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Lysosomes of Root Tip Cells in Corn Seedlings

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Summary. Nine acid hydrolases are present in lysosomes which are found in the mitochondrial fraction of a cell-free extract prepared from root tips of corn seedlings.

Light and heavy lysosomes can be distinguished. The latter are sedimentable in a sucrose-medium, the former only in sorbitol-medium. The fraction of heavy lysosomes is in turn composed of at least three populations of lysosomes differing in density and enzyme content.

Light lysosomes are membrane-bound particles with diameters from 0.3 to 1.5 μ. Electron micrographs of frozen-etched tissue and isolated particles provide evidence that light lysosomes are identical with small vacuoles. This type of lysosome is characterized by presence of transaminases in addition to that of hydrolases. Heavy lysosomes are small spheres (diameters from 0.1—0.3 μ) with membranes resembling those of vacuoles and of the endoplasmic reticulum. These lysosomes are characterized by high specific activities of two oxydoreductases known to occur also in the membranes of the reticulum.

The different types of particles are thought to represent stages of the development of the lysosomal apparatus; according to this hypothesis the large vacuole of parenchymatous cells represents the end product of this process.

I. Introduction

Vacuoles of meristematic root cells (MATILE, 1966) and of yeast cells (MATILE and WIEMKEN, 1967) contain a number of acid hydrolytic enzymes which characterize these vacuoles as lysosomes.

The relative uniformity of yeast cells has made it possible to isolate vacuoles from suitably lyzed protoplasts in an efficient manner. In contrast the diversity of the size of vacuoles in meristematic cells of higher plants renders the isolation of these organelles an experimentally difficult task. One possibility of preparing vacuoles from root-tip cells involves chopping plasmolized tissue and subsequent isolation of the liberated vacuoles by flotation (MATILE, 1966). However this method is inefficient and yields preparations of unsatisfactory purity for two reasons. Firstly the degree of vacuolation varies greatly among the cells of root tips; consequently chopping destroys the larger vacuoles and only small vacuoles are released. Secondly, the isolation of vacuoles by flotation is inefficient because pure suspensions of vacuoles can only be obtained if the conditions of centrifugation are such that no lipid granules occur among the flotated particles. The procedure therefore results in

isolation of only a fraction of relatively large vacuoles which move rapidly in the centrifugal field.

The experiments described in this report are based on the assumption that meristematic vacuoles of dimensions comparable to, or smaller than those of mitochondria and proplastids should be present in a conventional homogenate obtained upon grinding of the tissue in the presence of sand. The concept of fractionation of such extracts has been designed taking into consideration properties of vacuoles which had emerged from a previous study (MATILE, 1966). Biochemical tests combined with the morphological analysis of isolated particles using the freeze-etching technique, the high efficiency of homogenization, and a fractionation which includes both differential and density gradient centrifugation resulted in an isolation procedure on a scale large enough to study some of the most conspicuous features of meristematic vacuoles.

II. Material and Methods

1. Plant Material. Seedlings of corn (*Zea mays* L., variety Orla 266 F-1) were grown aseptically at 28° for 40 hours. Root tips, 5 mm long, were excised with a razor blade, submerged in ice-cold water, washed repeatedly and blotted on a filter paper.

2. Cell-free Extract. 5 g of root tips were ground in a mortar in the presence of 2.5 g of washed sand (grain size 0.1—0.8 mm) and 5 ml of a sorbitol medium (0.5 M sorbitol, 0.05 M Tris-HCl buffer pH 7.6, 1 mM EDTA); the resulting brei was diluted with 20 ml of sorbitol medium and the homogenate subjected to a low-speed centrifugation (10 min, 500× g). The turbid, yellowish, cell-free extract (supernatant) was subsequently subjected to the fractionation procedures described below.

3. Fractionation Procedures. A differential centrifugation at 20,000× g (15 min) and 150,000×g (30 min) yielded a mitochondrial, a microsomal and a soluble fraction. Flotating lipid was removed at the end of the first centrifugation. The mitochondrial fraction, consisting of a yellowish pellet with some starch at the bottom, was resuspended and subjected to a cleaning centrifugation (10 min, 500×g) which eliminated some of the aggregated starch. A second centrifugation at 20,000×g (15 min) yielded a pellet which was carefully separated from the starch still present at the bottom. After resuspension of the particles in sorbitol-medium (buffered with 0.01 M Tris-HCl at pH 7.6) the mitochondrial fraction was layered on linear density gradients of sucrose (buffered as the sorbitol-medium) ranging from 45—20% (w/v). Discontinuous gradients with steps of 40, 20 and 15% sucrose (w/v) were used for the separation of the lysosomes from mitochondria and other cell constituents. Gradients (4.0 ml) were loaded with suspension of particles (1.4 ml) and centrifuged for 2 hours in a Spinco SW 39 rotor at maximal speed. They were then divided into fractions of ca. 0.3 ml. In order to obtain large amounts of a certain class of particles the respective bands were collected from a number of gradients and the particles were sedimented in the presence of sorbitol-medium.

