Relation Between Basophilia and Fine Structure of Cytoplasm in the Fungus *Allomyces macrogynus* Em.

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ABSTRACT

In a fungus, *Allomyces macrogynus* Em., staining tests have revealed changes in the location of cytoplasmic basophilia following different phases of the developmental cycle. These variations in location were used to observe which fine structures correspond to basophile and non-basophile areas of the cytoplasm.

Hyphae, gametangia, zygotes, and plants were fixed at various developmental stages in OsO_4, pH 6.1, and embedded in vestopal. Sections were examined in the electron microscope.

Comparison of basophile and non-basophile cytoplasms leads to the conclusion that cytoplasmic particles of 150 to 200 A in diameter are responsible for basophilia. The possibility of these particles being ribosomes is discussed and confirmed.

The present paper also describes some observations on the fine structure of other cellular components of this fungus, such as nuclei, mitochondria, various granules, and flagella.

INTRODUCTION

The first electron microscopic studies of basophilic regions in different animal cells revealed two cytoplasmic components: one membranous and vesicular (endoplasmic reticulum) (28, 29) and the other particulate (24, 28, 29). The particles were thought to be responsible for basophilia as they were also found in some basophile cytoplasms where the membranous component is not prominent (29).

The basophile reaction of the cytoplasm is generally referable to the presence of ribonucleoproteins. This is usually ascertained by the use of specific tests such as enzymatic and acid digestion of RNA.

Observations made on material extracted from animal cells by Palade and Siekevitz (26, 27), from pea seedlings by Ts'o *et al.* (39) as well as from bacteria (11, 35, 38) and yeasts (9), have shown the existence of a particulate component formed of ribonucleoproteins. As pointed out recently by Tissières and Watson (38), the particles extracted from these varied sources show a similarity in sedimentation coefficient, size, and chemical composition.

Working on liver and pancreas, Palade and Siekevitz have compared, by electron microscopy, thin sections of intact tissue with thin sections of pellets of various cell fractions. They conclude that the particles either free, or attached to the endoplasmic reticulum, are the component containing most of the ribonucleoproteins (26, 27).

For these animal tissues the correlation between chemistry and morphology seems to be well established. Regarding plant tissues, however, systematic studies of the relation between basophilia and the fine structure have not been made since the early work of Brown *et al.* on bean root (4). At that time the thin-sectioning techniques were not good enough to permit a clear answer. Investigations of the fine structure of plant cells are far less numerous than studies on the fine structure of animal cells and the particles found in the cytoplasm are usually assumed to be ribonucleoproteins, simply by analogy with Palade's findings (5, 37). It is really not known, however,

whether particles will be found in the cytoplasm of all kinds of plant cells, in the large number and diverse forms found in the plant kingdom nor whether these particles are always related to basophilia. In the present paper we show, on the basis of cytochemical tests, that the correlation between dense cytoplasmic particles and ribonucleoproteins holds true for a fungus.

In the fungus *Allomyces*, the intracellular location of basophile material varies during the developmental cycle. This variation of location can be used to observe the fine structure which corresponds to the basophilia. It is an advantage of this system that this can be done *in situ*, without extraction of material from the cell. The fine structure of the gamete in *Allomyces* has already been partly described (45). During this phase the RNA basophilia is confined to a region close to the nucleus, in a so called nuclear cap (41). This basophile body has been found to consist of a dense matrix enclosed in a double membrane; no transverse, internal membranes have been observed.

This preliminary study has now been extended to the fine structure of other phases of the gametophytic cycle. The developing gamete, the germinating zygote, and the young plant have been investigated. In this study particular attention was paid to the appearance of the nuclear cap in the gamete and subsequently to its disappearance before germination.

Gametes in *Allomyces* are formed in male and female gametangia which have developed on gametophytic hyphae (12). During the formation of the gametes, the gametangia go through various stages of organization (43). One of these stages is quite typical: each of the numerous nuclei is encircled by lipid droplets in a crown-like fashion, while the surrounding cytoplasm is lightly and more or less uniformly basophile. If gametangia at this stage are dipped into a hypotonic medium, they very quickly form their gametes (in 1 to 1½ hours). The distribution of basophilia becomes less uniform, and later, during formation of the limiting cytoplasmic membranes, areas of rather strong basophilia are visible. Finally, the whole basophilia concentrates into the nuclear cap (43). As already mentioned, this basophile cap is composed mainly of RNA (41).

The liberated flagellate gametes swim in the medium and conjugate. The zygote germinates and gives rise to a young sporophytic plant with first a rhizoid and later a hypha.

At the time of germination, the zygote settles down, looses its flagella, and rounds up. The nuclear cap as an entity is no longer visible, but from then on, basophilia is observed to diffuse throughout the cell; at first the intensity of the basophile reaction is stronger in the center of the cell than at its periphery (42, 44). The cytoplasm of the plant, rhizoids, and hyphae, is entirely basophile.

The observations on the fine structure of basophile and non-basophile cytoplasm at these various phases of the cycle of development are dealt with in the present paper. We are adding observations made at the same time on some cell components, such as mitochondria, various granules, and flagella.

Material and Methods

Organism and Method of Culture:

A small piece of gametophytic hypha of *Allomyces macrogynus* Em. was inoculated onto Emerson's agar-containing medium (YpSs) (43) and cultured at 25°C. for 12 to 13 days.

Sampling of Material for Fixation:

Small blocks of solid medium carrying gametangia, that had reached the "lipid-crown" stage, were dipped into the hypotonic mineral solution of Machlis (43). They were allowed to mature for various times, then cut into one cubic millimeter cubes and fixed.

Motile cells and young plants were lightly centrifuged, then fixed, and finally delicately coated with agar (33) previous to embedding.

Fixation and Embedding:

The material was fixed with 1 per cent OsO_4 in Michaelis acetate veronal buffer at pH 6.1, without NaCl, and with addition of 0.001 M $CaCl_2$. Fixation was continued for about 16 hours at room temperature. The material was then washed with 0.5 per cent uranyl acetate for 2 hours, dehydrated with acetone, and embedded into polyester (usually vestopal W) according to Ryter and Kellenberger's method (32, 33).

Microtome and Electron Microscope:

Thin sections were cut with glass knives on a microtome designed by Kellenberger (17) and photographs taken with an RCA model EMU 2D microscope, fitted with Canalco apertures (10 mil) in the condenser and (2 mil) in the objective.

Image Interpretation:

The term "density" refers to the intensity of grey on the image. This grey results from both the concentration of matter and the specific electron-scattering power of this osmium-fixed matter. For example: highly concentrated polysaccharides, as in the gametangium wall (Fig. 3, *gw*), embedded in vestopal, give a very low "density" because polysaccharides have a lower electron-scattering power than vestopal.

Cytochemical Tests:

The presence of RNA in the nuclear cap of the gamete and the various locations of cellular basophilia have been previously determined by the use of basic dyes with ribonuclease and acid hydrolysis (41–43). The suggestion that RNA is responsible for the basophilia in the nuclear cap of the gamete and in the cytoplasm of the plant is also supported by experiments in induced fluorescence with acridine orange (2). They were carried out with the kind help of Dr. E. S. Anderson.

The controls on the location of basophilia were done on material fixed with formol 10 per cent-ethanol 1:1 and stained with acetic toluidine blue. Photographs were taken with a Wild microscope (objective Hi.100, yellow filter).

OBSERVATIONS ON THE FINE STRUCTURE OF THE CYTOPLASM

(a) During the Formation of the Gametes:

In the tip of the undifferentiated hypha, where basophilia is uniform, "dense" granules with a diameter from 150 to 200 A are present. These are evenly distributed in the whole cytoplasm, as shown in Fig. 1. To simplify, we shall hereafter refer to such granules as R particles.

Long, thin vesicles, that is cisternae, as well as small spherical ones are observed throughout. Usually, they do not contain R particles (Fig. 1). Since the distribution of basophilia in the very young gametangia is the same as in the hypha just cited, we have not considered them, but have started our observations on gametangia at the "lipid-crown" stage. Here, the distribution of basophilia is slightly patchy, but because of the coarse appearance of the cytoplasm, precise observations of this distribution are difficult (Fig. 6). Studies of the fine structure show that the cytoplasm again contains the dense granules of about 150 to 200 A in diameter; *i.e.* the R particles. They are not uniformly distributed, but form areas of varying concentration (Figs. 3 to 5). Cisternae and small vesicles are numerous and scattered throughout the cytoplasm. The striking appearance of the lipid crowns, as seen with the optical microscope (Fig. 6), is also shown by electron microscopy; in fact, large, dense inclusions of lipoidal nature (sudanophilic) (10) are apparent around each nucleus (Fig. 4).

When gametangia of this stage are dipped into a hypotonic solution, the maturation of gametes begins. In the 1st half hour, the size and number of vesicles increase (Fig. 7). It seems as if all the areas that previously had few R particles or none, are now encircled by a limiting membrane. Many rather large vesicles are seen in the cytoplasm. They are distributed among areas rich in R particles, but they do not contain R particles (Fig. 7).

