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Spermatozoan Morphology and Zygote Formation in Nematodes[1]

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Other than the studies on *Ascaris megalocephala* (Favard, 1961), *Ascaris lumbricoides* (Clark *et al.*, 1967; Foor, 1968), *Nippostrongylus brasiliensis* (Jamuar, 1966), and *Aspiculuris tetraptera* (Lee and Anya, 1967), there is relatively little information available regarding the ultrastructure of nematode spermatozoa. However, these studies have served to reemphasize the reports of early cytologists (see Chitwood and Chitwood, 1950, for review) who acknowledged the tremendous morphological diversity in the sperm of these organisms. In addition to being markedly different in structure from all other sperm previously described, there is little apparent uniformity in the spermatozoa within this large group, the only consistent features being the lack of a flagellum and a well defined acrosome.

The purpose of this report is twofold. First, it is intended to present a résumé of the ultrastructural aspects of the nematode sperm examined thus far. In this respect it must be admitted that our knowledge is only fragmentary. Of the 16 orders of nematodes (Hyman, 1951), we have information concerning only seven (all of which are exclusively parasitic), and several of these are represented by a single species. So far no attempts have been made to examine the spermatozoan ultrastructure of the numerous free-living forms. Since nematode spermatozoa lack a well defined acrosome, the second purpose of this report is to summarize what is known regarding zygote formation in this group.

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SPERM MORPHOLOGY

From ultrastructural information now available it is evident that, on the basis of their morphological characters, there are at least four types of nematode spermatozoa. For convenience, the terminology employed in this report designates the order from which each type was first described.

Ascaroid Type

The morphology of the spermatozoa present in the uterus of the ascarids *Ascaris megalocephala* (Favard, 1961), *Ascaris lumbricoides* (Clark *et al.*, 1967; Foor, 1968), *Polydelphis* sp., and *Toxocara canis*, is basically similar. Although the spermatozoa are extremely polymorphic, it is evident that they generally consist of a clear anterior region and a posterior region containing numerous organelles. The anterior cytoplasm, possessing only fibrillar elements, usually is quite broad and frequently is projected as pseudopodial processes (Fig. 1). The cytoplasm of the posterior region also is capable of forming pseudopods, even though it seems to be somewhat more rigid and usually maintains a triangular or conoid appearance. The organelles confined to the posterior cytoplasm consist of a dense, nonmembrane-bound nucleus which is encompassed by an electron-dense layer possessing small spheres of varying density and mitochondria. Numerous membranous elements containing microvillus-like processes

are present adjacent to, and apparently continuous with, the peripheral plasma membrane. The most outstanding feature of the posterior cytoplasm in the spermatozoa of all ascarids examined thus far is the presence of a lipid-like mass of variable size, shape, and electron density which, because it has a glistening appearance when seen in living sperm, has been called the refringent body (Figs. 1 and 7).

Because of their initial small size and synchronous formation within the spermatocytes, the development of both the membrane specializations and the refringent body has been the subject of controversy (see Nath, 1956, for review). In a detailed ultrastructural study of *Ascaris megalocephala* testes, Favard (1961) indicated that the droplets forming the refringent body as well as the membrane specializations (proacrosomal granules) originated from heterogeneous granules produced by the combined activity of the granular endoplasmic reticulum and Golgi apparatus during early spermiogenesis. Examination of the testes of *Ascaris lumbricoides* in our laboratory also has revealed the presence of heterogeneous granules which have both fibrillar and lipid-like components, but Favard's interpretation regarding their formation could not be confirmed. Images of the young spermatocytes in the latter species do not reveal extensive elaborations of either the granular endoplasmic reticulum or the Golgi apparatus, and, in those cells where these organelles are observed, they usually are confined to that area of the cytoplasm destined to form the residual body.

In *Ascaris lumbricoides*, the initial appearance of the membrane specializations is closely correlated with morphological changes of the mitochondria. In fact, sequential images of the developing spermatocytes indicate that the fibrillar portion of each granule begins to form within the mitochondrial matrix (Figs. 2, 3). The fibrils then expand to such proportions that the membranes are either disrupted or reorganized and are difficult to recognize as being of mitochondrial origin during further development of the granule (Fig. 4). Although it is evident also that the lipid-like material becomes associated with the fibrils at a very early stage (Figs. 4 and 5), the exact origin of this substance is not yet known. In any event, it is apparent that this complex comprised of membranous, fibrillar, and lipid-like elements remains intact and continues to increase in size throughout spermiogenesis.

After the developing spermatocytes have migrated almost to the seminal vesicle, it is apparent that the materials comprising the heterogeneous granules segregate, giving rise to two different cellular components. The fibrillar material again becomes incorporated into the adjacent membranous elements, and the lipid-like substance (now less electron dense) remains behind to form nonmembranous droplets (Fig. 6). Subsequent to their separation from the granules, the membranous elements migrate to the peripheral cytoplasm. As seen in Fig. 8, certain of these membranous structures fuse with the plasma membrane and release their contents, which now appear as whorls of flocculent material, into the lumen of the seminal vesicle. Most of the membranous elements, however, remain quite dense and do not associate with the plasma membrane at this time. On the other hand, the lipid-like droplets, although they sometimes fuse to form larger inclusions, retain the same general morphologic character.

The spermatids usually have a rounded appearance (measuring 10–12 μ in diameter) with the nucleus and mitochondria located in the central cytoplasm when they have reached their final development in the seminal vesicle of the male. The membranous elements (now considered as plasma membrane specializations) and the lipid droplets are confined to the peripheral areas. Many cells possess a single cytoplasmic projection, or pseudopod (Fig 9).

After insemination, the spermatids assume the typical conoid appearance. The remaining membrane specializations fuse with the

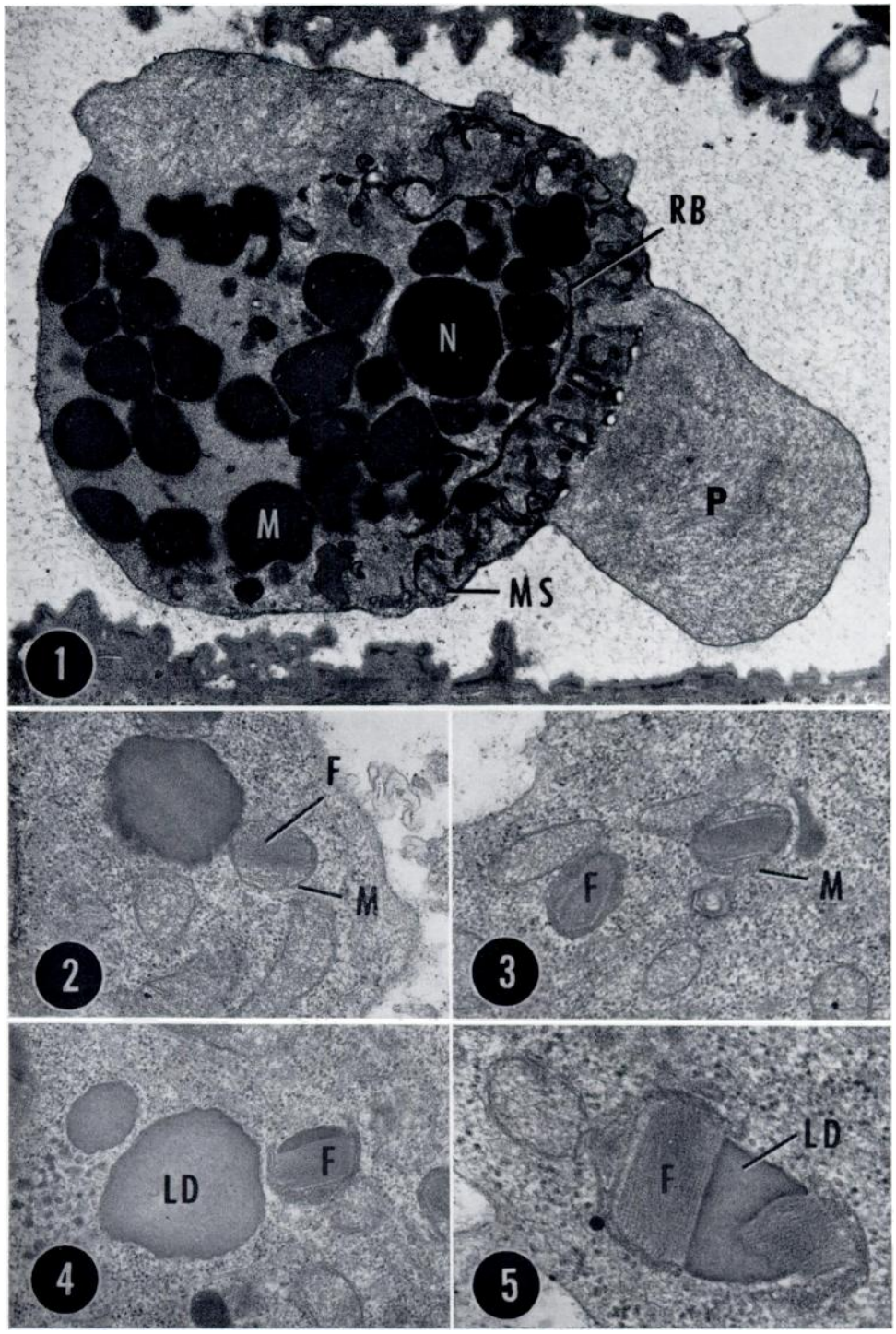


FIG. 1. *Ascaris* sperm in the female uterus, showing pseudopods (P), refringent body (R.B.), mitochondria (M), membrane specializations (M.S.), and dense nucleus (N). ×12,500.

FIG. 2–5. Series of micrographs illustrating the development of the heterogeneous granules in the spermatocytes of *Ascaris*. M, mitochondria; F, fibrillar elements; L.D., lipid-like material. Figs. 2, 3, and 4, ×30,500. Fig. 5, ×50,250.

