

## Sperm Morphology and Development in Two Acoel Turbellarians from the Philippines<sup>1</sup>

BARBARA CONTA BOYER<sup>2</sup> and GEORGE W. SMITH<sup>2</sup>

**ABSTRACT:** In this study we compare spermiogenesis and ultrastructure of the mature sperm in two species of acoel Turbellaria from the Philippines. Sperm development is divided into five stages: (1) the early undifferentiated state, with a large nucleus, sparse cytoplasm containing few organelles, and no inclusions; (2) spermiogenesis I, in which Golgi activity is prominent, dense bodies appear in the cytoplasm, and peripheral centrioles migrate toward the nucleus; (3) spermiogenesis II, in which a manchette of microrods forms around the nucleus, refractile bodies are produced by the Golgi, and free 9+0 flagella are seen between the cells; (4) spermiogenesis III, which is characterized by marked cell elongation, nuclear condensation, and flagellar elongation and incorporation into the developing spermatid shaft; and (5) the mature sperm, which has a proximal nucleus, a middle shaft region containing a central keel of microrods, laterally incorporated axonemes, and many inclusions such as refractile bodies, dense bodies, open vesicles, mitochondria, and a distal flagellar region containing the two 9+0 axonemes tapering to terminal basal bodies. We propose that the refractile bodies may function as acrosomes, that the central keel provides support, that the biflagellate condition is important in providing the motile force that moves the sperm through intercellular spaces, and that the 9+0 axonemes may contain some central structure. The microrods of the keel appear to be a previously undescribed cellular component. The peculiar morphology of these spermatozoa is probably an adaptation associated with locomotion through the interdigitated acoel parenchyma where an extremely elongate cell, propelled flagellar tip first by undulations, is particularly efficient.

THE SPERMATOZOA OF the turbellarian Platyhelminthes, which undergo internal fertilization, occur in a variety of forms, all of which are strikingly different from the primitive metazoan type of sperm described by Franzen (1970). This primitive form, associated with fertilization in a liquid medium, typically is tripartite with a short round or

conical head, a middle piece with four or five spherical mitochondria, and a tail consisting of a single free flagellum. It is not possible to characterize a typical turbellarian in this manner, and even within the acoel order, sperm morphology is variable.

However, some unifying concepts of the Acoela can be listed. The sperm usually has an elongate nucleus, is devoid of an acrosome, and has mitochondria, refractile granules, and dense bodies contained in a long shaft region. Paired axonemes with a 9+2, 9+1, or 9+0 microtubule configuration arise as free flagella in the young spermatid and become incorporated into the elongating cytoplasmic body (shaft and flagellar region) during spermiogenesis. This unusual process of spermiogenesis from a biflagellate spermatid to a long

<sup>1</sup>This research was conducted as part of the *Alpha Helix* Cephalopod Expedition to the Republic of the Philippines, supported by National Science Foundation grant PCM 77-16269 to J. Arnold. This study was also supported in part by a Marine Biological Laboratory Steps Toward Independence fellowship to B. C. Boyer and a Marine Biological Laboratory Macy fellowship to G. W. Smith.

<sup>2</sup>Union College, Department of Biology, Schenectady, New York 12308.

thread-shaped spermatozoan is described by Hendelberg (1969) for a number of acoele species. In order to clarify the observations of the present study, which support Hendelberg's findings, a short description and illustration of his conclusions regarding acoele spermiogenesis are presented in the Appendix. Henley (1974) describes acoele spermatozoa as either (1) very long (300–400  $\mu\text{M}$ ), slender, and threadlike, with paired axonemes that can be located within the spermatid shaft or in lateral undulating membranes; or (2) shorter (50–60  $\mu\text{M}$ ) and more compact, with paired axonemes located within undulating membranes. However, these basic patterns may be modified in a number of ways that appear to bear little relationship to taxonomic position. Hendelberg (1969) concludes that the "Turbellarian Archetype" as proposed by Ax (1963) probably had biflagellate sperm but perhaps evolved from the primitive monoflagellate metazoan sperm in conjunction with the evolution of internal fertilization.

The primitive members of the Acoela face unique problems in sperm transport because both male and female ducts are frequently absent, and therefore, the sperm must move through the interstices of the loose acoele parenchyma before reaching sites of storage or fertilization. The ultrastructure of the acoele parenchyma is not well understood and until recently the tissues were thought to be syncytial. However, electron microscopic studies (Pedersen 1964), which our work supports, have revealed complexly interdigitated cell membranes and highly vacuolated parenchymal tissues that present a more viscous medium in which sperm move than is usually the case in animals with internal fertilization. The peculiar morphology and unusual method of locomotion in which the flagellar end leads are probably adaptations associated with movement through tissue spaces.

Because of the evolutionary significance of the acoeles, which are widely accepted as the most primitive turbellarians and which, in many phylogenetic schemes, constitute some of the most primitive metazoa, the fine structure and development of the acoele sperm are of considerable interest. Particular problems of acoele spermatology concern the role of the

two flagella in sperm transport, the mechanics of motility of a 9+0 axoneme, the support and locomotion of a particularly long sperm, and the function of the refractile granules. These issues are addressed in this study, which reports on spermiogenesis and fine structure of spermatozoa in two species of acoeles from the Republic of the Philippines.

#### MATERIALS AND METHODS

Specimens of two acoele turbellarians from the family Convolutidae were collected during the 1979 expedition of the R/V *Alpha Helix* to the Republic of the Philippines. Animals that were black in color (*Convoluta* sp. 1) were washed off *Sargassum* collected near the Camotes Islands; the second group, consisting of green animals (*Convoluta* sp. 2), was obtained in the same manner from eel grass collected on a sand-coral tide flat south of Panglao.<sup>3</sup>

Whole animals were fixed for 1 hr at room temperature in 2.6% glutaraldehyde in seawater buffered with collidine-HCl prepared according to the method of Arnold and Williams-Arnold (1978) and then rinsed with two changes of filtered seawater at 15-min intervals. Specimens were postfixed in 2% OsO<sub>4</sub>, also prepared by the Arnold and Williams-Arnold (1978) method. After dehydration through a series of alcohols and propylene oxide, the acoeles were embedded in Epon and polymerized at 60°C. Thin sections cut with a diamond knife were stained with uranyl acetate and lead citrate and viewed with a Phillips 300 electron microscope. Thick sections of approximately 1  $\mu\text{m}$  were stained with toluidine blue for orientation and study of general morphology.

#### OBSERVATIONS

The sperm of acoeles originate from mesenchymal cells that develop as scattered clusters in paired dorsolateral regions of the body. In both species reported here we observed at

<sup>3</sup> *Convoluta* sp. 1 and sp. 2 are being described by Louise Bush (*Trans. American Microscopical Soc.*, in press) as *Convoluta boyeri* and *C. philippinensis* respectively; these names are conditional on their publication in the cited journal. (Note added in proof.)

least eight cells (probably spermatids) linked by cytoplasmic bridges (Figure 1) at a given stage and single sperm clusters containing groups at several different stages of development. We have divided sperm development into five stages: the early undifferentiated state; spermiogenesis I, II, and III; and the mature sperm. These stages are similar in the two acoel species observed, but some noteworthy differences are described below.

### 1. Early Undifferentiated State

#### a. *Convoluta* sp. 1 (Figure 2)

The early undifferentiated sperm cell is characterized by a large round or oval nucleus with a prominent nucleolus and a nuclear membrane punctuated by pores that frequently contain dense material. The relatively sparse cytoplasm, consisting primarily of free ribosomes, contains a few mitochondria, occasional profiles of rough endoplasmic reticulum, and, sometimes, one or two Golgi per section. Centrioles, when visible in favorable sections, are located at the periphery of the cell. Occasional small surface projections are seen from an otherwise smooth cell membrane.

#### b. *Convoluta* sp. 2

The early undifferentiated cells appear essentially identical to those of *Convoluta* sp. 1 in electron micrographs.

