AN ULTRASTRUCTURAL STUDY OF THE MATURE SPERMATOZOID OF *EQUISETUM*

By J. G. DUCKETT† and P. R. BELL‡

† *School of Plant Biology, University College of North Wales, Bangor, Wales*

‡ *Department of Botany and Microbiology, University College London*

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The male gamete of *Equisetum* is the largest and structurally most complex of those so far known in living pteridophytes.

The ultrastructure of the mature gametes, is described with particular reference to the influence of the multilayered structure (MLS) on its form. In *Equisetum* this organelle comprises a band of over 300 microtubules, underlain along its anterior edge by a lamellar strip, 15–20 μm in length, and forming a sinistral spiral of $2\frac{1}{2}$ gyres. The tubules extend from the strip, at an angle of about 40°, to form a broad sheath around the twisted pyriform nucleus located in the posterior half of the cell. From the anterior tip of the lamellar strip to the posterior end of the nucleus the gamete completes a helix of $3\frac{1}{2}$ gyres, traversed throughout by the microtubular band. As a result of growth of this band during spermatid metamorphosis, and the 40° angle between the plates of the lamellar strip and the microtubules, the strip is displaced anteriorly and laterally relative to the nucleus. In the mature gamete, although the strip and the nucleus remain interconnected by the microtubular band, only the posterior half of the strip lies directly above the anterior third of the nucleus. The precise interrelationship between nucleus and MLS is illustrated by reconstructions which display the spermatozoids as they would appear if uncoiled.

The 80–120 flagella are inserted outside that part of the microtubular band lying anterior to the nucleus. Their basal bodies retain the proximal cartwheel and stellate transition regions found already in spermatids, but in the mature gametes they are invested with collars of osmiophilic material. The axonemes depart at 10° tangentially from the helix and extend backwards parallel with the tubules of the microtubular band. In consequence of the overlapping gyres of the helix the flagella lie in a spiral groove, similar to that found in cycad spermatozoids. From this groove the plasma membrane closely follows the external surface of the microtubular band.

Contrasting with other archegoniates, maximal structural differentiation of the MLS is found in the mature spermatozoid. Flat-bottomed keels are present on the microtubules overlying the lamellar strip in which three distinct strata can be recognized. The two outer, consisting of alternating plates of electron-opaque and electron-transparent material, are separated by a continuous electron-opaque sheet. The innermost stratum comprises a continuous layer of finely granular material. Overlying the external anterior rim of the microtubular band is an osmiophilic crest. This retains the regularly banded substructure found in spermatids, but in mature spermatozoids is far more prominent than at any other time during spermatogenesis. It contains an electron-transparent lumen and is continuous with both the anterior ends of the microtubules and the anteriormost lamellar plates. Between the inner gyres of the MLS the crest is confluent with extensive sheets of smooth endoplasmic reticulum.

Underlying the lamellar strip is a spiral mitochondrion with prominent dilated cristae. The central cytoplasm contains at least 100 pleomorphic mitochondria, together with from 15 to 25 amyloplasts and a few microbodies. In the nucleus, in addition to condensed chromatin, are several spherical electron-opaque bodies and aggregations of membrane-bound vesicles. Structures identical in appearance with the former also occur in the cytoplasm, and it is suggested that they may be nuclear in origin, as are similar bodies in animal spermatogenesis. The vesicles may represent portions of redundant nuclear envelope whose extrusion into the cytoplasm was prevented by the ensheathing microtubular band. Pores are still present in the nuclear envelope, where this is not invested by the band.

The mature spermatozoids are liberated from antheridia within mucilaginous sacs bounded by fibrillar cell wall material, thought to contain lipid droplets promoting their dispersal when in contact with water. On escaping from the sacs the spermatozoids elongate slightly, and profiles of disrupted flagella are frequently encountered. Occasionally the microtubular band ensheathing the posterior part of the nucleus also becomes disorganized. There is no evidence of the utilization of amyloplast starch as an energy source during motility, and, in contrast to ferns and bryophytes, there is no sequestration of the central cytoplasm by the swimming spermatozoids.

Introduction

The motile male gametes of archegoniate plants all display extreme cellular asymmetry. Whether there are 2 flagella (bryophytes, *Lycopodium* and *Selaginella*) or about 40 (homosporous ferns), 80 (*Marsilea*), 120 (*Equisetum*), or 10000 (cycads), the spermatozoids are invariably coiled in a helix which is sinistral when viewed from the anterior (Duckett 1975*a*). The subcellular basis for this remarkable morphology, seen elsewhere in the Plant Kingdom only in the spermatozoids and zoospores of a few green algae (Graham & McBride 1975; Moestrup 1974; Pickett-Heaps 1975; Pickett-Heaps & Marchant 1972), lies in the development and geometry of the multilayered structure (MLS). This organelle, now identified as a characteristic feature of spermatogenesis in all the major phyla of the Archegoniatae (table 1) consists basically, of a band of regularly spaced microtubules, underlain along its anterior edge by a lamellar strip consisting of alternating plates of electron-opaque and electron-transparent material. The coiled form of the gametes, and in particular their left-handedness, orginates from the geometrical relationship between the microtubular band and the lamellar strip. If the whole MLS is envisaged as unrolled, with the microtubules uppermost, then, viewed from the anterior ends of the lamellae, the microtubules depart to the posterior from the right of the strip. Since the angle between the alignment of the microtubules and that of the lamellae is always acute (table 1) the coiling, with the lamellar strip on the inside, is necessarily sinistral.

Recent studies on ferns (Bell 1974) and mosses (Lal & Bell 1975) have shown that the MLS originates in the young spermatid within the matrix of the blepharoplast, almost simultaneously with the transformation of the pro-centrioles into centrioles. Its subsequent association with the nucleus, when the posterior edge of the lamellar strip touches the nuclear envelope, and the microtubules extend beyond this posterior edge, has been well documented in bryophytes (Carothers 1973, 1975; Duckett 1975*b*; Lal & Bell 1975; Paolillo, Kreitner & Reighard 1968*a*; Suire 1970), ferns (Duckett 1975*a*), and *Equisetum* (Duckett 1973*a*). The metamorphosis of the spermatids, involving elongation and spiralization of the nucleus, together with the displacement of the lamellar strip both laterally and anteriorly relative to it, has also been described in these archegoniates. However, at the present time, detailed accounts of the mature male gametes exist only for bryophytes, *Pteridium*, *Marsilea*, and cycads (for references see table 1). This paper presents a detailed account of the cytology of the mature spermatozoids of *Equisetum*, the largest and structurally the most complex of all pteridophyte male gametes examined hitherto. The relationship between the MLS and the nucleus is illustrated by reconstructions based on a quantitative study of over 2000 individual spermatozoids. A detailed analysis of spermatid metamorphosis may be found in Duckett (1973*a*).

Materials and methods

Gametophytes of *Equisetum hyemale* L. and *E. telmateia* Ehrh. were grown in axenic single-spore cultures on a modified Knop medium solidified with 1.5 % agar. Details of the general culture techniques are described elsewhere (Duckett 1970*a*, *b*, 1972).

For observations on unliberated mature spermatocytes, tips of actively growing male branches, containing antheridia in various stages of development, were fixed in 1 or 2 % glutaraldehyde (Taab Laboratories, Reading, U.K.) in 0.05 M phosphate or cacodylate buffer (pH 6.9) for 1–2 h at room temperature. Following repeated washing in buffer, the material was

postfixed for 2 h at 4 °C in 2 % osmium tetroxide, dehydrated in acetone, and embedded in either Durcupan ACM (Fluka AG, Switzerland) or Epikote (Shell, U.K.).

Male gametophytes were also submerged in distilled water for 15–30 min, after which the resulting spermatozoid suspension was fixed in 1 % glutaraldehyde in 0.05 M cacodylate buffer for ½–1 h at room temperature. The fixed gametes were centrifuged at 5000 rev/min for 2 min, the resulting pellet rinsed several times in buffer, and then embedded in 4 % agar. Subsequent osmication, dehydration, and embedding were the same as for the antheridial branches.

Fine sections, cut with diamond knife, were stained for 30 min each with saturated uranyl acetate and basic lead citrate (Reynolds 1963), and examined in a Siemens Elmiskop I or an A.E.I. 6G electron microscope.

Descriptive terminology follows that previously used by Duckett (1973*a*, 1975*b*). In both the figure captions and the text the term longitudinal refers to the axis of the spermatozoid helix. Planes containing this axis will cut the lamellar strip perpendicular to its plates. The individual plates are then clearly visible, while the microtubules, with their axes at 40° to the plates, are sectioned obliquely and thus present oval profiles. Sections which reveal perfectly circular transverse profiles of the microtubules, but in which individual lamellar plates cannot be clearly distinguished, are consequently inclined at about 40° to the axis of the helix.

Observations

(*a*) *The gross relationship between the multilayered structure and the nucleus*

Metamorphosis of the spermatids of *Equisetum* involves highly integrated spatial rearrangements of the MLS, consisting of a lamellar strip 15–20 μm in length and over 300 microtubules, and the nucleus. In the spermatids the lamellar strip embraces about two thirds of the periphery of the initially spherical nucleus, with one edge touching the envelope and the other free in the cytoplasm. The nucleus then elongates and simultaneously coils in contact with the continually lengthening microtubular band. At maturity the tubules form a sheath around the external surface of the nucleus lying at the posterior end of the cell. Anteriorly there is a separation of the lamellar strip beyond the tip of the nucleus. Thus, nucleus and strip remain inter-

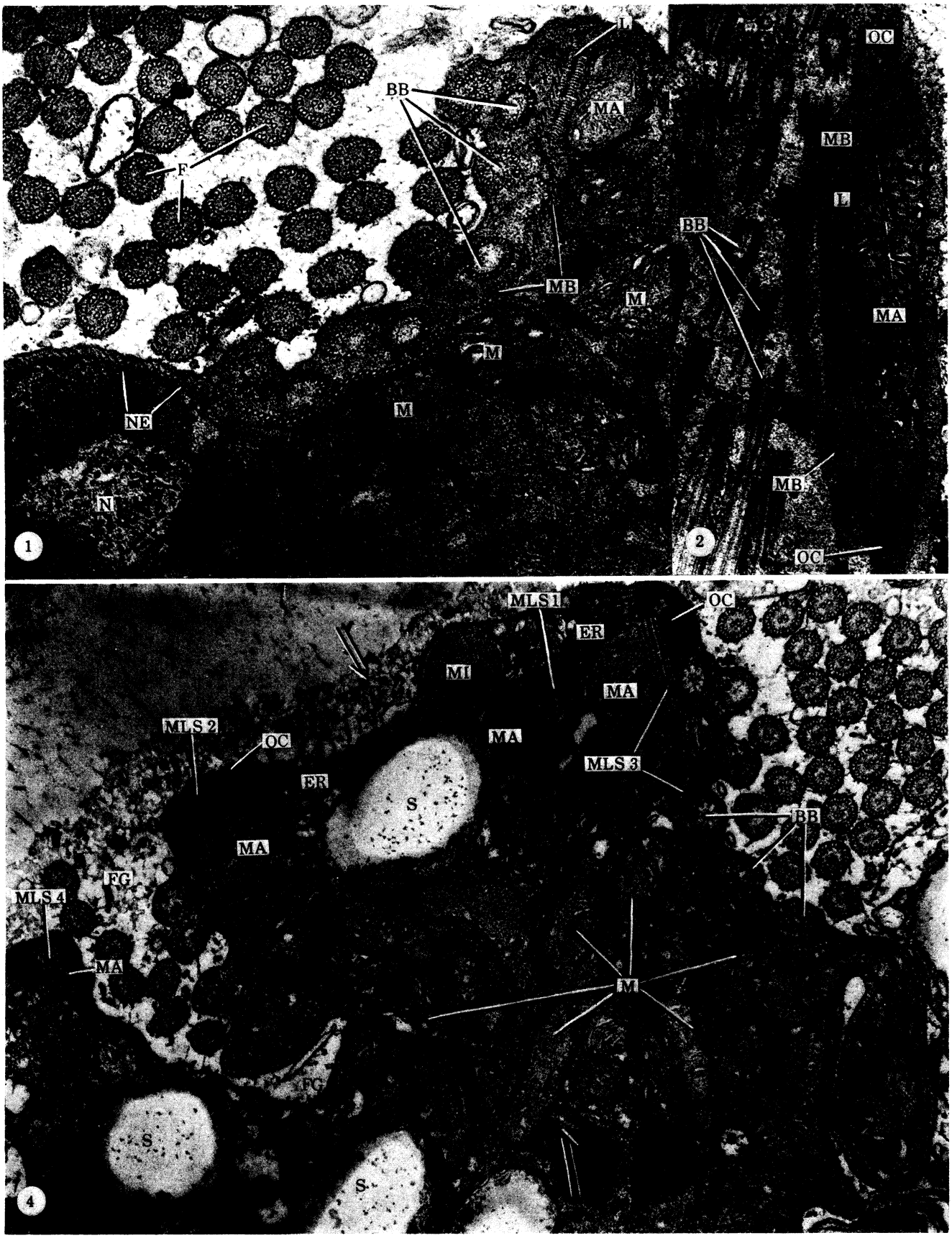
Description of plate 1

Figures 1 and 2. Half-differentiated spermatids.

Figure 1. Longitudinal section through the microtubular band midway along the length of the lamellar strip. The lamellar strip is connected to the nucleus by the microtubular band, on the outside of which lie several basal bodies embedded in a ribosome-free matrix. Note that the chromatin in the nucleus is still not wholly condensed. Ribosomes are conspicuous in the cytoplasm. (Magn. × 53000.)

Figure 2. Grazing longitudinal section through the MLS showing the 40° angle between the lamellar strip and the microtubular band. The flagellar bases are embedded in an electron-transparent matrix and the osmiophilic crest, showing its regularly banded substructure, is restricted to the external anterior edge of the microtubular band. (Magn. × 34000.)

Figure 4. Median longitudinal section through the anterior end of a mature spermatocyte. The microtubular band and lamellar strip are each sectioned 4 times (numbered MLS 1–4 from the anterior tip of the lamellar strip). The profiles of the microtubular band correspond approximately to positions 1, 5, 11 and 17 (not all the microtubular band of this last profile is included in the micrograph) in figures 13 and 14. Note that flagellar imbrication is clockwise on the right of the axis of the helix (arrowed) and counterclockwise on the left. Cartwheel and stellate profiles are visible in the basal bodies and sections through the bases of the axonemes reveal 9 + 0 microtubule configurations. The axis of the helix is indicated by the double arrows (⇒). (Magn. × 47000.)



FIGURES 1, 2 AND 4. For description see opposite.

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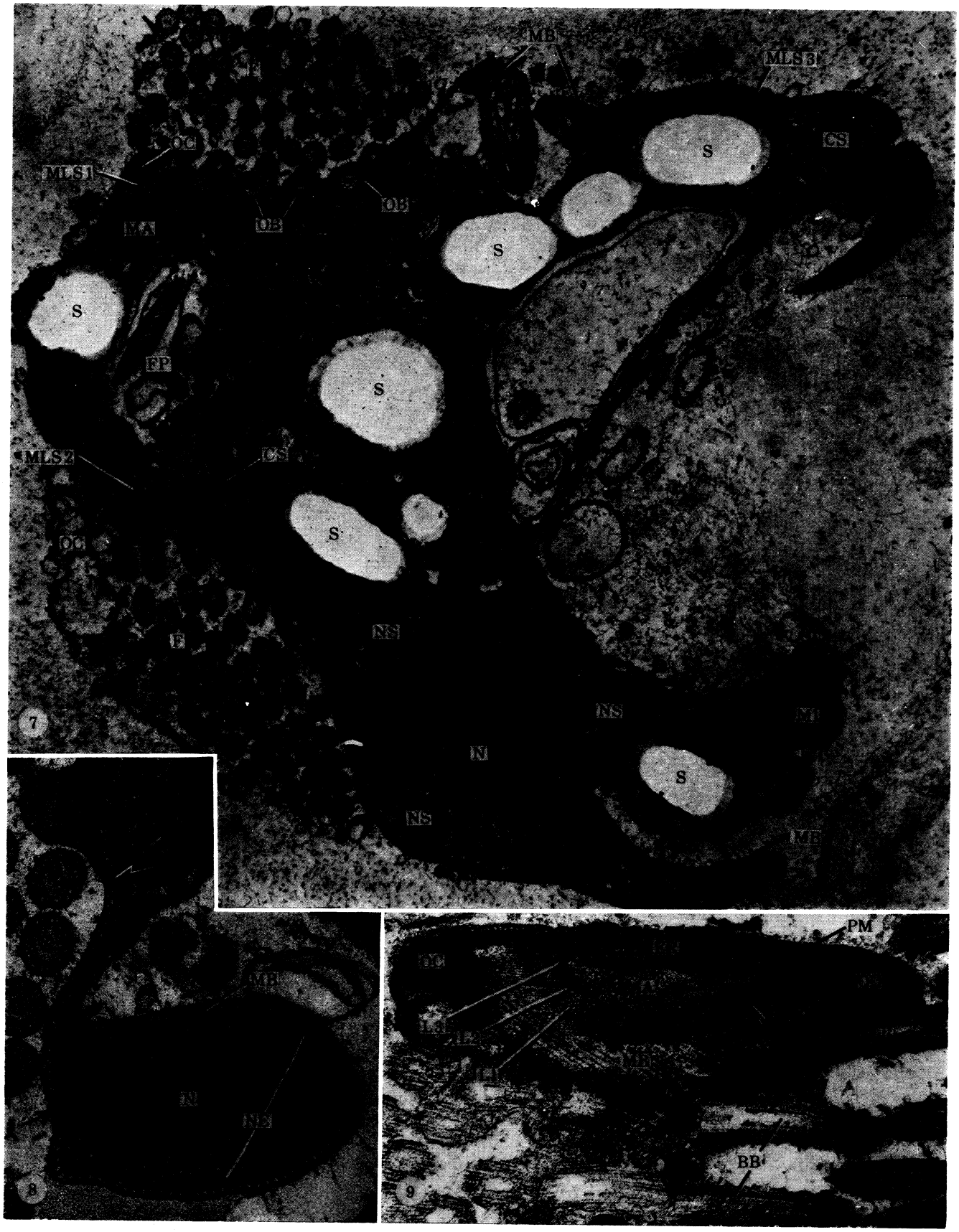

FIGURES 7-9. For description see opposite.

connected only by the microtubular band, a situation initially attained midway through the metamorphosis (figure 1, plate 1). Moreover, since the microtubules are orientated at approximately 40° to the axis of the lamellar strip (figure 2, plate 1), the separation process also leads to a displacement of the lamellar strip laterally relative to the nucleus such that only the posterior third of the strip directly overlies the nucleus.

The spiral lamellar strip and twisted pyriform nucleus complete $2\frac{1}{2}$ and $1\frac{1}{4}$ gyres respectively (figure 3), and from the anterior tip of the strip to the posterior end of the nucleus the gamete completes $3\frac{1}{4}$ gyres, traversed throughout by the microtubular band.

