Biochemical and Cytological Changes Accompanying Growth and Differentiation in the Roots of *Zea mays**

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ABSTRACT

The apical meristem of the root affords an excellent material with which to study changes in cellular components accompanying growth and differentiation. The ontogeny of cytoplasmic particles can be followed, since the younger cells are constantly dividing and reforming new cytoplasm. Electron microscope pictures of these newly formed cells reveal a dense background of microsomal granules and small, thin walled vesicles of the endoplasmic reticulum. Two types of mitochondria are noted and, as the cells enlarge, mitochondria regarded as immature can no longer be seen, but only mitochondria with well developed cristae. The development of these cristae was found to be associated with an increase in respiration of the tissue as well as with increased rates of oxidation and phosphorylation of isolated mitochondria. As the cells grow and mature, the mitochondria make up an increasing percentage of the total cytoplasmic protein, and this increase probably accounts to a great extent for the increase in tissue respiration. Concomitantly, there is a decrease in microsomal granules.

All these changes have been verified by electron microscope pictures of cells *in situ*, chemical analysis of isolated particulates, and metabolic studies of tissue and isolated fractions.

INTRODUCTION

A number of studies have shown that the activity and composition of root cells is not constant, but progressively changes as the meristematic cell enlarges and differentiates. Respiration rates per unit of nitrogen or per cell increase from the meristematic region through the region of elongation, and then decline (1-5). The respiratory quotient is highest in the region of elongation and early differentiation (1, 2, 4, 6). The respiratory increase produced by 2,4-dinitrophenol is least in this same region (7). The rate of potassium accumulation per unit protein nitrogen increases with distance from the root tip through the elongation zone (8). The mature regions of the root are most active in salt transfer to the xylem (9, 10). The protein content of root cells increases during

expansive growth (1, 3, 11, 12). Ribonucleic (RNA) and desoxyribonucleic acid (DNA) per cell have been reported maximal in the region of elongation (13, 11). Jensen (14) reports DNA/RNA ratios of 1:1 in the meristem and 1:3 in the elongation zone.

It seemed of importance to know if these changing attributes accompanying growth and differentiation of the root cell were paralleled by changes in the cytoplasm of the cell, with particular reference to the mitochondria. The plan of investigation has been to confirm the change in rate of respiratory metabolism accompanying growth, and to refer these changes to the amount of mitochondria and their respiratory activity. By use of electron microscopy, visual evidence of cytoplasmic changes has been sought to substantiate that obtained by the techniques of manometry, differential centrifugation, and chemical analysis. The initial results are reported here;

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a more detailed study of cytoplasmic changes accompanying growth is in progress.

Materials and Methods

Single cross-hybrid seed corn (WF9 X M14, Crow's Hybrid Corn Company, Milford, Illinois) was germinated in the dark for 4 days (26–27°C.) on paper saturated with 10^{-4} M $CaCl_2$. This method produced vigorous seedlings with primary roots 15 to 20 cm. and lateral roots 10 to 15 cm. long. Primary roots were used except for studies of mitochondrial respiration, in which lateral roots were also used to obtain sufficient amounts of tissue.

Electron Microscopy.—Intact roots and sedimented particulates from root homogenates were fixed, using a modification of the method of Palade (15). Three-tenths per cent $MgCl_2 \cdot 6H_2O$ was added to the 1 per cent osmium in veronal buffer, pH 7.6. Fixation was carried out for 30 minutes at room temperature. Specimens were washed for 30 minutes at 0–2°C. in 1 per cent $MgCl_2 \cdot 6H_2O$ with 3 changes of solution. The first 3 dehydration steps were for 30 minutes, followed by 2 to 3 changes of the absolute ethanol at room temperature over a period of 1 hour. Specimens were infiltrated for 2 to 3 hours with monomer methacrylate (1 part methyl to 4 parts n-butyl) with 1 per cent benzoyl peroxide. The material was embedded in pre-polymerized methacrylate. Sections were cut on a Sjöstrand ultramicrotome at 150 to 250 A, and were viewed on a RCA model EMU 2e microscope with rear focal plane objective aperture.

Physiological and Biochemical Observations.—Roots were sectioned into four regions starting at the tip: 0 to 0.5 cm., 0.5 to 1.5 cm., 1.5 to 2.5 cm., and 2.5 to 3.5 cm. These relatively large sections of tissue were used in order to obtain an adequate amount of tissue for analysis, and to encompass sufficient root length to examine the cytoplasmic particulates from mature cells. Microscopic measurement of cells in fixed, stained, and mounted longisections of these corn roots shows that cell elongation in the cortex is completed at a distance about 1 cm. from the root cap. The second section, then, encompasses the last stages of cell elongation.

1. Respiration of Tissue and Mitochondria.—Standard manometric techniques were followed (16) at 29°C. Twenty or twenty-five root sections were used per vessel in tissue respiration studies. Mitochondria were isolated from root sections by grinding for 1 minute in a glass homogenizer with a teflon pestle with a solution of 0.6 M sucrose, $\frac{1}{15}$ M potassium phosphate at pH 6.8, and 5 X 10^{-3} M ethylene diamine tetra-acetic acid (EDTA). The homogenate was clarified by centrifugation at 500 g for 5 minutes, and the mitochondria sedimented from the supernate at 15,000 g for 15 minutes. The pellet was suspended in grinding solution and resedimented. The mitochondria were then suspended in 0.5 M sucrose for addition to the manometer

vessels. Vessel additives are listed in Table I. Phosphorylation was determined by the disappearance of orthophosphate using the method of Fiske and SubbaRow (17). Nitrogen content of mitochondria was determined by digestion and nesslerization.

2. Isolation and Analysis of Cytoplasmic Particulates.—Sections from about 400 roots were blotted, weighed, and ground in glass homogenizer. Grinding media varied and are discussed later. All procedures were carried out in ice baths or in refrigerated centrifuges set for 0°C. The homogenates were centrifuged at 500 g for 5 minutes, and the supernate filtered through glass wool. Aliquots of the filtrate were subjected to differential centrifugation in a Spinco model L ultracentrifuge, each successive particulate fraction being obtained from the supernate of the preceding. The centrifugal forces, time, and the arbitrary designation of the isolated fractions were as follows: heavy mitochondria, 5,000 g for 15 minutes; light mitochondria, 20,000 g for 35 minutes; and microsomes, 80,000 g for 2.5 hours. The terms "heavy" and "light" mitochondria are used to identify the fractions, and do not imply the existence of morphologically distinct classes of mitochondria. Centrifugations for analytical work were made in the 30.2 rotor, those for electron microscopy in both the 30.2 rotor (Figs. 8 to 11) and the SW 39L rotor (Figs. 12 to 23). For this initial study, it was believed preferable to eliminate the losses of material that accompany washing even at the expense of a somewhat greater particle heterogeneity.

The pellets were drained of supernatant solution, homogenized in cold 0.2 N $HClO_4$, and aliquots taken for protein determination by the method of Lowry *et al.* (18). The precipitate was then sedimented, resuspended in 0.2 N $HClO_4$, and resedimented. The combined supernates were made to volume, and the soluble nucleotides estimated by absorption at 260 to 290 mμ, using adenosinemonophosphate as a standard. The acid precipitate was next extracted twice with 3:1 ethanol ether, the combined extracts evaporated, the residue digested in hot H_2SO_4 and H_2O_2, and orthophosphate determined. The results are reported as phospholipide phosphate. Nucleic acid was estimated by incubating the residue remaining after lipide extraction with 0.3 N KOH at 40°C. overnight. Aliquots of the hydrolysate were acidified with an equal volume of 0.5 N $HClO_4$. Absorption at 260 to 290 mμ of the cleared supernate was referred to a standard curve obtained from similarly treated yeast RNA.

RESULTS

Electron Microscopy of Root Cells.—The cytoplasm of root cells of the apical meristem possesses at least five types of structural entities: (1) small dense granules 8 to 20 mμ in diameter, (2) numerous vesicles with low density membranes, (3) two

kinds of elongate membranous profiles, (4) vacuole-like structures with thick, dense membranes, and (5) two kinds of mitochondria.

Small dense granules (8 to 20 mμ) occur throughout the cytoplasm of meristematic and vacuolating cells (Figs. 1 to 5). The high density of these granules in the cytoplasm obscures any association with vesicular or lamellar structures. Regions do occur in the cells in which the concentration of granules is low, and these regions are occupied by numerous vesicles. The granules are sedimented as part of the microsome fraction of tissue homogenates (Fig. 18), and analysis shows them to possess high RNA content (Table IX). In this respect they appear analogous to the ribonucleoprotein particles found in liver (19) and peas (20). During growth and maturation the number of these granules decreases (Figs. 5 and 6).

The vesicular elements of the cytoplasm are small (20 to 250 mμ) in the apical regions of the root (Figs. 1 to 4), but exhibit a greater range of profile dimensions (20 to 1000 mμ) in mature cells (Fig. 6). Round, ellipsoidal, and irregular profiles are to be found. These structures are similar, if not identical to structures described in animal cells (21) and in wheat root tips (22) as components of the endoplasmic reticulum. In some areas (Fig. 3) there is a high population of vesicles, some of which present flattened profiles which can be equated with cisternae (21, 23, 24).