About 15 minutes later, areas exhibiting a high concentration of R particles, together with mitochondria, lipid inclusions, and small vesicles, are found close to the nuclei (Figs. 8 and 10). The cytoplasm not occupied by these areas is free of R particles and is very poorly electron-scattering. Vesicles are smaller and not so numerous as in the preceding stage; they are most evident in the site of the forming cytoplasmic membranes and among the areas containing the R particles (Figs. 8 and 10). This stage in the formation of the limiting cytoplasmic membranes is easily identified in optical microscopy because of the obvious division of the cytoplasm; basophile reactions are stronger and limited to a few, rather large and well defined areas inside the future gamete (Fig. 9).

At a still later phase, all basophile material, except the metachromatic granules, is located in a crescent around each nucleus (Fig. 13). The fine structure of this basophile material has the appearance of a mass containing a high concentration of R particles (Fig. 12). It is devoid of mitochondria, vesicles, or lipids, and is delimited by a double membrane, which in Fig. 12 is incomplete. This mass represents the nuclear cap of the gamete. Non-basophilic cytoplasm lies beyond this mass and shows a very fine granulation (particle size much less than 100 A) of low density (Figs. 12 and 14). Numerous vesicles are distributed throughout this cytoplasm. They are almost spherical, vary in size, and have a content that scatters electrons even less than the surrounding cytoplasm (Fig. 14). The low density of this non-basophilic cytoplasm may be due to its chemical nature. A low electron-

scattering power has been observed in the case of polysaccharides, and indeed this cytoplasm gives a positive reaction with tests for polysaccharides (Hotchkiss-McManus test and Lugol staining). It also shows an affinity for acid stains. It may be added that this peripheral cytoplasm is bounded by a complete cytoplasmic membrane which separates it from the adjacent gametes (Fig. 12). The gametes at this stage are ready to be discharged into the medium through the papillae of the gametangium.

(b) On the Germinating Zygote and Young Plants:

The biflagellate zygote, resulting from the conjugation of two gametes, soon stops swimming, rounds up, and germinates, forming a small rhizoid. At the time of rounding up, the nuclear cap is no longer visible. Tests for basophilia give a strong reaction in areas in the center of the cell and a weak to negligible reaction towards the periphery (Fig. 16). By the time the germinating bud appears, the cytoplasm has already become uniformly basophilic, the intensity of the reaction being weaker than in the nuclear cap.

It is difficult to obtain satisfactory preparations of the zygotes because of the great fragility of the cells at this stage. Moreover, the preparations have always consisted of a mixture of swimming, spherical, and budding zygotes, as it is not possible to separate the individual phases. Thus, observations on the fine structure give different pictures, corresponding to different states of the cell. In some cells the limiting membrane of the nuclear cap is interrupted or replaced by a chain or area of small vesicles (Fig. 15). In other cells, instead of one nuclear cap, we observed two or more masses of R particles, surrounded by a more or less intact membrane. In some cases, the fine granulation of the surrounding cytoplasm is denser than in the gamete and a few R particles are sometimes observed in the cytoplasm (Fig. 15). The vesicles seem to be less numerous than in the gametes.

In the young plant, the situation is as follows: the whole cytoplasm is basophilic (Fig. 18). Numerous R particles are scattered uniformly throughout the cytoplasm. The cytoplasm contains many vesicles, either isolated or in clusters, which have various shapes and sizes; some are small and spherical, others narrow and elongated (cisternae) (Figs. 17 and 19). They seem not to display any specific orientation. Generally, R particles are not seen inside, but are found occasionally attached to the external surface of their limiting membrane (Fig. 19).

OTHER OBSERVATIONS AND RELATED DISCUSSION

Nucleolus and Nucleus:

In all the phases of the cycle studied here, the nucleolus has had the appearance of a dense mass of granules (Figs. 1, 3, 4, 12, and 17). These particles are about 100 to 130 A in diameter and are quite dense. A few areas having a lower concentration of granules are encountered sometimes in such masses (Fig. 3), but it is not yet possible to decide whether their presence bears any relation to the division of the nucleus. The latter is enclosed in a double porous membrane which has been described previously (45). The content of the nucleus has no defined structure. It appears either finely granular or sometimes finely filamentous.

Mitochondria:

In all cells described heretofore, the mitochondria have appeared as bodies limited by a double membrane and possessing an internal system of membranous protrusions, either ridge-like (*cristae*) (19, 23, 31), or, in some cases, tubular in form (36, 46).

In *Allomyces*, two different types of mitochondria have been found. The mitochondria of the motile cells (gametes, zygotes) are generally dumpy, with numerous characteristic cristae which penetrate deeply into the matrix and are arranged more or less parallel to one another (Figs. 12 and 14). In contrast, the mitochondria in the young plants, the rhizoids, and the hyphae are mostly elongate, and have few *cristae* which do not penetrate deeply into the matrix of the mitochondrion (Figs. 1, 17, 19). Cross-sections of what may be tubules and small vesicles are often present in the matrix (Figs. 1, 19). Mitochondria similar to this second type have been encountered often in plants and described as containing *sacculi* and tubules (5, 8, 14, 34, 37). We consider, however, that it is not at all certain that these mitochondria contain tubules and small vesicles instead of *cristae*. The *cristae* may be considered as kinds of flat pouches which are formed by folds of the internal limiting membrane of the mitochondrion. This is clearly shown in one of our photographs (Fig. 19). If such pouches are numerous, they are likely to be flattened in one dimension and packed in parallel. In section they will have the appearance found in mitochondria of gametes and zygotes.

Conversely, if only a few pouches or *cristae* are formed, it is easy to imagine that nothing will prevent them from following a sinuous course in the matrix and varying in volume. Hence, sections through a mitochondrion with such *cristae* will reveal only small parts of one or two *cristae*. They will present the appearance of sections through a mixture of short cristae, tubules and vesicles, exactly as is found in the mitochondria of the vegetative cells of *Allomyces*. This point of view is also supported by the fact that mitochondria which contain tubules, as in *Paramecium*, look quite different from our second type of mitochondria (46). Consequently, the main difference between the mitochondria of the motile cells and of the plants in *Allomyces*, would be in the size of the mitochondria and in the number and form of the *cristae*. It is not possible to decide whether there is a transformation from one type into the other or whether there is creation of a new type. The presence of intermediate types in maturing gametangia and germinating zygotes would suggest a transformation. The elongation of the mitochondria could cause a decrease in the number of *cristae* per unit volume, for example. We are inclined to think that the morphology of the chondriosome is related to its activity and may change, therefore, with changes in the activity. The location of the mitochondria is certainly also related to their activity. For example, in the cleavage stage of gametangia, the mitochondria are randomly distributed among the masses of R particles close to the nucleus, while in the gamete they mostly lie along the nuclear cap. In the germinating plant numerous mitochondria are found at both points of germination; that is, at places probably of high synthetic activity.

Possible Golgi Apparatus:

We have observed often in young plants, series of piled cisternae with many polar vesicles (Fig. 2). Though these cisternae are not closely packed and have some R particles attached to their external side, the whole complex bears some resemblance to the Golgi apparatus as described in plant cells (5, 6, 15, 37).

Other Cytoplasmic Inclusions:

Curious large granules, which look as if they consisted of concentric lamellae, have been observed in the hyphae, the gametangia, and the plants, but never in the motile cells (Fig. 1). Their dimensions vary greatly, from 38 to as high as 1750 millimicrons. Somewhat similar granules have been observed in the Malpighian tubes of *Gryllus domesticus*, and are thought to be urate deposits (3).

Still another type of granule was found in the gametangia; it has an average diameter of about 170 millimicrons, is strongly electron-scattering, and is seemingly contained in a vacuole (Figs. 3, 4, 8). Both kinds of granules may be products of metabolism which precipitate during fixation; their nature is unknown.

In addition, large, dark grains, of lipoidal nature stainable with Sudan (10), are found in the gametangia, the motile cells, and the very young plants. They are most conspicuous in the gametangia when they surround the nuclei like a crown (Fig. 4), and in the gametes where they are quite numerous.

Cytoplasmic Membrane Formation:

In gametangia at the "cleavage stage," double chains of vesicles are often visible between two segments of an already elaborated limiting membrane (Fig. 8). In mature gametangia, vesicles are also evident at the edge of the nuclear cap before the appearance of the complete limiting membrane (Fig. 12). Thus, it may be that the cytoplasmic (or plasma) membrane arises through the fusion of vesicles as has been shown in the formation of the cell plate in *Allium cepa* (30).

Flagella:

Sections through gametangia have shown the presence of flagella about 30 minutes after dipping gametangia at the "lipid-crown" stage into a hypotonic solution. In agreement with previous results of Manton *et al.* on dissociated flagella of *Allomyces* (21), we have observed on cross-section that these flagella have 9 peripheral filaments and 2 axial filaments and that each of the peripheral filaments is double (Fig. 11). This fine structure is similar to that reported to date for all kinds of animal and plant flagella (13, 20, 36). Filaments of flagella of *Allomyces* appear in cross-section to be tubules, as has also been observed in flagella of other organisms (36).