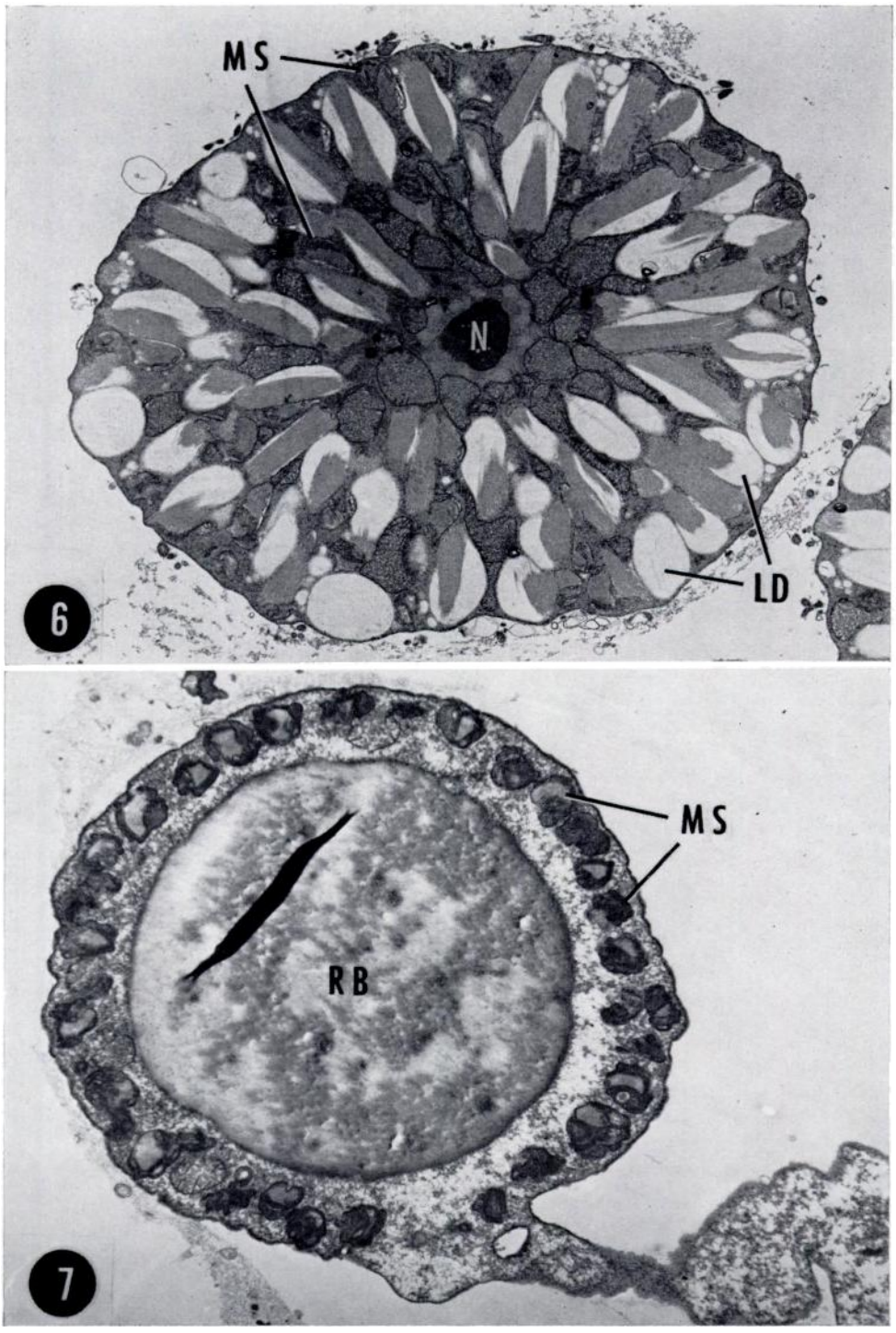


FIG. 6. Micrograph of *Ascaris* spermatocyte showing the separation of the membranes specializations (M.S.) and the lipid-like component (L.D.) which comprises the heterogeneous granules. N, nucleus. ×8,500.

FIG. 7. Sperm of *Polydelphis* sp. just after deposition in the female uterus. Note peripherally arranged membrane specializations (M.S.) and large refringent body (R.B.) formed from fusion of lipid-like droplets. ×7800.

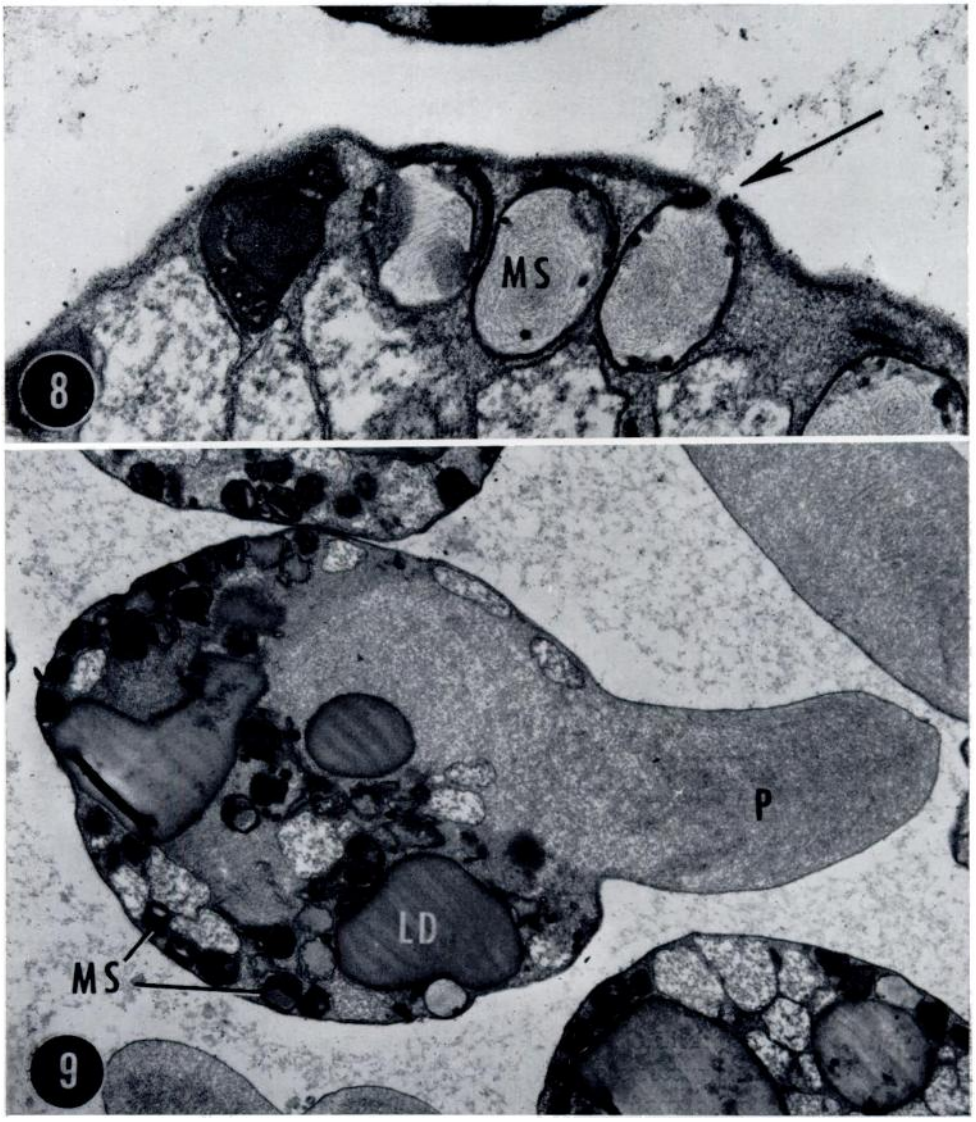


FIG. 8. Micrograph of *Ascaris* illustrating the morphological change in the material contained within the membrane specializations (M.S.) as well as its rarely observed release into the lumen of the seminal vesicle (arrow). ×25,000.

FIG. 9. Sperm (spermatid?) of *Ascaris* which has attained its final development in the male reproductive tract. This micrograph shows the individual lipid-like droplets (L.D.), and the membrane specializations (M.S.) which have not discharged their contents. Note the single pseudopod (P). ×10,500.

plasma membrane and release their contents, and the lipid-like droplets coalesce to form the large refringent body (Fig. 7). Although initially it is quite large, the refringent body undergoes a tremendous reduction in size and acquires an electron-dense appearance. Concomitant with the decrease in the size of the refringent body, there is a corresponding reduction in the size of the spermatozoa. Calculations based on approximate measurements indicate that certain *in utero* sperm seldom retain more than 50% of their initial volume.

The present observations support the conclusions of other investigators that the spermatozoa of ascarids do not acquire their final morphological appearance until they are deposited in the reproductive tract of the female. This latter phenomenon, first reported in *Ascaris megalocephala* (van Beneden and Julin, 1884) has since been confirmed (Cunningham, 1885; Favard, 1961) and noted in other nematode species (Nath and Singh, 1956; Nath *et al.*, 1961; Sommerville and Weinstein, 1964; Miller, 1966).

Other *in utero* nematode spermatozoa which possess an ultrastructural appearance comparable to that found in the ascarids appear in the spirurids, *Physaloptera* sp. and *Gnathostoma* sp., in the filarids *Dirofilaria immitis* and *Dipetalonema witei,* and, as shown by Beams and Sekhon (1967), in the rhabditid *Rhabditis*. The spermatozoa in these species, although somewhat different in size, are all ameboid and, in general, have a conical appearance. They contain peripheral membrane specializations which possess microvillus-like projections, numerous mitochondria, and a spherical nonmembrane-bound nucleus (Figs. 10 and 11). In contrast to the ascarid species examined, they all lack a single large refringent body. Lipid-like inclusions, possibly comparable to the refringent body or its precursors, are apparent in the sperm of the filarids *Dirofilaria immitis* and *Dipetalonema witei* as well as in the spirurid *Gnathostoma* sp. In the filarids, these structures consist of two or more dense bodies confined to the central cytoplasm (Figs. 11 and 33). They are evidently persistent features of the cytoplasm since they are still apparent subsequent to the spermatozoan's entry into the oocyte (Fig. 32). In contrast, the lipid-like droplets in *Gnathostoma* sperm (Fig. 10) are not consistent features of the cytoplasm, and it seems likely that the content of these inclusions gradually mixes with the cytoplasm prior to the sperm's entry into the oocyte. It should be mentioned also that while in the seminal vesicle of the male the membrane specializations of all ascaroid type sperm are usually not continuous with the plasma membrane and contain material having either an amorphous or a crystalline appearance (Fig. 9 and Fig. 11, insert).

Other than the ascarids, the development of the membrane specializations in species having ascaroid type sperm have yet to be examined in detail. However, images of spermatocytes in the testes of *Physaloptera* sp. reveal that, as in the ascarids, they make their initial appearance during early spermiogenesis and appear as membranous whorls closely associated with aggregations of a dense, fibrillar material (Fig. 12).

