Lysosomes

The question whether lysosomes are an independent category of cell organelles has long been a subject of discussion. While studying the intracellular localization of the «acid phosphatase», DE DUVE found this enzyme to be coupled in the ultracentrifugal sedimentation to particles differing from other cellular organelles. Besides the «acid phosphatase», many acid hydrolases were found whereas, in contrast to the mitochondria, the key-enzymes of the citric acid cycle (see fig. 43) do not occur. In homogenates of liver cells still more particles having a sedimentary behaviour differing from that of the lysosomes and containing enzymes not found in lysosomes have been isolated: acid D-amino-oxydase, uricase and catalase.

Lysosomes are nowadays regarded as a special kind of major particles of cytoplasm playing a special part in the intracellular digestion.

Owing to their sedimentary properties, lysosomes are supposed to have a size of 0.4 mi and a density of 1.15 (mitochondria 1.13) on an average. Dyeing in vivo is possible with acridin-orange and neutral red; lysosomal enzymes are inhibited by trypan blue. Agents destroying the cytomembranes release also the contents of lysosomes. Proteolytic and lipolytic ferments, mechanical traumata and low pH can release the acid hydrolases. Their transformation (after DE DUVE) is shown in fig. 44. The amount of the lysosomal enzymes is supposed to be large enough to dissolve the entire cellular organisation if a sudden release happens. This postulated lytical property finally led to the name «lysosomes».

Form and function

When phagocyted particles migrate through the cellular membrane, a lysosome appears at the inner limiting surface of the forming digestive cistern even before the outer membrane opening recloses. Constituents of lysosomes flow into the neighbouring tissue (regurgitation). The lysosomes are transported into the periphery of the cells by a regulatory function of the cytoplasmatic microfilaments (see fig. 21) and of the cytoplasmatic microtubuli originating from the centriole region.

The «digestive vacuole» formed after taking up foreign particles contains first no hydrolases and is called «phagosome». After taking up the acid hydrolases, it is sometimes referred to as heterophagous vacuole or phagolysosome. If the lysosomal outfit of enzymes is not capable of disintegrating foreign material, the latter will be stored.

The disintegration of foreign material (heterophagia) seems to proceed otherwise than the intracellular digestion of cellular constituents i.e. autophagia.

