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FINE STRUCTURE OF DEVELOPING CHROMOPLASTS IN OUTER YELLOW FRUIT PARTS OF *CUCURBITA PEPO* CV. *PYRIFORMIS*

Mit deutscher und kroatischer Zusammenfassung
Sa sadržajem na njemačkom i hrvatskom jeziku

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Introduction

In contrast to other plastids the chromoplasts exhibit a great variability in their fine structure. In spite of the considerable number of papers from this field of research for the majority of chromoplast types, it is not possible at this moment to give a clear picture of their development and structure.

The variously shaped and coloured fruits of the family *Cucurbitaceae* contain, according to the papers of Matienco (1964) and Grilli (1965a, 1965b and 1965c), also many types of chromoplasts. The majority of hitherto published papers deals with chromoplasts, which originate from chloroplasts. In the ornamental pumpkin *Cucurbita pepo* cv. *pyriformis* (briefly *C. p. pyriformis)* there is a part of the fruit, which is originally yellow, i. e. which has never been green, so that in this part the chromoplasts necessarily develop from proplastids. This phenomenon makes it possible to study the fine structure of such plastids from their developmental stage of the proplastid to the stage of the mature chromoplast in disintegration (lysis).

Material and Methods

For the present investigations the outer cell layers of the yellow portion of the fruits of *Cucurbita pepo* cv. *pyriformis* were used. The plants were grown in garden conditions. The samples for the investigations were taken several times: before the flowering, immediately after the flowering, in certain intervals during the growth and ripening of the fruit and finally just before the decay of the fruit. The material was investigated by the light microscope as well as by the electron microscope.

For electron microscopic investigations the material was fixed in 0,5% glutaraldehyde (in cacodylate buffer — pH = 7,2) for 30 minutes. After fixation it was washed for 1—2 hours in cacodylate buffer and then postfixed in 1% OsO_4 for 2 hours. The fixed material was dehydrated in ethanol. The procedure of fixation, washing, postfixation and dehydration was carried out at a temperature of 1°C. Before the embedding of the material in araldite the ethanol was replaced either by aceton or by propylenoxide. The sections were made on Reichert Om U2 ultramicrotome and stained by means of lead citrate (Reynolds 1963). All photographs were made with a Siemens Elmiskop I (at the Institute of Biology, University of Zagreb).

Results

Even before the flowering it is possible to see on the ovary the yellow zones from which the yellow parts of the fruit will later develop. In spite of the fact that this tissue is thouroughly yellow, there are no typical chromoplasts in it. In the light microscope only small plastids are visible, and it is difficult to state whether they are coloured or not. By electron microscope only propastids of normal structure with relatively big starch grains (Fig. 1) have been seen. After the flowering the fruit grows very rapidly. The yellow parts of the fruit turn now intensely yellow. By means of the light microscope it has been found that in the cells under the epidermis there are numerous, relatively big and clearly yellow chromoplasts. Proplastids are still present only in the epidermal cells. The electron microscope has shown that the chromoplasts are of oval shape and 5—6 µm long (Fig. 2). The stroma is dense and rich with ribosomes. In the section almost each chromoplast has peripherally located one or two clumps of tubules (Fig. 2) the so-called tubular complex (Newcomb 1967) or thylakoid plexus (Spurr and Harris 1968). In the remaining space of the chromoplast there are some perforated thylakoids. These thylakoids are sometimes present in many layers, but they never contain any formation, which would resemble granal structures. Unperforated thylakoids were rarely found, but they are sometimes present even in many layers. Plastoglobules are scarce, small and limited to the regions of tubular complexes (thylakoid plexuses).

