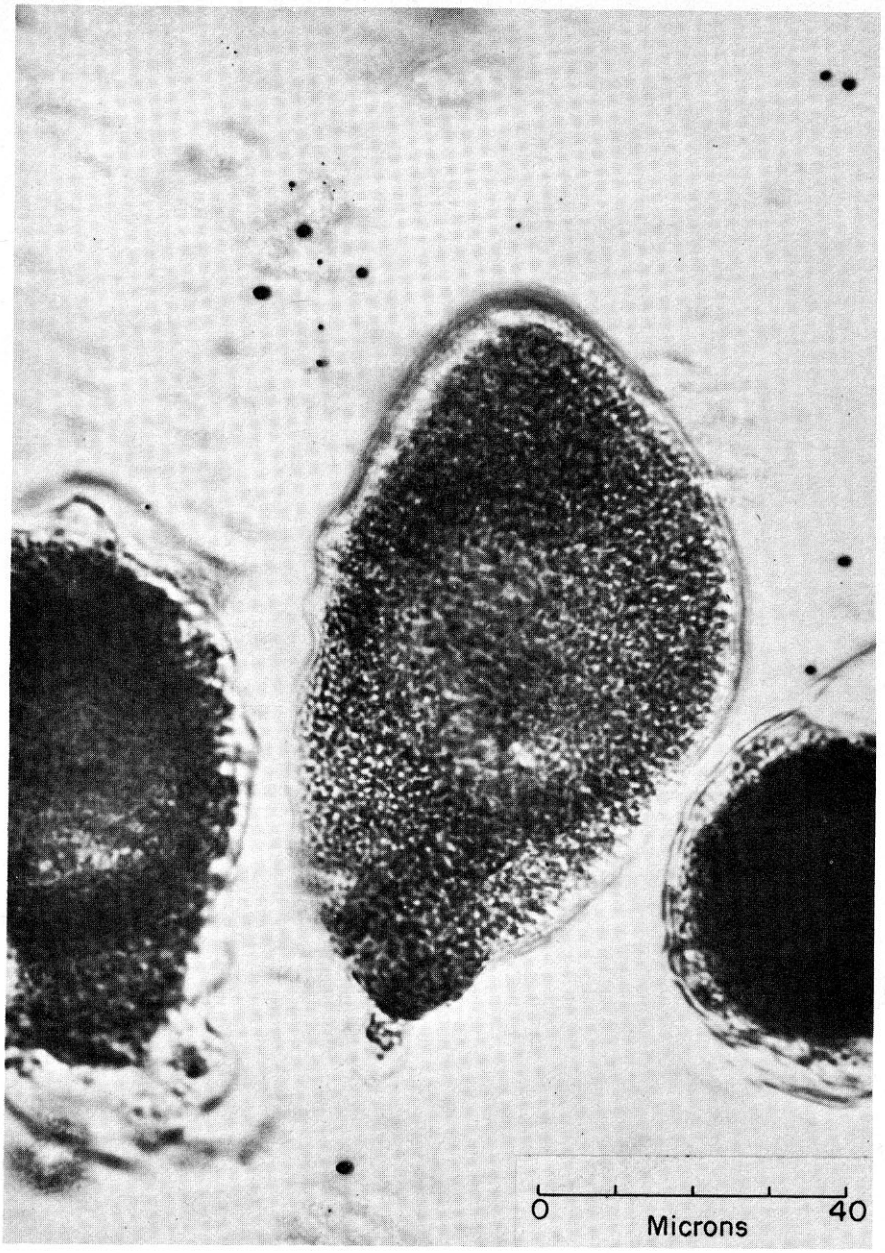

FIGURE 305.—Photomicrograph of an unfertilized egg of *C. virginica*. Janus green. Whole mount. Small rodlike inclusions seen along the periphery are mitochondria stained green; larger yolk granules are dark.

Allen, 1953; and Rebhun, 1960). The role of these almost submicroscopical bodies is not clear, but there is no doubt of their importance in the physiology and development of eggs. Recent publications of Dalcq (1960), Brachet (1960), and Rebhun (1960), and the reviews given by Novikoff (1961a, 1961b) should be consulted for ideas concerning the possible role of these elements in the morphogenesis of mosaic eggs in which they are concentrated in the posterior blastomeres.

