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Fine structure of lipotubuloids (elaioplasts) in *Ornithogalum umbellatum* L. I.

M. KWIATKOWSKA

Institute of Biochemistry and Physiology, University of Łódź, Laboratory of Plant Cytology and Cytochemistry, Łódź, Nowopołudniowa 12/16, Poland

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Abstract:

The bodies occurring in the ovary epidermis cells of *Ornithogalum umbellatum*, rich in lipids have been earlier described as elaioplasts. They consist of agglomerations of osmiophilic granules within the cytoplasm, caught in a network of, and interconnected by, a system of interesecting microtubules. These structures have been named lipotubuloids. They translocate in the cell by way of a progressive-rotary motion. Endoplasmic reticulum membranes, ribosomes and very scarse mitochondria and Golgi structures occur within the lipotubuloids.

INTRODUCTION

It was found earlier in intravital investigations (Kwiatkowska, 1966) that elaioplasts occurring in the ovary epidermis of *Ornithogalum umbellatum* are aggregations of numerous highly refractive granules ca. 0.4 μ in size, defined as osmiophilic granules. Cytochemical reactions revealed in these granules the presence of neutral lipids and phospholipids and of a protein component. Cytoenzymatic methods indicated the occurrence within the elaioplasts of hydrolytic enzymes such as acid phosphatase, lipase, nonspecific esterase as well as alkaline phosphatase (Kwiatkowska, 1966).

Observation of the fine structure gave results confirming the earlier advanced conclusions that the structures described in *O. umbellatum* are not plastides, neither have they any genetic connection with the latter. The investigations revealed a new element in the elaioplasts of *Ornithogalum*, a profuse system of microtubules closely connected with the osmiophilic granules known from intravital observations.

In view of the fact that the so far used term "elaioplasts" incorrectly suggests a plastide character of the structures discussed, a new name — lipotubuloids — has been suggested for them (Kwiatkowska, 1970). Under this name are meant osmiophilic granule aggregations webbed and joined by a system of microtubules, formed within the ground cytoplasm and translocating in it as a whole body.

MATERIAL AND METHODS

For investigation of the fine structure of lipotubuloids, tangential sections of the ovary epidermis of *O. umebllatum* L. were used at various development stages.

The material was fixed in OsO_4 after Palade, glutaraldehyde with supplementary fixation in OsO_4 after Sabatini et al. and in 2 per cent $KMnO_4$. Sections dehydrated with alcohol or acetone were embedded in methacrylate (after OsO_4 and glutaraldehyde) or in epon (after $KMnO_4$).

Ultrathin sections were prepared on an ultramicrotome (Freiberger Präzisionsmechanik) or another of Russian make. The sections were mostly stained in a uranyl acetate solution and partly also in lead nitrate.

The observations were made in UMW 100 and JEM-5 microscopes.

RESULTS

In all stages of development, the most numerous and most striking elements of the lipotubuloids are electron-dense (after OsO_4 fixation) different-sized granules. Between them some mitochondria and Golgi structures may be seen. All these elements approximate in size the granules visible in living lipotubuloids. The electron microscope revelaed moreover structures of the type of microtubules which owing to their small dimensions were not visible in the light microscope. The aggregations of the above described bodies lie in cytoplasm which does not differ in appearance from that in other regions of the cell.

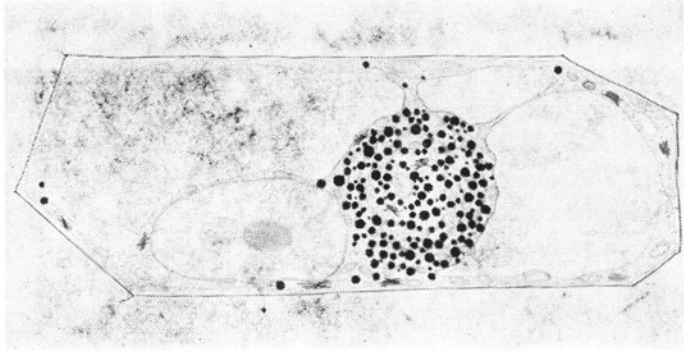


Fig. 1. Scheme of fine structure of lipotubuloid in ovary epidermis cell of *O. umbellatum*

Although the appearance of lipotubuloids in the spherical phase under the light microscope seemed to indicate the presence on their surface of a distinct homogeneous cover (Kwiatkowska, 1966), they are not encapsulated and separated from the cytoplasm. On all micrographs (notwithstanding the stage of development), the ground cytoplasm in the region of the lipotubuloid merges directly with the cytoplasm adjacent to the walls, and with that of the cytoplasmic filaments (figs 2 and 3).

The impression of a cover surrounding the lipotubuloid may be explained as follows: spherical lipotubuloids ca. 10–40 μ in diameter are situated in the cytoplasm of cells with large vacuoles and a thin ring of cytoplasm (ca 0.5–1 μ) adjacent to the wall. At the site of the lipotubuloid the tonoplast, together with a thin layer of cytoplasm stretches, caving into the vacuole (cf. scheme — fig. 1). in this way a large part of the lipotubuloid surface is covered with tonoplast, and sometimes also with a thin layer of cytoplasm free of osmiophilic granules.

STRUCTURE OF ELEMENTS OCCURRING WITHIN THE LIPOTUBULOIDS

The above discussed electron micrographs were obtained with the use of Palade's fixative which in these cases preserved best the lipotubuloids. In order to obtain fuller information concerning the structure of the elements composing the lipotubuloids, the material was also fixed in other ways. Although the results of these fixation methods were unsatisfactory as regards lipotubuloid preservation, they supplied data which proved useful in the interpretation of the pictures obtained.

1. Osmiophilic granules

After treatment with Palade's fixative, the osmiophilic granules have sometimes an almost ideal spherical shape, similar to that seen under the light microscope in living lipotubuloids (fig. 4). On some pictures their contour is less regular, sometimes irregularly stellate (fig. 3). The interior of the spheres is filled with finely granular electron-dense contents. In a series of equally fixed preparations, and sometimes even in the neighbouring lipotubuloids of the same section, the contents are not uniformly dark, but from almost black to light grey.

The osmiophilic granules are surrounded by a single membrane which is particularly well visualised if the contents of the sphere are weakly contrasting (e.g. figs 4 and 6).

In spite of the high variability in the appearance of the lipotubuloid granules from various cells, their great similarity within the same lipotubuloid is striking. It is only in older lipotubuloids that electron-transparent vacuoles appear (figs 4, 8 and 14) which the author considers as osmiophilic granules deprived of their contents (cf. Kwiatkowska, 1971 a).

In sections fixed in glutaraldehyde with secondary fixation in osmium tetroxide (embedding in methacrylate), the osmiophilic granules lose their well outlined contour and become blurred (fig. 8). In preparations dehydrated with acetone and fixed and embedded exactly as described above, some granules have a delimited homogenous electron-dense cortical zone and a light centre with a granular structure (fig. 7).

The lipotubuloids suffered the greatest deformation when fixed in potassium permanganate (embedded in epon). Then most of the osmiophilic granules became irregular, stellate in shape and difrentiated to a contrasting rather thick envelope

and a more electron-transparent interior (fig. 12, structures denoted L). Bodies of a similar appearance may also be found in the cytoplasm beyond the lipotubuloid, in epidermal cells and those of the stomata (fig. 13). Beside the above described structures, in the lipotubuloids fixed with $KMnO_4$, some oval or spherical bodies surrounded with a single membrane and filled with fine-grained contents are visible (fig. 12 — structures denoted S) resembling spherosomes. They can also be identified beyond the lipotubuloid in the cytoplasm of the cells shown in fig. 13.