Because of the coiling of the lamellar strip and the breadth of the microtubular band coming from it, fine sections of the mature spermatozoids of *Equisetum* are highly complex (figure 4†,

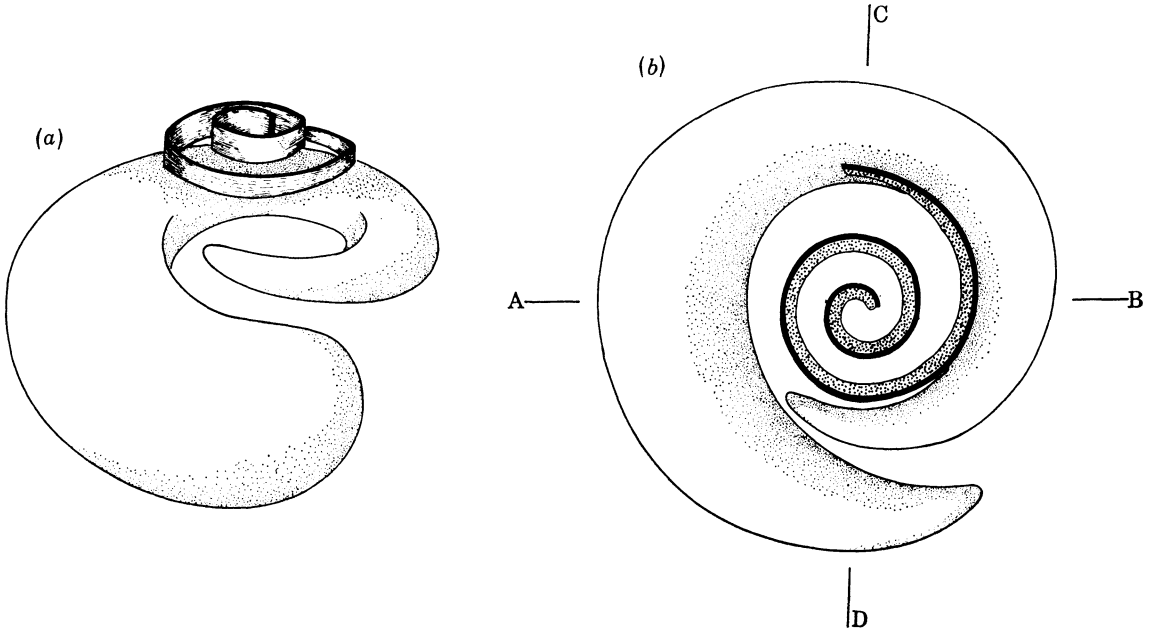

FIGURE 3. Diagrammatic reconstructions of the relationship between the nucleus and lamellar strip in mature spermatozoids of *Equisetum*. (*a*) Side view; (*b*) anterior view which also includes the spiral mitochondrion associated with the lamellar strip. The lines A–B and C–D refer to the planes of the diagrammatic median longitudinal sections portrayed in figures 15 and 16.

DESCRIPTION OF PLATE 2

FIGURE 7. Non-median longitudinal section through a mature spermatocyte. The MLS is sectioned three times, once anterior to the nucleus (MLS 1, corresponding to position 11 in figures 13 and 14) once through the nucleus (MLS 2, between positions 17 and 18 in figures 13 and 14) and once behind the nucleus (MLS 3 between positions 23 and 24 in figures 13 and 14). (Magn. × 27000.)

FIGURE 8. Section of the MLS corresponding approximately to position 15 in figures 13 and 14 showing lateral discontinuities in the microtubular band (arrows). (Magn. × 64000.)

FIGURE 9. Grazing longitudinal section through the MLS in a mature liberated spermatozoid. The 40° angle between the axes of the microtubules and the lamellar plates, seen in the spermatids, is retained in the motile gamete. The various strata of the lamellar strip can be distinguished by their differing degrees of electron opacity. The continuous layer of material separating L_1 and L_2 is arrowed. The osmiophilic crest separates the MLS from the cell membrane and sheets of smooth endoplasmic reticulum extend between the two profiles of the crest parallel to the plasma membrane. (Magn. × 55000.)

† In the course of revision figures 5 and 6 were omitted; it is regretted that complete renumbering was not possible.

plate 1; figure 7, plate 2). The key to the elucidation of the precise dimensions of the MLS lies in a critical examination of sections which are radial or parallel to the axis of the helix. In these it is possible to count the number of both microtubules and lamellar plates (table 2), and at the same time infer the overall shape of the microtubular band. Thus a detailed plan of the MLS from the anterior tip of the lamellar strip to beyond the posterior tip of the nucleus, can be obtained (figure 4, plate 1; figure 7, plate 2: plate 3). Grazing sections of the MLS provide the angle between the microtubular band and the lamellar plates (figure 2, plate 1; figure 9, plate 2.) Transverse sections of the anterior end of the gamete, show the lamellar layers as a continuous strip extending along the whole of the inside surface of the anterior edge of the microtubular band (figure 17, plate 4).

Figure 3 is a reconstruction, illustrating the shape of the nucleus and the lamellar strip. However, it is impossible here to show clearly the manner in which these are interconnected by the microtubular band. Thus, in figures 13 and 14 the relationship between them is shown as it would appear if the gametes were uncoiled. The numerical relationships, taken from fine sections upon which these reconstructions are based, are summarized in table 2, and representative micrographs are provided in plates 1–3. Beginning at the anterior tip of the lamellar strip (position 1, figures 13 and 14; figure 4, plate 1; figure 10, plate 3) the microtubular band is the same width as the lamellar plates. Thence the number of microtubules increases to a maximum of over 300 at the posterior tip of the lamellar strip where, in longitudinal sections, the most anterior tubule overlaps the most posterior one (position 17, figures 13 and 14).

Towards the posterior of the lamellar strip, in addition to the increasing amount by which the microtubular band extends downwards below the strip (as a result of the 40° angle and the increasing number of tubules) the profile of the microtubular band also changes. From nearly straight, at the anterior tip, of the lamellar strip, (positions 1 and 2, figures 13 and 14), it soon develops a V-shaped kink (figure 4, plate 1). The fold then becomes crozier-shaped (figure 4, plate 1; until by the mid-point of the lamellar strip (positions 10 and 11, figures 13 and 14, 150 microtubules in the band), the profile is that of a closed hook (figure 7, plate 2). Further along the strip, the tip of the nucleus appears in the eye of the crozier (position 12, figures 13 and 14, 180 microtubules in the band; figure 10, plate 3). From here to the posterior tip of the lamellar strip the nucleus increases in diameter and the number of tubules investing it likewise increases (figure 8; plate 2). However, below the posterior quarter of the lamellar strip the crozier opens out (positions 15–17, figures 13 and 14), and the anterior surface of the nucleus is no longer ensheathed by microtubules. Behind the posterior termination of the lamellar strip, the nucleus

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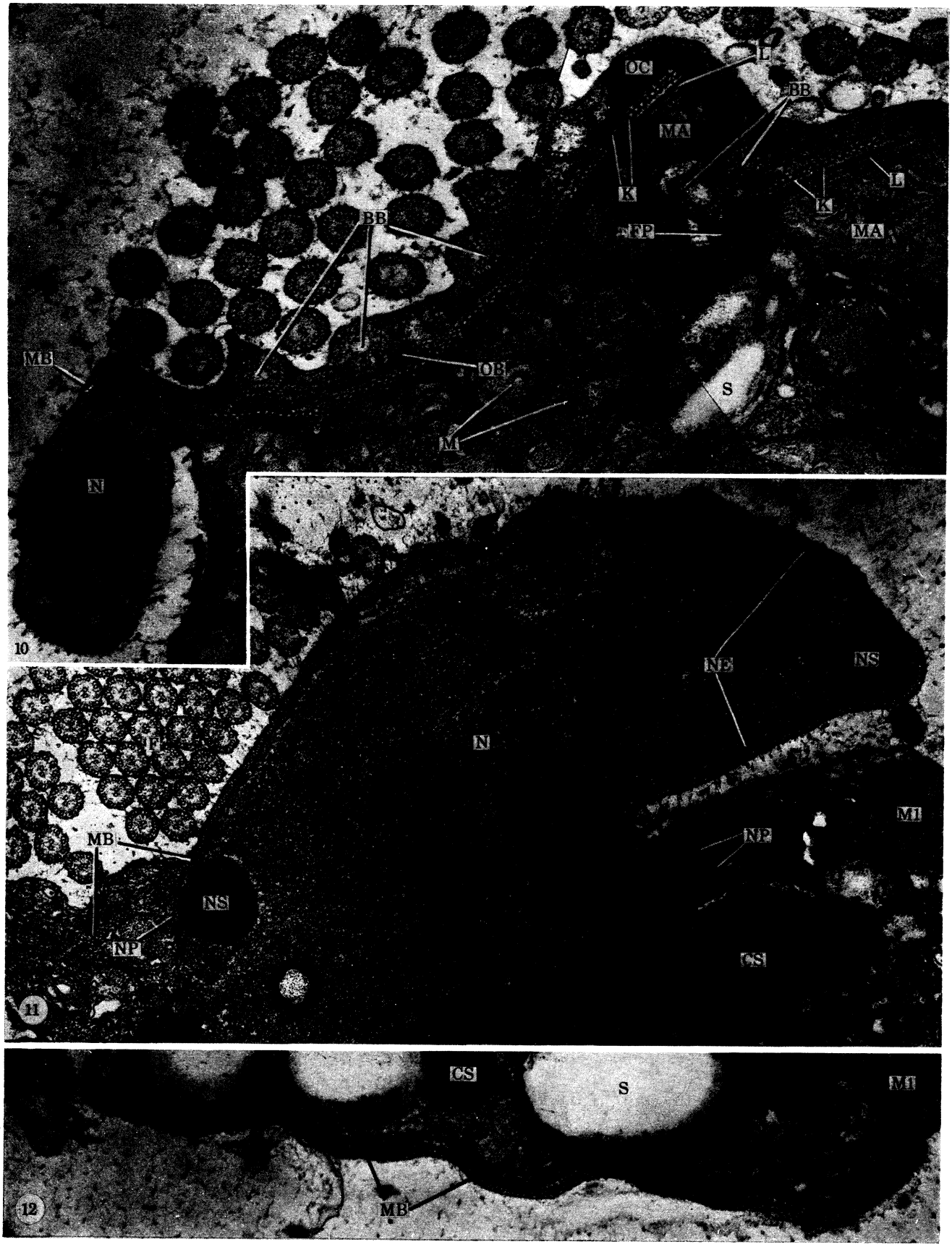
Description of plate 3

Figure 10. Section showing two profiles of the MLS; one very near the anterior tip of the lamellar strip (position 2 in figures 13 and 14; 30 microtubules) and the other with the microtubular band ensheathing the anterior tip of the nucleus (position 12 in figures 13 and 14; 165 microtubules). The extreme anterior tip of the flagella groove (FP) is visible adjacent to the two basal bodies outside the more anterior MLS profile. Keels are present on the microtubules overlying the lamellar strip. (Magn. × 55000.)

Figure 11. Section of the microtubular band corresponding approximately to positions 21 and 22 in figures 21 and 22. The band comprises 290 tubules of which only 24 are not included in the cradle around the nucleus. (Magn. × 37000.)

Figure 12. Section of the microtubular band just behind the posterior tip of the nucleus (between positions 23 and 24, figures 13 and 14; *ca.* 140 microtubules). (Magn. × 42000.)



FIGURES 10–12. For description see opposite.

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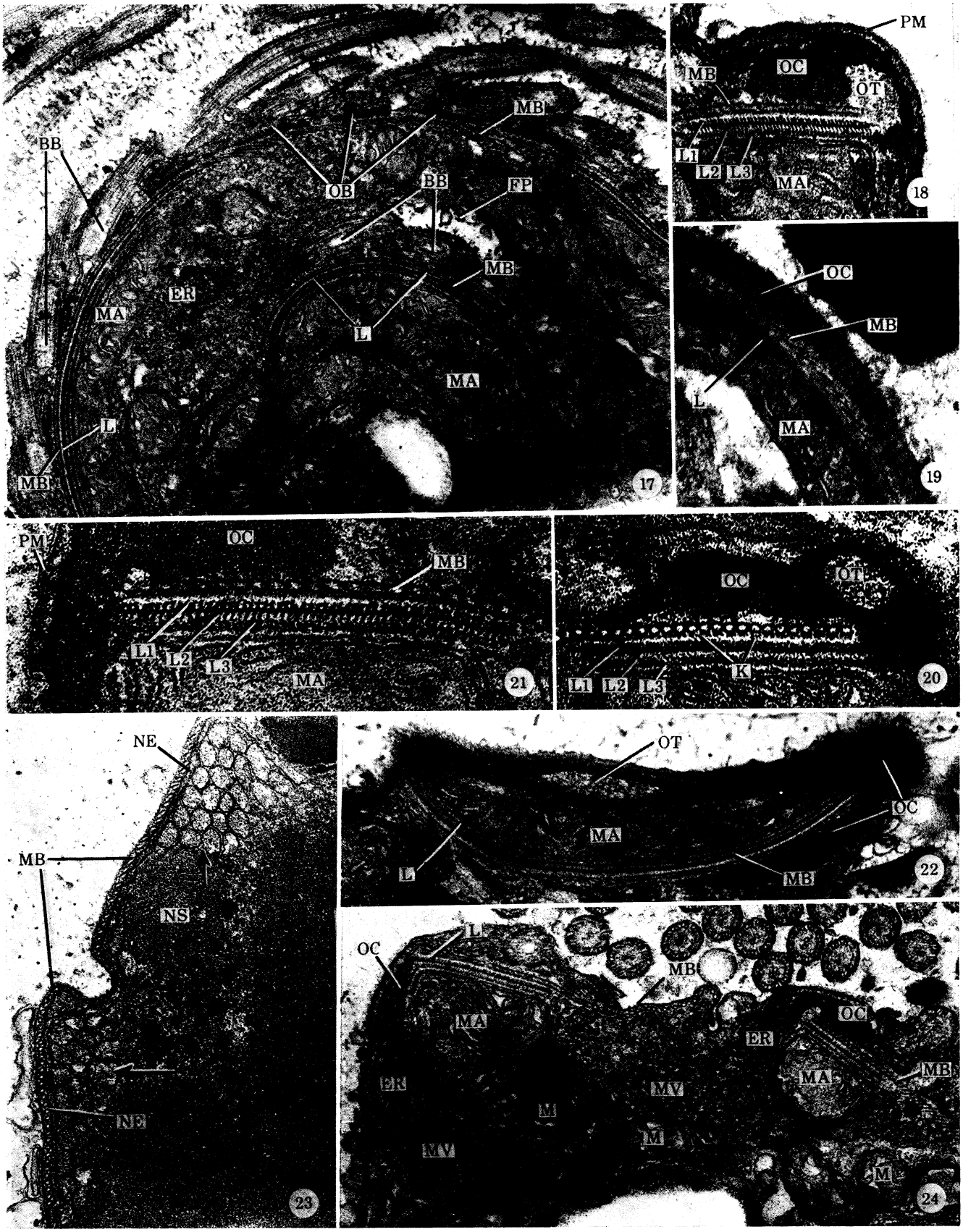

FIGURES 17–24. For description see opposite.

exhibits a further increase in diameter. The extent to which the microtubules invest its inner surface declines steadily, although the separation of its outer surface from the plasma membrane by the microtubular band, persists right up to its posterior tip (position 23, figures 13 and 14).

Where the anterior regions of the nucleus are free from the microtubular band they bulge inwards towards the axis of the helix (positions 18–21, figures 13 and 14; figure 7, plate 2; figure 11, plate 3). The posterior region of the nucleus, however, is flattened parallel to the long axis of the gamete. Coincident with the beginning of the flattening (between positions 21 and 22, figures 13 and 14) the width of the microtubular band, as a consequence of the termination of both anterior and posterior microtubules, begins to decline. The band, its width now even more rapidly diminishing, extends about $\frac{1}{4}$ gyre beyond the posterior tip of the nucleus (positions 24 and 25, figures 21 and 22; figure 7, plate 2; figure 12, plate 3), and then ceases altogether.

The expedient of displaying the mature spermatozoids in an uncoiled form illustrates the extent to which the lamellar strip has become displaced anteriorly and laterally relative to the nucleus during the differentiation of the gamete. Although separated vertically by a distance of 2–3 μm (equivalent to 80–100 microtubules in longitudinal sections), the gyres of the lamellar strip and the nucleus are approximately parallel.

Median longitudinal sections of *Equisetum* spermatocytes show several profiles of the microtubular band (figure 4, plate 1) whereas non-median ones reveal a lower number of band profiles since the tight inner coils are absent (figures 7, plate 2). The anteriormost profiles of the microtubular band are subtended by the lamellar strip alone, the middle by both the strip and the nucleus, and the posteriormost by the nucleus alone. However, the appearance of sections varies considerably depending upon their plane. For example a section may go through the

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Description of plate 4

Figure 17. Transverse section through the innermost gyres of the MLS at the anterior end of a spermatocyte. The basal bodies depart from the spiral of the microtubular band at an angle of about 10°. The collars of osmiophilic material around the proximal ends of the basal bodies extend as shortly rectangular plates along the surface of the microtubular band. (Magn. × 46 000.)

Figure 18. Longitudinal section through the MLS showing the central electron-transparent region of the osmiophilic crest in contact with both the microtubular band and the anterior edge of the lamellar strip. (Magn. × 78 000.)

Figure 19. *E. telmateia.* Section parallel to the long axis of the osmiophilic crest illustrating its regularly banded substructure. (Magn. × 60 000.)

Figure 20. Section of the MLS perpendicular to the long axes of the microtubules showing the presence of flat-bottomed keels overlying the lamellar strip. Even though this section is oblique to the long axis of the strip and thus does not show the individual plates, the three lamellar strata can still be distinguished by their different electron-opacities. A central electron-transparent lumen is present within the osmiophilic crest. (Magn. × 102 000.)

Figure 21. Section of the MLS showing details of the three lamellar strata and the relationships between the osmiophilic crest, the MLS, and the plasma membrane (left). (Magn. × 115 000.)

Figure 22. Transverse section through the MLS showing the osmiophilic crest extending over the anterior edge of the lamellar strip and subjacent mitochondrion. The crest contains an electron transparent lumen. (Magn. × 55 000.)

Figure 23. Section of the nucleus showing aggregations of membranous vesicles adjacent to its envelope (arrowed). (Magn. × 75 000.)

Figure 24. Longitudinal section through the anterior end of a mature spermatocyte showing two profiles of the MLS. Sheets of smooth endoplasmic reticulum are conspicuous radiating from the inner surface of the osmiophilic crest. The regions of the cytoplasm not packed with mitochondria contain numerous membranous vesicles (MV). (Magn. × 50 000.)

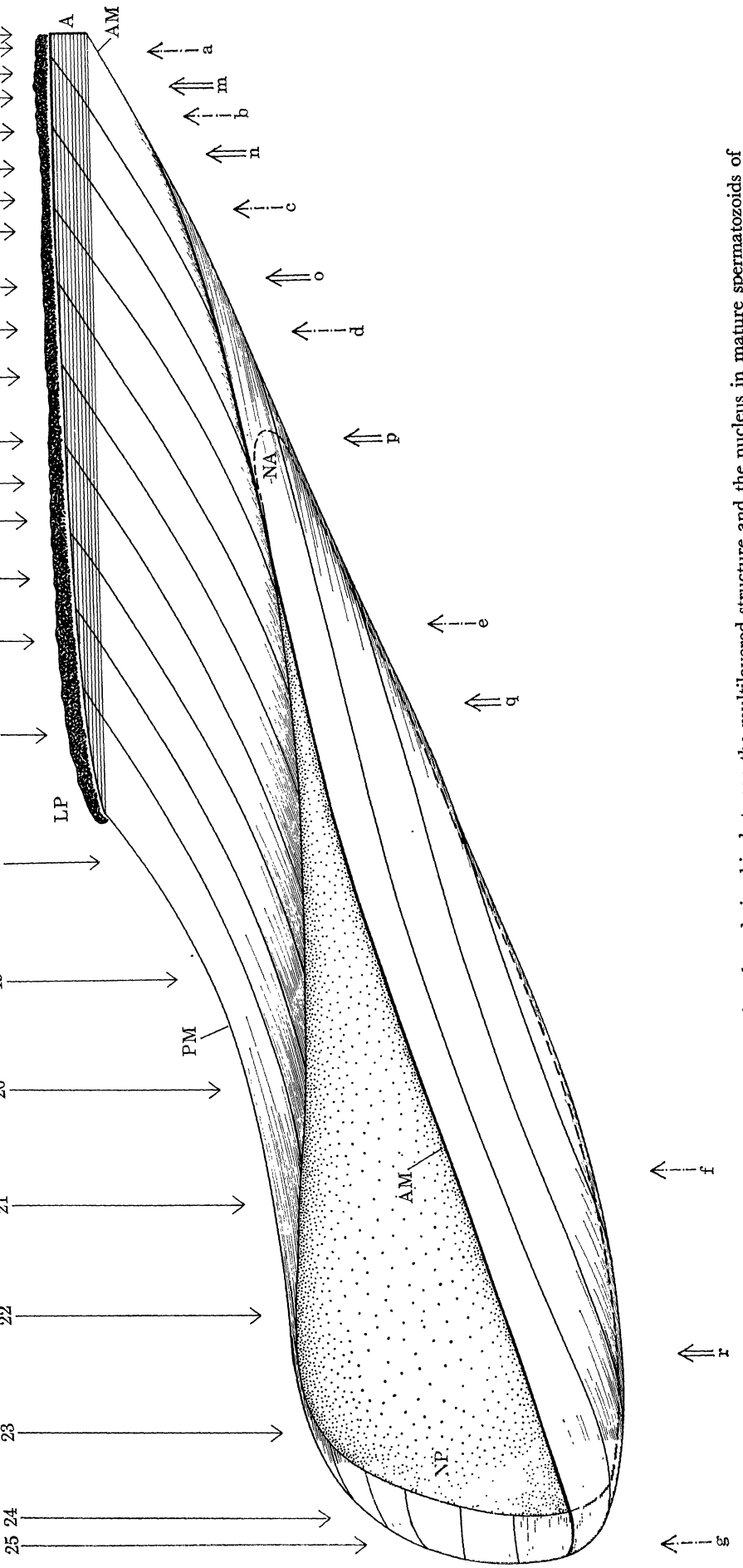


FIGURE 13. Diagrammatic reconstruction showing the relationship between the multilayered structure and the nucleus in mature spermatozoids of *Equisetum*. In order to illustrate clearly the manner in which the microtubular band forms a cradle ensheathing the nucleus, the spermatozoid is displayed in an uncoiled form.

