CHAPTER XIV

ORGANS OF REPRODUCTION

ANATOMY

The ripe gonad is a massive organ located near the surface of the body within a layer of connective tissue between the digestive diverticula on the inner side and the surface epithelium on the other. In sexually mature oysters it appears as many branching tubules (follicles) which merge along the dorsal side of the body to form one continuous structure encompassing the visceral mass and extending ventrally to the tip of the pyloric process (fig. 267).

The reproductive gland is not encapsulated, and its outlines are indistinct. At sexual maturity the surface covering of the body becomes so thin that a network of fine genital canals is clearly visible through it. The diameters of the canals gradually increase as they converge into a wider gonoduct through which the germ cells are discharged. Two separate systems of genital canals, one on each side of the body, are the only sign of the paired origin of the sex gland which in an adult oyster is completely fused into a single organ.

In many bivalves sex products are discharged through the kidneys, but in the oyster the gonoduct opens into a vestibule, or atrium, which also receives the urinal duct. This relationship can be seen on a series of slightly slanted sections of the lower part of a ripe gonad. One of these sections is shown in fig. 268. The female oyster from which the tissue was taken was preserved during the act of spawning. The follicles of the lower part of the ovary (left side of the figure) are almost empty of ripe eggs; a short ciliated passage between the ovarian follicle and the pear-shaped area of the kidney vestibule are at the right; two spawned eggs are near the outside opening of the vestibule, which is lined with ciliated epithelium. A transverse section of the kidney reservoir lined with typical secretory cells is in the upper right portion of the figure. The connection between the vestibule and the reservoir is located above this section. The follicles in the inner portion of a gonad lie at an angle to the genital ducts into which they discharge their content. The structure of spermary differs from that of the ovary only in that the follicles are filled with spermatozoa.

The degree of sexual development can be estimated roughly by measuring with calipers the thickness of a transverse section of the gonad layer. Since the gonad is not uniformly thick in all its parts, the section should be made at some selected place. In conformity with the practice used by the Biological Laboratories of the Bureau of Commercial Fisheries at Milford, Conn., Oxford, Md., and Gulf Breeze, Fla., the oyster is cut with a razor blade along a line extending from the lower corner of the labial palps across the stomach to the posterior end of the body. There is considerable variability in the gonad layer of oysters of known age and similar environment. In Long Island Sound the average maximum thickness of ripe gonads of 4- to 5-year-old oysters taken from three depth levels of 10, 20, and 30 feet was, according to Loosanoff and Engle (1942), about 2.4 mm. for the shallow water oysters and only about 1.5 mm. for those found in deeper water. Much greater gonadal development was recorded in other locations. Some fully mature Cape Cod oysters used in my experimental work had a layer of gonad from 6 to 8 mm. thick, and a similar degree of development was noted in oysters from a small tidal pool near the laboratory at Beaufort, N.C.

