ON THE PLASTIDS, MITOCHONDRIA, AND OTHER CELL CONSTITUENTS DURING OÖGENESIS OF A PLANT

LOTHAR DIERS

From the Botanisches Institut der Universität Köln, Germany

ABSTRACT

In the liverwort *Sphaerocarpus donnellii Aust.*, the behavior of the cell constituents, especially of mitochondria and plastids, was studied by electron microscopy during the development of the egg and its preceding cells. A degeneration and elimination of mitochondria and plastids was not found in any of the developmental stages. In all growth phases of the archegonium, the plastids may deposit starch which becomes especially frequent in the maturing egg cell. No indications have been observed that new mitochondria or plastids generate from the nuclear evaginations, which often penetrate deeply into the cytoplasm of the maturing and fully developed eggs. A quantitative investigation based on general micrographs elucidates the numerical aspects of the cell constituents during oögenesis. With the increase of cell volume, the numbers of dictyosomes, mitochondria, plastids, and lipid bodies increase. From the stages of the mother cell of the axial row up to that of the mature egg, the cell volume enlarges about 8 times and the nucleus volume about 15 times. Simultaneously, the numbers of mitochondria and plastids increase up to 8 to 15 times. On the basis of these findings, mitochondria and plastids with three-dimensional narrow constrictions are interpreted as divisional stages.

INTRODUCTION

According to the electron microscope investigations of Mühlethaler and Bell (1962), plastids and mitochondria degenerate and are formed de novo by evaginations of the nucleus during oögenesis of the fern *Pteridium aquilinum*. Mühlethaler and Bell also suggest that "this reconstitution of the plasm is valid not only for the ferns but also for the lower and higher plants and for the animals" (compare also Bell and Mühlethaler, 1962). This opinion is in strong contrast with the hypotheses of Meyer (1883) and Schimper (1885), that plastids possess a continuity from one plant generation to another. Investigations of Renner (1934) and of Schwemmle and coworkers (1938) strongly indicate plastid inheritance, which must mean plastid continuity, at least a genetical one. Based on their own work on plastid inheritance, Schötz (1962), Stubbe (1962), and Haustein (1962) have already emphasized this fact.

In the present electron microscope investigation of the developing archegonium and egg cell in the liverwort *Sphaerocarpus donnellii Aust.*, special attention was paid to the behavior of the plastids and mitochondria. If degeneration and neoformation of these organelles is generally valid, then this phenomenon should be observable in archegoniate plants other than *Pteridium*, for instance, in *Sphaerocarpus*.

MATERIALS AND METHODS

Normal ♀-gametophytes of the liverwort *Sphaerocarpus donnellii* were cultivated on agar in Petri dishes. The conditions of cultivation have already been described (Diers, 1965 *a*). Parts of the ♀-gametophytes were fixed (*a*) in 2% $KMnO_4$ for 2 hr at room temperature, (*b*) in 1 to 2.5% OsO_4 buffered solutions for 2 to 24 hr, or (*c*) in glutaraldehyde followed by postfixation in OsO_4. After dehydration in acetone or ethanol, the specimens were embedded in methacrylate, Araldite, or Epon. Sections were cut on a Leitz microtome with glass or diamond knives and examined in a Siemens Elmiskop I. The results presented here are based on more than 2000 electron micrographs. Moreover, ½ to ¾ μ thick sections of the fixed material were examined under the phase-contrast microscope.

RESULTS

On the Development of the Archegonium

The archegonia arise from epidermal cells on the dorsal side immediately behind the growing edge of the thallus (cf. Rickett, 1920). The development of the egg cell in the archegonia follows a definite pattern. Fig. 1 shows the characteristic stages (A through F) of this development. The primary axial cell of a young archeogonium (A) divides into the mother cell of the axial row and the cover cell which belongs to the wall cells at the tip of the young archegonium (B). By division of the mother cell of the axial row, the central cell and the neck-canal mother cell arise (C). During the growth of the central cell, the neck-canal mother cell divides into two neck-canal cells (D). Finally, the central cell divides unequally into the smaller ventral-canal cell and the larger egg cell (E). The involucre gradually forms around the archegonium during the maturation of the egg cell (F). In the material investigated here, this involucre reaches about halfway up the archegonium neck when the egg is mature, and the neck-canal is open to spermatozoids. A detailed description of archegonium development is given elsewhere (Diers, 1965 *a*, and *b*).

Development of Mother Cell

In the early developmental stages of the archegonium, all cells are similar in their fine structure. The plastids and mitochondria in the primary cell and in the mother cell of the axial row do not differ from those of the neighboring cells. Fig. 2, for example, shows a part of the mother cell of the axial row with a plastid. Like this plastid, the plastids of all cells possess 2 to 3 tightly packed thylacoids (Menke, 1961) or compartments (Weier, 1961), and occasionally small starchgrains (cf. also Diers, 1965 *a*, Figs. 1 to 7).

In the mother cell stage, the mitochondria and

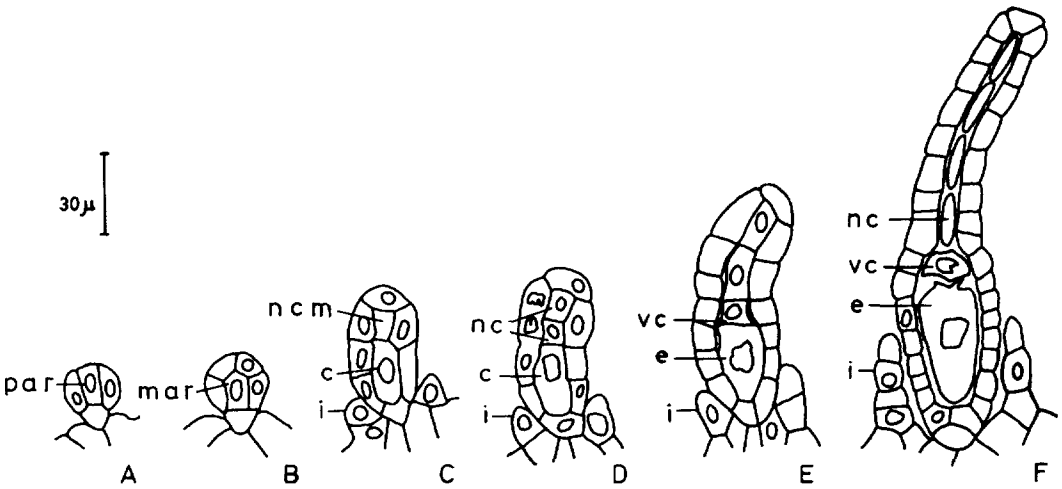

FIGURE 1 Successive developmental stages of the archegonium of *Sphaerocarpus donnellii*: *par*, primary cell of axial row; *mar*, mother cell of axial row; *ncm*, neck-canal mother cell; *c*, central cell; *nc*, neck-canal cell; *vc*, ventral-canal cell; *e*, egg cell; *i*, involucre. A to F are drawings of ½ to ¾ μ thick sections examined under the phase-contrast microscope. They were all prepared on the same scale with the aid of a drawing apparatus.

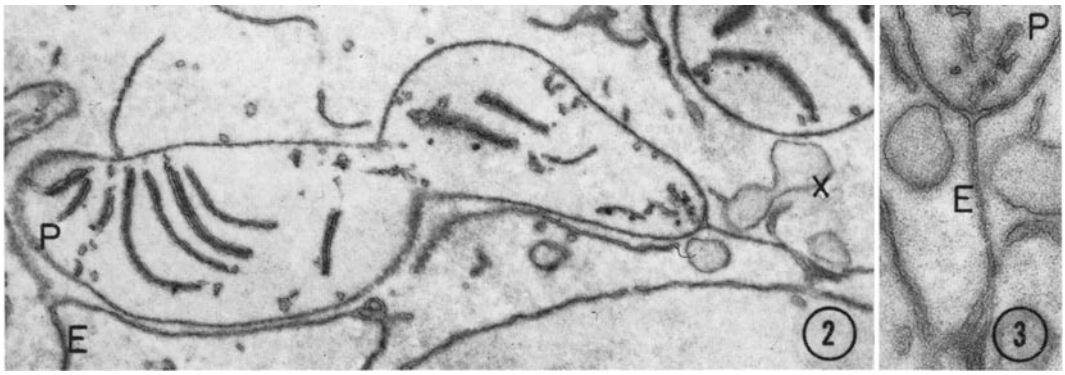

FIGURE 2 A portion of the primary cell of the axial row. The plastid (*P*) contains several thylacoids, some of them packed tightly together. Also seen are two bodies (*x*) with a single limiting membrane; one of them possesses a narrow constriction. Near the two bodies (*x*), the outer limiting membrane of the plastid is connected with membranes of the endoplasmic reticulum (*E*). × 20,000.