DISCUSSION

In all basophilic areas of the cytoplasm of the fungus *Allomyces*, we have found dense particles of 150 to 200 A in diameter; *i.e.* the R particles

These R particles are in high concentration in areas showing a strong basophile reaction, such as the nuclear cap of the mature gamete and the basophile masses close to the nuclei in the gametangium at "cleavage-stage." The cytoplasms, having a weaker but evenly distributed basophilic reaction, show a lower concentration of R particles which are evenly distributed. This is, for example, the case for young plants, where a vesicular component is also observed. Conversely, no R particles are found in non-basophile cytoplasms, such as the area surrounding the nuclear cap, although the vesicular component is present. Hence, in this fungus, the presence of basophilia is clearly related to the presence of the R particles.

Cytochemical tests, such as acid and enzymic (RNase) hydrolysis and experiments with induced specific fluorescence, have shown that in this fungus the basophilia is due to the presence of RNA. Consequently, these observations show that RNA is associated with the presence of R particles.

We know, furthermore, from the researches of Palade and Siekevitz (26, 27), that particles similar in size to the R particles have been found both *in situ* and in cell fractions from basophile cytoplasms, and chemical analyses have revealed that these particles are ribonucleoproteins. Particles of this size and chemical composition have now been found in cell fractions from various sources by several other authors (9, 11, 35, 38, 39).

On the basis of these findings and the fact that R particles found in cytoplasmic basophile areas of *Allomyces* are connected with the presence of RNA, it seems evident that these R particles are similar in nature to the ribonucleoprotein particles or ribosomes[1] found elsewhere and which are generally presumed to be a fundamental component of the cytoplasm.

As mentioned above, we have observed in both basophile and non-basophile cytoplasms a vesicular component. It appeared either as vesicles or as cisternae (long, thin vesicles). The cisternae were, however, not so often encountered as the vesicles. We think that these vesicles and cisternae are comparable to the profiles described by Palade as elements of a not highly organized endoplasmic reticulum (25). It may be mentioned that a similar rudimentary organization of the endoplasmic reticulum has usually been encountered in plant cells (5, 7, 34). Our observations have shown that these elements of the endoplasmic reticulum vary in form, size, and number during the successive steps of the life cycle. This variation is particularly striking during the formation of the gamete, but no explanation can yet be given for this phenomenon. In all of our observations of basophilic cytoplasm, we very infrequently encountered ribosomes attached to the external surfaces of the vesicles and cisternae of the endoplasmic reticulum, to produce such "rough surfaced" elements as described by Palade (25). The ribosomes are freely dispersed in the cytoplasm, as are generally observed in plant cells (5, 7, 34, 37). Such a situation has also been described in some rapidly growing animal cells (24).

Our observations on the formation of the gametes and the germinating zygotes suggest that the ribosomes migrate from the peripheral cytoplasm towards the nucleus to form the nuclear cap, and that the reverse process takes place to restore the basophilia in the whole cytoplasm, before the germination of the zygote. Electron microscopy cannot give here a definitive answer. It would only be possible by the use of other tehcniques, such as markers and autoradiography, to know whether this migration process really takes place, or whether the ribosomes disappear from certain regions of the cytoplasm, while new ribosomes are synthesized at a high rate in the adjacent areas.

Related observations on the fine structure of the main cellular components of *Allomyces* have shown them to possess a certain number of characteristics which have been already reported in most of the animal and plant cells observed up to now. These characteristics are: the double nuclear membrane pierced with pores, the granular mass of the nucleolus, the double limiting membrane of the mitochondria, and their internal ridges. We must also mention the vesicular and particulate components of the cytoplasm and the presence in flagella of nine peripheral and one axial pairs of filaments. Consequently, this investigation has shown that in this fungus, the fine structure of the main cellular components is similar to that found already in a great many cells of varied origins. A similar conclusion seems also to be valid for several other fungi (1, 16, 22, 40, 47), though, in some cases, technical difficulties of fixation and embedding apparently prevent a clear resolution of structures.

[1] Word suggested for the RNP particles at the First Symposium of the Biophysical Society. See Introduction *in* Microsomal Particles and Protein Synthesis, 1958, (R. B. Roberts, editor), Pergamon Press, Washington Academy of Sciences.

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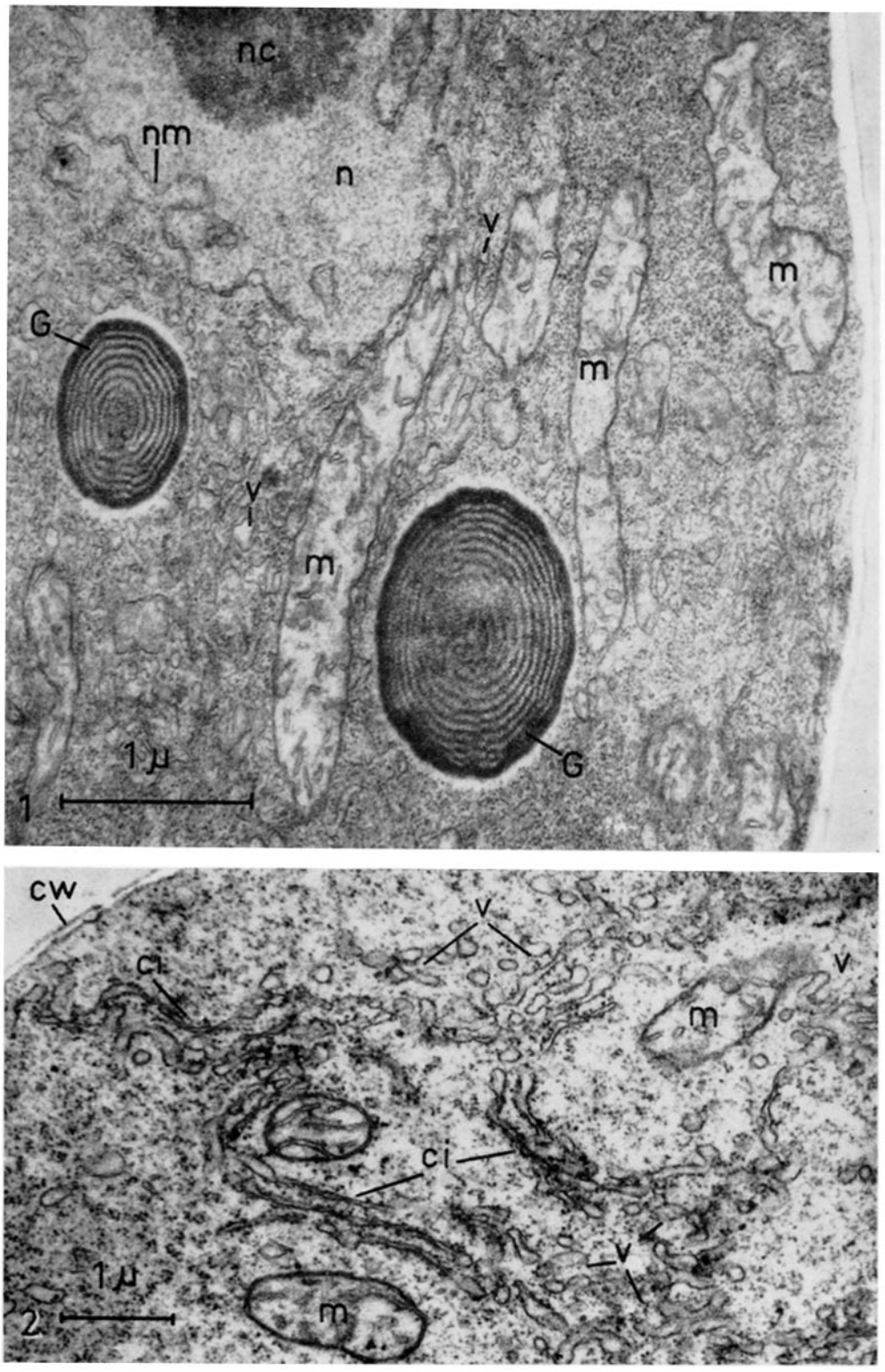
Explanation of Plates

Plate 48

Fig. 1. Section through the tip of an undifferentiated gametophytic hypha. Numerous R particles are visible; they are distributed throughout the basophile cytoplasm. Many small vesicles (*v*) and some cisternae can be seen, as well as two conspicuous large granules (*G*) which look as though they consist of concentric lamellae. The mitochondria (*m*) are elongated and show internal profiles of what may be sections through a few sinuous cristae. The nucleus (*n*) is limited by a double membrane (*nm*) and contains a dense mass of particles, the nucleolus (*nc*). × 29,000.

