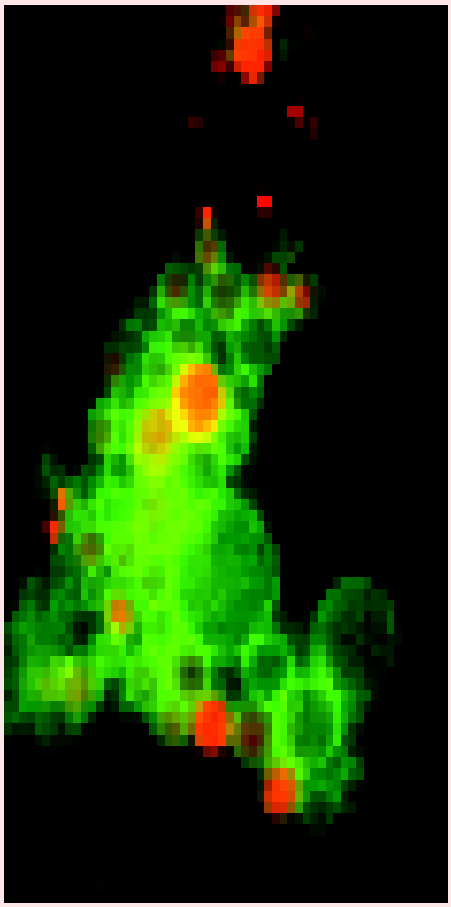
CHAPTER

8

Lysosomes



Lysosomes.

The lysosomes *(Gr., lyso*=digestive + *soma*=body) are tiny membrane-bound vesicles involved in intracellular digestion. They contain a variety of hydrolytic enzymes that remain active under acidic conditions. The lysosomal lumen is maintained at an acidic pH (around 5) by an ATP-driven proton pump in the membrane. Thus, these remarkable organelles are primarily meant for the digestion of a variety of biological materials and secondarily cause aging and death of animal cells and also a variety of human diseases such as cancer, gout, Pompe's disease, silicosis and I-cell disease.

HISTORICAL

During early electron microscopic studies, rounded dense bodies were observed in rat liver cells. These bodies were initially described as **"perinuclear dense bodies"**. **C. de Duve**, in 1955, renamed these organelles as 'lysosomes' to indicate that the internal digestive enzymes only became apparent when the membrane of these organelles was lysed (See **Reid** and **Leech**, 1980). However, the term lysosome means lytic body having digestive enzymes capable of lysis (*viz.*, dissolution of a cell or tissue; (**De Robertis** and **De Robertis, Jr.**, 1987).

Christian de Duve (Born 1917, won Nobel Prize in 1974).

Lysosomes were investigated according to following two schools : (1) **C. de Duve** and his coworkers (1963, 1964, 1974) worked in Belgium and their ap-

proach was biochemical one. (2) **Alex Novikoff** and his research group (1962, 1964) worked in United States and their approach was morphological and cytochemical. For the discovery of lysosomes and a brilliant series of experiments on them, **de Duve** shared the 1974 Nobel Prize for physiology with **Palade** and **Claude**, both were pioneer cell biologists.

OCCURRENCE

The lysosomes occur in most animal and few plant cells (Table 8-1). They are absent in bacteria and mature mammalian erythrocytes. Few lysosomes occur in muscle cells or in acinar cells of the pancreas. Leucocytes, especially granulocytes are a particularly rich source of lysosomes. Their lysosomes are so large-sized that they can be observed under the light microscope. Lysosomes are also numerous in epithelial cells of absorptive, secretory and excretory organs (*e.g.*, intestine, liver, kidney, etc.). They occur in abundance in the epithelial cells of lungs and uterus. Lastly, phagocytic cells and cells of reticuloendothelial system (*e.g.*, bone marrow, spleen and liver) are also rich in lysosomes.

