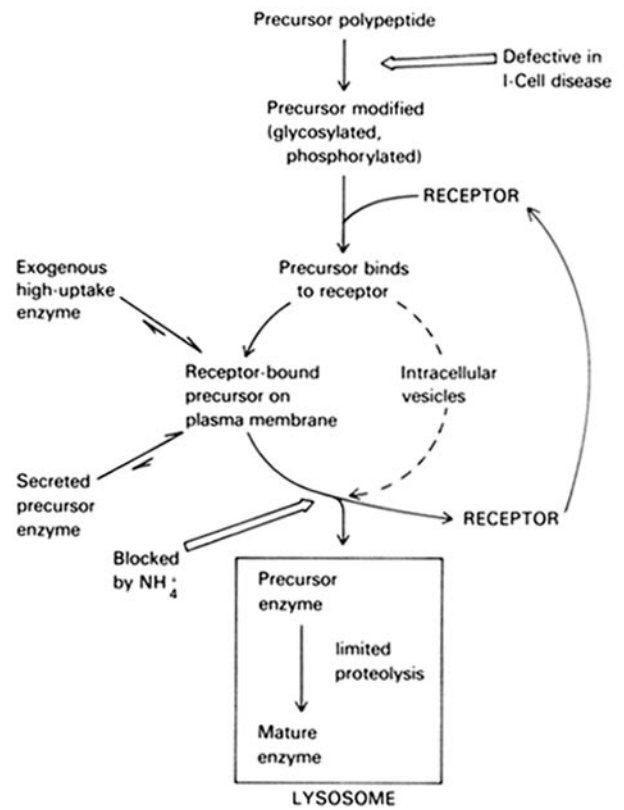

FIGURE 10 Schematic representation of the history of hydrolases in cultured fibroblasts. The present data indicate that precursor polypeptides are introduced into the endoplasmic reticulum, where they are glycosylated and phosphorylated. The precursor chains, presumably assembled at some point into enzyme molecules, bind to receptors, which convey them to lysosomes. Once inside organelles, the enzymes undergo restricted proteolysis. Small amounts of precursor can also be found in the extracellular spaces. (Courtesy of Elizabeth F. Neufeld [43]).
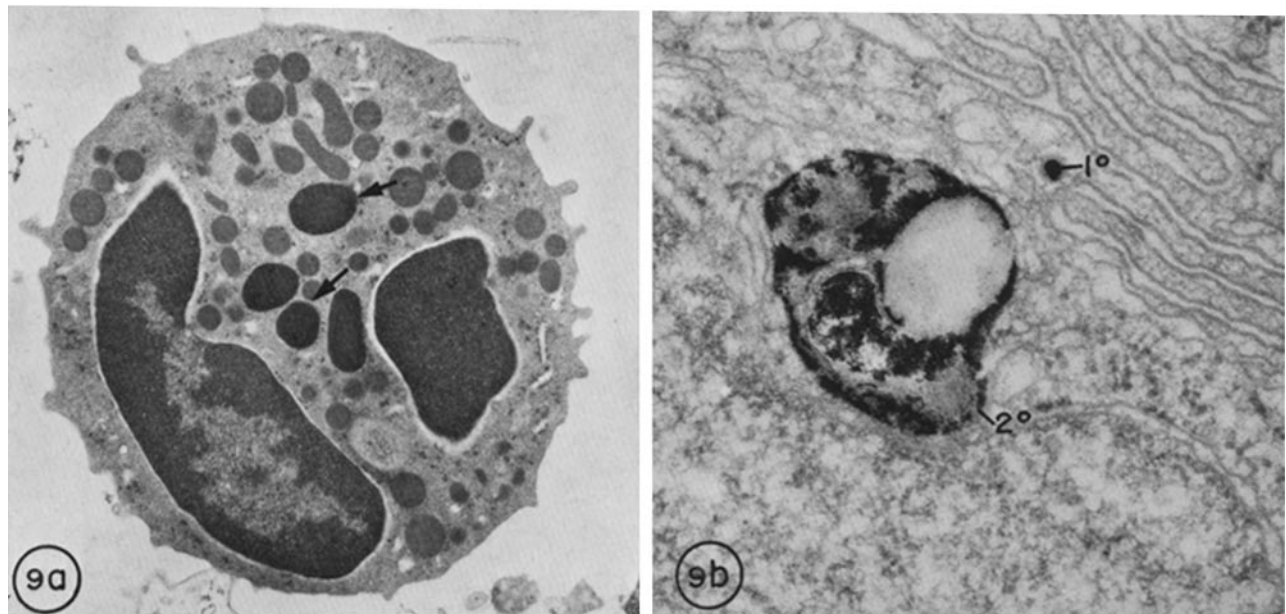

FIGURE 9 Two different forms of primary lysosomes. (a) Polymorphonuclear leukocyte. The cytoplasmic storage granules are morphologically and chemically distinct. Only the large, dense storage granules (arrows) contain acid hydrolases and correspond to the primary lysosomes of this cell type (see review, reference 46). It is now clear that relatively few cells store lysosomal enzymes in morphologically distinct structures recognizable as granules. In most cell types other than leukocytes, cytochemical staining has allowed the identification of the primary lysosome as small vesicles, so-called Golgi vesicles, which are sometimes coated. × 14,000. (b) Note the small acid-phosphatase-positive coated vesicle (1°) and a much larger secondary lysosome (2°). 9b × 60,000. (Courtesy of Dr. Daniel S. Friend.)