Possibly related to these vesicular bodies in comprising part of the endoplasmic reticulum are two kinds of elongate profiles (Figs. 2, 3, and 5). The first structure occurs singly or in loose parallel association (Fig. 3). These profiles appear as a chain-like series of vesicles about 20 mμ in diameter coalesced to form an organelle similar to the cytoplasmic lamellae found in *Nitella* (24). However, the habit is reminiscent in some respects of the rough surfaced membranes of the endoplasmic reticulum of animal tissues (19, 21). This type of cytoplasmic lamellae has not been observed in mature root cells.

The second type of elongate profile occurs in a multilamellar structure and is confined to meristematic cells (Figs. 2, 3). The constituent lamellae are about 15 mμ thick separated by an interval of 7 to 10 mμ. Superficially, these multilaminated organelles resemble dictyosomes of animal cells (25).

In meristematic and elongating regions there are a number of vacuole-like bodies with very

Respiration Rates of Root Sections

Respiration rates determined in 1 per cent sucrose and 0.001 M potassium phosphate, pH 6.5, for 30 minutes. Protein estimated by the method of Lowry *et al.* (18). Total protein estimated on cold 0.2 N perchloric acid precipitate of homogenates prepared in 0.6 M sucrose, 1/15 M potassium phosphate, pH 6.8, and 0.005 M EDTA, and cleared by centrifugation at 500 g for 5 minutes. Mitochondria sedimented at 20,000 g for 20 minutes from cleared homogenate.

Section No.	Distance from tip	Total protein (mg./ gm. fresh weight)	Mitochondrial protein (mg./ gm. fresh weight)	μl. O_2/hr.		
				Per gm. fresh weight	Per mg. total protein	Per mg. mitochondrial protein
	cm.					
1	0.0–0.5	24.1	5.0	1282	53	265
2	0.5–1.5	4.0	1.3	497	124	380
3	1.5–2.5	3.7	1.3	400	108	300
4	2.5–3.5	2.9	1.3	403	104	308

thick membranes (Figs. 3 and 4). The nature of these structures has not been determined.

Two kinds of mitochondria which probably represent two stages of development are present in meristematic cells (Figs. 1 to 4). The apparently mature mitochondria have a heterogeneous internal structure in which cristae can be recognized. In meristematic cells these mitochondria possess, in addition to cristae, a dense granular matrix which disappears in the mitochondria of elongating and maturing cells, leaving void areas (Figs. 5 and 6). The cristae are frequently seen to better advantage in isolated mitochondria (Fig. 12). In such preparations connections between cristae and the inner mitochondrial membrane are evident.

The second type of mitochondrion has been identified only in the meristematic regions. This type is characterized by a dense membrane surrounding a nearly homogenous matrix (Figs. 1 to 4) and exhibits a progressive development of internal vesicular structure, interpreted as cristae. It is the genesis of cristae that leads us to interpret these organelles as mitochondria (see Discussion). Occasionally, mitochondria of this intermediate type exhibit elongate profiles constricted in the middle, suggesting that they are undergoing fission (Fig. 4).

Physiological and Biochemical Observations.—

TABLE II

Respiration Rates of Mitochondria Isolated from Roots

Mitochondria isolated in 0.6 M sucrose, 1/15 M potassium phosphate, and 0.005 M EDTA, as described in Methods. Vessel contents were 25 micromoles K_2HPO_4, 40 micromoles α-ketoglutarate, 1.5 mg. adenosinetriphosphate, 0.33 mg. diphosphopyridine nucleotide, 0.5 mg. thiamine pyrophosphate, 0.1 mg. cytochrome *c*, 0.1 mg. coenzyme A, 2.5 micromoles $MgSO_4$, 110 micromoles glucose, 1.5 mg. hexokinase, and 0.5 ml. of mitochondrial preparation. Final sucrose concentration 0.16 M, total volume 2.5 ml., final pH 6.5, temperature 29°C. Respiration and orthophosphate disappearance measured over 30 minute period.

Section No.	Q_{O_2} (N)	PO_4 esterified per mg. N per hr.	P/O
		μM	
1	531	102	2.16
2	675	123	2.04
3	422	72	1.90
4	386	57	1.66

The electron micrographs of root cells give clear evidence of major changes in the morphology of mitochondria concomitant with growth and maturation. Studies of respiration rates of root sections were carried out to determine if any correlation could be found between these morphological changes and the capacity of the mitochondria to respire.

In agreement with previous observations (see Introduction), the respiration rates of root tissue on a protein basis increases to a maximum in a region where the cells have just achieved maximum elongation (Table I). A portion of the increase appears to be due to the fact that mitochondrial protein per unit of total protein has increased. In addition, however, part of the respiratory increase per unit protein in the second section appears to be due to a greater respiratory efficiency per unit of mitochondrial protein. This efficiency declines in the mature cells.

The suggestion that mitochondria from the elongating region of the root are more efficient was investigated further by determining the oxidation and phosphorylation of isolated mitochondria. The mitochondria from the second section proved to be more efficient in oxidation (Table II). There is a rather sharp decline in the oxidative efficiency of mitochondria isolated from the third and fourth sections. The amount of phosphorylation per milligram of nitrogen in

TABLE III

Fractionation and Analysis of Particulate Protein from Root Sections of Differing Maturity

Protein analysis by method of Lowry et al. (18). Total protein estimated on 10 per cent TCA precipitate of homogenates cleared by centrifuging at 500 g for 5 minutes. Soluble protein estimated by differences between total protein and the sum of sedimented particles.

Section No.	Total protein mg./ gm. fresh weight	Per cent total protein			
		Sucrose-phosphate-EDTA homogenate			
		Heavy mito-chondria	Light mito-chondria	Micro-some	Soluble protein
1	28.0	14.9	12.2	17.2	55.7
2	4.8	23.1	19.3	12.2	45.4
3	4.1	25.6	20.9	10.9	42.6
4	3.9	28.5	22.8	11.1	37.7
		Sucrose homogenate			
1	29.0	16.5	14.3	34.2	35.0
2	5.5	32.6	28.4	18.3	20.7
3	4.6	48.2	26.9	8.9	16.0
4	4.3	69.9	17.2	7.2	5.7

different regions of the root roughly parallels the rate of oxidation, but the P/O ratios show a gradual decline in phosphorylative efficiency of the mitochondria as the cells expand and mature.

The data of Table I suggest that in the more mature cells (sections 2, 3, and 4) a greater proportion of the protein nitrogen is in the form of mitochondria. This evidence was investigated further by differential centrifugation and protein analysis of particulates isolated from various regions of the root. Two homogenizing solutions were used: (1) 0.6 M sucrose, and (2) 0.6 M sucrose plus $\frac{1}{15}$ M potassium phosphate and 0.005 M EDTA (SPE). The inclusion of phosphate and EDTA was studied because of the marked effect of these additives in the isolation of mitochondria that would carry on oxidative phosphorylation (see below). A comparison of the results obtained with the different solutions is given in Table III. Results are expressed as percentage of protein precipitated from cleared homogenates with trichloracetic acid (TCA), in order to make the comparison independent of the effectiveness of tissue grinding.

There is an increase in the percentage of protein precipitated at low speeds as the cells mature. The increase coincides with the loss of protein

TABLE IV

The Effect of Phosphate and EDTA on the Amount of Nitrogenous Material Sedimented from Corn Root Homogenates

Five gm. of root sections taken between 1 and 3 cm. from the tip were ground in sucrose concentrations indicated, and particulates sedimented from cleared homogenate at 10,000 g for 15 minutes. Particles resuspended in sucrose or sucrose + phosphate + EDTA and resedimented at 10,000 g for 15 minutes.

	Particle N from 5 gm. tissue after resuspension and sedimentation in	
Homogenizing medium	Homogenizing medium	Homogenizing medium + phosphate + EDTA
	mg.	mg.
H_2O	1.97	1.24
0.25 M sucrose	2.06	1.13
0.6 "	1.74	0.89
1.0 "	1.18	0.53

from the microsome and soluble fractions. These data are in agreement with *in situ* observations that mitochondria are abundant in the cytoplasm of older cells, whereas the small granules of the microsome fraction are not. The magnitude of the change, however, is greatly dependent upon the homogenizing medium. Much less of the protein of the cytoplasm of mature sections is to be found in the low speed fraction if phosphate and EDTA are included in the homogenizing solution. This change was visible in the centrifuge pellet; sucrose grinding resulted in the precipitation of a streak of white, gelatinous material on the centrifugal side of the tube during the low speed centrifugation (Fig. 7). The amount of this adhesive substance was negligible in the heavy mitochondria from the tip of the root, but increased progressively through section 4 where the cells are mature and vacuolate. When homogenization took place in the presence of phosphate and EDTA, however, little or no white material was present. The data of Table IV show that the white material forms an appreciable part of the mitochondrial pellet sedimented from sucrose homogenates. Apparently, the white substance is a product of homogenization of mature cells, but in the presence of phosphate and the chelate ion it is dispersed and sediments as smaller particles or perhaps becomes solubilized (see soluble protein, Table III).

TABLE V

Oxidation and Phosphorylation of Mitochondria Isolated from Mature Root Tissue with and without Potassium Phosphate and EDTA

Experimental conditions as in Table II. Root material taken between 1 and 3 cm. back of tip. The pH of all solutions was adjusted to 6.8.

