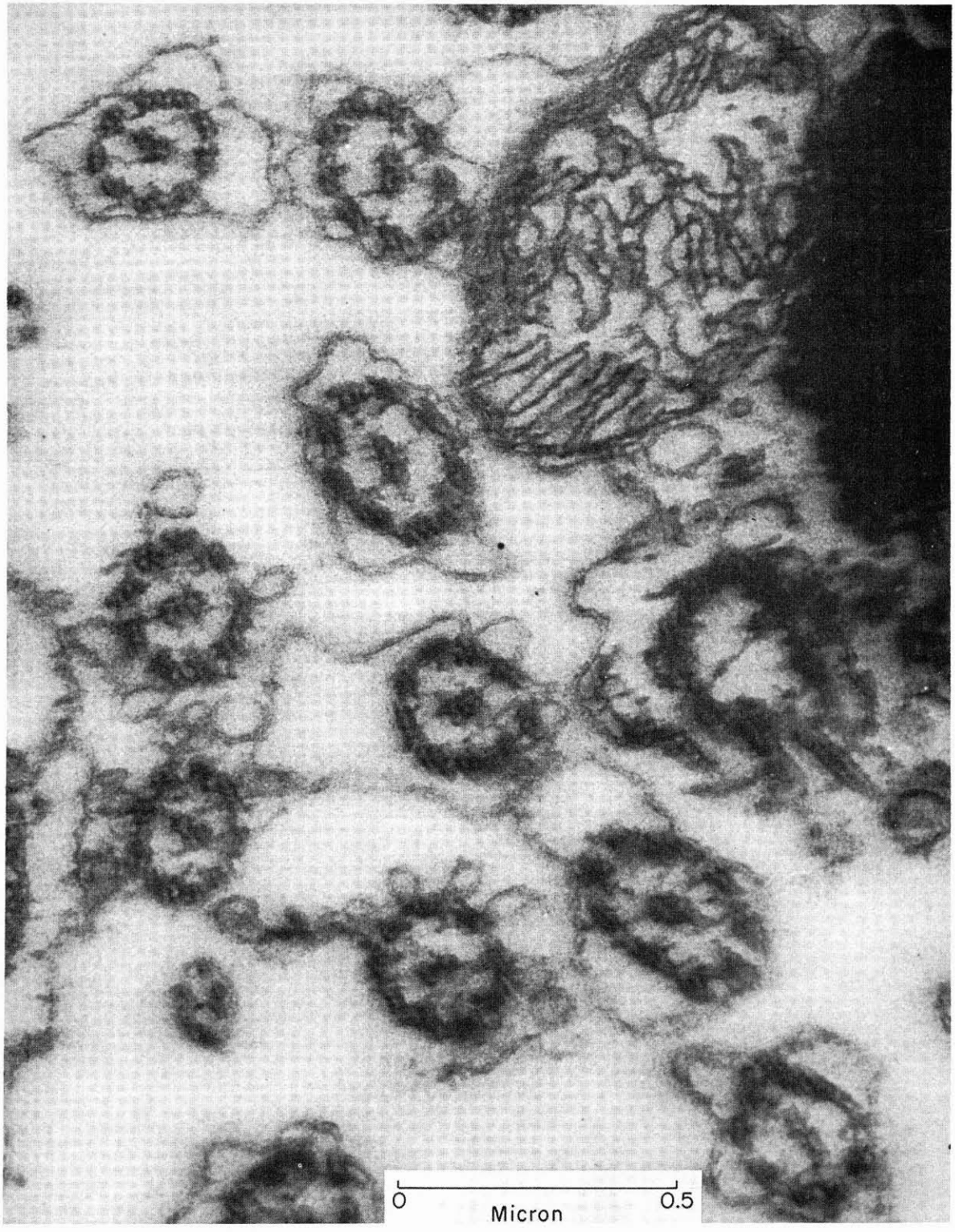

FIGURE 312.—Transverse section of the tails of oyster sperm of *C. virginica* slightly below the level of the middle piece. Electron micrograph.

