to obtain uniform distribution of sperm, and the finger bowl set aside for 5 hours, half submerged in running sea water. At the expiration of this period a sample was taken for examination and cleaved and uncleaved eggs were counted. In each case 300 eggs of the sample were examined. All tests were made in water of 31 to 32‰ salinity and 20.8° to 21.4° C. During the first 4 hours of aging the percentages of cleaved eggs declined from 90 to 70. After 5 or 6 hours the percentages dropped to 60. Then the fertilizability decreased to about 20 percent in 10 hours, and only a few eggs cleaved normally after 12 and 24 hours of aging.

It is common knowledge among embryologists that the fertilizing power of spermatozoa is not decreased if sperm is kept at 10° to 12° C. in a concentrated suspension in a tightly closed container. This is also true for oyster sperm. Its fertilizing power is affected by dilution and increased temperature. At room temperature in a dilute suspension, the spermatozoa lose their fertilizing ability within 4 to 5 hours. However, in a concentrated suspension, protected from evaporation, and stored in a refrigerator at about 10° C. the sperm remains active and retains its full fertilizing power for 24 hours and possibly longer.

The effect of cold storage on the fertilizability of eggs is not known. On several occasions ripe females with intact shells were kept for 3 to 4 days in a refrigerator (about 10° C.) and after that were successfully used in spawning experiments. The effect of cold storage on eggs of the excised ovary has not been studied.

POLARITY OF EGG

The polarity of all molluscan eggs apparently is determined while they are still attached to the wall of the ovarian follicle. Presumably the side on which the food reaches the growing ovocyte becomes the vegetative pole of the mature egg (Raven, 1958).

The metabolic gradient along the egg axis is indicated by a concentration of cytochrome oxidase which Kobayashi (1959) detected with M–Nadi reaction; the activity of the enzyme was observed using Gräff's modification of this method. (The reader not familiar with the reaction and its significance in cytochemical research is referred to the publications of Danielli (1958), Deane, Barrnett, and Seligman (1960), and to a review by Novikoff (1961a, p. 308).) In brief, the localization of oxidative enzymes and their presence in mitochondria can be determined by the staining reactions. The rate of respiration of eggs of *C. virginica* increases with fertilization by a factor of 1.4 (Ballentine, 1940) but in the eggs of the Sydney rock oyster, *C. commercialis*, the rate of respiration increases only at the onset of the first cleavage (Cleland, 1950).

CLEAVAGE

The spermatozoon may enter the oyster egg anywhere. Its path inside the egg cytoplasm toward the nucleus has not been described, and cytological details of the process leading to the fusion of the female and male pronuclei have not been studied. It is probable that the major features of these events are not different from those found in other mollusks. Maturation divisions occur in the oyster egg after the elevation of the fertilization membrane. The germinal vesicle (ovocyte nucleus) breaks down and moves toward the egg's periphery. At temperatures of 22° to 24° C. the first polar body is formed within 25 to 50 minutes after the addition of sperm. The reduction of the number of chromosomes probably takes place during the first meiotic division. This is to a certain extent corroborated by an examination of fertilized eggs of *C. virginica* stained in toto with Feulgen reagent or with acetic orcein. Unfortunately the results are not consistent enough to draw a final conclusion and the question remains unanswered, awaiting a complete cytological study.

The second polar body is formed shortly after the first, within 45 to 70 minutes after fertilization (at 22.5° to 24° C.). The two polar bodies remain attached to the surface of the egg (fig. 319) until the completion of cleavage and emergence of the trochophore.

The first cleavage following the formation of the second polar body divides the egg meridionally into two unequal cells designated as AB and CD (fig. 320). The inequality of the blastomeres is due to the occurrence of a polar lobe. Because the egg appears to consist of three cells this stage received the name trefoil.