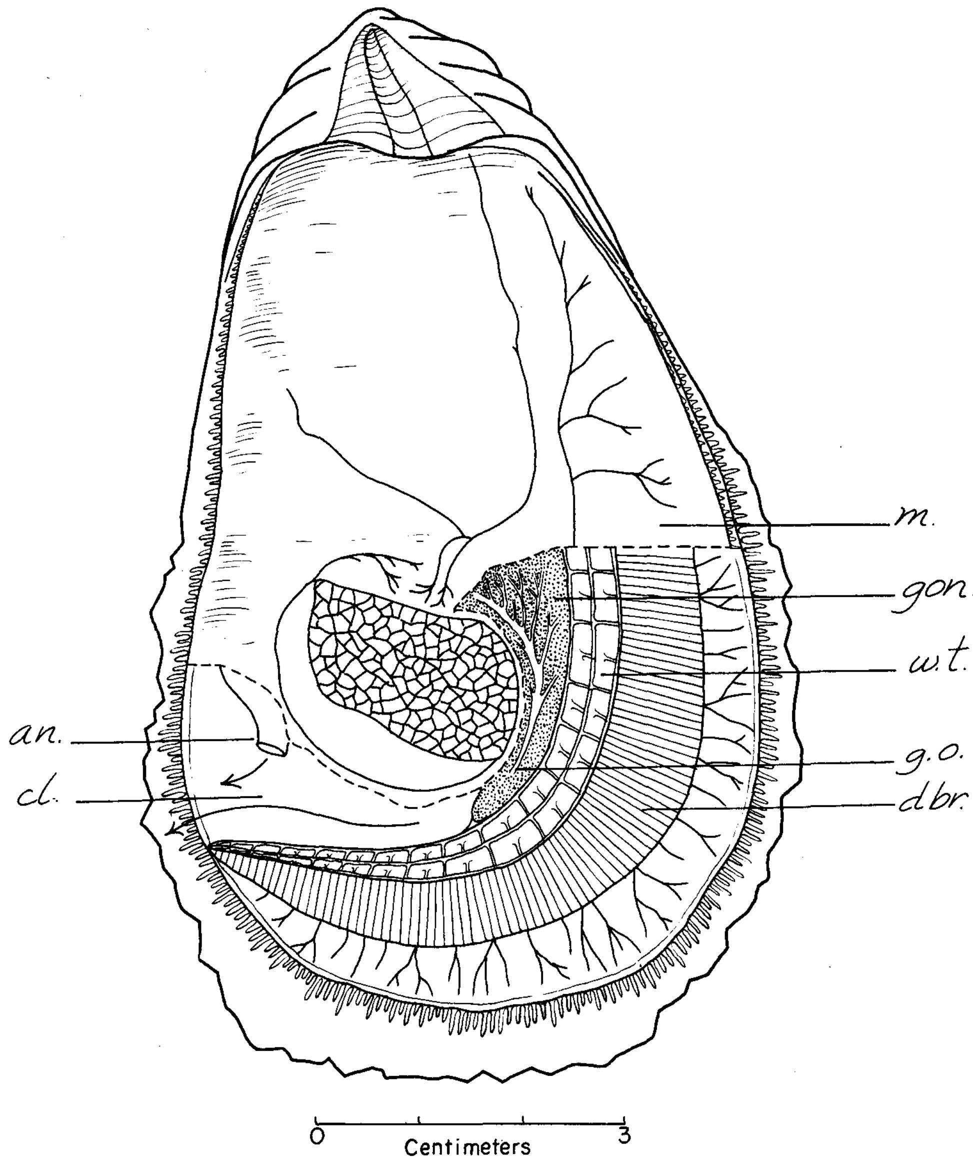

FIGURE 267.—Fully ripe *C. virginica*. The mantle of the right side was dissected above the epibranchial chamber and pushed to the right to expose the pyloric process. A network of canals leads to the single gonoduct through which the sex cells are discharged. Drawn from life. an.—anus; cl.—cloaca; d.br.—right demibranch; g.o.—genital opening; gon.—gonad; m.—mantle; w.t.—water tubes inside the gills.

Gonadal layers of about 10 mm. thickness were found in large *C. gigas* (8 inches in height) from Willapa Bay, Wash. (Galtsoff, 1930a, 1932).

In all species of *Crassostrea* the bulk of gonads varies from season to season, reaching its maximum shortly before the onset of spawning. The number of sex cells produced during one reproductive period varies, depending on the conditions of the environment. The greatest gonadal development is more likely to be found in the populations of oysters from the northern latitudes north of the Chesapeake Bay rather than in the south Atlantic and Gulf waters. This is apparently associated with the fact that the reproductive season in the northern latitudes is of short duration, 4 to 6 weeks, while in the warmer waters of the south gonadal formation and spawning, continue, with interruptions, for several months. In both groups the annual reproductive capacity (i.e., the number of eggs produced annually) maybe of the same order of magnitude or even greater in the southern oysters because of the longer reproductive period, but the greatest bulk of a ripe gonad is more likely to occur in oysters which have only one spawning period per year.

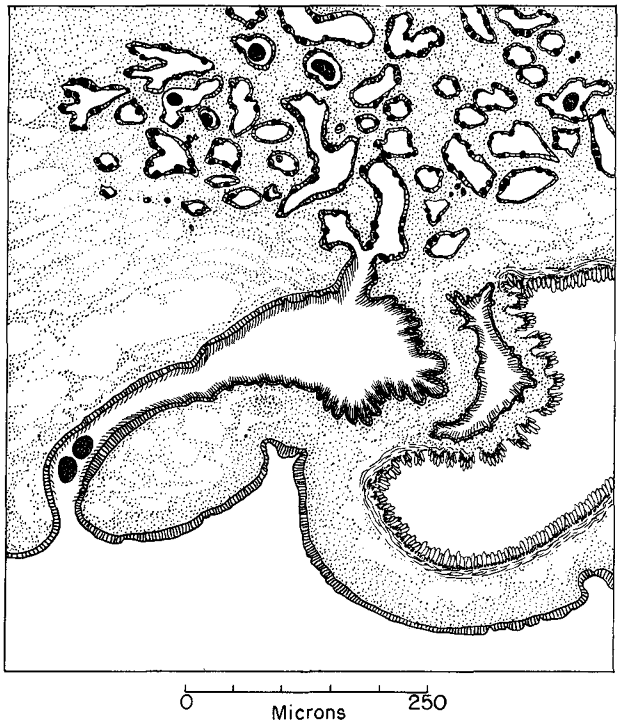

FIGURE 268.—Slightly slanted tangential section through the opening and adjacent portion of the ovary of *C. virginica*. Oviduct with two eggs at the lower right side. Kidney reservoir at upper right. Ovary follicles at left. Drawn semidiagrammatically from a photomicrograph of a preparation. Kahle, hematoxylin-eosin.

Several factors besides geography influence gonadal development. The most significant are temperature, depth (Loosanoff and Engle, 1942), salinity, available food, and pollution of oyster bottoms. Many examples of the suppression of gonadal growth by adverse conditions may be cited. For example, oysters living in waters highly polluted by various trade wastes have, as a rule, poorly developed gonads. Sometimes the development of the sex gland is suppressed to such a degree that only traces of follicles are found in the visceral mass, and the layer of the digestive diverticula is visible from the surface. These oysters have the greenish or brownish coloration typical for the digestive diverticula which in the sexually ripe oyster is not noticeable under a thick layer of gonad. Oysters with suppressed gonad development are found in the waters which receive continuous discharge of the pollutants from pulp and paper mills. Similar poor oysters are frequently encountered in waters of extremely low salinity or from areas where salinity increases to the highest limit of tolerance (34 to 40 $^{\circ}/_{\circ\circ}$). The determination of gonadal thickness described above lacks precision because variation in the compactness of the gonadal tissue cannot be measured, but the method is, nevertheless, useful for the practical purpose of estimating expected intensity of spawning.

