Appl. Entomol. Zool. **28** (3): 303–311 (1993)

Induction of Oocyte-Nurse Cell Differentiation in the Ovary by the Brain during the Initial Stage of Oogenesis in the Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)

Chitose ORIKASA,[1] Hideo YAMAUCHI,[2] Hiromichi NAGASAWA,[3,4] Akinori SUZUKI[4] and Masao NAGATA

Laboratory of Sericulture, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
[4]Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received 13 July, 1992; Accepted 1 April, 1993)

Co-culture of ovarian cells in young larvae with brain induced meiosis and endomitosis of the silkworm, *Bombyx mori*. This suggests that a substance secreted by the brain controls meiosis and endomitosis. When preparations from *Bombyx* heads were tested for meiosis-inducing activity, both the crude and highly purified preparations of bombyxin induced meiosis *in vitro*, but the crude preparation of prothoracicotropic hormone (PTTH) showed much lower activity. This indicates that bombyxin is the brain substance that induces meiosis. 20-Hydroxyecdysone also induced meiosis, but only at a low concentration.

Key words: *Bombyx mori*, oogenesis, meiosis, endomitosis, bombyxin

INTRODUCTION

Oogenesis in insects consists of three stages, oocyte-nurse cell differentiation, previtellogenesis and vitellogenesis. Previtellogenesis involves the growth of both oocytes and nurse cells before yolk deposition, while vitellogenesis includes the growth and maturation of the ovary.

In the silkworm, *Bombyx mori*, the oocyte-nurse cell differentiation in the germanium begins at the young larval stage. The mitotic descendant of oogonia, the cystoblast (KING, 1970), undergoes a sequence of three mitotic cell divisions, resulting in the formation of a cluster of eight siblings known as cystocytes (KING, 1970; TELFER, 1975). In *B. mori*, as is usual in Lepidoptera, all eight cystocytes initiate meiosis (TELFER, 1975). One cystocyte differentiates into the oocyte in which the meiotic pathway continues, while the remaining cystocytes divert to the endomitotic pathway and become nurse cells. The chromosomal structure in the meiotic prophase in *B. mori* has been studied in detail by RASMUSSEN and HOLM (1982).

[1] Present address: *Department of Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, 2–6 Musashidai, Fuchu, Tokyo 183, Japan*

[2] Present address: *Laboratory of Insect Physiology, Division of Forest Biology, Forestry and Forest Products Research Institute, Kukizaki, Inashiki, Ibaraki 305, Japan*

[3] Present address: *Laboratory of Molecular Biology of Marine Organisms, Ocean Research Institute, The University of Tokyo, Nakano-ku, Tokyo 164, Japan*

Previtellogenic growth of the oocyte, growth and differentiation of new follicles, is known to be affected by 20-hydroxyecdysone (20-HE) in *Tenebrio molitor* (LAVERDURE, 1970), *Heteropeza pygmaea* (WENT, 1978), and *Galleria mellonella* (SHIBUYA and YAGI, 1972). Vitellogenin synthesis in the fat body and its incorporation into the ovary during vitellogenesis are known to be regulated by juvenile hormone in some insect species (BELL, 1969; ENGELMANN, 1979, 1983; KOPPE et al., 1985), or by ecdysteroids in others such as *B. mori* (OHNISHI, 1987; CHATANI and OHNISHI, 1976; OGISO and OHNISHI, 1984).

In contrast to previtellogenesis and vitellogenesis, little is known about the hormonal control of oocyte-nurse cell differentiation. FURTADO (1979) reported that mitosis and meiosis in the ovary of the blood-sucking bug, *Panstrongylus megistus*, were controlled by the brain: induction of mitosis was regulated by the "A cells" in the pars intercerebralis (PI), while meiosis depended on the haemolymph level of ecdysteroids secreted by the molting gland after stimulation by a substance from the "A' cells" in the PI. In both *Roscius elongatus* and *R. brazzavilliensis*, meiosis might be controlled by the "A' neurosecretory cells" (ROBERT, 1979). These lines of evidence indicate the importance of the brain, especially the neurosecretory cells in the PI, in controlling the initial phase of oogenesis. The aim of this study is to elucidate the brain secretion that induces meiosis in *B. mori*.