Grinding solution	Mitochondria N mg./gm. tissue	Q_{O_2} (N)	P/O
0.6 M sucrose	0.27	10	0
+1/15 M potassium phosphate	0.14	105	0.46
+0.005 M EDTA	0.16	469	1.75
+0.005 M EDTA + 1/15 potassium phosphate	0.14	501	2.99

Electron micrographs showing successive layers in pellets of heavy mitochondria isolated from mature tissue homogenized in sucrose are presented in Figs. 8 to 10. The superficial layer of the white material sedimenting along the side of the centrifuge tube (Fig. 8) is composed of small vesicles or fragments of the endoplasmic reticulum. Deeper layers of the white material have larger vesicles and what appear to be mitochondrial fragments (Fig. 9). The mitochondrial pellet (bottom of the tube), resulting from sucrose homogenization, contains mitochondria, membranes, and small vesiculate elements (Fig. 10). This pellet may be contrasted with the mitochondrial fraction from the same material ground in SPE (Fig. 11). The mitochondria tend to be less irregular in profile; they have cristae which are more elongate and exhibit a more evident connection with the peripheral membranes. The reticular and granular material seen in sucrose preparations has largely disappeared.

In terms of the capacity to carry on respiration and phosphorylation, the mitochondria isolated in SPE must be considered more representative of those in the intact cell than those isolated in sucrose (Table V). The effectiveness of EDTA and phosphate in this respect is most evident in mature tissue; mitochondria isolated in sucrose alone from the tip half-centimeter of the root will respire and phosphorylate reasonable well (Table VI), but EDTA and phosphate are beneficial even here (cf. Table II).

Phosphate and EDTA must be present during

TABLE VI

Oxidation and Phosphorylation of Mitochondria Isolated in 0.6 M Sucrose from Sections of Corn Root

Experimental conditions as in Table II.

Root section	Q_{O_2} (N)	P/O
cm.		
0.0–0.5	385	0.92
1.5–2.5	73	0.0

TABLE VII

The Effect of Washing in Sucrose-Phosphate-EDTA on the Respiration of Mitochondria Isolated in Sucrose

Mitochondria isolated from root sections 1 to 4 cm. from tip. Experimental conditions as in Table II.

Mitochondrial preparation		Q_{O_2} (N)
Isolation medium	Washing medium	
Sucrose	Sucrose + phosphate + EDTA	39
Sucrose + phosphate + EDTA	Sucrose + phosphate + EDTA	550

tissue homogenization in order to be effective. As shown in Table VII, mitochondria isolated in sucrose alone will not respire well even if subsequently washed in SPE. Consequently, the white material which accompanies sucrose homogenization, and which is removed by washing in SPE (Table IV), is not in itself inhibitory to respiration. This has been confirmed by the addition of mitochondria isolated in sucrose to vessels containing mitochondria isolated in SPE; there was no significant change in the respiratory rate of the latter.

It is not believed that the lack of buffer during sucrose homogenization accounts for the deleterious effect on mitochondrial respiration. Typically, the pH of the tissue homogenates falls in the range of 6.8–7.1, irrespective of the presence of phosphate buffer.

These respiratory studies with isolated mitochondria were conducted with particles sedimenting between 500 g for 5 minutes and 15,000 g for 15 minutes. For the analysis of particles, a different schedule of centrifugation was followed in order to detect more closely any changes in the mitochondrial fraction. Both the "heavy" and "light" mitochondria used in analysis have the capacity to carry on oxidative phosphorylation (Table VIII). The light mitochondria are not as

TABLE VIII

Comparison of Oxidative Phosphorylation of Heavy and Light Mitochondria Isolated from Corn Root Sections

Experimental conditions as in Table II. Tissue homogenized in 0.6 M sucrose + 1/15 M K phosphate (pH 6.8) + 0.005 M EDTA. Heavy mitochondria sedimented between 500 g for 5 minutes and 5,000 g for 15 minutes; light mitochondria sedimented between 5,000 g for 15 minutes and 20,000 g for 35 minutes. Particles resuspended and resedimented from grinding medium.

Root section	Mitochondria	Q_{O_2} (N)	P/O
cm.			
0.0–0.5	Heavy	231	1.80
	Light	255	1.90
0.5–1.5	Heavy	427	1.76
	Light	171	1.28

efficient as the heavy except in the tip half-centimeter of the root.

A large number of electron micrographs have been examined for morphological characteristics of mitochondria *in situ* which are preserved by isolation in SPE, but lost in sucrose isolation. The one characteristic which appears to be most closely associated with respiratory activity is the shape and location of the cristae. Active mitochondria have elongated cristae, some of which in any one profile will show connection with the exterior membranes (Figs. 11 and 12). The cristae of sucrose-isolated mitochondria tend to be more rounded in profile (Fig. 10). Too little is known however, about the relationship between mitochondrial structure and function to estimate whether this correlation is more than fortuitous.

A series of electron micrographs was made of pellets sedimented from homogenates of sections 1, 2, and 3. Representative micrographs of the sucrose pellets are displayed in Figs. 12 to 20. These micrographs are of unwashed pellets, so as to show the type of material analyzed (Tables III and IX). The heavy mitochondria isolated in sucrose from the tip half-centimeter of the root are largely intact with well formed cristae (Fig. 12). Progressively through sections 2 and 3 (Figs. 13 and 14) there appears to be a greater degree of mitochondrial disintegration, and increasing amounts of granular and reticular material are sedimented together with mitochondria. Although intact mitochondria are found in

TABLE IX

Analysis of Particulate Matter Sedimented from Corn Root Homogenates

Section No.	5,000 g 15 min.		20,000 g 35 min.		80,000 g 150 min.		Non-sedimented material*	
	S	SPE	S	SPE	S	SPE	S	SPE
Acid-soluble nucleotide/mg. protein								
	μM	μM	μM	μM	μM	μM		
1	0.055	0.058	0.025	0.064	0.016	0.040	—	—
2	0.031	0.063	0.032	0.074	0.051	0.049	—	—
3	0.028	0.055	0.032	0.082	0.085	0.049	—	—
4	0.025	0.056	0.043	0.071	0.123	0.049	—	—
Lipide phosphate/mg. protein							Per cent of total phospholipide	
	μM	μM	μM	μM	μM	μM		
1	0.26	0.34	0.41	0.57	0.11	0.29	45	25
2	0.29	0.44	0.37	0.61	0.27	0.52	2	29
3	0.30	0.39	0.31	0.57	0.28	0.53	0	32
4	0.22	0.32	0.28	0.49	0.23	0.53	2	32
Nucleic acid/mg. protein							Per cent of total nucleic acid	
	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$		
1	73	106	163	127	426	227	0	65
2	121	115	166	104	195	162	25	61
3	125	103	153	116	108	173	18	59
4	118	90	109	121	99	138	17	60

* Per cent soluble calculated by difference between total sedimented particles and that precipitated by TCA. S = homogenization of tissue in 0.6 M sucrose. SPE = homogenization in 0.6 M sucrose + $\frac{1}{15}$ M potassium phosphate + 0.005 M EDTA.

sucrose homogenates of mature tissue (Fig. 10), quite often the exterior membranes seem to be disrupted, releasing vesicular matter reminiscent of cristae (Fig. 14). Much of the granular material is associated with membranes. The chief difference between these mitochondrial pellets and those isolated from SPE homogenates has already been noted—the granular and reticular material is much reduced (cf. Fig. 11).

The light mitochondrial fraction from all three sections is largely vesicular in nature, with some granular material which decreases as the tissue matures (Figs. 15 to 17). The pellet from the tip section (Fig. 15) possesses some small bodies with a dense, granular matrix, which superficially resemble the lysosomes of animal cells (26). Greater numbers of intact mitochondria are to be found in this fraction when the tissue is homogenized in SPE (Fig. 21). With respect to the microsome fraction, the pellets from the tip sections are rich in granules of about 15 mμ diameter (Fig. 18), which progressively decrease in number until

they are nearly absent from the tissue 1.5 to 2.5 cm. back of the root tip (Figs. 19, 20, and 23). This decrease in microsomal granules correlates well with the similar decrease noted *in situ*. The vesicles of the endoplasmic reticulum are in evidence in all three pellets, but seem to be larger with more distinct membranes in the more mature sections. The membranes are mainly of the smooth surfaced type. Rough surfaced membranes, *i.e.* membranes with adhering granules as described by Palade and Siekevitz (19), are not well defined, although in some instances the distribution of the granules is suggestive of their presence (Figs. 18 and 19). Phosphate and EDTA in the homogenizing solution affect a partial disintegration of the microsomal granules, resulting in a finer, more amorphous material dispersed throughout the pellet (Fig. 22). No consistent effect of phosphate and EDTA on the vesicular component of the microsome pellet has been observed.

The analysis of the pellets expressed on a unit

protein basis is presented in Table IX. The soluble nucleotide concentration of heavy mitochondria, isolated in sucrose, decreases with tissue maturity. This may be a contributing factor to their low oxidative capacity, since Siekevitz and Potter (27) have shown a relationship between nucleotide content and oxidation by mitochondria. The nucleic acid concentration of the microsome fraction decreases with tissue maturity with both homogenizations, supporting the visual evidence that the microsome granules, known to be high in RNA (20), decrease during growth and maturation. The low concentration of nucleic acid in the microsomes isolated in SPE from the tip section confirms the visual evidence that the granules have undergone dispersion (Fig. 22), as does the fact that 65 per cent of the nucleic acid appears in the soluble fraction. With all sections and all pellets, the phospholipide per unit protein is higher in SPE preparations.

DISCUSSION

This investigation was directed at determining whether there are changes in cytoplasmic particulates during growth and maturation of root cells which would correlate with changes in the composition and physiological activity of these cells. The electron micrographs clearly show that such changes in particulates do occur and suggest that further investigation of these transformations should prove profitable in the analysis of growth, respiration, and salt accumulation.