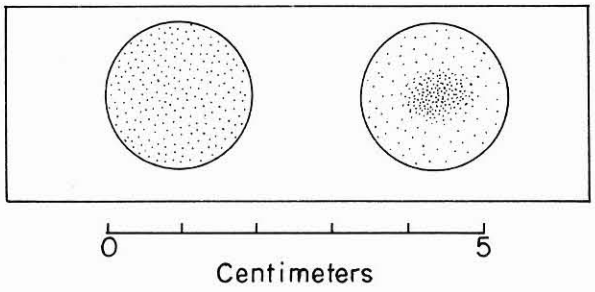

FIGURE 313.—Sperm suspension of *C. virginica* on a slide in sea water (left) and in sea water containing a small quantity of egg water (right). Drawn from life. Natural size.

Agglutination also occurs in the sperm of *C. gigas* and in *O. circumpicta* Pilsbry. Terao (1927) experimented with the latter species using egg water made by mixing 0.55 ml. of ripe eggs in 9 ml. of sea water and removing the eggs by centrifuging and filtering after they had stood for 20 minutes. The filtrate caused the isoagglutination of sperm even in a dilution of 1 to 10 millions. Heteroagglutination by the egg water of *O. circumpicta* has been observed in the suspensions of sperm of the bivalve *Arca*, sea urchin *Toxocidaris tuberculatus*, and starfish *Luidia quinaria*.

Lillie (1919) regarded the sperm agglutinating factor he discovered in *Arbacia* eggs as an essential to fertilization, and to the active substance of egg water he gave the name fertilizin. Tyler (1948) identified fertilizin with the jelly substance of the egg and on the basis of experimental data concluded that the presence of the jelly coat has a favorable effect on fertilization. By biochemical analysis of sea urchin eggs, Vasseur (1948a, 1948b) determined the composition of the jelly coat and found that 80 percent of it consists of polysaccharide and 20 percent of amino acids. The substance was found to exert a heparinlike action in a blood-clotting system (Immers and Vasseur, 1949). After removing the jelly coat with acidified water, Hagström (1956a, 1956b, 1956c, 1956d) found that the rate of fertilization was higher than in the presence of the coat. It is, therefore, apparent that the jelly coat is not essential for fertilization.

