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Scope and Method of Study: The method of study was a research of material in the library. The scope of study included the biochemical concept of the lysosome, its characteristics which afford visual identification, and its functions in physiology and pathology.

Findings and Conclusions: It was determined in the study that the lysosome is a polymorphic organelle of the cell which contains at least a dozen hydrolytic enzymes. It is on these enzymes that its functions depend, both pathological and physiological. The lysosome is essential in such physiological events as the resorption of the tadpole tail during metamorphosis, regression of the Mullerian ducts in embryos and perhaps it is essential in the fertilization process. The lysosome is also active in such diseases as muscular dystrophy, hypervitaminosis A and perhaps in old age.

ADVISER'S APPROVAL L. Herbert Bruneau

THE LYSOSOME

By

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THE LYSOSOME

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CHAPTER I

INTRODUCTION

The lysosome is one of the newest organelles to be brought to the attention of cytologists, biochemists, electron microscopists and cell anatomists. Its comparatively recent recognition as a resident of the cell is partly responsible for the fact that its functions and the mechanisms stimulating these functions, are not completely known. The heterogeneity of these organelles does not enhance studies dealing with what tissues they are found in.

Christian de Duve (1959) was the first person to suspect the existence of an organelle such as the lysosome. At that time he was working with mitochondrial enzymes involved in carbohydrate metabolism. His program called for an assay of these enzymes present in rat liver homogenate along with acid phosphatase which was used as a control. Upon analysis of enzyme concentrations in the homogenate he discovered that his control, acid phosphatase, was only one tenth the expected concentration. All fractions of the homogenate were stored for five days under refrigeration. At the end of this time another analysis revealed that the acid phosphatase was now at its expected level. After repeating the experiment several times he developed a theory for the enzyme concentration change. His theory suggested that the enzyme was contained in bag-like particles whose membrane could become disrupted

and release the enzyme (de Duve, 1963). His theory has been upheld with no serious opposition to the present day.

CHAPTER II

VISUAL IDENTIFICATION

Visual identification of the lysosome was dependent upon the enzyme acid phosphatase. This enzyme was capable of being stained by Gomorii's lead phosphate method making it possible to see the organelle under microscopes (Novikoff, 1961). It was not until 1955, six years after the first clue of their existence was obtained in 1949, that the lysosome was first identified in rat liver cells (de Duve, 1963). It was first seen in the rat liver in a position corresponding to that of the pericanalicular dense bodies. These dense bodies had been seen before but their functions were unknown. Because the pericanalicular dense bodies showed acid phosphatase activity they were assumed to be lysosomes. The dense bodies could not, with certainty, be called lysosomes but there are several generalizations and correlations which point to similarities between lysosomes and pericanalicular dense bodies. These are: 1) all methods leading to the isolation of lysosome rich fractions are equally successful in concentrating the dense bodies; 2) the observed dimensions of the dense bodies are in good agreement with those predicted for lysosomes; 3) at least some of the dense bodies show internal cavities and a well defined outer membrane, which also agrees with the postulated structure of lysosomes; 4) observations indicated that lysosomes belonged to parenchymatous cells of the liver

and the same appeared true of the dense bodies. If the dense bodies are not identical with lysosomes, the latter must be extremely rare, or have essentially the same structural properties as the mitochondria (Novikoff et al, 1956).

The knowledge of the structure of lysosomes is more precise in what it excludes than in what it includes. The structure excludes mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes and lipid droplets (Novikoff, 1961). It is known that the density of the particle is approximately 1.15 and the diameter ranges between 0.13 and 0.61 microns. The single unit membrane of the lysosome is 70 angstroms in thickness. Just beneath this membrane and separating it from the matrix is a clear zone. This clear zone is from 70 to 100 angstroms wide and remains intact even when distorted by materials in the matrix. The density of the matrix may be compared with that of the mitochondria. This matrix contains extremely fine granules with a diameter of 55 angstroms dispersed within it. Besides these granules the matrix contains one to several lumps varying in diameter between 0.03 and 0.12 microns. The lumps are usually located at the periphery of the body and separated from the limiting membrane by the clear zone. The lumps also show very fine granules with a diameter of approximately 20 angstroms. At times a cavity is seen in the organelle also (Daems and Rijssel, 1961).

