INSECT SPERM: THEIR STRUCTURE AND MORPHOGENESIS

DAVID M. PHILLIPS

From the Department of Anatomy and the Laboratories for Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts. Dr. Phillips' present address is the Department of Biology, Washington University, St. Louis, Missouri 63130

The thorough descriptive studies by the early cytologists Meves (1901, 1907), Retzius (1904, 1909), Bowen (1920, 1922 *a–c*, 1924), Pollister (1930), and Johnson (1931) provided later workers with a remarkably accurate picture of the microscopic anatomy of insect spermatozoa and of spermiogenesis in several insect species. Their light microscope observations were necessarily lacking in detail, however, and since the advent of the electron microscope, an increasing number of cell biologists have undertaken to extend our understanding of the structure of insect germ cells. Some have confined their attention to spermiogenesis or the morphology of mature spermatozoa of a particular species, while others have been concerned with the comparative morphology of single sperm organelles. Although there are by now many relevant papers of limited scope widely dispersed in the biological literature, there seems to be no single reference to which one can turn for an overview of the structure and development of the insect spermatozoon. In this review an effort is made to gain some perspective from a synthesis of these fragmentary accounts supplemented by the author's own observations based on electron microscopic examination of nearly two hundred insect species.[1]

[1] The testes of 195 insect species representing 15 orders have been examined. Most of the insects were collected in the vicinity of Boston, Massachusetts.

Organization of the Insect Testis

While butterflies and moths possess but a single testis, most species of insects have paired testes covered by a simple epithelium that is frequently pigmented. Indeed, the bright red, green, yellow, or brown color imparted by this pigmented epithelium is quite helpful in identifying the organ during dissection. The testicular epithelium in most insects encloses a number of follicles, each delimited by its own epithelium and connected independently to the genital duct. The number and shape of the follicles varies widely from one insect group to another (Imms, 1948; Wigglesworth, 1953; Ross, 1961). For example, in many of the Diptera, each testis consists of only one follicle, while in leafhoppers there are many spher-

Testes were dissected in cold fixative, usually within a few hours of being collected. Testes were usually fixed 2–6 hr in 3–6% glutaraldehyde (Eastman Kodak Co., Rochester, N.Y.) and buffered with 0.2 M collidine (Eastman Kodak Co.) at pH 7.2–7.6. Testes were then transferred through several changes of cold buffer (5–30 min) and were postfixed for 1–4 hr in 1% collidine-buffered OsO_4. After dehydration in alcohol, tissues were embedded in Epon (Luft, 1961), sectioned on a Sorvall MT-1 ultramicrotome (Ivan Sorvall Inc., Norwalk Conn.), were stained in 3% aqueous uranyl acetate (12–16 hr) and lead citrate (Venable and Coggeshall, 1965), and were examined in a Siemens Elmiskop I.

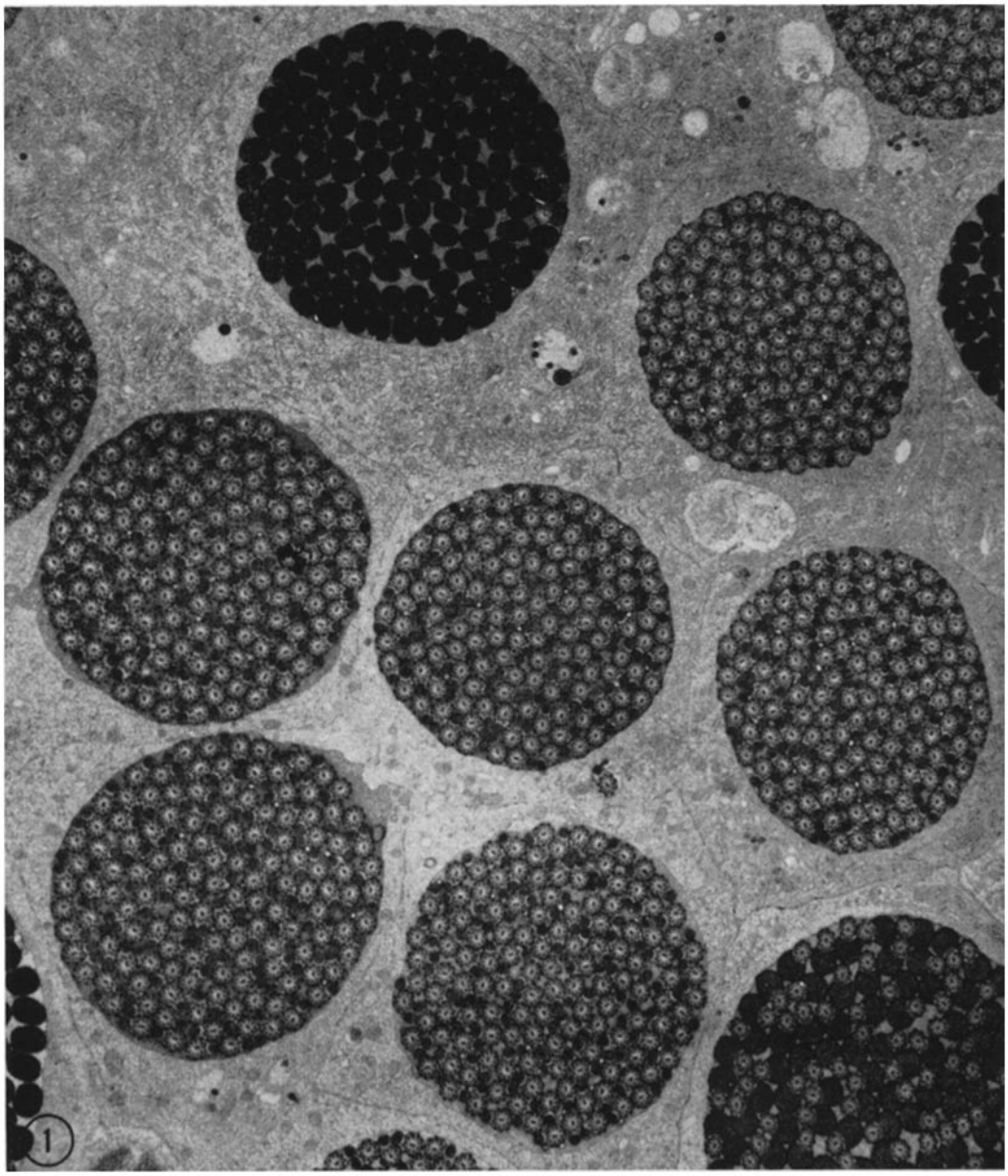


FIGURE 1 Low magnification electron micrograph of the imaginal testis of the caddis fly *Platycentropus* (Limnephilidae). All spermatozoa in this field appear in near transverse section. This indicates that neighboring cysts are oriented in the same direction. The spermatids within a cyst are also aligned in register so that a transverse section passes through all the cells within the cyst at nearly the same level. For instance, in some cysts all the spermatozoa are transected through the nucleus whereas in other cysts, which are transected farther posteriorly, no nuclei are observed. Most cysts contain exactly 128 (2^7) spermatozoa, which presumably arose from five synchronous mitotic and two synchronous meiotic divisions. The finding of nearly exactly 2^7 spermatozoa per cyst suggests that in insects very few germ cells die during spermatogenesis or spermiogenesis. × 7,500.

ical follicles in a cluster resembling a small bunch of grapes. The shape and arrangement of the multiple elongated follicles in the testis of some grasshoppers is reminiscent of a bunch of bananas.

The follicles contain many cysts, each of which consists of a clone of germinal cells embedded in large polyvalent epithelial cells (Baccetti and Bairati, 1964; Cantacuzene, 1968). The gonial and meiotic divisions are synchronous within a given cyst, and the cytoplasmic bridges that result from incomplete cytokinesis in these divisions make each clone a functional syncytium (Baccetti and Bairati, 1964; Hoage and Kessel, 1968). The number of spermatids per cyst is characteristic for each species and can be expressed as 2^n with "n" usually equal to 5, 6, 7, or 8. The number of divisions occurring in a cyst, therefore, appears to be constant for a given species. Two to the fifth power (i.e., 32) cells per cyst are characteristic of coccids (Hughes-Schrader, 1935, 1946; Nur, 1962) and some flies (Hess and Meyer, 1968) while 2^6 (i.e., 64) cells characterize the cysts of other flies (Hess and Meyer, 1968). The testicular cysts of many caddis fly species contain 2^7 (i.e., 128) spermatids per cyst, or very nearly that number (Phillips, unpublished) (Fig. 1). 256 (2^8) spermatids per cyst appear to be common to most beetles, butterflies, and moths (Phillips, unpublished).

Within a cyst, the interconnected spermatids differentiate synchronously (Smith, 1916; Bairati, 1967). Cysts containing spermatogonia, spermatocytes, or young spermatids are generally approximately spherical or polyhedral. As the germ cells elongate, the cysts also elongate and the spermatids become aligned parallel and in register along this length. The alignment of neighboring spermatids in the late stages is, in most cases, nearly perfect, so that a transverse section of a cyst containing late spermatids transects all the spermatids at approximately the same level (Fig. 1). The anterior portions of the heads of late spermatids are usually embedded in the polyvalent cells of the cyst wall. Posteriorly the cells are free in the cyst lumen where they are sometimes surrounded by amorphous or finely granular extracellular material which, in some species, displays considerable electron opacity.

Cysts within a follicle are generally systematically arranged with respect to the stage of development of the included germ cells. In insects in which the testicular follicles are spherical (leafhoppers, and many beetles, butterflies, and moths), cysts are arranged in order of increasing maturity from the periphery to the center of the testis. In grasshoppers, where the follicles are long and slender, cysts at the anterior end of the testis contain relatively early stages of the germ cells, and progressively more mature stages are found at successive levels toward the posterior end (Lima-de-Faria, 1959).

In most species of insects, gonial and meiotic divisions occur in pupal or nymphal stages of the life cycle. Therefore, the imaginal testis contains only spermatids and spermatozoa. In insects that live a very short time as adults, such as mayflies (Needham et al., 1935), caddis flies (Ross, 1944), and dark winged fungus gnats (Crouse, 1943; 1965), the testis of adults contains only spermatozoa. On the other hand, in some species that are relatively long lived as adults (e.g., many beetles and dragonflies), meiotic and even gonial divisions occur in the imaginal testis.

THE MITOCHONDRIA OF INSECT SPERM

Light Microscopic Analysis

Early students of insect spermiogenesis were particularly interested in the complicated, precisely ordered steps by which the numerous small spermatocyte mitochondria are transformed into giant mitochondrial derivatives, which occupy most of the volume of mature insect spermatozoa. Similar sequences of mitochondrial reorganizations were described by several investigators working with a number of different species. The most complete studies were those of Bowen (1920, 1922 *a*, *b*, 1924), Pollister (1930), and Johnson (1931). These and other investigators reported that by telophase of the second meiotic division, the previously spherical mitochondria become elongate structures located in the *zwischenkörper* between the two future spermatids. In some species the mitochondria appear to be pinched in half by the cleavage furrow (Meves, 1907; Duesberg, 1911; Bowen, 1920; Johnson, 1931) resulting in an approximately equal distribution of mitochondria to daughter cells at the second meiotic division (for discussion see Wilson, 1928).

Just after meiosis the mitochondria once again become spherical and are clustered together in one area of the cell. They then begin a complex series of rearrangements and fusions that lead to their integration into a large spherical mass, which

Retzius (1904) termed the *nebenkern*. In some insect species, the nebenkern is initially shaped like a ring rather than a sphere, and the mitochondria of this *ring nebenkern* subsequently take on a flattened shape and become arranged into a stack (Charlton, 1921; Pollister, 1930). Regardless of the configuration of the nebenkern, it is generally reported that the fusion of the mitochondrial subunits continues until the nebenkern consists of two large interlocking mitochondrial masses. These undergo a series of convolutions, and the nebenkern eventually separates into its two constituent mitochondria. Each large mitochondrion then assumes the form of an ellipse of revolution, and the two take positions directly behind the nucleus on opposite sides of the basal body of the spermatid flagellum. The mitochondria remain closely associated with the flagellum and subsequently elongate parallel to its long axis. In some hemipterans the two mitochondria spiral about the flagellum during this process (Bowen, 1922 *a*; Pollister, 1930). Bowen (1922 *a*), Pollister (1930), and Johnson (1931) each noted small ovoid refractile areas within the elongating mitochondria that give these organelles a beaded appearance. "Bleb-like swellings" were also reported along the length of the mitochondria of pentatomid spermatozoa by Bowen (1920, 1922 *a*, *b*).

Mitochondria become progressively longer and narrower during the course of spermiogenesis. Ultimately they attain a diameter that is about the same as that of the flagellum. The mitochondria of

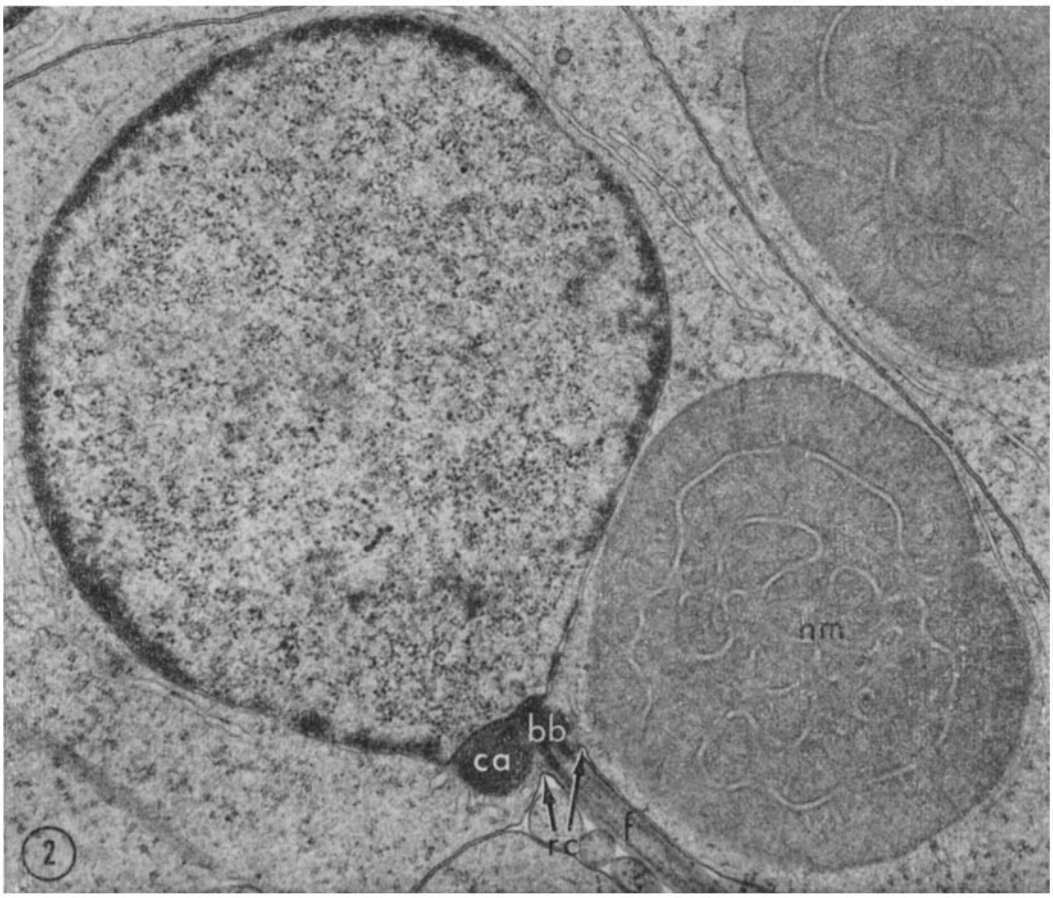


FIGURE 2 The nebenkern (*nm*) in a young spermatid of the stinkbug *Euschistus*. The nebenkern in the stage shown is composed of fusing interlocked mitochondria. The position of the nebenkern near the base of the flagellum (*f*) is characteristic of nearly all insect species that have been described. Ring centriole, (*rc*); centriole adjunct, (*ca*); basal body, (*bb*). × 17,000.