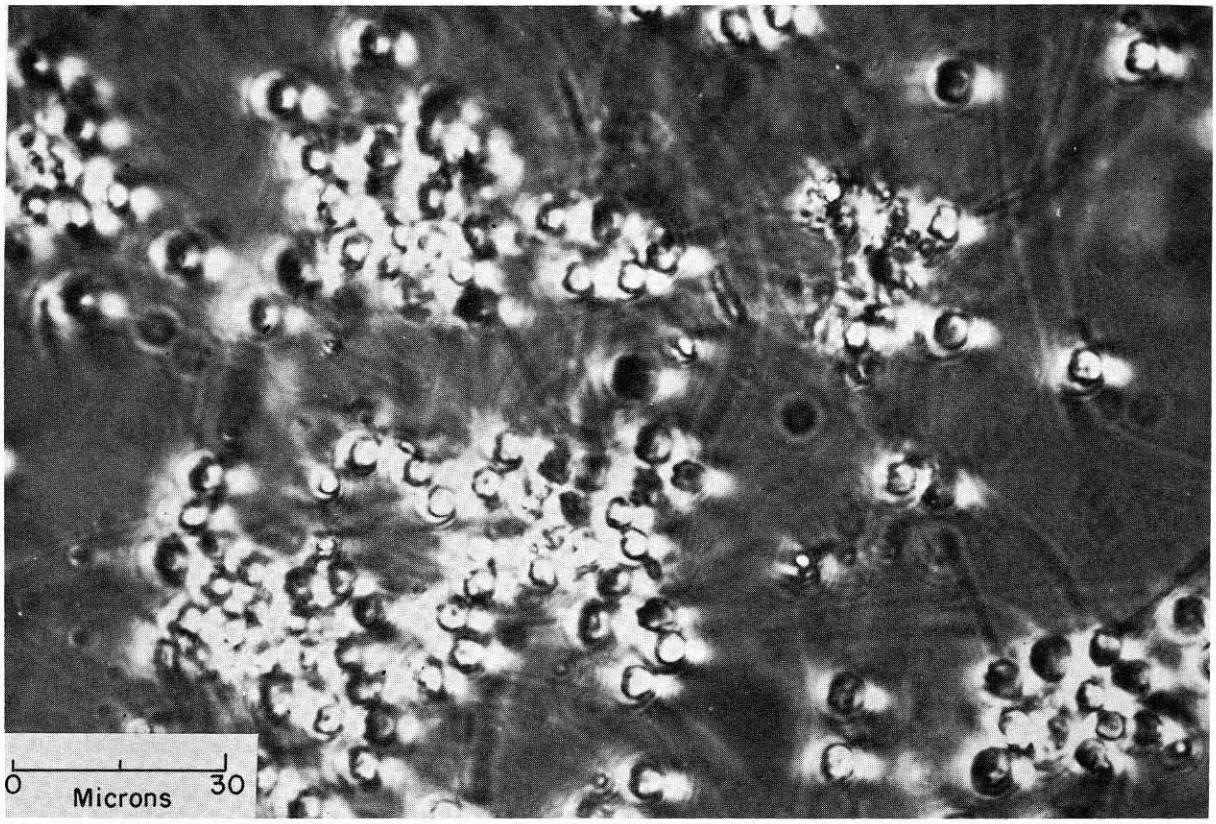

FIGURE 314.—Photomicrograph of sperm of *C. virginica* agglutinated by egg water of the same species. Phase contrast oil immersion.

This view agrees with the conditions found in oyster eggs which have no jelly coat.

According to the modern view discussed in the review of the problem by Runnström, Hagström, and Perlmann (1959), the jelly coat not only fails to improve fertilization but impedes it by acting as a sieve. Its action may be considered as an elimination process by which the number of spermatozoa capable of attaching to the cytoplasmic surface is substantially reduced.

Fertilizin of sea urchin eggs has two distinct properties: it agglutinates sperm suspension and activates the motility of free, single spermatozoa. Both of these properties are present in the fertilizin of an oyster egg.

ACROSOMAL REACTION

Spermatozoa of various invertebrates have been found to carry a substance of protein character, probably a lysine, capable of dissolving the vitelline membrane of the egg. Such lysine is present in the sperm of the giant keyhole limpet, *Megathura crenulata* (Tyler, 1939), in the sperm of *Mytilus*, where it is probably located in the acrosome (Berg, 1950; Wada, Collier, and Dan, 1956), and in other marine animals (Tyler, 1948, 1949).

Upon contact with the surface of an egg, the spermatozoon undergoes a so-called acrosomal reaction, which is described as the deterioration of the surface of the acrosomal region of the head followed by a projection of a stalklike filament. The acrosomal reaction and the discharge of the filament have been observed in starfish, holothurians, mollusks, and annelids. The reaction was studied by Colwin, A. L., and L. H. Colwin (1955) and Colwin, L. H., and A. L. Colwin (1956) in the annelid *Hydroides hexagonus* and enteropneust *Saccoglossus kowalewskii*. Using electron microscopy, the Colwins revealed many interesting details of the penetration of the spermatozoon into egg cytoplasm. In pelecypod mollusks the discharge of the acrosomal filament was observed in *Mytilus* and in the three species of oysters, *C. echinata*, *C. nippona*, and *C. gigas* (Wada, Collier, and Dan, 1956; Dan and Wada, 1955). The reaction can be induced by egg water as well as by the contact of a spermatozoon with the egg surface. The first sign of acrosomal reaction in oyster sperm is the flattening of the anterior surface of the spermatozoon. At the same time the head becomes extremely adhesive, the acrosome membrane bursts, and the filament is discharged. The reaction can be observed when a small drop of live sperm suspension is placed on a cover slip, a minute quantity of egg water is added, and the cover slip then inverted on a slide. The preparation is examined with phase contrast oil immersion lens using anisol (Crown oil) of refractive index 1.515 instead of cedar oil.