The solid arrows →, numbered 1–25, refer to the positions, along the microtubular band, of the diagrammatic longitudinal sections shown in figure 14.

The broken arrows - - →, labelled a–g, and the double arrows ⇒, labelled m–r, indicate the positions where the microtubular band is sectioned in figures 15 and 16 respectively.

A, anterior tip of multilayered structure; AM, anteriormost microtubule; PM, posteriormost microtubule; LP, posterior tip of lamellar strip; NA, anterior tip of nucleus; NP, posterior tip of nucleus.

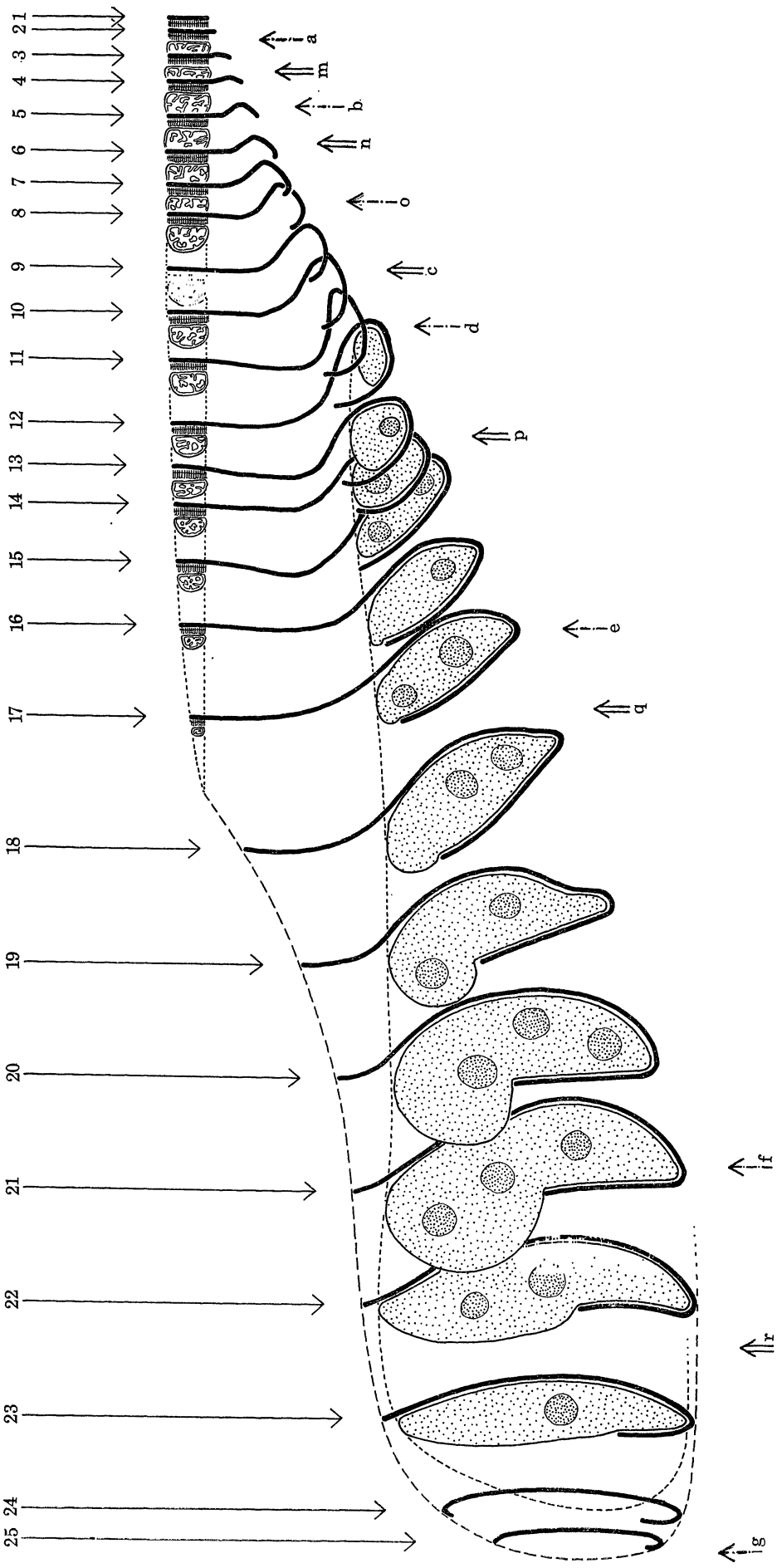


FIGURE 14. Diagram showing the appearance of longitudinal sections, through the nucleus and multilayered structure, at various points along the length of microtubular band in mature spermatozoids of *Equisetum*.

The solid arrows →, numbered 1–25, indicate the positions of the sections, along the length of the microtubular band, in the reconstruction shown in figure 13.

The broken arrows - - →, labelled a–g, and the double arrows ⇒, labelled m–r, indicate the positions where the microtubular band is sectioned in the diagrams of median longitudinal sections of the spermatozoids illustrated in figures 15 and 16.

anterior tip of the lamellar strip (figure 3*b*, profile A–B; figure 15). In this case the microtubular band is sectioned seven times, four times with the lamellar strip alone, twice with the lamellar strip together with the nucleus, and once alone behind the posterior tip of the nucleus. However, in the plane at right angles to this (figure 3*b*, profile C–D; figure 16) the microtubular band is sectioned only six times, and the lamellar strip and nucleus five and three times respectively. From these two sections (cf. figures 15 and 16) it can be seen that no two profiles through the band correspond exactly (also compare figure 4, plate 1 with figure 7, plate 2). In fact, just as there are an infinite number of radii to the helix so there are an infinite number of different profiles.

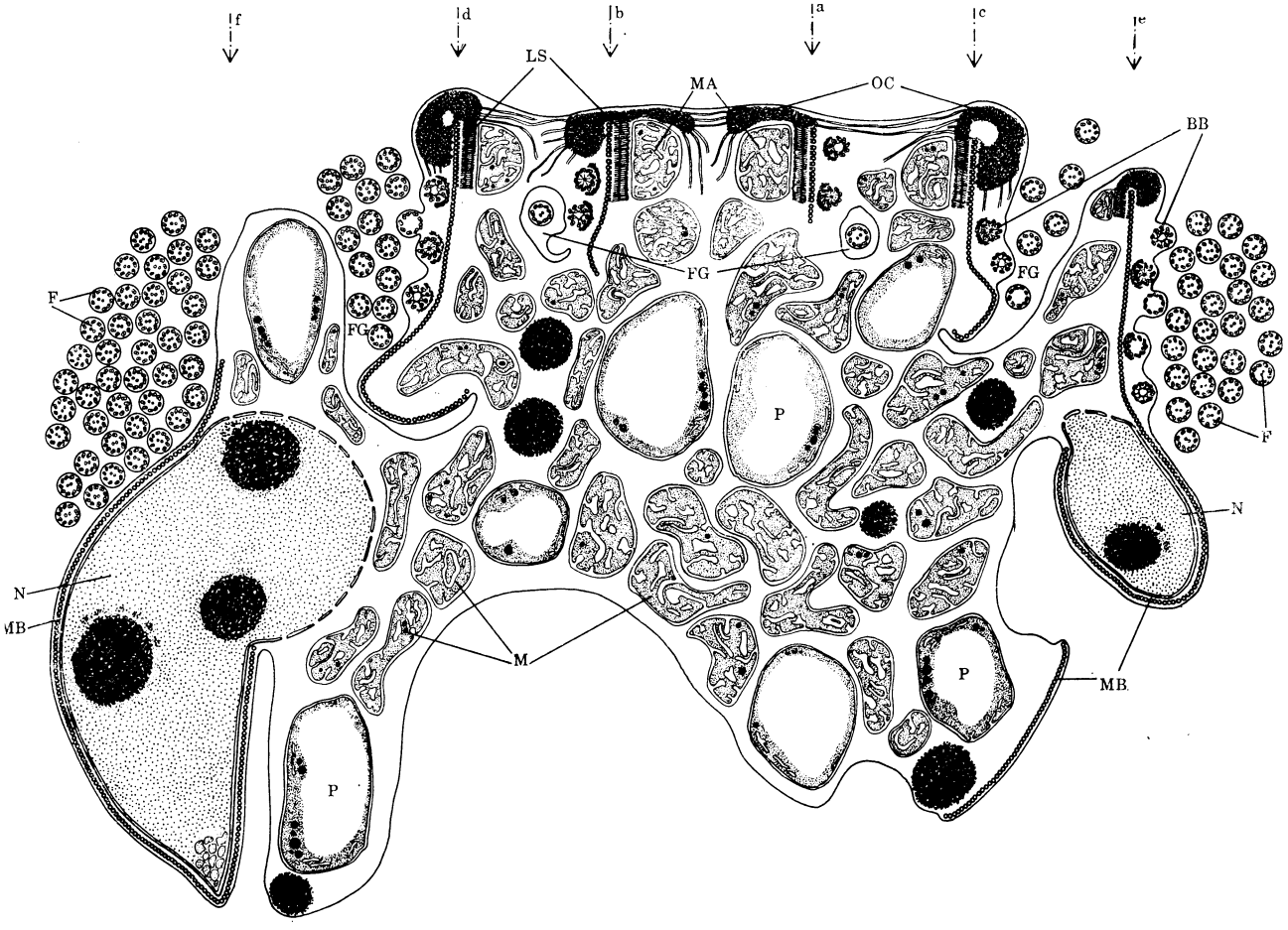


FIGURE 15. Diagrammatic median longitudinal section through a mature spermatozoid of *Equisetum*. The position of this section in relation to the coils of the spermatozoid is indicated by the line A–B in figure 3*b*. The broken arrows --→, labelled a–g, indicate the position of each section, through the multilayered structure and nucleus, along the length of the microtubular band in figures 13 and 14.

BB, basal bodies; F, flagella; FG, flagella groove; LS, lamellar strip; MA, spiral mitochondrion associated with the lamellar strip; M, pleomorphic mitochondria within the central cytoplasm; N, nucleus; OC, osmiophilic crest.

Although, in the mature spermatozoids of *Equisetum*, the amount of coiling of the lamellar strip and nucleus varies little, differences have been detected between the shapes of liberated and unliberated gametes. Spermatocytes within an antheridium are almost spherical. Their front end is flat, and the lamellar strip forms a plane coil for its first $1\frac{1}{2}$ gyres (figures 7 and 9,

plate 2). Behind this it pitches posteriorly at an angle of about 10°. The nucleus is almost a plane coil with its anterior tip barely overlapping, and situated within, the circumference of the much broader posterior gyre. The whole cell is approximately 22 μm long and 18 μm wide, the widest part being the posterior coil occupied by the nucleus.

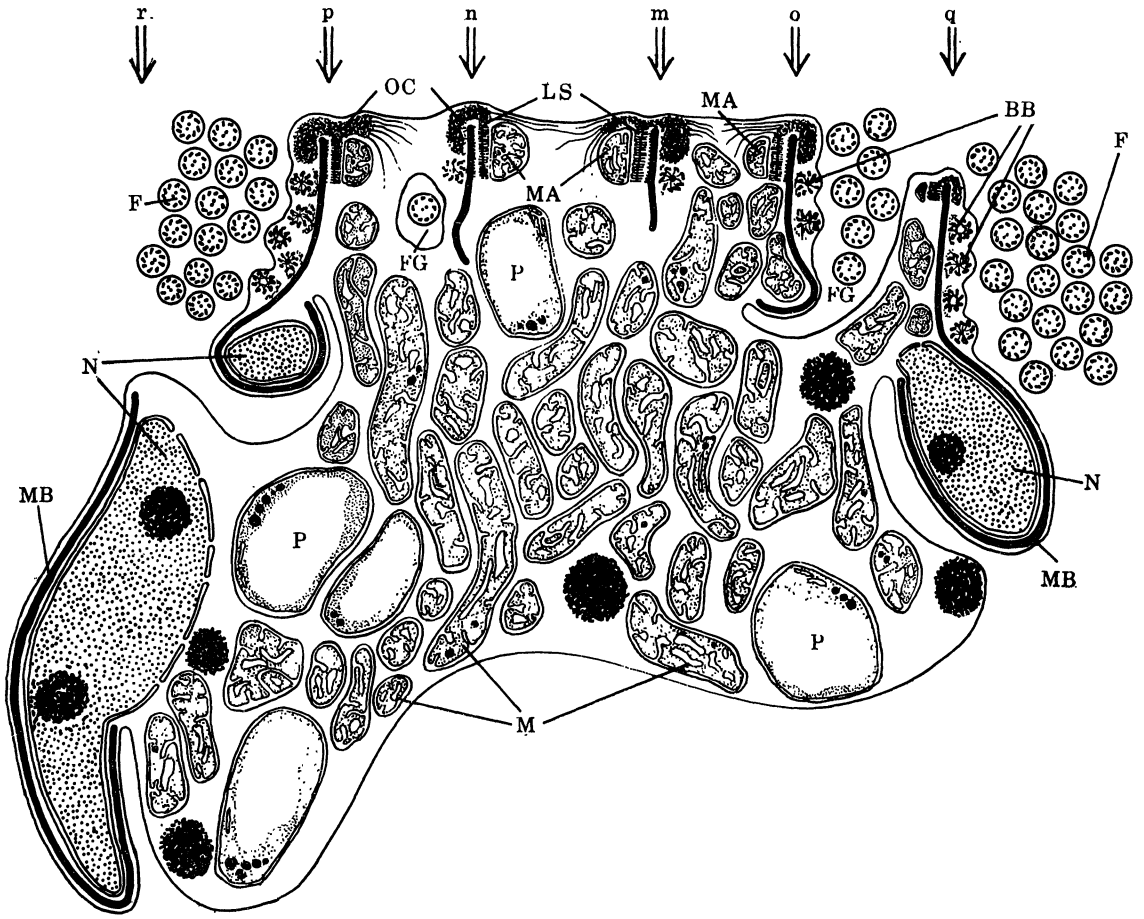


FIGURE 16. Diagrammatic median longitudinal section through a mature spermatozoid of *Equisetum*, in a plane at right angles to the section illustrated in figure 15. The position of this section in relation to the coils of the spermatozoid is indicated by the line C–D in figure 3*b*. The double arrows ⇒, labelled m–r, indicate the position of each section, through the multilayered structure and nucleus along the length of the microtubule band in figures 13 and 14.

The significance of the labelling is the same as in figure 23.

Spermatozoids fixed while swimming are, by contrast, often slightly elongated. The anterior coil of the lamellar strip remains almost in one plane, but the coils associated with the nucleus, form a helix with a much steeper pitch. It is interesting to note that the changes in shape during the swimming of *Pteridium* spermatozoids are likewise related to the pitch of the lamellar strip and the nucleus (Bell & Duckett 1976; Duckett & Bell 1971, 1972).

(*b*) *The substructure of the multilayered structure and associated components*

Several marked changes take place in the substructure of the MLS as the gametes of *Equisetum* near maturity. Although the microtubules have the same dimensions as in the spermatids (26–27 nm outside diameter, 13–14 nm inside), their regular spacing is rigorously maintained only

in the vicinity of the lamellar strip. Where the band is crozier-shaped, and around the posterior edge of the nucleus, gaps are sometimes encountered (figure 8, plate 2).

Overlying the lamellar strip the microtubules bear flat-bottomed keels (in the differentiating spermatids these are usually concave), about 15 nm wide and 10 nm deep (figure 10, plate 4; figure 18, plate 4). Between the keels and the lamellar plates is an apparently structureless,

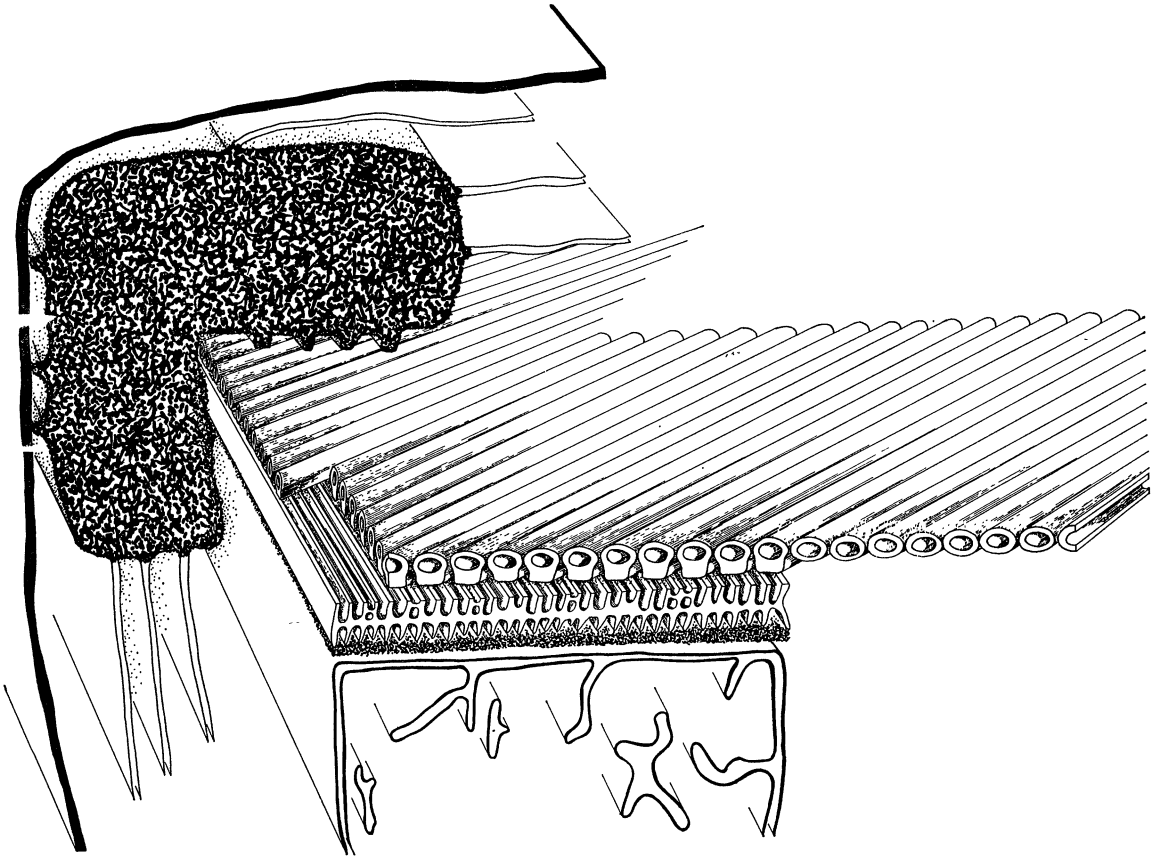


FIGURE 25. A three-dimensional reconstruction of the multilayered structure, approximately midway along the lamellar strip, in mature spermatozoids of *Equisetum*.

DESCRIPTION OF PLATE 5

FIGURE 26. Section of the nucleus showing aggregations of membrane-bound vesicles (arrowed) ensheathed by the posterior part of the microtubular band. Several spherical, electron-opaque bodies, surrounded by more transparent halos are scattered elsewhere. (Magn. × 36000.)

