

lying in the egg substance is, at this time, perfectly naked, *i. e.*, unaccompanied by archoplasm or radiation of any sort. As the sexual nuclei approach each other, radiations appear around the centrosome in the archoplasm; or these radiations appear first after the nuclei have met. *There can be no doubt in either case that the centrosome around which they appear is the egg center*, seeing that it lies within the archoplasm, which always accompanies the egg nucleus. The centrosome then divides in two, forming the amphiaster of the first cleavage.

The egg and sperm nuclei never fuse to form a single vesicular cleavage nucleus, but each forms its own group of sixteen chromosomes.

The study of the earlier stages shows, that the sperm head is accompanied soon after its entrance by a comet-like aster, with a minute centrosome. *This centrosome divides and forms an amphiaster, which entirely disappears in the late anaphase of the first maturation spindle. The sperm centrosomes never become functional again.* But a supernumerary central aster appears near the center of the egg during the metaphase of the second maturation spindle. This aster also disappears during the anaphase of the same spindle.

Thus the mode of fertilization in *Unio* agrees with that in *Myzostoma*, in so far as the centers of the cleavage spindles are derived from the ovum, but differs from it in as much as the spermatozoön in *Unio* brings in a centrosome, whereas the spermatozoön of *Myzostoma* does not introduce a centrosome into the ovum. *Unio* is thus in a certain sense intermediate between *Myzostoma* and those forms in which the sperm centrosome forms the active centers of the cleaving ovum.

*Centrosome and Middle-piece in the Fertilization of the Egg.* E. B. WILSON.

In an earlier paper the author had de-

scribed the sperm-aster in *Toxopneustes* as arising about the middle-piece of the spermatozoön as a center. Within the central mass thus formed no constant central granule could be found; the conclusion was therefore drawn that the middle-piece as a whole must be identified as the centrosome. Later studies on material differently fixed show that this conclusion was erroneous. In eggs fixed in picro-acetic and weak sublimate-acetic (1-5 per cent. acetic) the middle-piece stains intensely black and its entire history can be accurately followed in sections. As the sperm nucleus moves inward the middle-piece separates from the nucleus, is left behind, and finally breaks up and degenerates. The astral rays are thus found to focus at a point lying at the base of the nucleus, between it and the middle-piece. At this point is an extremely minute intensely shining granule, which undoubtedly is the centrosome, as described by Boveri, von Rath, Hill and Kostanecki. The centrosome occurs in the same form in *Arbacia* and *Asterias*. In some cases the sperm-aster and centrosome move away from the nucleus before the latter has separated from the middle-piece. These facts demonstrate that the middle-piece proper is not the centrosome, and that the latter is an infinitesimal granule which lies either inside the middle-piece or between it and the nucleus.

In *Arbacia* the sperm centrosome can be traced continuously though the first cleavage into the 2-cell stage, as in *Chaetopterus*, *Thalassema* and *Physa*; and precisely as in those forms in the late anaphases each cleavage centrosome, after doubling, gives rise to a daughter-amphiaster and central spindle which are, however, of extraordinary minuteness. In *Toxopneustes*, after exactly the same treatment, the result is apparently different, agreeing in substance with the author's earlier studies and with the accounts of Boveri and Reinke. In the 'pause,'

after fusion of the sperm-aster, a single 'centrosome' is found at the center of each aster. In later stages the center of the aster is occupied by a well-defined reticulated sphere, somewhat smaller than is the case after strong sublimate-acetic, and containing a group of distinct intensely shining granules (10-20 in number). The central sphere often has a sharp boundary and gives almost the appearance of a minute cell nucleus. Whether this appearance is normal remains to be seen, but the possibility must be born in mind that even related forms may differ considerably in respect to the morphology of the centrosome and centrosphere.

*Observations upon Fertilization in Gasteropods.*

H. E. CRAMPTON, JR.

The observations were made upon a species of *Doris* collected on the Pacific coast and upon a species of *Bulla* from Wood's Holl. A complete confirmation was obtained of the accounts of fertilization given by Wilson and Matthews, Boveri and Hill upon sea urchins, Mead upon *Chætopterus*, Kostanecki and Wierjeroski upon *Physa*. The sperm nucleus is preceded by the divided centrosome, although an aster is not formed till after the union of the germ nuclei. The first polar spindle of the egg has a double centrosome at the poles, while the second maturation spindle bears but a single centrosome at the pole. These, however, are very large, and the one remaining in the egg finally breaks up, the centrosomes of the cleavage spindle being derived from the sperm. The germ nuclei never fuse, but lie in very close contact to one another.

*The Maturation and Fertilization of the Eggs of Limax.* E. F. BYRNES.

After leaving the ovo-testis, the eggs of *Limax agrestis* are stored in the albuminous gland, where they are fertilized prior to the formation of the capsules.

By the time the egg reaches the albuminous gland the first polar spindle is already formed and occupies the middle of the egg (the stage of the 'archiamphiaster').

The center of the egg-astrosphere appears under widely different forms. In the stage of the archiamphiaster it appears as a central group of granules surrounded by two sharply outlined, homogeneous envelopes, an inner colorless envelope and an outer deeply staining one.

At the time of the formation of the first and second polar bodies the center of the astrosphere appears as a deeply staining center, surrounded by an almost colorless envelope from which the astral rays diverge.

After the extrusion of the first polar body it appears as a uniform finely granular sphere in which two tiny centrosomes are often distinguishable.

After the extrusion of the second polar body the center of the aster appears as a large, clear, spherical structure, traversed by a loose reticulum which connects, at the center of the sphere, with a large, deeply staining body. As the sphere increases in size the central body fades out, giving place to a reticulum which occupies the entire sphere. The egg-astrosphere then disappears.

The sperm enters the egg at the lower pole. As the sperm nucleus approaches the upper pole it keeps pace with the growth of the egg nucleus.

The centrosome of the segmenting egg enters the egg with the sperm, but the time of the appearance of the sperm-asters is variable.

1. *A New Microtome.*

2. *Laboratory Methods.* C. S. MINOT.

A new microtome was exhibited and its mode of working described; methods for polishing the edges of microtome knives, for storing pamphlets, and other matters of laboratory administration, were presented.