The acrosome reaction of *C. virginica* is similar to that observed by Dan and Wada in three other species of oysters. Under the effect of egg water the head becomes swollen and rounded and the filament is ejected from the acrosome. The discharged filament is wider than the tail and is about three to four times longer than the length of the head (fig. 315). In my observations only a small number of oyster spermatozoa suspended in egg water discharged acrosomal filaments.

The exact role of the filament in the fertilization of oyster eggs is still unknown. Investigations

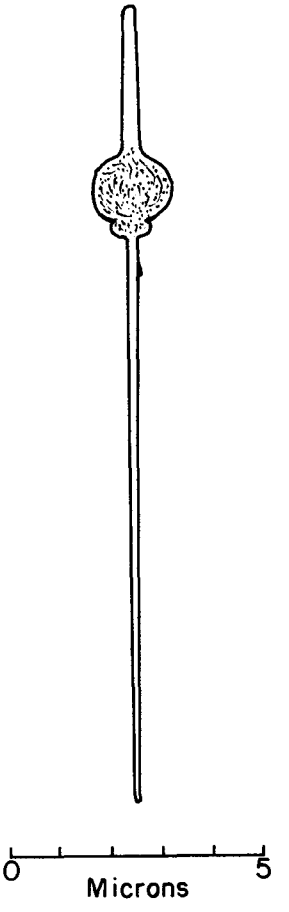

FIGURE 315.—Diagrammatic drawing of acrosomal reaction in the spermatozoon of *C. virginica* produced by egg water. Only a part of the sperm tail is shown in the drawing. Drawn from live preparation.

with eggs of other invertebrates suggest that the acrosome region of a spermatozoon is active during the first stages of fertilization and that it carries a lysine which facilitates the attachment of the spermatozoon to the egg membrane and its penetration into the cytoplasm.

The old view that spermatozoa penetrate the egg by a mechanical action of screw-borer movements of the pointed end (the perforatorium) has been abandoned. It is now generally accepted that the action of the sperm head is primarily chemical and that probably several enzymes are carried by the acrosome. Readers interested in the problem of fertilization are referred to comprehensive reviews of this subject by Runnström, Hagström, and Perlmann (1959), Colwin, A. L., and L. H. Colwin (1961a, 1961b), and Colwin, L. H., and A. L. Colwin (1961).

FERTILIZATION OF EGG

Eggs for fertilization experiments may be obtained in the laboratory by stimulating a single female spawn as described in chapter XIV. A suspension of eggs pipetted off the bottom of a laboratory tank is free of blood and other body fluids. Eggs may also be taken directly from the ovary by cutting off small slices from the surface of the gonad and mincing or shaking them in sea water. Cutting into the underlying layer of digestive diverticula should be avoided to prevent contamination with body fluids. The eggs must be washed several times in filtered seawater by decanting or by filtration through a fine sieve until the suspension is free of tissue cells and debris. After being in sea water for a short time, the eggs change their shape and become globular but their large germinal vesicles remain clearly visible (fig. 316).