Strongyloid Type

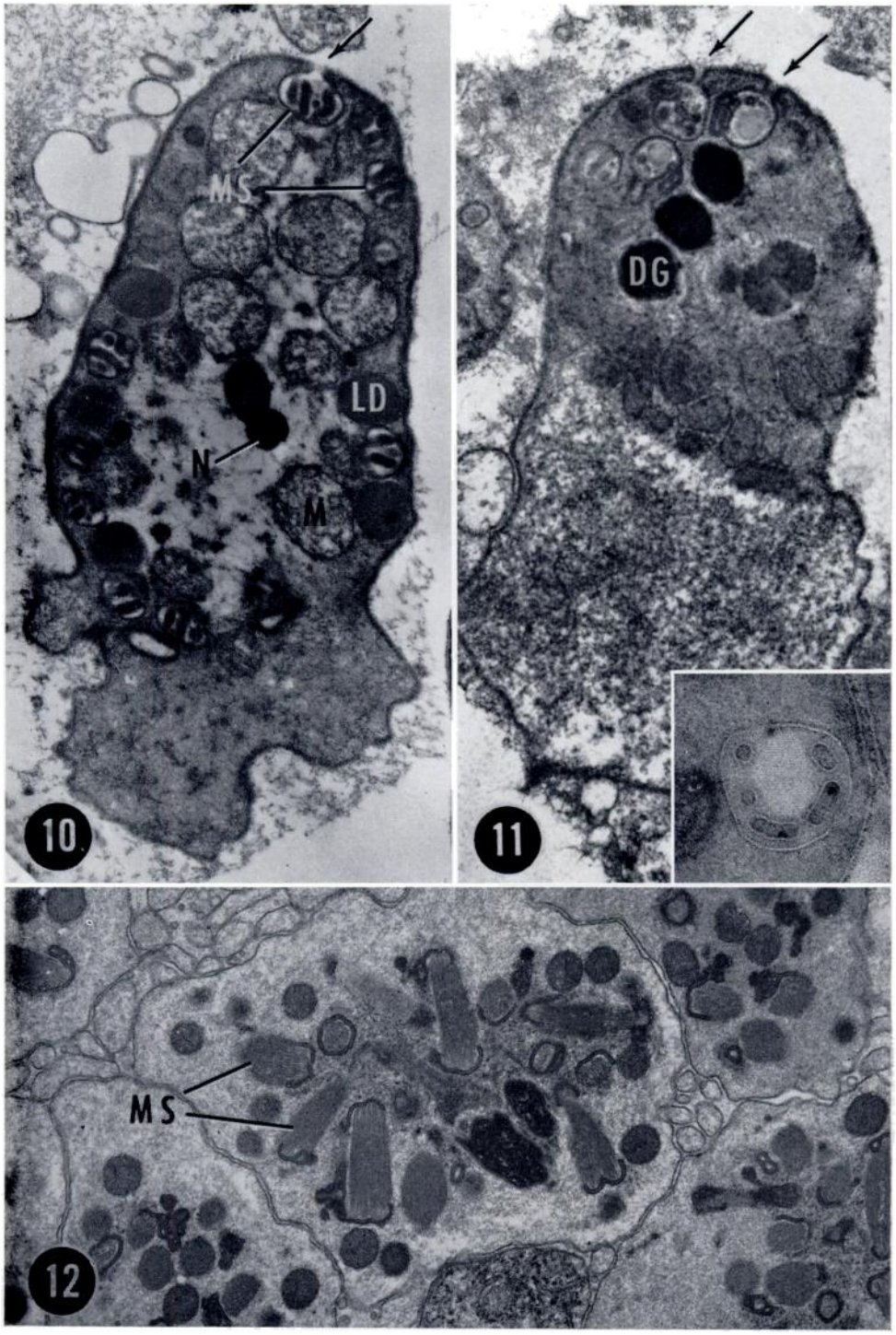
The strongyloids, represented by the trichostrongyle *Nippostrongylus brasiliensis* (Jamuar, 1966), and the strongyle *Ancylostoma caninum,* contain spermatozoa which differ from those of the ascaroid type in that they have distinct "head" and "tail" regions when present in the seminal vesicle of the male worm. For convenience, the spermatozoa of the closely related metastrongyle *Angiostrongylus cantonensis,* although not possessing this characteristic appearance, is also included in this group.

FIG. 10. Amoeboid *in utero* sperm of *Gnathostoma* sp. showing numerous lipid-like droplets (L.D.), mitochondria (M), and the small dense nucleus (N). The membrane specializations (M.S.) with porelike openings to the exterior (arrow) are confined to the conoid posterior cytoplasm. ×18,000.

FIG. 11. *In utero* sperm of *Dirofilaria immitis* showing membrane specializations open to the exterior (arrows) and dense granules (D.G.) within the posterior cytoplasm. ×23,500.

Insert: crystalloid material within the membrane specializations during sperm development in the male reproductive tract. ×61,000.

FIG. 12. Electron micrograph illustrating the developing membrane specializations (M.S.) in the spermatocytes of *Physaloptera* sp. ×12,500.



As seen in Fig. 13, the spermatozoa in the seminal vesicle of the male *Ancylostoma caninum* consists of a rather large anterior cytoplasmic region containing, in addition to filamentous elements, numerous dense membrane specializations and mitochondria. The elongate nucleus, which lacks a nuclear envelope and appears to be comprised of numerous dense filaments having a spiral-like arrangement, is confined to the smaller posterior or tail region. Two centrioles, each comprised of nine peripheral tubules and an electron lucid core, are arranged at right angles to one another and located immediately anterior to the nucleus (Fig. 13, C). A refringent body or other lipid-like inclusions are not observed. It is noteworthy that, other than the appearance and arrangement of the centrioles and the absence of filaments closely associated with the membrane specializations, the spermatozoa in the seminal vesicles of *Ancylostoma* are remarkably similar to those described by Jamuar (1966) in the male *Nippostrongylus*.

In contrast to their tadpole shape while in the seminal vesicle of the male, images such as are shown in Fig. 14 reveal that *Ancylostoma* spermatozoa are extremely polymorphic, or ameboid, with an obvious mixing of the nuclear and cytoplasmic regions, after they are deposited in the female. The membrane specializations, like those in spermatozoa of the ascaroid type, become confluent with the outer plasma membrane (Fig. 14, arrow) and lose their electron-dense matrix.

The metastrongyle spermatozoa observed in the seminal vesicles of *Angiostrongylus cantonensis* differ from those of *Ancylostoma* and *Nippostrongylus* in that they have a spherical appearance and contain a centrally located nucleus (Fig. 15). The nucleus, although rounded, is quite dense and contains filaments similar to those present in the nuclei of *Ancylostoma*. Elsewhere in the cytoplasm there are aggregations of filaments, mitochondria, and peripherally located membrane specializations. A refringent body is lacking. After they are deposited in the female worm the spermatozoa acquire an ameboid appearance somewhat similar to that observed in the ascarids. The mitochondria appear to be somewhat larger and less dense, and the membrane specializations become confluent with the plasma membrane (see Fig. 16, arrows).

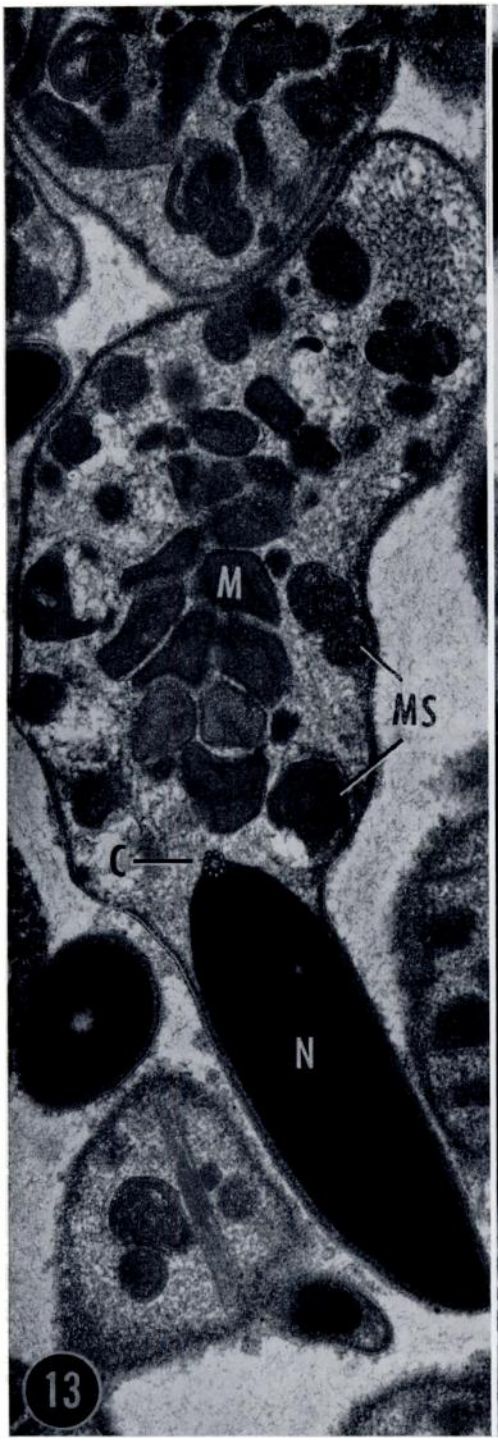
There appears to be good morphological evidence that the membrane specializations in the strongyloid type spermatozoa also originate from mitochondria as suggested by Jamuar (1966). As seen in Fig. 17, depicting a primary spermatocyte of *Angiostrongylus cantonensis,* the specializations are first apparent as groups of mitochondria in close association with aggregations of filamentous elements. One mitochondrion is flattened and curved, forming a semicircle about a bundle of filaments, and other mitochondria, usually having a rounded appearance, are located in the adjacent cytoplasm (Fig. 18). Subsequently, these groups of mitochondria appear to fuse (Fig. 19), forming membrane specializations with a characteristic rosette-like appearance (Fig. 15).

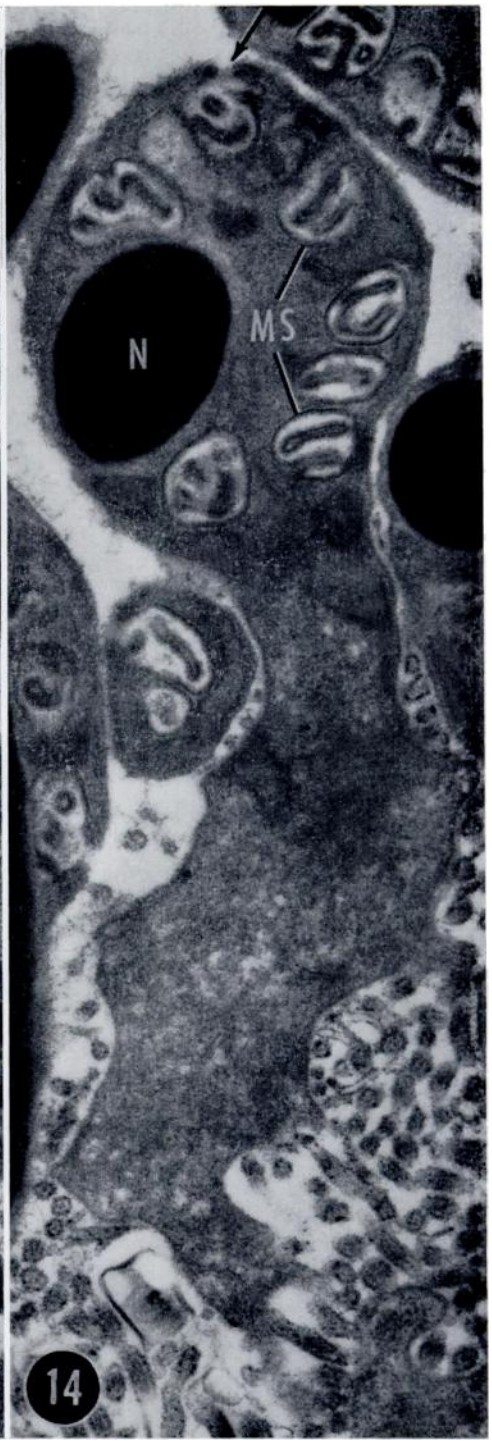
Dioctophymoid Type

In the seminal vesicle the spermatids of *Dioctophyma renale* appear either as short rodlike or rounded cells. The rodlike cells (Fig. 21) are immature and have not yet developed to their final form.