The plane of the second division, also meridional, is at a right angle to the first. Both blastomeres divide synchronously and separate into the four quadrants. In fig. 321, drawn from a photograph of a cleaving egg taken from the animal pole, the position of the spindles indicates the plane of new

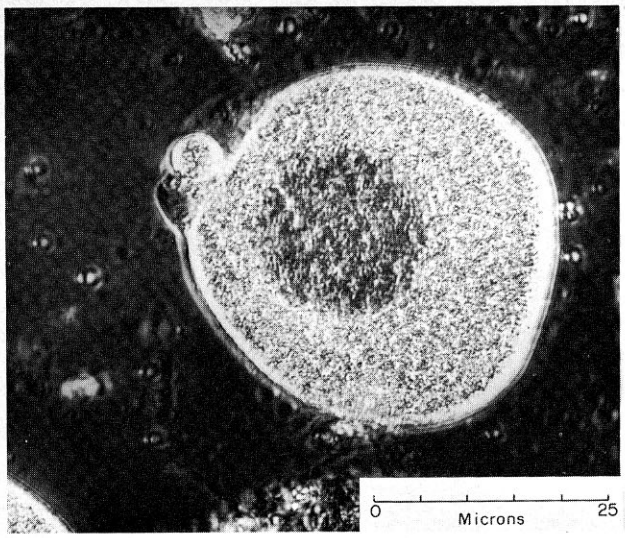

FIGURE 319.—Photomicrograph of a live fertilized egg of *C. virginica* after the formation of two polar bodies (top of egg). High-phase oil immersion lens.

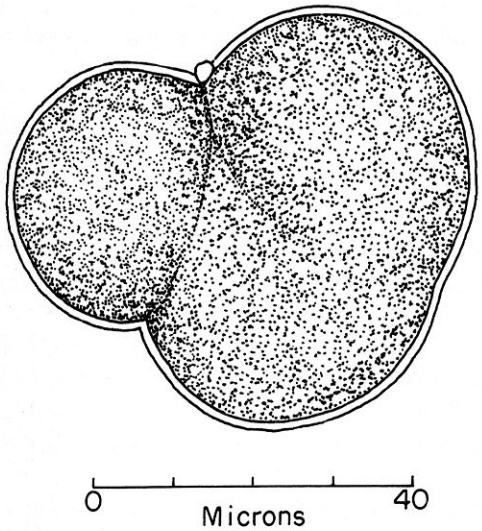

FIGURE 320.—First cleavage division of the egg of *C. virginica* 70 minutes after fertilization. Blastomere AB (left) and CD (right). Polar body on top.

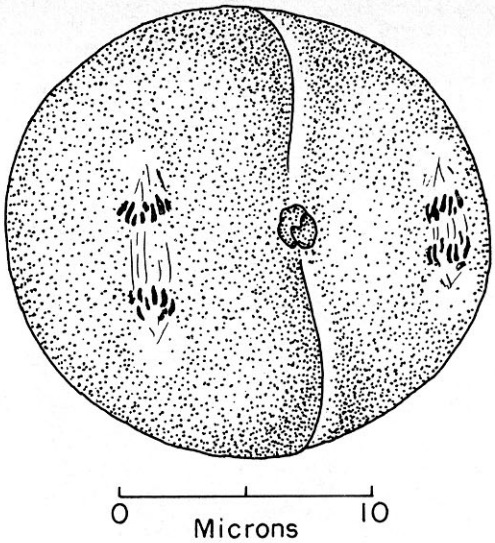

FIGURE 321.—Beginning of second cleavage of the egg. Viewed from the animal pole. Whole mount, Kahle, Feulgen stain.

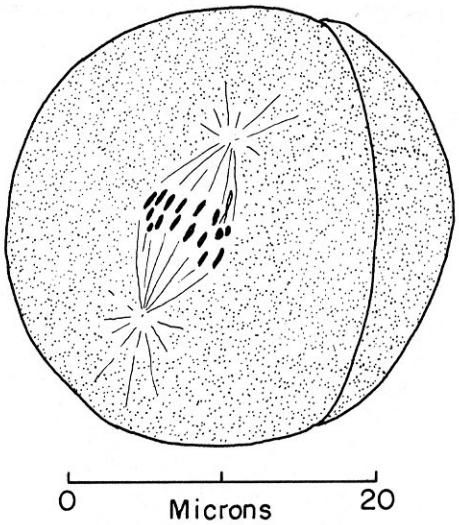

FIGURE 322.—Section of an egg of *C. virginica* at the second cleavage. Beginning of anaphase. Kahle, Heidenhain iron-hematoxylin.

furrows which will intersect the egg into four cells, A, B, C, and D. At this stage the mitotic figures are fairly large and the chromosomes are in a favorable position for examination. In the best sectioned preparations of the cleaving egg, eight daughter chromosomes were counted at the beginning of anaphase (fig. 322). It would be, however, premature to state that the diploid number of chromosomes in *C. virginica* is 8 because on other preparations 7, 9, and 10 were counted.