A sperm suspension may be obtained by any one of three methods. Male spawning can be induced by raising the water temperature or by adding a small amount of thyroid suspension, and live spermatozoa collected as they are discharged through the cloaca; small pieces of ripe spermary can be excised and the spermatozoa liberated in sea water by shaking; or a very ripe spermary can be pressed gently with the fingertip and the spermatic fluid pipetted as it comes from the gonoduct. Concentrated sperm suspension must be diluted for fertilization. I found it convenient to make a standard suspension using 0.2 g. of gonad material in 50 ml. of sea water and then

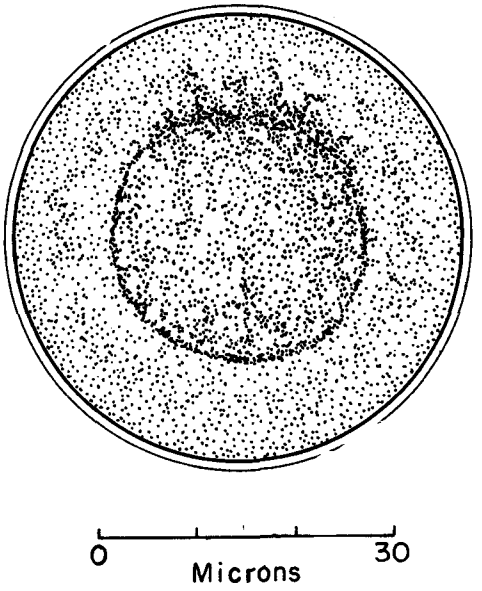

FIGURE 316.—Camera lucida drawing of naturally spawned but unfertilized egg of *C. virginica*.

diluting it, using 0.5 ml. for 100 or 150 ml. of water containing eggs.

Although several spermatozoa may attach themselves to an egg, (fig. 317), only one penetrates the cytoplasm. The others, called supernumeraries, eventually are cast off when cleavage

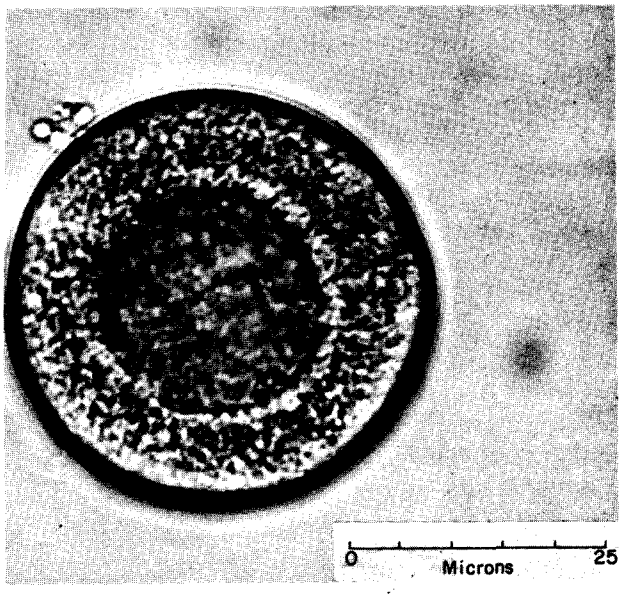

FIGURE 317.—Photomicrograph of a fertilized egg of *C. virginica* a few minutes after the formation of the fertilization membrane. Several spermatozoa are attached to the egg membrane but only one will penetrate it. The germinal vesicle is intact. Contrast phase oil immersion lens.

begins. If the sperm suspension is too concentrated, many spermatozoa enter one egg and cause polyspermy, a condition which may interfere with normal development of the egg.

The spermatozoon which succeeds in penetrating the egg's surface undergoes great changes. Its acrosome region becomes swollen and disrupted and the tail loses its motility; the head gradually penetrates the egg membrane as the sperm moves deeper into the cytoplasm. At the same time the fertilized egg contracts and assumes a globular shape if it was not round before; the cytoplasm becomes so dense that the germinal vesicle is no longer visible under the layer of yolk granules. A few seconds after the sperm head touches the egg's surface a thin, transparent fertilization membrane is elevated and under the light microscope appears to be homogeneous. This membrane apparently is formed from the pre-existing vitelline membrane and is underlined by a layer of subcortical particles (fig. 318). The two layers are optically separated. It is generally accepted (Runnström, 1952) that in *Arbacia* and many other species the fertilization membrane originates from the vitelline membrane because it fails to form after the vitelline membrane has been removed with potassium chloride, trypsin, or urea. No experimental work of this type has been done on oyster eggs.