FIG. 13. *Ancylostoma caninum* sperm in seminal vesicle of the male worm. Note the dense membrane specializations (M.S.) and the posteriorly located elongate nucleus (N). M, mitochondria; C, centriole. ×28,200.

FIG. 14. Micrograph illustrating the ameboid character of *Ancylostoma caninum* sperm in the uterus of the female worm. The anterior and posterior cytoplasms are no longer segregated, and the membrane specializations (M.S.), now continuous with the plasma membrane (arrow), have lost their electron-dense contents. ×35,500.





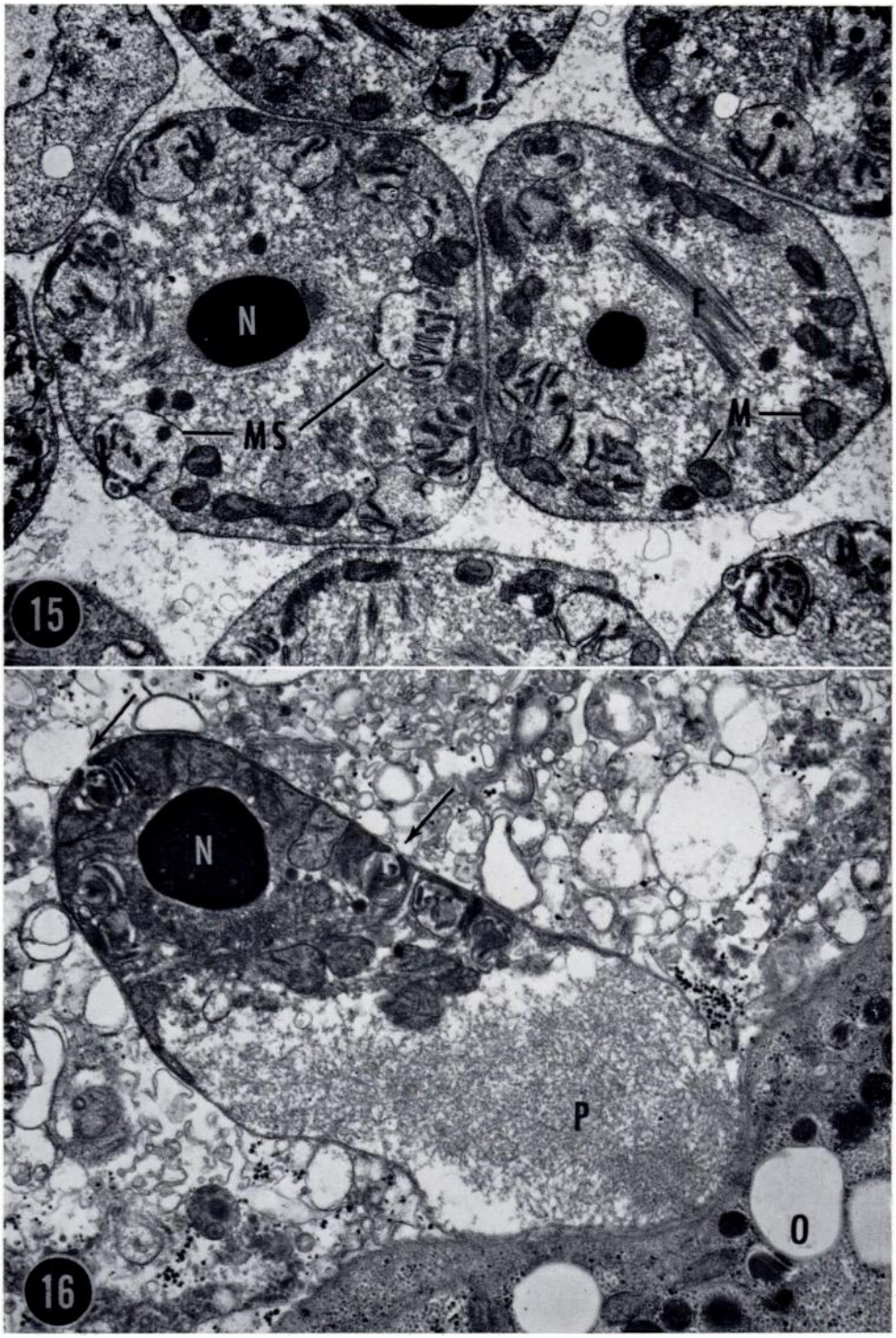
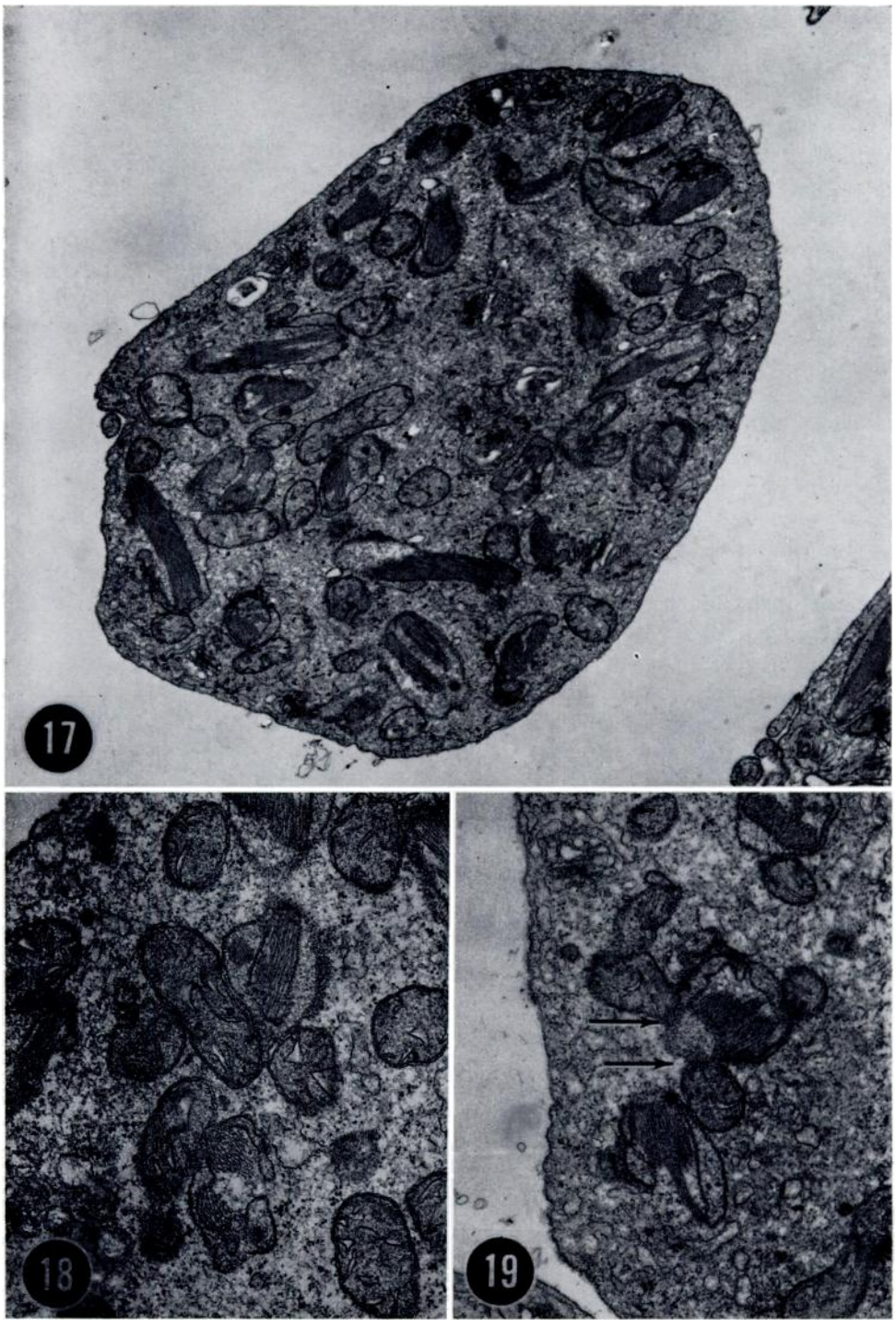


FIG. 15. *Angiostrongylus cantonensis* sperm in the seminal vesicles of the male worm, showing the centrally located nucleus (N) and peripherally arranged membrane specializations (M.S.). M, mitochondria; F, fibrillar elements. ×16,000.

FIG. 16. *Angiostrongylus cantonensis* sperm in contact with an oocyte (O) in the uterus of the female worm. Note large pseudopod (P) and continuity between the membrane specialization and the sperm plasma membrane (arrows). ×14,500.



FIGS. 17–19. Micrographs showing the development of the membrane specializations in spermatocytes of *Angiostrongylus cantonensis*. Figure 17 illustrates the numerous bundles of fibrillar elements within the mitochondria. Figures 18 and 19 are sequential images showing the aggregation and fusion (arrows) of mitochondria to form the rosette-like membrane specializations. Figure 17, ×15,200; Figure 18, ×25,000; Figure 19, ×22,500.

These spermatozoa lack membrane specializations comparable to those observed in the ascaroid and strongyloid types, but it is apparent that they also have a highly modified surface. As seen in Fig. 21, the spermatids are encompassed by two parallel membranes with the outermost, or plasma membrane, being separated from the inner one by a distance of 20 mμ. Between these two membranes is a thin layer of dense material often possessing a beaded appearance (Fig. 21, OM). The cytoplasm of these immature cells contains only centrioles, massive clumps of chromatin, and whorls or sheets of membranous elements which, in certain areas, appear to disrupt the well organized structure of the surface membranes (Fig. 21, arrows).

The rounded, more mature spermatozoa present in the seminal vesicles appear to be very similar to the *in utero* spermatozoan shown in Fig. 22. They differ from the immature forms in that the inner membrane which lies parallel to the plasma membrane is no longer present; the centrioles are no longer apparent; and the chromatin, now quite homogeneous, occupies the major portion of the cell. The membranous elements are more compact and are associated with tortuous channels which open to the exterior of the cell. It is interesting that, other than the formation of pseudopods, the spermatozoa of *Dioctophyma* undergo no obvious further development after insemination.

