THE STRUCTURE AND FUNCTION OF

CENTRIOLES AND THEIR SATELLITES IN

THE JELLYFISH *PHIALIDIUM GREGARIUM*

DANIEL SZOLLOSI, Ph.D.

From the Department of Biological Structure, University of Washington, Seattle

ABSTRACT

Testes of jellyfish *Phialidium gregarium* were fixed in 2 per cent OsO_4 in Veronal-acetate buffer at pH 7.4. Thin sections showed that in young spermatids the spindle fibers of the last maturation division are attached to satellites of the filament-forming centriole. In more mature spermatids this attachment is not observed. During the developmental phase, nine satellites can be observed emanating from the interspaces between the nine tubular triplets of this centriole. A circular region on each of the enlarged distal ends of the satellites attaches them to the cell membrane. The satellites apparently provide a firm anchor for the axial filament. Each of the epithelial cells covering the testis produces a single long flagellum. On the filament-forming centriole often a satellite can be observed to which tubules are attached. These tubules are 180 A in diameter and probably represent remnants of spindle fibers. It is suggested that the distal centriole has the ability to form several satellites or appendages at appropriate times during the cell cycle. These satellites are distinct from the daughter centrioles in that they are supportive structures: in certain phases of cell life, spindle fibers may attach to them, while in other instances the distal centriole and the flagellum it is forming are anchored by them.

Basal bodies, cilia, and flagella have been described in many different cell types. Morphological evidence has suggested that basal bodies are centrioles which give rise to cilia and flagella at certain stages of differentiation (5, 6). Soon after the first recognition of centrioles by means of electron microscopy and the description of their fine structure (1), small, dense bodies were observed which were connected to them by bridges (2, 3, 10). The terms pericentriolar body and satellite have been applied interchangeably to these dense bodies in the past. In this paper, evidence will be presented from electron microscope studies that two centrioles are present at the base of the sperm tail of the hydromedusa *Phialidium gregarium*, a distal or filament-forming centriole and a proximal centriole. The distal centriole forms the flagellum at a time when the spindle fibers of the last meiotic division are still associated with satellites projecting laterally from it. Further, the morphology and the functional role of satellites will be discussed.

MATERIALS AND METHODS

465

tered sea water, and fixed for 1 hour in a 2 per cent OsO₄ solution buffered by Veronal-acetate at pH 7.5. In some cases, the small hydromedusae were dropped directly into the fixative and the testes were dissected in 70 per cent ethanol. After ethanol dehydration was completed, small pieces of the tissue were embedded in Epon 812 (13). The specimens were mounted on metal chucks and oriented in such a manner that complete cross-sections could be obtained. Sections were cut, on a Huxley microtome with a diamond knife, at a thickness which gave silver to pale gold interference colors and mounted on
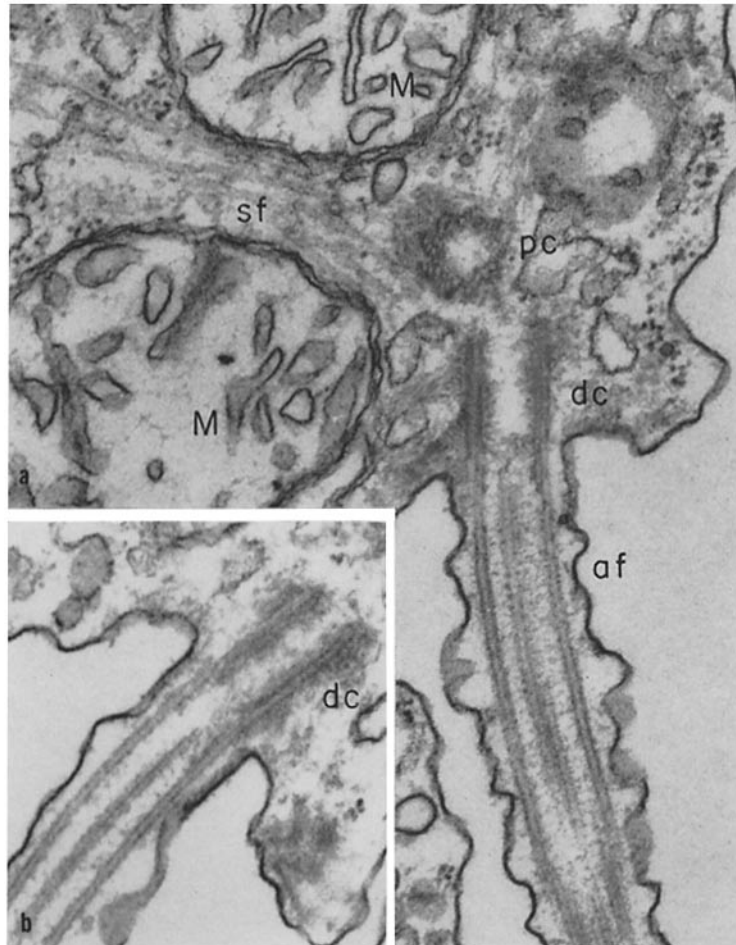
Abbreviations for Figures

af, axial filament
bp, basal plate
cr, ciliary rootlet
dc, distal centriole
f, flagellum
M, mitochondrion

N, nucleus
pc, proximal centriole
ps, perinuclear space
sf, spindle fibers
st, strands of satellites
S, satellite

For detailed descriptions of the figures see text.

FIGURE 1 *a* A spermatid with the proximal centriole (*pc*) and distal or filament-forming centriole (*dc*). Several spindle fibers (*sf*) converge toward them. × 61,000.

FIGURE 1 *b* The continuity is demonstrated of the tubules of the tail filament with the tubules within the wall of the distal centriole. × 58,000.

either 200 mesh or 75 X 300 mesh copper grids coated with a carbon film. All sections were stained with the alkaline lead technique of Millonig (15). Electron micrographs were taken with an RCA 2C electron microscope, and Dupont Ortho A lithographic sheet film with Cronar (polyester) base was used (23).

OBSERVATIONS

Centrioles and Flagellum of the Spermatid

Spermatocytes and spermatids of *Phialidium* are favorable objects for the study of centrioles because the cells are small and develop nearly synchronously in clusters; there is, hence, a good opportunity for locating in thin sections large numbers of centrioles in different functional stages. Spermatogenesis proceeds in the testis radially and

peripherally from the gastrodermis, and the developmental stages can be determined accurately (19). The protruding sperm-tail filament serves as an accurate marker for locating the centrioles in thin sections.

