From *Insect Neurochemistry and Neurophysiology • 1989* Edited by A. B. Borkovec and E. P. Masler


# CHANGES IN NUMBER AND SIZE OF CORPUS ALLATUM CELLS OF BLATTELLA GERMANICA DURING OOCYTE MATURATION

Ann-Shyn Chiang, Michal Gadot
and Coby Schal

Dept. of Entomology, Rutgers Univ.
New Brunswick, New Jersey 08903

The corpus allatum (CA) of the female German cockroach shows cyclic changes in both size and juvenile hormone III (JH III) biosynthesis during the gonotrophic cycle (Gadot et al., 1989). The increase in CA volume during oocyte maturation has been considered mainly due to increases in both cell number and cell size (Cassier, 1979; Tobe and Stay, 1985). To date, the number of nuclei in the CA has been estimated from sections of fixed thickness in fixed organs (Scharrer and von Harnack, 1958; Szibbo and Tobe, 1981; Tobe et al., 1984). Since nuclear size and shape vary with different developmental stages of the CA during the gonotrophic cycle in Diploptera punctata (Johnson et al., 1985) and other insects (Cassier, 1979), nuclear counts from sections may under- or over-estimate the total number of cells. The size of CA cells is usually presented as the nucleocytoplasmic ratio in fixed glands (Engelmann, 1957). We report on changes in number and size of CA cells of Blattella germanica during oocyte maturation by direct measurements of enzymatically dissociated cells.

Whole-mounts of partially dissociated CA monolayers (Fig. 1A) provided counts of the absolute number of cells in individual CA. Cell nuclei in the monolayer were intact and evenly distributed without overlap. Some nuclei were larger than others, especially in CA from females with mature oocytes. In contrast to reports of significant increases in CA cell number during oocyte maturation in D. punctata (Szibbo and Tobe, 1981; Tobe et al., 1984), cell number did

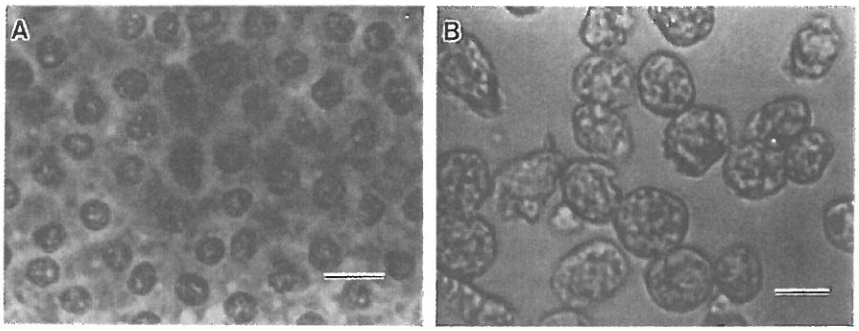


Fig. 1. (A) A whole-mount monolayer of CA cells. The CA from a day 0 female were desheathed by incubation with 0.1% collagenase, stained with 0.01% crystal violet in 0.1 M citric acid and then spread into a cell monolayer under a cover slip. (B) CA cells (from high-activity CA of day 6 grouped virgin females) in suspension. Individual CA were partially digested with 0.1% trypsin in cockroach saline and dissociated by vortexing. Bars = 10 μm.

not increase during this period in B. germanica. Cell number (mean ± SEM) per CA was 1927 ± 52 (n=12) on day 0, 2029 ± 63 (n=8) on day 3, and 2013 ± 68 (n=8) on day 6. Clearly, these changes in cell number do not account for the increase in CA volume and JH-III biosynthesis during oocyte maturation.

Changes in CA cell size during the period of oocyte maturation were directly measured in cell suspensions following enzymatic dissociation of freshly excised CA. Cells were randomly chosen by a hemocytometer grid and their maximum diameter was measured as an indicator of cell size. Dissociated CA cells were usually globular in suspension (Fig. 1B) and cell sizes from each of three age groups were normally distributed (Fig. 2). Cell diameter increased significantly during the period of oocyte maturation from a mean value of 8.9 ± 0.1 μm on day 0 (oocyte length < 0.5 mm) to 11.7 ± 0.2 μm on day 6 (oocyte length 1.6-1.9 mm) and to 12.7 ± 0.2 μm on day 7 (oocyte length 2.0-2.1 mm). Corpus allatum volume ($\times 10^6$ $\mu m^3$) increased from 0.87 ± 0.06 (n=8) to 1.22 ± 0.05 (n=12) and to 1.63 ± 0.09 (n=16) during the equivalent period. The pattern of increase in cell size in relation to oocyte length was similar to the increase in CA volume and JH-III biosynthesis.

