ULTRASTRUCTURE AND DIFFERENTIATION IN *CHARA* (*FIBROSA*)

IV. SPERMATOGENESIS*

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[*Manuscript received December* 12, 1967]

Summary

Spermatogenesis in *Chara* is described at the ultrastructural level. A large number of mitoses form spermatogenous threads, derived from the capitula inside the antheridium. Centrioles appear in the spermatogenous cells early as indistinct organelles that quickly become conspicuous. There is some evidence for the existence of a procentriole. Centrioles are subsequently associated in a normal fashion with the mitotic spindle. Intracellular differentiation commences after mitosis ceases. The nucleus moves to one side of the cell; a flat band of manchette microtubules is soon formed near it. The microtubules, which increase in number and elongate considerably, are inserted at one end into (and possibly extruded from) a densely staining, homogeneous inclusion, termed the manchette adjunct, which appears close to the centrioles. The centrioles, connected together by a spindle-shaped ciliary rootlet structure, move to the edge of the cell and start extruding flagella, which are covered in scales; another organelle, termed the vesicular adjunct and of unknown significance, appears near these centrioles. The manchette grows in length, and so assumes a spiral course in the cell; plastids then line up along the microtubules next to the nucleus. While still interconnecting cells, the cytoplasm shrinks steadily. With further elongation of the manchette, the flagella apparatus moves away from the nucleus, and mitochondria also line up along the manchette tubules between them. Lipid (?) bodies move near the plastids, which steadily accumulate starch. Golgi bodies show marked structural changes during differentiation; they are initially associated with a profusion of various vesicles, and later lose their identity, as does the endoplasmic reticulum which earlier interconnected cells through plasmadesmata. The nucleolus disappears, and later chromatin condensation gives the elongating nucleus an increasingly lamellate structure; finally these lamellae fuse to form a dense homogeneous nucleus. The cytoplasm continues to shrink, eliminating almost all cell organelles. The mature spermatozoid, tightly coiled in the cell, finally contains plastids and (lipoid) inclusions at one end, next to the dense, elongate nucleus, with linearly arranged mitochondria at the other end, and flagella inserted above the mitochondria. Manchette microtubules run the length of the organism as a flat band opposed to the nucleus and plastids, and finally as a tubular sheath partly enclosing the mitochondria. Four other tubules, possibly derived from a ciliary root structure, are also close to the mitochondria. The flagella are quite long by this stage. The observations are discussed in terms of the functions of cell organelles. In particular, it is suggested that centriolar movement in mitosis may be only one example of several morphogenic movements associated with microtubule organization; their function is in flagella formation, and not in synthesis of spindle, manchette, or other cytoplasmic microtubules.

* Part III, *Aust. J. biol. Sci.*, 1968, **21**, 255–74.

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I. Introduction

Chara has proved to be a very interesting subject for study of differentiation and morphogenesis. Some ultrastructural observations have already been presented (Pickett-Heaps 1967*a*, 1967*b*). The formation of the antheridium has been described (Pickett-Heaps 1968*a*), and was shown to be a developmental sequence in which precisely aligned cell divisions created the gross structure of the antheridium, after which intracellular differentiation quickly proceeded to alter the component cells drastically in their form and function.

This paper describes the development of the spermatogenous filaments and the intracellular differentiation that produces a motile spermatozoid. Many papers have been published on spermatogenesis in various organisms, but there are none, to the author's knowledge, that attempt to describe the similar developmental process in plant cells.*

Manton (1957, 1959) and Manton and Clark (1956) described the structure of antheridia and mature spermatozoids in *Fucus*, *Sphagnum*, and *Pteridium* but their studies were limited by the relatively poor fixatives (osmium) available at that time and the emphasis was not on development. (In *Chara*, osmium fixation alone has also been rather unsatisfactory). Nevertheless, several important observations were made particularly with respect to a band of tubular or fibrous structures close to the coiled nucleus, the manchette microtubules. These observations were confirmed recently in sperm of *Marsilea* and *Zamia* (Norstog 1967; Rice and Laetsch 1967), and a liverwort, *Sphaerocarpos* (Diers 1967).

Differentiation of spermatogenous cells is of great interest to the cytologist, since the original cell, with its quite normal appearance and constituent organelles, is transformed into a purely functional cell type, in which extreme cell asymmetry is normally produced, and in which cell organelles are either eliminated entirely or altered drastically to give a simple organism associated with a very simply defined cell function.

II. Methods and Materials

These have been described previously (Pickett-Heaps 1967*a*, 1967*b*). (Some specimens were also fixed in 4% unbuffered potassium permanganate for 1 hr at room temperature, in addition to those fixed in glutaraldehyde.) Antheridia of *Chara* have proved awkward to embed. The central region containing the spermatogenous threads causes no problems, but the outer shield cells almost always fill up with air bubbles during embedding, despite preventative measures. This phenomenon was in fact used with advantage because, following polymerization of the Araldite block, the antheridial contents could easily be removed as a solid ball from the hardened block by gentle manipulation, and these could then be re-embedded separately and in a labelled sequence that was related to their original position on the plant, and thus to the developmental stage reached by the constituent spermatogenous cells.

* *Note added in proof.*—Recently, Paolillo, Kreitner, and Reighard (1968*a*, 1968*b*) have described in detail spermatogenesis in *Polytrichum*, and also Carothers and Kreitner (1968) have investigated the blepharoplast structure in *Marchantia*.

III. Observations

The formation of the brightly coloured antheridium has already been described (Pickett-Heaps 1968*a*). The shield cells form an intricate pattern around the antheridium (Fig. 2), and at maturation they open into bright orange rosettes (Fig. 4) and release the spermatozoids. If crushed, the antheridium spills out its mass of spermatogenous threads (Fig. 3), the cells of which contain the spermatozoids (Fig. 5). The mature spermatozoid is tightly coiled within its mother cell wall (Fig. 5 — cf. Manton 1957), and the flagella are likewise coiled around it. Consequently thin sections show little of the overall spatial relationships to one another of the components of the spermatozoid, and some imagination is required to relate the two-dimensional sections with the three-dimensional structure of the cells, once they start to differentiate. A semi-diagrammatic representation of a maturing spermatozoid is shown in Figure 1. Mottier (1904, p. 253) estimates that the mature spermatozoid makes two and a half to three turns within the mother cell wall.

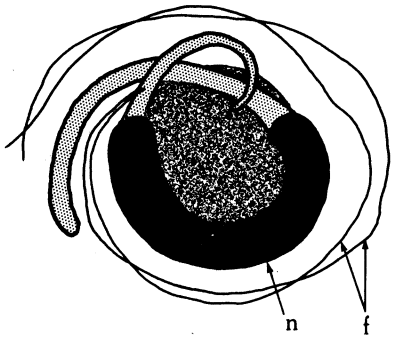


Fig. 1.—Diagrammatic representation of differentiating spermatocyte, roughly half-formed (comparable to Fig. 64) and showing coiled nature. Curved, condensing nucleus (*n*) has a projection at each end containing plastids, mitochondria, etc. with growing flagella (*f*) coiled around it. In the centre is the mass of shrinking cytoplasm (containing golgi bodies, endoplasmic reticulum, etc.).* After Mottier (1904).

The spermatogenous cells were quite difficult objects to preserve, judging by normally accepted criteria of electron-microscopists. At most stages of development, the image in the electron microscope suggested that plasmolysis (often severe) had occurred during fixation and subsequent processing. However, at later stages of differentiation the cell contents contract markedly to form a motile sperm which contains very little cytoplasm (Mottier 1904), so the appearance of plasmolysis is not always artifactual. The plasmalemma was often seen in convoluted profiles, which the author suspects to be usually a result of damage during processing. Light microscopists have also noted the marked liability and sensitivity of the spermatogenous threads to handling — for example, Karling (1926, p. 323) described how injury to one cell in a filament has a marked effect on others up to about 30 cells distant.

* Other abbreviations used in captions and on Figures 2–85 are as follows: *bb*, basal body (centriole extruding flagella); *c*, centrioles; *ch*, chromosome; *cp*, secondary capitula; *er*, endoplasmic reticulum; *fi*, flagella insertion; *g*, golgi body; L.S., longitudinal section; *m*, mitochondrion; *ma*, manchette adjunct; *nc*, nucleolus; *ne*, nuclear envelope; *p*, plastids; *sc*, shield cells of antheridium; *st*, starch; *t*, microtubules; T.S., transverse section; *va*, vesicular adjunct; *w*, cell wall.

(*a*) *Growth of the Spermatogenous Filaments*

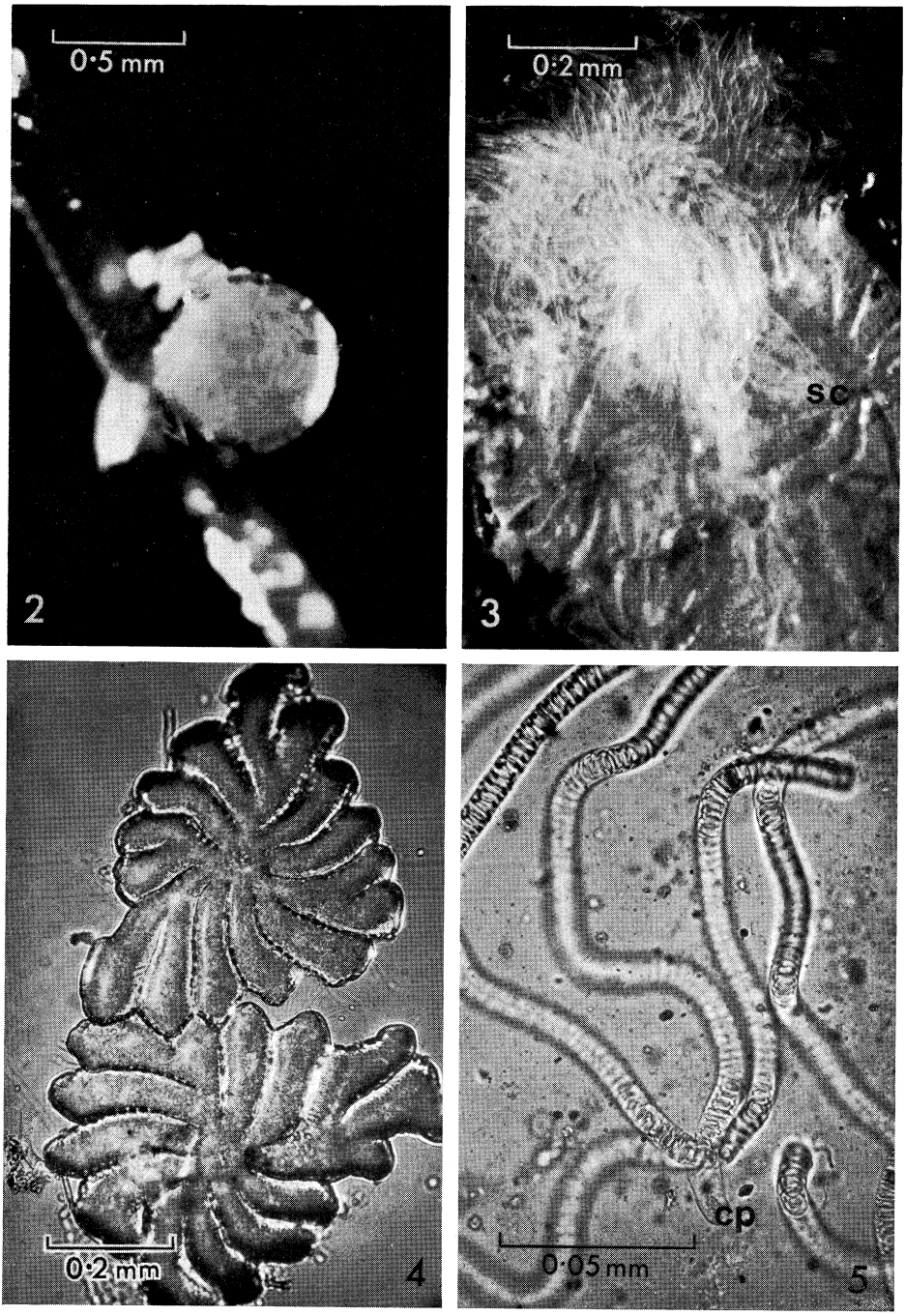
A large number of cell divisions cause the spermatogenous threads to elongate enormously and eventually to fill the interior of the antheridium as a tangled mass, immersed in a fine [mucilagenous (?)] material (see Pickett-Heaps 1968*a*). These mitoses were normally synchronous or progressive along the filaments (Karling 1928); at the ultrastructural level only shorter lengths were visible and these therefore showed cells essentially at the same stage of mitosis.

The cells of the young filaments were extremely osmiophilic, due partly to the very large number of ribosomes within them (Figs. 14, 17, etc.). This made observations on ultrastructure quite difficult. Mitosis in most respects seemed similar to that in vegetative cells (Pickett-Heaps 1967*b*).

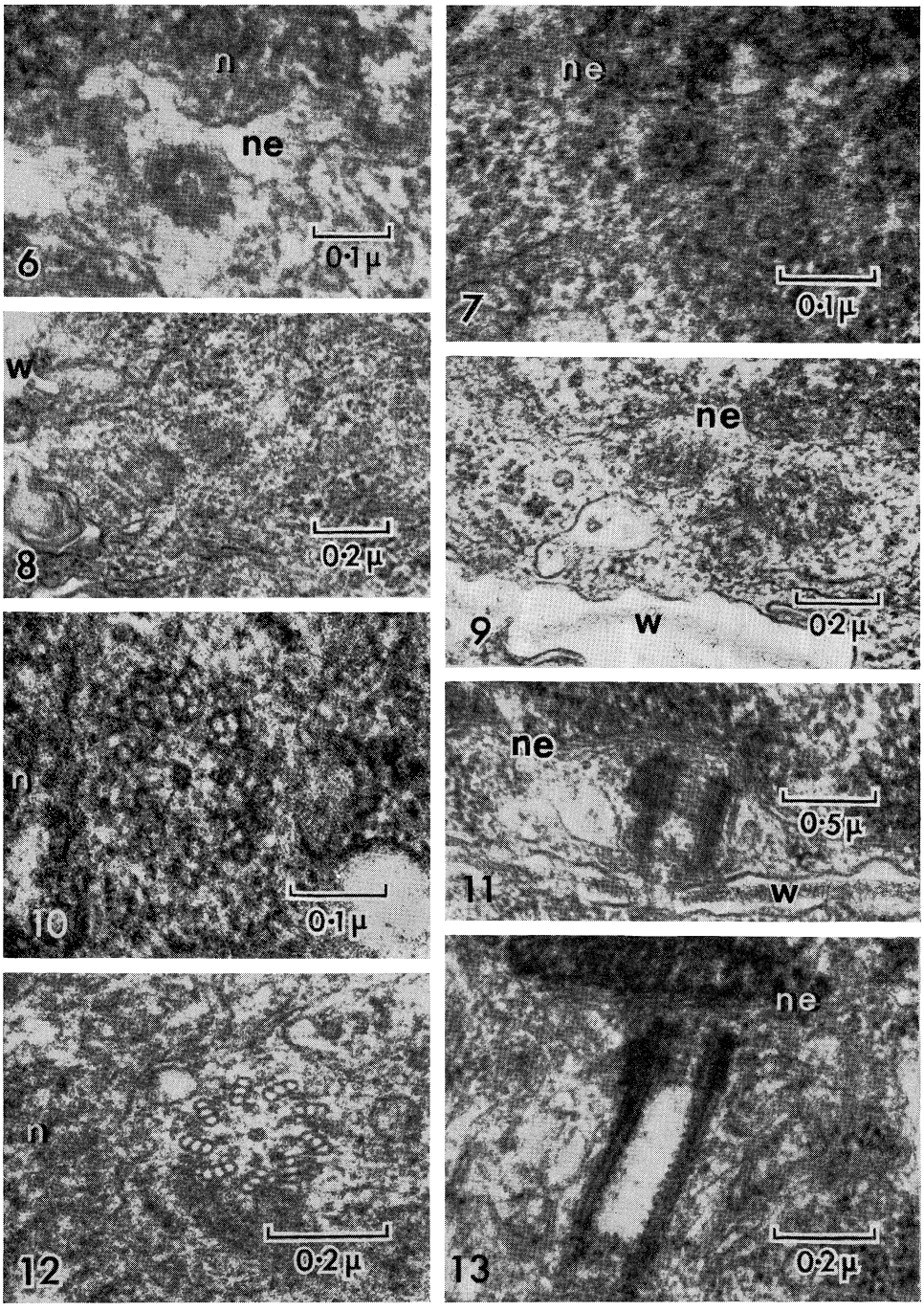
Of particular interest to the author was the appearance of centrioles. These were clearly visible in later development (Figs. 13 and 18) and yet in vegetative cells (Pickett-Heaps 1967*a*, 1967*b*) and in all other cells of the antheridium (Pickett-Heaps 1968*a*) no procentriole or possible precursor could be found or recognized. Somewhere along the developmental sequence between the secondary capitula and growing spermatogenous threads, the "formation" of centrioles should therefore be demonstrable. Unfortunately, the situation has not been clarified, despite intensive observations. Centrioles were first seen when the threads were quite short, then they appeared as ill-defined but nevertheless typically characteristic structures close to the nucleus (Figs. 8, 9, 10, and 12). At this stage they were short and extremely difficult to see in the cells. As growth of the filaments continued they stained heavily and became more distinct (Fig. 11), later becoming elongated (Fig. 13) after mitosis ceased. By the time the flagella were being extruded from the cells the centrioles were very conspicuous objects.

