MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXXII, No. 3 (Biology), Article 3, 1966

An Electron Microscope Study on the Development of Chloroplast in *Tradescantia virginiana*

By

Tatsuya TAMURA

Cytological Laboratory, Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan

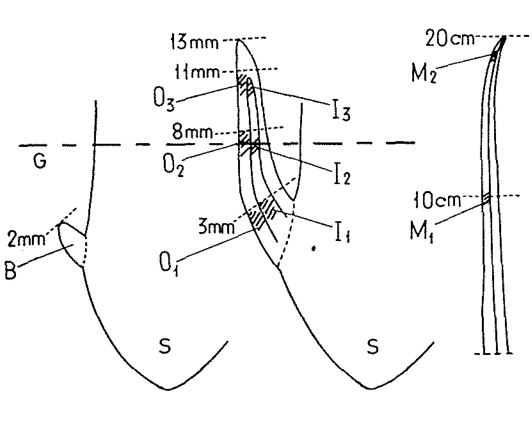
(Received December 21, 1965)

The chloroplast is one of the most important cell organellae, and its developmental process has been studied by several investigators by the aid of an electron microscope (MÜHLETHALER 1955, PERNER 1956a, 1956b, Von WETTSTEIN 1958, MÜHLETHALER et al. 1959, MENKE 1959, 1960a). Their views, however, are not always in accord with each other especially on the origin and early stages of chloroplast development, mainly because of the differences in the interpretations of electron micrographs they obtained.

In the present study, the electron microscopic structure of various particles found in the very young leaf cells was cleared and then on the basis of the results obtained, the developmental process of the chloroplast was studied.

Material and Methods

Mesophyll cells of the leaves of *Tradescantia virginiana* at the various stages of the development were used as materials. The parts of the leaves used



Text-fig. 1. Diagrams illustrating the parts which materials were taken from a bud, young leaves about 13 mm in length and a mature leaf.
G: subsurface part S: subterranean stem B: young bud covered by soil, white in color I and O: parts of the inner leaf and the outer leaf respectively I_1: white, with a orange tint I_2: same, but deeper than I_1 I_3: faint orange with a green tint O_1: white in color O_2: faint orange in color O_3: faint green in color M_1 and M_2: parts in mature leaf green in color

are shown in Text-fig. 1 ; they are very young buds covered by soil (B), young leaves (I and O) and green mature leaves (M).

Materials were fixed with 2% aqueous solution of KM_nO_4 for about 2 hours, dehydrated with ethanol and embedded in Epon. Some materials were fixed with the mixture of 2% O_sO_4 solution and phosphate buffer solution (PH 7.4) in equal parts for about 1 hour, and embedded in Epon after dehydration.

The epidermis was cut off from resin blocks by trimming. All particles in the cytoplasm surrounded by a membrane were studied without selection.

Results

Because KM_nO_4 is known as an excellent fixing agent of membraneous structures of cells, the following description is based mainly on the materials fixed with this agent. All the particles found in the cytoplasm and surrounded by a membrane are arranged in respect to their sizes in Table 1. Therefore, this table contains not only the particles which are regarded as the young chloroplasts, but those which are not. Particles in the first column are schematically shown nearly proportional to their sizes, and arranged in respect to their sizes. B, I_1, I_2 etc. mean the parts of leaves from which the materials were taken. Notations, B, I, O and M do not always indicate the order of cell ages in these parts ; but the cells in B are regarded younger than those found in I, O and M, and the cells in M are the oldest. I and O belong to different series, and I_1 is regarded as the youngest, and I_3 as the oldest in the series I. The same is true in the series O and M. The mark + means that a rather small number of particles was observed in a single section of a cell, and the mark ++ means that the particles are found abundantly.

Particle 1 in Table 1 is nearly round in cross section and is 0.5 -0.8μ in diameter. This particle is observed only in cells in B (the youngest bud). Fig. 1 shows an example of the particle 1. This particle is surrounded by triple layers (so-called double membrane) which consist of two electron dense layers (ca. 40 - 60 A thick) and one less dense layer (ca. 60 - 100 A thick) between them. The ground substance of the particle 1 shows, as a whole, medium electron density. Neither vesicles nor lamellae are observed in the ground substance.

MÜHLETHALER et al. (1959) reported that the smallest chloroplast initial found in *Elodea* cells are 0.02 - 0.2μ in diameter, but in *Tradescantia*, no particle of less than 0.5μ in diameter and surrounded by a double membrane was found.

It must be noted here that the double membrane structure as observed in the particle 1 is found in all the particles shown in Table 1 except the particle 2 which is surrounded by a single membrane.

The particle 2 in Table 1 is nearly round in cross section, and is 0.4 - 1.4μ in diameter. This particle is observed in the cells of nearly all parts of the leaves as shown in Table 1, but the number of the particles found in one

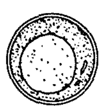
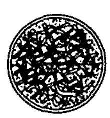
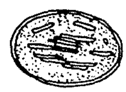
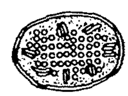
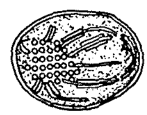
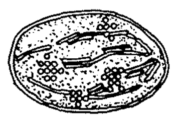
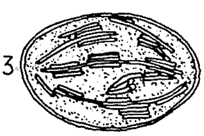
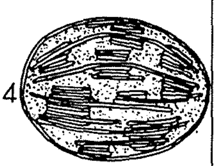
Particles											
Diagram		Size μ	B	I_1	I_2	I_3	O_1	O_2	O_3	M_1	M_2
1		0.5 ∫ 0.8	+								
2		0.4 ∫ 1.4	+	+	+	+	+	+	+		+
3		0.7 ∫ 1.4	++	+	+		+	+			
4		0.6 ∫ 1.5	++	+	+	+	+	+	+	+	+
5		1.4 ∫ 1.7		++	+		++	++			
6		1.1 ∫ 1.9	+	+	+		+	+	+	+	+
7		1.6 ∫ 2.0			++						
8		1.9 ∫ 2.3			++						
9		1.8 ∫ 3.1						++	++		
10		2.2 ∫ 2.6				++					
11		2.5 ∫ 3.1				++					
12		3.5 ∫ 3.9				+					
13		4.0 ∫ 5.0							++	++	++
14		4.5 ∫ 5.5								++	++

Table 1. Diagrams of particles surrounded by membrane and the parts where these particles are found in *Tradescantia*

cell is small. Fig. 2 shows an example of the particle 2. In this figure, the particle is surrounded by a dense membrane (ca. 60 A thick) which appears to be a single membrane. The density of the ground substance of this particle is medium, but it is higher than that of the particle 1. Similar to the particle

1, the particle 2 has neither vesicles nor lamellae in the ground substance.