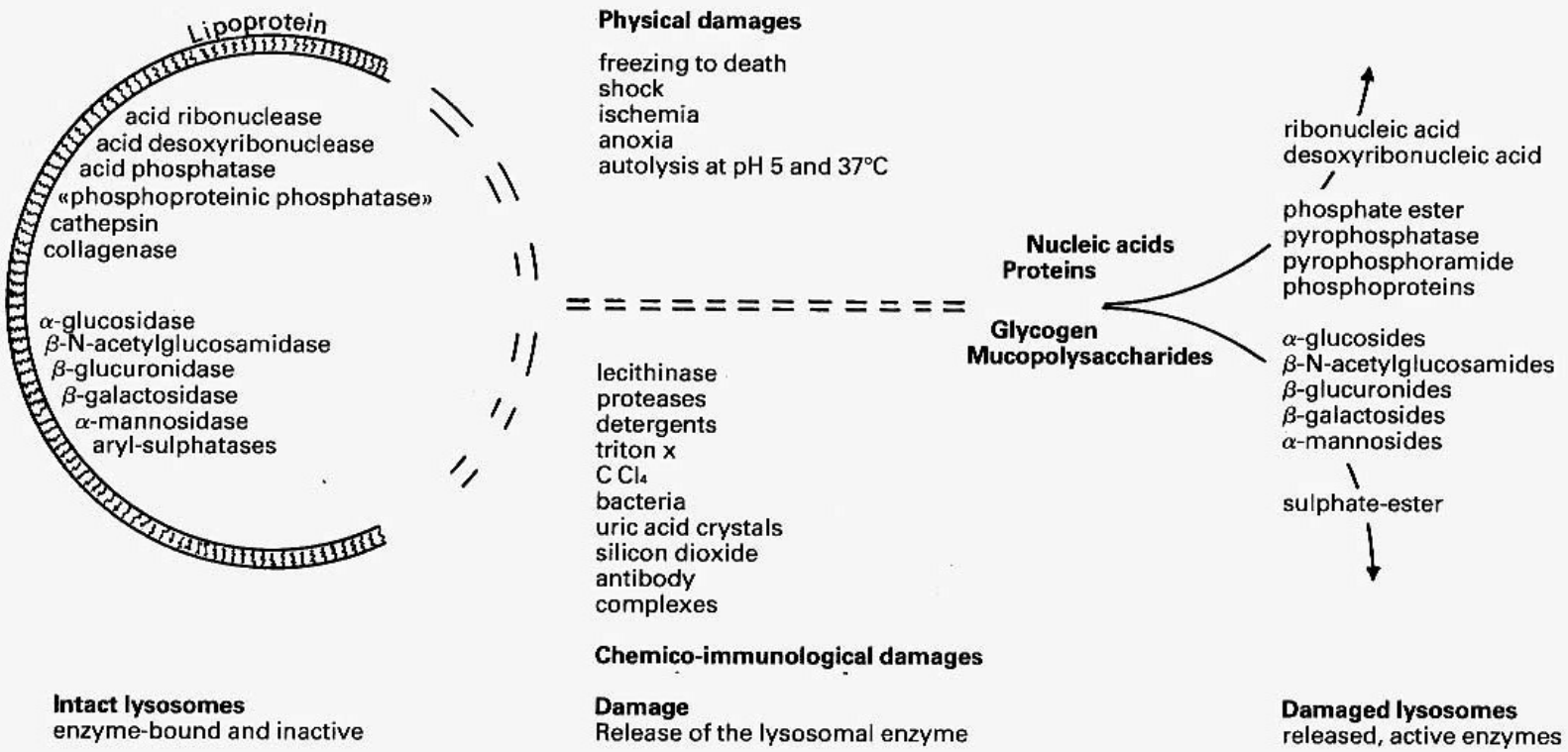

Fig. 44:
Lysosomes
On the left, the circle includes inactive, bound, lysosomal enzymes.
In the middle: physical and chemo-immunological noxious factors, which cause the release of lysosomal enzymes.
On the right, released, active lysosomal enzymes and their substrate target-substances.

Tab. 2: **Lysosomal enzymes**[1]

Oxidoreductases act upon hydrogen peroxide	**Hydrolases** acting upon ester compounds	**Hydrolases** acting upon glycosyl compounds	**Hydrolases** acting upon peptide compounds
Peroxidase	arylesterase triacylglycerol-lipase cholesterol-esterase phospholipase A_1 phospholipase A_2 acid phosphatase phosphatidase-phosphatase phosphoprotein-phosphatase phosphodiesterase I desoxyribonuclease II ribonuclease II sphingomyelin-phosphodiesterase arylsulphatases sulphatase A sulphatase B chondroitin-sulphatase	lysozyme neuramidase α-glucosidase β-glucosidase α-galactosidase β-galactosidase α-mannosidase β-mannosidase β-N-acetylglucosamidase β-glucuronidase hyaluron-glucosidase α-N-acetylgalactosaminidase α-N-acetylglucosaminidase α-L-fucosidase L-iduronidase	glutamate-carboxypeptidase carboxypeptidase A (cathepsin A) carboxypeptidase B (cathepsin B_2) carboxypeptidase C lysosom. dipeptidase dipeptidylpeptidase I dipeptidylpeptidase II kininogenin acrosin elastase cathepsin G neutral proteinase plasminogen activator cathepsin B (B_1 cathepsin D cathepsin E lysosom. collagenase renin
Hydrolases acting upon non-peptide carbo-nitric compounds			
acetylsphingosindeaclase aspartylglucosylaminase amino-acid-naphthyl-amidase benzoylarginin-naphthyl-amidase			
Hydrolases acting upon acid-anhydrides			
inorganic pyrophosphatase			
Hydrolases acting upon phosph.-nitrogen-compounds	**Hydrolases** acting upon sulphurated nitrogen		
phosphoamidase	heparin-sulphamidase		

[1] After data from ALTMANN and KATZ «Cell-Biology» 1976; BARRET A. J. (1972); DEAN R. T. (1975); HERS H. G. and VAN HOFF (1973); ROBINSON D. (1974). Certain enzymes were identified only in lysosomes of certain organs.

