Phil. Trans. R. Soc. Lond. B. **277**, 327–342 (1977) [327]
Printed in Great Britain

Ribosomes, membranes and organelles during meiosis in angiosperms

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[Plates 1–5]

Evidence is presented from both observational and analytical techniques indicating profound changes to take place in the ribosome population during male and female meiosis in some flowering plants. During microsporogenesis these appear to involve the elimination of the major part of the ribosome complement early in the meiotic prophase, and its subsequent restoration by the disintegration in the tetrad cytoplasm of 'nucleoloids', themselves synthesized in the nucleus during late prophase. In female tissue the process is essentially similar except for differences in the restoration of the ribosome population. Immediately before the eradication of the ribosomes in both sexes, a sizeable proportion of the meiocyte cytoplasm is encapsulated by double, or multiple unit membrane profiles. Significantly this cytoplasm remains unaffected by the agents responsible for the degredation of the ribosome population. These events are also reflected in the organelle populations where cycles of dedifferentiation and redifferentiation take place. In view of evidence from other organisms, it is considered unlikely that these cycles are in any way a prerequisite of meiosis, but more a characteristic of cells undergoing major changes of phase, where a cytoplasmic 'clean-up' is required before the next stage of growth may begin.

Introduction

Meiosis in the angiosperms is correlated in time with one of the most dramatic developmental changes known in plants, the transition from the sporophytic to the gametophytic phase of the life cycle. The sporophyte is the more elaborate of the two generations in morphology and biochemical capacities, and no doubt we see in it the expression of the greater part of the potentialities of the genome. The male and female gametophytes possess an independent metabolism, but they are highly reduced in morphology and range of functions, suggesting that a large part of the genome is repressed during this phase of the life cycle. This repression must be imposed by some event – or sequence of events – occurring in the meiocytes or in the early life of the spores they produce. Furthermore, diplophase information which might interfere with the development of gametophytic functions must presumably be expunged during the same period before the cytoplasm becomes host for the gametophytic nuclei after the quadripartition of the meiocyte (Dickinson & Heslop-Harrison 1970*a*; Heslop-Harrison 1971*a*, 1972). These changes associated with the alteration of generations cannot of course be linked in any causal sense with the chromosome cycle or with meiosis itself. The phenomena of apospory and apogamy prove that the mere states of haploidy and diploidy are not determining, and the fact that in the aposporous life cycle the transition from one phase to the other takes place through a somatic cell shows that there is no dependence on meiosis *per se*. Nevertheless, if the controls involved in the phase transition depend upon specific gene activations, a close timing

relationship with the events of the division is to be expected since the genome is available for transcription only at certain periods. It is our purpose in this paper to review recent evidence bearing upon the *cytoplasmic* changes correlated with the meiotic process in the flowering plants, and to show how certain events can indeed be interpreted as indicating some form or restandardization almost certainly related to the sporophyte–gametophyte transition. We consider both the ribosome cycle (Mackenzie, Heslop-Harrison & Dickinson 1967; Knox, Dickinson & Heslop-Harrison 1970; Dickinson & Heslop-Harrison 1970*a*; Williams, Heslop-Harrison & Dickinson 1973) and the organelle and membrane cycle (Marumaya 1968; Dickinson & Heslop-Harrison 1970*b*).

Historical perspective

The most extensive earlier investigations of events in the cytoplasm associated with meiosis in angiosperms were carried out by Guilliermond and his school (Guilliermond 1920, 1924; Py 1932), who examined both microspore and megaspore mother cells. Guilliermond held the view that the 'chondriome', which term covered both mitochondria and plastids, was composed of self-propagating elements, and that continuity was preserved through meiosis. Nevertheless he was aware that radical changes did take place during mother cell maturation, and his student, Py, described a 'granular' period during the meiotic prophase in several species during which neither plastids nor mitochondria could be distinguished. It was the existence of this phase which had caused Wagner (1927) to suppose that the chondriome was eliminated from the cytoplasm during mother cell maturation, to originate anew from the hyaline cytoplasm at the time of sporogenesis.

The early interest was principally in the organelles, but various cytologists did note that the general stainability of the meiocyte cytoplasm also underwent great changes during the division stages. Py (1932), for example, observed that the affinity for basic dyes fell during prophase, rising again in the young spores. Painter (1943) was first to appreciate that this reflected a substantial change in the RNA content of the cytoplasm, basing this conclusion on observations on the pollen mother cells of *Rhoeo discolor* (Commelinaceae).

In this period also striking observations were made on the behaviour of nucleoli during angiosperm meiosis which could not at the time be related to the other events in any consistent way. Gavaudan & Yu Chin Chen (1936) reported the presence of nucleolus-like bodies in the cytoplasm at the conclusion of meiosis II in the pollen mother cells in several monocotyledonous and dicotyledonous genera, and a detailed account of the behaviour of these was given by Frankel (1937) for various *Fritillaria* species. Later the corresponding bodies in *Pisum sativum* were studied by Håkansson & Levan (1942), who showed that cytochemically they were indeed very similar to the intranuclear nucleoli.

The recent biochemical and fine-structural evidence shows that the organelles and membranes of the meiocyte cytoplasm do undergo a cycle of change correlated with marked variations in the content of ribosomal RNA. The various earlier observations can be fitted readily into the picture that has emerged, and are still important in the basis they provide for generalization.

Cytoplasmic RNA and the ribosome population

Changes in the content of acetic-alcohol fixable RNA during meiosis in the pollen mother cells of *Lilium* spp. (Liliaceae) and *Trillium erectum* (Trilliaceae) were measured by Mackenzie

(Mackenzie 1968; Mackenzie *et al.* 1967), using the ultramicrochemical methods of Edström (1964). No estimates of a comparable precision appear to have been made subsequently in any plant species, so they remain the best available. The advantage of Edström's technique is that it permits measurements to be made on very small samples of cells, precisely identified without the presence of other contaminating tissues, and staged with reasonable accuracy in the meiotic sequence. As well as estimating RNA contents per cell, Mackenzie calculated base ratios after microelectrophoretic separation so that the type of RNA measured could be judged. The principal results are summarized in table 1 for *Lilium henryi* cultivar Midcentury. They show that the total RNA declines by some 50 % in the interval zygotene to pachytene, the amount per cell climbing again towards the end of the division. The corresponding fall in *Trillium erectum* was found to be some 39 %, rather less than in *Lilium*. During the downward fluctuation the base ratio shifts appreciably. In diplotene and diakinesis, at the lowest point, the A/G ratio rises, indicating that the residual RNA is less like somatic cell rRNA. From cytochemical evidence it seems that much of this residual RNA is associated with the chromosomes (Dickinson & Heslop-Harrison 1970*a*).

TABLE 1. CHANGES IN TOTAL RNA AND ADENINE/GUANINE RATIO OF RNA OF FIXED MALE MEIOCYTES OF *LILIUM HENRYI* CULTIVAR MIDCENTURY

meiotic stage	RNA per cell/pg	adenine/guanine ratio
leptotene	60 ± 5	—
zygotene	60 ± 7	0.99 ± 0.02
pachytene	31 ± 4	1.24 ± 0.04
diplotene	29 ± 3	1.67 ± 0.10
metaphase I	57 ± 11	—
anaphase I	—	0.95 ± 0.04
metaphase II (both dyad cells)	114 ± 6	—
young spore	—	1.03 ± 0.03

From Mackenzie, Heslop-Harrison & Dickinson (1967); determinations by A. Mackenzie (1968), using the microelectrophoretic technique of Edström (1964). ——, No data available.

The decline of cytoplasmic RNA in the zygotene–pachytene interval was measured cytophotometrically in the meiocytes of a dicotyledonous species, *Cosmos bipinnatus* (Compositae) by Knox *et al.* (1970). The reduction here was found to be rather more than 50 % (figure 2*a*).

Electron microscopy (figure 4)† shows with reasonable certainty that the decline in RNA content is related to the elimination of a major part of the ribosome complement of the cytoplasm (Dickinson 1968; Mackenzie *et al.* 1967; Dickinson & Heslop-Harrison 1970*a*). Ribosome counts for the male meiocyte of *Lilium* are given in figure 1. These were based upon electron micrographs of sections of standard thickness and relate to a period when volume growth is minimal. The main fall is in the interval zygotene–pachytene, just when the biochemical results indicate a drop in rRNA. However, the census shows a continuing decline in the population of free ribosomes right up to the end of the prophase. The observations are readily reconciled, however, as we shall shortly see.

Changes in the ribosome population of the female meiocyte (megasporocyte) are not so readily demonstrated as in the corresponding male cells, for not only is the tissue more

† Figures 4–31 appear on plates 1–5.

inaccessible but the rapid growth during early meiosis can make cursory impressions misleading. However, an elimination for the female ribosome population has been demonstrated in *Lilium longiflorum*. The main fall takes place between leptotene and zygotene. The electron microscopic image of a large proportion of the ribosomes fades during late leptotene, and subsequently the cytoplasm appears strikingly empty (figure 5). Microdensitometric observations on Azure B-stained cells show a corresponding decrease in total cytoplasmic RNA. The loss of stainability is readily detected visually, but the observed change is in part due to dilution during the growth of the meiocyte; the actual change in total fixable RNA per cell is seen when the data are

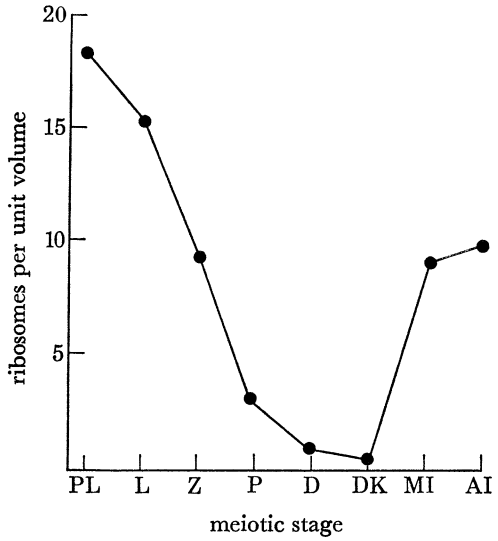

FIGURE 1. Changes in mean numbers of ribosomes per unit volume of cytoplasm in male meiocytes of *Lilium henryi*, estimated from electron micrographs. Meiotic stages: PL, preleptotene; L, leptotene; Z, zygotene; P, pachytene; D, diplotene; Dk, diakinesis; MI, metaphase I; AI, anaphase I.

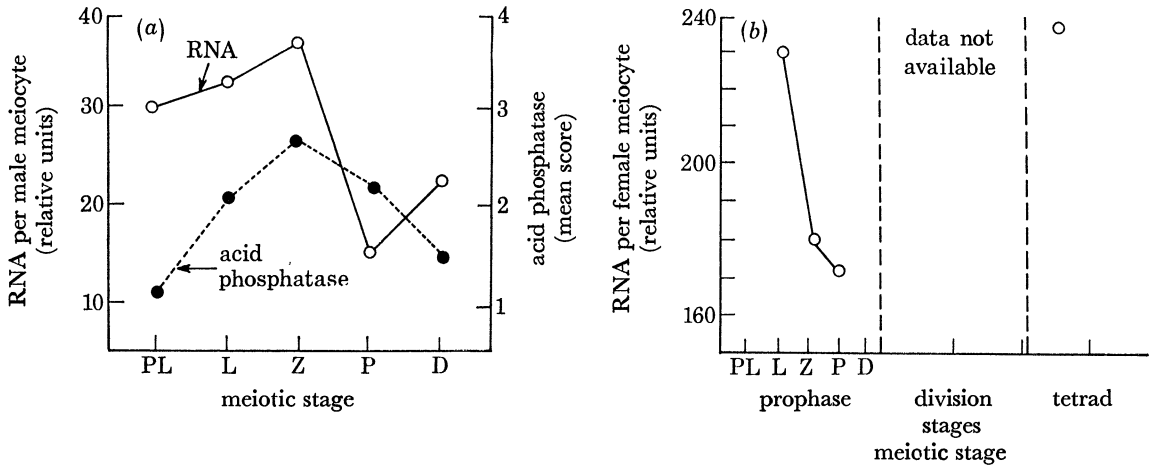

FIGURE 2(*a*) Changes in RNA content and acid phosphatase activity in glutaraldehyde-fixed male meiocytes of *Cosmos bipinnatus* (Compositae). The RNA curve is based from pyronin microdensitometry, and the acid phosphatase curve on a visual scoring method, as described by Knox, Heslop-Harrison & Dickinson (1970). Prophase stages as in figure 1.

(*b*) Changes in RNA content in glutaraldehyde-fixed female meiocytes of *Lilium longiflorum*, based upon Azure B microdensitometry, corrected for cell volume changes. Prophase stages as in figure 2. Due to technical difficulties, data could not be obtained during the interval between diakinesis and telophase II.

corrected for the growth in volume (figure 2*b*). One feature of the female meiocyte cytoplasm during this period not so conspicuously evident in the corresponding male cell is the presence of microbodies, present both before and during the degradation of the ribosomes (figure 5, inset). The content of these inclusions is at present unknown, and this information must be awaited before any suggestion that they may play a part in the degradation process can be advanced.

Counts from electron micrographs and microdensitometry both indicate that the cytoplasmic ribosomes reach their minimum numbers at the end of prophase. By this time, however, changes in the disposition of the residual population begin to appear, and an increasing number is to be observed in polysome groupings, rather than occurring freely in the cytoplasm (figure 6). By the dyad stage virtually no free ribosomes remain.

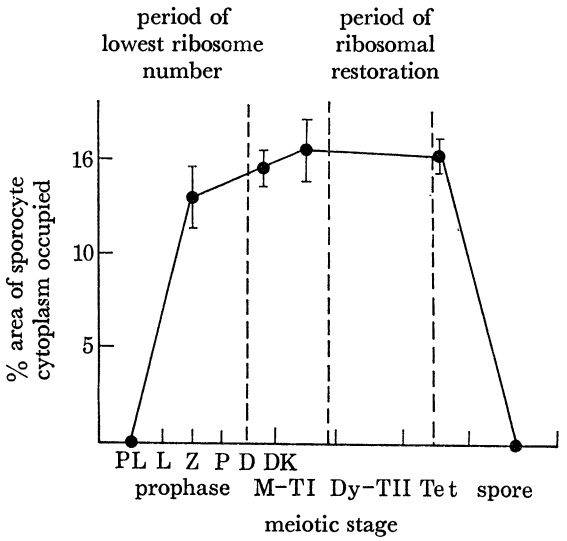

FIGURE 3. Proportion of cytoplasm encapsulated in double or multi-membrane bounded inclusions in the male meiocyte of *Lilium* through meiosis, data obtained as described by Dickinson & Andrews (1976). Prophase stages as in figure 1; M-TI, metaphase I to telophase I; Dy-TII, dyad to telphase II; Tet, tetrad.

In the male meiocyte, the amount of ribonuclease-extractable RNA rises after the diplotene diakinesis minimum. The values for the *Lilium henryi* cultivar to which the data of table 1 refer show that extractable RNA approached the leptotene level by metaphase I, the total amount in the dyad being nearly double this. The ribosome count per unit volume of cytoplasm was found to reach its minimum in diakinesis and to climb thereafter, achieving two-thirds of the leptotene level by anaphase I. The restoration of the ribosome population is evidently related to the formation of nucleolus-like bodies which are released into the cytoplasm of the meiocyte at anaphase I and anaphase II (Dickinson & Heslop-Harrison 1970*a*), and the origin of these has been followed in some detail in *Lilium longiflorum* (Williams *et al.* 1973).

Earlier studies of the meiotic nucleolar cycle in angiosperm male meiocytes showed that the mother cell nucleolus undergoes striking changes in form during prophase, ultimately losing basiphilia and becoming vacuolate (see, for example, Lin 1955). Furthermore, Das (1965) found in *Zea mays* that the mother cell nucleoli do not incorporate uridine after leptotene, although the chromosome associated synthesis rises during prophase to a maximum in pachytene, declining again through diakinesis. In *Lilium longiflorum* the pattern is very similar; incorporation of [^{3}H]uridine was found effectively to cease by mid-diplotene. However, an

autoradiographic investigation by Williams (1971) showed that RNA is synthesized actively in supernumerary nucleoli at the time of the pachytene–diplotene minimum in the cytoplasmic ribosome population, and that some synthesis is sustained through diakinesis. These supernumerary nucleoli are formed at the nucleolus organizing region of the nucleolar chromosomes during pachytene, at the time when the mother cell nucleolus is losing basiphilia and becoming vacuolate (figure 7). They are frequently found to lie in chains, the smallest in the chain having the highest basiphilia. Late diplotene nuclei contain supernumerary nucleoli not associated with the chromosomes, and these are probably derived by the detachment of the accessories from the NOR. The chromosomes in diplotene are of the lampbrush type (Grun 1958), with a diffuse sheath of basiphilic material which is almost entirely removable by RNase. After the dissolution of the nuclear envelope at the end of diakinesis, the supernumerary nucleoli cease to be distinguishable, presumably because their content becomes associated with the metaphase chromosomes. During anaphase, however, bodies with similar cytochemical properties and fine-structure become visible in the spindle region (figure 8). Most of these are released into the cytoplasm, to form the cytoplasmic 'nucleoloids' known from early accounts of angiosperm male meiosis and well described for *Fritillaria* by Frankel (1937). The residue enclosed within the envelope of the dyad nuclei is released into the cytoplasm during anaphase II.

There seems little doubt that the restoration of the cytoplasmic ribosome population at the end of the meiotic division is accomplished through the agency of the supernumerary nucleoli (figures 9 and 10). On this assumption it is possible to reconcile the changes in total fixable cell RNA, which includes both cytoplasmic and nuclear sources, and the corresponding ribosome counts. The steep decline between zygotene and diplotene results from the elimination of a large part of the cytoplasmic ribosome population, the residue being accounted for by a certain conserved fraction of ribosomes, mentioned further below, the remaining core of the parent cell nucleolus, and the chromosome-associated RNA. The continued fall in ribosome number recorded into diakinesis takes place at a time when there is active new synthesis in the super-

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Description of plate 1

Preparations are all of *Lilium longiflorum* or *Lilium henryi* cultivars, which do not differ in any significant feature in respect to the cytoplasmic and organellar events accompanying meiosis. All electron micrographs are from material fixed and prepared as described by Dickinson (1970).

Figure 4. Electron micrograph (EM) of a portion of a male meiocyte in early prophase. The contrast in ribosome density in the sporogenous (M) and tapetal (T) cells is well shown. Lipid globules (L) are frequent in the meiocytes at this time. (Magn. *ca.* × 9500.)

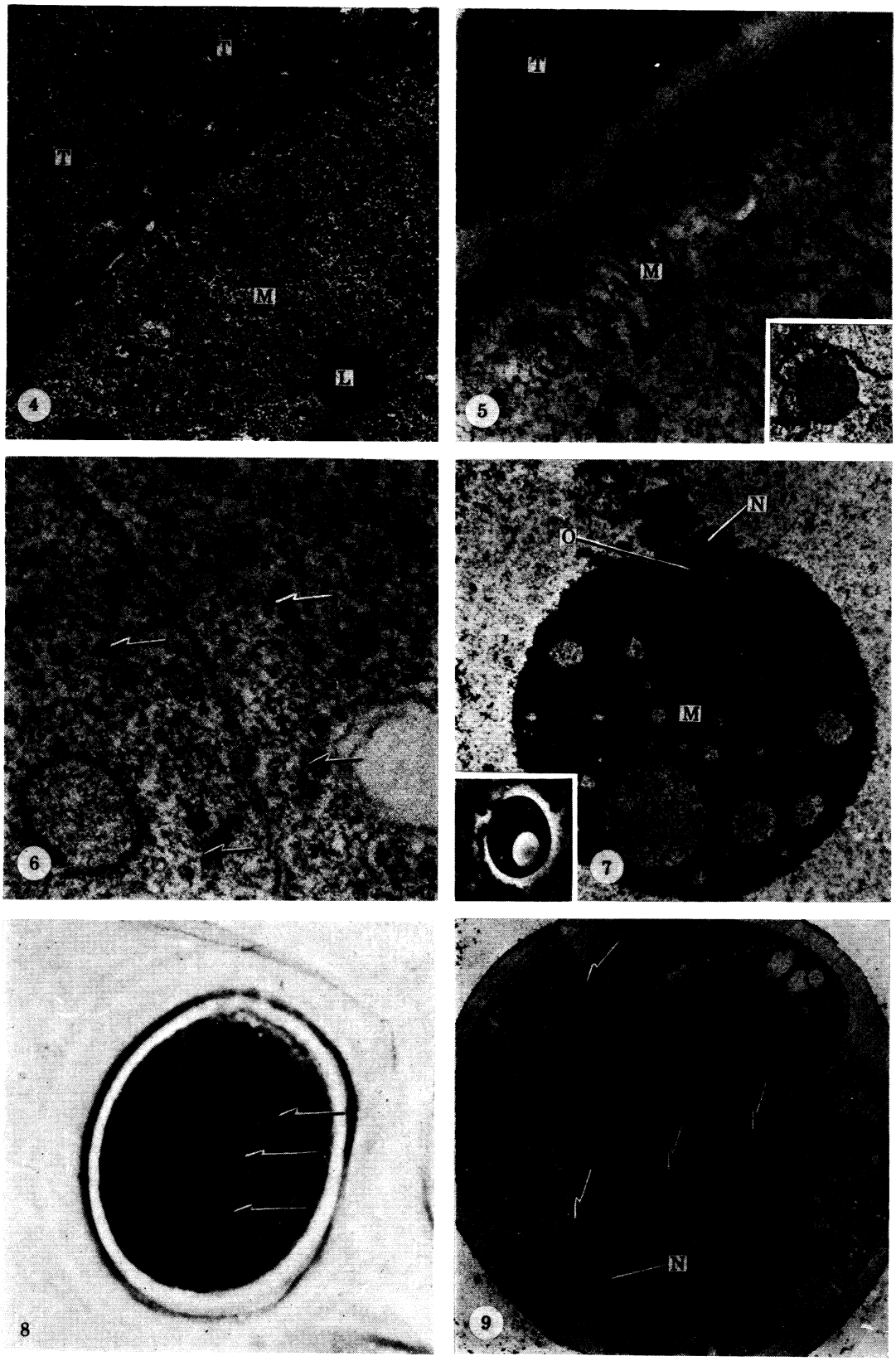
Figure 5. Female meiocyte in a stage corresponding to that of the male meiocyte of figure 4. Very few ribosomes can be distinguished in the megaspore mother cell (M), while the surrounding tissue (T) contains a normal complement. The vesicles at the cell periphery are probably concerned with callose deposition. Microbodies (inset) are frequent in the mother cells. (Magn. *ca.* × 42000; inset *ca.* × 8750; EM.)