Fig. 1 *a* shows a cross-section of the proximal centriole of a *Phialidium* spermatid demonstrating cytoplasmic tubules converging toward this centriole. Figs. 1 *a* and *b* show the distal centriole cut longitudinally and demonstrate that the peripheral tubules of this filament-forming centriole are continuous with the peripheral tubules of the flagellum. The flagellum emerges from a cup-shaped fold of the cell membrane at the distal end of the centriole, and at this level the basal plate can be seen. An appendage projects laterally at an angle from the filament-forming centriole (Fig. 2) and shows a slightly club-shaped termination.
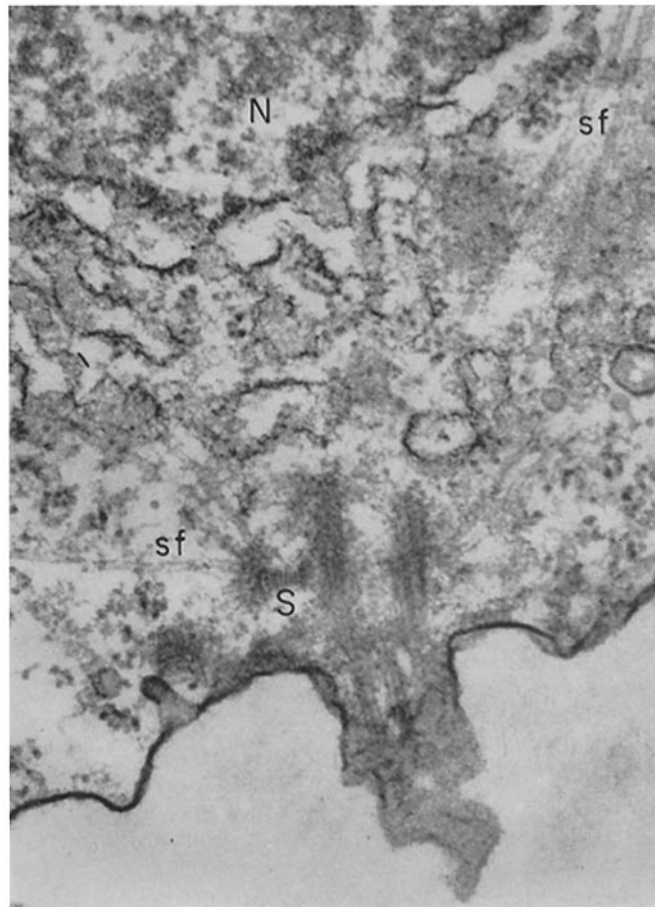

FIGURE 2 A young spermatid, showing three spindle fibers (*sf*) making contact with a cross-striated satellite (*S*). X 58,000.

This appendage will be referred to as a satellite. Tubular elements 180 A in diameter are associated with the enlarged terminal end of this satellite (Figs. 2 and 7). These and several other tubules also observed in the cytoplasm probably represent the spindle fibers or astral rays of the last maturation division. The satellite shows a cross-striation

In cross-section (Figs. 1 a, 3 a) the typical pinwheel-shaped arrangement of triplets of tubules can be recognized in the walls of both the proximal and distal centrioles (8, 16). Each one of the interspaces between the nine tubular triplets has a dense matrix which gives rise to a satellite displaying the same 120 A periodicity (Figs. 2 and 4).
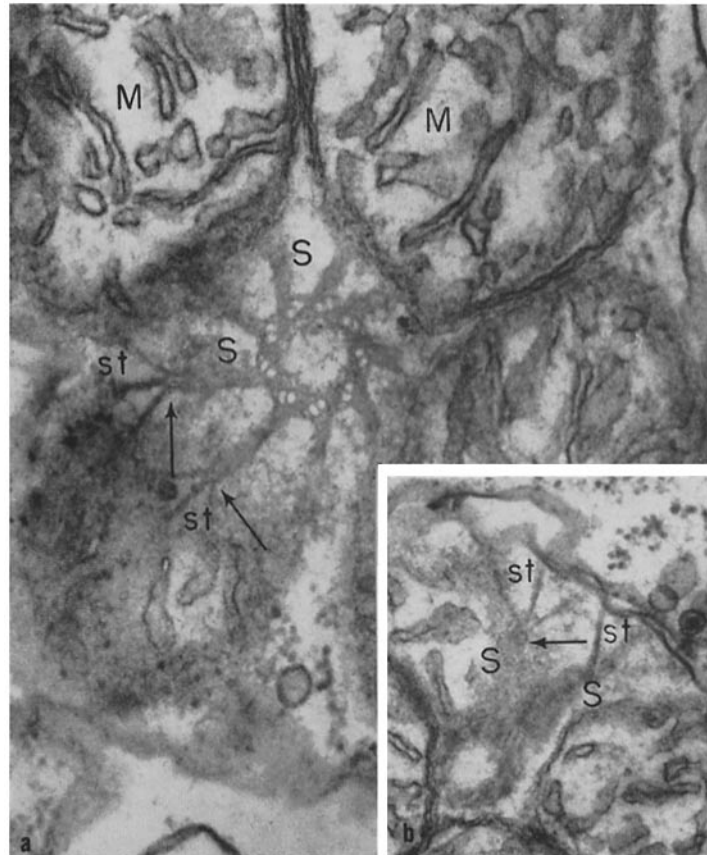
FIGURE 3 a   Cross-section of spermatid distal centriole with its nine tubular triplets and nine satellites. Two of the satellites demonstrate the circular profile (arrows) from which three strands (st) originate. One satellite (S) originates from the dense matrix between adjacent tubular triplets. X 73,000.

FIGURE 3 b   A spermatid centriole with two satellites and their strand-like (st) arborizations. At the arrow is seen the circular profile where the strands attach. X61,000.

with a 120 A separation between two dense components. This periodicity differs from that of the cross banding of ciliary rootlets in which the prominent bands are reported to be 850 to 900 A apart. Spindle fibers at metaphase of the second meiotic division attach to a dense structure near a centriole which is probably the club-shaped enlargement of a satellite (Fig. 14).

