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Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: LYSOSOMES, ULTRASTRUCTURAL ORGANELLES OF THE CYTOPLASM

Pages in Study: 19 Major Field: Natural Science

Candidate for Degree of Master of Science

Scope of Study: The study of lysosomes began after the advent of the electron microscope, and, as a part of the cell's ultrastructure, lysosomes have been an item of scientific conjecture as various researchers have offered hypotheses concerning the structure and function of these "organelles". Throughout the data gathering process no reference to lysosomes in plant tissue was found; all of the work surveyed concerned animal tissue, even when limited to lower organisms. This study is a discussion of the development of lysosome study as it has led from hypothesis to hypothesis, with a steady trickling of information from researchers in varied disciplines of the general field.

Findings and Conclusions: There is still no real conclusions by most current authorities as to the definite status of these organelles, or for that matter, whether they may be considered true and constant cell organelles. Lysosomes form a special group of cytoplasmic particles with a mean diameter of 0.4 microns and an average density of 1.15. They are characterized by a variety of acid hydrolases capable of degrading proteins, nucleic acids, and mucopolysaccharides. These enzymes are retained within the particles, and prevented from acting on surrounding substrates, by a lipoprotein membrane impermeable to these substrates. The simultaneous release of all internal enzymes in soluble and fully active form follows injury to the membrane. Whether or not they are finally classed as organelles, they are functionally important in many tissues and may hold the key to certain pathological phenomona of cell activities, and thus, of whole organisms.

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LYSOSOMES, ULTRASTRUCTURAL ORGANELLES OF THE CYTOPLASM

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1955

Submitted to the faculty of the Graduate School of the Oklahoma State University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE June, 1964

LYSOSOMES, ULTRASTRUCTURAL ORGANELLES

OF THE CYTOPLASM

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PREFACE

The study of lysosomes began after the advent of the electron microscope, and, as a part of the cell's ultrastructure, lysosomes have been an item of scientific conjecture as various researchers have offered hypotheses concerning the structure and function of these "organelles".

Throughout the data gathering process, I have failed to find experimental mention of lysosomes in plant tissue; all of the work surveyed concerned animal tissue, even when limited to lower organisms.

Indebtedness is acknowledged to Dr. L. Herbert Bruneau for his guidance and criticism during the writing of this report; to Dr. Margaret Brooks for her encouragement and inspiration; to Dr. H. S. Gallager, Pathologist, M.D. Anderson Tumor Institute, Houston, Texas, with whom I talked concerning current cell research and the chemotherapeutic approach to malignant neoplasms; and to the personnel of the biological science section of the Oklahoma State University Library, who so willingly aided me in obtaining necessary publications.

To my mother, I owe a debt of gratitude for her constant help and patience during this study and all of the previous years of my education.

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CHAPTER I

INTRODUCTION TO THE LYSOSOME CONCEPT

The cell is an integration of complex organelles, in every one of which specific enzymes are localized. As life of the cell involves constant interaction of these semi-isolated enzymes, so the growth of knowledge about the cell requires close co-operation among biologists, biochemists, and biophysicists (11).

Lysosome study thoroughly reflects this idea. The scope of the following report will be a discussion of the development of this study as it has led from hypothesis to hypothesis, with a steady trickling of information from researchers in varied disciplines of the general field. There is still no real conclusion by most current authorities as to the definite status of these organelles, or for that matter, whether they may be considered true and constant cell organelles. Whether or not they are finally classed as organelles, they are functionally important in many tissues, and they may hold the key to certain pathological phenomona of cell activities, and thus, of whole organisms.

Most biochemical work has been done with preparations in isotonic (0.25M) sucrose solution, fractionated on the centrifuge. Apart from the nuclear fraction, there are two

main fractions, the mitochondrial, containing large granules, corresponding in size to the cytoplasmic granules visible within the cell under the light microscope, and the microsomal, comprised of much smaller granules or microsomes and all remaining sedimentable cell debris. "Material remaining in the clear supernatant solution after prolonged high-speed centrifugation is generally regarded as coming from the extra-granular cytoplasm. This fraction could include material situated behind highly permeable membranes in the intact cell, e.g. in the nucleus. Enzymes found in this fraction would presumably be completely accessible to substrates in the intact cell." (5)