Figure 6. Clusters of polysomes (arrows) in the megaspore mother cell during prophase. (Magn. *ca.* × 57000; EM.)

Figure 7. Formation of an accessory nucleolus (N) at the nucleolus organising region (O) in the male meiocyte during pachytene. The mother cell nucleolus is still attached at this region (M). The inset shows the beginning of the formation of a chain of accessory nucleoli. (Magn. *ca.* × 10500; EM. Inset, *ca.* × 1500, phase contrast light micrograph, thick section of plastic embedded material.)

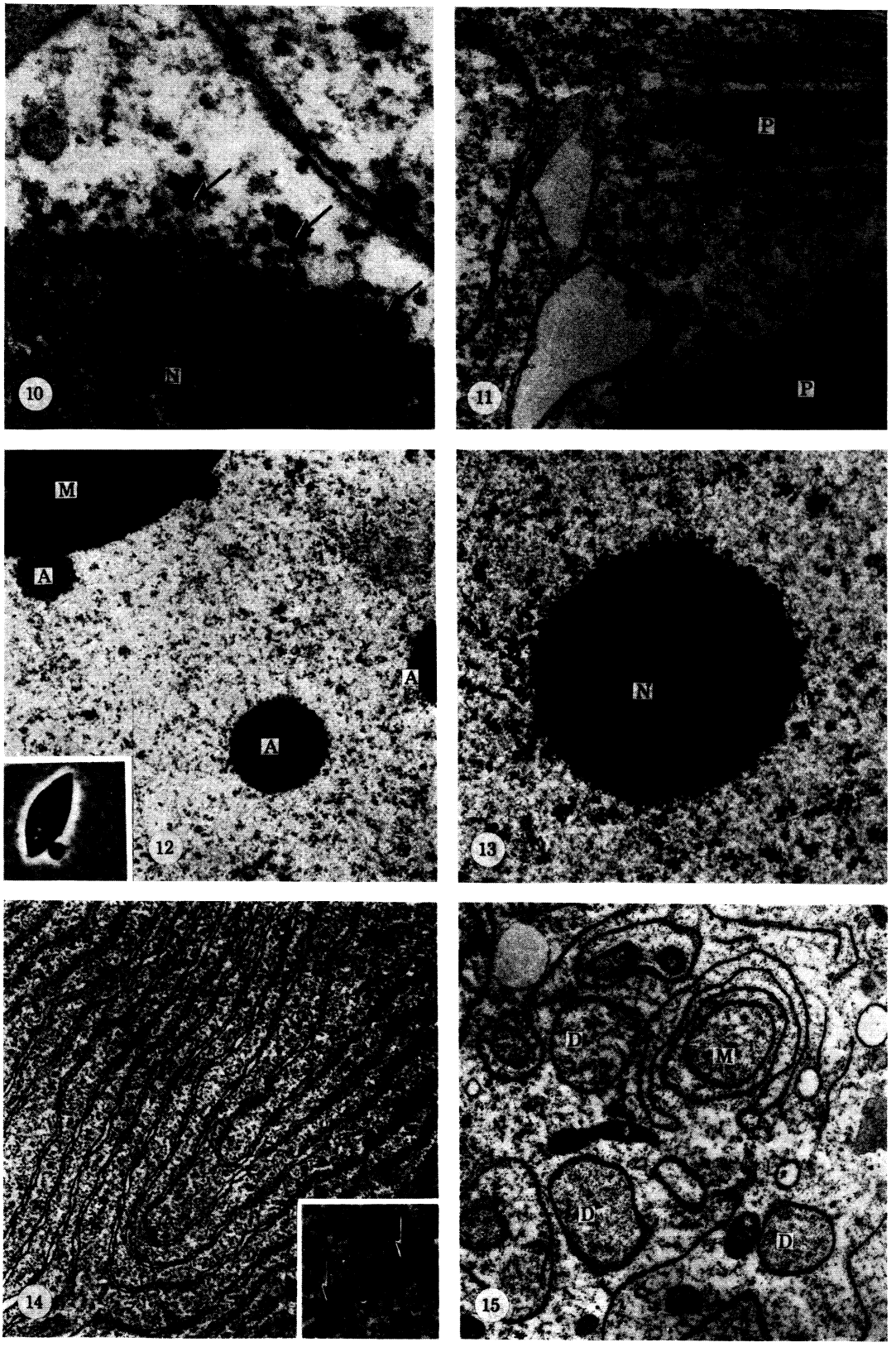
Figure 8. Cytoplasmic nucleoloids (arrows) in the spindle region, meiotic anaphase I. (Magn. *ca.* × 850, light micrograph, silver staining.)

Figure 9. Microspore tetrad showing a cytoplasmic nucleoloid(N). The arrows indicate early stages of primexine formation. (Magn. *ca.* × 950; EM.)



FIGURES 4–9. For description see opposite.

(*Facing p.* 332)



FIGURES 10–15. For description see opposite.

numerary nucleoli formed at the NOR, so that the value for total cell RNA does not decline in step.

In the female meiocyte, for which data were not available in the earlier studies, restoration of ribosomes apparently also begins at the dyad stage. The electron microscopic evidence shows an increase continuing from mid-dyad until leptotene levels of ribosomes are achieved in the latter period of the tetrad stage. Microdensitometry of these later tetrad stages confirms the structural evidence, but reliable measurements are not easy to obtain since the tissue is extremely vesicular and irregular in outline, making the calculation of changes in cell volume difficult and uncertain. The new ribosomes do not appear singly, but immediately become included into the increasing number of polyribosomes that characterize the female cytoplasm at this time. Some indication as to the activity of these polysomes may be given by the progressive increase of large proteinaceous masses in the cytoplasm. The nature of this protein is unknown, but it is present in paracrystalline array and its aggregations may exceed 2 μm in maximum dimension (figure 11).

The source of this new population of ribosomes is believed to be identical with that in the male tissue, but there are some differences in the process involved. Accessory nucleoli certainly appear to be formed at the surface of the mother cell nucleolus in pachytene (figure 12), presumably in conjunction with the nucleolar organizing region. However, neither the light nor electron microscope suggests that these bodies are formed in chains (figure 12, inset), but rather that they are instead released directly into the karyoplasm. Structurally, however, they are identical with the nucleoli formed at pachytene in microsporocytes (figure 13).

As in the male tissue, the supernumerary nucleoli disappear in late prophase, presumably again by association of their substance with the chromosomes. However, instead of condensing into cytoplasmic nucleoloids, this material appears to be transformed directly into cytoplasmic ribosomes during the periods of karyokinesis between monad and dyad, and dyad and tetrad. Some profiles indicating the presence of nucleoloids have been noted, but this is by no means such a regular phenomenon as in the male cells.

Regarded as a whole, particularly from the cytochemical evidence, this cycle of ribosome degradation and restoration in the female cells appears less comprehensive than that

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Description of plate 2

Figure 10. Periphery of cytoplasmic nucleoloid (N) comparable with that seen in figure 9, showing dispersal or ribosomes (arrows). (Magn. *ca.* × 112000; EM.)

Figure 11. Restoration of the ribosome population in the female meiocyte. Note aggregates of protein (P) in paracrystalline array. (Magn. *ca.* × 13500; EM.)

Figure 12. Formation of accessory nucleoli (A) in female meiocyte, some in close association with the mother cell nucleolus (M). The inset shows the mother cell nucleolus in the 'cap' conformation, with a single accessory nucleolus; chains, such as appear in the male meiocyte (figure 7) are not formed in the female. (Magn. *ca.* × 4200; EM. Inset, *ca.* × 1000, phase contrast light micrograph, thick section of plastic embedded material.)

Figure 13. Accessory nucleolus (N) in the karyoplasm of a female meiocyte. (Magn. *ca.* × 10500; EM.)

Figure 14. Stratified endoplasmic reticulum in the pre-prophase cytoplasm of the megaspore mother cell. The inset shows profiles (arrows) resembling nuclear pores in this membrane. (Magn. *ca.* × 26700; inset *ca.* × 68150; EM.)

Figure 15. The final stages in the formation of double (D) and multiple (M) membraned inclusions in the prophase cytoplasm pollen mother cells. (Magn. *ca.* × 8750; EM.)

characteristic of the male (compare figure 1 and 2*b*). However, as described further below, far more of the female cytoplasm is invested in membranes during this process. This encapsulation appears to confer some form of immunity to the cytoplasm contained within and, since the microdensitometer regards the cytoplasm as a whole, these protected areas would naturally be included in the cytochemical measurements. A more accurate representation of the extent of the elimination is furnished by the electron microscope, which shows that outside of the encapsulated regions it is quite as comprehensive as that occurring during microsporogenesis in *Lilium*.

Cytoplasmic membranes during meiosis

Fine-structural investigations of micro- and megasporocytes of *Lilium* have shown that during the meiotic prophase considerable parts of the cytoplasm come to be entirely enclosed within unit membranes. In the male meiocytes, volumes of cytoplasm are generally invested in double membranes (Dickinson 1971), while in the female meiocytes inclusions bounded by many such pairs of membranes are more common (Rodkiewicz 1965). Both double-membraned inclusions (d.m.i.) and multi-membraned inclusions (m.m.i.) are, however, to be found regularly in cells of either sex. Rodkiewicz proposed that in the female tissue these inclusions retained materials for subsequent use by the developing gametophyte, and we have suggested that constituents of the d.m.is are involved in the early development of the young pollen grain.

In cells of both sexes the inclusions are formed early in the meiotic prophase from membrane already present in the cytoplasm. In the pollen mother cells this membrane has previously been involved in a phase of protein synthesis (Dickinson 1968), and it is not unreasonable to propose that the same applies to that of the megaspore mother cell. Neither does the source of the membrane appear to differ between the sexes, for both in the male and female tissues the presence of nuclear pores indicates that it is generated from the nuclear envelope in a manner identical with the annulate lamellae of animal cells (figure 14).

Encapsulation of cytoplasm commences in late leptotene–early zygotene, just prior to the elimination of the cytoplasmic ribosomes. The membranes present lose the major part of their surface polysome populations, form cup-shaped profiles, and finally come to envelop a portion of the premeiotic cytoplasm (figure 15). This process appears completely non-selective, in that

Description of plate 3

Figure 16. Mutimembraned inclusion in the late prophase cytoplasm of a male meiocyte. Note the higher concentration of ribosomes in the centre (C) of this inclusion. (Magn. *ca.* ×23000; EM.)

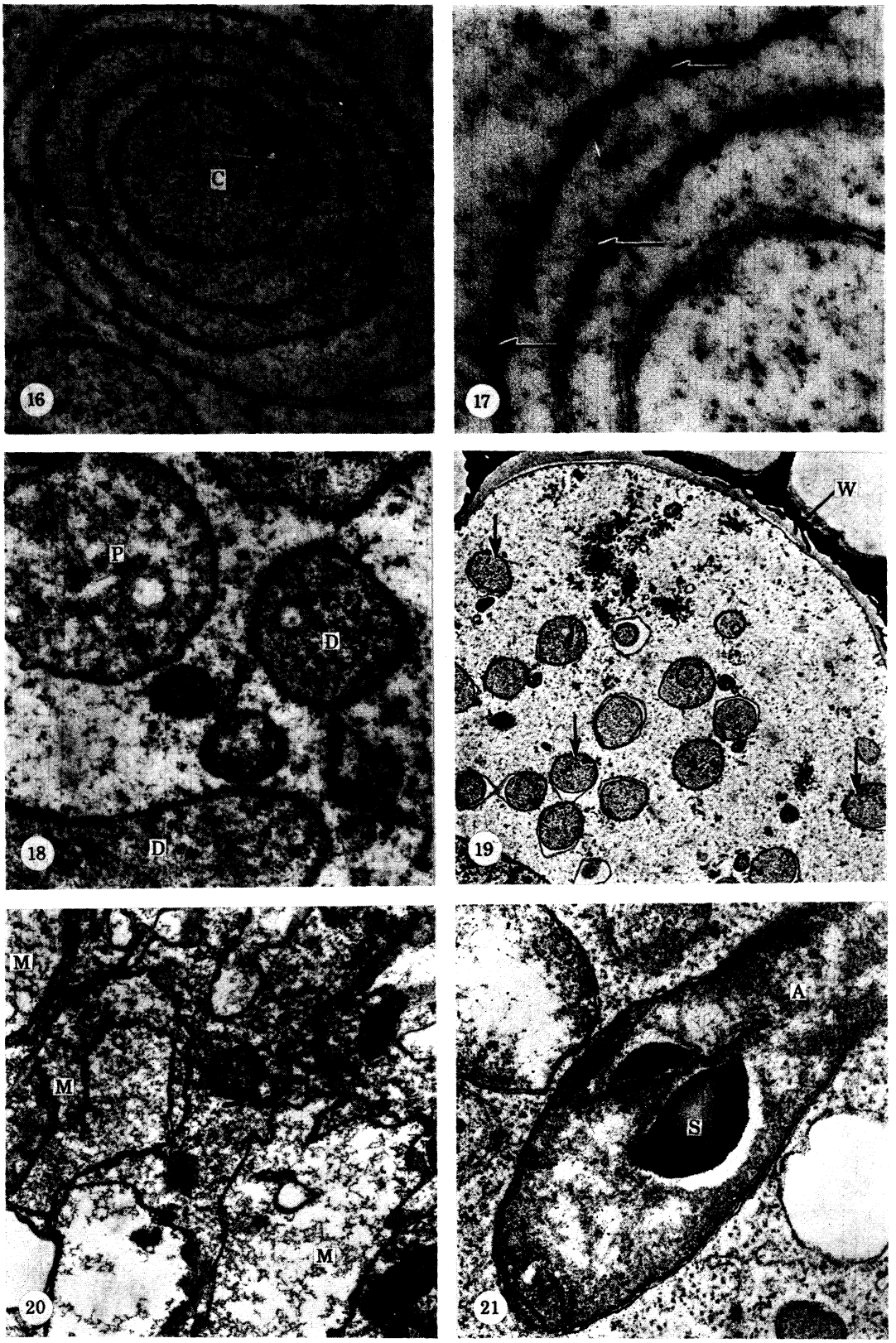
Figure 17. Detail of the membranes of an inclusion like that of figure 16. Note the particulate material (arrows) apparently adhering to the membrane surface. (Magn. *ca.* ×82050; EM.)

Figure 18. Double-membraned inclusions (D) and a de-differentiated plastic (P) in the cytoplasm of a female meiocyte. The two types of inclusion are superficially similar at this time, but the membranes differ. The concentration of ribosomes is greater in the DMI than in the surrounding cytoplasm, and many are aggregated in polysomes. (Magn. *ca.* ×26150; EM.)

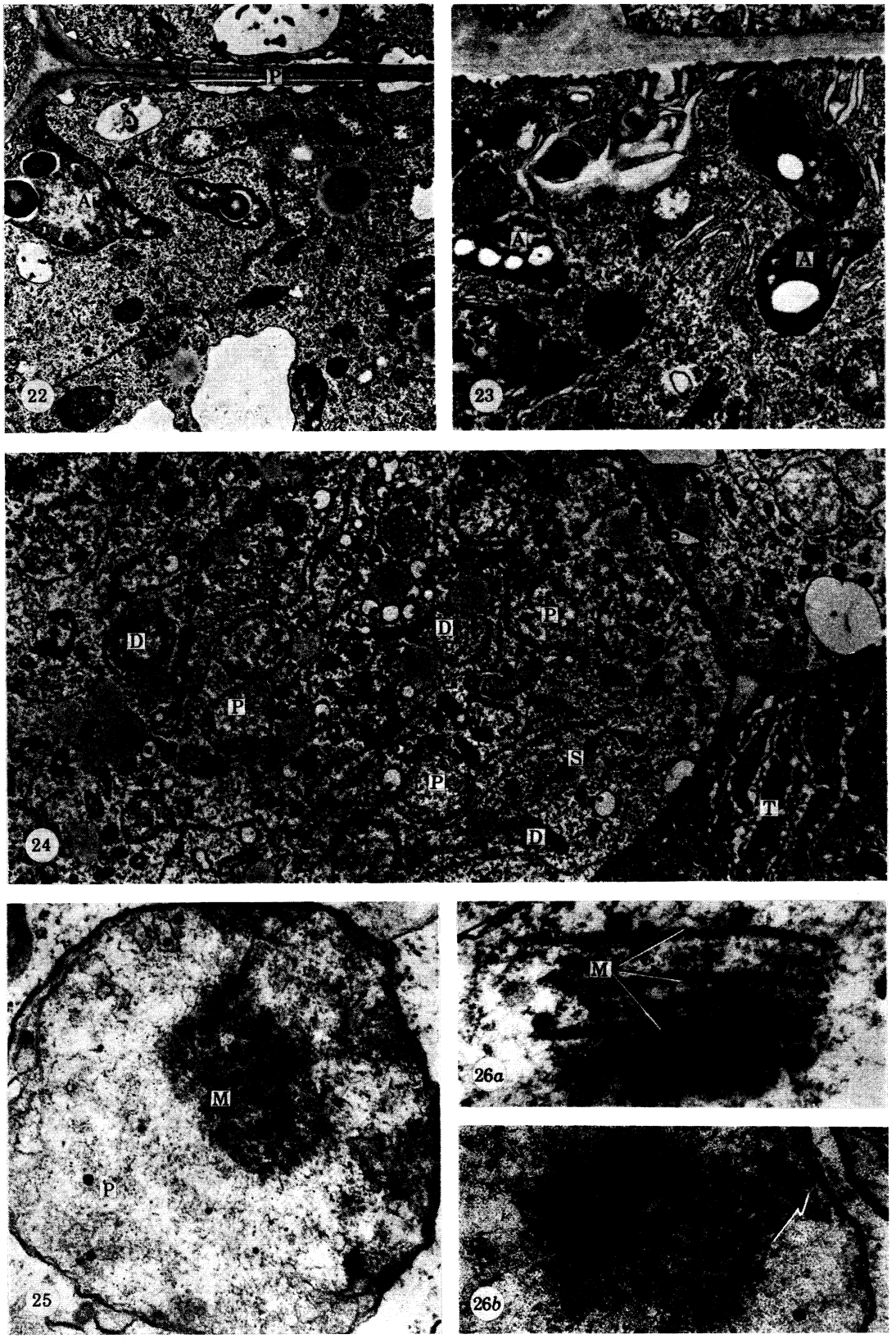
Figure 19. Early stages in the disintegration of d.m.is in the microspores. The areas of bounding membrane (arrows) destined to contribute to the pollen wall (W) are beginning to become distinguishable. (Magn. *ca.* 3700; EM.)

Figure 20. Early stages of disintegration of membrane-bounded inclusions in the female tissue (M). (Magn. *ca.* ×15300; EM.)

Figure 21. Amyloplast (A) in the pre-prophase cytoplasm of pollen mother cells. The electron opaque aspect of the starch grains (S) suggests amylase activity. (Magn. *ca.* 41000; EM.)



FIGURES 16–21. For description see opposite. (*Facing p.* 334)



FIGURES 22–26. For description see opposite.

lipid droplets, organelles, or even other m.is may be invested. Indeed it is this enclosure of d.m.is that gives rise to the m.m.is characteristic of the female cells. Why m.m.is are formed generally in the female and d.m.is in the male is not clear, but it may be noted that the megaspore mother cell is far larger than the male meiocyte, thus enabling the formation of larger inclusions. If physical considerations determine the minimal size of these inclusions (none are seen of a diameter less than 0.3 μm) the likelihood of m.m.is being formed would increase with the volume of cytoplasm and the area of membrane available.