STRUCTURE

The lysosomes are round vacuolar structures which remain filled with dense material and are bounded by single unit membrane. Their shape and density vary greatly. Lysosomes are 0.2 to 0.5µm in size. Since, size and shape of lysosomes vary from cell to cell and time to time (*i.e.* they are polymorphic), their identification becomes difficult. However, on the basis of the following three criteria, a cellular entity can be identified as a lysosome: (1) It should be bound by a limiting membrane; (2) It should contain two or more acid hydrolases; and (3) It should demonstrate the property of enzyme latency when treated in a way that adversely affects organelle's membrane structure.

Table 8-1. Examples of plant and animal cells, tissues and organs containing lysosomes (Source : Sheeler and Bianchi,1987).

A. Animal tissues	**B. Protozoa**
1. Liver	15. Leucocytes
2. Kidney	16. *Amoeba*
3. Nerve cells	17. *Tetrahymena*
4. Brain	18. *Paramecium*
5. Intestinal epithelium	19. *Euglena*
6. Lung epithelium	**C. Plants**
7. Macrophages (of spleen, bone marrow, liver and connective tissue)	20. Onion seeds
	21. Barley seeds
	22. Corn seedlings
8. Thyroid gland	23. Yeast
9. Adrenal gland	24. *Neurospora*
10. Bone	**D. Tissue culture cells**
11. Urinary bladder	25. HeLA cells
12. Prostate	26. Fibroblasts
13. Uterus	27. Chick cells
14. Ovaries	28. Lymphocytes

ISOLATION AND CHEMICAL COMPOSITION

Lysosomes are very delicate and fragile organelles. Lysosomal fractions have been isolated by **sucrose-density centrifugation** (or Isopycnic centrifugation) after mild methods of homogenization. Since the original de Duve's isolated lysosomal fractions were having contaminations of mitochondria, microsomes and microbodies, so, in 1960's it was investigated that rats injected with **dextran** or **Triton WR-1339**, incorporated these compounds into their lysosomes, thereby altering their density

and making their cleaner separation possible by differential centrifugation and density gradients (see **Reid** and **Leech**,1980).

Lysosomes tend to accumulate certain dyes (vital stains such as Neutral red, Niagara, Evans blue) and drugs such as anti-malarial drug **chloroquine**. Such 'loaded' lysosomes can be demonstrated by fluorescence microscopy.

The location of the lysosomes in the cell can also be pinpointed by various histochemical or cytochemical methods. For example, lysosomes demonstrate the property of **metachromasia** with toluidine blue and give a positive acid Schiff reaction (see Chapter 2). Metachromasia is the property exhibited by certain pure dyestuffs, chiefly basic stains,of colouring certain tissue elements in a different colour. Certain lysosomal enzymes are good histochemical markers. For example, **acid phosphatase** is the principal enzyme which is used as a marker for the lysosomes by the the use of **Gomori's staining technique** (**Gomori**,1952). Specific stains are also used for other lysosomal enzymes such as **B- glucuronidase**, **aryl sulphatatase**, **N-acetyl-B-glucosaminidase** and **5-bromo-4-chloroindolacetate esterase**.

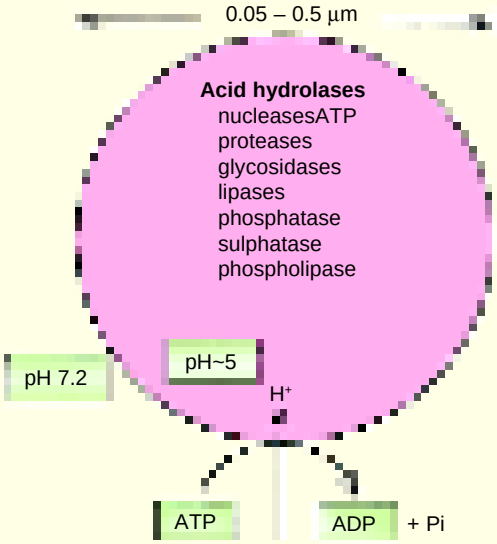


Fig. 8.1. A lysosome showing its various characteristics (after Albert *et al.*, 1989).