DETERMINATION OF VOLUME AND WEIGHT OF GONAD

The fully developed gonad is the largest organ of the oyster. The ovaries or spermaries can be separated from the underlying digestive diverticula by using small curved scissors. The excised pieces are weighed, and their volume is measured by displacement in a simple device made from a glass cylinder of appropriate dimensions (depending on the size of the sample) with a drain pipe at the bottom and a side glass tubing of about 5 mm. in diameter fused to the side at an angle of about 45° to record the water level. A convenient water level is selected and recorded, the tissue is introduced, and the water is then drained into a measuring vessel to the previous level. The body weights of adult New England oysters with fully developed gonads varied in my observations from 14.2 to 23.2 g. The gonads comprised from 31.2 to 40.7 percent of the total body weight exclusive of shell. The volume of the oysters' tissues ranged from 21 to 24 ml., with the gonads accounting for 32.8 to 33.4 percent of the total bulk. Oysters selected for these measurements were of the highest commercial quality and with maximum development of gonads. In poor oysters with light gonadal development the proportion of the weight of the gonad to body weight is only a small fraction of the figures given above.

HISTOLOGY

The gonads of the oyster originate from a group of primordial germ cells located in the mesodermal band on the ventral side of the pericardium in the vicinity of the visceral ganglion (Coe, 1943a). In embryos of bivalves primordial germ cells are identified by their relatively large size, round shape, and clear vesicular nucleus with one or two nucleoli (Okada, 1936, 1939; Woods, 1931, 1932). The primordium soon becomes separated into two groups which by continuous multiplication of the constituent cells extend symmetrically along both sides of the body. Each group grows anteriorly, surrounded by vesicular connective tissue of the visceral mass, and forms a system of profusely branching tubular follicles. The fusion of the branches along the dorsal side obliterates any remnants of the paired origin of the gonad.

The microscopical structure of the gonad varies, depending on the age of the oyster, degree of maturity, season, and environmental condition. In Ostreidae and in some other pelecypods (*Pecten*, *Mytilus*, *Volsella*) the follicles of a fully developed gonad consist almost entirely of primitive sex cells (gonia) at various stages of development with only a few minute follicular cells between them. Because of the absence of a capsule or membrane around the gonad, the sex cells are in direct contact with the surrounding tissues (fig. 269). It may be assumed that the role of follicular cells in the growth of the gonad of oysters is insignificant and that the gametogenic cells obtain their nourishment directly from the connective tissue which surrounds them. In *Mya*, *Teredo*, *Bankia*, and other Adesmacea the follicular cells are large and function as accessory nutritive cells.

In immature oysters and in the adults, that had completed the spawning period, the germinal epithelium consists of undifferentiated sex cells, some at the early stages of gametogenesis. Their sex can be recognized only by careful cytological examination. As the follicles grow and ramify they spread throughout the surrounding layer of connective tissue. The maturing sex cells inside

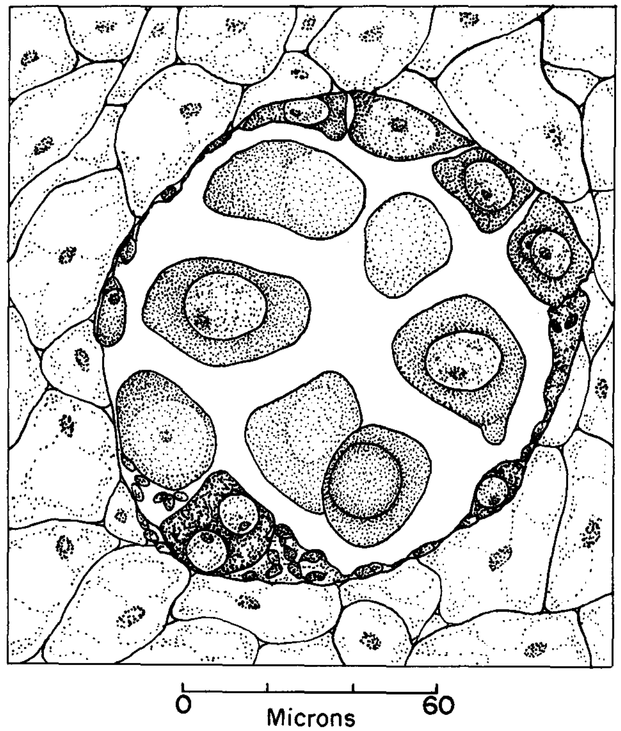

FIGURE 270.—Cross section of one surface follicle in a fully developed ovary of *C. virginica*. Bouin, hematoxylin-eosin.

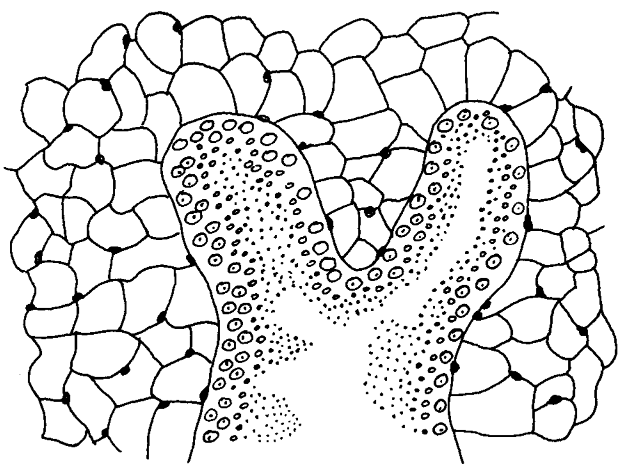

FIGURE 269.—Terminal portion of the follicle of a gonad of *C. virginica* at early stage of development (male phase). The follicle is surrounded by a large mass of connective tissue. Redrawn from Coe, 1936a.

them multiply, grow, and fill up the lumen (fig. 270). The follicles near the surface of the gonad are distinctly different. Their outer walls facing the body surface are lined with ciliated epithelium, and only the inner sides of the follicles retain the germinal cells (fig. 271). This differentiation of the germinal epithelium into two distinct types is probably common to all species of oysters. The follicles lined with ciliated cells, function as the genital canals through which mature sex cells are moved by ciliary action. They were first described by Hoek (1883) for *O. edulis* and subsequently were found in *O. lurida* and in several species of *Crassostrea*.

