Marking Experiments to Locate the Presumptive Endoderm in Fundulus heteroclitus

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Sixty-six living embryos of *Fundulus heteroclitus*, the killie fish, were marked at stage 12 or 13 of Oppenheimer (Anat. Rec. **68**: 1, 1937). A coloured mark was made on each blastoderm with cellophane which had been impregnated with Nile blue sulphate and neutral red, and carbon particles were jabbed into the mark with a glass needle. In only 3 out of 47 cases where both marks could be identified had one failed to move with the other. It was therefore assumed that this method was adequate.

Each specimen was drawn by camera lucida to show the position of the mark, and subsequently examined and redrawn at intervals during a period of up to three days. Forty-six were fixed in either Zenker's or Bouin's fluid, and serially sectioned. The blue stain was lost during histological preparation, but the carbon could usually be identified in the section. Usually the marks were placed in the region shown as presumptive endoderm by Oppenheimer (Quart. Rev. Biol. **22**: 105, 1947), on the posterior border of the blastoderm.

The blastoderm could be marked from its lower suface, after releasing its rim from the underlying periblast for a short distance, or from its upper surface. Marks were found in the gut more frequently when they had been applied from the lower rather than from the upper surface. This suggests that endoderm may already be invaginated at stage 12, but more experiments are necessary to establish or refute this. In more than half of all the stage 12 specimens carbon which had been placed at the hind end was later found in the notochord, somites or mesenchyme.

Oxygen Electrode Measurements on the Respiration of Echinoderm Gametes

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The oxygen electrode technique has been evaluted for measurements of oxygen uptake of eggs and sperms of the sea urchin (*Echinarachnius parma*) and the starfish (*Asterias vulgaris*). Gradual settling of the eggs to the bottom of the electrode chamber necessitates the introduction of mechanical stirring. A platinum micro-screw stirrer inserted directly through the teflon plug of the glass electrode chamber proved suitable. Fertilizability and development show that the eggs remain uninjured over a wide range of

stirrer speeds and durations of measurement. The jelly coat is gradually abraded off without adversely affecting respiratory rate or condition of the eggs. The electode response is, however, critically influenced especially by the speed of stirring. The stirring motor must thus be operated under rigidly controlled voltage conditions. The type of electrode is not critical. Open platinum-tipped electrodes ground flush with the glass surface are to be preferred to recessed or collodium-coated ones because of their rapid electric response and their ease of cleaning. The latter point is of considerable consequence with the employed protein-contaminated suspensions. Occasional disturbance of the electrode diffusion gradient through accidental contact between egg and electrode is of little importance, and is always of short duration. The second electrode is a silver wire loop. The circuit is kept constantly polarized.

Maximal exhaustion of oxygen is effected in 30 mins. by 300,000 fertilized sea urchin eggs in sea water in a 2-3 ml. electrode chamber. A strictly constant respiratory rate prevails all the way down to 7-10 mm. oxygen pressure. Then respiration rapidly comes to a stand-still. Unfertilized eggs and sperms behave similarly. Eggs may remain at this low oxygen pressure for periods up to at least 20 mins. without injury to development or fertilizability. Definition of egg suspension density is, however, difficult when repeated experiments on the same stock suspension are desired. Sperm suspensions are, on the other hand, considerably more promising in this respect.

The Pigment of the Jelly Coat Granules of Sand-dollar Eggs

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Pigment granules from the jelly coat of the sand-dollar (*Echinarachnius parma*) egg were obtained by: (1) solvation of the jelly layer in acid sea water, (2) isolation of the granules by differential centrifugation, and (3) repeated washing and resuspension of the granules in buffered sea water. The granules were thereafter packed at high centrifugal speed, lyzed in distilled water or weak buffer (pH 7), and the stroma separated away.

Chromatographic adsorption on $CaCO_3$ from an ether solution of acid treated extract reveals 3 main components. The main portion (80-90%) is dark purplish. The remaining two fractions, which eluate faster than the main fraction, are respectively greenish yellow and venetian red. The latter resembles optically and chemically echinochrome A, which has previously been described from sea urchin eggs and ovaries, whereas the main fraction differs on many essential points.

The native pigment is bound to protein. In neutral extracts it is reddish purple and very stable. At lower pH it turns bright cherry red. Boiling in 4 N-HC splits the pigment-protein bond. In alkaline solutions