Fig. 2. Area of cytoplasm of a young plant where some loosely packed cisternae (*ci*) are visible in three different places. Many vesicles (*v*) are also present in this region, and particularly at opposite ends of the cisternae, suggesting a resemblance to the Golgi apparatus encountered in other forms. R particles, mitochondria (*m*), and a portion of the cell wall (*cw*) are also shown. × 16,500.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

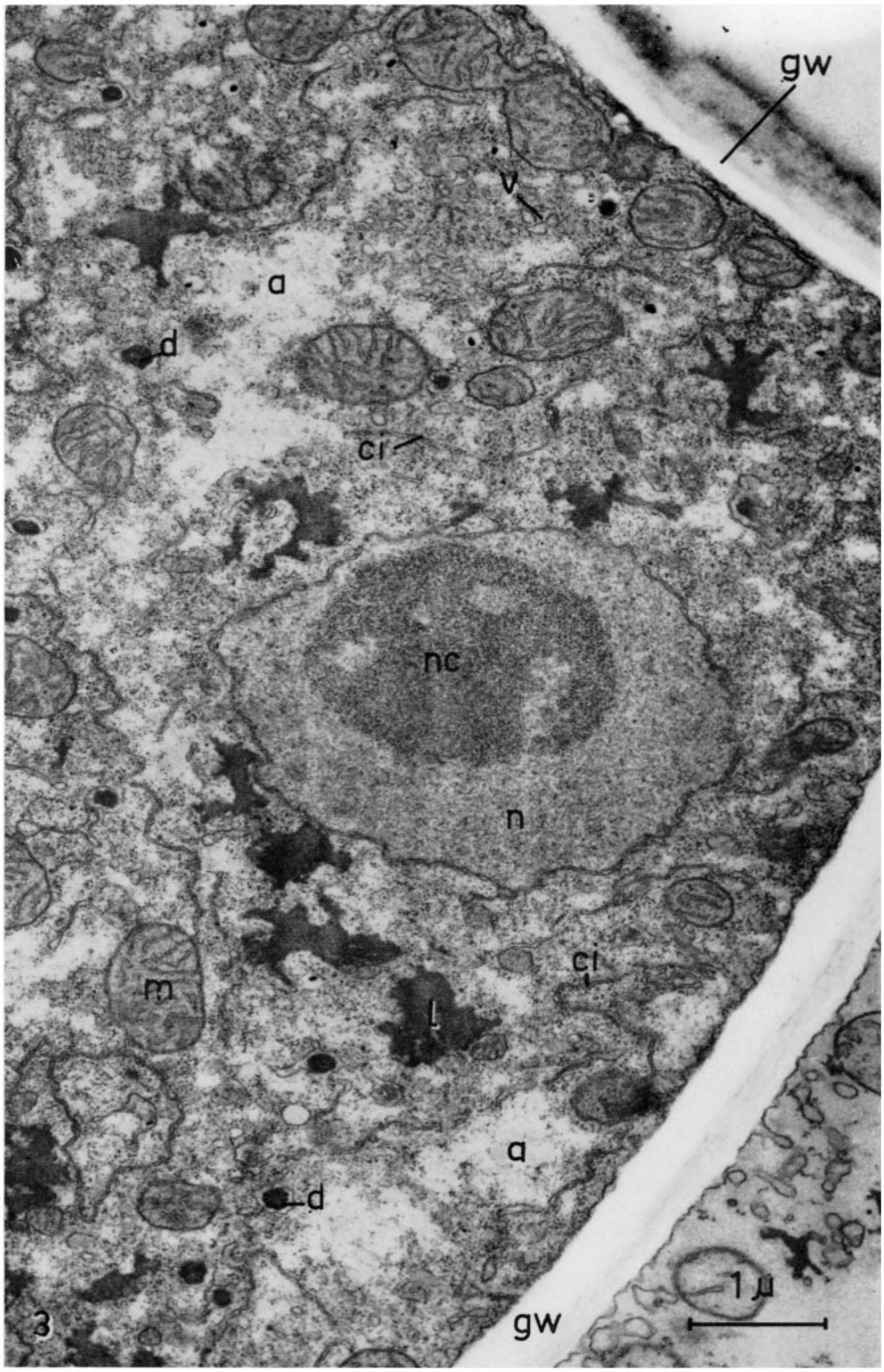
PLATE 49

FIG. 3. Area of a gametangium, fixed at the "lipid-crown" stage. The gametangium is limited by a thick wall (*gw*) and contains several nuclei; one of them is shown here (*n*). The nucleolus (*nc*) consists of a mass of "dense" particles of 100 to 130 A in diameter. A few areas with a low concentration of particles are clearly shown in the nucleolus.

The uneven distribution of R particles in the cytoplasm is obvious; some areas (*a*) are nearly deprived of R-particles. Cisternae (*ci*) and small vesicles (*v*) are numerous.

Several granules, 170 millimicrons in mean diameter and having a strong electron-scattering power, are enclosed individually in vacuoles (*d*).

Large, "dense" lipid inclusions (*l*) lie in the vicinity of the nucleus (*n*). Mitochondria (*m*) have numerous cristae that penetrate deeply into the matrix. × 20,000.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 50

FIG. 4. Detail of a gametangium, fixed at the "lipid-crown" stage. Large lipid inclusions, (*l*) having a strong electron-scattering power, are displayed around the nucleus (*n*) close to the nuclear membrane (*nm*). The plane of this section is particularly favorable for showing that the crown-like arrangement observed in light microscopy (Fig. 6) is equally found in electron microscopy.

"Dense" granules in vacuoles (*d*) can be seen as in Fig. 3. Nucleolus (*nc*). × 29,000.

FIG. 5. Gametangium fixed at the "lipid-crown" stage (same section as in Fig. 3).

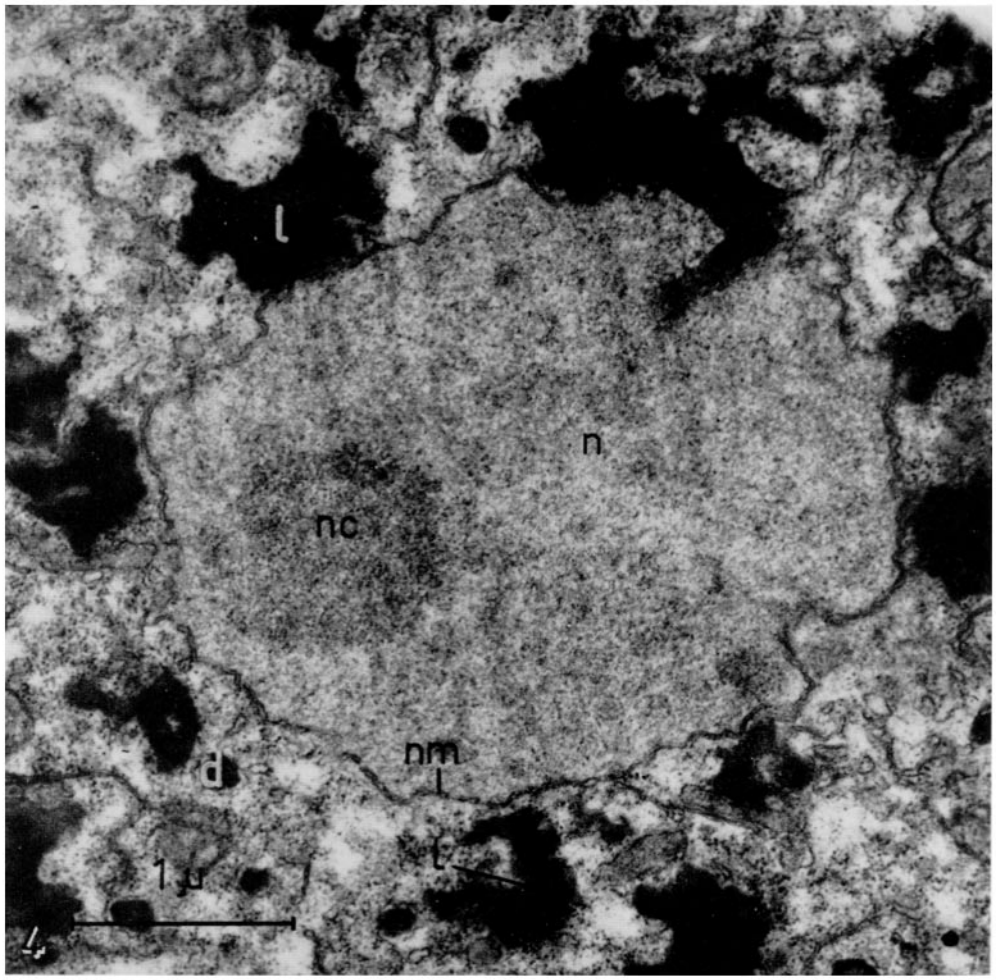
Detail of the cytoplasm which shows the uneven distribution of the R particles. Areas rich in R particles (*ba*) alternate with areas which contain few or no R particles (*a*).