The macroscopically visible yellow portion of the ripe fruit looks exactly like in the previous stage. The light microscope shows a nearly unchanged picture as well. The cell walls are thicker and the cells overcrowded with oval chromoplasts. The electron microscopic observations show however that similarity with the previous stage exists only in the shape and size of the plastids, the ultrastructure being essentially changed (Fig. 3). Along the border of the chromoplast in the stroma rich with ribosomes there are usually 3—10 layers (in the section) of characteristically perforated thylakoids (Fig. 5). In the cross section these thylakoids give a picture of series of more or less elongated vesicles (resembling beaded chains) the ends of which overlap a bit. In the central part of the chromoplast there are several single, also perforated thylakoids bent in different directions. On the electron micrographs the neighbouring thylakoid parts may be apparently in contact by special characteristic bridge-like connections (Fig. 3, 4, 5 and 6). In the regions of such connections the thylakoids usually change the direction of their plane so that it is possible to observe all transitions from perpendicular sections through to the parallel ones. Thylakoids sectioned perpendicularly to their surface show figures consisting of beaded chains i. e. series of vesicles with a diameter of about 20 nm apart from each other by about 12 nm. Thylakoids sectioned parallelly with their surface show, in the region of bridge-like connections, figures indicating the presence of longitudinally orientated formations resembling tubules (Fig. 4).

In addition to perforated thylakoids narrowly packed layers of unperforated thylakoids of different length also frequently occur (Fig. 6 and 7). With progressing senescence these layers are accumulating and are packed more and more densely so that finally the whole layer resembles a single thylakoid having a dark lumen without any striation. Along the border of the chromoplast single tubules, or rarely groups of them occur (Fig. 5).

The plastoglobules have a diametar of 0,2—0,5 μm and are rather numerous. They usually appear in groups separated by some thylakoids (Fig. 3 and 5). They have a low osmiophilie.

In some regions the stroma is denser and does not contain any ribosomes (Fig. 5). The origin and the function of this structure has not been explained.

Two or three months after the ripening of the fruit the outer cell layers begin to dry out. The colour of the fruit becomes darker. The light microscope does not show any distinct chromoplasts, the whole protoplast being homogeneous and yellow. The examination of ultrastructure shows that all lamellar systems are disintegrated by this process. The thylakoids are at first broken down to small isolated fragments, which later disappear completely. Eventually the outer membrane of the plastid is torn and the chromoplast disintegrated thoroughly (Fig. 8) the numerous plastoglobules remaining as the last conspicuous feature.

Discussion

The fine structure of the plastids from the originaly yellow part of *C. p. pyriformis* does not show any similarity with the chromoplast structure found by other authors (Grilli 1965a, 1965b, 1965c, Matienco 1964) in the fruits of *Cucurbitaceae*.

The chromoplasts of *C. p. pyriformis* are however very similar to the chromoplasts of the fruits of *Capsicum annuum* (Spurr and Harris 1968) in spite of their different origin. The chromoplasts of *Capsicum annuum* develop namely from normal green chloroplasts; those from the yellow part of the fruit of *C. p. pyriformis* originate however directly from the proplastids. This yellow part is originally yellow and does not contain chloroplast-like plastids in any developmental stage as they occur in the green parts of the fruit. The small, densely packed layers, existing in chromoplasts of the yellow part of the ripe fruit cannot be considered to be typical granal structures.

The similarity in the structure of the chromoplasts in *C. p. pyriformis* and *Capsicum annuum* exists mainly in the resemblance of fine structure of single thylakoide. In both kinds of fruits namely the chromoplast thylakoids are perforated. Spurr and Harris (1968) consider that the thylakoids contain perforations (holes) of a diameter of 18—36 nm. According to my own observations in *C. p. pyriformis* the size of the thylakoid perforations would vary to a great extent, the oblong holes being slot shaped.

The so-called bridge-like connections seem to be of a special interest. Schötz, Diers and Bathelt (1968) have found similar connections between thylakoids in some mutants of the genus *Oenothera,* and have solved their three dimensional view. But they do not describe any perforation in the region of the bridge-like connections. In the case of *Cucurbita,* however the thylakoids are obviously discontinued in the region of bridge-like connections.