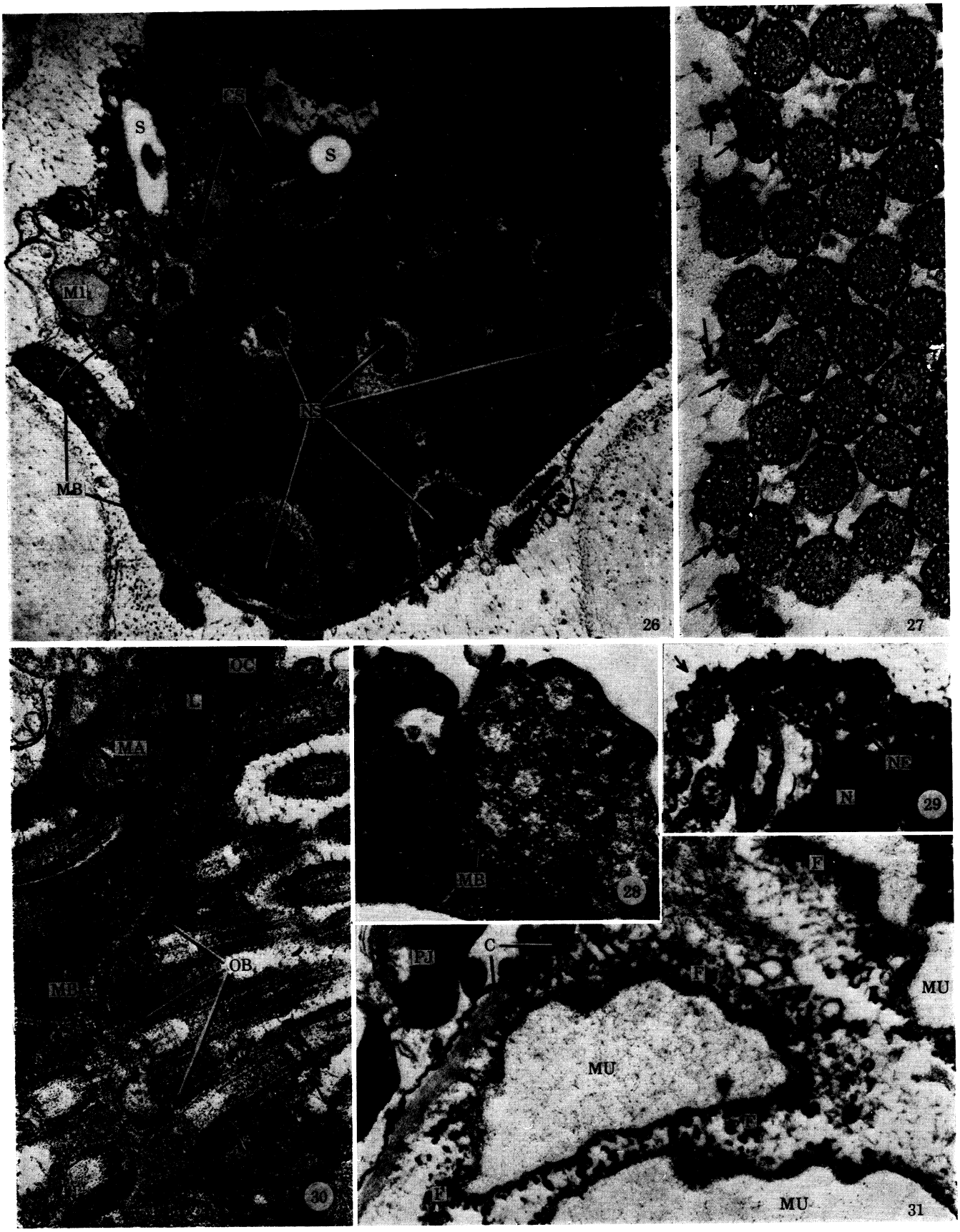
FIGURE 27. Transverse section of flagella. The incomplete 9+2 tubule configurations (arrowed) are sections through the flagellar tips. (Magn. = 82000.)

FIGURE 28. *E. telmateia.* Disrupted flagella from a motile spermatozoid. (Magn. × 70000.)

FIGURE 29. *E. telmateia.* Disrupted mucrotubular band around the posterior edge of the nucleus of a motile spermatozoid. The arrow indicates a disorganized flagellum. (Magn. × 40000.)

FIGURE 30. Longitudinal section through the flagellar bases showing collars of osmiophilic material around their proximal ends. The transition region of a basal body, containing the stellate profile, lies between the arrows. (Magn. × 58000.)

FIGURE 31. *E. telmateia.* Micrograph illustrating the substructure of the walls surrounding mature spermatocytes. MU, electron-transparent matrix surrounding each spermatocyte; F, finely granular layer containing osmiophilic globuli. Outside this is a network of fine fibrils (arrowed). The original middle lamella is no longer distinguishable. C, cellulosic wall of the antheridial jacket cells. (Magn. × 18000.)



FIGURES 26–31. For description see opposite.

(*Facing p.* 142)

electron-transparent strip, 20 nm wide. Three strata can be distinguished within the lamellar strip, whether the sections are perpendicular (figure 21, plate 4); parallel (figure 9, plate 2), or oblique (figure 18, plate 4) to its length. The two outer, consisting of a uniform series of alternating electron-transparent and electron-opaque plates, 20 nm and 25 nm wide respectively, and with a centre-to-centre spacing of 14 nm, are separated by a continuous sheet of electron-opaque material lying parallel to the upper and lower surfaces of the strip. The number of plates declines along the strip from an anterior maximum of about 60 (see table 2), but their spacing remains constant throughout. Thus, the width of the strip declines posteriorly, and the number of microtubules overlying it decreases, but a regular numerical relationship of 2.7 plates to 1 microtubule is maintained throughout.

The innermost stratum (farthest from the microtubules) comprises a continuous layer of finely granular material, about 20–30 nm in thickness (figure 21, plate 4) into which taper the inner lamellar plates. A three-dimensional interpretation of the MLS is shown in figure 25.

Intimately associated with the MLS is the osmiophilic crest, a strip of electron-opaque material, approximately equalling in length the lamellar strip, but overlying the exterior rim of the microtubular band (figures 18–22, plate 4). Although retaining the regularly banded substructure (figure 19, plate 4) seen in the younger spermatids (Duckett 1973*a*), the crest is most prominent in the mature gametes. Up to the late spermatid stage it is restricted to the external anterior surface of the microtubular band (figure 2, plate 1) and is approximately half of the width of the apposed lamellar strip. In the mature gametes, however, it extends centripetally around the anterior edge of the microtubular band and has a variable profile. Towards the posterior tip of the lamellar strip its inward projection ends at the strip (figure 10, plate 3), whereas in the first gyre it extends between the plasma membrane and the spiral mitochondrion lying subjacent to the strip (figure 4, plate 1). Near the anterior tip of the strip, the crest is restricted to this latter region (figure 24, plate 4). An elongate electron-transparent lumen is present within the crest near the middle of the lamellar strip (figures 18, 20, 22, plate 4), which anteriorly ends blindly within the matrix of the crest (figure 20, plate 4), but posteriorly opens out onto the microtubular band (figure 18, plate 4).

The osmiophilic crest and the microtubular band appear to be interconnected across an electron-transparent region 20 nm wide by columns of crest material (figure 21, plate 4). Similar bridges traverse the space between the plasma membrane and the crest. The anterior end of the microtubular band and the lamellae are embedded in crest material.

Running into the internal surface of the osmiophilic crest are several sheets of smooth endoplasmic reticulum (figure 4, plate 1; figure 24, plate 4). These are continuous across the innermost gyre of the MLS, lying parallel to the flat anterior surface of the gamete (figure 4, plate 1). Further along the lamellar strip they fan outwards towards the centre of the helix.

(*c*) *Flagella and basal bodies*

The basal bodies lie in a staggered arrangement along the outer surface of the microtubular band which is anterior to the nucleus, and are thus confined to the first $2\frac{1}{2}$ gyres of the helix. Near the anterior tip of the lamellar strip, where the microtubular band is narrow, usually only 1–3 appear in any one section (figure 4, plate 1; figure 10, plate 3), but the number increasess to a maximum of 6–8 near the posterior tip of the strip (figure 4, plate 1; figure 10 plate 3). The longitudinal axes of the basal bodies are approximately parallel to the axes of the microtubules, but diverge from the spiral at about 10° (figure 17, plate 4). Proximally the walls of the basal

bodies, surrounding central cartwheels 0.2 μm in length, are made up of nine slightly imbricating triplets, all nine being the same length. The outer C tubule is lost near the base of the stellate profile (0.5 μm in length) and beyond this region is a short length of 9 + 0 doublets (figure 4, plate 1) before the typical 9 + 2 axonemal configuration begins. Around the proximal half of each basal body is a collar of electron-opaque material, (figure 4, plate 1; figure 7, plate 2; figure 30, plate 5), continuous with a shortly rectangular plate lying between the basal bodies and the microtubular band (figure 17, plate 4).

As frozen-dried material examined in a scanning electron microscope demonstrates (Bilderback, Bilderback, Jahn & Fonseca 1973; Laroche, Guervin & Le Coq 1974), the flagellar bases are directed away from the anterior tip of the lamellar strip with the axonemes extending posteriorly, more or less in register with the helix. This interpretation is reinforced by an examination of the imbrication of the basal body microtubules. It is generally accepted that their imbrication is clockwise when the basal bodies are viewed proximally (for a review, see Paolillo 1974). Since the spermatozoid helix is sinistral, in longitudinal sections showing the MLS with the lamellar strip on the left and the basal bodies to the right of the microtubular band (i.e. looking towards the posterior tip of the strip) clockwise imbrication would be expected. Conversely, with the lamellar strip on the right and the basal bodies to the left of the band, the imbrication should be counterclockwise. From this reasoning it also follows that in any longitudinal section the basal body imbrication will be different on opposite sides of the axis of the helix (figure 4, plate 1; figure 7, plate 2). All the many observations made during the present study fall in with this expectation.

(*d*) *Cytoplasmic organelles*

As in the differentiating spermatids so in the mature gametes of *Equisetum* the lamellar strip is underlain throughout its length by a spiral mitochondrion. It has a dense matrix and is packed with dilated cristae displaying no regular orientation. Median longitudinal sections reveal profiles of 20–50 mitochondria from circular to highly irregular in form (figure 4, plate 1; figure 7, plate 2; figures 15, 16) in the central cytoplasm. The gamete as a whole probably contains at least 100 mitochondria.

Each mature spermatocyte contains between 15 and 25 spherical or ovoid plastids each almost filled with one (occasionally 2 or 3) massive starch grain (figure 4, plate 1; figure 7, plate 2). The peripheral stroma contains a few membranous fragments and scattered osmiophilic gloubli. In contrast, plastids of the antheridial jacket have well developed grana, between which are layers of closely packed osmiophilic globuli.

The small amount of ground cytoplasm that remains between the organelles is electron-opaque (figure 17, plate 4). Apart from the sheets of endoplasmic reticulum in contact with the osmiophilic crest, the extensive Golgi and endoplasmic reticulum systems characteristic of the differentiating spermatids are absent. However, in regions not packed with mitochondria, a reticulate membranous network of smooth endoplasmic reticulum (figure 24, plate 4) is sometimes encountered.

Two further components of the cytoplasm are both spherical, reaching up to 0.5 μm in diameter. From five to ten of these (figure 4, plate 1; figure 7, plate 2) have finely granular contents of medium electron-opacity, and are delimited by a single membrane. These are identified with microbodies, and they contrast sharply with ten to twenty bodies of similar diameter, but lacking a bounding membrane and consisting of finely granular, strongly osmiophilic material (figure 7, plate 2; figure 11, plate 3; figure 26, plate 5, figure 15, 16).

(*e*) *The plasma membrane and cell wall*

Although when mature the spermatocyte is still retained within an approximately spherical cell, the plasma membrane follows an extremely complex course. Except where it covers the flagellar bases and the osmiophilic crest, the membrane closely follows the external surface of the microtubular band. From the posterior limit of the band it runs anteriorly around the osmiophilic crest of the succeeding gyre. Since the gyres of the microtubular band overlap, this produces a deep spiral channel at the front of the gamete (figure 4, plate 1), which opens out only behind the posterior tip of the lamellar strip. Anteriorly the channel terminates as a closed pit just outside the innermost gyre of the lamellar strip (figure 7, plate 2; figure 10, plate 3). Most of the flagella emerge from this groove, and only those whose basal bodies are situated more posteriorly are completely free of its confines.

Less than half the volume delimited by the thin walls laid down after the last mitosis is actually occupied by the spermatocytes (figure 31, plate 5). Surrounding the spermatocytes is an electron-transparent structureless zone bounded by a layer of finely granular material containing osmiophilic globules. The gametes are liberated in sacs bounded by this layer, external to which is a network of fine fibrils. The original middle lamella, distinct up to the late spermatid stage (Duckett 1975*a*), is no longer visible.

The walls of the mature spermatocytes of *Equisetum* very closely resemble those of the fern *Ceratopteris* (Cave & Bell 1973) where lipid was demonstrated by staining and benzpyrene fluorescence. It thus seems likely that the osmiophilic globules in the walls of the *Equisetum* spermatocytes are also lipid. This, as in the bryophytes (Muggoch & Walton 1942), may cause their rapid dispersal on coming in to contact with water, before the freeing of the spermatozoids. Any such mechanism would widen the distribution of the gametes, and might be particularly important in *Equisetum* where gametophytes are commonly unisexual (Duckett & Duckett 1974).

(*f*) *The substructure of the nucleus*

A striking event of spermatogenesis in *Equisetum* is the very rapid development in late spermatids of a highly heterogenous substructure in the nucleus. Apart from the absence of a wall defined nucleolus (which apparently disappears at the last antheridial mitosis), the internal structure of the nucleus up to the late spermatid stage displays no marked differences, from interphase in the vegetative gametophyte cells. Regularly dispersed chromatin (figure 1, plate 1) occupies approximately one third of the nuclear volume (Duckett 1973*a*). The bulk of the nucleus in mature spermatocytes is filled with uniformly condensed chromatin (figure 7, plate 2; figure 11, plate 3; figure 26, plate 5). Scattered within which are from ten to twenty spherical electron-opaque bodies up to 0.5 μm in diameter (figure 11, plate 3; figure 23, plate 4; figure 26, plate 5), occupying about 20 % of the nuclear volume. They are more finely granular than the condensed chromatin, and identical in appearance with electron-opaque bodies lacking a bounding membrane in the cytoplasm. Within the nucleus these bodies are usually surrounded by an electron-transparent halo (figure 26, plate 5) in which granules up to about 100 nm in diameter are embedded. In several localized areas of the nucleus adjacent to the microtubular band are aggregations of membrane-bound vesicles, up to 200 nm in diameter, with more coarsely granular and slightly more electron-transparent contents than the general chromatin (figure 11, plate 3; figure 26, plate 5).

Adjacent to the microtubular band pores are completely absent from the nuclear envelope, although they can be seen along the internal surface of the nucleus (figure 11, plate 3). The perinuclear space is filled with electron-opaque material.

(*g*) *Morphological changes accompanying motility*

For several minutes after liberation the spermatocytes exhibit vigorous writhing movements until the encapsulating sheath is broken and motility begins (see also Bilderback *et al.* 1973). Extended periods of swimming have little effect on the overall morphology of the gametes. Even after 1 h at 20 °C there are no ultrastructural changes in the mitochondria and plastids. Furthermore, in contrast to the spermatozoids of *Pteridium* and *Marsilea*, there is no tendency in *Equisetum* for the central cytoplasm to be discarded.

In unliberated spermatocytes the flagella are closely packed adjacent to the cell surface and within the spiral groove, whereas in motile material much looser arrays are encountered.In addition, irregular arrays of doublet microtubules bounded by a common membrane are of frequent occurrence (figure 28, plate 5). As in fern spermatozoids trapped in archegonial necks (Duckett & Bell 1971), these are interpreted as being derived from the disruption of normal flagella. They are thus very different from the incomplete 9 + 2 profiles at the tips of some intact flagella, since these occur with equal frequency before and after motility (figure 27, plate 5).

Stacks of microtubules, interspersed with fragments of paired membranes, are sometimes encountered along the posterior edge of the nuclei of swimming spermatozoids (figure 29, plate 5). This probably indicates incipient degeneration of the microtubular band, fore-shadowing the death of the gamete.

Discussion

(*a*) *General considerations of the gross morphology of the multilayered structure*

This study demonstrates that, in line with other vascular archegoniates, the entire MLS persists in the mature spermatozoids of *Equisetum*. Both the overall dimensions of the lamellar strip and the number of microtubules subtending it remain the same as in the differentiating spermatids. Thus the mature male gametes of *Equisetum* are very different from those of bryophytes (Carothers 1975; Duckett 1975*b*; Kreitner & Carothers 1976; Lal & Bell 1975) where the lamellar strata disappear. It is also clear that there is only one lamellar strip in *Equisetum*, and not two, as implied by Polette & Ridgeway (1973). We are forced to the view that this latter conclusion was based on scrutiny of insufficient sections.

There is now general agreement that the MLS is of functional importance in determining the morphology of archegoniate spermatozoids. Not only does it figure prominently in the metamorphosis of the spermatid, but its geometry also determines the extent of the spiralization, and the dimensions and pitch of the helix of the mature gamete. This, in turn, is intimately related to the shape of the nucleus and the disposition of the flagella (Bell & Duckett 1976; Duckett 1973*a*, 1975*a*).

A comparison of *Equisetum* spermatozoids with all available data of other organisms which possess a MLS is set out in table 1. The gametes of *Equisetum* are intermediate between those of leptosporangiate ferns and cycads in respect of the length of the lamellar strip and the number of microtubules. *Equisetum* spermatozoids are also intermediate in both size (10 μm diameter in ferns; up to 1 mm in cycads), and the amount of nuclear coiling (3½ gyres in ferns; approxi-

mately spherical in cycads). Such comparisons provide clear indications that the size and complexity of a particular archegoniate gamete are directly related to the dimensions of the MLS.

Nevertheless, although the mature spermatozoids of *Equisetum* perhaps more closely resemble those of leptosporangiate ferns than of any other archegoniate, in that both have a nucleus ensheathed in a similar manner by microtubules, there are several notable differences between them which are difficult to attribute directly to the length of the lamellar strip and the amount of nuclear coiling. In ferns, only one quarter of the lamellar strip is displaced anteriorly beyond the anterior tip of the nucleus, whereas in *Equisetum* the corresponding displacement amounts to three quarters. In consequence the distance traversed by the anterior part of the microtubular band before it meets the nucleus is substantially greater. Since in both *Equisetum* and the ferns the flagella are inserted on the outside of this part of the band, its greater length in *Equisetum* may be related to the fact that about 120 flagella have to be accommodated in *Equisetum* compared with about 40 in *Pteridium*.

In ferns the posterior termination of the microtubular band corresponds with the posterior tip of the nucleus, whereas in *Equisetum* the band extends approximately $\frac{1}{3}$ gyre beyond it. Since the mature gametes are naked cells lacking any visible means of expelling water (Moestrup 1975), it is not clear what prevents osmotic bursting. The microtubular band may provide the equivalent of a rigid cell wall, the post-nuclear extension serving to protect the central cytoplasm which, in *Equisetum*, remains intact during motility.

The disposition of the flagella in a deep spiral channel is a feature which *Equisetum* spermatozoids share with cycads (Norstog 1967, 1968, 1974, 1975). Since the channel results from the overlapping anterior gyres of the lamellar strip, in leptosporangiate ferns (Bell & Duckett 1976), and *Marsilea* (Rice & Laetsch 1967), where the pitch of the helix is much steeper, the channel is absent and the flagella are all completely exposed.

The similarities and differences between groups which must be considered in any attempt to ascribe precise functions to the various components of the MLS are also brought out in table 1. Thus, if the lamellar strip is involved in highly ordered synthesis of regularly spaced microtubules, it would appear likely that the 35–45° angle of divergence between the strip and the microtubular band plays an integral part in the process (Bell 1974). It is found in several distantly related groups and, is maintained throughout spermatogenesis in *Equisetum* (see also Duckett 1973*a*). However, such a functional interpretation of the MLS must take into account the different angles found in cycads (16–18°) and the alga, *Klebsormidium* (about 90°), and the presence of a microtubular band without lamellae in charophytes. If the lamellae have a mechanical role in anchoring the microtubules in the mature gametes of pteridophytes and cycads, it is necessary to consider how this function may be performed in their absence in bryophytes (Paolillo 1974; Kreitner & Carothers 1976).