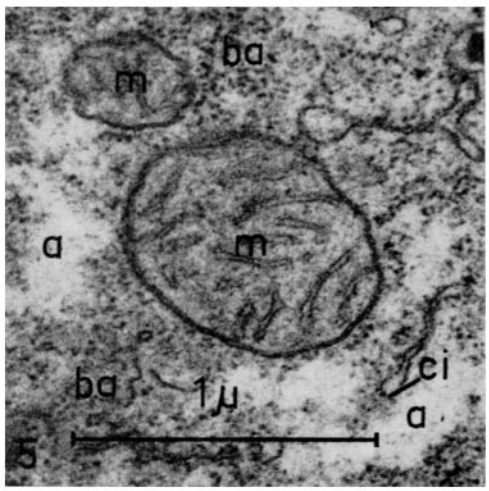
Cisternae (*ci*) and some vesicles, as well as two mitochondria (*m*) are also shown. × 40,000.

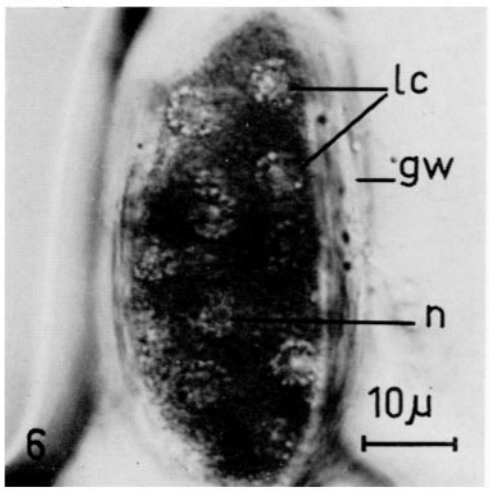
FIG. 6. Light micrograph of a gametangium at the "lipid-crown" stage, acetic toluidine blue–stained.

The cytoplasm has a coarse, granular appearance; the distribution of basophilia seems slightly patchy. A crown of non-stained granules (the lipid droplets) (*lc*), surrounds each nucleus (*n*).

The thick wall of the gametangium is shown at *gw*. × 1,185.







(Blondel and Turian: Basophilia and fine structure of cytoplasm)

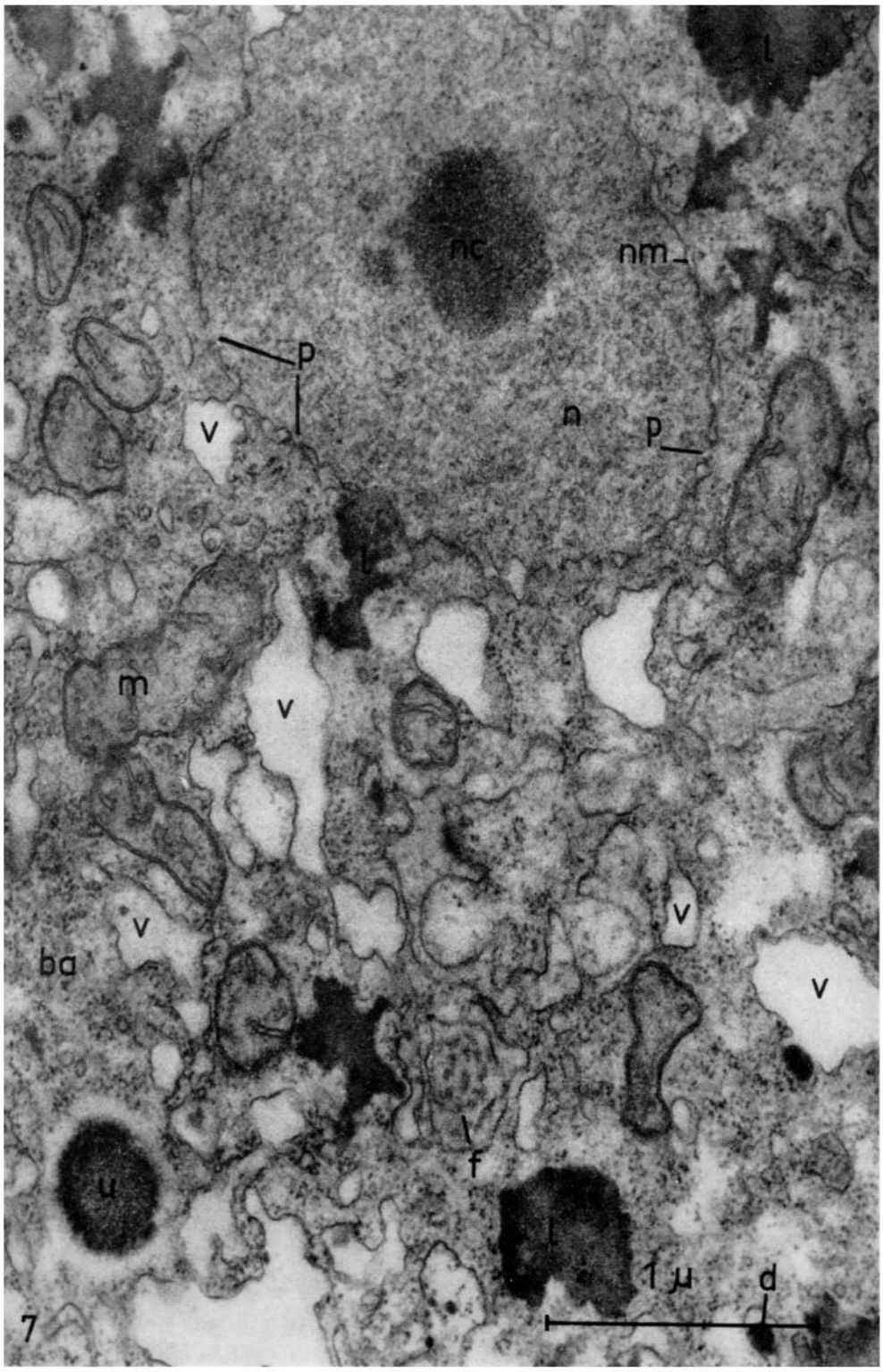
PLATE 51

FIG. 7. Section through a gametangium where the maturation of the gametes is underway (after 30 to 45 minutes' stay in Machlis' solution).

The cytoplasm now contains numerous vesicles (*v*). They are rather large and have no internal structure. Between them, the R particles are visible; their distribution is slightly uneven; they are particularly numerous in some areas (*ba*).

Flagella have formed, and a cross-section of one of them is apparent (*f*). In this section, the pores (*p*) of the nuclear membrane (*nm*) are particularly well shown. A granule (*u*) of about 400 millimicrons in mean diameter, surrounded by a clear zone, has been encountered in this section only.

Mitochondria (*m*) showing few cristae, nucleolus (*nc*), a small dense granule in a vacuole (*d*), and lipid (*l*). × 40,000.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 52

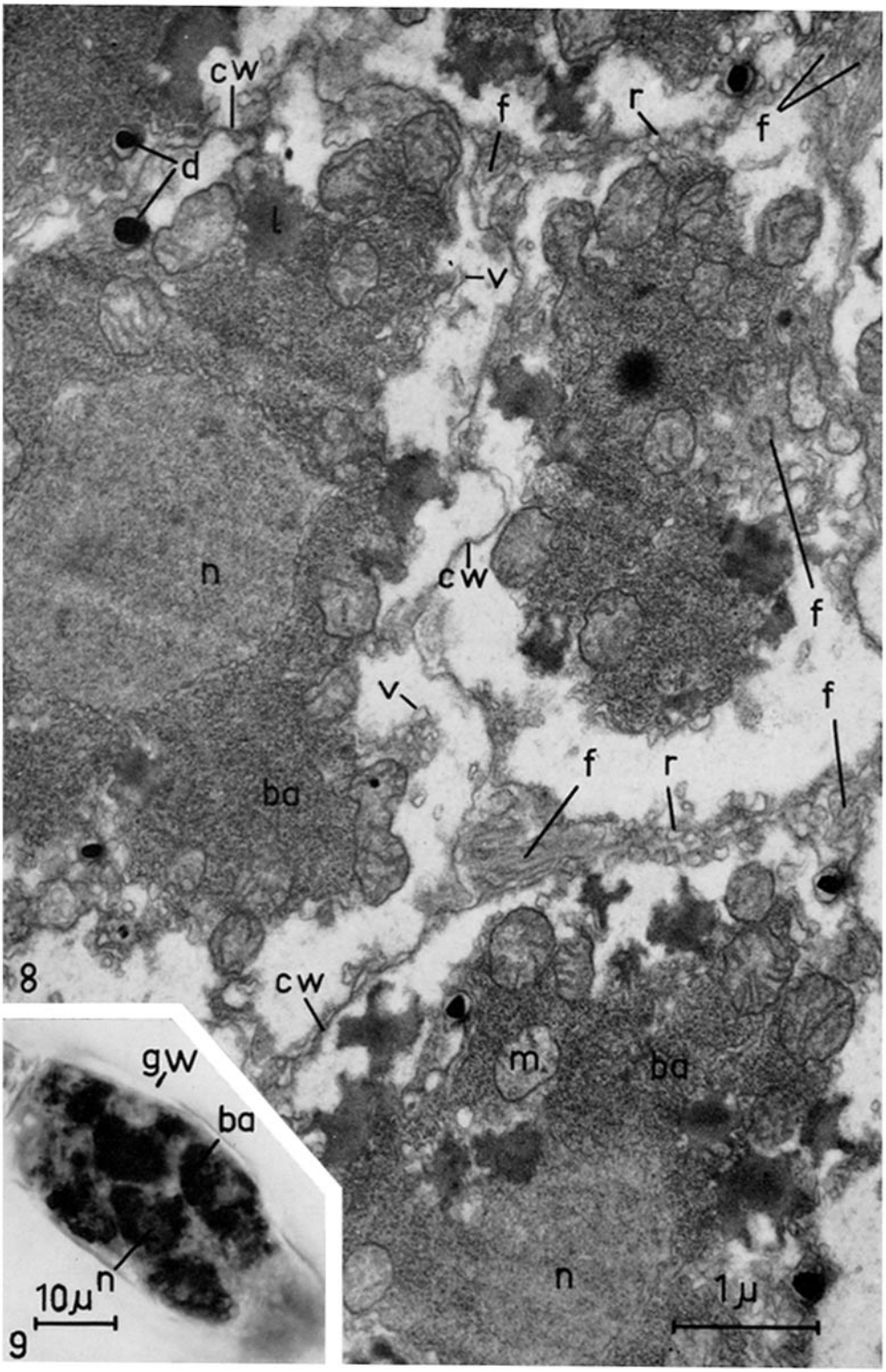
FIG. 8. Section through a gametangium fixed at the "cleavage stage" (after 45 minutes' to 1 hour's stay in Machlis' solution).