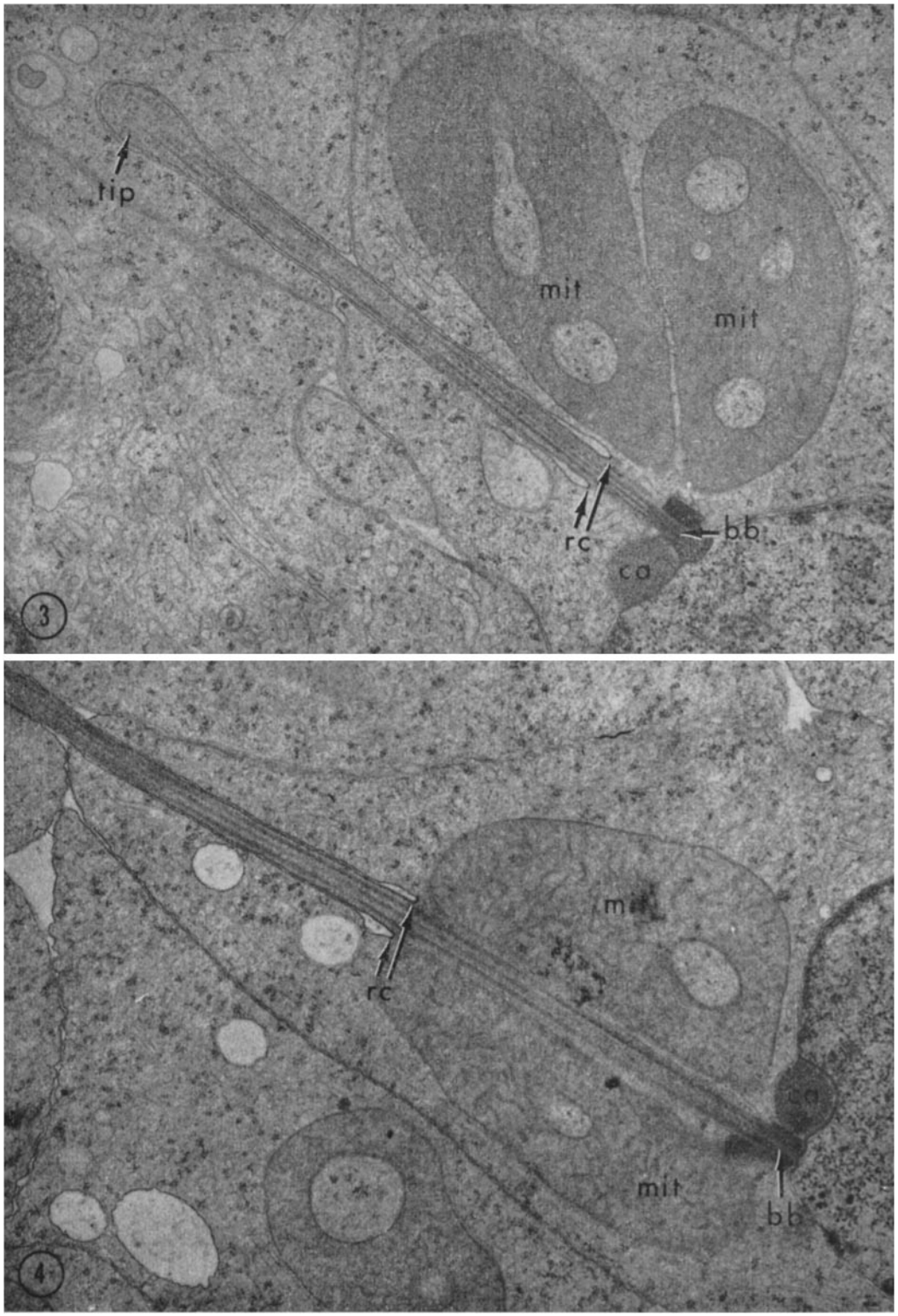


FIGURE 3 Spermatid of *Euschistus*, a later stage than shown in Fig. 2. The two large nebenkern derivatives (*mit*) are now positioned at the base of the elongating flagellum. The swelling at the tip of the flagellum (*tip*) is characteristic of growing insect flagella. The involuted cell membrane separates the flagellum from the rest of the cytoplasm. Ring centriole, (*rc*); basal body, (*bb*); centriole adjunct, (*ca*). × 13,000.

FIGURE 4 Spermatid of *Euschistus*, a later stage than shown in Fig. 3. The ring centriole (*rc*) and attached cell membrane have moved posteriorly and the two nebenkern derivatives (*mit*) have assumed positions on opposite sides of the spermatid flagellum. Basal body, (*bb*); centriole adjunct, (*ca*). × 13,000.

mature insect sperm generally extend from the base of the nucleus to very nearly the end of the sperm tail (Retzius, 1904, 1909).

Electron Microscopic Observations

Careful electron microscopic analyses of spermiogenesis in the butterfly *Pieris* by André (1959, 1962) and in the stinkbug *Murgantia* by Pratt (1966, 1967, 1968) exposed complexities in mitochondrial development, which were only suggested by early light microscopic studies. The reader is referred to Pratt (1968) for a detailed analysis of the progressive fusion of the numerous spermatocyte mitochondria into two interwoven labyrinthian nebenkern mitochondria and their ensuing rearrangements, configurational changes, and eventual segregation. There are indications from other electron microscopic studies that the process of nebenkern development and dissolution is essentially similar to that of *Pieris* and *Murgantia* in species of Apterygota (Werner, 1964), Odonata (Breland et al., 1966; Kessel, 1966), Orthoptera (Beams et al., 1954; Tahmisian et al., 1956; De Robertis and Raffo, 1957; Yasuzumi et al., 1958; Gatenby and Tahmisian, 1959; Bertaud and Gatenby, 1960; Kessel, 1967), Neuroptera (Breland et al., 1966), Coleoptera (Werner, 1965), and Diptera (Yasuzumi et al., 1958; Breland et al., 1966; Anderson, 1967; Meyer, 1968), as well as in other species of Hemiptera (Yasuzumi et al., 1960; Payne, 1966) and Lepidoptera (Yasuzumi and Oura, 1965). The two mitochondria of the nebenkern, though more complex in form, appear to be functionally as well as structurally similar to other mitochondria (Chance and Thorell, 1959; Perry et al., 1959).

The final realignment of the two derivatives of the nebenkern after they separate from each other is related to the growth of the spermatid flagellum. The sequence of events in the stinkbug *Euschistus* is illustrative of this relationship. In *Euschistus*, at the stage when the nebenkern appears as a spherical body consisting of two intertwined mitochondria, the flagellum is just beginning to grow outward from a centriole located immediately adjacent to the nucleus. The cell membrane is invaginated as far inward as the basal body as a closely fitting sheath around the lengthening fla-

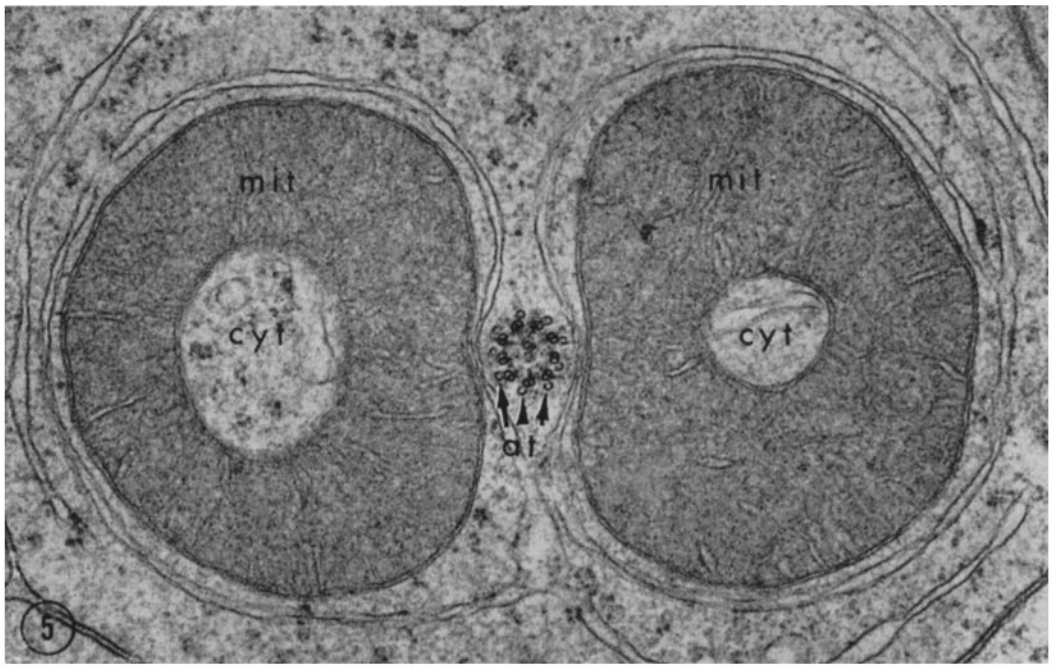


FIGURE 5 Transverse section of the flagellum and nebenkern derivatives (*mit*) in a spermatid of *Euschistus* at about the same stage as shown in Fig. 4. Areas of cytoplasm (*cyt*) inside the nebenkern derivatives probably correspond to refractile areas observed by Bowen (1922 *b*) in members of this genus. Note forming accessory tubules (*at*). × 30,000.

gellum. Therefore, two layers of plasma membrane separate the nebenkern from the flagellum (Fig. 2). A small ring of dense material, formerly termed the "ring centriole," encircles the flagellum at the innermost edge of the sleeve of invaginated cell membrane. This ring, or annulus, which is intimately associated with the cell membrane, behaves as if it were anchoring the cell membrane near the basal body during the early growth of the flagellum (Fig. 3) but later is released, slipping caudally along the flagellum, taking the attached cell membrane with it (Fig. 4). As the ring moves caudad the two derivatives of the nebenkern take up positions on either side of the spermatid flagellum (Figs. 4–6). The annulus of insect spermatids is undoubtedly analogous to the ring of urodele (Lommen, 1967) and of mammalian permatids (Meves, 1901; Duesberg, 1920; Burgos and Fawcett, 1955). The ring near the base of the flagellum in mammalian spermatids is much more prominent than that of insect spermatids but bears the same relationship to the invaginated plasma membrane and similarly moves posteriorly during spermiogenesis, permitting the mitochondria to come into closer association with the flagellum (Fawcett and Phillips, 1969 *c*).

After the two nebenkern derivatives in the insect spermatid have taken up positions on either side of the base of the flagellum, they begin a process of internal reorganization and differentiation that eventually endow the spermatid with either one or two highly ordered, elongate mitochondrial derivatives. The morphology of these is identical between individuals of the same species, but is highly variable from species to species. Whether the mitochondrial differentiation results in one or in two

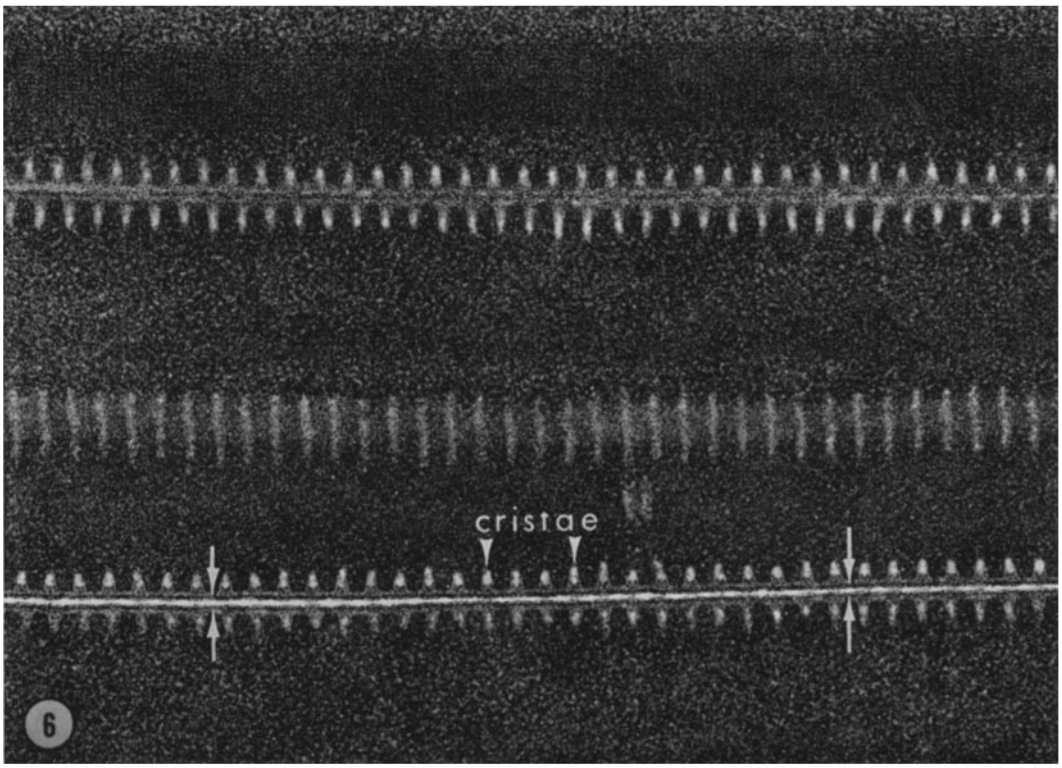


FIGURE 6 Longitudinal section through the mitochondrial derivative of three sperm cells of a checkered beetle (Cleridae). Mitochondrial cristae, which are more or less randomly arranged in the early nebenkern derivatives, are rearranged during spermiogenesis into regularly spaced lamellae, which generally extend only part way across the mitochondrion, leaving most of the space for crystalline material. In this figure, and in similar high magnification electron micrographs of sperm of other species, it appears that one of the mitochondrial membranes is absent or perhaps fused with the cell membrane. The plasma membranes of two of the spermatozoa shown in this figure are indicated by arrows. × 116,000.