The lack of mitochondria within the cytoplasm of mature *Dioctophyma* spermatozoa is in striking contrast to all other nematode sperm thus far examined. Although there are numerous mitochondria in these cells during early spermiogenesis, it is apparent that these organelles, as well as all other discrete membranous components, remain in the cytoplasm of the residual body during the last maturation division (Fig. 20). Since they appear to be formed subsequent to the loss of mitochondria and other cytoplasmic membranes, the exact origin of the membranous elements in the mature spermatozoa of this type remains to be elucidated.

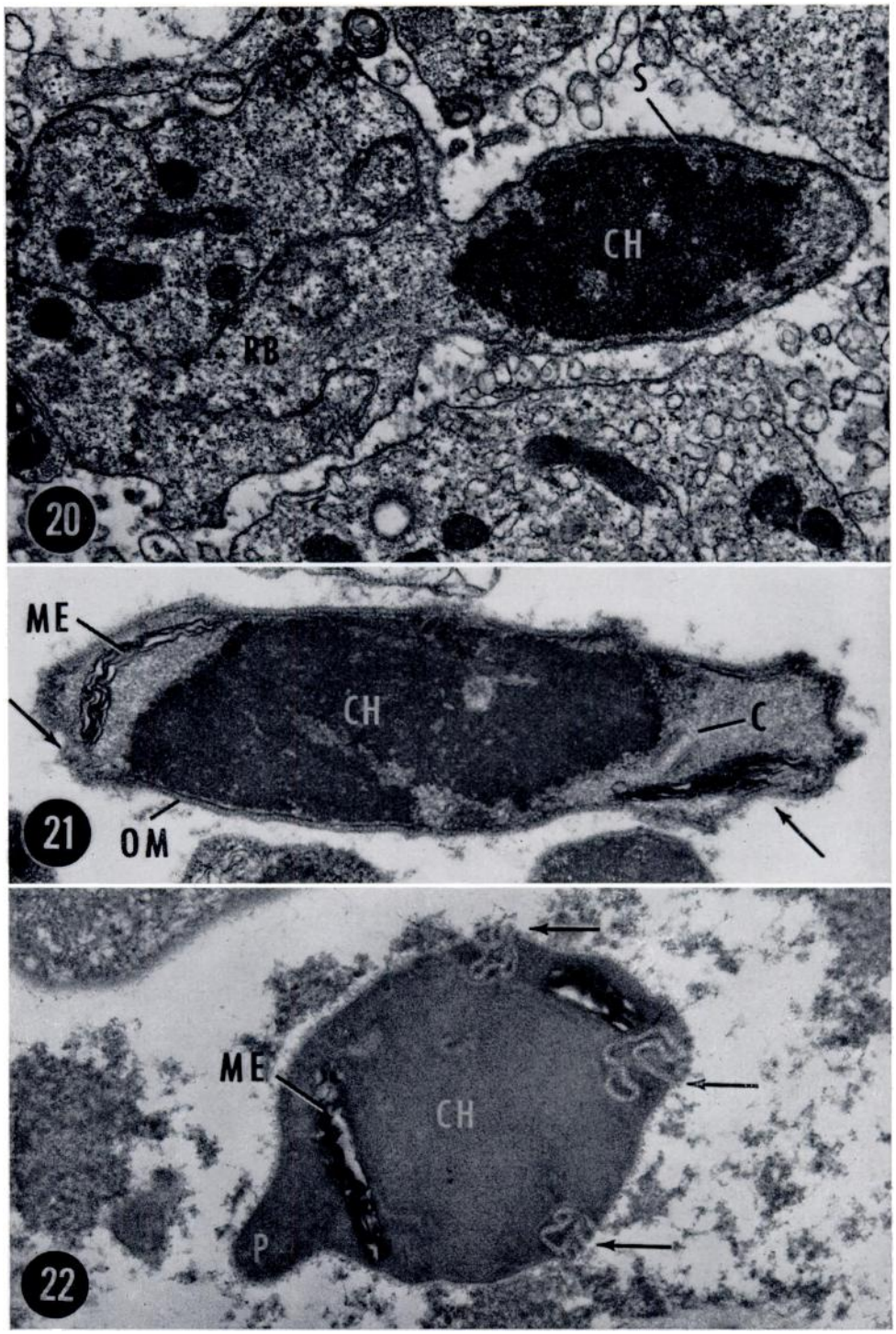
Oxyuroid Type

Lee and Anya (1967) have reported that the spermatozoa of the oxyurid *Aspiculuris tetraptera* are morphologically different from the spermatozoa of all other animals previously examined. Indeed, they do appear to be unique even when compared with those of the nematodes already described in this report. They possess a distinct head and tail and have a single, centrally located mitochondrion which extends throughout most of the elongate body. The nucleus is not encompassed by a nuclear envelope, and the DNA, present only in the tail region, is associated with an electron-dense sheath and bundles of microtubules. They lack an acrosome, a refringent body, and apparently do not contain structures comparable to the membrane specializations present in other nematode spermatozoa. Despite their unique appearance they are similar to other nematode spermatozoa in their ability to undergo ameboid movement.

ZYGOTE FORMATION

Despite the enormous numbers of eggs and spermatozoa present in the uterus of many nematodes, images of the uniting gametes, especially in an orientation favorable for interpretation, are difficult to obtain. However,

FIG. 20–22. Micrographs illustrating the development of *Dioctophyma renale* sperm. Figure 20, section through the male testes showing a spermatocyte (S) with massive chromatin (C.H.) still attached to the residual body (R.B.). ×23,000. Figure 21, immature sperm in the seminal vesicle of the male. Note the parallel surface membranes (OM) which are disrupted (arrows) in areas adjacent to the membranous elements (M.E.). C, centriole; C.H., chromatin; ×25,000. Figure 22, *Dioctophyma* sperm in the uterus of a female worm showing homogeneous chromatin (C.H.), membranous elements (M.E.), membrane specializations continuous with the plasma membrane (arrows), and a single pseudopod (P). ×40,000.



studies of the syngamy in *Ascaris* as well as preliminary observations of *Physaloptera* and *Angiostrongylus* indicate that in these species the fertilization process may be similar.

The primary oocytes of *Ascaris* are encompassed by a moderately electron-dense homogeneous layer approximately 50 to 100 mμ in thickness when they are first released into the upper portion of the uterus. The spermatozoa, frequently with the broad anterior region directed toward the oocyte, come to lie against the extraneous coat, and the pseudopods, often small and angular, are projected through the homogeneous layer. Subsequently, either by undulatory movement or cytoplasmic enlargement of the pseudopods, the extraneous layer is removed from that immediate area of the oocyte permitting direct contact between the sperm and oocyte membranes (Fig. 23).

Once direct contact has been established, certain of the gamete membranes appear to fuse almost immediately (Fig. 24). In other instances there is considerable interdigitation between the opposing gamete surfaces, and the sperm, presumably due to its continued ameboid movement, progresses to a position deep within the oocyte cytoplasm prior to membrane fusion (Fig. 25). The latter instances might indicate that there are no selected sites on the sperm membrane where fusion with the oolemma can occur. In these cases the fusion apparently takes place between the oolemma and the lateral, not the anterior, margins of the sperm. Subsequent to the fusion of the gamete membranes the underlying interdigitating membranes disappear and the entire contents of the spermatozoon enter the oocyte (Fig. 26).

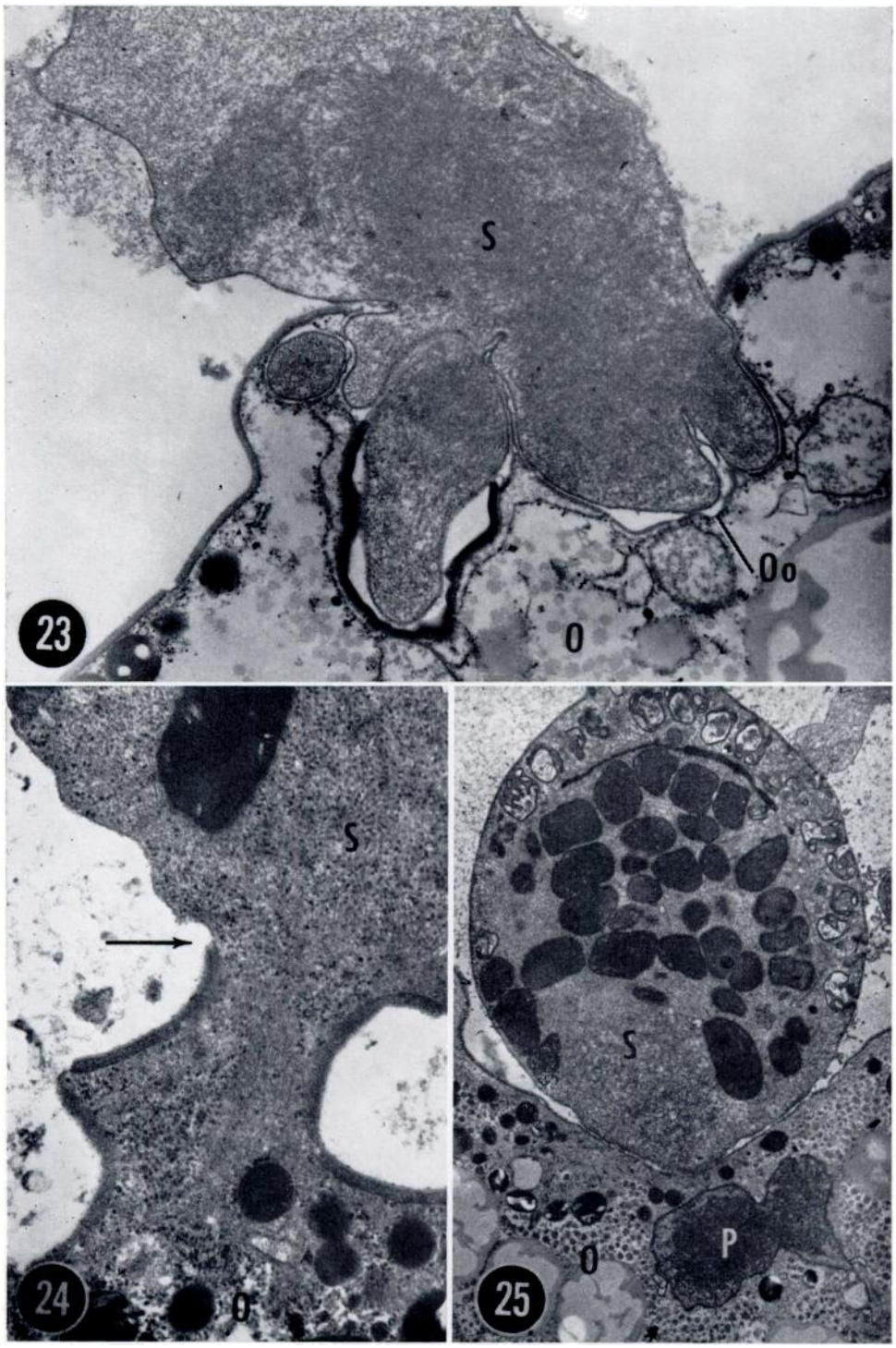
As reported in an earlier study (Foor, 1968), the dissolution of the membranes separating the gametes of *Ascaris* is marked by two concomitant changes within the spermatozoon, namely, the nucleus acquires a less dense particulate appearance, and there is a rapid proliferation of ribosomes throughout the cytoplasm. Although it appears that the nucleus is involved in their formation (Kaulenas and Fairbairn, 1968), the rate of synthesis of the ribosomes, as well as their arrangement within the cytoplasm, appears to be in direct correlation with the size of the refringent body contained within the newly penetrated sperm. In those spermatozoa having a small refringent body, the ribosomes are extremely abundant and scattered uniformly throughout the cytoplasm. However, in spermatozoa which possess a large refringent body, the ribosomes are initially less numerous and are arranged as linear strands adjacent to, or radiating from, the refringent body. (Compare Figs. 26 and 27.)