FIGURE 3 Higher magnification of a portion of the same section. The fusion of the membranes of the plastid (*P*) and the endoplasmic reticulum (*E*) is obvious. Approximately × 48,000.

dictyosomes of all archegonial cells show no remarkable pecularities in their fine structure. Single lipid bodies occur rarely (Diers, 1965 *a*, Fig. 2). Other bodies (*x*) about 0.4 to 1 μ in diameter with a single membrane are also seen (Figs. 2 and 3). They are commonly designated as spherosomes. Because this term is used for several types of cell constituents (cf. Schötz and Diers, 1965), the neutral expression "body (*x*)" is preferred.

CENTRAL CELL

In the central cell, which arises by division of the mother cell of the axial row, mitochondria of various sizes occur (Fig. 4). During maturation of the central cell, there is an accumulation of mitochondria just below the nucleus (cf. also Diers, 1965 *a*, Figs. 12 and 13). Dictyosomes, parts of the endoplasmic reticulum, and plastids peripheral to this region seem to occur. Larger mitochondria intermixed with plastids are found in the cytoplasm farther away from the nucleus (Fig. 4). In contrast with the mitochondria in the wall cells, most, if not all, of the mitochondria in the central cell possess small electron-opaque granules (Figs. 9 and 11), which are well known as intramitochondrial granules from studies on animal cells (Novikoff, 1961).

During the growth of the central cell, the membrane system is not so well developed in the plastids of the central cell as in those of the archegonial wall cells (Fig. 4) or of the preceding developmental stages (Fig. 2). The plastids in the central cell may possess starch grains (Fig. 12).

Occasionally, the central cell, the wall cells, and also the cells of younger archegonia (Fig. 1, A and B) contain undifferentiated plastids or young chloroplasts, the inner part of the limiting membrane of which seems to grow through the plastid interior to the opposite limiting membrane. In this manner, according to serial sections, the interior of the plastid may be nearly completely divided into two separated spaces (Fig. 5). Gantt and Arnott (1963) described this phenomenon as concentralization in the fully developed chloroplasts of the gametophyte in *Matteuccia struthiopteris*. They regard this concentralization as a method of chloroplast division. Schötz and Senser (1964) mention quite a corresponding formation in the chloroplasts of certain *Oenothera* hybrids. Neither Gantt and Arnott (1963) nor Schötz and Senser (1964) found a precise indication, in their electron micrographs, how chloroplast division is completed after concentralization. In contrast with *Matteucia* and *Oenothera*, in *Sphaerocarpus* this concentralization occurs also in plastids apparently not differentiated into chloroplasts (Fig. 5). Despite a thorough search, it was impossible to find such plastids which could be interpreted as the last stages of separation. At least for *Sphaerocarpus*, therefore, it is uncertain whether this concentralization may lead to a plastid division. Perhaps it is only associated with genesis of plastid membranes.

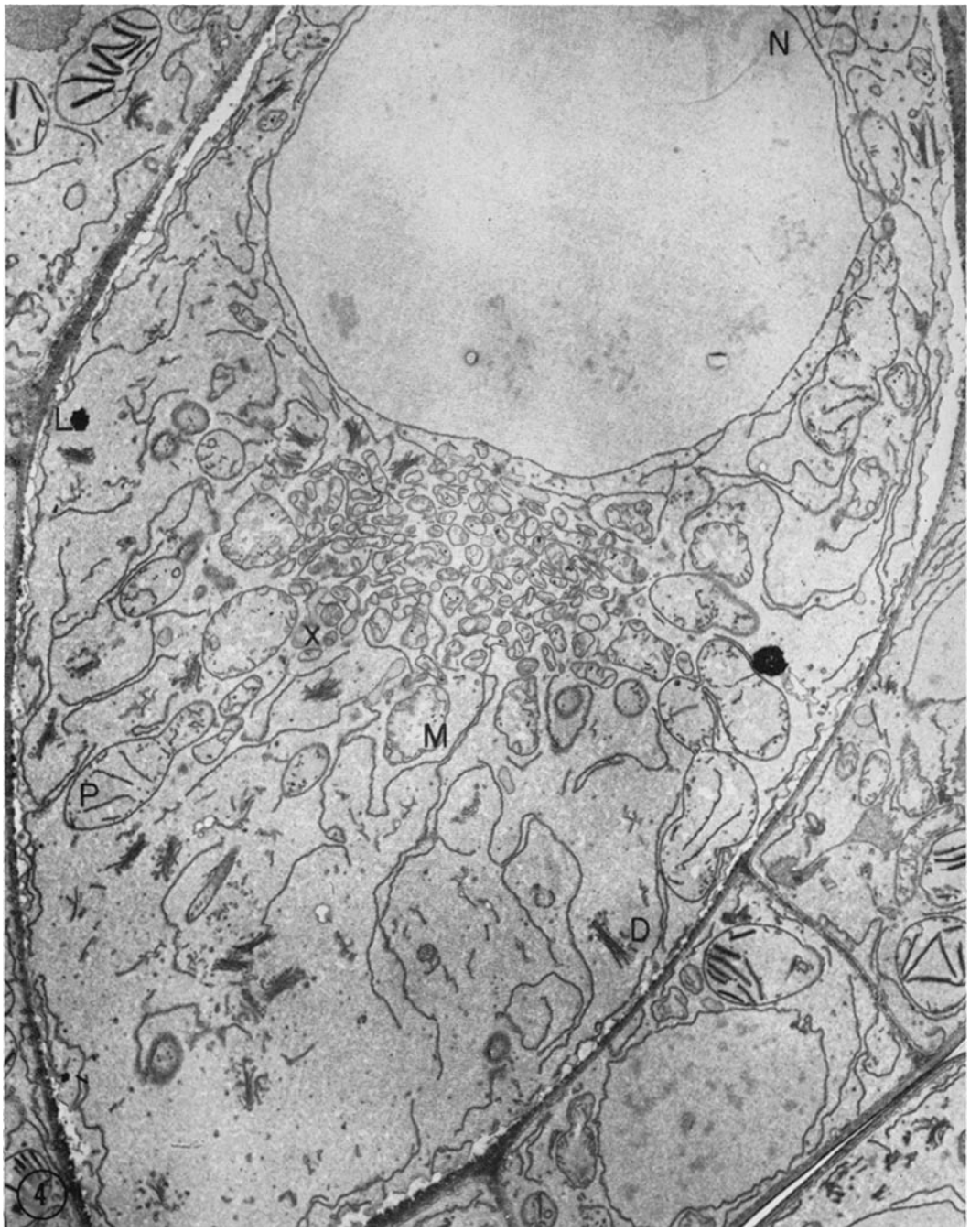

FIGURE 4 Central cell immediately preceding mitosis. Small mitochondria and bodies (*x*) with a single limiting membrane are gathered below the nucleus (*N*). Big mitochondria (*M*) and plastids (*P*) show an orientation towards the accumulation of mitochondria. The thylacoid system in the plastids of the central cell is only poorly developed as compared with that in the plastids of the ventral jacket cells. *D*, dictyosomes; *L*, lipid droplets. × 6700.

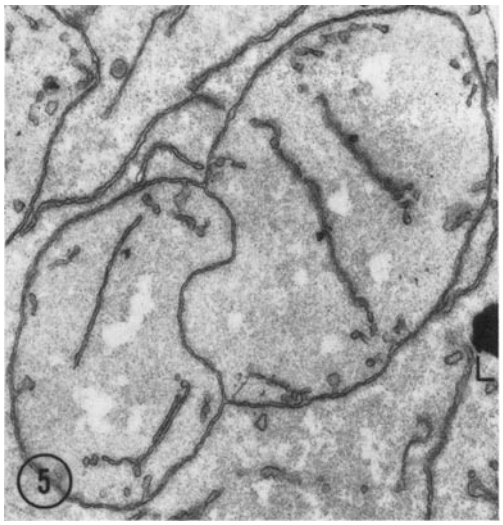

FIGURE 5 An undifferentiated plastid in the central cell. A few thylacoids, several vesicles, and small electron-transparent areas are visible in the two separated spaces. *L*, lipid droplet. × 25,000.