The third division of each quadrant cuts the cells in the equatorial plane and separates the first quartet of micromeres, small cells at the animal pole, from the macromeres, or larger cells at the vegetal pole (fig. 323).

At the fourth and fifth cleavages, resulting in 16- and 32-cell stages, the micromeres overgrow the macromeres. Only one of the macromeres is visible in the figure 324 and two in 325, which show a side view of an oyster egg at these two stages of development.

Cell lineage, or tracing the developmental history of the cleavage blastomeres through to

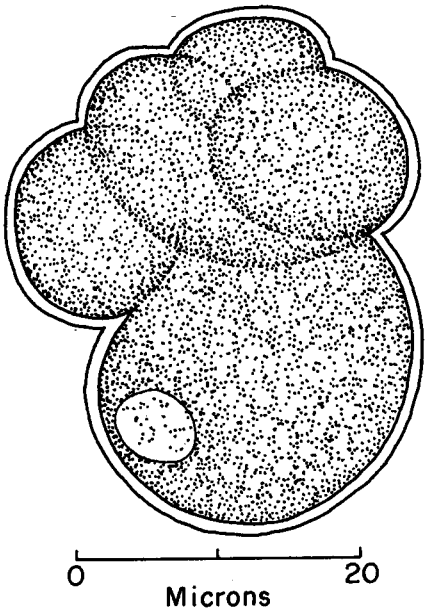

FIGURE 323.—Third division of fertilized egg of *C. virginica;* side view. Separation of micromeres (on top) from macromeres (bottom). Only one macromere is seen in the plane of view. Live egg.

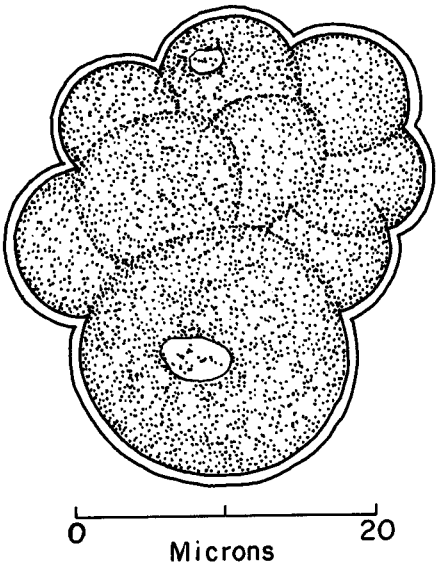

FIGURE 324.—Fourth cleavage of egg of *C. virginica* and the formation of 8 micromeres (2d quartet). Side view. Drawn from photomicrograph of live cell.

their ultimate fates as parts of the larva or adult, was first described by Whitman (1878) for the egg of *Clepsine,* and followed by Wilson (1892) for the egg of *Nereis.* The works of Lillie (1895) on the development of Unionidae, Conklin (1897, 1908) on *Crepidula* and *Fulgur,* Meisenheimer

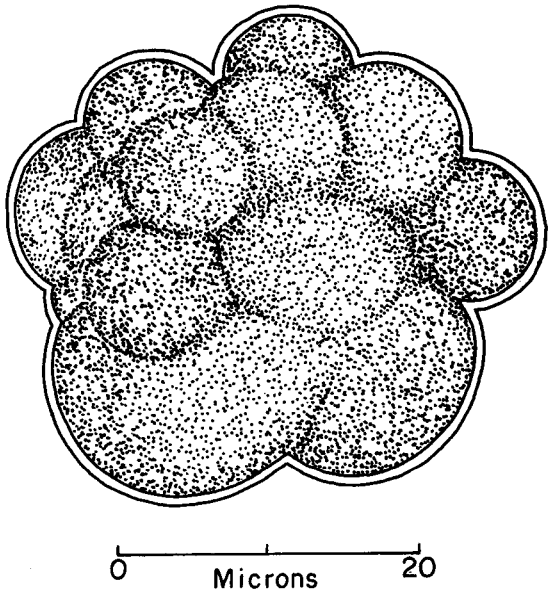

FIGURE 325.—Fifth cleavage of egg of *C. virginica* and the formation of the third quartet of micromeres (32-cell stage). Side view. Drawn from photomicrograph of live cell.

