The Institute of Biology's
Studies in Biology no. 84

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R. T. Dean

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R. T. Dean
M A Ph D
M R C Clinical Research Centre
Harrow Middlesex

Edward Arnold

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General Preface to the Series

It is no longer possible for one textbook to cover the whole field of Biology and to remain sufficiently up to date. At the same time teachers and students at school, college or university need to keep abreast of recent trends and know where the most significant developments are taking place.

To meet the need for this progressive approach the Institute of Biology has for some years sponsored this series of booklets dealing with subjects specially selected by a panel of editors. The enthusiastic acceptance of the series by teachers and students at school, college and university shows the usefulness of the books in providing a clear and up-to-date coverage of topics, particularly in areas of research and changing views.

Among features of the series are the attention given to methods, the inclusion of a selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the author or the Education Officer of the Institute.

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The Institute of Biology,
41 Queen's Gate,
London, SW7 5HU

Preface

Lysosomes are membrane-limited sacs present in most cells of animals and plants, which contain a diverse array of degradative enzymes. As was anticipated more than ten years ago by de Duve, the discoverer of the lysosome, they are important in the destructive phase of the continuous breakdown and resynthesis which most cellular material (such as proteins) undergoes. They are likewise concerned with the destruction of foreign material entering cells, and this constitutes a major element of the defence system of animals, for instance, against invading organisms. And in unicellular organisms, such as Protozoa, they serve as a main digestive system. More recently a wide range of less predictable functions of lysosomes (controlled incomplete degradative processes, intracellular transport and sequestration, and extracellular secretion) has become apparent. And because of the destructive capacity of lysosomal enzymes it is easy to appreciate that malfunction of the lysosomal system can have serious consequences.

This book attempts to provide a clear conceptual outline of the structure and function of lysosomes, and to indicate briefly their involvement in pathological disturbances: Chapters 1–5 describe the natural history of lysosomes, while Chapter 6 considers their functions, and mentions several examples of lysosomal pathology.

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R. T. D.

Glossary of Lysosomal Terminology

Autophagic Vacuoles: Membrane lined vacuoles containing morphologically recognizable cytoplasmic components. Comprise autolysosomes (which are secondary lysosomes, q.v.) and autophagosomes (which are vesicles sequestering cytoplasmic organelles which have not yet received lysosomal enzymes). Synonyms for autophagic vacuoles include cytolysome and cytosegresome.

Autophagy: The process of sequestration of intracellular components in vacuoles (including autophagic vacuoles, q.v.) which become lysosomes.

Crinophagy: A specialized form of autophagy in which secretory vesicles, normally carrying a cellular product to the exterior of the cell, instead fuse with lysosomes and are degraded.

Endocytosis: Internalization of formerly extracellular material, within a membrane-bound vesicle formed by invagination of the plasma membrance.

Exocytosis: Release of vesicular contents to the extracellular medium, by fusion of the vesicle membrane with the plasma membrane.

Heterolysosomes: Secondary lysosomes (q.v.) containing substrates derived from outside the cell by endocytosis (see Chapter 4), formed by fusion of primary lysosomes with heterophagosomes carrying the substrates.

Multivesicular body: Autophagic vacuole lined by a single membrane and containing inner vesicles resembling Golgi vesicles. They contain lysosomal enzymes, and so are a form of lysosome. They are formed by invagination of the external membrane, which then buds inwards to form an autophagic vacuole containing lysosomal membrane and some cytoplasmic material (see Fig. 5–1).

Phagocytosis: A form of endocytosis in which particulate material is taken up into large vesicles.

Pinocytosis: Endocytosis of soluble materials into small vesicles.

Primary Lysosomes: Lysosomes containing active acid hydrolases, which have not yet undergone fusion with other vesicles to bring them into contact with substrates.

Residual Bodies: Secondary lysosomes (q.v.) containing undigested residues (membrane fragments and whorls).

Secondary Lysosomes: The product of fusion of a primary lysosome with other intracellular vesicles containing substrates. Examples are heterolysosomes (q.v.) and autolysosomes.

Secretion: Release of cellular products into the extracellular space. Exocytosis is a specialized form.