The commonly observed increase in the respiratory rate per unit protein with cellular growth can be attributed to changes in the relative amount and efficiency of the mitochondria. The increase in oxidative efficiency parallels changes in mitochondrial structure. The meristematic cells contain mitochondria of two types. The first appears to be fully developed, inasmuch as it possesses cristae and is similar to mitochondria found in more mature cells. It is assumed that such mitochondria are functional in supplying energy for the endergonic processes of the meristematic cell. The second type of mitochondrion, interpreted as being an immature stage, is characterized by a homogeneous matrix in which cristae develop. The low respiratory efficiency per unit protein observed in mitochondria from the tip of the root can be explained by assuming that the immature mitochondria are not fully functional. In the elongating cells of the

root, where all mitochondria show internal differentiation, the respiratory efficiency is higher (Table I and II). In the regions of differentiation and maturation the mitochondria show a disorganization of internal structure, which parallels a diminution in respiratory rate. There is a consistent correlation between the presence of a system of well developed cristae mitochondriales and the capacity of the mitochondria to respire either in situ (Table I), or when isolated (Table II). Palade (28) has suggested that the number of cristae are related to the amount of oxidative enzymes.

The spatial separation of immature and mature cells in the root facilitates observation on the genesis of mitochondria. We have interpreted the homogeneous type of organelle as an immature mitochondrion because (a) it is the only clearly defined organelle of suitable size and number to represent an immature stage, (b) it shows the development of a cristae-like structure, and (c) it occurs only in the meristematic regions. In the meristem there are about equal numbers of immature and mature mitochondrial profiles. The granular matrix of these immature mitochondria is similar to that of the microbodies of regenerating liver which Rouiller and Bernhard suggest as precursors of mitochondria (29).

There is some evidence that these immature organelles multiply by fission, inasmuch as elongate profiles constricted in the central region are present (Fig. 4). Dempsey (30) has discussed the evidence gained from electron microscopy with respect to mitochondrial origin in animals. He concludes that evidence supporting fission is not adequate and presents evidence for a de novo origin from membranous structures. While membranous structures of the lamellar type are abundant in the root meristem, it is not yet clear that they function in mitochondrial genesis. Mitochondria have been observed to elongate and divide during meiotic division in the grasshopper spermatocyte (31). No evidence has been found in corn roots to support the view that mitochondria arise from microsomes (32). Further studies of mitochondrial genesis in the corn root are in progress.

There is evidence in Table I that a good share of the increase in tissue respiration per unit protein along the root axis is due to increases in the percentage of the cytoplasmic protein represented by mitochondria. The respiration rate of

the second section on a total protein basis increases 134 per cent over that of the first section. On the other hand, the rate of respiration on a basis of mitochondrial protein in section 2 is only 43 per cent greater than in section 1. Since the rate per unit of mitochondrial protein is a measure of efficiency, it can readily be seen that the increase in efficiency can only account for a small part of the increase in tissue respiration during growth. From these data it is concluded that the relative amount of mitochondria in the cytoplasm increases rapidly during elongation.

The data of Table III show that the amount of protein sedimenting between 500 g and 20,000 g increases as the cells grow and mature. The magnitude of this increase appears greater when the tissue is ground in sucrose than when ground in SPE. Since EDTA disperses a great deal of the material sedimenting in this fraction, it seems possible that this material represents fragments of mitochondria disrupted by the homogenizing process. However, some of the protein in this fraction (Fig. 14) is also represented by microsomal granules adhering to mitochondrial membranes, probably due to their basophilic nature (33, 34). Therefore, the real increase in amount of mitochondrial protein probably lies somewhere between the figures for the two homogenizing solutions. Further research is required to develop techniques and media which will preserve the fragile mitochondria in mature tissues during isolation. The inclusion of polyvinylpyrrolidone (35) or dextran (36) in the homogenizing medium may help in preserving these mitochondria. The use of density gradients during centrifugation (37, 38) might improve the homogeneity of this fraction.

There have been previous observations with plant material that the presence of EDTA and/or phosphate during homogenization aids in the isolation of mitochondria with high oxidative and phosphorylative capacity. The beneficial effects of phosphate in the isolation of active plant mitochondria have been reported by Millerd et al. (39) and Beaudreau (40). Lieberman and Biale (41) and Tager (42) found marked enhancement of oxidative activity of plant mitochondria by including EDTA in the isolation medium. On the other hand, EDTA in the medium has not always proved beneficial (43, 44).

The effect of EDTA is probably due to its ability to chelate the calcium ion which would be released from vacuoles of mature tissue. Calcium has been shown to inhibit oxidative phosphorylation (45-48). This could be a general effect of chelation of metals (47) which might be directly inhibitory to the phosphorylating mechanism. Other evidence indicates that the detrimental effect of calcium is due to a swelling or alteration of the morphology of the mitochondria (49-51).

A possible explanation of the conflicting reports on the effect of EDTA in preserving plant mitochondrial activity can be found in the experiments reported here. Mitochondria from the meristematic cells are not as dependent upon EDTA and phosphate as those from mature root tissue, presumably due to the lack of any extensive amount of vacuolation. Tissue with highly vacuolated cells, and in particular those which have accumulated large amounts of calcium, require EDTA during isolation of mitochondria.

The most striking change accompanying growth and maturation is the disappearance of the microsomal granules. The cytoplasm of the mature cells (Fig. 6) shows an exceedingly low density of granules in contrast to the masses present in the meristem (Fig. 1). An obvious conclusion is that the granules have been metabolized.

However, micrographs of mature cells show (Fig. 6) that not all of the microsomal granules have disappeared. A change in the association of the granules with other cytoplasmic elements is indicated by the microsomal pellets (Figs. 18 to 20). Inasmuch as the granules are too small for any appreciable portion to sediment independently at 80,000 g for 150 minutes in 0.6 M sucrose at 0°C.,[1] their appearance in the pellet must be the consequence of an aggregation or an association with the elements of the endoplasmic reticulum (Fig. 18). As the cells grow, these granules lose their association with microsomal vesicles and sediment in part with the membranous material of the mitochondrial fraction (Figs. 13 and 14) (Table IX), unless dispersed therefrom by EDTA and phosphate (Fig. 11). The membranous material of the mitochondrial fraction from mature cells may represent enlarged ele-

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[1] Calculations from formulae and specifications given in Spinco Ultracentrifuge Manual, Beckman Instruments, Inc., Belmont, California, show time required for complete sedimentation of 15 mμ particles of estimated 1.3 gm./cm.^{3} density to be in excess of 18 hours.

ments of the endoplasmic reticulum (Fig. 6), which together with associated granules sediment at low forces (Figs. 13 and 14).

The data of Table IX support the observation of pellet micrographs. All of the nucleic acid is sedimented from sucrose homogenates of tip sections, but 17 to 25 per cent fails to sediment from homogenates of basal sections. Evidently, the microsomal granules no longer adhere to the endoplasmic reticulum as the cytoplasm matures. The character of the membranes must change with growth.

The nature of the association between microsomal granules and the endoplasmic reticulum is probably not through a cationic bond. This is assumed by the fact that EDTA and phosphate causes a disassociation of the granules rather than their removal (Fig. 22). Ts'o, Bonner, and Vinograd (20) have reported that magnesium tends to aggregate the nucleoprotein particles from pea seedlings; it is probable that the chelation of magnesium or calcium by EDTA may be responsible for disintegration of the microsomal granules noted here.

SUMMARY

A study of the changes in cytoplasmic particulates in cells of differing maturity along the axis of corn roots has been made. Meristematic cells are characterized by immature and mature mitochondria and a dense mass of microsomal granules associated with an endoplasmic reticulum of small, thin membraned vesicles. With growth, the immature mitochondria develop cristae, and reach maximum oxidative efficiency in the region of cell elongation. The relative amount of cytoplasmic protein accounted for by mitochondria increases sharply with cell elongation and largely accounts for the increase in respiration per unit protein in this region. In mature cells the mitochondria tend to become disorganized internally, and are less efficient in respiration.

The microsomal granules appear to be partly metabolized during growth and maturation and lose their association with the vesicular elements of the endoplasmic reticulum. Concomitantly reticular elements become larger with more dense membranes.

Phosphate and EDTA in the homogenizing solution was noted to disperse the microsomal granules and to enhance the respiratory activity of the isolated mitochondria.

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EXPLANATION OF PLATES

PLATE 34

FIG. 1. Electron micrograph of a section through the cytoplasm of a meristematic cell immediately back of root cap. There is an extremely high density of microsome granules (g) which nearly obscures the small vesicles (V) of the endoplasmic reticulum. Immature mitochondria (IM) with relatively homogeneous internal structure are abundant. There are in addition some ill defined mitochondria (M) with a more heterogeneous internal structure. A small vacuole appears at Va. × 40,000.