### 2. Spermiogenesis I

#### a. *Convoluta* sp. 1 (Figure 3)

The round or ovoid nucleus in the undifferentiated spermatid is not visibly changed. The most significant feature at this stage appears to be an increased cytoplasmic/nuclear ratio which is probably the result of increase in cell size. This cytoplasmic area appears to accommodate a greater number of mitochondria, more endoplasmic reticulum (ER), numerous membrane-bound dense inclusions, and most notably, a marked increase in Golgi activity.

The membrane-bound dense inclusions are probably the same structures found in *Childia groenlandica* and described as dense bodies by Henley (1968). They consist of an electron-

opaque outer wall with an amorphous material in the center; some are completely opaque. Figure 4 illustrates a typical large Golgi complex closely associated with rough ER at the forming face. Many layers of sacculi are also present along with secretory vesicles at the maturing face. Centrioles are frequently seen near the nucleus or between the cell membrane and nucleus, suggesting centriolar migration to the nucleus. Cell shape varies greatly, perhaps due to packing of the developing stages.

#### b. *Convoluta* sp. 2 (Figure 5)

The first stage of spermiogenesis appears to be basically the same as in *Convoluta* sp. 1 with prominent Golgi activity and the appearance throughout the cytoplasm of dense bodies that cannot be distinguished from those of the black species. In many of the sections, two centrioles are observed in close proximity to the nucleus and free flagella (not yet incorporated into the spermatid cytoplasm) are seen between the cells. Some of these flagella appear to contain an indistinct central component.

### 3. Spermiogenesis II

#### a. *Convoluta* sp. 1 (Figures 6, 7)

At this stage the closely packed spermatids are irregularly shaped. As the nucleus condenses, it becomes flattened and elongate; a manchette forms which apparently consists of microrods that may be attached to the thickening nuclear membrane. In favorable sections, a centriole or membrane-bound axoneme with a 9+0 pattern of microtubules is seen either close to or within an indentation in the nucleus. This configuration is seen only in sections where the nucleus is not yet markedly condensed and the manchette is only beginning to form.

In the cytoplasm, Golgi activity is evident and more dense bodies are visible. An increase of rough ER, particularly at the cell periphery, is accompanied by the appearance of larger dark staining inclusions. The ER cisternae and Golgi saccules are filled with a diffuse material, similar to that seen in some of the new membrane-bound bodies. Other types of bodies possess either a dark electron-opaque

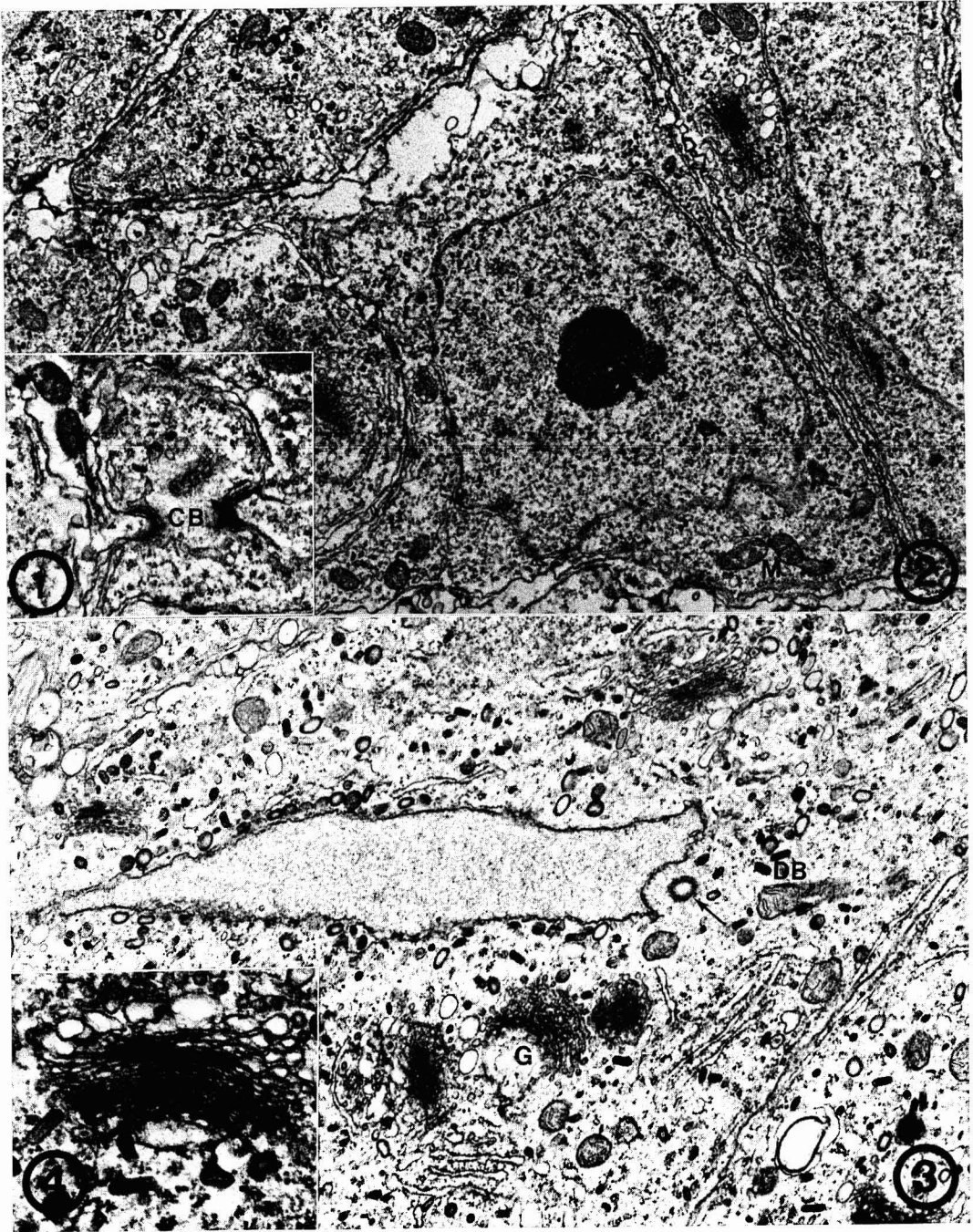


FIGURE 1. A cytoplasmic bridge (CB) linking developing spermatids,  $\times 18,300$ .

FIGURE 2. Early undifferentiated sperm cells of *Convoluta* sp. 1, with large nucleus and sparse cytoplasm including a few mitochondria (M) and Golgi (G),  $\times 14,000$ .

FIGURE 3. Spermiogenesis I in *Convoluta* sp. 1, showing some rough endoplasmic reticulum, Golgi (G), dense bodies (DB), and a centriole (arrow) near the nucleus,  $\times 16,800$ .

FIGURE 4. Spermiogenesis I in *Convoluta* sp. 1, showing typical Golgi,  $\times 32,700$ .