Further Reading

Introductory Material

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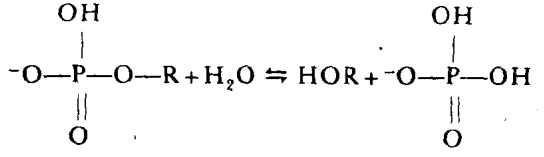
Contents

1 The Structure and Distribution of Lysosomes

1.1 The discovery of lysosomes

The biochemist tends to be interested in enzymes, proteins which function as biological catalysts. Usually one of his main problems is that his chosen enzyme is unstable, and rapidly ceases to be capable of catalysing its reaction when he tries to purify it, or even to study it in a tissue homogenate. Christian de Duve was faced with quite the opposite problem in the early nineteen fifties: when he let a homogenate of rat liver stand, his enzyme increased in apparent activity! The enzyme was acid phosphatase, which hydrolyses several organic phosphates, and works best under acid conditions:



Undeterred, de Duve showed that this slow activation could be produced much more rapidly by various physical tricks, such as freezing and thawing or adding detergents, and that it was accompanied by a transfer of the enzyme from a state in which it could be centrifuged into a precipitate ('sedimented') at about 20 000 g in 15 minutes, to one in which it was not even sedimented by centrifuging at 100 000 g for 1 hour. de Duve surmised that acid phosphatase was initially contained within a continuous membrane in a sedimentable particle. The enzyme activity in a homogenate is normally very low because the enzyme inside the particle cannot reach the substrate (organic phosphate) which is added to the solution outside the particle (the enzyme is said to be 'latent') but as the membrane breaks progressively on standing, or because of freezing and thawing, the enzyme gains access to the substrate, and activity is increased. Thus the latency is 'structure-linked', in that it depends on the intactness of the lysosomal membrane (Fig. 1–1). Having shown that the sedimentation characteristics of the particle were distinct from those of other known cellular entities (see section 1.2), de Duve proposed that they be called 'lysosomes', and that lysosomes are the sole location of acid hydrolases.

The term was logically chosen: lysosomal enzymes break up their substrates by means of water, i.e. hydro*lyse* them, and are contained

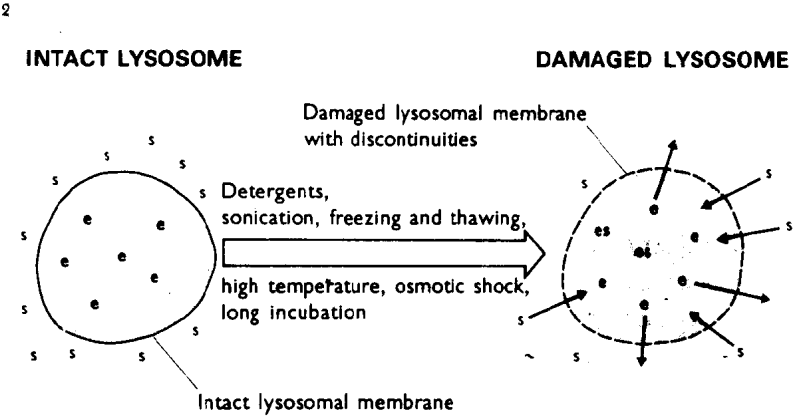


Fig. 1–1 Latency of lysosomal enzymes. In normal lysosomes the intact membrane prevents both access of substrates (s)—e.g. phosphate esters, nucleic acids, proteins, glycosides—to the interior of the lysosomes, and the exit of lysosomal enzymes (e) to the exterior of the lysosomes. Thus enzyme activity is hardly detectable until the membrane has been disrupted by one of the means indicated above.

within particles ('*somes*' from the Greek *soma*). Because of the potentially dangerous nature of lysosomal hydrolases, it is clearly desirable to compartment them, so that they do not have uncontrolled access to cellular materials. Indeed, when lysosomes within cells are broken specifically by experimental manipulation cell death ensues rapidly. However, lysosomes are far from being the 'suicide bags' as which they have been popularized in some quarters: they are 'behind bars' (de Duve), but in no pejorative sense! de Duve was awarded the Nobel Prize in 1974 in recognition of his discovery of lysosomes, and his subsequent work on these and other cellular structures. He gives a delightful description of the early development of the field in the first of the series of books on lysosomes edited by DINGLE and associates (see Further Reading).