The array of satellites also assumes a pinwheel configuration. Gibbons and Grimstone (8) reported, in their study on flagellates, the presence of a small fiber connecting the inner tubule (A) of one triplet with the outermost tubule (C) of the neighboring triplet. Due to the dense matrix between the tubular complexes within the wall of the centrioles in the spermatid of *Phialidium*, the
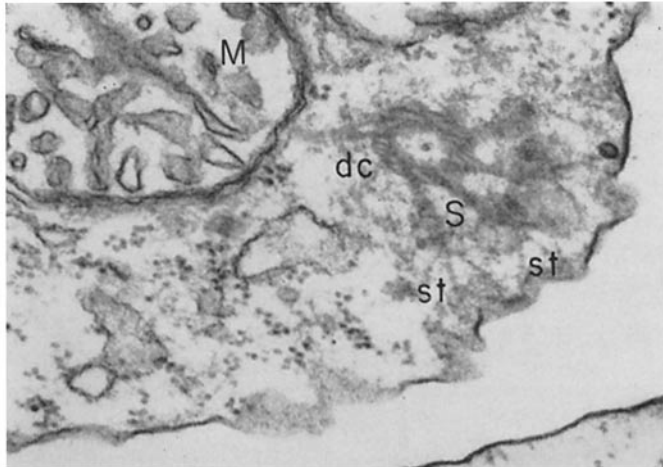
FIGURE 4   An oblique section through the spermatid filament-forming centriole (dc) with several satellites whose origin can be traced to the dense matrix between the tubular triplets. × 56,000.
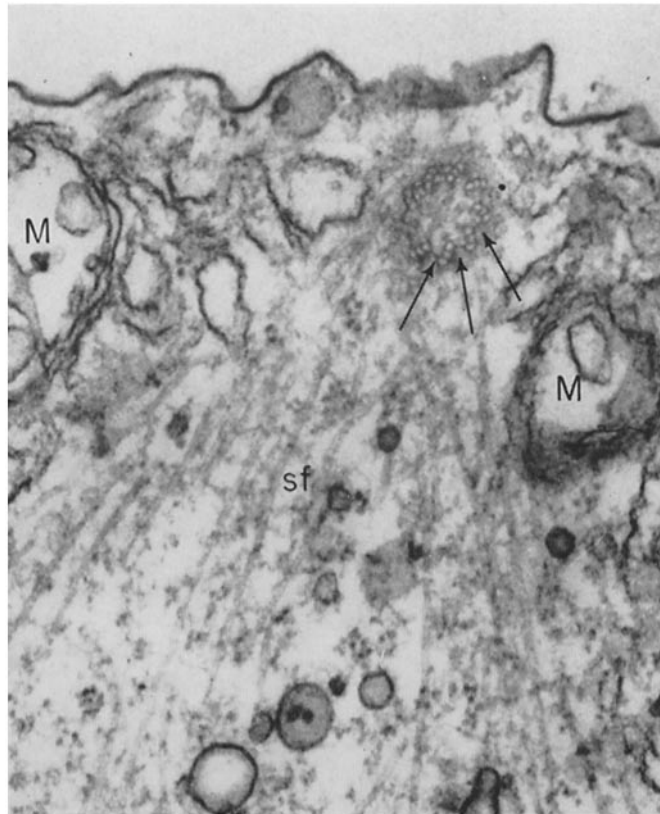

FIGURE 5   Metaphase of the second maturation division of a spermatocyte. The centriole at the spindle pole shows the fine fibrils between the A and C components of the adjacent tubular triplets (arrows). × 67,000.

fine fibrous connecting elements between the A and C components of adjacent triplets were not seen. In the centriole of a spermatocyte found in metaphase of the second maturation division, however, such fibers are observed (Fig. 5). In more mature spermatids fine strands project from circular profiles at the distal end of the satellite into the cytoplasm. These strands may extend along the cell membrane or, in other cases, may be firmly attached to the cell membrane. A dense granule is found in the center of the circular profiles (Figs. 3 and 6). A periodicity of 120 A is also observed

cases, three fine fibrils arranged like pigeon toes are seen per satellite. Since three is quite consistent for the number of fine strands observed thus far in many different planes of section, it is assumed that this is the usual case, though a larger number cannot be excluded. The plane of section in Fig. 6 is probably very close to the basal plate, the transition zone between the distal centriole and the sperm-tail filaments (see Fig. 8 and cross-section E in text; Fig. 3 in Gibbons and Grimstone, reference 8). Only two doublets of peripheral tubules are present and these are connected to one another
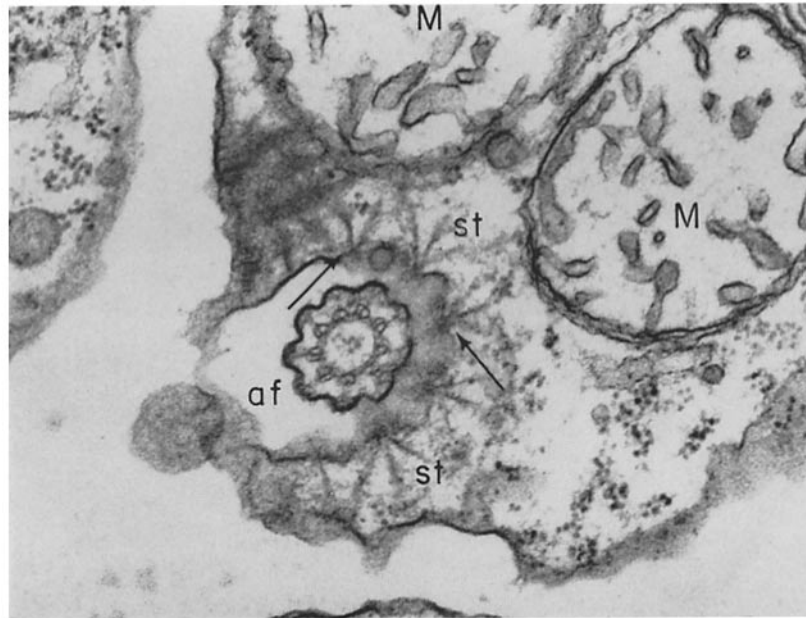

FIGURE 6   The circular profiles (arrows) of the satellites and their strands (st) are near the obliquely cut cell membrane of the spermatid. The tail filament (af) is sectioned close to the point where it emerges from the cytoplasm. X 56,000.

on some of the strands (Fig. 4). This complex, that is, the circular profiles and the fibrils emanating from them, seems to anchor the filament-forming centriole to the plasma membrane. In longitudinal sections, the satellites often form an angle of 55 to 60° with the long axis of the centrioles as they extend toward the cell membrane.