(1901a, 1901b) on *Dreissensia* and *Cyclas,* and Wilson (1904a, 1904b) on *Dentalium* and *Patella* constitute major contributions to the embryology of mollusks.

The nomenclature of the cleavage blastomeres, as developed by Wilson (1892), was progressively modified by Conklin (1897), Mead (1897), and Child (1900); the present system is based largely on the work of Robert (1902) on the development of the *Trochus* egg. The system is a combination of letters and numbers by which the blastomeres are identified.

The first four cells or macromeres are designated as A, B, C, and D; in the majority of cases studied D is the largest of the four and is situated at the side which will develop into the posterior portion of the embryo. When the first four blastomeres divide, their daughter cells are denominated 1a and 1A, 1b and 1B, 1c and 1C, and 1d and 1D, the small letters in each case referring to the micromere and the capital letter to the macromere.

In successive divisions 1A divides into 2a and 2A, 1B into 2b and 2B, and so on. When the micromeres divide, 1a is divided into $1a^1$ and $1a^2$, the superscript 1 denoting the daughter cell which is nearest to the animal pole and superscript 2 the one nearer to the vegetal pole. The nomenclature is capable of indefinite expansion, but certain confusion arises when the two daughter cells resulting

from the division of one cell lie at an equal distance from the pole. In this case the letters r for right and l for left are used. The practice is, however, not generally followed.

Descriptions of various types of cleavage can be found in volume 1 of MacBride (1914). The equal and unequal cleavages in the spirally cleaving eggs of annelids and molluscs are discussed by Costello (1955) in chapter 2 of Willier, Weiss, and Hamburger (1955).

During division the micromeres of a quartet, viewed from the animal pole, become slightly displaced because the spindles of the dividing cells (not shown in fig. 324 or 325) occupy an oblique position with respect to the egg's axis. At the following divisions the plane of separation of daughter cells is oriented approximately at a right angle to the preceding divisions. The pattern of such cleavage is called spiral. It gives rise to an irregular morula (sterroblastula according to Korschelt and Heider, 1895, from the Greek "sterros" meaning firm) found in annelids (*Nereis*), in some bivalves (Ostreidae, *Teredo*), and in gastropods (*Crepidula, Fulgur, Nassa*), and others. In all cases the sterroblastula arises from an unequal cleavage during which the micromeres overlie the macromeres, and at each division are slightly displaced to the right (dexiotropic cleavage) or to the left (laeotropic cleavage). Sometimes, as in the case of *Dreissensia*, the second dexiotropic cleavage is followed by a third dexiotropic cleavage after which the normal alternating course is established (Meisenheimer, 1901a). In the case of oyster eggs, as shown by Fujita (1929) for *C. gigas*, the cleavage is laeotropic.

The multicellular stages of a *C. virginica* egg are reached in the course of the sixth and ensuing cleavages (figs. 326 and 327) during which the micromeres divide much more rapidly than the macromeres, become progressively smaller and overgrow the vegetal pole. Approximately at this stage the sterroblastula of an oyster is formed.

Gastrulation begins with epibolic extension of the micromeres. At 22° to 24° C. the stage shown in fig. 328 is reached within 4 to 6 hours.

The cell lineage of *C. virginica* has not been studied; the stages of development of eggs of the species shown in figures 320 to 328 are similar to those previously described by Brooks (1898); Horst (1882) for *O. edulis;* Seno (1929) for *O. denselamellosa;* Hori (1933) for *O. lurida;* Yasugi (1938) for *O. spinosa*, and *C. gigas*. Yasugi