Both particles 3 and 4 are nearly round or ellipse in profile and have the same size, that is, the former is 0.7 - 1.4μ and the latter is 0.6 - 1.5μ in diameter. It must be emphasized that, while the particle 3 is observed only in the B, I_1, I_2, O_1 and O_2 parts of leaves, the particle 4 is found in all parts of leaves from B to M_2. Fig. 4 shows the particle 3 adjoining the particle 4 in a single cell. These particles are similar in structure, that is, both are surrounded by a double membrane, and their inner membranes invaginate the ground substance as indicated by arrows in Fig. 4.

The particle 3 is shown in Fig. 3. In this figure, it is seen that the particle has some vesicles in the ground substance. The membranes of these vesicles are about 60A in thickness. It is observed, moreover, in this study that the double membrane and the membrane surrounding the vesicle of the particle 4 show lower density than those of the membranes in the particle 3 (compare the particle 3 with 4 in Fig. 4). It is not impossible, therefore, to distinguish the particle 3 from 4. Electron dense minute globules (ca. 250 - 350 A) are found in the ground substance of the particle 3, while they are not found in the particle 4. These minute globules are also observed in the ground substance of the particles 5, 6, 7 and 9 as well. As will be discussed in later pages of this paper, the particle 3 and 4 are regarded as the young chloroplast and the mitochondrion respectively. This interpretation is quite in accord with that presented by MOLLENHAUER (1959) in *Zea mays*.

Particle 5 is nearly round or ellipse in cross section, and has a diameter of 1.4 - 1.7μ. This is found in I_1, I_2, O_1 and O_2 parts of leaves. Fig. 5 shows an example of the particle 5. In the ground substance of this particle, there are several vesicles with a linear arrangement. Similar vesicles are also found in the particle 3, but they are not arranged regularly. The thickness of the membranes surrounding these vesicles is about 60 A in both particles. In Fig. 5, are observed two electron dense lines (ca. 60 A width) which run parallel in pair and fuse at both ends. This structure is regarded as a profile of a sac or a tubule. It is highly probable to consider that this structure is developed from linearly arrayed vesicles which are found in the same figure (Fig. 5). This sac or tubule seems to be similar to those which has been called the "lamellar disc" by VON WETTSTEIN (1958), the "lamellar packet" by MENKE (1960b) and the "lamellae" by HODGE et al. (1956). In this paper, these sacs or tubules will be called the "lamellar packet" for convenience's sake and all membraneous complex in the ground substance will be called the "lamellar system".

Particle 6 is nearly round in cross section, and its diameter is 1.1 - 1.9μ. This particle is found in all parts of leaves except I_3, and is characterized by the presence of a large circle of 0.2 - 0.6μ in diameter in the ground substance. It is hardly possible in the KM_nO_4 fixed materials to distinguish the difference of density between the content of this circle and that of the ground substance of the particle 6, while in the O_sO_4 fixed materials, the former is denser than the latter.

Both particles 7 and 8 are nearly round or ellipse in cross section, and are nearly similar in size (ca 2.0μ in diameter). These particles are observed only in I_2 parts of leaves and are found more frequently than the other particles found in the same cell. Fig. 7 shows the particle 7. In this figure, it is seen that the lamellar packets distribute rather unevenly and aggregate in one or more parts of the ground substance. In Fig. 8 which shows the particle 8, it is seen that the lamellar packets distribute rather evenly throughout the ground substance taking a reticulate appearance.

Particle 9 which is found in O_2 and O_3 parts of leaves is nearly ellipse in outline. The longer axis of the particle 9 is 1.8 - 3.1μ. Both Figs. 9a and 9b show the particle 9. The particle in Fig. 9a is smaller than that in Fig. 9b and has a lamellar packet stack in an early developmental stage, while the particle in Fig. 9b shows the stack in advanced stage. In most cases, two to four lamellar packets run roughly parallel with the major axis of the ellipse, and the lamellar packet stacks are observed in some parts of the lamellar packets. The number of lamellar packets found in a single stack or granum is from two to seven in most cases. Generally speaking, however, lamellar packets constructing a single granum in a large particle are more numerous than those found in a small particle.

Particles 10 and 11 are nearly ellipse in profile. Generally, the particle 10 is somewhat larger than the particle 11; that is, the former is 2.2 - 2.6μ and the latter is 2.5 - 3.1μ in diameter. Both the particles 10 and 11 are found only in I_3 part of leaves so far as the present study is concerned. These particles have one or two prolamellar bodies which show a lattice structure. Similar body structure was reported by some investigators (Leyon 1954, Heitz 1954 and 1956, Hodge et al. 1956). Fig. 10b shows the particle 10. In this figure, a prolamellar body showing a lattice structure is seen in the central part of the ground substance. The details of the lattice structure are shown in Fig. 10a. It is clear in this figure that the unit composing the lattice is pentagonal or hexagonal in outline and that the wall which forms the unit of the lattice is electron dense. The wall is about 140 A in breadth. The joining point of these walls is electron denser than the walls themselves. The density of the part which is enclosed in the wall of the lattice unit is nearly the same as that of the ground substance of the particle. The distance between the center of a lattice unit and that of the next one is 630 - 780 A. While the number of free lamellar packets is small, many lamellar packet stacks are observed arround the prolamellar body or the lattice structure in the particle 10 (Fig. 10b). Contrary to the above case, in the particle 11, many free lamellar packets and stacks extending radially from the prolamellar body are observed, and the lamellar packet stacks are seen here and there in the lamellar system as shown in Fig. 11. It must be stated that in some particles beolnging to the particle 11, the surrounding membrane of the particle protrude to the cytoplasm. This protrusion or sac runs parallel with the long axis of the particle. Sometimes, this protrusion embraces a mitochondrion.

Particle 12 is nearly ellipse in profile, and its longer axis is 3.5–3.9μ. This particle is found only in I_3 part of leaves. Fig. 12 shows an example of the particle 12. In this figure the lamellar packets are more developed than those of the particle 11, that is, they traverse the particle in parallel with the longer axis having the lamellar packet stacks in some places. Moreover, in this figure the prolamellar bodies (the lattice structure) are seen, but they are smaller than those found in the particle 11.

Most particles observed in both M_1 and M_2 parts of leaves are the particles 13 and 14. These particles are nearly ellipse in profile, and the particle 13 is 4.0 – 5.0μ and the particle 14 is 4.5 – 5.5μ in the longer axis. Fig. 13 and 14 show the particle 13 and 14 respectively. Both particles are characterized by the fact that they contain a developed lamellar system in their ground substance. In Fig. 13 which shows the particle 13, it is seen that the arrangement of the lamellar packets which compose a granum is somewhat irregular. But in the particle 14 which is shown in Fig. 14, a majority of the packet stacks are composed of fifteen to twenty lamellar packets and are regularly stacked.