The cytoplasm of the future gametes is limited by a cytoplasmic membrane (*cw*), which is under formation. Double rows of vesicles (r) are visible in some parts of the future cytoplasmic membranes; in other places the limiting membranes are already completed. Longitudinal and cross-sections of flagella (*f*) are found most in the spaces between the gametes.

In each future gamete, a complex made up of the nucleus (*n*), areas of high concentration of R particles (*ba*), mitochondria (*m*), vesicles (*v*), and lipid inclusions (*l*), is observed. It is surrounded by a zone of very low "density." × 22,000. Corresponding light micrograph, Fig. 9.

FIG. 9. Light micrograph of a gametangium at the "cleavage stage," acetic toluidine blue–stained.

Strong basophilic masses (*ba*) surround the nucleus (*n*). The cytoplasm beyond them shows no basophile reaction. Gametangium wall (*gw*). × 1,185.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

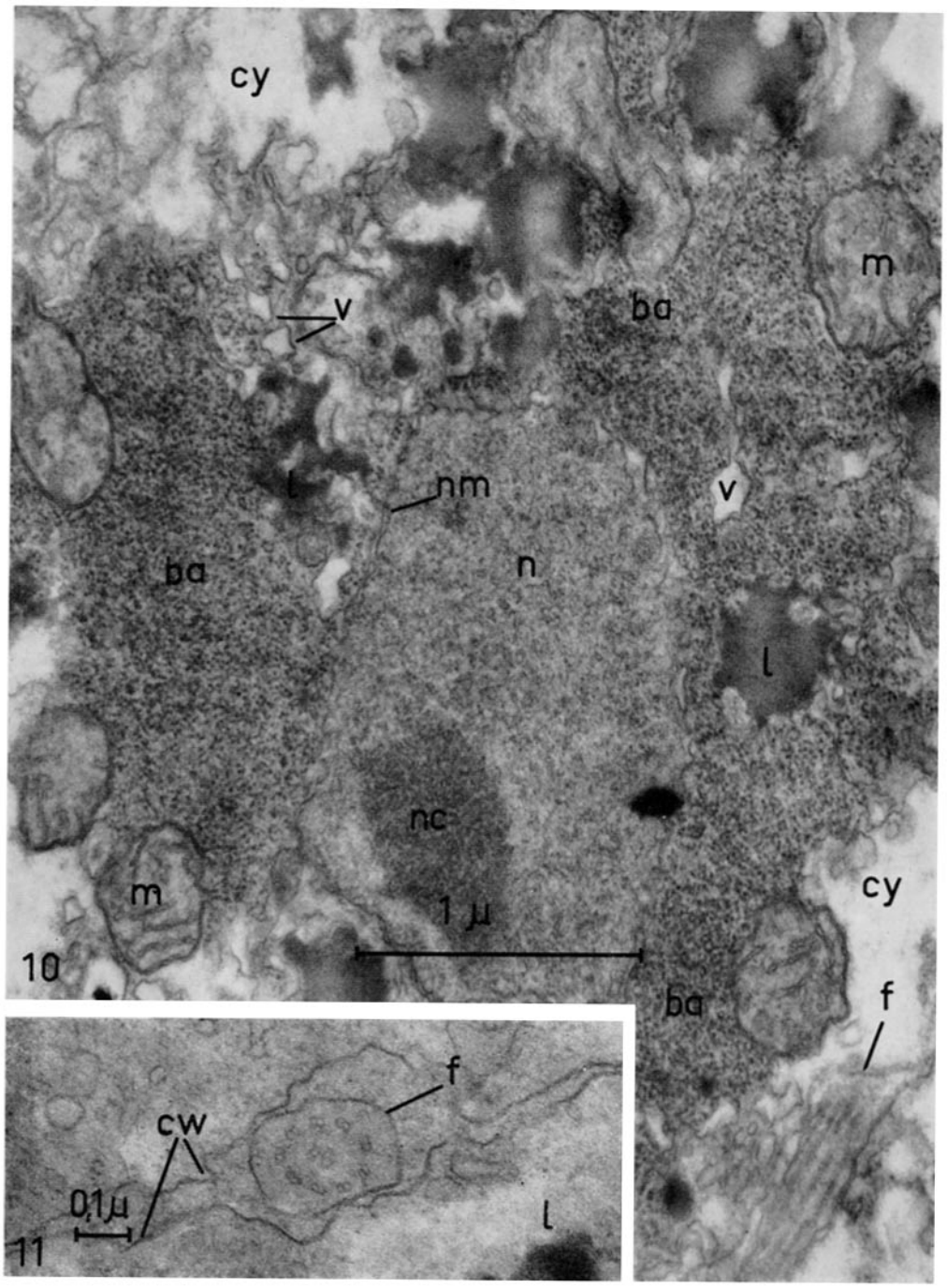
PLATE 53

FIG. 10. Detail of the central region of the future gamete, in a gametangium fixed at the "cleavage stage" (same stage as in Figs. 8 and 9).

Concentrated masses of R particles (*ba*) are observed close to the nucleus (*n*). They correspond to the basophile areas shown in Fig. 9. They contrast with the surrounding cytoplasm (*cy*) which contains no R particles. Small vesicles (*v*), lipid granules (*l*), and mitochondria (*m*) can be observed within and between the masses of R particles. Longitudinal section of a flagellum is indicated at *f*, nuclear membrane at *nm*, and nucleolus at *nc*. × 40,000.

FIG. 11. Area of a gametangium showing the cross-section of a flagellum (*f*). Nine double peripheral and two axial tubules can be observed within the flagellum, which is located between two cytoplasmic membranes (*cw*). These membranes delimit future gametes.

The material was here embedded in vinox. (18). × 72,500.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 54

FIG. 12. Detail of a mature gametangium (after 1 hour's stay in Machlis' solution).

The nucleus (*n*) is partly enveloped in a dense mass of R particles (*np*). This mass, which contains no other structures, corresponds to the strongly basophilic nuclear cap seen in Fig. 13. It is bounded by a layer of vesicles (*r*) of varying sizes, which make up the incomplete membrane. Beyond the nuclear cap lies a non-basophile cytoplasm.