MATERIALS AND METHODS

Insects. *B. mori* larvae of a hybrid race (N106 × Daizo), were reared on mulberry leaves at 25±1°C under a 12L–12D photoperiod.

Culture of ovaries. Larval body surface was sterilized by immersion in 70% ethanol for 30 s and then washed with sterile water. Ovaries were extirpated from the second or third instar larvae and washed twice with modified CARLSON's solution (NaCl 7.0 g, $CaCl_2$ 0.2 g, NaH_2PO_4 0.2 g, KCl 0.2 g, $MgCl_2 \cdot 6H_2O$ 0.1 g, $NaHCO_3$ 0.12 g and glucose 8 g/*l*). The ovaries were put into a CSM-2F medium (MITSUHASHI, 1968) in a disposable Petri dish (Lux, 35 mm dia.). The dish was left in a larger glass Petri dish (90 mm dia.) with a small volume of distilled water to humidify the chamber. Twenty ovaries were cultured with ten brains from larvae at the same developmental stage in 20 μl of the medium for 3–4 days at 25°C under 12L–12D. In the case of the third instar larvae, ovaries were placed on nylon mesh for exposure to air.

Test samples for the induction of meiosis. Bombyxin and prothoracicotropic hormone (PTTH) preparations used were those partially purified from *Bombyx* adult heads. The two bombyxin preparations were from the active fractions of step-8 and step-12 in the purification procedure previously published (NAGASAWA et al., 1979, 1984 a). The step-12 preparation was still impure and contained 2–3% bombyxin in terms of protein amount. The PTTH preparation was the active fraction of step-6 in the purification procedure for PTTH (KATAOKA et al., 1987). 20-HE was purchased from Sigma.

Microscopic observation. The cultured ovaries were washed with 0.05 M sodium cacodylate buffer (pH 7.4) containing 0.17 M sucrose. They were prefixed with 1% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4) for 3 h and rinsed with the same buffer three times. Then they were postfixed with 1% osmium tetroxide, dehydrated with ethanol and embedded in Spurr resin. The

embedded ovaries were sectioned at 650 nm with Poter-Blum Super-Microtome (Sorvall), and stained with toluidine blue. The sections were observed under a light-microscope and the number of cells in meiotic prophase (at zygotene stage) in a longitudinal plane of the ovary was counted in each section. Since it was difficult to determine the exact number of cells in meiotic prophase in one ovary, the total number of the cells at that stage which were summed up from all the sections was divided by the total number of sections ($N=70$).

RESULTS

Ovarian development in silkworm larvae

During the second larval instar, the germarium contained a population of oogonia that were characterized by dispersed chromatin. They underwent asynchronous mitotic division and increased in number. However, no cells in meiotic prophase were observed at this stage (Fig. 1 a). The cystocytes in leptotene and zygotene of the meiotic prophase