Comparison of the picture of lipotubuloids fixed in OsO_4 and $KMnO_4$ shows that the bodies with granular contents surrounded with a membrane which are visualised after fixing in potassium permanganate do not have morphologically distinguishable counterparts among the elements fixed with osmium.

There is only a certain resemblance between the above described bodies and the oval or elliptic bodies shown in fig. 11 (cf. fig. 13), however, bodies of this type were very rarely found after OsO_4 treatment, much less frequently than those described in the preceding paragraph (denoted S) in material fixed in $KMnO_4$. Neither could they be found in the cytoplasm outside the area of the lipotubuloid, where beside mitochondria and Golgi structures only osmiophilic homogeneous granules could be seen, differing from the lipotubuloid granules by the absence of microtubules around them.

Thus, the highly refractive osmiophilic granules forming the lipotubuloid occur also beyond it in the cytoplasm. In the living cell and in the electron microscope, after fixation in osmium tetroxide, these granules seem identical. Fixation in $KMnO_4$ shows, however, that within the osmiophilic granules population there exist two categories (both within the lipotubuloid and dispersed in the cytoplasm).

2. Microtubules

The microtubules in the elaioplasts of *O. umbellatum* were best preserved in material fixed after Palade. Potassium permanganate destroyed them completely, and after fixation with glutaraldehyde with supplementary treatment in OsO_4 (embedding in methacrylate) the microtubules are poorly visible, particularly in the close vicinity of the granules (fig. 8), and sometimes completely absent (fig. 7).

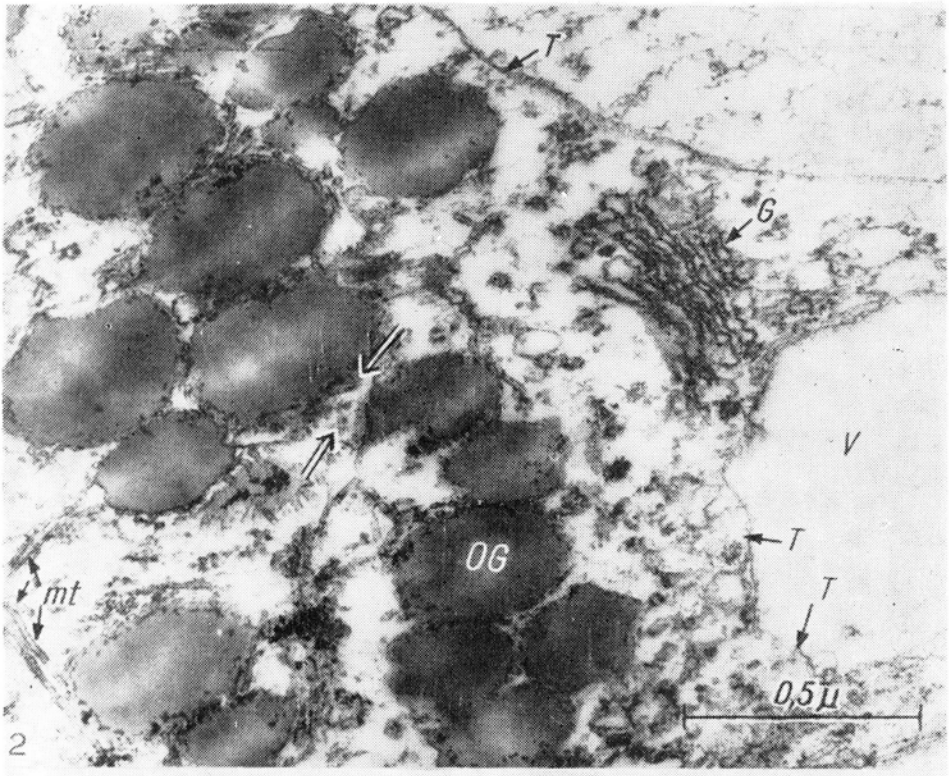
Plate I

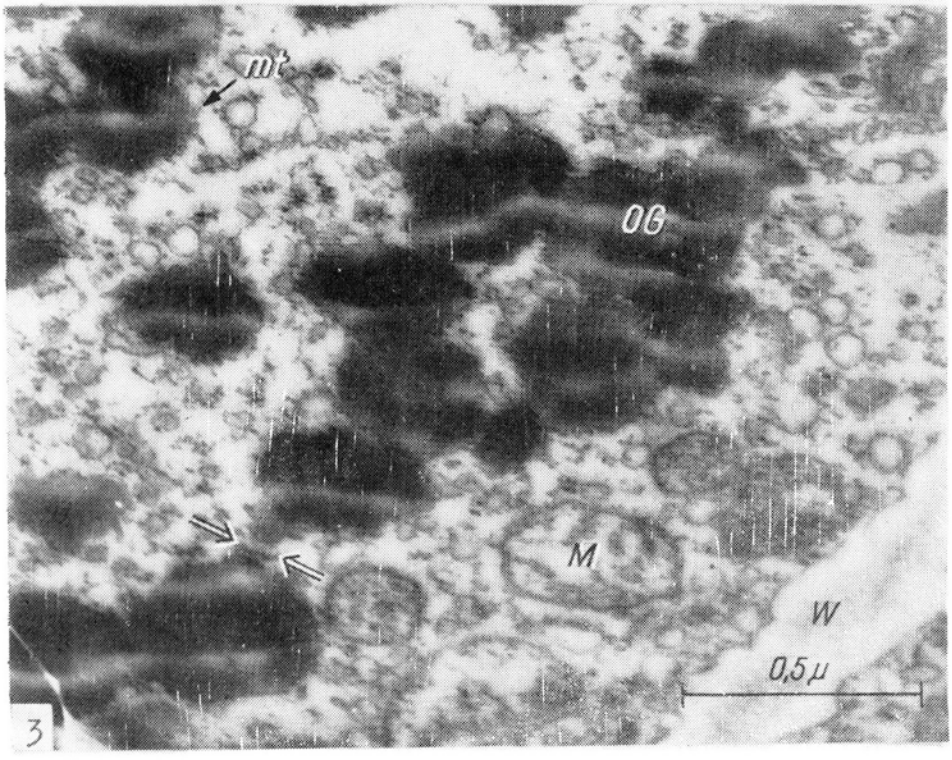
Figs 2 and 3. Fragments of lipotubuloids of *O. umbellatum*

2 — at the site of junction with the cytoplasmic filament, *3* — in the neighbourhood of the cell wall arrow denotes the site of distinct direct union of the lipotubuloid cytoplasm with that adjacent to the wall and the cytoplasm of the cytoplasmic filament

Fixed after Palade, embedded in methacrylate, stained with lead nitrate (fig. 2) and uranyl acetate (fig. 3). Photographed in JEM-5 and UMW-100 microscopes. Notations: *OG* — osmiophilic granules, *M* — mitochondria, *G* — Golgi apparatus, *V* — vacuoles, *T* — tonoplast, *W* — cell wall, *mt-* — microtubules.

