In vitro において化学不妊剤がヨトウムシの精子形成に及ぼす影響

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In Vitro Inhibition of Spermatogenesis by Chemosterilants in the Cabbage Armyworm, *Mamestra brassicae* L.[1]

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There have been many investigations examining inhibitory effects of chemosterilants on insect testes *in vivo* (Reviewed by LACHANCE et al., 1968; CAMPION, 1972). However, there have been few studies *in vitro*. Recently, several attempts have been made, by using the cultivation of testes or spermatocysts, to investigate the effects of ecdysone or juvenile hormone in some lepidopterous insects *in vitro*. In previous experiments, FUKUSHIMA and YAGI (1975) succeeded in cultivating the testes of diapausing pupae of the cabbage armyworm, *Mamestra brassicae*, in GRACE's medium and, further, demonstrated that spermatogenesis, especially spermiogenesis, was rapidly accelerated by α-ecdysone *in vitro*. This paper studies the direct effects of the chemosterilants, metapa and hempa, on the spermatogenesis of *Mamestra* diapausing pupae *in vitro*.

Both the culture method and the medium used in this experiment have been previously described (YAGI et al., 1969; FUKUSHIMA and YAGI, 1975). Tests taken from 6-day-old diapausing pupae of *Mamestra* were used for the culture. GRACE's medium containing 5 μg/ml of α-ecdysone (See FUKUSHIMA and YAGI, 1975) was used as a basic culture medium. Metepa and hempa were directly dissolved in the medium and their concentrations were adjusted to 0.5 μg and 1.0 μg/ml, respectively, which were adequate to show the inhibitory effects on cultivated wing discs of the same insect (NAKAYAMA et al., unpublished). On the 7th day after the onset of cultivation, the progress of spermatogenesis was examined by the method already described (YAGI and FUKUSHIMA, 1975).

FUKUSHIMA and YAGI (1975) reported that spermiogenesis in cultures of testes of *Mamestra* diapausing pupae was rapidly promoted by the addition of 0.5—5.0 μg/ml of α-ecdysone or active prothoracic glands and many elongated spermatocysts were observed in the testes after cultivation for 7 days.

In the present experiment, similar results were obtained (Fig. 1, Table 1). However, the spermiogenesis was inhibited in the medium containing metepa or hempa. A remarkable degeneration of spherical spermatocysts was also observed in the medium containing chemosterilants. On the other hand, minimal degeneration of spherical

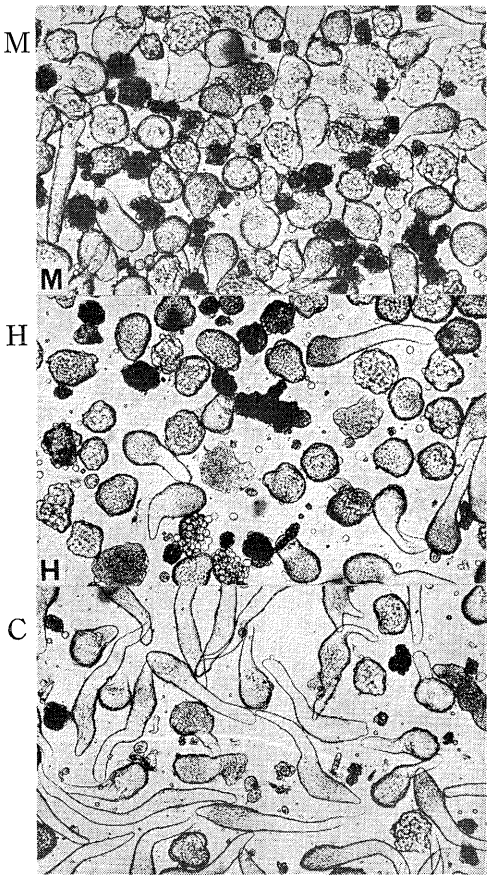

Fig. 1. Spermatocysts of *M. brassicae* 7 days after onset of cultivation of intact testes in Grace's medium containing 5 μg/ml of α-ecdysone with or without chemosterilants (×70). M: α-ecdysone plus 0.5 μg/ml of metepa. H: α-ecdysone plus 1.0 μg/ml of hempa. C: α-ecdysone alone.

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Table 1. Inhibition of Spermiogenesis in *M. brassicae* by Chemosterilants *in vitro*

Treatment[a]	No. of exp.	Spermiogenesis[b]					Degeneration of spherical spermatocysts[b]				
		−	±	+	++	+++	−	±	+	++	+++
α-ecdysone (control)	5	0	0	0	0	5	4	0	1	0	0
α-ecdysone+metepa	5	0	0	0	4	1	0	0	0	1	4
α-ecdysone+hempa	5	0	0	0	1	4	0	0	4	1	0

[a] Grace's medium containing 5 μg/ml of α-ecdysone was used as a basic culture medium and 0.5 μg/ml of metepa or 1.0 μg/ml of hempa was added to the medium.

[b] Seven days after cultivation, each testis was dissected to examine development or degeneration of spermatocysts.

Spermiogenesis:

−: spherical spermatocysts only; ±: mixture of spherical spermatocysts and pyriform spermatocysts; +: elongated spermatocysts are less than 10%; ++: elongated spermatocysts are more than 10% but less than 50%, +++: elongated spermatocysts are more than 50%.

Degeneration of spherical spermatocysts:

−: no degeneration; ±: degeneration <1%; +: 1%~10%; ++: 10%~50%; +++: >50%.

spermatocysts was observed in the chemosterilant-free medium. The effect of metepa on spermiogenesis and spherical spermatocysts was stronger than that of hempa (Table 1). In the α-ecdysone free medium, no spermatocyst was elongated and the spherical spermatocyst was degenerated. Results of dissection on the 14th day of cultivation also showed remarkable degeneration of spherical spermatocysts in the medium containing chemosterilants.

Therefore, it was suggested that the development from spermatogonia to spermatocyte was more inhibited than that of spermatocyte to spermatozoa (including spermiogenesis) by the chemosterilants. Grover et al. (1972) showed that by treatment with apholate, metepa and hempa at their larval stage, the size of testes in both larval and pupal stages of *C. pipiens fatigans* was reduced significantly. This reduction was more prominent in treatment with metepa rather than hempa. Present studies show that metepa and hempa directly affect testes *in vitro*. In his review Campion (1972) cited some examples of the effect of chemosterilants on the endocrine system. Further studies, are needed to clarify the interaction between hormones and chemosterilants on spermatogenesis *in vitro*.

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