

CENTRIOLE REPLICATION

A Study of Spermatogenesis in the Snail *Viviparus*

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ABSTRACT

This paper describes the replication of centrioles during spermatogenesis in the Prosobranch snail, *Viviparus malleatus* Reeve. Sections for electron microscopy were cut from pieces of testis fixed in OsO_4 and embedded in the polyester resin Vestopal W. Two kinds of spermatocytes are present. These give rise to typical uniflagellate sperm carrying the haploid number of 9 chromosomes, and atypical multiflagellate sperm with only one chromosome. Two centrioles are present in the youngest typical spermatocyte. Each is a hollow cylinder about $160 \text{ m}\mu$ in diameter and $330 \text{ m}\mu$ long. The wall consists of 9 sets of triplet fibers arranged in a characteristic pattern. Sometime before pachytene an immature centriole, or *procentriole* as it will be called, appears next to each of the mature centrioles. The procentriole resembles a mature centriole in most respects except length: it is more annular than tubular. The daughter procentriole lies with its axis perpendicular to that of its parent. It presumably grows to full size during the late prophase, although the maturation stages have not been observed with the electron microscope. It is suggested that centrioles possess a constant polarization. The distal end forms the flagellum or other centriole products, while the proximal end represents the procentriole and is concerned with replication. The four centrioles of prophase (two parents and two daughters) are distributed by the two meiotic divisions to the four typical spermatids, in which they function as the basal bodies of the flagella. Atypical spermatocytes at first contain two normal centrioles. Each of these becomes surrounded by a cluster of procentrioles, which progressively elongate during the late prophase. After two aberrant meiotic divisions the centriole clusters give rise to the basal bodies of the multiflagellate sperm. These facts are discussed in the light of the theory, first proposed by Pollister, that the supernumerary centrioles in the atypical cells are derived from the centromeres of degenerating chromosomes.

Spermatogenesis in the Prosobranch snails has attracted attention since von Siebold's (45) discovery of sperm dimorphism in the European snail, *Viviparus (Paludina) viviparus* (L.). In *Viviparus* the testis produces not only typical "hair-shaped" sperm similar to those of other gastropods, but also atypical "worm-shaped" sperm bearing multiple flagella. The atypical sperm are a regular feature of spermatogenesis in the sense that they are formed in enormous numbers by all male specimens, but it is unlikely that they ever fertilize

eggs (18, 36). Atypical sperm are probably found in all Prosobranchia except the Archaeogastropoda (3, 33). Although the mature atypical sperm of various species differ greatly in size and appearance, their development follows a common pattern. Ankel (3) stressed this point in his study of *Janthina*, a marine snail, which possesses what is perhaps the most bizarre sperm in the whole animal kingdom.

The first accurate account of atypical spermatogenesis was given by Meves (28) who made a

careful study of *Viviparus*. Meves was interested in the origin of the multiple flagella, and particularly in the relationship between the centrioles of the spermatocyte and the basal bodies (or blepharoplasts) of the flagella. He showed that each centriole of the atypical spermatocyte gives rise to a cluster of centrioles, and that several centrioles are transmitted to each spermatid, where they become the basal bodies of the multiple flagella. Meves's paper has long been cited in support of the theory that centrioles and basal bodies are homologous (the Henneguy-Lenhossek theory).

Meves also noted that most of the chromosomes fail to pass normally to the poles at the two meiotic divisions. In fact only one chromosome (chromatid) is included in the spermatid nucleus, the others remaining in the cytoplasm where they ultimately degenerate. Meves used the term *oligopyrene* (oligo = few, pyrene = nucleus) to describe these sperm and to distinguish them from the typical, or *eupyrene* sperm, containing the normal haploid complement. He also used the word *apyrene* in reference to certain sperm of the moth, *Pygaera*, which contain no chromosomes. Many Prosobranchia likewise produce apyrene sperm (3, 26).

The rather obscure changes occurring in these cells took on great theoretical interest from the observations of Pollister and Pollister (32, 34, 35). The Pollisters were impressed with the fact that the chromosomes in the atypical spermatocytes behave as if acentric; that is, as if they lack spindle attachment regions. They suggested that most of the chromosomes lose their centromeres during the meiotic prophase, and that the centromeres migrate through the nuclear membrane into the cytoplasm, where they transform into centrioles. Compelling evidence for this novel theory came from the demonstration that the number of extra centrioles arising during prophase is equal to the number of chromosomes which degenerate. The numerical correspondence was found to hold for *Viviparus malleatus*, the species most thoroughly studied, as well as for three other snails in the same family.

The present electron microscope study was begun in hopes that details of the centromere-centriole transformation might be obtained.

MATERIALS AND METHODS

Specimens of the Japanese live-bearing snail, *Viviparus malleatus* Reeve, were obtained from the

General Biological Supply House, Chicago. The testis was removed from the living animal, cut into small pieces, and fixed in 1 per cent OsO_4 at pH 7.4 prepared according to Caulfield (10). After fixation in the cold for 1 hour the material was rapidly dehydrated in an acetone series, and embedded in Vestopal W (Martin Jaeger, Geneva, Switzerland). Thin sections for electron microscopy were cut on a Porter-Blum microtome with glass knives and picked up on grids coated with a carbon film. Sections were routinely stained by floating the grids for a few minutes on the surface of a saturated aqueous solution of uranyl acetate; some sections were similarly stained with 1 per cent KMnO_4 . All pictures were taken with an RCA 3D electron microscope. The present study is based on approximately 1,500 micrographs. A total of 74 centrioles or centriole clusters have been studied, about half in serial sections.

Testes were also preserved in standard cytological fixatives, chiefly Hermann's, Flemming's with acetic, and Champy's, embedded in a 19:1 mixture of butyl and methyl methacrylates, and sectioned at 1 to 6 micra. The best demonstration of centrioles was obtained in Hermann-fixed material stained with Heidenhain's iron hematoxylin. Feulgen squashes were prepared from material fixed in ethanol-acetic acid (3:1).

RESULTS AND DISCUSSION

General Features of Spermatogenesis

The studies of Meves (28), Gatenby (15), and Pollister and Pollister (35) provide the classical description of spermatogenesis in both the typical and atypical lines. The following account is intended for orientation and also adds certain features not readily evident from light microscopy. Only a few corrections must be made to earlier accounts. The description given here agrees in most points with the recent electron microscope studies of Hanson, Randall, and Bayley (20), Kaye (24, 25), and Yasuzumi *et al.* (47-50).

Typical Series: Spermatogenesis in the typical series presents very few unusual features (Fig. 1 and Figs. 2 to 5). The spermatogonia are small cells with scanty cytoplasm. There is a Golgi mass on one side of the nucleus, two near-by centrioles, and scattered mitochondria. Sacs of the endoplasmic reticulum are rare, but free ribosome-like granules are plentiful (Fig. 2).

The primary spermatocyte is a somewhat

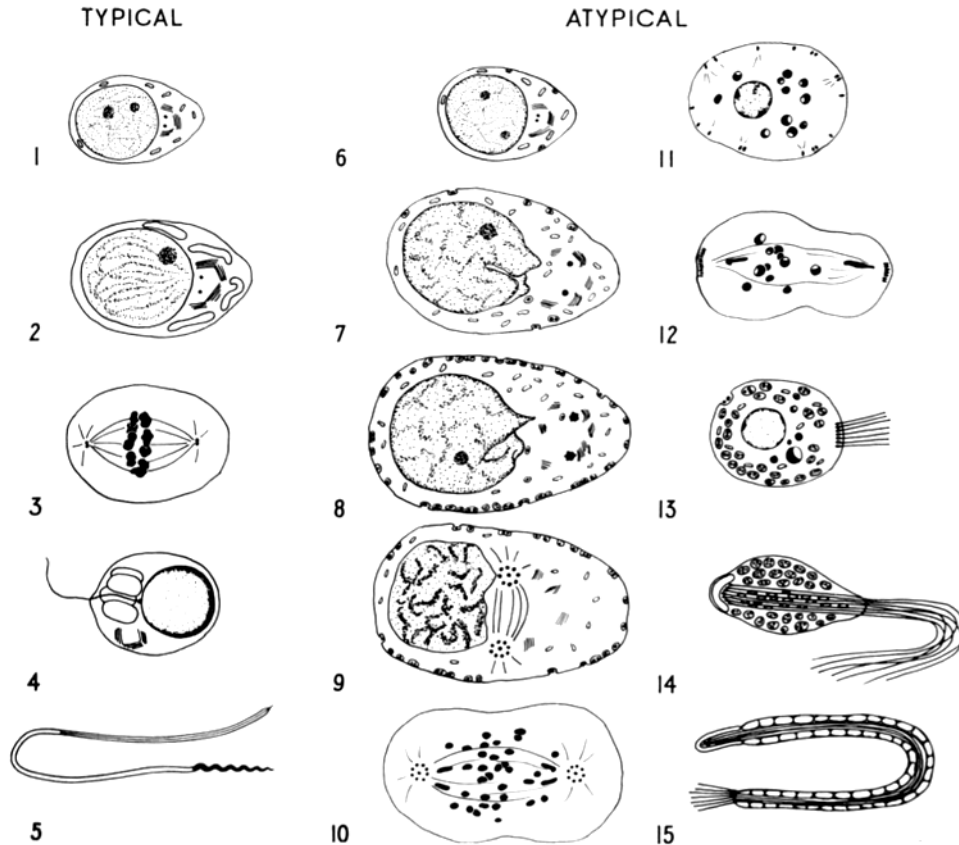


FIGURE 1

Stages in the development of the typical and atypical sperm of the snail, *Viviparus*. The drawings are semidiagrammatic and the relative sizes of certain structures have been purposely distorted to represent features resolvable only by electron microscopy. The centrioles in the atypical series have been drawn to illustrate the Pollister theory.

1-5. The typical series: 1, 2. Early and late primary spermatocytes. 3. First meiotic metaphase showing 9 bivalents. 4. Spermatid. 5. Mature sperm.

6-15. The atypical series: 6-9. Prophase of the first meiotic division. Note particularly the formation of the multiple centriole clusters. 10. First meiotic division with four normal, 32 lagging chromatids; centriole clusters at the spindle poles. The dense bodies at the periphery of the cell have been omitted for clarity in this and the two following stages. 11. Brief interphase between first and second meiotic divisions. 12. Second meiotic division. One chromatid passes to each pole. The centrioles are unequally distributed to a large and a small spermatid. 13-15. Spermatids and mature atypical sperm with multiple flagella.

larger, pear-shaped cell which possesses an eccentrically placed nucleus with two nucleoli. At the smaller end of the cell the cytoplasm contains a conspicuous Golgi region, two centrioles, endoplasmic reticulum consisting of separate vesicles, and a small number of enlarged mitochondria (Fig. 3). Granules are found free and in association with the sacs of the endoplasmic reticulum. At pachytene the nucleoli fuse and the

chromosomes display a marked bouquet orientation, their ends being directed toward the centrioles. In electron micrographs of this stage (Figs. 3 and 4) one may see the characteristic chromosome "cores" originally described by Moses (30). At metaphase of the first meiotic division 9 tetrads appear on the equator of a normal spindle. The two divisions result in the formation of four spermatids each containing the haploid number

of 9 chromosomes. In the cytoplasm of the spermatid the mitochondria fuse into four nebenkerne (15, 20, 24) which eventually elongate and wrap spirally around the flagellum (Fig. 5). The spermatid contains a single centriole located next to the nuclear envelope; it becomes the blepharoplast, or basal body of the flagellum. The flagellum, with its characteristic "9 plus two" arrangement of fibers, extends uninterruptedly from this centriole to the end of the sperm. The transformation of the early spermatid into the mature sperm involves complex changes in the nuclear material which have been described by Kaye (25) and Yasuzumi *et al.* (47-50) for *Viviparus (Cipangopaludina)*, by Grassé, Carasso and Favard (19) for *Helix*, and by Rebhun (37) for *Otala*. The mature sperm possesses a curious cork-screw nucleus and nebenkerne with a regular lamellar spacing (Fig. 1 and Fig. 15).

Atypical Series: The atypical multiflagellate sperm are derived from cells which can be recognized at least as early as the primary spermatocyte stage. Whether there are two types of spermatogonia, leading to the typical and atypical sperm respectively, remains a moot point. The earliest atypical spermatocytes, like the typicals, are rather small, pear-shaped cells containing an eccentrically placed nucleus with two nucleoli (Fig. 1). The cytoplasm, however, immediately distinguishes the two series. In the atypicals the endoplasmic reticulum is plentiful, consisting of large vesicles which give the cell a frothy appearance (Figs. 7, 8). Scattered between these vesicles are the mitochondria. They are numerous, round or somewhat elongate, and considerably smaller than the mitochondria of the typical spermatocytes (Fig. 8 and reference 15). One area, that known classically as the idiozome, is relatively

free of endoplasmic reticulum. It contains numerous small groups of Golgi lamellae, some very small vesicles, two centrioles, ribosome-like granules, and astral fibers (Figs. 7, 8, 27).

The later atypical spermatocyte is a much larger cell than the typical, although the nuclei in the two series are comparable in size. The nucleus in the atypicals becomes lobulated and puts out finger-like projections toward the idiozome (Fig. 8). The fine structure of the nuclear envelope is not unusual (Figs. 7, 8).

As Meves originally showed, the chromosomes fail to undergo normal meiotic changes. It is probable that pairing of homologues does not take place, since neither the bouquet arrangement nor "cores" are seen. In any event, by late prophase the nucleus contains about 36 chromosome masses. These presumably correspond to the 36 chromatids which ordinarily would be associated as 9 tetrads at this stage.

In the cytoplasm two conspicuous changes take place during the prophase (Fig. 1). The first is the appearance of numerous dense granules situated primarily at the periphery of the cell. The second is the transformation of the two original centrioles into mulberry-like bodies, consisting, according to the Pollisters, of 9 individual centrioles. Both changes will be discussed in more detail.