Plate I





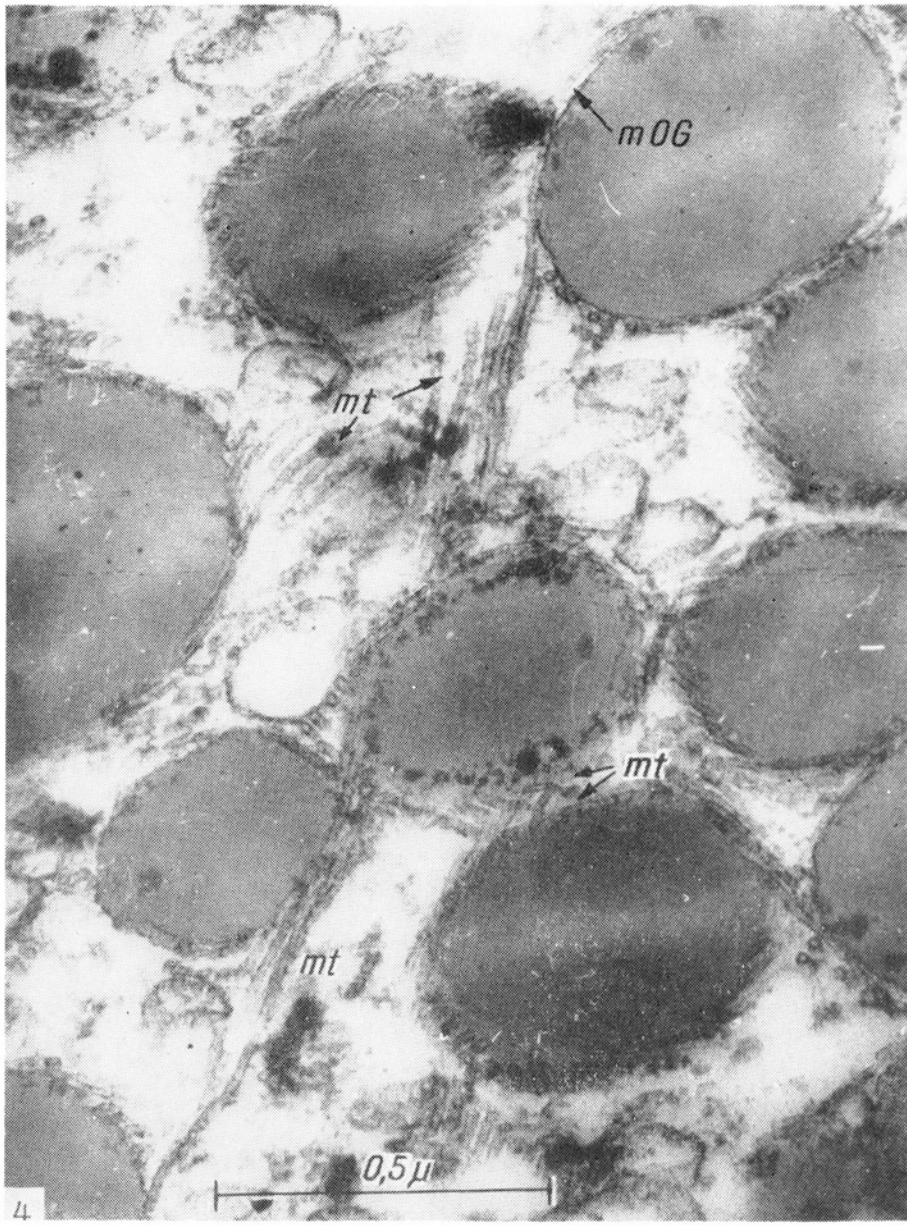
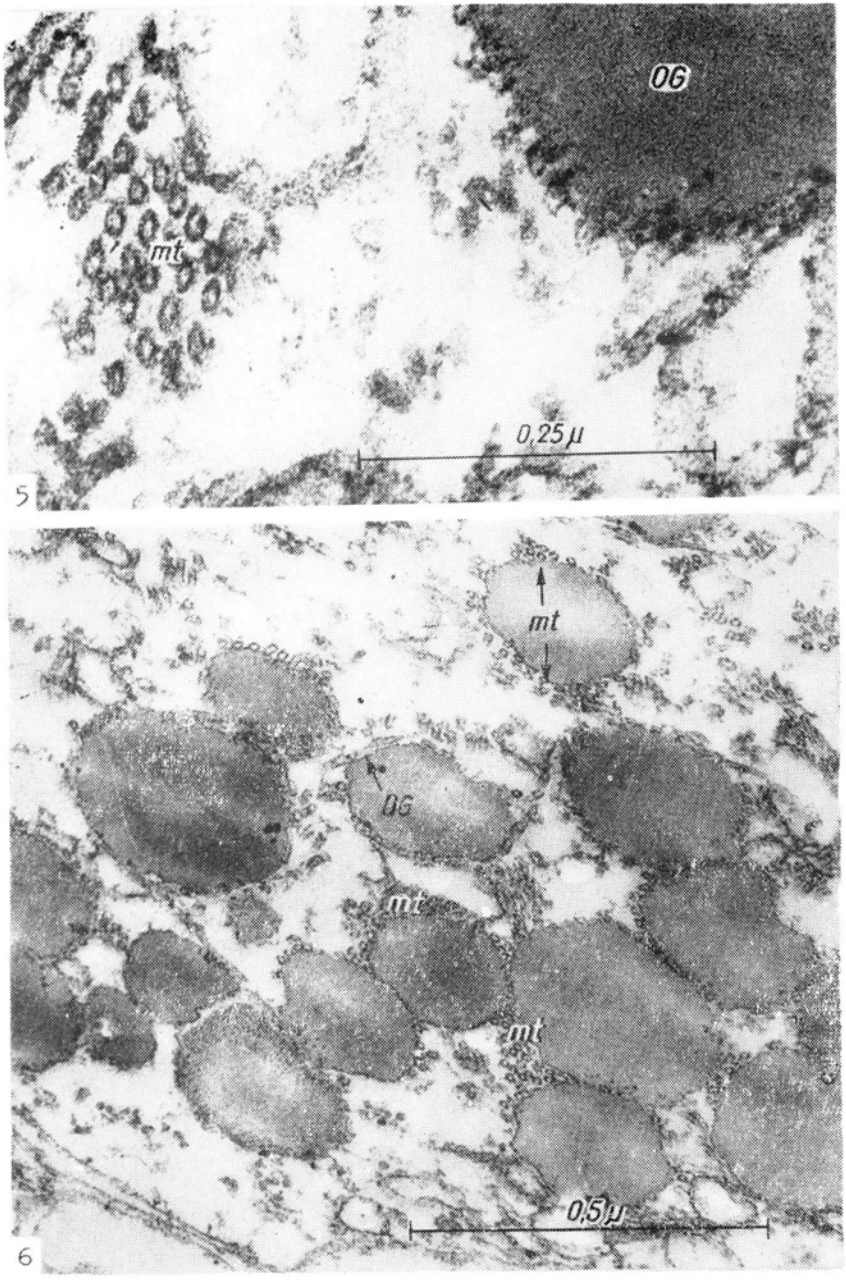


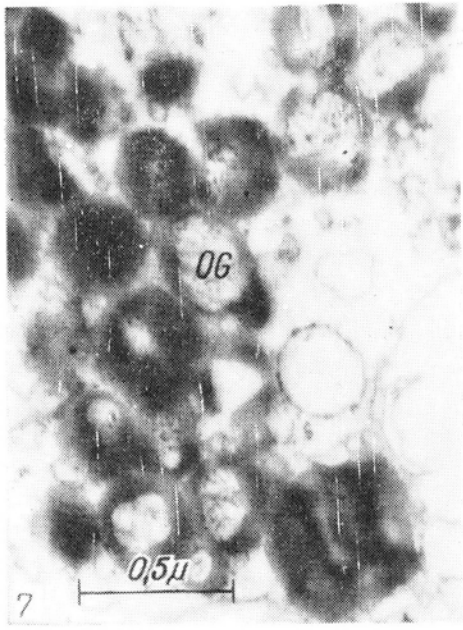
Fig. 4. *O. umbellatum*. Osmiophilic granules of lipotubuloid in a network of microtubule bands intersecting at various planes. Fixed according to Palade, stained with lead nitrate.