Since the transformation of germinal cells into ova and sperm is a gradual process which does not involve all the cells of the germinal epithelium at the same time, numerous undifferentiated or so-called residual cells are usually found along the inner periphery of a follicle. Some of them can be found even in a fully developed gonad (fig. 270).

The bulk of a functional ovary is made up of fully developed ova which fill up the lumina of

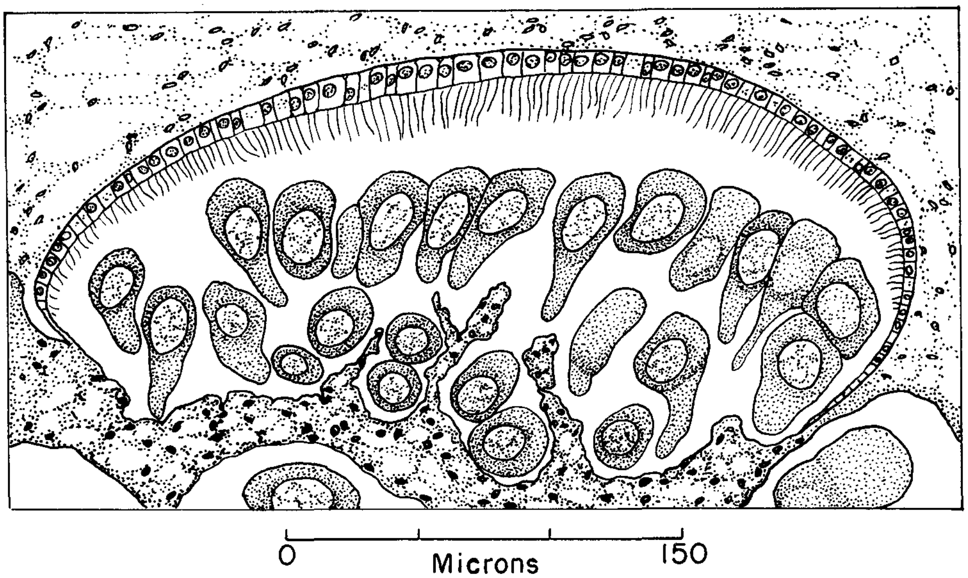

FIGURE 271.—Cross section of a surface follicle from a fully developed ovary of *C. virginica*. Germinal epithelium is formed by cells at different stages of ovogenesis and by small undifferentiated cells. Bouin, hematoxylin-eosin.