Ultrastructural information is now available on the male gametes of all the major archegoniate phyla (see table 1). However, it should be pointed out that only in *Pteridium*, and four bryophytes (*Anthoceros*, *Marchantia*, *Pellia* and *Polytrichum*), has the ontogeny of the male gamete been elucidated with the detail approaching that now available for *Equisetum*. Furthermore, within each major group, very few taxa have been investigated, and details of spermatozoid ultrastructure in Marattiales, Ophioglossales and Psilotales are unknown. Despite these limitations, the ultrastructure of the male gametes figures prominently in phylogenetic speculations on the origin of land plants (Moestrup 1974, 1975; Pickett-Heaps 1975). It is especially significant that the MLS is present in the motile cells of those green algae which, on the grounds of the nature of

TABLE 1. SUMMARY OF THE ARCHITECTURE OF THE MULTILAYERED STRUCTURE

Taxa where the MLS has been detected	maximum no. of tubules in the microtubular band	Length of the lamellar strip	maximum no. of lamellar plates	angle between the axes of the microtubules and the lamellar plates	no. of gyres occupied by the lamellar strip	association of the lamellar plates with a mitochondrion	reference
ALGAE							
**Chaetosphaeridium globosum*	60	2 μm	16†	*ca.* 45°†	‡	yes	Moestrup (1974)
**Coleochaete scutata*	unknown	unknown	80†	unknown	‡	no	Pickett-Heaps & Marchant (1972)
**Klebsormidium flaccidum*	20–36	unknown	18†	90°	‡	no	Marchant, Pickett-Heaps & Jacobs (1974)
**Trentopohlia aurea*	two bands, each with 6–8	two present, lengths unknown	unknown	45°	‡	yes	Graham & McBride (1974)
Chara corallina	30	not present	‡	‡	‡	‡	Moestrup (1970, 1975)
C. fibrosa	27	not present	‡	‡	‡	‡	Pickett-Heaps (1968)
Nitella missouriensis	21†	not present	‡	‡	‡	‡	Turner (1966, 1968)
BRYOPHYTES							
Anthocerotae							
Phaeoceros (Anthoceros) laevis	12	2 μm	40	45°	< 1	yes	Duckett (1975*b*), Moser (1970)
Hepaticae							
Blasia pusilla	18–20	2 μm	48†	45°	< 1	yes	Carothers (1973, 1975)
Lejeunea patens	unknown	unknown	unknown	unknown	unknown	yes	Duckett (unpublished observations)
Marchantia berteroana	16	unknown	unknown	unknown	unknown	yes	Pickett-Heaps (1975)
M. polymorpha	17	2 μm	50†	48°	< 1	yes	Carothers & Kreitner (1967, 1968)
Nowellia curvifolia	unknown	unknown	unknown	45°	unknown	yes	Simone (1973)
Pellia epiphylla	15	2 μm	50†	45°	< 1	yes	Suire (1970)
P. fabbroniana	15	unknown	unknown	unknown	unknown	yes	Heitz (1959)
Preissia quadrata	unknown	unknown	unknown	unknown	unknown	yes	Heitz (1959)
Reboulia hemispherica	15–20	unknown	unknown	45°	unknown	yes	Turner (1966)
Sphaerocarpos donnellii	16	unknown	unknown	45°	unknown	yes	Diers (1967), Heitz (1959, 1960), Zimmerman (1973)

Musci							
Bryum capillare	15	unknown	unknown	unknown	< 1	yes	Bonnot (1967)
Physcomitrium coorgense	24	2 μm	47†	45°	< 1	yes	Lal & Bell (1975)
Polytrichum formosum	12†	unknown	32†	unknown	< 1	yes	Genevès (1967, 1968)
P. juniperinum	12	2 μm	30†	45°	< 1	yes	Paolillo (1965), Paolillo (1968*a*)
Sphagnum sp.	unknown	unknown	unknown	unknown	unknown	unknown	Manton (1957)
Splachnum rubrum	*ca.* 20	unknown	unknown	unknown	unknown	yes	Heitz (1960)
PTERIDOPHYTES							
Lycopsida							
Isoetes lacustris	*ca.* 40	unknown	unknown	unknown	unknown	yes	Thomas & Duckett (unpublished observations)
Lycopodium complanatum	> 40†	unknown	> 30†	45°	> 1†	yes	Carothers *et al.* (1975)
Selaginella kraussiana	17	unknown	unknown	45°	unknown	unknown	Robert (1974)
Sphenopsida							
Equisetum (*arvense, hyemale, ramosissimum, telmateia*)	> 300	15–20 μm	57–*60*–54	45°	$2\frac{1}{2}$	yes	Duckett (1973*a*) and this publication
Pteropsida							
Osmunda cinnamomea	> 180	2–10 μm	*ca.* 50	45°	*ca.* 2	yes	Duckett (unpublished observations)
Ceratopteris thalictroides	150	5–10 μm	45–50	35–45°	$1\frac{3}{4}$	yes	Duckett & Cave (unpublished observations)
Dryopteris filix-mas	150	5–10 μm	45–50	35–45°	$1\frac{3}{4}$	yes	Duckett (1975*a*)
Polypodium vulgare	unknown	unknown	unknown	unknown	unknown	yes	Vazart (1964)
Pteridium aquilinum	142–*150*–156	5–10 μm	41–*45*–56	35–45°	$1\frac{3}{4}$	yes	Duckett (1975*a*)
Marsilea vestita	25 and *ca.* 50	1 μm	unknown	unknown	< 1	yes	Rice & Laetsch (1967), Myles (1975), Myles & Bell (1975)
GYMNOSPERMS							
Ginkgo biloba	unknown	unknown	unknown	unknown	unknown	yes	Gifford & Lin (1974)
Zamia integrifolia	60000	1.5–2 mm	> 100†	16–18°	$4\frac{1}{2}$	no	Norstog (1967, 1968, 1974, 1975)

* Zoospores.
† Estimated from published electronmicrographs.
‡ Not applicable.

their cell division (Pickett-Heaps & Marchant 1972), and of their glycolytic enzymes (Frederick, Gruber & Tolbert 1973), are considered to be close to those which gave rise to land plants.

On the other hand, features of the MLS appear to be of little value so far in suggesting affinities between archegoniate phyla. The very different spermatozoid morphologies encountered in the various groups fully support palaeobotanical evidence that the phyla have long been isolated. At the lower levels of classification, this study reveals that there is complete uniformity in spermatozoid morphology between the subgenera of *Equisetum*, otherwise clearly distinct in both sporophyte and gametophyte morphology (Duckett 1970*a*, 1972, 1973*b*).

This contrasts sharply with bryophytes, where notable intergeneric differences exist. Similarly in the Pteropsida, the spermatozoids of leptosporangiate ferns are wholly dissimilar from those of *Marsilea*.

(*b*) *The substructure of the multilayered structure*

The lamellar strip in the mature spermatozoids of *Equisetum* is far more clearly stratified than in spermatids. Although three lamellar layers can be distinguished in the latter (Duckett 1973*a*), no horizontal membrane separates the two outer strata. In young spermatids the innermost lamellar stratum consists of plates or tubules, but is an amorphous osmiophilic strip at maturity. That the lamellar strip should exhibit maximal differentiation in the mature gamete sharply distinguishes *Equisetum* from other archegoniates, with the possible exception of cycads (Norstog 1974) where, unfortunately, no ontogenetic data is yet available. In bryophytes the whole lamellar strip disappears during spermatid maturation (Carothers 1973, 1975; Paolillo, Kreitner & Reighard 1968*a*, *b*); in ferns there is partial occlusion of the transparent regions of the outermost lamellar stratum (Bell, Duckett & Myles 1971), and in *Lycopodium* (Carothers, Robbins & Haas 1975) this stratum is transformed into a thinner layer of osmiophilic material. A similar change also takes place in the MLS of *Marchantia* (Kreitner & Carothers 1976), *Blasia* (Carothers 1973), and *Anthoceros* (Duckett 1975*b*) just prior to the complete disappearance of the lamellae. In bryophytes (Kreitner & Carothers 1975) and *Lycopodium* (Carothers *et al.* 1975) such morphological changes in the lamellae may be correlated with the synthesis of the overlying microtubules, but in *Equisetum* the structural modifications coincide with the termination of this process. Thus, we are now in the perplexing position of having a growing body of information on the development and phyletic diversity of the MLS, but little knowledge of its function. The understanding of the relationship between the lamellar strip and the manner of growth of the microtubular band would undoubtedly be advanced if it becomes possible to extract and purify the MLS and study the incorporation of tubulin *in vitro*. The recent demonstration that the isolated basal body complex of *Chlamydomonas* acts as an initiating centre for the assembly of tubulins into microtubules makes such a goal especially inviting (Snell *et al.* 1974).

The presence of keels on the microtubules, overlying the lamellar strip, is another feature of the MLS in the differentiating spermatids of *Equisetum* retained at maturity. Indeed, such keels appear to be universal in archegoniates, having now been described in *Lycopodium* (Carothers *et al.* 1975), bryophytes (Carothers & Kreitner 1967), ferns (Duckett 1975*a*), and cycads (Norstog 1974, 1975). However, in the present study we have no evidence that the microtubular band possesses other appendages such as the vertical outer ridges in *Lycopodium* (Carothers *et al.* 1975) and the cross-linking demonstrated in *Pteridium* (Bell 1975). Great importance is currently attached to the presence of arms and cross-bridges in considerations of coordinated microtubule synthesis and function (Heath 1974; McIntosh 1974; Tilney 1971; Tucker 1974). A critical investigation of these features in relation to the microtubular band of archegoniate gametes would

TABLE 2. NUMERICAL RELATIONSHIPS OF THE MULTILAYERED STRUCTURE IN SECTIONS PERPENDICULAR TO THE LONG AXIS OF THE LAMELLAR STRIP

(Mean numbers in each case are calculated from counts of at least ten sections. The data is derived from mature spermatocytes of both *Equisetum hyemale* and *E. telmateia*. No significant differences were detected between the two taxa.)

reference points along the MLS	total number of tubules in the microtubular band	number of electron-opaque plates in the lamellar strip	number of microtubules overlying the lamellar strip	ratio of the lamellar plates: overlying micro-tubules	number of microtubules extending beyond the lamellar strip	number of microtubules ensheathing the nucleus	section numbers in reconstructions
anterior tip of lamellar strip	26–*29*–33	55–*59*–64	22–*25*–28	*2.8*	0–*3*–6	—	1–2
	28–*31*–35	57–*60*–64	20–*22*–25	*2.9*	5–*10*–14	—	2–3
	34–*37*–43	53–*58*–62	19–*22*–25	*2.7*	9–*13*–19	—	3–4
	38–*56*–61	47–*53*–59	18–*21*–24	*2.6*	19–*34*–40	—	4–5
	46–*62*–68	45–*52*–61	17–*20*–22	*2.7*	36–*41*–47	—	5–6
	67–*70*–76	45–*51*–55	18–*19*–21	*2.7*	46–*51*–56	—	6–7
	79–*85*–90	46–*51*–57	19–*20*–21	*2.8*	58–*63*–69	—	7–8
	94–*106*–113	45–*49*–54	18–*19*–21	*2.7*	73–*88*–95	—	8–9
	116–*127*–139	38–*43*–48	13–*16*–18	*2.8*	103–*112*–122	—	9–10
	131–*152*–161	33–*37*–42	11–*13*–17	*2.7*	125–*139*–183	—	10–11
	149–*160*–173	28–*34*–38	9–*11*–14	*2.9*	138–*146*–164	—	11–12
anterior tip of nucleus	154–*169*–187	25–*29*–35	8–*10*–12	*2.7*	146–*157*–180	18–*27*–46	11–12
	158–*179*–198	22–*27*–36	7–*9*–12	*2.8*	149–*168*–186	34–*52*–78	12–13
	183–*202*–226	18–*22*–30	6–*8*–10	*2.7*	178–*194*–218	74–*90*–115	13–14
	198–*218*–234	14–*18*–26	5–*7*–9	*2.8*	193–*210*–226	94–*107*–126	14–15
	194–*226*–244	12–*15*–19	4–*7*–8	*2.6*	188–*213*–239	110–*134*–154	15–16
	231–*278*–298	8–*11*–14	4–*5*–8	*2.5*	228–*272*–292	142–*194*–233	16–17
	269–*293*–305	3–*6*–8	1–*3*–4	*2.2*	269–*290*–299	179–*204*–244	17–18
posterior tip of lamellar strip	274–*312*–332	—	—	—	—	169–*217*–257	17–18
	257–*283*–320	—	—	—	—	197–*238*–267	18–19
	273–*297*–329	—	—	—	—	238–*257*–278	19–20
	287–*304*–331	—	—	—	—	263–*284*–316	20–21
	271–*291*–323	—	—	—	—	258–*285*–297	21–22
	217–*228*–241	—	—	—	—	211–*219*–233	22–23
	173–*184*–192	—	—	—	—	173–*182*–187	23–24
posterior tip of nucleus	118–*145*–168	—	—	—	—	—	23–24
	34–*76*–124	—	—	—	—	—	24–25

greatly enhance our understanding of both the development of the band and its interaction with the plasma membrane and the nuclear envelope.

(*c*) *The osmiophilic crest*

The mature spermatozoids of *Equisetum* possess a more complex osmiophilic crest than any hitherto described in bryophytes and pteridophytes. However, its possible homology with the electron-opaque cap covering the apical cone in the male gametes of cycads (Norstog 1967, 1968) should not be overlooked. The banded substructure, the electron-transparent central strip, the continuity with sheets of endoplasmic reticulum, and the extremely variable profile are all unique to *Equisetum*. The last feature is particularly striking where the crest extends as a broad wedge between the cell membrane and the mitochondrion subjacent to the lamellar strip, since in *Pteridium* (Duckett 1975*a*), *Anthoceros* (Duckett 1975*b*), and *Marchantia* (Kreitner& Carothers 1975) the crest is restricted to the external anterior edge of the microtubular band.

By virtue of its intimate association with the anterior end of the microtubular band, the osmiophilic crest, like the lamellar strip, has been considered as both a synthetic and structural component of archegoniate gametes (Bell & Duckett 1976; Duckett 1973*a*, 1975*a*; Kreitner & Carothers 1976). Although it clearly resembles the microtubule organizing centres found in diverse organisms (Hepler & Palevitz 1974), its persistence and maximal differentiation at maturity in *Equisetum* (and similarly in *Pteridium*) strongly suggests that a mechanical role in maintaining the structural integrity of the anterior end of these gametes is more likely. Its physical continuity with both the lamellar strip and microtubular band internally, and its links with the plasma membrane externally are further features consistent with the view that it is an intracellular cement.

(*d*) *Flagella and basal bodies*

The substructure of the flagella and their basal bodies in the mature spermatozoids of *Equisetum* is essentially identical with that seen in the spermatids (Duckett 1973*a*). Both cartwheel and stellate portions of the basal bodies are retained in an apparently unaltered form. Such developmental uniformity is exceptional in other archegoniates. In the moss *Polytrichum*, the electron-transparent matrix in the arms of the stellate profile becomes occluded with dense material (Paolillo 1974; Paolillo *et al.* 1968*a*, *b*), and in leptosporangiate ferns (Bell & Duckett 1976; Duckett 1975*a*) a plug of osmiophilic material, continuous with the osmiophilic reticulum in which the basal bodies are invested, enters the proximal end of the basal body, and the cartwheel disappears. However, in common with ferns, all the triplet microtubules in the basal bodies of *Equisetum* are of the same length. In bryophytes (Paolillo 1974), and probably cycads (Norstog 1974) certain of the triplets are longer than the remainder.

The tips of the flagella in *Equisetum* closely resemble those in *Pteridium* and *Anthoceros* (Duckett, Moser & Carothers, unpublished observations). The two central tubules disappear first and, as the tip tapers, the outer doublets gradually cease until only a few remain. It is noteworthy that this situation is also found in *Vaucheria* (Ott & Brown 1974), while in some members of the Chlorophyceae (for example, *Chlamydomonas* (Ringo 1967)) the nine doublets disappear before the central pair. Since *Chlamydomonas* appears to be far from the green algae related to archegoniates (Pickett-Heaps 1975; Pickett-Heaps & Marchant 1972), it would be pertinent to know the kind of axonemal tips which occur in those green algae which are probably closer and which possess a MLS (see table 1). An *Equisetum*-like flagellum tip is present in *Chara* (Pickett-Heaps 1968), suggesting that this feature may prove significant in phylogenetic studies.

The semi-circular collars of electron-opaque material investing the basal bodies in *Equisetum* appear to be unique. In leptosporangiate ferns the basal bodies continue to lie in an osmiophilic reticulum, whilst in *Marsilea* (Rice & Laetsch 1967) a cylinder of dense material with a transparent centre descends from the lower end of each basal body towards the microtubular band. The basal bodies of cycads are embedded in an electron-opaque matrix (Norstog 1967, 1968, 1974, 1975), and in *Lycopodium* (Carothers *et al.* 1975) are invested by material with a globular texture. The most likely role of this osmiophilic material is anchorage of the basal bodies (Duckett & Bell 1971; Carothers *et al.* 1975), and possibly the transmission of stimuli necessary for coordinated flagellar motion.

(*e*) *Antheridial plastids*

In the presence of amyloplasts in the central cytoplasm of the mature spermatozoid, *Equisetum* resembles other archegoniates (Duckett 1975*a*, *c*; Paolillo 1974) and many green algae (Moestrup 1975). However, in contrast to the ferns (Duckett & Bell 1971; Myles 1975), this cytoplasm, which must add considerably to the mass of the gamete, is not shed during swimming. There is no evidence that the starch is mobilized as an energy source, or that the grains are involved in geoperception. It is possible that it ultimately contributes to the food reserves of the zygote, but this has yet to be demonstrated.

Considerations of function apart, there are considerable differences in the number and arrangement of the starch grains in different groups of archegoniates. In the monoplastidic gametes of bryophytes (Paolillo 1974) the amyloplast may contain one (for example, *Anthoceros* (Duckett 1975*c*) and *Sphagnum* (Manton 1957)), or several grains which may, in turn, be clustered (for example, *Physcomitrium* (Lal & Bell 1975) and *Sphaerocarpos* (Zimmermann 1973)), or linearly disposed (for example, *Pellia* (Suire 1970)). In the multiplastidic spermatozoids of vascular archegoniates one starch grain in each plastid is normal in *Pteridium* (Duckett & Bell 1971) and *Equisetum*, but several are found in *Osmunda* (Duckett, unpublished data) and *Marsilea* (Rice & Laetsch 1967; Myles 1975). These features may be found to have phyletic significance, as have those of the amyloplasts of sieve tubes in angiosperms (Behnke 1973).

In contrast to those of the spermatozoid, the amyloplasts of the antheridial jacket in *Equisetum* contain well-developed thylakoid systems. However, these display certain features not hitherto described from other parts of the gametophyte (Duckett, unpublished observations), or the sporophyte (Whatley 1971). Whereas the mature chloroplasts normally contain several grana, those in the cells surrounding the spermatocytes, usually display only one or two very large grana. The closely packed osmiophilic globuli are presumably the sites of accumulation of the carotenoid pigment (Hauke & Thompson 1973) responsible for the pink or red coloration of the antheridial branches (Duckett 1970*a*, 1972). A similar accumulation of osmiophilic globuli is correlated with the change from green to orange in maturing antheridia of *Anthoceros* (Duckett 1975*c*).

(*f*) *Mitochondria*

In common with the spermatozoids of all other pteridophytes and bryophytes, but contrasting sharply with cycads and some algal zoospores (table 1), the lamellar strip in *Equisetum* lies above a specialized mitochondrion. This is invariably restricted to the inner surface of the lamellar strip, and thus decreases in width as the number of lamellar plates declines posteriorly along the strip. However, in the ferns *Pteridium*, *Dryopteris* (Duckett 1975*a*; Bell & Duckett 1976) and *Osmunda* (Duckett, unpublished observations), this mitochondrion remains the same

width throughout its length and, as the lamellar strip narrows posteriorly, extends into direct contact with the microtubular band.

As in ferns the maturation of the spermatozoids of *Equisetum* is accompanied by an increase in the size and number of the mitochondrial cristae, and an increase in the electron opacity of the matrix. However, no regular orientation of the cristae can be detected in *Equisetum* whereas in *Marsilea* the cristae lie perpendicular to the microtubular band (Myles & Bell 1975), and in bryophytes, these two components are parallel (Carothers 1975; Paolillo *et al.* 1968*b*; Zimmermann 1973).