A clearly defined catherine-wheel structure was visible inside the nine sets of three tubular structures at this and later stages (Figs. 10, 12, 60, etc.) (Manton 1964*a*, 1964*b*; Ringo 1967). Further scrutiny of cells indicated that the central catherine-wheel structure might have been the procentriole; following intensive examination of sections of very young cells a number of similar objects were seen, but their structure was always ill-defined (Figs. 6 and 7). Though sometimes similar in appearance to nuclear pores, many micrographs suggest that these objects were clear of the nuclear membrane, though always close to it. The source of such presumptive procentrioles remains totally obscure.

The behaviour of the centriole was quite difficult to follow during mitosis. The proportion of filaments containing cells in division in any antheridium was small, and the number of cells showing recognizable stages of division as well as centrioles was smaller still. In some prophases a centriole was found at a pole in association with spindle microtubules (Fig. 16), apparently typical of the structural organization in other centriole-containing dividing cells. In one clearly defined metaphase (Fig. 14) a paired set of centrioles was found at one pole in a corner of a cell, and associated spindle microtubules were probably attached to the chromosomes (Figs. 14 and 15). This indicates, as expected, that centriole replication occurs at least before metaphase. Division of the cell was always symmetrical even though diagonal spindles were quite common (Figs. 14 and 17).



Figs. 2–5.—Live material photographed (originally in colour) under a coverslip: *2*, Bright orange antheridium, almost mature. Note pattern formed by the external shield cells (see Fig. 4); *3*, the same antheridium, shown squashed by pressure on the coverslip. The mass of enclosed spermatogenous threads has spilled out of the shield cells (*sc*); *4*, at maturation, the orange shield cells split open to release the spermatozoids. Two such shields are shown; *5*, almost mature spermatozoids are seen coiled in the spermatogenous threads, which are attached to secondary capitula cells (*cp*).



Figs. 6 and 7.—Possible ill-defined procentrioles seen very close to the nuclear envelope (*ne*) of the very young spermatogenous cells.

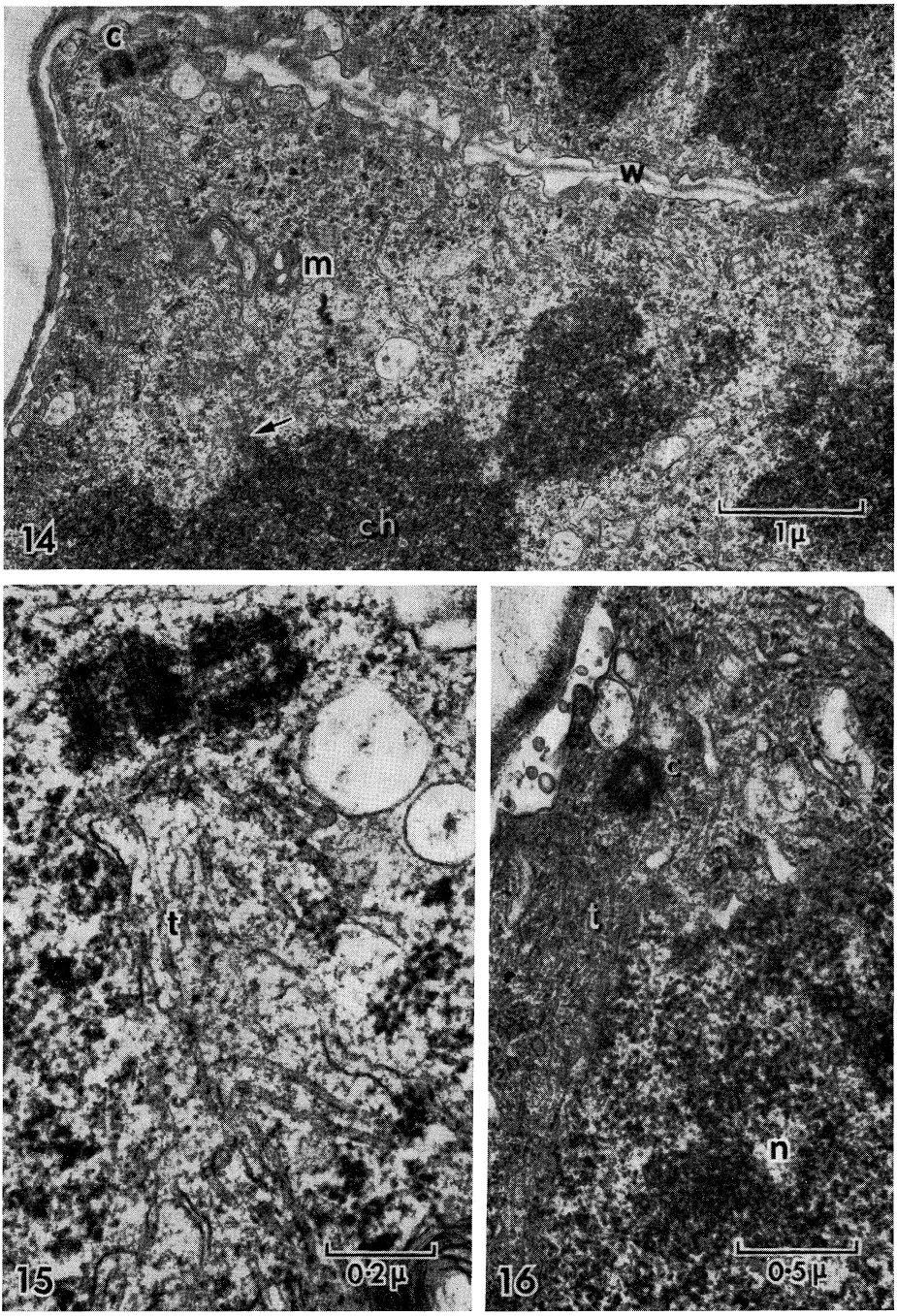
Fig. 8.—Poorly staining centrioles, seen in L.S. at prophase pole of very young spermatogenous cell.

Fig. 9.—Very young spermatogenous cell, centriole seen in T.S.

Fig. 10.—As for Fig. 8; catherine-wheel structure more visible and outer triplets possibly being formed.

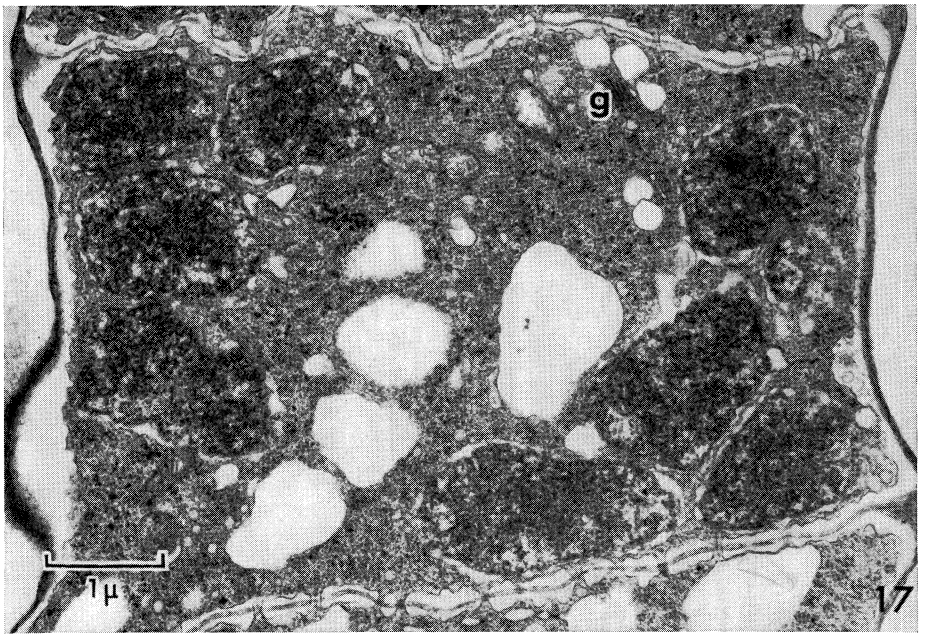
Fig. 11.—Short, clearly visible centriole in spermatogenous cells that was actively dividing.

Fig. 12.—Compare with Figure 10. Basic centriolar structure appears fully formed, but stains very lightly.



Figs. 14–16.—Mitosis in the growing spermatogenous filaments: *14*, metaphase (L.S.) showing paired centrioles (*c*) at pole which often (as here) is in the corner of the cell (cf. Fig. 17), and spindle microtubules attached to chromosomes (*ch*) at arrow; *15*, detail of Figure 14, showing spindle tubules (*t*) approaching the centriolar region; *16*, prophase, typical concentration of spindle microtubules (*t*) at pole occupied by centrioles.

Fig. 13.—Compare with Figure 11. Typical; centriole close to nuclear envelope (*ne*), elongated after cell division has ceased in the spermatogenous filaments.



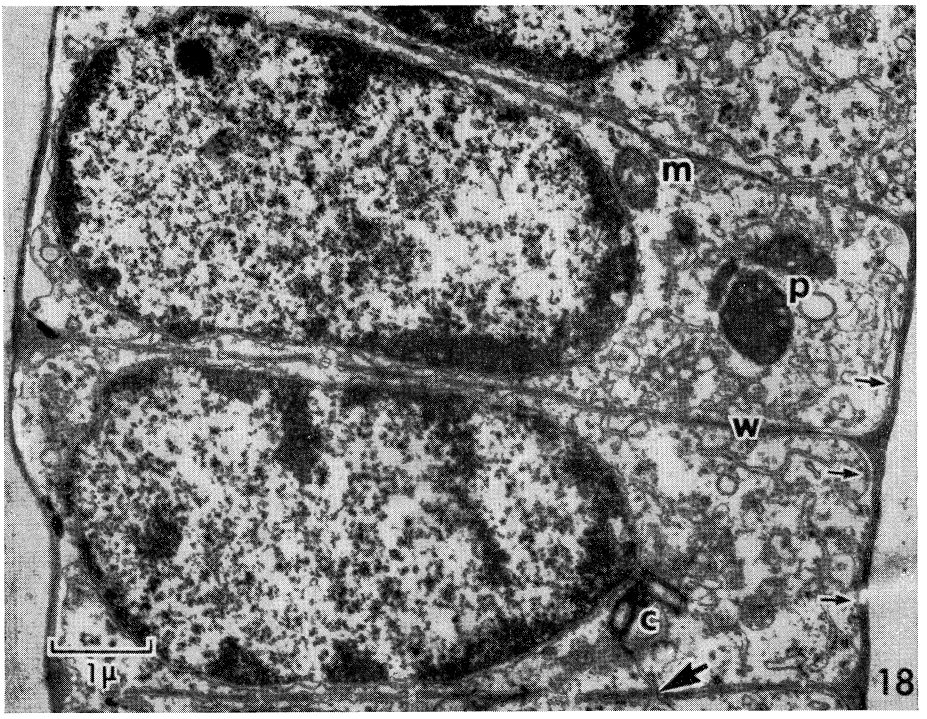


Fig. 17.—Mitosis in growing spermatogenous filaments. Telophase (L.S.); cell plate formation not yet initiated. The highly lobed nuclei are typical (Pickett-Heaps 1967*b*). Although daughter nuclei are in opposite corners (cf. Fig. 15), the cell would have been partitioned horizontally (see also Karling 1926). Golgi bodies (*g*) are associated with large vesicles (see Figs. 20 and 22).

Fig. 18.—Spermatogenous thread (L.S.). First stage of differentiation after mitosis finished. Ground cytoplasma more disperse (cf. Fig. 17). Nuclei moved to one side of cell, but centrioles (*c*)

During earliest stages of filament growth, characteristic association of membranes with each other (probably the endoplasmic reticulum) and with spherosomes and other smaller vesicular components was very frequently observed (Pickett-Heaps 1968*a*). The filament cells always contained mitochondria (often very elongated), endoplasmic reticulum, plastids, and well-developed Golgi bodies. Typical plant "wall" microtubules were always present.

After cell division was completed (i.e. when no more dividing cells were found in the threads) the cells underwent differentiation to form the spermatozoids.

(*b*) *Differentiation*

Intracellular differentiation will first be described very briefly in general, and then in greater detail by referring to changes in individual organelles.

Differentiation starts with a dispersion of the cytoplasm, and the nucleus moves to one side of the cell (Fig. 18). Formation of the manchette and extrusion of flagella then commence (Fig. 36). The cytoplasm shrinks steadily, and plastids line up along the manchette tubules (Figs. 45 and 46). As the manchette grows, assuming a spiral configuration within the cell wall, nuclear and cytoplasmic condensation continue. Mitochondria line up near the flagella and along the manchette microtubules (Fig. 48). After further elimination of cytoplasmic components, the spermatozoid consists basically of a row of plastids, a dense, coiled nucleus, and a row of mitochondria arranged linearly along the coiled manchette. Long flagella are also coiled within the cell, being inserted above the mitochondria.

(i) *Cytoplasmic Matrix*

This somewhat loose term is included to emphasize a marked change that was observed in the overall cytoplasmic texture of the cell. During growth of the spermatogenous threads the cytoplasm generally was very dense (Figs. 6–17). After division ceased a marked dispersion was observed (Fig. 18). Later still, during cytoplasmic shrinkage, the ground cytoplasm again became very dense (Figs. 48, 52, etc.) until cytoplasmic remnants were eliminated from the mature spermatozoid (Fig. 76). Figure 84 shows an abnormality where cytoplasmic condensation was not evident.