AGING OF EGGS AND SPERM

The longevity of eggs of marine invertebrates, i.e., their ability to form fertilization membrane and undergo cleavage, was observed in the sea urchin (*Arbacia*) and in other common species (Harvey, 1956). Oyster eggs also undergo aging changes and lose their ability to be fertilized. This has been demonstrated in a number of tests made in the Bureau's shellfish laboratory at Woods Hole. Because of wide individual variability in fertilization capacities only one female and one male were used in each series of tests. The following technique was used: Suspension of eggs was made by shaking 0.5 g. of ripe ovary tissue in about 200 ml. of sea water: eggs released by this action were permitted to settle on the bottom and the supernatant water was decanted; the remaining eggs were rinsed twice in sea water and transferred to a beaker filled with 500 ml. of filtered sea water. The beaker was kept half submerged in running sea water to prevent heating to room temperature. Samples of eggs were taken for fertilization every hour during the first 4 hours, then at 2-hour intervals for the next 6 hours, and finally one sample was taken each time after 12 and 24 hours. Eggs were collected at random from the bottom of the beaker and placed in a finger bowl in 100 ml. of filtered sea water. To fertilize them 0.5 ml. of dilute stock suspension of sperm was used; the water was gently stirred

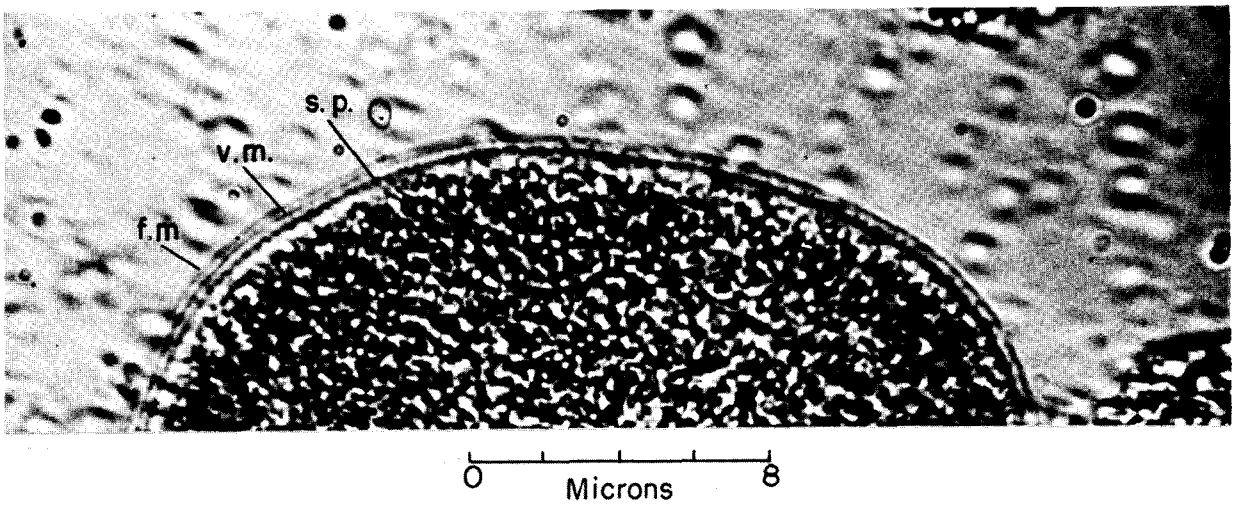

FIGURE 318.—Photomicrograph of a portion of fertilized egg of *C. virginica* shorly after the attachment of sperm. Fertilization membrane (f.m.) (outside layer) is underlined by the vitelline membrane (v.m.) with a dense row of subcortical particles (s.p.). Live preparation. Oil immersion phase contrast lens.