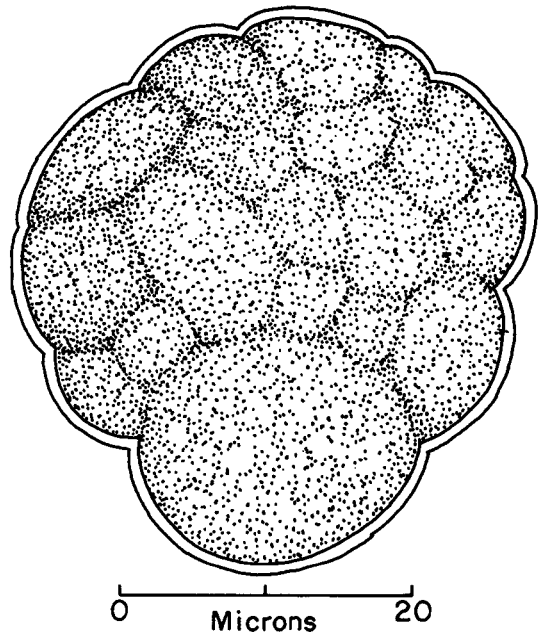

FIGURE 326.—Formation of sterroblastula in *C. virginica* egg; of the four macromeres only two are visible from the side. Micromeres begin to overgrow the vegetal pole. Drawn from photomicrograph of live egg.

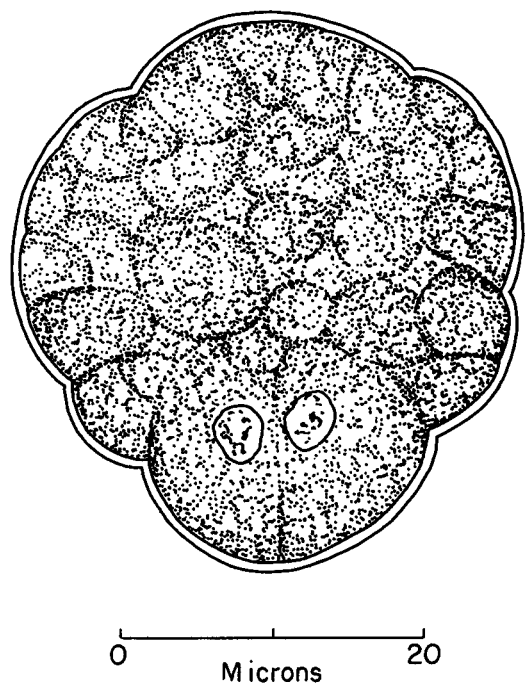

FIGURE 327.—Advanced sterroblastula stage of *C. virginica*. One of the macromeres has divided into two daughter cells of equal size. Drawn from photomicrograph of live egg.

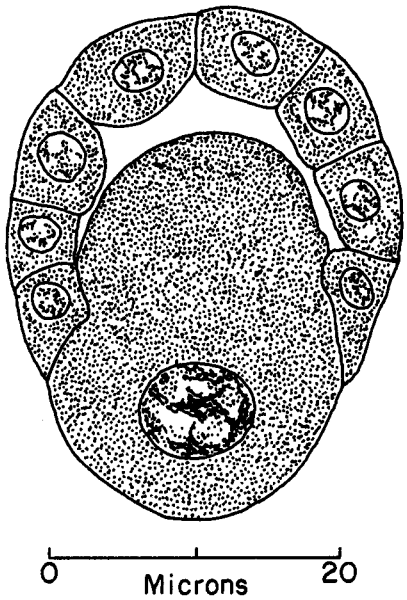

FIGURE 328.—Early stage of gastrulation in the egg of *C. virginica*. Seen at a section of an egg cut along its axis. Heidenhain, iron-hematoxylin.

found that equal cleavage can be induced artificially in eggs of the Japanese oyster by centrifuging for 2 minutes at 1,500 r.p.m. and at the centrifuge radius of 14 cm.

Fujita (1929) gives a brief account of the cell lineage of the eggs of *C. gigas* and states that the mode of cleavage of this species is identical to that of *C. virginica*. The main features described by him are as follows. The first polar body in the fertilized egg of *C. gigas* appears 15 minutes after insemination. At the two-cell stage (fig. 329) the two blastomeres of unequal size, AB and CD, are separated along the meridional plane. Their position corresponds to the anterior (Ant.) and posterior (Pst.) ends of the embryo. The second division, also meridional, separates the four blastomeres A, B, C, and D (fig. 329b).

