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NEMERTEA

Cerebratulus lacteus

LIVING MATERIAL:

Adults are found near or below the low-water mark, burrowing in mud, sandy mud, or sand in sheltered bays, harbors and estuaries. If not available at Woods Hole they can be shipped in from Maine or from north of Cape Ann, Mass. The sexes are separate. Sexually ripe males are bright red, ripe females dull, brownish red; in spent animals these colors fade (Coe, 1895).

BREEDING SEASON:

May and June in Long Island Sound, July in the Woods Hole region, and July and August in Massachusetts Bay and on the Maine coast (Coe, 1937).

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The sexes are best segregated. The female Cerebratulus may be kept for three weeks or more in the laboratory, but the eggs are less suitable for experimental purposes after the first week. The worms should be kept in tall battery jars, over the tops of which gauze covers are securely tied; the worms are active and will escape down the drain unless this precaution is taken. A sea water hose should extend through the gauze to the bottom of the jar, to insure a continuous supply of fresh sea water. A low temperature (around 10° C.) is optimal, but it is difficult to maintain with running sea water at Woods Hole.

B. Procuring Gametes: A continuous supply of gametes may be obtained by removing successive portions from the posterior end of a ripe individual. A large female, for example, may produce upwards of fifty million eggs.

Female gametes: Females kept in the manner described above will shed some eggs spontaneously, but to obtain large numbers a posterior piece, an inch long, should be cut off and placed on a five-inch square of cheesecloth in a fingerbowl of cool sea water. With scissors or scalpel make a slit on either side of the mid-dorsal line. The muscular contractions of the fragment will soon force the ripe ova into the water. The cheesecloth retains the slime and body section and allows the eggs to filter through. When they have settled, decant the water and add fresh, filtered sea water.

Male gametes: To obtain sperm, a half-inch posterior fragment of a male is placed in a dish of clean sea water and a puncture made through the dorsal body wall. A drop of sperm suspension, drawn up into a fine-mouthed pipette, should be diluted in 40 cc. of sea water.

C. Preparation of Cultures: When first shed, the egg contains a germinal vesicle, and fertilization at this time results in polyspermy and abnormal development. Allow the eggs to stand until microscopic examination shows that the germinal vesicles have ruptured and the eggs have reached the metaphase of the first maturation division (a matter of 10 to 30 minutes, depending on the temperature and the ripeness of the eggs). Now add one drop of freshly prepared, dilute

sperm suspension. It is important to avoid over-insemination. The eggs remain fertilizable for as long as five hours after shedding.

The cultures should stand undisturbed for half an hour after insemination; then decant the upper layers of water and add a fresh supply of sea water. All cultures should be kept on the water table, at temperatures not exceeding 20° C.

When swimming larvae are formed, they should be decanted daily to fresh sea water. If they are to be followed through to metamorphosis, diatom feeding must be initiated after the first few days.

D. Methods of Observation: Eggs and larvae can be examined by mounting them in a drop of sea water under a supported coverslip. A very small amount of chloral hydrate added to the preparation will usually narcotize the swiftly moving larvae.

NORMAL DEVELOPMENT:

A. The Unfertilized Ovum: The egg is about 120 microns in diameter and is contained in a chorionic sac considerably larger than the egg itself. The egg is dark brown and opaque, due to the presence of large masses of radially arranged yolk globules. At one pole of the egg, and also in the overlying chorion, a small, nipple-like protrusion is present. This marks the point of former attachment to the ovarian wall, and lies immediately opposite the region where the polar bodies are extruded. The prominent germinal vesicle is often located somewhat excentrically, near the animal pole (Wilson, 1900).

When first shed, the eggs are surrounded by a transparent jelly-layer, but this soon dissolves upon contact with sea water. Each egg is surrounded by a thin chorion, which begins to lift from the surface immediately after shedding. This membrane is soft and can easily be cut away or removed by shaking. Ten to twenty minutes after shedding, the germinal vesicle breaks down and its substance flows toward the animal pole, appearing as a diffuse lighter area. The chromosomes become arranged on the first maturation spindle and remain at metaphase until fertilization.