The following important question is, in which structures the pigments are localized. Macroscopically the colour of the yellow fruit part does not change during its development, though big changes in the fine structure occur. The tissue is namely equally yellow when its proplastids have only some tubules and few orimental thylakoids and when the ripe fruit contains chromoplasts with abundantly developed thylakoid system. Futhermore, the fruit tissue is equally yellow even when in the disintegrating chromoplasts there are still only plastoglobules.

In this connection the use of the term »proplastid« for the plastids in young *C. p. pyriformis* fruits apears to be doubtful. Should they be called proplastids according to their ultrastructure or should they be called chromoplasts as they may contain pigments giving the tissue the clear yellow colour?

Summary

The changes in the yellow part of the fruit of *Cucurbita pepo* cv. *pyriformis* were followed by means of light and electron microscopy. In the earliest stages only proplastids have been found though the tissue appears thouroughly yellow. The proplastids develop into chromoplasts, which have a well developed clump of tubules and very few thylakoids. The chromoplasts of the ripe fruit contain a well developed system of specially perforated thylakoids which are connected in some places by means of characteristic bridge-like formations. The explanation of their structure in three dimensions is attempted. During the decay of the fruit the thylakoids disintegrate at first, and finally the structure of the whole chromoplast breaks down.

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ZUSAMMENFASSUNG

FEINBAU DER ENTWICKLUNGSSTADIEN VON CHROMOPLASTEN DER ÄUSSEREN GELBEN FRUCHTPARTIEN VON *CUCURBITA PEPO* CV. *PYRIFORMIS*

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Veränderungen des Feinbaues im gelben Teil der Frucht von *Cucurbita pepo* cv. *pyriformis* wurden vor dem Blühen bis zur Fruchtverwesung verfolgt. In den frühesten Stadien wurden nur Proplastiden vorgefunden, obschon das Gewebe völlig gelb ist. Parallel mit dem Fruchtwachstum entwickeln sich die Proplastiden in Chromoplasten, die sich durch gut entwickelte Knäuel von Tubuli und sehr spärliche Thylakoide auszeichnen. Die reife Frucht enthält Chromoplasten mit einem reich entwickelten System von mehrschichtigen und einschichtigen perforierten Thylakoiden. Diese Thylakoide sind gegenseitig durch charakteristische Übergänge verbunden. Es wurde versucht den räumlichen Bau dieser Verbindungen zu erklären. Während der Verwesung der Frucht kommt es zunächst zur Fragmentierung der Thylakoide in kleinere Abschnitte und daraufhin zu ihrem völligen Abbau, so dass in seinem Endstadium der Chromoplast nur noch Plastoglobuli enthält.

SADRŽAJ

FINA GRAĐA RAZVOJNIH STADIJA KROMOPLASTA VANJSKOG ŽUTOG DIJELA PLODA VRSTE *CUCURBITA PEPO* CV. *PYRIFORMIS*

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Praćene su promjene fine građe u žutom dijelu ploda vrste *Cucurbita pepo* cv. *pyriformis* od prije cvatnje pa do ugibanja ploda. U najranijim stadijima pronađeni su samo proplastidi, iako je tkivo posve žuto. Usporedo s rastom ploda proplastidi prelaze u kromoplaste koji imaju dobro razvijeno klupko tubula, a vrlo malo tilakoida. Zreli plod sadrži kromoplaste s dobro razvijenim sistemom višeslojnih i jednoslojnih perforiranih tilakoida. Ovi tilakoidi se međusobno povezuju karakterističnim vezama. Pokušala se razjasniti njihova prostorna građa. Propadanje ploda ima za posljedicu najprije trganje tilakoida u manje odsječke a potom njihovu potpunu razgradnju, tako da plastidi sadrže samo plastoglobule.

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Explanation of figures

Fig. 1.—8. *Cucurbita pepo* cv. *pyriformis*. Plastids from the outer cell layers of the yellow part of the fruit.