The autophagous vacuoles seem to originate from bulges of the endoplasmatic reticulum, which first develop baggy bulges and finally form complete digestive cisterns. Their hydrolytic enzymes originate probably from the vesicles rich in enzymes of the endoplasmatic reticulum or are supplied by connection with lysosomes. Their activity can disintegrate whole endogenic mitochondria.

Form and function – biological magnitudes independent of each other, whose interrelation has always raised fascinating questions in biology – are different:

1. In the granules of the polymorphonuclear granulocytes, probably freshly formed enzymes are stored and then used for intracellular digestion.
2. Endogenic (degenerated, alterated) cell constituents are autolysed in the lysosomes, apart from the rest of the cell.
3. As «digestive cisterns» («digestive vacuoles» is misleading because no empty spaces are in question) they disintegrate phagocyted material of extracellular origin.

In granulocytes (microphages) just as well as in monocytes (macrophages), alveolar macrophages and eosinophiles, foreign particles and autogenous substances «estranged from the body» have been disintegrated. Especially when bacteria are phagocyted, lysosomes (fig. 45–47, 20) releasing their contents by autolysis gather round the intracellu-

Tab. 3: **Mechanisms and regulation of the release of lysosomal enzymes from polymorpho-nuclear leukocytes** (Cell biology 1976)

Mechanism	Stimulus for release	Regulation factors
release during phagocytosis	bacteria zymosane immunocomplexes soluble insoluble immunoglobulin-aggregations crystals of calcium-pyrophosphate	
release without phagocytosis	superficial immunglobulin aggregations superficial immunocomplexes chemotactic factors	contractile proteins microtubuli microfilaments serinesterase energy metabolism cyclic nucleotides calcium
cytotoxic release (destruction after taking particles)	complement components bacteria uric-acid crystals silicon-dioxide crystals	
destruction by membrane-active agents	leukocidin streptolysin O vitamin A antineutrophil antibodies	