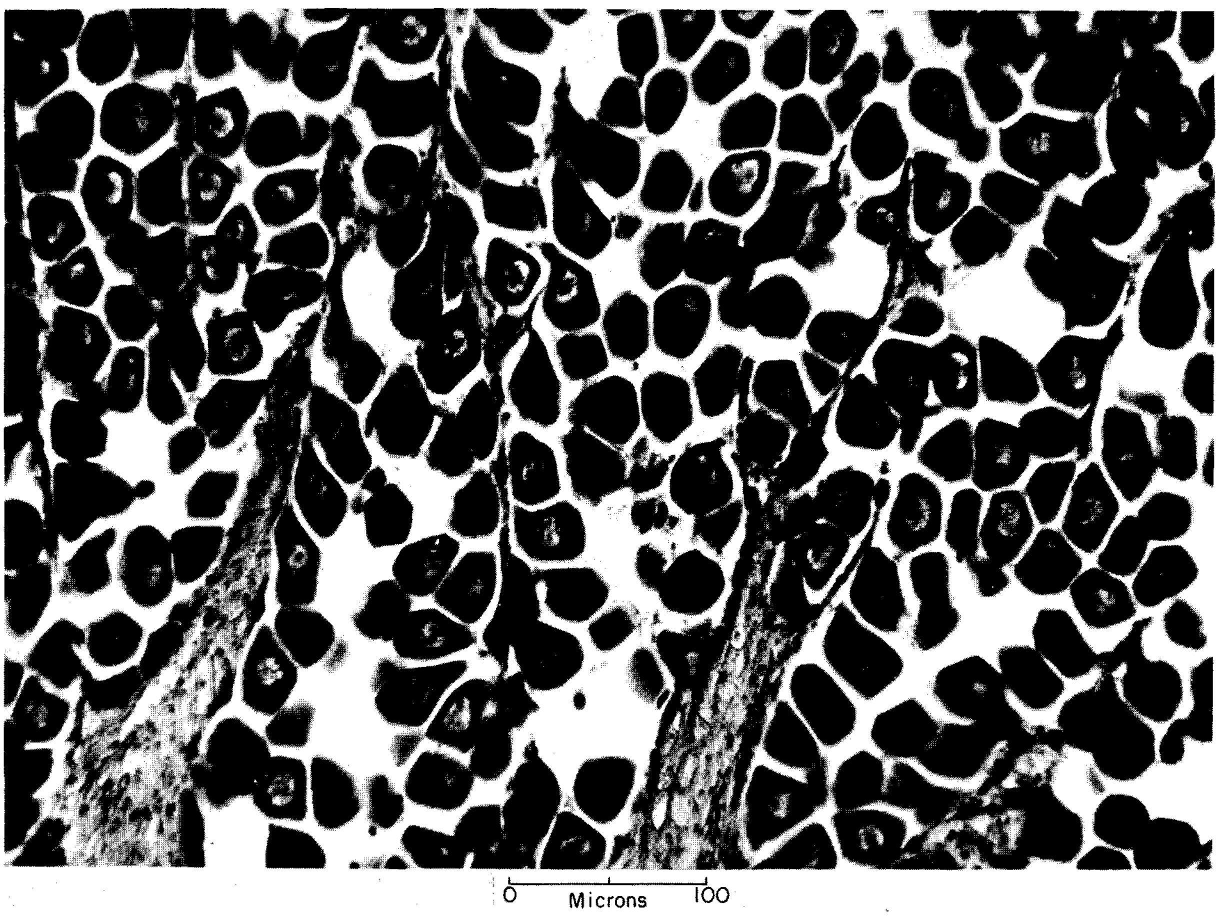

FIGURE 272.—Photomicrograph of an oblique section of a ripe ovary of *C. virginica* shortly before spawning. Bouin, hematoxylin-eosin.

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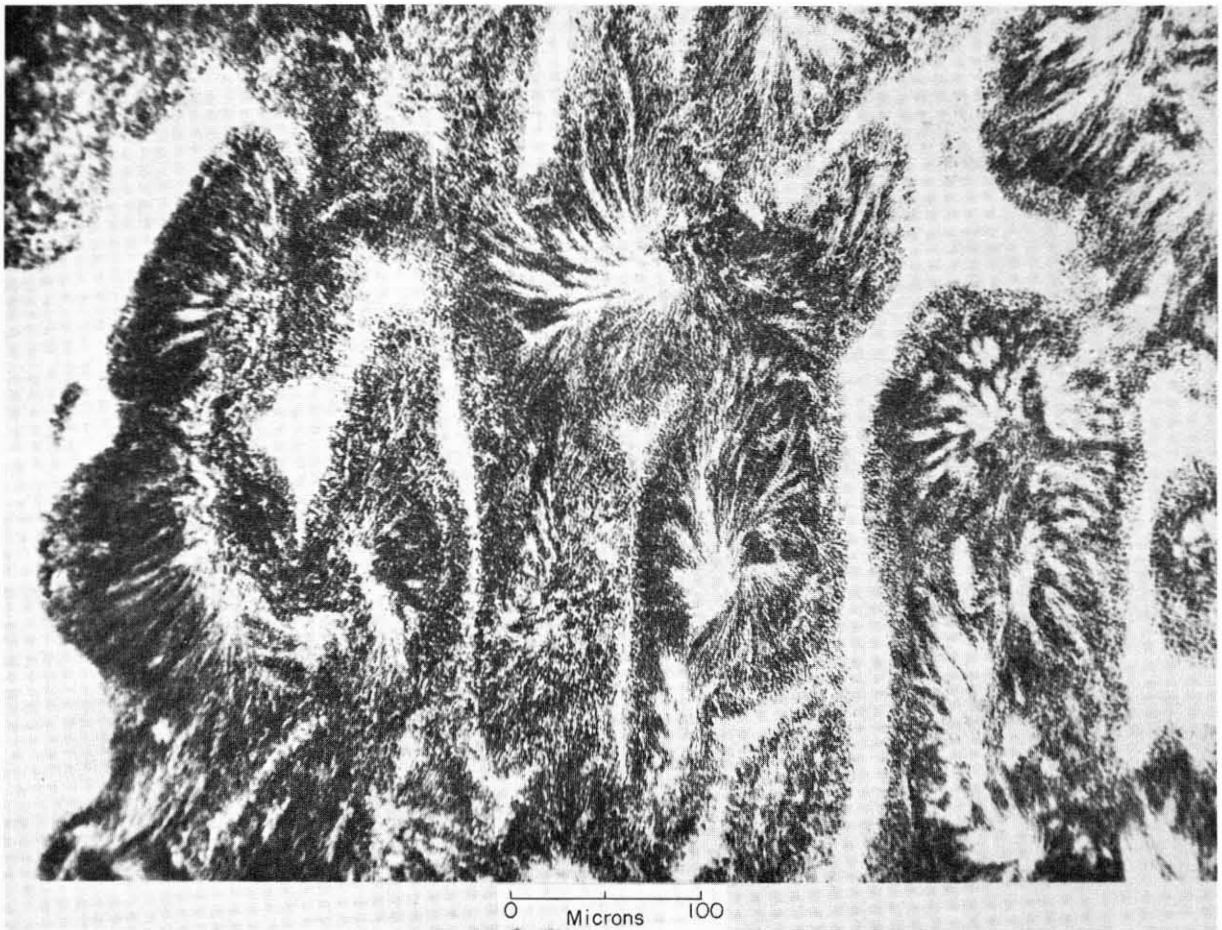

FIGURE 273.—Photomicrograph of a cross section of a ripe spermary of *C. virginica*. Bouin, hematoxylin-eosin.

the follicles and of a number of cells at different stages of the development nearer to the walls. The eggs are attached to the walls of the follicles by elongated peduncles, giving them a pear-shaped appearance. At the height of sexual development the layer of undifferentiated germinal epithelium is reduced to a very narrow band of small cells hardly visible at low magnification (fig. 272). The connective tissue between the follicles also very nearly disappears.