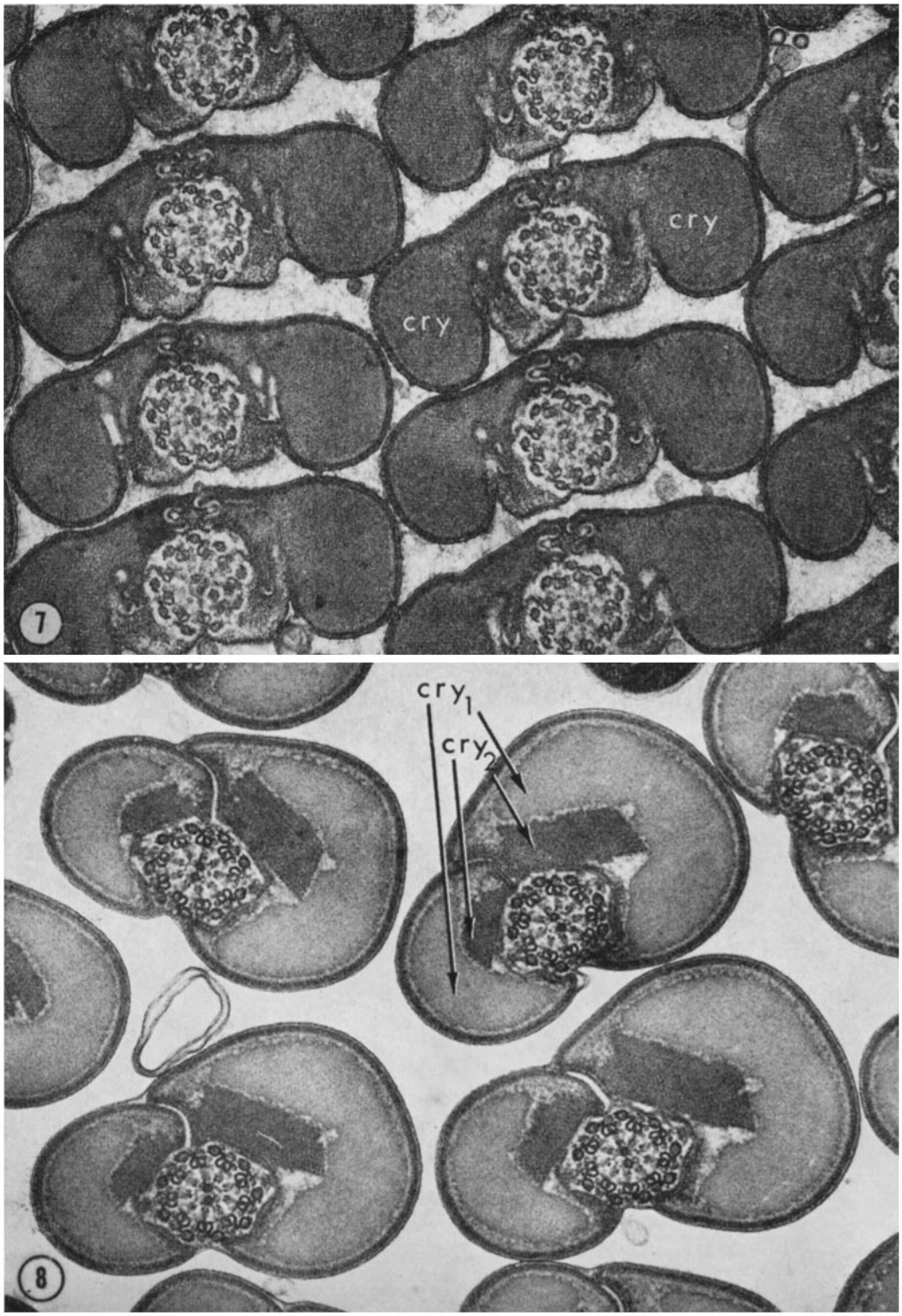


FIGURE 7 In spermatozoa of some insect species only one of the two mitochondrial derivatives persist. In other species, such as the lacewing *Chrysopa* (shown here), both mitochondrial derivatives persist. Intramitochondrial paracrystalline material, (*cry*). × 61,000.

FIGURE 8 The mitochondrial derivatives of sperm of the stilt bug *Jalysus* are unusual in possessing two morphologically distinct types of crystalloids (cry_1), (cry_2). × 61,000.

mitochondrial derivatives depends on the insect species. In mature sperm of some there are two mitochondrial derivatives of equal size but are enantiomorphic (Tandler and Moriber, 1966; Herold and Munz, 1967) (Figs. 7 and 9). In other species, spermatozoa possess two mitochondrial derivatives of unequal size, with one usually extending farther posteriorly or anteriorly than the other (Thompson and Blum, 1967; Yasuzumi and Oura, 1965) (Figs. 8 and 10). In still other insect species only one mitochondrial derivative persists in the mature sperm (e.g., in many Trichoptera, Phillips, unpublished) (Fig. 11). To what degree this characteristic is constant among members of the same family or order is difficult to say because mature sperm of very few of the hundreds of thousands of insect species have been examined. Of the 18 species of Lepidoptera we have studied, all apparently have two mitochondrial derivatives of unequal size. Sperm from the Diptera families Sciariadae, Mycetophilidae, and Ptychopteridae, have just one. In other Diptera families (Drosophilidae, Syrphidae, Sepsidae, and Muscidae), two mitochondrial derivatives of unequal size persist and in others (Culicidae and Simuliidae), two mitochondrial derivatives of apparently equal size are found in mature sperm.

Elongation of nebenkern derivatives begins even before they move into their definitive positions adjacent to the flagellum and it continues during most of the remaining period of sperm maturation. In the butterfly *Pieris*, André (1959, 1962) described a precisely ordered realignment of the mitochondrial cristae during this period of elongation. During spermiogenesis, the cristae gradually assume the configuration of evenly spaced parallel folds or lamellae disposed perpendicular to the long axis of the cell and extending from only one side of the organelle. Because of this orientation to the long axes of the developing tail, the cristae are not observed in transverse sections. Although the process of mitochondrial differentiation has not been carefully followed in other species, the alignment of cristae in mature mitochondrial derivatives of many insects appears to be similar to that in *Pieris* (Fig. 6) (Werner, 1964, 1965; Yasuzumi and Oura, 1965; Kessel, 1966). A remarkably similar process of mitochondrial differentiation also takes place during spermiogenesis in some species of pulmonate snails (Kaye, 1958; André, 1962).

Shortly after the mitochondrial cristae are realigned, small areas of structured material appear in the mitochondrial matrix. In some species this material, when viewed in transverse section, first resembles a hexagonal grid or a honeycomb-like structure (Phillips, 1966 *a*). In other insects it appears in cross-section as a hexagonal pattern of punctate profiles (see Fig. 3; Phillips, 1969 *a*). This paracrystalline material continues to accumulate until the mitochondrial matrix is largely replaced by it. The paracrystalline component was first described by André (1962) as periodically striated when viewed in longitudinal section. In a subsequent careful study of negatively stained mitochondrial crystalloids from sperm of a number of *Drosophila* species, Meyer (1964) described different major and minor periods in the striated paracrystalline material of different species. He found major periods of 260 A in *D. melanogaster*, 320 A in *D. bifurca*, and 300 and 400 A in *D. hydei* X/O males. In spermatozoa of *D. hydei* and *D. melanogaster*, Meyer reported that the paracrystalline material sometimes appears in longitudinal sections as a herringbone pattern with a periodicity of about 450 A (Meyer, 1964, 1966; Hess and Meyer, 1968). The corresponding component in the fungus gnat *Sciara* also presents a herringbone pattern with a 450 A periodicity when sectioned longitudinally, but its pattern differs slightly from that of *Drosophila*. In transverse section, the crystalloid of *Sciara* sperm has the appearance of hexagonally packed circles or hexagons with 90 A center-to-center spacing, and in oblique section it presents a pattern of parallel lines with 90 A periodicity (Phillips, 1966 *b*). A similar herringbone pattern has been reported by Herold and Munz (1967) in *Peregrinus* (Homoptera, Delphacidae), and we have observed it in intramitochondrial crystalloids in many other insects, including orders of Odonata, Dermaptera, Psocoptera, Hemiptera, Homoptera, Neuroptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera (Figs. 7–9) (Phillips, 1969 *a*, and unpublished).

Mitochondrial derivatives in sperm of some insect species contain two morphologically distinct types of paracrystalline material (Fig. 9). Although it has been reported that the mitochondria of some insect species contain dense amorphous material instead of the usual crystalloid (Bawa, 1964; Breland et al., 1966; Thomson and Blum, 1967), it is likely that higher resolution micrographs of thinner sections would reveal a periodic structure in this material.

The complex process of mitochondrial aggrega-

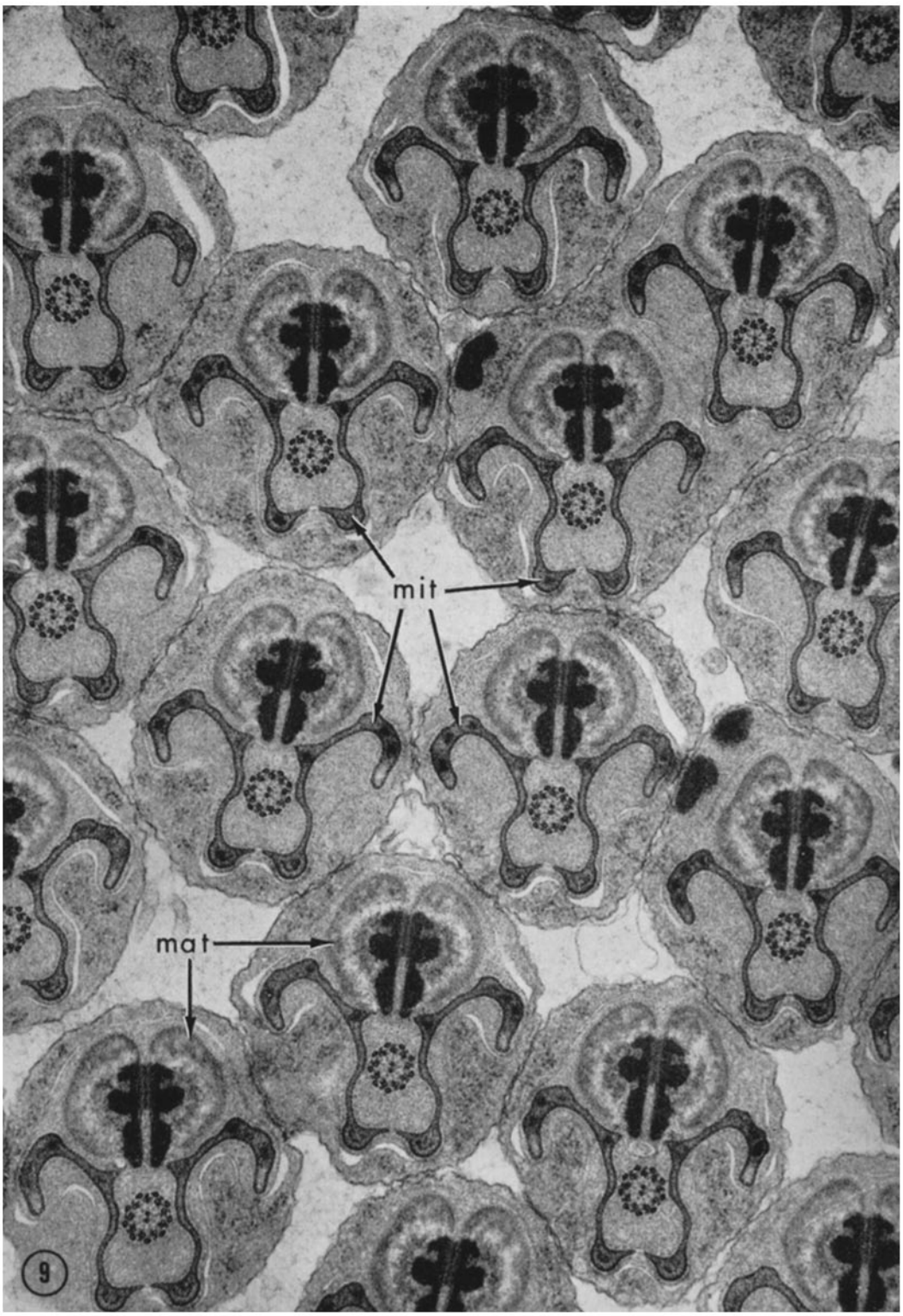


FIGURE 9 Transversely sectioned spermatids of the plant hopper *Acanalonia*. Though the forms of the two mitochondrial derivatives (*mit*), which appear here to either side of the flagellum, are highly irregular, the mitochondrial profile is virtually identical in all cells transected at a given level. The shape assumed by the mitochondrial derivatives, though not always so complex, is highly specific for each insect species. The ordered material (*mat*) seen above the flagellum in this figure is characteristic of plant hopper spermatozoa. × 25,000.

tion and fusion to form a nebenkern followed by dissociation of two mitochondria, and their elongation and internal reorganization is without a parallel in the biology of somatic cells. The functional significance of this remarkable series of transformations remains a mystery. It culminates in the mature insect spermatozoan in one (Fig. 11) or two (Figs. 7–9) mitochondrial derivatives with periodically spaced parallel cristae extending into the lumen from one side of the mitochondrial derivative. The matrix is packed with paracrystalline material which, in some insects, comprises most of the mitochondrial volume (Figs. 7 and 8). The mitochondrial derivatives assume precise, species-specific shapes, so that in transverse section they present highly characteristic and sometimes bizarre profiles (Fig. 9).

Exceptional Mitochondrial Forms

Doyle (1933) observed that in the fungus gnat *Sciara* the large spherical mitochondria of young spermatids never form a nebenkern, but instead arrange themselves individually along the spermatid flagellum and later fuse into a giant mitochondrial derivative. We have confirmed this and find further that the matrix of the mitochondrial derivative of *Sciara* spermatids initially consists mainly of homogeneous proteinaceous material that was present in the mitochondria before fusion. Later, a paracrystalline material also appears in the matrix. Differentiation of the mitochondrial derivative is not completed in the male tract. In the female genital tract after insemination, the proteinaceous material is cast off, leaving only the paracrystalline material surrounded by mitochondrial membranes (Phillips, 1966 *a*, *b*).

The destinies of specific chromosome groups during spermatogenesis in coccids have been carefully worked out (Hughes-Schrader, 1946, 1948; Brown and Nur, 1964), and the fine structure of coccid spermatozoa has been studied by electron microscopy (Robison, 1965, 1966, 1968 *a*, *b*; Ross and Robison, 1969; Moses and Coleman, 1963; Moses et al., 1968), but the fate of cytoplasmic organelles during differentiation has not been closely followed. Robison (1965, 1966) found no mitochondria or structures resembling mitochondrial derivatives in mature spermatozoa of *Parlatoria oleae*. Sperm of other coccids also appear to lack mitochondrial derivatives (Robison, 1968 *a*, *b*; Ross and Robison, 1969; Moses and Coleman, 1963; Moses et al., 1968), but at what point in sperm development they are lost has not been reported.

THE SPERM FLAGELLUM

A 9 + 2 pattern of tubules characterizes cilia and flagella of numerous plant and most animal species (Manton, 1952; Fawcett and Porter, 1954; Afzelius, 1959; Gibbons and Grimstone, 1960). It is not surprising, therefore, to find this the prevailing arrangement of tubules in the flagella of insect sperm as well. In occasional insect species, however, there are variations in the central elements of the axonemal complex (Phillips, 1969 *a*, *b*), but the doublet tubules appear to be a constant feature of all motile sperm tails. As in other cilia and flagella, one member of each doublet of the insect sperm flagellum is slightly smaller than the other and often possesses a more electron-opaque center. This member, which has been designated as subfiber A by Gibbons and Grimstone (1960), has two small arms directed toward the larger member (subfiber B) of the adjacent doublet (André, 1961; Kaye, 1964; Phillips, 1966 *b*, *c*).

In some insect species, subfiber A and subfiber B are dissociated from each other near the end of the flagellum. In transverse sections of the flagellum posterior to the point of their separation, the nine subfibers A appear as complete circles while the nine subfibers B appear as C-shaped profiles (Phillips, 1966 *c*). It is likely, therefore, that the doublet tubules in insect sperm are composed of one complete tubule and one that is incomplete and shares a portion of the wall of subfiber that is common to both. A similar phenomenon can be observed in sperm of the bat *Myotis lucifugus* as described by Fawcett and Ito (1965) (see their Fig. 17).