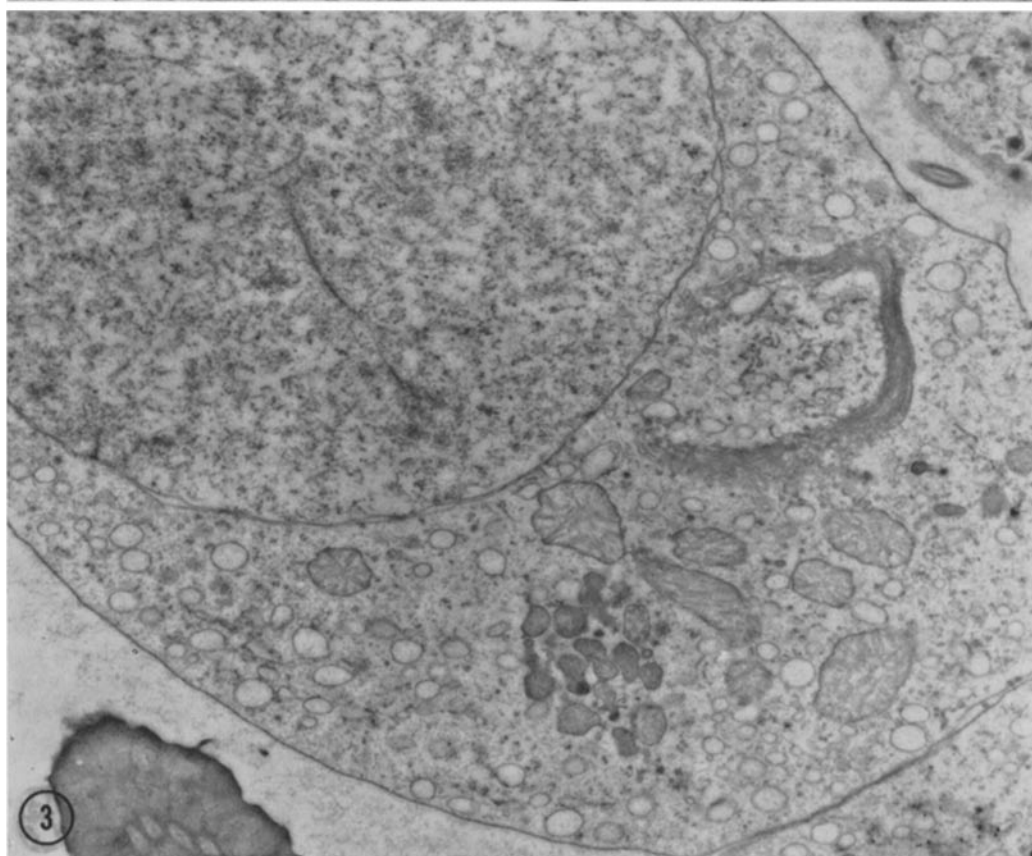
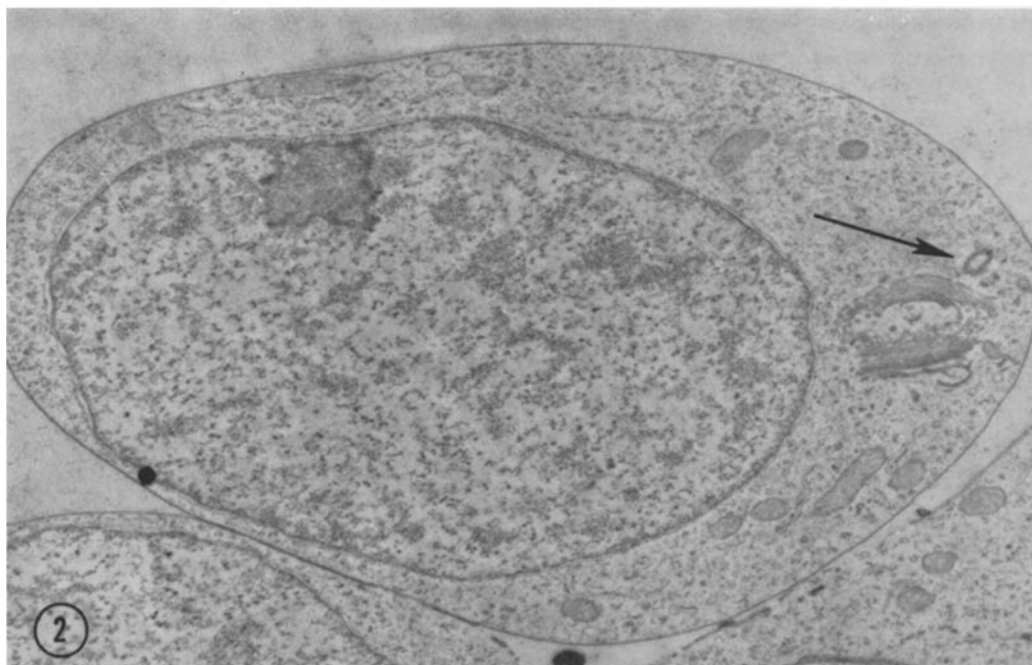
The two meiotic divisions are highly irregular (Fig. 1). At metaphase I the two centriole clusters migrate to the poles of the spindle. Thirty-two of the 36 chromosomes (chromatids) behave abnormally: they fail to form a typical metaphase plate and ultimately segregate randomly to the two secondary spermatocytes. Four chromatids appear to undergo more normal movements, two going to each pole. The nucleus of the secondary spermatocyte

FIGURE 2

Spermatogonium showing scanty cytoplasm and very few sacs of the endoplasmic reticulum. The arrow points to a centriole and its daughter pro-centriole next to the Golgi region. $\times 15,000$.

FIGURE 3

Pachytene in a spermatocyte of the typical series. The cytoplasm contains numerous vesicular elements of the endoplasmic reticulum, a prominent Golgi region (idiozome), and a small number of very large mitochondria. The dark granules at lower center are of unknown significance. Within the nucleus may be seen the chromosome cores which are diagnostic of this stage. $\times 15,000$.



cyte is reconstituted from these normal chromatids. The abnormal chromatids form small vesicles in the cytoplasm, which possess a double "nuclear envelope" (Fig. 9). At the second meiotic division, the two normal chromatids pass to opposite poles. According to the Pollisters, the number of centrioles at each pole increased from 9 to (presumably) 18 during the first meiotic division. These scatter to the periphery of the secondary spermatocyte and later aggregate into two groups at the poles of the second meiotic spindle. In *V. malleatus*, but not in *V. viviparus*, the second meiotic division is unequal, there being formed a large and a small spermatid (29, 35). The smaller group of centrioles generally passes to the smaller cell.

The spermatid thus consists of a nucleus with a single chromatid, and cytoplasm in which are found the following components: degenerating vesicular chromosomes, a variable number of centrioles, sacs of the endoplasmic reticulum, Golgi membranes, and numerous dense bodies. Flagella grow out from the centrioles, which lie at first on the cell periphery (Fig. 10). Later as the spermatids elongate the centrioles come to rest in a depression at the posterior margin of the nucleus (Figs. 12 to 14). The mitochondria take up a position between the flagella while the dense bodies migrate to the cell surface (Figs. 11, 15).

The Dense Particles: All cells in the atypical series, from the earliest spermatocytes to the mature sperm, contain dense cytoplasmic particles. They have been described by many previous workers under a variety of names: granula, trophochromatic substance, mitochondria of Benda, albuminous bodies, etc. (2-6, 31, 38, 46). Frequently they have been mistaken for the mito-

chondria, as in Meves's original account and later by the Pollisters. Gatenby (15) distinguished them from the true mitochondria on the basis of fixation and staining tests.

They are first seen in the spermatocytes of the atypical series before the multiplication of the centrioles (Fig. 7). At this time they are small and each is surrounded by a membrane which stains heavily with uranyl acetate. The membrane also shows a clear "triple-layered" appearance after KMnO_4 staining. In both these respects it more nearly resembles the cell membrane than the sacs of the endoplasmic reticulum. The particles apparently can pass through the cell surface, as they are found both in the cytoplasm and free in the extracellular space (Fig. 8). During later stages, especially after the two meiotic divisions, the dense particles increase in size, total number, and number per sac (Fig. 11). They are easily seen by light microscopy in stained preparations of spermatids and they are a conspicuous feature of fresh material observed by phase contrast. They eventually fuse into large masses surrounding the flagellar bundle (Figs. 12, 15).

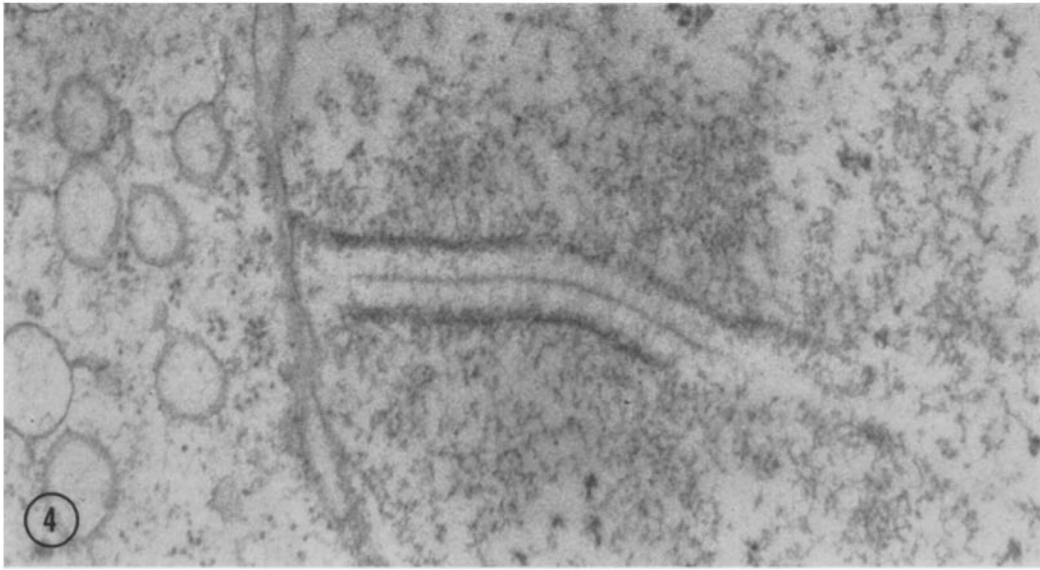
The significance of the particles is obscure. Yasuzumi and Tanaka (48), who were the first to describe their migration through the cell surface, claim that they are Feulgen-positive breakdown products of the chromosomes. However, Hanson *et al.* (20) state that the particles are Feulgen-negative, and my own preparations confirm this observation. Pollister and Pollister (35), who used the Feulgen reagent, presumably would not have mistaken the particles for mitochondria had they appeared Feulgen-positive. The fact that they arise in early spermatocytes, long before the degeneration of the chromosomes, is likewise incom-

FIGURE 4

Pachytene in a typical spermatocyte showing a median sagittal section through a chromosome core. Note the ladder-like arrangement of filaments (?) extending from the thin central line to the two heavy lateral lines; also the radial orientation of the chromosomal fibers outside the heavy lines. The lateral limits of the chromosome pair coincide with the top and bottom of the micrograph. The orientation of the chromosome ends perpendicular to the nuclear envelope is characteristic of this stage of meiosis $\times 55,000$.

FIGURE 5

Early spermatid of the typical series. The mitochondria have now fused into four nebenkerne, of which three are seen here surrounding the flagellum. The large Golgi mass below the nucleus will eventually be cast off with most of the cytoplasm. $\times 20,000$.



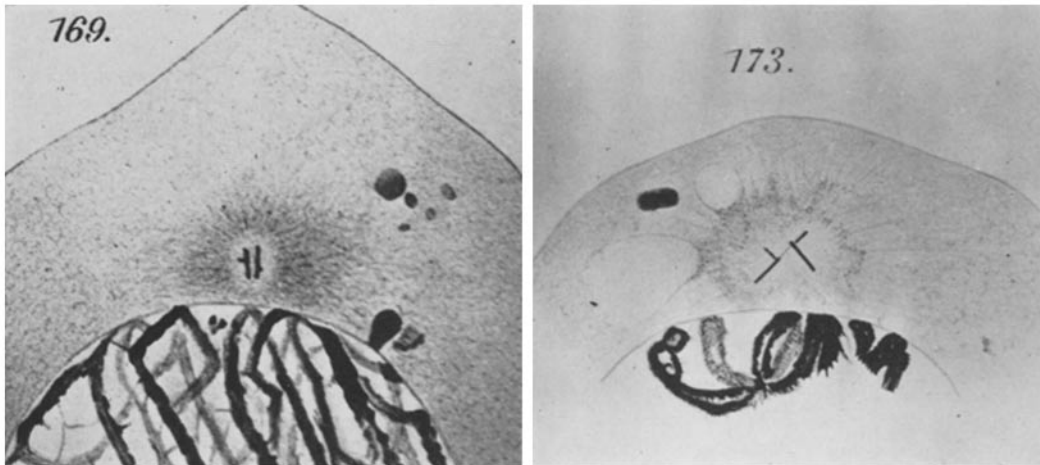


FIGURE 6

Drawings of centriole duplication in spermatocytes of the hagfish, *Myxine*, published in 1905 by A. and K. E. Schreiner (43). The procentrioles appear as buds on the parent centrioles in the pachytene cell (left). By late diplotene (right) the daughter centrioles have reached about half their mature length.

patible with Yasuzumi's view. Yasuzumi's Fig. 17 (48) shows a spermatocyte containing numerous particles, but the cell is incorrectly identified as a spermatid.

Hanson *et al.* (20) find no RNA, DNA, or lipoids in them, but report a positive periodic acid-Schiff test for polysaccharides. The latter test is also positive after treatment with salivary amylase. Battaglia (5, 6) states that the atypical sperm of *Columbella* give a positive polysaccharide test but show very little ultraviolet absorption. My own observations agree with these previous findings. The particles are Feulgen-negative, show no RNA with azure B at pH 4 (14), and give a positive periodic acid-Schiff test.