Fig. 1. Proplastid of a normal ultrastructure from the yellow part of the ovary before flowering. 32 000 : 1.

Fig. 2. Chromoplast from the yellow part of the fruit immediately after flowering. Besides two tubular complexes (tc) localized peripherically, there are only few single tubules and thylakoids. 23 000 : 1.

Fig. 3. Chromoplast from the ripe fruit. Along the border there are multilayered perforated thylakoids, and single perforated thylakoids in the center. Both contain characteristic bridge-like connections (↑). Small bundles of unperforated thylakoids (t) as well as a few groups of plastoglobules (g) are also present. 26 500 : 1.

Fig. 4. Detail of Fig. 3. Bridge-like connections. 60 000 : 1.

Fig. 5. Chromoplasts from the ripe fruit with single and multilayered perforated thylakoids. On the border of the chromoplast a group of tubules (tu). Region of stroma without ribosomes (s). Plastoglobules in groups. 50 000 : 1.

Fig. 6. Portion of a chromoplast from a ripe fruit. Beside perforated thylakoids and bridge-like connections (↑) there are also densely packed unperforated thylakoids between them. 56 000 : 1.

Fig. 7. Same as in Fig. 6. 40 000 : 1.

Fig. 8. Plastid from a fruit before its complete decay. The thylakoids are thoroughly disintegrated. Only plastoglobules are present. 29 000 : 1.