EGG CELL

The central cell divides unequally into the smaller ventral-canal cell and the considerably larger egg cell (Fig. 1 E and Diers, 1965 *b*, Figs. 1 to 6). In late telophase and in the very young egg, the cell constituents show the same fine structure as in the central cell. The plastids contain a poorly developed membrane system and thereby are clearly distinguished from the plastids of the neighboring archegonial wall cells. Occasionally they possess small starch grains. Indications of a vacuolization which was found by Bell and Mühlethaler (1962) in the plastids of the corresponding developmental stages in *Pteridium aquilinum* have not been observed. Mitochondria are randomly distributed in the cytoplasm of the young egg cell. They often show the intramitochondrial granules. A degeneration as described by Bell and Mühlethaler (1964 *a*) in the same developmental stages of the egg cell in *Pteridium* has not been found here. With the growth of the young egg cell, the number of plastids with starch grains multiplies so that finally plastids quite often show 3 to 6 starch grains in one section (Fig. 6).

The egg nucleus begins to show an irregular surface at the stage of starch storage in the plastids. During the further maturation of the cell, the protuberances of the nucleus become more and more distinct. Sometimes these evaginations penetrate deeply into the cytoplasm. Near the nucleus, one finds sections through such evaginations which seem to be separated from the nucleus (Diers, 1965 *b*, Figs. 8, 10, and 11). In *Pteridium*, similar evaginations were reported by Mühlethaler and Bell (1962) and Bell and Mühlethaler (1962, 1964 *a*) who went on to describe the development of mitochondria from such evaginations. Mühlethaler and Bell (1962) and Bell and Mühlethaler (1964 *b*) mention that plastids also are generated from such nuclear evaginations. In *Sphaerocarpus*, the great majority of these evaginations which seem to be separated from the nucleus are shown in serial sections to be connected to the nucleus. In a very few cases, there are some signs that evaginations may indeed be detached from the nucleus. But there was no indication in *Sphaerocarpus* that plastids or mitochondria may develop out of these separated nuclear parts (cf. Diers, 1964).

The nearly mature and the fully developed egg cells contain plastids with few membranes and several starch grains (Fig. 7). Many mitochondria are present, which show no intramitochondrial granules. One observes lipid droplets frequently lying together in groups (Diers, 1965 *b*). In contrast to the plastids in the maturing and fully developed egg cells, the plastids in the cells of the archegonial venter are differentiated into chloroplasts with a distinct internal membrane system (Fig. 8).

Mitochondria and Plastids with Narrow Constrictions

In the young and maturing eggs and in all preceding developmental stages of the archegonium, one sometimes finds in a single section plastids and mitochondria with narrow constrictions. Serial sections indicate that these constrictions are partly deceptive, because in deeper sectional planes these obviously narrow constrictions develop into broad parts of the organelle. The series in Fig. 9, for instance, shows a big mitochondrion, which possesses a constriction according to one section. But in another plane of sectioning, this constriction broadens to the full width of the mitochondrion. The model (Fig. 10) demonstrates how the picture of such a narrow constriction may arise by sectioning a mitochondrion in a certain plane. Similar observations were made on some plastids. But in other cases, series of adjacent sections show that the constriction of a

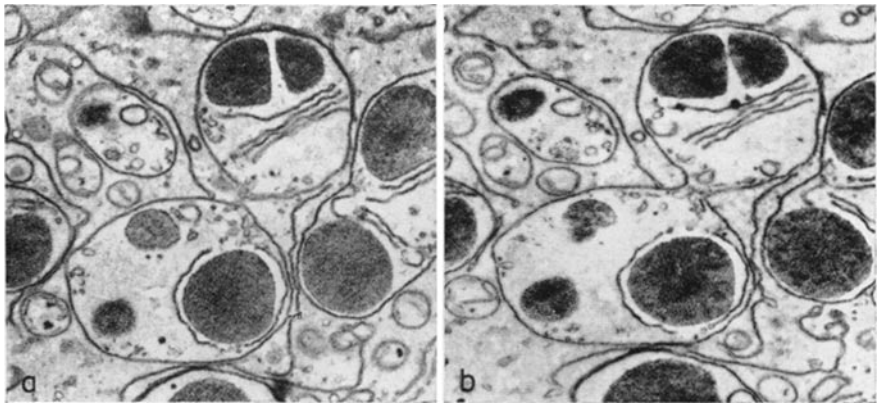

FIGURE 6 Two sections of a long series of successive sections through big starch-containing plastids in an egg cell of nearly the same age as that in the archegonium of Fig. 1 F. The plastid possesses a narrow, three-dimensional constriction, which can be observed only on five sections. × 15,000.

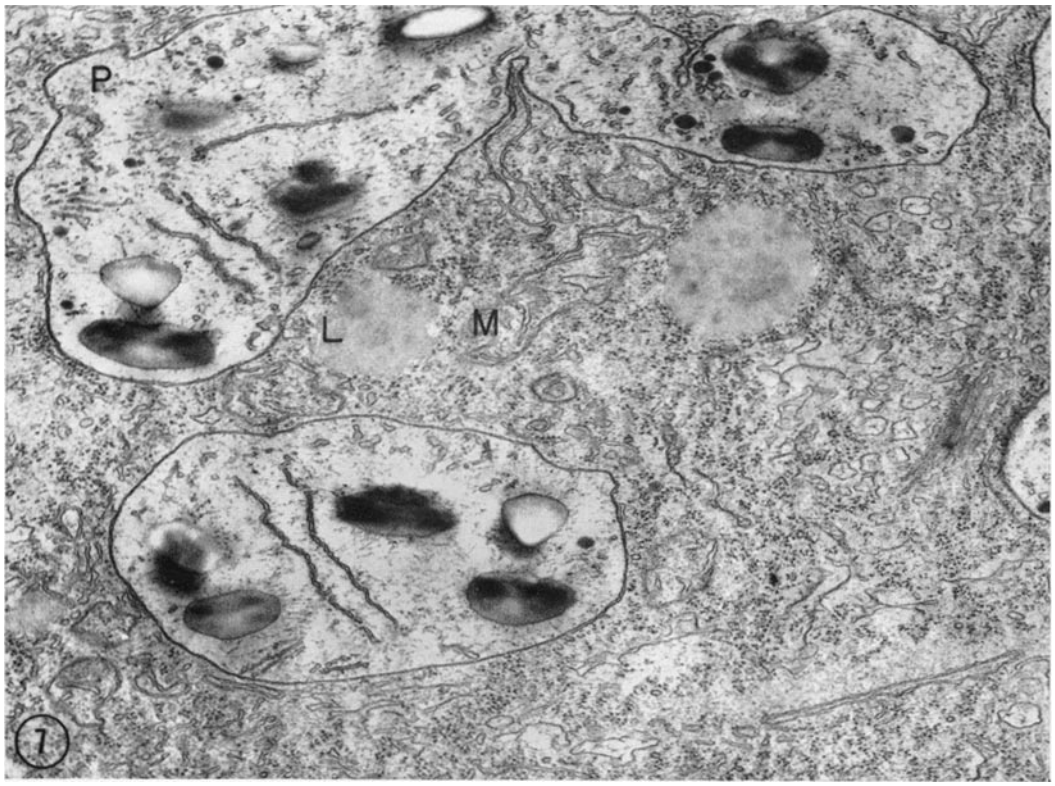

FIGURE 7 A portion of an egg cell somewhat older than the egg cell of Fig. 6, after fixation in OsO_4. In the plastids (*P*) are a few thylacoids, several starch grains, osmiophilic globuli, and small dark particles which show a high similarity to ribosomes. The cytoplasm contains, above all, ribosomes appearing as small dark dots, and, in addition, mitochondria (*M*), lipid droplets (*L*), and membranes of the endoplasmic reticulum. × 30,000.

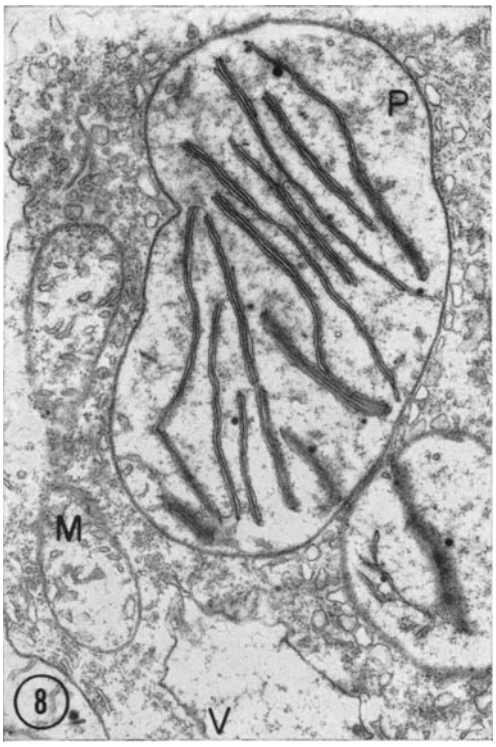

FIGURE 8 A portion of a jacket cell from the archegonial venter after OsO_4 fixation. Compare the well developed platelike grana, each consisting of 2 to 4 thylacoids here, to the few thylacoids in the plastids of the egg. *P*, plastids; *M*, mitochondrion; *V*, vacuole. × 20,000.

plastid or mitochondrion remains and is detectable only on one or a few succeeding sections. Consequently, a three-dimensional constriction is formed here (Figs. 6, 11, and 12). Such plastid and mitochondrial shapes may be regarded as the last phases of organelle division or the early stages of organelle fusion. One has to take into consideration both possibilities, because findings have been published on the division and coalescence of mitochondria (Drawert and Mix, 1961; cf. also the survey of Novikoff, 1961) and on the division (Granick, 1961; Green, 1964) and fusion (Epstein and Schiff, 1961) of plastids. A third explanation for the narrow constrictions of the mitochondria and plastids may be the plasticity of these organelles. The constrictions may be regarded as reversible variations in the plastid or mitochondrial shape which may appear or disappear, independent of division (Novikoff, 1961; Esau, 1944). The micrographs alone may not decide which of these three interpretations is right in our material. But, the calculations given in the following sections, based on the developmental history of the egg cell, allow a decision which of the three interpretations has to be accepted as the most probable here.

