LYSOSOMAL STORAGE DISEASES

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INTRODUCTION

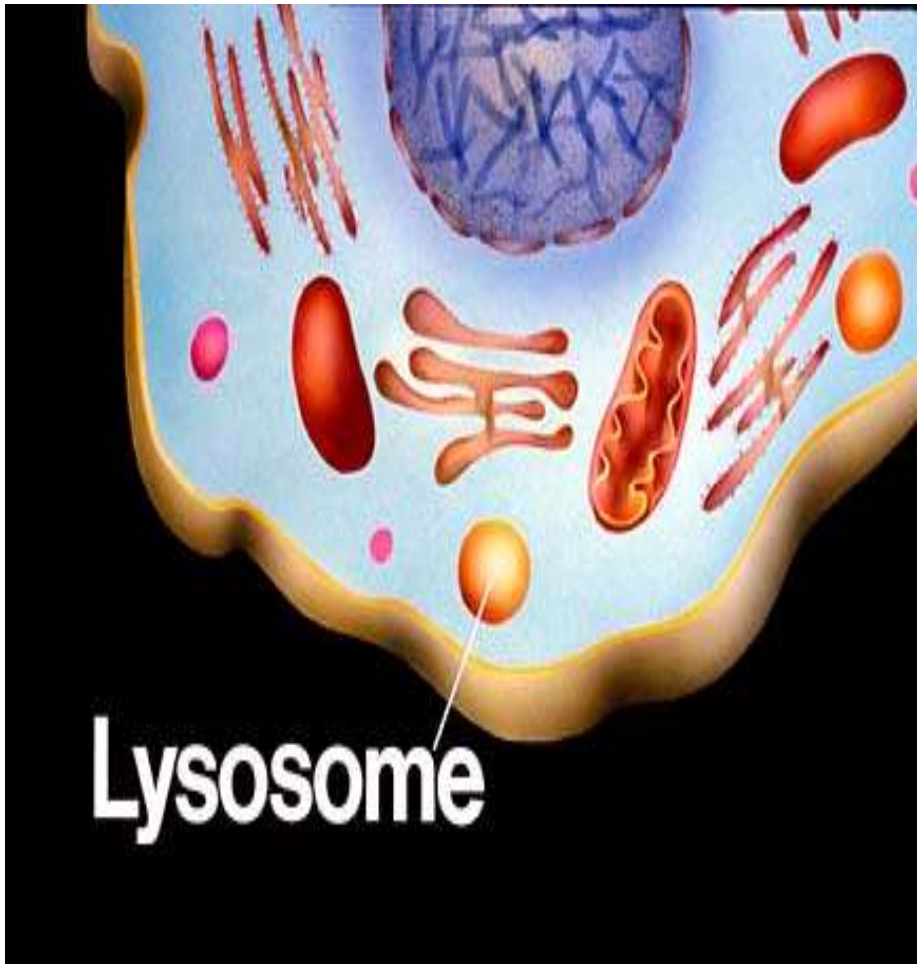
- Lysosomes are membrane-bound **organelles** that function as the "stomachs" of **eukaryotic cells**. They contain about fifty different **enzymes** that break down all types of biological molecules including **proteins**, nucleic acids, **lipids**, and **carbohydrates**.

- Cells transport material into lysosomes, the material is digested by the enzymes, and the digested molecules are moved back into the **cytosol** for use by the cell. Both extracelluar materials brought into the cell by Endocytosis and obsolete **intracellular** materials are degraded in the lysosome. they serves as the "recycling center" for the cell.

- The name *lysosome* derives from the Greek words *lysis*, which means dissolution or destruction, and *soma*, which means body. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their role in Autolysis.

- Lysosomes were discovered by the Belgian cytologist Christian de Duve in 1955.

- At pH 4.8, the interior of the lysosomes is acidic compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons (H+ ions) from the cytosol across the membrane via proton pumps and chloride ion channels.