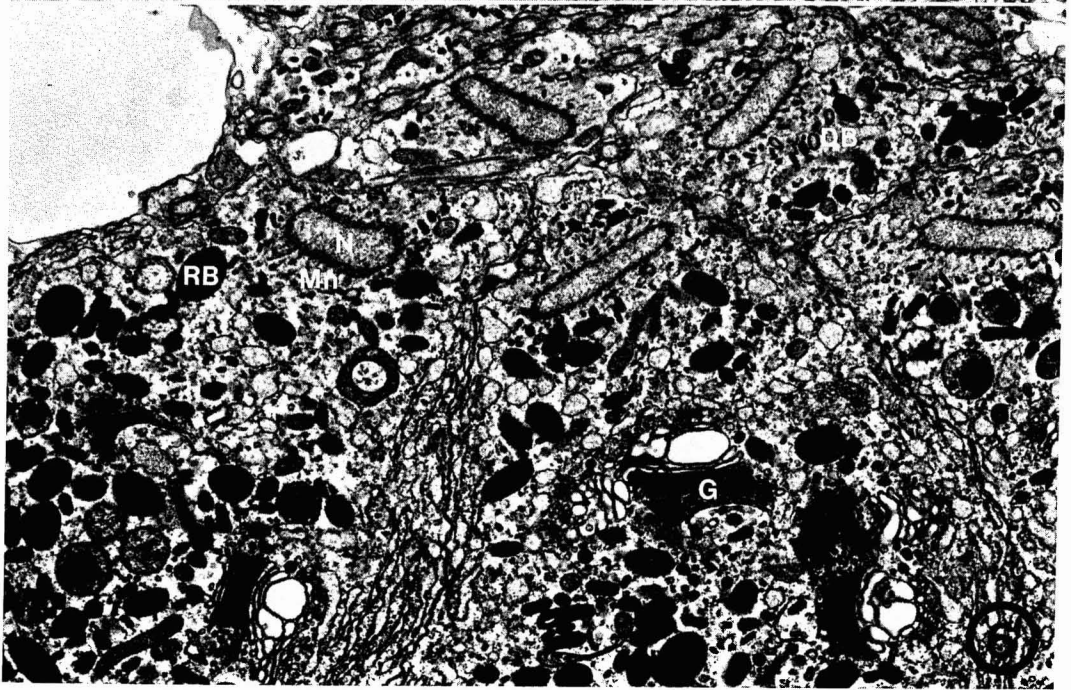
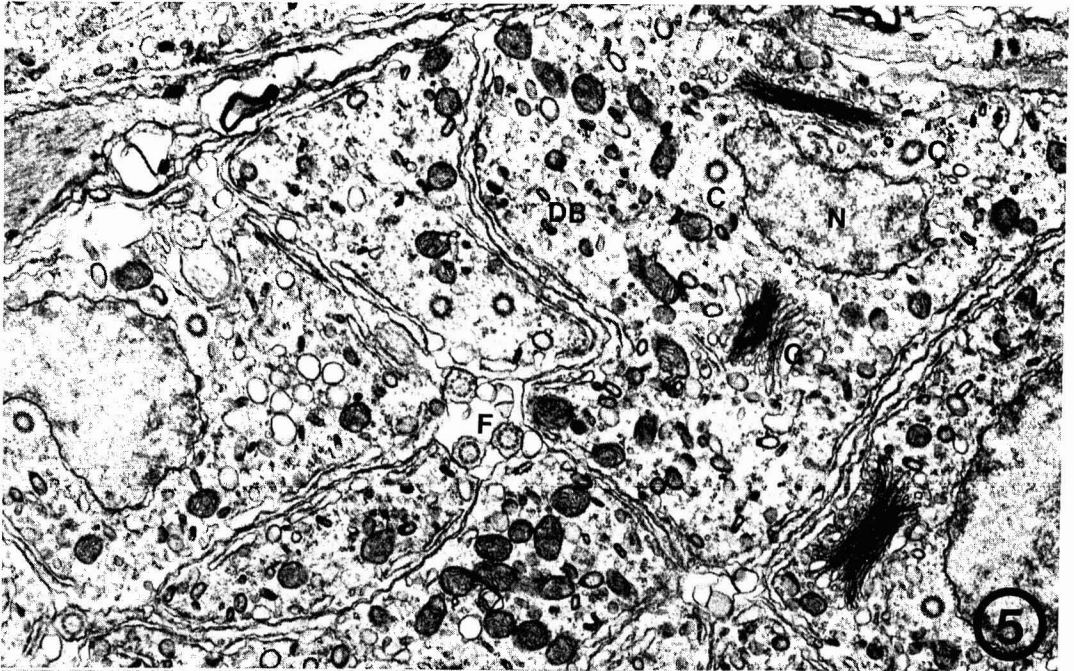


FIGURE 5. Spermio genesis I in *Convoluta* sp. 2, showing two centrioles (C) on opposite sides of the nucleus (N), Golgi (G), dense bodies (DB), and intercellular free flagella (F),  $\times 16,800$ .

FIGURE 6. Spermio genesis II in *Convoluta* sp. 1 with nucleus (N) surrounded by a manchette (Mn) of microtubules, and cytoplasm containing refractile bodies (RB), dense bodies (DB), and Golgi (G),  $\times 13,400$ .

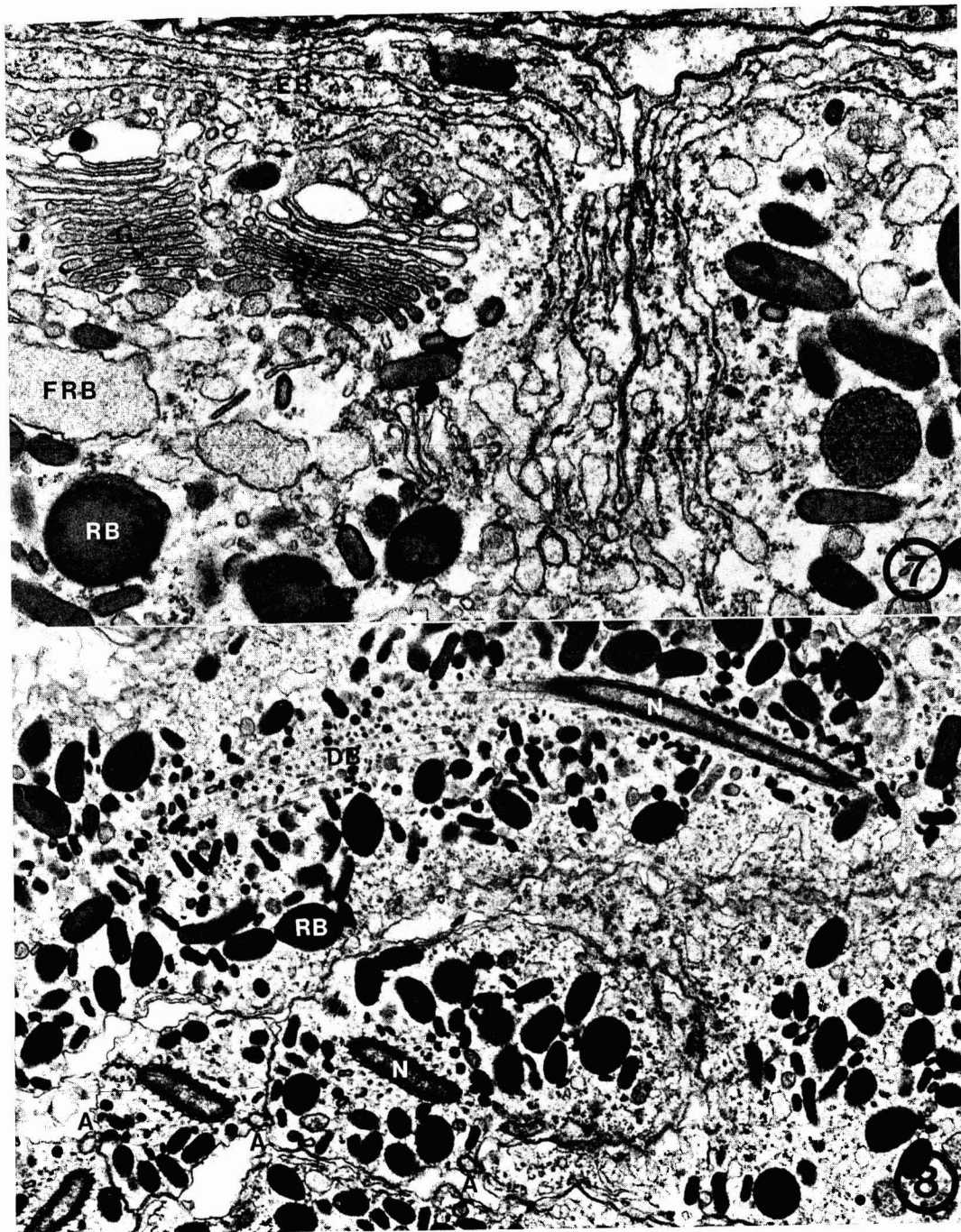


FIGURE 7. Spermiogenesis II in *Convoluta* sp. 1, showing association of endoplasmic reticulum (ER), Golgi (G), forming refractile bodies (FRB), and mature refractile bodies (RB),  $\times 28,000$ .