4. Biochemical Analysis. The estimation of hydrolytic enzymes was carried out as described previously (MATILE, 1966; SEMADENI, 1967). The activity of DNAse was assayed according to BURTON (1956). β-Amylase: the dinitrosalicylic acid reagent (SUMNER and HOWELL, 1935) was used for the estimation of the production of

reducing sugars from Toronto-starch at pH 5.0. The degradation of the starch by α-amylase at pH 7.0 was estimated by recording the decrease of absorbance at 570 nm of the starch-iodine complex. α-Glucosidase: the substrate *p*-nitrophenyl-glucoside was buffered with citrate-phosphate at pH 6.25. The activity of carboxy-peptidase was measured according to ZUBER (1964); CBO-leucyl-phenylalanine in citrate buffer pH 5.3 was the substrate. The estimation of protein in TCA precipitates was carried out according to LOWRY et al. (1951). Total lipid was assayed using the method of ZÖLLNER and KIRSCH (1962). Phosphorus (phospholipids, ATPase) was estimated according to FISKE and SUBBAROW (1925), reducing sugars using the reagent of NELSON (1944).

5. Morphological Aanalysis. Samples of isolated subcellular particles were sedimented in the presence of sorbitol-medium; the pellets were resuspended in a small volume of 30% glycerol and submitted to the freeze-etching procedure according to MOOR (1964). Fragments of tissue were prefixed in buffered glutaraldehyde; after 1 hour the fixative was replaced by 30% gylcerol. The impregnation of the fixed tissue was completed within 1 hour, and small pieces of tissue were subsequently frozen and etched.

III. Results

1. Sedimentability of Hydrolytic Enzymes. The sedimentability from cell-free extracts is one of the criteria frequently used to distinguish structurally bound and free enzymes. This criterium is obviously only valid if the relative density of a certain cell constituent allows sedimentation in the respective medium. According to previous observations (MATILE, 1966) free meristematic vacuoles are not sedimentable in the presence of 0.4 or 0.5 M sucrose; however, they sediment easily in media of 0.5 and even 0.75 M sorbitol or mannitol. In order to check the occurrence of vacuoles in the extract from root tips ground with sand the sedimentation of hydrolytic enzymes known to occur in vacuoles was measured both in the presence of 0.5 M sorbitol and 0.5 M sucrose. Aliquots of a cell-free extract prepared in 0.5 M sorbitol were supplemented with an equal volume of either 0.5 M sorbitol or 1.0 M sucrose. Table 1 shows the activities of several enzymes in the particulate and in the soluble fraction after high speed centrifugation (30 min at 150,000 × g). A much smaller pellet is formed if sucrose is added to the extract; compared with the material sedimented in the presence of sorbitol alone only about half as much protein is found in this sediment. The difference in protein concerns only certain enzymes; cytochrome oxidase and glucose-6-phosphate dehydrogenase, used as examples of mitochondrial and soluble proteins, were completely sedimentable and soluble, respectively, under either condition. In contrast the activities of hydrolases present in the pellet are much higher if the sedimentation is carried out in the presence of sorbitol alone. Another enzyme which has been reported to occur in preparations of isolated vacuoles (MATILE, 1966) as well as of mitochondria (SEMADENI, 1967), glutamate-oxalacetate transaminase, is also somewhat better sedimentable in the absence of sucrose.

Table 1. *Sedimentability of hydrolytic and other enzymes as dependent on the composition of the medium. Cell free extract prepared in 0.5 M sorbitol medium. Equal aliquots of extract supplemented with sucrose (final concentration 0.5 M) or sorbitol medium. Centrifugation: 30 min at 150,000 × g. Enzyme activities in arbitrary units*

Medium	0.5 M Sorbitol			0.5 M Sucrose 0.25 M Sorbitol		
	sedimented	soluble	% sedimented	sedimented	soluble	% sedimented
Protein mg	0.95	1.28	42.6	0.53	1.84	22.4
Protease	57.6	98.5	36.9	38.4	122.0	24.0
DNAse	13.5	21.8	38.2	6.5	30.6	17.5
RNAse	12.8	34.4	27.2	5.9	36.7	13.8
Phosphatase	3.58	4.32	45.3	3.17	4.77	40.0
Glutamate-Oxalacetate-Transaminase	9.9	17.3	36.4	8.6	19.4	30.7
Cytochrome oxidase	14.9	0	100	15.3	0	100
Glucose-6-phosphate Dehydrogenase	0	4.61	0	0	4.76	0

These findings indicate the presence in cell-free extracts from root tips of structures which carry lysosomal enzymes and which are not sedimentable in the presence of sucrose. Another portion of lysosomes seems to be sedimentable in a sucrose medium.