Quantitative Investigation on the Cell Constituents during Oögenesis

As mentioned above, the development of the egg cell may be precisely followed from its inception to maturity. This makes it possible to calculate the relative proportions of the cell constituents from the stage of the primary cell of the axial row up to that of the mature egg. The purpose of this quantitative investigation is to compare the relative proportions of organelles contained in unit areas of cytoplasm as the primary cell differentiates into the egg.

The conditions for such a quantitative analysis are satisfied. The egg and its preceding cells possess only few and small vacuoles. With the exception of the lipid bodies in some developmental stages of the egg, the organelles during oögenesis show a random distribution. Only in the maturing egg does one occasionally observe small regions at the cell periphery which seem to contain less organelles than other cell areas. A calculation showed that these deviations from the random distribution are negligible. Cells immediately before mitosis (Fig. 4) or in the early stages of mitosis were not considered because in these cells the organelles are not randomly arranged. Since the various fixatives and embedments produce different tissue volume changes (Menke, 1957; Berzborn and Menke, 1964; Kushida, 1962), all calculations were made on archegonia fixed in $KMnO_4$ and embedded in Araldite.

To obtain comparable statements on the nucleus and cell area in the successive developmental stages, as many sections as possible were evaluated which showed the whole cell sectioned in the median longitudinal direction. With respect to organelles, the results were strengthened by the evaluation of additional sections which showed the nucleus tangentially sectioned or only a larger sector of the cell without nucleus.

In each longitudinal section, the areas of the cell and of the nucleus were measured with a planimeter and the organelles were counted. The nuclear area was substracted from the total cell

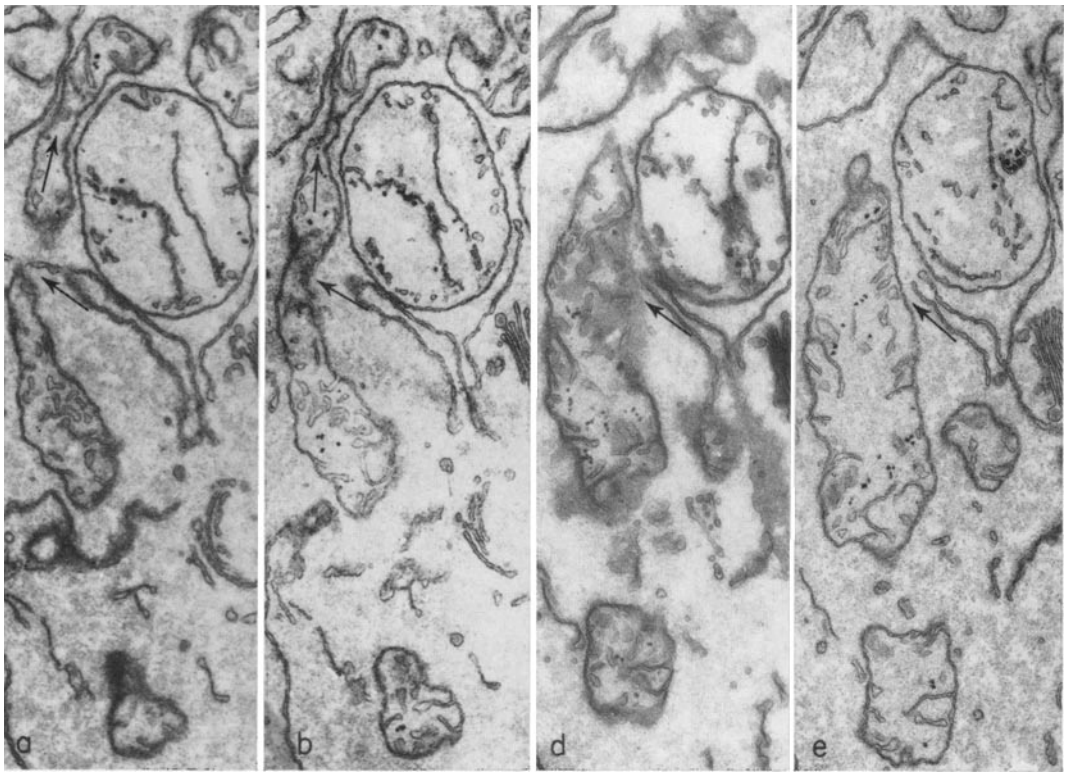

FIGURE 9 A series of adjacent sections through a mitochondrion with two narrow constrictions (arrows) in a central cell. In section *a*, upper arrow indicates a constricted region; lower arrow, a break in the continuity of the mitochondrion. In section *b*, upper arrow shows a very narrow constriction; mitochondrion has joined at lower arrow where a constriction is visible. In sections *d* and *e*, mitochondrion is much broadened at lower arrow. One section (*c*) has been omitted. Intramitochondrial granules are located as electron-opaque dots in the matrix of the mitochondrion. × 20,000.

area and the number of organelles was related to the remaining cell area, which is called henceforth cytoplasmic area. In order to use a uniformly comparable cytoplasmic area for all developmental stages and sections, the values of the number of organelles in each section were calculated for a cytoplasmic area of 100 μ^2. From these values, the mean was determined for each developmental stage.

With respect to the numbers of cell constituents per 100 μ^2 cytoplasmic area, the statistical validity of differences was always calculated according to the Student's or t test (Mather, 1946; van der Waerden, 1957). The indices of t indicate the degrees of freedom. Fig. 13 *a* shows the result of this calculation which includes the values for dictyosomes, bodies (x) with a single limiting membrane, lipid bodies, mitochondria, and plastids. The developmental stages from the primary cell of the axial row up to the mature egg are indicated on the abscissa from left to right. Fig. 13 *b* enables one to compare the sizes of the cell and nucleus in the various stages of archegonial growth. The classification of stages on the abscissa is the same as in Fig. 13 *a* and, moreover, contains some notes about the identification of the various phases of archegonial growth. Some further comments are added to characterize more precisely the evaluated preparations. The growth and the maturation of the egg cell were studied in several archegonia of closely succeeding developmental stages. Having a height of 96 μ, the archegonium of stage VIII is about half as tall as an adult archegonium. In this growth stage, the ventral-canal cell begins to be separated from the egg cell (cf. Diers, 1965 *b*, Fig. 6). The egg cell in stage IX is somewhat older. The ventral-canal cell does not yet show any indication of degenera-

tion, although it has completely separated from the egg cell. The ventral-canal cell of the succeeding stage X shows an obvious degeneration, but it is still recognizable as a cell. In an archegonium containing a nearly mature egg cell (stage XI), the ventral- and the neck-canal cells are more or less completely disintegrated; however, the neck-canal is not yet open. In the last stage XII, we see the mature egg in an archegonium with the open neck-canal. For details of the maturation of the egg cell, see Diers (1965 *b*).

What conclusions may be drawn from the quantitative analyses shown in Fig. 13?

In Fig. 13 *b* it is apparent that the cell area increases in size about 5-fold, from about 160 to 170 μ^2 up to 900 to 1000 μ^2, during oögenesis. In the archegonium of stage X, the egg cell shows an extraordinary size of 1250 μ^2. There are several indications from other preparations that this value has to be regarded as an exceptional size of no special importance. It is remarkable that, despite the unusual size in cell area, the numbers of cell constituents per 100 μ^2 agree, in part, rather well with those of the preceding and following developmental stages. The nuclear area also increases considerably in size, from about 40 to 45 μ^2 up to 200 to 240 μ^2.