The numerous mitochondria in the central region of the *Equisetum* spermatozoid show no regular arrangement. This differs from the situation in *Pteridium* (Duckett 1975*a*), where some of the mitochondria are closely appressed to the inner surface of the microtubular band between the nucleus and the lamellar strip. These mitochondria are not discarded with the remainder of the cytoplasm, apparently being held in position by a sheet of osmiophilic material which is lacking in *Equisetum*. Also absent in *Equisetum* is the osmiophilic strip extending from the anterior edge of the MLS around the specialized anterior mitochondrion, in *Pteridium* (Duckett 1975*a*) and *Anthoceros* (Duckett 1975*b*). This strip may serve to anchor the mitochondrion and protect it from osmotic forces, to which, because of the retention of the cytoplasm, the corresponding mitochondrion in *Equisetum* would not be exposed.

On the assumption that the number of mitochondrial profiles is related to their actual number, we estimate that the mature spermatozoids of *Equisetum* contain not less than one hundred of these organelles. However, recent studies have demonstrated the presence of a single mitochondrial reticulum at specific times in the life cycle of several algae and fungi (for a review, see Atkinson, John & Gunning 1974). A fusion process which results in a single mitochondrion characterizes spermatogenesis in many animals (Fawcett 1970) and bryophytes (Duckett 1975*b*). Thus, the possibility that the mature male gametes of *Equisetum* contain a smaller number of much branched mitochondria cannot be discounted. Nevertheless, in terms of mitochondrial volume, the spermatozoids of *Equisetum* are intermediate between those of ferns (50–60 mitochondrial profiles (Bell & Duckett 1976) and cycads (several thousand (Norstog 1975)).

(*g*) *The nucleus*

In common with *Pteridium* (Duckett 1975*a*) the condensation of the chromatin in the spermatid nucleus, as it is seen in electron micrographs, takes place very quickly. In the several hundred antheridia examined, partially condensed chromatin was never encountered. Chromatin condensation in bryophytes however, is much more gradual. (Carothers 1973, 1975; Genevès 1970; Kreitner 1970; Lal & Bell 1975; Paolillo 1974; Paolillo *et al.* 1968*a*, *b*; Suire 1970; Zimmermann 1974). Here a variety of chromatin structures of increasing magnitude appear sequentially from soon after the association of the nucleus and the MLS in the young spermatids. Thus, plant spermatozoids appear to share with those of animals considerable diversity in the behaviour of the chromatin (Kaye 1969; Kaye & McMaster-Kaye 1975). Moreover, in both kingdoms, it is equally difficult to interpret morphological changes at the ultrastructural level in terms of macromolecular reorganization in the nucleus, in particular the rearrangement of the DNA. Some kind of alignment of the DNA is readily demonstrable in the nuclei or archegoniate spermatozoids with polarized light (Pfeiffer 1949), but it is not known whether the ordering of the chromatin is accompanied by changes in the nucleoproteins similar to those seen in animals (Kaye & McMaster-Kaye 1974, 1975; Gledhill 1975).

A most unusual feature of *Equisetum* spermatozoids is the heterogenous internal structure of the nuclei, a situation readily seen with the light microscope (Sharp 1912). Peripheral, membrane-bound, bodies have not been reported in any other archegoniate spermatozoids, and naked electron-opaque globules have been found elsewhere only in the nuclei of *Lycopodium* (where they amount to about 30 % of the volume) (Z. B. Carothers & R. R. Robbins, personal communication) and *Osmunda* (about 10 %) (Duckett, unpublished observations). At present we do not know the origin or composition of these bodies, but animal reproductive cells perhaps provide certain clues (see Franke (1974) and Kessel (1973) for reviews). During spermatogenesis in many animals blebs of nuclear envelope are released into the cytoplasm (the so-called redundant nuclear envelope), possibly a device for reducing the area of the nuclear envelope in step with the changing nuclear shape. In *Equisetum* the membrane-bound vesicles within the nucleus are perhaps similar portions of redundant nuclear envelope whose elimination into the cytoplasm has been prevented by the ensheathing microtubular band. The naked electron-opaque bodies in the nucleus may be identical with similar bodies in the cytoplasm. Massive extrusion of finely granular, electron-opaque material, so far of unknown composition, frequently accompanies chromatin condensation in animal spermatogenesis (Bawa 1975; Kessel 1973; Schrankel & Schwalm 1974), and a similar process may occur in *Equisetum*. Here, however, the total elimination of these bodies from the nucleus is perhaps prevented by the rapidity with which the chromatin condenses.

A final noteworthy feature of the spermatozoid nucleus in *Equisetum* is the persistent clarity of its envelope. A perinuclear space, delimited by clear unit membrane profiles, is always present and, in the regions not ensheathed by the microtubular band pores remain visible. These features are rarely discernible in other archegoniate spermatozoids, a situation which is particularly striking in the liberated gametes of *Pteridium* (Bell 1975; Bell *et al.* 1971), where the nuclear envelope condenses to a single thin osmiophilic layer. This difference in the behaviour of the nuclear envelope is again most probably to be related to the continued protection of the nucleus by the cytoplasm throughout the life of the *Equisetum* spermatozoid.

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Key to abbreviations used on plates and figures

All plates relate to *Equisetum hyemale* unless otherwise stated. BB, basal bodies; CS, spherical, cytoplasmic, electron-opaque bodies; ER, endoplasmic reticulum; F, flagella; FG, flagella groove; FP, closed pit at the anterior end of the flagella groove; K, microtubular keels; LS, lamellar strip; L_1, L_2, L_3, outer, middle and inner lamellar strata respectively; M, mitochondria; MB, microtubular band; MI, microbodies; MA, spiral mitochondrion subjacent to the lamellar strip; MLS, multilayered structure; N, nucleus, NE; nuclear envelope; NP, nuclear pores; NS, spherical, intranuclear, electron-opaque bodies; OB, collars of osmiophilic material around the proximal ends of the basal bodies; OC, osmiophilic crest; OT, electron-transparent central region of the osmiophilic crest; PJ, plastids of antheridial jacket cell; PM, plasma membrane; S, starch grains.

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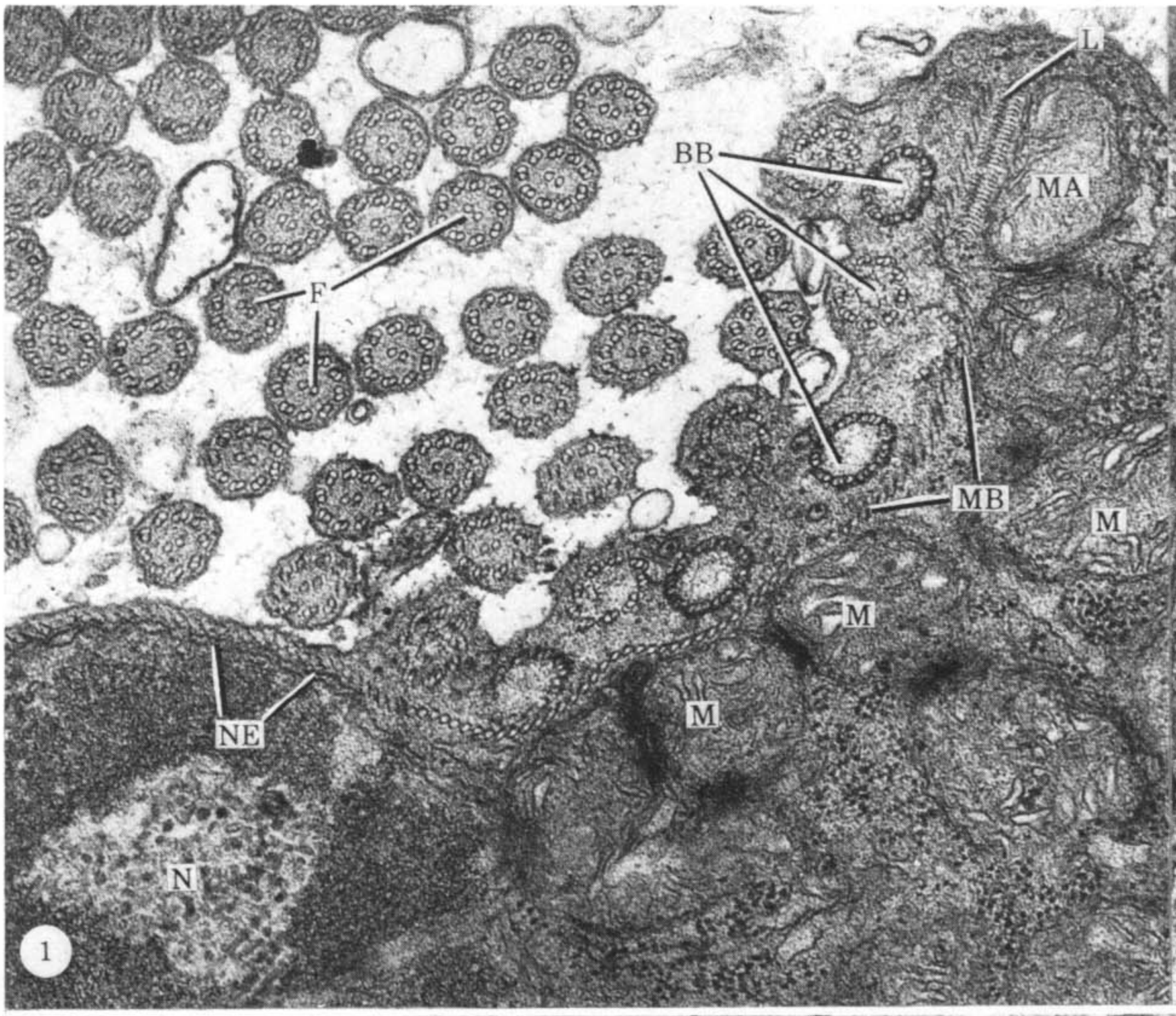
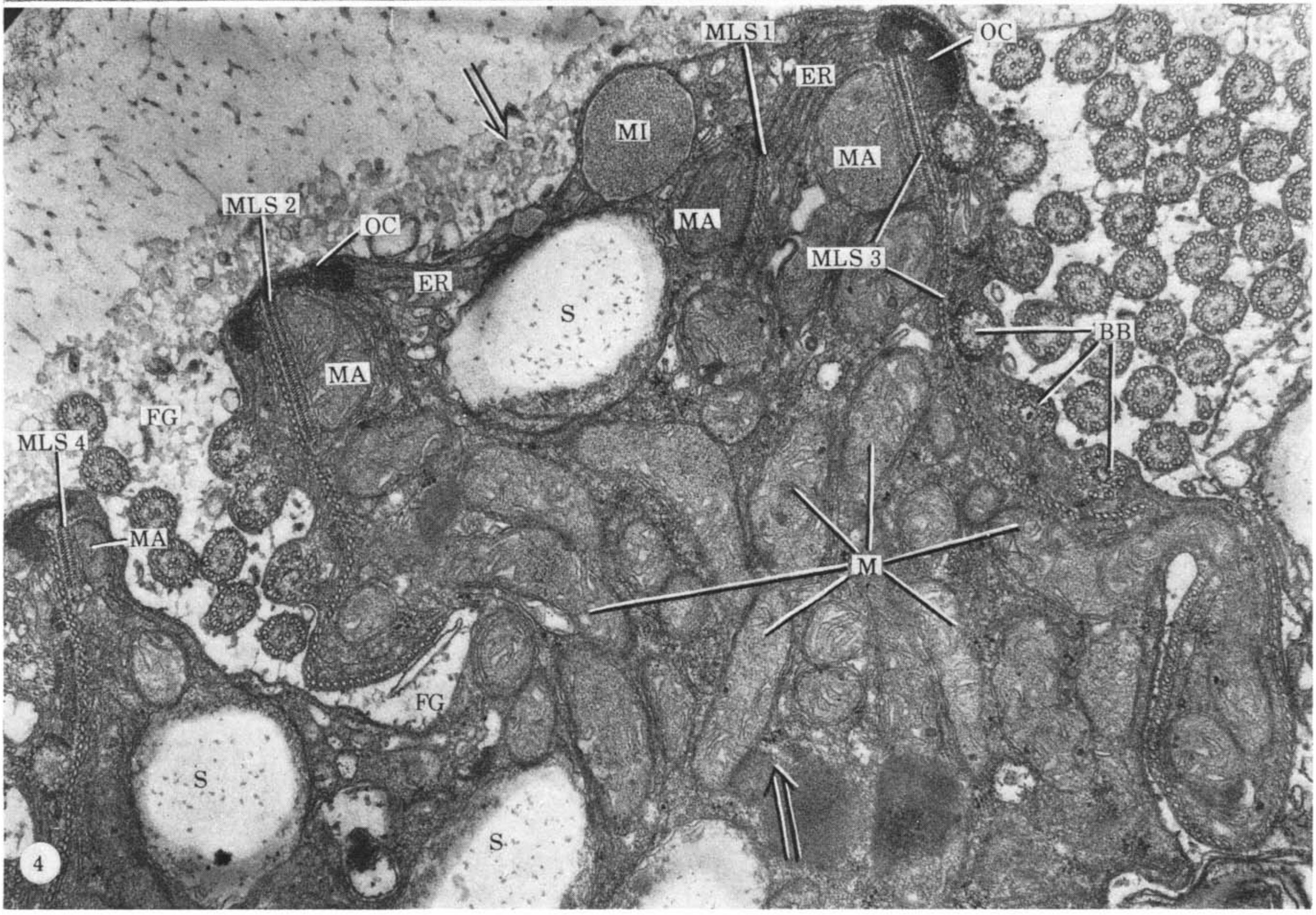
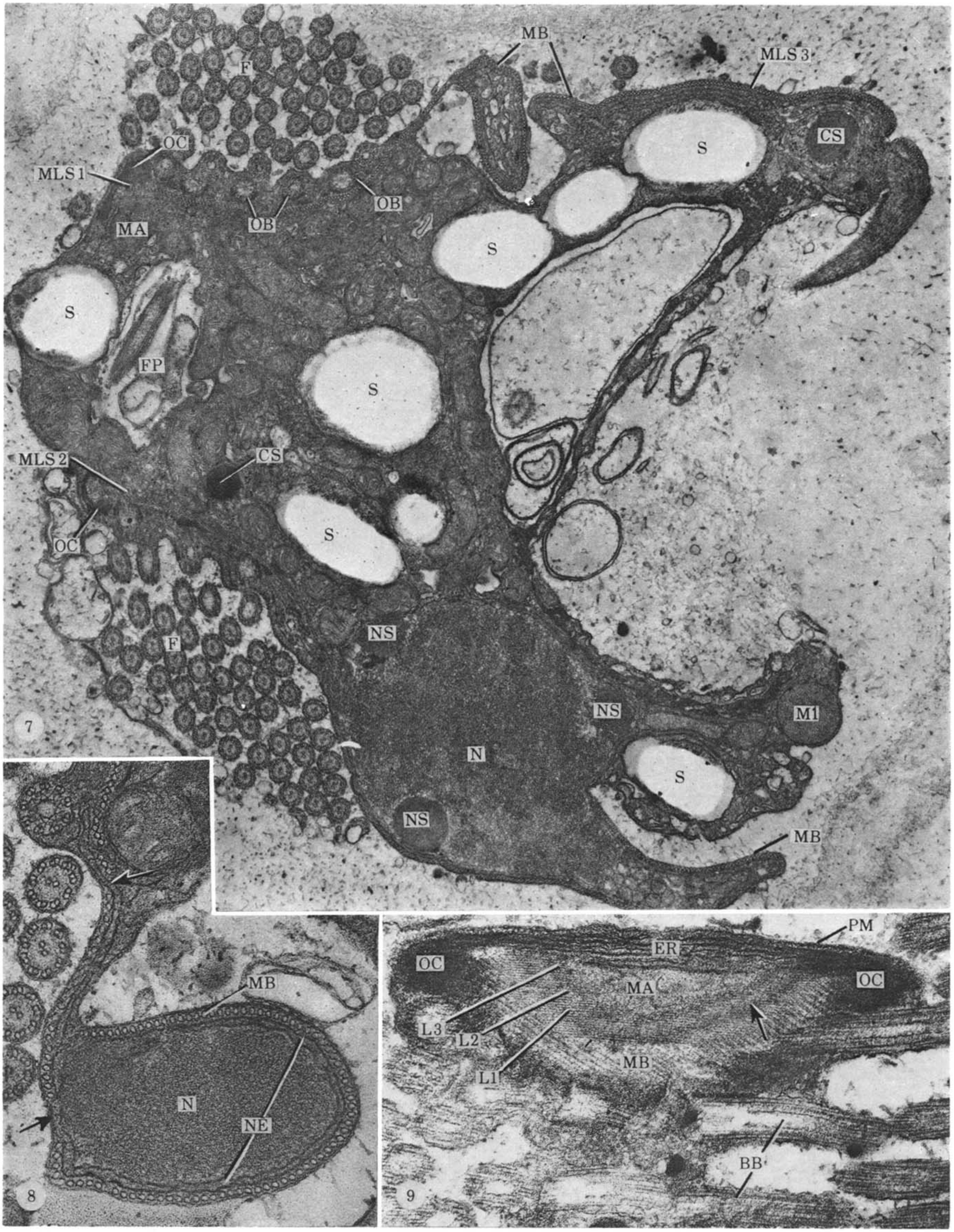
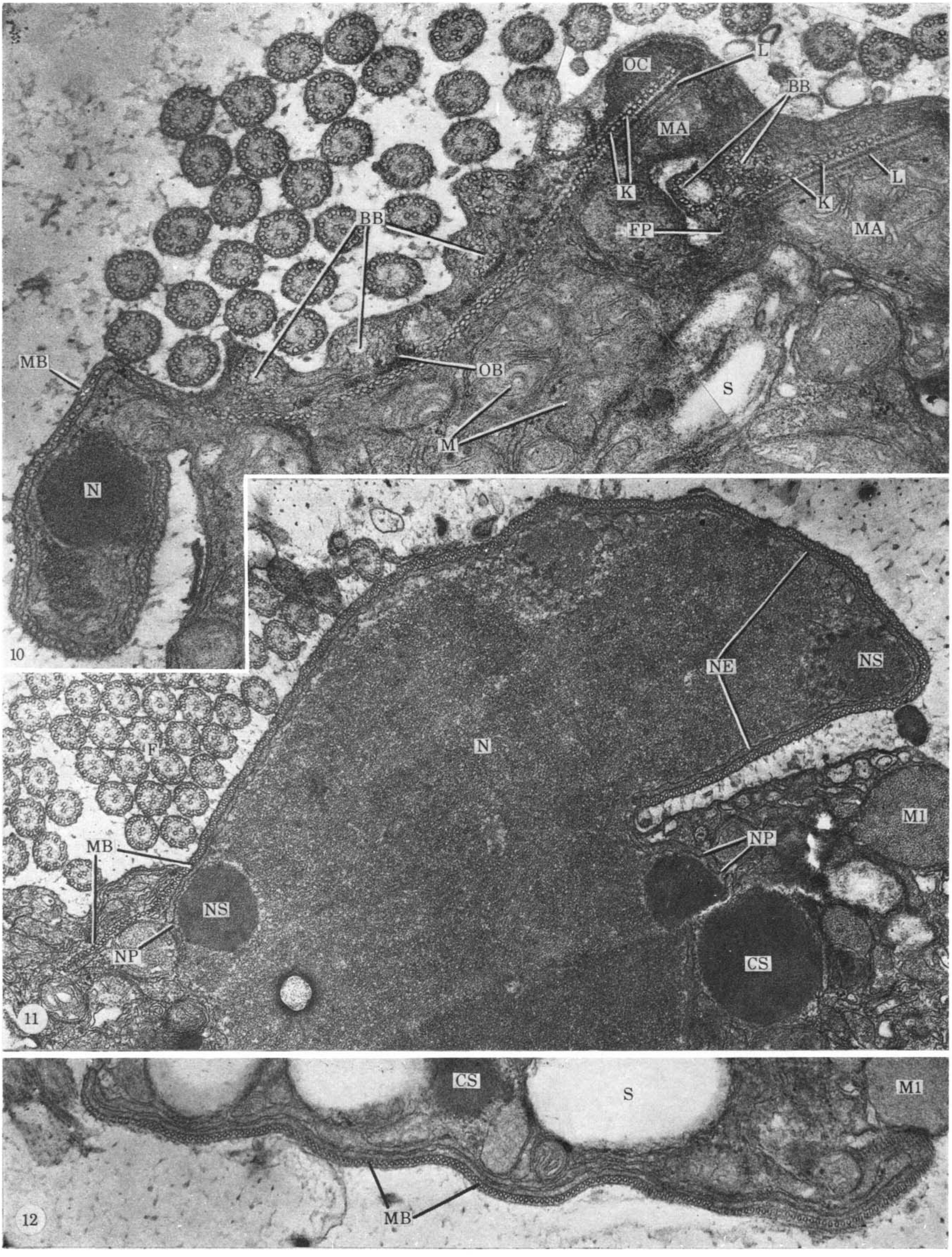