The results of the observation described above are based on the materials fixed with KM_nO_4. Generally speaking, the structure of the lamellar system found in the O_sO_4 fixed materials is similar to that observed in the KM_nO_4 fixed materials, but there are some differences between these two materials. In the O_sO_4 fixed materials, there are several dense globules, which are found in the particles 7, 8, 10, 13 and 14. It is highly probable to consider that they are carotinoid globules which were isolated from *Spinach* chloroplast by MURAKAMI and TAKAMIYA (1962). Another difference is found in the grana parts. In the O_sO_4 fixed cells, electron dense minute droplets are found at both edges of the grana, though the nature of these droplets is not considered in the present study.

Discussion and conclusion

The process of the chloroplast development is discussed on the basis of the following two assumptions: (1) the size of chloroplasts increase accompanied by their development: (2) new chloroplasts are not formed from chloroplast initials in mature leaves.

As shown in Table 1, of all the particles observed in the present study, the particles 2, 4 and 6 are found in all or nearly all parts of leaves (B, I, O and M).

Particle 2 is surrounded by a single membrane and has a somewhat dense content. Similar particles have already been observed in several plants and are regarded as spherosomes (FREY-WYSSLING et al. 1963 and others). Chloroplasts, unlike the spherosomes, are surrounded by a double membrane which is an important characteristic of this cell organ. It is hardly possible, therefore, to regard the particle 2 as an initial of the chloroplast.

Particle 4 and particle 3 are similar in appearance; that is, they are about 1μ in diameter and are surrounded by a double membrane. The inner membrane of these particles invaginate the ground substance. Moreover they have a few small vesicles in the ground substance. So, it is very difficult to distinguish the particle 4 from the particle 3 only by these aspects. The structural characteristics of these two particles stated above are similar to those of mitochondria in plant cells. Results of the present study show that the particle 4 is found in all parts of leaves from B to M_2 (see Table 1). It is not probable to regard this particle 4 as the chloroplast initial, because this particle is found not only in young leaves but also in mature leaves (contrary to the assumption 2).

Particle 3, on the other hand, is found only in B, I_1, I_2, O_1 and O_2 parts of leaves. Electron density of the membrane of this particle is higher than that of the particle 4, and is similar to that of all other particles in the Table 1 except the particle 4.

On the bases of the facts stated above, the particle 4 is regarded as a particle other than the chloroplast initial and may be considered as a mitochondrion, so far as the particles known in plant cells are concerned. The particle 3 is, as discussed later, considered to be the chloroplast initial.

Particle 6 has a large circle in its ground substance, and is found in all parts of leaves except I_3. This fact means that the particle 6 can hardly be regarded as the chloroplast initial, because new chloroplasts are not formed from chloroplast initials in mature leaves (the second assumption). From the above discussion, it is hardly probable that the particles 2, 4 and 6 are immature chloroplasts.

Contrary to these particles, particles 1, 3, 5, 7, 8, 9, 10, 11, 12 and 13 are regarded as the chloroplast initials or the immature chloroplasts, because they have a double membrane and are found only in certain limited parts of the leaves (see Table 1).

As far as the present study is concerned, the particle 1 which is found in B is the smallest. It has been assumed that the size of the chloroplasts increases accompanied by their development (the first assumption). Therefore, the particle 1 is regarded as one of the chloroplast initials in the youngest stage of development.

As will be discussed below, the particle 1 is considered to develop into the particle 3, because the particle 3 is found not only in B but in I_1, I_2, O_1 and O_2 parts of leaves, and is larger in size than the particle 1 (cf. the assumption 1). To put it in the other words, the particle 1 develops into the particle 3 accompanied by the invagination of the inner membrane into the ground substance. The small vesicles found in the ground substance of the particle 3 is probably formed by blebing off the invaginated membrane (Fig. 4). This view is quite in accord with that presented by MÜHLETHALER et al. (1959), MENKE (1960b) and others. MÜHLETHALER et al. found particles of 0.02 — 0.2μ in diameter in apical meristem cells of *Elodea*, and regarded them as the particles at the early stage of chloroplast development. These particles are similar in structure to the particle 1 found in *Tradescantia* in the present study except for the particle size.

The developmental process of the chloroplasts from the particle 3 to mature chloroplasts may be considered from two main view points.

In the first place, the developmental process of the chloroplasts is considered only on the basis of the particle size. According to the assumption 1, the process of the development is as follows: 1 - 3 - 5 - 7 - 8 - 9 - 10 - 11 - 12 - 13 - 14. This process is called the S_1 - process in this paper for the sake of convenience. On the basis of the inner structure of the particles arranged in this process, it is found that there are some gaps in the serial changes of the development. These gaps or discontinuities are found between the particle 8 and 9, and also between the particle 9 and 10. Consequently, it is not reasonable to consider the developmental process only from the view point of the particle size.

Secondly, the developmental process may be considered on the bases of the particle size and the parts of leaves where the particles are found. In leaves about 13 mm in length (I and O), it may be generally stated that cell age of the

(B) -(I) -(M)