Using the radiochemical assay for JH-III biosynthesis in vitro, during a 4 h incubation dissociated CA cells incorporated L-[methyl-$^{3}$H]methionine into JH-III at rates comparable to intact glands (2.16 ± 0.19 and 2.77 ± 0.22 pmol/hr/gland, respectively). HPLC analysis confirmed that JH-III was the only JH produced by these dissociated cells. Since methionine can not be utilized by homogenized CA for JH biosynthesis (Schooley and Baker, 1985), it is not plausible that released enzymes from disintegrated cells would account for this activity. These results indicate that the dissociation procedure did not impair the JH-III biosynthetic activity of CA cells and that the basal lamina and intercellular junctions between CA cells of adult female B. germanica are not required in the biosynthesis of JH-III, at least in short-term incubations.

In conclusion, we demonstrate methods to directly determine cell number and cell size in the CA. Total cell number in the corpora allata of B. germanica adult females does not increase as juvenile hormone synthesis and CA volume increase during oocyte maturation. Rather, cell size increases significantly during this period. Thus, CA activation in the first ovarian cycle of this species is associated mainly with an increase in cell size with minor changes in cell number. In addition, we demonstrate, for the first time, de novo synthesis of JH-III in a suspension of

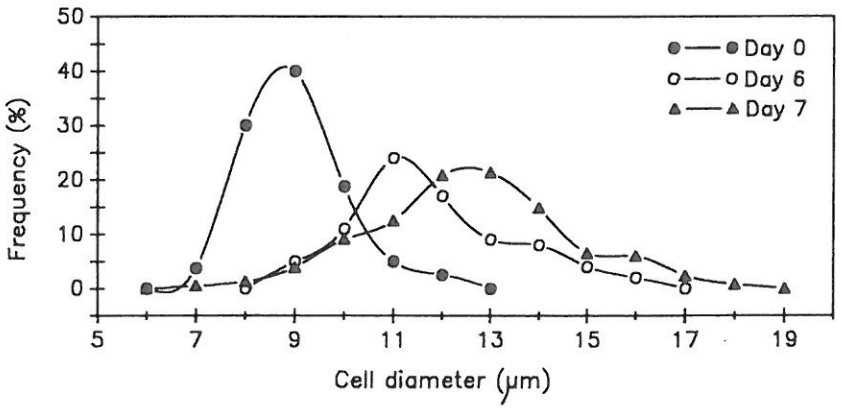


Fig. 2. Distributions of cell diameters during oocyte maturation. Each distribution was generated from a sample of 10 cells per gland from 8 different glands taken from 4 insects.

CA cells, which has important implications to future preparation of primary cell cultures.

Acknowledgments - We thank Edina Burns for technical assistance. Supported by PHS-NIH grant HD-21891 (to C. Schal) and the Thomas C. Headlee Fellowship (to A. Chiang). New Jersey Agricultural Experiment Station publication No. J-08170-01-89 supported by State funds and by the U.S. Hatch Act.

## REFERENCES

Cassier P. (1979) The corpora allata of insects. Int. Rev. Cytol. **57**, 1-73.

Engelmann F. (1957) Die Steuerung der Ovarfunktion bei der ovoviviparen Schabe Leucophaea maderae (Fabr.). J. Insect Physiol. **1**, 257-278.

Gadot M., Chiang A. and Schal C. (1989) Farnesoic acid-stimulated rates of juvenile hormone biosynthesis during the gonotrophic cycle in Blattella germanica. J Insect Physiol. (in press).

Johnson G.D., Stay B. and Rankin S.M. (1985) Ultrastructure of corpora allata of known activity during the vitellogenic cycle in the cockroach Diploptera punctata. Cell Tissue Res. **239**, 317-327.

Scharrer B. and von Harnack M. (1958) Histophysiological studies on the corpus allatum of Leucophaea maderae. I. Normal life cycle in male and female adults. Biol. Bull. **115**, 508-520.

Schooley D.A. and Baker F.C. (1985) Juvenile hormone biosynthesis. In Comprehensive Insect Physiology, Biochemistry and Pharmacology (Edited by Kerkut G.A. and Gilbert L.I.), Vol. 7, 1st Edn, pp. 363-389, Pergamon press, Oxford.

Szibbo C.M. and Tobe S.S. (1981) Cellular and volumetric changes in relation to the activity cycle in corpora allata of Diploptera punctata. J. Insect Physiol. **27**, 609-613.

Tobe S.S. and Stay B. (1985) Structure and regulation of the corpus allatum. Adv. Insect Physiol. **18**, 305-432.

Tobe S.S., Clarke N., Stay B. and Ruegg P. (1984) Changes in cell number and activity of the corpora allata of the cockroach Diploptera punctata: a role for mating and the ovary. Can. J. Zool. **62**, 2178-2182.