2. Isolation of Lysosomes. As shown by the fractionation of cell-free extracts by differential centrifugation the acid hydrolases are present in the mitochondrial and microsomal fractions as well as in the supernatant which contains the soluble protein. From the data listed in Table 2 it appears that the specific activities of these enzymes (based on the amount of protein present in the respective fraction) are relatively high in the mitochondrial fraction. In some cases (β-amylase, α-glucosidase and esterases) the highest activities occur in the soluble fraction. It is noteworthy that the microsomal fraction also contains appreciable activities of hydrolases. These enzymes must be contained in particles of this fraction since other enzymes known to be soluble are completely absent (glucose-6-phosphate dehydrogenase) or scarcely present in both the mitochondrial and the microsomal fraction (α-amylase).

Preliminary experiments have shown that most of the structurally bound hydrolases which are not sedimentable in sucrose occur in the mitochondrial fraction. Therefore, washed particles of this preparation

Table 2. *Specific activities of some acid hydrolases in subcellular fractions obtained by differential centrifugation of a cell-free extract. The amount of reaction product produced in 60 min of incubation at 37° is referred to 1 mg of protein of the respective enzyme preparation*

	Cell free extract	Mitochondrial fraction	Microsomal fraction	Soluble fraction
Protease μg Tyrosine-equivalents	34.8	95.5	52.5	36.6
DNAse μg DNA	907	1790	925	1720
RNAse μg RNA	455	696	437	625
Phosphatase μM P	4.71	13.6	5.2	11.1
β-Amylase μM Maltose	6.43	13.7	7.38	19.8
α-Glucosidase μM Glucose	0.359	0.529	0.417	0.592
p-Nitrophenylacetate Esterase, μM Acetic Acid	0.978	0.60	0.69	2.06
α-Naphtylacetate Esterase μM Acetic Acid	1.25	1.80	1.51	2.86
Glucose-6-phosphate Dehydrogenase ΔE_{340}/10 min	2.01	0	0	2.92
α-Amylase ΔE_{570}/10 min	3.88	0.50	1.31	4.74

were further fractionated using discontinuous gradients of sucrose. Because of the differential sedimentability of lysosomes in sorbitol and sucrose media a large fraction of particles carrying acid hydrolases, is trapped, upon contrifugation at the interface between sucrose and the overlayered sorbitol medium. Fig. 1 illustrates the distribution of several enzymes in a discontinuous gradient with steps of 40, 20 and 15% sucrose which had been overlayered with particles of the mitochondrial fraction suspended in sorbitol medium. It appears that at least three different types of lysosomes occur in this fraction. One type of particle is trapped at the interface between sorbitol and 15% sucrose. It is characterized by the presence of several hydrolytic enzymes (Fig. 1A). In addition, this fraction contains transaminases and very low activities of diaphorase and cytochrome *c* reductase (Fig. 1B). Two other types of lysosomes, trapped at the interfaces between 15 and 20%, and between 20 and 40% sucrose, respectively, contain the same hydrolases. The relative activities