FIGURE 8. Spermiogenesis III in *Convoluta* sp. 1 in which the nucleus (N) has elongated and condensed. Manchette microrods with dense bodies (DB) between are seen in longitudinal section, the spermatid shaft is filled with refractile bodies (RB), and axonemes (A) are seen in the nuclear region,  $\times 12,000$ .

central core with a diffuse periphery or a very dark periphery with a more overall homogeneous density (Figure 7). These new inclusions probably represent stages in the development of refractile bodies observed by Henley (1968) in *Childia* and also seen forming from the Golgi in *Anaperus* (Silveira 1967). In late stage II cells, the cytoplasm is filled with several Golgi, mitochondria, dense bodies, and refractile bodies in various stages of formation (Figure 6).

*b. Convoluta* sp. 2

The manchette forms and refractile bodies appear in the cytoplasm.

#### 4. Spermiogenesis III

*a. Convoluta* sp. 1 (Figure 8)

Cell elongation is most evident during this stage, and is accompanied by further nuclear elongation and condensation. The manchette, consisting of longitudinally oriented micro-rods, is well developed and the nucleoplasm has condensed into a very dense ring with a less dense central region. Dense bodies are seen between the microrods in longitudinal sections.

Few inclusions are formed in the cytoplasm near the proximal end of the nucleus. Proceeding distally, the cytoplasm is packed with mature refractile and dense bodies. The spermatid shaft region contains refractile bodies in various stages of formation; they are very electron-dense and almost homogeneous in appearance except for their darker periphery and occasionally darker core. Golgi and ER are less prominent here than in stage II; 9+0 axonemes are seen near the cell membrane, running parallel to the long axis of the cell on opposite sides of the flattened nucleus.

*b. Convoluta* sp. 2

Nuclear elongation, an extensive manchette, and a dense core of condensed nucleoplasm just inside the wrinkled nuclear membrane also characterize stage III in *Convoluta* sp. 2. There is extensive Golgi activity at the nuclear level, while peripheral mature refractile bodies and medial dense bodies are distributed along the long axis of the nucleus. Paired lateral axonemes, which appear to be 9+0, are visible in many sections

at the nuclear level as well as below in the spermatid shaft.

#### 5. Mature Sperm

Figure 9 illustrates the mature sperm of *Convoluta* sp. 2, for which the conventional designations of head, middle piece, and tail are not applicable. It is best described as having a distal nuclear region and a proximal cytoplasmic region that tapers to a tip containing only axonemes. This cytoplasmic region represents the former spermatid shaft described in earlier stages. We now designate parts of this mature sperm as the *nuclear*, *shaft*, and *flagellar* regions.

*a. Convoluta* sp. 1 (Figures 10–13)

The nucleoplasm, while not visibly different from stage III, consists of a thick, dense band inside the nuclear membrane and a less dense granular core. The manchette has disappeared. No cytoplasmic inclusions are visible at the proximal end of the nuclear region. Adjacent to the distal part of the nucleus is a ring of mitochondria interspersed with dense bodies (for interpretation, see Figure 9). A few refractile bodies are seen in the nuclear region but are more prominent and larger in the proximal part of the shaft region. Axonemes observed in the nuclear region probably represent the distal ends of flagellar tips. See Figure 17 (Appendix) for an interpretation of how flagella are incorporated into the spermatid shaft.

The shaft is very complex. The proximal part is composed of membrane-bound axonemes and a centriolelike structure in the median position (Figure 11). Further distad along the shaft, this medial structure forms a ring of nine microrods, and in the most distal part or the shaft region, it is seen as two rows of four microrods which may be lined up as pairs or skewed (Figure 12, white arrow; the microrods are more clearly visible in the micrograph of *Convoluta* sp. 2, Figure 15).

The cytoplasm is filled with refractile bodies, dense bodies, membrane-bound open vesicles, and mitochondria, which do not seem to be arranged in any particular pattern. More distally, approaching the flagellar region, the shaft consists of the two lateral

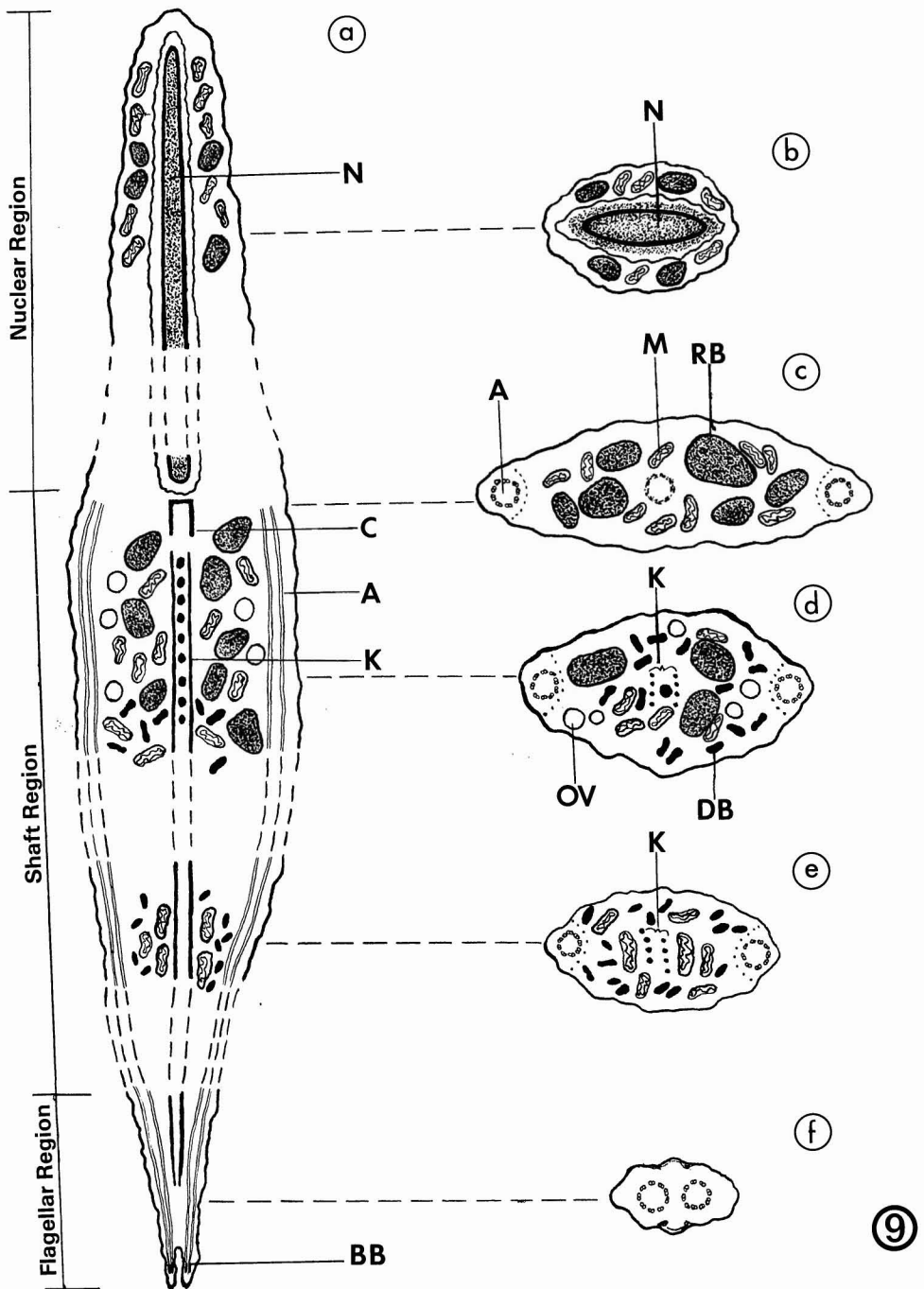


FIGURE 9. The mature sperm of *Convoluta* sp. 2. *a*, longitudinal section illustrating various regions, where dashed lines indicate great length of the sperm; *b*, nuclear region (N) with cytoplasmic refractile bodies and mitochondria; *c*, upper shaft region showing the keel (K) as a centriolelike structure (C), refractile bodies (RB), mitochondria (M), and paired 9+0 axonemes (A); *d*, middle shaft region showing the keel (K) of paired microtubules and central core, refractile bodies, dense bodies (DB), open vesicles (OV), mitochondria, and paired axonemes (A); *e* lower shaft region showing a skewed keel (K), dense bodies, mitochondria, and paired axonemes; *f*, flagellar region with only the paired axonemes, ending in basal bodies (BB).