In addition to the 9 + 2 pattern of tubules, insect sperm flagella generally possess nine outer singlet tubules (Fig. 11), sometimes referred to as accessory fibers (Kaye, 1964; Cameron, 1965). These accessory tubules extend farther anteriorly than do those of the axonemal complex (Fig. 15), and toward the end of the tail they terminate before the posterior termination of the doublets and central pair (Phillips, 1966 *b*, *c*). Accessory tubules characterize spermatozoa of Apterygota (Bawa, 1964; Werner, 1964), Ephemeroptera (Phillips, 1969 *a*), Odonata (Kessel, 1966), Orthoptera (Shay and Biescle, 1968; Yasuzumi et al., 1958; Kaye, 1964; Bassot and Martoja, 1966;

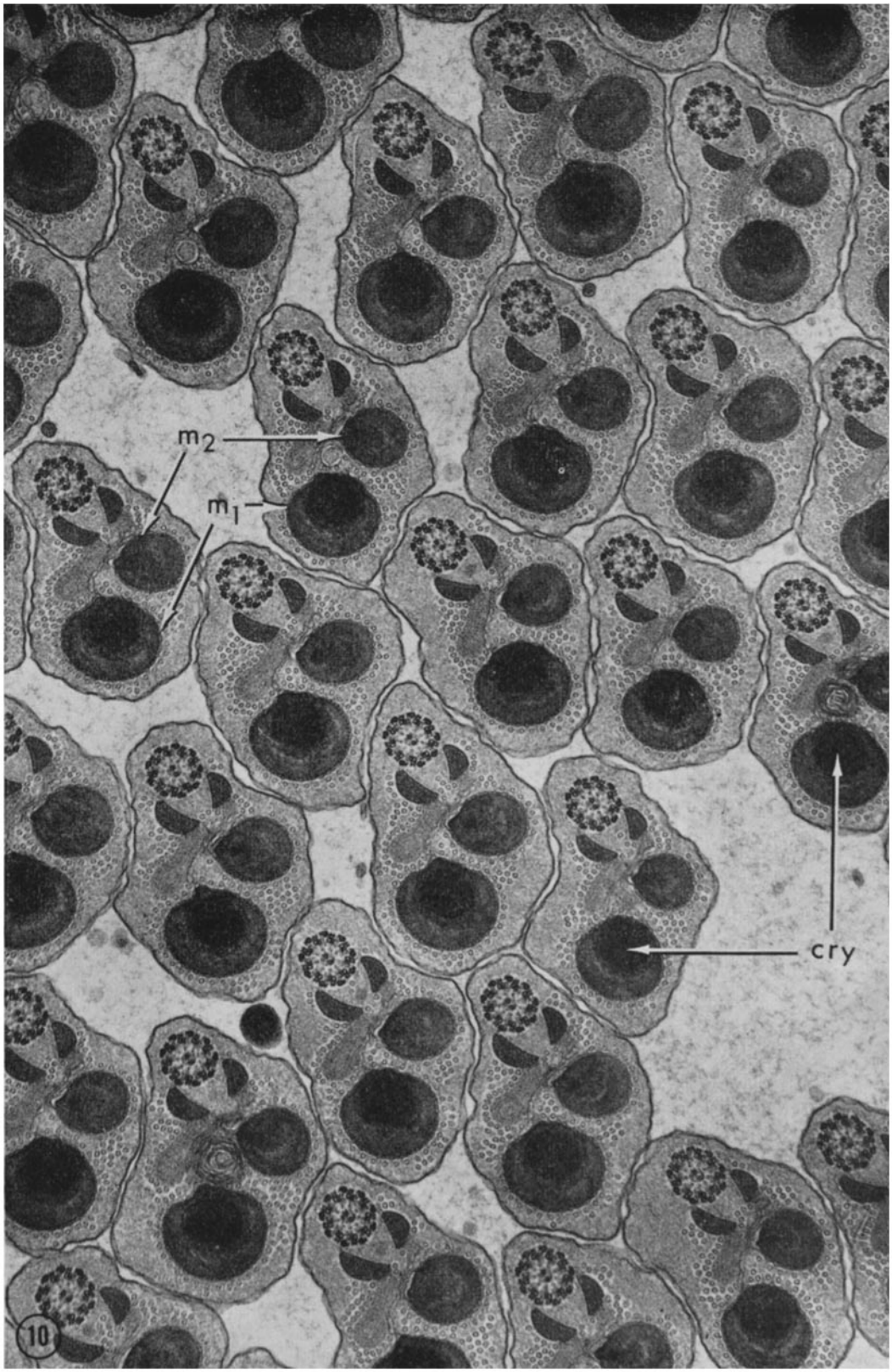


FIGURE 10 Two mitochondria appear in each of these transversely sectioned snout beetle (Curculionidae) spermatids. At anterior levels one (m_1) is larger than the other (m_2) and contains a more prominent paracrystalline inclusion (*cry*). Both mitochondria persist in mature spermatozoa of this species, but one remains smaller at anterior levels. The mitochondrial derivatives and other elongating structures are surrounded by microtubules. × 29,000.

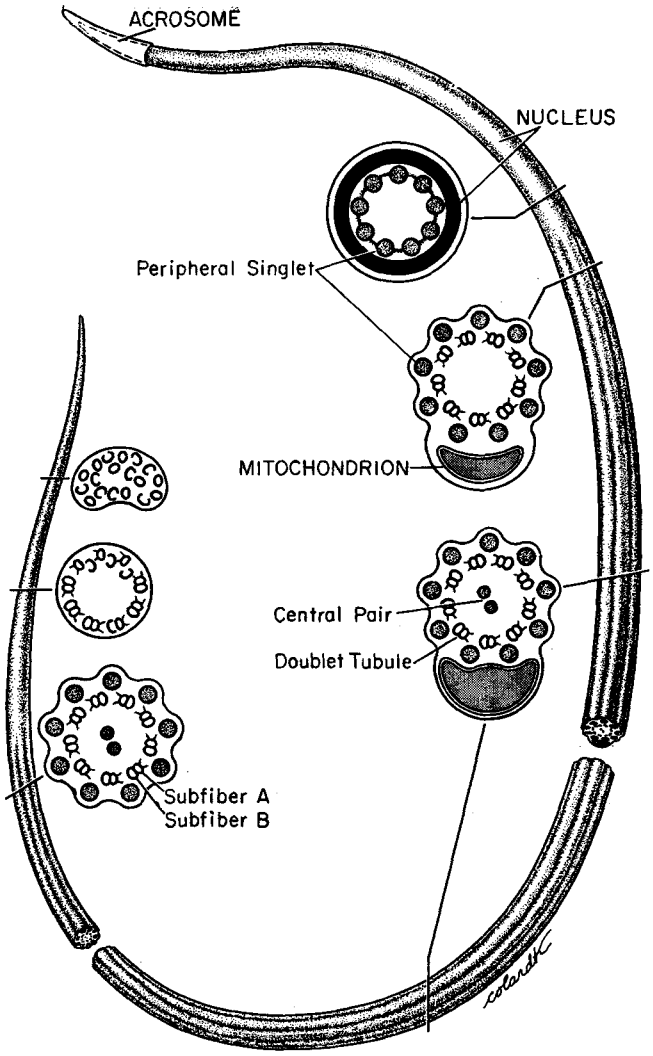


FIGURE 11 Diagram of a spermatozoon of the caddis fly *Neuronia* illustrating some of the features common to insect sperm.

Breland et al., 1968; Kessel, 1967) (Fig. 14), Dermaptera, Psochoptera (Phillips, 1969 *a*), Mallophaga (Ito, 1966; Baccetti et al., 1968 *a*, 1969 *b*), Hemiptera (Barker and Riess, 1966; Furieri, 1963 *b*; Tandler and Moriber, 1966) (Figs. 5 and 8), Homoptera (Herold and Munz, 1967; Phillips, 1969 *a*) (Fig. 9), Neuroptera (Baccetti et al., 1968 *b*, 1969 *a*) (Fig. 7), Coleoptera (Cameron, 1965) (Fig. 10), Trichoptera (Phillips, 1969 *b*), Lepidoptera (André, 1961, 1962; Yasuzumi and Oura, 1965) (Figs. 21 and 22), Diptera (Yasuzumi et al., 1958; Nunez, 1963; Baccetti and Bairati, 1964; Bairati and Baccetti, 1964; Breland et al., 1966; Phillips, 1966 *b*, *c*, 1969; Anderson, 1967; Behnke and Forer, 1967; Hess and Meyer, 1968; Meyer, 1968), and Hymenoptera (Rothschild, 1955; Furieri, 1963 *a*; Wilkes and Lee, 1965; Tompson and Blum, 1967; Hoage and Kessel, 1968). Accessory tubules are absent from spermatozoa of two species of Siphonaptera that have been examined (Phillips, 1969 *a*; S. Ito, personal communication) and are also lacking in two species of Trichoptera (Phillips, 1969 *a*)and from sperm of the mecopteran *Panorpa annexa* (Baccetti et al., 1969 *a*). In contrast to the unchanging character of the flagellar doublets, the size and appearance of the accessory tubules is quite variable from species to species. In sperm flagella of some psocids and mayflies, they are smaller than either member

of the doublets or the central pair (Phillips, 1969 *a*). In other species they are larger.

In many cases the central pair of flagellar tubules exhibits the same species specific morphology as the accessory tubules. In the Lepidoptera, where the accessory tubules often appear solid rather than tubular (André, 1961; Yasuzumi and Oura, 1965), the central pair of tubules frequently also possess a dense center (Fig. 21). In sperm of some insects, both the central tubules and the nine accessory tubules show in cross-section a central dot that appears to represent a slender dense rod running through the center of the tubule (Cameron, 1965; Kessel, 1967). Accessory fibers and central tubules of the black scavenger fly *Sepsis* (Phillips, 1966 *c*) and the beetle *Tenebrio* (Cameron, 1965) contain a central tubule in place of the rod. The most elaborate intratubular structure has been described by Kaye (1964) in the cricket *Acheta*, in which the center of the accessory fibers and central tubules is packed with 14 regularly arranged slender tubules with center-to-center spacing of about 45 A. Evidence has been presented suggesting some chemical as well as morphological similarity between accessory tubules and the central pair. Behnke and Forer (1967) found that in sectioned material of the crane fly *Nephrotoma suturalis* the central tubules and accessory fibers are clearly seen whereas the doublet tubules are no longer present. The central and accessory microtubules, therefore, seem to be more resistant to digestion than the doublets. The similarity in structure and perhaps in chemistry between the central tubules and the accessory tubules suggest a similar function.

In electron micrographs in which transverse sections of flagellar tubules are clearly resolved, the walls of the flagellar tubules appear to be composed of regular subunits (André, 1961; Kaye, 1964). The walls of the accessory tubules and of the central pair consist of 13 evenly spaced subunits in sperm flagella of the black scavenger fly *Sepsis* (Phillips, 1966 *c*). Each subunit is approximately 70 A in diameter and, in transverse section, appears to possess an electron-lucent center 30–40 A across. This is in agreement with reports of others that the flagellar doublet tubules of *Chlamydomonas* are each composed of 13 subunits, 3 of which are shared with the adjoining member (Ringo, 1967) and that cytoplasmic microtubules of plant and animal cells are composed of "12 or 13 subunits" (Ledbetter and Porter, 1964; Gall, 1965, 1966; Behnke and Zelander, 1967; Fuge, 1968). On the other hand, Pease (1963) found that each of the central tubules of negatively stained rat sperm flagella consists of only 10 filaments, and André and Thiéry (1963), using similar techniques, determined that the central tubules of human sperm are composed of only 10 or 11 subunits. These results are not necessarily to be regarded as contradictory. It is possible that generic differences may exist in the number of subunits per flagellar tubule. Indeed, such differences may be responsible for the differing sizes of tubules in sperm flagella of different species and differing tubule sizes in the same flagellum, as for example in the accessory fibers and central pair in some Trichoptera.

Exceptions to the 9 + 9 + 2 Tubule Pattern

In a recent paper (Phillips, 1969 *a*) we reported that the tubule pattern of the flagella of a number of insect species differs markedly from the typical 9 + 9 + 2 tubule pattern. In the sperm of the psocid, *Psocus*, the doublet tubules and accessory tubules are not straight as in typical flagella, but are disposed in a long-pitched helix so that they form an angle of about 8° with a single central dense rod that replaces the central pair. Helical flagellar tubules also occur in the flea *Ctenocephalides felis*, but here the flagellum is more complex in its structure than in *Psocus*. Not only do the doublet tubules spiral around the central pair but, in addition, the entire flagellum describes a larger helix around a centrally located rod (Phillips, 1969 *a*, *b*, Smith, 1969). In the wasp, *Dahlbominus fuscipennis*, the flagellum also spirals around the mitochondrial derivative, but the flagellar tubules in this instance appear to be parallel to the central pair (Lee and Wilkes, 1965; Wilkes and Lee, 1965).

The tubule pattern of sperm flagella of other insect species differs from the typical 9 + 9 + 2 in the number of central elements. The most striking example is the replacement of the central pair of tubules by seven symmetrically arranged central tubules in two species of caddis flies. We have observed a 9 + 3 tubule pattern in one fungus gnat species (Phillips, 1969 *b*). 9 + 3 flagella are also characteristic of some spiders (Reger, 1969). Breland et al. (1966, 1968), studying spermatozoa of a species of mosquito, reported a single central tubule, and we have also found a 9 + 9 + 1 pattern to be characteristic of two other

species of mosquitoes, as well as the unusual spermatozoa of *Psocus* previously mentioned. A 9 + 9 + 0 tubule pattern has also been observed in sperm of four species of mayflies (Phillips, 1969 *a*, *b*, and unpublished).

Possibly the most peculiar sperm flagellum thus far described is the giant flagellum of the fungus gnat *Sciara* (Phillips, 1966 *b*; Makielski, 1966). This flagellum, which arises from a giant centriole (Phillips, 1966 *a*, 1967), consists of 70–90 doublet tubules, each flanked by accessory singlets. In cross-sections near the nucleus, the flagellar tubules of mature testicular sperm are disposed in an oval. Posteriorly, the oval becomes discontinuous, and the row of doublets coils like a scroll, so that at posterior levels it forms a spiral in profile. In the female genital tract, sperm undergo further extensive changes, which include uncoiling and subsequent recoiling of the axial filament complex into a different configuration (Phillips, 1966 *b*).

Coccid spermatozoa also possess a highly unusual tubule arrangement. The motile apparatus is composed of evenly spaced microtubules (Moses and Coleman, 1963; Robison, 1966; Ross and Robison, 1969) in concentric rings or, in certain species, an even more complex arrangement (Robison, 1968 *a*, *b*; Ross, 1968).

The microtubules in coccids do not form in relation to a centriole (Moses et al., 1968) and are not doublets. Therefore, the motile apparatus in these forms should probably not be considered a true flagellum but a motile apparatus composed of microtubules comparable to the microtubular motile apparatus of flatworm sperm described by Christensen (1961) and by Silveria and Porter (1964).

The flagellum of sperm in the louse *Pediculus humanus corporis* contains two parallel axonemal complexes, each composed of a typical 9 + 9 + 2 tubule pattern (Ito, 1966). Double axial filament complexes also characterize spermatozoa of certain flatworm species (Sato et al., 1967; Burton, 1967, 1968) and in some fish (Boisson et al., 1967; Stanley, 1965). Louse sperm are among the few spermatozoa that have been studied cinematographically. The form of their flagellar beat appears to be spiral (Ito, 1966).

Baccetti et al. (1969 *b*) have recently described a most peculiar sperm flagellum in the thrip *Cryptothrips latus*. This flagellum consists of what appear to be two 9 + 2 axial filament complexes that have become dissociated. In some transversely cut sperm, 18 doublet and 4 singlet tubules can be counted but they appear to bear no regular spacial relationship to each other.

SYMMETRY OF FLAGELLUM AND MITOCHONDRIA

The position and shape of the mitochondrion or mitochondria with respect to the flagellum bestow bilateral symmetry upon the insect spermatozoan, e.g. Figs. 7 and 21 (see also André, 1961, Fig. 1; Yasuzumi and Oura, 1965, Fig. 14; Werner, 1964, Fig. 22). However, in most species a plane that passes through the center of both central tubules does not coincide with the general axis of symmetry of the flagellum. Such a plane is, instead, slightly oblique to the plane of symmetry established by the mitochondrion (Fig. 21) or mitochondria (Fig. 7). When the arms of subfiber A are directed in a clockwise direction, i.e. when the flagellum is viewed from base to tip (Gibbons and Grimstone, 1960), a line passing through the center of both central tubules is inclined to the right in some species and to the left (passing through eleven o'clock and five o'clock) in other species (Fig. 7). In all the micrographs of a given species, we note that lines passing through the central pairs are always directed to the same side (either all to the right or all to the left). Inverting the figure does not change the side to which the line passing through the central pairs is directed with respect to the axis of symmetry imparted by the mitochondrion or mitochondria.