Lysosomal Enzymes

According to a recent estimate,a lysosome may contain up to 40 types of hydrolytic enzymes (see **Alberts** *et al*.,1989). They include **proteases** (*e.g.*, cathepsin for protein digestion), **nucleases**, **glycosidases** (for digestion of polysaccharides and glycosides), **lipases**, **phospholipases**, **phosphatases** and **sulphatases** (Table 8-2). All lysosomal enzymes are acid hydrolases, optimally active at the pH5 maintained within lysosomes.The membrane of the lysosome normally keeps the enzymes latent and out of the cytoplasmic matrix or cytosol (whose pH is about ~7.2), but *the acid dependency of lysosomal enzymes protects the contents of the cytosol (cytoplasmic matrix) against any damage even if leakage of lysosomal enzymes should occur*.

The so-called **latency** of the lysosomal enzymes is due to the presence of the membrane which is resistant to the enzymes that it encloses. Most probably this is due to the fact that most lysosomal hydrolases are membrane-bound, which may prevent the active centres of enzymes to gain access to susceptible groups in the membrane (see **Reid** and **Leech**,1980).

Table 8-2. **Some lysosomal enzymes and their substrates (Source : Sheeler and Bianchi, 1987).**

Enzyme	Substrate
A. Proteases and peptidases	
1. Cathepsin A,B,C,D and E	Various proteins and peptides
2. Collagenase	Collagen
3. Peptidases	Peptides
B. Nucleases	
4. Acid ribonuclease	RNA
5. Acid deoxyribonuclease	DNA

Enzyme	Substrate
C. Phosphatases	
6. Acid phosphatase	Phosphate monoesters
7. Phosphodiesterase	Oligonucleotides, phosphodiesters
D. Enzymes acting on oligosaccharide chains of glycoproteins and glycolipids	
8. b-galactosidase	b-Galactosides
9. Acetylhexosaminidase	Acetylhexosaminides, heparin sulphate
10. b-Glucosidase	b-Glucosides
11. a- Glucosidase	Glycogen
12. a-Mannosidase	a-Mannosidase
13. Sialidase	Sialic acid derivatives
E. Enzymes acting on glycosaminoglycans	
14. Lysozyme	Mucopolysaccharides, bacterial cell wall
15. Hyaluronidase	Hyaluronic acid, chondroitin sulphates
16. b-Glucuronidase	Polysaccharides, mucopolysaccharides
F. Enzymes acting on lipids	
17. Phospholipase	Lecithin, phosphatidyl ethanolamine
18. Esterase	Fatty acid esters

Lysosomal Membrane

The lysosomal membrane is slightly thicker than that of mitochondria. It contains substantial amounts of carbohydrate material, particularly **sialic acid**. In fact, most lysosomal membrane proteins are unusually highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration (**Lucy**,1969). Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. On the contrary, the cortisone, hydrocortisone and other drugs tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue.

The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H^+ inside the organelle (**Reijngond**,1978). Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

KINDS OF LYSOSOMES (POLYMORPHISM IN LYSOSOMES)

Lysosomes are extremely dynamic organelles, exhibiting polymorphism in their morphology. Following four types of lysosomes have been recognized in different types of cells or at different times in the same cell. Of these, only the first is the **primary lysosome**, the other three have been grouped together as **secondary lysosomes**.

1. Primary Lysosomes

These are also called **storage granules**, **protolysosomes** or **virgin lysosomes**. Primary lysosomes are newly formed organelles bounded by a single membrane and typically having a diameter of 100 nm. They contain the degradative enzymes which have not participated in any digestive process. Each primary lysosome contains one type of enzyme or another and it is only in the secondary lysosome that the full complement of acid hydrolases is present.