Novikoff, et al, in 1952-53, began sub-fractionation of mitochondrial and microsomal fractions after noticing diverse morphological types revealed by phase-contrast microscopy. Marked enzymic differences were found among the eight sub-fractions studied. The most marked differences noted were in the distribution of acid phosphatase and uricase among the so-called microsomal fractions. de Duve, in Louvain, Belgium, had by now concluded that the particle density was high, and from studies of acid phosphatase, he and his co-workers concluded that the particles behave as typical osmotic systems, surrounded by a semipermeable membrane. Finally, from nitrogen assay data, it was concluded that they must be very few in number, accounting for four percent or less of the cell's nitrogen.

Novikoff's group again began to examine these fractions

with the electron microscope. To their admitted amazement, "In every fraction where de Duve and Beaufay found biochemical evidence of lysosomes, we found distinctive particles with a mean diameter of .37μ, and over fifty percent of the particles seen possessed large internal cavities indicating the semi-permeable membrane postulated by de Duve." (12)

The most distinctive feature of these cytoplasmic particles is the minute granules which they contain. These granules are more dense to the electron beam, and smaller than those of the ergastoplasmic membranes. Virtually identical bodies have been observed by Bernhard (15) in tissue sections of liver, which emphasizes that they are real and not products of the procedure of isolation.

The possible relationship of these granules to mitochondria is quite indefinite, and from the papers surveyed, this author could find no noticeable accord among current researchers in their ideas of this relationship. The most likely relationship seems, to this author, to be one of "cat and mouse", the lysosomes functioning as hydrolytic sacs in which the mitochondria are degraded. However, Rouiller and Bernhard (15) feel that lysosomes, "microbodies", may be precursors of mitochondria. Rhodin (12) says there is no kinship; and de Duve (8) suggests that until there is more definite morphological certainty concerning lysosomes no correlation is valid, and that such a relationship, if it exists, cannot alter the fact that lysosomes are different from mitochondria, as defined on the basis of functional and

biochemical criteria.

Lysosomes, then, form a special group of cytoplasmic particles characterized by a variety of acid hydrolases capable of degrading proteins, nucleic acids, and mucopolysaccharides. These enzymes are retained within the particles, and prevented from acting on surrounding substrates, by a lipoprotein membrane impermeable to these substrates. de Duve and co-workers, in 1955, first described these bodies with the proposed name "lysosomes", calling attention to their richness in hydrolytic enzymes. (9)

CHAPTER II

MORPHOLOGY AND ENZYMIC ACTIVITY OF LYSOSOMES

The schematic diagram, from de Duve (8), on the following page is representative of the information, discussed in this report, concerning recent concepts of lysosome structure and function. It stresses the following:

1. A mean diameter of 0.4μ and an average density of 1.15.
2. Enzymic equipment lacking several key enzymes of oxidative metabolism, but comprising a number of easily soluble hydrolases, having in common an acid pH optimum.
3. A surrounding membrane of lipoprotein nature which effectively prevents the enzymes from escaping, as well as their respective substrates from penetrating into, the particles.
4. The simultaneous release of all internal enzymes in soluble and fully active form, following injuries to the membrane. (8)

The morphology of lysosomes seems to be varied, according to the species, and especially according to the physiological function of the tissue in which they are found. Novikoff (11) and Holt (10), as early as 1952, described peribiliary "dense bodies" with ferretin-like granules in liver tissue. Straus (16) linked kidney "droplets" with high concentrations of acid phosphatase, and other hydrolases de Duve had found in "lysosome" fractions of liver. Novikoff also suggests that the "microbodies" described by Rouiller and