(ii) *Nucleus*

Soon after division ceased the nucleolus decreased in size and disappeared (Fritsch 1935, p. 458), and the chromatin became arranged around the periphery of the nucleus, the remainder of the nucleus appearing coarsely granular (Fig. 18). This granulation became finer and then the lamellate structure (see below) appeared in the nucleoplasm.

An early sign of differentiation was the movement of the nucleus to one side of the cell (Fig. 18). This occurred before manchette initiation (see later) was detected. The shape of the nucleus after the manchette started growing often suggested that the nuclear membrane was held firmly against the microtubules of the manchette (Fig. 36). As the manchette grew further in length, the nucleus elongated and started

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still removed from walls. Note poorly developed plastids (*p*), endoplasmic reticulum (interconnecting cells at large arrow), nucleolus disappearing, and chromatin becoming arranged around the periphery of the nucleus. Wall microtubules (small arrows) just visible—see Figure 25.

to condense. The chromatin initially became more disperse (Fig. 66) and then assumed a fibrillar texture. Further elongation of the nucleus gave it a spiral course in the cell. With continuing condensation the fibrillar texture of the chromatin turned into a heavily staining lamellate structure (Figs. 67–69, 71, and 72). The thicker lamellae then appeared to fuse together (Figs. 73–75) to give finally the condensed, homogeneous nucleus typical of sperm cells (Figs. 76 and 78).

(iii) *Microtubules and Manchette Formation*

Microtubules appear to be important in fundamental morphogenetical processes in plant cells. In spermatogenesis, manchette microtubules seem to be the prime agents responsible for the overall spatial reorganization of the cytoplasm. This is a feature of differentiation and results in the highly specialized form of the cell.

Typical wall microtubules were invariably present in undifferentiated spermatogenous cells (Figs. 18 and 25) and some others were present in random arrays in the cytoplasm, often close to the nucleus. It was at first thought that wall microtubules became bunched together to form the manchette, but it now appears that a special organelle is associated with manchette formation.

As the centrioles moved to the edge of the cell and began to extrude flagella, a densely staining object was seen near them (as in Fig. 37, etc.). This structureless component was ill-defined and of rather variable shape. For the purposes of this paper, it will be termed the manchette adjunct; the author has not yet found a reference to this specific organelle in the literature (see Section IV). Its significance was appreciated when slightly older cells were examined. In these manchette formation had commenced, the manchette consisting of (initially) about 3–5 microtubules (Figs. 38 and 39) close packed into a flat band which was being formed along one side of the cell, and against which the nucleus was situated (Figs. 36, 45, 50, etc.). Further examination of such cells showed without any doubt that these tubules, even when quite short, were invariably inserted into the manchette adjunct near the centrioles (Figs. 29, 37, and 47). It is not absolutely clear whether manchette microtubules were inserted into a manchette adjunct from the very first, as some pictures suggest that the adjunct was present right from the beginning, while others suggest that a couple of tubules were present first. Wall microtubules were always seen near the manchette microtubules later in development, but, because of the characteristic organization of the latter, were initially clearly distinguishable from them (Fig. 40).

The number of manchette microtubules increased during further development from about 3–6 to a maximum of about 27 (Figs. 38–42, 49, 50, 71, and 72). Some wall microtubules may have been incorporated into the growing manchette, but the impression was gained that most wall microtubules disappeared during this growth. The manchette adjunct itself disappeared much later in development.

Fig. 22.—As for Figure 20; note intercisternal elements (arrowed), seen in all golgi bodies that are suitably sectioned.

Fig. 23.—Nuclear envelopes (*ne*) directly interconnected through plasmadesmata (cf. Fig. 24).

Fig. 24.—Endoplasmic reticulum (*er*) is very frequently seen passing through plasmadesmata (see also Fig. 18).

Fig. 25.—Typical wall microtubules (*t*) seen in T.S. at outer wall of spermatogenous cells (see also Figs. 18 and 40).

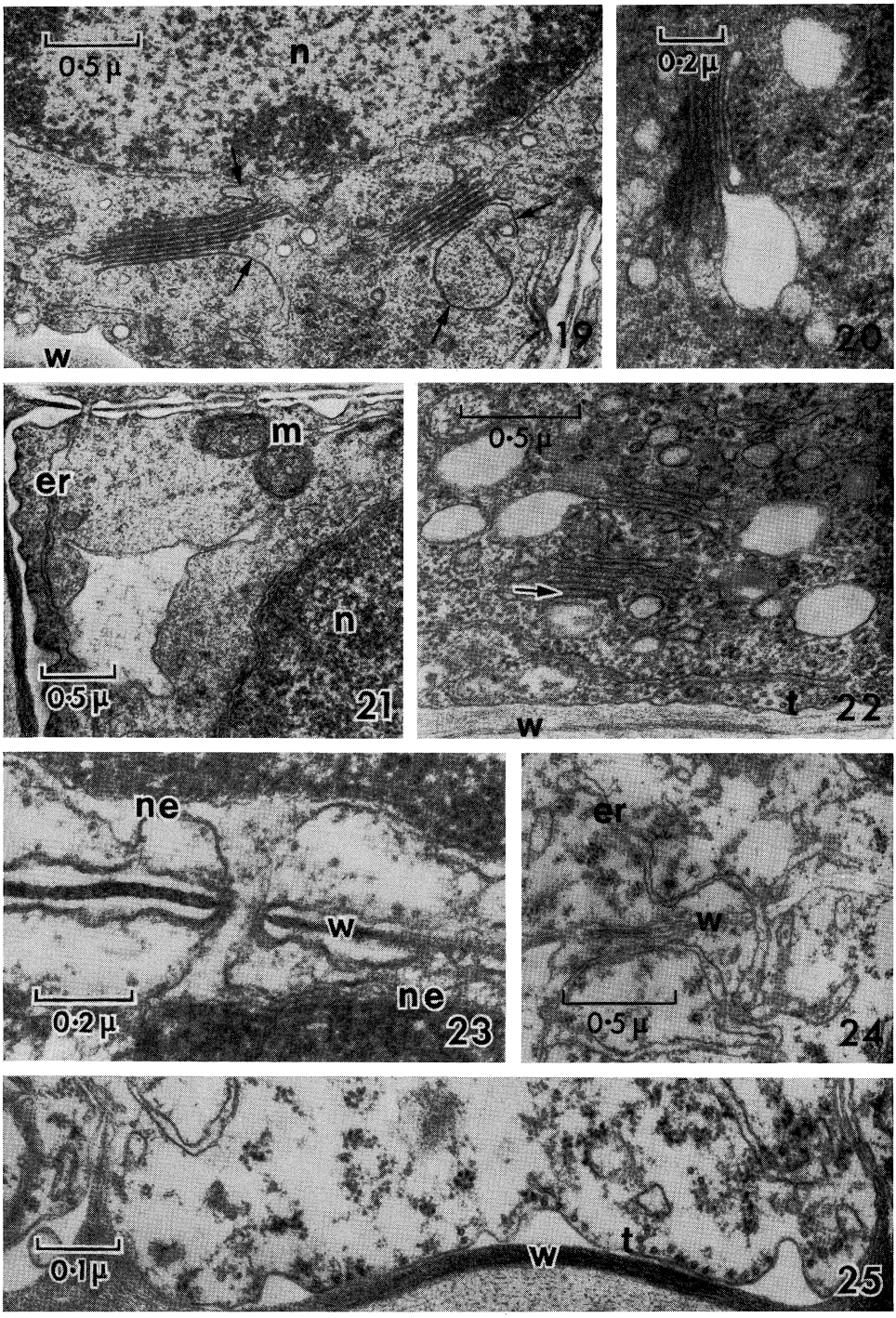
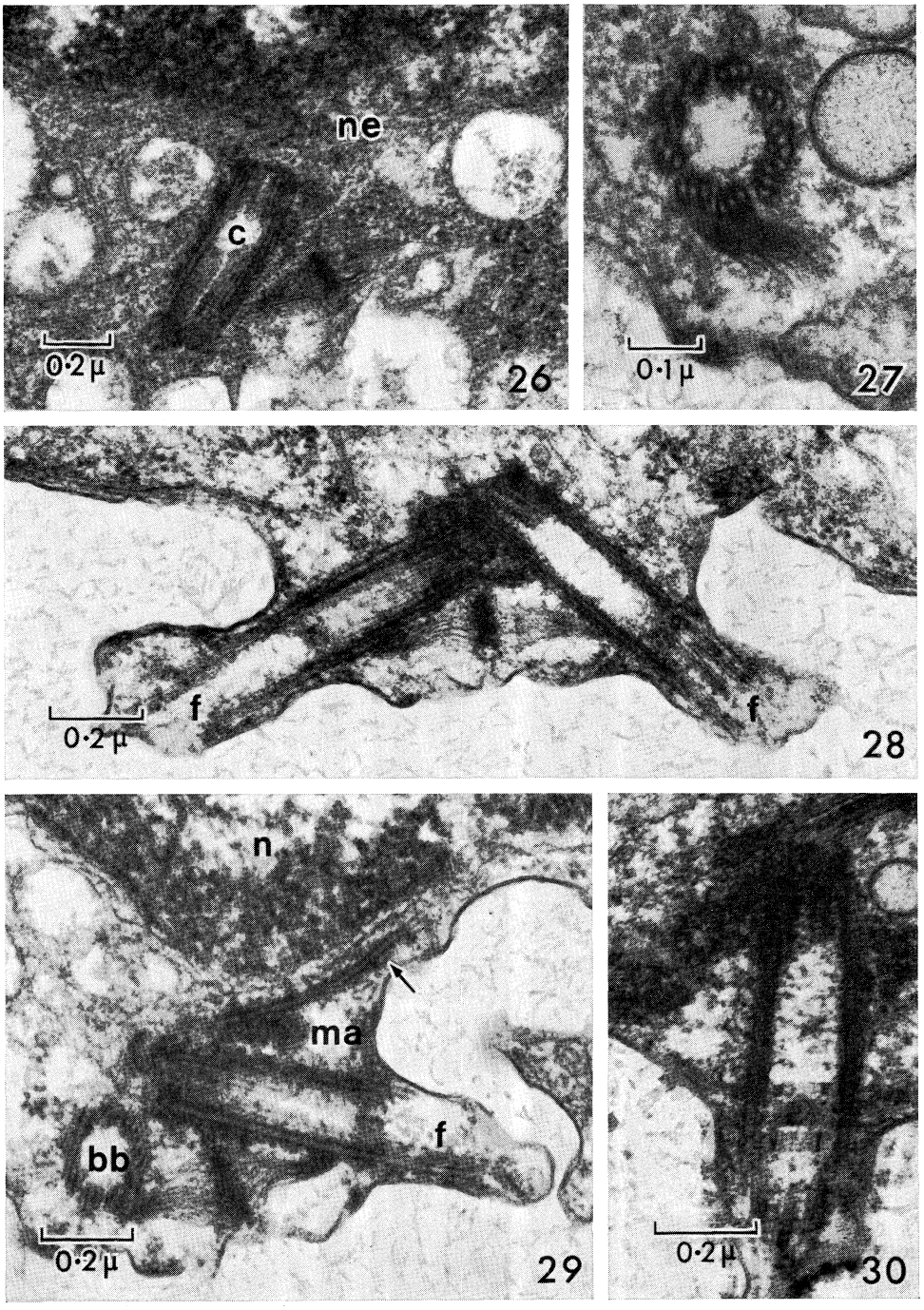


Fig. 19.—Golgi bodies, growing spermatogenous thread. Cisternae (arrowed) are frequently observed in this drawn-out fashion.

Fig. 20.—Some golgi bodies in both mitotic and young post-mitotic cells appear to be forming large, electron-transparent vesicles (cf. Pickett-Heaps 1967*a*, fig. 30).

Fig. 21.—Dilated cisterna of endoplasmic reticulum (*er*) in young, actively dividing spermatogenous cells (cf. Pickett-Heaps 1967*a*, figs. 18 and 30).



Figs. 26–29.—Spindle-shaped, fibrillar component of ciliary root structure: *26*, first appearance of this structure, connected to centriole of cell that has just ceased to divide (these were never seen in dividing cells); cytoplasm was still very dense and cell was therefore probably younger than that in Figure 18; in both these cases, the centrioles (*c*) were situated far from the outer cell membrane; *27*, centriole near to outer cell membrane; fibrillar connection is seen slightly spread

Three to four other microtubules were always found near the manchette–basal body complex (Figs. 37 and 39). These were heavily staining and may have been part of a ciliary root structure (Hoffman and Manton 1962, 1963; Ringo 1967). It was difficult to follow these tubules during further growth; however, in much older cells there were always seen near the mitochondria and basal bodies four tubules distinct from the 27 of the manchette itself (Figs. 70–72).

The continuing elongation of the manchette microtubules during growth was closely associated with the developing spiral form of the spermatozoid (see Fig. 67). With the nucleus appressed to the manchette, a striking morphogenic movement occurred as the plastids also moved to the manchette (Fig. 45), becoming linearly arranged along the tubules (Figs. 46–48, 52, and 53) next to the nucleus. The next rearrangement of cell organelles occurred with further elongation of the manchette after the nucleus, previously situated close to the basal bodies, moved further away from them. Most, but not all, of the mitochondria in the cell now also moved to the manchette microtubules between the basal bodies and nucleus (Figs. 48, 49, 51, 52, etc.). The exact relationship to the manchette of the flagella insertion became very difficult to follow (see below); later, the flagella were obviously parallel and situated over the manchette tubules (Figs. 71, 72, 77, etc.). With continuing cytoplasmic shrinkage the flat band of manchette tubules began to enclose partially the linearly arranged mitochondria (Figs. 71 and 72), finally tailing off into a row of tubules enclosed by the plasmalemma.

During final stages of differentiation, the manchette microtubules tended to split up slightly, often into groups (Figs. 74, 78, and 79). These still remained very closely appressed to the plastids, condensed nucleus, and mitochondria, the number over one end of the nucleus and the plastids decreasing progressively to zero (Figs. 77 and 79). Although liberated spermatozoids were not examined (see Section IV), it is highly probable that the manchette microtubules were present in the mature organism.

(iv) *Basal Bodies*

In the young spermatogenous threads the centrioles were short, with characteristic substructure (catherine-wheel, etc.) at one end, the rest of the interior being "empty" (Fig. 11). The 9 by 3 outer tubular structure grew in length, as did the central region (unstained except for an inner fibrillar edge which was sometimes visible — Fig. 13). The centrioles were initially situated approximately at right angles to each other, very close to the nuclear envelope, and were orientated away from the nucleus (Figs. 13 and 18). Their orientation became more tangential as they moved to the edge of the cell and extrusion of flagella commenced (Figs. 28, 29, and 36;) later, they were seen at a considerable distance from the nucleus, following further

out; *28*, basal bodies (i.e. centrioles extruding flagella) at typical obtuse angle; extrusion of flagella just commencing, and basal bodies interconnected by the fibrillar structure; *29*, as for Figure 28, but one basal body has been cut in T.S.; appearance of the ciliary-root structure the same; manchette adjunct (*ma*) just above, probably just starting to form manchette tubules (arrow).

Fig. 30.—Substructure of basal body, seen after flagella already extruded approximately one turn in the cell (cf. Fig. 51).

elongation of the manchette (Fig. 48). A symmetrical spindle-shaped ciliary rootlet structure appeared, sometimes early in differentiation, connecting the centrioles (Fig. 26). The "points" of the fibrous spindle were sometimes seen to spread slightly around the centriole (Fig. 27). These ciliary rootlets (Figs. 28, 29, and 65) were not seen after flagella extrusion was almost complete.