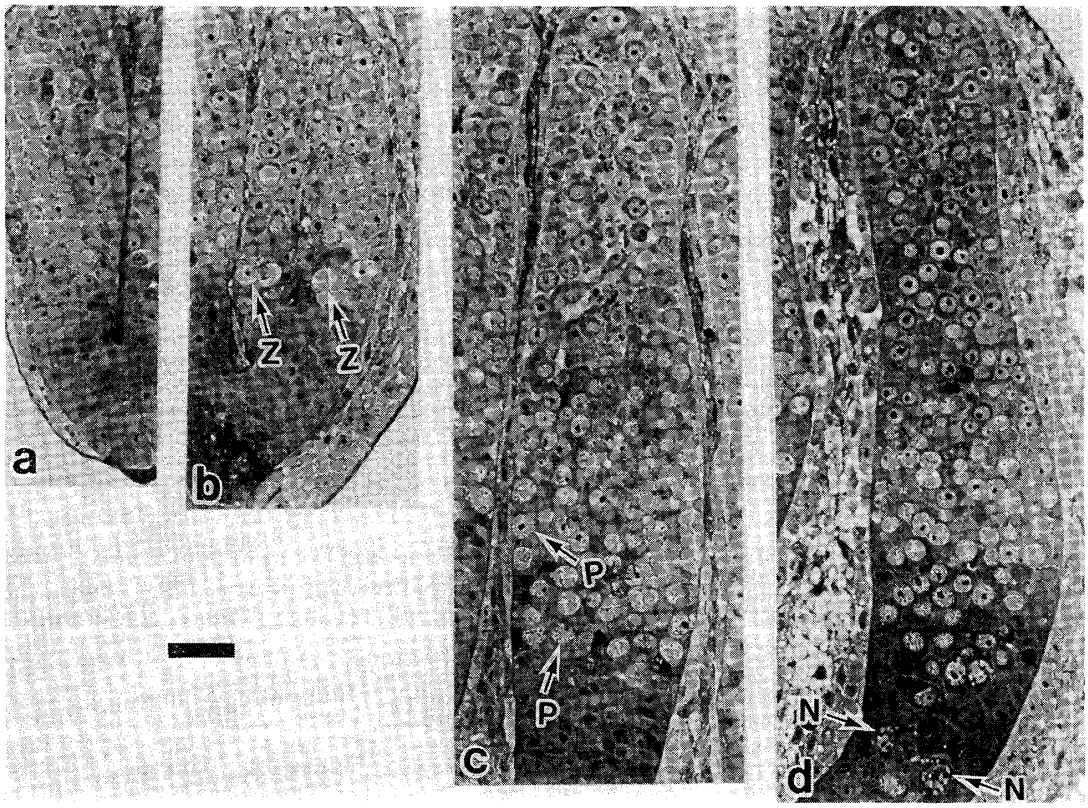

Fig. 1. Ovarian development in the second and third instar larvae of *B. mori*. a: The second larval instar. Only mitotic cells of oogonia were observed in the ovariole. b: The first day of third larval instar. The cells in meiotic prophase were first observed at the distal portion of the ovariole, and the chromosomes of these cells indicated that the cells are at the stage of zygotene (Z, arrows). c: The third larval instar. The cells in meiotic prophase were observed in the ovariole. The chromosome configurations indicate that the cells are at the stages of zygotene and pachytene (P, arrows) but nurse cells had not appeared at this stage. d: The time of head capsule slippage at the 3rd instar. Nurse cells (N, arrows) first appeared at the terminus of the ovariole, and endomitosis was observed. Scale indicates 20 μm in a, b, c and d.

Table 1. Effect of brain on initiation of meiotic prophase in the ovary of *B. mori*

Stage of ovaries	Culture condition	Number of cells in meiotic prophase/section
One day before first ecdysis	Ovary	0
	Ovary+Brain	0
First day of second instar	Ovary	2
	Ovary+Brain	26
Second day of second instar	Ovary	2
	Ovary+Brain	23

The ovaries were cultured in the CSM-2F insect culture medium for 3–4 days. The average numbers of cells in meiotic prophase per section were calculated (n=3).

first appeared at the basal portion of the germarium during the period between the head capsule slippage (about one day before ecdysis) in the second instar and the first day of the third instar. They could be morphologically distinguished from oogonia, because the chromosomes in leptotene became dispersed in the nucleus. The chromosomes in zygotene assumed an appearance typical for this stage (Fig. 1 b). During the third instar, the cystocytes in leptotene, zygotene and pachytene increased progressively in number (Fig. 1 c). Nurse cells in endomitosis first appeared at the base of the germarium between the head capsule slippage in the third instar and the first day of fourth instar. They showed condensed chromosome masses scattered in the nucleus (Fig. 1 d). After the fourth instar, the germarium was divided into four zones of cells, as reported by MIYA et al. (1970).

Effect of brain on initiation of meiotic prophase in ovary in vitro

The ovaries were extirpated from first instar larvae at the time of the head capsule slippage and cultured *in vitro* with or without the brain. No cells in meiotic prophase were observed in either case (Table 1).