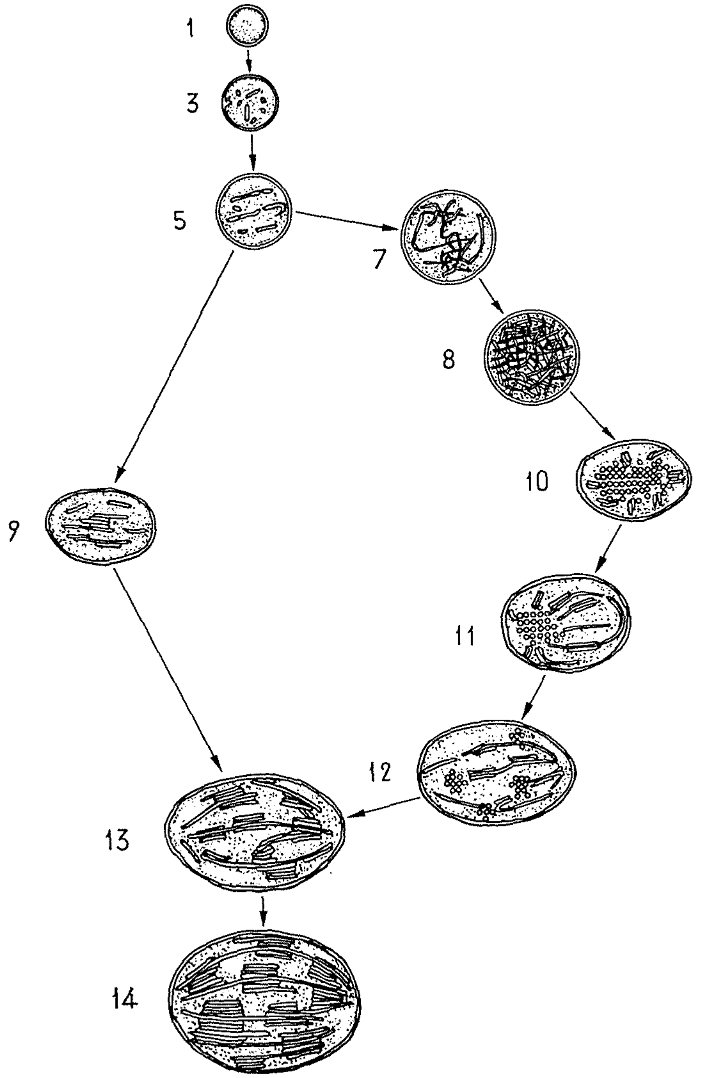
B	(1)	(3)								
I_1		(3)	(5)							
I_2		(3)	(5)	(7)	(8)					
I_3						(10)	(11)	(12)		
M_1									(13)	(14)
M_2									(13)	(14)
	(1)	-(3)	-(5)	-(7)	-(8)	-(10)	-(11)	-(12)	-(13)	-(14)……S_2-process

(B) -(O) -(M)

B	(1)	(3)				
O_1		(3)	(5)			
O_2		(3)	(5)	(9)		
O_3				(9)	(13)	
M_1					(13)	(14)
M_2					(13)	(14)
	(1)	-(3)	-(5)	-(9)	-(13)	-(14)…………………………………… S_3-process

Table 2. Young and mature chloroplasts are arranged with respect to their size, and parts of leaves in which particles are found. B : bud, I and O : inner and outer leaves about 13 mm length, M: mature leaves

outer leaf (O-series) of the leaves is older than that of the inner leaf (I-series), but whether I_3 is younger than O_1 or not, is not confirmed in the present study. Therefore, the developmental process may be studies independently, that is, series B-I-M including the inner leaf and the series B-O-M including the outer leaf. On the bases of the particle size and the parts of leaves where the particles are found, the particles may be arranged in series B-I-M or S_2-process and in series B-O-M or S_3-process as shown in Table 2



Text-fig. 2. Schema of processes of chloroplast development.

and Text-fig. 2.

As shown in Text-fig. 2, there is no severe structural deformation and rearrangement in both the S_2 and S_3 processes. The main difference between these two processes is found in the point that the aggregation of lamellar packets (formation of the prolamellar body) takes place in the process S_2, while such an aggregation is not found in the process S_3.

MÜHLETHALER and FREY-WYSSLING (1959) has concluded that under growth conditions with sufficient exposure to light, the development of the grana and stroma lamellae proceeds without interruption. If the plants are kept in the dark, small vesicles are formed which accumulate in the prolamellar body. After illumination these elementary vesicles merge to form membranes which evolve into grana and stroma lamellae.

The view presented by these authors is in accord with the result obtained in the present study in the foundamental process, but it is not stated decidedly here, whether the S_2 process takes place only in the leaves which are insufficently illuminated or not.

The processes of the chloroplast development in *Tradescantia* is tentatively concluded as follows: there are two different processes, S_2 and S_3, in the chloroplast development (see Text-fig. 2). These processes are constructed on the bases of the particle size and the parts of the leaves where the particles are found.

Particle 1 (Fig. 1) is regarded as one of the youngest chloroplast initials because it is the smallest particle and surrounded by a double membrane. Moreover, it has no vesicle in the ground substance. As the particle size increases the inner membrane of this double membrane begins to invaginate the ground substance of the particle. Then the protrusion blebs off and formes several vesicles (P_3 in Fig. 3). These vesicles, fusing end to end, seem to form the lamellar packets (P_5 in Fig. 5). Following this stage, the behavior of the lamellar packets in the young chloroplasts of inner leaves (I) are different from that of outer leaves (O). In the S_2-process which is found in the inner leaves, the process of the chloroplast development is accompanied by the formation of the prolamellar body which is formed by the aggregation of the lamellar packets (P_7 and P_8 in Fig. 7 and 8, respectively). Then the stacks of lamellar packets and free lamellar packets are found surrounding the prolamellar body (P_{10} in Fig. 10) which changes into lamellar packets locally having grana stacks (P_{11} in Fig. 11). During these changes, the prolamellar body becomes smaller and gradually disappears accompanyed by the increase of the packets in number. In this stage, the packets are arrayed traversing the chloroplast, and form both the grana stacks and intergranal packets (P_{12} in Fig. 12). In this way, the whole lamellar system is completed in the chloroplast (P_{13} and P_{14} in Fig. 13 and 14 respectively).

In the S_3-process which is found in the outer leaves, the prolamellar body is not formed during the chloroplast development. That is, the lamellar packets in the particle 5 increase in number followed by the lamellar packet stacking in some places (Fig. 9, particle 9). As the particle grows larger, the lamellar stacks increase in number and develop into grana stacks (Fig. 13, particle 13). Through this developmental process, the orderly arranged lamellar system in the mature chloroplast is completed (Fig. 14, particle 14).

It must be noted that the origin of the youngest chloroplast initial or the particle 1 in this study has been discussed by MÜHLETHALER et al. (1962), BADENHUIZEN (1962), MENKE et al. (1964), DIERS (1964) and others, but there seems to be no agreement in their views. In the present study, a particle surrounded by a double membrane smaller than the particle 1 was not found.

Summayr

(1) The developmental process of *Tradescantia* chloroplast was studied with an electron microscope.

(2) All the particles surrounded by a membrane found in the mesophyll cells of leaves from young to mature were studied.

(3) Particles which were regarded as the young chloroplast were arranged from the viewpoint of the particle size, and parts of leaves where the particles were found.

(4) Two processes, the S_2 and S_3 processes, at least, are found in the chloroplast development. In both cases, small particles (ca. 0.5 – 0.8μ in diameter) surrounded by a double membrane are regarded as the youngest chloroplast initials. In the S_2-process, these initials develop to the mature chloroplasts accompanied by the formation of prolamellar bodies, while in S_3, the development is completed without the formation of these bodies. Causal analysis of the occurrence of these two processes is under investigation.

Acknowledgement

The author wishes to express his sincere thanks to Prof. N. SHINKE for his valuable advice and guidance throughout this work.