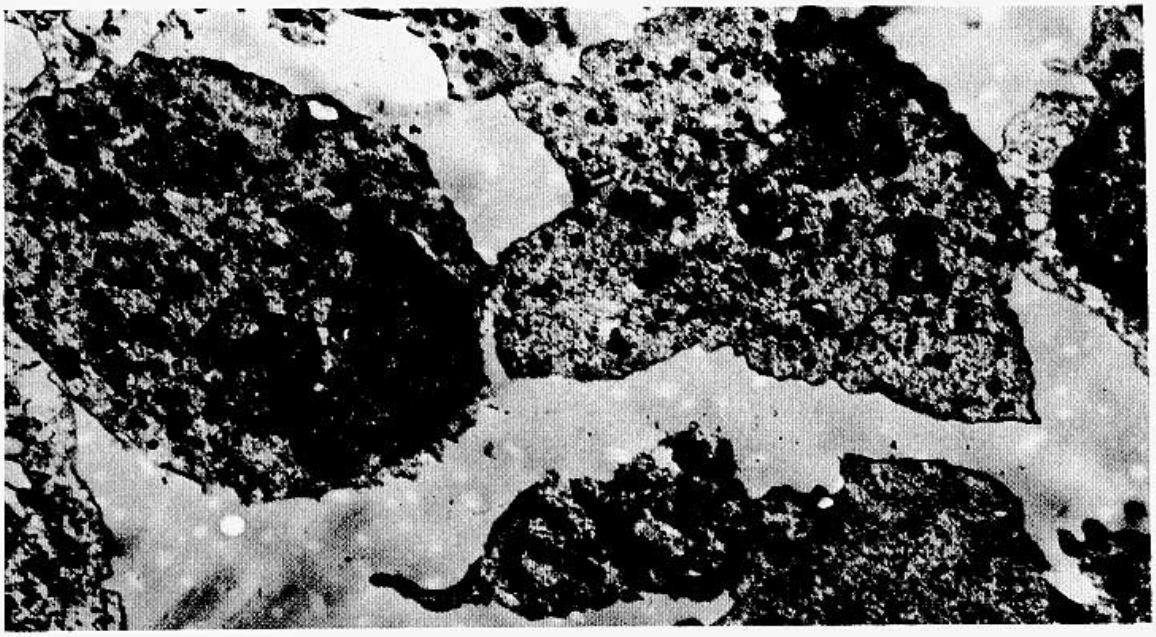

Fig. 45:
Coarse «granulafraction» in polynuclear cells (peritoneal exudate, magnif. 1:5000).

Fig. 46:
Abundance of *lysosomes* in a digestive cistern of a monocyte. Disintegration of a segment nuclear. Lysosomes compact, homogenous, circular (1:40,000).

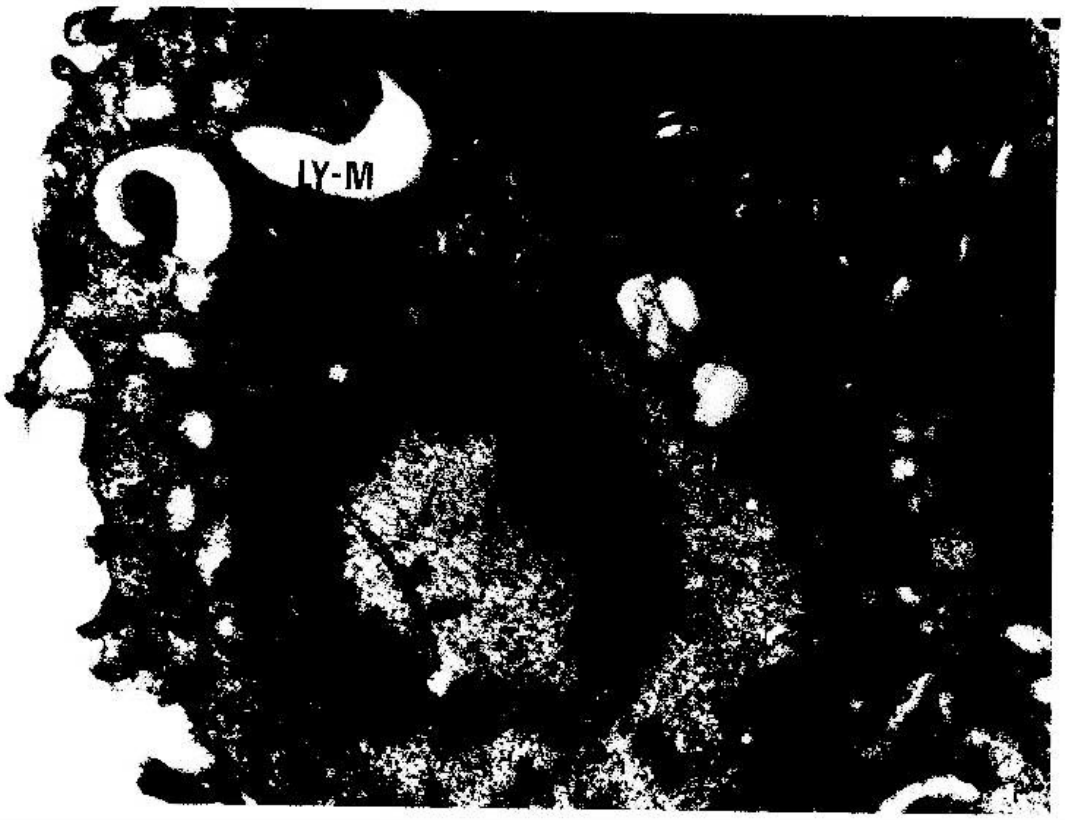

Fig. 47:
Beside the *digestive cistern* of a monocyte, 2 lysosomes and a somewhat larger structure like the «myelin-figures» Ly-M. 1:20,000.

lar digestive cisterns. The hydrolytic enzymes are released (fig. 44) so that the disintegration of the bacteria is quickly effected. The released substance (in question is probably not one but a plurality of substances) has sometimes been called phagocytin (HIRSCH J. G.; MILLER F.).