In one of the first studies of cilia with thin sectioning, Fawcett and Porter (1954) observed that, where a group of transversely cut cilia appear in a micrograph, lines drawn through the center of both tubules of the central pair are nearly parallel in neighboring cilia. From a study of gill cilia of lamellibranch it was concluded by these authors and later by Gibbons and Grimstone (1960) that lines drawn through the central pairs of ciliary tubules are perpendicular to the direction of ciliary beat. These findings were subsequently challenged by Satir (1963), who reported that the central tubules are regularly oriented in nonbeating cilia of *Elliptio* but vary in their orientation in beating cilia instantly immobilized by the fixative.

Recently Fawcett (1968) has demonstrated that the central tubules of guinea pig sperm flagella are oriented in the same direction in each spermatozoon and that a line drawn through the center of both tubules of the central pair is slightly

oblique to the plane of flattening of the sperm head. In insect spermatozoa, Thompson and Blum (1967) measured the angle subtended by a line drawn through the central pair and the line through the plane of symmetry determined by the position of the mitochondrion. They found that the angle varied between 0° and 90°, with no particular angle occurring with high frequency. Our observations are not in accord with this finding. On the contrary, in spermatozoa that appear to us to be well fixed, the axis of the central pair always deviates from the plane of symmetry by a few degrees, and the direction of the displacement of the axis of the central pair is characteristic of the species. In spermatozoa that appear to us to be poorly fixed, the angle by which the axis of the central pair is displaced can be quite variable. It is possible that the axis of the central pair of tubules may change when the flagella are beating as reported by Satir, but it appears that variation of the position of the central pair can be produced artificially during tissue preparation.

Flagellar Formation

There are apparently two common modes of initiation of development of flagella and cilia in plant and animal cells. In some cell types a spherical vesicle appears in association with the end of the basal body from which flagellar tubules will arise. As the flagellar tubules elongate, the vesicle membrane over their tip becomes invaginated and thus encloses the tubules in its cup-shaped indention. The vesicle then elongates as the flagellar tubules elongate and eventually comes in contact with the cell membrane and fuses with it. This type of flagellar formation has been carefully detailed in the water mold *Allomyces arbusculus* by Renaud and Swift (1964) and has also been described in mammals (Sorokin, 1962, 1968; Martinez and Deams, 1968) and insects (Sakai and Shigenaga, 1967; Anderson and André, 1968).

In the second type of flagellar formation the basal body is initially located directly beneath the plasma membrane, and the flagellar fibers extend into an evagination of the cell membrane (Sorokin, 1968; Dingle and Fulton, 1966; Dirksen and Crocker, 1966; Outka and Kluss, 1967; Thornhill, 1967; Steinman, 1968; Johnson and Porter, 1968). Both types of cilia formation occur in the same cell type in mammalian lung epithelium (Sorokin, 1968). In both modes of flagellar formation, later stages involve an elongating flagellum with a swelling at the growing end (Sorokin, 1962, 1968; Renaud and Swift, 1964; Outka and Kluss, 1967) (Fig. 4).

In the two insects in which the early stages of flagellar formation have been closely followed, the neuropteran *Chrysopa* (Friedlander and Wahrman, 1966) and the apteran *Lepisma* (Werner, 1964), flagellar formation is initiated deep within the cytoplasm and utilizes the vesicle system. We have noted vesicles in association with forming flagella in early spermatids of other insect species (Fig. 13) but have not followed their development closely. Flagellar formation of the type that involves direct interaction between plasma membrane and centriole has also been observed in insect germ cells. It occurs during a process termed "centriole blebbing" (Hoage and Hessel, 1968) in honeybee spermatocytes. The flagella formed during the process are, however, very rudimentary (Hoage and Kessel, 1968).

The process of flagellar formation in spermatids of *Sciara* is unique. Here the tubules are not initially associated with a vesicle or plasma membrane but extend directly into the cytoplasm (Phillips, 1966 *a*) from a centriole located deep in the cytoplasm.

The accessory tubules of insect flagella form after the flagellar doublets and central pair (Kaye, 1964; Cameron, 1965; Phillips, 1966 *a*; Meyer, 1963). The details of accessory tubule formation have been carefully studied in the mealworm, *Tenebrio molitor*, by Cameron (1965). The earliest stage in the process is the appearance of an arm extending from subfiber B of each doublet. These arms were noted by others (Kaye, 1964; Phillips, 1966 *b*; Kessel, 1967) but they were not recognized as precursors of the accessory fibers. Cameron reported that they elongate by accretion to their free margin and soon appear in cross-section as curved C-shaped structures. They subsequently detach from the doublet and close the gap in the "C" to form complete tubules. We have followed a similar process of outer tubule formation in other species (Figs. 5 and 14). What appear to be successive stages in the process are seen presented in Fig. 14. Cameron noted that central elements inside the tubules form some time after the walls of the tubules are complete. This appears to be true in other insects as well (Kaye, 1964; Oura and Yasuzumi, 1966). Behnke (1967) has reported that forming microtubules in mammalian blood platelets are also initially

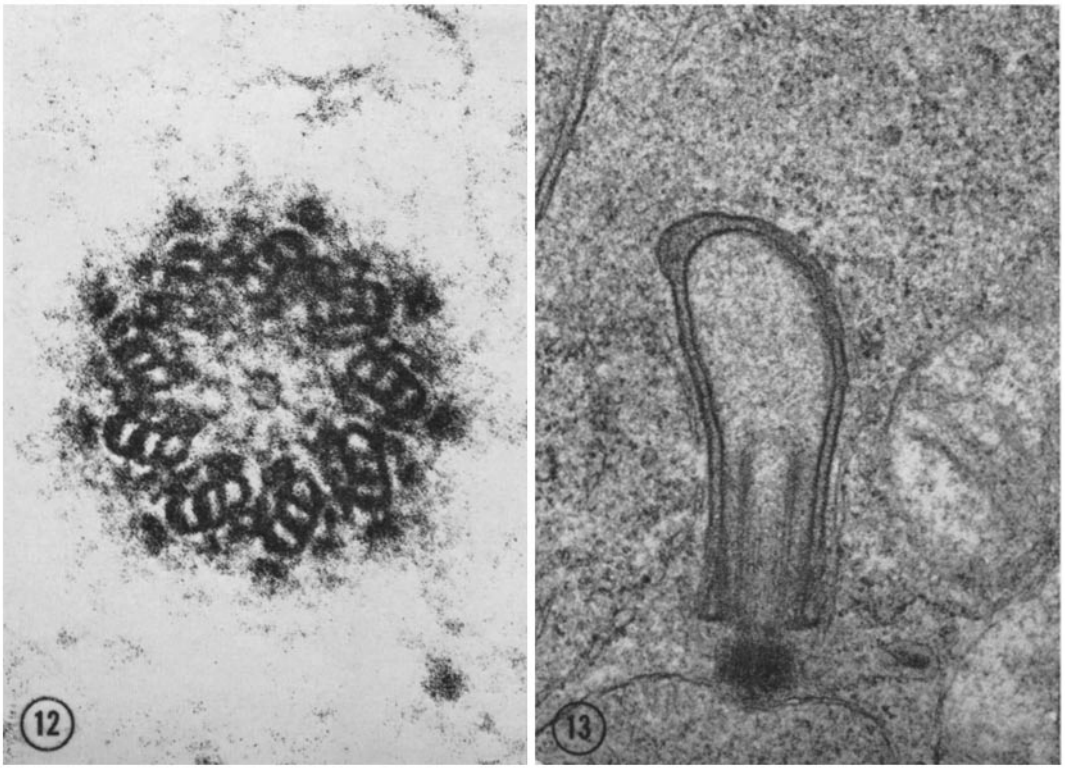


FIGURE 12 Transverse section of a centriole in a spermatocyte of the mosquito *Culex* sp. The centriole is composed of a triplet of tubules and generally is similar in appearance to centrioles that have been described in animal and plant cells. The tubules on one side are seen in near perfect cross-section, while those on the opposite side are transected slightly obliquely. This indicates that the triplets are not parallel but describe a gentle helix. × 193,000.

FIGURE 13 Longitudinal section of an arising flagellum in a spermatid of *Culex*. The flagellum extends into a flattened, cuplike vesicle. × 39,000.

C-shaped and later close to become complete tubules; thus formation of tubules by lateral accretion of subunits to a free edge is not confined to the accessory tubules of flagella.

CENTRIOLES

With the exception of the giant centrioles of *Sciara* the centrioles found in insect spermatocytes are not unusual and have attracted little investigative attention. To our knowledge the only published micrographs clearly demonstrating the structure of centrioles in insect germ cells are those of Hoage and Kessel (1968) and Anderson and André (1968) that show typical centrioles with nine triplets in their wall. We have also observed centrioles of typical structure in spermatocytes of six insect species (Fig. 12). In some micrographs in which the triplet tubules on one side of the centriole appear in true transverse section, those in the opposite wall of the centriole are cut obliquely (Fig. 12), suggesting that in insects, as in many other organisms (André and Bernhard, 1964; Fawcett, 1966), the centriolar triplets may not be straight but probably have a long pitched helical course.

Young spermatids of insects contain two centrioles, one of which serves as a basal body for the growing flagellum while the other is oriented at right angles to the first (Werner, 1964; Friedlander and Wahrman, 1966; Breland et al., 1968; Smith, 1969). In all species of insects that we have examined, both centrioles disappear during spermio-

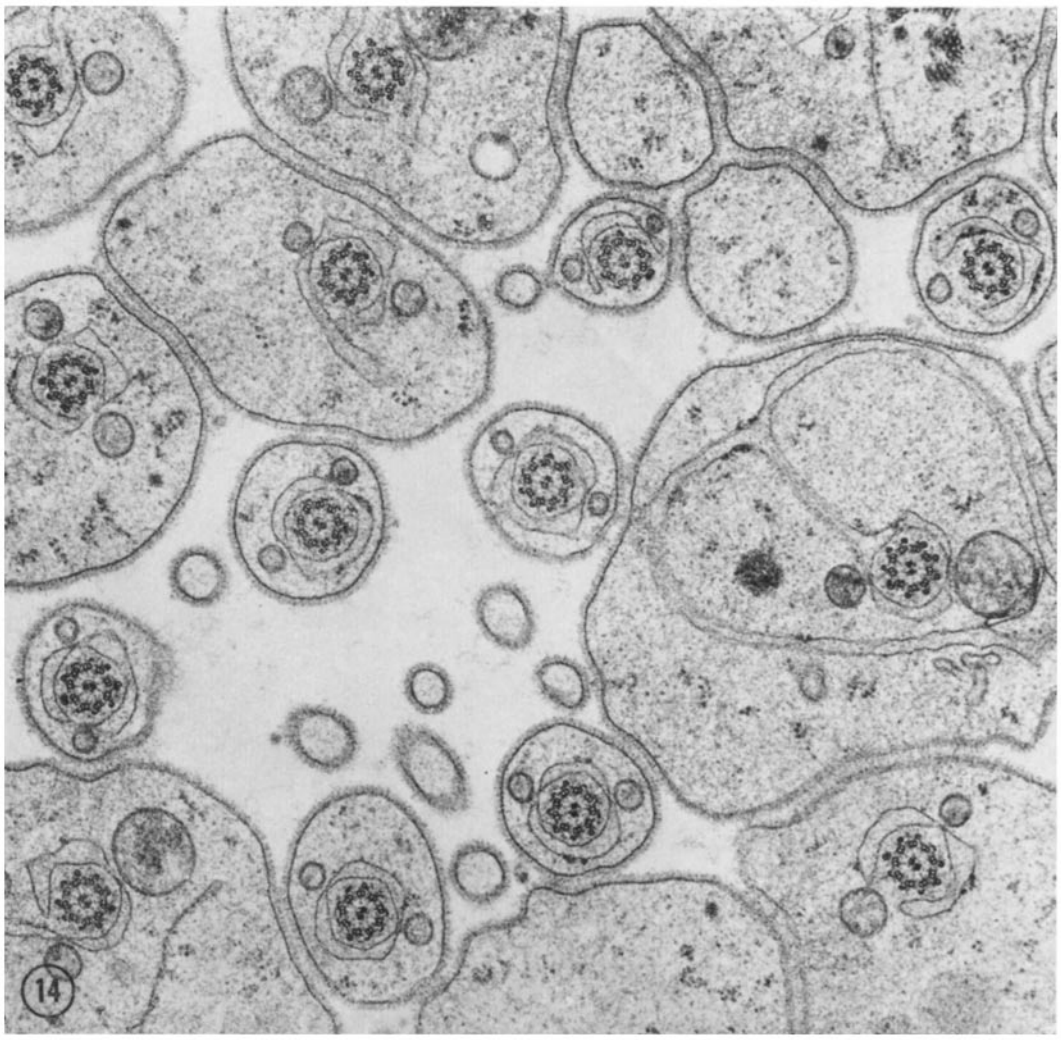


FIGURE 14 The accessory fibers of insect sperm form as curved arms which extend from subfiber B of each doublet. These arms subsequently become detached from the doublet and close to form complete tubules. Stages of this process are seen in these spermatids of the spur-throated grasshopper, *Melanoplus*. × 28,000.

genesis. In many species a mass of granular material that has been termed the centriole adjunct (Gatenby and Tahmisian, 1959; Breland et al., 1966), forms around the centriole that is oriented perpendicular to the axis of the flagellum. This centriole becomes obscured by the density of the material in which it is embedded and eventually can no longer be distinguished.

The nuclear envelope in the region of the centriole is modified. A thin band of dense material is deposited on its inner aspect giving it a thickened appearance (Figs. 3–5). In a number of species, intranuclear material, possibly modified chromatin, aggregates in the nucleoplasm opposite the basal body. In Fig. 15 a number of late spermatids of the moth *Crambus* have been transected through various levels at or very near the base of the flagellum. Because these spermatids are in nearly perfect register, adjacent cells are transected at approximately the same level. The peripheral singlets are seen to extend anteriorly into an indentation in the nucleus and beyond

the proximal termination of the doublets. The doublets terminate near the posterior margin of the nuclear indentation, and the central pair terminates somewhat farther posterior. No centriole is observed at the base of these flagella. No triplets are observed, and the tubules that terminate at the base of the flagellum at different levels appear identical in fine structure to the tubules seen at more posterior levels. Thus, it is apparent that no centriole occurs at the base of the flagellum in this species, and no centrioles have been found in spermatozoa of any of the other insect species we have studied. It is difficult to be certain that there are no centrioles in all species examined. In some species, we have seen many sperm transected through the region at the base of the flagellum, but in others we have observed just a few cells cut through this region. In at least nine species representing the orders Coleoptera, Ephemeroptera, Diptera, Trichoptera, Lepidoptera, Homoptera, and Hemiptera, we are convinced that there are no centrioles present in mature sperm, and in others we feel fairly sure that this is the case. Since we have also not seen centrioles in micrographs of mature sperm published by other authors, it may be generally concluded that mature insect sperm possess no centrioles.