The centrioles were initially at an obtuse angle to one another and consequently the flagella were also initially extruded at a similar angle (Fig. 28). It has been impossible, however, to follow precisely the subsequent movement of the basal bodies (this term is used to describe centrioles associated with flagella). They eventually became parallel (Figs. 59, 72, etc.), close to the manchette microtubules.

The substructure of the basal body has not been examined in detail, this being beyond the scope of the present article. It appears to contain some of the characteristic features of such organelles, including a stellate pattern (Figs. 30, 51–60 — Manton 1964*a*, 1964*b*; Manton and Parke 1965; Manton *et al.* 1965; Ringo 1967). Some of these features were not apparent during the initial extrusion (Figs. 28 and 29).

During later development the basal bodies lost their identity to an increasing extent. In the more mature spermatozoid they were difficult to identify, although the structure of the flagellum itself was always obvious (Figs. 71 and 72). It appeared as if the basal bodies had lost their distinguishing characteristics.

Another previously unidentified "organelle" appeared close to the basal bodies in young cells. This was quite different to the manchette adjunct (see above), though initially it appeared somewhat similar. It had a thick saucer shape, was composed of a densely staining, homogeneous material (Fig. 61), and was distinguished by the invariable ring of vesicles surrounding it at its edge and separated from it by a small distance (Figs. 58, 59, and 61). The author has not seen reports of a similar organelle elsewhere in a regrettably limited search through the literature. For the purpose of this paper, the organelle will be termed the "vesicular adjunct". It appeared early during formation of the flagella, and was always seen in very close proximity to the basal bodies (Fig. 59) throughout all but the final stages of intracellular differentiation, when it disappeared.

(v) *Flagella*

The two flagella were in most respects entirely typical. Their outer membrane was loosely fitting in young cells, became tighter during growth, and was covered by a thin even layer of material (Figs. 43 and 44), on the outside of which were arranged a typical pattern of tiny scales (Figs. 65, 67, 82, 84, etc. — cf. Manton and Parke 1965; Manton *et al.* 1965). These scales resisted the destructive effects of permanganate fixation. Extrusion of the flagella commenced by the outer paired tubules being pushed into the plasmalemma which grew to accommodate them (Figs. 28 and 29); there was no evidence of extrusion into a large vesicle (Renaud and Swift 1964).

Fig. 33.—Spiral ribosomes were frequently seen in L.S. along one face of the golgi bodies (arrows).

Fig. 34.—Cell fixed in permanganate: cytoplasm shrunken (cf. Fig. 64), wall (*w*) almost invisible; golgi body very conspicuous.

Fig. 35.—Lumen of endoplasmic reticulum becomes filled during differentiation with fibrillar material. Possible early stage of this process shown; arrows denote fibrils.

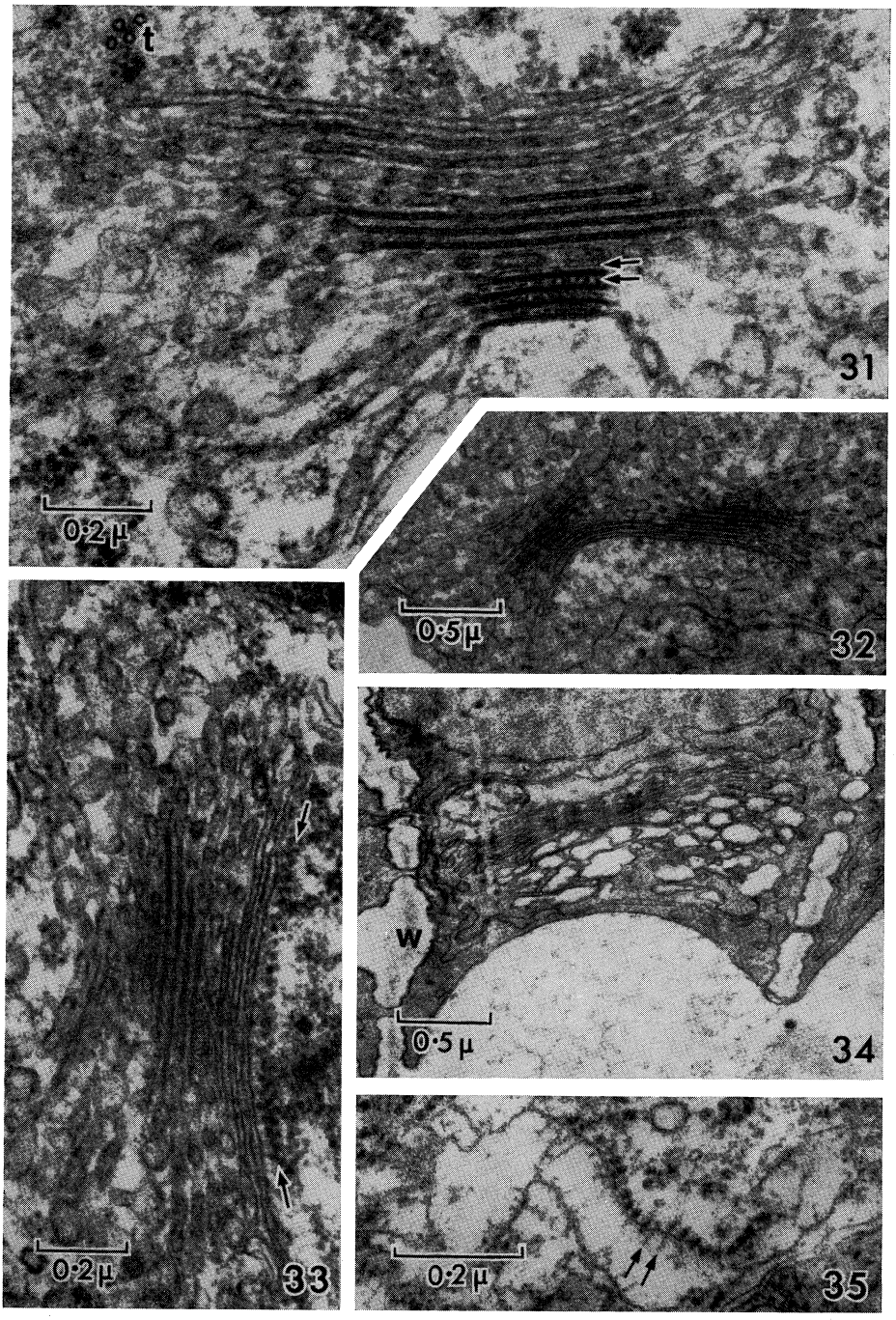


Fig. 31.—Typical golgi body in young differentiating cell. Cisternae slightly dilated on one side, becoming increasingly compacted and highly reticulate at the other face. Intercisternal elements visible (small arrows). The four microtubules (*t*) may have been part of a cilia root structure (see Figs. 37, 39, etc.).

Fig. 32.—Golgi bodies interconnected by some cisternae (see Pickett-Heaps 1967*a*, figs. 27 and 29), possibly replicating by fission.

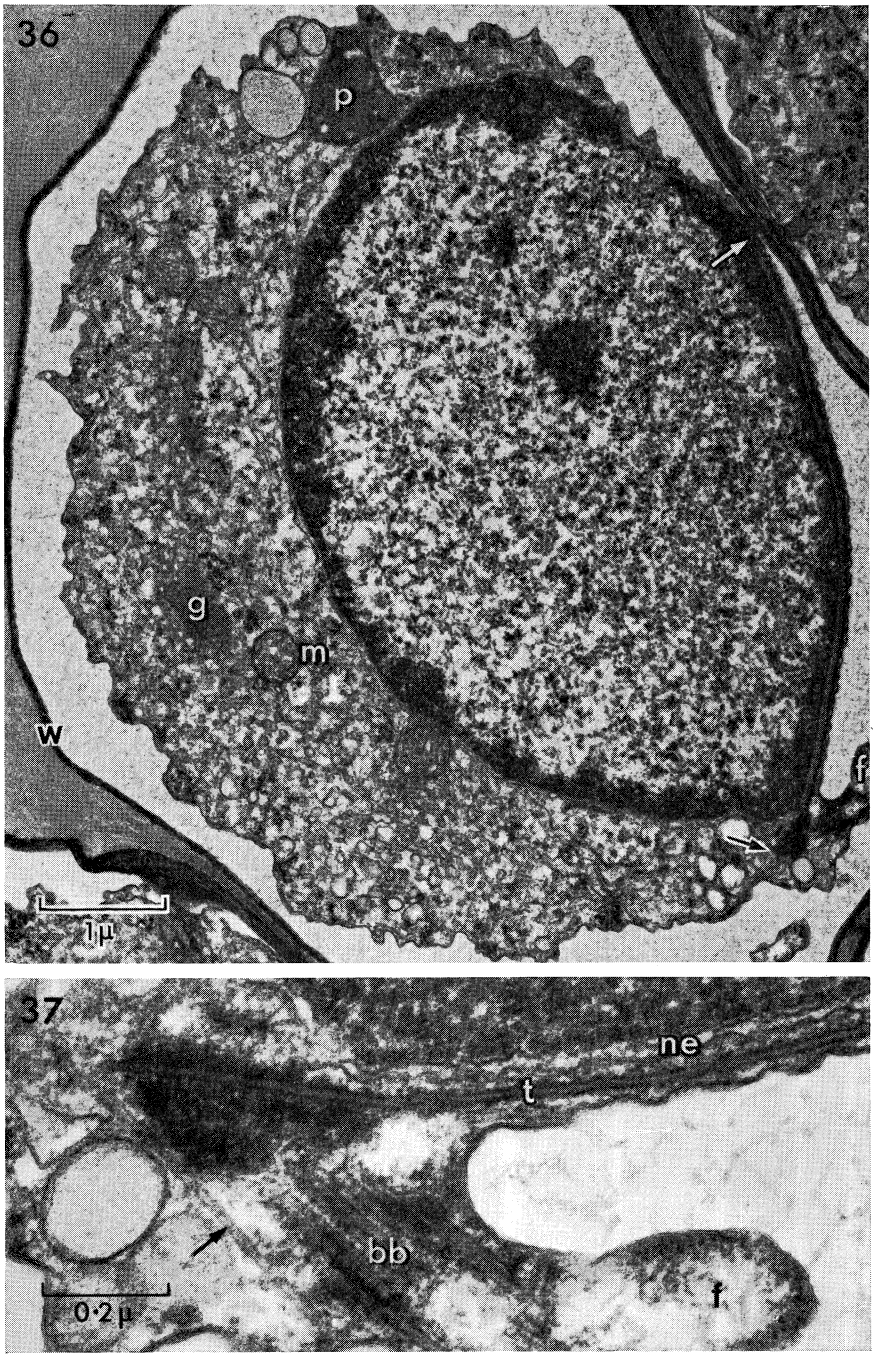


Fig. 36.—Young cell, cytoplasm starting to shrink, in T.S. (cf. Fig. 18). Basal body now at cell surface just starting to extrude flagella (*f*). Manchette adjunct immediately beside basal body. One manchette tubule can be traced from the manchette adjunct (black arrow) some distance up the side of the nucleus (to white arrow). Shape of nucleus suggests it is attached to the band of manchette tubules. Plastids (*p*) and mitochondria (*m*) scattered about cell (compare with Figs. 46 and 48 for movement of these organelles to the manchette).

Sections of flagella were sometimes found with an incomplete complement of tubular structures (Figs. 43 and 44), probably representing sections taken near the tips. The central doublet tubules appeared to be formed a short distance away from the basal body (Fig. 55 — see Silveira and Porter 1964, fig. 15); sections in this region often showed none (Fig. 56) or only one central tubule (Fig. 54), though in one case (Fig. 57) three tubules were present.

In the mature spermatozoid, the flagella were quite long (about two and a half turns inside the cell — Fig. 76). A small densely staining body was sometimes seen under the flagellar membrane (Fig. 81), which on close examination showed a crystalline structure (Fig. 82).

(vi) *Plastids*

These organelles, with a very poorly developed internal structure, were always present in young spermatogenous cells (Fig. 18). After division was completed the cells had about four plastids each, initially dispersed throughout the cytoplasm. However, a most striking and characteristic morphogenic movement of these organelles occurred as the manchette microtubules grew, the plastids lining up close together flat against the tubules next to the nucleus, staying thus throughout further development (Figs. 45–47, 53 *et seq.*).

Soon after this movement, the plastids began to accumulate material (almost certainly starch) which filled the plastids by maturation (Figs. 48, 69, 76, 77, and 79). The author has no evidence suggesting that these plastids were discarded at liberation of the spermatozoid (cf. Manton 1957).

(vii) *Lipid Bodies and Spherosomes*

Spherosomes were very common in capitula and very young spermatogenous cells (Pickett-Heaps 1968*a*) but became less evident as the spermatogenous threads grew. Small lipoidal inclusions became quite numerous later and differentiating cells always contained some. Soon after the plastids moved to the manchette most of these also moved close to the plastids (Fig. 48), staying near them throughout further differentiation.

(viii) *Mitochondria*

Cells invariably contained mitochondria, and these were often considerably elongated during mitosis and growth of the filaments. During differentiation they were quite small and oval-shaped. In another characteristic morphogenic event, most mitochondria later moved to the manchette microtubles (Figs. 48, 49 *et seq.*), this occurring some time after the nucleus and plastids had taken up their position on the manchette (see above). As with the plastids, the mitochondria were packed together linearly, and later the tubules began to encircle them (Figs. 70–73). A few other mitochondria were generally found near the nucleus and plastids (Fig. 77).

(ix) *Plasmalemma*

The plasmalemma of adjacent cells was continuous through plasmadesmata (Figs. 23, 24, 63, etc.), and remained thus for a considerable period during differentiation, until cytoplasmic shrinkage (Fig. 62) finally broke such connections (Fig. 71).

Fig. 37.—Detail of Figure 36 showing manchette adjunct and embedded manchette microtubule (*t*). Another microtubule (arrow) is possibly one of the group of three or four often seen near the basal bodies (*bb*) (see Fig. 39, etc.).

(x) *Endoplasmic Reticulum*

Membranes of endoplasmic reticulum were always conspicuous in cells until quite late in their development, when both the endoplasmic reticulum and golgi apparatus lost their identity and disappeared (e.g. Fig. 66). In very young differentiating cells the lumen of the endoplasmic reticulum was sometimes considerably distended (Fig. 21 — cf. Pickett-Heaps 1967*a*, figs. 18 and 30). Ribosomes were present on the membranes, sometimes close to fibrous material in the lumen (Fig. 35). During the middle stages of cytoplasmic shrinkage, the lumen of the endoplasmic reticulum (and nuclear envelope) was filled with such material (Figs. 45 and 46). The membranes of the endoplasmic reticulum in adjacent cells were often continuous through plasmadesmata (Figs. 18 and 24); one micrograph showed a direct link between the nuclear envelopes in two such cells (Fig. 23).

(xi) *Golgi Apparatus*

The golgi bodies were always conspicuous objects in the younger cells, but they progressively lost their identity during late maturation (Fig. 66). Reticulate cisternae and intercisternal elements were generally visible (Fig. 31, etc.), and in some cases the golgi bodies were interconnected, possibly dividing by splitting (Fig. 32).

Marked changes were seen in the appearance of the golgi bodies during differentiation. In growing spermatogenous filaments they were fairly small, although some cisternae were giving rise to very large, clear vesicles (Figs. 17, 20, and 22). As intracellular differentiation started, the cisternae increased in number, and a few were greatly attenuated, moving into the cytoplasm (Fig. 19). Some cisternae then seemed to become compact and decrease in size, as the other faces became more reticulate (Fig. 31). The golgi, normally two to four in number, were then associated with a profusion of vesicles or tubular membranes or both (Figs. 31, 33, 34, and 52). An association of (spiral) ribosomes with one face of the golgi was frequently observed (Fig. 33). During cytoplasmic shrinkage, the golgi bodies often became paired in the more densely staining cytoplasm (Fig. 52).