References

BADENHUIZEN, N. P., 1962. Canad. J. Bot., **40**: 861.

DIERS, L., 1964. Ber. Dtsch. Bot. Ges., **27**: 369.

FREY-WYSSLING, A., E. GRIESHABER and K. MÜHLETHALER, 1963. J. Ultrastr. Res., **8**: 506.

HEITZ, E., 1954. Exp. Cell Res., **7**: 606.

——————, 1956. Experientia, **12**: 467.

HODGE, A. J., J. D. MCLEAN and F. V. MERCER, 1956. J. Biophys. Biochem. Cytol., **2**: 597.

LEYON, H., 1954. Exp. Cell Res., **7**: 265.

MENKE, W., 1959. Z. Naturforsch., **14**: 394.

——————, 1960a. Z. Naturforsch., **15**b: 479.

——————, 1960b. Z. Naturforsch., **15**b: 800.

MENKE, W. und B. FRICKE, 1964. Z. Naturforsch., **19**b: 520.
MOLLENHAUER, H. H., 1959. J. Biophys. Biochem. Cytol., **6**: 431.
MURAKAMI, S. and A. TAKAMIYA, 1962. Insti. Appli. Microbio. Symp., **3**: 223.
MÜHLETHALER, K., 1955. Protoplasma, **45**: 264.
MÜHLETHALER, K. und A. FREY-WYSSLING, 1959. J. Biophys. Biochem. Cytol., **6**: 507.
MÜHLETHALER, K. und P. R. BELL, 1962. Naturwiss., **49**: 63.
PERNAR, E. S., 1956a. Z. Naturforsch., **11**b: 560.
——————, 1956b. Z. Naturforsch., **11**b: 567.
WETTSTEIN. D. VON, 1958. Brookha. Symp. Biol., No. **11**: 138.

Explanation of Plates

Abbreviations

e; endoplasmic reticum, g; golgi apparatus, gr; grana stack, lm; lamellar packet stack, ls; lattice structure, n; nucleus, ne; nuclear envelope, t; tonoplast, v; vacuole, w; cell wall

Plate I

Fig. 1. The particle 1 (P_1) in Table 1. The particle 1 surrounded by a double membrane has no vesicle in the ground substance. The electron density of the ground substance is medium, nearly the same as that of the cytoplasm. x 3, 100

Fig. 2. The particle 2 (P_2) in Table 1. The particle 2 surrounded by a single membrane has no vesicle. Density of the ground substance is medium but higher than that of the particle 1. x 25, 000

Fig. 3. The particle 3 (P_3) observed in I_1. Some vesicles are observed in the ground substance. x 30, 000

Fig. 3a. An enlargement of a lower left part of the particle 3 (P_3). The inner membrane invaginates, and the protrusion blebs off. x 65, 000

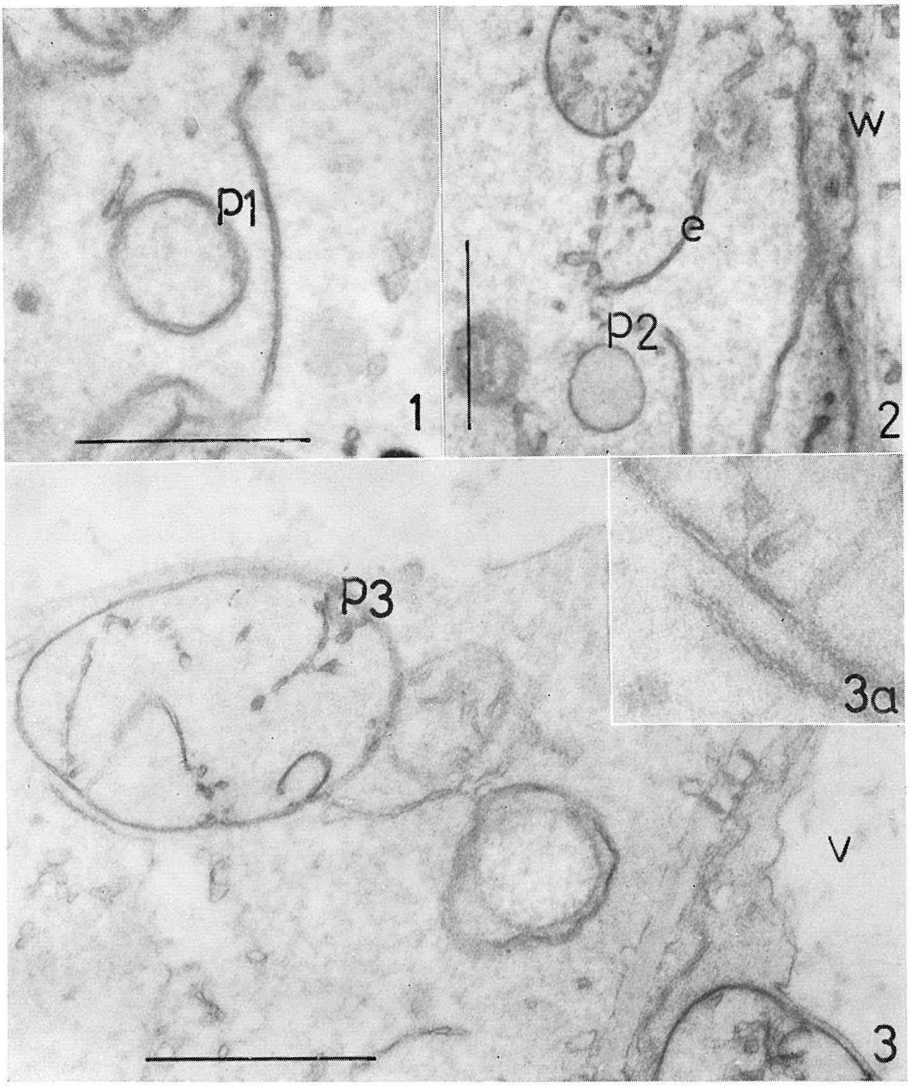

P1
1
W
e
P2
2
P3
3a
V
3

Plate II

Fig. 4. The particle 3 (P_3) adjoining the particle 4 (P_4) in a single cell observed in B. Both particles are surrounded by a double membrane, and parts of their inner membrane are clearly observed to invaginate. (indicated by arrows), The double membrane and the membrane composing the vesicle of the particle 4 are low in electron density, in comparison with those of the particle 3. x 63,500.