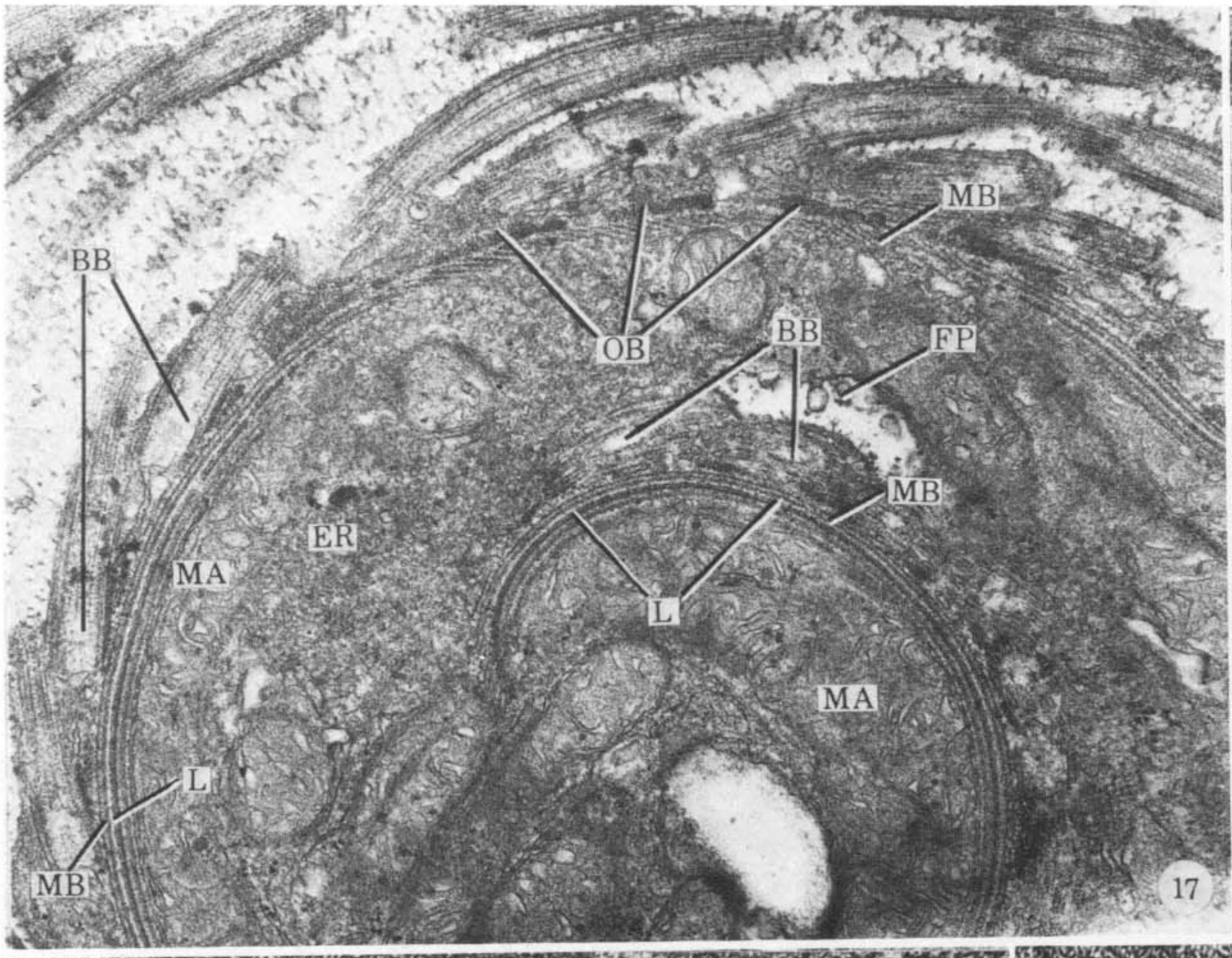
FIGURES 1, 2 AND 4. For description see opposite.

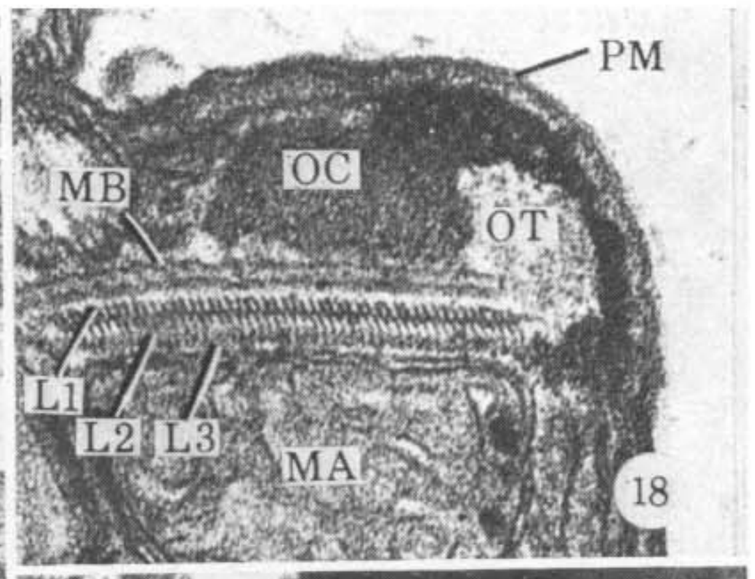


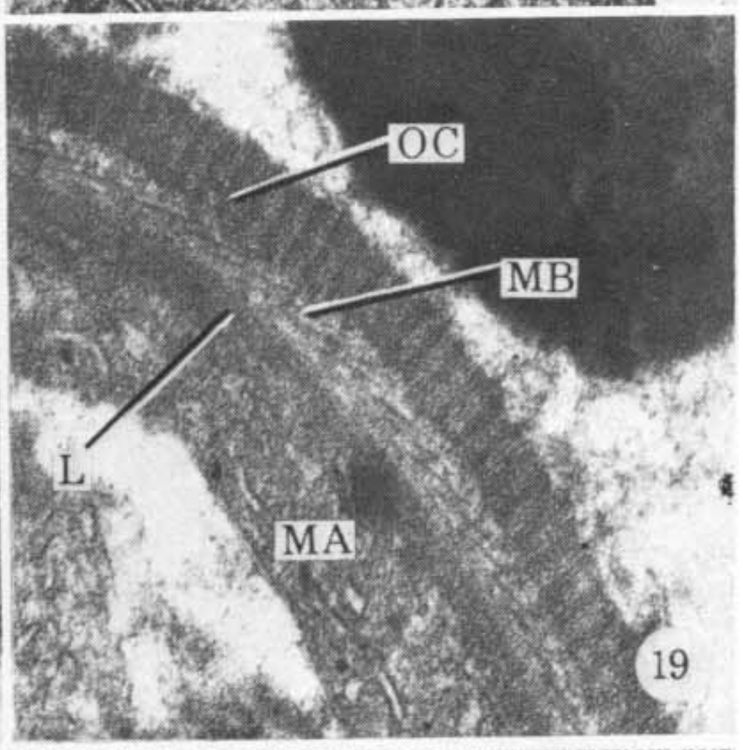
FIGURES 7-9. For description see opposite.

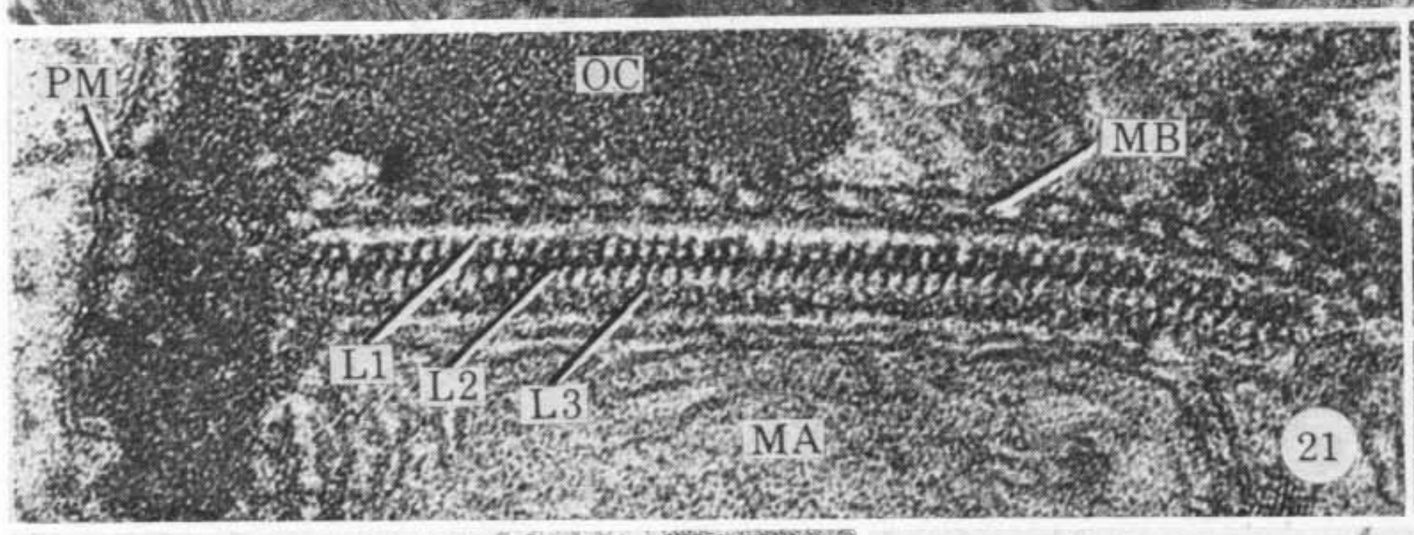


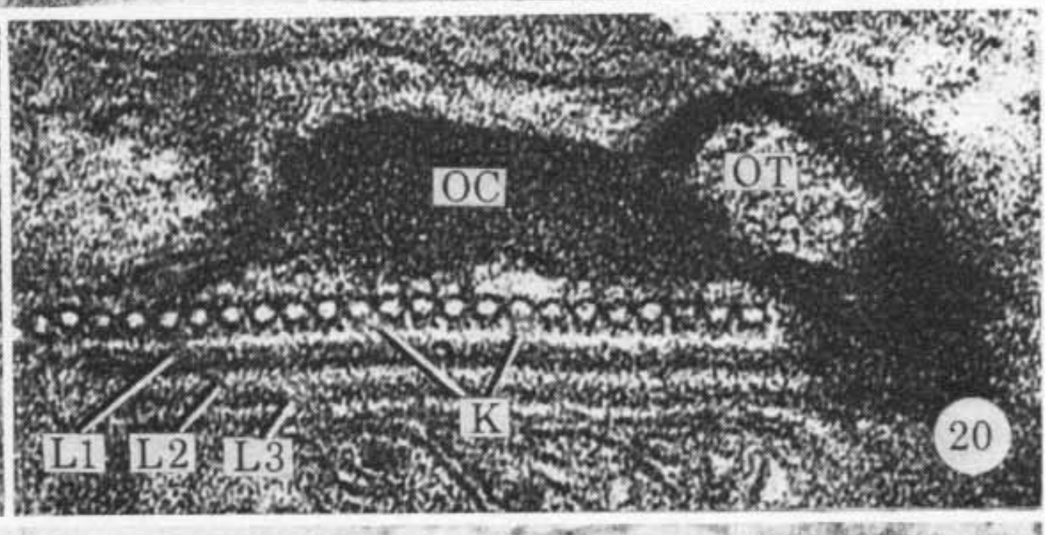
FIGURES 10–12. For description see opposite.