It was very difficult to ascertain the function of the golgi vesicles (cell wall synthesis had apparently ceased). Occasionally, in young cells, the vesicles were quite large and by further swelling may have been involved in a process of vacuolation (Figs. 20 and 22 — cf. Pickett-Heaps 1967*a*, 1967*c*). The fate of the smaller vesicles is unknown. No convincing evidence could be obtained indicating an involvement of the golgi in production of the scales which covered the outer surface of the flagella. Occasionally micrographs suggested that scales might have been present in much larger vesicles near the flagella base (Fig. 65).

Fig. 40.—Manchette band now 12 tubules across. Section is taken along the manchette some little distance away from the basal body (flagella has no central tubules — cf. Figs. 55 and 56). Note typical wall microtubules (arrows) still present although cytoplasm has shrunk from walls.

Fig. 41.—Manchette adjunct with about 11 tubules inserted into it.

Fig. 42.—As for Figure 41, but cell is older; the number of tubules in the manchette adjunct is now about 20 (full complement is about 27 — see Figs. 49, 71, etc.). Vesicular adjunct (*va*) also visible (Figs. 58, 59, and 61).

Figs. 43 and 44.—Incomplete complement of component fibres in flagella, probably resulting from section being taken at the tip. Note typical layer of material surrounding flagella membrane.

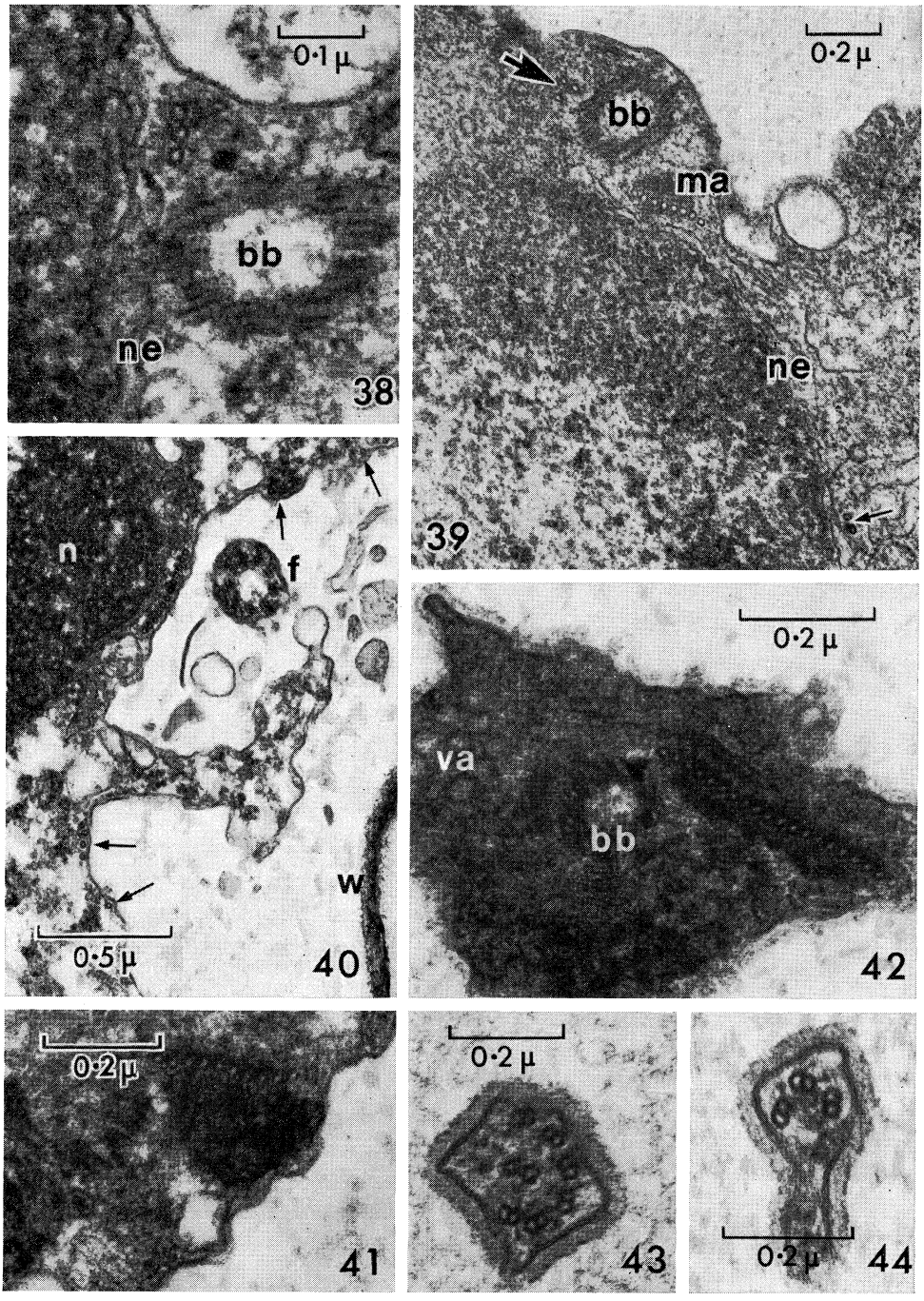
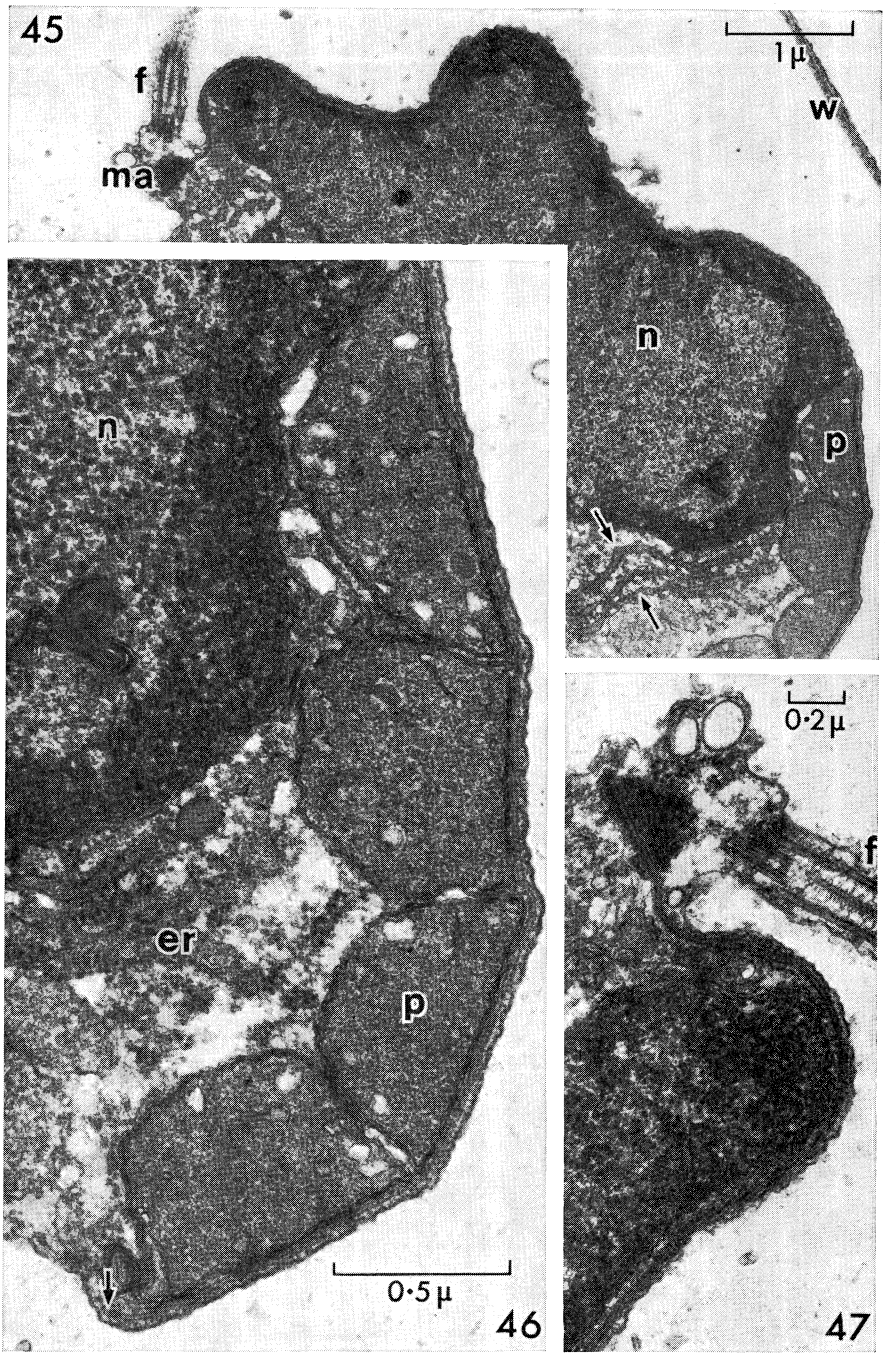


Fig. 38.—Possible very early stage in formation of band of manchette tubules close to the nucleus and basal body (*bb*); three tubules seen in a row.

Fig. 39.—Manchette band now composed of eight microtubules, most of these being embedded in the manchette adjunct (*ma*). Note characteristic group of three (or often four) tubules near basal body (large arrow). Other microtubules (small arrow) are often seen apparently at random near the nuclear envelope (*ne*) in young cells.



Figs. 45–47.—Elongation of the manchette, and morphogenic movement of plastids—compare with Figures 18, 36, 48, etc. *45*, cell in T.S. Low-power view shows manchette adjunct near flagella (note scales on flagella). Manchette tubule(s) can be traced from the adjunct to end of plastids (Fig. 46). Cytoplasm of cell now considerably shrunken. Nucleus slightly lobed (this seemed to be only a temporary condition, consequent on manchette elongation). Endoplasmic reticulum (arrows) filled with staining material. *46*, Detail of Figure 45. Immature plastids seen aligned along manchette tubules, which end at arrow. Note absence of starch in plastids (cf. Figs. 48, 69, etc.). *47*, Detail of Figure 45, showing, as in Figure 37, insertion of manchette microtubule into manchette adjunct near flagella.

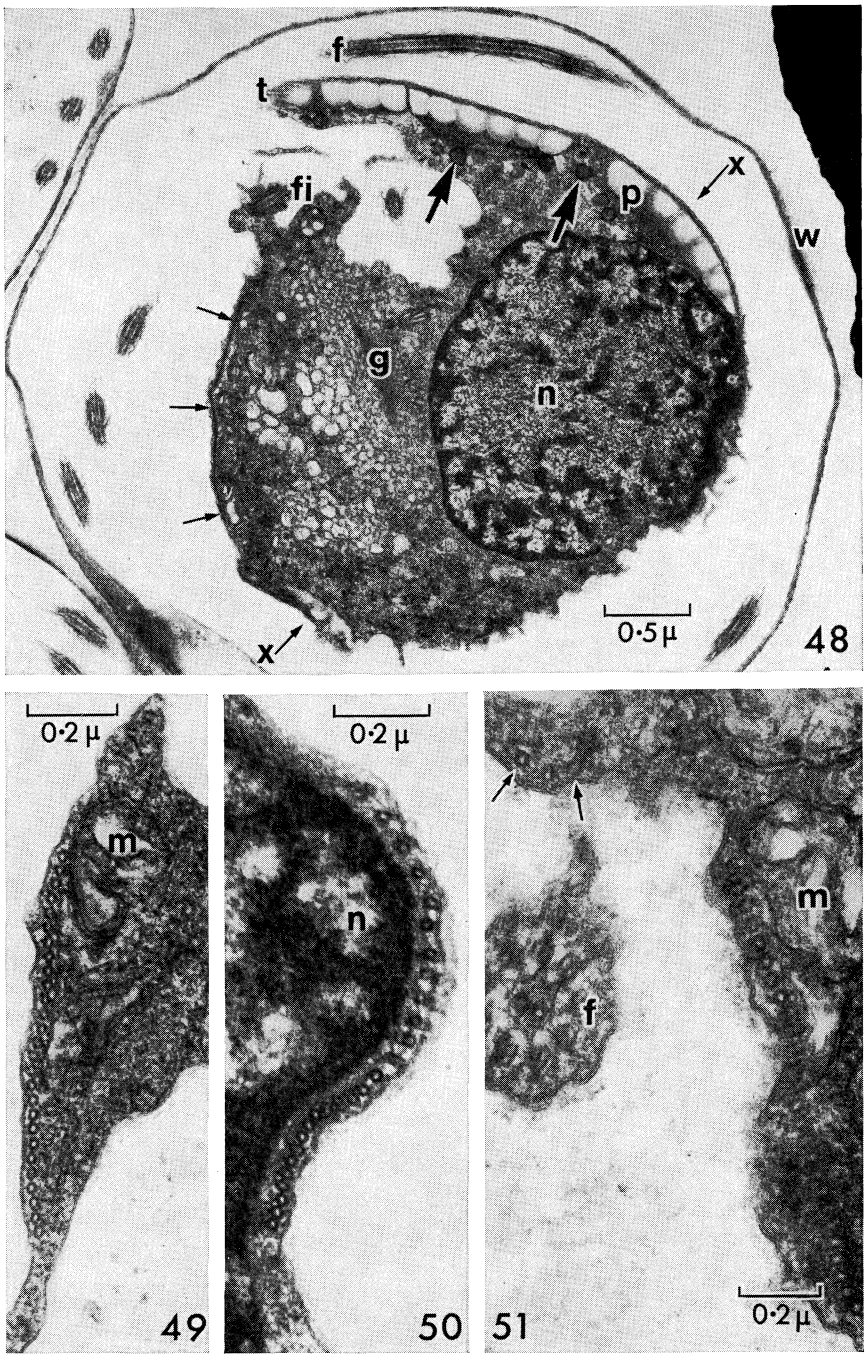
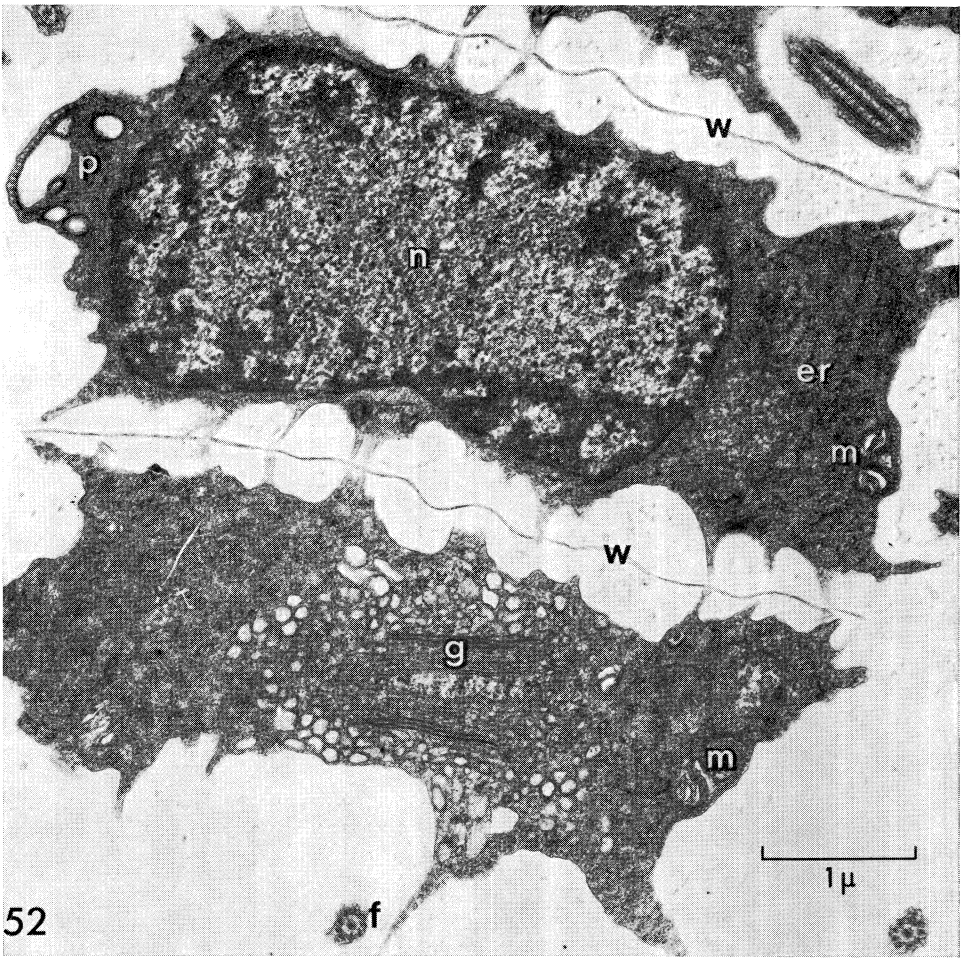


Fig. 48.—Further elongation of manchette, showing partly coiled cell in T.S. Flagella insertion now has moved a considerable distance from the nucleus (cf. Fig. 45) which is rounded. Mitochondria beginning to line up along manchette tubules (small arrows). Plastids are accumulating starch, and lipid (?) bodies are moving near them (large arrows). Cytoplasm considerably shrunken. Figure 52 is a section roughly in the plane x—x.