FIG. 2. Same region as in Fig. 1, showing the development of cristae mitochondriales (c) in the immature mitochondria (IM). In this section a multilamellar structure (ML) appears which superficially resembles the dictyosome of animal cells (25). × 40,000.
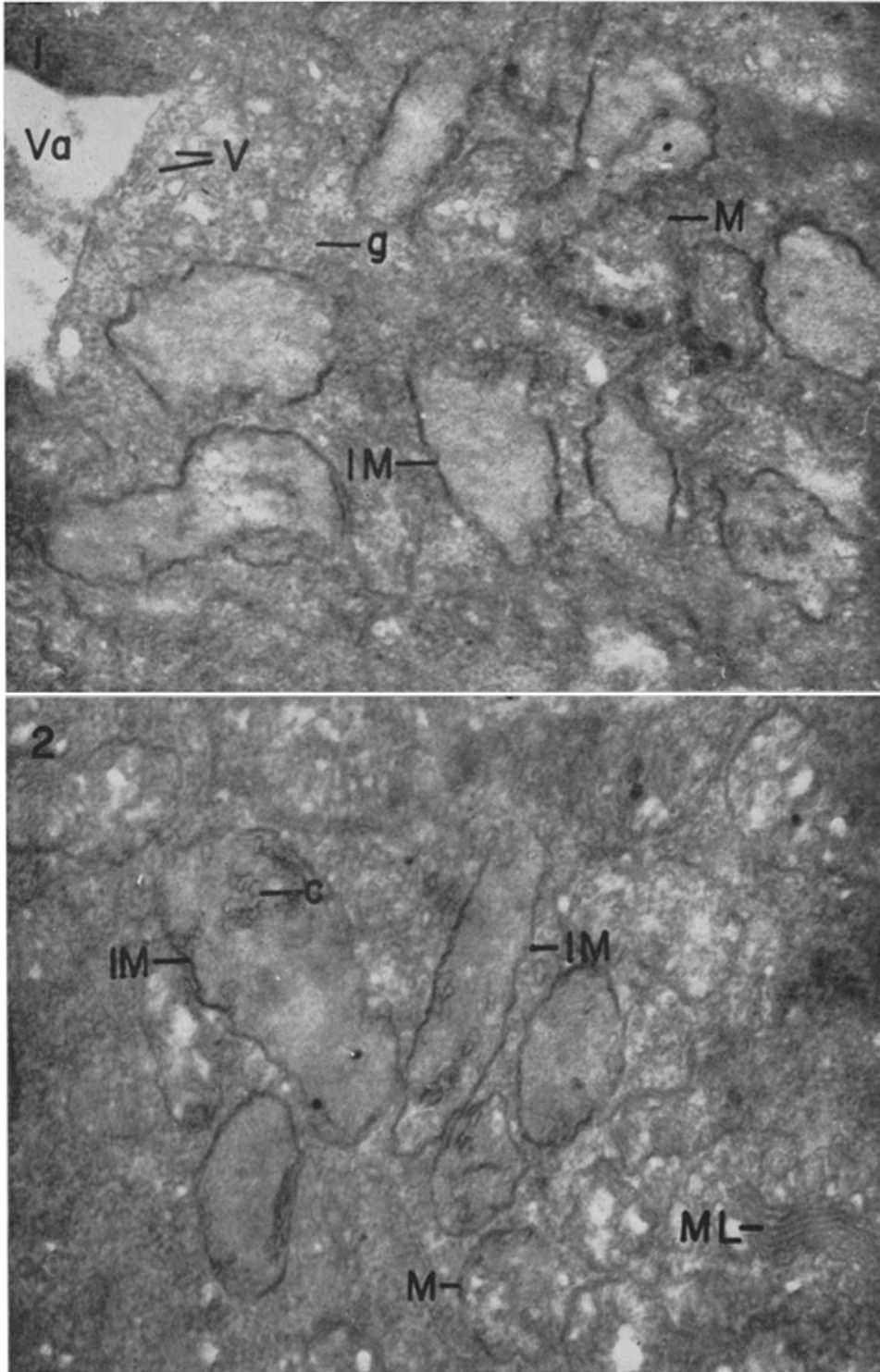
(Lund *et al.*: Changes in growth of *Zea Mays* roots)

PLATE 35

FIG. 3. Same region as Figs. 1 and 2. There are numerous lamellar elements of the endoplasmic reticulum (E), which in some profiles appear as a chain of small vesicles. The ends of the multilamellar structures (ML, see Fig. 2) are associated with small vesicular elements (V). This is particularly noticeable in tangential profiles (see upper left). Cristae (c) are more evident in the mature mitochondria (M) of this section. Thick membraned vesicles (T) occur in the cytoplasm of meristematic cells; these may be fat droplets or elaioplasts. $\times$ 40,000.
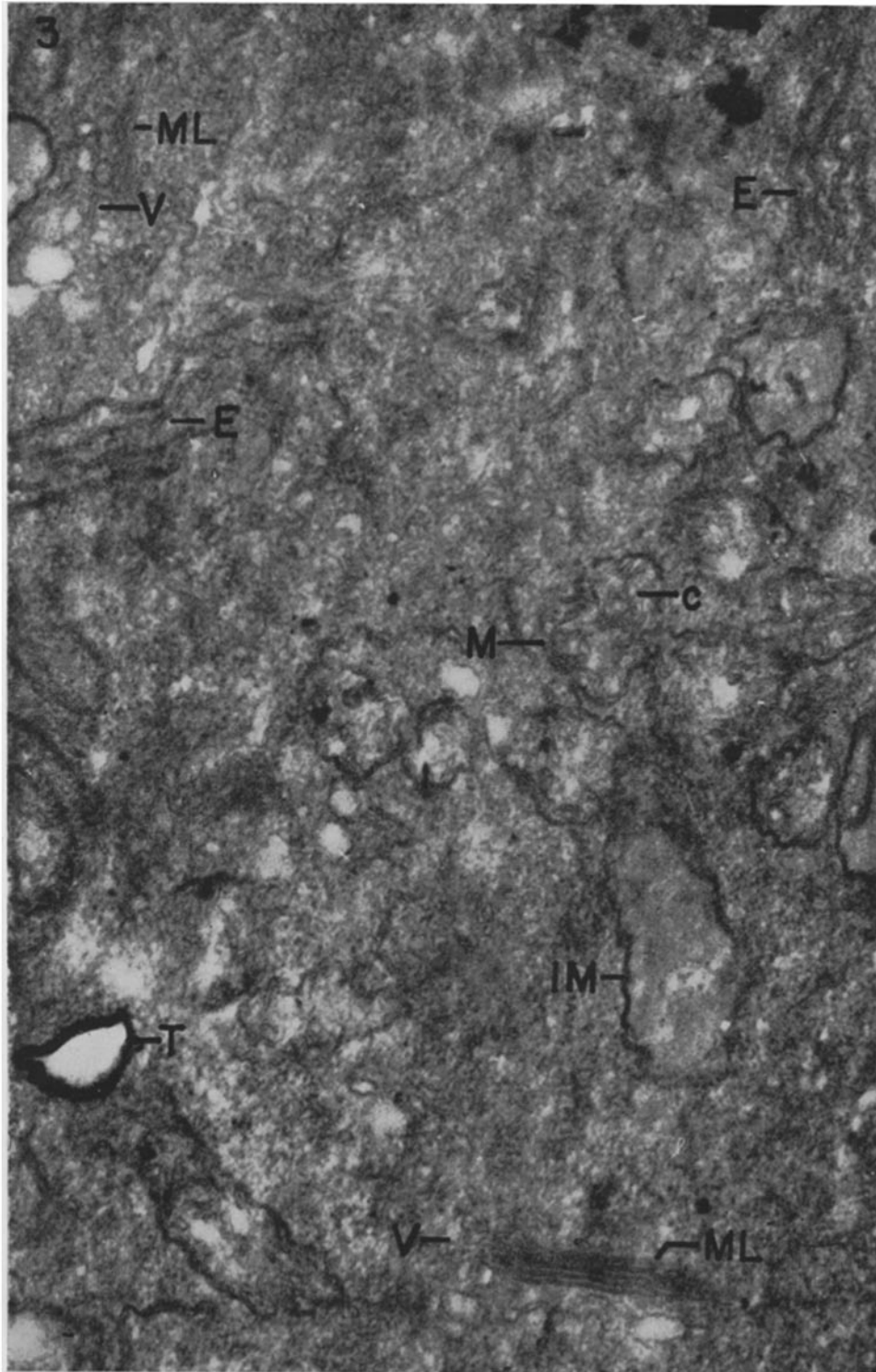
(Lund *et al.*: Changes in growth of *Zea Mays* roots)