Attempts to correlate the observed syngamy in *Ascaris* with the events of zygote formation in other nematode species have met with only limited success. In many uteri the oocytes are completely surrounded by spermatozoa which give no indication that they are attempting to enter the oocytes. However, micrographs of the *in utero* gametes in *Physaloptera* and *Angiostrongylus* indicate that in certain instances pseudopods emanating from the spermatozoa also make first contact with the oocyte (Figs. 16 and 28). Although interdigitation of the gamete membranes in these species has yet to be observed, images of what is thought to be the initial fusion of *Physaloptera* gametes (Fig. 29) give some

FIG. 23. Micrograph showing interdigitation between the oocyte oolemma (Oo) and the plasma membrane of a penetrating sperm in *Ascaris*. S, sperm; O, oocyte. ×11,500.

FIG. 24. Illustrates the rapid fusion of the gamete cytoplasms in certain uniting *Ascaris* gametes. The extraneous coat is still present on the oolemma at the fusion site (arrow) of the oolemma and the sperm plasma membrane. S, sperm; O, oocyte. ×18,000.

FIG. 25. Micrograph showing sperm (S) with pseudopods (P) deep within the oocyte (O) cytoplasm prior to gamete fusion in *Ascaris*. ×7,500.



indication that zygote formation in this species might be accomplished in much the same manner as described for *Ascaris*. However, from observations of other *in utero* gametes such as *Dirofilaria immitis* (Fig. 33) one gains the impression that in certain instances syngamy might be facilitated by a phagocytic mechanism of the oocyte.

Images of the fertilized oocytes in all species having the ascaroid type spermatozoa have revealed the absence of a nucleus, as well as the presence of mitochondria and vesicles derived from the membrane specializations, within the newly penetrated sperm cytoplasm (Figs. 30–32). Similarly, with the exception of a somewhat slower rate of nuclear disintegration, this same general appearance of the spermatozoan cytoplasm has been observed in fertilized oocytes of the strongyloid nematodes (Fig. 31).

The most remarkable similarity between the newly penetrated spermatozoa of all other species and those of *Ascaris* is the obvious rapid proliferation of ribosomes within their cytoplasms. This observation is especially noteworthy in view of the fact that a well defined refringent body, which presumably contains the precursors for ribosomal synthesis in ascarids, has not been observed in other nematode spermatozoa. Thus, if the materials contained within the refringent body in ascarids are indeed utilized for ribosomal synthesis, then it seems reasonable to assume that comparable materials must also be present in the cytoplasm of other nematode spermatozoa in some other form.

DISCUSSION

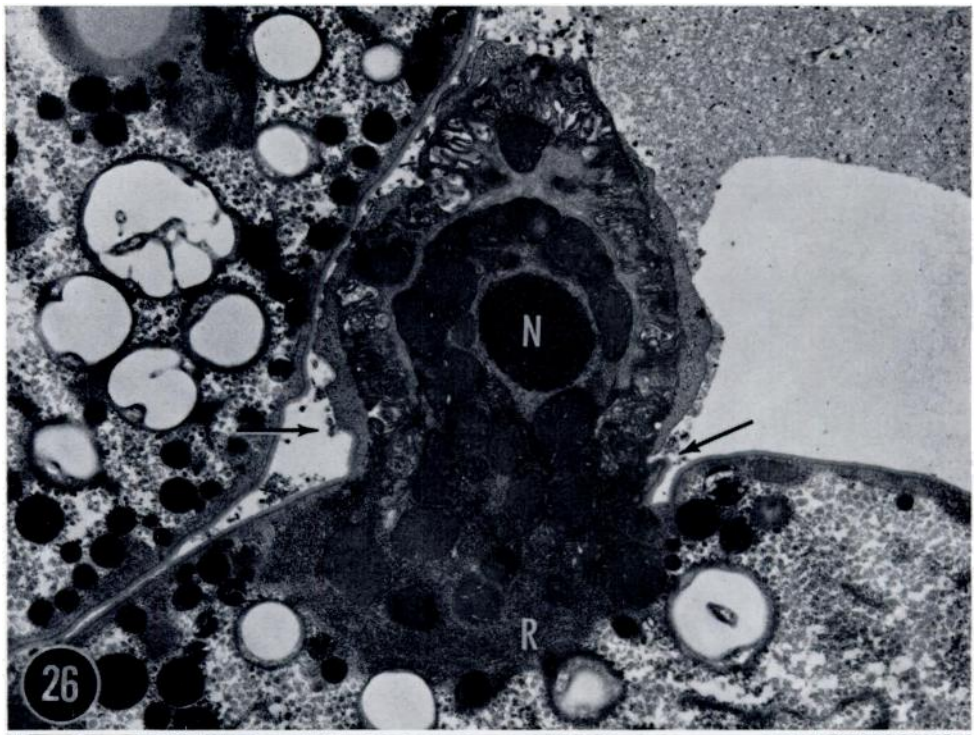
Few gametes have been more intensively studied than have those of the intestinal nematodes *Parascaris equorum (Ascaris megalocephala)* of the horse and *Ascaris lumbricoides* of swine. This has been due largely to the biological impact of the observations of van Beneden and Boveri, who were first to elucidate both the meiotic process and the genetic continuity of chromosomes, respectively, while employing developing *Parascaris equorum* eggs as a research model (see Wilson, 1928, for review).

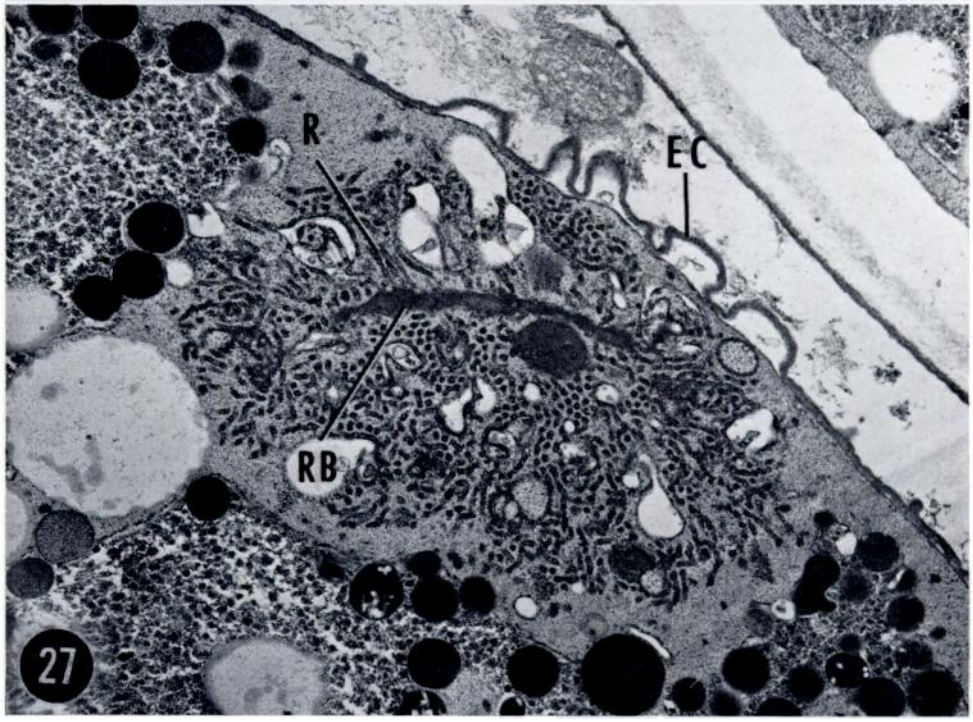
Despite this intensive study of ascarids and, later, of other nematodes, the cytological details of the gametes themselves have remained relatively obscure. Studies of the female gametes have been complicated by the fact that the final maturation and fertilization of the oocytes occur simultaneously; in many cases the sperm enters the oocyte prior to the first meiotic division. The spermatozoa, being regarded as a simple modification of the flagellated type present in other animals, have been equally confusing in thir morphological diversity. They have been described as either ameboid, flagellated, or hollow cells which often exhibit distinct dimorphism in size (Chitwood and Chitwood, 1950).

Studies of the gametes have been further complicated by the fact that the tubular gonads of nematodes are of two types, namely, telogonic and hologonic. In the telogonic gonads the germ cells reportedly originate from a germinal zone located at the blind terminal end, whereas in hologonic gonads, limited to the orders Trichuroidea and Dioctophymoidea, the germinal areas extend throughout the entire length of the reproductive tract. This diversity in the origin of the germ cells has been further expanded by reports (Cobb, 1925, 1928) that in the free living nematode *Spirina parasitifera* each

FIG. 26. Sperm entering the primary oocyte of *Ascaris*. Fusion of the gamete membrane is seen at arrows. Note the dissolution of the sperm nucleus (N) and the rapid proliferation of ribosomes (R) in the newly penetrated sperm cytoplasm. ×9,500.

FIG. 27. Newly penetrated *Ascaris* sperm with linear strands of ribosomes (R) radiating from the lipid-like refringent body (R.B.). Note the distorted appearance of the extraneous coat (E.C.) adjacent to the site of sperm penetration. ×12,500.





spermatid, by both amitotic and mitotic divisions, gives rise to 128 spermatozoa. Although Chitwood and Chitwood (1950) considered Cobb's observations to be in error, preliminary ultrastructural studies indicate that packets of developing spermatozoa occur also in *Trichinella spiralis* (personal observations). Thus it appears that, at least in certain nematodes, the formation of the spermatozoa might be similar to that reported in certain arachnids (Warren, 1930) and insects (Nur, 1962).