Figs. 49 and 50.—Mitochondria seen lining up against the band of manchette tubules (see Fig. 52) which is now at its full complement of *c.* 27 (Fig. 49); tubules seen lying against nucleus in Fig. 50.

Fig. 51.—Section through the manchette near the basal bodies; 26 tubules are estimated to be present in the band with mitochondria close to them. Note stellate pattern in the flagella, and also a group of three nearby microtubules (arrows). Compare with Figures 39 and 70–72.



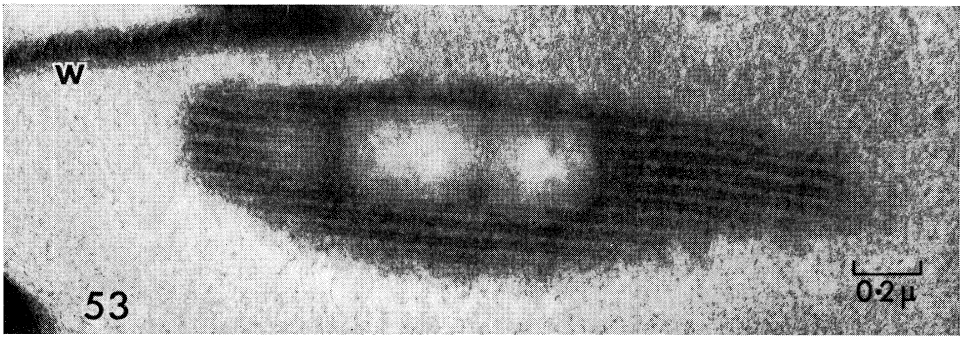


Fig. 52.—Top cell is roughly equivalent to that in Figure 48, sectioned in the approximate plane x—x. Cytoplasm pulling away from walls, but is still interconnecting cells. Sections of the curved bands of manchette microtubules clearly visible at mitochondria (*m*) and starch-filled plastid (*p*). The lower cell was sectioned "under" the nucleus and reveals paired golgi bodies (*g*) — clearly defined granules are often seen between them. Endoplasmic reticulum (*er*) just visible.

Fig. 53.—Section tangential to the curved band of manchette, showing parallel tubules, underlying plastids just visible (see "*p*" in Fig. 52).

(xii) *Other Organelles*

Other organelles or objects whose significance is obscure were sometimes seen in the cytoplasm. Little comment on such observations is warranted at this stage. However, an interesting, myelin-sheath-like structure was often present alongside almost mature organisms (possibly a product of cytoplasmic elimination) (Fig. 83).

(xiii) *Abnormal Development*

Some abnormalities in development were encountered — Figure 84 shows a cell with similar cytoplasmic features to adjacent cells of normal appearance, but its cytoplasm is disperse instead of being dense (e.g. Figs. 52, 64, etc.). In Figure 64, nuclear division had apparently been incomplete — this was a relatively common abnormality. Some organisms had only one flagellum (Fig. 80).

IV. Discussion

Spermatogenesis is a very interesting process that has been studied in a wide variety of animal and insect cells. Spermatogenesis occurs in few plants and is unusual in several important respects in that:

(1) Plant cells are contained within fairly rigid cell walls and the formation of a motile spermatozoid represents a departure from this general property.

(2) Cells of higher plants do not possess centrioles, whereas many lower forms of plants (e.g. algae) do. *Chara* seems to fall between these two categories.

(3) Plant cells have some organelles not found in animal cells (e.g. plastids) and some organelles whose properties and functions appear different or modified compared with their equivalents in animal cells (e.g. plant wall microtubules). Therefore the transformation of a cell typical of plants into a cell more typical of many animal cells might be expected to show some fundamental similarities or disimilarities in cell ultrastructure and function.

In animal sperm cells, some functions of manchette microtubules seem quite easily understood. They appear to be involved with movement (e.g. undulatory) of the mature sperm (Robison 1966) and in the formation and maintenance of cell asymmetry (i.e. the highly elongate form of spermatozoid — Silveira and Porter 1964; Kessell 1966; Robison 1966; Anderson 1967; Anderson, Weissman, and Ellis 1967; etc.). In some recent elegant work, McIntosh and Porter (1967) showed that microtubules appear successively in two very distinct arrangements about the nuclei of fowl spermatids; firstly, they were present as a double helix wrapped closely around the elongating nucleus, then they disappeared and were replaced by a sheath of longitudinal tubules. Furthermore, some abnormalities in development could be related to abnormal microtubule organization.

Manton and Clarke (1956) and Manton (1957, 1959) showed a fibrous band in the coiled spermatozoids of *Pteridium*, *Fucus* (the "proboscis fibres"), and *Sphagnum*, which they concluded was significant in maintaining structural stability. Diers (1967) and Rice and Laetsch (1967) reported such microtubules in *Sphaerocarpus* and *Marsilea*, while Norstog (1967) associated these microtubules with "euglenoid" movement in *Zamia* sperm. Such tubules may also be involved in nuclear condensation with the concurrent elimination of some nuclear component inside a

vesicle (Kessell 1966; Anderson, Weissman, and Ellis 1967). Evidence of a similar direct involvement in nuclear condensation has not been obtained in *Chara*, but organization and growth of the manchette microtubules seem certain to be associated with (and responsible for) the development of the coiled, elongated form of the mature spermatozoid, and highly specific reorganization of cellular components (plastids, mitochondria, and basal bodies).

Mottier reports that the first sign of this cytoplasmic differentiation in *Chara* "appears in the form of a very delicate thread or band extending partly around the cell and embracing the nucleus in its arc. This thread seems to be merely a differentiation of the plasma-membrane" (Mottier 1904, p. 248). The author concludes that this "thread" or "band" resulted from the formation of manchette microtubules close to the plasmalemma. Mottier also comments on the appearance of a "conspicuous row of granules" which must be the plastids lining up along the manchette microtubules. The manchette is probably also part of the "blepharoplast" described by Fritsch (1935, p. 458). There is no indication as to why the plastids (and later the mitochondria) move to the microtubules as they do. This organized morphogenic movement might possibly be similar to the highly directional movement of much smaller cell-wall vesicles that seem guided by spindle microtubules into the cell plate (Esau and Gill 1965; Pickett-Heaps and Northcote 1966*a*, 1966*b*) or by wall microtubules into xylem wall thickenings (Pickett-Heaps 1967*d*, 1968*b*).

The relationship of manchette microtubules to the formation, structure, and function of the mature spermatozoid of *Chara* appears equivalent in most respects to that of other sperm cells. Manchette microtubules may be involved in movement of the mature spermatozoid (cf. Robison 1966; and others), but the author can add little further evidence. Once released from the antheridia, the spermatozoids are very difficult to catch. The author has watched only one (possibly injured) live spermatozoid under phase-contrast. The organism, still quite coiled, showed rapid vibrations and some undulations along its length. However, it has yet to be proven that the manchette microtubules still exist in such released spermatozoids, although this seems likely. Kessell (1966, 1967) found that manchette microtubules disappeared from around sperm heads after spermiogenesis was completed in the dragonfly and grasshopper.

The formation of the manchette is of special interest. As stated previously, the author at first concluded that the wall microtubules were directly involved. However, the manchette adjunct seems implicated in the synthesis or organization of this important structure or in both processes. A search through the literature has not yet revealed an identical phenomenon in plants described elsewhere. However, Burgos and Fawcett (1955) found a ring-like structure in cat spermatids that is probably similar to the manchette adjunct, observing that "the filaments comprising the

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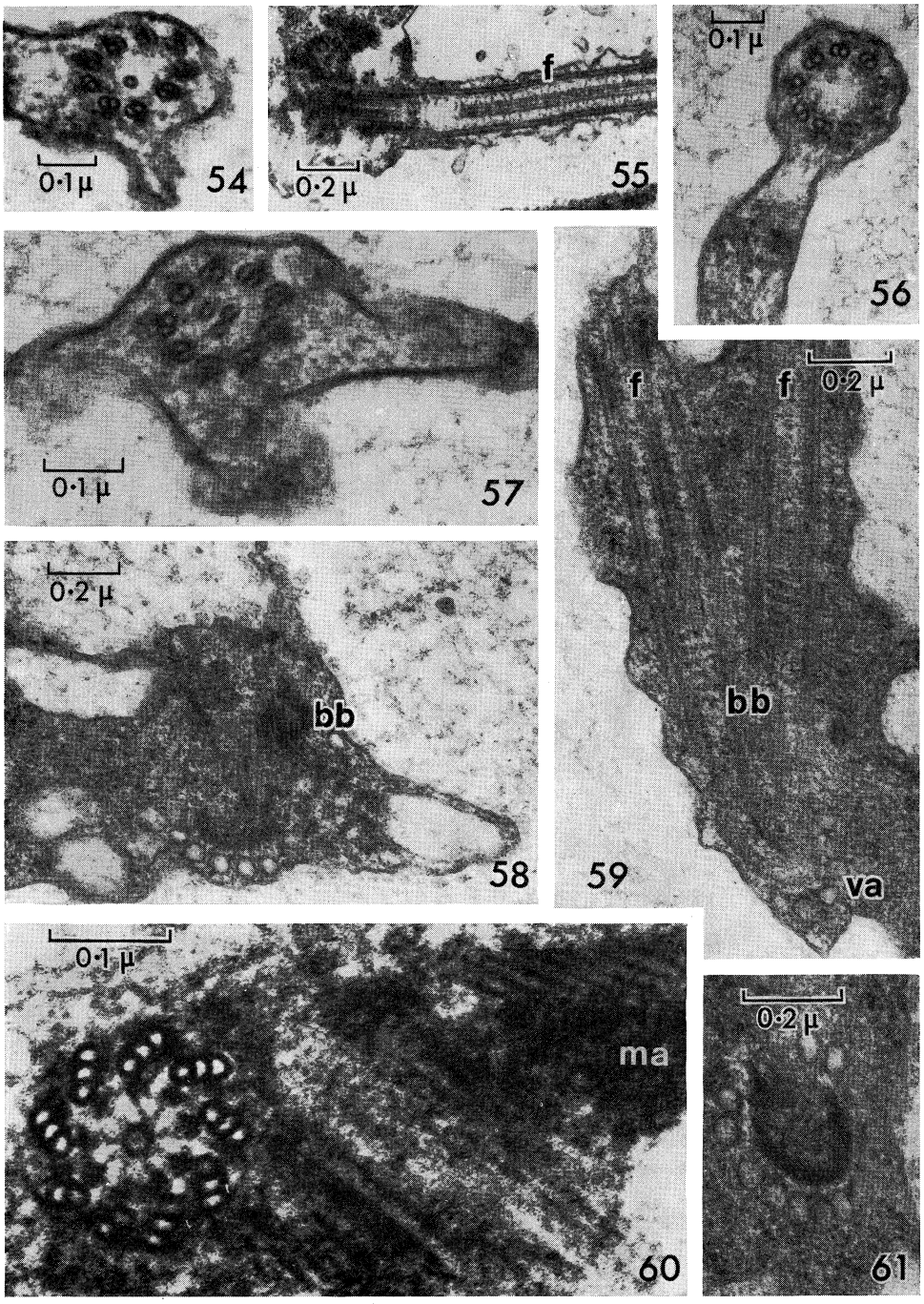
Fig. 58.—Typical section of vesicular adjunct; part of basal body (*bb*) is also visible.

Fig. 59.—Flagella insertion in older organism; vesicular adjunct (*va*) in normal position. Compare angle between these older flagella, and that in Figure 28.

Fig. 60.—T.S. through basal body, flagella quite long. Note characteristic wheel-like structure inside basal body. Edge of manchette adjunct (*ma*) visible.

Fig. 61.—Another characteristic section of the vesicular adjunct.



Figs. 54, 56, and 57.—T.S. through flagella near insertions at basal bodies (see Fig. 55). Note variation in number of central tubules. The three central tubules (Fig. 57) probably represent an abnormality. Note also loose membrane around flagella.

Fig. 55.—L.S. through flagella near insertion at basal body. Note that central tubules begin a short distance from the flagellar insertion.