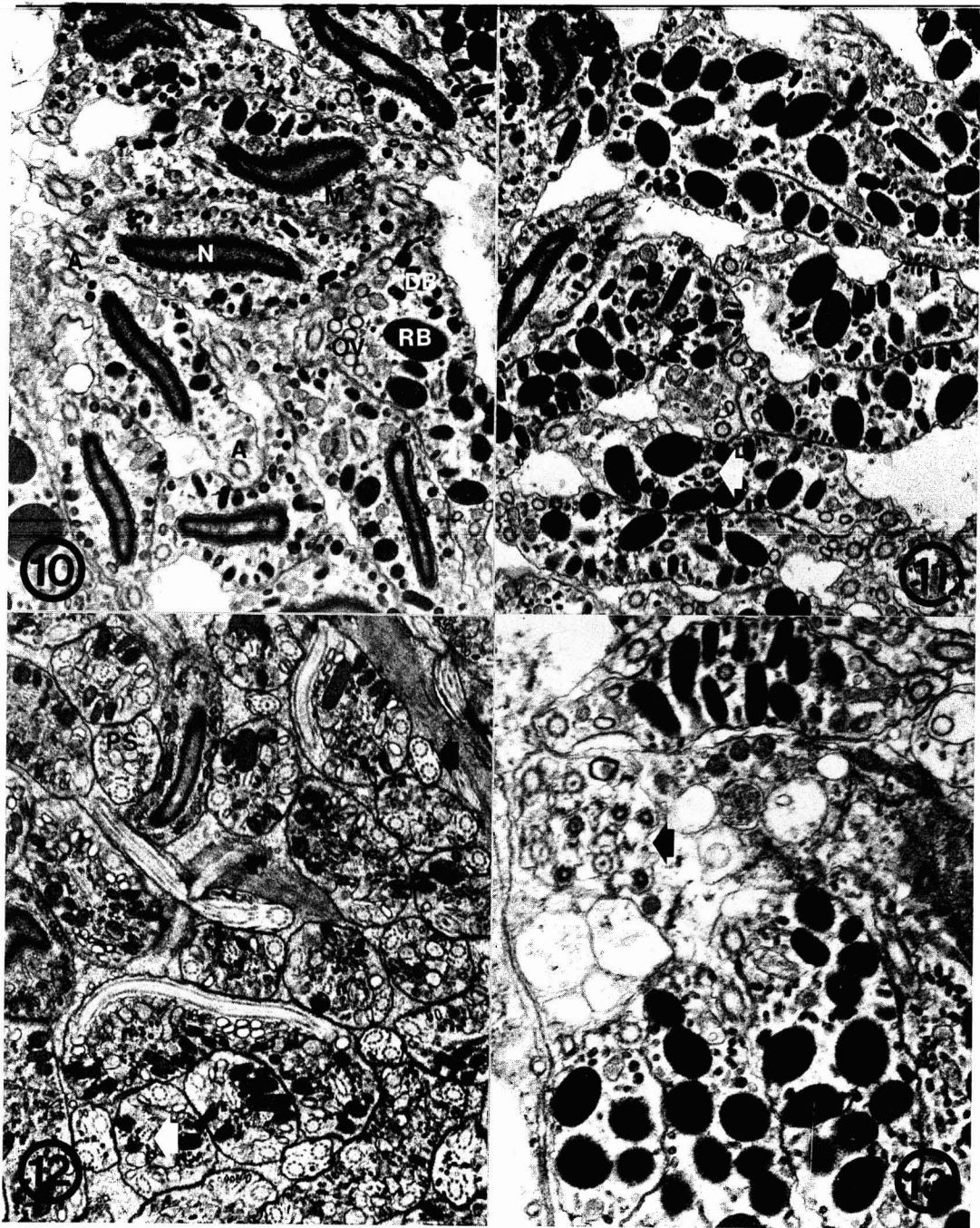


FIGURE 10. Mature sperm of *Convoluta* sp. 1, showing cross sections through the nuclear region with paired axonemes (A) at opposite sides of the nucleus (N) and a section through the upper shaft region with refractile bodies (RB), dense bodies (DB), and open vesicles (OV),  $\times 10,400$ .

FIGURE 11. Mature sperm of *Convoluta* sp. 1, showing cross sections through the upper shaft region, including the medial centriole-like structure (arrow),  $\times 9600$ .

FIGURE 12. Mature sperm of *Convoluta* sp. 1, with cross sections and oblique sections through several regions. The white arrow (lower left) indicates the paired microtubules of the keel; the black arrow (upper right) points to a section through the flagellar region; PS is a section through the posterior shaft after termination of the keel;  $\times 8700$ .

FIGURE 13. Mature sperm of *Convoluta* sp. 1. The arrow indicates sections through the flagellar region after the axonemes have separated, acquired a central component, and apparently terminate in basal bodies,  $\times 12,000$ .

axonemes and dense bodies with occasional mitochondria interspersed between. The medial axonemalike complex is not seen at this level (Figure 12, PS).

The flagellar region tapers to just the two 9+0 elements, void of any other cytoplasmic inclusions (Figure 12, black arrow). Figure 13 (arrow) strongly suggests that the two axonemes separate near the tip, acquire a central component, and terminally grade into a basal body.

*b. Convoluta* sp. 2 (Figures 14–16)

The mature sperm of *Convoluta* sp. 2 is very similar to that of *C.* sp. 1 but differs in a few details. Refractile bodies seem identical to those of *C.* sp. 1 but are more abundant in the distal nuclear region where they are interspersed with mitochondria. Almost no dense bodies are seen. The most proximal tip of this region contains only the nucleus. Axonemes are not visible in sections at the nuclear level, indicating that, unlike *C.* sp. 1, the flagella terminate at the base of the nucleus.

Refractile bodies and mitochondria are the only inclusions seen in the proximal end of the shaft region; further down, dense bodies appear along with a number of open vesicles surrounded by a thick membrane. Toward the distal end, refractile bodies and open vesicles disappear, and only mitochondria and dense bodies remain in the shaft.

*Convoluta* sp. 2 has a particularly well developed medial centriolelike component consisting of four pairs of very distinct 450 Å microrods, and an even larger central solid core is seen in some sections (Figure 14). The absence of the core in many cross sections plus evidence from longitudinal sections suggest that the core is an aggregate of solid spheres that do not continue through the entire length of the shaft region. However, the microrods of the central component appear to extend considerably farther down the shaft region than in *C.* sp. 1. In sections of the distal shaft where refractile bodies are not seen, the aligned microrods typically have a mitochondrion associated with each row (Figure 15) and often are skewed so that they no longer appear paired in register.