Eggs of the surf clam *S. solidissima* contain a heparinlike blood anticoagulant which was also extracted from the tissues of this clam (Thomas, 1954). Whether substances with similar activity are present in oyster eggs is not known.

The nucleus of a mature egg is surrounded by a nuclear membrane which can be clearly seen on sectioned and stained preparations of the ovary (fig. 300). A spherical, dense nucleolus is excentrically located; its diameter varies from 4 μ to 6 μ.

FIGURE 306.—Unfertilized egg of *C. virginica* centrifuged for 10 minutes at 4,000 times gravity after staining with toluidin blue. Dark yolk granules are at the lower (centrifugal) pole while the mass of small inclusions consisting of lavender particles and bluish mitochondria are at the opposite end of the egg.

The chromosomes appear as fine threadlike structures near the nuclear membrane. Using methyl green and pyronin B stains, Kobayashi (1959) found that the nucleolus in the eggs of *C. gigas* and *O. laperousi* consists of two parts, the karyosome,

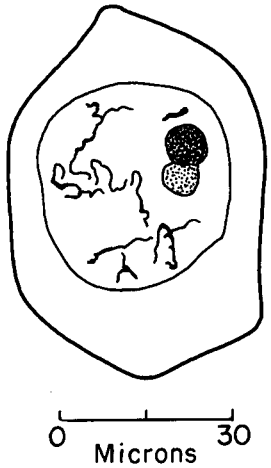

FIGURE 307.—Ovocyte of *O. laperousi* stained with methyl green and pyronin B after Navashin fixation. Dark globule is the karyosome, light shaded area is the plasmosome. From Kobayashi, 1959.

shown in solid black in fig. 307, and the plasmosome, lightly shaded, in close contact with each other. The definite KP axis (Karyosome-Plasmosome) of the unfertilized egg changes after fertilization by the turning of the karyosome around the plasmosome. The existence of this axis in the eggs of other species of oysters has not been described.

STRUCTURE OF THE MATURE SPERMATOZOON

The spermatozoon of bivalve mullusks appears under the microscope (Franzén, 1956; Lenhossék, 1898; Retzius, 1904) to consist of an oval or round head with a pointed front, a middle piece at the lower end of the head, and a long tail (flagellum) with a narrow "end piece" which is longer than the width of the head. The middle piece consists of four, sometimes five, oval-shaped bodies clustered around the tail; and a minute "central granule" or centriole located in the center at the point of attachment of the tail. The sharply outlined oval bodies are mitochondria; they are strongly osmiophilic and can be deeply stained with rosanilin. The head of the spermatozoon is formed by a compact nucleus capped with the apical body or acrosome with a pointed tip (perforatorium), which apparently assists the spermatozoa in penetrating the egg membrane at fertilization (Wilson, 1928). The features listed above may be seen in properly fixed and stained preparations of the sperm of *C. virginica* and in live spermatozoa examined with phase contrast oil immersion lenses. In live preparations the nucleus appears to be dark while the acrosome and middle piece are light (fig. 308). The center of the spermatozoon head is occupied by an axial body, a relatively large, light-refracting structure which is separated from the acrosome.

The dimensions of normal, uncytolyzed spermatozoa have been measured by means of an eyepiece micrometer of a light microscope. The head varies from 1.9 μ to 3.6 μ in length (median value 2.7 μ) and between 1.0 μ and 2 μ in width. The tail is from 27 μ to 39 μ long (median value 36 μ). The tails are usually slightly curved; specimens with straight tails are rarely found.