1.2 Isolation

Although de Duve was able by 1960 to show that lysosomes were distinct particles, using centrifugation techniques, and that several hydrolases most active under acid conditions besides acid phosphatase were lysosomal, he did not obtain completely pure lysosomes.

The general problems of isolating cellular organelles in an undamaged state, have been discussed in previous volumes (nos. 9 and 31). Some of the difficulties are: how to break cells without breaking the organelle of interest, how to maintain the organelles intact once they are removed from their normal cellular milieu, and how to separate the organelles from each other. By now we have a fair idea of how best to deal with the first two points, though no method could be described as ideal. As far as

lysosomes are concerned, the problem of how to completely separate the organelle from others remains serious.

Centrifugal techniques depend for success primarily on differences in size and/or density of the particles to be separated. Unfortunately lysosomes are similar in both these respects to mitochondria, of which there are rather more in most cells. The differential centrifugation scheme used by de Duve in 1955 in characterizing lysosomes is shown in Fig. 1–2, which indicates the similar behaviour of mitochondria and lysosomes.

However, certain functional characteristics of lysosomes can be used to modify their behaviour in centrifugation, and obtain their complete separation from mitochondria (and other structures). For instance, as discussed in Chapter 4, some substances can enter cells by the process of phagocytosis, in which they become encapsulated in a membrane-bounded vesicle which fuses with lysosomes. If the substance has a very low or very high density, its accumulation in lysosomes will alter their density sufficiently for it to be distinct from that of mitochondria. Thus if particles are centrifuged to equilibrium on density-gradients (usually of sucrose) mitochondria and modified lysosomes can be separated, and pure lysosomes obtained (Figs. 1–3 and 1–4). Alternatively, there are now means of altering the density of mitochondria to the same end. Lysosomes show such physical and chemical diversity (e.g. Figs. 1–6 and 1–7) that any purified lysosomal fraction is unlikely to be fully representative of the lysosomal system. Some of the enzymatic criteria which, in conjunction with morphological examination, are used to assess the purity of such preparations, are illustrated in Fig. 1–5, which describes a particular preparation of rat liver lysosomes.

It is now clear that most acid hydrolases are almost entirely restricted to lysosomes, as de Duve anticipated. β-Glucuronidase is a notable exception, showing substantial activity in the endoplasmic reticulum of most cell types. Other lysosomal enzymes do show low, but detectable activity in endoplasmic reticulum (section 5), but this may be merely due to enzyme in transit to lysosomes after synthesis on rough endoplasmic reticulum (see section 3.1).

1.3 Morphology

In electron micrographs of tissue sections there can be seen numerous heterogeneous cytoplasmic vacuoles with single membranes, which are clearly distinct from mitrochondria, and from those small vacuoles with crystalline inclusions (peroxisomes). Many are lysosomes, but they can only be conclusively identified as such by means of enzyme staining (see Figs. 1–6 and 1–7 for examples of such sections).

Acid phosphatase was originally most often used, but now the activity of several other acid hydrolases can be demonstrated at both the light and electron-microscope levels. For several enzymes, it is difficult to obtain