When the ovaries taken out of newly ecdysed second instar larvae were cultured alone, only a few cells were observed in meiotic prophase, but the basal membrane of the ovariole degenerated and the oogonial cells disappeared (Fig. 2 a). In contrast, the co-culture of the ovaries with brains induced meiotic prophase in many cells. The basal membrane remained in the same condition as observed in the ovary from a normal larva (Fig. 2 b). Similar effects of co-culture were obtained with the ovaries of 2-day-old second instar larvae. Table 1 summarizes these results, which strongly suggest that the brain secreted a substance into the culture that stimulated oocyte to enter the meiotic prophase.

Effects of bombyxin, PTTH and 20-HE on the induction of meiosis in ovary

The preparation of crude brain extract was prepared by homogenizing 60 brains of second instar larvae was followed by heating and centrifugation and testing for the meiosis-inducing activity. The preparation, however, interfered with normal development and produced no meaningful results. The crude brain extract was then partially purified either to bomyxin or to PTTH (NAGASAWA et al., 1979; NAGASAWA et al., 1984 a; KATAOKA et al., 1987). When the ovaries of newly ecdysed second instar larvae were cultured in the medium containing the bombyxin preparation at the 8th step of puri-

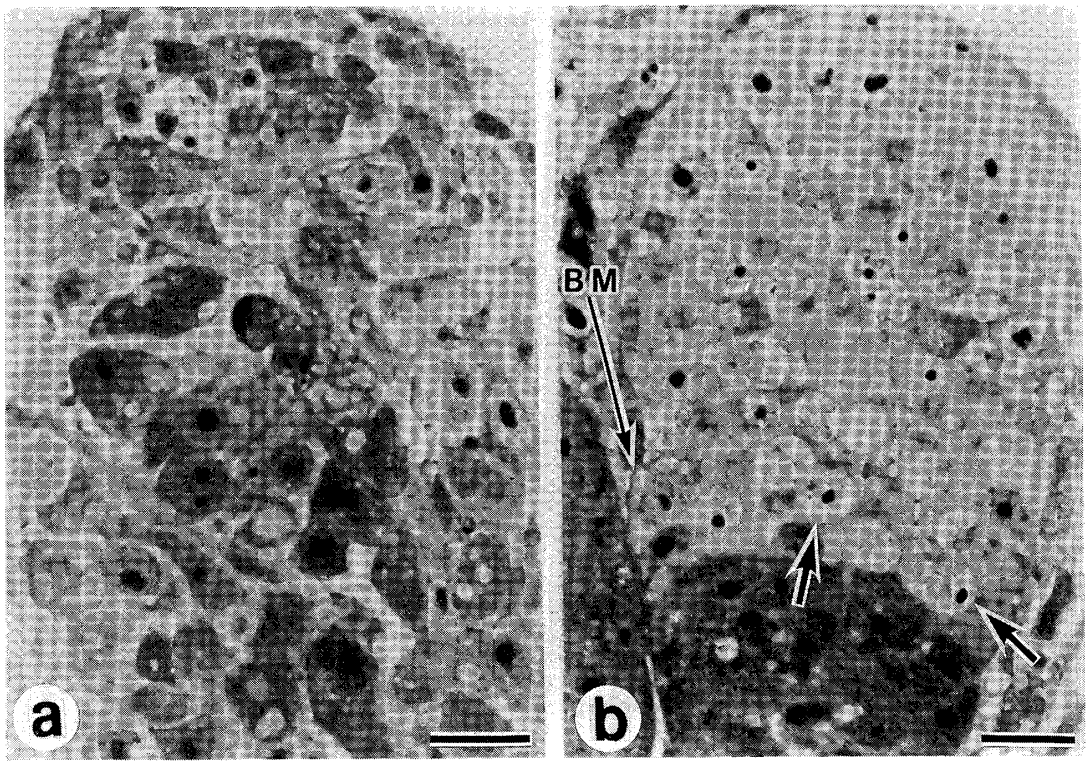

Fig. 2. Effect of the brain on the initiation of meiotic prophase in the ovary of *B. mori*. Ovaries from larvae at the first day of second instar were cultured. a: The ovary cultured alone. The cells in meiotic prophase were not observed. The ovary degenerated and the ovariole membrane disappeared. b: Culture of the ovary with brains from larvae at the same developmental stage; the cells at the stage of zygotene (arrows) of meiotic prophase were observed and the ovariole membrane was retained. Scales in a and b indicate 10 μm. BM indicates a basal membrane of ovariole (arrow).