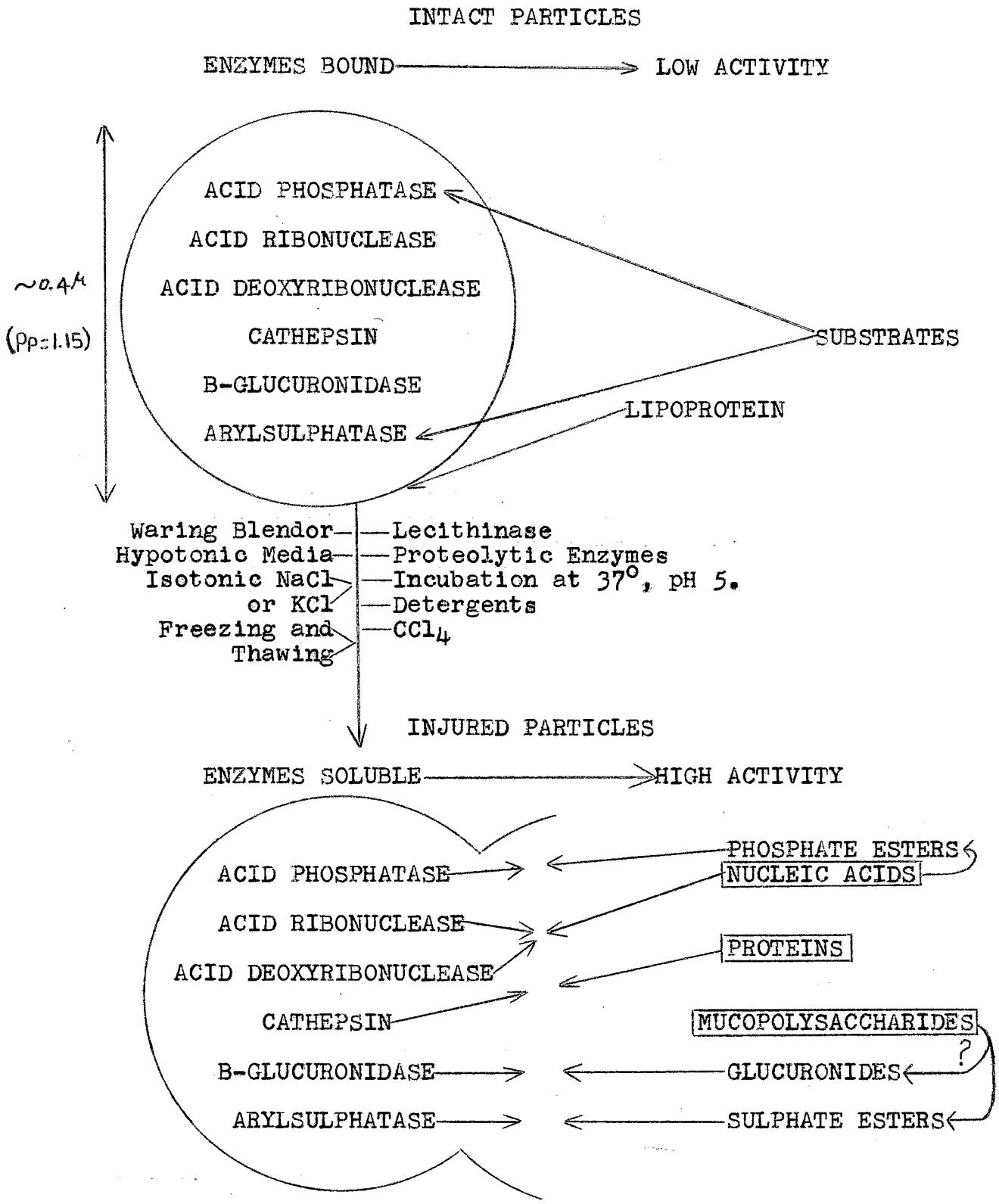

Fig. 1. SCHEMATIC REPRESENTATION OF THE LYSOSOME CONCEPT(8)

Bernhard (15), along the canaliculi of hepatic cells, may not be lysosomes because they are non-granular; however he (15) describes lysosomes that act in formation of squamous epithelium which are non-granular because "the acid-phosphatase has now become soluble and left the cytolysosome."

Beaufay (3) has reported the probability of lysosomes in rat and cat brains from results of fractionation studies. Doyle (6) has located most of the acid phosphatase of the spleen in the macrophages, and these cells contain large numbers of lysosome-like bodies with granules.