The microtubule configuration of the lateral axonemes in both acoels is described as

9+0, because it appears that this region has neither the two central microtubules typical of acoel somatic cell cilia nor the elaborate single central element observed in many flatworm sperm (as reviewed by Henley 1974). Faint central components are suggested in some sections (Figure 15, arrows), but since they are not seen in all micrographs, it is possible that they occur as a series of disconnected filaments rather than continuous rods or tubules.

Figure 16 shows mature spermatozoa of *Convoluta* sp. 2 in various planes of section loose in the parenchyma tissues, perhaps as they migrate prior to fertilization. Particularly notable is the wavy appearance of the nucleus, which is not evident when the sperm are closely packed. The diffuse and interdigitated nature of the acoel parenchyma is evident in this section.

## DISCUSSION

### *Spermiogenesis*

The most complete studies of turbellarian spermiogenesis are those of Hendelberg (1969, 1974), in which some of the earlier controversies regarding number, position, and direction of flagella of acoel sperm are resolved. Other studies of the acoels *Polychoerus* and *Childia* by Costello and Henley (Henley 1974) are in agreement with those of Hendelberg and with our observations. In all cases, two free flagella are formed by a round spermatid and later become laterally incorporated into the spermatid shaft as the cell elongates during spermiogenesis (see Figure 17, Appendix).

Though previous studies provide little information on nuclear changes during acoel spermiogenesis, a manchette of microtubules temporarily ensheathing the nucleus is reported by Costello, Henley, and Ault (1969) in *Childia*. Both *Convoluta* sp. 1 and *C.* sp. 2 have an unusual manchette during spermiogenesis II and III in which the longitudinal elements appear to be solid and may be attached to the nuclear membrane. They are not present in the mature sperm and perhaps are involved in nuclear elongation and/or

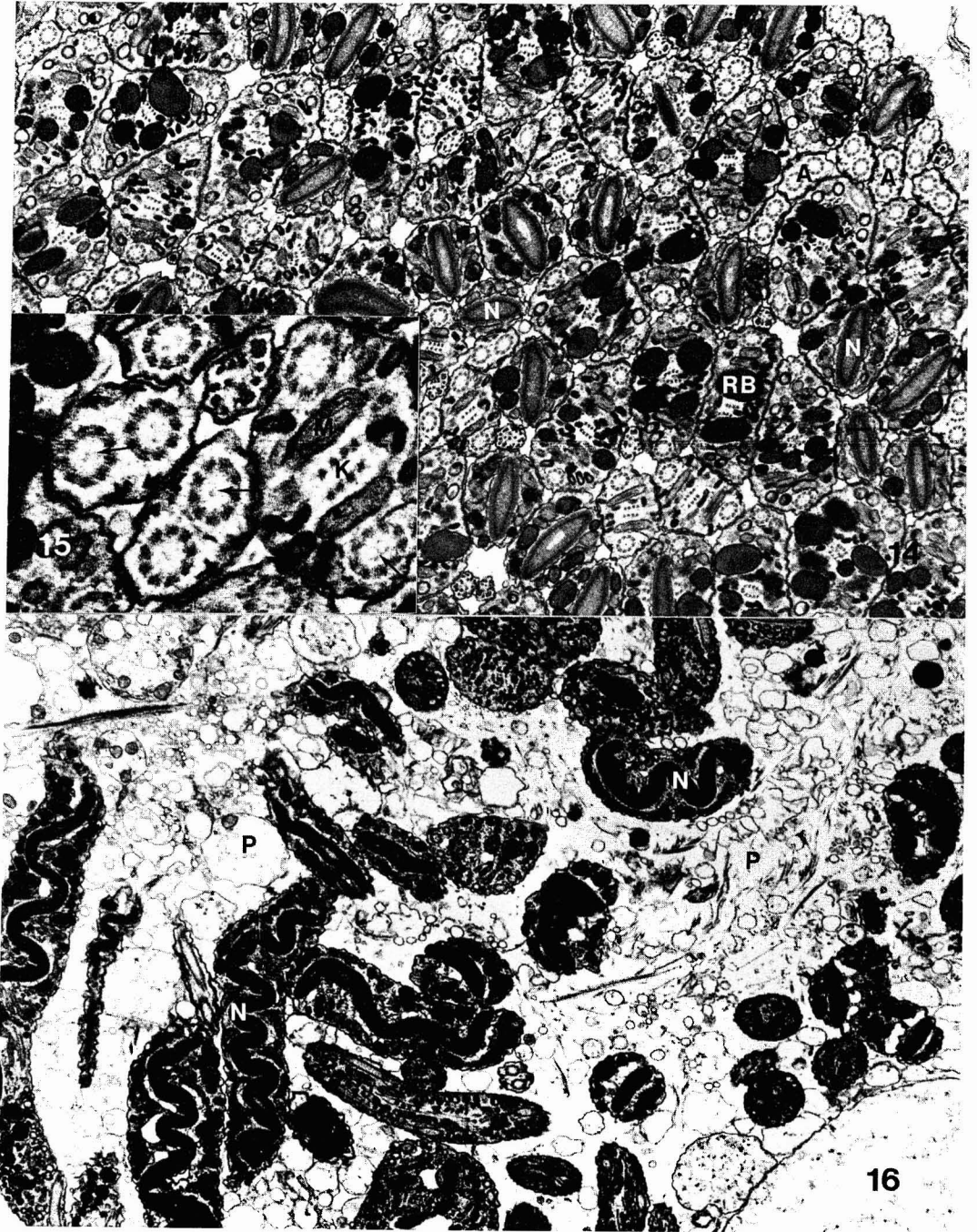


FIGURE 14. Mature sperm of *Convoluta* sp. 2, showing cross sections through all regions and including the nucleus (N), refractile bodies (RB), axonemes (A), and the central core of the keel (arrow, upper left),  $\times 12,000$ .

FIGURE 15. Mature sperm of *Convoluta* sp. 2, showing a cross section through the lower shaft where mitochondria (M) flank the paired microrods of the keel (K). Arrows point to faint central elements in the axonemes,  $\times 38,000$ .

FIGURE 16. Mature sperm of *Convoluta* sp. 2 loose in the parenchymal tissue (P). Note wavy appearance of nucleus (N) in longitudinal section,  $\times 7200$ .

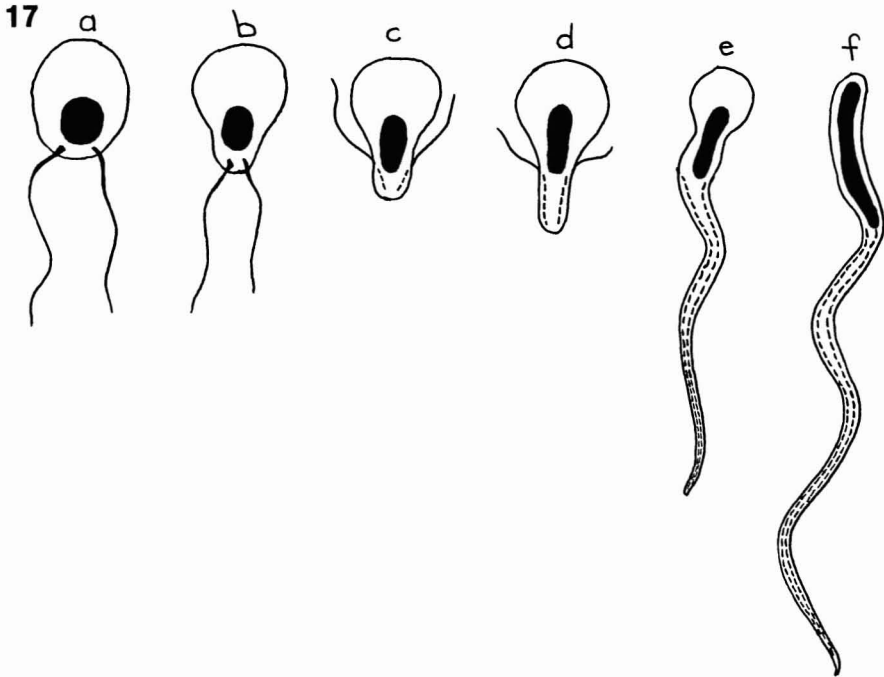


FIGURE 17. Stages of spermiogenesis showing elongation and flagella incorporation. For further explanation see the Appendix. Redrawn and modified from Hendelberg (1969).

shaping, as suggested by Phillips (1974a). Attachment to the nuclear membrane may be related to the extreme nuclear elongation characteristic of spermiogenesis of acoel spermatozoa. However, the extensive movement of cytoplasm into the spermatid shaft may also depend on manchette elements, another manchette function suggested by Fawcett, Anderson, and Phillips (1971).