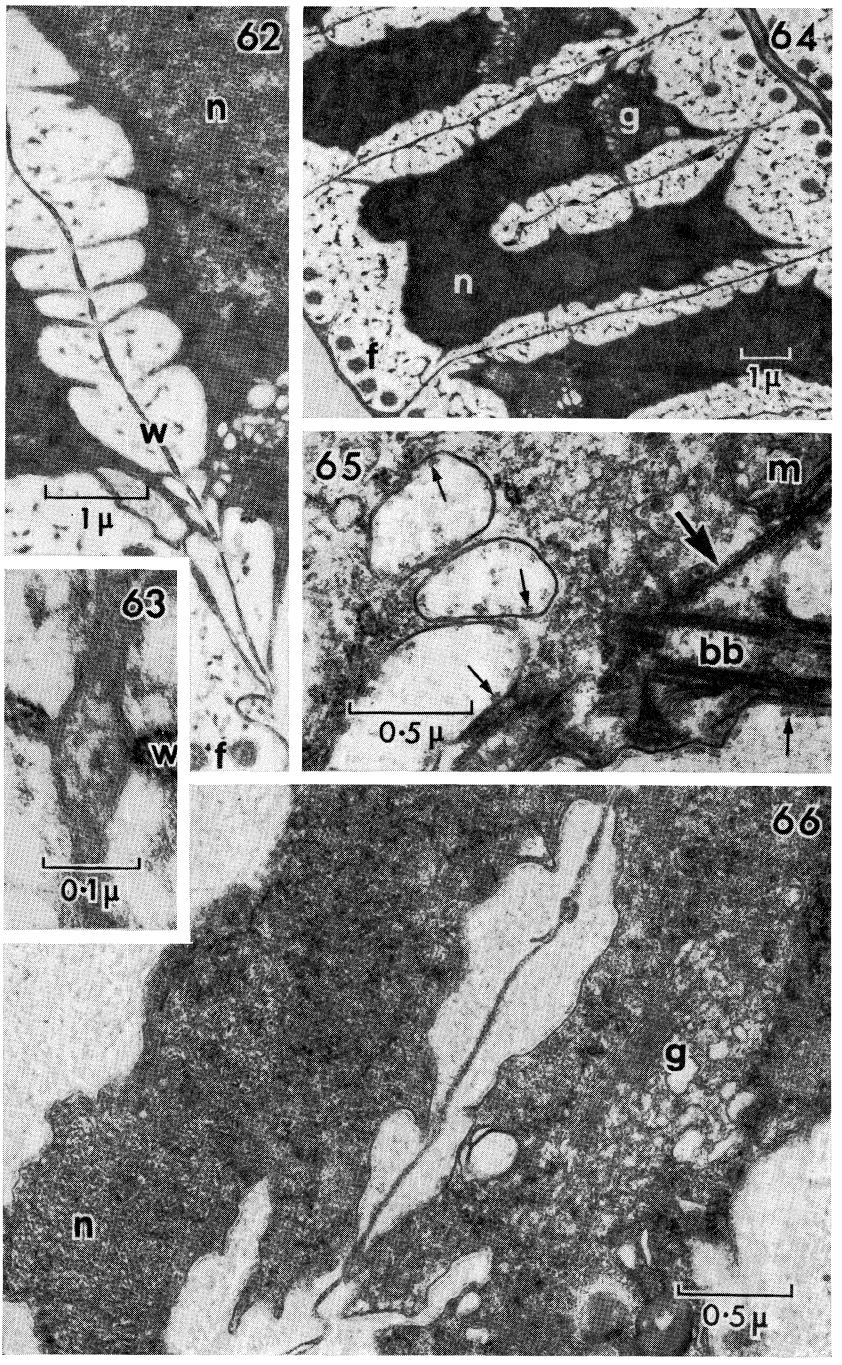
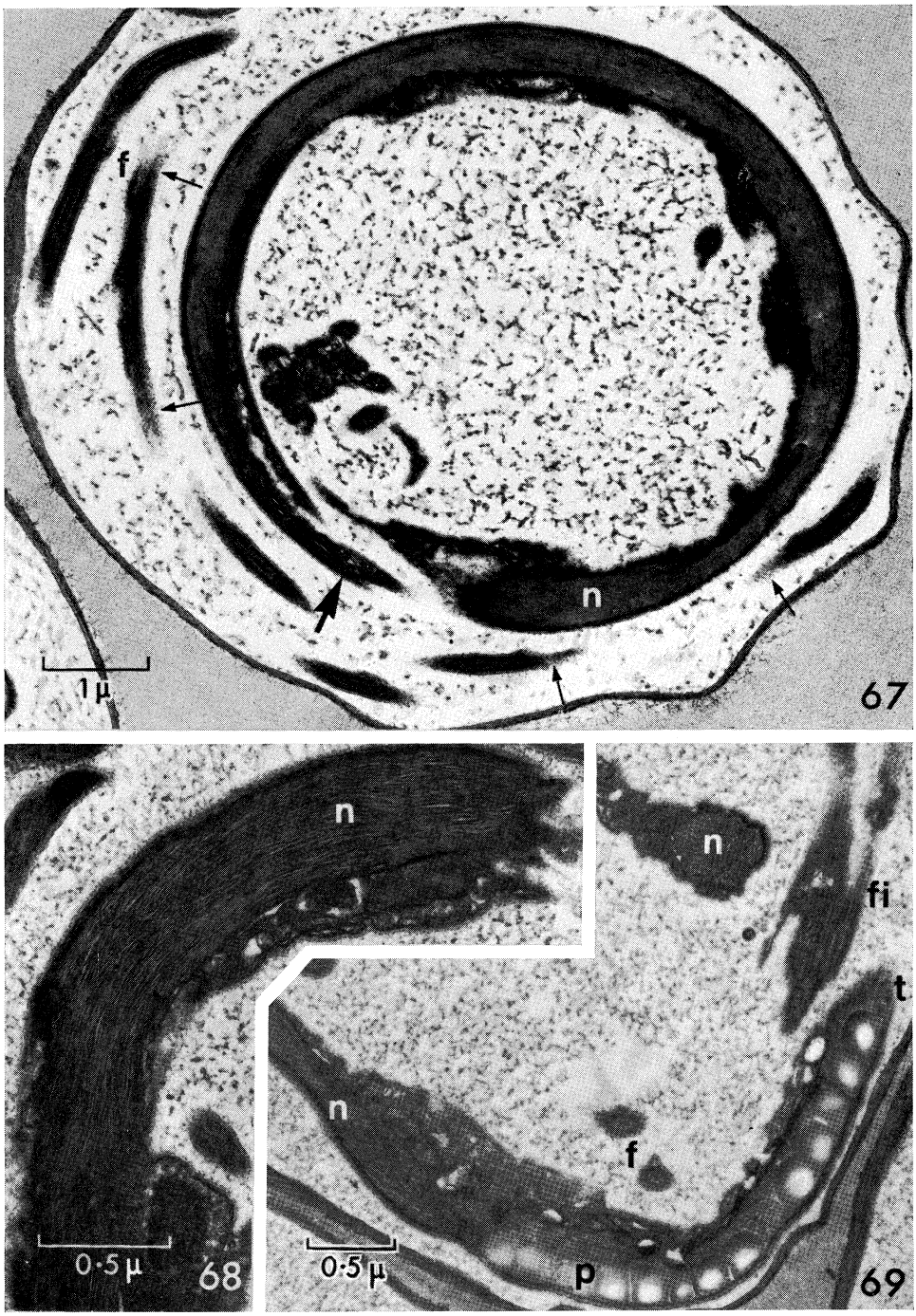


Fig. 62.—Cytoplasmic shrinkage becoming very marked. Note strands of cytoplasm which still interconnect adjacent cells through plasmadesmata in the wall.

Fig. 63.—As for Figure 62; plasmalemma continuous through wall (*w*).

Fig. 64.—Spermatogenous threads in L.S.; abnormality with nuclei of adjacent cells incompletely separated. Note typical dense cytoplasm and sections of looped flagella (*f*).

Fig. 65.—Mitochondrion (*m*) near manchette tubules (large arrow); spindle-shaped ciliary rootlet structure still present in these maturing spermatocytes. Scales (small arrows) are visible on external membrane (see Fig. 85) and also possibly on inside of large cytoplasmic vesicles.



Figs. 67–69.—Nuclear condensation and elongation. *67*, Nucleus now highly coiled. Mitochondria and flagella insertion just visible (large arrow); plastids out of plane of section. Flagella covered in scales (small arrows) coiled outside nucleus which is now a layered structure. *68*, Portion of coiled nucleus showing dense, layered texture. Note remains of cytoplasm still present. *69*, As for Figure 67, showing starch-containing plastids lined up along manchette tubules (*t*), adjacent to nucleus. Portions of flagella insert (*fi*) visible.

Fig. 66.—Nuclear condensation: nuclei elongated and beginning to coil in these cells. Texture of nucleus becoming granular. Some cytoplasmic organelles loosing their structure — note poorly defined golgi body (*g*).

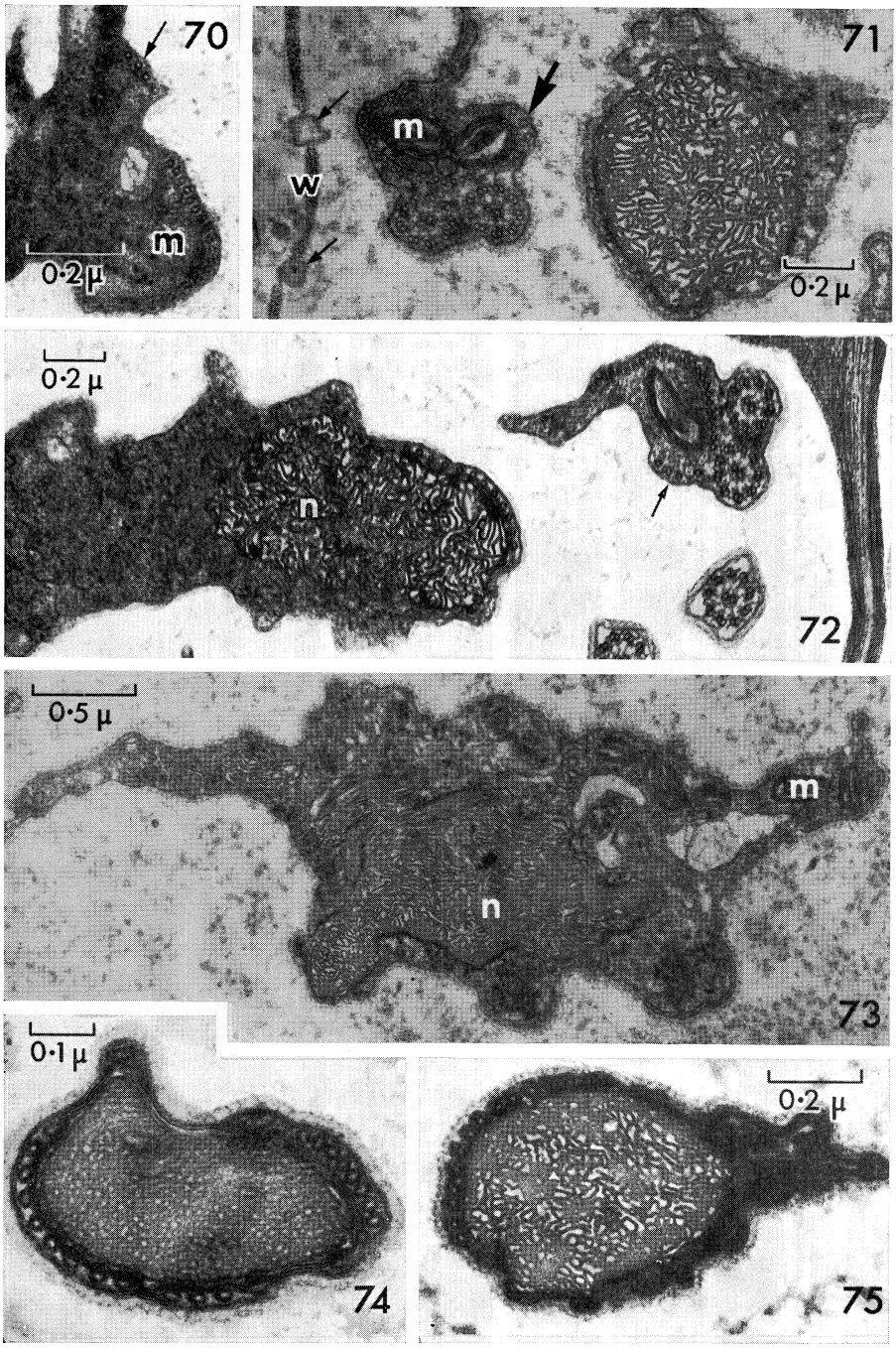


Fig. 70.—T.S. of manchette, past flagella insertion. Manchette tubules near mitochondria (*m*) as usual — note group of four (sometimes three) tubules separate from others (arrow).

Fig. 71.—Continuing nuclear shrinkage; lamella beginning to fuse. Flagella insertion seen in T.S.; note, as in Figure 70, groups of four tubules (large arrow) separate from manchette tubules. Traces of membrane still attached to plasmadesmata (small arrows).

manchette arise from it (i.e. the ring) . . . . . ." (Burgos and Fawcett 1955, p. 292). Anderson (1967, p. 261) also finds that manchette microtubules arise from "dense pericentriolar material". Hoffman and Manton (1962, 1963), Manton (1964*b*), and Ringo (1967) report small rows of microtubules near the basal bodies in various algae but these were termed ciliary rootlets, etc. (see below). Paolillo (1965) describes the "dreiergruppe" in the androcyte of *Polytrichum*; it is a more complicated structure than the manchette adjunct and he quotes references which state that the "dreiergruppe" is not the analogue of the fibrous band described by Manton (1957, 1959) (Paolillo 1965, pp. 674–5).

The author's interest in the manchette adjunct was stimulated by its evident relationship through microtubules to development and morphogenesis during spermatogenesis. Individual observations on the manchette adjunct removed from the context of sequential development are of far less significance. As to the actual functions and nature of the adjunct, speculation is premature at this stage. It seems most likely involved in a special form of microtubule synthesis where exact spatial configuration of the tubules might be important (perhaps similar to the basal body–flagella relationship), although many other possibilities exist.

True "ciliary root microtubules" are perhaps also present in early and late stages during *Chara* spermatogenesis (Figs. 37 and 39 — cf. Hoffman and Manton 1962, 1963; Manton 1964*b*; Ringo 1967). If so, these are either eliminated during further development (suggesting that they are in this case an expression of a primitive form of the cell, rendered obsolete during evolution) or else they are incorporated into the manchette tubules, perhaps being represented by the characteristic group of four tubules seen close to the mitochondria in more mature organisms (Figs. 70–72).

The appearance of the centriole is of considerable interest during spermatogenesis in *Chara*. The vegetative cells or other cells in the antheridia of this alga have never yet been seen to contain a centriole or recognizable precursor (Pickett-Heaps 1967*a*, 1967*b*, 1968*a*). The impression gained from a long and rather frustrating search into centriole formation was that the catherine-wheel structure might be the procentriole (cf. Gall 1961); however, the evidence presented above is hardly convincing at this stage. The wheel-like structure has been reported in a very wide variety of centrioles and basal bodies. The problem of accounting for centrioles which "appear" during differentiation is quite common, being often avoided by authors through lack of information. For example, Dingle and Fulton (1966) in their study of the flagella apparatus of *Naegleria* say little about this. For the same organism, Schuster (1963) suggested that aggregations of endoplasmic reticulum might be involved in formation of kinetosomes (i.e. basal bodies). In an insect,

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Fig. 72.—Coarsely lamellate nucleus (younger than that in Fig. 71). Note dense, rather formless cytoplasm, and as usual, the four tubules separate from the others at the manchette (arrow).

Fig. 73.—As for Figure 71. The mitochondria (*m*) are sectioned some distance away from the flagella insert, but the group of four tubules mentioned above is not now visible. Typical formless, disintegrating cytoplasm.

Fig. 74.—Continuing fusion of lamellae in maturing sperm nucleus. Manchette tubules have split up slightly into groups.

Fig. 75.—As for Figure 71 but note outer membrane shrinking over the individual manchette tubules. Outer membrane typically covered with even layer of material.

Barker and Reiss (1966) suggest that material, termed the "centriolar adjunct", moves into the cytoplasm from the nucleus, and that this may contain a (second) centriole (cf. Kessell 1967, figs. 6 and 7), but the situation is not very clear. In an interesting paper on a water mold (*Allomyces*) Renaud and Swift (1964, figs. 1, 4, and 6) illustrate the existence of a small procentriole which looks similar to the procentriole postulated to exist in *Chara*. They conclude that the centriole does not arise *de novo*. Mizukami and Gall (1966, pp. 109–10), in their study of centriole replication in two fairly primitive plants (*Zamia* and *Marsilea*), have shown that a very large spherical blepharoplast developed which is comprised of hundreds or thousands of procentrioles; fragmentation of the blepharoplast releases the procentrioles (these again are not unlike those shown above in *Chara*) which migrate to the cell surface to form basal bodies. Again, the ultimate origin of the blepharoplast is obscure. Since such procentrioles are very difficult to see, they may well have been present in the cytoplasm all the time. The author supports this view, and cannot categorically deny their existence under such circumstances in vegetative cells of *Chara* (or even in the cells of *Triticum*).