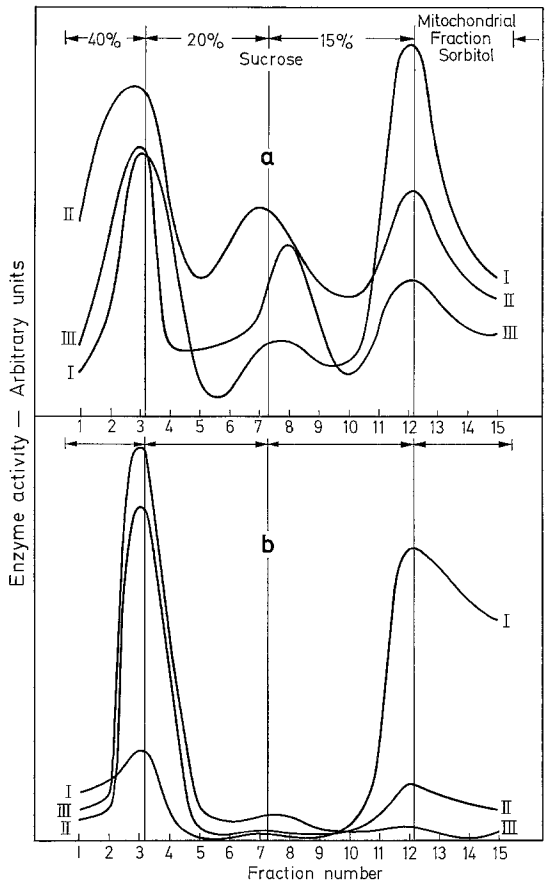

Fig. 1. Subfractionation of a mitochondrial preparation obtained upon differential centrifugation of a cell-free extract. A discontinuous gradient of sucrose with steps of 40%, 20% and 15%, loaded with particles of a mitochondrial fraction suspended in sorbitol medium, was centrifuged for 2 hrs. in a Spinco SW 39 rotor at 39,000 rpm. a. Distribution of acid hydrolases along the gradient. I: Phosphatase, II: protease, III: RNAse. b. Distribution of glutamate-oxalacetate transaminase (I), $NADH_2$-dichlorophenolindophenol diaphorase (II) and $NADH_2$-cytochrome *c* reductase (III)

of lysosomal enzymes found in the two types of particles are however remarkably different. The differences are even more pronounced if the relative activities of the enzymes of Fig. 1 B are compared: transaminase activity is present almost exclusively in the type of lysosomes trapped at 15% sucrose, whereas the bulk of the oxydoreductases occurs in the type trapped at 40% sucrose.

It should be noted that some hydrolytic activity is also present in the pellet which contains the mitochondria, with all of the cytochrome oxidase activity and

the mitochondrial transaminases. It is however very likely that this sedimentable activity is contained in particles aggregated with a dense material of unknown nature; if the pH of the media is raised from 7.6 to 10 the sedimented activity is greatly reduced. Since certain hydrolases (e.g. carboxypeptidase) are inactivated if exposed to an alkaline medium the cell fractionation has been carried out routinely at pH 7.6. However, these conditions have the shortcoming that exact quantitative results concerning the distribution of the various lysosomes in the cell-free extract cannot be obtained.

For reasons of experimental convenience the subsequent examination of lysosomes was carried out with two preliminary fractions referred to as to *light* and *heavy* lysosomes. Using discontinuous gradients with steps of 45 and 15% sucrose light lysosomes were collected from the interface between 15% sucrose and the loaded suspension and heavy lysosomes were trapped on 45% sucrose.

3. Constituents of Heavy and Light Lysosomes. A complete list of enzymes and substances found in heavy and light lysosomes is given in Table 3. These particles contain nine different acid hydrolases; they include enzymes attacking well defined natural substrates such as proteins, peptides, DNA, RNA and starch, as well as enzymes assayed by their action upon artifical substrates (phosphatase, esterases, α-glucosidase). With the exception of α-glucosidase, the specific activities, based on the amount of protein present in the respective fraction, are higher in the fraction of the light lysosomes than that of the heavy ones. If related to the activities in the cell-free extract the light lysosomes contain up to 12 times as much lytic activity per unit of protein (Table 3A).

The activity of five additional enzymes could be demonstrated in isolated lysosomes: transaminases, oxydoreductases and ATPase (Table 3B). The transaminases are mainly localized in the light lysosomes (see also Fig. 1B). In a previous study evidence for their localization in the membranes of meristematic vacuoles has been presented (MATILE, 1966); therefore it may be concluded that light lysosomes are identical with vacuoles. $NADH_2$-cytochrome *c* reductase and $NADH_2$-dichlorophenolindophenol diaphorase, oxydoreductases known to occur in the membranes of the endoplasmic reticulum, are present in preparations both of light and heavy lysosomes; their specific activity in the heavy lysosomes is remarkably high. Finally, an ATPase, not yet characterized in detail, occurs in preparations of either kind of lysosomes (Table 3B).