2. Heterophagosomes

They are also called **heterophagic vacuoles**, **heterolysosomes** or **phagolysosomes**. Heterophagosomes are formed by the fusion of primary lysosomes with cytoplasmic vacuoles containing **extracel-**

lular substances brought into the cell by any of a variety of endocytic processes (*e.g.*, pinocytosis, phagocytosis or receptor-mediated endocytosis, see Chapter 5). The digestion of engulfed substances takes place by the enzymatic activities of the hydrolytic enzymes of the secondary lysosomes. The digested material has low molecular weight and readily passess through the membrane of the lysosomes to become the part of the matrix (Fig. 8.2).

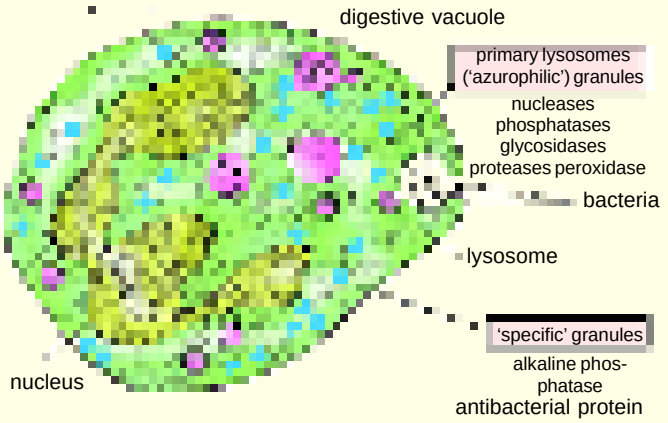


Fig. 8.2. Diagram of a white blood cell (neutrophil) ingesting bacteria. Two types of granules fuse with the phagocytotic vacuoles and contribute digestive enzymes and other components.

3. Autophagosomes

They are also called **autophagic vacuole**, **cytolysosomes** or **autolysosomes**. Primary lysosomes are able to digest **intracellular structures** including mitochondria, ribosomes, peroxisomes and glycogen granules. Such autodigestion (called **autophagy**) of cellular organelles is a normal event during cell growth and repair and is especially prevalent in differentiating and dedifferentiating tissues (*e.g.*, cells undergoing programmed death during metamorphosis or regeneration) and tissue under stress. Autophagy takes several forms. In some cases the lysosome appears to flow around the cell structure and fuse, enclosing it in a double membrane sac, the lysosomal enzymes being initially confined between the membranes. The inner membrane then breaks down and the enzymes are able to penetrate to the enclosed organelle.In other cases, the organelle to be digested is first encased by smooth ER, forming a vesicle that fuses with a primary lysosome (Fig. 8.4). Lysosomes also regularly engulf bits of cytosol (cytoplasmic matrix) which is degraded by a process, called **microautophagy**.

As digestion proceeds, it becomes increasingly difficult to identify the nature of the original secondary lysosome (*i.e.*, heterophagosome or autophagosome) and the more general term **digestive vacuole** is used to describe the organelle at this stage.

4. Residual Bodies

They are also called **telolysosomes** or **dense bodies**. Residual bodies are formed if the digestion inside the food vacuole is incomplete. Incomplete digestion may be due to absence of some lysosomal enzymes. The undigested food is present in the digestive vacuole as the residues and may take the form of whorls of membranes, grains, amorphous masses, ferritin-like or myelin figures (Fig.8.3).

Residual bodies are large, irregular in shape and are usually quite electron-dense. In some cells,