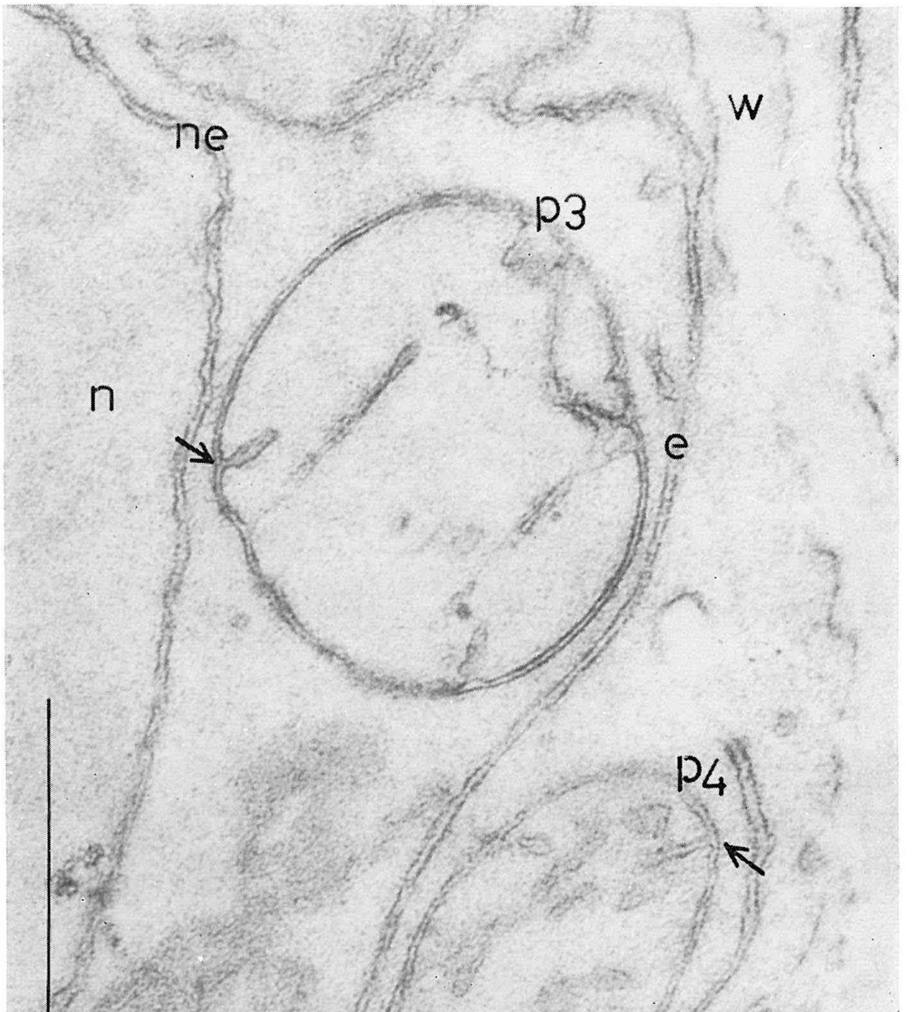
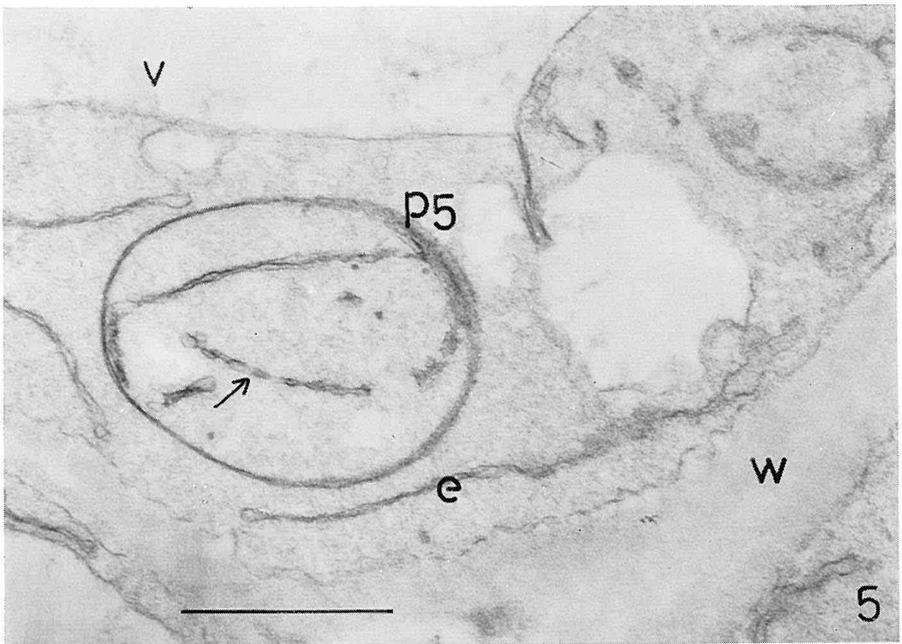

ne
w
p3
n
e
p4
4

Plate III

Fig. 5. The particle 5 (P_5) in Table 1. In the ground substance, there are several vesicles with a linear arrangement (indicated by an arrow) and a structure regarded as a profile of a sac or a tubule. x 28,000

Fig. 6. The particle 6 (P_6) in Table 1. The characteristic of this particle is that it has in its ground substance a large circle. In ground substance electron less dense portions are seen. x 20,000


v
P5
e
w
5

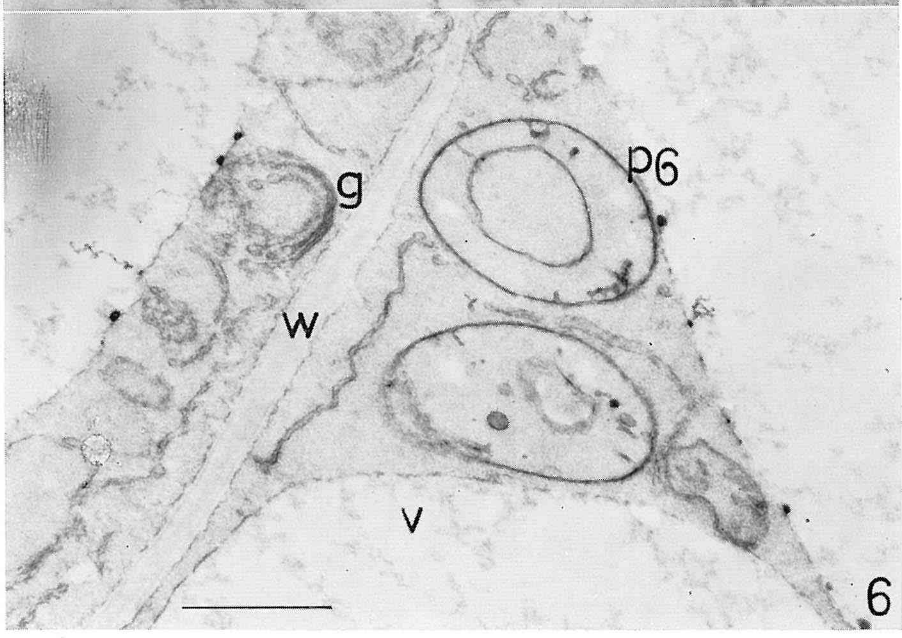

g
P6
w
v
6

Plate IV

Fig. 7. The particle 7 (P_7) in Table 1. In the ground substance of this particle, the distribution of the lamellar packets is uneven and they are seen to aggregate in a part or some parts. x 25, 000