In a slightly tangential cross-section (Fig. 6) through the cup-shaped region where the flagellum emerges from the cytoplasm, the relationship of the satellite attachment complex is particularly clear. The circular profiles apparently attach to the cell membrane while their fine strand-like arborizations bend back toward the cytoplasm. In most

by a continuous double membrane. This circular connecting membrane corresponds to the *cylindre ou manchon fibreux intraflagellaire* of Noirot-Timothée (16). Each double tubular complex is linked with the flagellar membrane by a homogeneous dense mass or by small fibers. At the axis there are remnants of two tubular elements, the proximal ends of the central flagellar tubules. The cell membrane forming the basal cup of the flagellum and the flagellar membrane itself are scalloped to form nine semicircles, corresponding to the nine subunits of the centrioles and the nine satellites. The scallopings of the adjacent membranes coincide. Attachments of the satellites and their fibrils are
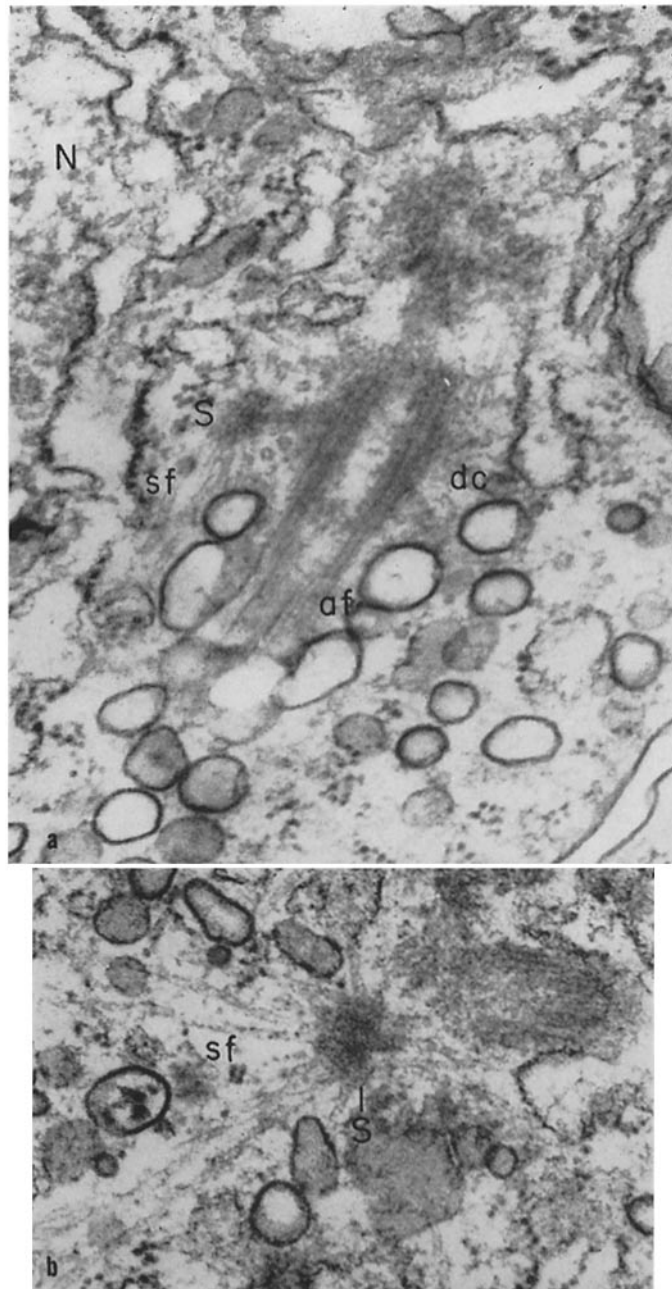
FIGURE 7 *a* A young spermatid, showing spindle fibers (*sf*) attached to a satellite (S) of the filament-forming centriole (*dc*). × 64,000.

FIGURE 7 *b* The spindle fibers (*sf*) of the spermatid converge to the club-shaped end of a satellite (S) and attach to it. × 66,000.

found in the valleys between the outer semicircles of the basal cup, whereas the tubules of the tail filament are situated *at the level of the corresponding arcs.*

In very young spermatids the tail-forming centriole can be recognized to possess a satellite (Figs. 2, 7 *a*). It is clear that the spindle fibers or astral rays converge toward the enlarged distal portion of

The ciliary rootlet is similar to those described in the literature (5, 6, 17, 20) (Figs. 9, 11, and 12). It often splits into two components. The largest rootlet diameter measured in this study was 1600 A, but often rootlets or branches were seen to be narrower. One branch of the rootlet bends at the level of the proximal centriole and projects laterally. This rootlet shows 8 to 12 longitudinal fibril-
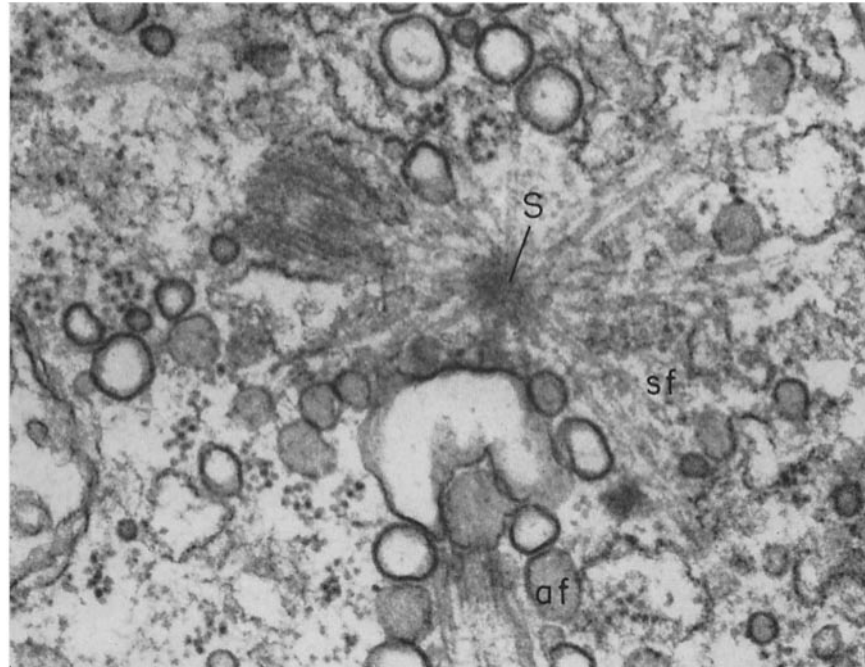

FIGURE 8   The spermatid spindle fibers (*sf*) form a star-shaped figure as they converge toward a satellite (*S*). × 64,000.

the satellite and that they are not continuous with the tubular elements of the centrioles themselves (Figs. 2, 7 *a* and *b*). If a section just grazes the satellite in a plane parallel to the long axis of the centriole, a star-shaped figure is formed by the converging spindle fibers (Fig. 8).