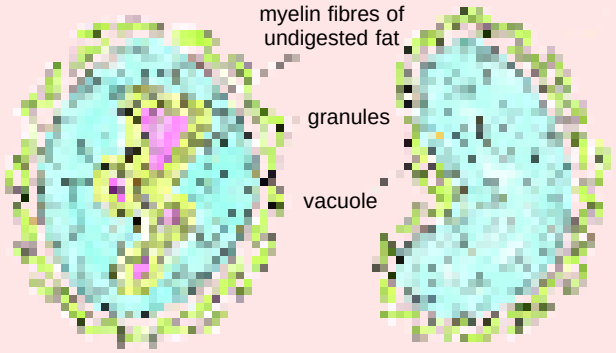
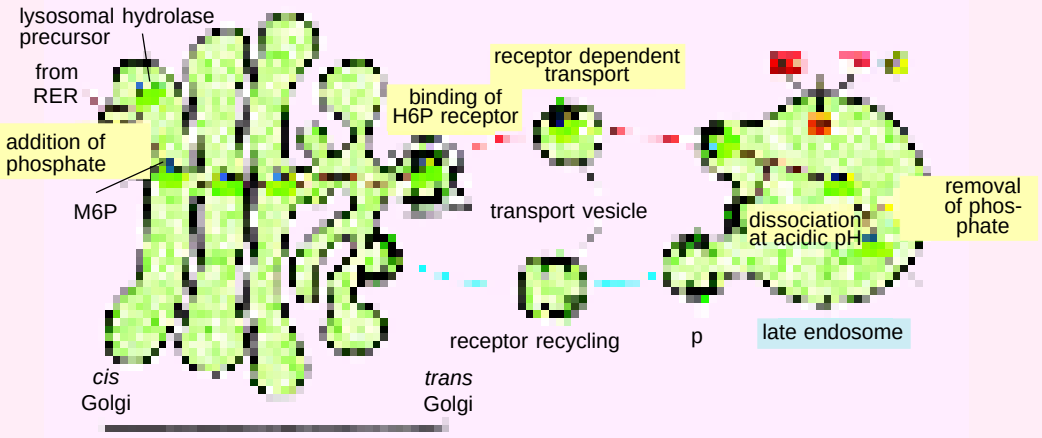


Fig . 8.3. Lysosomes of the kidney cells of rat, showing the presence of residues (A).

such as *Amoeba* and other potozoa, these residual bodies are eliminated by **defecation**. In other cells, residual bodies may remain for a long time and may load the cells to result in their aging. For example, pigment inclusions (age pigment or **lipofuscin granules**) found in nerve cells (also in liver cells, heart cells and muscle cells) of old animals may be due to the accumulation of residual bodies.

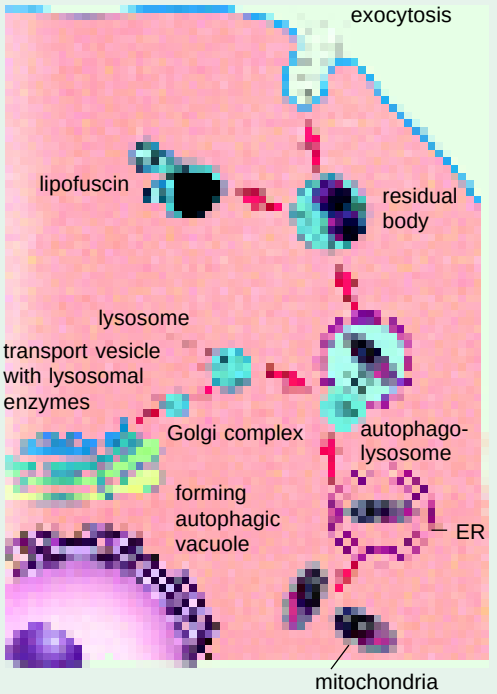
ORIGIN

The biogenesis (origin) of the lysosomes requires the synthesis of specialized lysosomal hydrolases and membrane proteins. Both classes of proteins are synthesized in the ER and transported



A summary of the autophagic pathway.

through the Golgi apparatus, then transported from the trans Golgi network to an intermediate compartment (an **endolysosome**) by means of **transport vesicles** (which are coated by clathrin protein; Fig. 8.4). The lysosomal enzymes are glycoproteins, containing N-linked oligosaccharides that are processed in a unique way in the cis Golgi so that their **mannose** residues are phosphorylated. These **mannose 6-phosphate (M6P)** groups are recognized by **M6P-receptors** (which are transmembrane proteins) in the trans Golgi network that segregates the hydrolases and helps to package them into budding clathrin-coated vesicles which quickly lose their coats. These transport vesicles containing the M6P-receptors act as shuttles that move the receptors back and forth between the trans Golgi network and endolysosomes. The low pH in the endolysosome dissociates the lysosomal hydrolases from this receptor, making the transport of the hydrolases unidirectional.