fication procedure (the step-8 bombyxin preparation) at concentrations of 0.0125, 0.025 and 0.05 *Samia* units/μl medium, meiosis was induced in all cases and the cells entered the zygotene stage of meiotic prophase in three days (Table 2). On the other hand, the step-6 PTTH preparation at the concentrations of 0.0125 and 0.025 *Bombyx* units/μl did not induce cells in meiotic prophase (Table 2). Only at a higher concentration (0.05 *Bombyx* units/μl) did it induce a considerable number of cells in meiotic prophase.

The step-8 bombyxin preparation was then further purified (the step-12 bombyxin preparation, previously called "highly purified bombyxin" or "highly purified 4K-PTTH" (NAGASAWA et al., 1984 b)) and tested for meiosis-inducing activity (Table 2). This bombyxin preparation, although inducing meiosis, appeared to have a slightly lower activity than the step-8 bombyxin preparation. At all three concentrations examined (Table 2), the basal membrane of ovariole degenerated and the cells of the mesodermal origin were elongated into germarium, though they both were observed in the ovaries cultured with brains or the step-8 bombyxin preparation as in the normally developing ovary.

When the ovaries were cultured with 20-HE at the concentration of 650 pg/μl corresponding to the maximum concentration of the haemolymph ecdysteroid in the

Table 2. Effects of Bombyxin, PTTH and 20-HE on the initiation of meiotic prohase in the ovary of *B. mori*

Sample cultured with		Dose	Number of cells in meiotic prophase/section
None (medium only)			2
Bombyxin	Step 8	0.0125 unit/μl	29
		0.025	26
		0.05	48
	Step 12	0.0125 unit/μl	22
		0.025	17
		0.05	39
PTTH	Step 6	0.0125 unit/μl	0
		0.025	0
		0.05	12
20-HE		6.5 pg/μl	18
		650.0	0

The ovaries were taken from the larvae on the first day of 2nd instar and cultured in the CSM-2F insect culture medium for 3–4 days. The average numbers of cells in meiotic prophase per section were calculated (n=3).