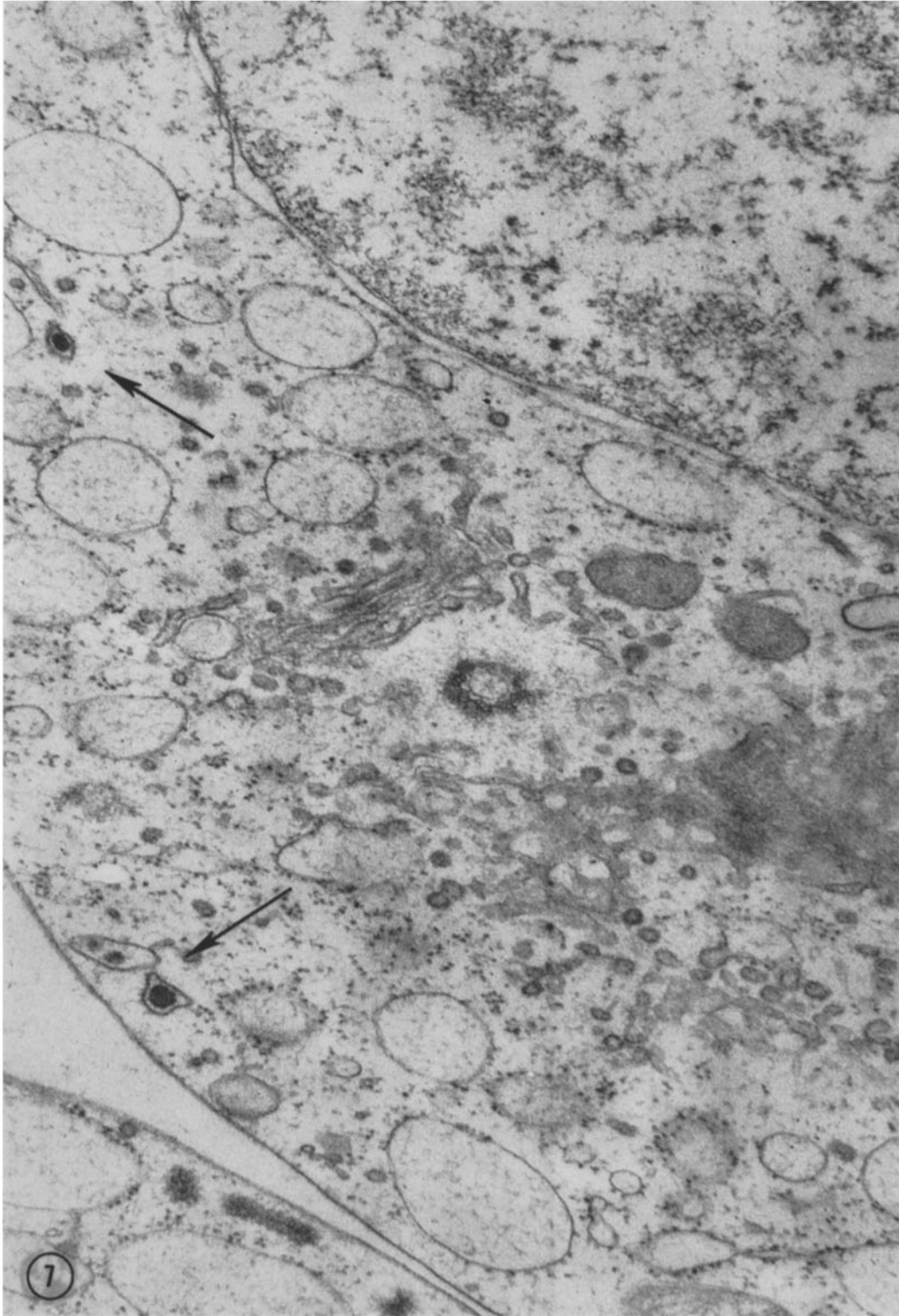
Centriole Duplication in Spermatogonia and Typical Spermatocyte

The centrioles of *Viviparus*, in both the typical and atypical lines, are similar to centrioles and

basal bodies of other organisms (1, 7, 8, 9, 13, 17, 22, 39, 40). They are short hollow cylinders averaging 160 $m\mu$ in diameter and 330 $m\mu$ in length. Their wall consists of 9 complex fibers running parallel to the long axis of the cylinder (Figs. 16 to 31). These are divided into subunits whose number is commonly three but sometimes appears to be two or four. The subunits will be referred to as *subfibers* and the group of three as a *triplet* fiber. The terminology adopted throughout this description is that used by Gibbons and Grimstone (17) in their excellent account of basal bodies in flagellates. The subfibers have a tubular appearance in transverse section. Their wall is about 5 $m\mu$ thick and surrounds a less dense core 12 to 13 $m\mu$ in diameter. They are thus similar to the subfibers of the flagellar doublets (Fig. 14), with which they are in direct continuity (13, 16, 17). They also resemble the fine fibers or tubules seen in the spindle (7, 41, 42) as well as the astral fibers (Figs. 16, 20, 27). It should be noted that

FIGURE 7

A very early spermatocyte of the atypical series, showing one of the two centrioles present at this stage (*cf.* Fig. 28 for enlargement). This cell may be distinguished from an early spermatocyte of the typical series by its larger size, the absence of chromosome cores, the abundant vesicular elements of the endoplasmic reticulum, the small mitochondria (not shown here) and particularly the dense cytoplasmic particles enclosed in a membrane (arrows). $\times 60,000$.



adjacent subfibers are quite intimately associated; in fact they appear to share a common wall (Fig. 28). The over all dimensions of a triplet fiber, as seen in transverse section, average about $47\text{ m}\mu$ by $23\text{ m}\mu$. The ratio of these two dimensions is almost exactly 2, whereas for completely separate subfibers the ratio would be 3. Similarly longitudinal sections of a triplet show four dark lines separated by three lighter spaces (Fig. 29). The longitudinal axes of the subfibers lie in a single plane, the *axial plane* of the triplet. The axial planes of all the triplets are inclined in the same direction at about 30 to 45° to the circumference of the centriole (Fig. 28), so that transverse sections of the centriole have a spiral or pinwheel appearance.

One often sees connecting lines between subfibers in adjacent triplets. If we designate the three subfibers as A, B, and C, reading from the center to the periphery, we may say that AA and AC connections are the commonest (Fig. 28). It is not known whether the lines seen in transverse sections represent fibers or the cut edges of longitudinal plates. The lumen of the centriole generally appears "empty." Occasionally, however, one sees an elaborate central structure, which appears like a cart-wheel with an indistinct hub and 9 radial spokes projecting to the wall (Fig. 28). An almost identical structure was described by Gibbons and Grimstone (17) in the proximal region of the basal bodies of *Trichonympha* and *Pseudotriconympha*.

According to the descriptions of Meves and the Pollisters a spermatogonium or typical primary spermatocyte of *Viviparus* contains two centrioles located in or near the idiozome; these later occupy the poles of the first meiotic spindle and divide at

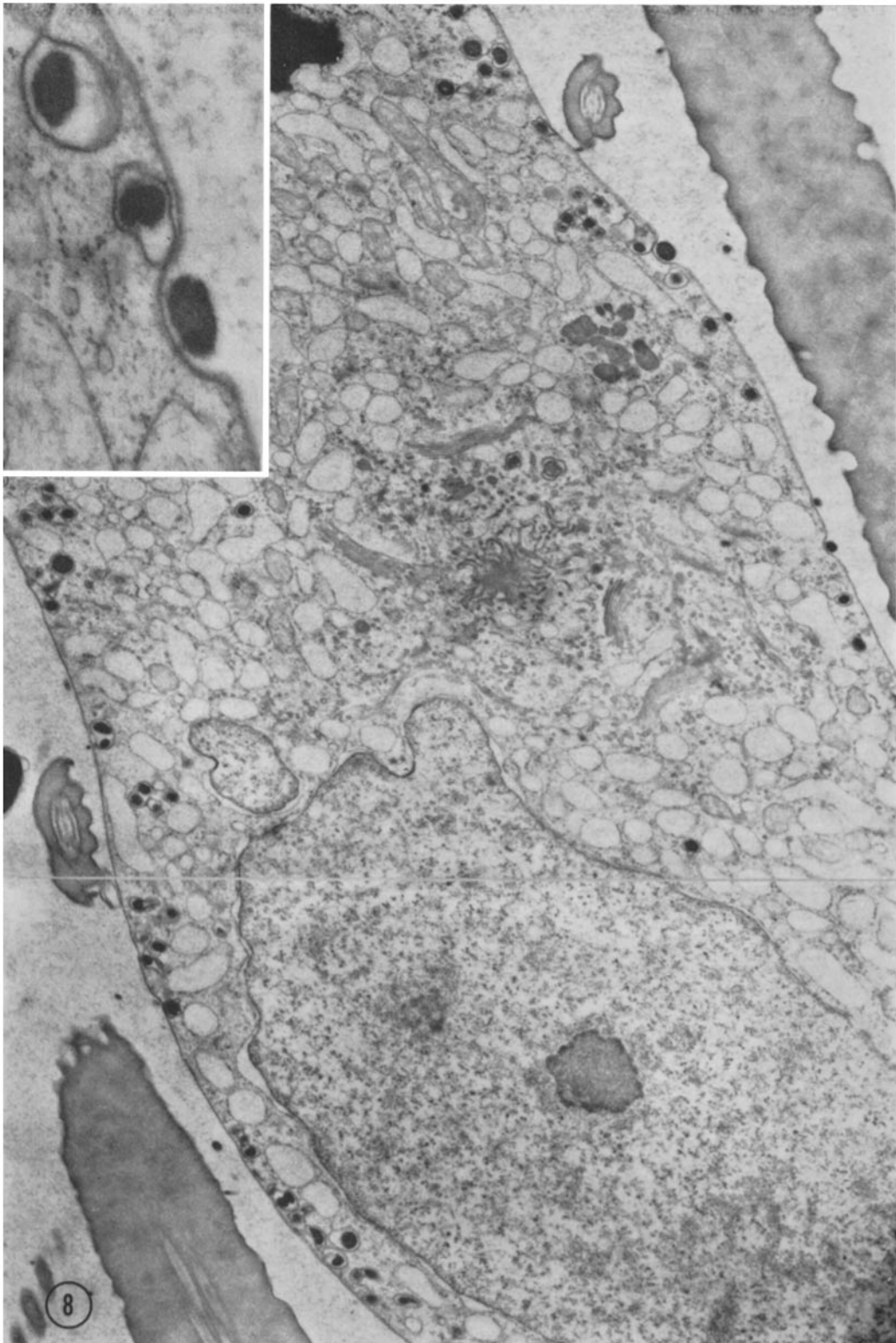
anaphase. The electron microscope shows the situation to be somewhat more complex. In zygotene and pachytene cells each centriole is accompanied by a smaller structure which is the precursor of the daughter centriole (Figs. 17 to 26). This *procentriole*, as it will be called, is similar to a mature centriole except for length. That is, it consists of fibers arranged in a circle of about $150\text{ m}\mu$, but the length of the fibers averages only $70\text{ m}\mu$. Measurements on favorably oriented procentrioles show that their diameter is slightly less than that of mature centrioles. However, the difference is not so large as appears from a casual inspection of the micrographs. Many mature centrioles are surrounded by dense amorphous bodies which add greatly to their apparent diameter (Figs. 40, 41, 43). Conversely, procentrioles are seldom seen face-on, and longitudinal sections generally do not include the full diameter. The procentriole typically lies at a distance of about $70\text{ m}\mu$ from one end of the parent centriole, and its longitudinal axis is perpendicular to that of its parent. After maturation of the procentriole, the parent and daughter lie at right angles in the form of an L or V.

Serial sections have been valuable in defining the structure of the annular procentriole, since single sections may be difficult to interpret. For instance, sections which cut the edge of a procentriole may show only an indistinctly fibrous or fuzzy mass near the parent (Figs. 17, 23), and a face-on view might be mistaken for a transverse section of a mature centriole (Fig. 42). In such cases the adjacent sections provide the necessary clarification. Although the general organization of the procentriole is evident, very few of the micrographs permit a count of the fibers, and none

FIGURE 8

A spermatocyte of the atypical series, somewhat older than that shown in Fig. 7. The dense particles are now quite numerous and may be seen both inside and outside the cell, in the latter case often in a depression of the cell surface. Finger-like projections of the nucleus characteristically point toward the Golgi region (idiozome), an area considerably larger and with a less orderly arrangement than the Golgi region in a typical spermatocyte (*cf.* Fig. 3). $\times 15,000$.

Inset: Presumed stages in the passage of particles through the cell surface. It appears that the membrane surrounding a particle may fuse with the cell surface at one point, then open out to extrude the particle. It is also possible that the particles are entering the cell from outside, in which case their membrane would be a pinched off portion of cell membrane. There is no definite evidence concerning the direction of particle movement. $\times 60,000$.



of the available face-on views show the component subfibers. Since subfibers may be seen in some of the longitudinal sections of procentrioles (Figs. 21, 23), it is assumed that thinner or more favorably oriented transverse sections would show them likewise. Suggestions of a central hub with radiating spokes have been seen occasionally (Fig. 42).

The transformation of the procentriole into a mature centriole was not followed in typical spermatocytes, since stages later than pachytene were rare in the testes examined. Judging from the sequence of events in the atypical cells, and in other organisms (*e.g.* Fig. 6), we may assume that the procentriole grows longer without altering its orientation relative to the parent.

An interesting feature of the centriole is the polarization of its axis. The two ends of a centriole differ structurally or behave differently in at least four ways: (*a*) one end produces the flagellum; (*b*) one end is associated with the daughter procentriole at the time of duplication; (*c*) one end is itself derived from a procentriole; and (*d*) the two ends differ because of the asymmetry of the triplet fibers.