A summary of the autophagic pathway.

FUNCTIONS OF LYSOSOMES

The important functions of lysosomes are as follows:

1. Digestion of large extracellular particles. The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the latter to devour the foreign proteins, bacteria and viruses.

2. Digestion of intracellular substances. During the starvation, the lysosomes digest the stored food contents, *viz.*, proteins, lipids and carbohydrates (glycogen) of the cytoplasm and supply to the cell neccessary amount of energy.

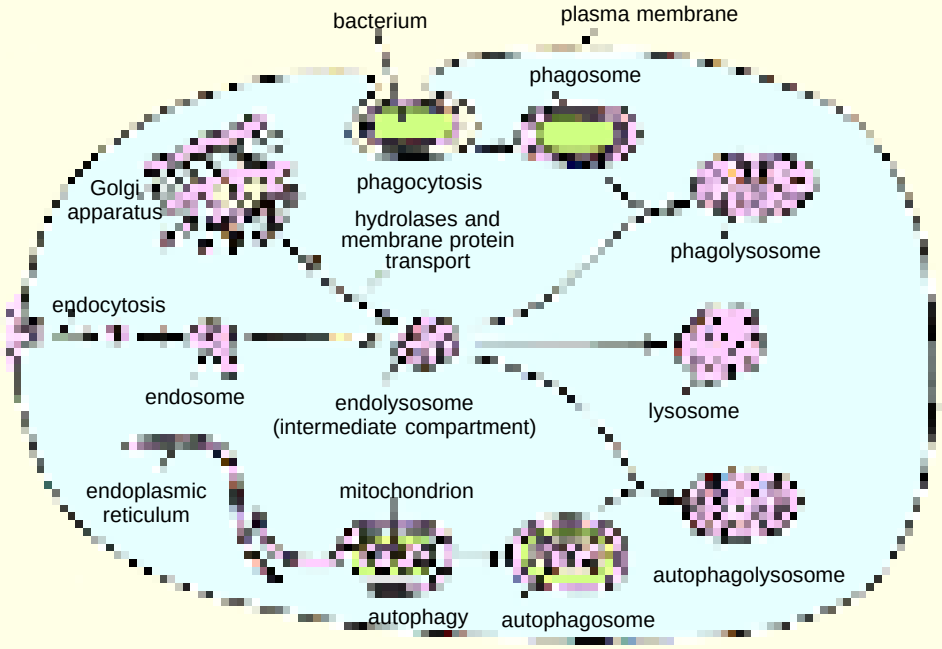
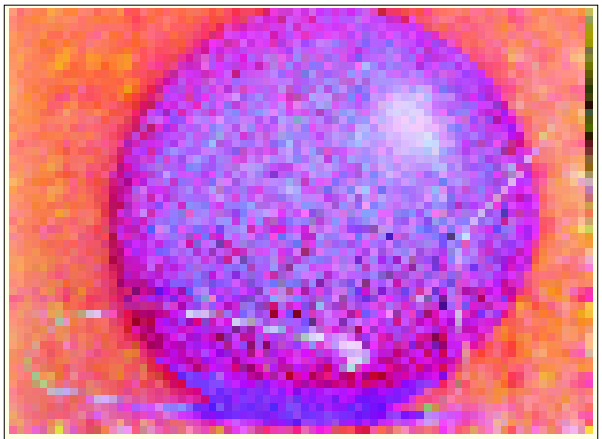


Fig. 8.4. Recently understood mechanism of the origin of three types of lysosomes : phagolysosome, lysosome (the classical secondary lysosome) and autophagolysosome. Transport vesicles (the classical primary lysosomes) originate from trans Golgi network to fuse with endolysosome which contains already endocytosed materials for digestion (after Alberts *et al*., 1989).