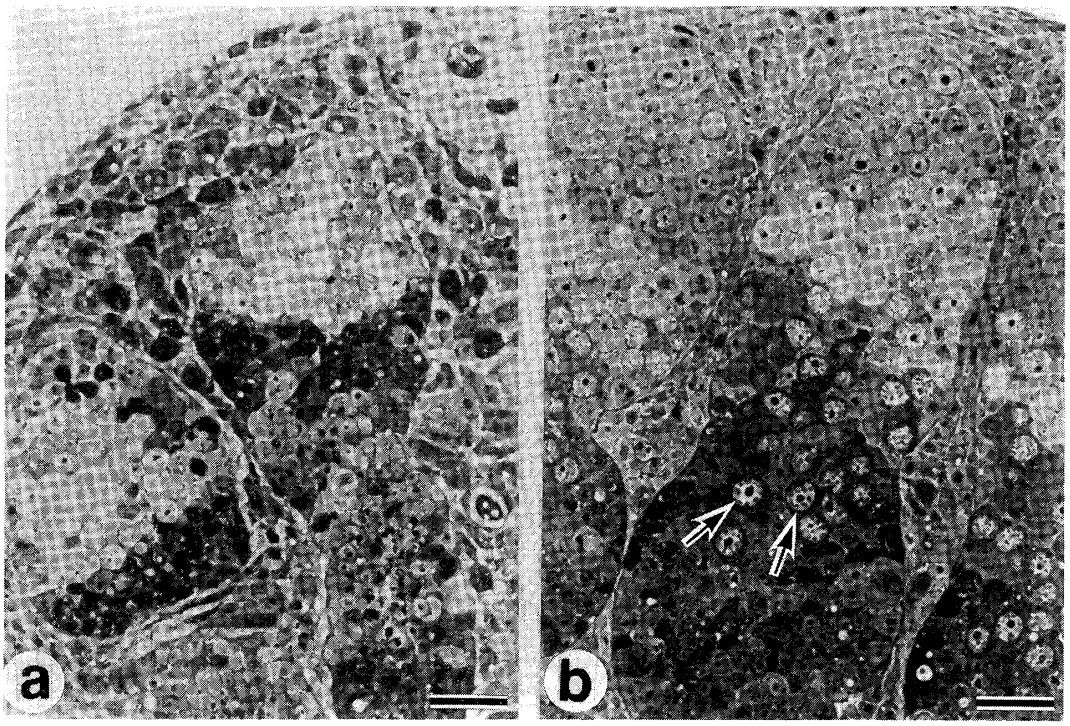

Fig. 3. Effect of the brain on the endomitosis in the ovary of *B. mori*. Ovaries from larvae at the 1st day of the third larval instar were cultured. a: The ovary cultured alone. The cells at the zygotene and pachytene stages of meiotic prophase were observed but the nurse cells had not appeared. b: Culture of the ovary with the brain from larvae at the same developmental stage. Endomitosis occurred and the nurse cells (arrows) were observed at the terminal part of the ovarioles. Scales in a and b indicate 20 μm.

second instar larvae, meiosis did not occur. In contrast, very low concentration of 20-HE (6.5 pg/μl) induced considerable meiosis (Table 2).

Effect of brain on the endomitosis in the ovary

The ovary at early third instar was occupied by the cells at the pachytene stage in the first meiosis, but endomitosis, a process for differentiation to nurse cells, has not started. When the ovaries taken out of newly ecdysed third instar larvae were cultured alone on nylon mesh, endomitosis did not occur and the basal membranes of ovariole and follicle cells degenerated completely (Fig. 3 a). Endomitosis was observed in the ovaries cultured with brains after 4-day cultivation (Fig. 3 b). In addition, the basal membranes of ovariole and follicle cells did not degenerate. These results indicate that the brain also induces endomitosis in the ovary through its secretions.

DISCUSSION

The present results suggest that the meiosis and endomitosis in the ovarian cells of *B. mori* are under endocrine control during oogenesis and that the cell cycle is promoted by a secretion(s) from the brain. The fact that the two bombyxin preparations with different purities induced meiosis *in vitro* might indicate that the meiosis-inducing substance is bombyxin. Another brain secretion also seems to exist that induces endomitosis. It remains to be determined if they are the same.

In *P. megistus*, the brain plays two roles in the initial stage of oogenesis in the ovary: one to directly induce mitosis and another to stimulate the molting gland to produce ecdysone, which seemingly induces meiosis (FURTADO, 1979). But the mitosis in *B. mori* does not seem to be controlled by the brain, because the anterior part of the germarium of the ovariole (where mitotic cell division occurs) did not expand when the ovary was cultured with brain. On the other hand, meiosis was obviously induced by a brain secretion, when the ovary from a first-day larva in the second instar was cultured with brain. Before this stage, meiosis was not observed even when the ovary was incubated with brain, indicating that initiation of meiosis is stage-specific.

When the effects of the two bombyxin preparations that induced the meiosis were compared, the effects on the ovariole membrane were distinct; the 8-step bombyxin preparation retained the basal membrane, while the 12-step bombyxin preparation did not. Therefore, it is highly possible that there is another factor influencing basal membrane retention, one that is discarded during the purification of bombyxin from step 9 to step 12.

Though bombyxin was first isolated as a prothoracicotropic hormone from the silkworm *B. mori*, it showed hormonal activity to the erisilkworm *Samia cynthia ricini* at an extremely low dose (0.1–0.4 ng/debrained pupa), but not to *B. mori* itself. Bombyxin has been completely chemically characterized, and belongs to the insulin family of peptides. In vertebrates, it is known that insulin-family peptides have a variety of functions. Among them, insulin and insulin-like growth factors exert effects on meiotic maturation in the oocyte (EL-ETR et al., 1979). The structural similarity of bombyxin to vertebrate insulin and levels of hormonal activity to a multitude of insect species allows us to assume that bombyxin might have some important functions other than the prothoracicotropic one in *B. mori*. Our present study indicates that one of the intrinsic functions of bombyxin in *B. mori* may be to induce meiosis in the ovary.