Electron microscopy reveals much greater complexity in the structure of the spermatozoon (Galtsoff and Philpott, 1960). Study was made of small sections of ripe spermary preserved in cold 1 percent osmium tetroxide buffered to pH

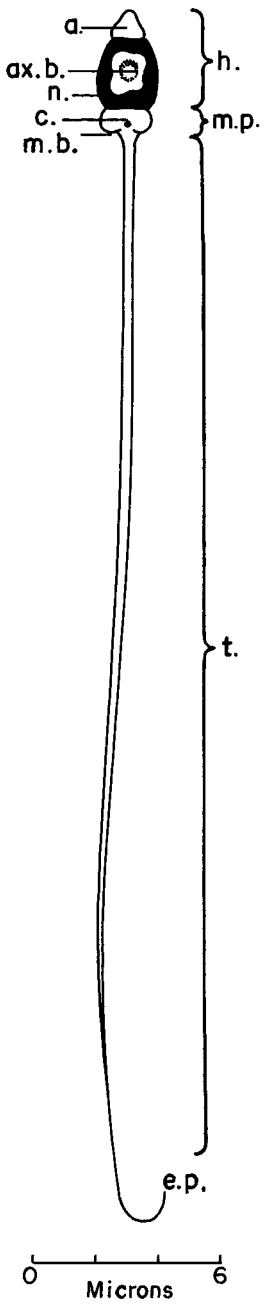

FIGURE 308.—Live spermatozoon of *C. Virginica* examined under light microscope with phase contrast oil immersion lens. A—acrosome; ax. b.—axial body; C—centriole; e.p.—end piece of tail; h.—head; m.p.—middle piece; mt.—mitochondrial bodies; N—nucleus; t.—tail.

7.2–7.4 and embedded in a mixture of three parts butyl and one part methyl methacrylate. To increase the contrast, some of the material was placed in 1 percent alcoholic phosphotungstic acid for 5 to 6 hours before embedding. The embedded material was sectioned on Philpott's microtome (Philpott, 1955), using a diamond knife. Since the preserved pieces consisted of a multitude of spermatozoa arranged in a central mass with their tails pointing outward, the individual spermatozoa were always cut at random in different planes, regardless of how the embedded tissue may have been oriented on a microtome block. The resulting electron micrographs showed a number of sperm heads cut at different planes and many transverse sections of tails (fig. 309). The entire structure of the head was diagrammatically reconstructed by bringing various elements together and placing them in their relative positions (fig. 310). The oval-shaped head consists of slightly granular, homogeneous material covered with an osmiophilic membrane made of two layers. The apical portion of the nucleus is occupied by a caplike acrosome of highly osmiophilic substance. The acrosome is clearly separated from the nucleus by a sharply defined membrane. An egg-shaped body in the central part of the nucleus extends from the apex of the acrosome almost to the base of the nucleus. This structure, named axial body (Galtsoff and Philpott, 1960), has a central stem of fibrous material which emerges from the flattened bottom and extends about two-thirds of the total length of the axial body. The indented base of the nucleus is near the base of the axial body. The caved-in space formed by this indentation consists of material of lesser electron density and extends under the nucleus to the upper surface of the centriole, which is surrounded by four mitochondrial bodies. Only two of them are shown in fig. 310.

The centriole of the sperm of *C. virginica* is a hollow, cylindrical structure with walls made of nine bands; these can be seen in cross section (fig. 311). The side view (fig. 310) shows that the centriole is formed in several alternating and slightly constricted layers which connect with the four mitochondrial bodies. These bodies have the typical appearance of twisted lamallae enclosed in a membrane which encompasses the centriole and continues over the tail.

The tail consists of a pair of axial filaments surrounded by a ring of nine double filaments spaced at equal intervals along the periphery (fig. 312). The filaments are interconnected by delicate strands. The axial filaments begin near the basal plate (fig. 310) where the tail emerges. Radial trabeculae connect the ring filaments to the outside wall of the tail and form nine separate

