With the larvae of *Dendraster eccentricus* development was much more rapid and vigorous. Segmentation was approximately twice as rapid as in the sea urchins. In 48 hours the 4 armed plutei were formed in 10 to 12 days, and in 10 days the third pair of arms appeared. This last seemed to be a critical step because if the third pair of arms did not appear within two weeks, the larva remained juvenile. Almost immediately after the appearance of the third pair of arms, the fourth pair began to develop from the oral plate, and were complete within the following week. Subsequently, growth in size and development of the echinus element were striking phenomena. There appeared from this time on ever increasing differences in rate of development between individuals in the same culture. Until metamorphosis, the 8-armed plutei swam freely near the top or fed on the bottom. Differentiation in the sense of shortening the arms did not normally occur. The pluteus exhibited no orientation to contact, but the newly metamorphosed animal showed the stereotropic reactions of the tube feet characteristic of the adult. The earliest metamorphosis occurred 35 days after fertilization. Dimensions of metamorphosed sand urchin were: diameter, .367 mm.; depth, .334 mm. Length of pluteus before metamorphosis, .785 mm. Shortest time required for metamorphosis, 35 days.

[1] Allen, E. J., and Nelson, E. W., *J. Marine Biol. Assn.*, 1910, viii, 421.

3684

Nature of Hyaline Membrane in Fertilized Egg of Sea Urchin.

A. R. MOORE.

From the Department of Animal Biology of the University of Oregon, Eugene, Oregon, and the Hopkins Marine Station of Stanford University.

The inner or hyaline membrane of the fertilized egg of the sea urchin, *Strongylocentrotus purpuratus*, which serves to hold the blastomeres together, is rendered permanent by keeping the developing eggs in a solution composed of 35 cc. sea water + 15 cc. 3/8 M $Ca\ Cl_2$. Such cultures show the following facts: (1) The outer or fertilization membrane around the inert blastula disappears normally, *i. e.*, within 24 hrs. It is therefore not broken by the hatching blastula, as is generally supposed. (2) So long as the larvae remain within the Ca-membrane they retain the form of blastulae, but segmentation goes on, and eventually the blastocoel is filled with mesenchyme cells; invagination does not occur. (3) After 3 to 5 days the

larvae burst the membranes by pressure; the point of breaking has no reference to the axis of the animal. (4) After escape, the larvae invaginate and form more or less abnormal plutei; the insoluble membranes lie about on the bottom of the dish. The fertilized eggs of the sand urchin, *Dendraster eccentricus*, since they have no hyaline membrane, do not show similar reactions. In the calcium sea water solution they develop normal gastrulae and plutei only a little more slowly than the controls. In this sand urchin, therefore, the blastomeres must be held together by a mechanism different from that of the sea urchin.

If the fertilized eggs of sea urchin are put into sea water which has been brought to pH 3.5 by means of HCl, the hyaline membrane disappears, and in the course of a few minutes the perivitelline space becomes filled with hyaline droplets exuding from the cytoplasm. In volume the droplets may come to equal three-fourths the volume of the cytoplasm, but neither the diameter of the egg (.072 mm.) nor of the sphere enclosed by the outer membrane (.098 mm.) is immediately altered. Therefore the droplets must largely consist of the sea water of the perivitelline space transferred into the droplets, presumably by osmosis. The whole process is suggestive of the Spiro-Henderson effect. If now the eggs are returned to normal sea water of alkaline reaction, the hyaline droplets return into the cytoplasm, *i. e.*, the process is reversible. If, however, the eggs in the acidulated sea water are centrifuged, the hyaline droplets are broken away from the cytoplasm. Such eggs upon being returned to normal sea water continue their development, but the hyaline droplets remain lying free in the perivitelline space, and when the fertilization membrane breaks, they fall out and may be seen spread over the bottom of the dish. This proves that resorption of the droplets in the uncentrifuged eggs depends upon the physical connection of the droplets with the cytoplasm. The larvae resulting from both centrifuged and uncentrifuged eggs develop to normal early plutei. Those resulting from cytoplasm minus droplets are slightly smaller in size than normal. The results prove that the hyaline substance lost from the cytoplasm in the centrifuged eggs is not essential to normal development.

The hyaline membrane behaves like an ion protein compound formed from an exudate of the blastomeres. This substance in sea water pH 8.2 reacts with Ca to form an insoluble Ca-proteinate membrane. In acidulated sea water pH 3.5, the Ca-proteinate cannot exist; consequently, the membrane disappears. The hyaline membrane, therefore, behaves like an ion-protein compound, in the sense used by Loeb.