The neurosecretory cells producing bombyxin were identified immunohistochemically in PI of the brain, which seems to be consistent with the fact that in *P. megistus* neurosecretory cells in PI are responsible for oogenesis (FURTADO, 1979). These cells are stained throughout the stages from larval hatching to adult eclosion (MIZOGUCHI et al., 1990), indicating that bombyxin is present in all stages. Since the germariun of *Bombyx* ovary exists up to the adult stage, when egg formation has completed, bombyxin may continuously stimulate the cells of the germarium in the ovary to differentiate into the oocyte-nurse cell complex from larval to adult stages.

The crude preparation of PTTH had a much lower meiosis-inducing activity. It is possible that this low activity is due to bombyxin present in the PTTH preparation. If so, PTTH may not be involved in meiosis.

The hemolymph ecdysteroid titer increased and reached its maximum before the first molting in *B. mori* (NAGATA et al., 1987). But this increase does not seem to directly cause ovarian meiosis, high concentration of 20-HE, comparable to the maximum titer in the first instar, did not. A low concentration of 20-HE induced considerable meiosis. It has been shown that the ovary itself synthesizes ecdysteroids in several insect species (HAGEDORN, 1985). HANAOKA and HAGEDORN (1980) also demonstrated that in the mosquito, *A. aegypti*, the ovaries themselves produce a large amount of ecdysone when incubated with a head extract. Also in *B. mori*, ecdysteroids were produced and secreted by the ovaries of fourth instar larvae when cultured *in vitro* (unpublished). All these data lead us to propose that bombyxin secreted by the brain stimulates the ovary to produce ecdysteroids, which in turn induce meiosis. Determination of ecdysteroid titer in the ovary after stimulation by bombyxin should provide further information.

REFERENCES

BELL, W. J. (1969) Dual role of juvenile hormone in the control of yolk formation in *Periplaneta americana*. *J. Insect Physiol.* **15**: 1279–1290.

CHATANI, F. and E. OHNISHI (1976) Effect of ecdysone on the ovarian development of *Bombyx* silkworm. *Dev. Growth & Differ.* **18**: 481–484.

EL-ETR, M., S. SCHORDERET-SLATKINE and E.-E. BAULIEU (1979) Meiotic maturation in *Xenopus laevis* oocytes initiated by insulin. *Science* **205**: 1397–1399.

ENGELMANN, F. (1979) Insect vitellogenin: Identification, biosynthesis, and role in vitellogenesis. *Adv. Insect Physiol.* **14**: 49–108.

ENGELMANN, F. (1983) Vitellogenesis controlled by juvenile hormones. In *Endocrinology of Insect* (R. G. H. DOWNER and H. LAUFER, eds.). Alan R. Liss, Inc., New York, pp. 259–270.

FURTADO, A. (1979) The hormonal control of mitosis and meiosis during oogenesis in a blood-sucking bug *Panstrongylus megistus*. *J. Insect Physiol.* **25**: 561–570.

HAGEDORN, H. (1985) Role of ecdysteroids in reproduction. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 8 (G. A. KERKUT and L. I. GILBERT eds.). Pergamon Press, Oxford, pp. 205–262.

HANAOKA, K. and H. H. HAGEDORN (1980) Brain hormone control of ecdysone secretion by the ovary in the mosquito. In *Progress in Ecdysone Research* (J. A. HOFFMANN, ed.). Elsevier/North-Holland, Amsterdam, New York & Oxford Biomedical Press, pp. 467–480.

KATAOKA, H., H. NAGASAWA, A. ISOGAI, S. TAMURA, A. MIZOGUCHI, Y. FUJIWARA, C. SUZUKI, H. ISHIZAKI and A. SUZUKI (1987) Isolation and partial characterization of a prothoracicotropic hormone of the silkworm, *Bombyx mori*. *Agric. Biol. Chem.* **51**: 1067–1076.

KING, R. C. (1970) *Ovarian Development in Drosophila Melanogaster*, Academic Press, New York.

KOEPPE, J. K., M. FUCHS, T. T. CHEN, L.-M. MUNT, G. E. KOVALICK and T. BRIES (1985) Role of

juvenile hormone in reproduction. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 8 (G. A. KERKUT and L. I. GILBERT, eds.). Pergamon Press, Oxford, pp. 165–204.