Gibbons and Grimstone (17) defined a *clockwise* basal body as one "in which, to an observer looking along the basal body from the proximal end, the subfibers are encountered in the sequence C, B, A when moving clockwise." They found that the basal bodies in 6 different individuals of *Trichonympha* were clockwise. The definition is not directly applicable to a free centriole, since we do not generally know which end is proximal; *i.e.*, which end would be opposite the flagellum. It is nonetheless easy in theory to distinguish the two

ends of a free centriole: viewed from one end the fibers are encountered in the order C, B, A when moving clockwise, from the other end they appear in this order when moving counterclockwise. We may thus speak of the *clockwise end* of any centriole or basal body, and define the whole structure as clockwise if the clockwise end is also the proximal end. Unfortunately, it has not been possible to distinguish the centriole ends in any of the present serial sections, as proper regard for orientation was not taken when the sections were placed on the grids.

The procentriole is always located at one end of the parent. In the typical cells there is no way to determine whether this is the proximal or distal end, as defined by the flagellum. However, a fortunate circumstance permits us to say (on the basis of two cases only) that the procentrioles cluster around the proximal end in the atypical cells (see next section).

The oriented growth of the centriole provides the other criterion of polarization. One end, the procentriolar, is "old," the opposite is "new." In *Viviparus* we do not know whether the procentriolar end is proximal or distal, since the flagella are produced long after the relative orientation of parent and daughter is lost. Fortunately the orientation can be determined in the primary spermatocytes of the Lepidoptera (28). The centrioles in these cells complete their duplication during the prophase and come to lie as two V-shaped structures at the cell surface. The electron microscope shows that each V is in fact a pair of normal centrioles situated at right angles and partially embedded in a dense matrix (un-

FIGURE 9

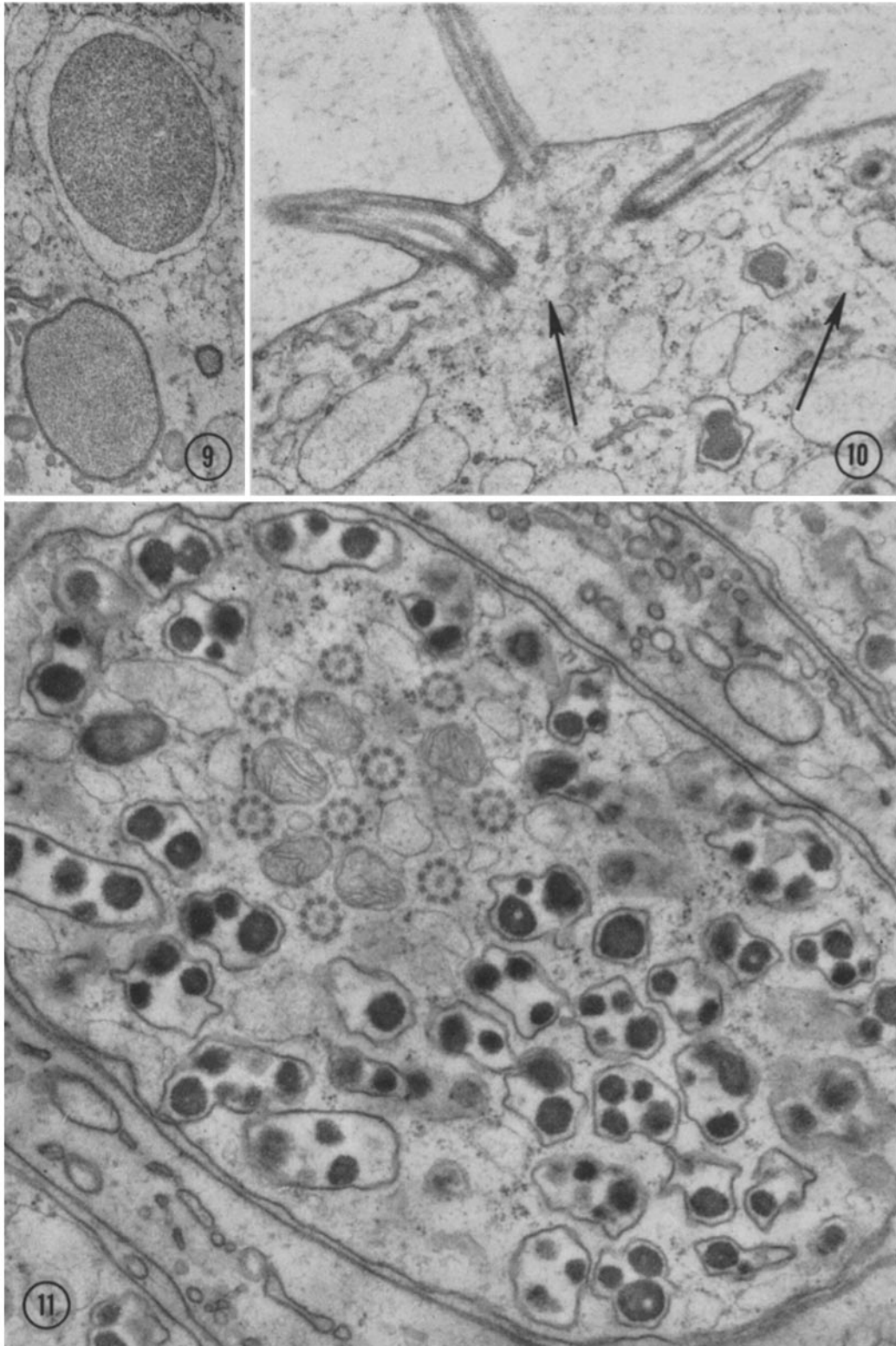
Vesicular, degenerating chromosomes from metaphase or anaphase of the first meiotic division of an atypical spermatocyte. $\times 25,000$.

FIGURE 10

Atypical spermatid immediately after the second meiotic division before the centrioles have migrated away from the cell surface. Longitudinal sections of two centrioles and portions of three flagella are shown. Note also the dense particles enclosed in a membrane and the astral fibers (arrows). $\times 35,000$.

FIGURE 11

Transverse section of an atypical spermatid with 9 flagella. In the atypicals the mitochondria do not fuse into nebenkerne, but individually migrate to the region of the flagellar bundle. The dense particles are now very numerous and occur in clusters of up to a dozen. $\times 40,000$.



published observations on *Samia walkeri*). Each V puts out two flagella before taking up its position at one pole of the first meiotic spindle. The flagella grow out from the tips of the V and thereby define the distal ends of the centrioles. Since the growth of the daughter is oriented we know that its pro-centriolar end is proximal. If the same is true of the parent, then it follows that parent and daughter are juxtaposed at their proximal or pro-centriolar ends. There is a possibility that the cart-wheel arrangement described earlier characterizes the proximal end. The cart-wheel has been seen in pro-centrioles (Fig. 42) and the best micrograph of it is from a section which just clipped the end of a centriole (Fig. 28). In *Trichonympha* and *Pseudotriconympha* the cart-wheel occurs only in the proximal region of the basal body. If this situation is generally true, we have still another way to distinguish the ends.

Earlier investigators using the light microscope recognized that centriole replication involves a growth period. "Budding" centrioles may be seen

particularly well in the careful drawings of Heidenhain (23) and Schreiner and Schreiner (43). In the hagfish, *Myxine*, studied by the Schreiners, not only is a growth period evident, but the orientation of the new centriole is diagrammatically clear (Fig. 6, reproduced from (43)). More recently Cleveland (11, 12) has described elaborate growth stages for the "centriole" of several flagellates, although the structure with which he is dealing undoubtedly includes more than a centriole in the sense used here. It has also been known for a long time that centrioles may duplicate in prophase (see the listing in (35)), although conventionally they are supposed to "divide" at metaphase or later. Two of the finest examples from the older literature have already been mentioned, the primary spermatocytes of the hagfish (43) and of the Lepidoptera (28).

Earlier studies with the electron microscope showed the cylindrical organization of the centriole, and its resemblance to the basal body of a

FIGURE 12

Longitudinal section through the head of a mature atypical sperm. The nucleus, which contains only 1 chromosome, appears as an elongated horseshoe of low density. It possesses a double envelope of normal appearance. The centrioles, each with an anterior cap of dense material, are located far forward in the head; the flagella extend uninterruptedly from the centrioles to the end of the sperm. To the left are a few of the large bodies formed by fusion of the dense particles. The lines *aa*, *bb*, and *cc* define the planes of the sections shown in Figs. 13, 14, and 15 respectively. $\times 45,000$.

FIGURE 13

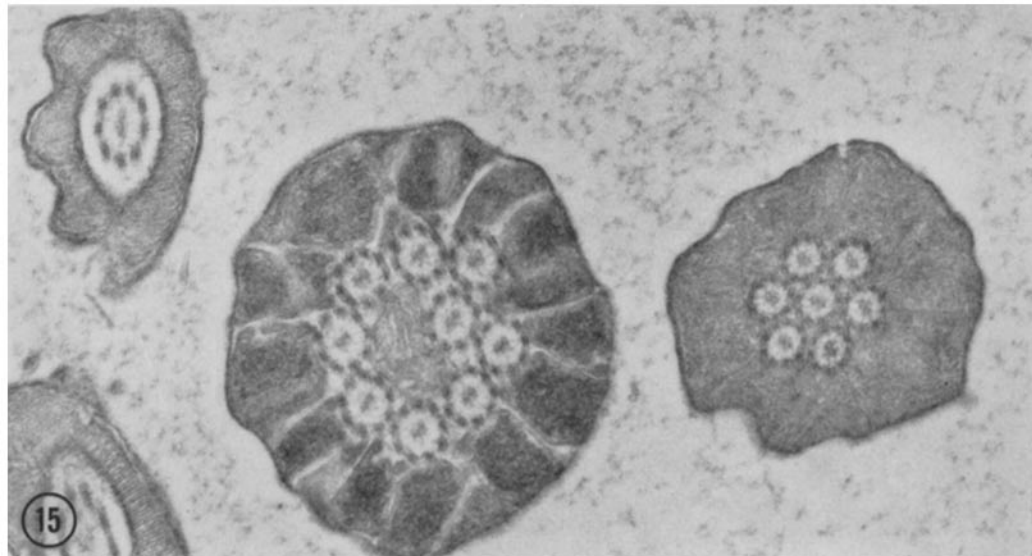
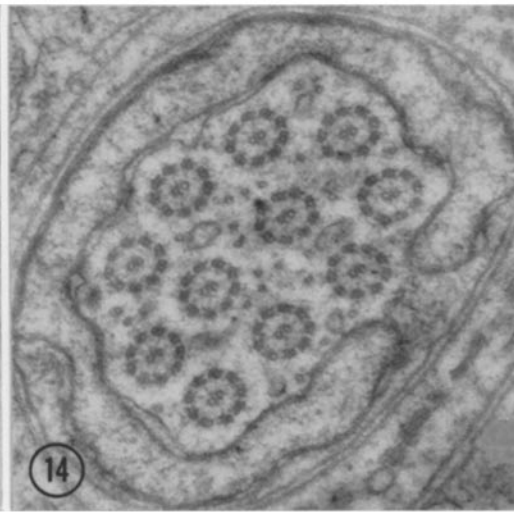
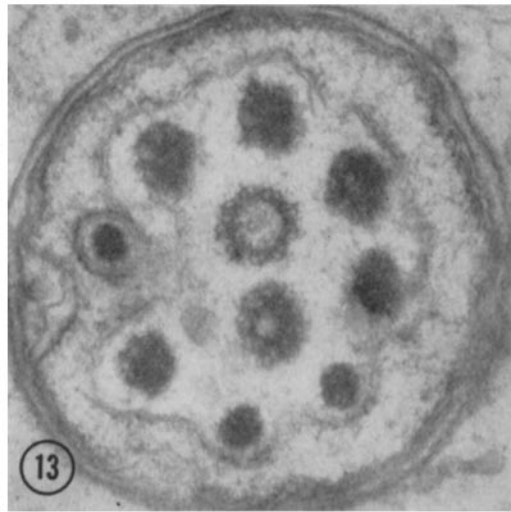
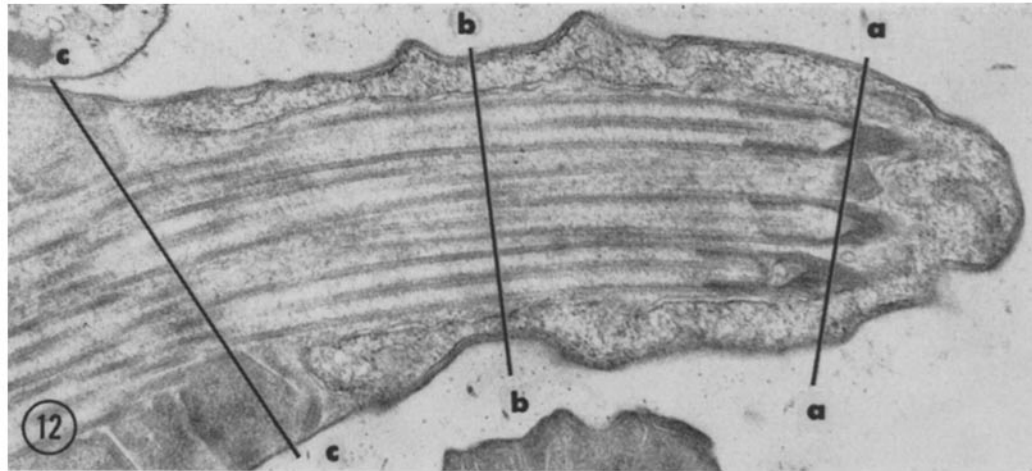
Transverse section through a mature atypical sperm at the level of *aa* in Fig. 12. At the center of the sperm is a section through the body of a centriole; elsewhere only the dense anterior caps of the centrioles are seen. The less dense peripheral area is the nucleus. $\times 75,000$.