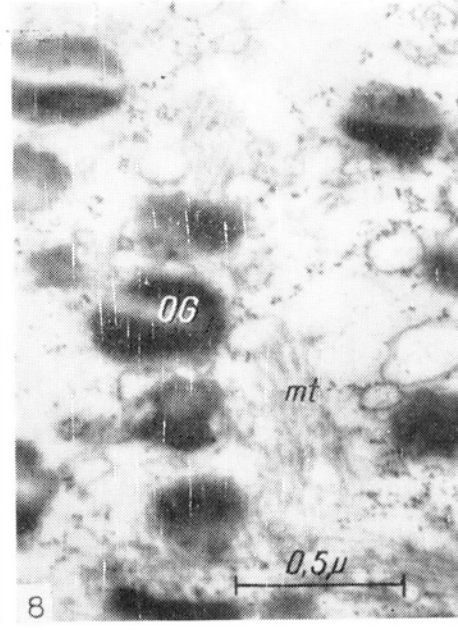
JEM-5. mOG – membrane of osmiophilic granule. Notations as in preceding figures

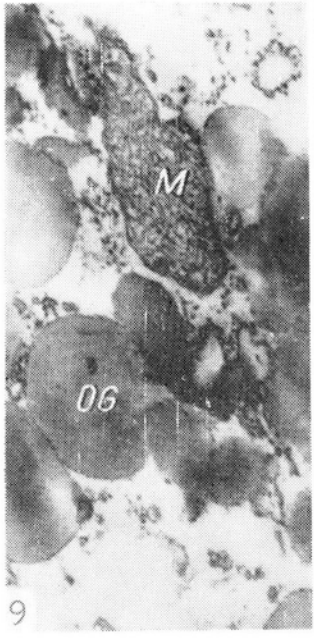


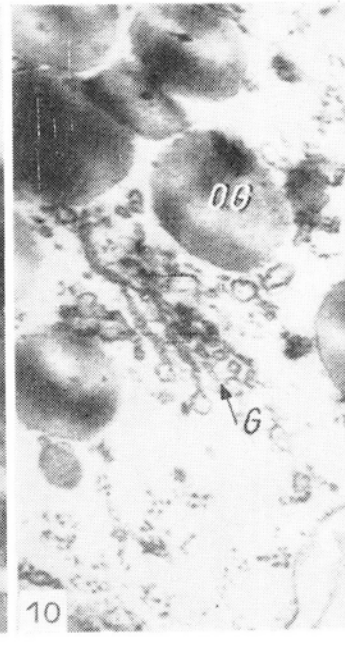
Figs 5 and 6. *O. umbellatum.* Microtubule bundles in lipotubuloid in cross section. 5 — microtubules in space between osmiophilic granules; 6 — microtubules adhering to osmiophilic granules and forming "hoods" on one or more sides of the granule.

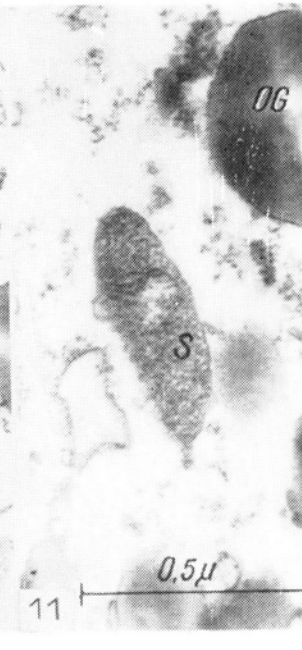
Fixed after Palade, staided with lead nitrate. Notations as in preceding figures. JEM-5