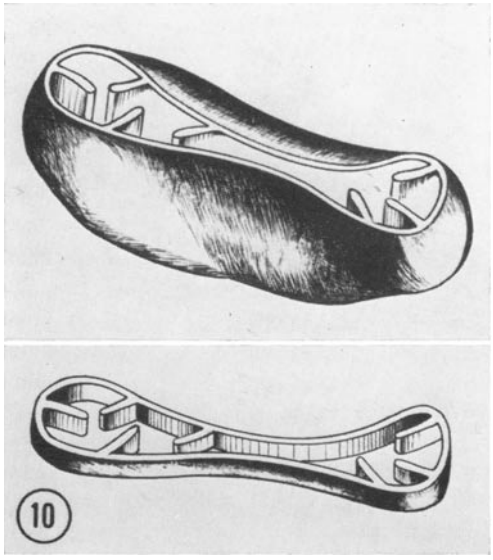

FIGURE 10 A general reconstruction of a mitochondrion, which is sectioned in such a plane that a narrow constriction of the organelle appears. If the mitochondrial length is considered to be 1.5 μ and the thickness of the thin section considered to be 500 A, then to the same scale the succeeding section from the model will be as thick as that shown in the figure and possess a narrow constriction like that in the electron micrographs in Fig. 9.

Horizontal sections show that the diameters of the egg and its preceding cells and of the nucleus in all these cells are not reduced during archegonial development. This observation together with the earlier results (Fig. 13 *b*) indicates an increases considerably in size, from about 40 to 45 μ, up to 200 to 240 μ^2,.

DICTYOSOMES

According to Fig. 13 *a* the number of dictyosomes per 100 μ^2 cytoplasmic area varies between 5 and 8. The higher value of 9 for stage IV and the lower value of about 4 for stage XII are very probably of no importance because of their only slight deviation from 8 and 5, respectively. The nearly constant value of 5 to 8 dictyosomes per 100 μ^2 cytoplasmic area shows that there is no diminution of these organelles during oögenesis. On the contrary, a multiplication of them must occur because, according to Fig. 13 *b*, the area and correspondingly the volume of the cell increase considerably. How the reproduction of the dictyosomes may occur is uncertain (cf. Whaley, 1965). Some micrographs indicate that a propagation by division is possible, as suggested by Buvat (1958).

BODIES (x)

The number of the bodies (x) with a single limiting membrane varies from 4 to 5 per 100 μ^2. Only in stages V to IX does it drop, varying from 2 to 3 and 3.5 per 100 μ^2. The difference is not great, and hence it is not possible to draw definite conclusions. However, it is striking that one observes a distinct decrease between stages IV and V and a slow increase during the succeeding developmental stages VI to IX. The difference in the values for stages IV and V can be statistically evaluated with $t_{(17)} = 6.05$, i.e., the probability for a highly significant uniformity is far below 0.001. This value, 0.001, would have been reached with $t_{(17)} = 3.97$. Therefore, the difference in the number of bodies (x) in stages IV and V is statistically significant. Consequently, the possibility cannot be eliminated that some of these bodies disappear during the division of the central cell. It is possible to imagine that these bodies play an essential but still unknown part in

the metabolism of mitosis, in the course of which they are partly used up. In any case, during maturation of the egg (stages VI to XII), the bodies increase in number little by little up to the former value of 4 to 5 per 100 μ^2. Obviously it would seem that there is a propagation of the bodies in the cell because, according to Fig. 13 *b*, the whole cell area and correspondingly the cell volume enlarge. The origin and reproduction of the bodies with a single limiting membrane during oögenesis in *Sphaerocarpus donnellii* are not yet elucidated. Despite an intense search, no micrographs have been found which indicate that the bodies originate from the endoplasmic reticulum or the dictyosomes. But occasionally one observes bodies with narrow constrictions (Fig. 2). Such findings suggest that these bodies may multiply by their division.

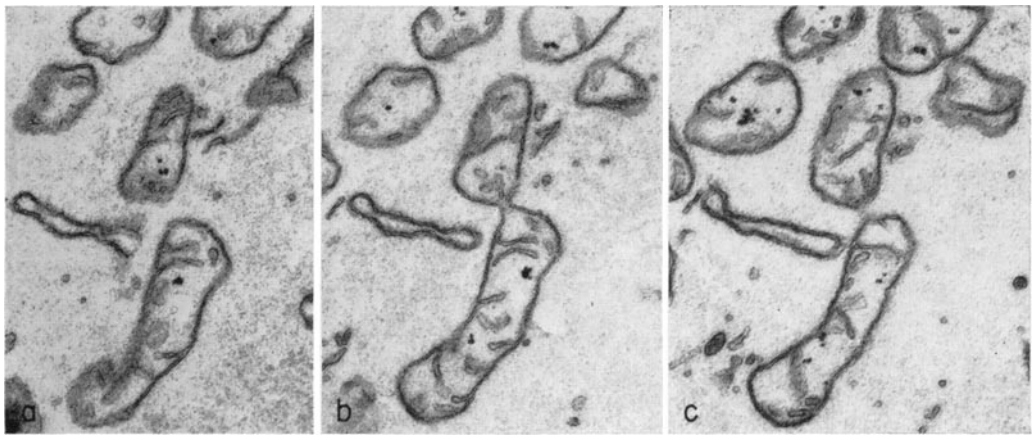

FIGURE 11 Three successive sections of a longer series with a narrow, three-dimensional constriction of a mitochondrion in a central cell. This constriction is discernible only on one section (*b*). Intramitochondrial granules can be seen in the mitochondria. × 25,000.

LIPID BODIES

A statistical analysis of lipid bodies shows that, during the growth of the egg cell, the lipid bodies become more frequent at a significant level (cf. the general micrographs, Diers 1965 *a* and *b*). The values for stages X and XII seem to be exceptionally high. This increase is partially accounted for by the aggregation of lipid bodies into large groups. Therefore one must not place any special importance on these high values. As in stages IX and XI, values of about 6 to 8 per 100 μ^2 are thought to be normal values in all the older developmental stages. These values (6 to 8) are considerably higher than those of 0.5 to 2 per 100 μ^2 in stages I to VII. The statistical calculation with the highest value for stages I to VII and with the lowest value for stages VIII to XII results in a highly significant difference with $t_{(12)} = 7.85$. This means that the probability error, P, is below 0.001, because 0.001 is already reached with $t_{(12)} = 4.32$. Consequently, during oögenesis, an increase in the number of lipid bodies per 100 μ^2 occurs. The total number of lipid droplets increases all the more in the cell because, according to Fig. 13 *b*, the egg cell shows an apparent enlargement of its volume.

MITOCHONDRIA

According to Fig. 13 *a* the number of mitochondria per 100 μ^2 cytoplasmic area does not decrease but rather increases during oögenesis. The values fall into three ranges: between 13 and 15 in stages I to III, between about 23 and 26 in stages IV to IX, and between 32 and 36 in the last stages, X to XII. The difference in values for stages III and IV is statistically significant with $t_{(13)} = 12.23$, while $t_{(13)} = 4.22$ is sufficient for $P = 0.001$. Another highly significant difference in the values occurs for stage IX and the succeeding stages. Consequently, a considerable propagation of mitochondria must occur.

PLASTIDS

During oögenesis, nearly all the numbers of plastids fall between 6 and 9 per 100 μ^2. However, the number present during stages IV and V is