In recent years, interest has focused on the chemistry and biosynthesis of lysosomal enzymes. All lysosomal enzymes are glycoproteins, with the exception of cathepsin B1 and lysozyme (if the latter is indeed a true lysosomal enzyme). Although more than 50 different hydrolytic enzymes have been detected in lysosomes, only a few have been purified to homogenity. There are no known amino-acid sequences of lysosomal enzymes. So far, the one most fully characterized is β-glucuronidase. All of the limited number of lysosomal enzymes studied thus far contain mannose, galactose, and perhaps surprisingly, glucose. Almost all additionally contain fucose (49). What is known about biosynthetic routes of lysosomal enzymes—e.g., (a) How is the polypeptide formed? (b) Are "pre" and "pro" forms involved? and (c) What are the kinetics of this process? A few inroads have currently been forged in this area in the laboratories of Neufeld, Figura, Blobel, Sabatini, and Kornfeld.

In brief, hydrolase transport to lysosomes can now be regarded in the general context of the transport of secretory proteins. As glycoproteins, acid hydrolases would be expected to enter cisternae of the rough endoplasmic reticulum (RER); this has been verified by in vitro translation of cathepsin D (50). Thus, the nascent enzymes should be equipped with signal peptides to facilitate their entry into the RER. Such a signal has been found in the study of cathepsin D by Erickson and Blobel.[1] Where are the precursor polypeptides shortened? Neufeld and her co-workers have shown that the process is relatively slow; in fact, the slowness of the pace suggests that it may occur only after the hydrolases have become lodged in lysosomes (43, 51). Thus, the details of glycosylation, phosphorylation, and proteolytic cleavage and their kinetics are just beginning to emerge (see Fig. 10 and refs. 43, 51–53).

One major question involves the mechanism of delivering the recently synthesized enzymes to the lysosomes and sorting them out of the normal secretory pathway. It is possible that the manner of sorting is carried out by receptors. This is an area in which ideas are in flux. Although receptors for lysosomal enzymes were first encountered on the plasma membrane surface (43, 54), Sly and his co-workers (55) at Washington University in St. Louis have recently discovered that the majority of high-affinity receptors for β-glucuronidase are intracellular. This led them to propose that most newly synthesized lysosomal enzymes rely on the phosphomannosyl recognition marker for intracellular segregation from other products of the RER. From this viewpoint, receptor-bound enzymes would gather in specialized vesicles derived from the ER or Golgi complex and be delivered to lysosomes presumably by fusion. It is also possible that the vesicles could fuse with plasma membrane, exposing receptor-bound enzyme to the exterior of the cell, and that portions of the membrane carrying receptor-bound enzyme might subsequently be internalized through endocytosis (43). Binding of the hydrolases to receptors on the membrane seems to be mediated by an ionic signal, mannose-6-phosphate (54, 55). George Jourdian and his associates at the University of Michigan in Ann Arbor are well underway in their isolation and characterization of the liver-cell membrane receptor that binds β-galactosidase (56). All of these synthetic pathways are still little explored, but can be anticipated to result in significant new information in the near future.[2]

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[1] Erickson, A., and G. Blobel. Personal communication.

[2] Varki and Kornfeld have recently found the precise location of phosphorylated mannose residues on oligosaccharides (1980. *J. Biol. Chem.* 255:10847–10858).
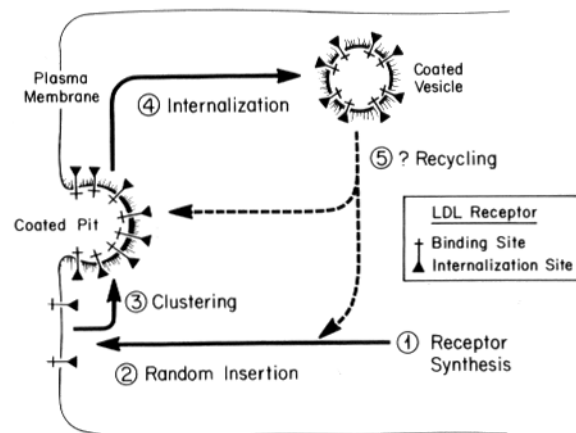

FIGURE 11 Working model for the mechanism by which LDL receptors cluster in coated pits on the plasma membrane of human fibroblasts. The postulated steps are as follows: (1) synthesis of LDL receptors on polyribosomes; (2) insertion of LDL receptors at random sites along noncoated segments of plasma membrane; (3) clustering of LDL receptors in clathrin-containing coated pits; (4) internalization of LDL receptors occurs as coated pits, which invaginate to form coated endocytic vesicles; and (5) recycling of internalized LDL receptors back to the plasma membrane. Step 5 may occur in lysosomes. (Courtesy of J. S. Goldstein et al., [58]).

Finally, one new aspect of lysosome function is now being charted—their role in the intracellular degradation of physiologically important molecules that regulate growth, nutrition, and differentiation in cells. Receptor-mediated endocytosis is now known to occur in many cell types for selective and efficient uptake of macromolecules (57, 58). These include certain transport proteins, such as low-density lipoprotein (LDL), transferrin, and transcobalamin II, as well as peptide hormones such as insulin and epidermal growth factor, asialoglycoproteins, and lysosomal enzymes. It is now clear that receptor-mediated endocytosis occurs in a great variety of cell types, and that many internalized proteins are delivered to lysosomes and degraded there, whereas others are not degraded, but instead are delivered to cellular structures other than lysosomes (see Fig. 11). The compartments responsible for this selective sorting of internalized proteins are presently being investigated (58, 59). In conclusion, it has become evident that the lysosomal system is not just a garbage dump. Rather, through the process of selective endocytosis, multiple biologically active substances, such as hormones, enzymes, LDL, antibodies, and toxins are herded into the cell and may or may not be degraded by lysosomes (58).

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