Centrioles, Flagellum and Ciliary Rootlet of Epithelial Cells

The testes of *Phialidium* are covered by large epithelial cells, each of which possesses a single long cilium which also may be termed a flagellum (Fig. 9). The point of emergence is, as in the spermatid, a cup-shaped indentation of the cell membrane at the basal body. Two centrioles are found at this point. The ciliary rootlet emerges from the filament-forming centriole and has typical cross-striations about 850 to 900 A apart.

lar subunits. The number of such subunits varies, but they are always separated by a space of about 150 A. Thus, in the thicker region of the rootlet more fibrillar subunits are found.

In electron micrographs of the fresh water mussel *Elliptio*, a basic periodicity of 550 to 700 A in ciliary rootlets has been reported; this was established by prominent alternating dense and light bands, the light bands being transected by two to four less prominent dense lines (5, 6). The banding pattern of the ciliary rootlets of *Phialidium* is even more complicated. The center-to-center distance between the "major periods" of prominent bands is about 850 to 900 A. Each major dense band, however, can be seen to consist of three distinct components: two denser bands limit laterally a fainter central band. This complex measures 250 A across. Between the prominent dense bands five faint

cross-lines can be resolved, with every second band being slightly more dense. In the case of *Phialidium*, the bands of the ciliary rootlet are perpendicular to the long axis of the rootlet. The dimension of the general banding pattern reported here coincides well with the periodicity on ciliary rootlets in the electron micrographs published by Olsson (17) and is diagrammatically represented in Fig. 13.

These tubules are of the same dimensions as spindle fibers.

An incidental observation deserves mention here. In longitudinal sections of the sperm tail and the epithelial cell's flagellum the contained tubules show periodically recurring lateral projections which apparently interlink adjacent pairs of tubules as well as the peripheral pairs with the
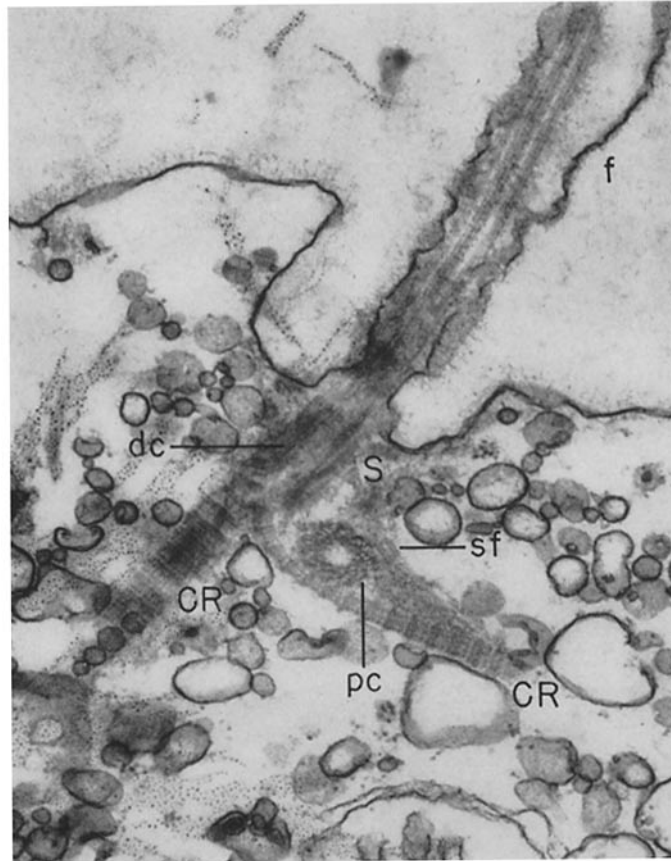

FIGURE 9   Testicular surface epithelial cell, showing a flagellum, centrioles, rootlets, and a satellite. The distal centriole (*dc*) forms the flagellum as well as a rootlet (*cr*) which divides into two branches near the proximal centriole (*pc*). A satellite is shown with two tubular elements. × 45,000.

Satellites are associated with the distal, *e.g.* filament-forming, centriole in the epithelial cells covering the testes, as described above for spermatids, and seem to be identical to the satellites in spermatids (Figs. 2, 7). They show the same cross banding and have a club-shaped enlargement to which the tubules attach. In Fig. 11, some of the centriolar appendages are associated with the plasma membrane. In Figs. 9 and 10 the association of tubules with the satellite is quite clear.

central pair of tubules. This periodicity is approximately 300 A (sperm tails, Fig. 15 *a* and *b*; flagellum of epithelial cell, Fig. 9).

DISCUSSION

The general features of centrioles in the spermatid and the testicular surface epithelial cell of jellyfish *Phialidium gregarium* are similar to those described by Gibbons and Grimstone (8) in ciliated protozoa *Trichonympha* and *Pseudotrichonympha*. One of the

new observations in the present study has been the finding that the satellites originate from the dense matrix between the tubular triplets of the distal centriole. Although only a few perfect cross-sections are available, it can be stated that, at least in some cases, the number of satellites is nine. One satellite originates from the matrix between adjacent triplet structures of the centriole. Bernhard and de Harven (2) take issue with Bessis's claims (3) that nine dense masses are found regularly
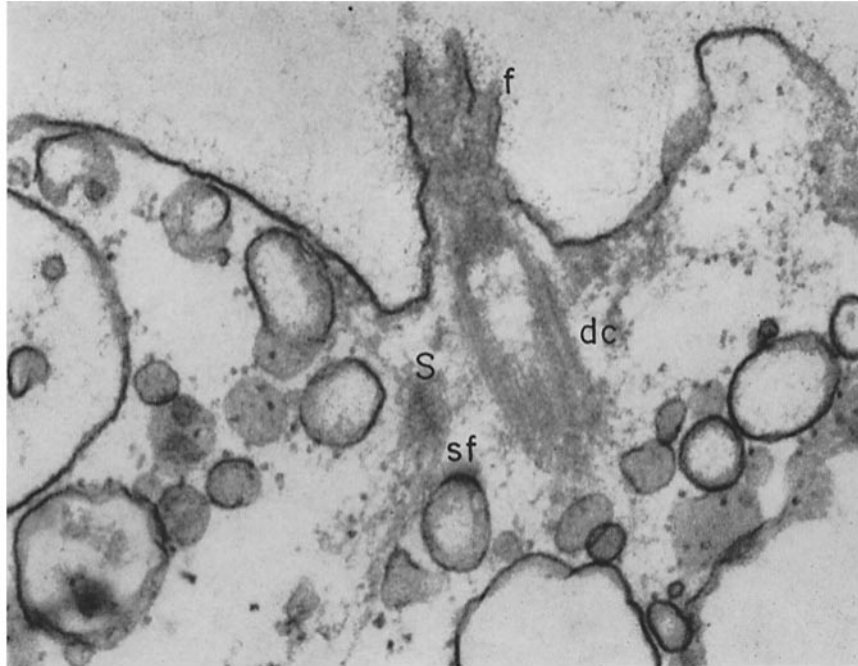