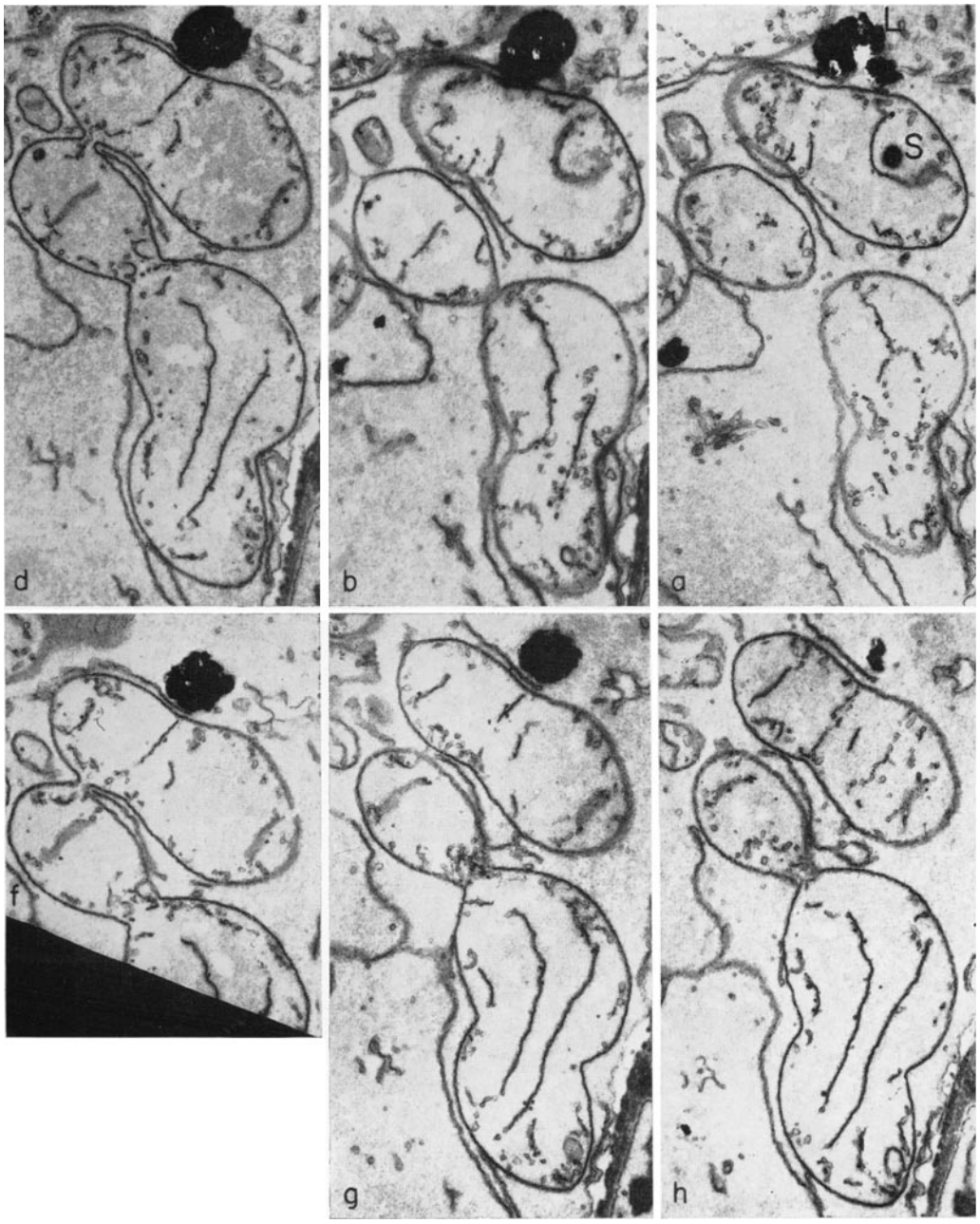

FIGURE 12 The same central cell as in Fig. 4. A part of a longer series of adjacent sections through a plastid with a narrow, three-dimensional constriction and a broader constriction (sections *c* and *e* omitted). The narrow constriction is discernible in three successive sections (*d* to *f*). One of the three portions of the plastid contains a starch grain (*S*) in the first (*a*) section and in the preceding (unpublished) sections. *L*, lipid droplet. × 15,000.

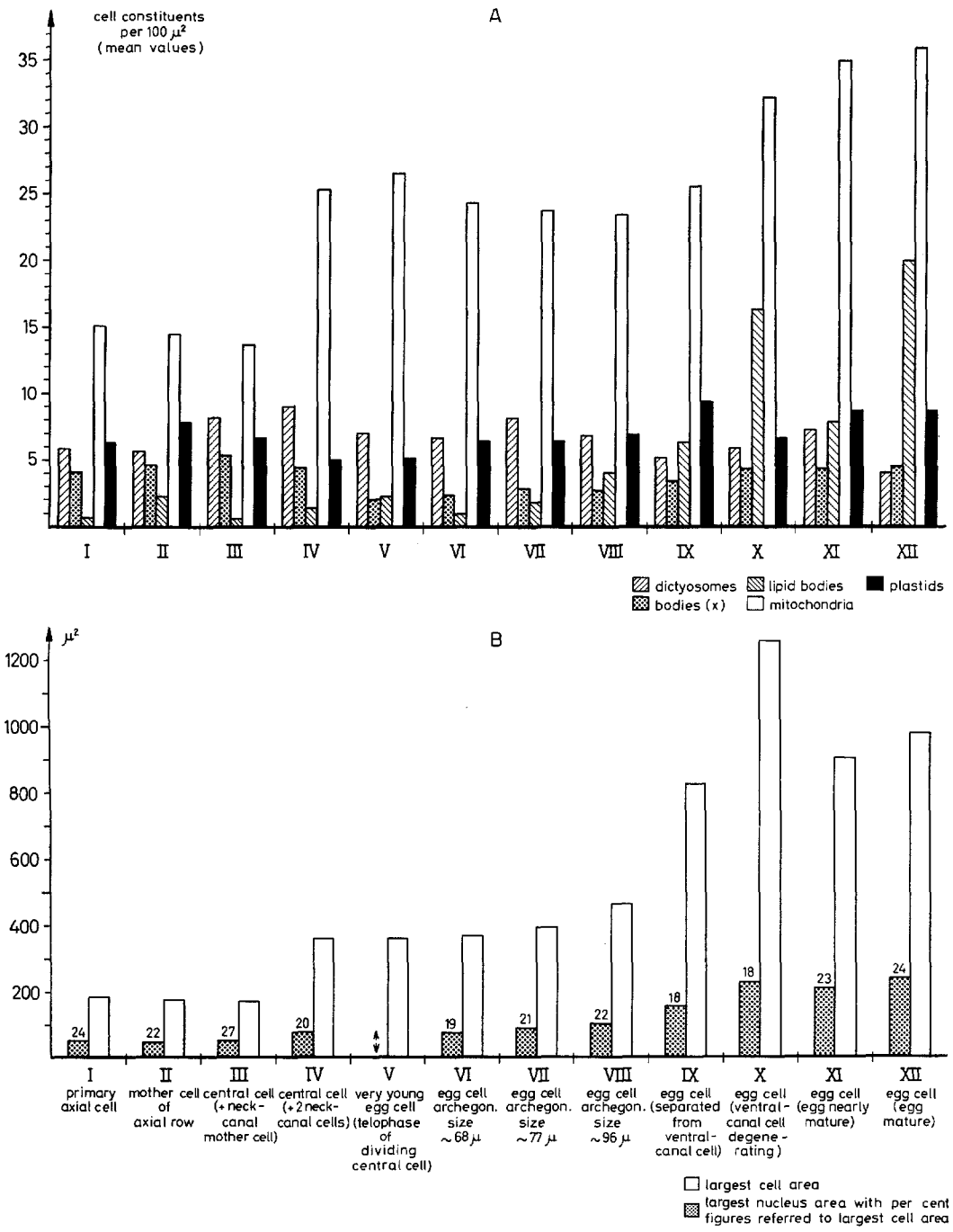

FIGURE 13 Developmental stages of the egg and its preceding cells.

FIGURE 13 *a* Cell constituents per 100 μ^2 cytoplasmic area during oögenesis in *Sphaerocarpus donnellii Aust.*

FIGURE 13 *b* The largest cell and nuclear areas in median longitudinal sections during oögenesis in *Sphaerocarpus donnellii Aust.*

somewhat more than 5, and the number present during stage IX is about 9.5, but these values are not significant statistically, or they are just at the limit of statistical significance, for $P = 0.001$. One may conclude that these small differences have no importance. In contrast to the lipid bodies and mitochondria, but corresponding to the dictyosomes, the number of plastids per 100 μ^2 cytoplasmic area is nearly constant during oögenesis. Because the cell size enlarges (Fig. 13 *b*), the plastid number must, however, also increase. Therefore, a propagation of plastids has to take place in the whole cell during formation and maturation of the egg.

The quantitative investigation leads to the conclusion that a reproduction of mitochondria and plastids must occur during oögenesis. Based on this result, one may now interpret the narrow, three-dimensional constrictions of plastids and mitochondria which were observed in several of the developmental stages investigated (Figs. 6, 11, and 12). As mentioned above, such plastids and mitochondria look like organelles which are in division or fusion or are undergoing reversible variations in shape because of their plasticity. Plastids and mitochondria do not coalesce at this stage, nor can the three-dimensional constrictions be accounted for simply by a change in shape. Only the division of preexisting plastids and mitochondria could account for the increasing numbers of mitochondria and the constant number of plastids per 100 μ^2 of cytoplasm concomitant with the increase in cell size and consequently of volume of cytoplasm. Therefore, in all probability, profiles showing narrow, three-dimensional constrictions must be interpreted as division figures.