FIGURE 14

Transverse section through a mature atypical sperm at the level of *bb* in Fig. 12. Note that the axes of the 11 flagella are orientated in the same direction, approximately 45° to the edge of the micrograph. The cell surface of the sperm, and of the cell which completely invests the sperm, appears "triple layered" after KMnO_4 staining. $\times 50,000$.

FIGURE 15

Transverse sections through two atypical sperm at the level of *cc* in Fig. 12. The large masses lining the periphery of the cell are formed by fusion of the dense granules seen at earlier stages. To the left are transverse sections through two typical sperm. The lower one shows orientated lamellae within an elongated nebenkern; the upper one, though not exactly transverse to the sperm axis, shows that there are four nebenkerne. $\times 40,000$.



cilium or flagellum (*e.g.*, 9, 13, 21, 40). The most detailed recent accounts of centriole structure are those of Bessis, Breton-Gorius, and Thiéry (8), de Harven and Dustin (22), and Bernard and de Harven (7), all of whom have described the spiral arrangement of multiple fibers. Similar descriptions of triplet fibers in basal bodies have been given by Rhodin and Dalhamm (39) and Afzelius (1) who have studied tracheal cilia and sea urchin sperm tails respectively. Gibbons (16) and Gibbons and Grimstone (17) have presented a thorough account of basal body structure in several species of flagellates, in *Paramecium*, and in the retinal rods and tracheal epithelium of the rat.

Recently Bessis *et al.* (8) described amorphous or somewhat fibrillar bodies found near the centriole which they called "massules" and "ponts." Somewhat similar structures can be seen in Figs.

25 and 40. Bernard and de Harven (7) and de Harven and Dustin (22) published micrographs of "pericentriolar" structures, and pointed out that some of them can be interpreted as stages in the formation of daughter centrioles. Their excellent micrographs were the first to show the structure of immature centrioles. They also suggested that the commonly seen perpendicular arrangement of two centrioles, in both mitotic and interphase cells, is related to the peculiar mode of duplication.

Mazia, Harris, and Bibring (27) have recently studied the formation of mitotic centers during cleavage of the echinoderm egg. They conclude, from an analysis of mitotic inhibition by mercaptoethanol, that the centrioles duplicate in the interphase or possibly in the preceding telophase. They draw distinctions on experimental grounds between "duplication," "splitting," and "sepa-

FIGURE 16

Transverse section through a centriole from a spermatogonium. The astral fiber (tubule) on the right appears to connect directly with one of the 9 triplet fibers making up the wall of the centriole. $\times 100,000$.

FIGURES 17 TO 20

Four sections from a series of 9 through the pair of centrioles in a pachytene cell of the typical series. The nuclear envelope runs vertically through each micrograph. $\times 75,000$.

FIGURE 17

Section No. 1: Transverse section of a centriole and longitudinal section through the edge of its accompanying procentriole. Note the 9 triplet fibers in the mature centriole and the suggestion of fibers or tubules in the procentriole. Within the nucleus is part of a chromosome core which can be followed in Fig. 18. Note that this core, as well as that in Fig. 19, points toward the centrioles. This orientation of the chromosome ends (bouquet arrangement) has long been recognized as a feature of meiotic prophase.

FIGURE 18

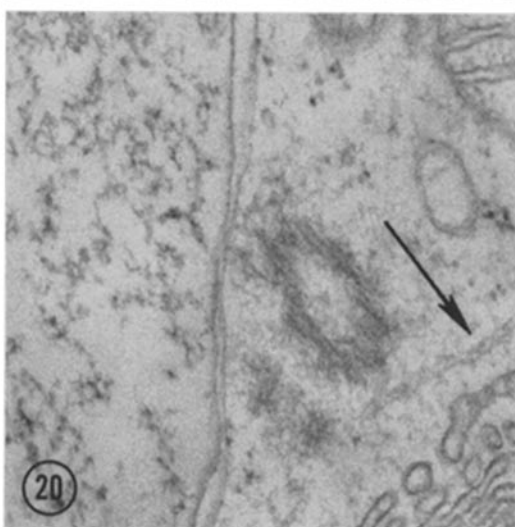
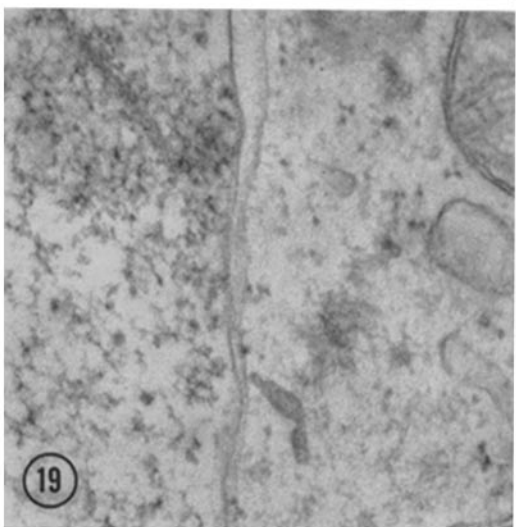
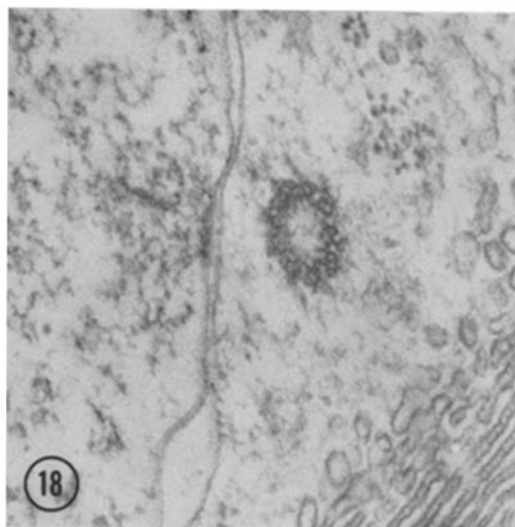
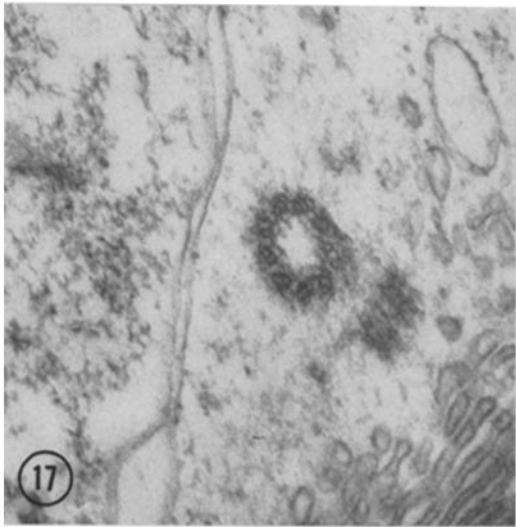
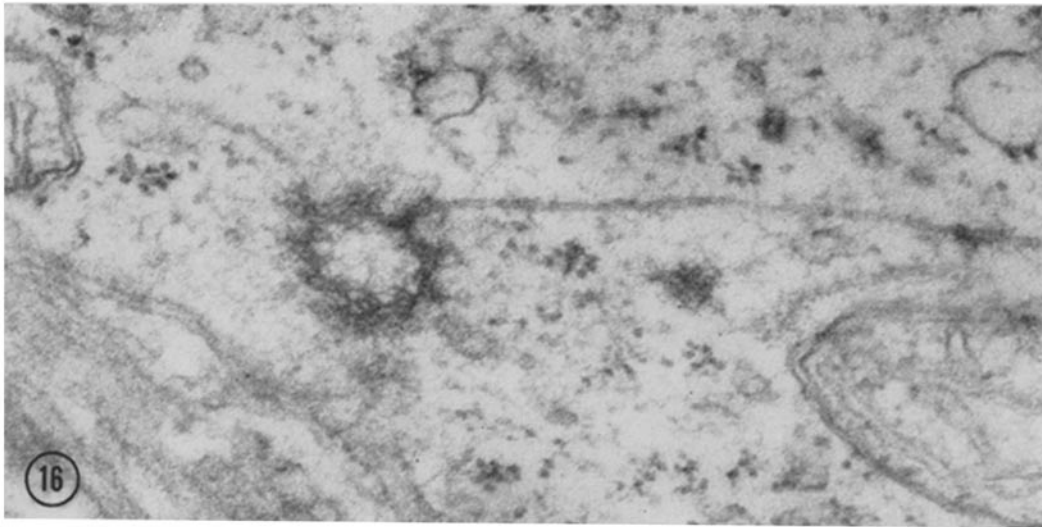
Section No. 2: The procentriole is no longer present.

FIGURE 19

Section No. 6: Space between the two centrioles; note the chromosome core.

FIGURE 20

Section No. 9: Oblique section of second centriole and longitudinal section of its accompanying daughter. The latter is cut through its center and so appears as two dense masses separated by a lighter area. An astral fiber (tubule) is visible to the right (arrow). Note that the procentrioles in Figs. 17 and 20 lie at right angles to their parents. Since the parents themselves are at right angles (a common though not invariable condition) the axes of the two procentrioles are perpendicular. In Fig. 17 the axis is approximately 135° , in Fig. 20, 45° .



ration." It seems probable that their "duplication" corresponds to procentriole formation, or possibly to an earlier macromolecular event. "Splitting" and "separation" probably concern the physical disjoining of parent and mature daughter.

Centrioles have often been cited in discussions of cytoplasmic inheritance, since they appear to undergo periodic "self-duplication" or "self-reproduction" like the chromosomes. On morphological grounds alone this duplication is seen to be a two step process involving first the formation of the procentriole, and second its growth into a mature daughter. The first of these steps is certainly the more relevant to discussions of self-reproduction, yet at the moment is obscure. We have already seen that the mature centriole is a polarized structure in which we can recognize a distal end, concerned with the production of the flagellum (and perhaps also with astral rays and spindle-fibers), and a proximal end, derived from the procentriole. We have also seen that the procentriole which appears at the time of duplication is associated with the proximal end of the parent. It is tempting to suppose that the procentriole remains as a "germinal center" in the mature centriole and that it alone is concerned with duplication. The first step in centriole reproduction

might be, for instance, the production of a new procentriole from the old one by some sort of template mechanism. Under these circumstances the centriole could well possess independent hereditary properties.

However, there are reasons for thinking that centrioles do not always arise from pre-existing centrioles (35). For instance, a centriole appears in the sperm-forming cells of ferns and mosses, although none is demonstrable by light microscopy in earlier stages (44). Cases of this sort could be explained by assuming that procentrioles are always present, but mature only during gametogenesis. It is also conceivable, as emphasized by the Pollisters, that centrioles may be derived from centromeres under exceptional conditions. Now that we know of the existence of procentrioles and of their complex organization, we can begin to look for them in some of the cases of puzzling centriole behaviour.

Centriole Multiplication in the Atypical Spermatoocytes

As a generalization we can say that centriole multiplication in the atypical series is similar to

FIGURES 21 AND 22

Two adjacent sections through a centriole and its accompanying procentriole in a pachytene cell. In Fig. 21 one can see individual subfibers (tubules) in the procentriole wall. Their total length is about 80 $m\mu$. Fig. 22 includes the less dense center of the procentriole and affords proof of its annular shape. $\times 110,000$.

FIGURES 23 TO 26

Four sections from a series of 17 through the pair of centrioles in a pachytene cell. $\times 110,000$.

FIGURE 23

Section No. 1: Two subfibers about 80 $m\mu$ long in the wall of the procentriole.

FIGURE 24

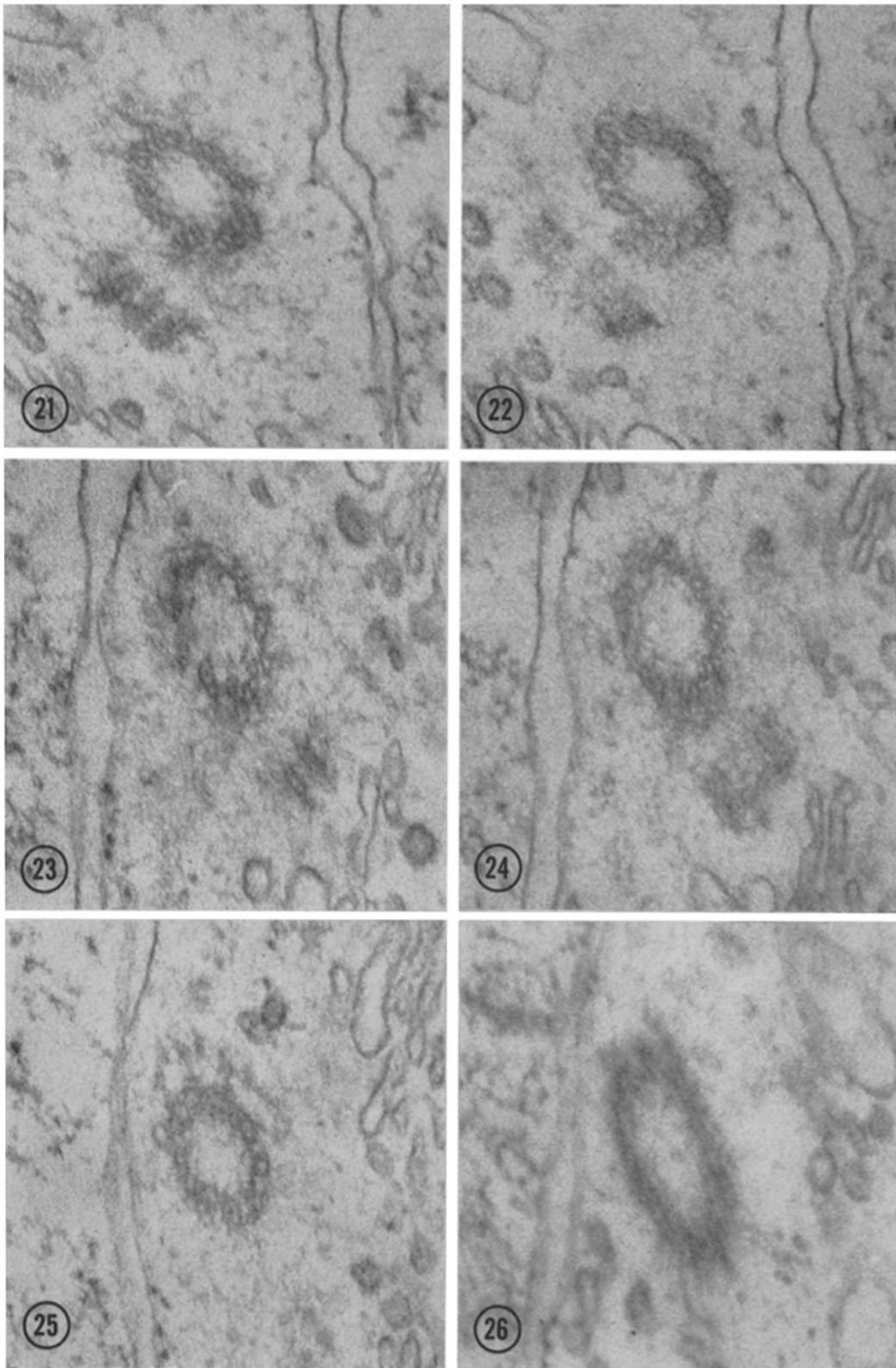
Section No. 2: Cuts through the center of the procentriole.