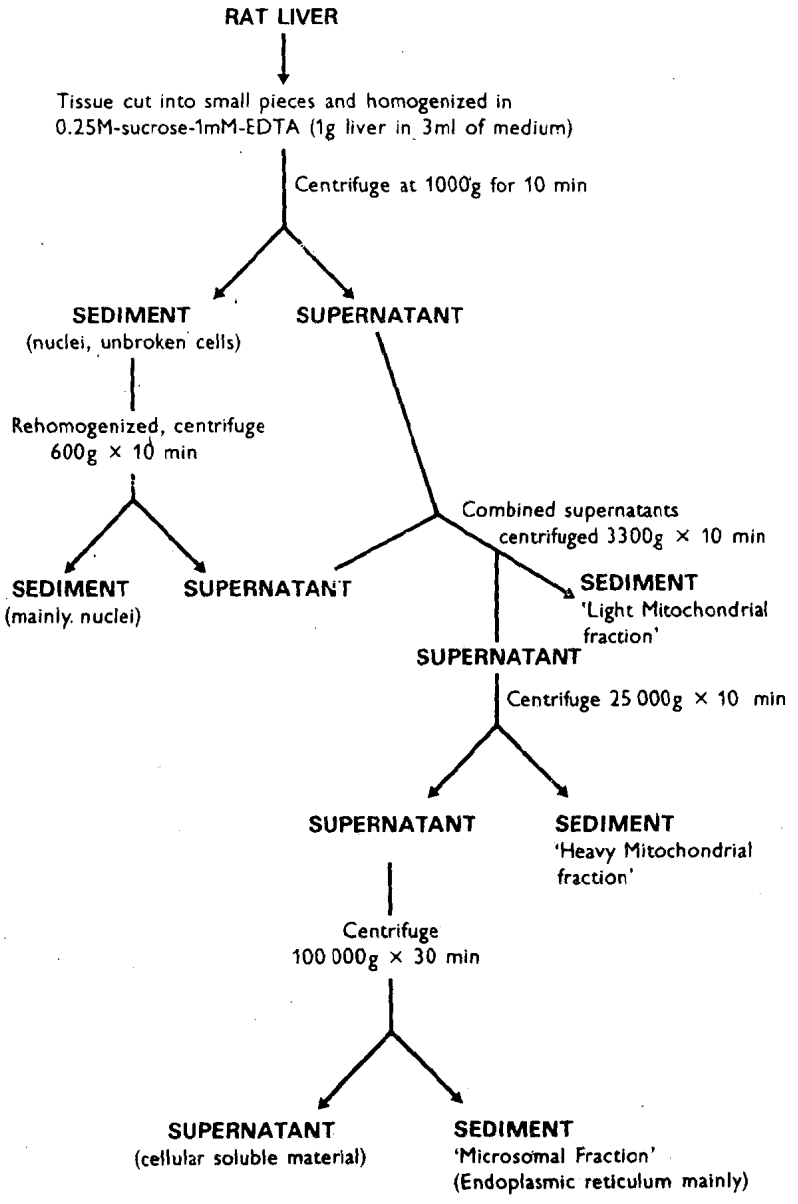


Fig. 1–2 Partial purification of subcellular components from rat liver by differential centrifugation. 0.25 M-Sucrose provides osmotic protection (see text), while EDTA (ethylene diamine tetra-acetic acid) as its sodium salt, sequesters trace metals which inhibit or destabilize some enzymes. All operations are conducted at 4°C. As the names 'Light' and 'Heavy Mitochondrial Fraction' imply, two fractions are obtained which consist largely of mitochondria. The first of these contains a higher proportion of lysosomes, and constitutes a partially purified lysosomal fraction. Such a fraction is often used for further purification.