A major difficulty in identifying lysosomes is their polymorphism. This polymorphism is explainable as a result of their digestive activities. The presence of a mitochondrion within the limiting membrane is sure to cause a distortion in the organelle (de Duve, 1963).

CHAPTER III

BIOCHEMICAL IDENTIFICATION

Although the dimensions of the lysosome seem to give useful basis for their identification, the figures cannot be depended on because of the lysosome's polymorphism. The lysosome was proposed to exist from biochemical analysis and biochemistry is still the most sure way of identifying the organelle. To determine if lysosomes are present in a given tissue, one must know to what extent the acid hydrolases are sedimentable in a tissue homogenate. Precautions must be used at every step in this process. As soon as a sample of tissue is removed from an animal it must be placed on ice but not frozen to prevent its decomposition. During the homogenizing it is necessary to use a gently homogenizer with a cool buffered medium. The isolation of this particle from the homogenate requires great dexterity in the use of the centrifuge. The major difficulty in the isolation procedure lies in the fact that the size of the lysosome overlaps that of the mitochondria and the microsomes. The size, density, and other known properties of the lysosome have enabled the development of a method for the isolation of purified lysosomes which is based on differential and density gradient centrifugation. Details of this procedure are given in Table I following. In batch preparation this method yields 60 milligrams of lysosomal protein from 160 grams of rat liver. Purified lysosomes show an enzyme

TABLE I

ISOLATION OF PURIFIED LYSOSOMES

Fraction	Centrifugal Force for the Fraction Collection	Increase in Specific Activity*	% Yield
I	Between 40,550 and 320,000 (0.25 M-sucrose)	5	38
II	At 97,000 (0.3 M-sucrose)	15	25
III	At 291,000 (through a gradient of 0.45 to 0.7 M-sucrose)	20	18
IV	Between 177,000 and 340,000 0.7 M-sucrose	65	14

* Compared with homogenate.

concentration 60 to 70 times that of the homogenate (Tappel et al, 1964). With this type of isolation biochemists have been able to recognize at least a dozen enzymes present in the lysosome. These enzymes are: acid phosphatase, acid deoxyribonuclease, acid ribonuclease, aryl sulfatases A and B, phosphoprotein phosphatase, β-galactosidase, β-N-acetylglucosaminidase, α-mannosidase, cathepsin, β-glucuronidase, and collagenase (Novikoff, 1961). The presence of these enzymes is not absolute proof that lysosomes exist in the tissue. Some of the "lysosomal" enzymes exist in other cell organelles. An example of this is the presence of β-glucuronidase in the microsomal fraction (de Duve, 1963). All the enzymes mentioned require an acid pH and have great lytic properties. For this reason the organelle was given the name lysosome meaning "lysing" body."

CHAPTER IV

STRUCTURE-LINKED LATENCY AND ACTIVATION OF LYSOSOMAL ENZYMES

The membrane of the lysosome prevents the indiscriminate destruction of cell parts by the powerful hydrolytic enzymes in the lysosomes. By the use of proteases and lecithinase it has been determined that the membrane is of a lipoprotein nature. Under normal circumstances the membrane is impermeable to both enzymes and their substrates (Giese, 1963).

It is assumed that the membrane of the lysosome limits the spread of active enzymes present within its boundaries but only a slight amount of damage is necessary to release the enzymes in soluble active form into the cytoplasm. Koenig (1962) disagrees with this assumption. He says that lysosomes may be solid complexes in which various enzymes are retained by ionic conjugation with acidic glycolipids and that this binding is responsible for the latency of the intact particle and not the integrity of the membrane. In support of this hypothesis, Koenig mentions that lysosomes do not look like bags in the electron microscope, as well as a number of experimental results obtained mostly on the brain lysosomes, but also on kidney and liver particles. He also mentions the fact that the enzymes can be released by relatively low concentrations of various organic and inorganic cations.