A and B represent the anterior, and C and D the posterior halves of the embryo, while B and C form its left and A and D its right halves (fig. 329b). The ensuing cleavage starts with the blastomere B and proceeds in laeotropic order to C, D, and A; the resulting daughter cells, the micromeres a_1, b_1, c_1, and d_1, retain the shape of the mother cells but are smaller. The macromere D and micromere d_1 are respectively the largest. The four daughter cells a_1 through d_1 form the first quartet of micromeres located between the macromeres on the dorsal side of the embryo.

The 12-cell stage is initiated by the division of the macromere D; the ensuing larger cell d_2 (fig. 329c) is generally known as the first somatoblast X. (In the system of nomenclature used by American and European embryologists (see p. 346) the 1D cell gives rise to 2d and 2D and the 2d is the X cell.).

The cleavage is continued laeotropically, and the second generations of micromeres a_2, b_2, and c_2 are smaller than the first macromeres. They lie on the outside of the macromeres. The third cleavage of macromeres A, B, and C continues in laeotropic order and results in the micromeres a_3, b_3, and c_3; they are larger than other micromeres. After the third cleavage the macromeres make no further contribution to the formation of micromeres and in the course of development become the entoderm. The first somatoblast (X cell) gives rise to many organs of ectodermal origin. At the 18-cell stage of the embryo the position of cell X and its first divisions mark the beginning of the transition from spiral to bilateral symmetry (fig. 329d).

The mesoderm begins to form at about the 32-cell stage with the appearance of cell 4d, the second somatoblast, also designated as cell M. In bivalves the cell M remains at the surface for a long time, then divides into the two mesodermal teloblasts which sink into blastocoel (Raven, 1958, p. 117). The formation of mesoderm in *C. gigas* has not been followed in detail, but as a rule the mesoderm bands in bivalves remain rather rudimentary (Raven, 1958). Fujita states that the establishment of the three germinal layers in *C. gigas* is completed at the 30-cell stage (fig. 329 e and f).

The gastrula stage is reached in 4 to 6 hours. The cell lineage of *C. gigas* is generally comparable to that described for other bivalves (see: Raven, 1958, p. 70), but for details the reader should consult Fujita's (1929) original text and his drawings.

In about 4 to 6 hours after fertilization, an egg of *C. virginica* reaches the stage (fig. 330) when a few large cilia become visible at the vegetal pole, the oval-shaped body is covered with very