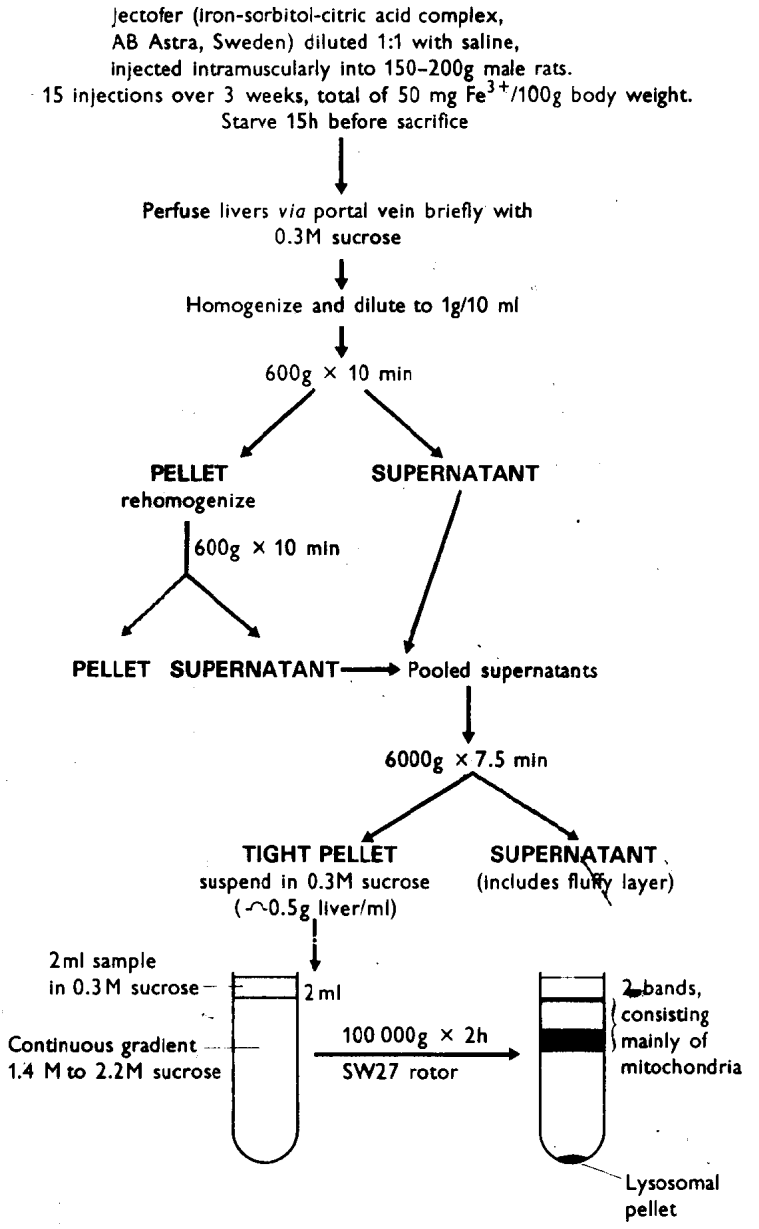


Fig. 1–3 Isolation of rat liver lysosomes loaded with colloidal iron (after ARBORGH *et al.* (1973). *FEBS Lett.*, **32**, 190–194).

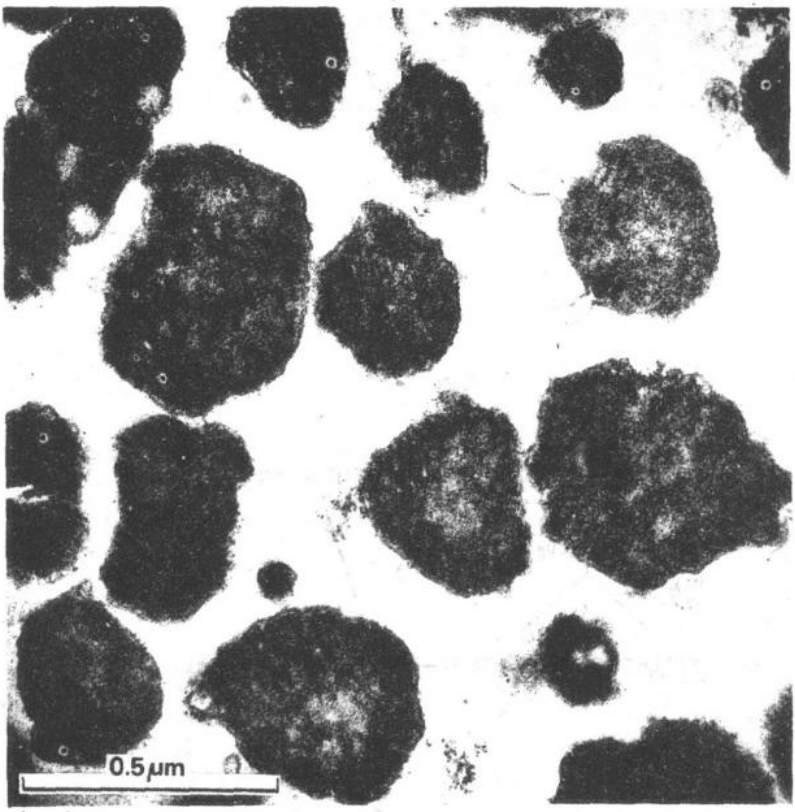


Fig. 1–4 Purified iron-loaded lysosomes. The lysosomes show diverse shapes and highly irregular membrane contours. No contaminating organelles, such as mitochondria, are apparent. Electron micrograph of Dr H. Glaumann, reproduced with his kind permission.