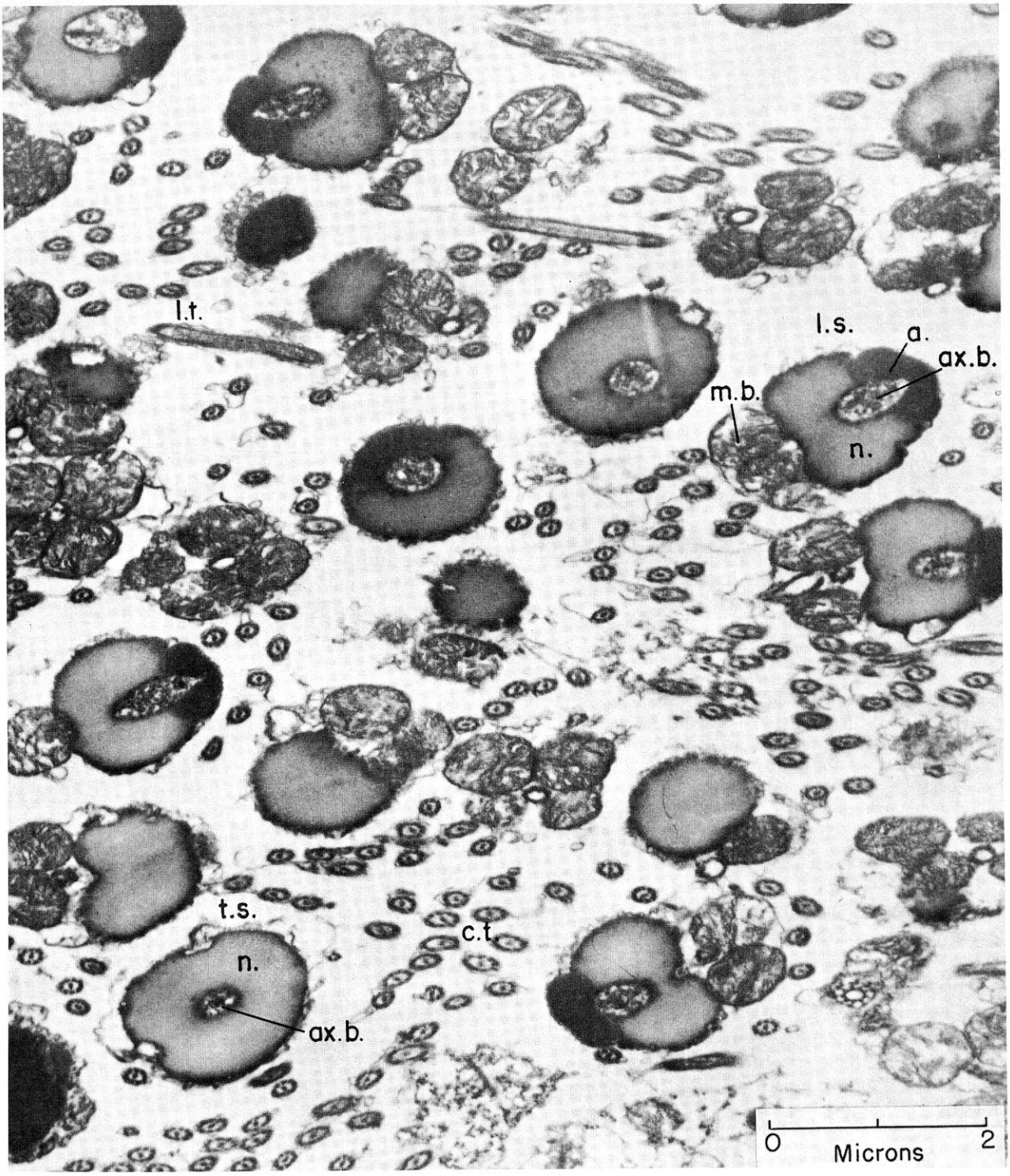

FIGURE 309.—Electron micrograph of a section of ripe spermary of *C. virginica*. Longitudinal and transverse sections of sperm heads and tails can be seen in various parts of the micrograph. A—acrosome; ax. b.—axial body; c.t.—cross section of tails; l.s.—longitudinal section of sperm head; l.t.—longitudinal section of part of a tail; m.b.—mitochondrial bodies; N—nucleus; t.s.—transverse section of head.