Estimation of the proportion of cytoplasm included by this process is not easy, for measurements have to be corrected for cell and nuclear size changes. Measurements have, however, been made of the areas of cytoplasm invested by m.is in the male meiocytes of *Lilium longiflorum*, and these are shown in figure 3. It can be seen that by the mid-prophase some 15 % of the cell cytoplasm, as estimated from area, is invested by double or multiple membrane systems. A similar estimate has yet to be made for the female tissue, but simple inspection of electron micrographs suggests that a far higher proportion of female cytoplasm must be encapsulated.

During the degradation of ribosomes during prophase, the cytoplasm invested within the inclusions reacts differently from that without. In these membrane-bound lacunae, the ribosomes appear to be far less affected by whatever agents effect the eradication; this is most striking in m.m.is, where a gradation of ribosome concentration can be seen between the different layers of the inclusions, ribosomes being most abundant in the central inclusion (figure 16).

The few ribosomes remaining on the surface of the investing membrane are of course degraded, but it is noteworthy that a granular deposit remains after their eradication, perhaps indicating that it is only one component of the ribosome that is eliminated (figure 17).

By late prophase, when ribosome levels are at their lowest, it is often difficult to distinguish between the d.m.is of the male tissue and the undifferentiated plastids, described further in a later section, also in the cytoplasm. Measurements indicate that little significant change overcomes the proportion of cytoplasm invested from late prophase until the breakdown of the inclusions in the post-tetrad stage (figure 3). In fact very little change at all takes place within these inclusions until their break-up. The most striking concerns the ribosomes contained in the m.i. which, while mainly free in early stages, later regularly become associated in polysomes (figure 18).

Description of plate 4

Figure 22. Male meiocytes in early prophase. The amyloplasts (A) are becoming conspicuously pleiomorphic. The cells are still interconnected by plasmodesmata (P), and cytomictic channels have not yet been formed. (Magn. *ca.* × 10200; EM.)

Figure 23. Female meiocytes in a phase corresponding to that of the male meiocytes in figure 22. Amyloplasts are still evident (A). (Magn. *ca.* × 13650; EM.)

Figure 24. Pollen mother cell cytoplasm in early prophase, showing the similarity between the de-differentiated plastids (P) and the double-membraned inclusions (D). The contrast in ribosome concentration between sporogenous tissue (S) and tapetum (T) is well shown. (Magn. *ca.* × 12250; EM.)

Figure 25. Re-differentiating plastid (P) in microspore, showing the membrane-particle association (M), the membrane not seen in this section. (Magn. *ca.* × 43000; EM.)

Figure 26 (*a*) Membrane-particle association in a megaspore plastid, showing the membranous component to advantage (M). (*b*) as (*a*) microspore, showing part of the membranous component of the membrane-particle association (arrow) and detail of the associated particles in a plastid like that of figure 25. (Magns: figure 26*a*, *ca.* × 62000; figure 26*b*, *ca.* × 65500; EM.)

Perhaps the most conspicuous difference between the m.is in male and female tissues concerns the manner of their degradation in the post-tetrad stage. In the young microspores of *Lilium*, the d.m.is persist until early in the young spore stage, when striking alterations take place in their bounding membranes. Regions of these membranes become more electron opaque and coated with ribosomes, while the remainder of the envelope becomes tenuous and large electron lucent spaces appear between the individual elements (figure 19). The content of the inclusions becomes diffuse and finally disappears, although early in the degradative process it may appear concentrated by the intrusion of the inner membranous element.

Following the removal of the content, the main part of the investing membranes is lost, leaving only the 'dark' regions of membrane, which now join their inner and outer elements at the edges to form 'plates' of membrane coated with ribosomes. These plates, apparently under the influence of cytoplasmic microtubules, move to the surface of the young spore where they play a major part in the synthesis of elements of the nexine pollen wall layer (Dickinson & Heslop-Harrison 1971; Dickinson & Andrews 1976). In the female the demise of the m.m.i. is far less spectacular. The bounding membranes first appear tenuous and their interstitial spaces irregular. The membrane finally becomes discontinuous, mixing the now-diffuse content with the general cytoplasm (see figure 20). Elements of the membrane itself remain free in the cytoplasm for some time subsequent to these events.

The cytoplasmic organelles during meiosis

Variations in the optical-microscopic appearances of the 'chondriome' during meiosis in angiosperm microsporocytes led Guilliermond (1920, 1924) and Py (1932) to suppose that the organelles of the cytoplasm pass through a granular period in prophase before restoration in the young spore. Bal & De (1961) studied this phase electron microscopically, and concluded that the organelle lineage might actually be broken at this point, with a subsequent restoration *de novo*. More detailed investigations using better fixation technique have proved this last interpretation to be incorrect, but have shown that cycles of dedifferentiation and redifferentiation do occur in both plastids and mitochondria of the microsporocytes (Marumaya 1968; Dickinson & Heslop-Harrison 1970*b*). Our own earlier observations on *Lilium* male meiocytes have now been supplemented by studies of the megasporocyte, and it is clear that the events in the two sexes are closely equivalent (table 2).

Description of plate 5

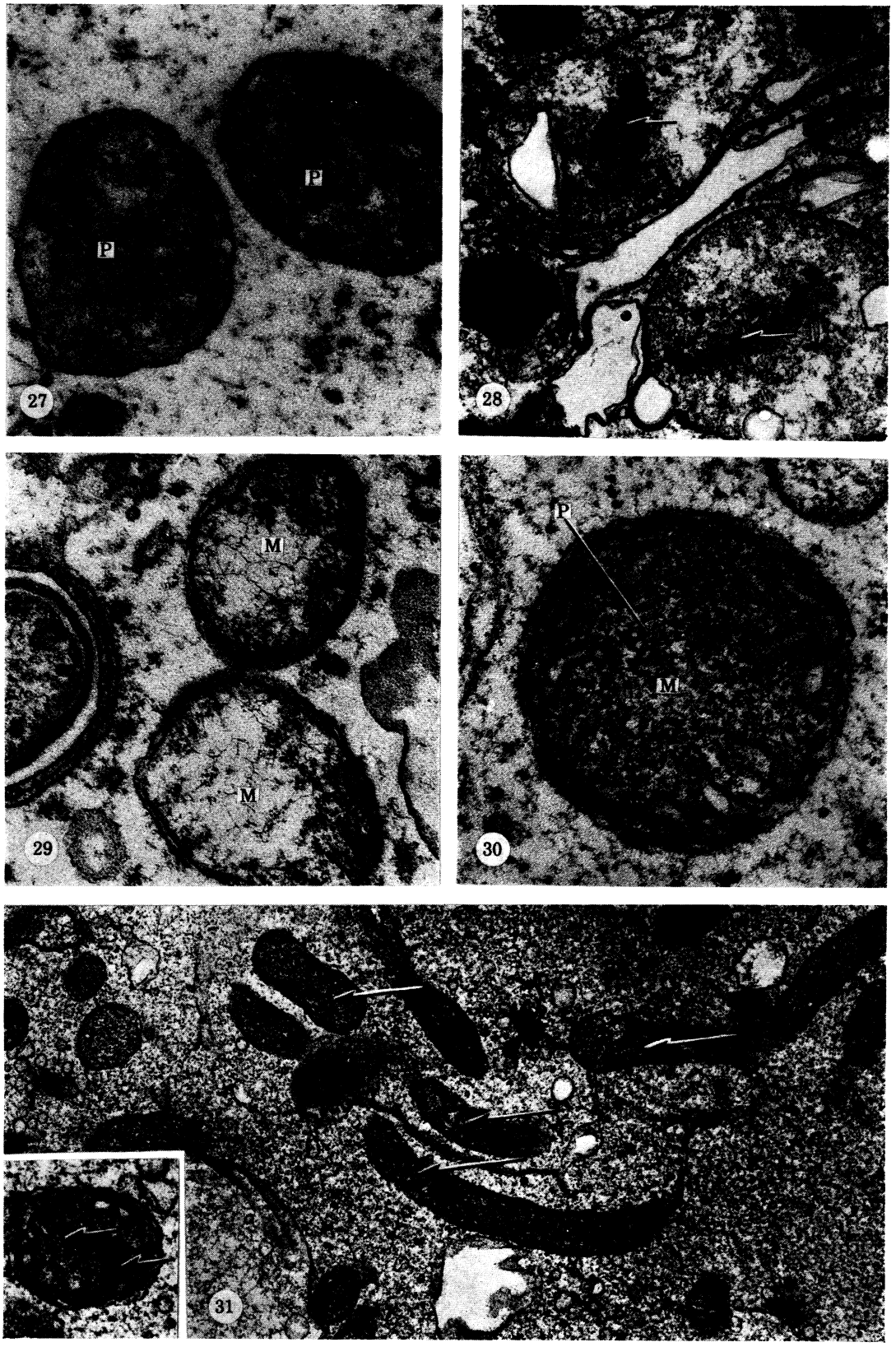
Figure 27. Re-differentiating plastids (P) in microspores. The stroma is beginning to darken following the disperal of the membrane-particle associations. (Magn. *ca.* × 16850; EM.)

Figure 28. As figure 24, but a later stage of re-differentiation in young embryo sac. Starch is beginning to form, even although the membrane-particle associations have not yet completely dispersed. (Magn. *ca.* × 12500; EM.)

Figure 29. Mitochondria (M) in the premeiotic pollen mother cells. (Magn. *ca.* × 41000; EM.)

Figure 30. Promitochondrion (P) in the female meiocyte, showing the dense stroma characteristic of the mid-prophase to metaphase I period. (Magn. *ca.* × 96000; EM.)

Figure 31. Re-differentiation of the mitochondria (arrows) in a female meiocyte at the end of meiosis I. Each organelle has inclusions of electron-opaque material, seen in more detail in the inset. (Magn. *ca.* × 20500, inset *ca.* × 51000; EM.)



FIGURES 27–31. For description see opposite. (*Facing p.* 336)

The plastids

The premeiotic microspore and megaspore mother cells contain normal amyloplasts (figure 21), measuring between 0.5 and 1.5 μm, and containing one or two starch grains, a full complement of osmiophilic globuli, ribosomes and the fibrils normally identified as DNA. As the cells enter prophase the starch grains are eroded, and the organelles enter a phase of division,

TABLE 2. SUMMARY OF THE CHANGES IN PLASTIDS AND MITOCHONDRIA DURING MEGA- AND MICROSPOROGENESIS IN *LILIUM LONGIFLORUM*

	plastids					
stage	starch	osmiophilic globuli	membrane particle associations	ribosomes	other features	mitochondria
synchronous premeiotic stage	+	+	−	+	amoeboid profiles frequent, suggestive of division stages	normal, but with some pleiomorphic profiles indicating division
leptotene	(+)	+	−	+	lamellar system regressing; occasional aspects suggestive of division	isodiametric 'expanded' mitochondria
zygotene	−	+	−	(+)	plastids tending to spherical or ellipsoidal shapes; division aspects declining	condensation to small promitochondria
pachytene	−	+	−	−	period of maximum structural simplification; ribosomes not discernible; shapes irregularly spherical or ellipsoidal	promitochondria
diplotene/diakinesis	−	+	(+)	−	as in pachytene	promitochondria
metaphase I – dyad	−	+	+	(+)	first appearance of m.p.as; microtubules occasionally visible	electron-opaque granular areas, some amoeboid profiles
metaphase II – early tetrad	−	+	+	(+)	m.p.as present probably in all plastids	electron-opaque granular areas, pleiomorphic
late tetrad	♂ ♀ −(+)	+	(+)	+	beginning of m.p.a. dispersal; density of stroma increasing; occasional paracrystalline bodies	as in early tetrad
early spore male and later tetrad, female	♂ ♀ (+)+	+	−	+	lamellar system redifferentiating as starch reappears; profiles suggestive of division frequent	normal configurations restored

indicated by striking pleiomorphism (figures 21–23). The starch is lost altogether during late leptotene, and the organelles subsequently round off to become isodiametric. During the interval when the ribosomes are lost from the cytoplasm, the plastids lose almost all internal structure, including most of the plastid ribosomes. By late zygotene the plastids are completely dedifferentiated and are almost indistinguishable from the double-membraned

inclusions characteristic of the male cytoplasm at this stage (figure 24). With so great a loss of character, it is small wonder that Bal & De (1961) suggested that the plastid lineage terminated at this point. Osmiophilic globuli are not lost, however, and the presence of these bodies, combined with the still characteristic aspect of the plastid membranes, does permit their positive identification. In addition, no ribosomes can be distinguished in the plastids, whereas, as we have seen, a population of polysomes is conserved within the d.m.is.

The plastid population remains relatively unaltered throughout the remainder of prophase and through the metaphase of meiosis I. A new structure then becomes evident in the stroma in both male and female tissues. This takes the form of an association between small granules (figure 25) and a section of membrane (figure 26*a*, *b*). The granules measure between 15 and 60 nm in maximum dimension, and they may be interspersed with a small number of osmiophilic droplets of much the same dimensions. Diffuse masses of fibrils are also present. The membrane-particle association (m.p.a.) is probably a regular feature of all plastids (Dickinson & Heslop-Harrison 1970*b*). With rare exceptions only one m.p.a. is present in each plastid at the dyad stage, but in the late tetrad stage in microsporocytes when plastid division figures are to be seen there is invariably a synchronous division of the m.p.a.

Some observations on the sensitivity of the components of the m.p.a. to enzymic digestion have been made. The first results suggest that the granules contain RNA and protein, while the fibrillar material is removed by DNase. These findings fit in well with the ultrastructural observations, since the granules are often morphologically indistinguishable from ribosomes. Marumaya (1968) in his study of *Tradescantia* used $KMnO_4$ as a fixative and so was unable to distinguish changes in the plastid ribosomes, but he did report 'clear areas' in the stroma which he considered to be the sites of the organellar DNA.

The m.p.as disappear from the plastids in the post-tetrad stage. When the tetrad ruptures and the young microspores are released into the thecal fluid, the stroma of the plastids darkens swiftly and obscures the m.p.as, individual elements of which appear to be dispersed throughout (figure 27). In the female cells, late in the tetrad stage, the m.p.a. expands slowly, becomes more diffused and is gradually obscured by the darkening stroma (figure 28). Closely following the darkening of the stroma, ribosomes become visible again throughout and the organelles enter a phase of active division. Perforated lamellae then arise and the synthesis of starch begins. In the young microspores this synthesis continues for an extended period so that by the time of pollen mitosis the cytoplasm is characterized by the presence of large distorted amyloplasts, each containing about six starch grains. While this accumulation is not so rapid in female tissues, the organelles do subsequently behave as normal somatic amyloplasts and undergo no further cycles of differention.

The mitochondria

The changes that occur in the mitochondrial population are in no way so striking as those affecting the plastids, but they are extremely regular and are equally closely synchronized with nuclear and other cytoplasmic events.

The cytoplasm of both male and female cells entering meiotic prophase contain normal 'somatic' mitochondria (figure 29). These are expanded organelles, measuring some 1.0 μm in maximum dimension. During the leptotene they begin a phase of division, revealed by a range of lobed profiles; thereafter they contract to form, by zygotene, smaller, mostly isodiametric, organelles, about 0.3 μm in maximum dimension. They retain small numbers of cristae, and with standard fixation and staining procedures reveal a very electron-opaque matrix, rich in

ribosomes (figure 30). This reduction and simplification of the mitochondria was observed by Bal & De (1961) in *Tradescantia*, who referred to the small organelles as 'promitochondria'.

The promitochondria remain unchanged throughout meiotic prophase and early metaphase I, at which time a new inclusion regularly appears within them, in the form of an accumulation of electron opaque material in the matrix, of variable size, but rarely exceeding 200 nm in maximum dimension (figure 31). This material is largely removed by RNase, but the accumulation does not resemble the membrane-particle association of the plastids present during the same period. During later meiotic stages the material becomes dispersed throughout the mitochondria, which themselves become pleiomorphic and even undergo division while still retaining the condensed aspect (figure 31). Towards the end of the four-nucleate stage in the female and in the young spore in the male the mitochondria enlarge once more and recover the normal somatic aspect.

Conspectus

Taken with earlier reports based upon light microscopy and various other electron-microscopic observations the evidence reviewed above shows that the cytoplasm and organelles of the angiosperm meiocyte undergo radical reorganization during the division, and that the changes are closely locked with the chromosome cycle. As we have noted in the introductory paragraphs, the events are undoubtedly best interpreted in relation to the transition from the sporophytic to the gametophytic phase of the life cycle. A major part of the cytoplasmic ribosome population of the diploid mother cell is eliminated during the meiotic prophase, to be restored at the time of the quadripartition of the cell which gives the spores. The new population evidently arises through fresh activity at the nucleolus organizing regions during the pachytene and diplotene stages of the division, and the process does not involve the parent cell nucleolus. Such a cycle would be intelligible were it a requirement for the sporophyte–gametophyte transition that residual long-lived, ribosome-associated mRNA from the sporophytic parent should be removed before the initiation of new transcription in the haploid spore nuclei (Heslop-Harrison 1971*a*). The activation of these nuclei follows shortly upon the cleavage of the meiocyte, and the genetic individuality of the spores and the young gametophytes is quickly expressed, as for example in those species where there is a segregation of *S* (incompatibility) alleles in the products of the male meiosis. During the period when the spores begin their separate lives, they are enclosed within the callose wall of the meiotic tetrad, and without plasmodesmatal or other cytoplasmic contact with each other or with parent tissue. The tetrad wall evidently provides some degree of isolation (Heslop-Harrison & Mackenzie 1967), and this may be essential for the early establishment of genetic independence (Heslop-Harrison 1964). It is noteworthy that Linskens & Schrauwen 1968) found a dramatic reduction in the numbers of polysomes in the diplotene and diakinesis intervals in *Lilium henryri* male meiocytes, followed by a rapid increase again at the end of the division. They concluded that the cycle was related to the need for the haploid phase to build up its own protein synthesis system after the elimination of that of the diploid mother cell.

Various other cases of degradation and resynthesis of ribosomes in relation to developmental changes have been reported, perhaps the most noteworthy in the present context being that described by Siersma & Chiang (1971) in *Chlamydomonas reinhardtii*. In this unicellular chlorophycean alga the cytoplasmic ribosomes are conserved during vegetative growth. At the time of gametogenesis, however, about 90 % of the pre-existing 'vegetative' phase ribosomes are

eliminated, and, during the period of degradation, a new population is synthesized, ultimately to be distributed to the gametes. In this instance the major developmental transition from vegetative phase to sexual phase is not correlated in time with meiosis, the reduction division taking place later during the germination of the zygote. Siersma & Chiang consider the possibility that the ribosomes themselves may possess a certain degree of specificity related to their function in the two stages of the life cycle. An alternative interpretation would be that mentioned above, namely that the elimination phase is related to the inactivation of persistent vegetative phase information.

The autophagic activity involved in the cytoplasmic restandardization of the meiotic prophase is presumably due to degradative enzymes. Cytochemical observations on *Lilium* and other meiocytes show that the activity of various lytic enzymes does increase spectacularly during mid-prophase. The change in acid phosphatase activity in meiocytes of *Cosmos bipinnatus* is shown in figure 2*a*, and corresponding trends have been observed in cytochemically detectable ribonuclease. The enhancement of activity precedes the ribosome elimination phase slightly, and the conclusion that the degradation is mediated by an activation of lysosome-like enzymes seems inescapable. It may be noted in passing that these substantial variations in enzyme activity associated with cytoplasmic changes occur coincidentally with the nuclear processes of meiosis, and must be taken into account in assessing the results of observations on isolated meiocytes. Any assumption that phase-specific changes in enzyme activity must necessarily be related to intranuclear events could obviously lead to serious misinterpretation.

A proportion of the angiosperm meiocyte cytoplasm (in *Lilium*, some 15 % in the male, more in the female) is conserved in the d.m.is and m.m.is through the ribosome elimination phase. This would seem to provide a mechanism for transmitting a sample of the diploid mother cell cytoplasm, unchanged, to the haploid spores. There is good reason to suppose that this transfer may be essential for the conduct of the events in the period immediately following upon meiosis, for in both male and female cells the interval is one of intense activity, on the male side in the production of the complex pollen grain wall, and on the female in nuclear division and growth. The most persuasive evidence that some of the early activities of the spores and the gametophytes they produce are governed by agencies carried forward from the parental meiocytes comes from observations on pollen wall morphogenesis. As mentioned above, the d.m.is of the microspore are certainly involved directly in the synthesis of the wall (Dickinson & Heslop-Harrison 1971; Dickinson & Andrews 1976). The principal events of wall morphogenesis occur in the period following immediately upon meiosis when the complex patterns later to be seen in the mature pollen grain wall are established, but strikingly enough they do not depend on the presence of the haploid nuclei, since they proceed in fragments of cytoplasm deprived of nuclei altogether (Heslop-Harrison 1971*b*). Evidently, then, the meiocyte cytoplasm receives morphogenetic information *before* the meiocyte cleavage, and it seems a reasonable surmise that this is conserved and carried through the ribosome elimination phase into the spores within the membrane-bounded inclusions.

The cycle of dedifferentiation and redifferentiation in the plastids and mitochondria is presumably also connected with the sporophyte–gametophyte transition. Although much is still to be learned about the nature and extent of the organellar dedifferentiation, it is evident that while there is a severe regression in the case of the plastids, which are reduced to the barest elements, the mitochondria are not degraded to a similar degree, and presumably remain functional through the division phases.