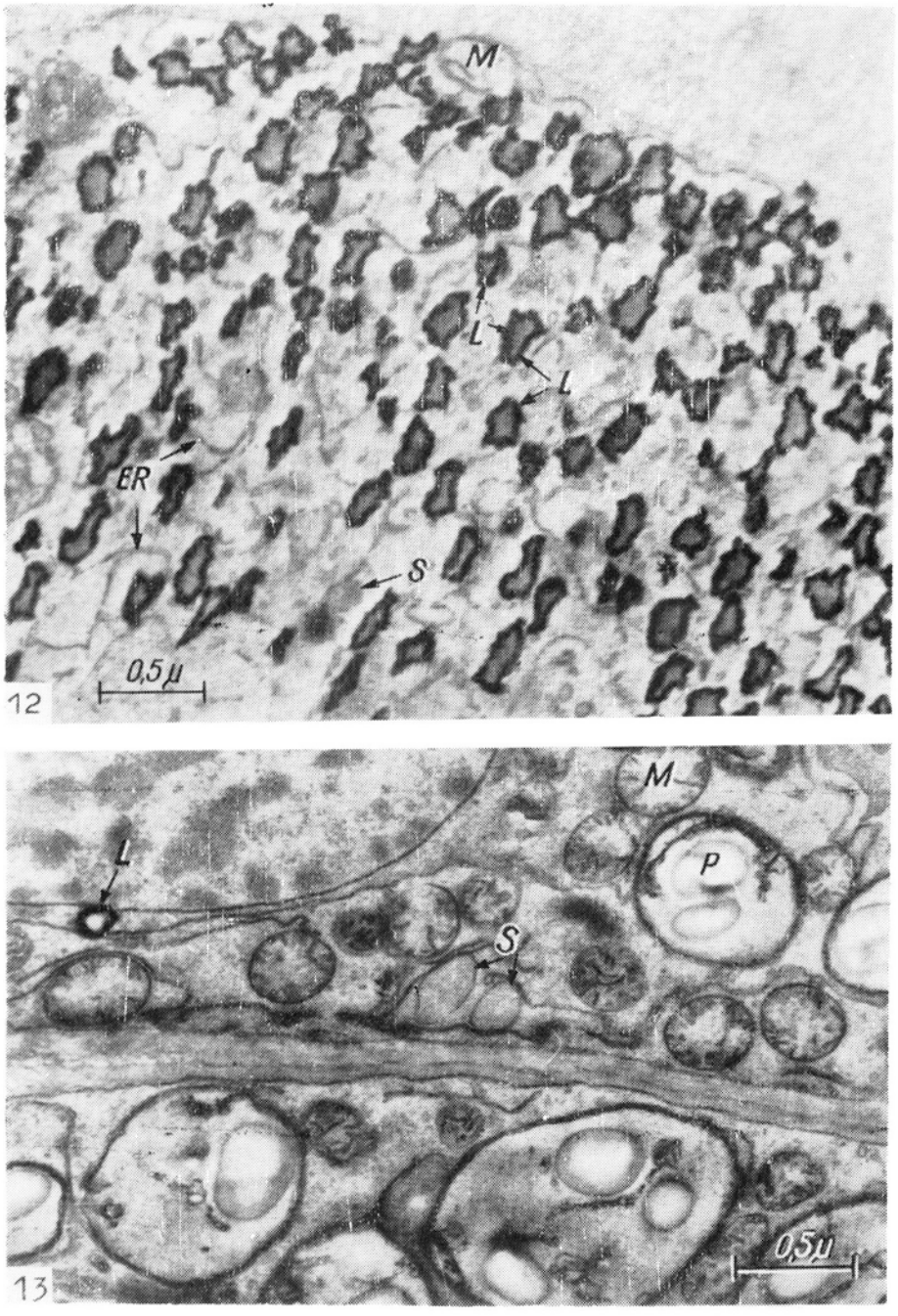






Figs 7 and 8. *O. umbellatum*. Lipotubuloids after fixation in glutaraldehyde and osmium tetroxide, embedded in methacrylate, dehydrated with acetone (fig. 7) and alcohol (fig. 8). UMW 100
Figs 9–11. *O. umbellatum*. Mitochondrium (fig. 9), dictyosome (fig. 10), and body resembling spherosome in appearance (fig. 11) within the lipotubuloid

Fixed after Palade, stained with uranyl acetate. UMW 100



Figs 12 and 13. *O. umbellatum*. Fragment of lipotubuloid (fig. 12) and stoma cell from epidermis' fixed with 2% $KMnO_4$ (embedded in epon)

Photographs taken in Tesla BS 242 microscope. *S* — spherosome-like structures. *L* — structures of lipid droplet character *ER* — endoplasmic reticulum, *P* — plastides other notations as in preceding figures

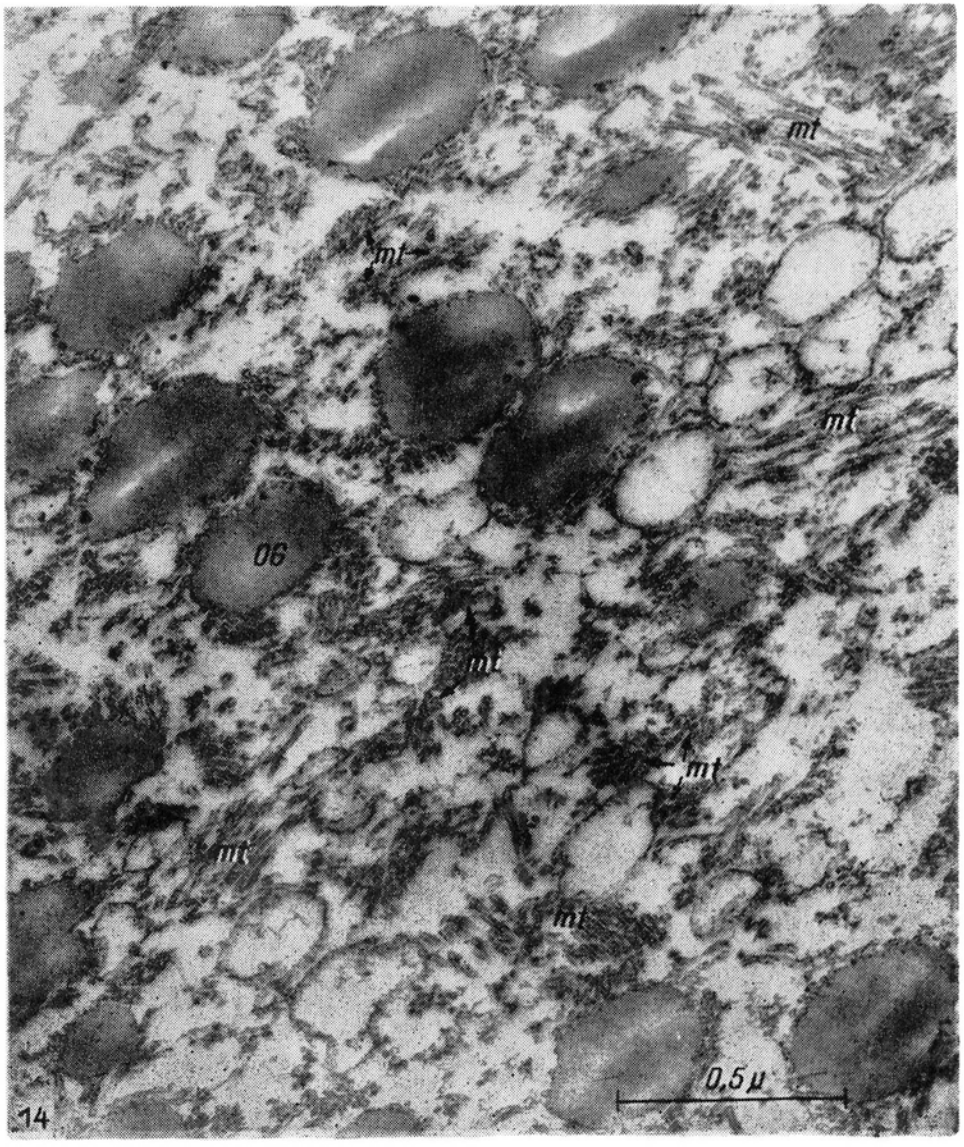


Fig. 14. *O. umbellautm.* Lipotubuloid with profuse microtubular system in spaces between osmiophilic granules. Fixed after Palade, stained with lead nitrate. JEM-5

The microtubules of the lipotubuloids are ring-shaped on the cross section and 180 Å in diameter. The walls of this ring are more electron-dense than the interior. On some cross sections, 8 or 9 units forming the tubule wall may be distinguished (fig. 5). The tubules are very seldom branched. On sections on which the microtubules are longitudinally oriented the latter vary in length in dependence on the cross section plane. Their walls are parallel and on their surface sometimes darker diagonal stripes are visible (fig. 4). The lipotubuloid tubules exhibit, thus all the characteristics of microtubules.

The microtubule system in the lipotubuloid is very characteristic and the same on all photographs taken at an appropriate enlargement. The microtubules are closely connected with the osmiophilic lipotubuloid granules. The lumen of some of the microtubules is connected with the interior of the osmiophilic granules, and mostly their walls closely adhere to the outer surface of the granule membrane. This is particularly well visible on cross sections passing transversally through the microtubules (figs 4 and 6). The walls of the neighbouring microtubules do not adhere to each other, they are separated by a 100–200 Å space visible both on cross sections and between the microtubules arranged parallelly to the cross section plane.

The microtubules are usually grouped by a dozen or some dozens in bands or bundles (figs 5, 6 and 14). Several such bands running in various planes adhere to one granule. Close to the granule the microtubule band bends slightly parallelly to the granule surface, and when it intersects other bands running in planes parallel to the other sides of the granule, they form a kind of basket interwoven around the granule (fig. 4). Around each granule some 30–60 microtubules can be distinguished at various angles to the cross section plane. Only around the most peripherally situated lipotubuloid granules, the microtubules are scarse or sometimes completely absent (fig. 6).