FIG. 4. Cytoplasm of a daughter cell in a late stage of division. Many of the vesicular elements are greatly expanded, perhaps forming precursors of the vacuole, since some seem to be coalescing (V). There are two elongate, immature mitochondria (IM) which appear to be undergoing fission (arrows). $\times$ 60,000.
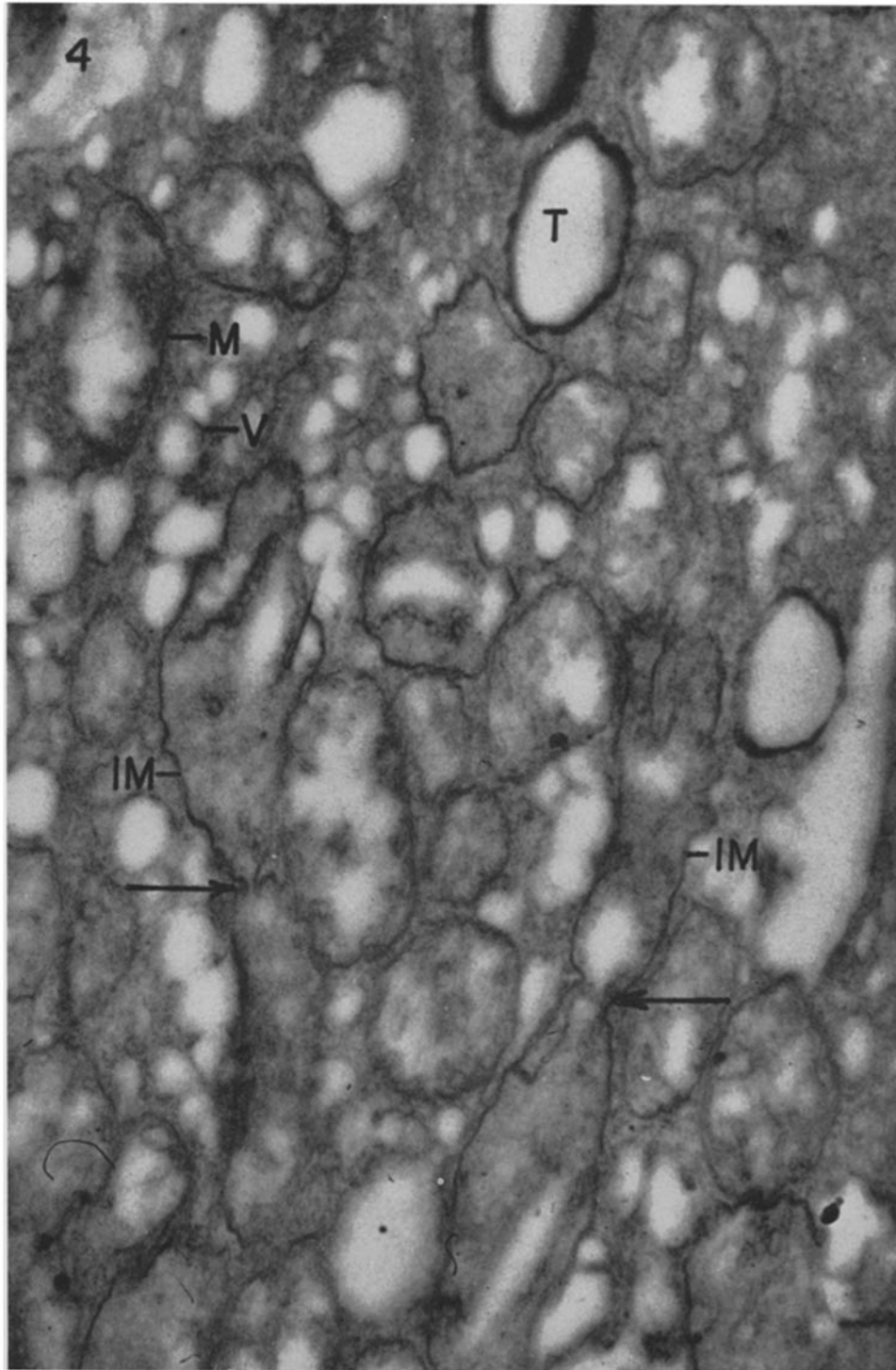
(Lund *et al.*: Changes in growth of *Zea Mays* roots)

PLATE 37

FIG. 5. Portion of an elongating cell. Vacuoles (Va) have a well defined tonoplast (Tp). There has been a decrease in the relative abundance of small dense granules (g) as compared with the meristematic cells (Figs. 1, 2, and 3). Cell wall (W). × 40,000.

FIG. 6. Cytoplasm of a mature cortical cell about 2 cm. behind root tip. There is a paucity of granules (g) as compared to younger cells. The mitochondrial matrix has almost disappeared. The most prominent feature of the mature cytoplasm is the presence of numerous large vesicles. The outer membrane, or plasmalemma (PL), may be continuous with those of the vesicles. × 40,000.
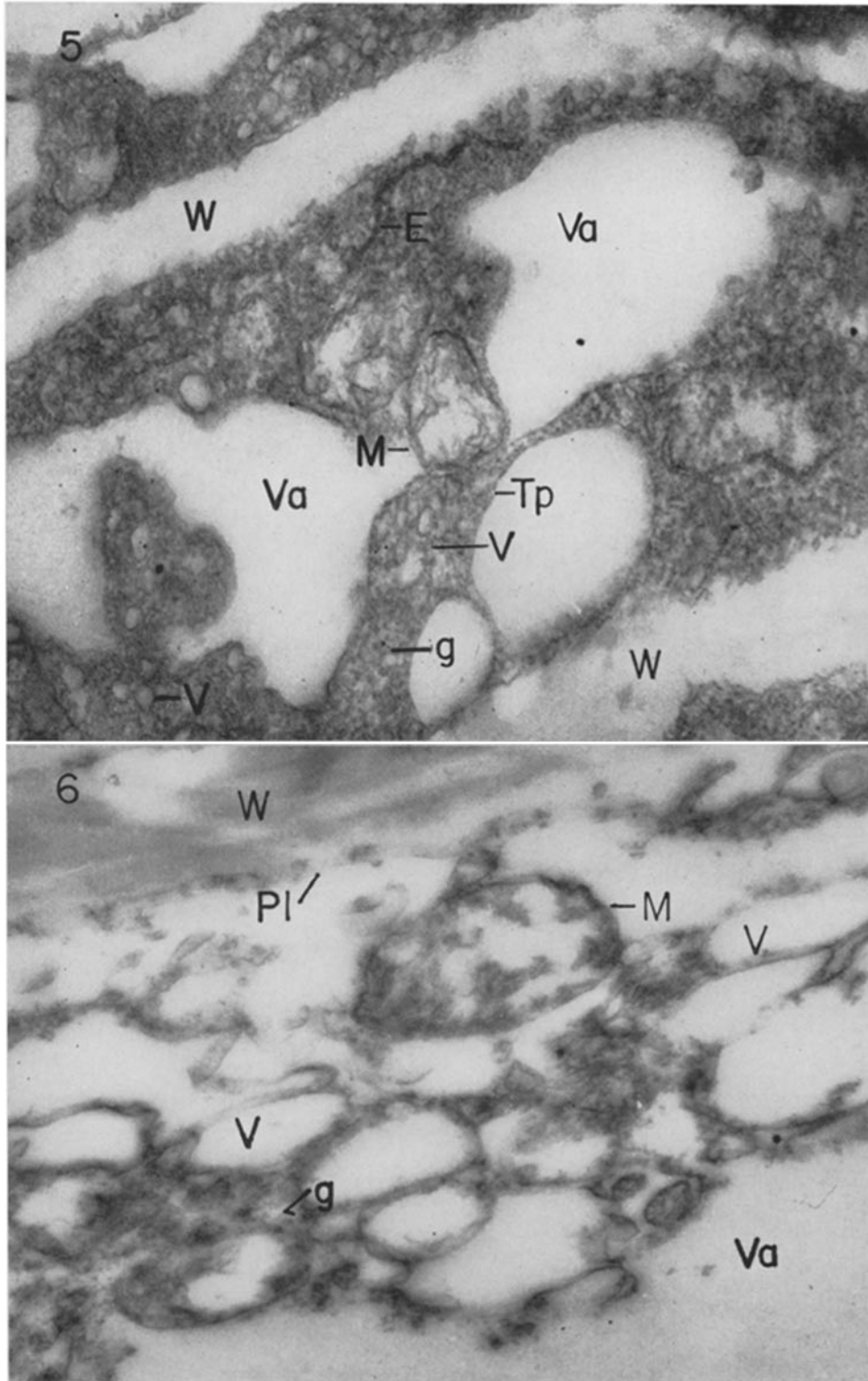
(Lund *et al.*: Changes in growth of *Zea Mays* roots)

PLATE 38

Fig. 7. Photograph showing the streak of white material which sediments on the side of the tube at 5,000 g for 15 minutes from homogenates of mature tissue prepared in 0.6 M sucrose. Figures 8 to 10 are from this type of pellet.

Fig. 8. Superficial layer of white material sedimenting on the side of tube. This material is composed of vesicular elements and a few granules. $\times$ 40,000.

Fig. 9. Section of a deeper layer of white material on the side of tube. The material is composed of larger vesicles, membranous structures, and possibly mitochondrial fragments (MF). $\times$ 40,000.

Fig. 10. Section of pellet at bottom of centrifuge tube, showing profiles of mitochondria (M). Note the irregular shape of the mitochondria and the ellipsoidal profile of the cristae. Microsomal granules (g) are occluded with the vesicular elements. $\times$ 40,000.