Lysosomes are ruptured rapidly in the absence of oxygen. There

are three possibilities concerning how anoxia can activate the lysosome. These are: 1) the membrane is a dynamic structure which is continually rebuilt with the help of oxidative metabolism; 2) the integrity of the membrane depends on the maintenance of its components in the oxidized state; 3) anoxia released one or more enzymes which break down the membrane. As to the third case, a particularly simple mechanism could be set up by the anoxia lowering the intracellular pH, which might be sufficient to accelerate a cathepsin rupture of the lysosomal membrane from within (de Duve, 1959).

Ultraviolet rays have a very powerful effect on lysosomes. Experimental results indicate that ninety per cent of the proteolytic activity of the lysosomes can be released within thirty minutes of ultraviolet administration (Weissman and Dingle, 1961).

Excess vitamin A is also responsible for the release of lysosomal enzymes. The hypervitaminosis A condition is suspected of releasing the catheptic activity of the particle (Thomas, 1964).

CHAPTER V

PHYSIOLOGICAL FUNCTIONS

The functions of the lysosome, whether pathological or physiological, are all associated with its compliment of hydrolytic enzymes. A few of the physiological functions of the lysosome along with the mechanisms of their actions will be discussed to be followed by some pathological functions.

Denise Sheib (1964), an experimental embryologist has done some work linking the lysosome to the regression of Mullerian ducts in the chick embryo. When the male chick embryo reaches the ninth day of incubation, the Mullerian ducts begin to regress in a caudo-cranial direction. Their regression is completely achieved within forty-eight hours. To establish the theory that lysosomes are responsible for this degeneration, Sheib had first to prove that the enzymes characteristic of the lysosome were present in the Mullerian duct tissue. This was easily achieved by homogenizing Mullerian ducts and analyzing for lysosomal enzymes. All the ducts were found to contain lysosomal enzymes so the next step was determining the mechanism of their activation. Her work showed that the sex hormones testosterone and estrogen were responsible for liberation and retention of the lysosomal enzymes. The male hormone will cause necrosis of explanted ducts within four hours of application. In these explants only one microgram of hormone per

duct was necessary to cause tissue degeneration. Since the gonads have differentiated by the ninth day it is, in all probability, the male hormones which causes the tissue degeneration. The female hormone stimulates the lumens of explanted ducts to grow larger and it is also able to prevent the degeneration of the male ducts. In chicks it has been suggested that the male hormones activate the necrosis of the left Mullerian duct while the female hormones activate the necrosis of the right duct. This last statement is partially supported by the fact that only the right duct atrophies in female chick embryos.

The recession of tadpole tails during metamorphosis is also due to lysosomal activity. If the tail of a metamorphosing frog is stained by Gomorii's lead phosphate technique very intense lysosomal activity is seen. It is interesting that the cathepsin increases in concentration until eighty per cent of the tail has been digested and it does not reach premetamorphosis concentration until ninety per cent of the tail has been digested. Studies of the tail regression indicate that its rate is directly proportional to the amount of thyroxin present. This is not positive proof that thyroxin is the triggering chemical since other chemicals also stimulate lysosomal breakdown (Weber, 1964). Vitamin A alcohol is one of the chemicals capable of causing lysosomal breakdown. Vitamin A alcohol is so effective that it can cause resorption of tadpole tails prior to the time of metamorphosis (Jackson, 1964).

It has been suggested that, in the process of fertilization, spermatozoa may depend on the release of lysosomal enzymes to dissolve some of the structures that surround the egg cell. Subsequent changes in the egg seem in turn to involve the release of enzymes from the

cortical granules that cover the inner surface of the cell. As a result the outer layers of the cell are broken down; a new membrane resistant to such an attack is built up underneath, and the metabolism of the egg is geared toward division and development. The cortical granules of the egg may also belong to the lysosome family. They can be ruptured by an injury such as a pin prick, hence the digestive action of these bodies may have something to do with parthenogenesis (de Duve, 1963).