Most frequently, only a single row of microtubules adheres to the osmiophilic granules, sometimes, however, a second layer adjoins to the microtubule layer contacting the granule surface, to these another is attached and so on, giving on the cross section the appearance of a "hood" on one or several sides of the granule (fig. 6).

One band of microtubules adheres to the surface of several neighbouring granules and is interwoven with numerous bands oriented in various directions and planes. Owing to this the lipotubuloid granules are linked with each other by microtubules and the lipotubuloid forms a compact whole.

In some lipotubuloids the microtubules are very numerous and predominate over the granular elements. Large numbers are then seen in the spaces of the lipotubuloid free of osmiophilic granules (fig. 14). On the micrographs comprising a great part of the lipotubuloid, it is visible that the microtubule bundles have a tendency to arrange themselves rather in parallel direction to the lipotubuloid surface, although they run in various planes. This arrangement resembles wool strands wound up in a ball.

3. Endoplasmic reticulum

After fixation of the epidermis after Palade, the endoplasmic reticulum in the lipotubuloids mainly appears in the form of minute vesicles with smooth membranes. Numerous ribosomes occur freely in the cytoplasm, only some of them are attached to the reticulum membranes. Within the intensively enlarging lipotubuloids, numerous minute vesicles of smooth reticulum are visible, frequently arranged in chains of three to four, sometimes interlinked by bridges (fig. 3). The vesicles are about 300–400 Å in diameter. Some are transparent to electrons, others filled with contents similar to those in the osmiophilic granules. The vesicles are absent in lipotubuloids which have ceased to grow and are undergoing vacuolisation (cf. Kwiatkowska, 1971 a,). Some vesicles are connected with the microtubules (fig. 4).

In material fixed with glutaraldehyde, vesicles with smooth membranes and scarse ribosomes on the surface are also visible (fig. 8), and sometimes elongated canals of rough reticulum.

The appearance of reticulum in preparations fixed in $KMnO_4$ is quite different. Between the deformed osmiophilic granules of the lipotubuloids there are numerous elongated pairs of reticulum membranes running in various directions and sometimes connected to one another (fig. 12), between them scarse minute vesicles. On some pictures the connections between the osmiophilic granules and the reticulum are visible. In the close vicinity of the membranes lie bodies resembling spherosomes. The connections between the granules and the reticulum were not so distinct after fixation of the lipotubuloids in OsO_4. Since the reticulum membranes underwent fragmentation in osmium tetroxide, it seems possible that in the course of fixation, the connections of the osmiophilic granules with the membranes were also destroyed.

4. Mitochondria

Scarse mitochondria were found at the periphery of the lipotubuloid, sometimes grouped by several next to each other, they were less frequent within the lipotubuloid (fig. 9). These mitochondria are somewhat larger than the osmiophilic granules, spherical, oval or elongated. After osmium fixation they show greatly developed cristae and granular matrix. They do not differ from the mitochondria not connected with the lipotubuloid, neither do they show any signs of injury or degeneration.

The mitochondria in lipotubuloids fixed in $KMnO_4$ have much less developed cristae and a lighter matrix.

5. Golgi apparatus

The Golgi apparatus like mitochondria are scarse in the lipotubuloids. They may be identified both in preparations fixed after Palade and with $KMnO_4$. The number of dictyosomes within the lipotubuloids varies in various preparations,

however, on the basis of the observations to date, it would be difficult to decide whether their number is dependent on the developmental stage of the lipotubuloid. Like mitochondria, the Golgi apparatus lies most frequently on the lipotubuloid periphery (fig. 2), but it occurs also within them (fig. 10). The cisterns of dictyosomes localised at the periphery of the lipotubuloid are usually arranged parallelly to its surface.

DISCUSSION

As results from the foregoing description, lipotubuloids do not possess any specific structures. All the elements distinguished in them occur in the cells also beyond the lipotubuloids. Characteristic for them are the different numbers of the particular organelles, osmiophilic granules and microtubules being the constant and most numerous components. Mitochondria and dictyosomes occur only sporadically. Specific are also interconnections of the microtubules and the osmiophilic granules, owing to which the lipotubuloid constitutes an entity with a definite fine structure. In the light of the results concerning the lipotubuloids of *O. umbellatum*, the opinion of Guilliermond (1922) and other authors that elaioplasts are simply conglomerates of ergastic substances in the form of lipid droplets held together solely by the force of adhesion, would not seem correct.

To what cellular structure do the osmiophilic granules correspond? Numerous authors believed that the granules forming the elaioplast are lipid droplets (to mention: Hieronymus 1892; Guilliermond, 1922). Tourte (1966) considers the elaioplast granules of *Haemanthus albiflos* to be lipid droplets surrounded with an endoplasmic reticulum membrane, and compares them with the liposomes of animal cells.

In a number of papers the suggestion was advanced that the granules forming the elaioplast are similar to spherosomes (Wałek-Czernecka, Kwiatkowska, 1961; Górska-Brylass, 1962; Kwiatkowska, 1964). In recent years the views concerning spherosomes have been modified, however, in the literature a conclusive opinion as to their character is still lacking. An exhaustive review of literature on spherosomes is given by Wałek-Czernecka (1965) and Olszewska (1967).

In conformity with the views presented by the above mentioned authors, spherosomes are considered here as organelles characterised by a high light refraction coeficient, size from the limits of visibility to 1μ, spherical shape, the presence of proteins beside lipids, the occurrence of hydrolytic enzymes characteristic for lysosomes of animal cells. As demonstrated by Wałek-Czernecka (1962, 1963, 1965), acid hydrolases may be considered as enzymatic markers of spherosomes which allow to distinguish them from lipid droplets of the same size, with which they have many common traits.

The physical characteristics of the lipotubuloid osmiophilic granules fully correspond to spherosomes. Also the presence of proteins in the osmiophilic granules

is beyond all doubt. They have been demonstrated after complete extraction of lipids with pyridine after the exclusion of nonspecific staining in the reaction for protein (Kwiatkowska, 1966). As regards the presence of hydrolytic enzymes in the osmiophilic granules of *O. umbellatum*, it could be revealed by cytoenzymatic methods only in some of them. After the reaction for one or two enzymes (detected jointly in one preparation), the majority of the osmiophilic granules remains colourless (Kwiatkowska, 1966), thus, only a part of them possess the above mentioned properties of spherosomes. It should be added that acid phosphatase was demonstrated (at the electron microscope level) in dictyosomes of plant cells (Poux, 1963; Pickett Heaps, 1967 b; Dauwalder et al., 1968) like in the dictyosomes of animal cells. This circumstance makes the identification of spherosomes by enzymatic methods difficult in the light microscope, where it is not always possible to distinguish minute dictyosomes from spherosomes of a similar size. The same is true as regards lipotubuloids in which scarse dictyosomes occur. They are generally larger than spherosomes, thus it is possible that the irregular bodies stained with the reaction product, larger than the osmiophilic granules and situated generally on the surface of the lipotubuloids may correspond to dictyosomes.