Lysosome

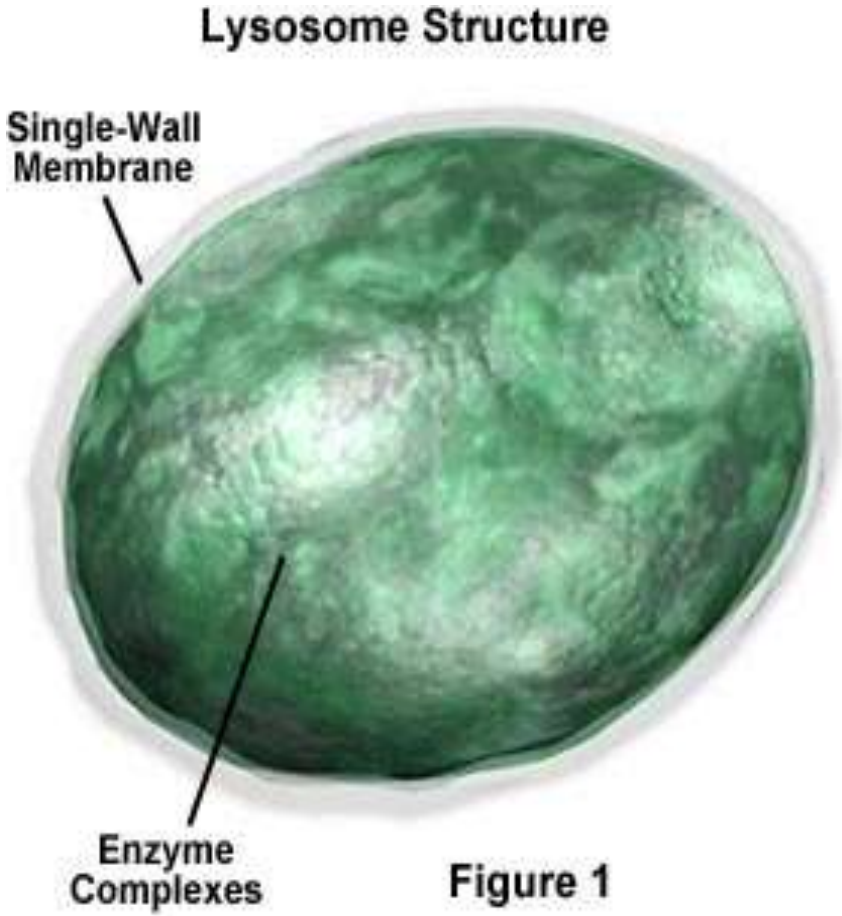
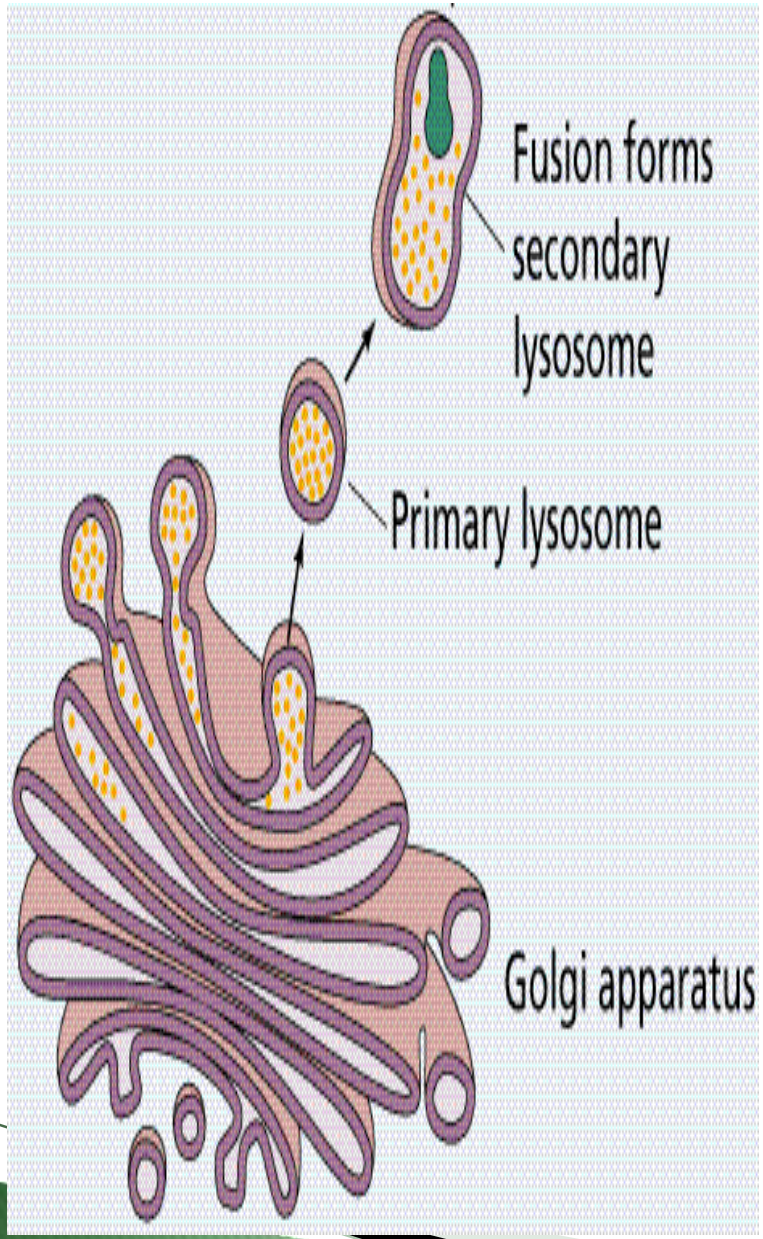
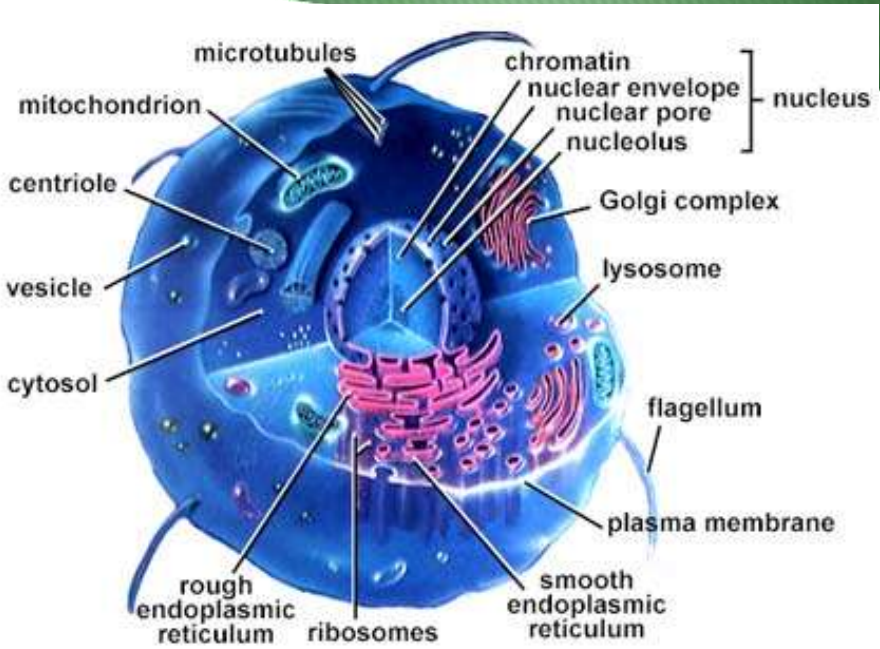
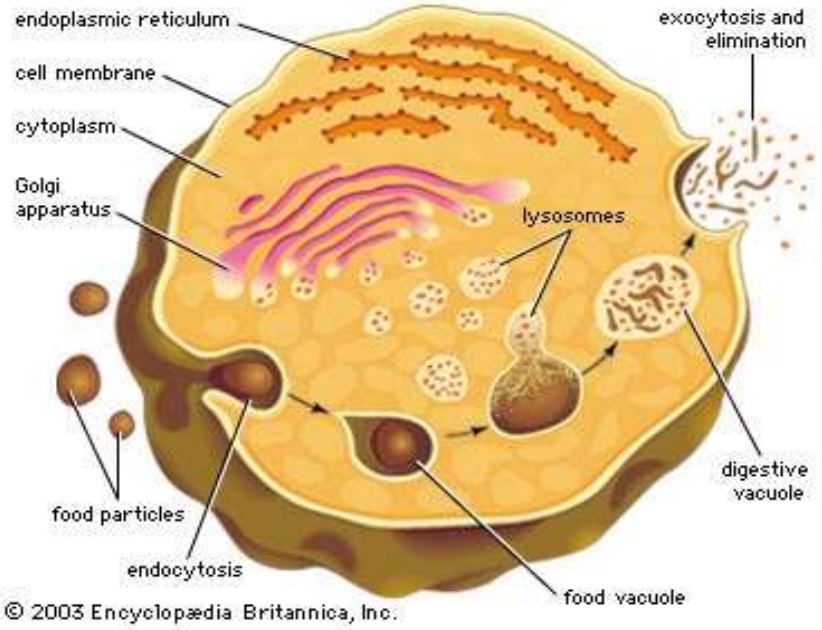

Lysosome Structure
Single-Wall Membrane
Enzyme Complexes

Figure 1


Fusion forms
secondary
lysosome
Primary lysosome
Golgi apparatus


microtubules
chromatin
nuclear envelope
nucleus
mitochondrion
nuclear pore
nucleolus
centriole
Golgi complex
lysosome
vesicle
cytosol
flagellum
plasma membrane
rough
endoplasmic
reticulum
ribosomes
smooth
endoplasmic
reticulum


endoplasmic reticulum
exocytosis and
elimination
cell membrane
cytoplasm
Golgi
apparatus
lysosomes
digestive
vacuole
food particles
endocytosis
food vacuole
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- Like other micro bodies, lysosomes are spherical organelles contained by a single layer membrane. This membrane protects the rest of the cell from the lysosomes' harsh digestive enzymes that would otherwise damage it. The lysosomes are also the "clean up crew" of the cell because they break down the old and damaged organelles.

- Lysosomes originate in the Golgi apparatus, but the digestive enzymes are manufactured in the rough endoplasmic reticulum. Lysosomes are found in all eukaryotic cells, but are most numerous in disease-fighting cells, such as white blood cells.

- Some human diseases are caused by lysosome enzyme disorders. **Tay-sachs disease** is caused by a genetic defect that prevents the formation of an essential enzyme that breaks down a complex lipid called **ganglioside**. An accumulation of this lipid damages the nervous system, causes mental retardation and death in early childhood. Arthritis inflammation and pain are related to the escape of lysosome enzymes.

ENZYMES

Some important enzymes found within lysosomes include:

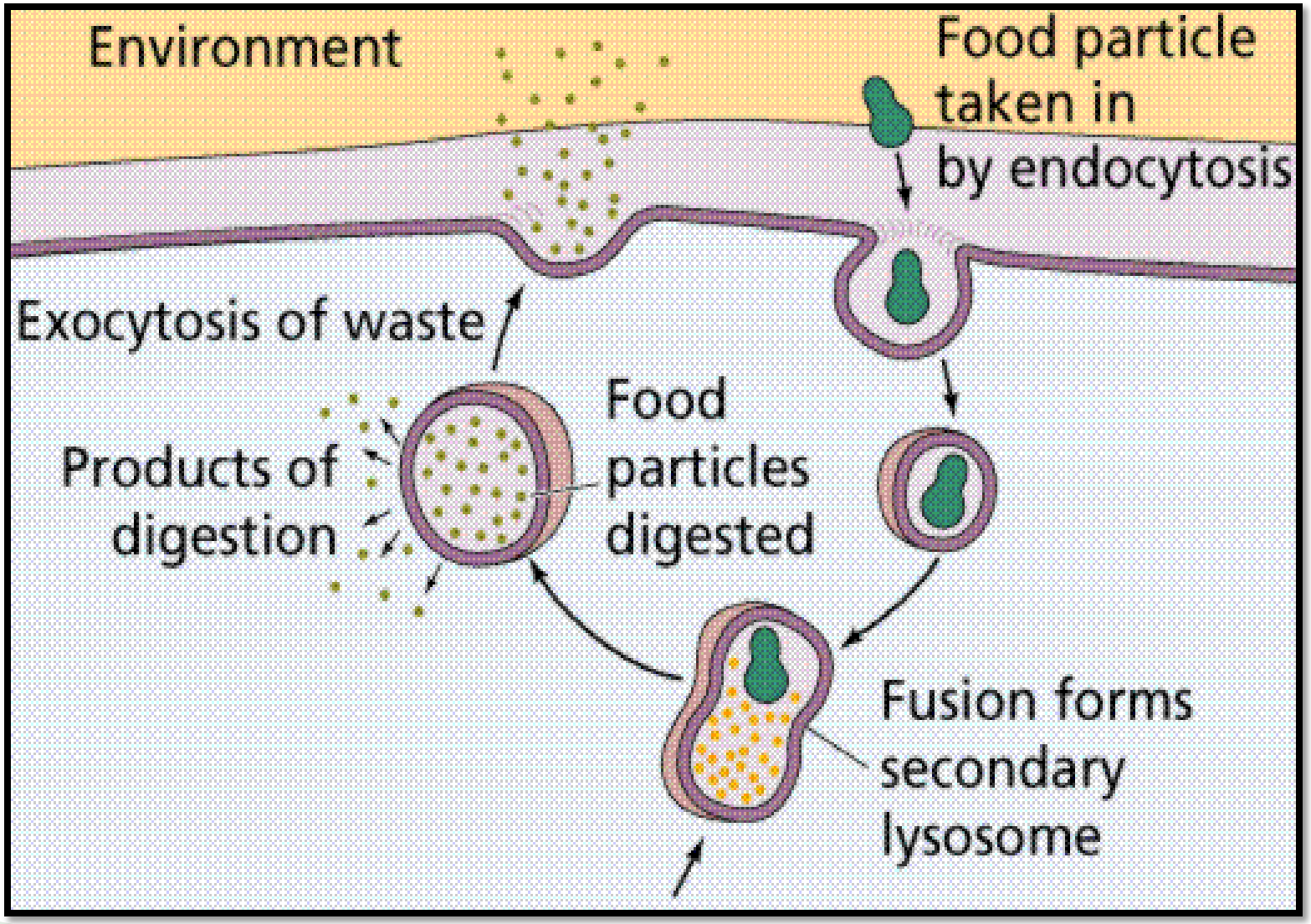
- Lipase, which digests lipids
- Carbohydrase, which digest carbohydrates (e.g., sugars)
- Proteases, which digest proteins
- Nucleases, which digest nucleic acids
- phosphoric acid monoesters.
- Lysosomal enzymes are synthesized in the cytosol and the endoplasmic reticulum,

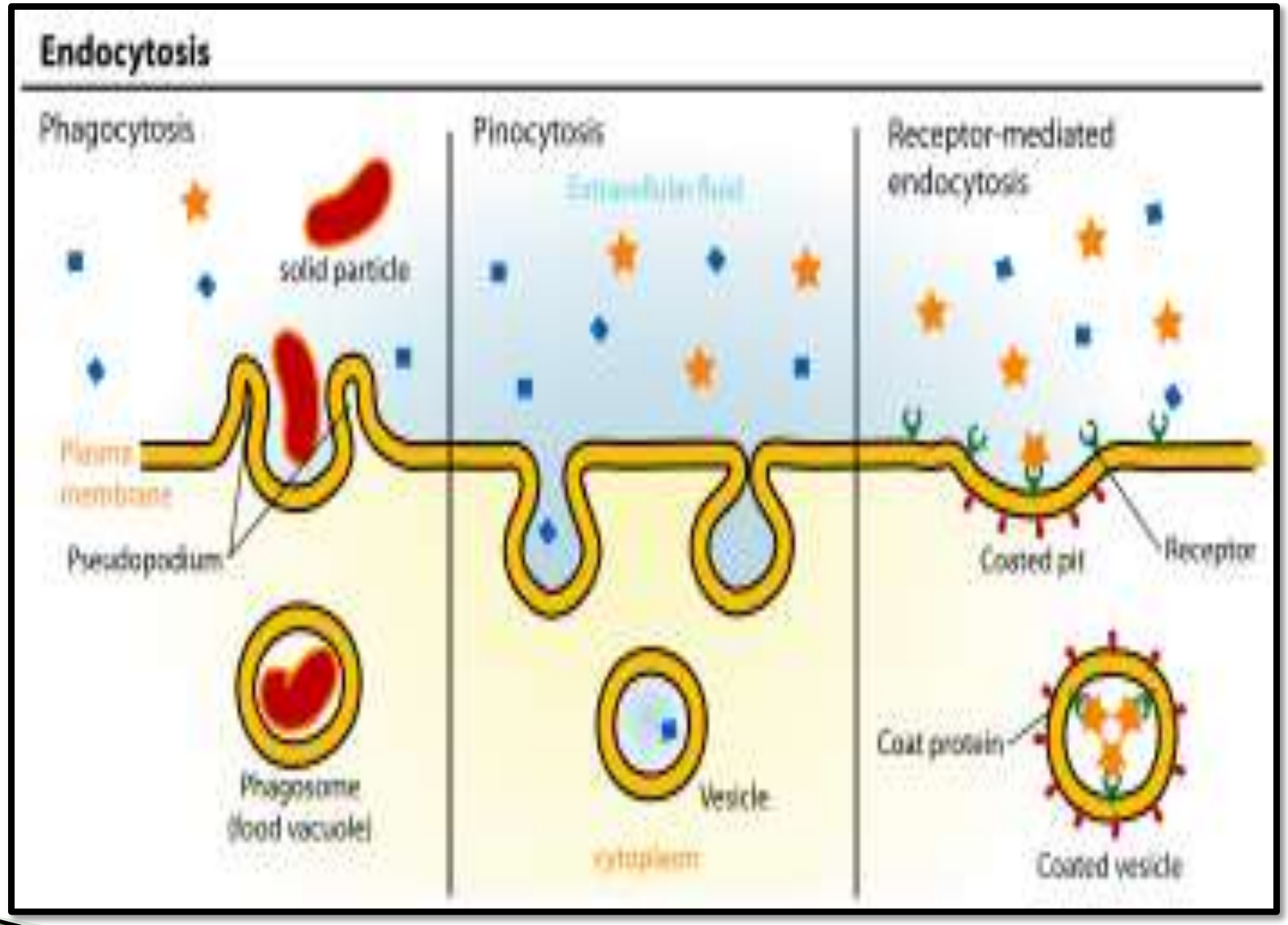
FUNCTIONS

- Lysosomes are used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracelluar material, like foreign invading microbes), Endocytosis (where receptor proteins are recycled from the cell surface), and Autophagy (wherein old or unneeded organelles or proteins, or microbes that have invaded the cytoplasm are delivered to the lysosome).