specific substrates with which to demonstrate the localization of the enzyme unambiguously, because other enzymes also transform the substrates. Therefore 'immunological' techniques for localization of lysosomal enzymes are becoming increasingly important. These techniques rely upon the fact that when foreign molecules are injected into higher animals, such as man, the animal synthesizes molecules (called antibodies) which circulate in the blood, and are capable of reacting specifically with the injected molecule and not with other molecules. They constitute a normal defence mechanism of animals against foreign materials and organisms (such as bacteria). This so-called 'immune response' is exploited medically to give protection against diseases (by means of vaccinations) and is also exploited scientifically. If a

Fig. 1–5 Characteristics of purified iron loaded lysosomes from rat liver (data from ARBORGH *et al.* (1973) *FEBS Lett.*, 32, 190–194).

Component (organelle represented)	*Recovery in whole procedure* %	*Yield in lysosomal fraction* %	*Relative specific activity in the lysosomal fraction*	*Likely contributions to protein of lysosomal fractions* %
Protein	100.5	.34	1	
Acid phosphatase (lysosomes)	103.0	11	33	
Aryl sulphatase (lysosomes)	112.0	12	33	
Cathepsin D (lysosomes)	107.0	13	35	
NADPH-cytochrome C reductase (microsomes)	N.G.	.007	.022	0.5
Succinate-Cytochrome C reductase (mitochondria)	N.G.	.033	.091	1.8
D-Amino acid oxidase (peroxisomes)	N.G.	.23	.615	1.5
Summed contaminants				⩾3.8

Important biochemical criteria of purifications are as follows: the summed recoveries of enzymes and protein in the whole procedure should be determined, and should be between 90–110% if interpretation is to be meaningful. Enzyme recoveries of greater than 100% often indicate the separation of enzyme from inhibitors; on the other hand, a low enzyme recovery usually indicates an unstable enzyme. Obviously the absence of an unstable mitochondrial enzyme from a lysosomal fraction is not satisfactory evidence for the absence of mitochondria. However, when stable enzymes which are characteristic of known organelles are shown to be absent, and yet recovered elsewhere, it can be claimed that the lysosomal preparation is free of contamination by the organelle concerned. Such information is usually presented as the enzyme relative specific activity (RSA) in the fractions: specific activity (SA) is activity per unit of protein, while RSA is the ratio of SA of the enzyme in the fraction to its SA in the homogenate. Thus in a lysosomal preparation, one requires lysosomal enzymes to show high RSA values (three are shown above) and enzymes from other organelles to show low RSA values (three are shown above). Such data can also be used to estimate the amount of contaminating protein each organelle could contribute. In this table contaminants seem only to account for about 4% of the protein of the lysosomal fraction, but there are two shortcomings to the data. Firstly, no 'marker' enzyme for the cell membrane was assessed, and so the degree of contamination by plasma membrane is unknown. Secondly the total recoveries of the three marker enzymes for non-lysosomal structures were not given (N.G.).