The arrangement of cells in a ripe spermary (fig. 273) is similar to that in the ovary. Fully formed spermatozoa are massed together inside the follicle with their tails inward. They become separated as the sperm moves through the genital canals, which are frequently distended by the accumulation of spermatozoa ready to be ejected. In *O. edulis* and *O. lurida* the sperm in the lumen of a follicle form distinct balls which retain their shape until they are discharged by the oyster. A number of sex cells remain in the follicles at the completion of spawning. Consequently, at the end of the reproductive season the gonads contain mature ova and sperm as well as undifferentiated cells; many of them are pycnotic and detached from the germinal lining of the wall. Phagocytosis becomes pronounced as large numbers of leucocytes invade the follicles to digest and cytolize the remaining sex cells (fig. 274). The connective tissue between the follicles becomes disorganized. After the reabsorption of the gonad is completed only a narrow band of germinal epithelium remains in a few follicles and the entire layer has shrunk to such a thin band that it is not visible to the naked eye. The oyster is now at an indifferent stage. Its sex can be recognized in some individuals in which young ovocytes or spermatocytes are present, but in many others

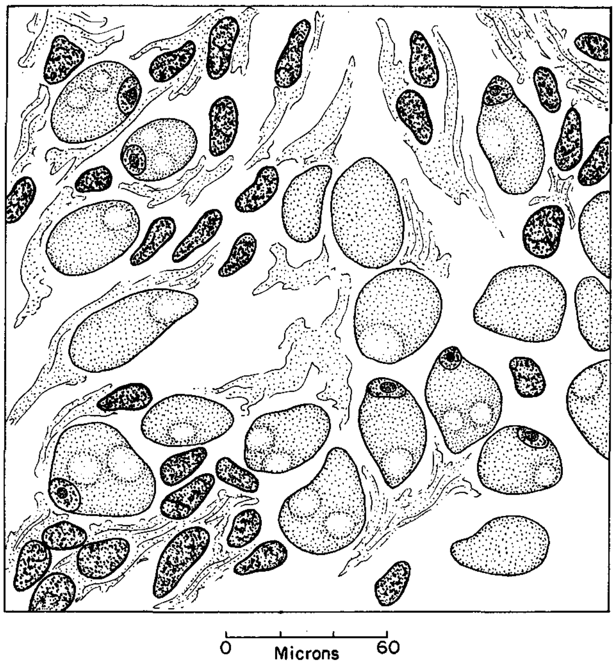

FIGURE 274.—Ovary of *C. virginica* shortly after completion of spawning. Unspawned ova are cytolized. Note the invading phagocytes and the disorganization of connective tissue. Bouin, hematoxylin-eosin.

the young sex cells are not sufficiently differentiated and their sexes can not be identified. Ovocytes, when sufficiently developed, can be distinguished from spermatocytes by their large nuclei and granular cytoplasm.

During spawning the sex cells are moved inside the genital canals by ciliary motion of the epithelium. How they are released from the follicles and reach the genital canals located near the surface of the sex gland is not known. Histological examination discloses no contractile elements in the tissues surrounding the follicles, and no contraction of the gonad or part of it could be detected during spawning. Upon reaching the gonoduct the released cells continue to be moved by the powerful cilia of the duct and vestibule (fig. 275). Longitudinal and circular muscle fibers are found under the basal membrane of the vestibule and appear to be better developed in the male gonad than in the female. Roughley (1933) refers to the presence of sphincter muscles in the gonoducts of *Ostrea* (*Crassostrea*) *commercialis*, but no structure resembling a sphincter is found in *C. virginica* (Galtsoff, 1938a). It is conceivable that a contraction of circular muscle fibers of the spermiduct to a certain degree controls the passage of sperm through the genital opening, but no such action has been detected under experimental conditions.

The epithelium of the distal end of the gonoduct and of the urinogenital vestibule contains, besides the ciliated cells, a large number of mucous cells (MC) not present in the lining of the canals. In the ovary the opening of the oviduct into the vestibule is marked by a small ridge of ciliated cells (Galtsoff, 1938a, 1938b).

SPAWNING

The sexual apparatus of oysters is of the simplest type for it lacks the accessory sex organs which in some mollusks are used for mutual excitation, storage of sex cells, copulation, and secretion of egg capsules. In spite of the structural simplicity of the reproductive organs, the spawning of the female oyster is a rather complex action which involves coordination of the gills, nervous system, mantle, and the adductor muscle, while the sexual act of the male is much simpler. It is, therefore, more convenient to describe separately the phenomena involved in the spawning of the two sexes. Under natural conditions simultaneous release of sperm and eggs, essential for successful reproduction of the species, is attained through mutual stimulation.

SPAWNING REACTIONS OF THE FEMALE

Spawning of the female proceeds in several consecutive steps which, in the order of their participation, involve the ovary, the gills, the mantle, and the adductor muscle. The behavior of these organs follows a distinct pattern, one action succeeding the other in a precise sequence which finally terminates in the dispersal of eggs in the surrounding water.

The first step is ovulation, i.e., the discharge of eggs from the ovary into the epibranchial chamber. The moment the eggs appear in the epibranchial chamber the two edges of the mantle come together and effectively seal the pallial cavity and cloaca. This peculiar behavior of the mantle may be observed in a spawning female oyster placed close to the wall of a rectangular tank. In an actively feeding animal the space between the two mantle edges is wide open; the pallium and the tentacles are extended outward parallel to the surface of the valves and the gills, exposing the side of the adductor muscle, the rectum, and the inner part of the cloaca (fig. 276). A different picture is seen in a spawning female. A few minutes before ovulation the edges of the