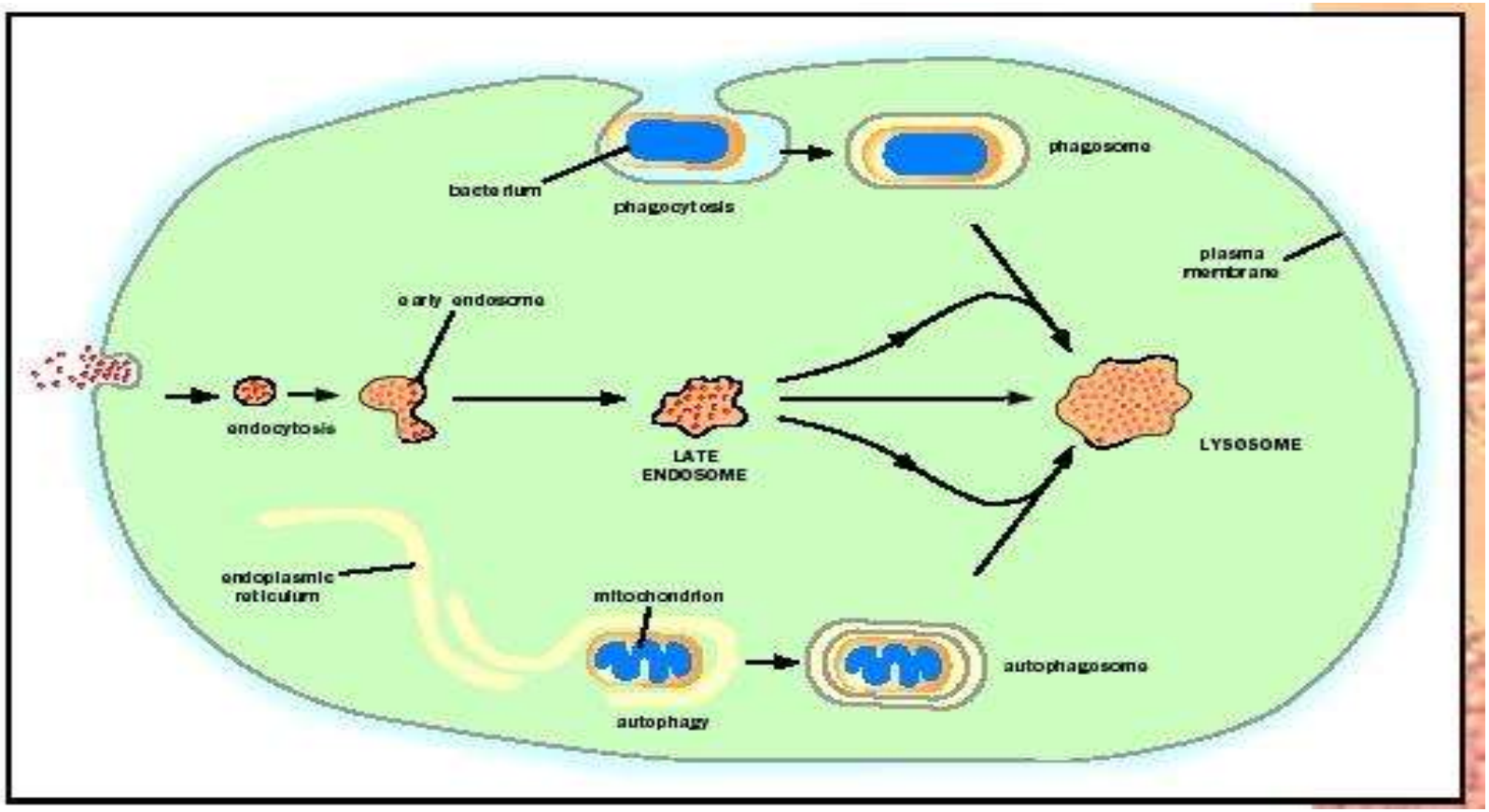
- Autophagy may also lead to autophagic cell death, a form of programmed self-destruction, or autolysis, of the cell, which means that the cell is digesting itself.

- Other functions include digesting foreign bacteria (or other forms of waste) that invade a cell and helping repair damage to the plasma membrane by serving as a membrane patch, sealing the wound.


Environment
Food particle taken in by endocytosis
Exocytosis of waste
Products of digestion
Food particles digested
Fusion forms secondary lysosome


Endocytosis
Phagocytosis
Pinocytosis
Receptor-mediated endocytosis
solid particle
Plasma membrane
Pseudopodium
Coated pit
Receptor
Phagosome (food vacuole)
Vesicle
cytoplasm
Coat protein
Coated vesicle

Three routes to degradation in lysosomes



LYSOSOMAL STORAGE DISEASES

- The lysosomal storage disease are a group of over forty human genetic disorders that result from defects in lysosomal function. The diseases are relatively rare and together have an incidence of approximately 1 in 7000-8000 live births.

- It is now recognized that LSDs are not simply a consequence of pure storage, but result from perturbation of complex cell signaling mechanisms

- These in turn give rise to secondary structural and biochemical changes, which have important implications for disease progression and therapy.

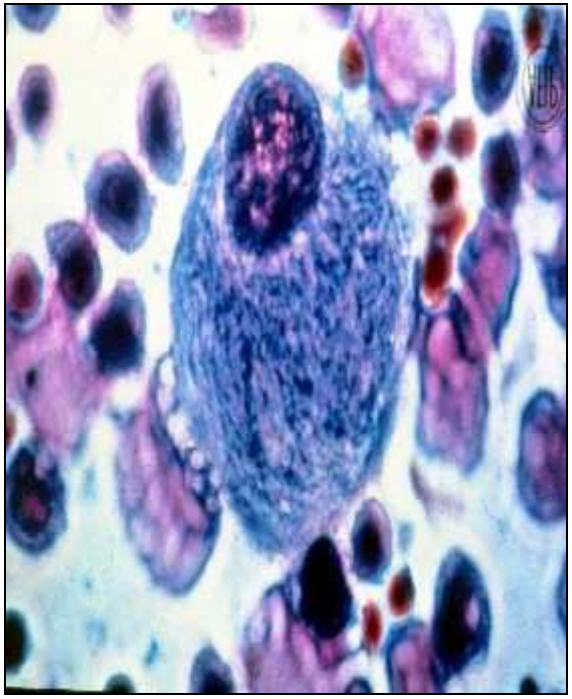
- Significant challenges remain, particularly targeting treatment to the central nervous and skeletal systems.

History of the LSDs

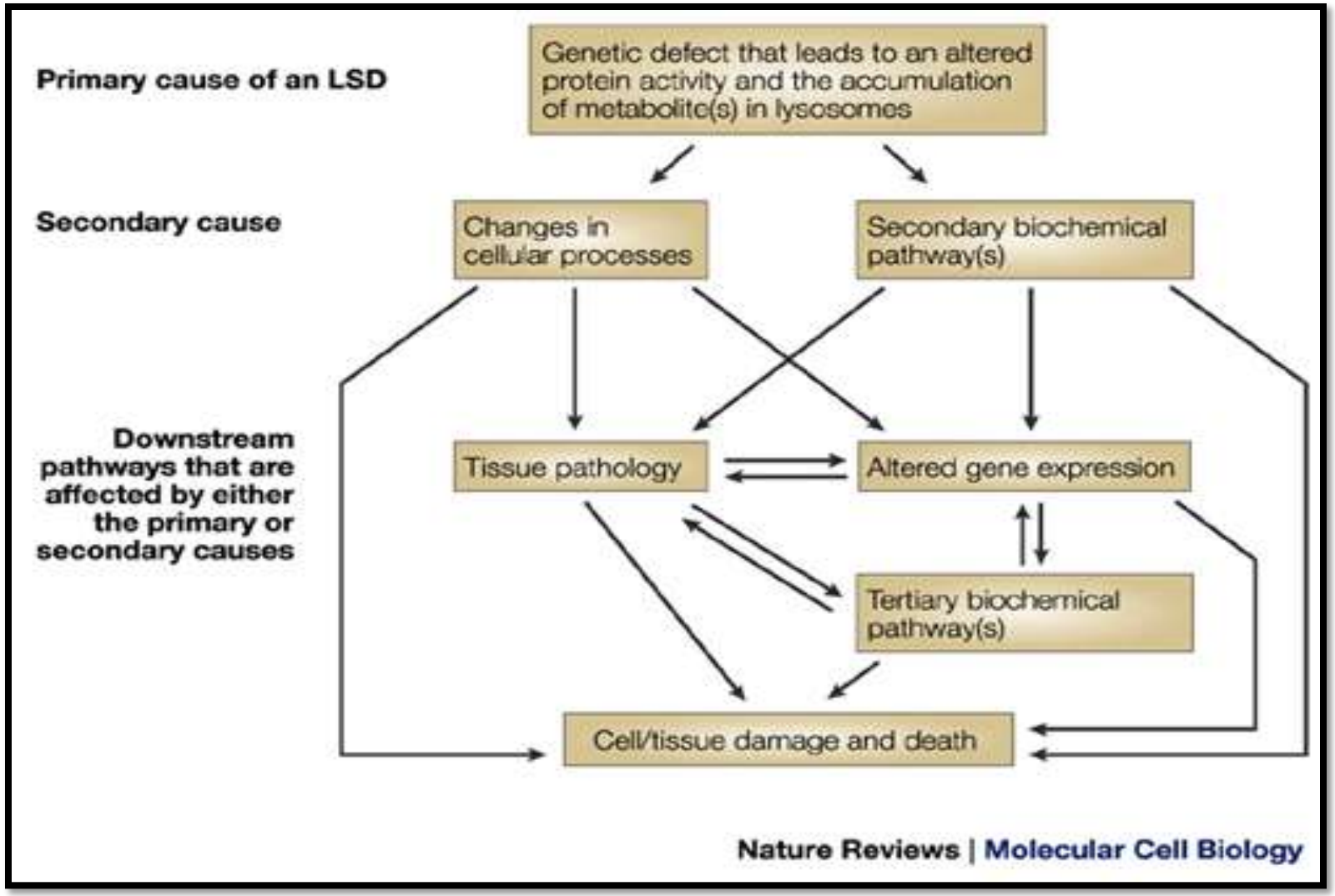
- Tay-Sachs disease was the first lysosomal storage disorder (LSD) described, in 1881
- Gaucher disease was the second, in 1882
- The first link between an enzyme deficiency and a LSD (α-glucosidase and Pompe disease) was published in 1963.
- The successful treatment of a LSD, Gaucher disease with β-glucosidase, became available in the early 1990s .