3. Autolysis. In certain pathological conditions the lysosomes start to digest the various organelles of the cells and this process is known as **autolysis** or **cellular autophagy**. When a cell dies, the lysosome membrane ruptures and enzymes are liberated. These enzymes digest the dead cells. In the process of metamorphosis of amphibians and \tunicates many embryonic tissues, *e.g*., gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells.

4. Extracellular digestion. The lysosomes of certain cells such as sperms discharge their enzymes outside the cell during the process of fertilization. The lysosomal enzymes digest the limiting membranes of the ovum and form penetration

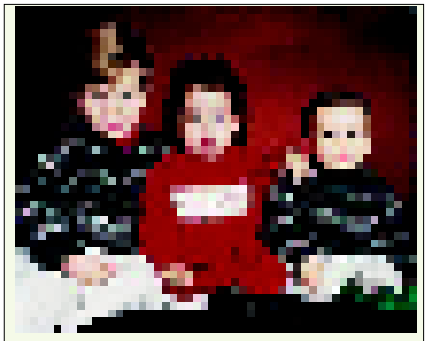


The lysosomes of sperms discharge their enzymes outside the cell during the process of fertilization. Here a human sperm is being seen fertilizing an egg.

path in ovum for the sperms. Acid hydrolases are released from **osteoclasts** and break down bone for the reabsorption; these cells also secrete lactic acid which makes the local pH enough for optimal enzyme activity. Likewise, preceding ossification (bone formation), **fibroblasts** release cathepsin D enzyme to break down the connective tissue (**Dingle**, 1973).

LYSOSOMES AND DISEASE

Malfunctioning of lysosomes often results in various pathological disorders affecting the life of the cell or an individual. Some of these are **inborn diseases**, caused by gene mutation (*e.g.*, I-cell disease, gout, Pompe's disease, Tay-Sach's disease, etc.) and others are induced by some environmental pollutants (*e.g.*, silicosis). Typically, the accumulated materials (*e.g.*, low-molecular weight materials, drugs, dyes, etc.) may cause **malignant** transformation of cells by bringing about leakage of lysosomal enzymes that attack the genetic material in the DNA.



Two out of the three siblings in this picture are suffering from Pompe's disease, a rare inborn disease.

LYSOSOMES IN PLANTS

Plants contain several hydrolases, but they are not always as neatly compartmentalized as they are in animal cells. Many of these hydrolases are found bound to and functioning within the vicinity of the cell wall and are not necessarily contained in membrane-bound vacuoles at these sites. Many types of vacuoles and storage granules of plants are found to contain certain digestive enzymes and these granules are considered as lysosomes of plant cell (**Gahan**, 1972). According to **Matile** (1969) the plant lysosomes can be defined as membrane-bound cell compartments containing hydrolytic digestive enzymes. **Matile** (1975) has divided vacuoles of plants into following three types:

1. Vacuoles

The vacuole of a mature plant cell is formed from the enlargement and fusion of smaller vacuoles present in meristematic cells; these **provacuoles**, which are believed to be derived from the ER and possibly the Golgi and contain acid hydrolases. These lysosomal enzymes are associated with the tonoplast of large vacuole of differentiating cells. Sometimes, mitochondria and plastids are observed inside the vacuole suggesting autophagy in plants (**Swanson** and **Webster**, 1989).

2. Spherosomes

The spherosomes are membrane-bounded, spherical particles of 0.5 to 2.5 µm diameter, occurring in most plant cells. They have a fine granular structure internally which is rich in lipids and proteins. They originate from the endoplasmic reticulum (ER). Oil accumulates at the end of a strand of ER and a small vesicle is then cut off by constriction to form particles, called **prospherosomes**. The prospherosomes grow in size to form spherosomes. Basically, the spherosomes are involved in lipid synthesis and storage. But, the spherosomes of maize root tips (**Matile**, 1968) and spherosomes of tobacco endosperm tissue (**Spichiger**, 1969) have been found rich in hydrolytic digestive enzymes and so have been considered as lysosomes. Like lysosomes they are not only responsible for the accumulation and mobilization of reserve lipids, but also for the digestion of other cytoplasmic components incorporated by phagocytosis.