FIGURE 25

Section No. 5: Cuts beyond the limits of the procentriole. Note the 5 or 6 "pericentriolar masses" just above the centriole.

FIGURE 26

Section No. 17: An oblique cut through the second centriole.



that in the typical except for the number of daughters produced.

The earliest atypical spermatocytes can be recognized by their large size, the absence of the usual meiotic nuclear phenomena, and the presence of dense cytoplasmic particles. In these cells one finds a pair of centrioles in or near the Golgi region (Figs. 7, 27, 28). These centrioles are indistinguishable from the ones found in spermatogonia and the various stages of typical spermatogenesis (Figs. 28 to 31). At a somewhat more advanced stage the two centrioles migrate away from the nucleus and become surrounded by a cluster of annular procentrioles (Figs. 32 to 42). The compound body thus formed has been referred to in the past as the mulberry-like centriole (Meves) or as the centrosphere (Pollister).

The procentrioles composing a cluster appear to be identical to the procentrioles of typical spermatocytes. They are about $150\text{ m}\mu$ in diameter, but only $70\text{ m}\mu$ in length. In most micrographs the details of their structure are obscure, although it is clear that the wall is divided into smaller units, probably 9 in number. In a few cases a hub with radial spokes is seen within the procentriole (Fig. 42).

It will be recalled that the procentriole in the typical cells does not touch the parent, but lies about $70\text{ m}\mu$ from it. In the atypical cells the situation is similar in that the procentrioles lie on an imaginary surface drawn at a distance of 60 to $80\text{ m}\mu$ from the parent. They lie flat on this surface; that is, with their longitudinal axes perpendicular to it. Furthermore, the procentrioles are clustered around one end of the parent, a fact which is best seen in sections cut parallel to the longitudinal axis of the parent (Figs. 34, 37, 38). The end around which they are clustered is the proximal. This statement is based on micrographs of two late prophase clusters in which the parent centriole was capped by a mass of dense material identical to that seen on spermatid and sperm centrioles (Figs. 12, 13). The daughters were closest to the end with the dense cap. Since the cap is opposite the flagellum in the later cells, it almost certainly identifies the proximal end in

these prophase clusters. It thus appears that the arrangement of parents and daughters is governed by similar factors in both typical and atypical cells; the final configuration is dictated in part by the number of daughters.

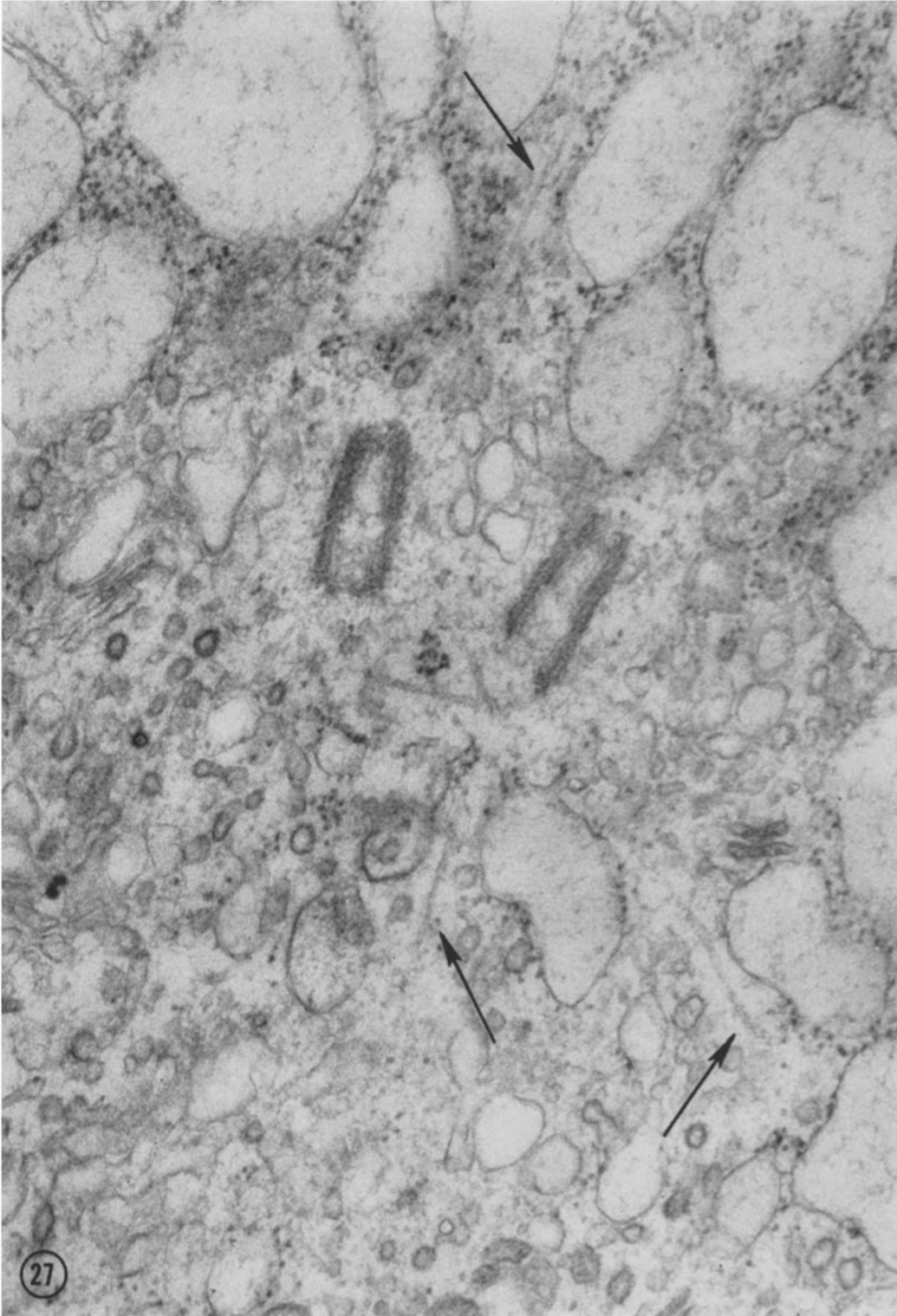
Single sections generally show 5 to 8 procentrioles (Figs. 32 to 34, 43). For technical reasons it has been impossible to determine the exact number of procentrioles per cluster. Nevertheless, minimal estimates of 10, 11, and 13 have been obtained in three favourable sets of serial sections, and in each case it is clear from the symmetry of the cluster that additional procentrioles would be seen if the series were complete. For instance, 11 separate procentrioles are shown in Figs. 35 to 37. It is possible that some procentrioles are wholly included in the three sections between Figs. 36 and 37 (which lay on bars of the grid), and it is almost certain that two or three more face-on views would be seen in the sections beyond Fig. 37. The total number probably lies between 15 and 20. It should be emphasized that procentrioles have never been seen outside the immediate vicinity of a mature centriole.

The later history of the mulberry cluster is known in broad outline, although certain important details are missing from the electron microscope record. Before the end of prophase some of the procentrioles have elongated. It is possible that all of them have done so, but serial sections of mature clusters have not been obtained and a definite statement cannot be made. Fig. 43 shows a cluster of 8 short daughters surrounding the parent centriole. Two other clusters of this same maturity have been seen; they were cut in the same plane and likewise showed 8 daughters uniformly spaced around the parent. It is interesting that these three cases, which at present constitute the only evidence relating to partially mature clusters, show the number of daughters predicted by the Pollister theory (see later). Since the number of procentrioles seen in earlier stages is certainly greater than 8, these clusters presumably contain additional centrioles or procentrioles.

According to the classical accounts the two centriole clusters are located at the poles of the

FIGURE 27

An over all view of the idiozome region from a very early atypical spermatocyte. The two centrioles have not yet migrated apart nor are they surrounded by daughter procentrioles. A few astral rays (tubules) are evident at the arrows. $\times 80,000$.



aberrant first meiotic division (Fig. 1). Unfortunately no sections of this stage have been found. They would be of considerable interest since the Pollisters say that the number of centrioles seen by light microscopy doubles during telophase. After the first meiotic division the centrioles disperse and take up positions at the periphery of the cell. Several electron micrographs have confirmed the migration, although centriole counts are manifestly impossible once the clusters have begun to break up. The second meiotic division involves a reaggregation of the centrioles at the poles. Only one set of serial sections of this stage has been obtained; in it the cluster at the spindle pole contained 9 centrioles. Many pictures have been taken of the immediately following stages, when the centrioles, still at the cell surface, begin to put out flagella (Fig. 10). All steps in the transforma-

tion of the spermatid into the mature atypical sperm have been seen and recorded, a few representative views being shown in Figs. 11 to 15. The centrioles come to lie in the depression at the posterior border of the cup-shaped nucleus. Anteriorly each is capped by a dense mass (Figs. 12, 13), while posteriorly they are directly continuous with the flagella. There are no unusual features of flagellar structure (Fig. 14).

A word should be added here about the origin of the flagellum from the centriole. According to both Meves and Pollister the centriole in the spermatid divides into two, a proximal centriole which remains attached to the nuclear membrane, and a distal centriole which takes up a position at the cell surface. The two are said to remain connected by an "axial filament" during the elongation of the spermatid. It was known to Meves that

FIGURE 28

Transverse section through a mature centriole from a very early atypical spermatocyte (see Fig. 7 for the same at lower magnification). One can see the three component subfibers which make up each of the 9 fibers in the centriole wall. This micrograph also shows a central "hub" with radial "spokes" as well as connecting lines between subfibers in adjacent triplets. The fading of structure in the upper right part of the centriole suggests that the knife just barely clipped the end of the centriole. $\times 140,000$.

FIGURES 29 TO 31

Three sections from a series of four through a centriole in an early atypical spermatocyte. $\times 110,000$.

FIGURE 29

Section No. 1: Longitudinal cut through three triplets in the centriole wall (parentheses). The triplet on the right consists of four parallel lines separated by three lighter spaces. This arrangement is expected for a median longitudinal section through three closely appressed tubules. The fact that the lines can be followed for nearly the whole length of the centriole indicates that the tubules are straight.