HISTORY OF THE LSD's

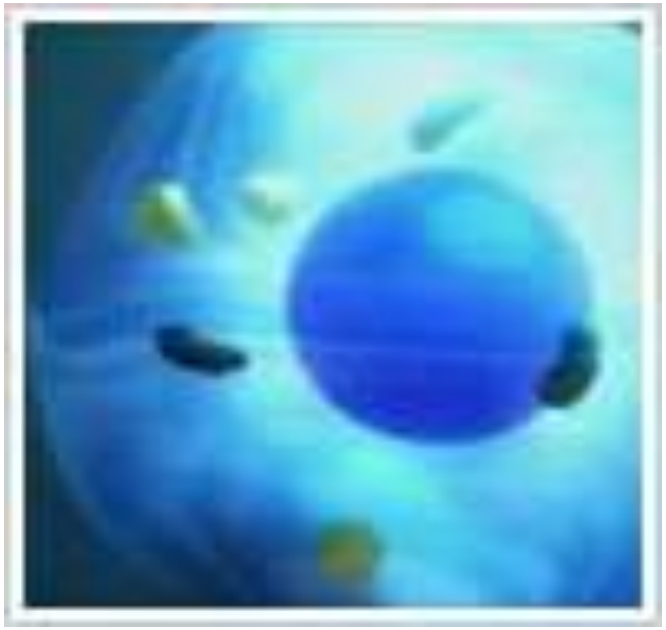
Ernest GAUCHER
(1854-1919)



Gaucher cell 1882


Primary cause of an LSD
Genetic defect that leads to an altered protein activity and the accumulation of metabolite(s) in lysosomes
Secondary cause
Changes in cellular processes
Secondary biochemical pathway(s)
Downstream pathways that are affected by either the primary or secondary causes
Tissue pathology
Altered gene expression
Tertiary biochemical pathway(s)
Cell/tissue damage and death
Nature Reviews | Molecular Cell Biology

- When a lysosomal enzyme (or another protein that directs it) is deficient or malfunctioning, the substrate it targets accumulates, interfering with normal cellular activity.



Healthy cell vs. LSD cell with accumulated substrate

LSD SUB CATEGORIES

- Sub-categories are based on the type of enzymatic defect and/or stored substrate product.

- For example, the Mucopolysaccharidosis (MPS) are grouped together because each results from an enzyme deficiency that causes accumulation of particular glycosaminoglycans (GAG) substrates.

I ~ Defective metabolism of glycosaminoglycans
" the Mucopolysaccharidosis"

- MPS I (Hurler, Hurler~Scheie, Scheie)
- MPS II (Hunter)
- MPS III (San filipo Types A,B,C and D)
- MPS IV (Morquio type A and B)
- MPS VI (Maroteaux~Lamy)
- MPS VII (Sly)
- MPS IX (Hyaluronidase deficiency)
- Multiple Sulfatase deficiency

II ~ Defective degradation of glycan portion of glycoproteins

- Aspartylglucosaminuria
- Fucosidosis, type I and II
- Mannosidosis
- Sialidosis, type I and II

III ~ Defective degradation of glycogen

Pompe disease

IV ~ Defective degradation of sphingolipid components

- Acid sphingomyelinase deficiency (Niemann~Pick A & B)
- Fabry disease
- Farber disease
- Gauchers disease, type I, II and III
- GM1 gangliosidosis, type I, II and III
- GM2 gangliosidosis (Tay~Sachs type I, II, III and Sandhoff
- Krabbe disease
- Metachromatic Leukodystrophy, type I, II and III

V ~ Defective degradation of polypeptides

Pycnodysostosis

VI ~ Defective degradation or transport of cholesterol, cholesterol esters, or other complex lipids

Neuronal ceroid lipofuscinoses, type I, II, III and IV

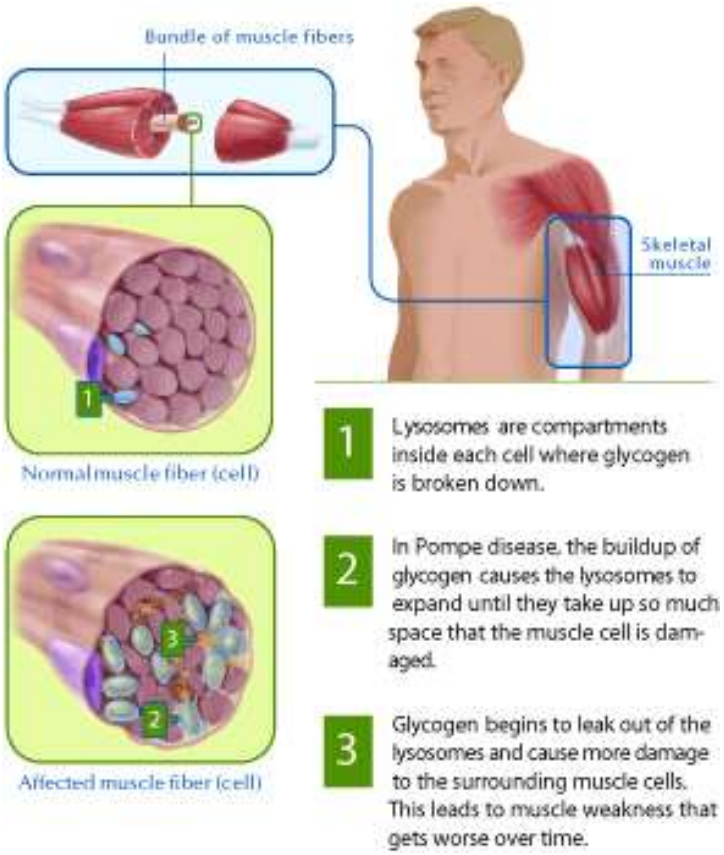
VII - Multiple deficiencies of lysosomal enzymes

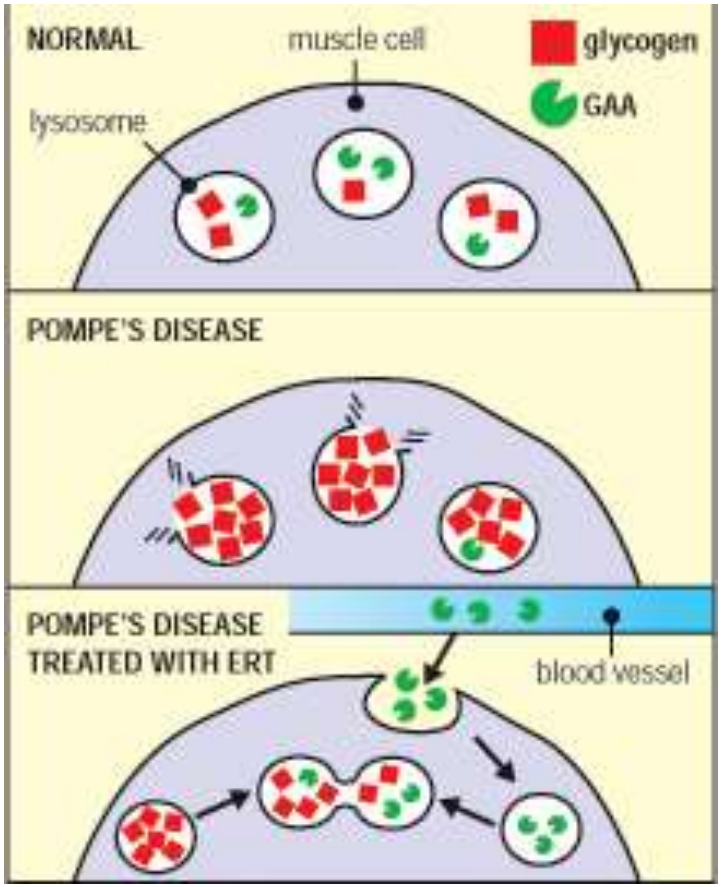
Galactosialidosis
Mucolipidosis, type II and III