FIGURE 10 Epithelial cell showing the filament-forming centriole (dc) with a club-shaped satellite (S) to which spindle fibers (sf) attach. X 64,000.
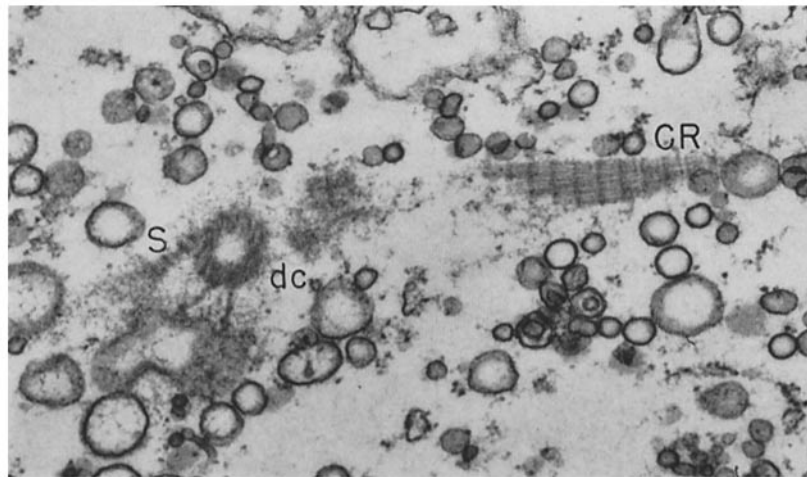

FIGURE 11 Epithelial cell showing the filament-forming centriole with one satellite having typical cross-banding, while other smaller centriolar appendages attach to the cup-shaped fold of the cell membrane at the base of the flagellum. X 55,000.
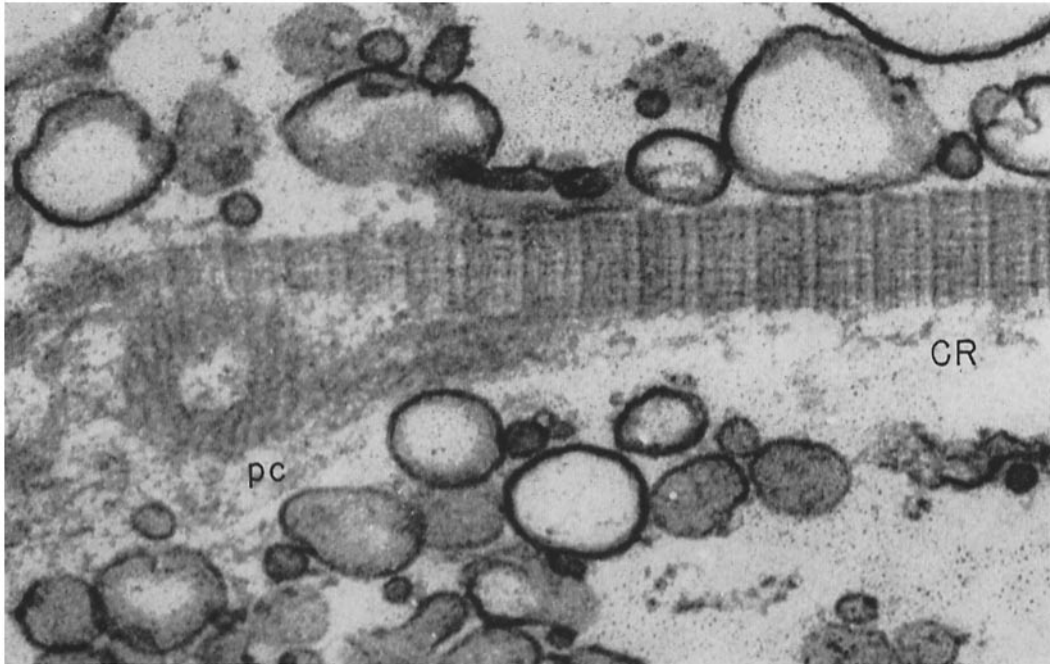
FIGURE 12 A ciliary rootlet of epithelial cell demonstrating the great complexity of its substructure. × 85,000.
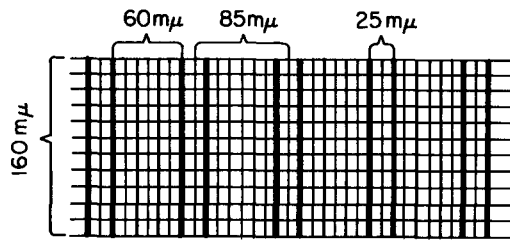

FIGURE 13 Diagrammatic representation of the banding pattern of the ciliary rootlet.

around centrioles, and they point out that this number may depend on the particular physiological state of centriolar activity. No serial sections of centrioles at spindle poles have been analyzed; therefore, it is not known how many satellites actually exist or whether the number varies. It is undeniable, however, that in certain periods of physiological activity nine satellites are present. This is documented in Fig. 3 of the present paper; as well as in a micrograph presented by Maillard (Reference 14, Plate G-d) of a centriole in the A-cell of the anterior pituitary gland in fetal rats. In these cases, the connections to the centrioles can-

not be traced exactly. Satellites have not been demonstrated, thus far, on the proximal centriole.

A periodicity on the satellite has been previously described by Sorokin (21). The satellites he described were also associated with the filament-forming centriole. Similarly, a repeating periodic banding is evident in electron micrographs of ciliary derivatives published by several other investigators (see e.g., Figs. 5 and 6 of Bernhard and de Harven, reference 2; Plate VI of Carasso, reference 4; Hendrickson, reference 11; and Kelly, reference 12).