Many workers in this field have commented upon the heterogeneity in enzymic composition and size of the particles with which the 'lysosomal' enzymes are associated. "Franklin (1962), from a comparison of changes in the activity of acid phosphatase and arylsulphatase-B in ageing and ischaemic rat kidney, concluded that the two enzymes were contained within different classes of particles or within different parts of the same particle. Greenbaum, Slater and Wang (1960) found that B-glucuronidase, acid ribonuclease and cathepsin in sucrose homogenates from lactating rat mammary gland were sedimentable. All three enzymes were activated by freezing and thawing, or by the addition of Triton X-100. Their total activities, however, varied independently during lactation, which could perhaps reflect heterogeneity in the composition of the particles." (5)

Conchie and Levvy (5), therefore, propose that the adjective "lysosomal" should be applied to a property rather

than to a particle, since it describes a group of hydrolytic enzymes that are associated with cytoplasmic granules and display latent activity. Latency, however, is not unique to the lysosomal enzymes and will be discussed in further detail in Chapter IV.

Concerning the heterogeneity in size of the particles described as lysosomes, recent work by Beaufay and Berthet (2) has provided further support for the existence of lysosomes as a separate group of particles entirely distinct from mitochondria.

The density of subcellular particles may be expected to depend upon the composition of the suspending medium; thus the distributions obtained with a given biological preparation subjected to isopycnic centrifugation will be found to vary according to the nature of the gradient used.

In the work cited (2), preparations known to contain mitochondria and two additional populations of cytoplasmic particles which were defined mainly on the basis of their enzymic properties as the lysosomes, characterized by a number of acid hydrolases, and the newly identified particles containing catalase, urate oxidase and D-amino acid oxidase, were studied to determine the behaviour of each as a function of medium composition. Experiments were performed using gradients of varying concentrations of sucrose in H_2O. "In 2.04-3.15 molal sucrose gradients cytochrome oxidase and the hydrolases showed a peak density around 1.22; using gradient limits of 1.75-3.42 molal sucrose in

H_2O, cytochrome oxidase had peaks at 1.19 and 1.22. If the particles to be separated were layered on top of the gradient in 0.264 molal sucrose, instead of being distributed throughout the tube, the results for the lysosomal enzymes and for urate oxidase were comparable to those obtained in the previous experiments, but the mitochondria were now found to be distributed around a single peak of modal density 1.19 and were thus largely separated from the other particles." (2)

It appears that mitochondria, when exposed to high concentrations of sucrose, assume a 'dense state', presumably because of partial dehydration of the matrix. These results not only lend support for the existence of lysosomes as distinct particles, but provide preparative applications for the study of these particles.

CHAPTER III

TYPES OF LYSOSOMES AND THEIR PHYSIOLOGICAL SIGNIFICANCE

Novikoff (11) catagorizes lysosomes according to their morphology as well as enzymatic action as follows:

1. Pinocytosis vacuoles- pinosomes; using Chaos chaos, small acid phosphatase rich pinosomes were found and it was thought that small dense bodies seen contacting the vacuolar membrane were transferring digestive enzymes into it. Pinosomic protein intake may be an important process in cell physiology and could indicate the method of entrance as well as breakdown mechanism for proteinaceous macromolecules.

2. Vacuoles resulting from engulfing solid particles- phagosomes; the ingestion of red blood cells by macrophages is found within ascite tumor cells in the peritoneal cavity of tumor injected rats. The characteristic single limiting membrane of the phagosome is evident, and in stained sections erythrocytes within various stages of digestion can be seen. These bodies also demonstrate a high acid phosphatase activity.

This phagosomal-type action is known to occur in the atrophy of Kupffer cells of rat liver, the corpus luteum, macrophages of the uterus particularly after parturition, thymus tissue at puberty, etc. Very little attention has

ever been given to these normal physiological autolytic processes, however, it is known that acid hydrolases are increased in many of these situations and that "lysosome" structure is found in large numbers of bodies present in the few instances observed.

Ashford (1), in studies with glucagon perfused rat liver, reports that the number of recognizable lysosomes increased greatly after perfusions with glycagon (a protein catabolic hormone). Within almost every lysosome there was found a mitochondrion in some stage of hydrolytic breakdown, as if they were the favorite object for lysis. The situation just mentioned becomes more plausible when realizing that in cells undergoing autolysis the lysosomes become quite large. Ashford suggests that lysosomes of liver cells may only represent portions of cytoplasm (mitochondria included) set aside for hydrolysis to provide the protoplast with breakdown products for physiological reorientation.