VIII - Transport and trafficking defects

Cystinosis
Danon disease
Mucolipidosis type IV
Niemann-Pick type C
Infantile sialic acid storage disease
Salla disease

Pompes disease(glycogen storage diseases)

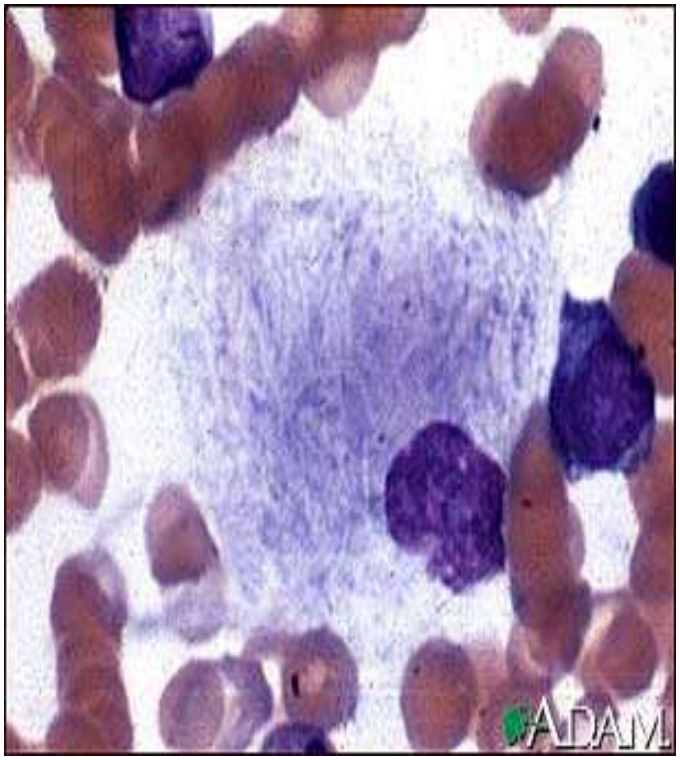




The enzyme acid alpha-glucosidase (GAA) breaks down glycogen to glucose which is then released in the blood. But in pompes disease(glycigen storage diseases) this enzyme is absent leading to accumulation of glycogen

GAUCHER'S DISEASE

- It is called a "lipid storage disease" where abnormal amounts of lipids called "glycosphingolipids" are stored in special cells called reticuloendothelial cells.
- Gaucher disease is caused by decreased amounts of an enzyme called beta-glucocerebrosidase.
- This enzyme deficiency leads to the build-up of a fatty substance, primarily in the liver, spleen, and bone marrow.
- The nucleus is pushed off to the side and the remainder of the cell is filled with abnormal lipids.



- There are no cures for lysosomal storage diseases and treatment is mostly symptomatic, although bone marrow transplantation and enzyme replacement therapy (ERT) have been tried with some success

- In addition, substrate reduction therapy, a method used to decrease the accumulation of storage material, is currently being evaluated for some of these diseases

Biochemical and Cellular basis of lysosomal storage disorders

- Most mutations result in the delivery of a defective enzyme with a reduced catalytic activity to lysosomes
- Another (activator) protein required for optimal hydrolase activity is defective or absent
- A mutation that causes misfolding results in defective transport of a lysosomal hydrolase out of the endoplasmic reticulum
- Alternatively, defective transport of a lysosomal hydrolase out of the ER occurs because a multi-enzyme complex that is required for transport cannot form (Cathepsin A / sialidase / β-Galactosidase)

- In the Golgi, defective glycosylation could result in an enzyme with reduced catalytic activity
- Alternatively, defective glycosylation with mannose-6-phosphate in the Golgi could produce an enzyme that cannot reach lysosomes
- Defects in other transport steps from the Golgi could also lead to an LSD
- Defects in integral lysosomal membrane proteins with transporter roles
- Defects in proteins that are involved in other vital regulatory events of lysosomal function (LAMP2, lysosomal associated membrane protein 2)

- The most frequently encountered human lysosomal storage diseases consist of three major groups of human metabolic disorders that have been categorized according to the chemical nature of the accumulating materials.

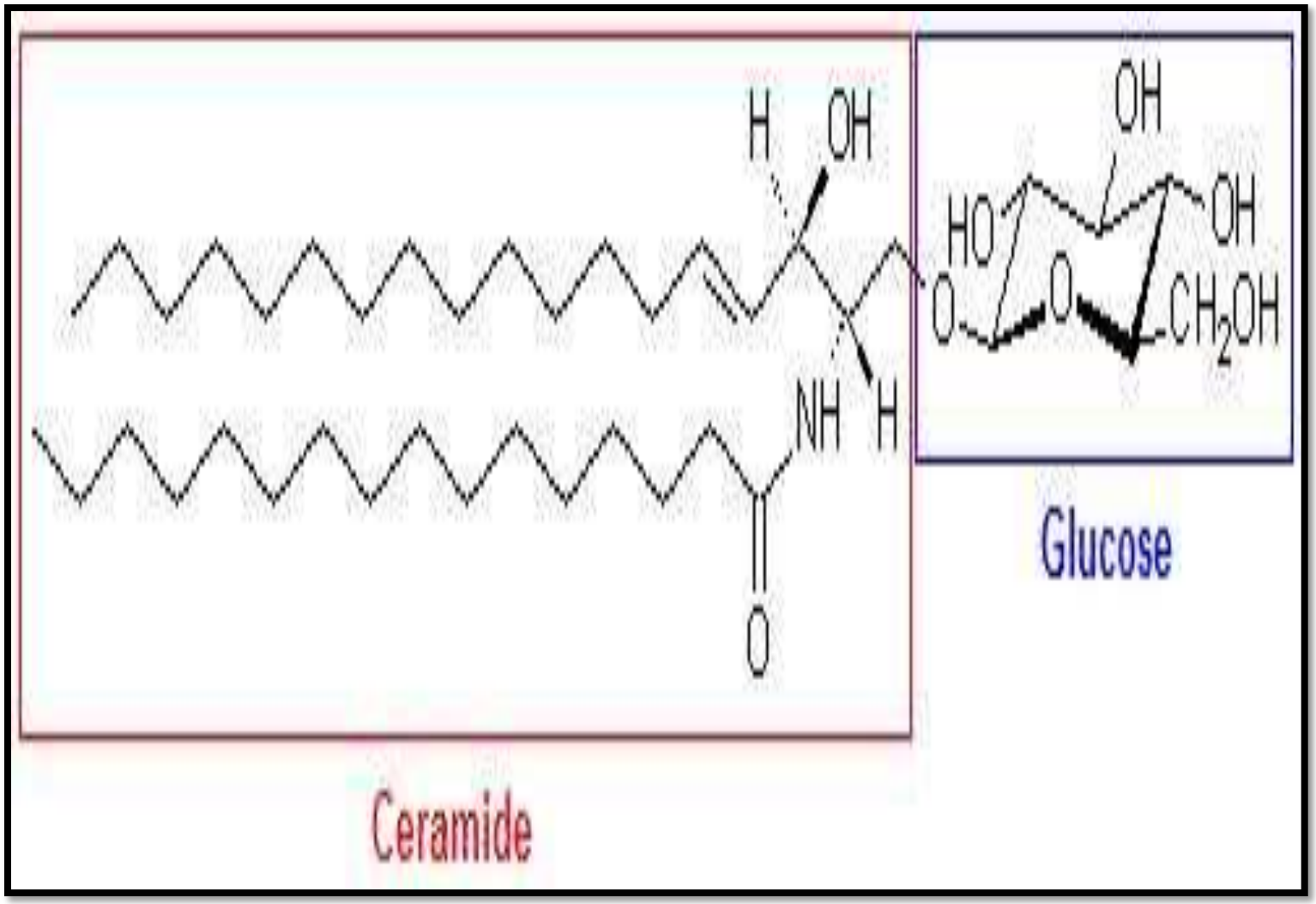
- The first group consist of individuals with lipid storage disorders such as Gauchers disease, Niemann-pick disease, Fabry disease and Tay-sachs disease.