The lack of an acrosome within the nematode sperm has been particularly confusing to investigators, and attempts to establish a functional relationship between either the peripheral membrane specializations (Favard, 1961; Clark *et al.*, 1967) or the refringent body (Bowen, 1925) of *Ascaris* sperm and the acrosome of flagellated sperm have met with little success. Although membrane specializations appear to be a consistent feature of nematode sperm (with the possible exception of *Aspiculuris*), the refringent body is not. In any event, ultrastructural studies show that in those spermatozoa in which they occur, both organelles are confined to the posterior cytoplasm. Furthermore, as pointed out in an earlier study (Foor, 1968), they undergo no obvious morphological change immediately prior to contact with the oocyte and during syngamy, they enter the oocyte intact and remain apparent for some time in the zygote cytoplasm.

Repeated observations of the disparity in the initial appearance of ribosomes within the newly penetrated sperm cytoplasm of *Ascaris* have given morphological credence to previous reports that the refringent body consists of a highly specialized RNA-containing protein (ascaridine) (Faure-Fremiet, 1913; Filhol, 1937; Panijel and Pasteels, 1951) and possibly a lipid moiety (Faure-Fremiet, 1913; Nath, *et al.*, 1961). Presumably in those spermatozoa which possess a large refringent body the rate of ribosomal synthesis is dependent upon the rate at which the precursor material is released (or unmasked) from the lipid moiety. However, in those sperm having a small refringent body much of the precursor material is probably unmasked prior to penetration, thus enabling ribosomal synthesis to progress more rapidly. In any event, it now seems evident that the massive ribosomes present in the cytoplasm of newly penetrated sperm are derived exclusively from the precursor materials and the genome contained within the sperm (Kaulenas and Fairbairn, 1968).

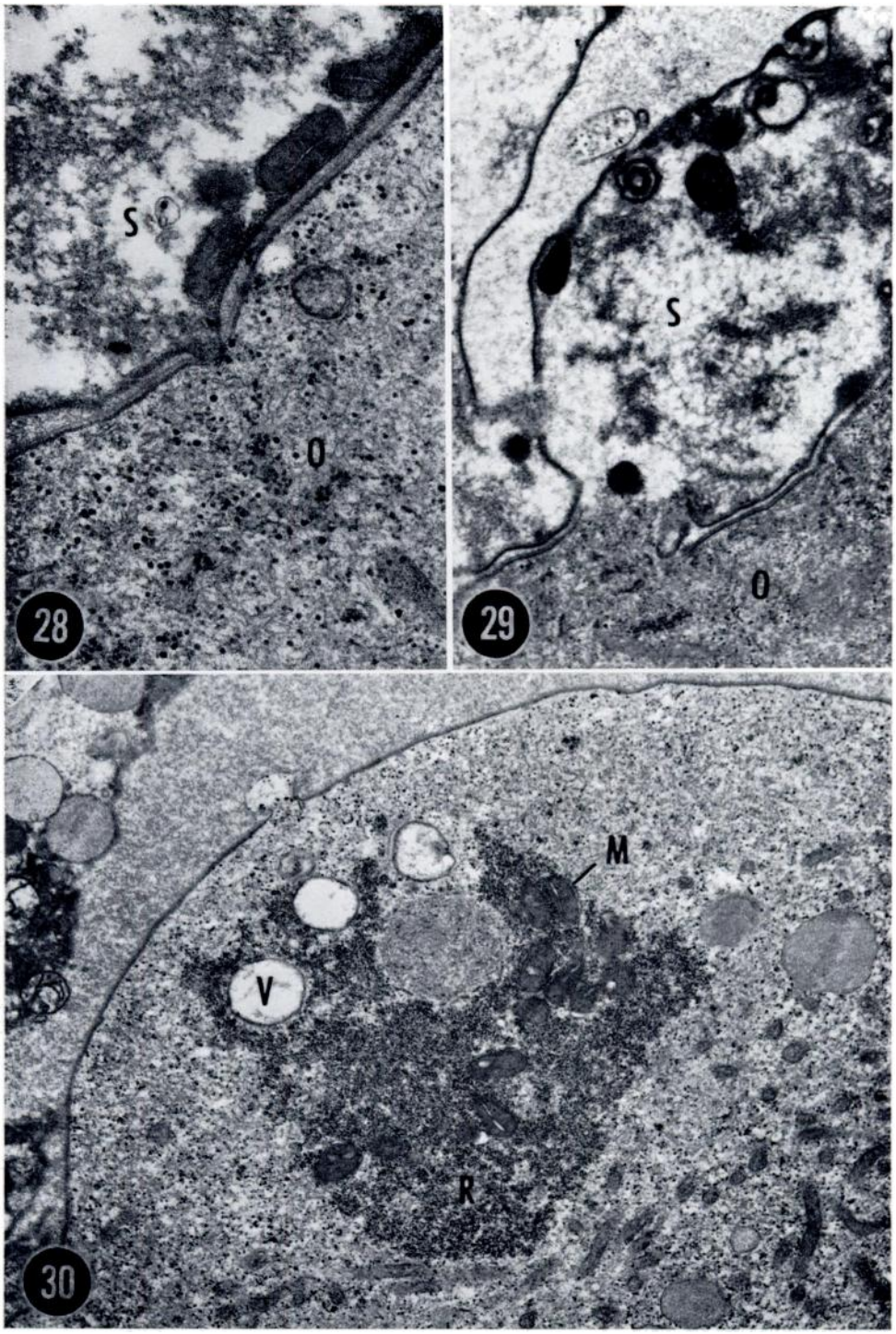
Although evidence indicates that the proteinaceous (ascaridine) material of the refringent body usually becomes distributed throughout the sperm cytoplasm prior to its fusion with the oocyte, it is difficult to formulate a conjecture regarding the gradual disappearance of the lipid-like portion. There is as yet no evidence that the latter component influences either the longevity or viability of the *in utero* sperm, and it seems especially presumptuous to suggest that it is utilized as an energy source since other tissues of adult ascarids are normally incapable of catabolizing lipids (Fairbairn, 1957).

Before terminating the discussion of the refringent body, it should be mentioned that examinations of electrophoretically homogeneous prepartions of its protein content have revealed that it is comprised of acidic

FIG. 28. Initial contact between the sperm (S) and oocyte (O) of *Physaloptera*. Note the thin extraneous coat overlying the oolemma. ×40,000.

FIG. 29. Micrograph illustrating what is thought to be the initial fusion of *Physaloptera* gametes. S, sperm; O, oocyte. ×19,000.

FIG. 30. Complete fusion of the sperm and oocyte cytoplasms in *Physaloptera*. The sperm cytoplasm contains only ribosomes(R), mitochondria (M), and vesicles (V) derived from the membrane specializations. ×16,500.



proteins containing large amounts of aspartic acid and tryptophane with lesser amounts of other aliphatic and dibasic acids (Panijel, 1950). In more recent investigations cytoplasmic bodies somewhat reminiscent of the refringent body have been described in the spermatids of the rat (Vaughn, 1966) and the water snake (Sud, 1961), as well as in the spermatozoa of certain crustaceans (Chevaillier, 1967), insects (Moriber, 1956), and marine echuroid worms (Das *et al.*, 1967). Chemical analysis of these latter structures, however, have revealed that they contain basic proteins (histones) rich in lysine and arginine, and are believed to represent either constituents of the acrosome (Moriber, 1956; Das *et al.*, 1967) or somatic nuclear histones sloughed at the time of histone transition during spermiogenesis (Chevallier, 1966; Vaughn, 1966; Vaughn *et al.*, 1969). Thus the importance of these contrasting observations lies in the fact that even at the molecular level the formation of the refringent body in *Ascaris* appears to be different from either the acrosome or similar morphological structures thus far noted in other sperm.

The involvement of the mitochondria in the formation of the membrane specializations, as well as at least a portion of the material comprising the refringent body in *Ascaris*, is supported by observations of the developing spermatocytes in other nematode species. Crystalline structures are often observed within the developing membrane specializations of filarid spermatozoa, and mitochondria-associated fibrillar bundles are present in the spermatocytes of the strongyloid nematodes.

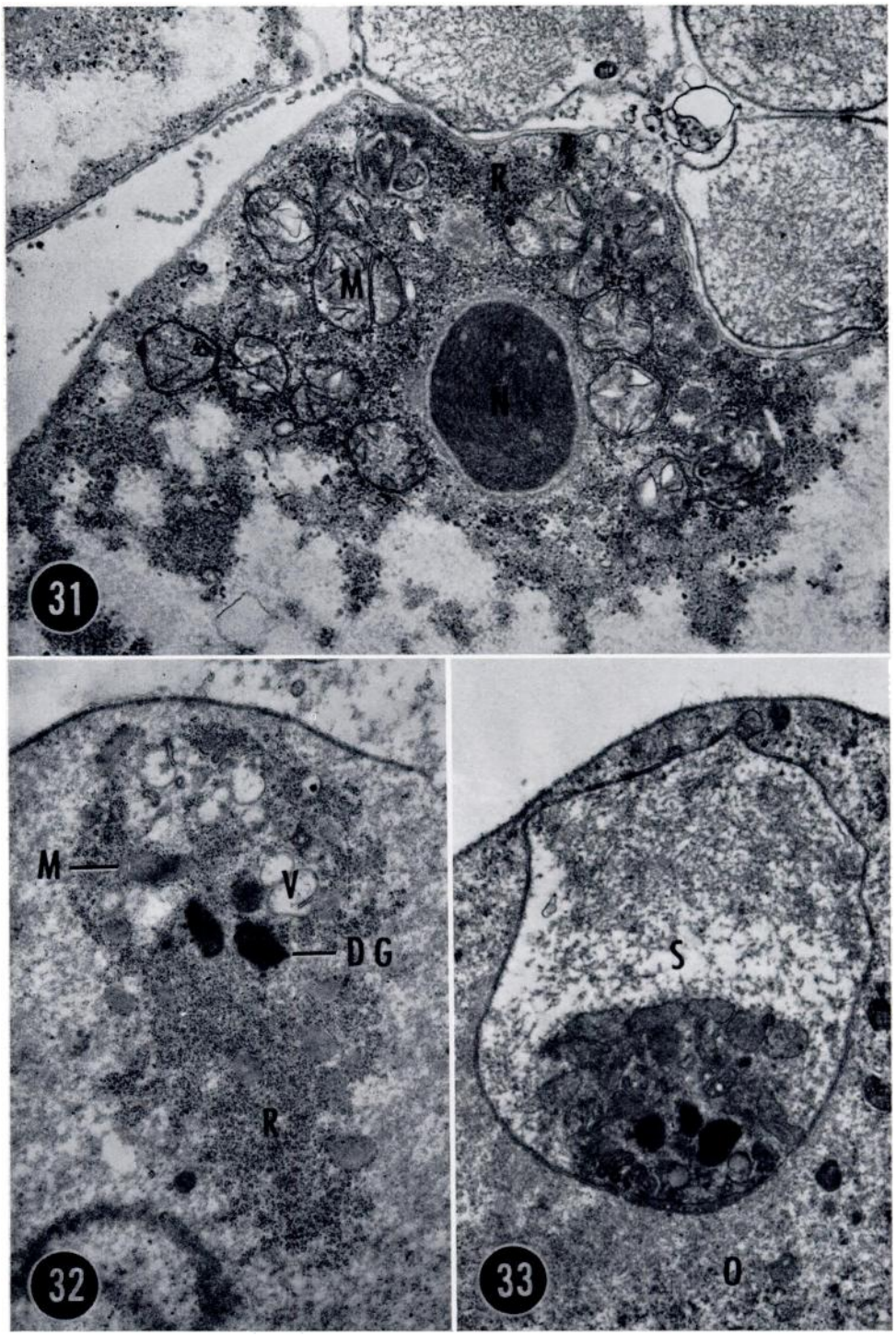
The presence of fibrillar or crystalline structures within the mitochondria of male gametes is by no means restricted to nematodes. Mitochondrial cross striations have been reported in spermatozoa of the honeybee (Rothschild, 1955), and paracrystalline mitochondrial formations have been described in the gastropod *Testacella* and the lepidopterans *Pieris* and *Macroglossum* by André (1962, 1963), in *Drosophila* by Meyer (1964), and in *Sciara* by Makielski (1966) and Phillips (1966). André (1962) and Meyer (1964) suggested that the crystalline material might represent a condensed state of the respiratory protein, possibly providing support for the sperm. An alternative interpretation is that the intramitochondrial substances, at least in nematodes, might be a reflection of some maturation phenomenon which, like the fusion of the mitochondria in flagellate sperm, is involved in reducing the number of functional mitochondria within the cytoplasm. From all indications it appears that the membrane specializations in both the ascarids and strongyles do indeed represent abortive mitochondria which release their contents (see also Jamuar, 1966), and in this manner these spermatozoa more nearly approach the condition of *Aspiculuris*, which possesses but a single mitochondrion. One can further postulate that this segment of the maturation process in nematodes has reached its zenith in *Dioctophyma* spermatozoa in which mitochondria are totally absent.