It is not known how long the spindle fibers remain attached to the satellites after meiotic or mitotic divisions, nor whether spindle fibers are associated with all satellites. This latter point might, however, reflect difficulties with the alignment of this small organelle for sectioning in a preferential plane. When the spermatid chromatin starts to compact and the distal centriole reaches the proximity of the cell membrane, the spindle fibers apparently lose their contact with the satellite. Many tubular elements 180 A in diameter can be found in the cytoplasm, to be sure, but they can no longer be traced to the club-shaped enlargements
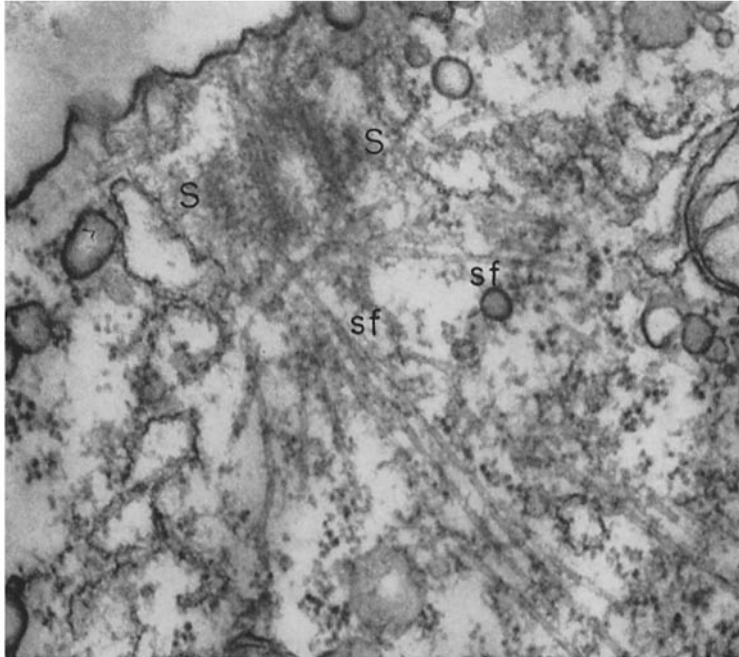
FIGURE 14 A longitudinal section of a centriole at metaphase of the second maturation division of spermatocyte. The spindle fibers converge to a dense structure lateral to the centriole. This dense structure may be the club-shaped enlargement of a satellite. × 64,000.
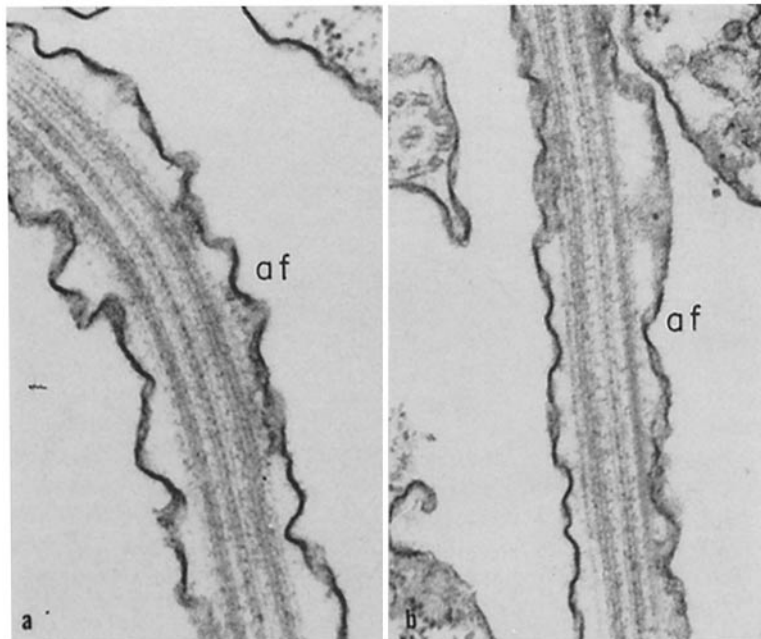

FIGURE 15 a and b Longitudinal sections of the sperm tail demonstrating the periodically recurring lateral projections of the individual tubules. These projections apparently interlink the adjacent pairs of tubules. × 56,000.

of the satellites. It may be necessary to find a different system for the study of these relationships; *i.e.*, one in which the formation and dispersion of the spindle can be controlled or timed better.

The fine strands emanating from the club-shaped enlargement of the satellite were never found to exist simultaneously with spindle fiber attachments. They seem to appear only after the flagellum has emerged from the cytoplasm and the centrioles become located near the cell membrane. It is suggested that the circular profiles at the distal end of the satellites and their newly
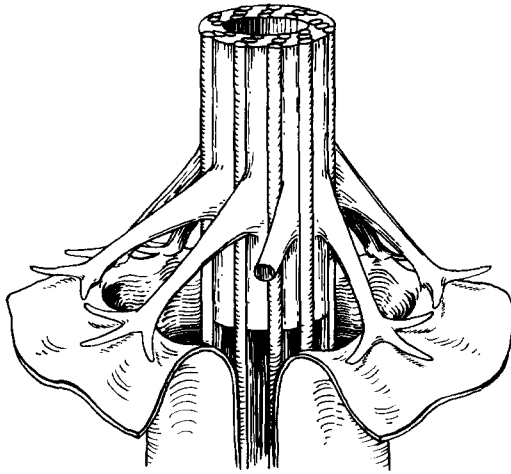

Figure 16   The three-dimensional reconstruction of the spermatid filament-forming centriole and its nine satellites providing a firm anchoring for the tail filament. A slit is left in the cell membrane and plasma membrane of the tail filament to show more structural detail.

formed strands establish an attachment with the cell membrane around the base of the flagellum. Such an arrangement would lend a great structural rigidity to the centriole and the flagellum it is forming. The scalloped cell membrane, shown in Fig. 6, would therefore be a reflection of a firm attachment between the circular profiles of the satellite and the plasma membrane, thus reinforcing the interpretation that the satellite serves, in this case, a structural role. An attempt has been made to reconstruct diagrammatically the centriole in this phase of activity (Fig. 16).