For many years the fine structure of spherosomes has been the subject of studies. It seemed at first that at the electron microscope level there are no more difficulties in distinguishing spherosomes from lipid droplets. Spherosomes, namely have a distinct single limiting membrane, and fine-grained or filamentous contents, whereas the lipid droplets are usually deformed, without a distinct limiting membrane, and after fixation with osmium their contents are dark and homogeneous (Mühlethaler, 1955; Perner, 1958; Peveling, 1962; Frey-Wyssling et al., 1963; Mikulska, 1964; Woodking, Northcote, 1965; Mikulska and Gabara, 1968; Schulz, Jensen, 1969). Lipid droplets have a different appearance after fixation in potassium permanganate and in osmium. Generally, only the outer layer of the granule, visible in the form of an irregular serrulated ring of high electron density, is preserved. All authors, however, who found the above described two categories of structures did not interpret them in the same way. Some investigators referred to the spherical or irregular homogenous bodies, called by earlier authors lipid droplets, as spherosomes. For the sake of simplicity the present author denotes them as type II spherosomes (in contradistinction to the above described ones — type I) (to mention: Hohl, 1960; Drawert and Mix, 1962; Heslop-Harrison, Dickinson, 1967; Peveling, Lichtenthaler, 1967; Jones, 1969a, b, c).

The spherosomes of *Campanula* sp. demonstrated by Sorokin and Sorokin (1966) are very similar. In their material, after fixation with glutaraldehyde and osmium tetroxide, a light core and dark cortical part were revealed.

At the same time bodies with features of type I spherosomes were described under various names, e.g. lysosomes or "dense bodies" (Leyon, 1954; Geneves et al., 1958; Setterfield et al., 1959; Avers 1962). They were also named "component A" (Falk, 1962), "granular bodies" (Sorokin, Sorokin, 1966), cytosomes (Sitte, 1958; Mollenhauer et al., 1966) or "microbodies" (Falk, 1962; Frederick et al., 1968; Frederick and Newcomb, 1969; Schnepf, 1969; Jones, 1969c).

Sometimes in the cells of various plant species, no bodies of spherosome type I were found. Then various bodies, for instance vesicles transparent to electrons (Matile et al., 1965, 1966; Engelman, 1966; Mikulska et al., 1969) were called spherosomes of type III.

Lately a number of papers have appeared in which the name spherosome is used as a synonym of lipid droplets, to quote Hölzl (1969): "Sphärosomen – Lipidtropfen = Lipidvakuolen". The term spherosome is applied in this sense by Yatsu, (1965), Sauter (1968), Spichiger, (1969), Hölzl, (1969) and others to dark, homogeneous after fixation in OsO_4, bodies or to the vesicles with a dark envelope transparent to electrons in preparations fixed in $KMnO_4$.

Thus, at present there exists no unanimity as regards the fine structure of spherosomes. It is to be expected that a number of doubtful problems concerning this structure will be solved by cytoenzymatic studies at the electron microscope level, which would confront the results of enzymatic investigations obtained in the light microscope with those found in the electron microscope. So far the presence of acid phosphatase has been demonstrated in spherosomes (at the electron microscope level) only by Mikulska and Gabara (1968) and Olszewska (1970). Some of the spherosomal enzymes, for instance arylsulphatase, have been discovered in bodies denoted as fragmosomes (Poux, 1963), acid phosphatase was found in structures known as lysosomes or "lysosome-like" structures (Berjak, 1968; Crang, Miles, 1969; Gahan, Maple, 1966; Gahan and Lean, 1969), which probably correspond to type I spherosomes. The presence of hydrolases was also revealed in the vesicles and vacuoles.

The osmiophilic granules of *O. umbellatum* lipotubuloids are surrounded with a single membrane with homogeneous electron-dense contents. Thus they resemble type II spherosomes, and sometimes those described by Sorokin and Sorokin (1966), they differ, however, from spherosomes of types I and III. Most authors consider structures like osmiophilic granules on electron micrographs of plant cells as lipids. (According to the here adopted definition of spherosomes as organelles containing hydrolytic enzymes, all authors who call lipid droplets spherosomes should be classified to this group). The lipid droplets in animal cells have a similar appearance, particularly in brown adipose tissue and in early development stages of white adipose tissue (e.g. Napolitano, 1963, 1965) and in cells of the subsynovial fat pads of the knee joint (Luckenbill and Cohen, 1966). In *O. umbellatum*, however, no other structures are visible, either in the lipotubuloid or in the cell beyond it, in material fixed with OsO_4 or glutaraldehyde and OsO_4, which would correspond by their properties to spherosomes of type I seen for instance in *Clivia* sp. or to type III spherosomes. On the other hand, in *O. umbellatum* structures resembling spherosomes of type I may be found in preparations fixed with $KMnO_4$. Some granules from the lipotubuloids are similar to them, they preserve a smooth contour, a spherical or oval shape, they are surrounded by a single membrane and filled with fine-grained contents; identical bodies are seen within the cytoplasm beyond the lipotubuloid. The remaining lipotubuloid granules undergoing deformation resemble rather lipid droplets.

The results of the earlier discussed enzymatic reaction also indicate that various kinds of granules occur in the *O. umbellatum* lipotubuloids, some of which, less numerous, are carriers of the hydrolytic enzymes investigated, and the remaining, more numerous ones are probably deprived of them.

Comparison of these results with electron microscope observations leads to the conclusion that the hydrolytic enzymes are localised in the less numerous granules which after fixing of the material in $KMnO_4$ look like type I spherosomes. On the other hand, the granules unstained after enzymatic reaction probably correspond to structures with properties similar to the lipid droplets.

The possibility of distinguishing in the electron microscope, by means of $KMnO_4$ fixation, of a population of bodies which cannot be revealed after treatment with glutaraldehyde and osmium has been lately discussed by Jones (1969 a, b and c). In centrifuged cells (from barley endosperm) fixed in $KMnO_4$, between the mitochondrial fraction and the structures which author refers to as "spherosomes" (meaning by this term lipid droplets), a small number of bodies somewhat smaller than mitochondria may be distinguished, surrounded by a single membrane with a delicate granular matrix and designated as "micro-bodies". Jones considers the "microbodies" as carriers of hydrolytic enzymes, thus the term "microbodies" is used in the sense in which in the present paper we use the word spherosomes. After fixing with osmium, in corresponding barley endosperm cells subjected to centrifugation, no "microbody" fraction could be distinguished.

Since the lipotubuloid granules representing probably spherosomes and the granules corresponding to lipid droplets do not differ morphologically, either in living material or after fixation of the cells in OsO_4, the bright granules visible in living cells and the spherical homogeneous bodies seen in ultrathin sections fixed with osmium are denoted here by the general name of osmiophilic granules.

The second component element of lipotubuloids of *O. umbellatum* — the tubules — exhibit characters typical for microtubules widely described in plant and animal cells since the appearance of the paper by Ledbetter and Porter (1963). *O. umbellatum* microtubules frequently occur in groups arranged parallelly to each other at distances of ca. 100–200 Å like the tubules described by Ledbetter and Porter (1963), this suggesting that the microtubules are surrounded by a special zone. The microtubules, however, arround the osmiophilic granule are not surrounded by this characteristic light zone at the site of contact with the membrane of the osmiophilic granule where these two structures adhere closely to one another. In the microtubule walls of *O. umbellatum* about eight to nine component units can be distinguished.

