Effects of Ecdysterone on Cultivated Ovaries of the Greater Wax Moth Larvae (Lepidoptera : Pyralidae)¹

On the cultivated gonads of the final instar larvae of greater wax moth, *Galleria mellonella*, LENDER and DUVEAU-HAGEGE (1963) have reported that oogonia promptly multiplied, oocytes entered into previtellogenesis, and both nutritive cells (trophocytes) and follicular cells became differentiated in the culture medium free of any insect hormone. More recently, LAVERDURE (1969) has succeeded to cultivate the ovaries of early pupal stage of the yellow mealworm, *Tenebrio molitor*, in the presence of ecdysone, and suggested that the ecdysone caused remarkable growth and development of both ovaries and oocytes *in vitro*. In the present studies, we intend to know the effects of the moulting hormone, ecdysterone, on the cultivated ovaries taken from the last instar larvae of the greater wax moth.

Both culture method and medium used in this experiment were the same as those mentioned previously (YAGI *et al.*, 1969). Greater wax moth larvae were reared on the mass of honeybee comb at 25°C, and ovaries of 8-day-old last instar larvae were used for the culture. The surface of the insects were sterilized by submersion in 70% ethanol for one minute and by rinsing with three changes of sterilized distilled water. Then the ovaries taken from the sterilized larvae were placed in the medium containing 3 µg ecdysterone per ml of the medium. Ovaries were also cultivated in the same medium without inclusion of ecdysterone as controls. The cultures were kept at 25°C.

About 3 days after the onset of cultivation, some changes occurred in the ovaries subjected to ecdysterone. They enlarged in size and became transparent. Their enlargement was due to swelling of each ovariole, especially inside of the pedicel. These changes became more and more clear with the lapse of time. Most notable morphological changes of the ovaries have occurred when they were cultivated for 10 days or longer. In this stage the oocytes, trophocytes and follicular cells were differentiated in the basal part of the ovariole (Fig. 1B). The nuclei of trophocytes were large in size and irregular in outline; whereas those of the oocytes were smoo-

Fig. 1. A: Longitudinal section of *Galleria* ovary on 10th day after onset of cultivation, showing no visible changes in the medium lacking ecdysterone ($\times 200$).

B: Longitudinal section of *Galleria* ovary on 10th day after onset of cultivation, showing the swelling of the pedicel (PE) and the differentiation of the oocytes (OC), trophocytes (TC) and follicular cells (FC) in the presence of ecdysterone ($\times 200$).

C: High magnification of a part of the ovariole shown by arrows in Fig. 1B ($\times 700$).

Ovary was fixed in CARNOY's fluid, embedded in paraffin, sectioned at 5 µ and stained with DELAFIELD's hematoxylin-eosin.

ther in outline (Fig. 1C). Both oocytes and trophocytes were surrounded by follicular cells. The ovaries cultivated in the absence of ecdysterone, however, showed no visible changes, although they were well maintained for as long

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as 3 weeks (Fig. 1A). In the cultures with ecdysterone, active contraction was observed in both ovaries and muscles attached to the ovaries for 2 weeks or longer. No contraction was observed in the control cultures. From the present experiments it became evident that the ovaries of the 8th day of the final instar was unable to develop *in vitro* without ecdysterone. This result does not agree with that of LENDER and DUVEAU-HAGEGE (1963), and this difference was thought to be due to the different stages of the materials. In our experiments, ovaries of the final instar larvae just before cessation of feeding, were used and it was sure that at this period any ovarian development could not be promoted without a moulting hormone, whereas it is likely that they might use materials beyond this critical period.

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Ichiro SHIBUYA
Shigemi YAGI

Laboratory of Applied Zoology
Faculty of Agriculture
Tokyo University of Education
Meguro-ku, Tokyo, 153 Japan

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