FIGURE 30

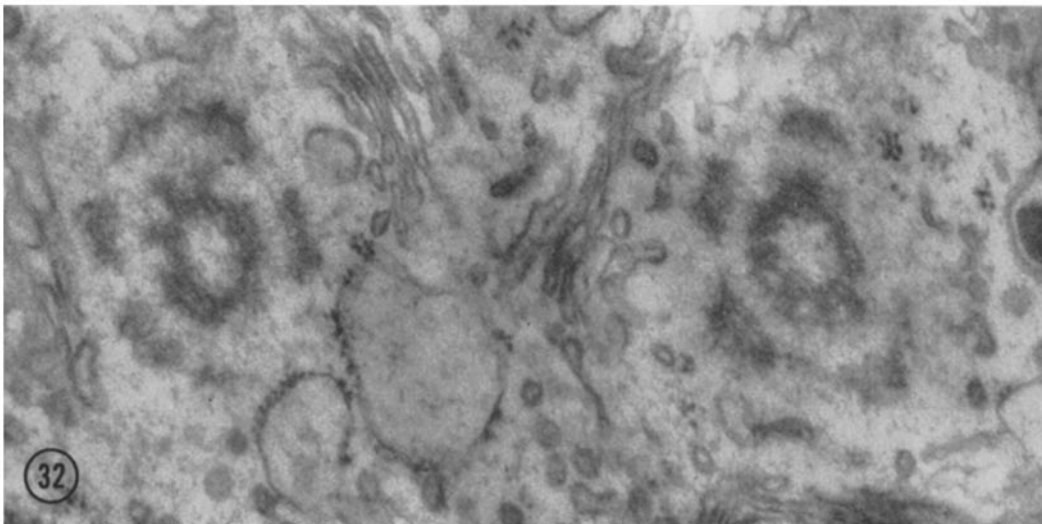
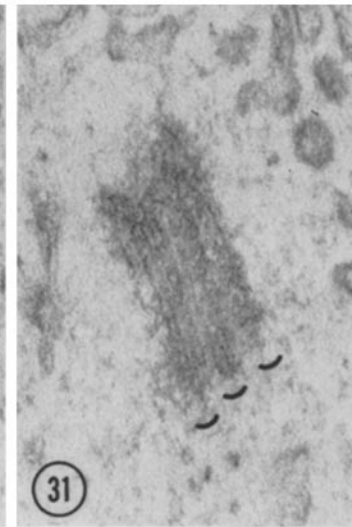
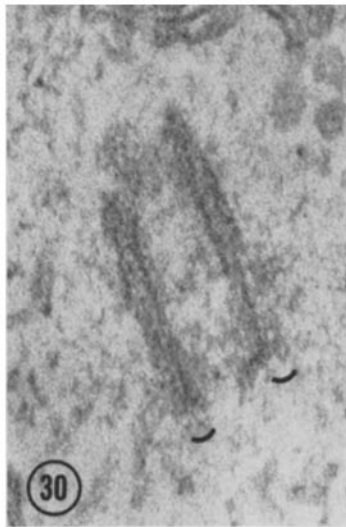
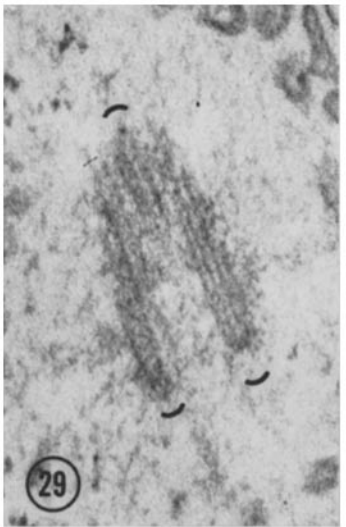
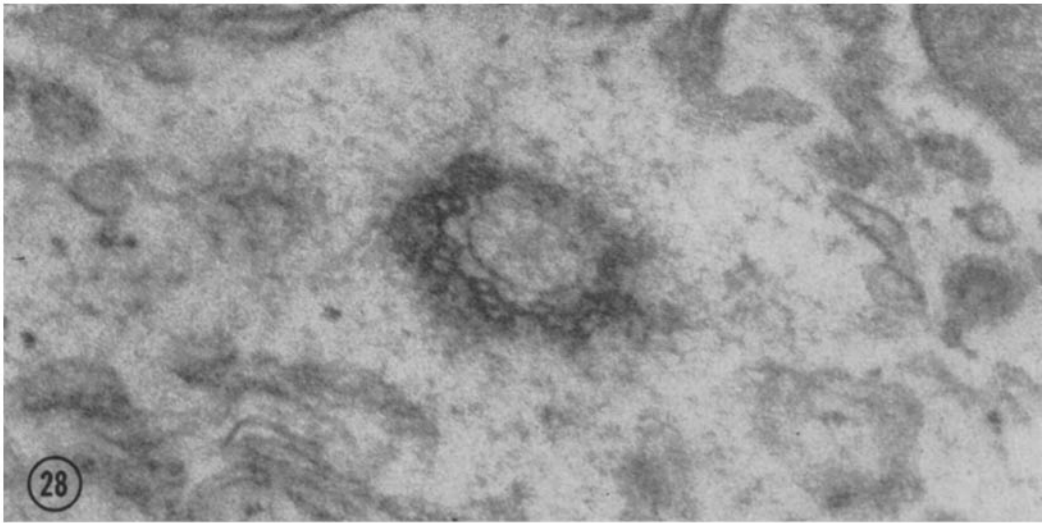
Section No. 3: Nearly median section showing parts of 2 triplets (parentheses).

FIGURE 31

Section No. 4: Tangential to the centriole surface and includes parts of three triplets (parentheses).

FIGURE 32

The two mature centrioles from an early atypical spermatocyte each surrounded by a cluster of daughter procentrioles. Between the parent centriole and the procentrioles can be seen some amorphous dense masses similar to the "massules" and "ponts" described by Bessis *et al.* (8). $\times 75,000$.



the flagellum, defined as the *free* filament extending posteriorly from the distal centriole, actually shortens in the final stage of spermatid development (Fig. 1). He was led to suppose, therefore, that the flagellum is a direct extension of the axial filament and that the distal centriole is a ring which progressively slips down the length of the flagellum. Meves was correct in claiming direct continuity of flagellum and axial filament. However, the distal centriole appears to be an optical illusion, or perhaps a region of more densely staining cytoplasm, since the electron microscope reveals no special structure where the flagellum leaves the posterior border of the cell. Yasuzumi and Tanaka's (48) description of two centrioles at the *anterior* end of the flagellum, *i.e.* at the nuclear surface, is certainly in error. In their Fig. 21, PC is apparently a portion of the nuclear envelope, while DC is the single centriole.

Origin of the Procentrioles in the Atypical Line

Meves (28) showed that the atypical spermatids of *Viviparus* contain several centrioles, each of which becomes the basal body, or blepharoplast

of a flagellum. He was able to demonstrate that the extra centrioles arise in the primary spermatocyte and he assumed, quite logically, that they are produced by successive duplications of the two original centrioles.

The whole sequence of events was placed in a new light when the Pollisters (32, 35) suggested that the extra centrioles are transformed centromeres (kinetochores). On this theory centromeres leave the chromosomes and migrate to the cytoplasm early in the prophase of the atypical first meiotic division. The gradual enlargement of the centrioles (prior to a distinct mulberry stage) is explained by assuming that centromeres are added to them one or two at a time. The finger-like projections of the nucleus are supposed to represent the actual points of centromere extrusion. A strong point of the theory is that it provides a reasonable explanation of the aberrant chromosome movements, which do in fact resemble those of artificially produced acentric fragments.

Centriole counts in the spermatids provided the main evidence for the theory. The counts showed (by extrapolation) that the number of

FIGURE 33

Transverse section through a mature centriole and parts of at least 7 daughter procentrioles. Note the relatively constant separation of daughters and parent. $\times 75,000$.

FIGURE 34

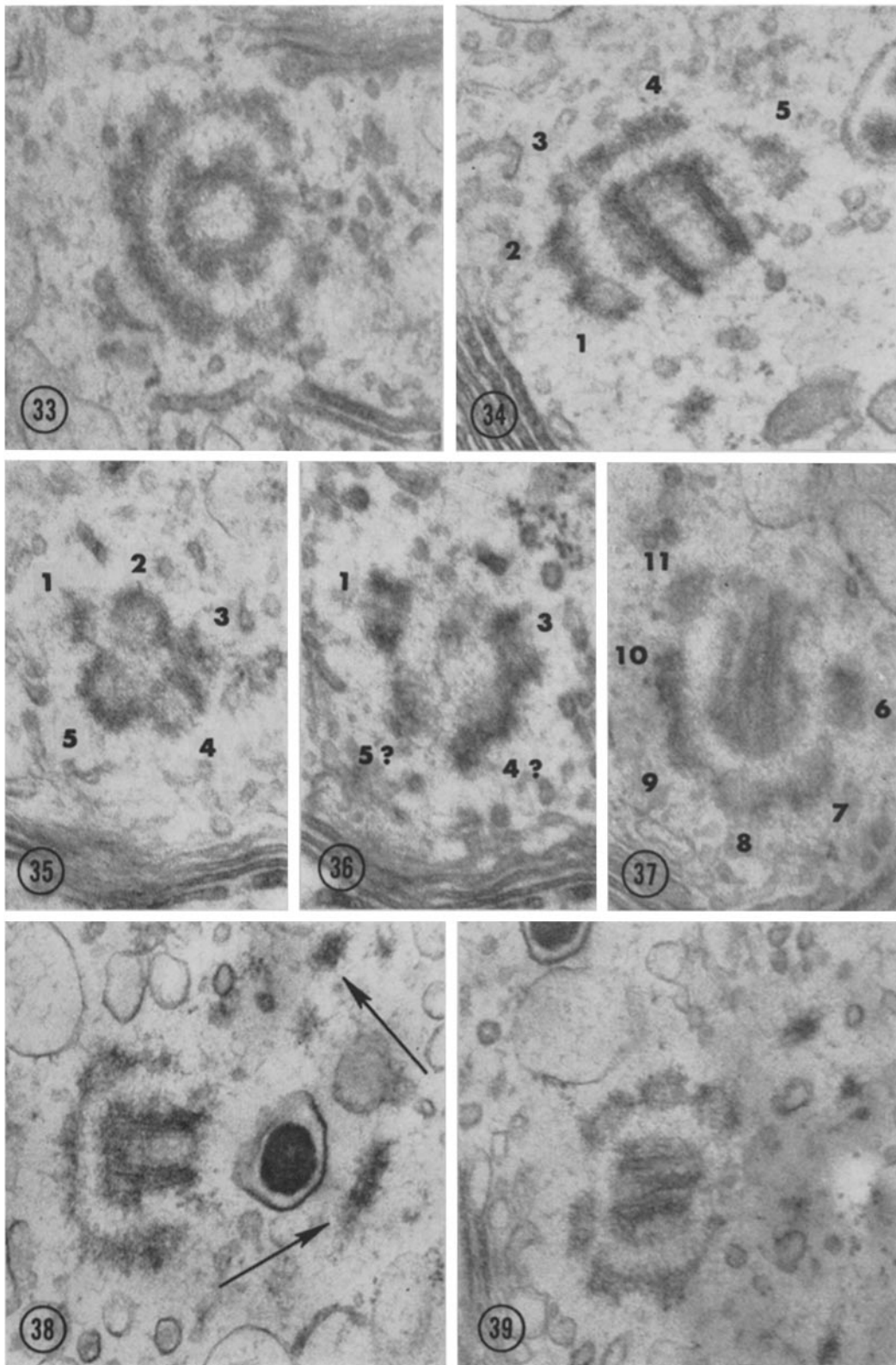
Longitudinal section through a mature centriole and parts of at least 5 daughter procentrioles. The latter are annuli. They appear as amorphous or slightly fibrillar smudges (Nos. 2 and 4), as two dark masses separated by a lighter space (3 and 5), or as an annulus (1), depending on whether the section passes through the edge, the center, or full face. Note that the procentrioles cluster about one end of the parent, and that a relatively uniform space separates centriole and procentrioles. $\times 75,000$.

FIGURES 35 TO 37

Serial sections through a mature centriole and its cluster of daughters. The sections are respectively Nos. 1, 2, and 6 from a row of 6. The annular form of the procentrioles is particularly clear in Fig. 35 where Nos. 2, 4 and 5 are cut in full face. In Fig. 36, Nos. 1 and 3 show the lighter center and dark edges expected for a median longitudinal section through an annulus. In this series one can count a minimum of 11 separate procentrioles. Others may well be included in the sections between Fig. 36 and 37, which fell on grid bars, and several more procentrioles almost certainly lie on the "back" side of Fig. 37. $\times 75,000$.

FIGURES 38 AND 39

Two non-adjacent sections through a mature centriole and its daughters. Note the close association with the dense granules enclosed in a membrane, and also the "pericentriolar" masses at the arrows. $\times 75,000$.



extra centrioles appearing during prophase must equal the number of degenerating chromosomes. Complete data were given for *V. malleatus* and three other Viviparid snails, each with a different chromosome number. Numerical correspondence was said to exist in another three species for which detailed counts were not published (34).

The present study has provided two important facts about centriole multiplication in the atypical cells: first, this multiplication is very similar to that in the typical cells, involving the formation of procentrioles; and second, all the procentrioles appear to arise at the same time and mature together.

It is reasonable, therefore, to rule out sequential duplication as envisaged by Meves, as the explanation for the increased centriole number. An evaluation of the Pollister theory is more difficult. We know now that the apparent increase in size of the prophase centrioles, as viewed by light microscopy, is due to the growth of their component procentrioles, present from an early stage. We can also be fairly confident from electron microscope observations that centromeres are smaller than mature centrioles and otherwise differ from them in fine structure (7, 42). It is impossible, therefore, to maintain the Pollister theory in its simplest form, namely that centromeres are equivalent to centrioles and are added periodically to the prophase cluster.

The problem would be considerably simplified if we had detailed information on the organization of centromeres. It is conceivable, for instance, that

centromeres and procentrioles may turn out to be similar structures. In this case we could accept the essence of the Pollister theory by assuming that the chromosomes supply the initial cluster of procentrioles.