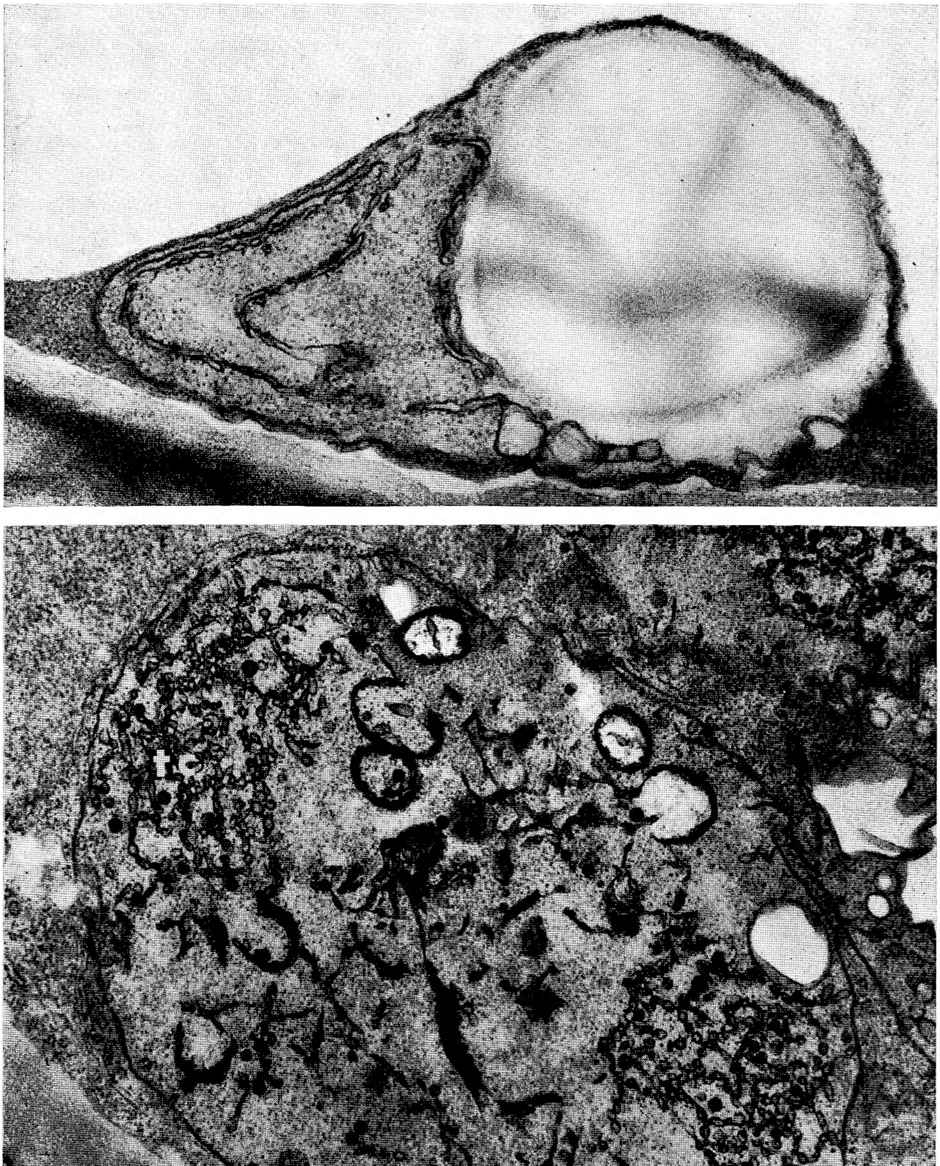

Fig. 1—2.

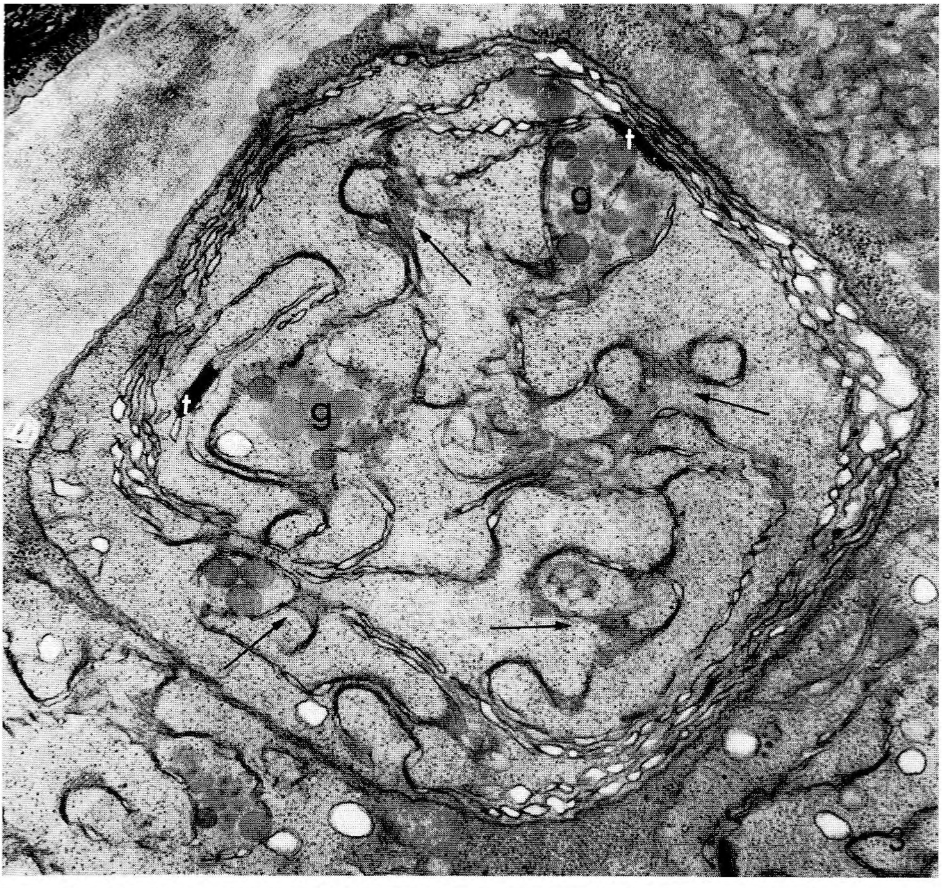

Fig. 3—4.