Fig. 11. Section through mitochondrial pellet isolated from sucrose-phosphate-EDTA homogenate. The mitochondria (M) tend to be more regular in outline and possess elongate cristae, as compared with those isolated in sucrose (Fig. 10). There are fewer microsome granules in this pellet. $\times$ 40,000.
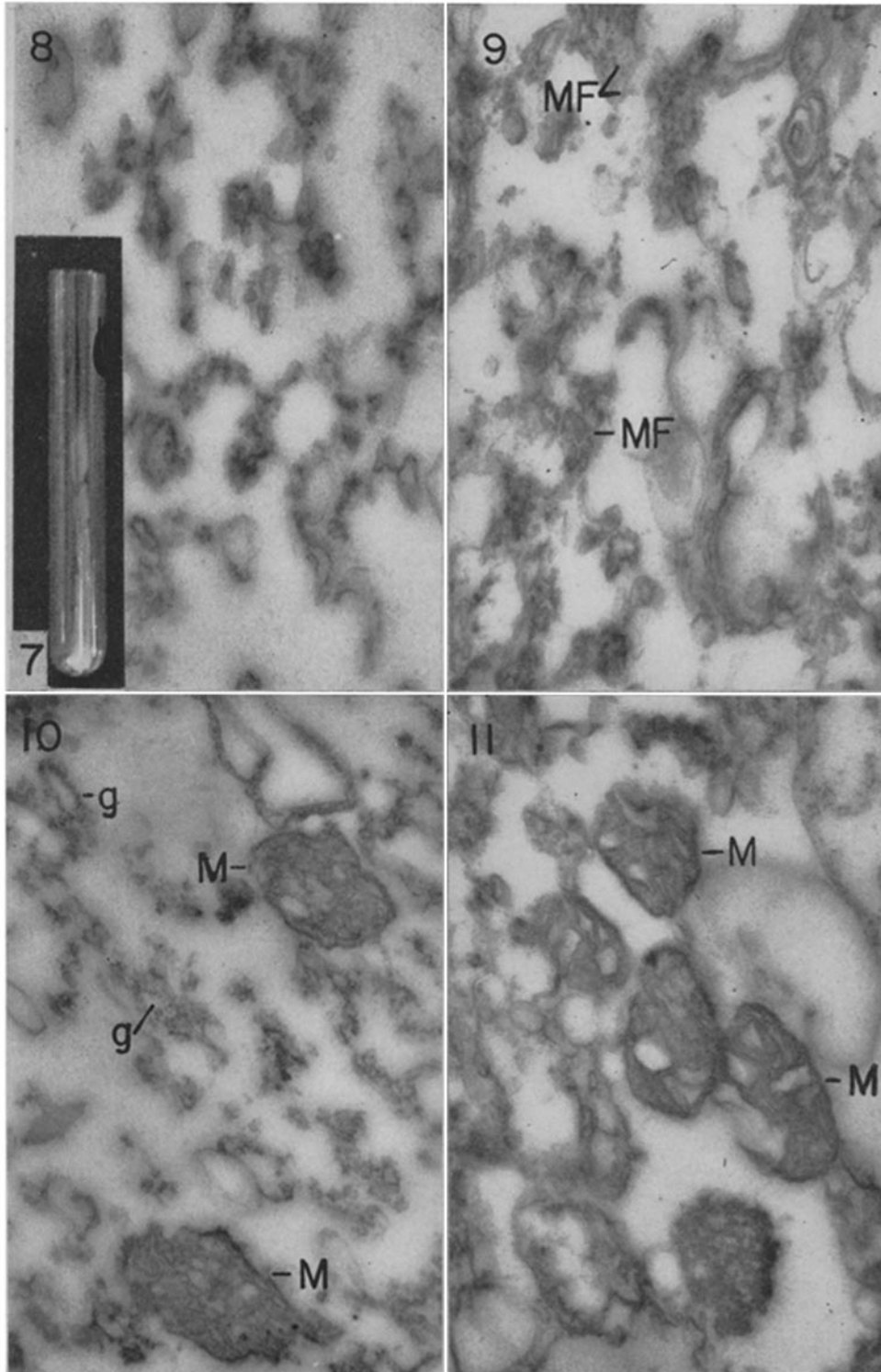
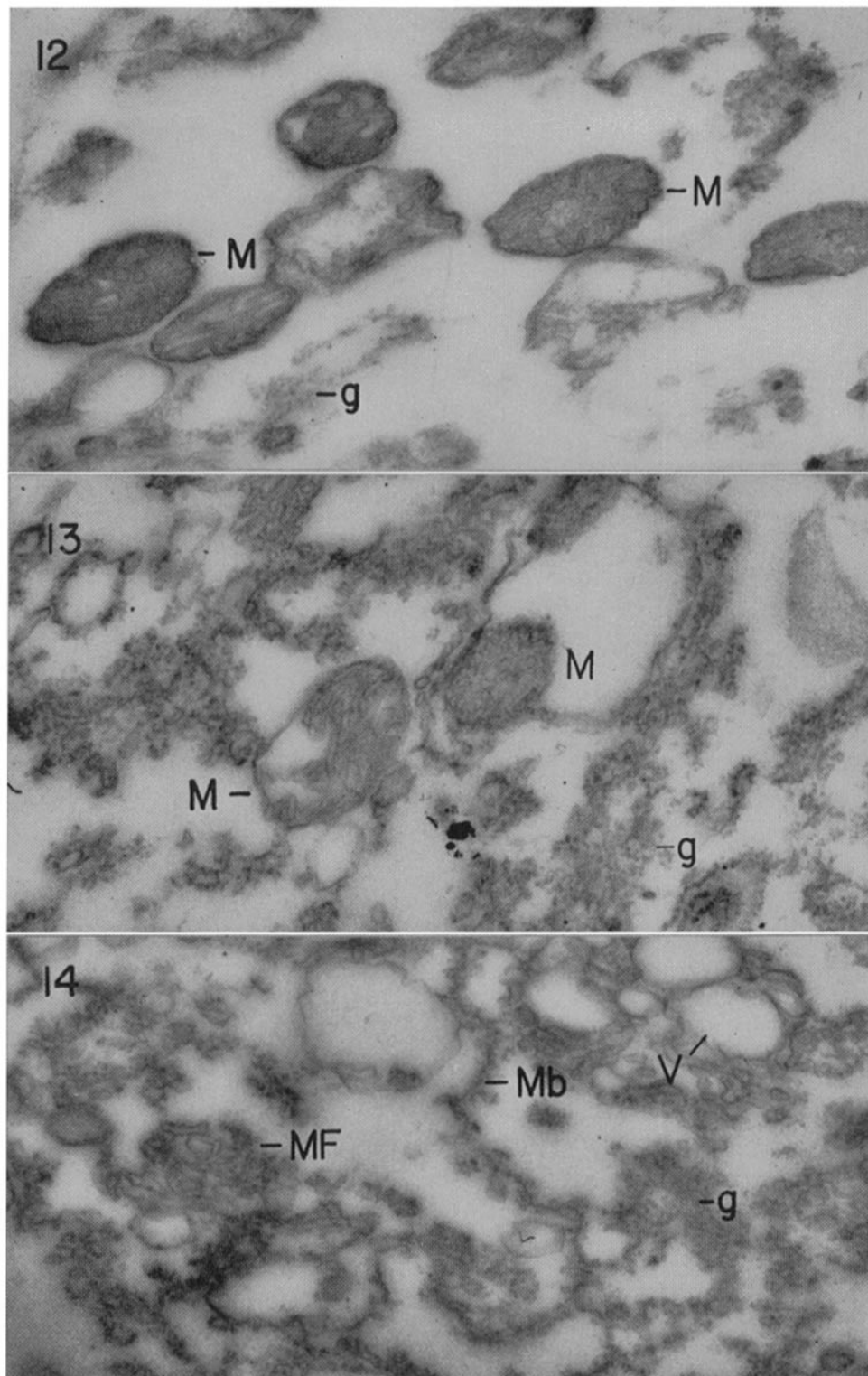(Lund *et al.*: Changes in growth of *Zea Mays* roots)

FIGS. 12 to 14. Sections through pellets sedimented between 500 g for 5 minutes, and 5,000 g for 15 minutes from 0.6 M sucrose homogenates of maize root sections. $\times$ 40,000.

FIG. 12. Mitochondria (M) with well defined cristae isolated from the tip $\frac{1}{2}$ cm. of the root. Occasional vesicular elements with associated granules (g) can be seen.

FIG. 13. Mitochondrial pellet from a region $\frac{1}{2}$ to $1\frac{1}{2}$ cm. back of the root tip. Note the increase in aggregated and occluded microsomal granules (g). Mitochondria (M) have suffered some loss of structural integrity during isolation.

FIG. 14. Mitochondrial pellet from the region $1\frac{1}{2}$ to $2\frac{1}{2}$ cm. back of the root tip. There is evidence that mitochondria (MF) are being disrupted during isolation. There is an abundance of microsomal granules (g), which are aggregated or associated with membranes (Mb).

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FIGS. 15 to 17. Sections through pellets sedimented between 5,000 g for 15 minutes, and 20,000 g for 35 minutes from 0.6 M sucrose homogenates of maize root sections. × 40,000.

FIG. 15. Light mitochondrial fraction isolated from the tip ½ cm. of the root. This fraction is composed of vesicles of varied size. Lysosome-like bodies (Ly) are found in this fraction.

FIG. 16. Light mitochondrial fraction isolated from the region ½ to 1½ cm. back of the root tip.

FIG. 17. Light mitochondrial fraction isolated from the region 1½ to 2½ cm. back of the root tip. Fewer microsomal granules are associated with the membranes of the vesicles.