As previous studies with meristematic vacuoles have shown, the above enzymes, in contrast to the acid hydrolases, are localized in the membrane of the isolated particles. The existence of cytoplasmic membranes in preparations of lysosomes is further indicated by the relatively high content of phospholipid-phosphorus in these preparations. Per unit of protein the heavy lysosomes contain more phospholipid-P than the light particles. Both types of lysosomes are remarkably rich in total lipid; its amount equals roughly the amount of protein. RNA is also contained in both light and heavy lysosomes; per unit of protein its

Table 3. *Enzymes and chemical constituents present in isolated lysosomes. Light and heavy lysosomes were obtained from a mitochondrial fraction by centrifugation in a discontinuous sucrose gradient. A. Acid hydrolases; specific activities refer to 1 mg of protein. Incubation period: 60 min at 37°. B. Transaminases, oxydoreductases and ATPase; the activities refer to 1 mg of protein present in the respective preparation. ATPase was assaied at 37°, the other enzymes at 25°. C. Chemical constituents*

Table 3A

	Cell free extract	Light lysosomes	Heavy lysosomes
Protease μg Tyrosine-equivalents	34.8	197	116
Carboxypeptidase μM Phenylalanine	1.42	11.55	6.10
DNAse μg DNA	890	7130	4540
RNAse μg RNA	394	4650	1260
Phosphatase μM P	7.17	53.5	16.2
β-Amylase μM Maltose	6.72	49.5	23.7
α-Glucosidase μM Glucose	0.411	1.28	1.55
p-Nitrophenylacetate-Esterase μM Acetic Acid	0.978	2.97	1.79
α-Naphtylacetate-Esterase μM Acetic Acid	1.25	6.69	2.62

Table 3B

	Cell free extract	Light lysosomes	Heavy lysosomes	Mitochondria
Transaminases				
ΔE_{340}/10 min				
Glutamate-Oxalacetate	25.8	10.6	0.70	41.2
Glutamate-Pyruvate	23.8	19.0	0.88	27.4
Oxidoreductases				
$NADH_2$-Cytochrome *c* Reductase ΔE_{550}/10 min	38.0	26.4	302.5	60.5
$NADH_2$-Dichlorophenolindophenol Diaphorase, ΔE_{600}/10 min	28.4	14.1	54.4	34.5
ATPase, μM P/60 min	—	15.05	10.4	—

amount is almost three times as high in the former than in the latter lysosomes. Preparations of lysosomes finally contain reducing substances reacting with NELSON's (1944) reagent for reducing sugars. The nature of this material, which characterizes mainly the light lysosomes, has not yet been investigated (Table 3C).

Table 3C

	Light lysosomes	Heavy lysosomes
Protein μg/0.1 ml of suspension	38.2	39.6
Phospholipid-P μM P/mg Protein	1.08	1.60
Lipid mg Oleic acid-equivalents/mg Protein	0.922	1.171
RNA mg/mg Protein	0.283	0.108
Reducing substances μM Glucose-equivalents/mg Protein	8.67	4.08

4. Subfractionation of Heavy Lysosomes. Preparations of heavy lysosomes obtained by centrifugation of a mitochondrial fraction in a discontinuous gradient with steps of 45 and 15% sucrose were further analized in continuous gradients ranging from 45 to 20% (w/v) sucrose. In order to overlay such suspensions it was necessary to lower their density to an appropriate degree by addition of sorbitol medium. Upon centrifugation for 2 hours in a Spinco SW 39 rotor at maximal speed a distinct band near the top of the gradient is formed; the rest of the particulate material was distributed in a more or less diffuse manner, with a center of turbidity appearing in the region corresponding to about 30% sucrose. The distribution of lysosomal enzymes along the gradient is illustrated in Fig. 2. Evidently three populations of particles which contain these enzymes can be distinguished. The particles which equilibrate near the top of the sucrose gradient (Fig. 2, A and B; fraction 11) correspond to the material obtained at the interface between 20 and 15% sucrose in discontinuous gradients. The existence of two further types of lysosomes is indicated by peaks or shoulders of hydrolytic activities in fractions 5 and 8. Again it appears that the three types of lysosomes differ with respect to the relative activities of hydrolases; thus the lightest particles (fraction 11) contain a relatively high activity of RNAse but only a low activity of α-glucosidase whereas the material of fraction 8 is characterized by high activities of β-amylase and α-glucosidase.