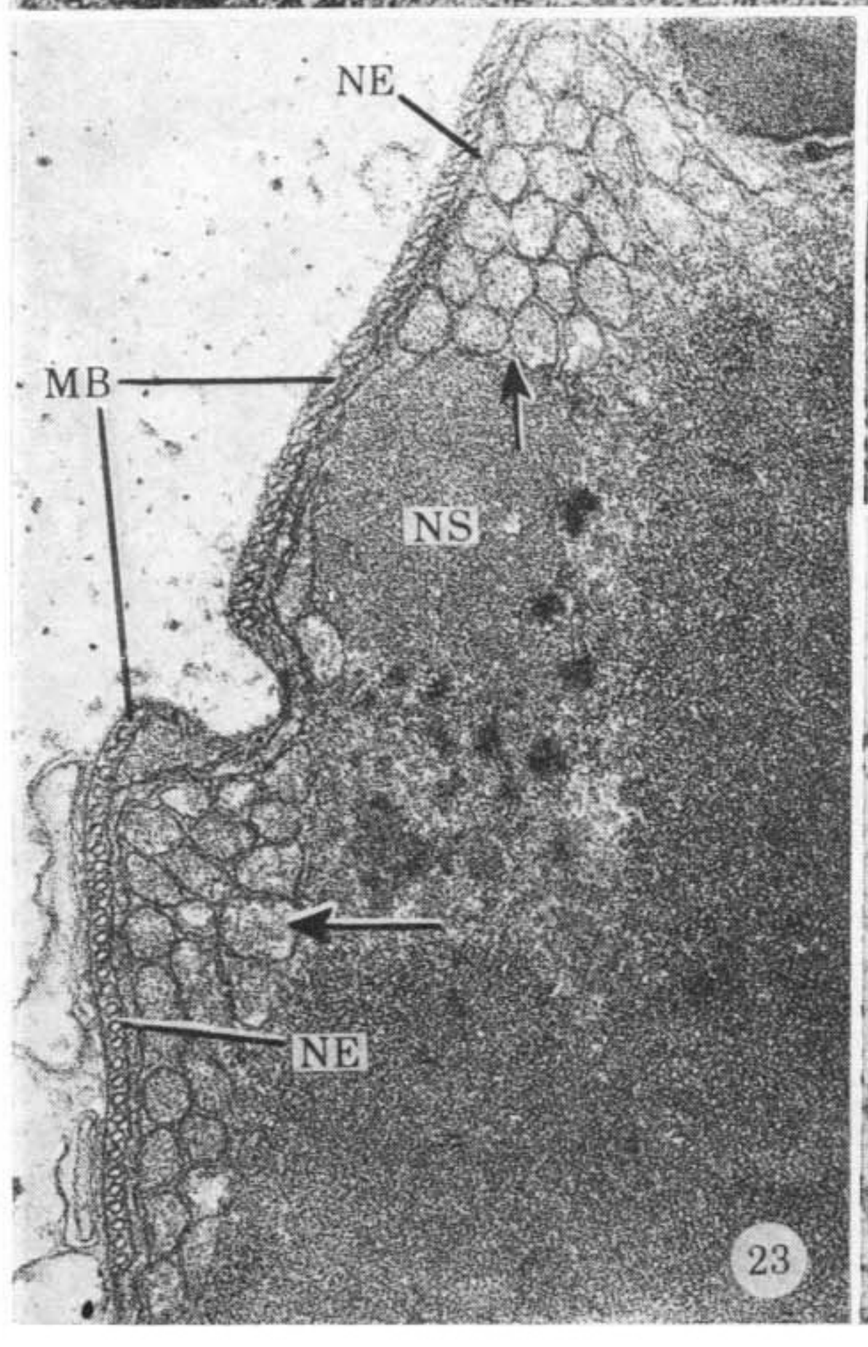


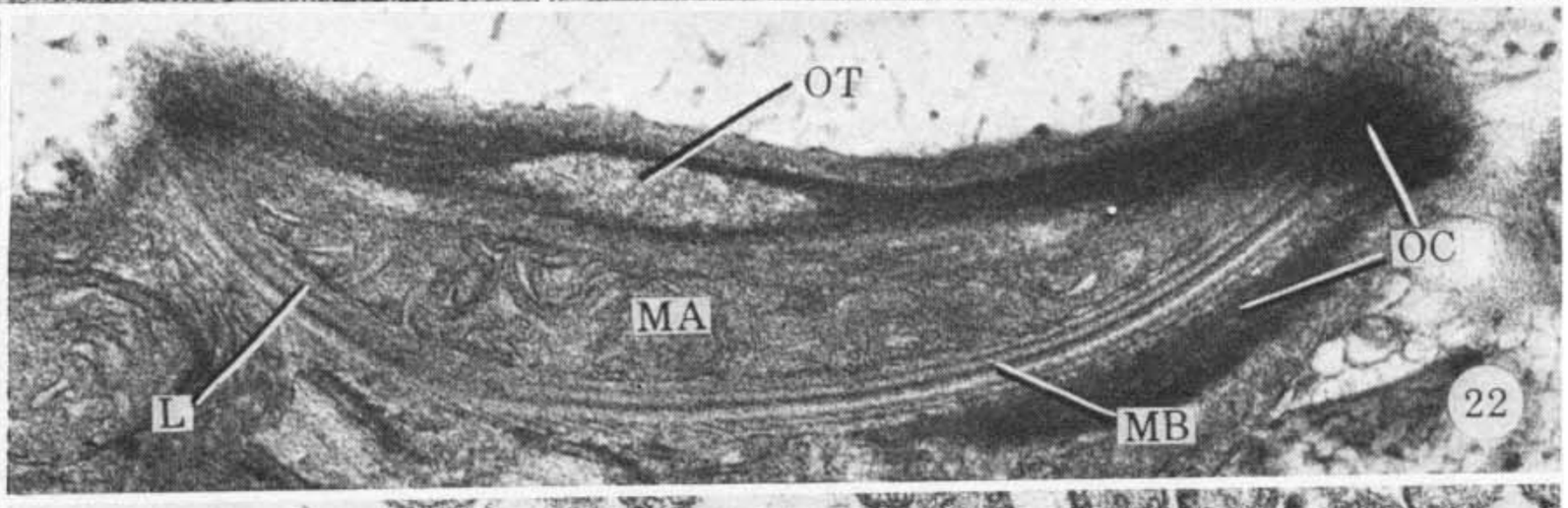


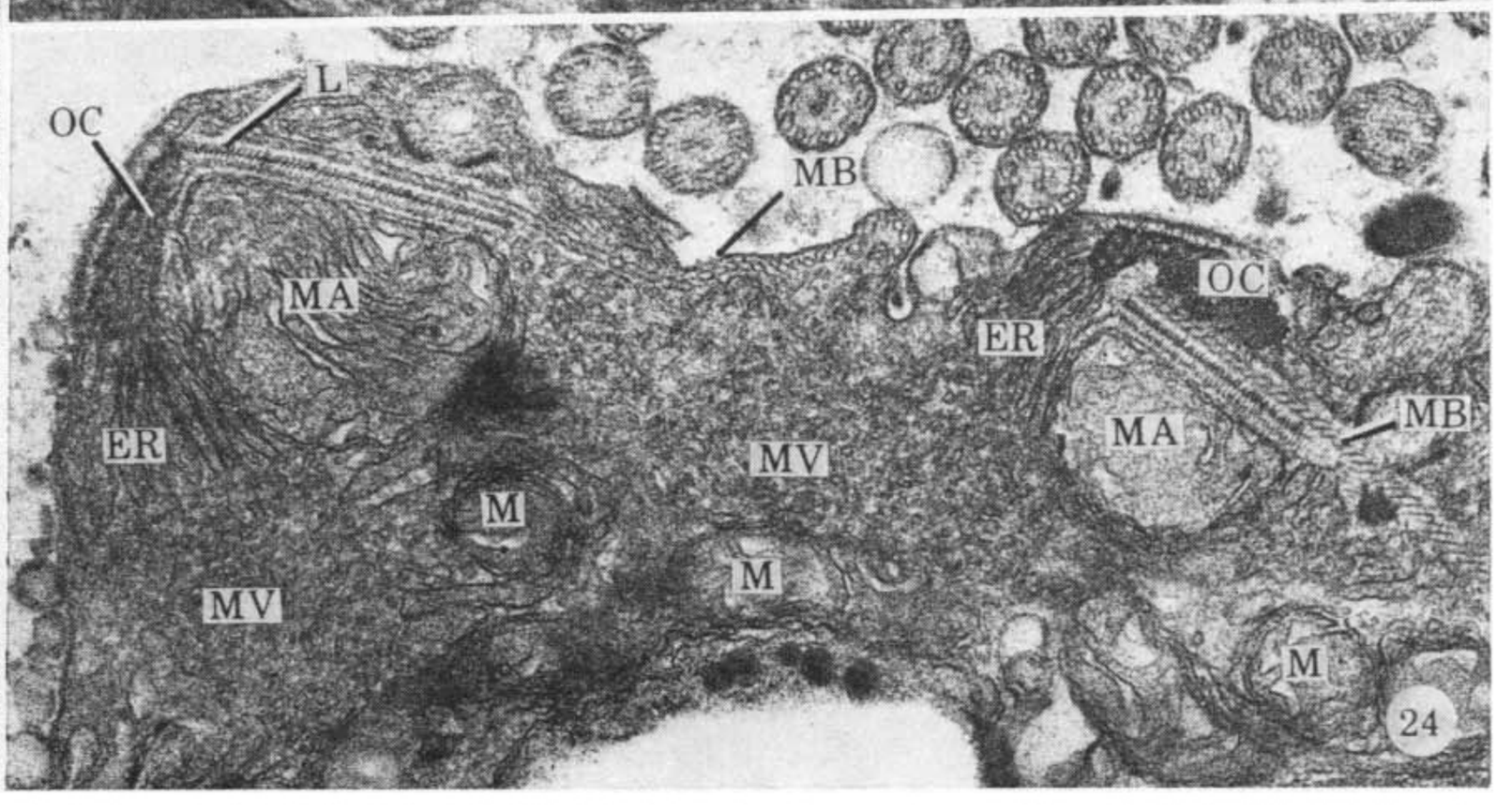




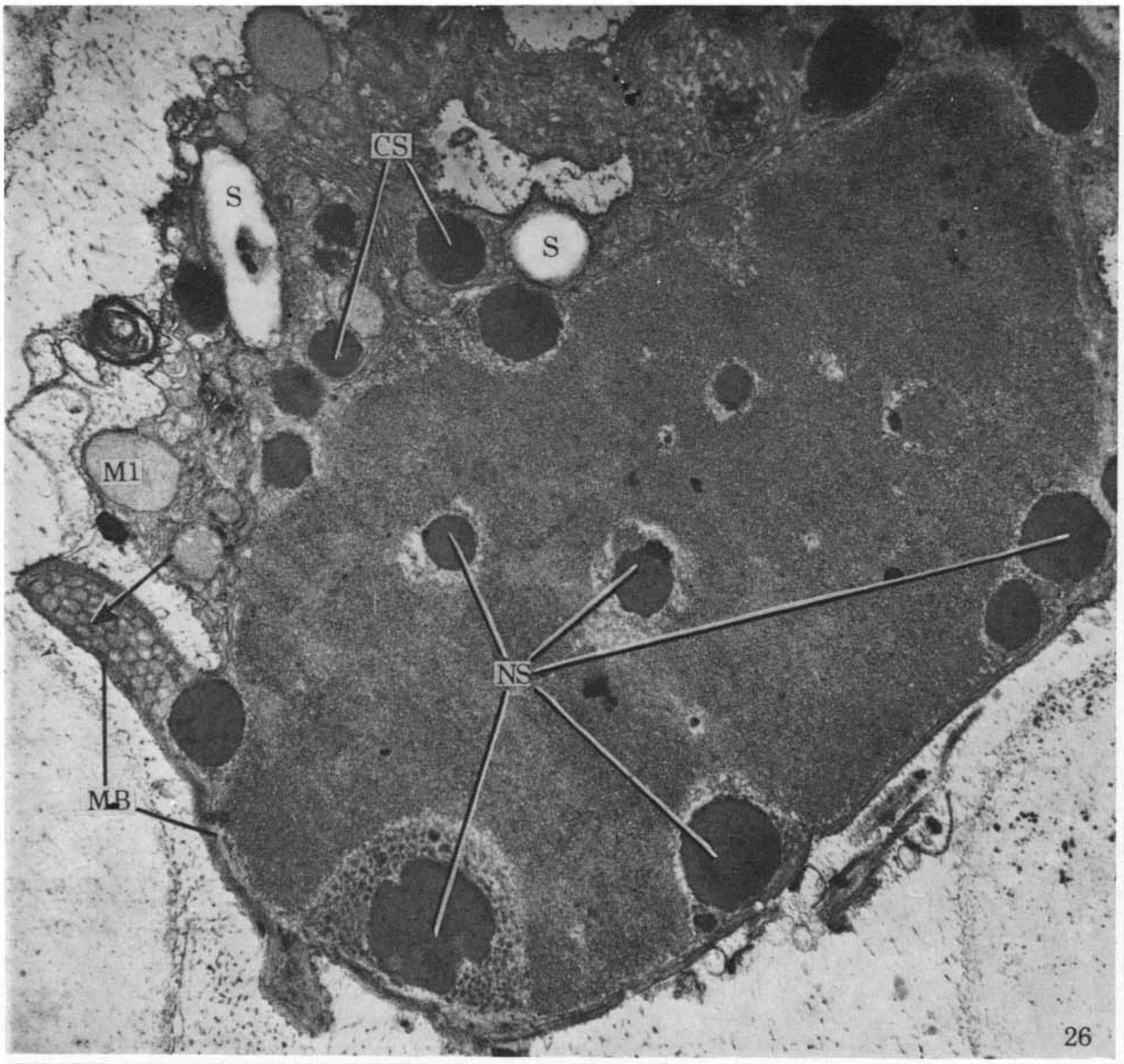


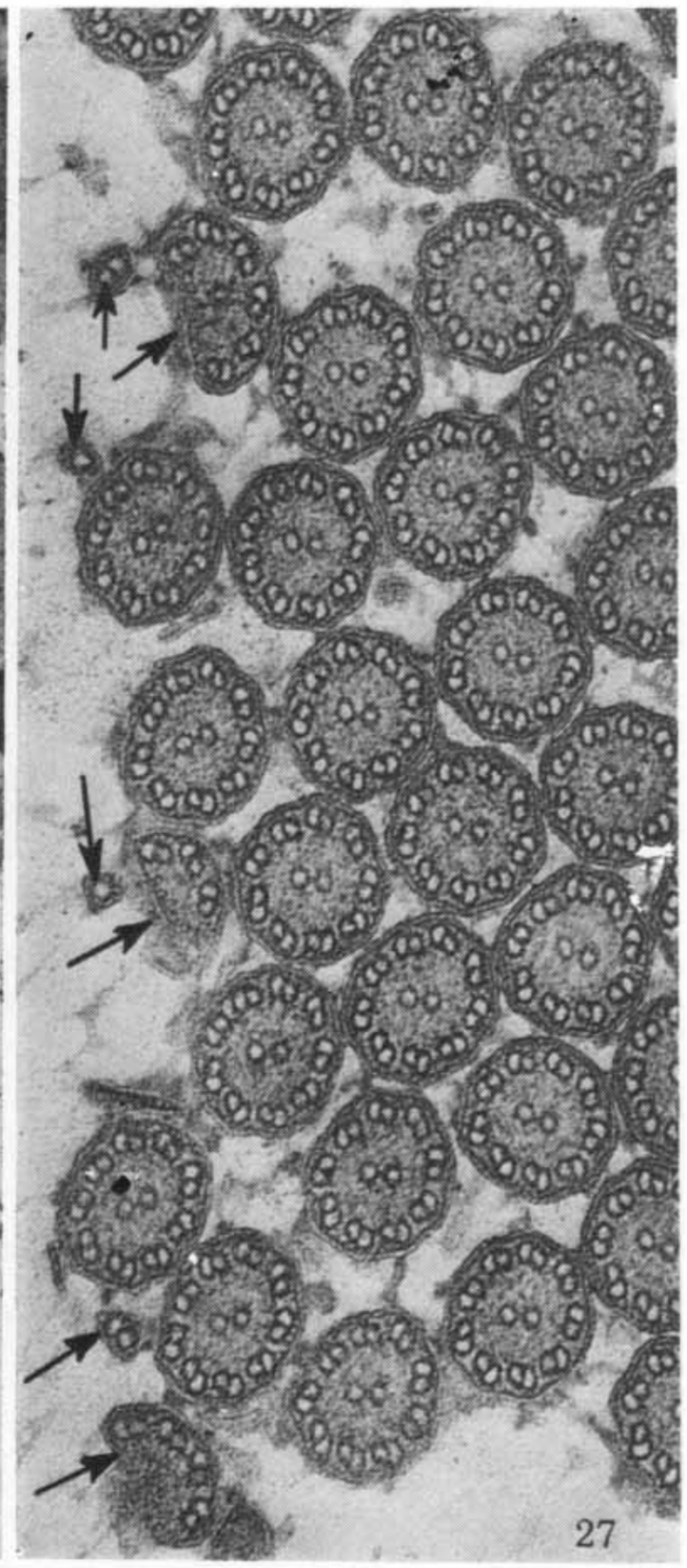


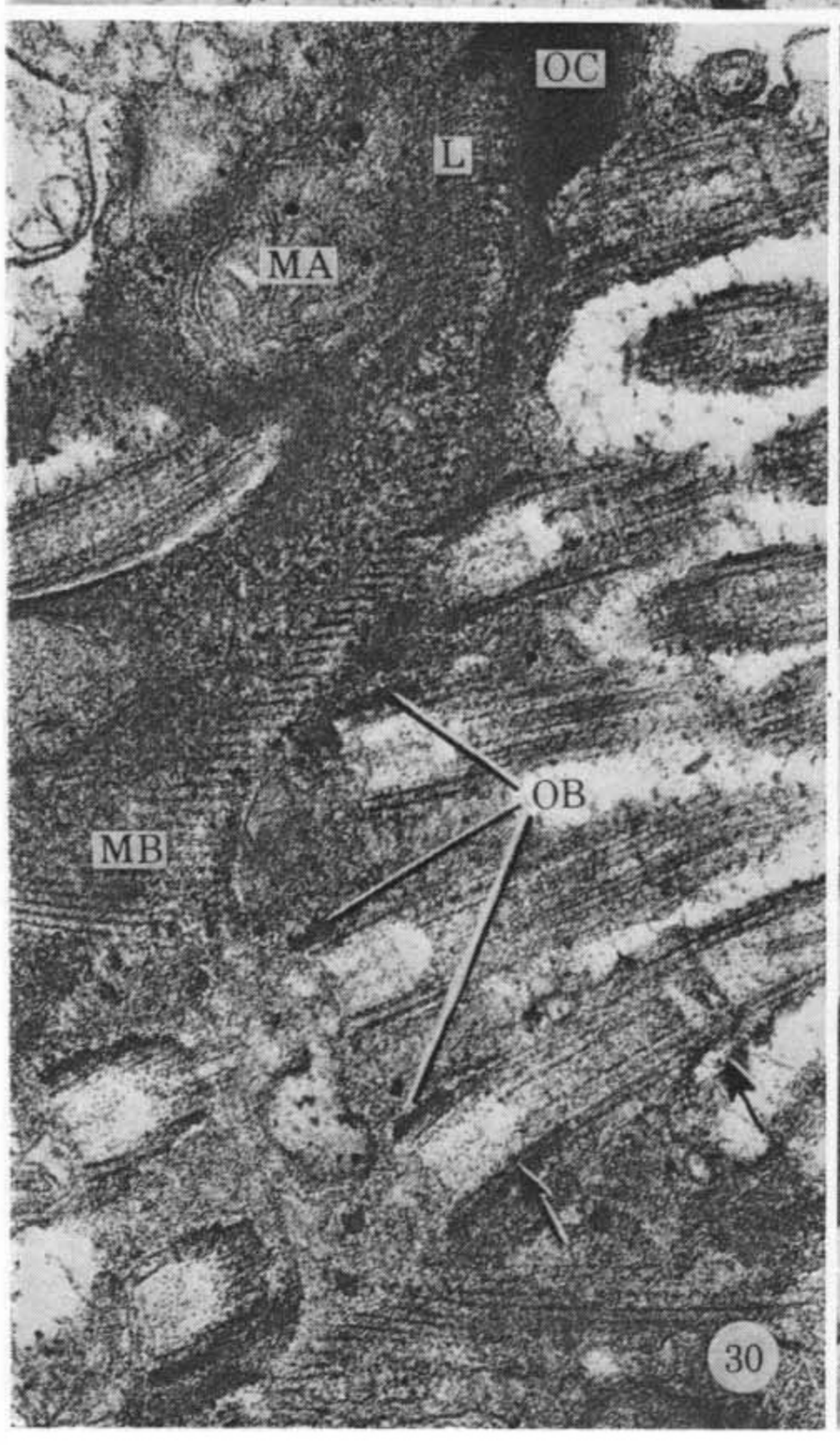


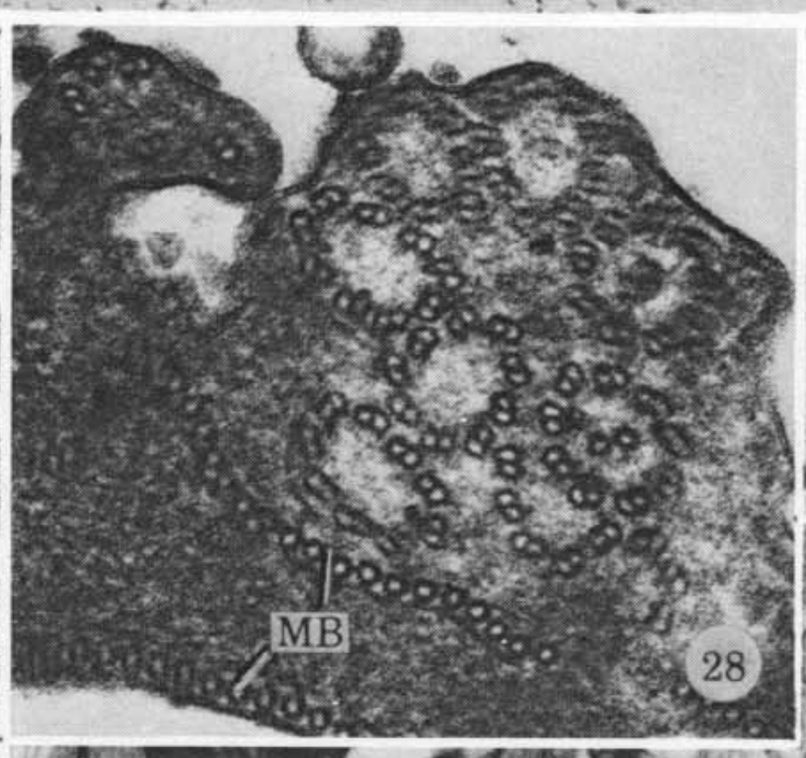


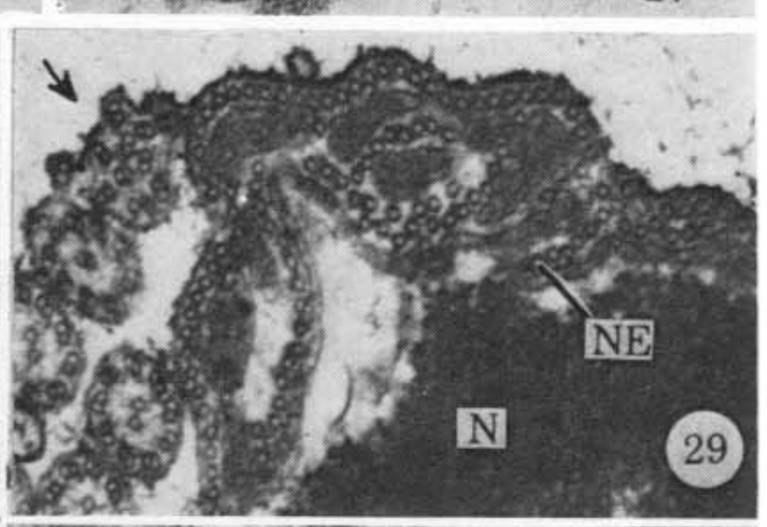
FIGURES 17–24. For description see opposite.

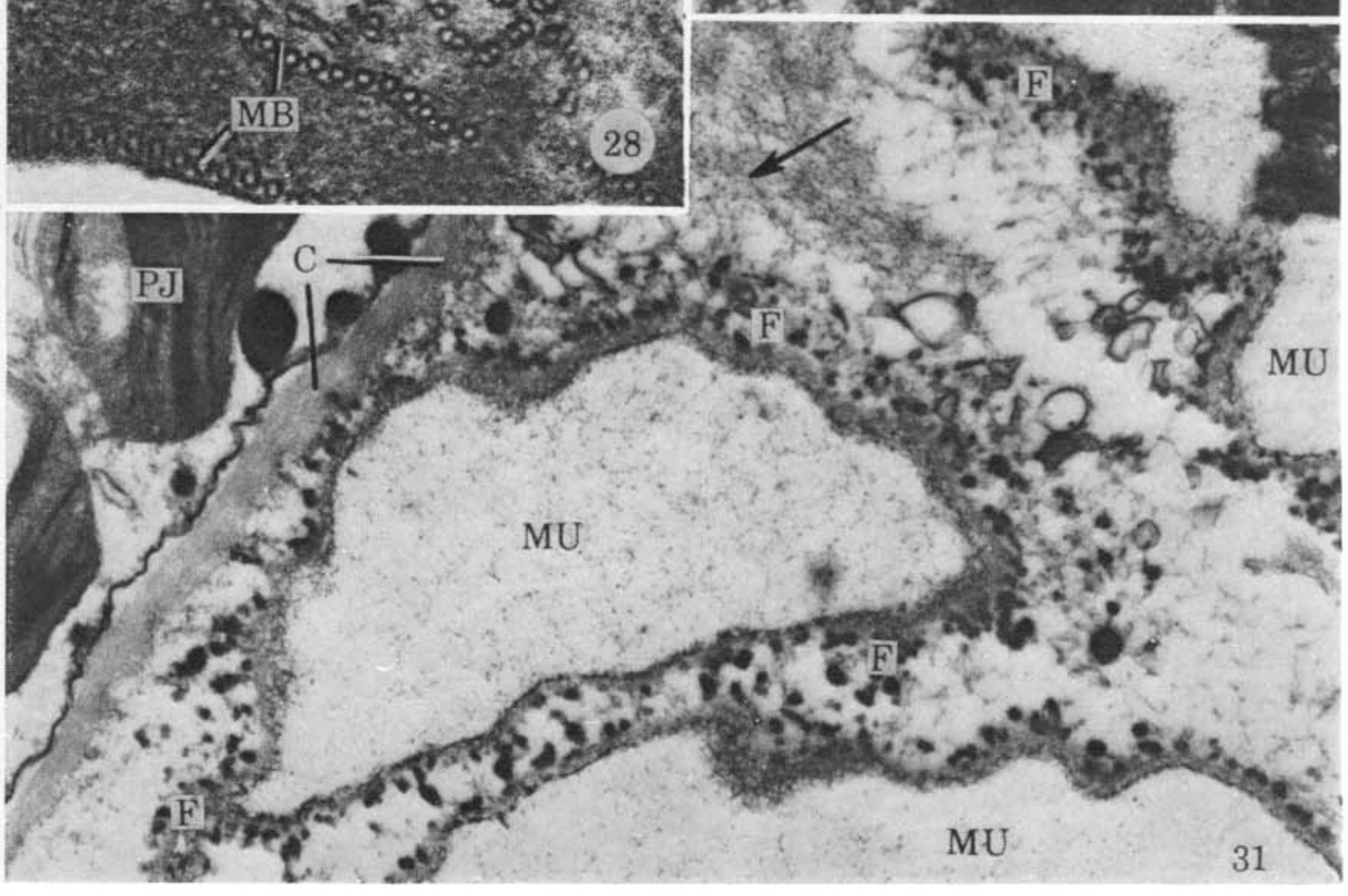












FIGURES 26–31. For description see opposite.