The lysosome plays an important role as the digestive system of the cell. When a particle of food is taken into the cell by endocytosis, it is contained in a "phagosome." It is not readily apparent how this phagosome obtains the digestive enzymes of the lysosome (de Duve, 1963). An experiment performed by Straus (1964) shows us a possible mechanism for the enzyme entrance into the phagosome. In his experiments endocytosis produced phagosomes containing a material which would take a blue stain. In these same cells the lysosomes were stained red. Thirty minutes after both phagosome and lysosome were stained the lysosomes began to fuse with the phagosomes. After twelve hours most of the granules showed a combination of red and blue stains. After forty-eight hours the blue stain began to disappear, and after seventy-two hours most of the granules appeared red and only a few showed a blue tint. De Duve (1963) says that when the material in the phagosome has been digested and all useful components diffused into the cytoplasm, the waste products and lysosomal enzymes are excreted by exocytosis if possible. It is apparent that in multicellular animals excretion of the waste products could not easily occur since each cell is in close

apposition to the next. In this case the enzymes are used over and over again and the waste products are contained in "dense bodies" located throughout the cell.

The leucocytes also employ the mechanism of endocytosis in protecting the body from disease producing organisms. Upon the ingestion of a bacterium the leucocyte loses its cytoplasmic granules or lysosomes. The degree to which the lysosomes disappear is dependent upon the number of ingested particles in the cell. Studies have shown that degranulation occurs within 0.1 second and that it occurs only when the granules are in proximity to the ingested particle or in an area where another granule has ruptured. The disappearance of the lysosome may be caused by these two possibilities: 1) a local change in pH and/or; 2) the fusion of the granule with the phagosome. The cells seem never to recover from the process of degranulation and die soon after the ingested material has been digested (Cohn et al, 1964).

Lysosomes may have some function in iron metabolism or in the storage of iron in the form of ferritin. Lysosomes isolated from the liver and spleen contain more ferritin crystals than those isolated from other organs such as the thymus. Since the liver and spleen are involved in the destruction of erythrocytes and the preservation of iron it is then reasonable to assume that they have some role in iron metabolism (Rahman, 1962). The presence of iron is based only on what appears to be ferritin molecules in the lysosomes. The iron concentration is too weak to give sufficient color for its visualization when staining procedures are used (Novikoff, 1961).

The lysosome is capable of removing foreign particles from the

cell milieu. This is exemplified by the accumulation of lipofuscin (a general term for complex, nonuniform substances) within the lysosomal membrane. When the pigments are first deposited within the cell they are first seen within the lysosomes (or structures analogous to them) surrounded by a single membrane. Later as the accumulation of pigment proceeds, the entire lysosome may be transformed into a pigment granule and the membrane is no longer discernable (Barka and Anderson, 1963). By using Gomorii's lead phosphate technique it may be shown that some acid phosphatase activity still exists around the edge of the granule (Essner and Novikoff, 1961).

Another function suggested for the lysosome is active transport. Bennett (1956) theorized that these organelles may be concerned with active transport across liver endothelial and parenchymal cells. It has been shown that carbon and colloidal dye particles injected intravenously in frogs are transported across the liver endothelial and parenchymal cells into the bile canaliculi. Observations indicate that the particles transporting these materials are lysosomes. Beaufay (1959) has examined the possibility that lysosomes may play a role in the segregation of substances which are especially concentrated in the bile. His results were negative with bromosulfalein and with an iodenated opacifying agent (biligraphin), which was recovered almost entirely in the final supernatant of liver homogenates of animals killed ten to thirty minutes after intravenous injection of these compounds. With neutral red, on the other hand, part of the dye was retained in the particulate fractions, the highest concentration being found in the fraction richest in lysosomes.