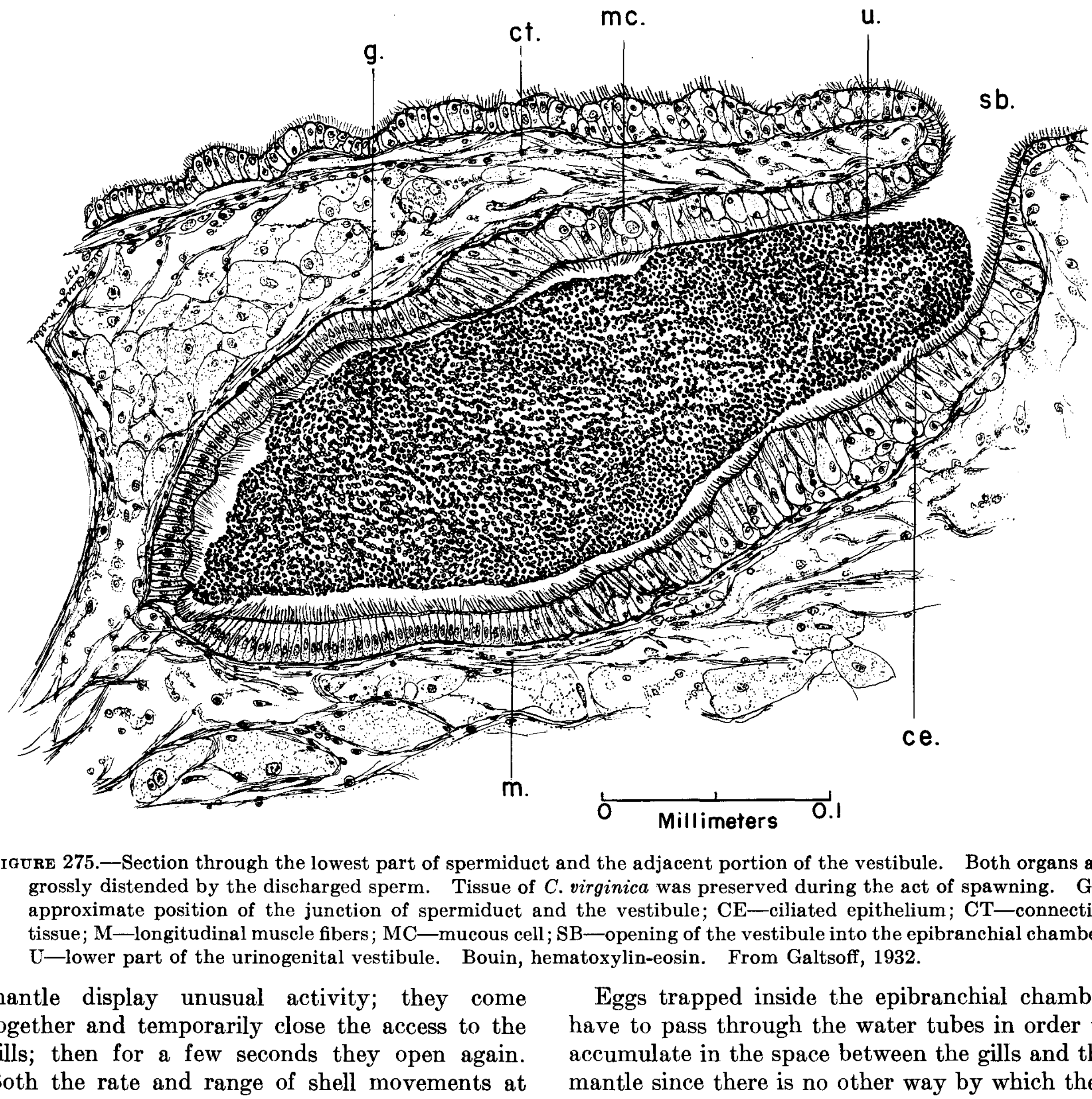

FIGURE 275.—Section through the lowest part of spermiduct and the adjacent portion of the vestibule. Both organs are grossly distended by the discharged sperm. Tissue of *C. virginica* was preserved during the act of spawning. G—approximate position of the junction of spermiduct and the vestibule; CE—ciliated epithelium; CT—connective tissue; M—longitudinal muscle fibers; MC—mucous cell; SB—opening of the vestibule into the epibranchial chamber; U—lower part of the urinogenital vestibule. Bouin, hematoxylin-eosin. From Galtsoff, 1932.

mantle display unusual activity; they come together and temporarily close the access to the gills; then for a few seconds they open again. Both the rate and range of shell movements at this time gradually increase. Finally, the entrance to the gill cavity closes completely except for one small opening or "window" as shown in fig. 277. Soon a white cloud of unfertilized eggs appears at the window, the adductor muscle contracts sharply, and the eggs are discharged and dispersed several inches away from the oyster (fig. 278). The opening between the edges of the mantles may be formed at any place along their periphery but once formed its position remains unchanged throughout the duration of spawning. Spawning may last from a few minutes to nearly 1 hour, depending on the amount of mature eggs in the ovary.

Eggs trapped inside the epibranchial chamber have to pass through the water tubes in order to accumulate in the space between the gills and the mantle since there is no other way by which they can reach this area (Galtsoff, 1938a). This conclusion was confirmed by microscopic examination of a section of the gills of a female preserved during the act of spawning (fig. 279). Any other minute particles suspended in water pass through the ostia and water tubes into the exhalant chambers and are swept by the outgoing current. The eggs released through the genital pore, however, take the opposite course when they enter the water tubes of the gills.