The morphological findings so far discussed fail either to confirm or contradict the Pollister theory in a decisive fashion. Let us turn then to the major point on which the original claim rested—the numerical correspondence between the new centrioles and degenerating chromosomes. In *V. malleatus* 16 of the 18 chromosomes present in the primary spermatocyte ultimately degenerate. According to the Pollisters each mulberry cluster in late prophase contains 9 centrioles; of these one is the original and 8 are new, giving a total of 16 new centrioles supposedly derived from the chromosomes. Sometime during or after the first division, the 9 centrioles passed on to each secondary spermatocyte duplicate. Eighteen centrioles are then apportioned to two spermatids (Fig. 1). In *V. malleatus*, but not in *V. viviparus*, the second division is unequal, so that large and small spermatids are formed in equal numbers (29). The large spermatids usually receive more centrioles than the small, and the large atypical sperm consequently have more flagella. From counts of centrioles and flagella in 241 cells the Pollisters showed that the small sperm receive from 3 to 9 centrioles, the large from 9 to 15. Cells with 9 centrioles are rare, but may be either large or small. Small sperm with 8 centrioles are as common as large ones with 10, 7's are as common as

FIGURES 40 AND 41

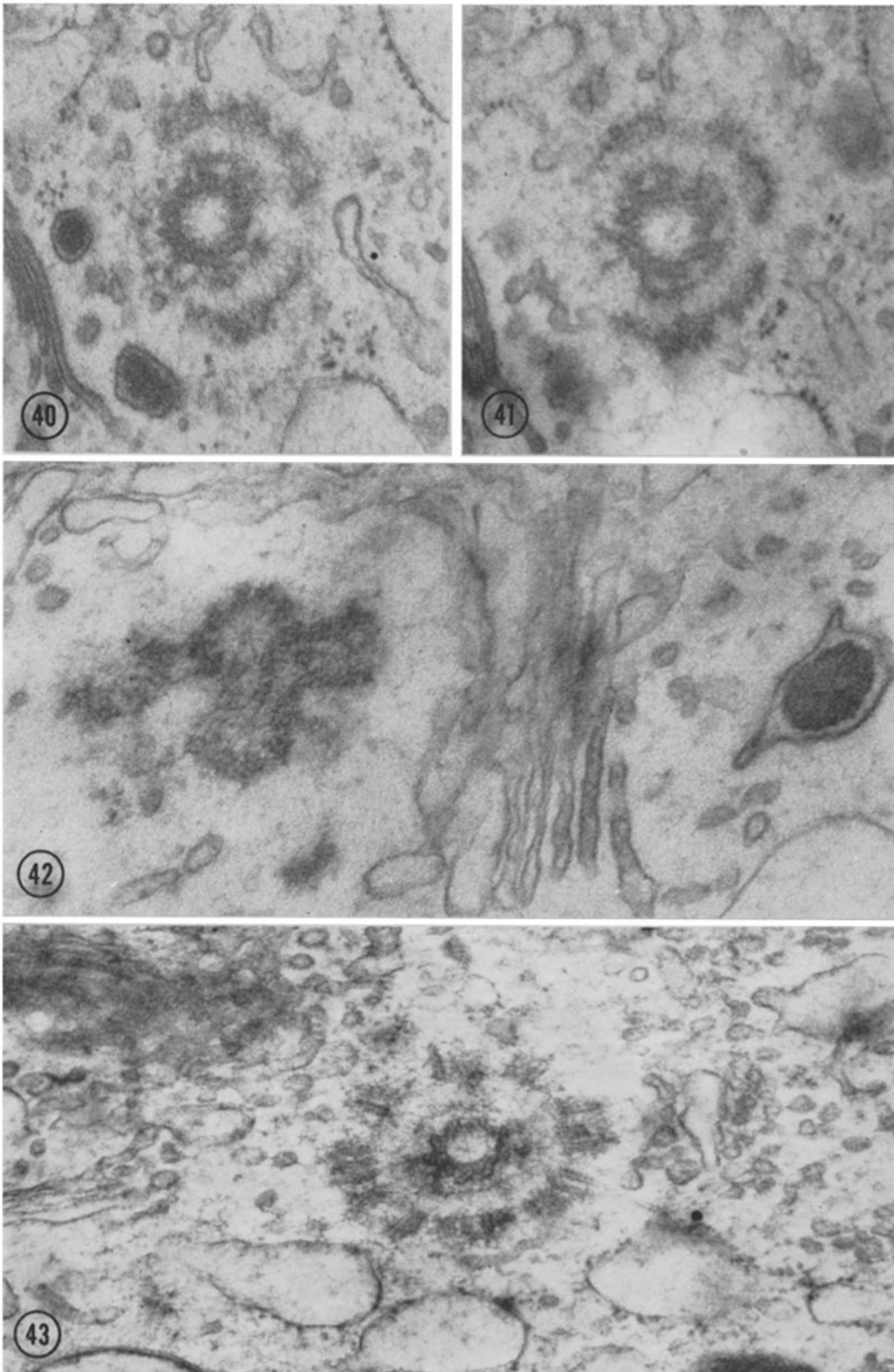
Two adjacent serial sections through a mature centriole and its daughter procentrioles. There are a number of amorphous masses immediately surrounding the mature centriole. These resemble the "massules" and "ponte" described by Bessis *et al.* (8). $\times 75,000$.

FIGURE 42

Parts of four procentrioles, two of which are shown in full face. About 9 units can be counted in the wall of the topmost procentriole and there is also a suggestion of a central "hub" and radiating "spokes." A dense granule enclosed in a membrane is included at the right. $\times 100,000$.

FIGURE 43

A partially mature mulberry cluster showing the parent centriole in the center and 8 daughters around it. Subfibers are evident in the walls of the daughters and the spiral or pinwheel arrangement of the triplet fibers is well displayed by the mature centriole. The dense material between parent and daughters is organized into a fairly definite ring. $\times 75,000$.



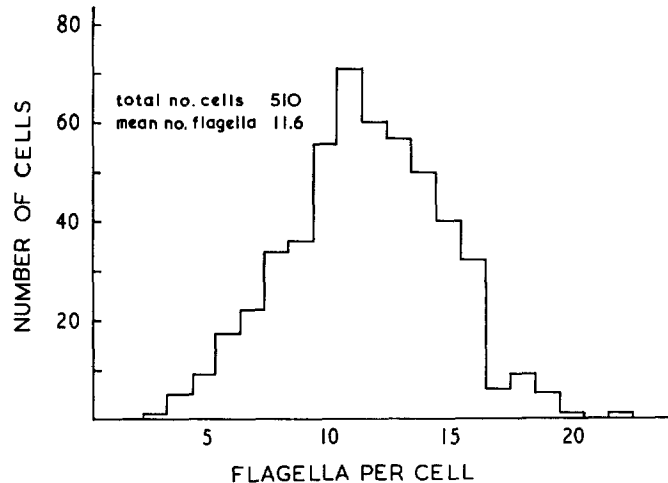


FIGURE 44

Frequency histogram showing the number of flagella on atypical sperm and spermatids of *V. malleatus* (large and small sperm not distinguished). Data from randomly selected electron microscope fields.

11's, and in general complementary classes adding to 18 are equally abundant.

As already mentioned, the number of procentrioles in each cluster is certainly greater than 8. This fact is not necessarily in contradiction to the Pollisters' account. The typical centriole was originally described as single until telophase, yet consists of a centriole and its daughter as early as pachytene. Similarly the atypical cluster is said to contain 9 centrioles, but it could consist of 17 procentrioles plus the parent, giving a total of 18. These might mature in pairs that are not resolvable by light microscopy, or 8 might mature early, 9 later. As mentioned earlier, sections of three partially mature clusters, including the one shown in Fig. 43, displayed 8 daughters symmetrically placed around the parent. In this regard it is interesting that Meves showed 17 and 19 centrioles respectively in the two clusters of a late prophase cell (his Fig. 68), while his drawings of earlier clusters show about half this number.¹

The uncertainty about the procentrioles made it desirable to repeat the Pollisters' analysis of flagellar numbers. The chromosome number was first determined from aceto-orcein and Feulgen squashes of testes fixed in ethanol-acetic acid (3:1). Typical meiotic metaphase plates consistently showed 9 bivalents. Atypical divisions

¹ Meves studied *V. viviparus* ($n = 7$); on the Pollister theory each of these clusters should contain 14 centrioles.

displayed about 36 chromosomes, the number expected if the chromatids are completely unpaired.

Flagellar counts were next made on spermatids and mature atypical sperm. Fields containing the appropriate cells were randomly selected at low magnifications of the electron microscope. From the enlarged micrographs 510 cells were counted. As can be seen in Fig. 44, cells with as few as three and as many as 22 flagella were found. The mean number of flagella was 11.6 and the mode 11. The large and small atypicals could not be readily distinguished in these sections and so are lumped together. Quite clearly the data do not agree with those published by the Pollisters. The Pollisters obtained a bimodal distribution with a mean of 9, as demanded by their theory. The fact that the distribution in Fig. 44 is unimodal is, in itself, of little concern. This probably indicates that the second meiotic division often produced equal spermatids in my material. But the mean flagellar number would be 9 if these data conformed to the Pollister scheme.

In hopes of clarifying this contradiction, new snails were purchased from a second source (L. Haig, Ltd., Newdigate, Surrey, England). Fresh testis smears were made, fixed in OsO_4 vapor, and examined by phase microscopy. In such preparations the flagella can be counted easily and the large and small sperm distinguished. Data from 400 cells are given in Fig. 45. The mean number

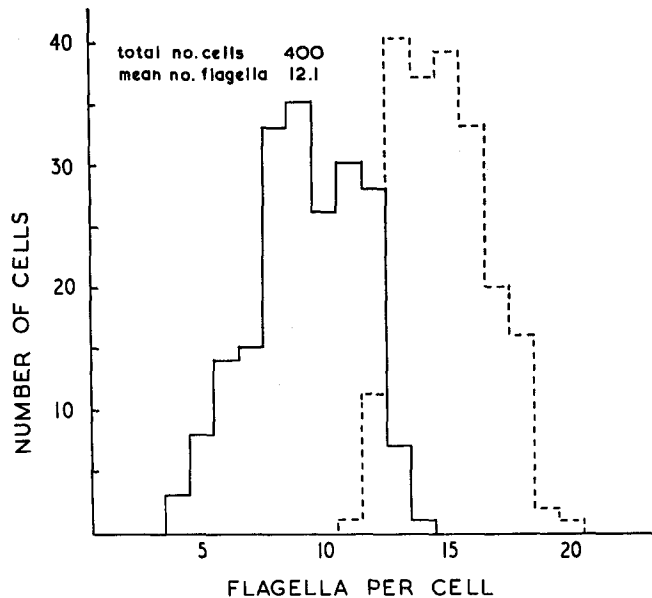


FIGURE 45
Frequency histogram showing the number of flagella on atypical sperm of *V. malleatus*. Small sperm shown by the solid line, large ones by the dotted. Since small and large sperm occur in a 1:1 ratio, equal numbers have been included. Counts made on fresh testis smears, fixed in OsO₄ vapor, and observed by phase contrast microscopy.

of flagella is 12.1, very close to that obtained from the electron micrographs. Equal numbers of large and small sperm were included in the histogram in order to compensate for the bias which arises from the fact that small sperm are more often countable than large.

Data were also collected from specimens of the European *V. viviparus*. The haploid chromosome number of this snail is 7 according to Meves (28), Popoff (36), and Ankel (3); this number was confirmed in each specimen used for flagellar counts. According to Meves, one chromosome goes to each spermatid. On the Pollister theory, therefore, there should be an average of 7 flagella per sperm. Counts on 480 sperm from three individuals are pooled in Fig. 46. The average number of flagella is 10.8. Sperm with 7 or fewer flagella account, in fact, for only 5 per cent of the total.

Meves states that the number of "cilia" in the mature sperm of *V. viviparus* is 12, the implication being that all sperm have this number. He also gives 12 as the number of axial filaments and as the number of centrioles at each pole of the second meiotic division. In view of the care with which his work was done, some weight must be given to his statements. At the very least, 12 must have been a common number in his material. Hanson *et al.* (20) say, "the number of tails is commonly about 10 but may be as few as 8 or as many as 16."

Despite these contradictions, the Pollisters' extensive counts establish beyond question a

remarkable correlation between the number of flagella and the number of degenerating chromosomes in individuals of four different species. The conflicting information presented here may perhaps be reconciled in the following manner. The Pollisters counted centrioles and flagella in *spermatids*, and used the counts to infer the number of extra centrioles arising in the *spermatocyte*. This procedure will be valid, of course, only so long as the behaviour of the centrioles during and after the meiotic divisions is regular. Apparently this was true for the Pollisters' specimens. On the other hand, irregularities after the first prophase which lead to increase or decrease in the number of centrioles will invalidate counts made on spermatids or sperm. Such irregularities may have characterized my material. The Pollister theory must, therefore, be tested by direct counts on the mulberry clusters in the spermatocytes. It is curious that the Pollisters themselves did not emphasize this point, since they knew of cases where the number of flagella is not correlated with the number of chromosomes. For instance, in *Janthina* the atypical sperm is a gigantic cell almost a millimeter in length and it bears about 2,000 flagella (3).² Very large atypical sperm are also found in *Strombus* (3, 38) where again the number of

² It is suggestive, though perhaps merely coincidental, that the flagella in the posterior part of the cell are aggregated into 14 or 15 bundles, while the chromosome number is said to be "etwa 16."

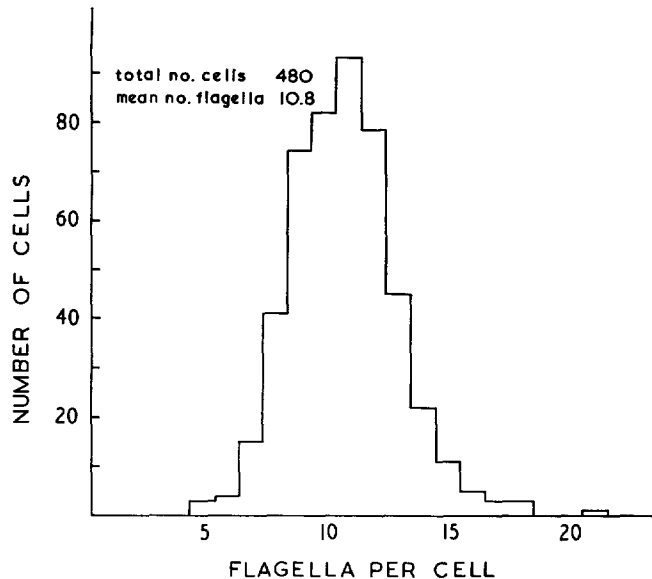


FIGURE 46
Frequency histogram showing the number of flagella on atypical sperm of *V. viviparus*. The atypical sperm of this species do not fall into large and small size classes, as in *V. malleatus*. Cells selected on basis of countability only. Data from three specimens pooled. Fresh testis smears, fixed in OsO₄ vapor, observed by phase contrast microscopy.

flagella is certainly greater than the chromosome number. Finally the converse situation exists in other species. In *Vermetus sp.* and *Conus mediterraneus*, studied by Ankel (3) and Kuschakewitsch (26), the haploid chromosome number is about 14. All the chromosomes degenerate at the first meiotic division but the apyrene sperm bear only two flagella.

In summary, the information derived from this study has not confirmed the Pollister theory, nor has it provided decisive contrary evidence. Since events after the first meiotic prophase may alter any numerical relationship originally existing between acentric chromosomes and extra cen-

trioles, unequivocal evidence can only come from prophase centriole counts. Such counts are not easy to obtain by either light or electron microscopy. Finally, if the Pollister scheme is correct, we should expect centromeres to be similar to procentrioles. Unfortunately high resolution micrographs of centromeres are not yet available.

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