A similar cycle of chloroplast ribosome degradation and reformation has been described by Siersma & Chiang (1971) in *Chlamydomonas reinhardtii* during the transition from the vegetative to the gametic phase: here again the radical development switch seems to require a removal of both cytoplasmic and plastid ribosomes. These authors report that the degradation of the plastid ribosomes begins about five hours after that of the corresponding process in the cytoplasm. In *Lilium*, the plastid ribosome cycle seems to proceed more or less in step with that in the cytoplasm, so far as can be judged from the electron microscopic evidence.

The redifferentiation of the plastids is associated with the appearance of the m.p.as, and these are seemingly the sites of formation of the new plastid ribosome population; the m.p.a. may perhaps be said, therefore, to represent a kind of rudimentary plastid nucleolus. Pursuing the same argument as that advanced above for the cytoplasmic cycle, the replacement of the plastid ribosome complement is probably to be looked upon as a means of removing sporophytic parental influences in preparation for a new differentiation governed by the haploid gametophyte genome. Evidence is already available that the enzyme complements of plastids are brought immediately under haplophase control in the classical case of waxy and non-waxy segregation in *Zea mays*, where the two plastid types can be detected among the young spores. This example suggests that the plastid ribosomes are available to receive fresh haploid-nucleus generated messenger as soon as redifferentiation begins, without any parental carry-over.

The mitochondrial regression is less extreme than that that overtakes the plastids, and ribosomes persist in the stroma throughout the period of elimination in cytoplasm and plastids. This is in keeping with the high degree of independence attributable to these organelles in eukaryotes. Evidently their metabolic functions remain very much the same throughout through the sporophyte-gametophyte cycle.

The far-reaching changes in the angiosperm sporocyte cytoplasm, now established for both sexes, are presumably manifestations of the re-standardization postulated by Mather (1965) as likely to be associated with sexual reproduction. In the foregoing, the rejuvenation has been considered principally in relation to the radical developmental switch from sporophyte to gametophyte, but one can envisage other functions. Should the cytoplasmic 'clean up' extend to foreign nucleic acids, it would provide a control over the transmission of viruses through meiosis and into the spores (Heslop-Harrison 1972). Such transmission through the gametophytes is rare. The embryo sac is especially well guarded: it arises from a spore, and later enjoys a degree of physical protection unparalleled in other organs of the plant, being invested in callosic and cutinized membranes and lacking plasmodesmatal contact with neighbouring parental tissues. Suggestively enough, in diplosporous and aposporous apomicts the isolation of the embryo sac and the developing embryo is maintained precisely as in sexual species, indicating that there is selective advantage in the concomitants even when meiosis itself is abandoned. Diplosporous apomicts should offer the opportunity to investigate the cytoplasmic and organellar events in mother cells without the complication of a meiosis taking place over the same period.

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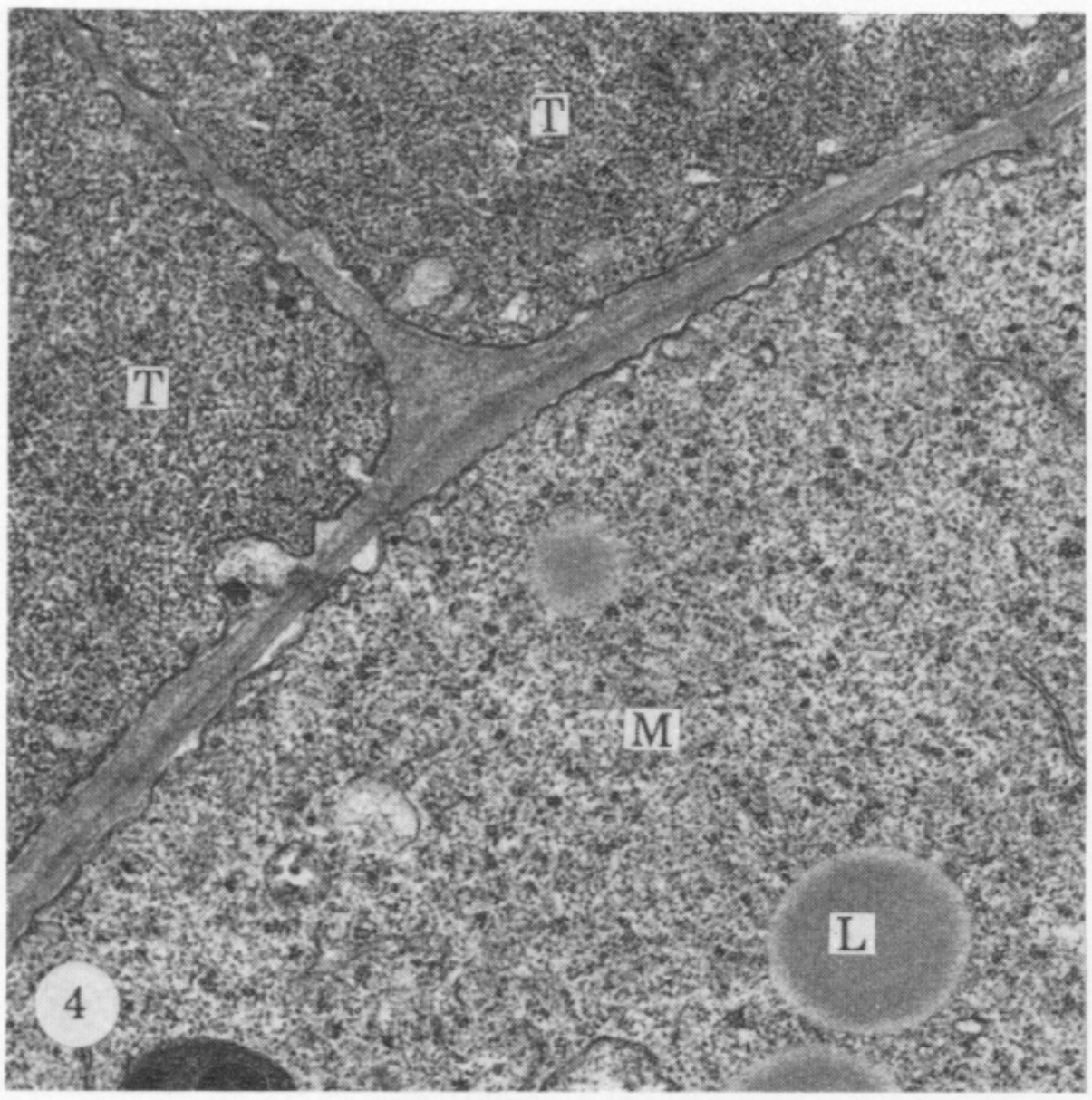
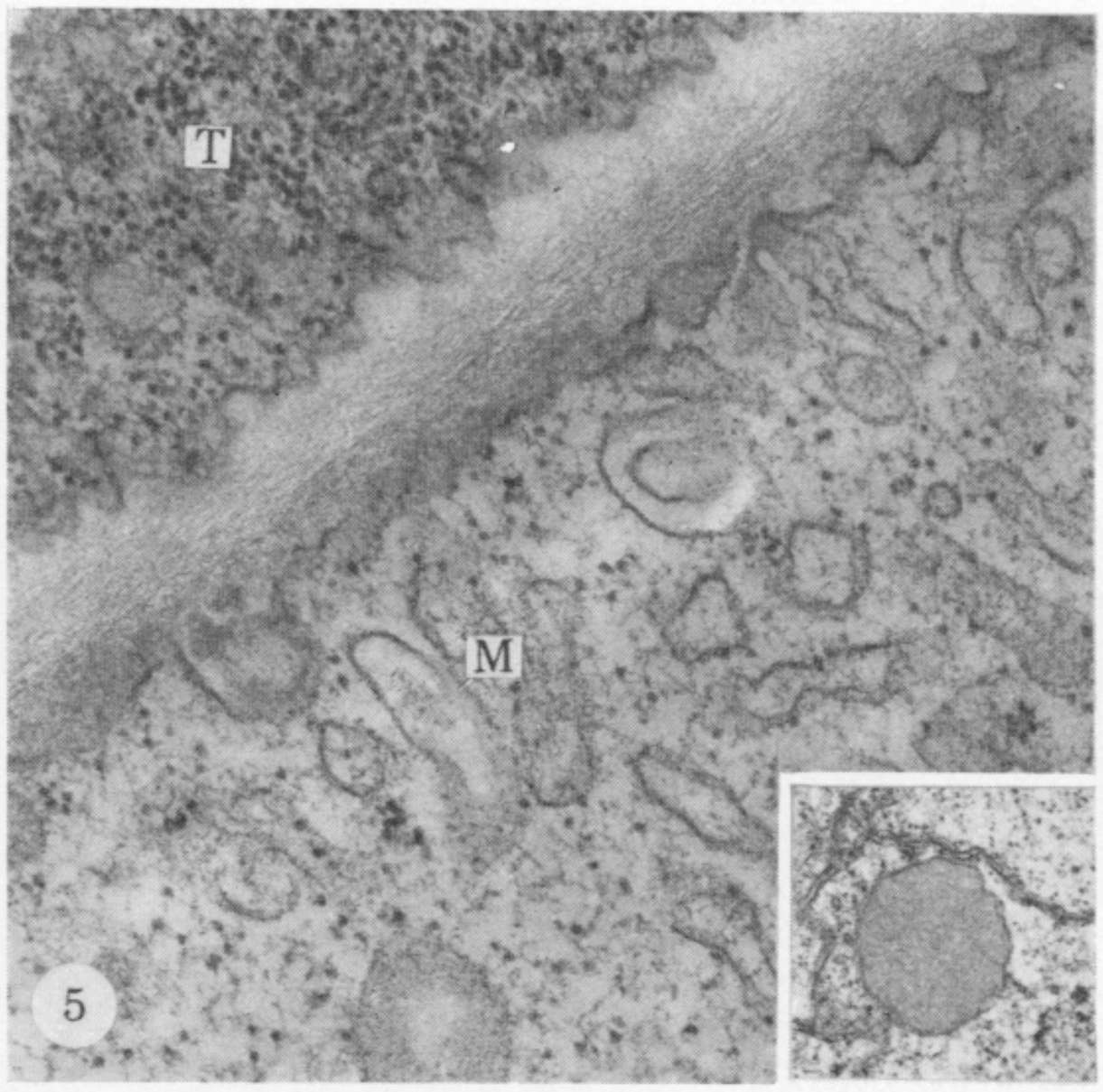
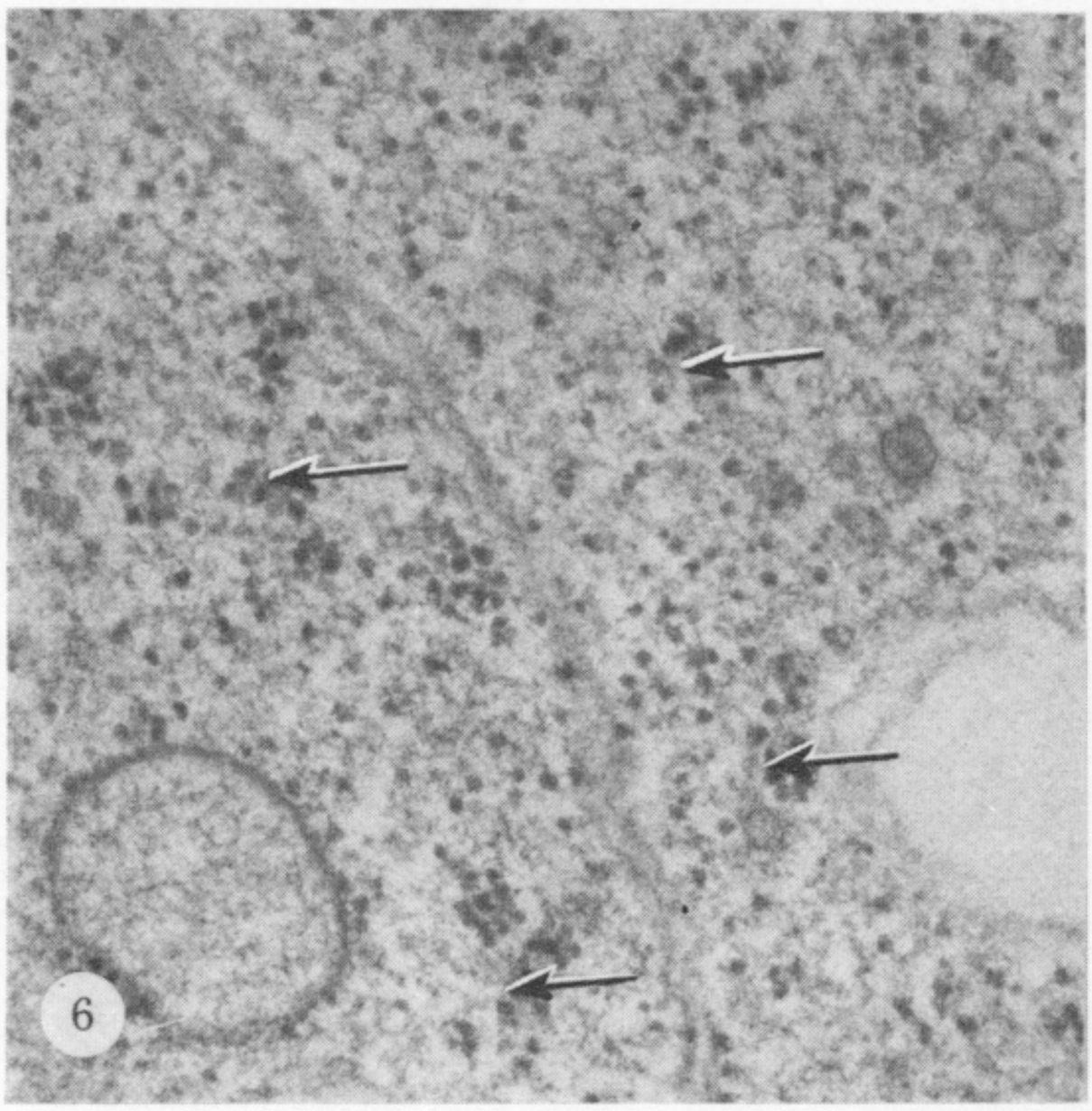
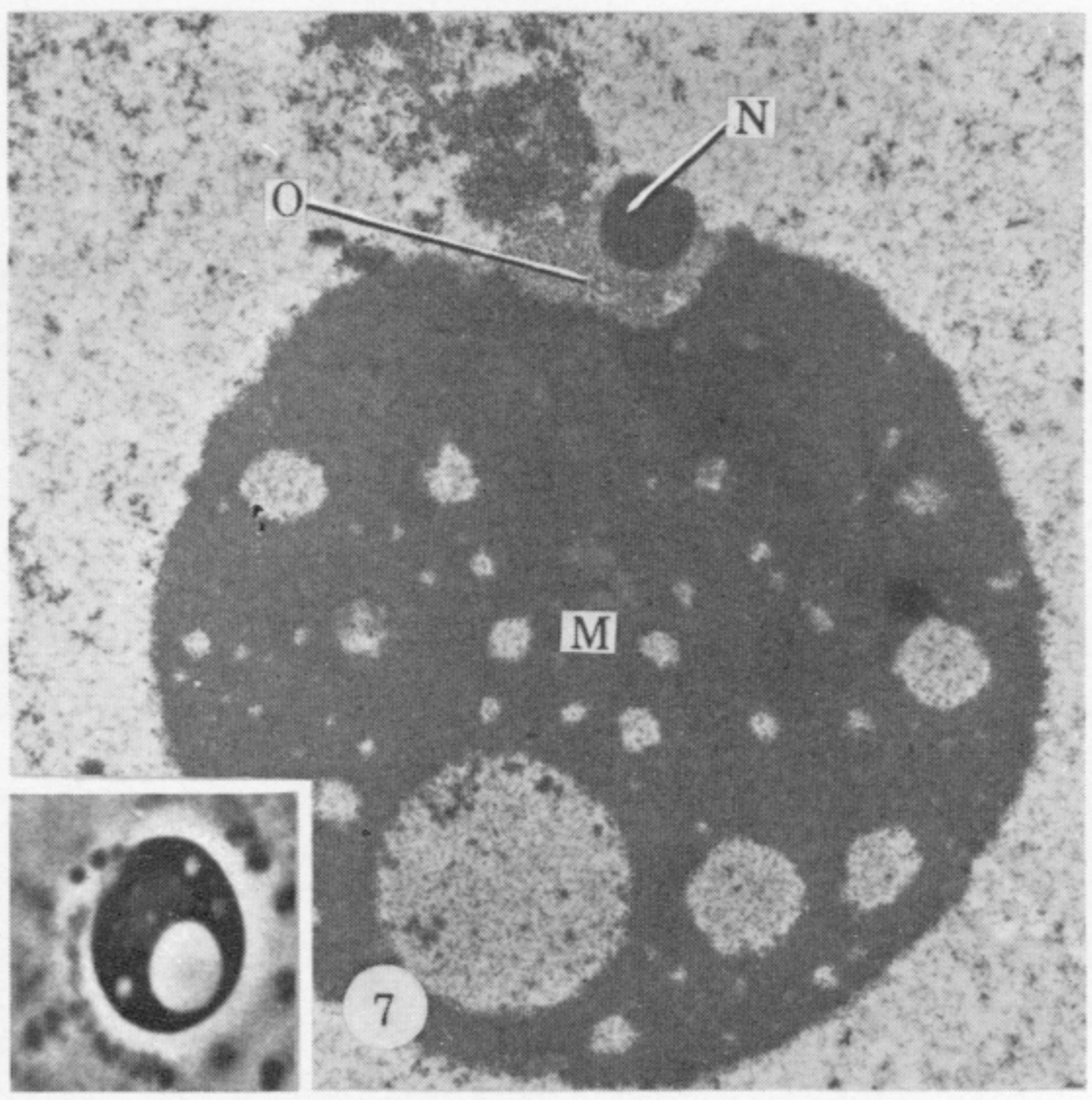
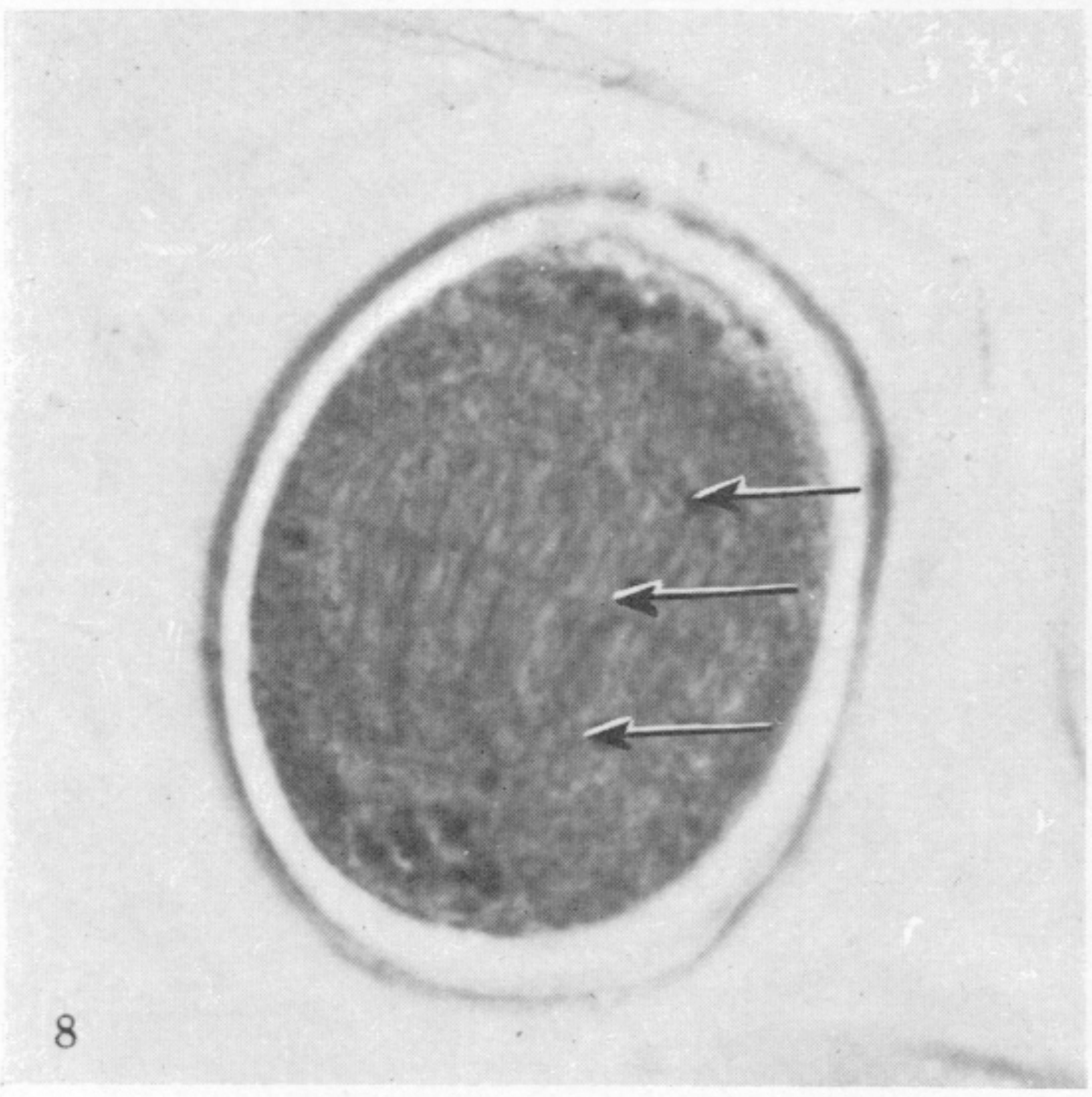
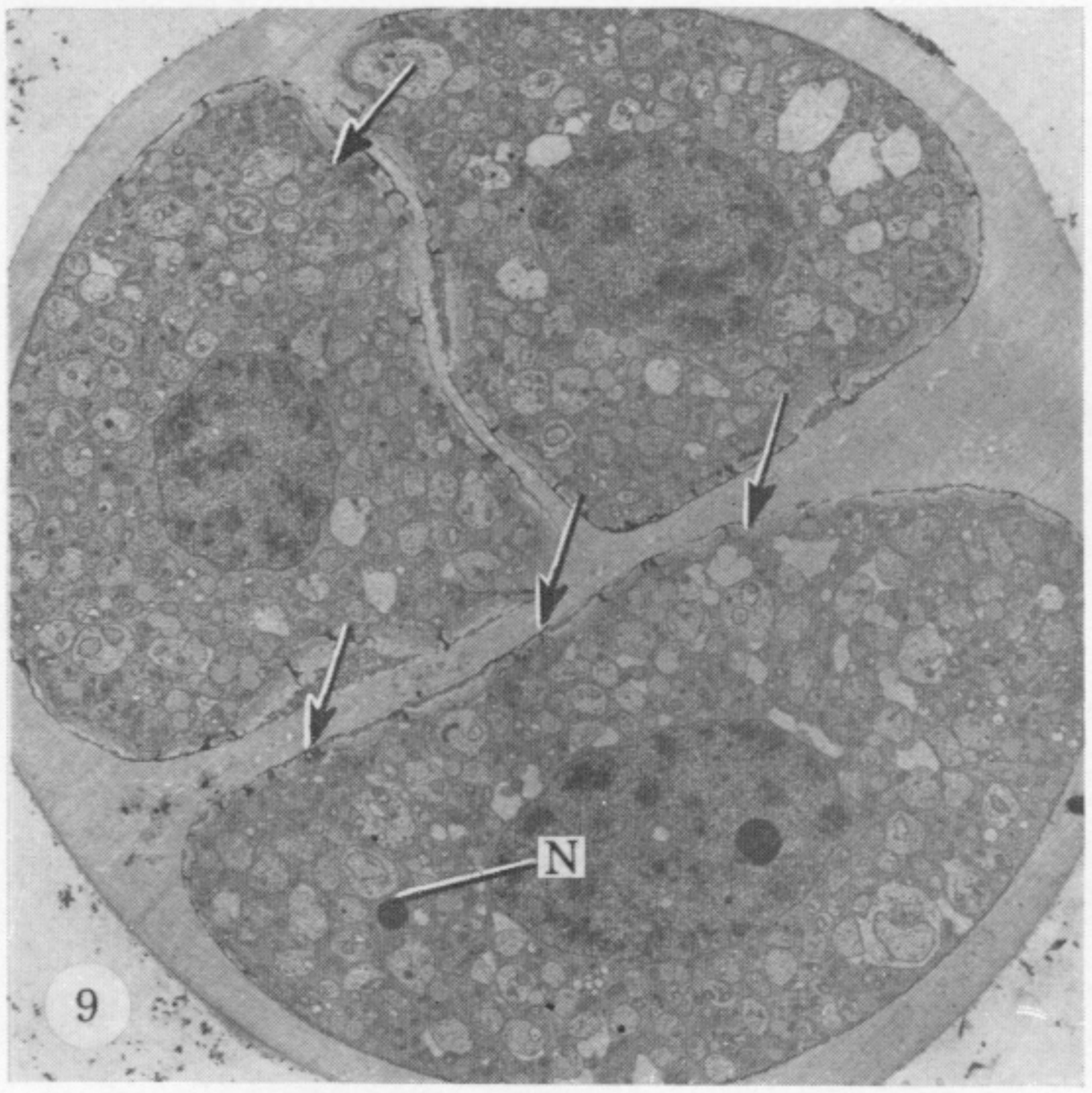