B. Fertilization and Cleavage: The sperm are relatively large, and have a long, sickle-shaped head, and a long tail which propels them through the water with rather slow powerful strokes. While several sperm may penetrate the outer membrane of freshly inseminated eggs, only one normally will bore its way into the egg substance (Wilson, 1900). Apparently the entire sperm enters the egg (Yatsu, 1909). No fertilization cone or membrane is formed.

About 75 minutes after insemination the egg flattens slightly at the animal pole in preparation for polar body formation. During this interval the protrusion at the opposite pole slowly diminishes. This decrease continues throughout maturation and the lobe is usually completely withdrawn by the time the second polar body is given off. After the formation of the first polar body, the egg rounds up and becomes spherical, but again flattens when the second polar body is extruded, a process which occurs about 20 minutes later.

The egg continues to elongate in a direction perpendicular to the animal-vegetal axis. The first cleavage cuts through the egg along the axis marked by the polar bodies, dividing the ovum into two equal cells. The second cleavage furrow appears at right angles to the first. The third cleavage, which quickly

follows, is horizontal, and, since it passes through the egg slightly below the equator, produces four large upper cells and four smaller lower cells. In spite of their larger size the upper cells are considered to be the first quartet of "micromeres," the lower cells being the "macromeres." This third cleavage is clearly dexiotropic. Following it, at least six quartets of micromeres are cut off by alternating laeotropic and dexiotropic divisions, faithfully following the pattern of spiral cleavage as seen in annelids and molluscs. (See figures in the paper by Wilson, 1900.)

The blastula is a nearly spherical, ciliated structure composed of a single layer of cells surrounding a large segmentation cavity. Although not visible in the living embryo, those cells which are destined to invaginate and become the endoderm are distinctly taller than those at the aboral pole. About the time when these cells start to invaginate, a small plate of cells near the aboral pole becomes conspicuous and, while a two-layered, pyramidal gastrula is forming, these cells sink in slightly and produce a cluster of cilia. The cilia elongate enormously and fuse to form an apical flagellum, which is probably sensory in nature. At the time of gastrulation, the mesodermal mother cells migrate inward from the region of the blastoporal lip. Towards the end of gastrulation, the prototroch appears as a band of long cilia encircling the oral surface of the larva. (See the paper by Wilson, 1900, for diagrams of these stages.)

C. Time Table of Development: The following schedule of development is compiled from the data of Wilson (1900). No temperature was recorded. Times are given from insemination.

Stage	Time
First polar body	75 minutes
Second polar body	95 minutes
First cleavage	135 minutes
Second cleavage	155 minutes
Third cleavage	170 minutes
Blastula	15 hours
Gastrula	20 hours
Hatching, young pilidium	38 hours
Well-formed pilidium	108 hours

D. Later Stages of Development and Metamorphosis: Pilidium larvae of three days have a characteristic helmet shape, due to the extension of the edges of the oral surface to form two large, rounded lappets bordered with powerful cilia. The apical organ and its associated flagella persist. Through the transparent outer ectoderm the inner organs are clearly visible. The mouth leads into a wide, ciliated oesophagus which is separated by a slight constriction from the globular stomach. Neither proctodeum nor intestine develops in the free-swimming larva. Spanning the internal cavity, which is the remnant of the old blastocoele, the developing muscle fibers can be seen, the most conspicuous being those which extend from the apical organ to the digestive tract and marginal lappets. Scattered mesenchyme cells are also present in the blastocoele cavity. See the diagrams of Wilson (1900) and Coe (1943) for further details of the structure of the pilidium.

Relatively few larvae reared in the laboratory reach metamorphosis. There is a free-swimming period of about 12 days before this remarkable metamorphosis

sets in. When the future worm has differentiated, utilizing only the larval intestine and the material from four ectodermal invaginations which sink in and surround it, the larval ectoderm, prototroch, lappets and apical organ are cast away and the little worm sinks to the bottom. The cap-like larval rudiment swims about for a time and then, unable to feed and exhausted, it dies. (Further details can be found in the text-book by MacBride, 1914, pp. 118–127.)

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