Spawning Control in Starfish

In starfish, spawning occurs when a hormonal polypeptide, a gonad-stimulating substance (GSS), is released from the nervous system into the coelomic cavity where the ovary is suspended[1–4]. This hormone acts on the ovary to produce 1-methyladenine, which functions as a meiosis-inducing substance (MIS) and acts on the surface of oocytes to induce maturation[5,6]. Subsequently, discharge of oocytes from the gonophore takes place through dissolution of cementing matter among the eggs and the follicular envelope, followed by contraction of the gonadal wall[7].

On the other hand, the presence of spawning inhibitors antagonizing the action of GSS has been demonstrated in the ovary[8]. The chemical nature of these substances is group-specific, i.e., L-glutamic acid is the inhibitory factor for *Asterina pectinifera*[8] and steroidal glycosides, asterosaponins A and B, for *Asterias amurensis*[9]. HEILBRUNN et al.[10] reported the occurrence in the ovary of *Asterias forbisi* of an antimeiotic substance, which they assigned as heparin or a heparin-like substance. Further, they concluded that the same compound blocks the mitotic division of eggs of *Chaetopterus* and *Arbacia*. Considering these situations, we investigated the antimeiotic effect of the ovary extract for oocytes of *Asterias amurensis* and noticed the presence of a novel inhibitor(s), probably a steroidal glycoside(s) other than the asterosaponins. This paper is a preliminary description of experimental results thus far obtained.

It is known that in *Asterias amurensis* oocytes undergo spontaneous maturation without any treatment when isolated artificially in seawater[11]. In this experiment, therefore, oocytes were torn from an ovarian fragment with forceps and immediately transferred into seawater containing a definite amount of the test sample kept in a small Petri dish. The number of immature oocytes still possessing intact germinal vesicles was counted after 1 h. As controls, oocytes were kept in calcium-free seawater, where they remain in the germinal vesicle stage[11], as well as in normal seawater. Though asterosaponins A and B did not inhibit spontaneous maturation at doses of 10–1,000 µg/ml, the seawater extract of the ovary revealed significant inhibition at doses of 5–20 mg dry tissue/ml. This coincided with the report by HEILBRUNN et al.[10] concerning the ovary extract. Then, we attempted isolation and characterization of the active principle causing the antimeiotic effect.

Through preliminary trials, 20% aqueous acetone, 0.1 *M* pyridinium acetate (pH 6.1), acidic seawater (pH 3.0) and normal seawater were found to be equally suitable for extraction from the ovary of the antimeiotic substance, which was insoluble in anhydrous acetone.

Purification of antimeiotic and antispawning substances from the ovary of *Asterias amurensis*

Preparation	Weight (g)	Dose required for spawning inhibition (mg/ml)	Dose required for 50% inhibition of meiosis (mg/ml)
Lyophilized ovary	620	—	—
Acetone powder	570	—	—
20% acetone	172	20	30
80% acetone	40.5	5	15
n-Butanol extract	4.1	0.6–0.2	3.2
Sephadex G-25			
Fraction No. 8	0.22	0.04	> 50
Fraction No. 10	0.72	> 50	0.3

Then, the purification of the active principle was carried out in the following way (Table). An acetone powder was prepared at first from lyophilized ovaries of 220 females collected early in the breeding season. The powder was extracted with 20% aqueous acetone, to which was added anhydrous acetone to make an 80% acetone solution. After standing overnight in the cold, the solution was centrifuged to separate precipitates. Biological activity was found solely in the supernatant, which was evaporated in vacuo. The aqueous residue was acidified with dilute sulfuric acid to pH 3.0 and was partitioned with an equal volume of *n*-butanol. The butanol layer was separated and evaporated in vacuo to dryness. The residue was dissolved in 11 ml of 0.1 *M* pyridinium acetate buffer (pH 6.1) and was applied onto a Sephadex G-25 column (3.3 × 65 cm), which was developed with the same buffer (fraction size, 13.5 ml; flow rate, 40 ml/h). The gelfiltration clearly separated the desired antimeiotic substance (fraction No. 10) from the antispawning substance (fraction No. 8), identified as asterosaponins A and B[9].

The seawater extract of the ovary containing the antimeiotic substance at concentrations of 0.2–40 mg dry tissue/ml was applied to oocytes of *Asterina pectinifera*[12]. None of the solution tested here inhibited meiotic division of the oocytes induced by MIS simultaneously applied at the essential minimum of dose (2×10^{-7} *M*). Thus, the antimeiotic substances should be considered to arrest meiosis at the germinal vesicle stage before MIS acts on the oocytes.

Preliminary examination on the chemical nature of the antimeiotic substance revealed that it belongs to a kind of steroidal glycoside, showing no heparin-like properties. Further investigation from chemical and biological standpoints is in progress[13].

Zusammenfassung. Aus Extrakten von Ovarien des Seeigels *Asterias amurensis* konnte mittels Gelfiltration ein Meiosehemmstoff, vermutlich ein Steroidglycosid, und eine die Ablage von Eiern hemmende, Asterosaponin A und B enthaltende Fraktion abgetrennt werden.

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[12] As the test organisms in this experiment, oocytes of *Asterina pectinifera* are most suitable, since the oocytes isolated in seawater never undergo maturation unless treated with MIS.
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