The significance of this subject (*de novo* synthesis of centrioles) has further ramifications. The involvement of centrioles in spindle structure of older spermatogenous cells in *Chara* resembles that in animal and other cells, yet in vegetative *Chara* cells, and cells of higher plants generally, no centrioles are seen in association with the mitotic spindle (the possible existence of ill-defined procentrioles, although unlikely, cannot be ruled out). This is of some importance, since there is a common belief that centrioles are vitally concerned in the establishment of polarity prior to division. This was stressed recently by Brinkley, Stubblefield, and Hsu (1967, pp. 1 and 15–16) who, however, also say that centriole movement might be related to microtubule formation (Brinkley, Stubblefield, and Hsu 1967, p. 16). In particular, many authors have cautiously suggested that centrioles might be involved in (spindle) microtubule formation (see, for example, de-Thé 1964; Krishan and Buck 1965; Murray, Murray, and Pizzo 1965) and there can be no doubt of the close association of the two organelles (e.g. Robbins and Gonatas 1964; Szollosi 1964; de-Thé 1964). Anderson, Weissman, and Ellis (1967, p. 19) have suggested that the centriole might even be involved in the polymerization of the manchette microtubules during spermatogenesis, and Anderson (1967) considers the centriole vital in both establishing cell polarity and organizing the manchette microtubules during spermatogenesis in *Drosophila*. Such views are difficult to reconcile with related phenomena in plant cells, where microtubules are polymerized to form part of spindle structures (Ledbetter and Porter 1963; Pickett-Heaps and Northcote 1966*a*, 1966*b*) and are also synthesized and moved about in xylem wall differentiation (Pickett-Heaps 1967*d*), for example, without any apparent intervention of a centriole or procentriole. It seems far simpler and consistent to regard the association of the centrioles with the mitotic spindle as a convenient means of ensuring invariable

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tubules seems to decrease progressively along the organism towards the plastids; *79*, as for Figure 77, but nine separated manchette microtubules present.

Fig. 80.—Flagellum insert, abnormal spermatozoid with only one flagellum (cf. Figs. 71, 72, etc.). Complement of tubules seems approximately normal.

Fig. 81.—L.S. of flagellum: dense inclusion under flagellum membrane (see Fig. 82).

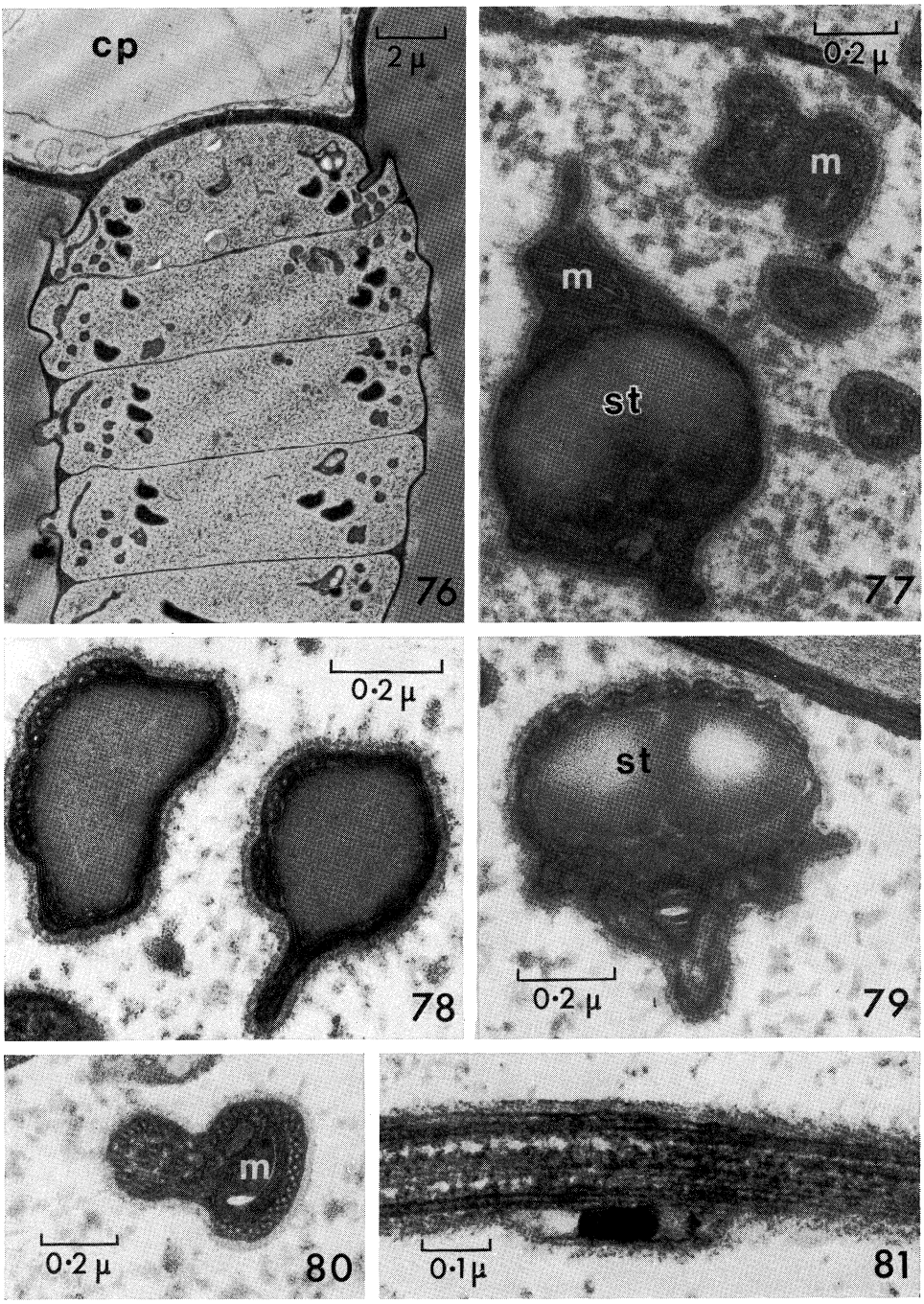
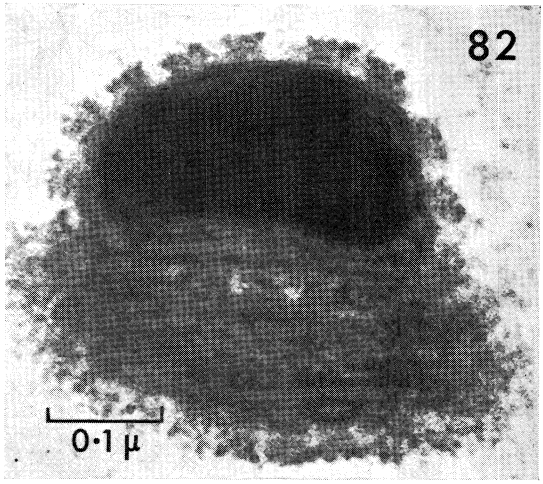
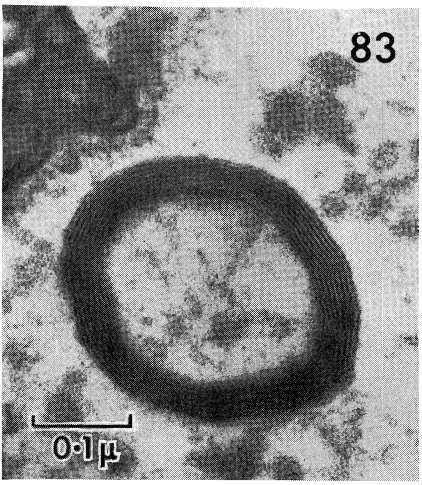


Fig. 76.—L.S. of spermatogenous thread attached to secondary capitula (*cp*). Spermatozoids virtually mature (cf. Fig. 5). Note sections of sperm nuclei, flagella, flagella insert and plastids.

Figs. 77–79.—Mature spermatozoids: *77*, plastid containing starch (*st*), and mitochondria; this was probably right near the tip of the organism as no microtubules are present. Flagella insert and mitochondria above; *78*, note dense homogeneous nucleus of spermatozoid. Number of





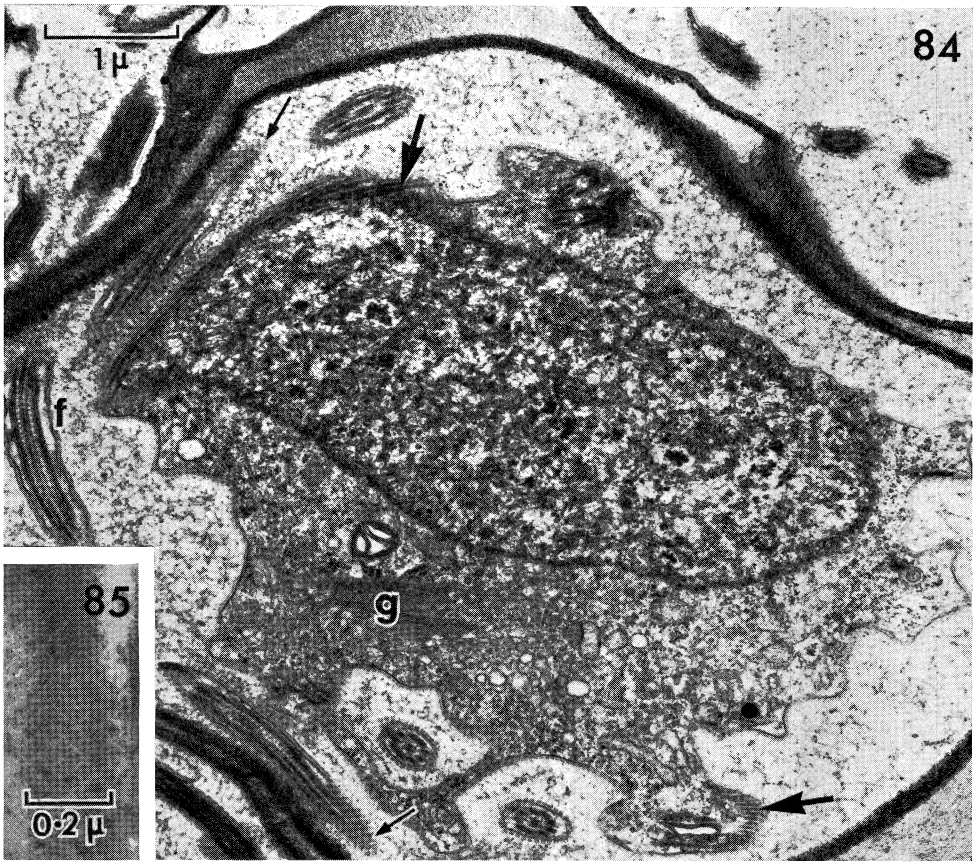


Fig. 82.—As for Figure 81; inclusion seen at higher magnification, shows evidence of layered or crystalline structure. Note scales around outer membrane.

Fig. 83.—Myelin-like figure frequently found in almost mature cells.

Fig. 84.—Most unusual abnormality. All surrounding cells were very dense and shrunken (as in Figs. 62 and 64). This cell remained disperse, though possessing apparently all of the structural characteristics of adjacent cells, including manchette tubules (large arrows); section cut skew. Note flagella scales (small arrows).

Fig. 85.—Tangential section of flagellum, showing pattern of scales on surface.

equal partitioning of the organelles during cell division. The evidence suggests that centrioles are intimately associated with a "microtubule-organizing region." Thus, their movement in animal cells before prophase is more logically a response to, but not a cause of, the development of polarization. Further, such a morphogenic movement of centrioles related to a reorganization of microtubules can then be roughly equated with many other such movements of cell organelles — for example, the movement of plastids and mitochondria as described above. This interpretation is supported by the obvious inference that it is the 9 by 2 tubular structure of flagellar components that is given by the template of 9 by 3 tubules in the basal body — there is no similar template for the central two tubules, and recently Behnke and Forer (1967) have shown clear differences in the chemical properties of these and some other microtubular structures (see also Shelanski and Taylor 1967). There can be no doubt as to the fundamental requirement for centrioles or basal bodies in the formation of flagella, but there seems considerable doubt as to the requirement of these organelles for the synthesis and organization of other cytoplasmic microtubules.

The morphogenic movement of mitochondria to the manchette microtubules appears to be very characteristic of spermatogenesis (e.g. Anderson 1967; Anderson, Weissman, and Ellis 1967; Kessell 1967; and many others) and, being situated near the cilia, they are obviously well placed to provide these organelles of motility with their energy requirements. In *Chara* the movements of plastids and lipoid bodies towards a clearly defined region on the manchette (Figs. 45, 46, and 48) and the subsequent accumulation of starch which remains intact in the mature sperm (at least till release) suggests that the organism needs these storage organs for future viability. Similarly, other sperm cells may have some storage for glycogen, for example in the cilia (Anderson, Weissman, and Ellis 1967). Manton (1957) says that the plastids are jettisoned once locomotion starts in spermatozoids of *Sphagnum*; the present author cannot yet dispute a similar event happening in *Chara* but it seems unlikely that the plastids would be carefully retained and then filled with starch to be cast off in a similar fashion. Fritsch (1935, p. 458) says: "The liberated sperm appears prominently elongated, the accompanying cytoplasm being clearly marked at the anterior and posterior extremities . . .". Rice and Laetsch (1967) also found starch-containing plastids in *Marsilea* sperm. Some of the bodies seen at this region of the cell may be concerned with fertilization, but no data has yet been obtained on this point.

Nuclear condensation, where the chromatin becomes fibrous and then increasingly lamellate before these lamellae fuse, seems typical of spermatogenesis in other cells (e.g. Silveira and Porter 1964; Kessel 1967).

A function of the golgi apparatus in the production of flagellar scales has not been clearly demonstrated, though it was expected following the classic results of Manton and her colleagues (Manton and Parke 1965; Manton *et al.* 1965; etc.). Presumably cell wall synthesis had ceased during spermatogenesis, so the proliferation of vesicles around golgi bodies and other changes in the appearance of these organelles must have some other significance. In young, virtually undifferentiated spermatogenous cells, for instance, the lamellae of the golgi bodies were often swollen and

some large, electron-transparent vesicles were formed (cf. Pickett-Heaps 1967*a*, 1967*c*), but once differentiation commenced production of these large vesicles ceased. Other marked changes in the appearance of the golgi bodies, and their association with spiral ribosomes, cannot yet be explained.

The function of the vesicular adjunct, which is always seen close to the basal bodies, is not known. Its spatial association with basal bodies during initial and later stages of flagellar extrusion suggest that it is somehow involved in this process, but no direct supporting evidence has been forthcoming.

Bands of striated fibres interconnecting basal bodies are very well known. Such striated fibres are also found in algae (see Ringo 1967, pp. 565–7). The spindle-shaped ciliary rootlet structure seen in *Chara* (Figs. 26–29, 65) is not the same, however, since it contains only one central broad striation (probably an inappropriate term) and a symmetrical array of fibres which interconnects the basal bodies. These organelles appear early in differentiation (before extrusion of the flagella commences) and apparently disappear well before final maturation of the spermatozoid. It seems unlikely therefore, that they are associated with flagellar movement; they might be involved in the orientation of the basal bodies, or even (perhaps like the ciliary root microtubules — see above) be an equivalent of embryological gill-slits in higher animals (i.e. primitive features of evolutionary significance which appear and disappear without being important to the final organism). Until the function of these organelles is known, such ideas must remain conjectural.

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