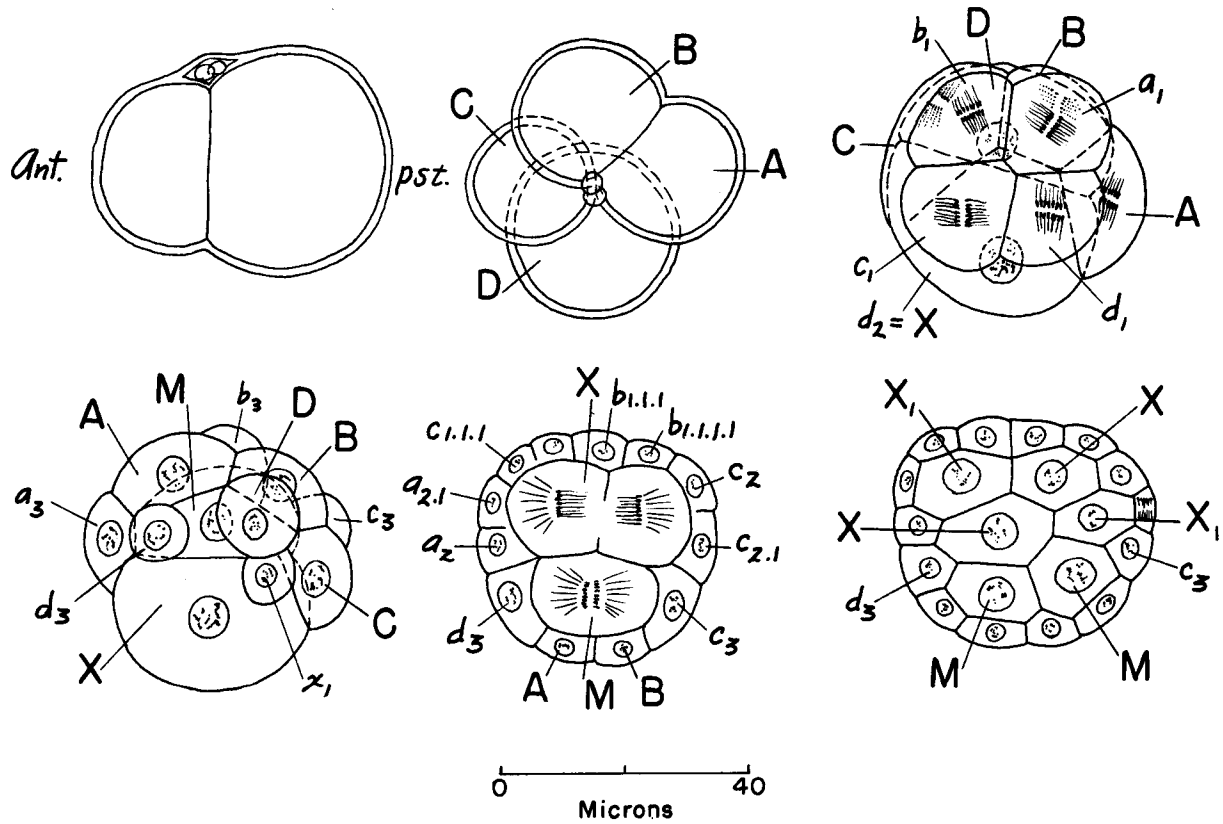

FIGURE 329.—Several stages of development of the egg of *C. gigas*. Redrawn from Fujita, 1929. a—Two-cell stage, Ant.—anterior, Pst.—posterior ends; b—Four-cell stage, formation of blastomeres A, B, C, and D; c—12-cell stage and the formation of the first somatoblast (cell X), viewed from the animal pole; d—embryo viewed from vegetative pole after the formation of the mesomere M; e—cleavage of mesomere M and the first somatoblast X, posterior view optical section; f—advanced stage of development showing the arrangement of the mesomeres M, M, and the somatoblasts, X, X, X_i, X_i, posterior view optical section. Cleavage nomenclature as given by Fujita.

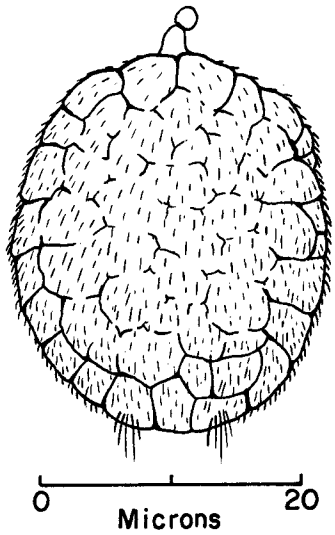

FIGURE 330.—Larva of *C virginica* ready to hatch. Drawn from photomicrograph of live larva.

fine ciliation, and two polar bodies still remain attached to the animal pole. The beating of the cilia is not coordinated at this stage, and the movements of the larva are irregular and spasmodic. A few minutes later a girdle of powerful cilia is formed, the polar bodies are lost, and the larva begins to swim upward (fig. 331). In a finger bowl containing cleaving eggs, the newly hatched larvae appear as white columns rising from the layer of fertilized eggs on the bottom of the container (fig. 332). The larvae can be pipetted off easily, transferred into larger containers and provided with suitable food.

The time required to complete the development of an oyster egg varies, depending on condition of eggs, temperature, salinity, oxygenation of water, and other environmental factors. Records of three sets of observations made in the Woods

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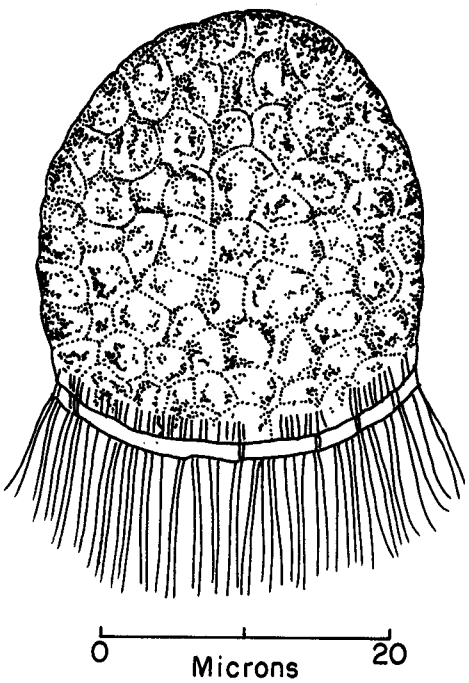