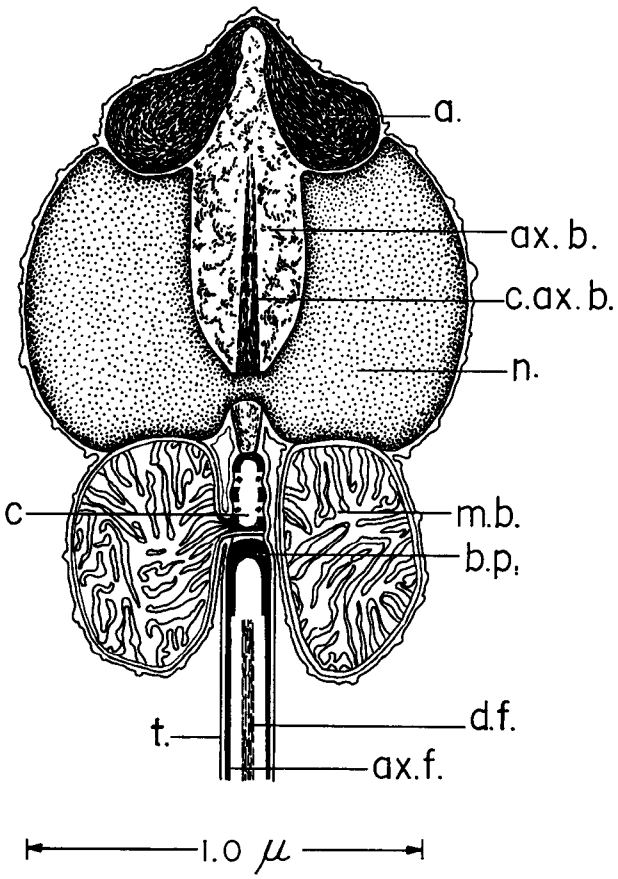

FIGURE 310.—Reconstruction of the sperm head of *C. virginica* made from a large number of longitudinal sections. a.—acrosome; ax. b.—axial body; ax. f.—axial filament of the tail; b.p.—basal plate of the tail; c.—centriole; c. ax. b.—core of the axial body; d.f.—double filament on the periphery of the tail; m.b.—mitochondrial body; n.—nucleus; t.—proximal part of the tail.

compartments filled with material of lesser electron density. The central radial strands are similar to the "spokes" described by Afzelius (1955) for the sperm of the sea urchin *Psammechinus miliaris*. They are not present in the proximal portion of the tail where there are no axial filaments, but otherwise the ultrastructure of the sperm tail is similar to that of cilia and flagella of various animals and plants.

FERTILIZATION

The spawned eggs of *C. virginica* and *C. gigas* are heavier than water and quickly sink to the bottom. The time they remain in suspension may be prolonged by horizontal currents and upward movements of the water, and consequently the chances of fertilization are increased. Because spawning is usually initiated by the males, the water into which the eggs are discharged already contains active spermatozoa and fertilization takes place within a few minutes following ovulation. It is obvious that the success of reproduction of an oyster population in which spawning is mutually stimulated by the discharge of sex cells is dependent on close proximity of the sexes and their simultaneous response to spawning stimuli.

Eggs and sperm secrete substances called gamones which play an important role in fertilization. Secretion from an unfertilized egg has a significant effect on spermatozoa. This effect can be observed if a suspension of eggs is permitted to stand for 15 to 20 minutes and the supernatant fluid is decanted or filtered and added to the suspension of sperm. The resulting so-called "egg water" (Lillie, 1919) causes the agglutination of sperm. To observe the agglutination reaction with the naked eye, a drop of egg water must be added to a sperm suspension, which shortly forms irregular lumps (fig. 313). Under a high-power light microscope one sees that the heads of agglutinated spermatozoa stick together to form large aggregates (fig. 314).

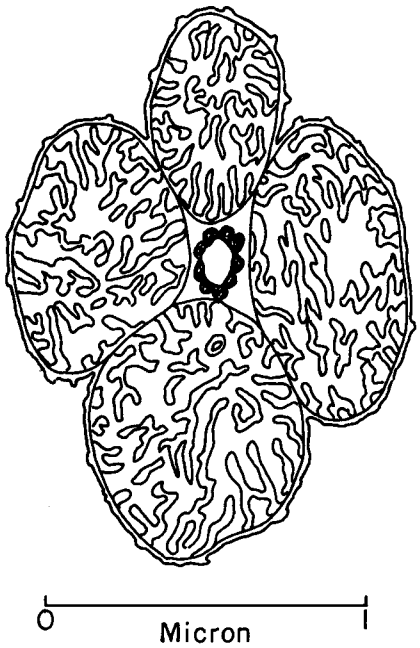

FIGURE 311.—Drawing based on electron micrographs of the cross section of the lower part of the middle piece of the spermatozoon of *C. virginica*. Centriole, at the center, is surrounded by four mitochondrial bodies.