Fig. 8. The particle 8 (P_8) in Table 1. Lamellar packets distribute rather evenly throughout the ground substance taking a reticulate appearance. x 22, 000

Figs. 9a and 9b. Particle 9 (P_9). The particle in Fig. 9a is smaller than that in Fig. 9b and has a lamellar packet stack in an early developmental stage, while the particle in Fig. 9b shows the stack in advanced stage.

Fig. 9a x 22, 000 Fig. 9b x 25, 000

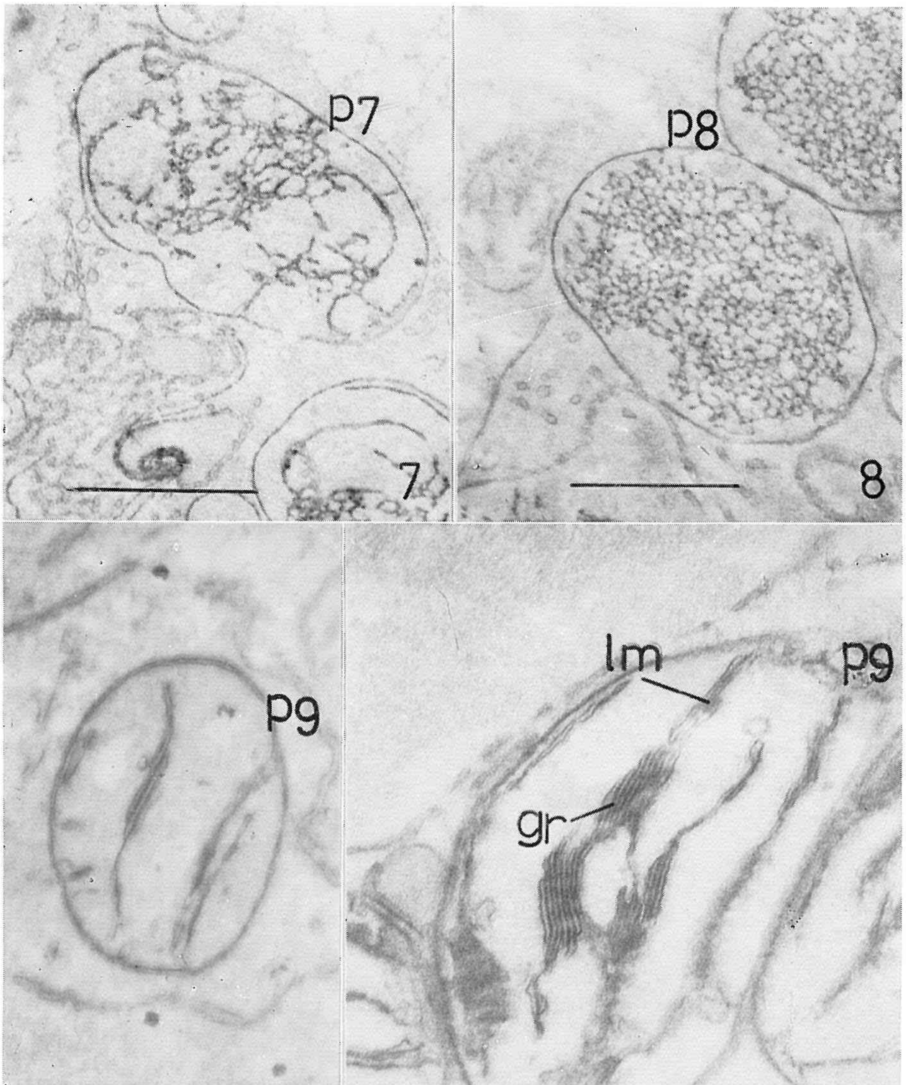

p7
7
p8
8
pg
9a
lm
pg
gr
9b

Plate V

Fig. 10a. Lattice structure in the particle 10 (P_{10}). The unit composing the lattice structure in the particle 10 is pentagonal or hexagonal in this profile. x 38,000

Fig. 10b. The particle 10 (P_{10}). Grana stacks are observed around the lattice structure. x 28,000

Fig. 11. The particle 11 (P_{11}). Lamellar packets and packet stacks extend radially from the prolamellar body. x 30,000

Fig. 12. The particle 12 (P_{12}). A comparatively developed lamellar system traverses the ellipse particle, and the grana stacks are seen in some portions, but the lattice structure are small and are found locally in the lamellar system (indicated by arrows). x 13,000