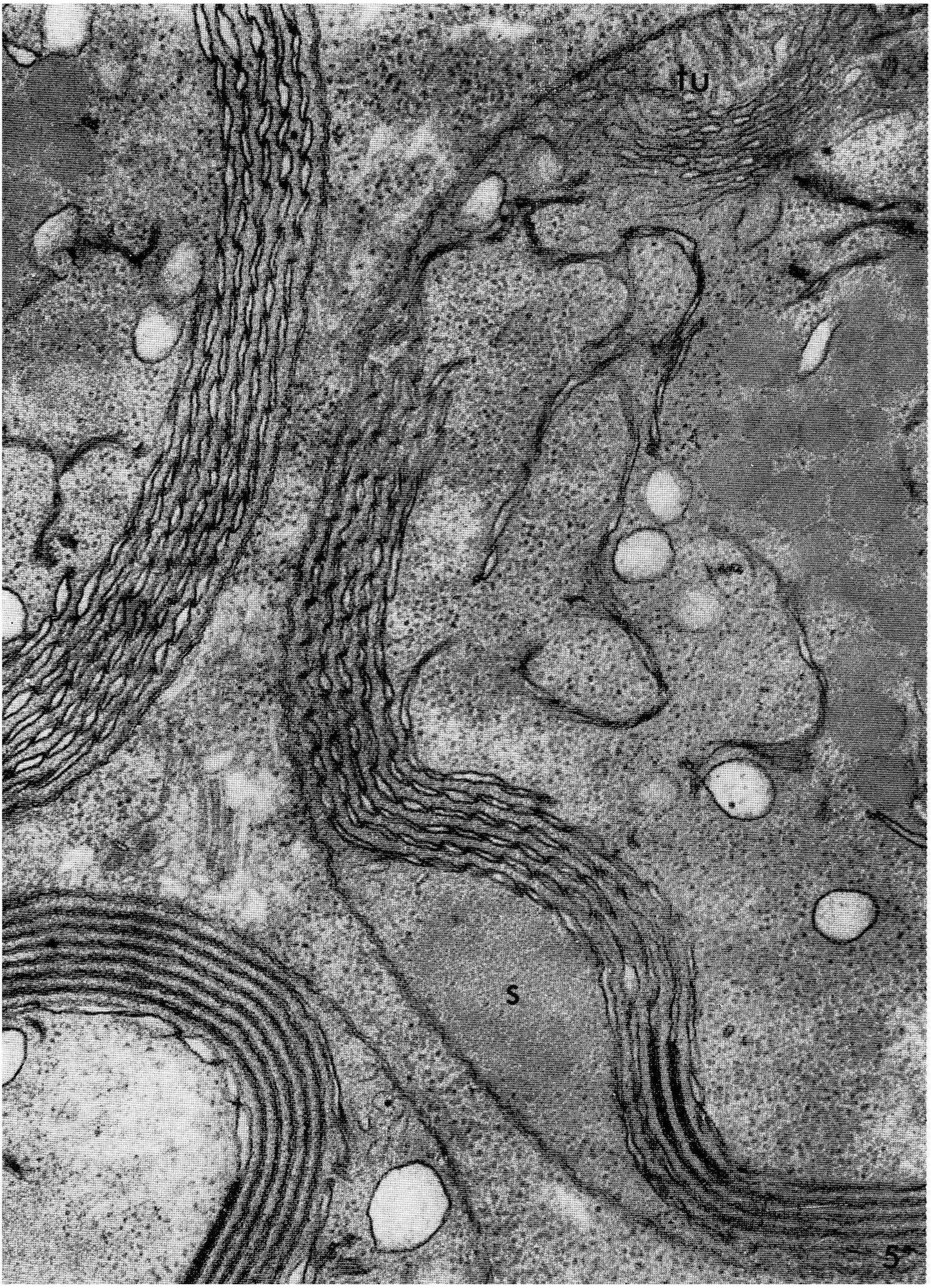

Fig. 5.