The role of sperm-derived centrioles in cleavage of fertilized eggs was the subject of much controversy among early cytologists and embryologists. In many species of animals, sperm-asters seem to form around the neck or middle-piece of sperm in the fertilized egg. This led to the belief that the centrioles of the adult organism were in part derived from the centriole introduced by the sperm. Indeed, Boveri theorized that the introduction of a centriole was the primary function of the sperm in fertilization. The issue long remained unresolved owing to the difficulties of tracing with the light microscope the minute centrioles that can only be observed in fixed material and are easily overlooked even in serial sections. The confusion about the role of sperm centrioles in fertilization was compounded by the fact that sperm-asters are much more difficult to visualize in some species than in others and that they generally disappear before the asters of the first cleavage appear. Moreover, formation of cytasters can be induced by agents other than sperm (for discussion see Wilson, 1928). Since insect spermatozoa do not contain centrioles it must be concluded that, at least in insects, introduction of one or more centrioles into the egg by the sperm is not necessary for initiation of cleavage.

THE CENTRIOLE ADJUNCT

In an early electron microscopic investigation of spermiogenesis, Gatenby and Tahmisian (1959) diagrammed the morphogenesis of a dense component around the basal body in spermatids of *Melanoplus differentialis* and *Nemobius* sp. (Orthoptera). This structure probably corresponds to the pericentriolar structure that early students of spermiogenesis referred to as a *pseudoblepharoplast* (Bowen, 1920) or *postnuclear body* (Johnson, 1931). Modern morphologists have referred to this structure in various insect speceis as the juxtanuclear body (Sotelo and Trujillo-Cenoz, 1958), basophilic centriole (Kaye, 1962), flagellar accessory structure (Kaye and Kaye, 1966), perinuclear granular aggregate (Kessel, 1967), granular material (Kessel, 1966), dense fibrous material (Phillips, 1966 *a*), dense material (Anderson, 1967), and as the centriole adjunct (Nũnez, 1963; Schin, 1965; Breland et al., 1965, 1966, 1968). Confusion is compounded by the fact that this structure has been mislabeled "ring centriole" in a widely used cytology text (De Robertis et al., 1965).

The early history of this structure, which we shall refer to as the centriole adjunct, is similar in many species. The dense material first appears in intimate association with the basal body and increases in amount during early spermiogenesis. In some species, e.g. *Culiscta inornata* (Diptera, Culicidae) (Breland et al., 1966), *Sciara coprophila* (Diptera, Sciaridae) (Phillips, 1966 *a*), *Gryllus domesticus* (Orthoptera, Gryllidae) (Kaye, 1962), and *Melanoplus differentialis differentialis* (Opthoptera, Acrididae) (Kessel, 1967), more than a square micron of area may be occupied by this material in sections of early spermatids. It sometimes appears granular (Kessel, 1966, 1967) (Figs. 4 and 5), and in other species it appears fibrous (Kaye and Kaye, 1966; Phillips, 1966 *a*). Breland et al. (1966) have shown in several species of mosquitoes that the centriole adjunct, which is quite extensive in spermatids, decreases markedly in size during spermiogenesis and in mature sperm is a relatively small, very dense structure. In certain insects, namely *Sciara* (Phillips, 1966 *a, b*), some species of Lepidoptera (Fig. 15) and some trichoptera, the centriole adjunct disappears entirely during spermiogenesis. This organelle of insect sperm is located in a

position corresponding to that of the columns of the connecting piece in mammalian spermatozoa, and it has been proposed (Breland et al., 1966; Fawcett and Phillips, 1969 *a*), that the centriole adjunct may serve to secure the flagellum to the sperm head.

MICROTUBULES

Microtubules in large numbers first become apparent in spermiogenesis at the onset of elongation. Rows of microtubules are then observed encircling the spermatid nucleus (Werner, 1966; Phillips, 1966 *a*; Kessel, 1966, 1967; Shoup, 1967), the mitochondrial derivatives (Yasuzumi and Oura, 1965; Kessel, 1966; Shoup, 1967), and the acrosome (Tandler and Moriber, 1966). Microtubules parallel to the long axis of the cell but not associated with particular organelles also appear in the cytoplasm at this time (Phillips, 1966 *a*; Behnke and Forer, 1967).

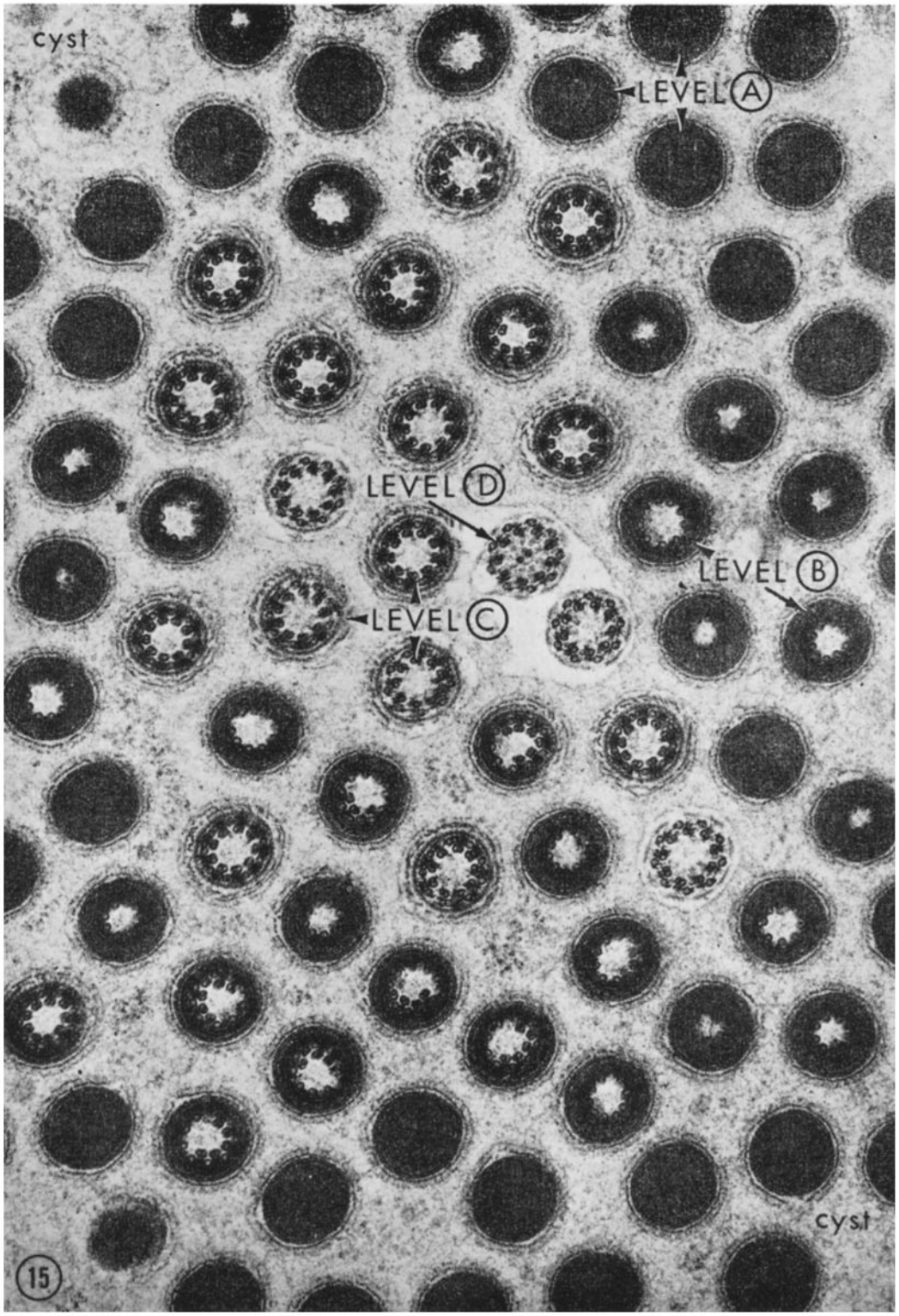
In early stages of spermatid elongation in the dragonfly *Aeschna grandis*, Kessel observed that rows of microtubules oriented parallel to the long axis of the cell are situated in deep indentations of the nucleus. The furrows in the nucleus deepen as the chromatin condenses, and when condensation is complete the microtubules disappear (Kessel, 1966). Kessel concluded from these observations that microtubules may play a role in elongation of the spermatid nucleus. Perinuclear microtubules are observed during the period of nuclear condensation and elongation in many insect species, but the dispositon of the tubules and the shape of the nucleus are often quite different from those described by Kessel in *Aeschna*. In *Sciara*, the condensing spermatid nucleus has two lateral furrows, but, contrary to the situation in *Aeschna*, the tubules are absent from the furrows and appear instead on all other sectors of the nuclear surface (Phillips, 1966 *a*). In *Drosophila melanogaster*, microtubules occur in particular locations parallel to the long axis of the cell and generally along convex surfaces of the irregularly shaped nucleus (Shoup, 1967). Microtubules disappear in later stages of sperm formation in all insect species studied. There are no reports of tubules around nuclei of mature sperm except the microtubules of the motile apparatus of coccids.

Observations by Shoup (1967) on the translocation heterozygote T(1:23)25(20)y/25/FM6 of *Drosophila melanogaster*, which is characterized by male sterility, support the idea that microtubules play a role in the differentiation of the sperm head. Nuclei of spermatids in males carrying this translocation do not complete condensation and elongation (Lindsley et al., 1960), and the earliest recognizable morphological abnormality of these spermatids is the absence of microtubules around the nucleus.

In the spermatids of many animal species examined with the light microscope, a sheath called the manchette may be observed surrounding the caudal pole of the nucleus and extending posteriorly in the spermatid cytoplasm (Wilson, 1928). The manchette is found, in electron micrographs of spermatids of mammals (Burgos and Fawcett, 1955; Fawcett and Phillips, 1967), birds (McIntosh and Porter, 1967), reptiles (Boisson and Mattei, 1965), annelids (Bradke, 1963; Anderson et al., 1967; Anderson and Ellis, 1968), and platyhelminthes (Silveira and Porter, 1964), to consist of microtubules. The perinuclear tubules of insect spermatids are undoubtedly analogous to the manchette of other animals, though whether their principal function is in nuclear shaping and condensation is, at present, conjectural.

The role of microtubules surrounding elongating

FIGURE 15 Although young insect spermatids have two centrioles, one of which serves as a basal body for the flagellum, we have observed no centrioles in late spermatids or sperm of any of the species we have studied. Most of the late spermatids shown in this micrograph of the moth *Crambus* have been transected at the base of the flagellum where one would expect to observe centrioles if any were present; however, none are observed. Circular cross-sectioned profiles of electron-opaque nuclei are observed in the most anteriorly sectioned cells (*A*). In spermatids that are presumably cut at slightly more posterior levels (*B*), accessory fibers are observed within a ring of dense chromatin. At still more posterior levels (*C*) the doublet tubules are also observed, and in the cell which we presume has been cut at the most posterior level (*D*) the central pair is also seen. The spermatid heads are embedded in epithelial cells that compose the cyst wall (*cyst*). × 36,000.


cyst
LEVEL (A)
LEVEL (D)
LEVEL (B)
LEVEL (C)
cyst
15

mitochondria (Fig. 10) and those free in the cytoplasm is likewise uncertain. But inasmuch as tubules have been implicated in the process of cell elongation in other cell types (Byers and Porter, 1964; Overton, 1966), one might expect that they play a similar role in differentiation of insect spermatids.

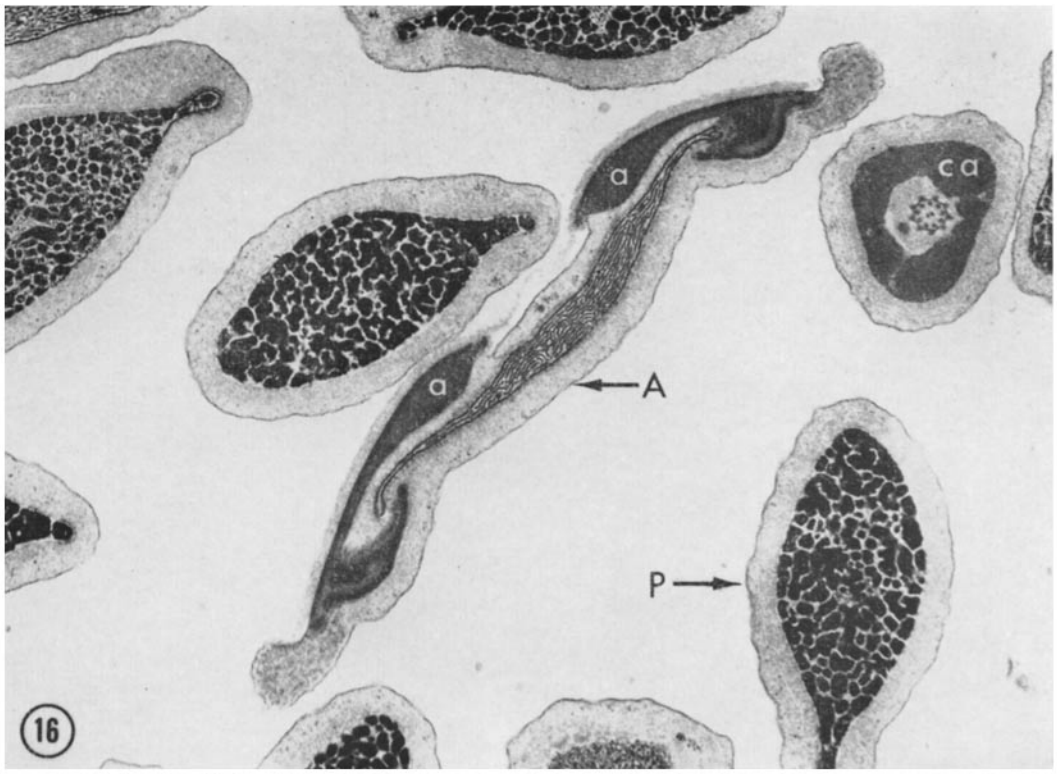
NUCLEAR CONDENSATION

In the period when the nucleus is decreasing in size and chromatin is becoming more compacted, concomitant chemical changes are known to take place in the nucleus. In a number of organisms there is a transition from lysine-rich to arginine-rich histones or protamines (Pollister and Mirsky, 1946; Felix et al., 1956; Alfert, 1956; Bloch and Hew, 1960; Das et al., 1964; Bloch and Brack, 1964; Kaye and Kaye, 1966). Bloch and Brack (1964) reported that, in the grasshopper *Chortophaga*, arginine-rich histone is synthesized in the cytoplasm fairly late in spermiogenesis and subsequently migrates into the nucleus. Dass and Ris (1958) felt that there was a correlation between the shift in nuclear basic proteins and a change in the appearance of chromatin fibers. They found that the chromatin of young spermatids of the grasshopper *Chorthippus* was made up of 200–250 A fibers, each composed of two 100 A subunits. During spermiogenesis it appeared that these 100 A subunits underwent further division into subunits of 40 A diameter. Unfortunately the precise temporal relationship between these changes in chromatin structure and the shift in basic proteins in unclear. The structural reorganization undergone by the chromatin during nuclear condensation certainly does not follow the same course in all insect species. Though the chromatin is generally very diffuse in early spermatids and is extremely dense and devoid of visible substructure in mature insect sperm, the intermediate stages vary widely in appearance in different species. The course of events in many species appears to be quite the opposite of the reorganization of chromatin into thinner filaments during spermiogenesis reported in *Chorthippus* by Dass and Ris. In the house cricket, Kay and Kaye (1966) found that chromatin fibers became *thicker* early in spermiogenesis and that the thickening occurred prior to the change in character of the basic proteins. We have observed that in some species, such as the spur-throated grasshoppers, chromatin filaments appear to become progressively thicker during spermiogenesis, and at any given stage the appearance of the chromatin is uniform throughout the nucleus. In other species, chromatin condensation is nonuniform. For example, in the treehopper *Ceresa* (Fig. 17), chromatin at some stages is farther advanced in condensation around the periphery of the nucleus than in the center. In the bush katydid *Scudderia* (Fig. 16) the chromatin at the anterior end of the nucleus is less condensed than in posterior regions. In such species, chromatin strands of several different diameters can be measured in the same nucleus. Generally, we find that strands of chromatin become progressively thicker as spermiogenesis proceeds rather than thinner as Dass and Ris reported.