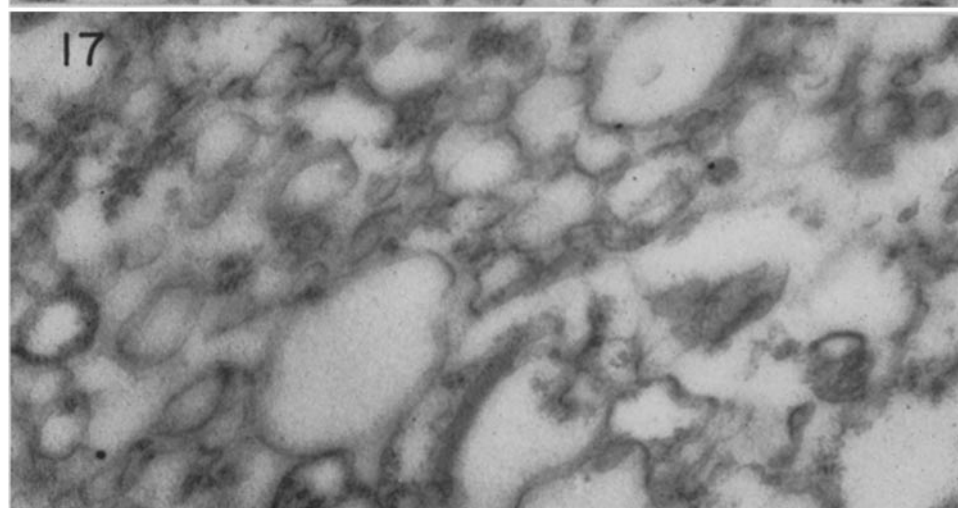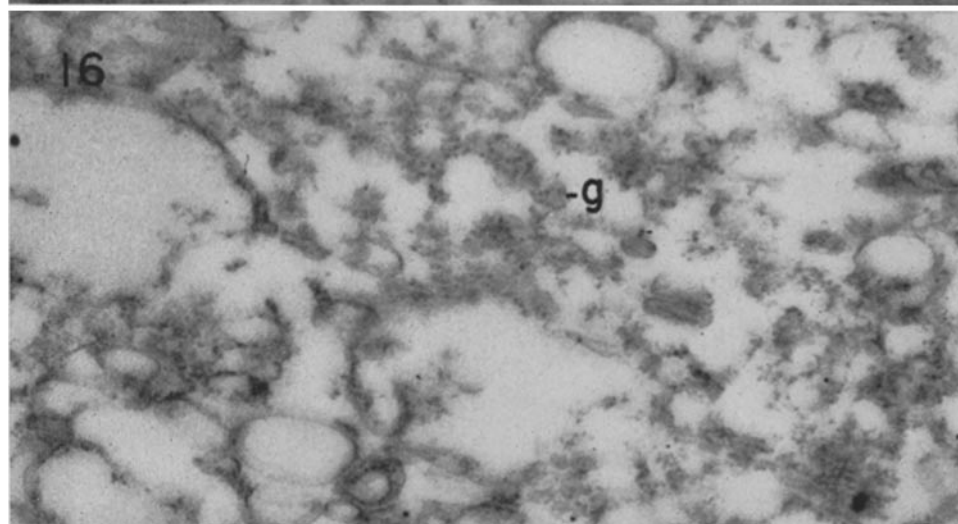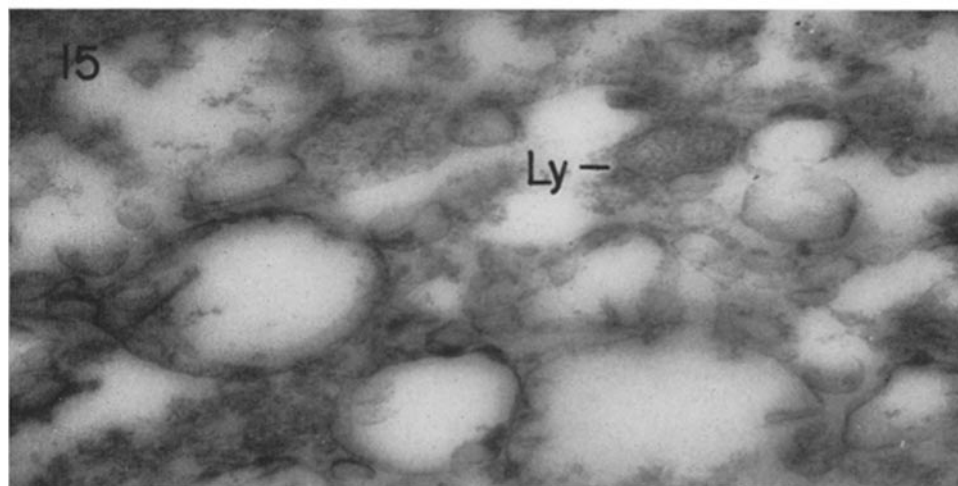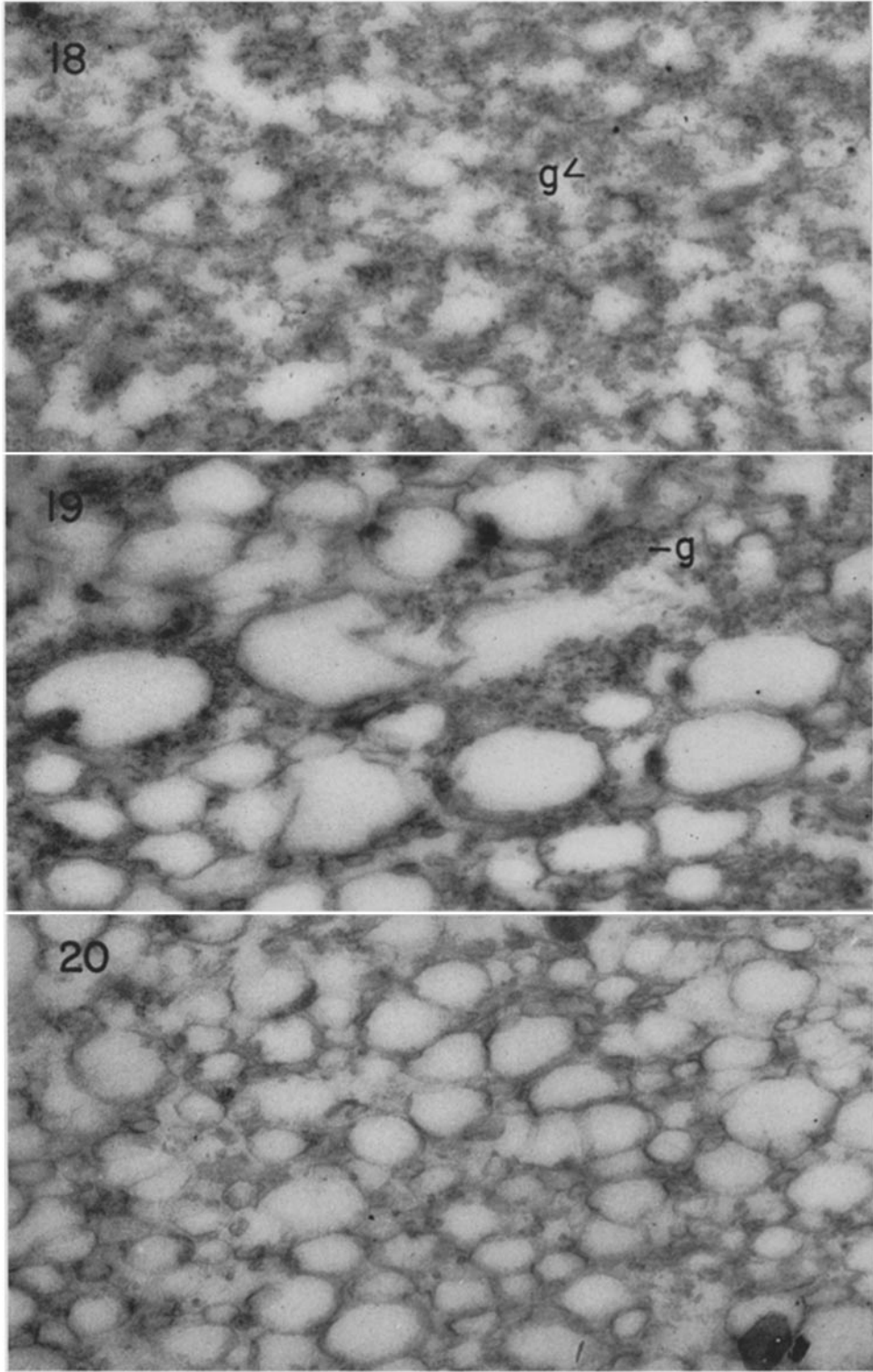(Lund *et al.*: Changes in growth of *Zea Mays* roots)

PLATE 41

FIGS. 18 to 20. Microsomal pellets sedimented between 20,000 g for 15 minutes, and 80,000 g for 150 minutes from 0.6 M sucrose homogenates of maize root sections. Note the progressive decrease in granules (g) in the following microsomal fractions. $\times$ 40,000.

FIG. 18. Microsomal fraction from the tip $\frac{1}{2}$ cm. of the root consisting of small, thin membraned vesicles and numerous granules.

FIG. 19. Microsomal fraction isolated from the region $\frac{1}{2}$ to $1\frac{1}{2}$ cm. back of the root tip.

FIG. 20. Microsomal fraction isolated from the region $1\frac{1}{2}$ to $2\frac{1}{2}$ cm. back of the root tip. The pellet consists almost entirely of vesicular matter.

PLATE 42

FIGS. 21 to 23. Sections through pellets isolated from homogenates of maize roots prepared with sucrose-phosphate-EDTA. $\times$ 40,000.

FIG. 21. Light mitochondrial fraction from the tip $\frac{1}{2}$ cm. of the root (between 5,000 g for 15 minutes, and 20,000 for 35 minutes). Mitochondria (M) are present. Compare with those isolated in sucrose alone (Fig. 15).

FIG. 22. Microsome fraction from tip $\frac{1}{2}$ cm. of root; as compared with Fig. 18, the granules are less numerous and ill defined.

FIG. 23. Microsome fraction from the region $1\frac{1}{2}$ to $2\frac{1}{2}$ cm. back of the root tip. As in Fig. 20, this fraction consists solely of vesicular matter.

(Lund *et al.*: Changes in growth of *Zea Mays* roots)