While the eggs pass through the gills the ostia are wide open and the ciliary currents along the filaments are neither inhibited nor reversed. The eggs, therefore, flow against the current produced

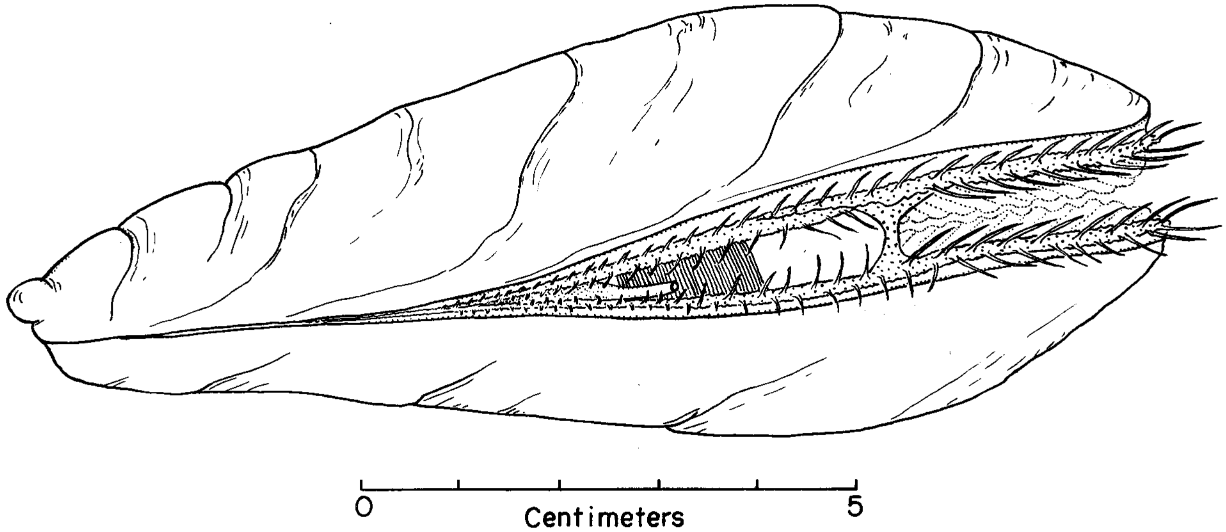

FIGURE 276.—Actively feeding *C. virginica.* The valves are wide open, the pallium and the tentacles are extended outward and the cloaca, the adductor muscle, and rectum are clearly visible. Drawn from life. The opening between the valves is slightly exaggerated.

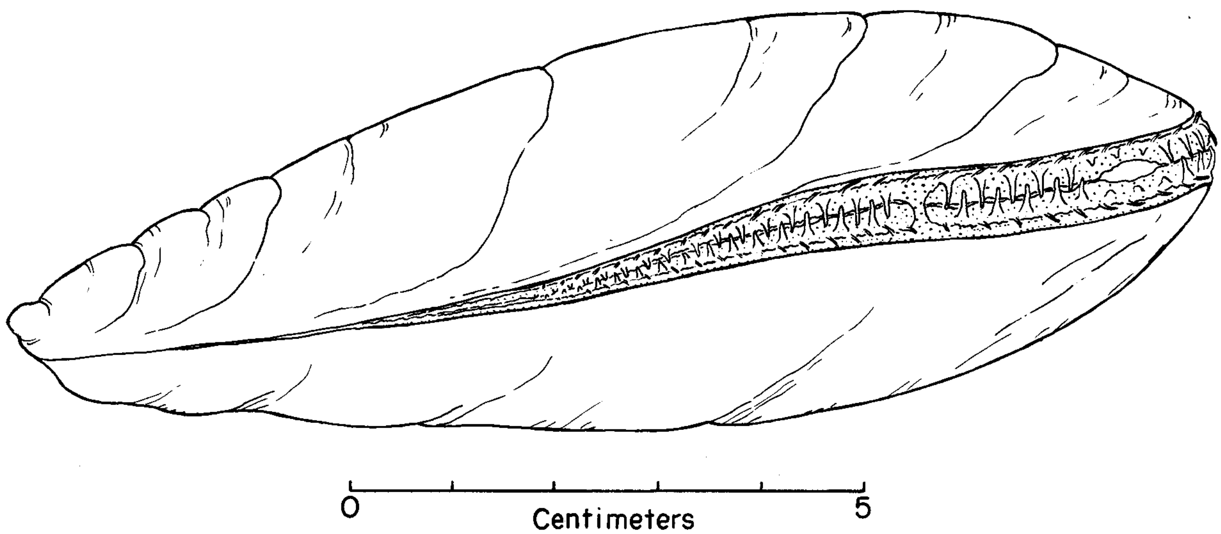

FIGURE 277.—Position of the mantle and tentacles of *C. virginica* shortly before the discharge of eggs through a small window left open (right side of figure). Drawn from life.

by the lateral cilia. Laboratory observations show that when the valves open the gill lamellae also expand and the water tubes dilate. At the same time the closing of the cloaca and of the mantle cavity cuts off the access of water from the outside. As a result, a suction is produced inside the water tubes by the expansion of gill lamellae, forcing the eggs into the water tubes and through the ostia to the surface of the gills.

Eggs of larviparous oysters (*O. lurida* and *O. edulis*) also pass through the gills but are retained in the pallial cavity until fully developed larvae are formed and escape from the mother's body.

Stafford (1913) thought that the eggs of *O. lurida* are too heavy to be carried by the respiratory current and so fall into the water tubes and are forced through the ostia by the pressure of their own mass. The correctness of such an explanation seems doubtful. The act of spawning in the larviparous species has not been studied adequately, probably because the ovulation and passage of eggs into the pallial chamber proceed without any outward indication (Yonge, 1960). Eggs of these species are fertilized inside the body