THE ACROSOME

Studies with the light microscope established that the acrosome in insects, as in other animals, is a product of the Golgi complex (Bowen, 1920; Pollister, 1930; Johnson, 1931). Initially a spherical body, termed the proacrosome granule, appears in the Golgi complex. As this granule becomes larger it becomes intimately associated with the nucleus. The Golgi complex subsequently migrates into the caudal cytoplasm. The proacrosome granule gradually changes in shape in subsequent development, attaining its final form very late in spermiogenesis.

Electron microscopic studies have confirmed and added some detail to earlier observations. In the insect species in which acrosome formation has been described, the proacrosome granule is situated on the concave face of the Golgi complex, between its innermost cisternae and the spermatid nucleus (Gatenby and Tahmisian, 1959; Kaye, 1962; Phillips, 1966). This relationship prevails in many species (Fig. 18). This is consistent with numerous reports, in many somatic cell types, that secretory granules are formed on the concave face of the Golgi complex (Grassé, 1957; Policard et al., 1958; Mollenhauer, 1963; Daniels, 1964; Friend and Murray, 1965; Friend, 1965). However, in one insect species, the meadow grasshopper *Conocephalus*, the proacrosomal granule appears to arise on the convex side of the Golgi complex, which is situated between the proacrosome granule and the nucleus (Fig. 19). The only other instance in which granules have been reported to form on the convex side of the Golgi complex is in developing rabbit polymorphonu-



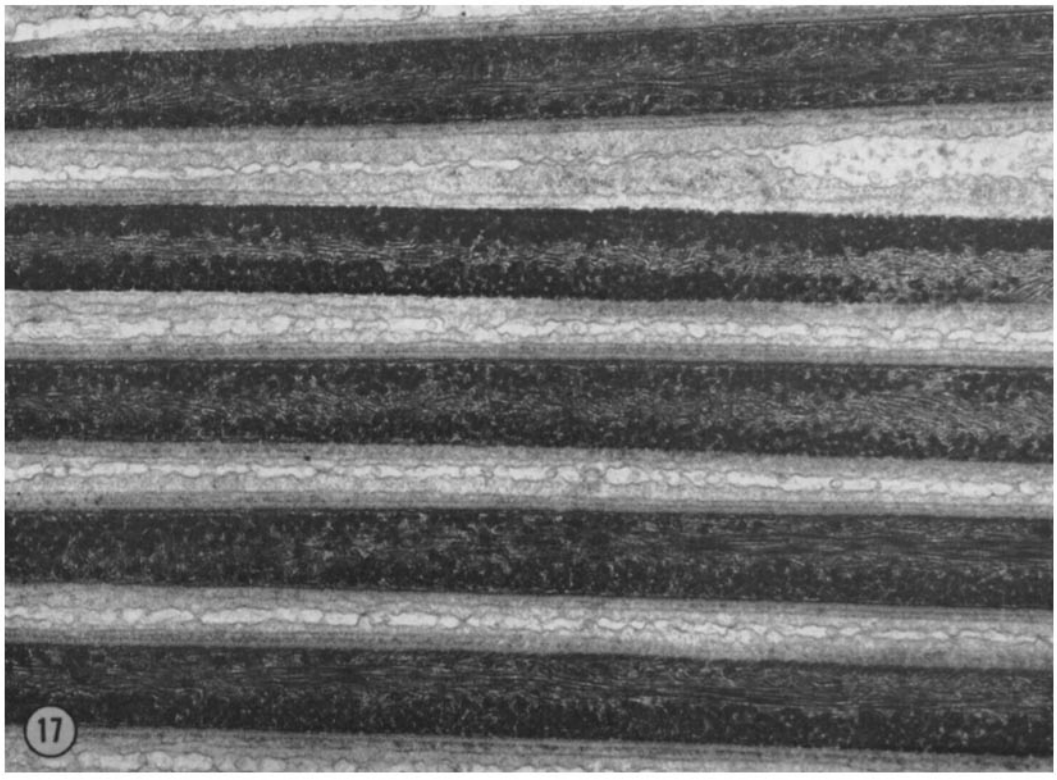


FIGURE 16 Transversely sectioned spermatid heads of the bush katydid *Scudderia*. In the cell sectioned most anteriorly (*A*), the chromatin appears as thin lamellae, whereas in nuclei sectioned farther posteriorly (*P*), the chromatin is clumped. Acrosome, (*a*); centriole adjunct, (*ca*). × 16,000.

FIGURE 17 Longitudinal section of spermatid heads of the treehopper (Membracidae) *Ceresa diceros*. Chromatin around the periphery of the elongate, cylindrical nuclei is condensed into large clumps while the central chromatin appears as narrower filaments. × 18,000.

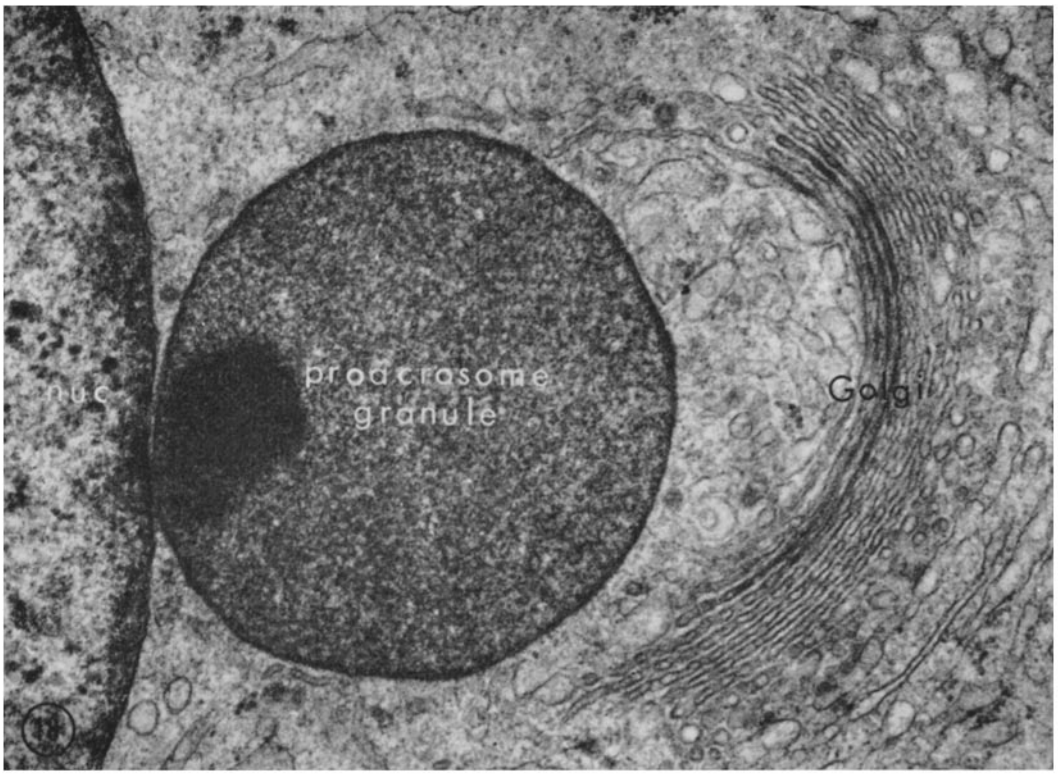


FIGURE 18 Acrosome formation in a young spermatid of *Euschistus* (Hemiptera, Pentatomidae). A proacrosome granule contiguous with the nucleus lies in its characteristic position below the concave side of the Golgi complex. × 53,000.

clear leukocytes. Bainton and Farquhar (1966) demonstrated clearly that azuorphil granules, the earlier of the two types of granules that form during development of these cells, are formed on the concave side of the Golgi complex, whereas the specific granules, which are synthesized later, form on the convex face of the Golgi complex. Here, as in insect spermatids, the granules that form on the concave face of the Golgi complex are situated between the Golgi complex and the nucleus, whereas the granules that form in association with the convex side are located peripheral to the Golgi complex. Thus it appears that granules can form on either side of the Golgi complex. In insect spermatids the proacrosomal granule originates on the concave face in most species but arises on the convex face in at least one insect, the meadow grasshopper *Conocephalus*.

The size, shape, and internal substructure of fully formed acrosomes show wide species variations ranging from very large and often highly structured acrosomes of some hemipterans (Payne, 1966; Tandler and Moriber, 1966) to the minute homogeneous acrosome of *Sciara* (Phillips, 1966 *b*). Spermatozoa of some species of caddis fly possess very small acrosomes consisting of a thin rim of material around the anterior edge of the nucleus. Two caddis fly species we have studied, *Polcentropus* and *Hydropsych*, appear to possess spermatozoa with no acrosome at all (Fig. 20).

CELL SURFACE MODIFICATIONS

Elaborate laminated appendages project radially from the plasma membrane like blades on a paddle wheel and extend from the anterior tip posteriorly along most of the length of lepidopteran spermatozoa. Such appendages were first described by André (1959, 1961) in sperm of two butterflies, *Pieris* and *Macroglossom*. André termed these structures "appendices laciniae." Similar structures were observed in the silkworm *Bombyx mori* by Yasuzumi and Oura (1965), and we have

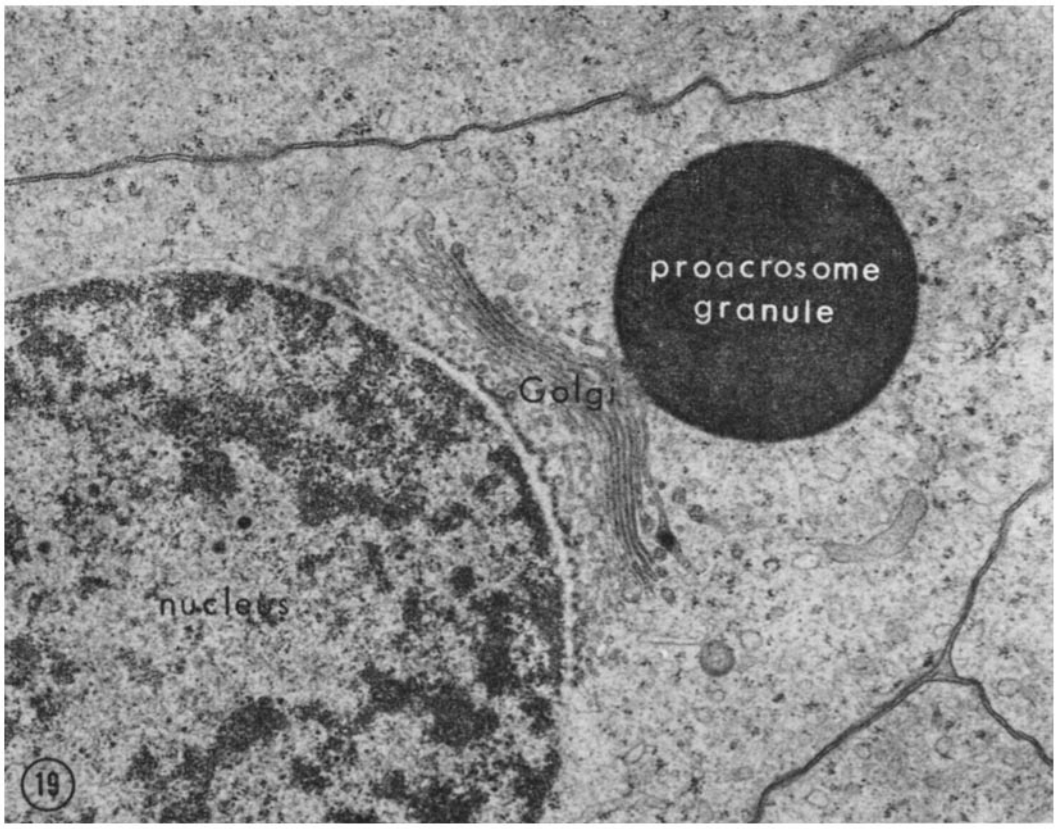


FIGURE 19 In the meadow grasshopper *Conocephalus* sp. the proacrosome granule forms in relation to the convex side of the Golgi complex, and the Golgi complex is situated between the spermatid nucleus and proacrosome granule. We have observed this spatial relationship between the proacrosome granule and the Golgi complex in testis of several individuals of this species, but the relationship illustrated in Fig. 18 prevails in all other insect species studied. × 18,000.

found them on testicular sperm of all 18 lepidopteran species examined (Fig. 21). When lepidopteran sperm are viewed in transverse section, all but one of the radial surface projections appear to consist of regularly spaced thin laminae with a 90 A periodicity. The internal substructure of one appendage differs from that of the others in having a reticular fine structure. The reticular appendage is consistently located on the cell membrane adjacent to flagellar doublet number 6 (according to the conventional system of numbering flagellar tubules—see Fawcett 1961, 1966).

The shapes of the bladelike appendages, as viewed in cross-sections, vary somewhat along the length of the spermatozoan and may be quite different in different lepidopteran species. In some, for example, they are columnar, in others they appear more nearly trapezoidal (Fig. 21), and in still others they may appear clavate, pyramidal, or rectangular.

In seven of the lepidopteran species from which we have taken testicular sperm, we have also examined spermatozoa in the ejaculatory ducts where spermatozoa are stored as they pass from the testis down the male genital tract. In sperm of all seven species examined, the appendices laciniae undergo marked configurational rearrangements. In the sperm of ejaculatory duct, the appendages consist of a single sleeve of material that surrounds the entire sperm cell (Fig. 22). The size of the sleeve varies among species and along the length of the cell in any one species. A single appendage attached to the sleeve probably represents the reticular appendage described above.

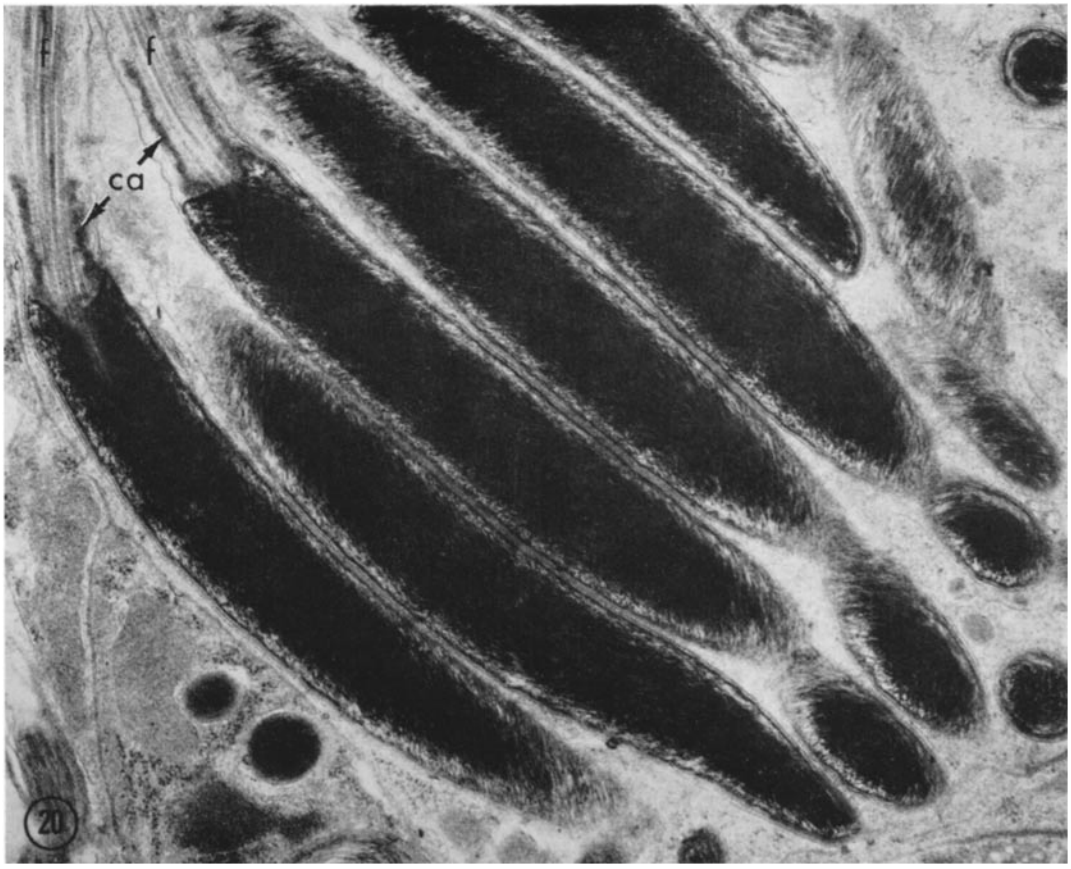


FIGURE 20 Mature sperm of *Polycentropus* sp. We have examined imaginal testes of several individuals of the trumpet net caddis flies *Polycentropus* sp. and *Hydrosyche* sp. The chromatin in spermatozoa always appears as a thin filament. The filaments are similar to prokaryote rather than eukaryote chromatin. We have never observed a completely condensed nucleus. These sperm also appear to lack an acrosome. We have never observed acrosomes in micrographs such as the one shown here of longitudinally transected spermatid heads. Flagellum, (*f*); centriole adjunct, (*ca*). × 16,000.