- Accumulation of large quantities of sphingolipids throughout the body or in the nervous system.

- Sphingolipids are membrane lipids. They are named so because they consist of long chain amino alcohol known as sphingosine $[CH_3\text{~}(CH_2)_{12}\text{~}CH{=}CH\text{~}CH(OH)\text{~}CH(NH_2)\text{~}CH_2OH]$ to which long chain fatty acid is linked by an amide bond to the nitrogen atom on carbon two of sphingosine.

- This combination of sphingosine and fatty acid is called ceramide and it is common in all accumulating materials in lipidosis.

- Ex: In Gauchers disease the lipid that accumulates is glucocerebrosidase. It is composed of ceramide plus single molecule of glucose linked by a beta glycosidic bond to ceramide. where as sphingomyelin, the lipid that accumulates in Niemann picks disease is comprised of ceramide plus phosphocholine


H
OH
NH
H
O
OH
HO
OH
O
O
CH2OH
Glucose
Ceramide

- The **second** group of lysosomal storage disorders are Mucopolysaccharidosis.
- Large chains of sulfated oligosaccharides accumulate and damage cells in which they occur in excess.
- **Signs and symptoms**: mental retardation, skeletal defects, corneal clouding and enlargement of the spleen and liver.
- The **third** major group consists of **Glycogen storage disorders**
- A number of other infrequently encountered metabolic disorders such as sialidosis, Glycoproteinosis, mucolipidosis and, neuronal ceroid lipofuscinoses fall in to the classification of lysosomal storage disorders.

- The lysosomal storage diseases are classified by the nature of primary stored material involved, and can be broadly broken in to the following:

- Lipid storage disorders (including Gauchers disease and Niemann~pick disease)
- Gangliosidosis (including Tay~ sachs disease)
- Leukodystrophies
- Mucopolysaccheridoses (including Hunter syndrome and Hurler syndrome)
- Glycoprotein storage diseases
- Mucolipidoses

SIGNS AND SYMPTOMS

- Fabry disease: causes kidney and heart problems and skin rash. (ceramidetrihexosidase deficiency)

- Gauchers disease : causes the spleen enlargement, anemia and bone lesions if untreated. (glucoserebrosidase)

Hurlers syndrome: causes deformities of the skeleton and facial features, enlargement of liver spleen, joint stuff ness, clouding of the cornea, mental retardation and deafness.

- Niemann ~pick B disease: leads to enlargement of the liver and spleen, as well as lung disease.

- Tay~sachs disease: a lysosomal storage disease that occurs more commonly in people of eastern European Ashkenazi descent and causes degeneration of the brain in infants.

GANGLIOSIDOSIS

- A disease of the accumulation of Gangliosides is called gangliosidosis which is a form of lipid storage disorder.
- Mutations in genes coding for enzymes of Gangliosides metabolism cause severe lipid storage disease called gangliosidosis.

LEUKODYSTROPHY

- Leukodystrophy refers to a group of disorders characterized by progressive degeneration of white matter of the brain.
- The leukodystrophies are caused by imperfect growth or development of the myelin sheath. The fatty covering that acts as an insulator around nerve fibers.

MUCOPOLYSACCHARIDOSIS

- Mucopolysaccharidosis is a group of autosomal recessive metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down the molecules called glycosaminoglycans (long chain of sugar carbohydrate in each of our cells that help build bone, cartilage, tendons, cornea, skin and connective tissue).

- Glycosaminoglycans are also found in the fluid that lubricates our joints.

- The result is permanent, progressive cellular damage which affects appearance, physical abilities, organ and system functioning.

GLYCOPROTEINOSIS

- Glycoproteinosis (glycoprotein storage disorder) are lysosomal storage disease affecting glycoprotein's, resulting from defects in lysosomal function.

TREATMENT

- There are no cures for lysosomal storage diseases and treatment is mostly symptomatic, although bone marrow transplantation and enzyme replacement therapy (ERT) have been tried with some success.

- In addition, umbilical cord blood transplantation is being performed at specialized centers for a number of these diseases.

- In addition, substrate reduction therapy, a method used to decrease the accumulation of storage material, is currently being evaluated for some of these diseases. Furthermore, , a technique used to stabilize the defective enzymes produced by patients, is being examined for certain of these disorders. The experimental technique of gene therapy may offer cures in the future.

ENZYME REPLACEMENT TRIALS IN LIPID STORAGE DISEASES

- Enzyme replacement therapy is a medical treatment replacing an enzyme in patients in whom that particular enzyme is deficient or absent. Usually this is done by giving the patient an intravenous (IV) infusion containing the enzyme.

- Enzyme replacement therapy is currently available for some lysosomal diseases: Gaucher disease, Fabry disease, MPS I, MPS VI and Glycogen storage disease type II.

- Enzyme replacement therapy does not "treat" the underlying disease, only the symptoms

- Delivery of deficient enzymes to the organs in which the accumulating lipids were stored.

  ex: Arylsulfatase A in patients with metachromatic Leukodystrophy

- **Enzyme isolation procedures** to provide enzymes that were pure for human administration.

  ex: β- hexosaminidase A isolated and infused intravenously in to patient with sand Hoff form of Tay-sachs disease.

Naglazyme (galsulfase) for MPS VI

- **Naglazyme** (galsulfase) is an enzyme replacement therapy for the treatment of Mucopolysaccharidosis VI (MPS VI), an inherited life-threatening lysosomal storage disorder caused by a deficiency of the lysosomal enzyme *N*-acetylgalactosamine 4-sulfatase. Naglazyme provides a recombinant version of this enzyme to individuals diagnosed with MPS VI

SUBSTRATE REDUCTION THERAPY

- It offers a novel approach to the treatment of lysosomal storage disorders. By **reducing the rate of macromolecule synthesis** to a level where the residual degradative activity in the cell is sufficient to prevent substrate accumulation, it should be possible to reverse storage and storage-related pathologies.

- Ex: **Miglustat** is an N-alkylated imino sugar that acts against a number of enzymes involved in processing glycoconjugates, including the ceramide-specific glucosyltransferase, which catalyzes the initial committed step in glycosphingolipid synthesis.

- Miglustat could therefore be used for substrate reduction therapy in glycosphingolipid lysosomal storage disorders. Thus **miglustat** for treatment of type 1 Gaucher's disease, as well as related neuronopathicglycosphingolipidoses.

APPROACHES FOR TARGETING

- The use of macromolecular delivery systems that are targeted to endocytic machinery enable both cell specific as well as organelle specific, in this case the lysosomes interactions resulting in more efficacious treatment for these disease states.

MECHANISMS FOR LYSOSOMAL DELIVERY

- In an effort to expand the therapeutic potential of macromolecular drug delivery systems, targeted approaches have been investigated for their use in chemotherapy and enzyme replacement therapy.

- The endolysosomal path serves as a scaffold for intracellular movement leading to fusion with lysosomes to allow for the breakdown of macromolecules and vesicular content.

ENDOLYSOSOMAL PATHWAY

- The endolysosomal pathway is of direct relevance to targeted intracellular drug delivery because not only does Endocytosis allow for macromolecular internalization but it enables receptor and lysosomes specific localizatoin

- Two different mechanisms are utilized for internalization and trafficking to the lysosomes, namely:

✓ Fluid-phase Endocytosis (FPE)

