PRESERVATION OF HORSESHOE CRAB EMBRYO IN LOW TEMPERATURE AND CULTURES OF EMBRYOS WITH A PUNCTURE IN THEIR CHORION

Tomio ITOW, Shin'ichi IWASAKI and Tatsuya MASUDA

Department of Biology, Faculty of Education, Shizuoka University, Ohya 836, Shizuoka, 422 Japan

In our studies to describe and analyze the developmental roles of a cell-mass at the posteior end of the embyronic area, from which body segments are originated, some techniques are needed to be established. We need to preserve eggs for a long time, and need to introduce a material into embryos.

[A] Preservation of embryos in low temperature (Fig. 1)

The embryos during stage 5 to stage 8 (neighbour stages of germ disc appearance) can be preserved at 5°C for a long time without losing their developmental capacity. After preservation for 3 months, embryos developed normally when transferred to a room temperature.

When embryos beyond stage 9 were treated for long time at 5

C, some of them developed into malformations lacking the posterior part or with a distorted body pattern. No other type of malformations was obtained from the treatments at those stages.

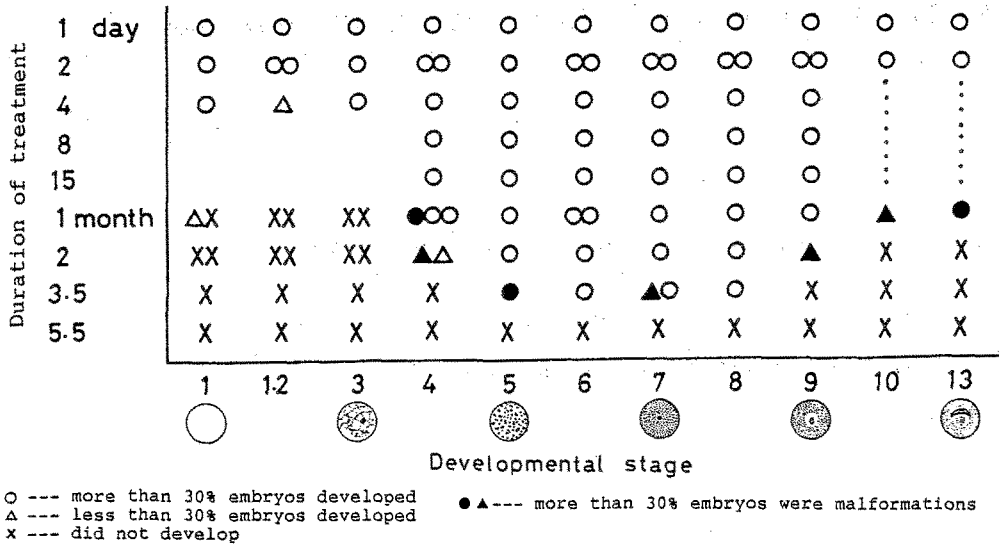

Fig. 1. Treatment at 5°C.

[B] Cultures of embryos with punctured chorion (pinhole diameter 0.1-0.2mm)

Embryos were cultured in sea water including several antibodies after the treatment.

Most of the embryos before stage 7 (the stage of appearance of germ disc) died, although some embryos survived if treated immediately after fertilization. Puncture in extra-embryonic area did not affect embryos after stage 8.

When embryos were punctured in the posterior region of embryonic areas, the rate of survival was the same as in the case of puncturing in extraembryonic area. The treated embryos deve-

loped ones losing posterior parts of embryos (Fig. 2).

When punctured embryos (stages 15) were treated with polyethyleneglycol, they formed into ones with supernumerary abdominal appendages.

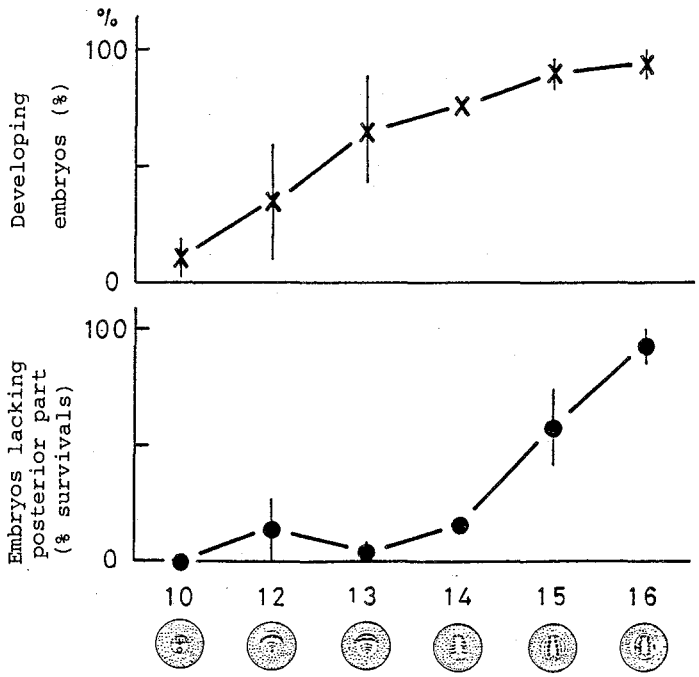

Fig. 2. Cultures of embryos which were punctured in the posterior region of embryonic area.