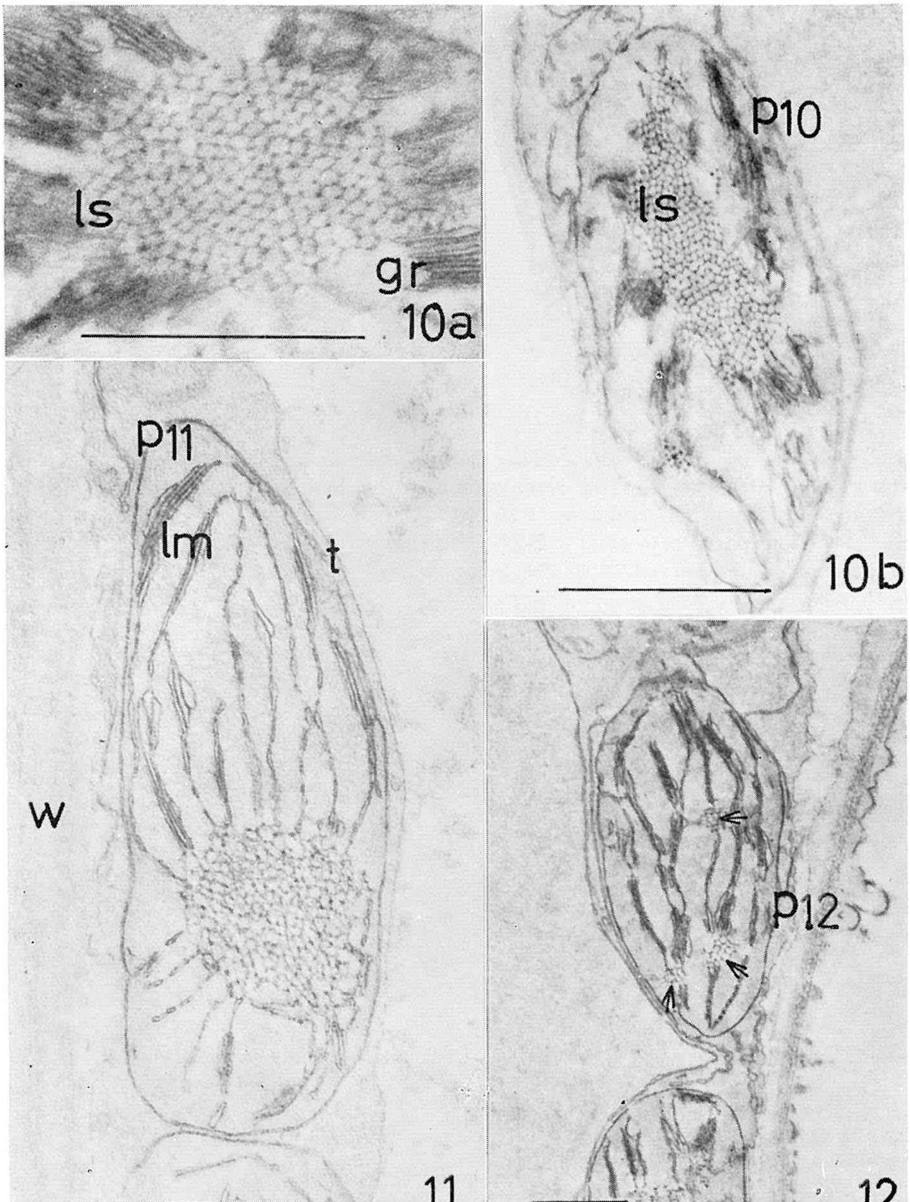
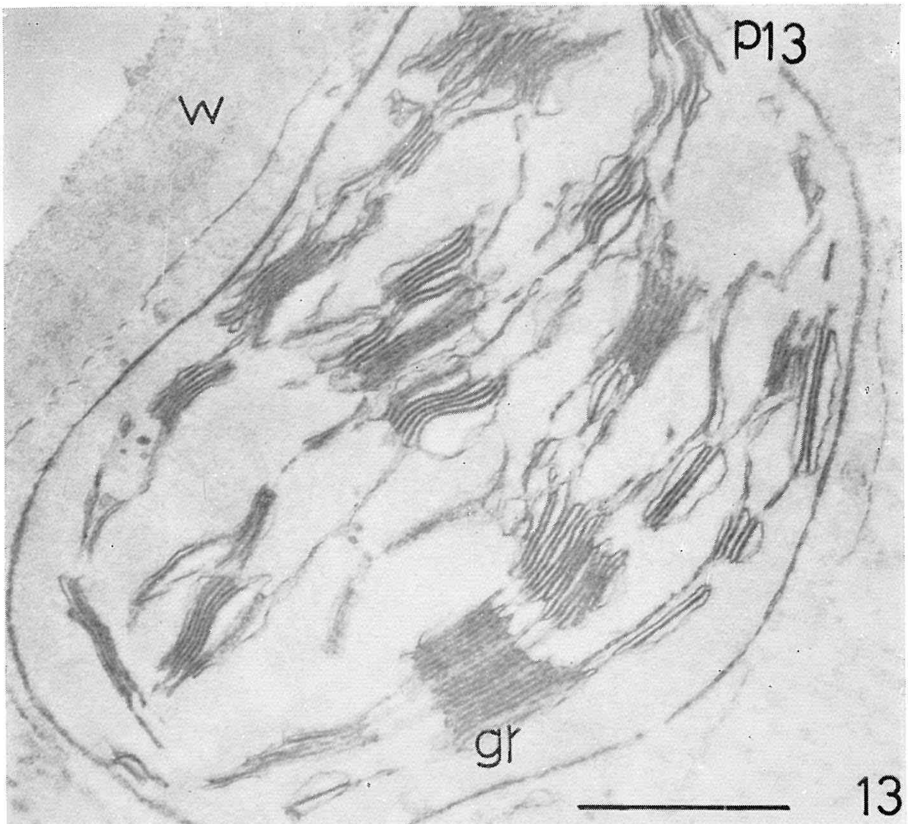
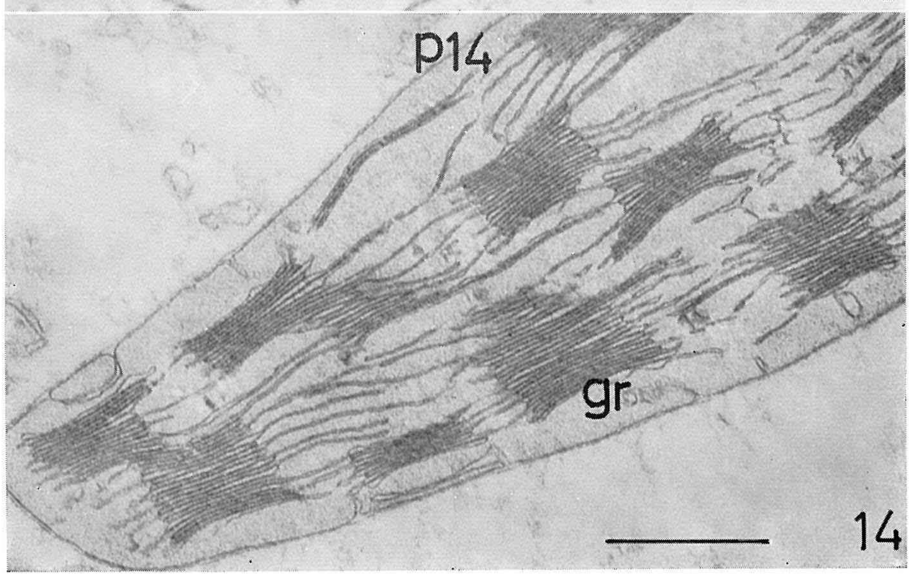

ls
gr
10a
P10
ls
10b
P11
lm
t
w
11
P12
12

Plate VI

Fig. 13. The particle 13 (P_{13}). This particle has a developed lamellar system in the ground substance. But the arrangement of the lamellar packets which compose a granum is somewhat irregular. x 26, 000

Fig. 14. The particle 14 (P_{14}). In the lamellar system of this particle developed grana stacks are observed. x 20, 000


P13
W
gr
13


P14
gr
14