

SUPERNUMERARY SEGMENTS INDUCED BY DNA SYNTHESIS INHIBITORS AND  
THE CANCELLING EFFECT OF EGG EXTRACT IN THE HORSESHOE CRAB,  
TACHYPLEUS TRIDENTATUS (CHELICERATA: MEROSTOMATA: XIPHOSURIDA)

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Why hand has 5 fingers ? Why insect has 6 legs ? Why does the horseshoe crab have 10 abdominal segments ? We can not reply these questions as yet. The mechanism of determination of number and differentiation of homologous structures in an individual is an exciting problem in developmental biology. We can feel that the mechanism of determination of number and differentiation of segments will become clear in near future (Lewis, 1978; Wareing & Phillips, 1981; Beachy et al., 1985).

Hydroxyurea, an inhibitor of DNA synthesis, changes the differentiation of abdominal segments of the horseshoe crab, Tachypleus tridentatus and increases the number of segments in 90-100% of the surviving embryos (Itow et al., 1984). We examined these monsters from that year on, and obtained some instructive results. This report deals with these results.

- (1) Cell proliferation of treated embryos. The cell proliferation of treated embryos was stopped. This fact suggests that hydroxyurea stops cell cycles at S period in horseshoe crab embryos.
- (2) Effects of the other DNA synthesis inhibitors. The inhibitors of DNA synthesis other than hydroxyurea, such as aphidicolin, fluorodeoxyuridine, fluorouracil or thymidine, also induced the embryos with supernumerary segments at a high rate. The type of the monsters was same as that induced with hydroxyurea.
- (3) The effect of M-period blockers. Treatment with 10 to 100 ug/ml colchicine stopped cell cycles at M-period. Besides, it stopped embryonic deve-

lopment. However, the treated embryos never developed into embryos with supernumerary segments, and 3-(1-anilinoethylidene)-5-benzylpyrrolidine-2,4-dione (TN-16) did not induce supernumerary segments, neither. That is, M-period blockers did not induce supernumerary segments.

Low temperature (5°C) stopped cell proliferation and embryonic development, and the treatment at 5 C never induced supernumerary segments.

In summary, only inhibitors of DNA synthesis (blockers of S-period of cell cycle) induced supernumerary segments.

- (4) Electrocauterization of the posterior end of embryonic area. The cell-mass under the posterior end of embryonic area was electrically cauterized, and then the cauterized embryos were treated with hydroxyurea. The segments of these embryos did not increase in number. It means that the normal segments and supernumerary ones are formed from the cell-mass of posterior end of embryonic area.
- (5) The cancelling effect of the embryo extract. The egg extract counteracted the effects of hydroxyurea. When the extract was applicated immediately after the treatment of hydroxyurea, the embryos developed normally. It means that the effect of hydroxyurea appears after the treatment. Of course when the extract was stimulatory treated with hydroxyurea, the experimental embryos developed normally. But in these cases the extract did not cancel the delay of development and the stop of cell cycle by hydroxyurea. The extract could not cancel the effect of fluorodeoxyuridine to form supernumerary segments. The active substances of the extract seemed to be amino acids.

#### References

- Beachy, P. A., S. L. Helfand and D. S. Hogness (1985) *Nature*, 313:545-551.
- Itow, T., E. Iizuka and H. Inoue (1984) *Proc. Arthropod Embryol. Soc. Jpn.*, 1984.
- Lewis, E. B. (1978) *Nature*, 276:565-570.
- Wareing, P. F. and I. D. J. Phillips (1981) *Growth and differentiation in plants*. Pergamon Press, Oxford.