The ingestion of foreign material is said to involve an increased metabolism of glycolysis, which is believed to account for the acid medium and for the release of the enzymes from the leukocyte granules. The vitamins A, D and K soluble by lipoids reduce the stability of the lysosomes in vitro while hydrocortisone is believed to support this effect.

Heterophagous and autophagous «vacuoles» are sometimes referred to as secondary lysosomes because they form compact residual particles, often in the shape of so-called myelin figures (fig. 47).

Many viewpoints and experimental findings (SCHEIB D.; DEAN R. T.) suggest that the lysosomes play a great biological part in the development and transformation of the rapidly changing tissue formations in the embryonic and foetal life where tissue must be developed and dissolved quickly.

Biochemical data

Lysosomal membranes – according to cell biology 1978 – are permeable to

many monovalent salts at 0° C;
monosaccharides (incl. sedoheptulose);
most of the amino-acids and peptides (mol. weight $< \sim 200$);
neutral forms of weak bases (mol. weight > 500);
3 iodotyrosin (monoiodotyrosin);

impermeable to

many monovalent salts at 37° C;
disaccharides;
hexitol, hexonic and hexoronic acids;
most of the peptides (mol. weight $> \sim 200$;
various forms of weak bases.

The pH of the lysosomes measured so far in the external medium are about 7.0, those found in the lysosomes range from 4.5 to 6.6.

For the analysis of the non-enzymatic and enzymatic fractions of the lysosomes, the findings rely much on animal material.

The lysosomal enzymes are listed in table 2, their functional relations appear from fig. 44.

Regulatory factors

The effectiveness of lysosomal enzymes and substrates depends on their release. On the one hand, the cell must under resting conditions be protected from the influence of the lysosomal enzymes; on the other hand, endogenic substances foreign to the body or estranged must rely on the disintegration by the lysosomal enzymes. A survey of the known mechanisms of regulating and releasing in the leukocytes having polymorphous nuclei is shown in table 3.

Lysosomal and functional defects are of importance in many so-called storage diseases, especially in mucopolysaccharidosis and sphingolipidosis (see the chapter of lysosomal disorders).

Cytoskeleton

Following the discussions of the last two decades it is now accepted, that the cytoplasm of the most cells is a complex gel, structured by a variety of intracellular filaments, especially *microfilaments, intermediate filaments* and *microtubules* (KOCH, G. L. E., 1981). These units form the socalled *cytoskeleton,* responsible for cell-stability, movements, transport-processes and cell-division (mitosis). In a larger sense also the *Desmosomes* (fig. 1) for the connection between the cells, stability and elasticity of tissues belongs to the cytoskeleton. Whereas the microtubules show a radially orientation with the centriole as centre, the other filaments have a threedimensional architecture.

The present knowledge is limited to certain cell-types of single species. Therefore any categorization is fragmentary (DAVISON, P. F., 1981).

The interrelations between the microtubules-system and the centrioles are considered before (see chapter «Centrioles», pag. 34). In the class of 10 nm

intermediate filaments by the antiseratechnic are to distinguish the following basic elements:

Vimentin (fibroblast cytoskeletal protein);

Desmin or *Skeletin* (in muscles, skeleton);

Prekeratin (epidermal tonofilaments);

GFAP (glial fibrillary acidic protein).

GFAP (50 kD) of 50,000 Daltons weight, Vimentin (58 kD) and Desmin (56 kD) are homopolymeres, single proteins, which forms their corresponding filaments by association. The tonofilaments of epidermal cells seems to be heteropolymeres, consisting of a mixed population of protomeres (STEINERT, P. H., IDLER, W. W. and WANTZ, M. L., 1980). Components of the *neurofilaments* in vertebrates (fig. 273) are three proteins of 2109,155 and 70 kD, which comigrate with the slowest wave of axoplasmic flow (HOFFMAN, P. N. and LASEK, R. J., 1975).