3. Altered lysosomes in cytolysing cells - cytolosomes; These illustrate the physiological lytic processes also. Lysosomes are found in abundance in cells of lytic tissue, for example, in the reabsorbing tail of Rana pipiens tadpoles there is a marked rise in cathepsin. Brachet (4) describes this lysosome action, and in other papers, reports the marked rise in lysosomal enzymes when the mullerian duct of the chick embryo regresses.

Lysosomes may be involved in endocrine secretion; the two processes above are known to be hormonally controlled.

Abundant lysosomes have been found in all of the endocrine glands studied by Novikoff, et al (11) which includes the pancreas, thyroid, and adrenal medulla.

Many other normal tissues studied have acid phosphatase containing granules-- "lysosomes"; one exception being striated muscle. They appear in all extensions of the neurons of normal rat brain cells studied (12), and it is suggested that they may have a general role in nerve transmission, perhaps in the hydrolysis of acetylcholine.

In embryonic tissue it is thought that the many lysosomes may be correlated with the high rate of turnover due to growth processes.

In all normal tissues studied lysosomes were found in less abundance than mitochondria.

The release of all lysosomal enzymes occurs in an almost perfectly parallel fashion; but their distributions, whether determined on the basis of sedimentation rate or of density, are not identical.

One study on rat thymus tissue after whole body x-irradiation (13) showed that the lysosomal bodies increased after x-ray exposure, and, although not an intended result of the experiment, that author noted the obtaining of better separation of lysosomes after x-irradiation since they seemed to be located more abundantly in the cytoplasm of the radioresistant group of cells in the thymus.

CHAPTER IV

THE RELEASE OF ENZYMIC ACTIVITY OF LYSOSOMES

The list of hydrolytic enzymes, found by biochemical methods, contained within the "mixed fraction" (that intermediate to the main microsomal and mitochondrial sub-fractions) includes acid phosphatase, ribonuclease, deoxyribonuclease, cathepsin, and B-glucuronidase. The distribution of these enzymes differs markedly from the homogenous distribution of the mitochondrial oxidases; and these five show a similar distribution, lending strong support to the provisional conclusion that they belong to granules of a common class.

All of these hydrolases were found to be unreactive towards added substrates when present within intact granules, and simultaneously activated and solubilized by treatments which damage the particles. This parallel action, too, suggests that these enzymes are all associated with granules of the same group.

Table I shows the approximate intracellular distribution of enzymes noted by de Duve (9) in studies using fractionation methods upon rat liver tissue.

TABLE I

ENZYMIC ACTIVITY OF THE CELL

Cell Fraction	Percent of Total Activity	Enzymic pH Optimum
Mitochondrial	40	7.4
Microsomal	20	5.2
Lysosomal	10-20	below 5.2
Other	20-30	____

Experimental evidence indicates that the sole factor in latency and activation of lysosomal enzymes is the integrity of a membrane-like barrier. A membrane of lipoprotein composition is indicated by the nature of several of the releasing agents, in particular, the enzymic ones. An obvious corollary of inactivity in fresh tissue, intact particles, is that the activities actually observed must be due to free enzymes, and should therefore be recovered quantitatively in high-speed supernatant of this preparation. This never happens, but can be explained- since the assay conditions favor autolysis of the particles so that additional enzyme molecules may become free to participate in the reaction.

de Duve (9) has previously shown that bound protease can be released from lysosomes by such means as detergents, repeated freezing and thawing, or simple incubation at 37° C at an acid pH. Recently he has found that hydrocortisone added <u>in vitro</u> inhibited the release of lysosomal enzymes by

the last method. Weissmann (17) has found that energy from ultraviolet irradiation also causes release of catheptic activity from sub-cellular particles; whether by direct action upon these granules, or upon an enzyme system responsible for maintaining their integrity, he did not ascertain.

There is little doubt that the breakage of these particles and release of their enzymes is the main factor responsible for autolysis which takes place in tissue dispersions and mitochondrial preparations.

How, then, are lysosomes ruptured in intact cells? In experiments with liver slices (8), rupture was found to occur more rapidly in the absence of oxygen. How anoxia affects the lysosomal membrane is not known, but some interesting possibilities are given below.