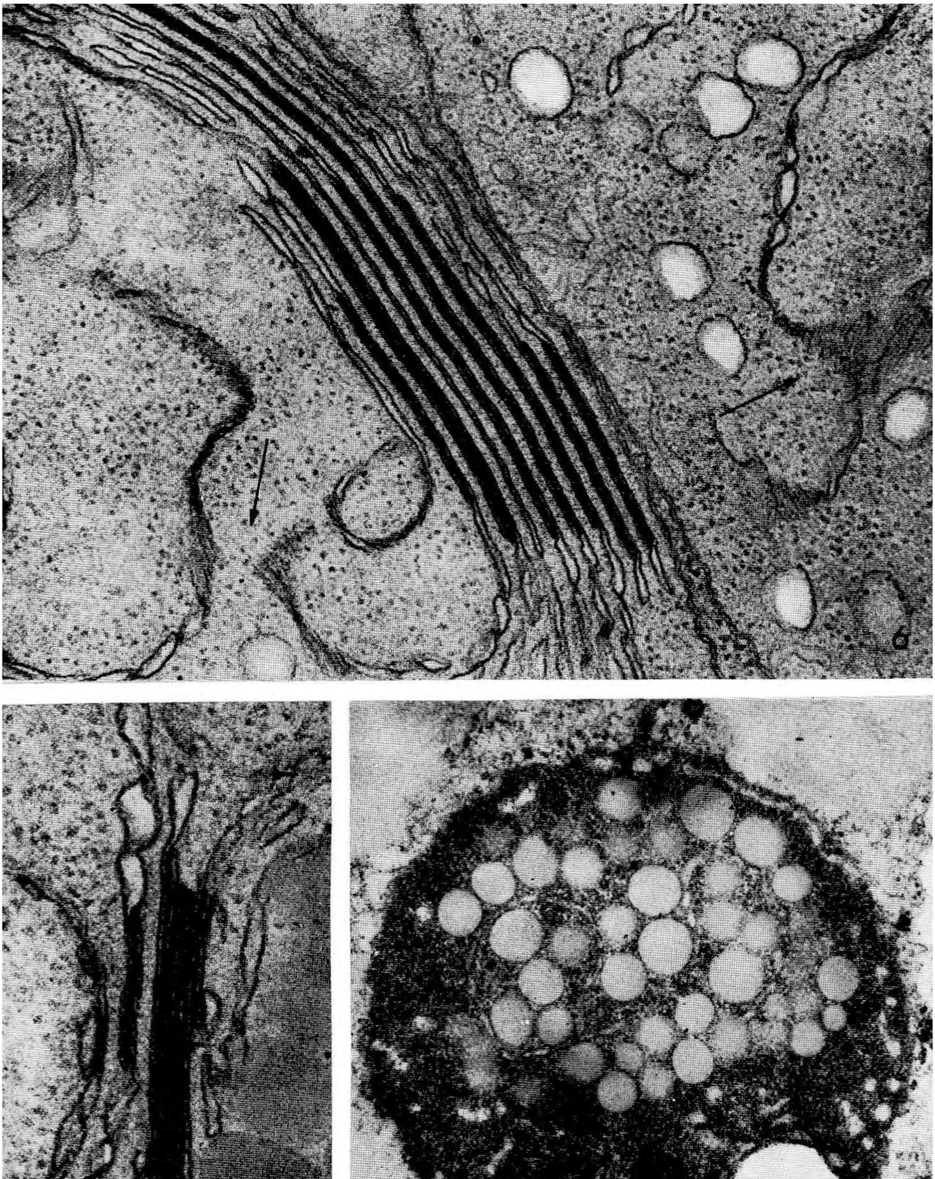

Fig. 6—8.