CHAPTER VI

PATHOLOGICAL FUNCTIONS

Lysosomes are not always beneficial to the cell or organism. They sometimes are active elements in diseases. It has been suggested that one disease in which the abnormality of lysosomes may be at work is disseminated lupus erythematosus. This is a disease in which it is conceivable that hyperlability, or hyperactivity, of lysosomes may be responsible for the aggregation of tissue lesions in the disease. The excess vulnerability to ultraviolet of patients with this disease may be one example. It might also be suggested that the multiplicity of autoantibodies that characterize disseminated lupus erythematosus may represent a secondary phenomenon having nothing to do with pathogenesis of the disease itself. Because of the occurence of denaturation of native tissue components through the action of lysosomal enzymes, patients of disseminated lupus develop antibodies against their own nuclear material as well as against many proteins contained in their tissues (Thomas, 1964).

Excess vitamin A causes a condition known as hypervitaminosis A. In rabbits excess vitamin A causes depletion of metachromatic material from the matrix of cartilage, loss of hair, dermatitus characteristic of the disease, and even death if vitamin A doses are large enough (Thomas, 1964). When vitamin A is given in excess there occurs the

development of bone fractures apparently without cause. It is suggested that lysosomes are stimulated in some manner by the vitamin A and they dissolve or digest portions of the bone causing weaknesses to develop which later appear as spontaneous fractures (Weissman and Dingle, 1961).

Lysosomes are also suggested as being the cause of muscular dystrophy. There are two types of muscular dystrophy, genetic and nutritionally induced. It is possible to demonstrate large increases in the lysosomal enzymes in the muscles of genetically dystrophic mouse and chick. Further, using the vitamin-E-deficient rabbit, a study was made of the increases in lysosomal enzymes and these increases are correlated with the excretion of tissue breakdown products in the urine and observed histological changes. The primary effect of this vitamin E deficiency might be lipid peroxidation damage of the cells and their subcellular constituents including lysosomes. Rupture of the lysosomes and release of lysosomal enzymes would cause further damage to the cell. Cell death and tissue damage would be followed by an invasion of macrophages and other phagocytic cells with increase in lysosomal enzymes, catabolism of muscle and muscle dystrophy. In the case of the genetically dystrophic animal the relationship between the gene and lysosomal enzyme increase may be a simple one, but if it is not, the primary effect may be synthesis of an abnormal protein resulting in cell damage or death and subsequent macrophage invasion and increase in lysosomal enzymes (Tappel et al, 1964).

Lipofuscin granules (altered lysosomes) also are detrimental to the health of animals. It is assumed that these granules are a factor in old age, therefore, they are of interest to gerontologists. Of the

several cytological changes described as correlated with aging, the most widely accepted is the accumulation of lipofuscin granules. That lipofuscin accumulates with age in human myocardium has been shown quantitatively by several workers, who consider that, because of its absence in the very young, its presence without exception in the aged hearts studied, its lack of correlation with specific cardiac diseases and heart failure, and its large displacement of myocardial volume, the accumulation of lipofuscin in the human myocardium seems to meet the criteria set forth for a basic biological aging process (Novikoff, 1961).

CHAPTER VII

CONCLUSION

In conclusion we may say that the lysosome has been tagged as being responsible for many events occuring in the body to which no other organelle could be assigned. The lysosome may be defined as follows: 1) its average diameter is 0.4 microns and its density is 1.15 with both values subject to wide variations; 2) it contains no enzymes of oxidative metabolism but only hydrolytic enzymes surrounded by a single lipoprotein membrane; 3) it is vital in the digestion of food in cells but it is also a factor in many disorders; 4) its control may be hormonal but very little is known about this aspect. Taken as a whole very little is known about this potentially important organelle but the scope of the knowledge is broadening rapidly. Improved methods of differential centrifugation will lead to greater purification of lysosomal fractions enabling us to determine a more exact description of the enzyme content. New staining methods will enable cell physiologists to break away from their dependence on the single enzyme, acid phosphatase, and use the many other enzymes present in the organelle for its location. In the future knowledge of how to control the lysosome should give scientists and man the control over such diseases as muscular dystrophy. Perhaps in the future their control may mean the control of the aging process and make possible the fountain of youth.

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