This appendage is apparently less modified during passage through the male tract than the appendices laciniae. Riemann (1969) has recently reported similar observations in a moth, the cabbage looper *Trichoplusia ni*.

Sperm of some species of grasshoppers also possess cell surface amplifications but these are not nearly so spectacular as those of the Lepidoptera. The surface of orthopteran sperm is covered by a fine radially striated fibrillar coat. The fibrillar mantle of sperm of *Melanoplus differentialis differentialis* presents a honeycomb pattern when sectioned parallel to the cell membrane (Roth, 1957). The pattern was found by Kessel (1966) to be made up of fibers 300–350 A long packed together to make a repeating hexagonal pattern with 130 A center-to-center spacing.

LOSS OF CYTOPLASMIC ORGANELLES

Mature insect spermatozoa lack ribosomes, Golgi complex, and many of the other cytoplasmic organelles present in young spermatids. It is assumed that the mechanism by which cytoplasmic organelles are generally eliminated from somatic cells is intracellular degradation involving the acid hydrolases of lysosomes (Altmann, 1955; de Duve, 1959; Majno et al., 1960; Novikoff, 1961) or areas of focal cytoplasmic degradation

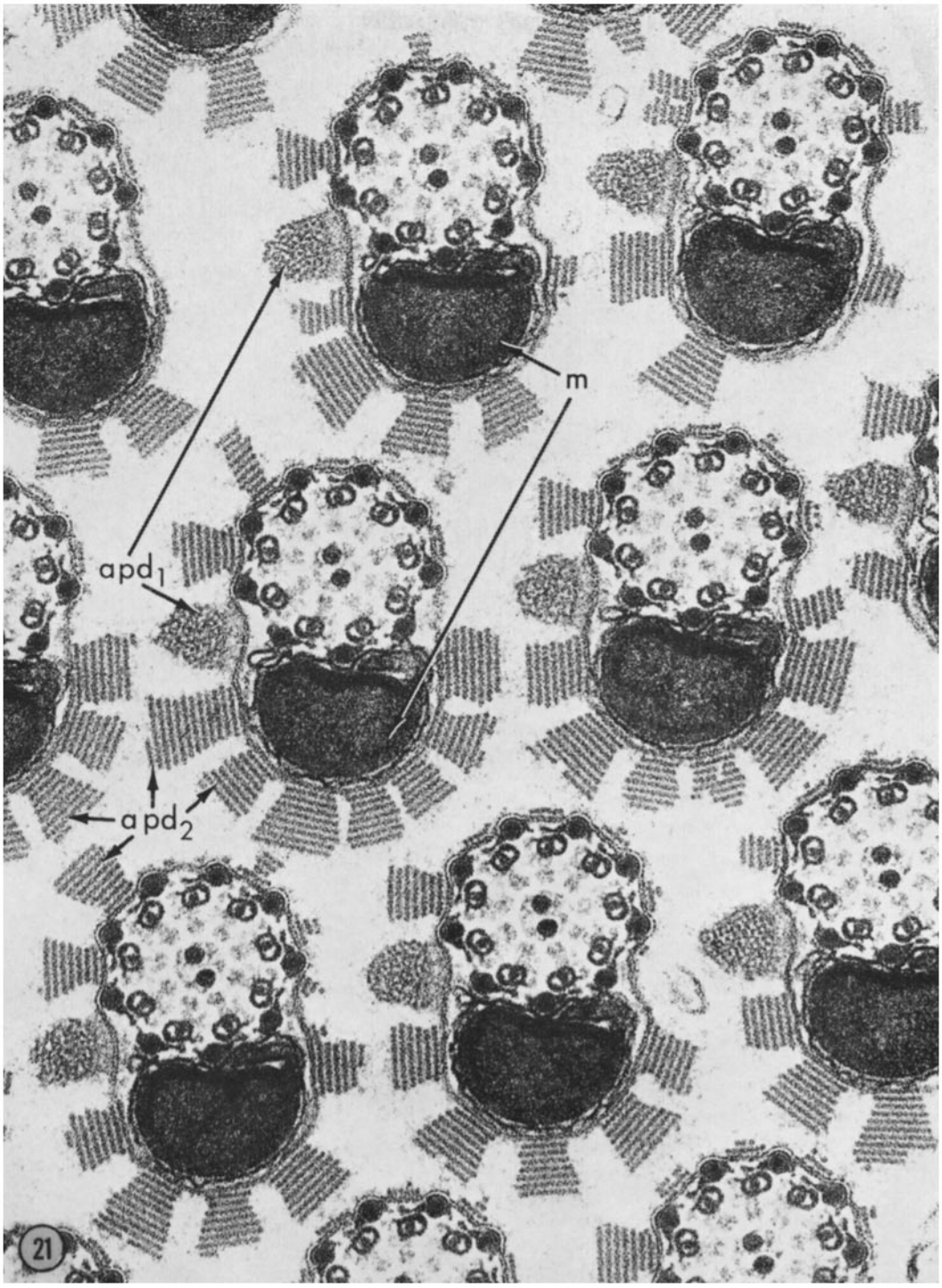


FIGURE 21 Transversely sectioned sperm of the butterfly *Ancyloxypha* (Hesperidae) showing the appendages of the cell membrane characteristic of lepidopteran spermatozoa. One of the appendages (*apd 1*), always located peripheral to doublet number 6 of the flagellum, is dissimilar to the others. The other appendages (*apd 2*) are seen as broad columns composed of evenly disposed light and dense bands with center-to-center spacing of 90 A. Mitochondrial derivative (*m*). × 123,000.

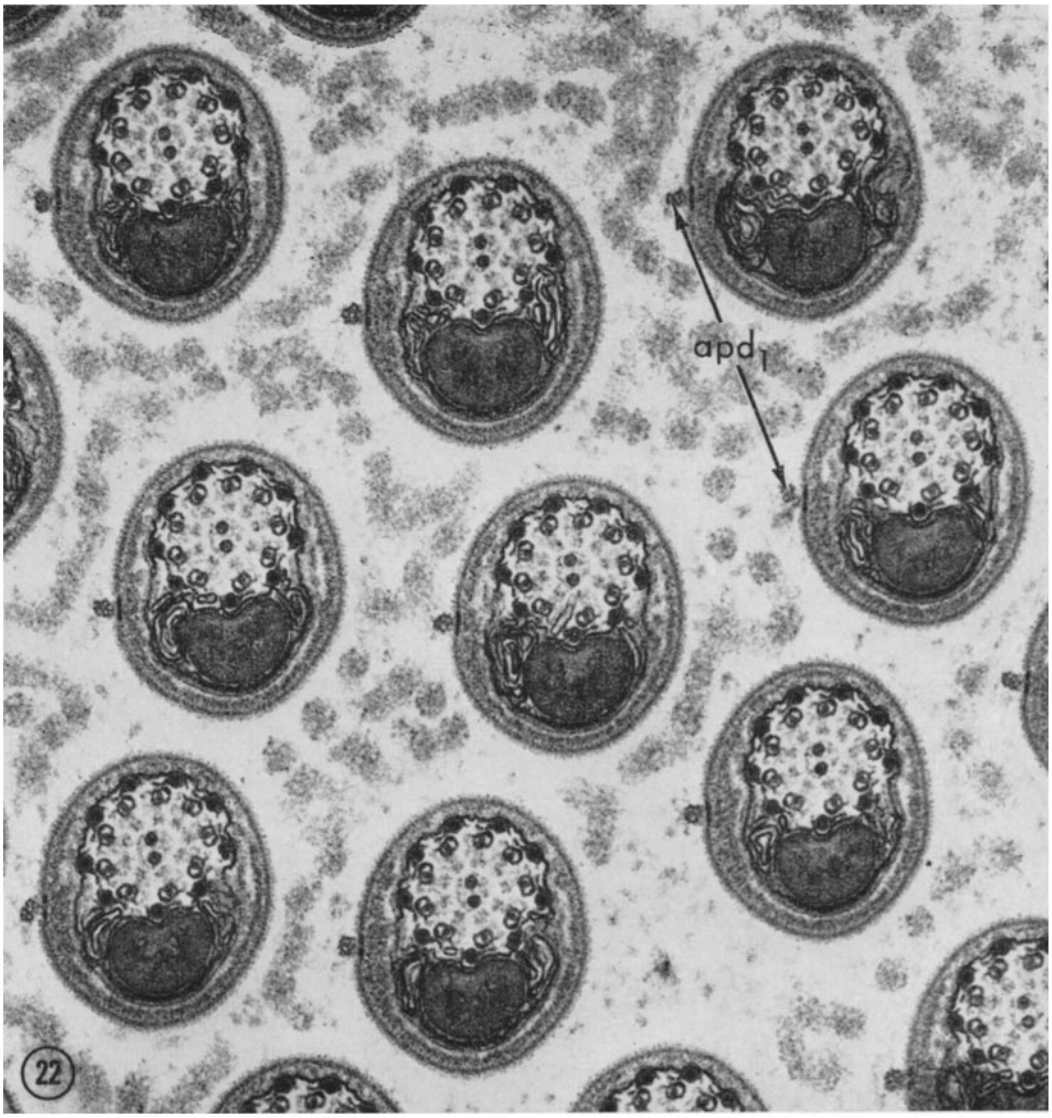


FIGURE 22 Transected sperm from the ejaculatory duct of the moth *Desmia funeralis* (Pyralidae). A single sleeve of fibrous material, probably derived from the appendices lacinies, surrounds the entire cell. The single reticular appendage (*apd 1*) appears virtually unaltered. × 84,000.

(Hruban et al., 1963; Swift and Hruban, 1964). However, lysosome-like bodies and other evidence of cytoplasmic degradation are conspicuously absent from micrographs of insect spermatids. Sloughing off of excess cytoplasm, rather than intracellular degradation, appears to be the means whereby spermatids divest themselves of superfluous organelles. In *Sciara* spermatids, areas of cytoplasm surrounded by cell membrane and containing many ribosomes are often segregated from the main body of the spermatid and connected to it by only a thin strand of cytoplasm (Phillips, 1966 *a*). In the grasshopper *Melanoplus*, large whorls of membrane enclosing bits of cytoplasm are sometimes seen in continuity with the spermatid cell body (Fig. 23). These are presumed to be manifestations of the process of abscission of excess cytoplasm. A similar process also occurs during maturation of mammalian spermatids. In mammals the "residual body" or "sphere chro-

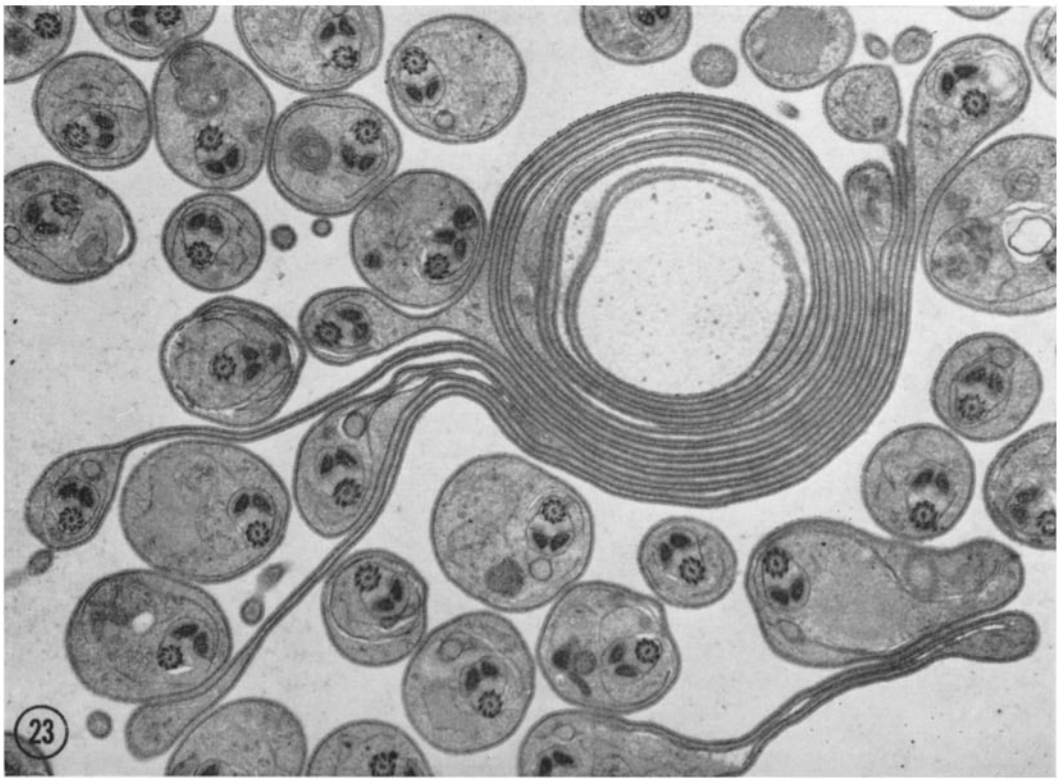


FIGURE 23 Cell membrane and cytoplasm sloughed off from *Melanoplus* spermatids forms structures vaguely reminiscent of the myelin sheath. × 8,500.

matophile" containing RNA (Daoust and Clermont, 1955; Kingsley-Smith and Lacy, 1959), histone (Vaughn, 1966), protein (Sud, 1961), and glycogen is left behind when maturing spermatids are released from the germinal epithelium (Fawcett and Phillips, 1969 *b*).

CONCLUDING REMARKS

In the preceding pages, the author has attempted to illustrate the variations in spermiogenesis and in mature sperm in the Insecta. It is hoped that this review, though limited in its scope, will help to promote the utilization of this interesting material for the investigation of general cytological problems, as well as provide a background for further morphological studies.

This work was begun in the laboratory of Dr. Hewson Swift who first stimulated my interest in insects. Most of the research reported here was carried out in the laboratory of Dr. Don W. Fawcett. I am very grateful to him not only for the use of his facilities, but also for encouragement and guidance and for his help in the preparation of this review. I would also like to thank Doctors Jean-Paul Revel and Susumu Ito for their encouragement and help.

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