The Total Numbers of Plastids and Mitochondria in Whole Cells of Various Developmental Stages

Based on the median longitudinal sections, one may evaluate the volumes of the cell and the nucleus. The cells which contain only small vacuoles were considered as a cube, a cylinder, or a conical frustum. The dimensions necessary for the calculation were taken from the median longitudinal section which showed the largest cell area. The nucleus was regarded as a sphere. The radius was calculated from the largest planimetered nuclear area, which was then considered as a circular area.

TABLE I

The Cell and Nuclear Volumes and the Total Numbers of Mitochondria and Plastids per Cell in Some Characteristic Developmental Stages of the Egg and its Preceding Cells

Stages of development	Cell vol	nucleus vol	Total mitochondria	Total plastids
	μ^3	μ^3		
II Mother cell of axial row	1300	100	60–90	20–35
IV Central cell (2 neck-canal cells)	3200	300	250–400	30–50
VI Egg cell archegonial size 68 μ	3300	300	250–400	40–65
VIII Egg cell archegonial size 96 μ	6000	450	450–650	80–120
XI Egg cell (egg nearly mature)	10300	1200	1000–1600	160–270
XII Egg cell (egg mature)	9800	1500	800–1300	150–260

The lengths of the longest plastids and mitochondria were measured on the sections. On the average, these measurements, rounded off to the next higher figure, come to 3 to 5 μ for the plastids and 1.5 to 3 μ for the mitochondria. The very rare, unusually long plastids and mitochondria were not considered. For each developmental stage, the mean values are known which give, on the average, the numbers of sectioned plastids or mitochondria per 100 μ^2 cytoplasmic area (Fig. 13 *a*). If one multiplies 100 μ^2 by the length measurements for the plastids (3 to 5 μ) or mitochondria (1.5 to 3 μ), then it is apparent that the known mean values per 100 μ^2 (Fig. 13 *a*) also are approximately the numbers of whole plastids and whole mitochondria in 300 to 500 μ^3 and 150 to 300 μ^3 cytoplasmic volumes, respectively. These values are related to the whole cytoplasmic volume which is equal to cell volume minus nuclear volume. And thus one obtains the estimated total numbers of plastids and mitochondria in the whole cell (Table I). Because the cells contain

numerous plastids and mitochondria which are smaller than 3 to 5 μ and 1.5 to 3 μ, respectively, the results in Table I are more or less minimum numbers. If these numbers are multiplied by 1.5 to 2, they should give nearly maximum numbers.

Table I shows that in all stages the nuclear volume averages about $\frac{1}{7}$ to $\frac{1}{14}$ of the total cell volume. A definite relation between nucleus and cytoplasm is maintained during the whole development of the egg cell. This would be already suggested from the relation between the largest cell area and the largest nuclear area (Fig. 13 *b*). From the early stage of the mother cell of the axial row (stage II) up to the stage of the mature or nearly mature egg (stages XI and XII), the volume of the cell increases about 8-fold, and the volume of the nucleus about 15-fold. Simultaneously, the numbers of plastids and mitochondria also increase about 8-fold to 15-fold. Therefore the relation between the nuclear or cell volume and the total numbers of plastids and mitochondria in the cell is maintained relatively strictly during oögenesis.

DISCUSSION

The plastid in Fig. 2 shows a peculiarity which was observed only once in all the archegonia examined. At one point the plastid membrane is immediately connected with a cisterna of the endoplasmic reticulum (Fig. 3). This suggests that there may be very close functional interactions between the endoplasmic reticulum and the plastids, or, alternatively, that relations exist between the membranes of the plastids and the endoplasmic reticulum during their development. For the present, both interpretations have to remain speculative; they seem to be unlikely, however, because of the rare occurrence of this fusion of membranes. A simpler explanation is more probable, that occasionally cell constituents are pushed firmly against one another by cytoplasmic streaming, resulting in a coalescence of membranes observable in the electron microscope. The possibility of fusion of the outer plastid membrane and the membranes of the endoplasmic reticulum suggests a certain similarity in the structure and the composition of these membranes. Robertson (1964, Figs. 16 and 17) has pointed out adequate connections between the membranes of mitochondria and the membranes of the endoplasmic reticulum.

In the late developmental stages of the central cell (stage IV) and in the dividing central cell, one observes electron-opaque, intramitochondrial granules. Their composition and function are still unknown. The same black dots are visible in mitochondria in the young egg cell. Because these intramitochondrial granules become rarer during maturation of the egg, one may suppose that in certain developmental phases of the central cell and also of the subsequent egg cell, the metabolism in the cell or in the mitochondria alters, and that this becomes apparent in the appearance and disappearance of intramitochondrial granules. According to the investigations of Peachey (1964), the intramitochondrial granules become increasingly prominent when the cells of toad urinary bladder or the isolated mitochondria of rat kidney are kept in a medium with divalent cations, barium, strontium, or calcium.

Small electron-transparent regions occasionally occur in the matrix of mitochondria and of plastids during all developmental stages. Such electron-transparent areas are widespread, especially in undifferentiated plastids and in mitochondria of meristematic tissues (cf. Whaley et al. 1960; Diers and Schötz, 1965). These regions may contain components which are easily leached out during fixation and dehydration, or they may be caused by a slight swelling of the organelles. But it is highly improbable that they are the first stages of a degenerative vacuolization.

In *Sphaerocarpus*, the micrographs show no indication of degeneration and elimination of plastids or mitochondria during oögenesis. If a degeneration of these organelles took place in *Sphaerocarpus*, as Mühlethaler and Bell (1962) describe for the young egg of *Pteridium aquilinum*, such a degeneration would be expressed by a dramatic decrease in the actual numbers of these organelles in, at least, some stages of development. According to Fig. 13 *a*, however, the numbers of the mitochondria and plastids show no diminution even while the cell volume simultaneously enlarges. The result of the quantitative analysis in the present investigation leads to the conclusion that, in *Sphaerocarpus*, no degeneration and elimination of plastids and mitochondria occurs during oögenesis. But since, according to Bell and Mühlethaler (1964*a*), the degeneration stage of the mitochondria occupies an estimated period, lasting about 1 to 2 hr, the objection could be raised that this stage has been missed in the present study. To invalidate this objection, the time of archegonium

and egg cell development was determined on living gametophytes. Beginning with the earliest detectable elevation on the upper side of the thallus or with the stage of the dividing central cell up to the mature egg, this development is completed within 5 to 6 or, at the most, 4 days, respectively, in our material. Therefore, it is possible to seize the whole oögenesis in temporally close successive stages. Because more than 80 archegonia were studied under the electron microscope, we may conclude that all important developmental stages were obtained. Above all, the developmental stages of the central cell and the very young egg cell were investigated in detail because, according to Bell and Mühlethaler (1962), it is in these stages of archegonial development in *Pteridium* that vacuolization and degeneration of plastids and mitochondria take place. Despite an intense search, such degeneration was not observable in *Sphaerocarpus*. Furthermore, one must bear in mind that degeneration stages of organelles as large as mitochondria and plastids could not vanish without leaving their traces. Such degeneration stages must be recognizable in the cytoplasm for a somewhat longer time.

In *Pteridium*, according to Bell and Mühlethaler (1964*a*), immediately after or perhaps somewhat before the end of the mitochondrial degeneration, the egg begins to form new mitochondria from detached nuclear evaginations. In *Sphaerocarpus*, long before the egg nucleus shows evaginations which occur in developmental stages VIII to IX, one observes a distinct increase in the numbers of mitochondria and plastids (Fig. 13 *a* and Table I). Consequently, this reproduction of the organelles certainly cannot occur by detachment of nuclear evaginations. The most convincing explanation of this augmentation of the numbers of mitochondria and plastids is that the already existing plastids and mitochondria divide. This is supported by the appearance of narrow constrictions of these organelles, which in all probability have to be interpreted as divisional stages as mentioned above.

Besides the reports on *Pteridium aquilinum*, in recent years several other electron microscope investigations on egg cell development in other plants have been published. Menke and Fricke (1964) studied oögenesis in the fern *Dryopteris filix-mas*. Like *Pteridium*, this species belongs to the Filicales. Therefore, both species are more or less closely related to one another. Though in *Dryopteris* the alterations in the fine structure of the cells taking part in oögenesis show a high similarity to the findings in *Pteridium aquilinum*, Menke and Fricke (1964) did not observe in *Dryopteris* a degeneration of plastids and mitochondria and did not recognize a neoformation of these organelles from nuclear evaginations during the development and maturation of the egg.