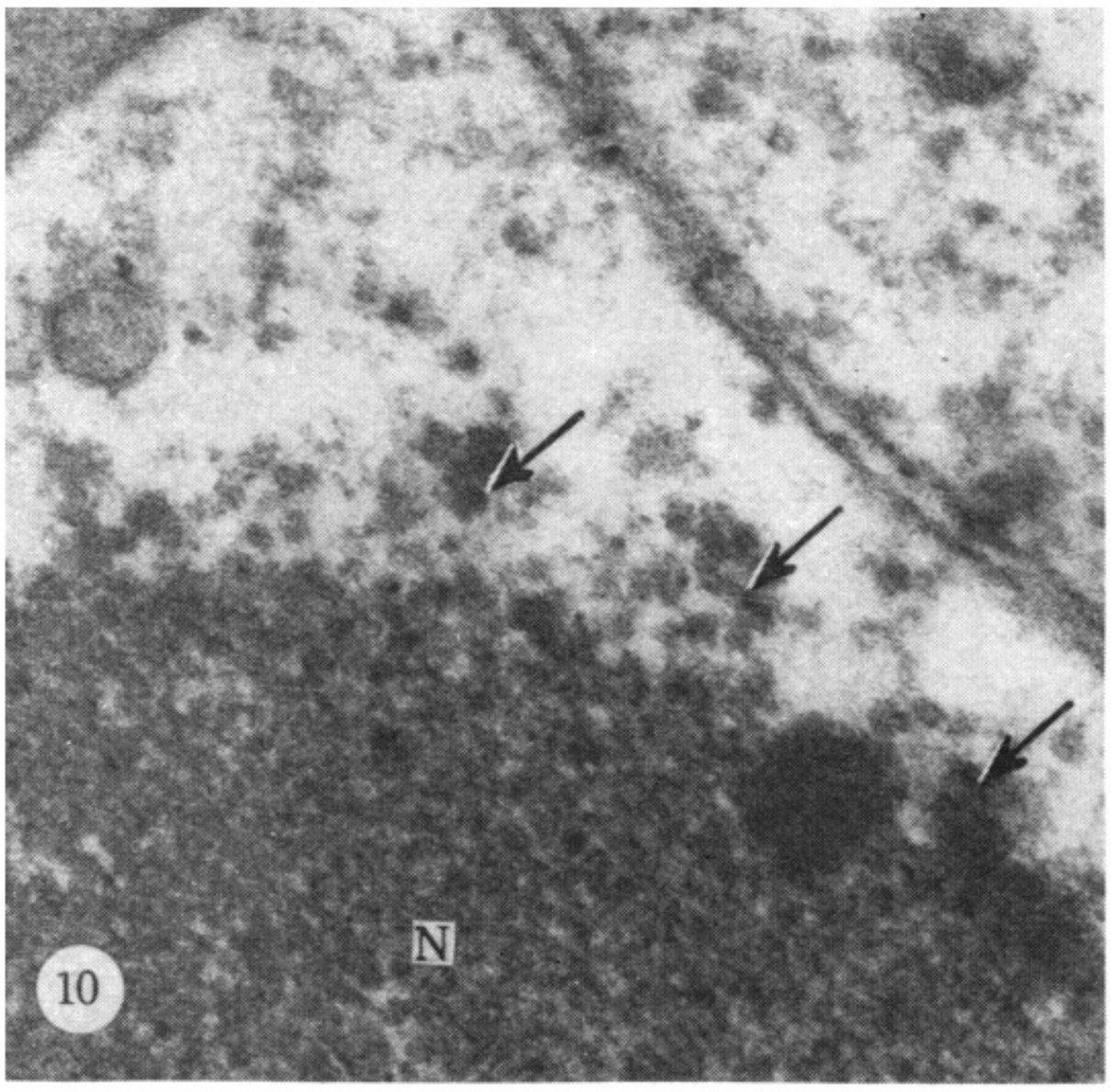


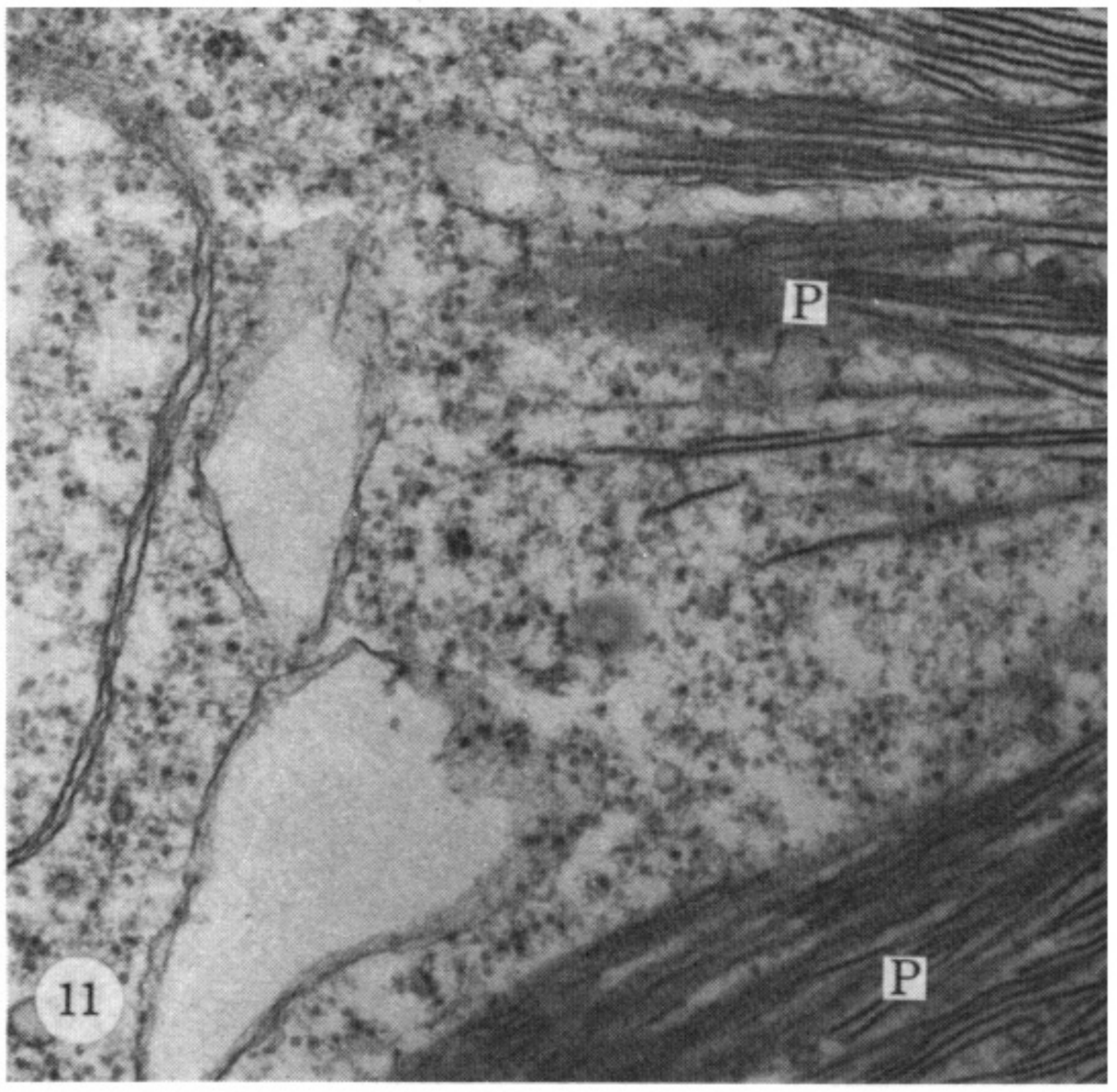


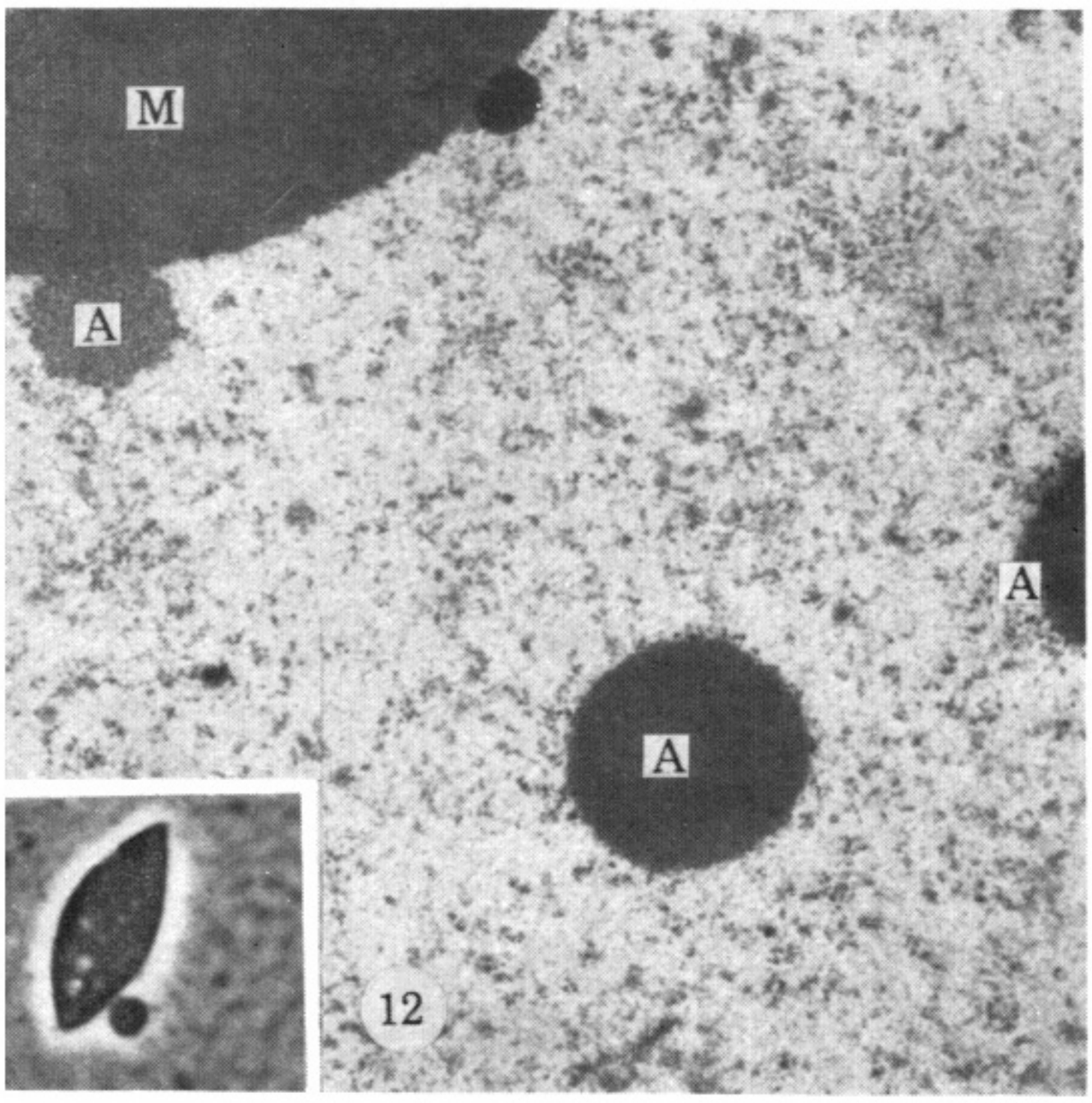


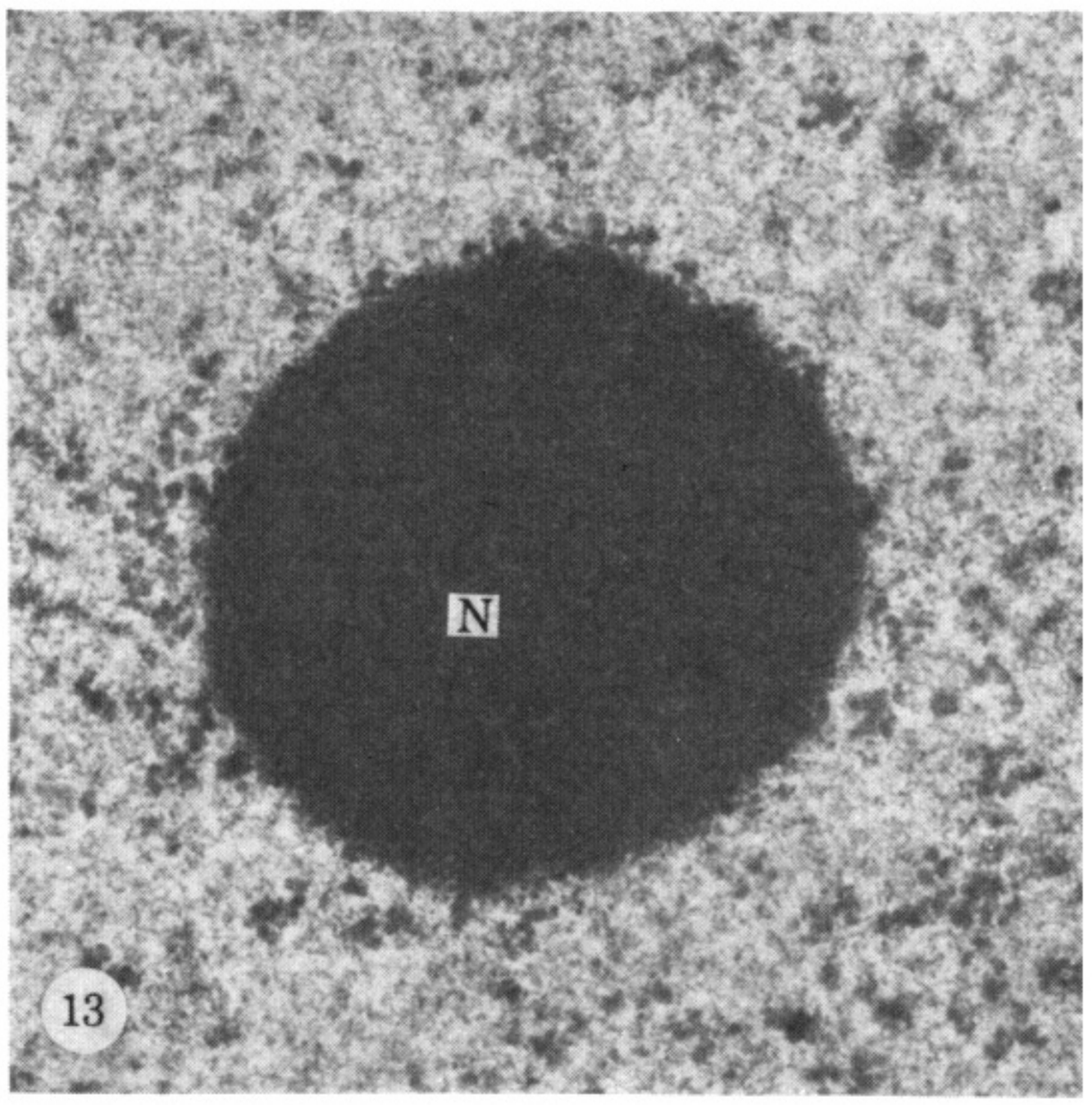


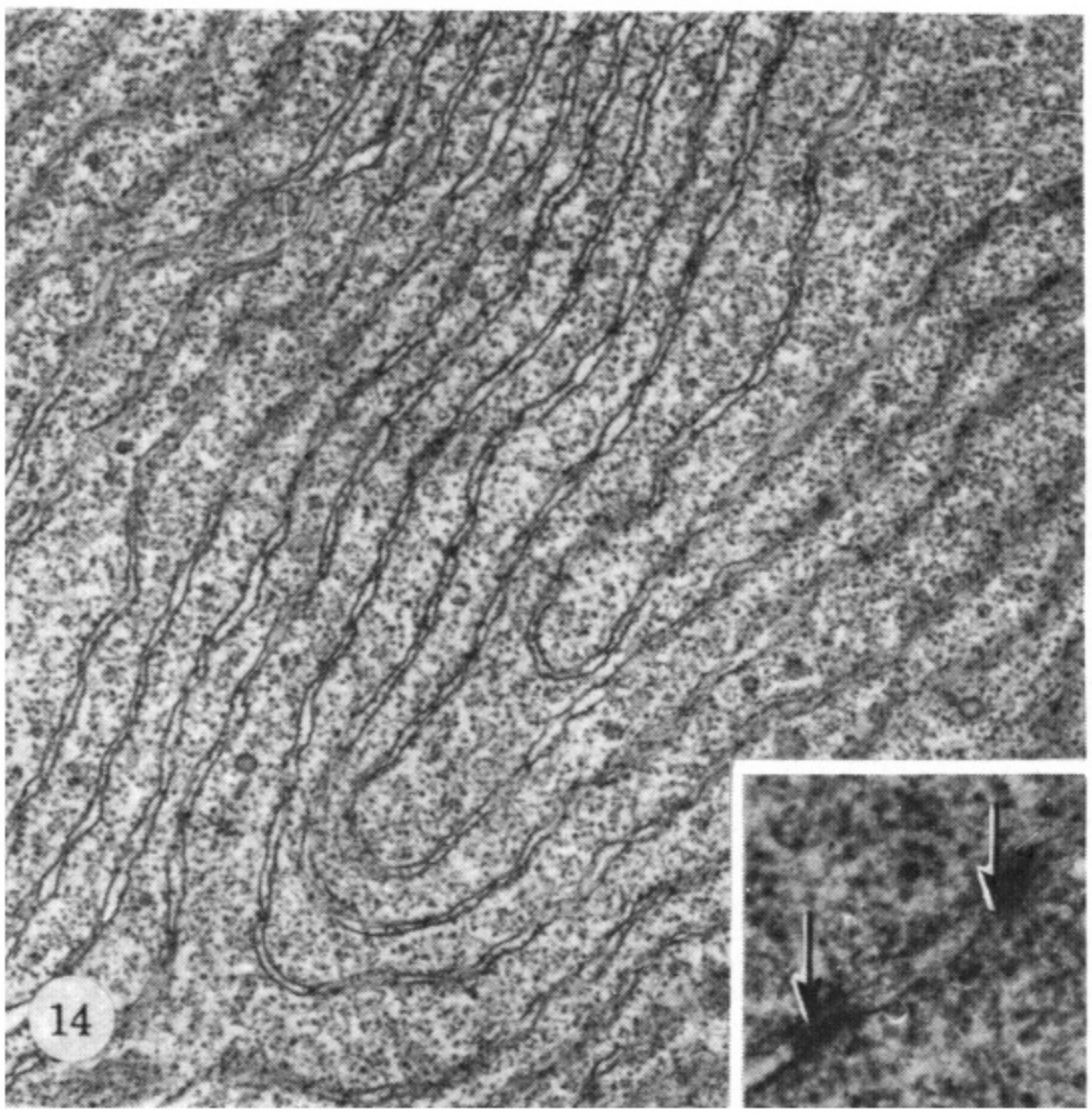
FIGURES 4–9. For description see opposite.