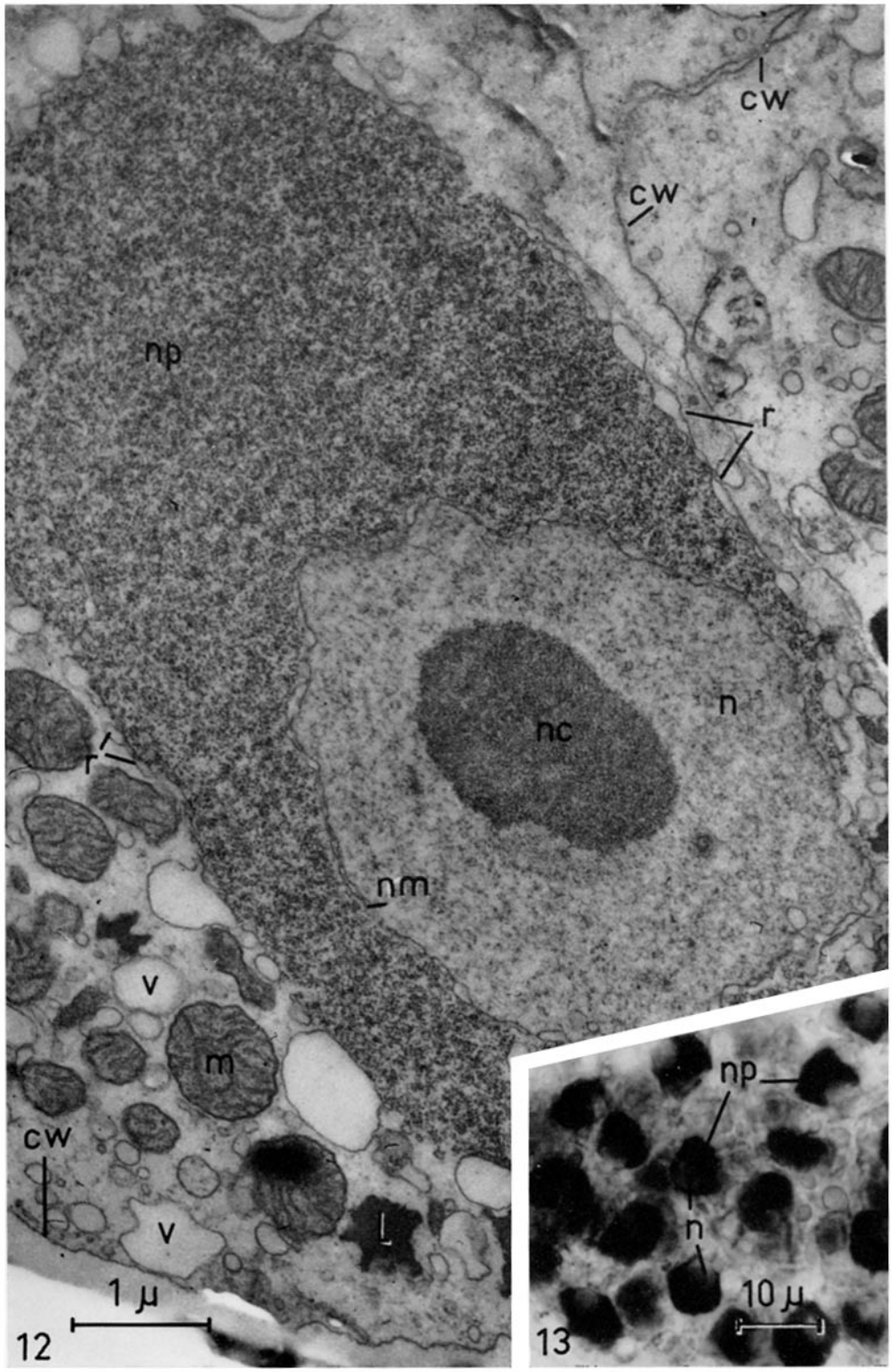
Numerous vesicles (*v*) can be seen throughout the cytoplasm. Lipid inclusions are at *l*.

The dumpy mitochondria (*m*) possess many cristae which penetrate deeply into the matrix. A cytoplasmic membrane (*cw*) delimits each gamete. × 20,000.

FIG. 13. Light micrograph of a mature gametangium, acetic toluidine blue–stained.

A strong basophilic crescent or nuclear cap (*np*) partly surrounds each nucleus (*n*). The cytoplasm beyond the nuclear cap gives no basophile reaction.

In some nuclei, a darker nucleolus is visible. × 1,185.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 55

FIG. 14. Detail of non-basophilic cytoplasm of a gamete (part of a mature gametangium).

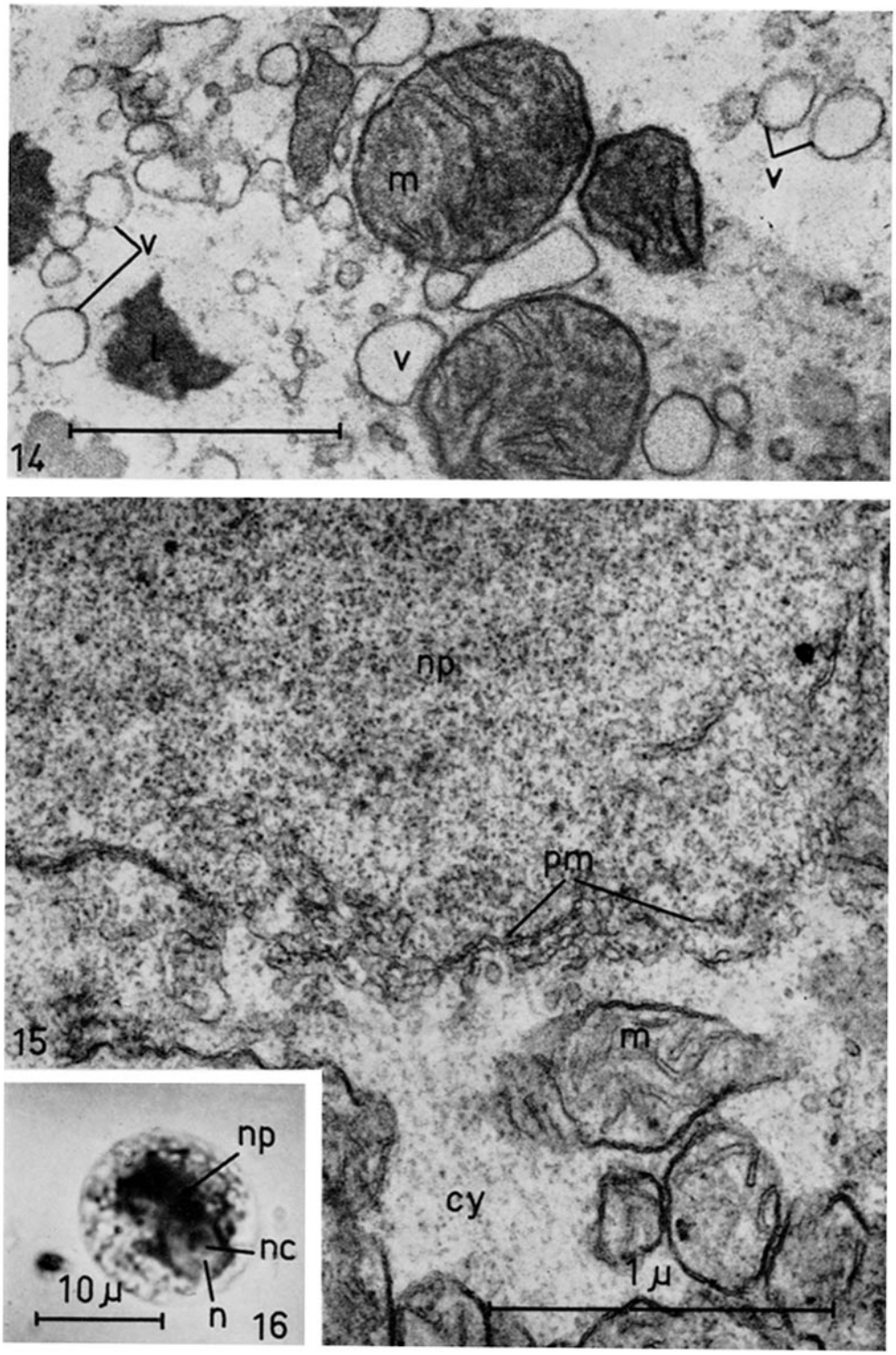
This cytoplasm presents a scarcely visible granulation of low "density." The vesicles (*v*) are numerous and many almost spherical. The mitochondria (*m*) have many cristae, which are arrayed more or less parallel to one another. Two lipid granules (*l*) can be seen. × 40,000.

FIG. 15. Part of a rounded zygote before germination. The upper half shows the mass of R particles of the nuclear cap (*np*); its limiting membrane (*pm*) is no longer continuous and intact. Segments of it are replaced by areas of very small vesicles.

The fine granulation of the adjacent cytoplasm (*cy*) is "denser" than in the gamete (Fig. 14), and a few R particles can be noted within. Mitochondria (*m*). See Fig. 16 for the light microscope picture of this stage. × 50,000.

FIG. 16. Light micrograph of a rounded zygote, acetic toluidine blue–stained.