Cytoplasmic inclusions of the shaft region seem to develop in a typical acoel manner. Refractile bodies (Figure 7) appear to be produced by Golgi in close association with endoplasmic reticulum; they are diffuse when released from the Golgi, but become more compact and electron-dense with maturity. A similar sequence of events seems to occur in the maturation of dense bodies (Figure 4). The development of inclusions in these two species appears to be very similar to that of *Anaperus* (Silveira 1967) and both *Childia* and *Polychoerus* (Henley 1968, 1974).

Our results confirm previous work on flagellar number and support other studies of

growth and incorporation. With the exception of *Nemertoderma*, which has a uniflagellate spermatozoon (Tyler and Rieger 1975), paired flagella seem to be the rule in turbellarians. In acoels, they grow as free flagella from the round spermatid and subsequently become incorporated into the growing spermatid shaft (see the Appendix). Thus, in the mature spermatozoon, the nucleus is located at the slender end, and naked axonemes reside laterally within the shaft and flagellar regions, with the flagella tips pointing toward the nucleus and the basal bodies located near the tip of the flagellar region. Costello also observed a medial tail "keel" structure of microrods in *Polychoerus*, which he postulated to be centriolar derivative (Henley 1974). Our studies show a similar morphology of axoneme and keel in both *Convoluta* sp. 1 and *C.* sp. 2, suggesting similar developmental processes.

Many questions concerning centriolar derivation and migration in acoels arise from our studies. Conventional spermatogenesis involves two orthogonally oriented centrioles

in mature spermatozoa. The proximal centriole is located perpendicular to the axoneme plate, and the distal centriole is in line with the axoneme plate. In mammals, the proximal centriole forms a microtubular structure called the *centriolar adjunct* at its one end (Fawcett and Phillips 1969). The distal centriole organizes tubulin for the axoneme (tail). It is not known how one pair of centrioles can organize the three derivatives (two flagella and one keel structure) found in these acoels. Perhaps the answer lies in the fact that both centrioles can function as organizers of flagellar protein. Assuming the keel to be tubulin or tubulinlike protein, the proximal centriole could organize the flagellar axonemes from both ends, replicate, and migrate to the distal end of the cell, while the distal centriole gives rise to the keel substructure. Localization of the centrioles in the most distal part of the cell offers tacit approval of this idea; it is probable that these centrioles are visible in Figure 13 near the arrow. However, we must emphasize that we cannot be sure of the sequence of events leading to flagellar initiation.

#### *Mature Sperm and Motility*

The mature sperm produced by the peculiar process of spermiogenesis involving flagellar incorporation have been described previously by Hendelberg (1969) for several species of acoels. Locomotion in these sperm is unusual in that the narrow flagellar region leads, so that the nuclear end is functionally posterior, although it corresponds morphologically to the head of a conventional spermatozoon. Wave propagation and undulation begin at the flagellar region where the basal bodies are located and proceed in the direction of the nucleus; thus, flagellar movement is normal in that they beat from the base to the tip, but the sperm moves "backwards," with the nuclear region being dragged along. This backward movement apparently is related to the position of the basal bodies in the tip of the flagellar region where the undulatory wave begins.

In many acoel species, copulation is by hypodermic impregnation in which the penis penetrates the epidermis of the partner and

sperm are deposited in the parenchyma, requiring sperm movement through the tissues to reach mature eggs. Even in these two species of *Convoluta*, which have a female genital pore and bursa for sperm storage, the spermatozoa still must wander from the bursa through the parenchyma to the eggs. Therefore, sperm structure and function in acoels must in part reflect the particular challenge of these migrations. In addition, Fawcett (1970) postulates that a long middle piece and associated increase in number of mitochondria may be an adaptation to meet the greater energy requirements of locomotion in the viscous medium of the female reproductive tract in animals with internal fertilization. The long shaft region of acoel sperm, with its many mitochondria, therefore may be related to the more formidable problem of migrating through interstitial tissue spaces.

To meet the greater energy requirements of locomotion in the viscous parenchymal tissue of the female, two axonemes probably are more effective than a single one, and if so, we have additional support for the suggestion of Hendelberg (1969) that the biflagellate condition is correlated with the evolution of internal fertilization. Dual flagella may have originated in the primitive Acoela, because the problems of sperm locomotion in this group are especially difficult, and then been retained in other turbellarians even after the acquisition of passages for sperm transport.

In addition to mitochondria, the shaft region is occupied by large numbers of refractile and dense bodies which, as Henley (1974:294) states, are probably not "merely going along for the ride." Since an acrosomal reaction has been found to be a prerequisite for fertilization in most species studied so far (Karp and Berrill 1981), the absence of a conventional acrosome in turbellarian sperm suggests that some other sperm component may function in a similar manner. Evidence from our study and that of Silveira (1967) indicates that the refractile bodies form in association with the ER and Golgi. These granules have been analyzed in *Childia* by Henley (1968) and found to contain acid mucopolysaccharides in the central region surrounded by neutral or basic polysaccharides; phosphatase tests

were equivocal, and no nucleic acids were found. Though we were not able to perform histochemical analysis of the large granules found in both *Convoluta* sp. 1 and *C.* sp. 2, their development from Golgi in stage II and distribution in the shaft region strongly suggest they are homologous to the granules described in other acoels. The histochemical properties of the refractile bodies, which indicate a primitive lysosomelike function, and the backward movement of the sperm (so that the shaft region where these granules are concentrated presumably contacts the egg before the nuclear region) suggest that the refractile bodies function in a manner similar to acrosomes by releasing lytic substances.

The number and arrangement of microtubules in acoel sperm vary. The typical 9+2 axoneme has been observed in the sperm of *Mecynostomum lutheri* and *Convoluta saliens* (Hendelberg 1969). A 9+1 pattern for an acoel sperm has been described in which an elaborate central structure was present (Afzelius 1966), and a 9+0 microtubular arrangement was observed in the sperm of *Childia*, *Polychoerus carmelensis*, *P. caudatus*, and *Anaperus gardineri* (Costello et al. 1969; Henley and Costello 1969; Henley, Costello, and Ault 1968).

The sperm of both *Convoluta* sp. 1 and *C.* sp. 2 also have 9+0 axonemes in which a distinct central component seems to be lacking, although the former is similar to *C. psammophila* (Bedini and Papi 1970) in that the axonemes separate near the tip of the flagellar region and acquire a single central component before emerging from a basal body at the extreme tip (Figure 13). All these spermatozoa are motile, refuting earlier predictions that 9+0 patterns occur only in nonmotile sensory cilia and flagella. Phillips (1974b), however, speculates on whether the 9+0 flagella of acoels might actually contain a moderately electron-dense center such as observed in the sperm of the scorpion, or whether the micrographs depicting no central element are of spermatids rather than mature sperm. The present studies support Phillips' concern. As can be clearly seen, favorable sections suggest the presence of an indistinct central element(s) in some of the axonemes in the shaft region of mature sperm (Figure 15).