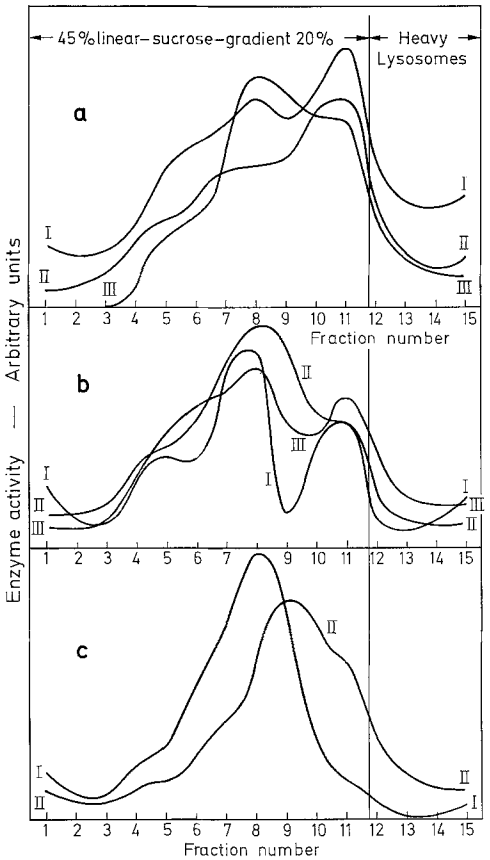

Fig. 2. Subfractionation of heavy lysosomes obtained upon fractionation of a mitochondrial preparation using a discontinuous sucrose gradient with steps of 45% and 15%. A linear density gradient ranging from 45—20% sucrose, loaded with a preparation of heavy lysosomes, was centrifuged for 2 hrs. in a Spinco SW 39 rotor at 39,000 rpm. a. Distribution of the acid hydrolases RNAse (I), protease (II) and DNAse (III) along the gradient. b. Distribution of the acid hydrolases β-amylase (I), α-glucosidase (II) and phosphatase (III). c. Distribution of $NADH_2$-cytochrome *c* reductase (I) and $NADH_2$-dichlorophenolindophenol diaphorase (II)

The distribution of oxydoreductases present in preparations of heavy lysosomes is somewhat puzzling. Apparently the lightest particles are low in both cytochrome reductase and diaphorase. The bulk of these enzymes seems to be contained in the lysosomes of fraction 8; however the distribution curve of the diaphorase suggests that an additional type of particles is present, equilibrating between fractions 8 and 11.

In conclusion the lysosomes present in a mitochondrial fraction are heterogenous concerning their relative density with respect to sucrose as well as in their equipment of acid hydrolases, oxydoreductases and transaminases.

5. Morphological Analysis of Lysosomes. Replica of frozen-etched root tips show cells at different stages of vacuolation. This process seems to be very complicated; it will be described in a separate publication (MATILE and MOOR, in preparation).

Cells from the region of beginning vacuolation are characterized by a large number of small vacuoles (Fig. 3a). The identification of these structures in freeze-etchings requires information concerning the fine structure of the vacuolar membrane. This information must be derived from etchings in which the cross-fractured content of the vacuole (large ice crystals) and a view on either the inner or the outer surface of the membrane are visible in the same, individual organelle (Fig. 3b and c). The inner membrane appears to be sculptured with globules which cover a majority of its surface; small areas which are free of particles are completely smooth. The outer membrane visible on the etching in Fig. 3b is also sculptured with globular particles which often occur in clusters.

Isolated light lysosomes in freeze-etchings appear as spheres with diameters ranging from ca. 0.3—1.5 μ (Fig. 3d). Heavy lysosomes corresponding to the particles of fraction 8 (see Fig. 2) are much smaller, (diameters ca. 0.1—0.3 μ). Particles corresponding to the fractions 5 and 11 closely resemble that of fraction 8 (Fig. 3f).

In isolated light lysosomes the inner surface of the membrane has a similar fine structure as the meristematic vacuoles (Fig. 3d, e). The outer surface carries giant globules which seem to form aggregates. The number of particles which on freeze-etchings sculpture the surfaces of the lysosomal membrane is different in individual organelles; this lack of uniformity may be due to the cutting of the frozen material which causes detachment of particles.

The fine structure of heavy lysosomes resembles closely that of light lysosomes. It should be noted that the membranes of the endoplasmic reticulum have the very same structure as the lysosomal membranes (MATILE and MOOR, in preparation). The above findings suggest that the lysosomes are identical with the tiny vacuoles of meristematic cells.

IV. Discussion

Evidence for the existence of lysosomes in seedlings of maize has been presented by MATILE et al. (1965) and SEMADENI (1966); these lysosomes