Jensen (1963) studied the ultrastructure of the megagametophyte of the cotton plant and observed that the cytoplasm of the egg contains well recognizable mitochondria and starch-containing plastids. Camefort (1962) investigated some stages of egg cell development in *Pinus laricio var. austriaca* and observed a more or less distinct degeneration of the mitochondria and an extensive deformation of the plastids in the mature egg. Rodkiewicz and Mikulska (1963) and Mikulska and Rodkiewicz (1964) reported a degeneration of many mitochondria in the developing megasporocyte of *Lilium candidum*. These investigations on *Pinus* and *Lilium* were made with material fixed only in OsO_4.

In any case, the findings already published on oögenesis in plants prove that the suggested general validity of elimination of plastids and mitochondria and their neoformation from the egg nucleus is not always realized.

The author gratefully acknowledges stimulating discussions with Professor W. Menke. The work was supported by the Deutsche Forschungsgemeinschaft.

Received for publication 22 July 1965.

BIBLIOGRAPHY

BELL, P. R., and MÜHLETHALER, K., 1962, The fine structure of the cells taking part in oögenesis in *Pteridium aquilinum* (L.) Kuhn, *J. Ultrastruct. Research*, **7**, 452.

BELL, P. R., and MÜHLETHALER, K., 1964 *a*, The degeneration and reappearance of mitochondria in the egg cells of a plant, *J. Cell Biol.*, **20**, 235.

BELL, P. R., and MÜHLETHALER, K., 1964 *b*, Evidence for the desoxyribonucleic acid in the organelles of the egg cells of *Pteridium aquilinum*, *J. Mol. Biol.*, **8**, 853.

BERZBORN, R., und MENKE, W., 1964, Zur Beurteilung der Kaliumpermanganat-Fixierung von Chloroplasten, *Z. Naturforsch.*, **19 b**, 763.

BUVAT, R., 1958, Nouvelles observations sur l'appareil de Golgi dans les cellules des végétaux vasculaires, *Compt. rend. Acad. Sc.*, **246**, 2157.

CAMEFORT, H., 1962, L'organisation du cytoplasme dans l'oosphére et la cellule centrale du *Pinus laricio* Poir. (*var. austriaca*), *Ann. Sc. Nat.*, **3**, 265.

DIERS, L., 1964, Bilden sich während der Oogenese bei Moosen und Farnen die Mitochondrien und Plastiden aus dem Kern?, *Ber. bot. Ges.*, **77**, 369.

DIERS, L., 1965 *a*, Elektronenmikroskopische Beobachtungen zur Archegoniumentwicklung des Lebermooses *Sphaerocarpus donnellii* Aust. Die Entwicklung des jungen Archegons bis zum Stadium der fertig ausgebildeten Sekundären Zentralzelle, *Planta*, **66**, 165.

DIERS, L., 1965 *b*, Elektronenmikroskopische Untersuchungen über die Eizellbildung und Eizellreifung des Lebermooses *Sphaerocarpus donnellii* Aust., *Z. Naturforsch.*, **20 b**, 795.

DIERS, L., und SCHÖTZ, F., 1965, Über den Feinbau pflanzlicher Mitochondrien, *Z. Pflanzenphysiol.*, **53**, 334.

DRAWERT, H., und MIX, M., 1961, Licht- und elektronenmikroskopische Untersuchungen an *Desmidiaceen*. VIII Mittlg. Die Chondriosomen von *Micrasterias rotata*, *Flora*, **151**, 487.

EPSTEIN, H. T., and SCHIFF, J. A., 1961, Studies of chloroplast development in *Euglena*. 4. Electron and fluorescence microscopy of the proplastid and its development into a mature chloroplast, *J. Protozool.*, **8**, 427.

ESAU, K., 1944, Anatomical and cytological studies on beet mosaic, *J. Agric. Research*, **69**, 95.

GANTT, E., and ARNOTT, H. J., 1963, Chloroplast division in the gametophyte of the fern *Matteuccia struthiopteris* (L.) Todaro, *J. Cell Biol.*, **19**, 446.

GRANICK, S., 1961, The chloroplasts: inheritance, structure and function, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press Inc., 1961, **2**, 489.

GREEN, P. B., 1964, Cinematic observations on the growth and division of chloroplasts in *Nitella*, *Am. J. Bot.*, **51**, 334.

HAUSTEIN, E., 1962, Die Kontinuität der Plastiden und die Beobachtungen von Mühlethaler und Bell, *Z. Vererbungslehre*, **93**, 531.

JENSEN, W. A., 1963, Cell development during plant embryogenesis, *Brookhaven Symp. Biol.*, **16**, 179.

KUSHIDA, H., 1962, A study of cellular swelling and shrinkage during fixation, dehydration and embedding in various standard media, *J. Electronmicr.*, **11**, 135.

MATHER, K., 1946, Statistical Analysis in Biology, London, Methuen & Co., Ltd., 1946.

MENKE, W., 1957, Artefakte in elektronenmikroskopischen Präparaten. I. Mitt.: Anisotrope Volumänderungen von Chloroplasten, *Z. Naturforsch*, **12 b**, 654.

MENKE, W., 1961, Uber die Chloroplasten von *Anthoceros punctatus*, *Z. Naturforsch.*, **16 b**, 334.

MENKE, W., und FRICKE, B., 1964, Beobachtungen über die Entwicklung der Archegonien von *Dryopteris filix-mas*, *Z. Naturforsch.*, **19 b**, 520.

MEYER, A., 1883, Das Chlorophyllkorn in chemischer, morphologischer und biologischer Beziehung, Leipzig, 1883.

MIKULSKA, E., and RODKIEWICZ, B., 1964, The cytoplasmic structure of the megasporocyte and embryo sac of a lily as seen under an electron microscope, *Acta Soc. Bot. Polon.*, **33**, 619.

MÜHLETHALER, K., und BELL, P. R., 1962, Untersuchungen über die Kontinuität von Plastiden und Mitochondrien in der Eizelle von *Pteridium aquilinum* (L.) Kuhn, *Naturwissensch.*, **49**, 63.

NOVIKOFF, A. B., 1961, Mitochondria (Chondriosomes), *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press Inc., 1961, **2**, 299.

PEACHEY, L. D., 1964, Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granules, *J. Cell Biol.*, **20**, 95.

RENNER, O., 1934, Die pflanzlichen Plastiden als selbständige Elemente der genetischen Konstitution, *Ber. sächs. Akad. Wissensch. Math.-Phys. Kl.*, **76**, 241.

RICKETT, H. W., 1920, The development of the thallus of *Sphaerocarpus donnellii* Aust., *Am. J. Bot.*, **7**, 182.

ROBERTSON, J. D., 1964, A review with recent new studies of experimental alterations and a new subunit structure in synaptic membranes, *in* Cellular Membranes in Development, (M. Locke, editor), New York, Academic Press Inc., 1964, 1.

RODKIEWICZ, B., and MIKULSKA, E., 1963, Electron microscope observations of cytoplasmic changes in developing megasporocyte of *Lilium candidum*, *Flora*, **154**, 383.

SCHIMPER, A. F. W., 1885, Untersuchungen über die Chlorophyllkörner und die ihnen homologen Gebilde, *Jahrb. wissensch. Bot.*, **16**, 1.

SCHÖTZ, F., 1962, Zur Kontinuität der Plastiden, *Planta*, **58**, 333.

SCHÖTZ, F., und SENSER, F., 1964, Untersuchungen über die Chloroplastenentwicklung bei Oenothera. III. Der pictirubata-Typ, *Planta*, **63**, 191.

SCHÖTZ, F., und DIERS, L., 1965, Elektronenmikroskopische Untersuchungen über die Abgabe von Plastidenteilen ins Plasma, *Planta*, **66**, 269.

STUBBE, W., 1962, Sind Zweifel an der genetischen Kontinuität der Plastiden berechtigt? Eine Stellungnahme zu den Ansichten von Mühlethaler und Bell, *Z. Vererbungslehre*, **93**, 175.

SCHWEMMLE, J., HAUSTEIN, E., STURM, A., und BINDER, M., 1938, Genetische und zytologische Untersuchungen an *Eu-Oenotheren*. *Z. indukt. Abstammungs-u. Vererbungsl.*, **75**, 358.

VAN DER WAERDEN, B. L., 1957, Mathematische Statistik, Berlin, Springer-Verlag, 1957.

WEIER, T. E., 1961, The ultramicro structure of starch-free chloroplasts of fully expanded leaves of *Nicotiana rustica*, *Am. J. Bot.*, **48**, 615.

WHALEY, W. G., 1965, Proposals concerning replication of the Golgi apparatus, *in* Probleme der biologischen Reduplikation, (P. Sitte, editor), Berlin, Springer-Verlag, 1965, in press.

WHALEY, W. G., MOLLENHAUER, H. H., and LEECH, J. H., 1960, The ultrastructure of the meristematic cell, *Am. J. Bot.*, **47**, 401.