The basophile mass of the nuclear cap is no longer well defined, but there is a strong basophile reaction in the center of the cell (*np*), without precise delimitation. The periphery of the cell gives no reaction. × 1,875.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 56

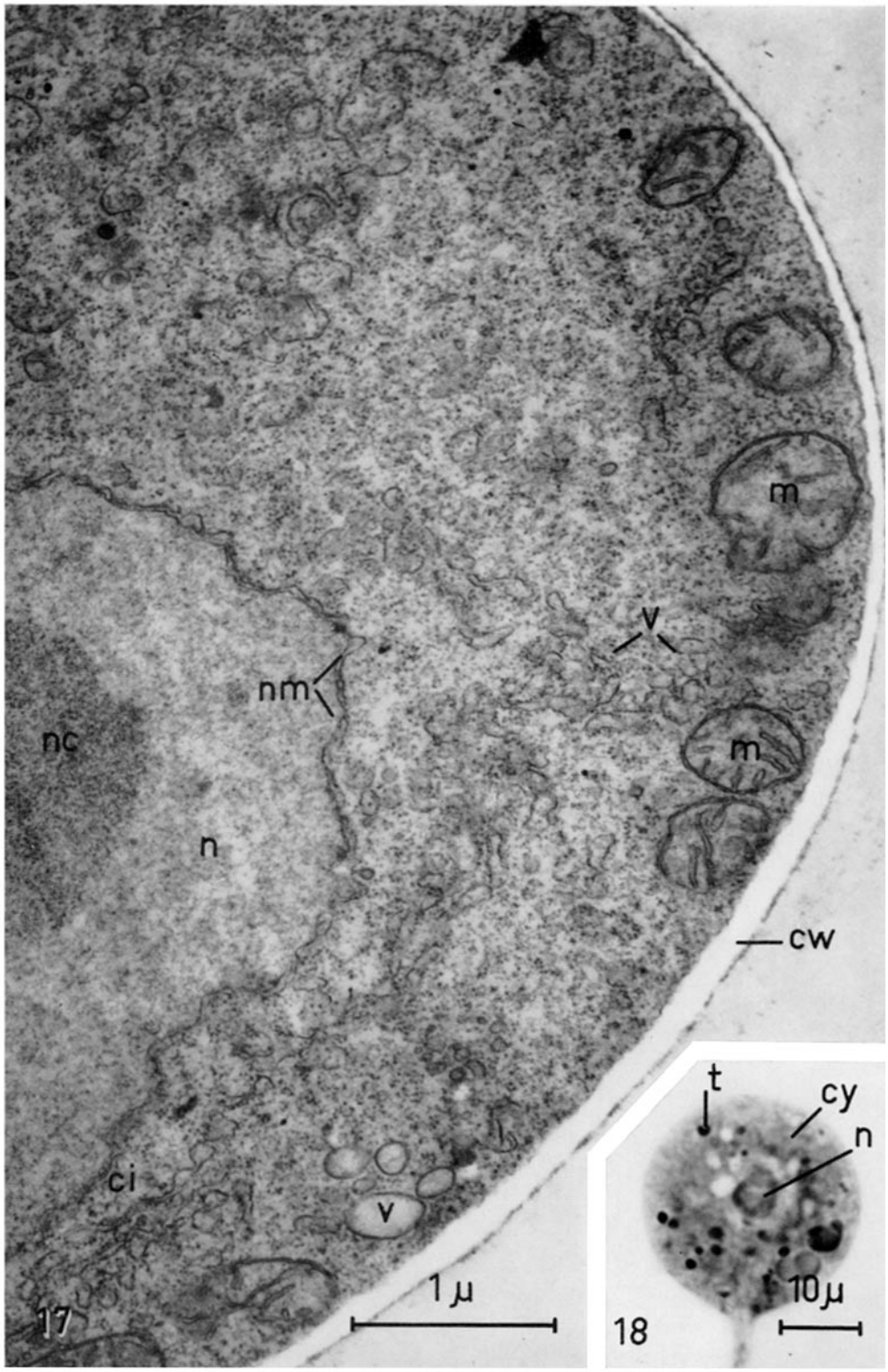
FIG. 17. Young plant. A cell wall (*cw*) limits the basophile cytoplasm, which contains R particles scattered throughout. Vesicles (*v*) and cisternae (*ci*) are apparent.

Mitochondria are in evidence at the periphery and most of them have short cristae. A double membrane (*nm*) limits the finely granular content of the nucleus (*n*), within which the dense mass of particles (of about 100 to 130 A in diameter) of the nucleolus (*nc*) is apparent. × 30,000.

FIG. 18. Light micrograph of a young plant; acetic toluidine blue–stained.

The basophile reaction is light and uniform throughout the cytoplasm (*cy*), except in the numerous metachromatic granules, where it is stronger (*t*).

In the center, the nucleolus (*n*) surrounded by the clearer zone of the nucleus is visible. A small part of the rhizoid is also included. × 1,185.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)

PLATE 57

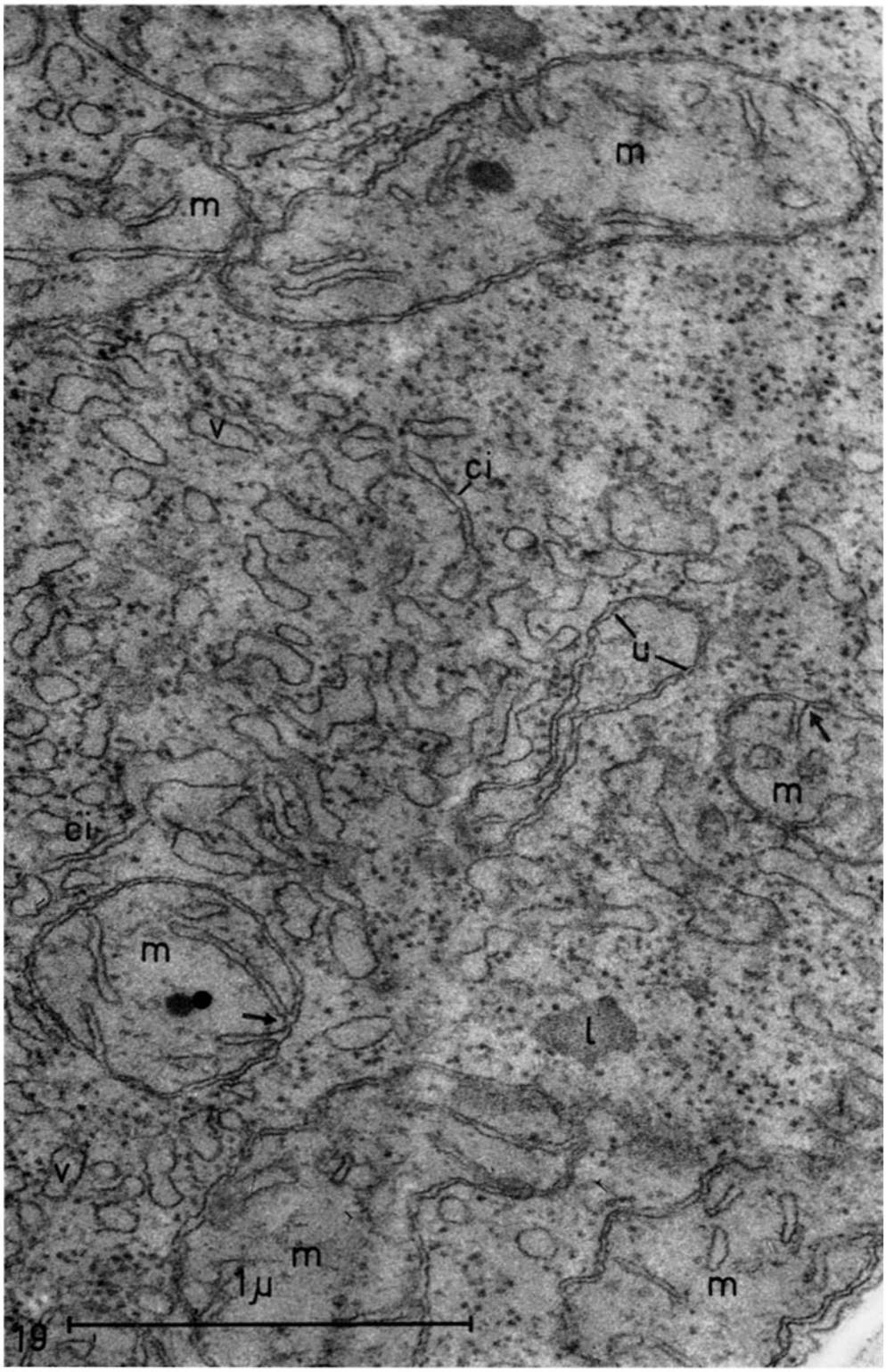
FIG. 19. Young plant. Detail of the basophile cytoplasm of the rhizoid, in the region close to the plant.

Particles of 150 to 200 A in diameter, *i.e.* R particles, are scattered throughout the cytoplasm.

Vesicles (*v*) and cisternae (*ci*) are close to one another. Very few R particles are attached to the external surfaces of their limiting membrane.

The double limiting membrane of the mitochondria is evident, and the fact that the cristae are infoldings of the internal membrane is clearly shown (arrows). In the mitochondria sections through short cristae and small vesicles are also shown. These internal profiles can be interpreted as sections through a few cristae having a sinuous course in the matrix. Two mitochondria each contain a "dense" granule.

The profile (*u*) belongs probably to a mitochondrion. × 60,000.



(Blondel and Turian: Basophilia and fine structure of cytoplasm)