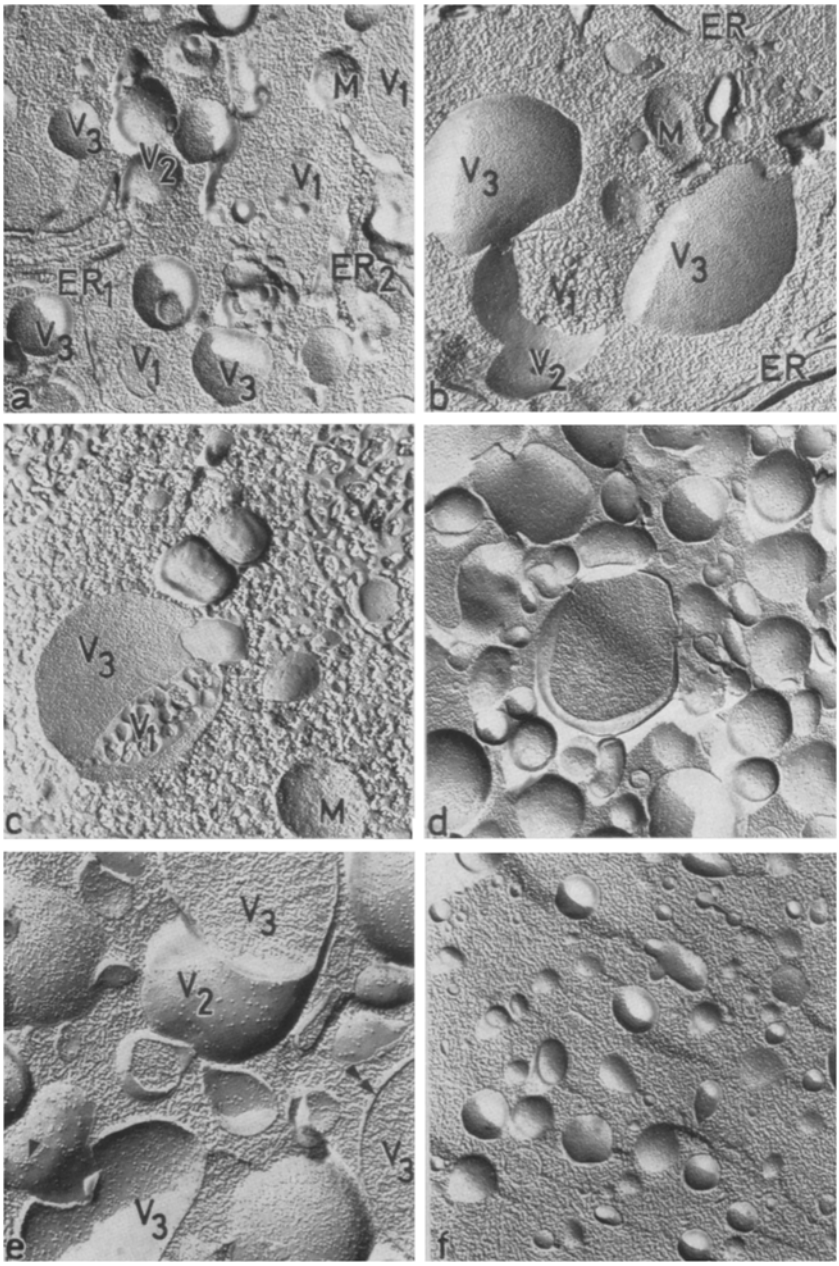

Fig. 3. Frozen-etched preparations of cells from root tips (distance from the tip approximately 2 mm) and of lysosomes isolated therefrom. a. View on the cross-

have been identified with spherosomes which are very numerous in certain tissues such as the coleorrhiza and the scutellum (FREY-WYSSLING et al., 1963). It has been supposed that the spherosomes correspond to the primary lysosomes (DE DUVE and WATTIAUX, 1966) of animal cells. In electron micrographs of meristematic cells of corn rootlets an organelle with the typical appearence of spherosomes is absent (GRIESHABER, unpublished results); therefore the lysosomes described in the present report represent a different class of particles than spherosomes.

The freeze-etchings of both meristematic root cells and of particles isolated from these cells suggest the identity of one type of lysosomes with small vacuoles. Although the size of these vacuoles corresponds, roughly to that of spherosomes there are some differences the most remarkable of which concerns the behaviour of isolated vacuoles in sucrose media. In contrast to spherosomes, the isolated vacuoles do not sediment in the presence of even relatively low concentrations of sucrose. Apparently, these light lysosomes are equipped with membranes which must be completely impermeable for sucrose molecules. Although they contain more RNA and less lipid per unit of protein they have a lower appearent density than heavy lysosomes which sediment readily in 0.4 M sucrose.