1. The dynamic structure of the membrane must be continually rebuilt with the help of oxidative energy.

2. The integrity of the membrane depends upon the maintenance of some of its components in an oxidized state.

3. Anoxia releases one or more enzymes which break down the membrane.

4. Perhaps anoxia lowers the intercellular pH sugficiently to accelerate a catheptic rupture of the lysosomal membrane from within. (Autolysis, <u>in vitro</u>, has been shown to be strongly pH dependent).

de Duve (9), speaking of intracellular digestion, says that for an enzyme to survive it must be continually active upon its substrate. Perhaps this is the basis for a positive survival value of turnover for organisms.

Another possibility concerns the phenomenon of latency.

If latency in a broken cell preparation reflects a real phenomenon in the living cell, it may be a device for regulating the activity of enzymes *in vivo*. The fact that the lysosomal enzymes are apparently situated behind barriers of differing permeability in different tissues, and in some tissues may be found free in the soluble fraction, offers the possibility of selective control by specific enzyme inhibitors (5).

CHAPTER V

PATHOLOGICAL ASPECTS OF LYSOSOMES

Lysosomes are thought also to have a necrotic function when an excessive release of their enzymes occurs within a cell. The lysosomal enzymes are capable of destroying the most important tissue constituents, and the basic defense of the cell against this attack is the integrity of the lysosomal membrane.

A most significant effect observed in animals treated with carcinogenic dyes was an increase in total cathepsin and acid nucleases, and in the free or unsedimentable activities of all lysosomal enzymes in the precancerous livers and in those containing tumors. There seems to be a strong correlation between the intensity of necrotic phenomena and the proportion of lysosomal enzymes found in free form in homogenized tissue. It is not clear whether they should thus be considered as potential killers or "suicide-bags" (8), since the observed breakdown could be a postmortal phenomenon. That they can act as scavengers to help clear tissues of dead cells is strongly indicated. This and other work by de Duve suggests that lysosomes could be made of use in chemotherapy, either directly to kill the cells, or indirectly to release a lethal agent from a non-toxic precursor.

Increased catabolism of muscle in both nutritional and genetic dystrophy appears to have a direct correlation with increases in lysosomal enzymes (18). In vitamin E deficient dystrophic rabbits the "free" and "total" activities of these enzymes were increased, some as much as one thousand times. Somewhat smaller increases were found in liver. This was found also in leg muscle of genetically dystrophic chickens. "These results suggest that increased lysosomal enzymes are the cause of muscular dystrophy." (18).

"Beaufay and de Duve (1959) showed that lecithinase from Clostridium welchii destroys 'lysosomal membranes.' This may reflect a general mechanism by which a pathogen can accomplish the death of the host." (5).

It has also been suggested in some recent literature that ionizing radiations destroy function through a breakdown of intracellular membranes.

CHAPTER VI

CONCLUSIONS

Most bodies studied by electron microscopy and described as microbodies, cytosomes, and "large granules", are probably lysosomes, defined as: Cytoplasmic organelles delimited by single outer membranes and possessing high levels of acid phosphatase, and presumably other hydrolases, with acid pH optima.

The problem of obtaining clean cut lysosomal fractions for study has not yet been overcome, since, by their great range in size, they have a sedimentation range overlapping those of both the mitochondria and the microsomes. This situation was a deterrent to elucidation of these particles for years as researchers considered the dissociation between acid phosphatase and cytochrome oxidase to indicate mitochondrial and microsomal enzyme heterogeneity; but de Duve (7) interpreted this to mean that acid phosphatase was attached to a special group of particles entirely distinct from oxidizing mitochondria.

The thesis now set forth is that the isolated fractions are heterogenous and contain a mixture of at least two distinct populations of particles in different proportions; but that there is homogeneity in enzymic content of the particles

themselves (at least they cannot be separated by centrifugation). The above status is referred to as statistical, or first approximation, homogeneity.

Aided by reliable staining methods researchers in the future may be able to distinguish different enzymatic types among lysosomes and correlate these with their characteristic fine structure. The future should also bring quantitative data into which these qualitative descriptions can be fitted.

de Duve, in 1959, (8) in his discussion of lysosomes, says, "It is too early to decide whether lysosomes represent a cell organelle of general significance or are restricted to some kinds of cells only." In the light of his, and many other studies since that time, this author feels certain that it can now be said; lysosomes are cell organelles of significance and are found in many tissues under varying physiological conditions and may be extremely important entities in certain pathological phenomena as well.

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