The centriolar organization found in the ciliated epithelial cells covering the testes is similar to that described above for the spermatid. Certain lateral appendages of these centrioles are associated with

tubular elements; some others make contact directly with the cell membrane. Micrographs of the ciliary rootlets disclose a great complexity of their organization but do not add to our understanding of their function. The cilia of the testicular surface epithelium are very long, and some investigators call them flagella. It is impossible to decide, on a morphological basis alone, which term should be used. One distinction apparently is the type of beat (5, 6), but no observations have been carried out on the testes of living *Phialidium*. The rootlet is usually referred to as a "ciliary rootlet," but in multiciliated cells in which rootlets have been described previously only the filament-forming centriole is present. In isolated instances, a rootlet has been found also in spermatids, where it is found to be compressed between the cell membrane and one of the gigantic mitochondria at the base of the nucleus, making morphological distinction even more difficult. Cilia and flagella may differ morphologically in that only the filament-forming centriole is found in case of cilia, while two centrioles, at right angles to each other, are present at the base of flagella.

The significance of the banding pattern of the satellite is not clear at the moment. On cursory examination the periodicity is different from that of the ciliary rootlet. It is possible, however, that the basic elements of the satellites are essentially similar to those of the ciliary rootlet and that the difference is to be sought in the degree of aggregation or polymerization. The cross-banding pattern of the satellites is certainly the most prominent pattern, for only rarely are there indications of longitudinally oriented fibers.

The electron micrographs presented in this study show that in the case of *Phialidium* the spindle fibers converge to one of the satellites (or possibly to a few satellites) rather than to the centrioles directly (Figs. 7 *a* and *b*). At this time nothing can be added to describe the details of this attachment or the nature of its origin that would not be purely speculative. It seems clear, however, that the spindle fibers do not just end blindly near the centrioles but rather that they make contact with the club-shaped enlargement of the satellites. The mode of attachment of the spindle fibers to the centrioles has puzzled many microscopists in the past. In previously published electron micrographs, it has been shown that the spindle fibers reach only the vicinity of the centrioles or end a certain distance from them (2, 4, 9,

18). The axes of the centrioles at the spindle pole are arranged in such a manner that the spindle fibers could not be continuous with the tubular elements of the centrioles themselves.

Thus far, satellites have been implicated in the role of centriolar duplication. Bernhard and de Harven (2, 10) and Gall (7) showed clearly that this is the case for at least some of the lateral projections. Examination of their published micrographs discloses that certain lateral projections do look like small centrioles (reference 2, Fig. 7), while others seem to be club-shaped (reference 2, Figs. 4 to 7). The material for their studies consisted of young, rapidly dividing cells, and it is reasonable to presume that two different centriolar functions were being expressed simultaneously: the club-shaped satellites for spindle attachment and other projections representing stages in the production of daughter centrioles. Spermatids do not usually divide and, accordingly, in no case of *Phialidium* spermatids were daughter centrioles observed as lateral projections. In the case of a viviparous snail, however, it has been shown that the atypical spermatids show daughter centrioles which correspondingly form several sperm-tail filaments (7). Two distinct functions may be performed simultaneously by the centrioles of young *Phialidium* spermatids and the centrioles of the flagellated epithelial cells: the formation of the sperm tail or the flagellum, respectively, and the attachment of the spindle fibers. Spindle fibers may be replaced by a fibrous arborization of the satellites to form the specialized anchoring system at a more advanced stage of differentiation. Thus, the significance of lateral projections described by other authors as being involved in centriole duplication appears to be quite different from that of lateral projections found in *Phialidium* spermatids. The idea is attractive that a relationship between spindle fibers and satellites is a general feature of dividing cells. It must be emphasized, however, that the present observations were limited to a few meiotic and some post-meiotic cells in a single species and that such a conclusion may not be borne out by further investigation.

Centrioles may be capable of forming a large number of satellites or lateral projections in different physiological states. The proximal centriole of photoreceptors, for example, forms a satellite with a large club-shaped ending, but, at the same time, on a different level several small fibers may form anchorage to the cell membrane (11, 12, 22). A most important property of centrioles thus may be the ability to form one to several satellites or projections at appropriate times during the cell cycle to serve different functions.

Organelles associated with centrioles are then of at least two kinds. The term "satellite" should be reserved for the supportive or structural elements involved in anchoring either flagella, cilia, or spindle fibers, while the side projections in replication phases of the centriole should be called "daughter centrioles."

REFERENCES

1. BERNHARD, W., and DE HARVEN, E., *Compt. rend. Acad. sc.*, 1956, **242**, 288.
2. BERNHARD, W., and DE HARVEN, E., *4th Internat. Congr. Electron Microscopy, Berlin,* 1960, **2**, 217.
3. BESSIS, M. and BRETON-GORIUS, J., *Compt. rend. Acad. sc.*, 1958, **246**, 1289.
4. CARASSO, N., and FAVARD, P., *in* Traité de microscopie Électronique, (C. Magnan, editor), Paris, Hermann, 1961, 1104.
5. FAWCETT, D. W., *in* Frontiers in Cytology, (S. Palay, editor), New Haven, Yale University Press, 1958.
6. FAWCETT, D. W., *in* The Cell, (J. Brachet and A. E. Mirsky, editors), Academic Press, Inc., 1961, 3, chapt. 4.
7. GALL, J., *J. Cell Biol.*, 1961, **10**, 163.
8. GIBBONS, I. R., and GRIMSTONE, A. V., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 697.

9. HARRIS, P., and MAZIA, D., Interpretation of Ultrastructure, New York, Academic Press, Inc., 1963, 297.

10. DE HARVEN, E., and BERNHARD, W., Z. Zellforsch., 1956, 45, 378.

11. HENDRICKSON, A., personal communication.

12. KELLY, D. E., Am. Scientist, 1962, 50, 597.

13. LUFT, J. H., J. Biophysic. and Biochem. Cytol., 1961, 9, 409.

14. MAILLARD, M., J. Micr., 1963, 2, 81.

15. MILLONIG, G., J. Biophysic. and Biochem. Cytol., 1961, 11, 736.

16. NOIROT-TIMOTHÉE, C., Ann. Sc. nat. Zool. et Biol. animale, 1959, No. 12, 1, 266.

17. OLSSON, R., J. Cell Biol., 1962, 15, 596.

18. DE ROBERTIS, E., NOWINSKI, W. W., and SEAZ, F. A., General Cytology, Philadelphia, W. B. Saunders Co., 3rd edition, 1960, 334.

19. ROOSEN-RUNGE, E. C., Pacific Sc., 1962, 16, 15, and personal communication.

20. SATIR, P., Scientific Am., 1959, 204, 108.

21. SOROKIN, S., J. Cell Biol., 1962, 15, 363.

22. TOKUYASU, K. and YAMADA, E., J. Biophysic. and Biochem. Cytol., 1959, 6, 225.

23. WOOD, R. L., Norelco Reptr., 1960, 7, 23.