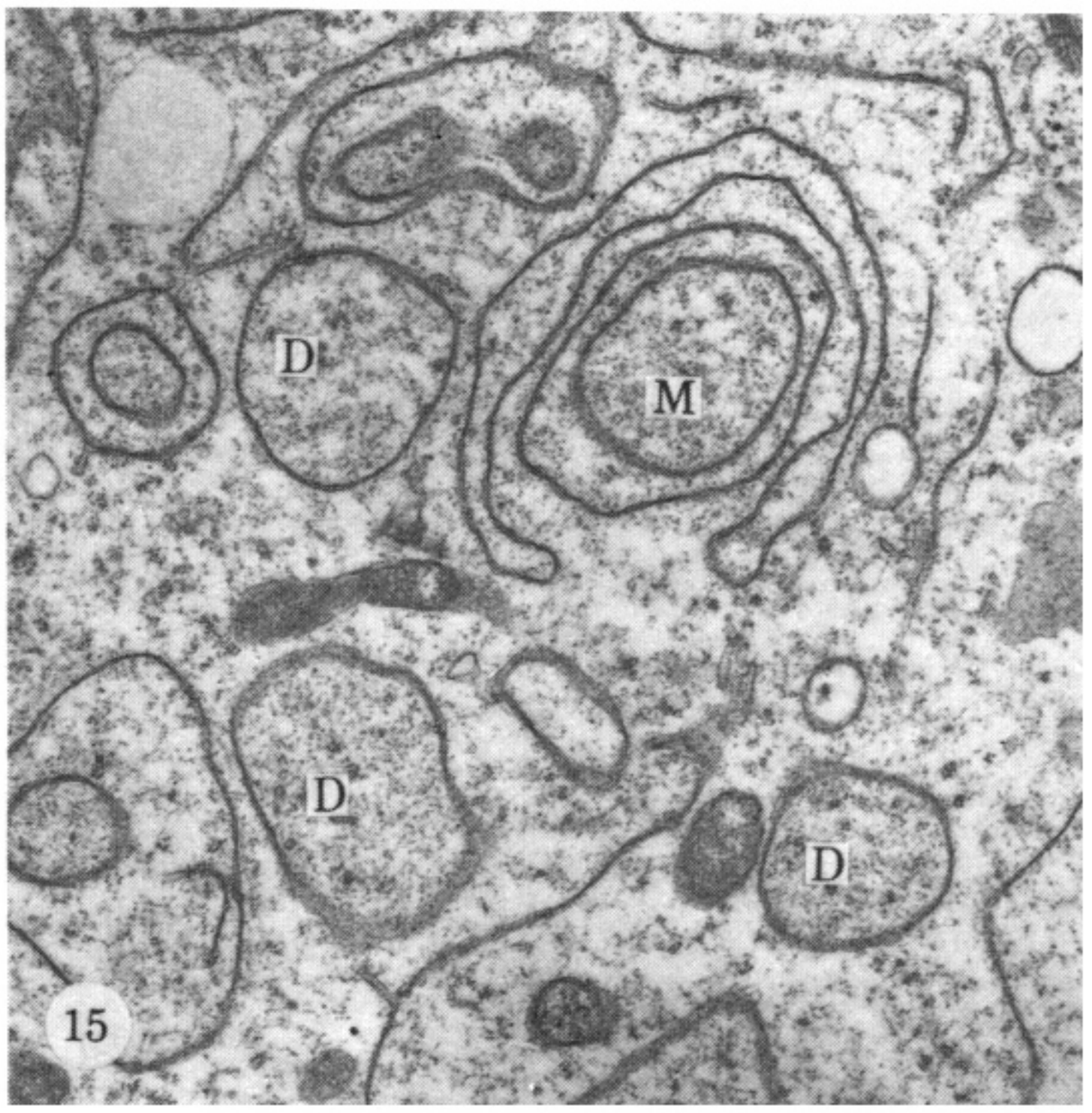




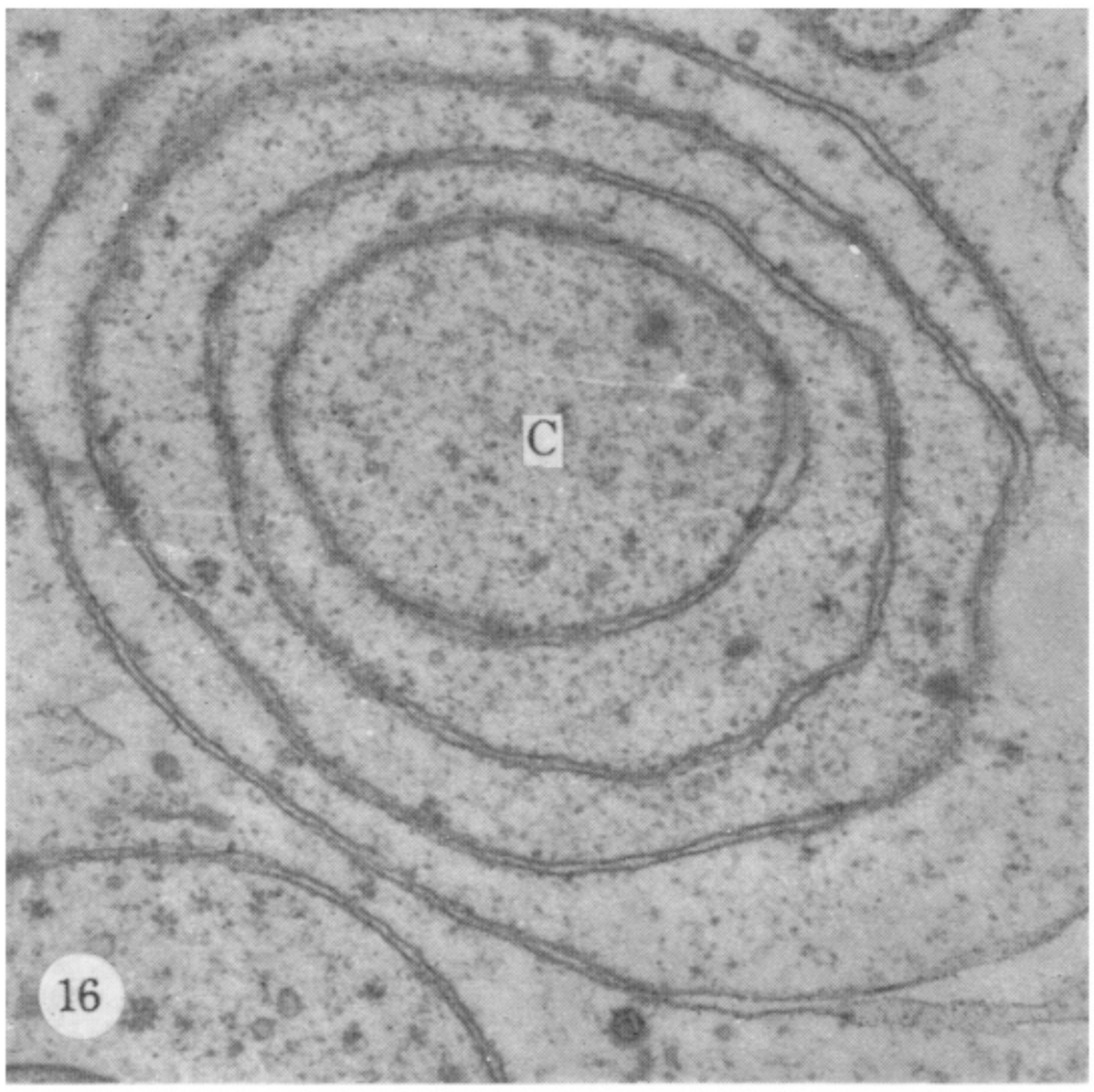


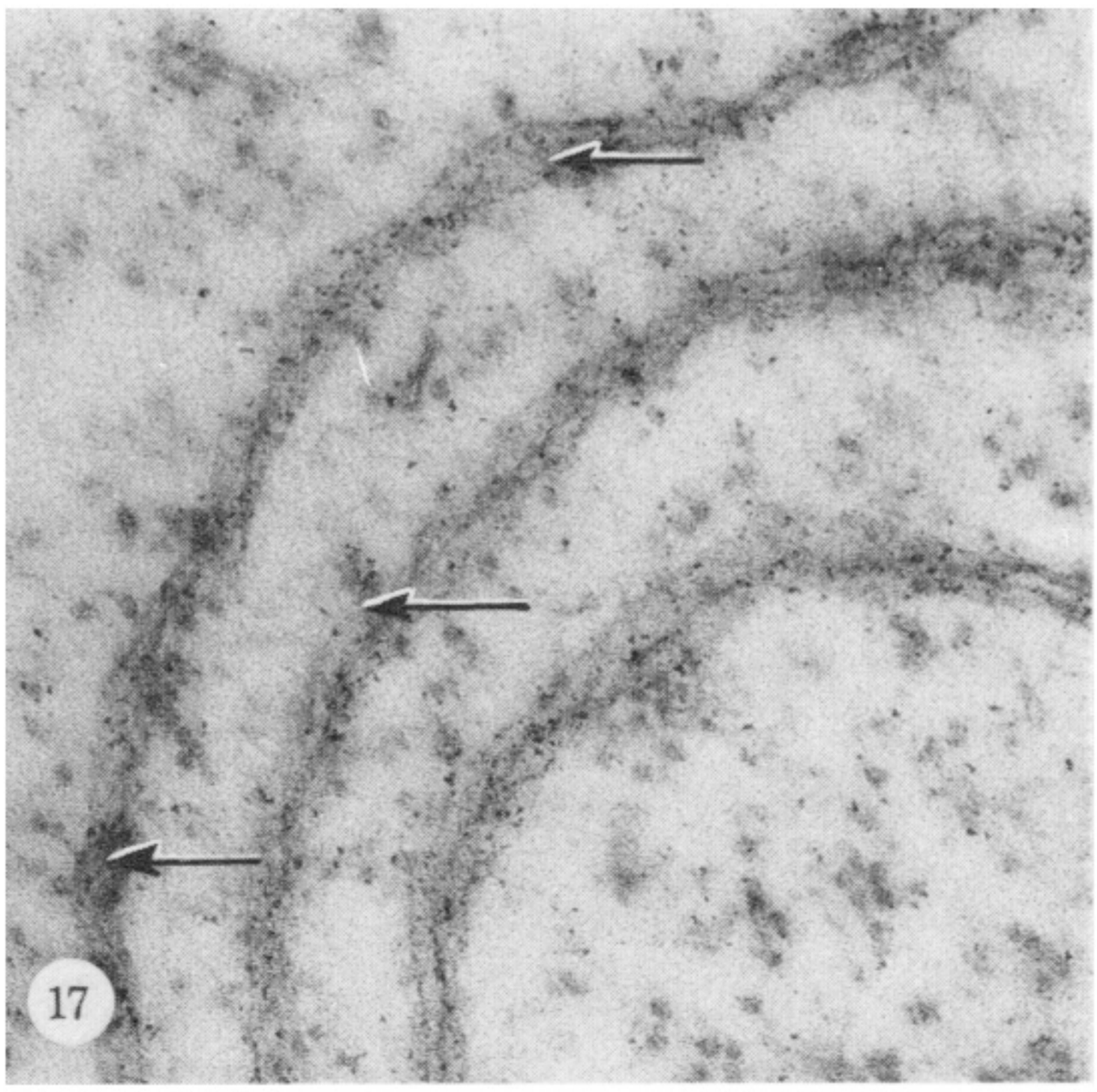


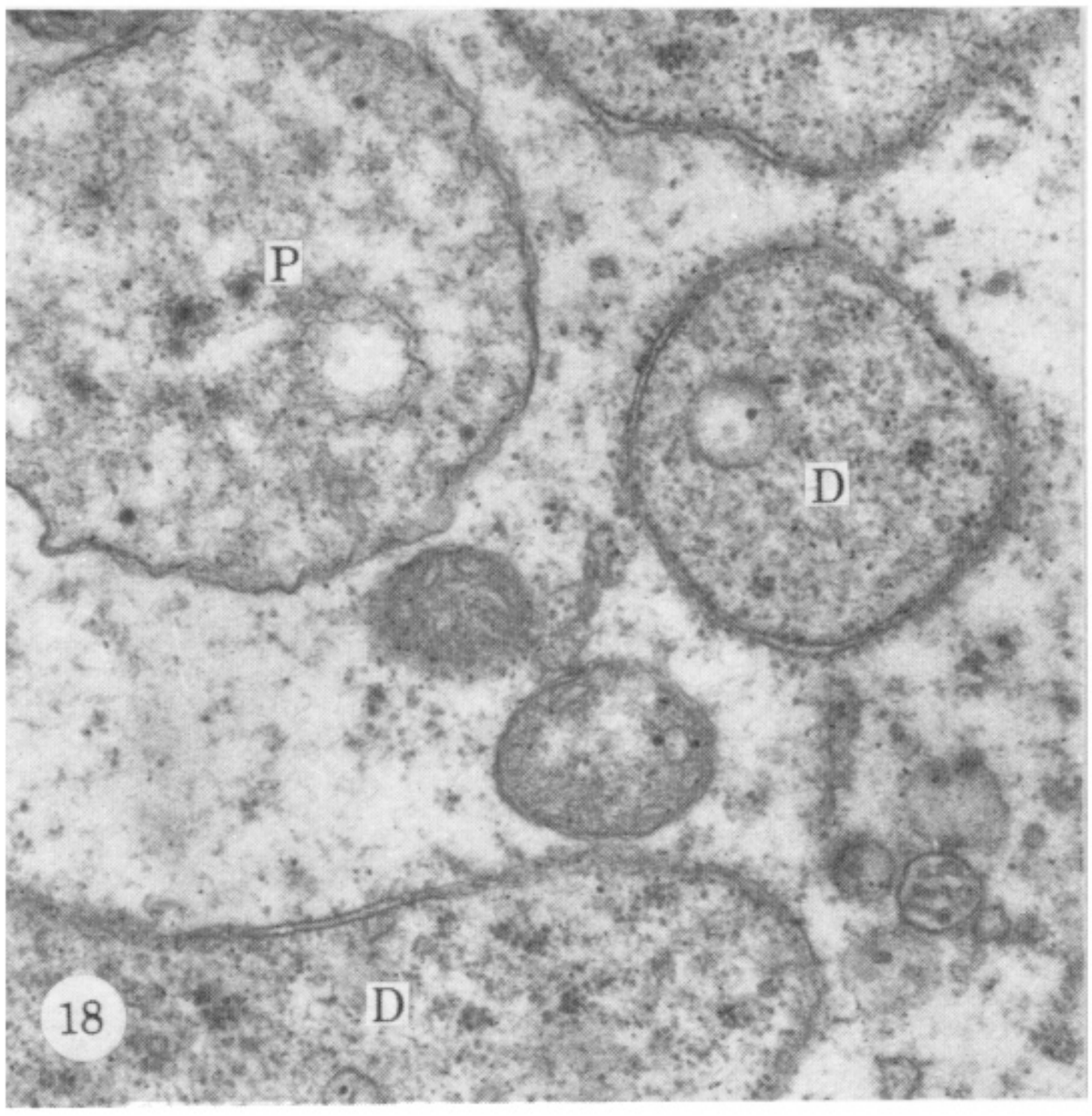


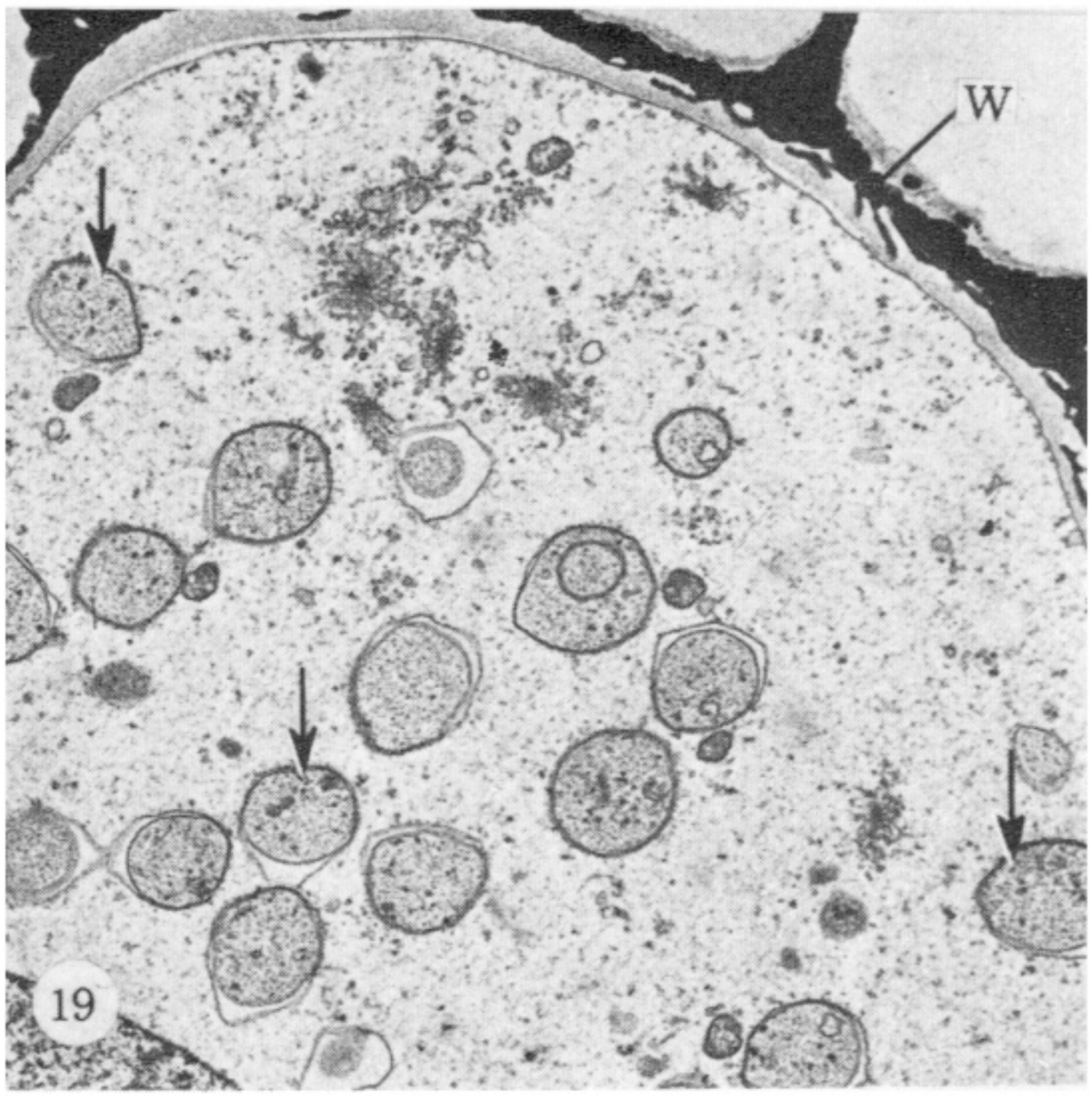


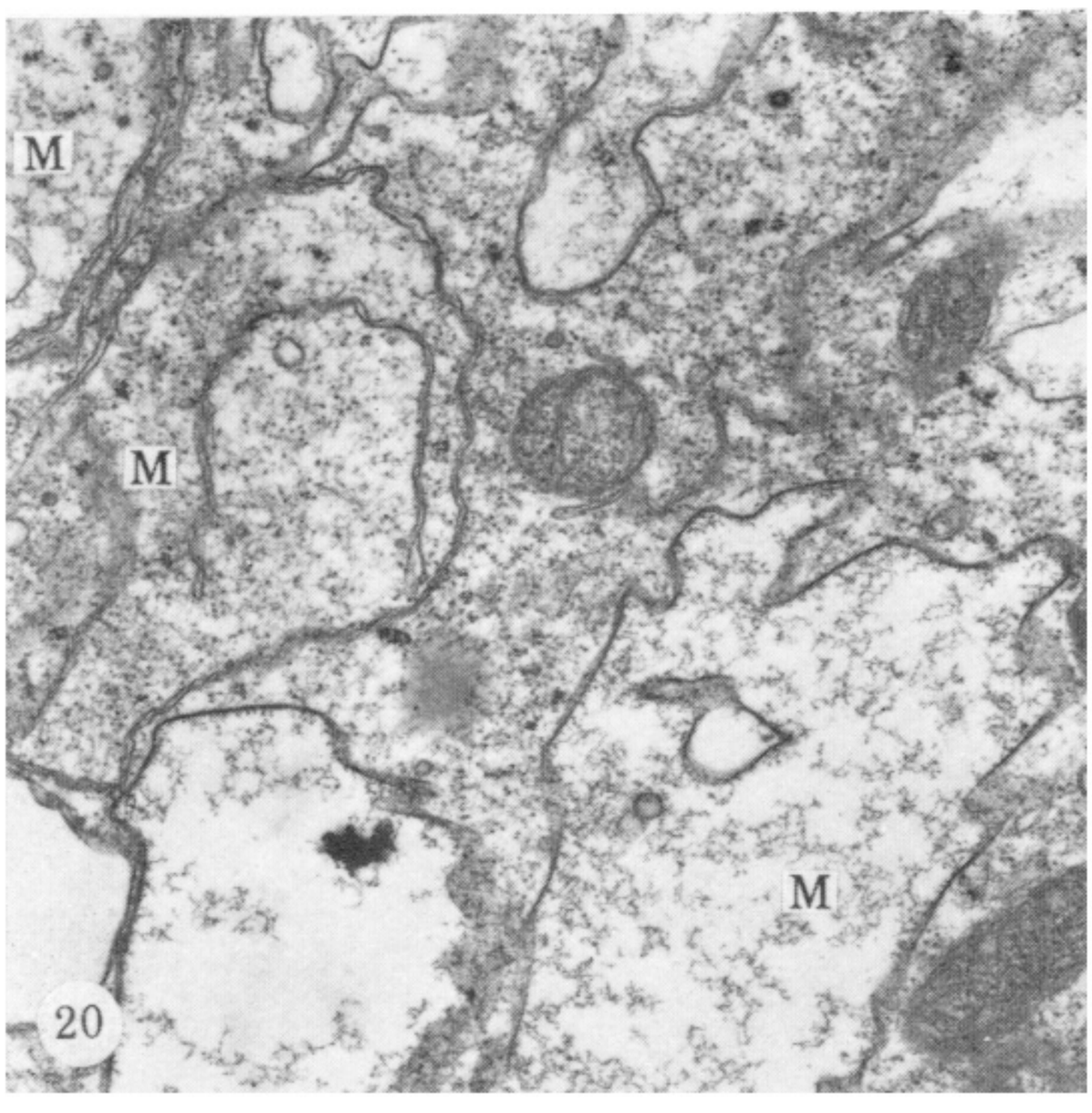