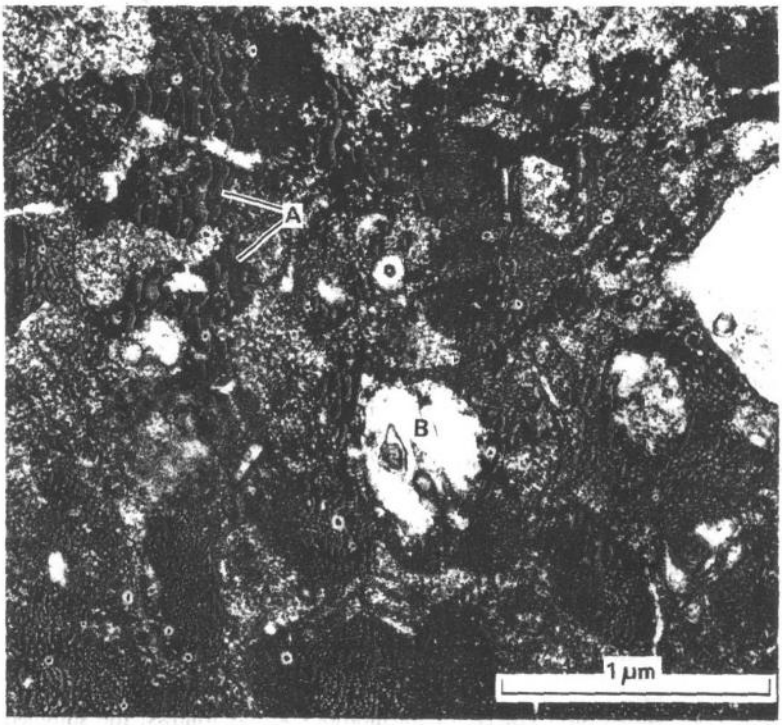


Fig. 1–6 Autophagy and the morphological heterogeneity of lysosomes. This section of a histiocyte from the subcutaneous region of a mouse injected with peroxidase (given subcutaneously) has been stained for acid phosphatase. A lysosome apparently 'wrapping' round some cytoplasm (A), contains enzyme activity through the matrix within the single membrane. The acid phosphatase of a second lysosome (B), perhaps the product of such a wrapping process, is found only between its pair of membranes, the inner of which will eventually be degraded, allowing the lysosomal enzymes to attack the entrapped substrates. Several other lysosomes are visible. By kind permission of Dr K. Ogawa.

single material is injected, then the antibodies will react only with that material. Thus antibodies specific for lysosomal enzymes can be obtained (with considerable effort!) and if coloured or electron absorbing materials are chemically linked to the antibodies, the combination can be used to localize the lysosomal enzyme either by light microscopy (with a coloured material) or electron microscopy (with an electron absorbing material). Fig. 1–8 shows the localization of the lysosomal proteinase (see section 1.4) cathepsin D in rabbit fibroblasts, demonstrated by light-microscopy using a sensitive modification of this method.

Other characteristics often used to identify lysosomes, for instance

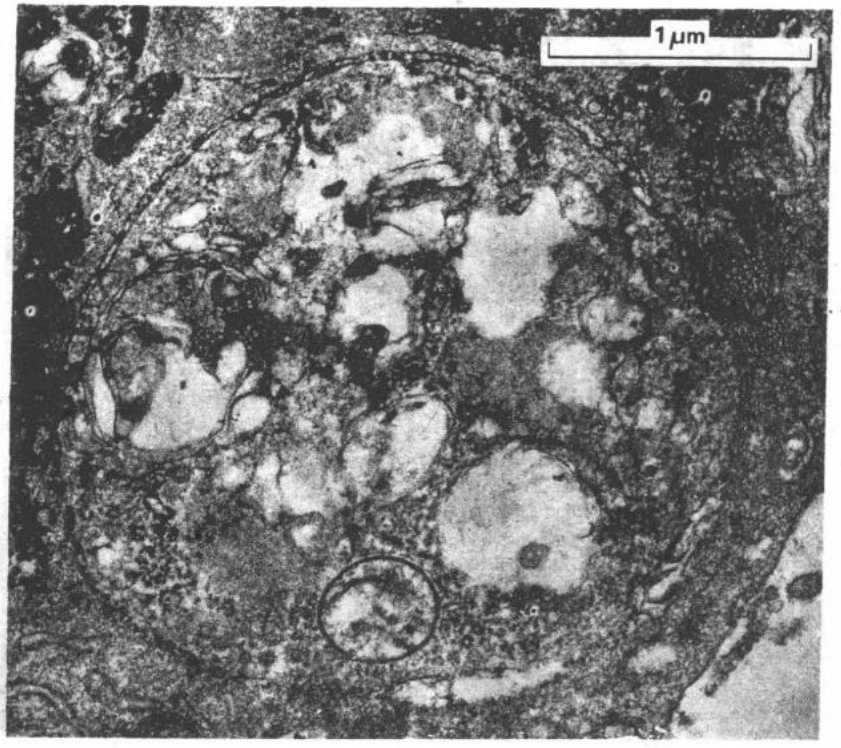


Fig. 1–7 An autophagic vacuole with two membranes and acid phosphatase only between the membranes. A wide range of cellular membrane fragments, about to undergo digestion, are present within the lysosomal membranes. From a section of a mouse liver histiocyte, animal treated as for Fig. 1–6, and the plate kindly provided by Dr K. Ogawa.

staining by heavy metal ions (for electron microscopy) and with fluorescent dyes such as acridine orange (for fluorescence microscopy), are not always satisfactory as other organelles may stain.

