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Growth and Differentiation of Daphnia Magna Eggs *in vitro*.

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In the course of some ultra-centrifuging experiments there arose the need of rearing the parthenogenetic eggs of Cladocera *in vitro*. A review of the literature revealed no record of the successful growth of eggs of this group of animals outside the brood-chamber of the mother. We have succeeded in rearing the eggs of *Daphnia magna* in tissue culture slides and inasmuch as the embryos are transparent, this enabled us to make observations on some of the sequence of events in the course of the development of the embryos without the necessity of staining and sectioning.

A number of different media were used in an endeavor to grow *Daphnia magna* eggs *in vitro*. A modified Ringer's solution utilized successfully for physiological work on isolated organs of Cladocera by Levy,[1] proved injurious to the eggs. The growth of eggs in a medium consisting of the fluid obtained from the brood-chamber of animals in which there were developing eggs showed no advantage over a simpler medium consisting of sterile pond water. This is of considerable interest in view of the fact that it has been held (Dearborn,[2] Birge[3]) that the brood-chamber of Cladocera secretes a fluid which serves as nourishment in the course of the development of the embryos. Careful controlled experiments revealed that the parthenogenetic eggs in the brood-chamber of *Daphnia magna* completed their development in about 46 hours at 25°C. When the parthenogenetic eggs of this animal were reared in sterile pond water on tissue culture slides, they too completed their embryonic history from eggs to free swimming independent organisms in the same period of time. This demonstrates very clearly that the eggs of this animal are self-sufficient with regard to the nutritive material already stored in them at the time of their deposition.

A *Daphnia magna* female, the eggs of which were to be removed from the brood-chamber, was placed in a depression slide with round polished cavity 15 mm in diameter and 3 mm deep filled with sterile

1 Levy, R., *Compt. Rend. Soc. Biol.*, 1927, **97**, 1600.

2 Dearborn, G. V. N., Chapter on protoplasm and simple animal functions. *Human Physiology*, Philadelphia and New York, Lea & Febiger, 1909.

3 Birge, E. A., Chapter on water fleas, *Fresh Water Biology*, Ward and Whipple, New York, John Wiley & Son, 1918.

pond water. With the aid of 2 fine needles the animal was held in place in the field of the binocular microscope and the ventral edges of the carapace were carefully spread apart. By gently moving the body of the animal back and forth with the needles, the eggs were made to roll out of the chamber without being subjected to any pressure. The eggs were transferred to a new depression slide containing sterile pond water and were incubated at 25°C. The chorion and the vitelline membranes of the eggs are very easily injured. The removal of eggs from the brood-chamber of the mother without injury to them determines the degree of success of rearing the eggs of this animal *in vitro*.

We have obtained several complete series of photomicrographs of *Daphnia magna* eggs taken at 3-hour intervals throughout the course of their development *in vitro*. We have made observations on some of the more prominent changes in the course of the development of the living material as observed by gross microscopical examination. The first sign of movement of the body was observed in embryos 30 hours old. At this stage also the heart exhibited its first pulsations. The first external evidence of brain development appears as a blastodermic thickening mid-dorsally in the cephalic region of 21-hour-old embryos. In embryos 27 hours old there comes into prominence, dorsal to the prospective brain, a double mass of granular substance, representing the material for the development of the eye. In adult individuals the eye is a single organ placed in front of the head but embryologically this structure has a double origin. Other details in the course of the development of the embryos *in vitro* were also followed. Embryos reared on depression slides when transferred to bottles containing the standard amount of the culture medium (Banta[4]) become sexually mature and produce young.

[4] Banta, A. M., *Physiology, Genetics and Evolution of Cladocera*, Carnegie Institution Publication No. 513, 1939.