Other differences between meristematic vacuoles and spherosomes concern the presence of certain enzymes. Somes enzymes are either completely absent (transaminases) in spherosomes, or are present in very low amounts (DNAse, RNAse, β-amylase). In contrast these enzymes occur in isolated vacuoles together with other hydrolases which are found in spherosomes (protease, phosphatase, esterase). On the other hand spherosomes and vacuoles are similar in having a high amount of lipid and RNA, and in containing oxydoreductases; the latter characterize mainly the heavy lysosomes described in this report.

fractured cytoplasm of a cell containing many small vacuoles (V). Some of the vacuoles are crossfractured (V_1) others are fractured along the outer (V_2) or the inner surface (V_3) of the membrane. Endoplasmic reticulum is visible in a crossfractured situation (ER_1). Surface views of membranes of the reticulum are also marked (ER_2). M: mitochondrion. (17,300). b, c. Vacuoles fractured along the outer (V_2) or the inner surface (V_3) of the tonoplast. Large ice crystals characterize the crossfractured cell sap (V_1). On the inner surface of the vacuolar membrane small smooth aereas are surrounded by large aereas which are densely sculptured with globules. (b: 18,000; c: 26,300). d. View on a frozen-etched preparation of isolated light lysosomes (17,800). e. Isolated light lysosomes fractured along the outer (V_2) or the inner surface (V_3) of the membrane. The fine structure of both surfaces is characterized by adjacent globular particles. Particles of the outer surface frequently form clusters (arrows). The distribution of particles on the inner surface closely resembles that in vacuoles. The lysosomes are bounded by a single membrane (double arrow). (34,500). f. View on a frozen-etched preparation of isolated heavy lysosomes corresponding to particles of fraction 8 in Fig. 2. (14,100)

Acid-phosphatase-positive granules which have been observed repeatedly in meristematic root cells (GAHAN, 1965; AVERS and KING, 1960; JENSEN, 1956) are most probably identical with the tiny vacuoles described in the present study. The same enzyme has been demonstrated to occur in meristematic vacuoles of cells of the shoot apex (POUX, 1963).

At least four populations of lysosomes occur in a mitochondrial fraction prepared from a cell-free extract of root tips. They differ not only in their relative density in sucrose but also with respect to their content of enzymes. Transaminases occur almost exclusively in the particles which are not sedimentable in sucrose (light lysosomes); $NADH_2$-cytochrome *c* reductase and $NADH_2$-dichlorophenolindophenol diaphorase, oxydoreductases which characterize the membranes of the endoplasmic reticulum (ERNSTER et al., 1962), are mainly localized in the heavy lysosomes. Furthermore, the ratios of the specific activities of the hydrolytic enzymes present in heavy and light lysosomes are different.

The lysosomal enzymes not only occur in the mitochondrial fraction (which is the object of this study), but also in the microsomal and soluble fractions. From the localization of these enzymes in meristematic vacuoles it may be concluded that the large vacuoles of differentiating or fully expanded cells contain the same enzymes: undoubtedly the large vacuoles originate from the tiny vacuoles of meristematic cells (GUILLIERMOND et al., 1933). Upon grinding the tissue with sand the large vacuoles are destroyed and the liberated hydrolases are released and mixed with the enzymes of the ground cytoplasm (e.g. α-amylase, glucose-6-phosphate dehydrogenase) in the soluble fraction. This hypothesis is supported by the finding that the lysosomal enzymes localized in the vacuole of the yeast cell are completely soluble in extracts obtained upon the rupture of the cells in a glass-bead homogenizer (MATILE and WIEMKEN, 1967).

The functional significance of the lysosomes described in the present report may be visualized as follows. The different types of particles represent stages of differentiation of the lysosomal apparatus. From the point of view of comparative organellography (FREY-WYSSLING, 1965) the lysosomes seem to be homologous to the endoplasmic reticulum (presence of typical oxydoreductases, fine structure of membranes); heavy lysosomes may represent primary derivatives of the reticulum. These primary lysosomes are possibly particles analogous to the prospherosomes of other tissues (FREY-WYSSLING et al., 1963, MATILE et al., 1965; SEMADENI, 1967). It is likely that synthesis of lysosomal enzymes continues as the organelle develops and differentiates; its content of RNA (ribosomes ?) could be related to such a process. The occurrence of transaminases in the membranes of meristematic vacuoles (MATILE, 1966) and light lysosomes, respectively, may indicate that these structures have reached the functional status of a secondary lysosome. In fact, electron

micrographs of cross-fractured vacuoles of this type often show the presence of structured material within the vacuole. Intracellular digestion taking place in such autophagic vacuoles involves the action of lytic enzymes, the breakdown of macromolecules leading to the accumulation of various kinds of micromolecules. Functionally the transaminases may be involved in the transfer of amino groups from amino acids produced in the lysosome to acceptor molecules present in the cytoplasm. A similar significance in active transport may be attributed to the ATPase.

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