Although lysosomes vary greatly in size and shape (Figs. 1–6 and 1–7) there are some common morphological features: the membrane (and that of organelles with which lysosomes interact by fusion (section 3.4) such as plasma membranes) is thicker than that of other organelles, and immediately within it there is usually an electron-lucent area. Several morphological variants are described in later sections; the transformations, following phagocytosis, for instance, can be used to introduce identifiable materials into lysosomes (thorotrast (thorium dioxide) or colloidal gold particles, or the enzyme horse-radish peroxidase).

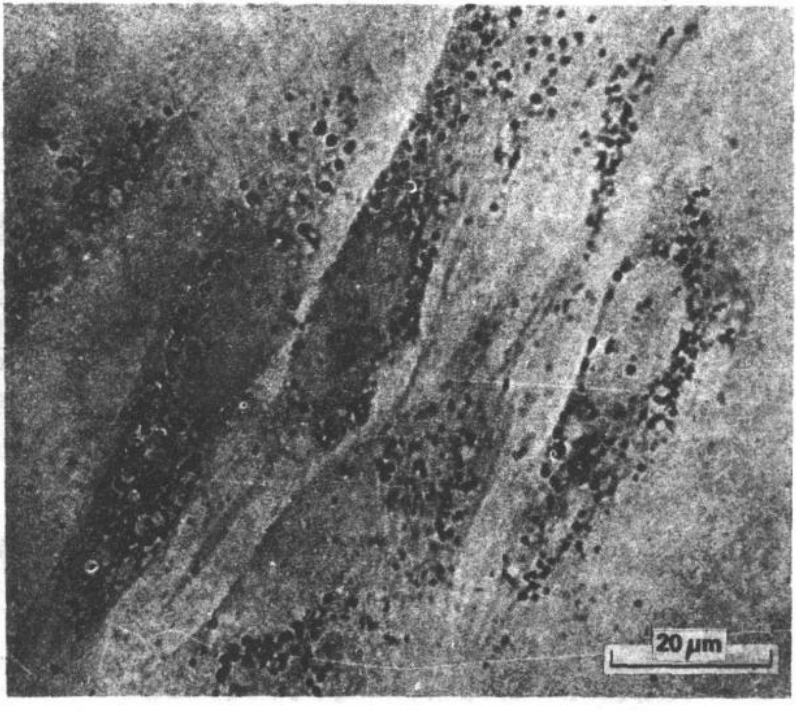


Fig. 1–8 Localization of cathepsin D in rabbit fibroblasts. The sites of the enzyme were revealed by means of a double-antibody technique, in which the fixed cells were incubated first with a sheep antibody preparation from a specific antiserum to rabbit cathepsin D, and secondly with a pig antibody conjugated with horseradish peroxidase, in which the antibody was obtained from an antiserum to sheep IgG. After washing, the cells were reacted for peroxidase and examined by bright-field microscopy. Particulate staining is observed, indicative of a lysosomal distribution of cathepsin D. n, nucleus. Photograph kindly provided by Dr A. R. Poole.

1.4 Composition

Although it is clear that the lysosomes of some cell types contain some specialized enzymes, most animal cells possess a common but diverse array of lysosomal enzymes, almost all of which are hydrolases. About 60 enzymes are known to be present in the lysosomes of one or more cell types: there are several proteinases, glycosidases, nucleases, phospholipases, phosphatases and sulphatases. These classes of lysosomal hydrolases are characterized in Fig. 1–9. The pH optima of the enzyme activities are normally in the acid range, although some enzymes active at neutral pH are known.