FIGURES 10–15. For description see opposite.

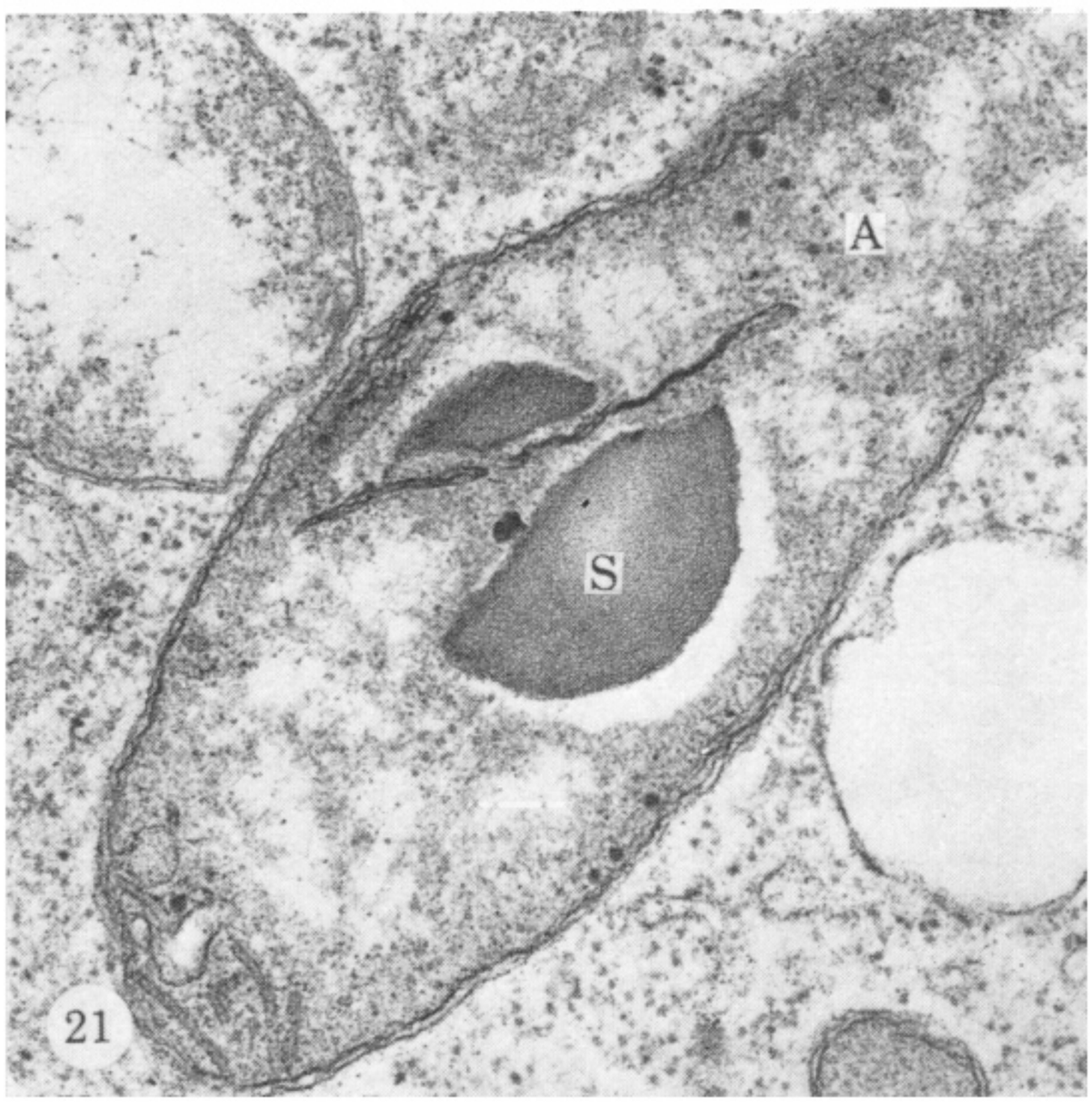




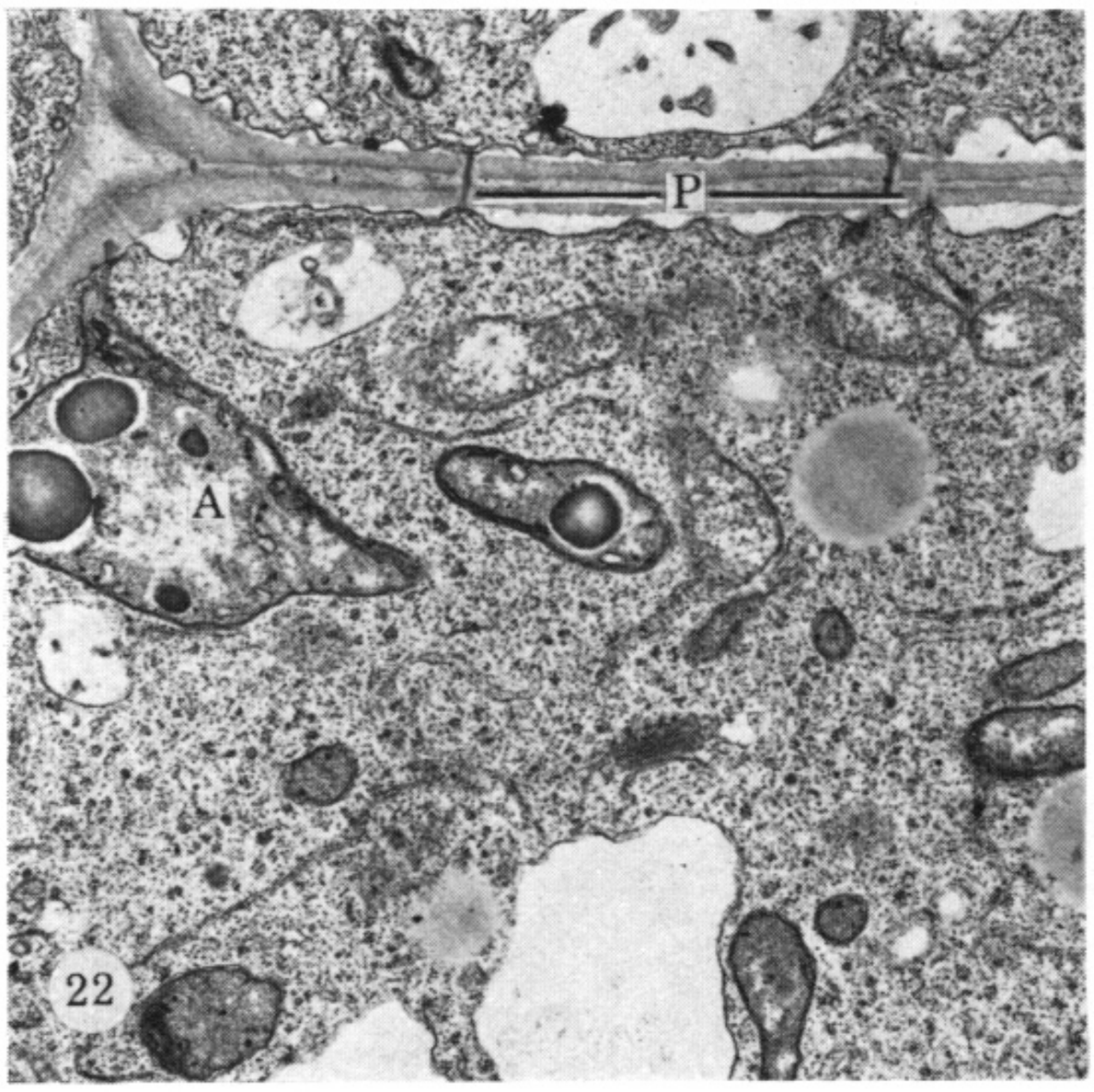




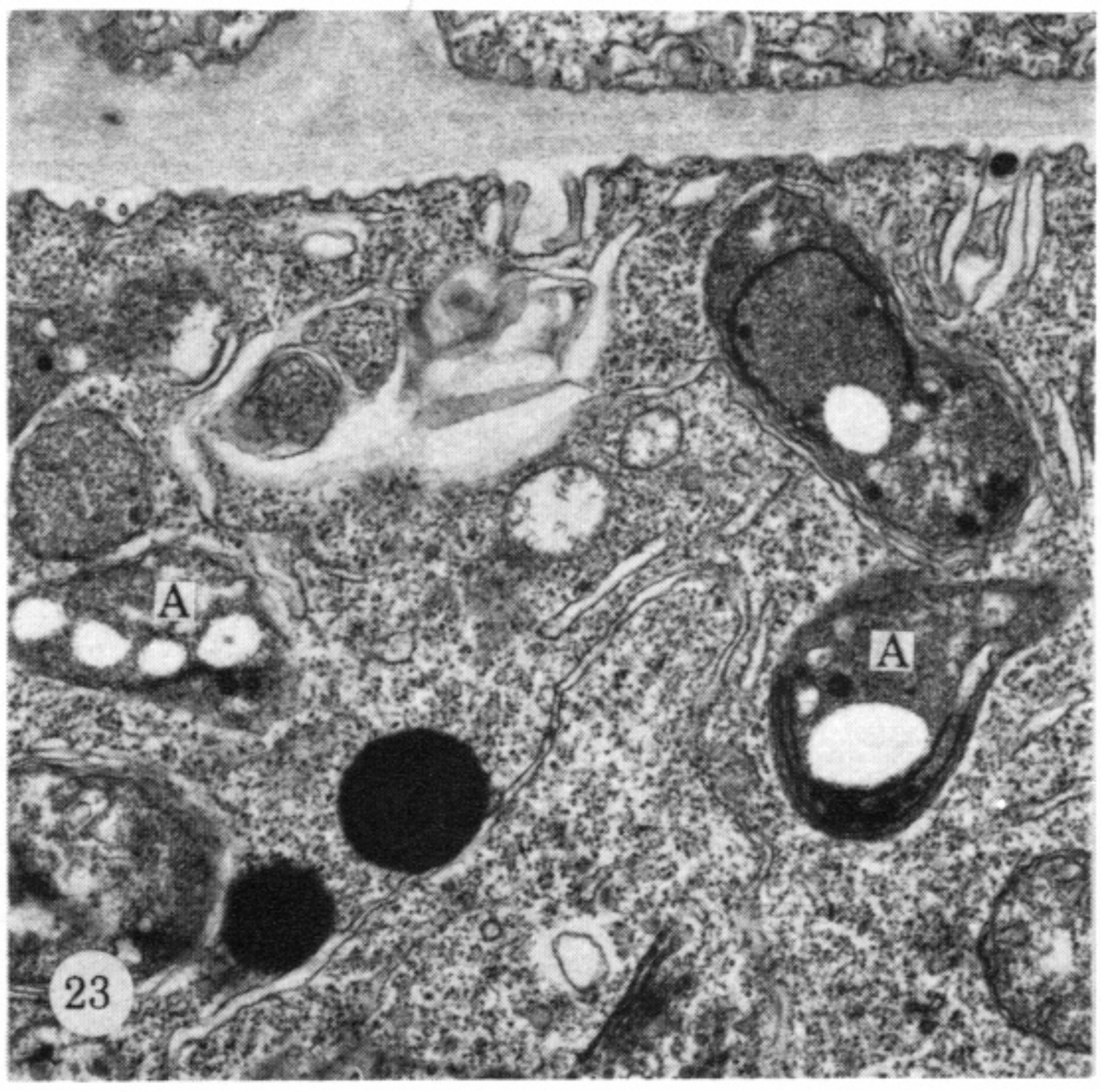




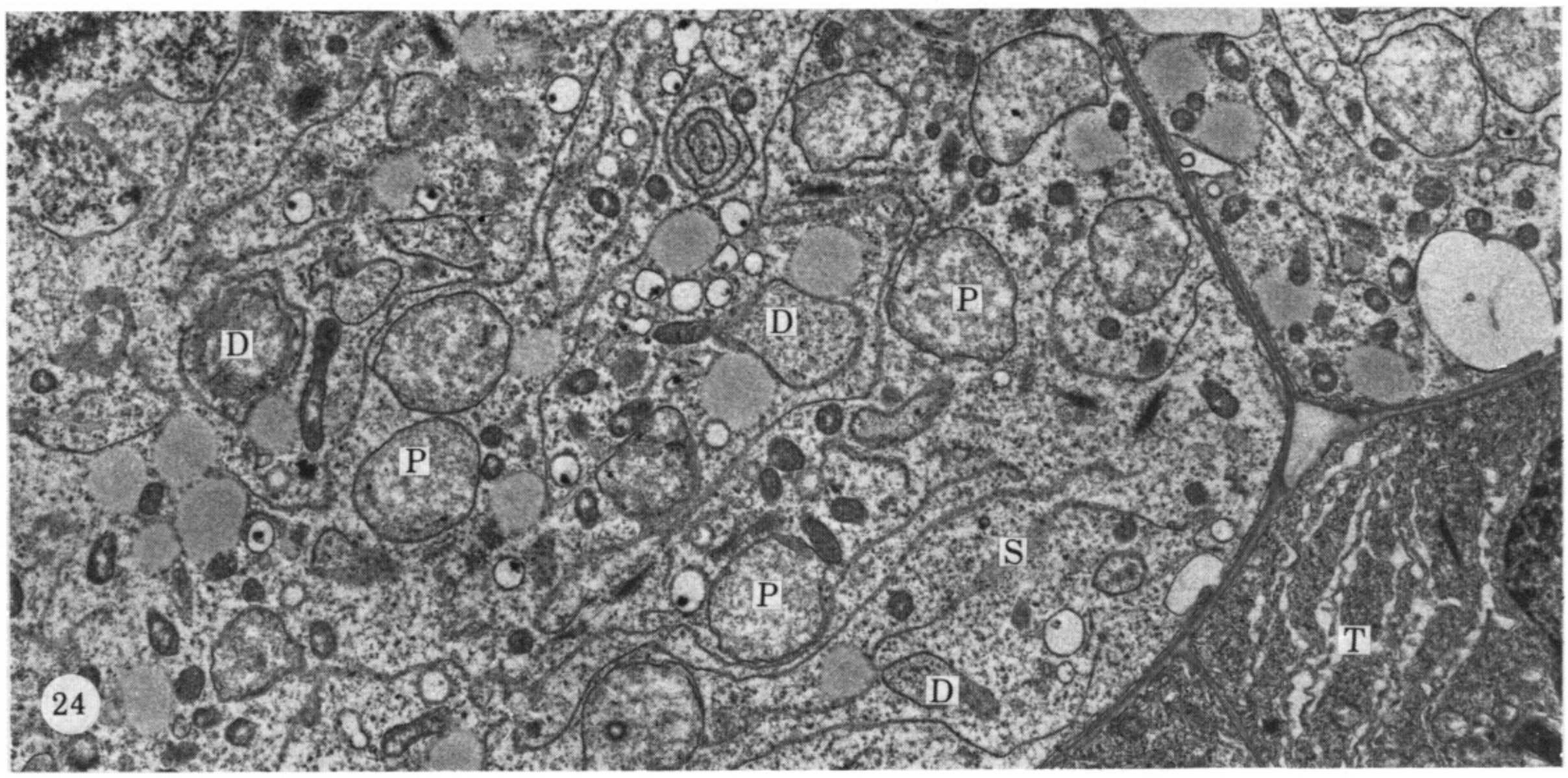
FIGURES 16–21. For description see opposite.