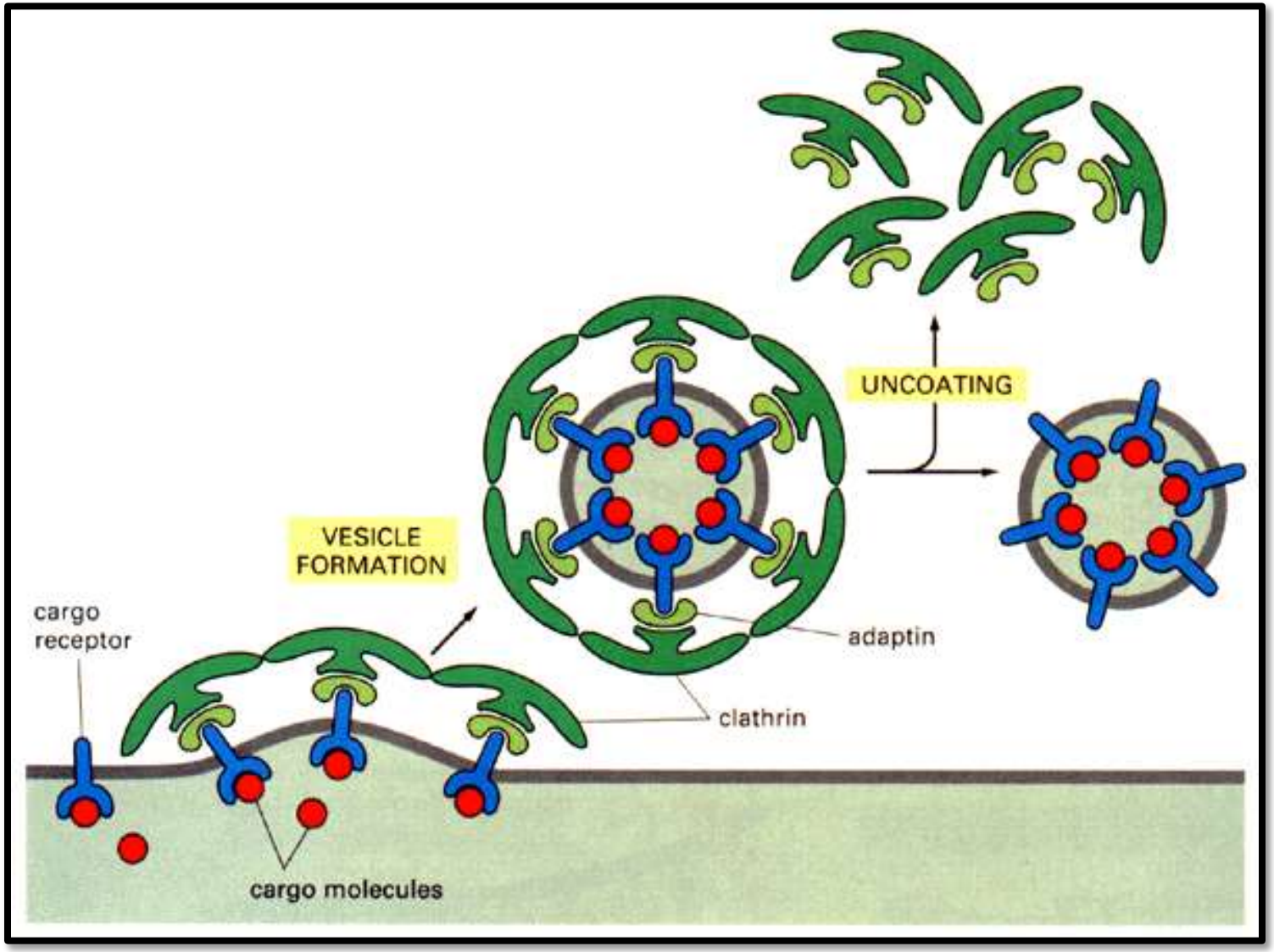
✓ Clathrin dependent Endocytosis

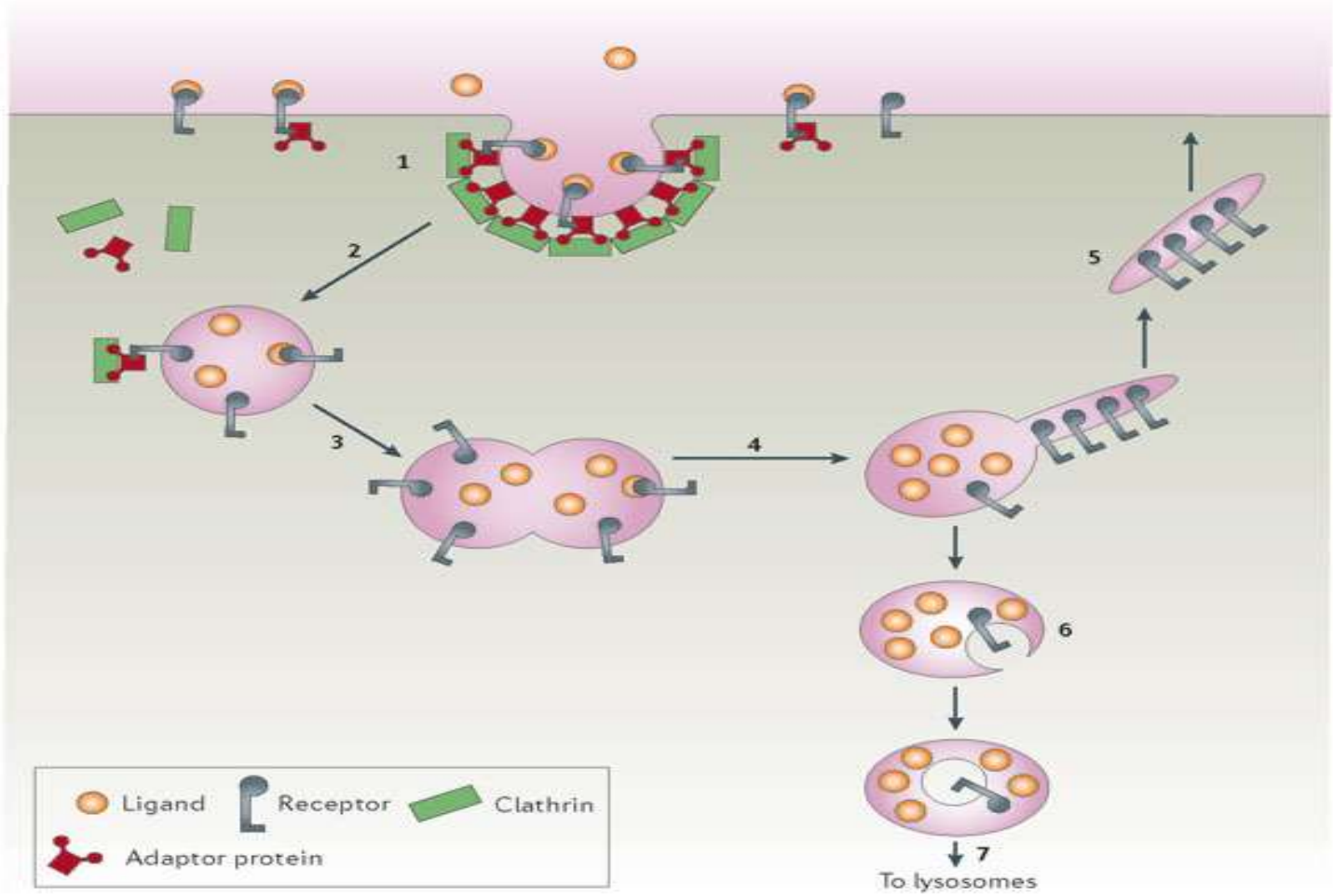
Occurring in a receptor-dependent and independent manner respectively.

- FPE is a non-specific adsorptive pinocytic mechanism which allows for the cellular incorporation of molecules contained in the extra cellular fluid. molecules absorbed via this pathway avoid direct binding with membrane constituents.

- Clathrin dependent Endocytosis serves as the main mechanism of internalization for macromolecules and plasma membrane constituents for all cell types.

- Invaginations in the membrane that are coated by triskelion protein.


UNCOATING
VESICLE
FORMATION
cargo
receptor
adaptin
clathrin
cargo molecules



THERAPY OF METABOLIC DISORDERS THAT INVOLVE THE CNS

- For patients with brain and peripheral nervous system damage one must develop a method to deliver effective quantities of enzyme to the involved cells in the nervous system.
- Injecting enzymes intravenously, intrathecally,or intracisternally doesn't solve the problem.
- So need to **alter** the BBB so that molecules as large as enzymes can enter the brain.
- Intracarotid injection of HYPEROSMOLAR solution of **mannitol** or **arabinose** solves the problem .
- Shrinking of endothelial cells- allows molecules as large as albumin 67,000 Daltons achieved.

CONCLUSION

- Enzyme replacement therapy is still in experimental stage of investigation on basis of early indications of clearance of accumulated lipids in Fabry and Gauchers disease.

- A successful outcome of this approach will have a profound effect on the role of enzyme replacement for therapy of host of human metabolic disorders.