LAVERDURE, A. M. (1970) The action of ecdysone and the methyl ester of farnesol on the nymphal ovary of *Tenebrio molitor* grown *in vitro*. *Ann. Endocrinol.* **31**: 516–524.

MITSUHASHI, J. (1968) Tissue culture of the rice stem borer, *Chilo suppressalis* WALKER (Lepidoptera: Pyralidae): III. Effect of temperatures and cold-storage on the multiplication of the cell line from larval hemocytes. *Appl. Entomol. Zool.* **3**: 1–4.

MIZOGUCHI, A., M. HATTA, S. SATO, H. NAGASAWA, A. SUZUKI and H. ISHIZAKI (1990) Developmental changes of bombyxin content in the brain of the silkmoth, *Bombyx mori*. *J. Insect Physiol.* **36**: 655–664.

MIYA, K., M. KURIHARA and I. TANIMURA (1970) Electron microscope studies on the oogenesis of the silkworm *Bombyx mori* L. Fine structure of ovary in the early stage of fifth instar larva. *J. Fac. Agric. Iwate Univ.* **10**: 59–83.

NAGASAWA, H., A. ISOGAI, A. SUZUKI, S. TAMURA and H. ISHIZAKI (1979) Purification and properties of the prothoracicotropic hormone of the silkworm, *Bombyx mori*. *Dev. Growth & Differ.* **21**: 29–38.

NAGASAWA, H., H. KATAOKA, Y. HORI, A. ISOGAI, S. TAMURA, A. SUZUKI, F. GUO, X. ZHONG, A. MIZOGUCHI, M. FUJISHITA, S. Y. TAKAHASHI, E. OHNISHI and H. ISHIZAKI (1984 a) Isolation and some characterization of the prothoracicotropic hormone from *Bombyx mori*. *Gen. Comp. Endocrinol.* **53**: 143–152.

NAGASAWA, H., H. KATAOKA, A. ISOGAI, S. TAMURA, A. SUZUKI, H. ISHIZAKI, A. MIZOGUCHI, Y. FUJIWARA and A. SUZUKI (1984 b) Amino-terminal amino acid sequence of the silkworm prothoracicotropic hormone: Homology with insulin. *Science* **226**: 1344–1345.

NAGATA, M., K. TSUCHIDA, K. SHIMIZU and N. YOSHITAKE (1987) Physiological aspects of nm-g mutant: an ecdysteroid-deficient mutant of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **33**: 723–727.

OHNISHI, E. (1987) Growth and maturation of ovaries in isolated abdomens of *Bombyx mori*: Response to the ecdysteroids and other steroids. *Zool. Sci.* **4**: 315–321.

OGISO, M. and E. OHNISHI (1984) Uptake and metabolism of [^{3}H]ecdysone in cultured ovaries of the silkworm, *Bombyx mori*. *Mol. Cell. Endocrinol.* **38**: 13–19.

RASMUSSEN, S. W. and P. B. HOLM (1982) The meiotic prophase in *Bombyx mori*. In *Insect Ultrastructure*. Vol. 1 (R. C. KING and H. AKAI, eds.). Plenum Press, New York and London, pp. 61–85.

ROBERT, A. (1979) Les premiers stades de l'ovogenese et les variations de la neurosecretion cerebrale chez deux especes sympatriques *Roscius elongatus*, SATL et *R. brazzavilliensis*, ROBERT (Heteroptera: Pyrrhocoridae). *Int. J. Insect Morphol. & Embryol.* **8**: 11–31.

SHIBUYA, I. and S. YAGI (1972) Effect of ecdysterone on cultivated ovaries of the greater wax moth larvae. *Appl. Entomol. Zool.* **7**: 97–98.

TELFER, W. H. (1975) Development and physiology of oocyte-nurse cell syncytium. *Adv. Insect Physiol.* **11**: 223–319.

WENT, D. F. (1978) Ecdysone stimulates and juvenile hormone inhibits follicle formation in a gall midge ovary *in vitro*. *J. Insect Physiol.* **24**: 53–59.