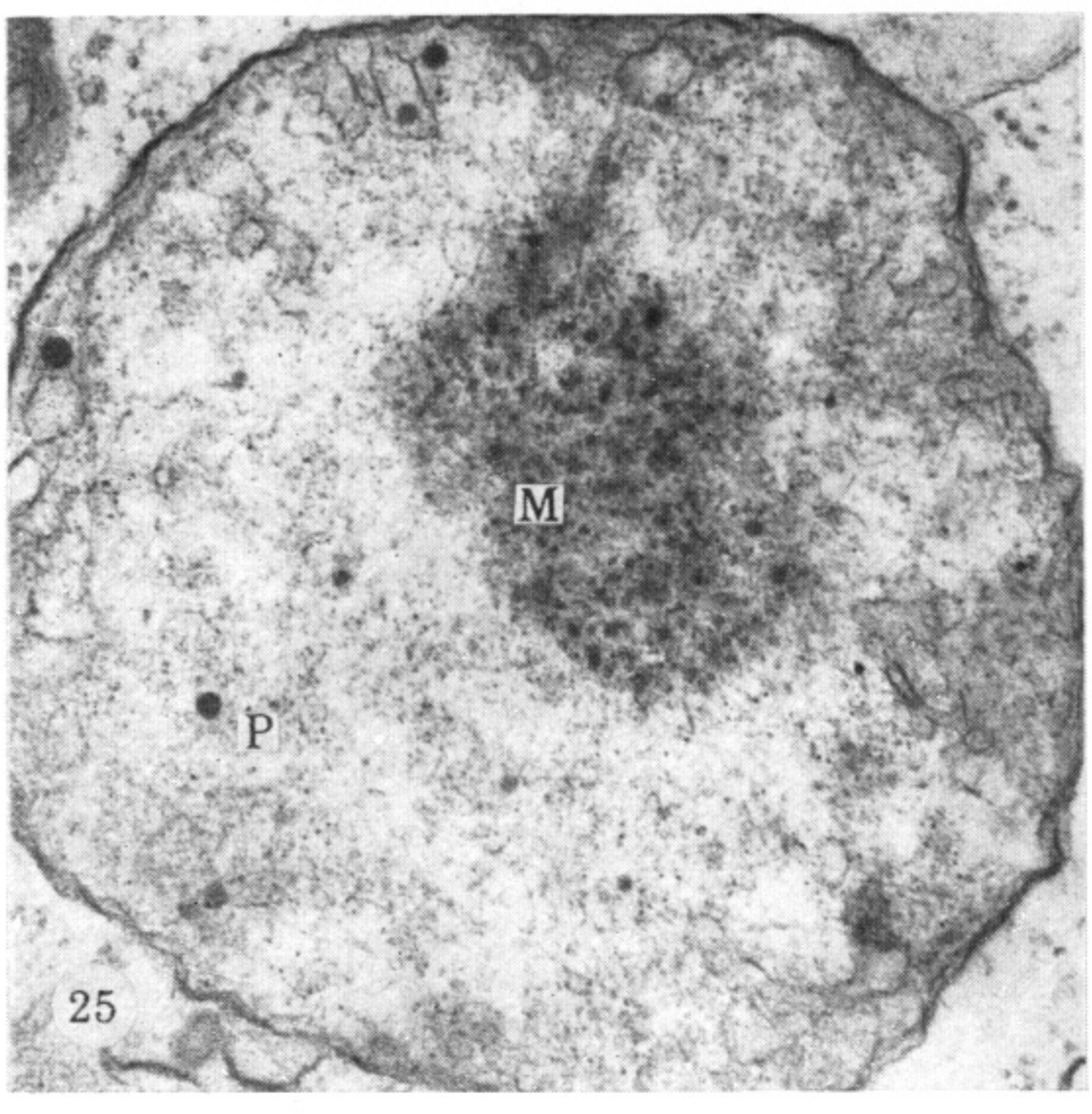
22



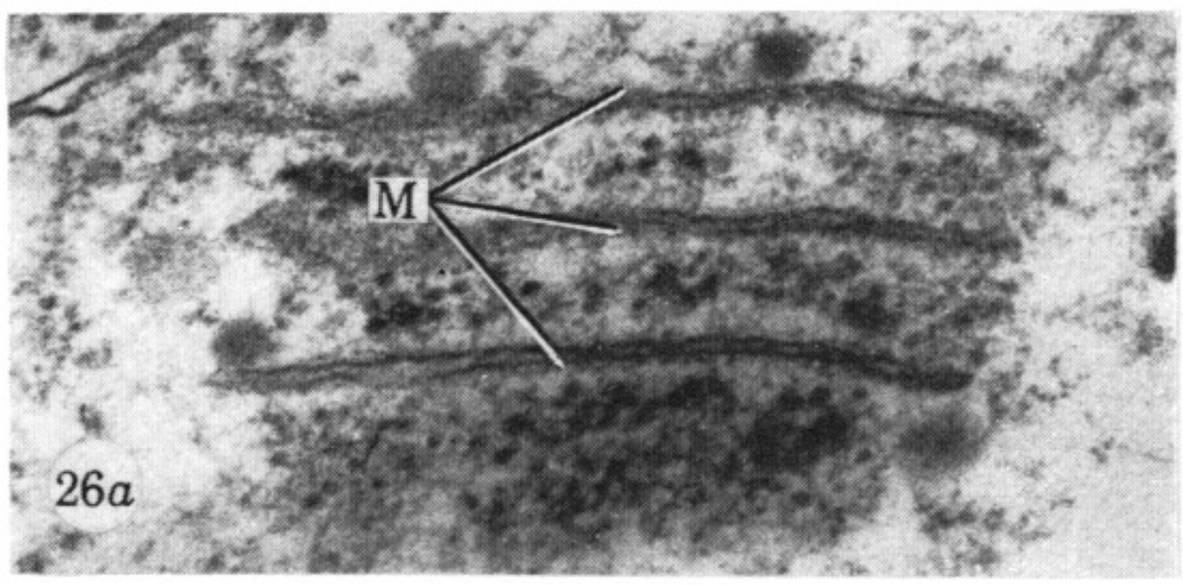
23



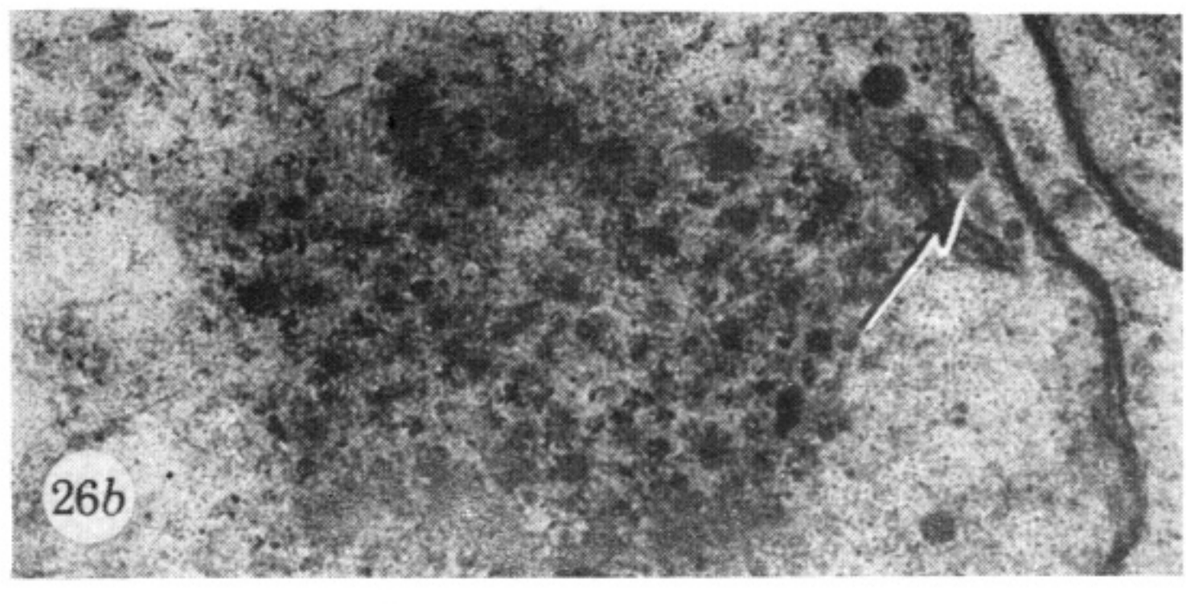
24



25

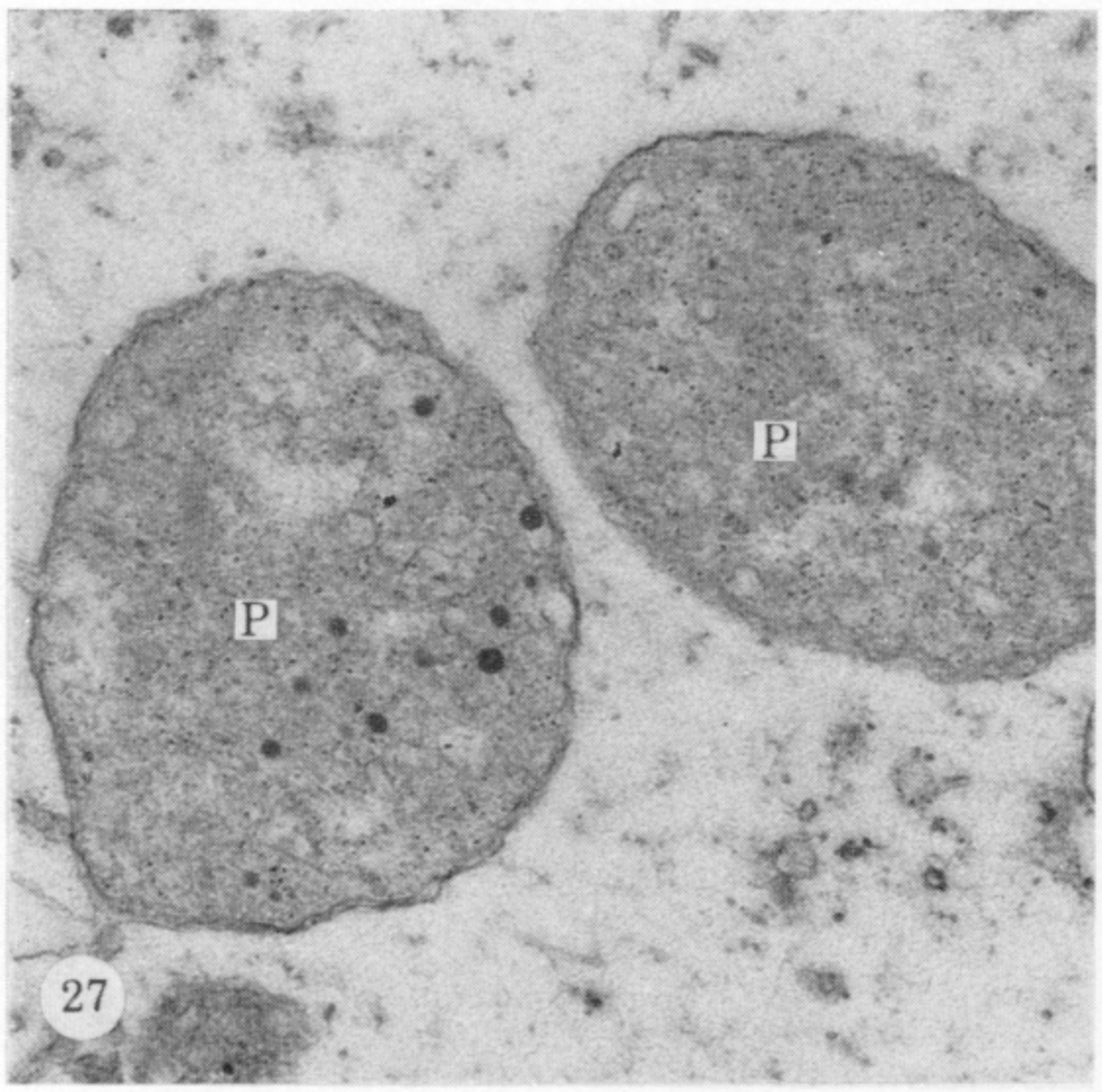


26*a*

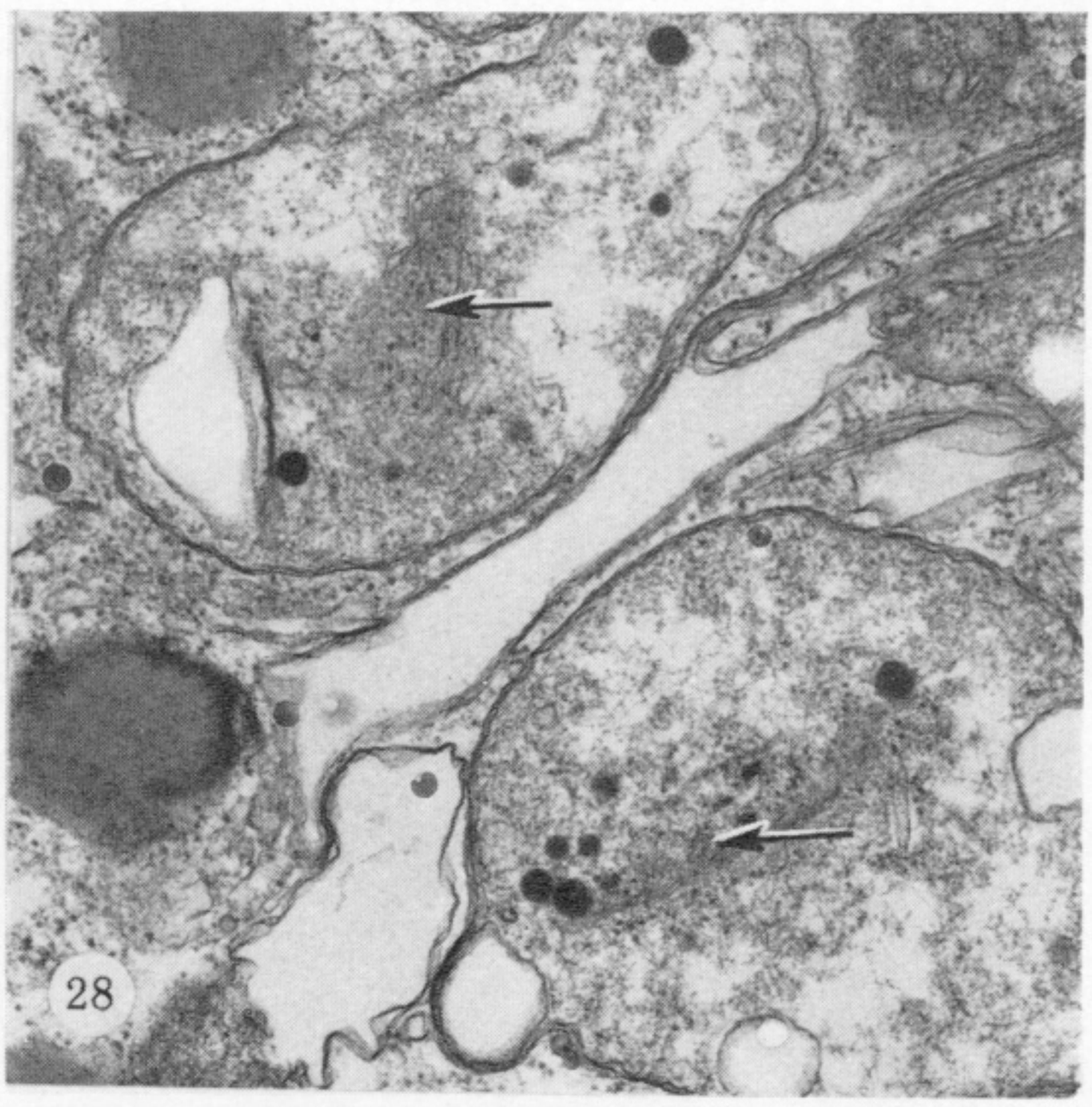


26*b*

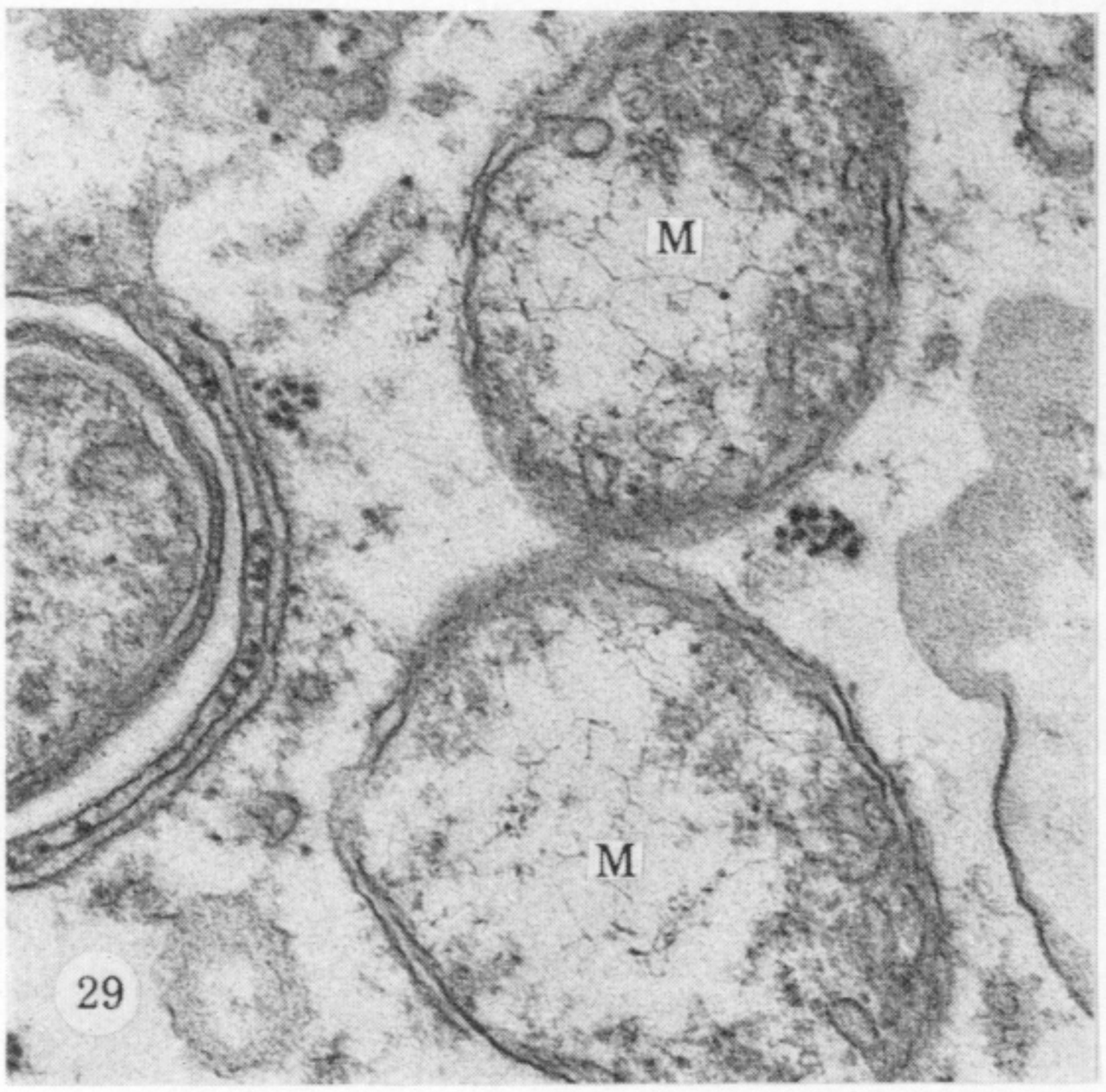
FIGURES 22–26. For description see opposite.



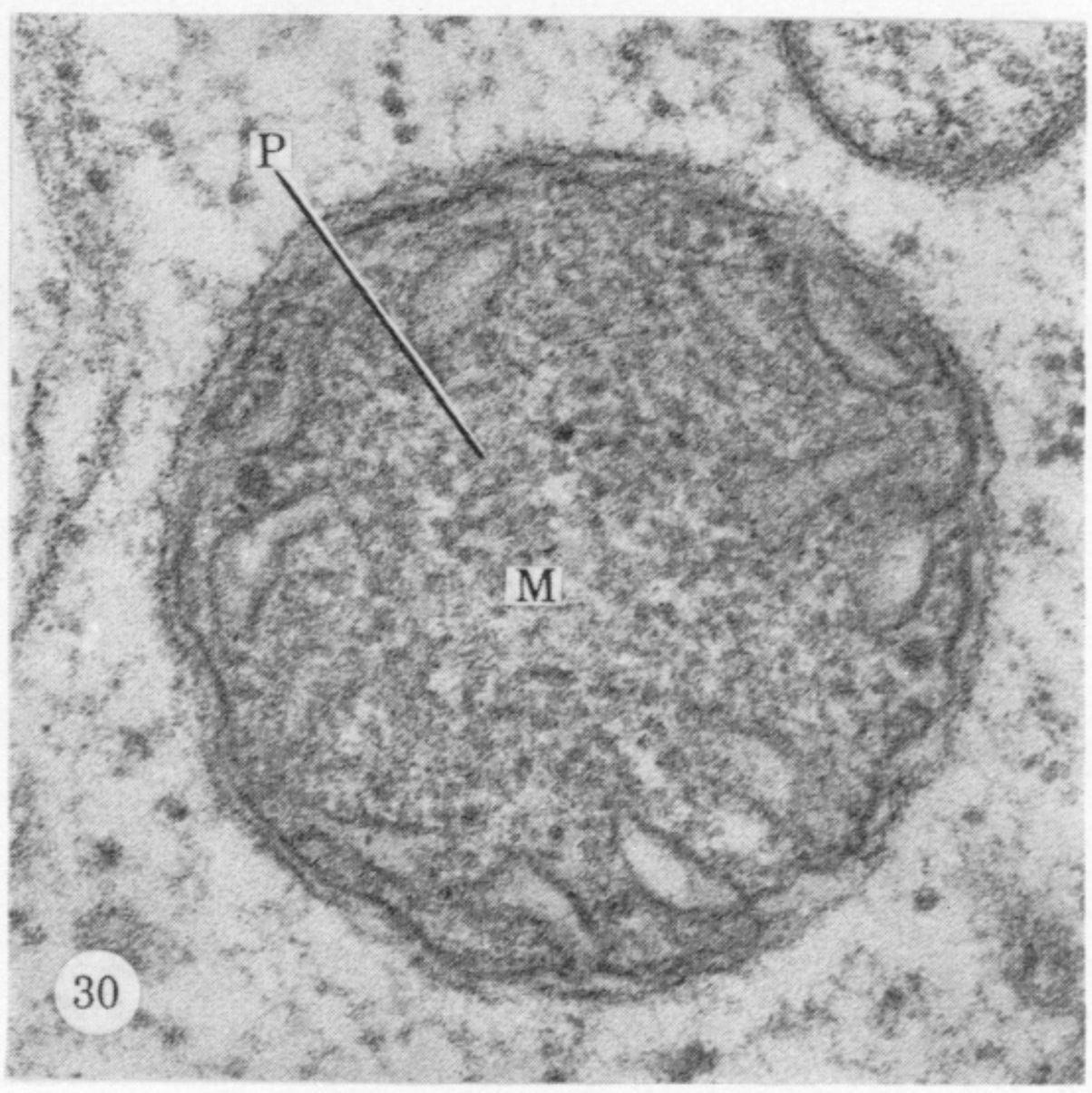
27



28



29



30

31

FIGURES 27–31. For description see opposite.