The importance of central elements in axonemes can be understood in terms of the functions of the central pair, radial spokes, and nexin links in explaining the "sliding filament model" of cilia and flagella movement. The model allows for the movement of outer doublets relative to one another so as to produce localized bending due to restraining forces of central elements and the radial spokes. This model is dependent on the attachment of the outer doublets to some structure in the axoneme center. Phillips (1974b: 390) states that "convincing evidence for a motile flagellum with no central elements of any sort without accessory tubules has not been demonstrated." No accessory tubules outside the axonemes are present in the sperm of these acoel species, but the faint central elements within the axonemes in some sections may be sufficient to explain motility by the sliding model.

The unusual axonemelike medial component of paired microrods in the shaft of *Convoluta* sp. 1 and extending into the flagellar region of *C.* sp. 2 is probably homologous to the third atypical axial complex of *C. psammophila* reported by Bedini and Papi (1970) and the central keel of *Polychoerus* (Henley 1974). Costello has suggested that the keel may serve as a skeletal component in long sperm (Henley 1974). It seems plausible that such a structure may function not only in maintaining shape but also in coordinating movement of the axoneme pair by serving as a support for the entire shaft-axoneme region. Evidence for this suggestion is difficult to obtain in static micrographs, but the displacement of the microrods so that they are skewed rather than paired in register suggests some functional involvement in both support and movement (Figure 14). Also, it seems unlikely that the faint central components within each lateral axoneme could provide the necessary mechanical strength for these spermatozoa, as Silveira and Porter (1964) suggest may be the role of the thick, dense central unit in the triclad 9+1 sperm. The microrods of the keel, which measure approximately 450 Å (about two times the diameter of a microtubule), in both acoel species appear to be previously undescribed cellular components. The unique solid structure of these apparently supportive

elements is of particular interest and deserves further investigation.

In conclusion, we suggest that a very elongate sperm is particularly well adapted for movement through the narrow, viscous interstitial environment of the acoel parenchyma, that two axonemes are necessary to propel such a sperm, that the keel structure provides support and maintains shape, and that the refractile bodies may serve a lytic function in fertilization. The 9+0 axonemes are motile and, though they may have some central structure, are quite different from the conventional 9+2 axoneme. Such unusual sperm morphology may represent the prototype for the elongate biflagellate turbellarian sperm that evolved in association with movement through animal tissues.

#### APPENDIX

The conclusions of Hendelberg (1969) regarding spermiogenesis of thread-shaped sperm in acoels are summarized as follows: Young spherical spermatids, connected in groups by cytoplasmic bridges, develop two flagella that arise near the nucleus (Figure 17a). Soon, a small pointed protrusion, referred to as the *spermatid shaft*, forms between the bases of the two flagella (Figure 17b), which become shorter as the protrusion elongates (Figure 17c, d). When the spermatid shaft has reached a certain length, the two flagella can no longer be seen (Figure 17e). Hendelberg concludes that the two flagella become incorporated beneath the cell membrane on opposite sides of the elongating shaft and thus are no longer "free." The basal bodies of the flagella remain with the growing end of the shaft so that the tips, which were free at an earlier stage, come to lie near the nucleus at the end of spermiogenesis. As the flagella merge with the cytoplasm, the nucleus elongates and most of the spermatid cytoplasm moves into the shaft except for a residual lump which detaches from the young spermatozoon (Figure 17e, f). During locomotion, undulations of the sperm always begin at the narrow tip end and proceed in the direction of the broad nuclear end so that flagellar movement is initiated at the basal ends of the flagella and proceeds toward their tips.

#### ACKNOWLEDGMENTS

We would like to thank Louise Bush of the Gray Museum, Marine Biological Laboratory, Woods Hole, Massachusetts, for identifying the acoel specimens; David Phillips and John Boyer for helpful discussion; JoAnn Buchanan for cutting thin sections; Katie Martin for preparing Figure 9, and Pat Williams for the final typing of the manuscript. B. C. Boyer is particularly grateful to John Arnold for the opportunity to participate in the *Alpha Helix* expedition, for helping with specimen preparation, and for continued interest, support, and encouragement.

#### LITERATURE CITED

- AFZELIUS, B. 1966. *Anatomy of the cell*. University of Chicago Press, Chicago.
- ARNOLD, J. M., and L. D. WILLIAMS-ARNOLD. 1978. Spermiogenesis of *Nautilus pompilius*. I. General survey. *J. Exp. Zool.* 205: 13–26.
- AX, P. 1963. Relationships and phylogeny of the Turbellaria. Pages 191–224 in E. C. Dougherty, ed. *The lower Metazoa*. University of California Press, Berkeley and Los Angeles.
- BEDINI, C., and F. PAPI. 1970. Peculiar patterns of microtubular organization in spermatozoa of lower Turbellaria. Pages 363–369 in B. Bacetti, ed. *Comparative spermatology*. Academic Press, New York.
- COSTELLO, D. P., C. HENLEY, and C. R. AULT. 1969. Microtubules in spermatozoa of *Childia* (Turbellaria, Acoela) revealed by negative staining. *Science* 163: 678–679.
- FAWCETT, D. W. 1970. A comparative view of sperm ultrastructure. *Biol. Reprod. Suppl.* 2: 90–127.
- FAWCETT, D. W., and D. M. PHILLIPS. 1969. The fine structure and development of the neck region of the mammalian spermatozoon. *Anat. Rec.* 165: 153–184.
- FAWCETT, D. W., W. A. ANDERSON, and D. M. PHILLIPS. 1971. Morphogenetic factors influencing the shape of the sperm head. *Develop. Biol.* 26: 220–251.
- FRANZEN, A. 1970. Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. Page 3–46 in B. Bacetti,

- ed. Comparative spermatology. Academic Press, New York.
- HENDELBERG, J. 1969. On the development of different types of spermatozoa from spermatids with two flagella in the Turbellaria with remarks on the ultrastructure of the flagella. *Zool. Bidrag. Uppsala* 38: 1-52.
- . 1974. Spermiogenesis, sperm morphology and biology of fertilization in the Turbellaria. Pages 148-164 in N. W. Riser and M. P. Morse, eds. *Biology of the Turbellaria*. McGraw-Hill, New York.
- HENLEY, C. 1968. Refractile bodies in the developing and mature spermatozoa of *Childia groenlandica* (Turbellaria: Acoela) and their possible significance. *Biol. Bull.* 134:382-397.
- . 1974. Platyhelminthes (Turbellaria). Pages 267-343 in A. Giese and J. Pearse, eds. *Reproduction of marine invertebrates*. Vol. 1. Academic Press, New York.
- HENLEY, C., and D. P. COSTELLO. 1969. Microtubules in spermatozoa of some turbellarian flatworms. *Biol. Bull.* 137:403.
- HENLEY, C., D. P. COSTELLO, and C. R. AULT. 1968. Microtubules in the axial filament complexes of acoel turbellarian spermatozoa as revealed by negative staining. *Biol. Bull.* 135:422-423.
- KARP, G., and N. J. BERRILL. 1981. *Development*. McGraw-Hill, New York.
- PEDERSEN, K. J. 1964. The cellular organization of *Convoluta convoluta*, an acoel turbellarian: A cytological, histochemical, and fine structural study. *Zeit. Zellforsch.* 64: 655-687.
- PHILLIPS, D. M. 1974a. *Spermiogenesis*. Academic Press, New York.
- . 1974b. Structural variants in invertebrate sperm flagella and their relationship to motility. Pages 379-401 in M. Sleight, ed. *Cilia and flagella*. Academic Press, New York.
- SILVEIRA, M. 1967. Formation of structured secretory granules within the Golgi complex in an acoel turbellarian. *J. Microsc.* 6:95-100.
- SILVEIRA, M., and K. R. PORTER. 1964. The spermatozooids of flatworms and their microtubular systems. *Protoplasma* 59: 240-265.
- TYLER, S., and R. M. RIEGER. 1975. Uni-flagellate spermatozoa in *Nemertoderma* (Turbellaria) and their phylogenetic significance. *Science* 188:730-732.