3. Aleurone Grain

The aleurone grains or protein bodies are spherical membrane-bounded storage particles occurring in the cells of endosperm and cotyledons of seeds. They are formed during the later stages of seed ripening and disappear in the early stages of germination. They store protein (*e.g.*, globulins)

and phosphate in the form of phytin. **Matile** (1968) has demonstrated that aleurone grains from pea seed contain a wide range of hydrolytic enzymes including protease and phosphatase which are required for the mobilization of stored protein and phosphate, although the presence of other enzymes such as β-amylase and RNAase suggest that other cell constituents may also be digested. Thus, like spherosomes, aleurone grains store reserve materials, mobilize them during germination and in addition form a compartment for the digestion of other cell components (**Hall** *et al*., 1974). The aleurone grains are derived from the strands of the endoplasmic reticulum.

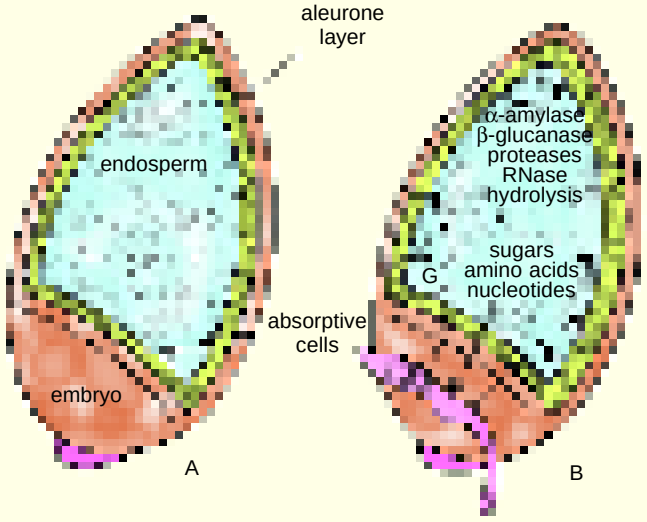


Fig. 8.5. Role of hormone and hydrolytic enzymes in seed germination. A—Ultrastructure of seed before germination; B—During germination, gibberellins (G) pass from the embryo to the aleurone layer, where the *de novo* synthesis of hydrolytic enzymes is induced. These enzymes break down the macromolecular stored reserves and the low molecular weight products are transported to the embryo, where they function as nutrients (after Thorpe, 1984).

During germination of barley seed, the activity of hydrolases is found to be controlled by hormones such as **gibberellic acid** (Fig. 8.5). Gibberellic acid, a plant growth hormone, is released by the embryo to the aleurone layer where, in turn, the hydrolases are released to the endosperm. This hormone operates by derepressing appropriate genes in the aleurone cells, which then begin to crank out new hydrolytic proteins (see **Thorpe**, 1984).

Extra-cellular Digestion by Plants

Plant cells are generally unable to engulf large particles, presumably because of the restrictions imposed on the cell by cell wall. The secretion of hydrolases to carry out extracellular digestion, therefore, becomes an important process. Hydrolases are commonly secreted by fungi, enabling the organism to degrade and grow on macromolecules it cannot transport into the cell. Higher plants also secrete hydrolases, a notable example being the insectivorous pitcher plants, which produce a proteinase-containing liquid in which victims are trapped and digested.

REVISION QUESTIONS

1. What are the lysosomes ? Describe their origin, structure and function.
2. Describe the method of isolation of lysosomes in the cells of plants and animals. Add a note about histochemical marking of lysosomal components : membrane and enzymes.
3. Describe the process of autophagy. What is the ultimate fate of the digestive vacuole ?
4. Write short notes on the following :
   (i) Polymorphism in lysosomes;
   (ii) Lysosomes and disease;
   (iii) Lysosomal enzymes;
   (iv) Lysosomes of plants.