FIGURE 331.—Larva of *C virginica* at the time of its emergence 6 to 6½ hours after fertilization. Drawn from a photomicrograph of a live larva.

Hole laboratory at room temperatures varying from 22.5° to 24.5° C. and salinity of water of 32.2 °/oo are given in table 37. To obtain records of rates of development at different temperatures, several hundred artificially fertilized eggs were placed in each Syracuse dish filled with fresh sea water and covered to prevent evaporation. The debris was removed, and the water contained no unfertilized or cytolyzed eggs.

TABLE 37.—*Observations on the time required for artificially fertilized eggs of* C. virginica *to reach trochophore stage*

All observations were made at Woods Hole in July at room temperatures varying from 23° to 25° C. The time required to reach different stages varied in different groups of eggs. The observations are arranged in two groups: A and B, which differ primarily in the duration of time required to reach rotating blastula and trochophore stages.

Stage of development	STAGES A	STAGES B
Fertilization membrane	5 min	10 to 25 min.
First polar body	40 min	25 to 52 min.
Second polar body	1 hr. 10 min	40 to 65 min.
First cleavage	1 hr. 12 min	45 min.
Second cleavage		52 to 120 min.
Third cleavage	2 hr. 10 min	55 to 195 min.
Morula stage		135 min.
Rotating blastula	4 hr	6 hr. 30 min.
Trochophore	5 hr	8 to 9 hr.

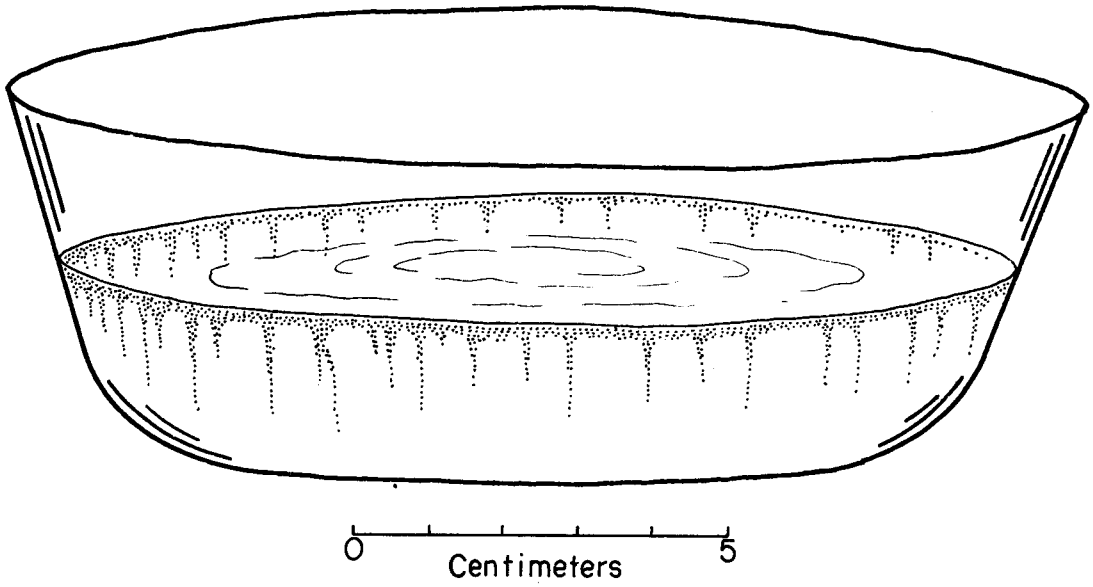

FIGURE 332.—The emergence of larvae of *C. virginica* from fertilized eggs kept in a finger bowl. The free-swimming larvae form columns, which tend to disperse at the surface. Drawn from life.

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