A review of the literature has revealed that, in addition to the intramitochondrial formations mentioned above, there are other instances where the mitochondria appear to form fenestrated saccules, as in rotifers (Koehler, 1965), or become associated with whorllike arrangements of cytomembranes

FIG. 31. Newly sperm of *Angiostrongylus cantonensis*. The nucleus (N) is beginning to break up, and there are numerous ribosomes (R) in the adjacent cytoplasm. M., mitochondria. ×22,500.

FIG. 32. Newly penetrated sperm of *Dirofilaria immitis* showing ribosomes (R), mitochondria (M), vesicles (V) derived from membrane specializations, and dense granules (D.G.). ×16,500.

FIG. 33. *Dirofilaria immitis* spermatozoan (S) which appears to have been engulfed by some phagocytic mechanism of the oocyte (O). The gamete membranes have not fused, and the spermatozoon cytoplasm has not been "activated." ×13,750.



(Reger, 1963) in mature spermatozoa of ticks. Similarly, the elimination of part or all of the mitochondria from the spermatid, as noted in *Dioctophyma,* has been reported in the Iceryine coccids (Hughes-Schrader, 1946) *Procambarus* (Moses, 1961), and *Callinectes* (Brown, 1966), certain isopods (Reger, 1964), mealybugs (Ross and Robinson, 1969), and many others (Nath, 1956). More comparative studies are needed to reveal whether or not the mitochondrial variations in other organisms can be considered homologous to those noted in nematode sperm. In any event, any attempt to interpret the numerical and morphological diversity of the mitochondria within the spermatozoa of any organism is particularly challenging and, at the present time, speculative.

The interpretations of other structural features of the nematode spermatozoa pose additional problems. As seen in the ascarids and strongyles, the nucleus is compact and highly condensed whereas in *Aspiculuris* it is dispersed and associated with bundles of microtubules. In *Dioctophyma* it is very diffuse and apparently comprises the major portion of the gamete. Whether or not these striking differences in the appearance of the nucleus might be a reflection of variations in either the genetic information or the histone content within each nucleus (Bloch and Brack, 1964) is unknown.

Collectively it appears that all nematode spermatozoa lack both an acrosome and a flagellum, and their cytological organization is vastly different from that reported in flagellated spermatozoa. Even in those species in which the spermatozoa have a distinct "head" and "tail," this resemblance to flagellated spermatozoa is purely superficial since critical studies have shown that the tail is usually occupied by nuclear material and appears to be incapable of movement (Jamuar, 1966; Lee and Anya, 1967).

Although it has been generally considered that the aflagellate sperm was consistent with the absence of cilia or flagella within the entire nematode group, this has not proved to be the case. Recent studies have shown that cilia are present in the sense organs of certain free-living and parasitic nematodes, and there is reason to believe that this observation can now be extended to include all larval and adult forms (Hope, 1965; Roggen *et al.,* 1966; Ross, 1967; Kozek, 1968).

Despite the presence of centrioles and an abundance of microtubules during the developmental stages of the nematode spermatozoa, it is apparent that they lack the ability to organize these elements into motile organelles. In most cases it appears that the microtubules are concerned either with maintaining the developing spermatocytes in an asymmetrical configuration or, as in the case of *Ancylostoma,* are involved in the process of compartmentalizing the cell constituents which facilitate cytoplasmic reduction in a manner similar to that described during the course of spermiogenesis in the earthworm (Anderson *et al.,* 1967).

It seems apparent that (again with the possible exception of *Aspiculuris*) the microtubular elements in most nematode spermatocytes, like the microtubules forming the manchette in certain other spermatocytes (Anderson *et al.,* 1967), are progressively lost during the final stages of maturation.

There can be little doubt that in those nematode spermatozoa in which they occur, the coalescence of the refringent granules and the release of the contents of the peripheral membrane specializations, as well as the apparent initiation of motility in most species thus far examined, are morphological indications that the final maturation of the spermatozoa occurs only after they are deposited in the female reproductive tract. In addition to providing further evidence that the sperm maturation in the female uterus in nematodes is not peculiar to ascarids, the present observations take on a new meaning in view of the recently disclosed phenomenon of sperm capacitation in mammals (Chang, 1951, 1958; Austin and Bishop, 1958; Bedford, 1963, 1964).

If the physiological and structural altera-

tions of the *in utero* nematode sperm can be equated with those which occur in mammalian sperm subsequent to their deposition in the female reproductive tract, it seems likely that studies of nematode sperm will offer a new avenue of approach in forming a clearer concept of the capacitation phenomenon.

Indeed, the acquisition of motility, as well as the structural alterations of *in utero* nematode spermatozoa, do appear comparable to certain aspects of the capacitation process discussed elsewhere in this issue (see Bedford).

In discussing motility, it should be mentioned that previous studies of nematodes have failed to reveal any movement of the spermatozoa (Jamuar, 1966; Sommerville and Weinstein, 1964). On the other hand, Lee and Anya (1967) have noted that *Aspiculuris* sperm recovered from the seminal vesicles of the male worm were capable of putting out pseudopodia when placed in Tyrode's balanced salt solution. Thus from this indirect evidence it seems likely that the forces responsible for movement are not intrinsic within the spermatozoa and that the acquisition of motility is probably influenced by the uterine fluids of the female.

If this hypothesis proves to be correct, then the function of the uterine fluids in nematodes might closely parallel Barros and Austin's (1967a,b) observations that the follicular fluid of mature ovarian follicles can induce the acrosomal reaction in hamster sperm.

This latter phenomenon is certainly not characteristic of all organisms since studies of the millipede *Polydesmus* (Reger and Cooper, 1968) as well as of the nematode *Dioctophyma* mentioned in this report reveal that in these species the spermatozoa present in the female reproductive tract are more or less identical with those present in the spermatic ducts or seminal vesicle of the male.

It is expected, however, that future comparative studies of the reproductive tracts of various animals will expand the growing list of organisms in which the spermatozoa mature (or become capacitated) after leaving the male organs. Similarly, it seems likely that before a final concept of capacitation can be formulated, it will have to be expanded to include the spermatozoa of both vertebrate and invertebrate organisms and, as implied in this report, acrosomeless as well as acrosome-containing sperm.

Zygote Formation

The present studies give evidence that immediately prior to zygote formation in nematodes the spermatozoa do not (1) undergo a vesiculation of the plasma membrane, (2) form acrosomal filaments, or (3) release substances which lyse the extraneous coat surrounding the oocyte. Thus, at least in these respects, it appears that the initial stages of syngamy in nematodes differ from those of certain mammals and other invertebrates discussed elsewhere in this issue.

The observations of *Ascaris* are in accord with a previous study (Foor, 1968) which indicated that pseudopods emanating from the spermatozoon usually make first contact with the oocyte. In addition, it appears that by their physical movement the pseudopods are responsible for the localized removal of the extraneous coat covering the oolemma. Thus this pseudopodial activity seems to be comparable to the acrosomal preliminaries described in flagellated sperm since it allows for direct contact between the gamete plasma membranes (Franklin, 1970).

Once the gamete membranes come into direct contact there appears to be little uniformity regarding their actual fusion. In some gametes the fusion occurs almost immediately. In others the fusion apparently is delayed, and the sperm, with their pseudopods interdigitating with the oolemma, form deep depressions in the oocyte. Interestingly, at this stage of syngamy the interdigitation of the gamete membranes of *Ascaris* is somewhat similar to that reported between the acrosomal filaments and the egg membrane in *Hydroides* (Colwin *et al.*, 1957).

In *Ascaris*, there is little reason to doubt that the gametes fuse following the inter-

digitation process. Intact sperm have not been observed completely within the oocyte, and there is no evidence that this close association between the sperm and the oocyte is aborted once it has been established. It appears that the opposing membranes disappear and the gamete cytoplasms become continuous.

The dearth of observations concerning the syngamy (as well as the morphology of the gametes) in species other than *Ascaris* prevents any generalized description of the zygote formation in nematodes. Despite the fact that all nematode spermatozoa examined thus far are capable of forming pseudopods, certain observations give indications that all factors governing the gamete fusion have not been taken into account in the rather simple description of sperm penetration in *Ascaris* as outlined above. For example, in many uteri the unfertilized oocytes appear to be surrounded by spermatozoa which give no indication that they are attempting to penetrate the oocyte surface. In still other instances (such as in *Dirofilaria*, Fig. 33), it appears that the spermatozoa may be engulfed by some phagocytic mechanism of the oocyte. From these observations, as well as images of *Ascaris* which show that certain gametes fuse almost immediately whereas others undergo considerable interdigitation prior to fusion, one gains the impression that the conditions regulating syngamy in nematodes involve a series of complex processes which are directly dependent upon a rather synchronous maturation of the gametes themselves. In this regard, it would appear then that certain aspects of the syngamy in nematodes are reminiscent of those described in other species. This is especially true when one considers that recent studies indicate that the syngamy in both mammals (Bedford, 1970; and Zamboni, 1970) and sea urchins (Anderson, 1968; Aketa *et al.*, 1968) involves certain inherent characters of both the mature sperm plasmalemma and the extraneous coat and oolemma surrounding the egg.

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