It is characteristic for *O. umbellatum* lipotubuloids that the microtubules occur in close contact with the osmiophilic granules: they adhere by their walls to the outer surfaces of the membranes of the latter granules, and sometimes single microtubules are seen to be connected with their interior. One band of microtubules contacts several neighbouring osmiophilic granules, intertwined with bands of microtubules running in other planes and directions. In this way every granule in encased in

a microtubule „basket", and the entire lipotubuloid forms a system of linked elements.

In the available cytological literature concerning both animal and plant cells no description of such a closely linked granular-tubular system could be found by the author. The only similar picture was that of subsynovial adipose mulberry cells occurring under the knee joint in the chick (Luckenbill and Cohen, 1966). In these cells, after fixation with glutaraldehyde and osmium tetroxide, profuse fibrils occur, intercrossing and forming a network around the lipid droplets as do the microtubules in the lipotubuloids. The lipid droplets form aggregations in the shape of mulberries, resembling lipotubuloids. The fibrils united with them differ, however, markedly from the microtubules by their dimensions (70–100 Å) and do not exhibit a tubular structure. It is therefore difficult to establish a strict analogy between these bodies and the lipotubuloids of *O. umbellatum*.

In the elaioplasts of the flower plants described by Tourte (1964, 1966) microtubules were not visualised. Neither was their presence revealed in the oil bodies of *Hepaticae* (Pihakaski, 1966, 1968; Hölzl, 1969). The microtubules surrounding the osmiophilic granules were found, on the other hand, in the elaioplasts of *Malva*, which in view of this were classified to lipotubuloids (Kwiatkowska, 1971 b). On account of the lability of the microtubules and their small dimensions, it cannot be affirmed categorically that they are absent when appropriate conditions of fixation are not observed as in the case of the elaioplasts described by Tourte (1966). It seems probable, therefore, that among the elaioplasts of nonplastide origin, further examples of lipotubuloids may be found.

SUMMARY

Electron microscopic investigations showed that the lipotubuloids of *Ornithogalum umbellatum* are aggregations of osmiophilic granules and microtubules within the cytoplasm, with scarse mitochondria and Golgi apparatus sometimes within them.

These aggregations are not separated from the cytoplasm by a membrane.

The fine structure of the elements found in the lipotubuloids is described.

a) The osmiophilic granules in material fixed with OsO_4 or glutaraldehyde with supplementary fixation in OSO_4 are surrounded by a single membrane with homogeneous electron-dense contents. The size of the granules varies within the limits of 0.04–0.6 μ, the most frequent size being 0.3–0.4 μ. After fixation with $KMnO_4$, the osmiophilic granules exhibit two different categories. Most of them, lose their spherical shape, their cortical zone is very dark and the interior light so that they resemble lipid droplets. Only few of the lipotubuloid granules are spherosome-like: they are surrounded by a single membrane and filled with fine-grained matrix. The number of these bodies in the lipotubuloid corresponds approximately to the number of granules containing hydrolytic enzymes as visible in the light microscope. Both these kinds of structures occur also in the cytoplasm beyond the lipotubuloid.

b) The microtubules of the lipotubuloids have a typical structure: they consist of unbranched tubules ca. 180 Å in diameter parallelly arranged with dark wall and a lighter core. On cross sections about 9 units may be distinguished forming the walls. The microtubules are parallelly arranged in the lipotubuloid forming bundles and bands of several tens of microtubules which run in various directions and planes intersecting with each other. The microtubules are closely associated wiht the osmiophilic granules (i.e. probably with lipid droplets and spherosomes), they

adhere to their surface by their walls forming baskets surrounding the granule on all sides. Some microtubules are directly connected with the lumen of the granules. Beyond the elaioplast no microtubules could be found.

c) The endoplasmic reticulum within the lipotubuloid has the form of minute vesicles with smooth membranes, ribosomes are generally freely distributed. After fixation in KMnO, the endoplasmic reticulum is visible in the form of elongated membranes which are sometimes linked with the osmiophilic granules and adhere to them.

d) Golgi apparatus and mitochondria occur in lipotubuloids more frequently on the periphery than in deeper layers, they do not differ in fine structure from those found in the cells beyond the lipotubuloid.

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Ultrastruktura lipotubuloidów (elajoplastów) Ornithogalum umbellatum L.

Streszczenie

W wyniku badań w mikroskopie elektronowym stwierdzono, że lipotubuloidy *Ornithogalum umbellatum* są znajdującymi się na terenie cytoplazmy skupieniami osmofilnych granul i mikrotubul, niekiedy widoczne są w nich nieliczne mitochondria i struktury Golgiego. Skupienia te nie są oddzielone od cytoplazmy membraną.

Opisano ultrastrukturę elementów znajdujących się w obrębie lipotubuloidów.

a) Osmofilne granule w materiale utrwalanym OsO_4 albo aldehydem glutarowym z dopełniającym utrwalaniem OsO_4 otoczone są membraną pojedynczą i zawierają gęstą dla elektronów homogenną treść. Ich wielkość waha się we wszystkich etapach rozwojowych w granicach od 0,04 μ do 0,5 μ, najliczniejsze są granule od 0,3 do 0,4 μ. Po utrwaleniu obiektu $KMnO_4$ osmofilne gra-

nule mają dwojaki charakter. Większość z nich traci kulisty kształt i wykazuje strefę korową bardzo ciemną i jasne wnętrze, przybierając wygląd podobny do kulek lipidowych. Nieliczen granule lipotubuloidów mają postać sferosomów: otoczone są pojedynczą membraną i wypełnione drobnoziarnistą matrix. Liczba tego rodzaju utworów w lipotubuloidzie odpowiada w przybliżeniu liczbie ziarenek zawierających enzymy hydrolityczne widocznych w mikroskopie świetlnym. Obydwa rodzaje struktur występują także w cytoplazmie poza lipotubuloidem.

b) Mikrotubule lipotubuloidów mają typową dla tych struktur budowę: są nierozgałęzionymi rureczkami o średnicy ok. 180 Å, równoległych, ciemnych ściankach i jaśniejszym rdzeniu. Na przekrojach poprzecznych w ściankach mikrotubul można wyróżnić ok. 9 podjednostek. Mikrotubule układają się w lipotubuloidzie równolegle w wiązki i pasma składające się z kilkudziedięciu mikrotubul, które przebiegają w różnych kierunkach i płaszczyznach oraz krzyżują się ze sobą. Mikrotubule są ściśle powiązane z osmofilnymi granulami (tzn. prawdopodobnie z kuleczkami lipidowymi i sferosomami), przelegają do ich powierzchni ściankami tworząc koszyczki oplatające granulę ze wszystkich stron. Niektóre mikrotubule łączą się bezpośrednio ze światłem granul. Poza obszarem elajoplastu nie znaleziono mikrotubul.

c) Retikulum endoplazmatyczne w obrębie lipotubuloidu ma postać drobnych pęcherzyków o gładkich membranach, rybosomy rozmieszczone są na ogół sswobodnie. Po utrwaleniu obiektu w $KMnO_4$ endoplazmatyczne retikulum widoczne jest w formie wydłużonych membran,które łączą się niekiedy z osmofilnymi granulami, bądź przelegają do nich.

d) Struktury Golgiego i mitochondria występują w lipotubuloidach częściej na ich peryferii niż w głębi, nie różnią się ultrastrukturą od tych, które są obecne w komórce poza lipotubuloidem.