Cell Tissue Res. 197, 453–461 (1979)

Cell and Tissue Research

Ultrastructural Characteristics of Insect Corpora Allata in Relation to Larval Diapause*

C.-M. Yin** and G.M. Chippendale
Department of Entomology, University of Missouri, Columbia, Missouri, U.S.A.

Summary. The ultrastructure of active and inactive corpora allata from last instar larvae of the southwestern corn borer, *Diatraea grandiosella,* was examined. Active glands were obtained from pre-, early, and mid-diapausing larvae; inactive ones from late and non-diapausing larvae. Each gland contains 13 to 18 cells which have the following common features: well developed smooth endoplasmic reticulum, scattered rough endoplasmic reticulum and free ribosomes, microtubules, vacuolated nucleoli, and interlocking plasma membranes. The gland contains intercellular deposits, and is supplied by regular and neurosecretory axons.

Special ultrastructural features of the corpus allatum from the five groups of larvae examined were as follows: *pre-diapause:* extensive vesicular smooth endoplasmic reticulum, numerous cup-shaped mitochondria and Golgi bodies with stacked cisterns and vesicles, few small lipid droplets, large nuclei with dispersed chromatin, absence of lysosomes; *early diapause:* stacked, whorled, and vesicular smooth endoplasmic reticulum of equal abundance, numerous rod-shaped mitochondria, some Golgi bodies but without distinct stacks of cisterns, few lipid droplets and lysosomes, chromatin dispersed and also attached to the nuclear envelope; *mid-diapause:* similar to early diapause except for the presence of more stacked, smooth endoplasmic reticulum, chromatin in large chunks mostly attached to the nuclear envelope; *late diapause:* whorled smooth endoplasmic reticulum and rod-shaped mitochondria predominating, complicated Golgi bodies with stacks of cisterns and large empty sacs, few large lipid droplets, some lysosomes containing mainly whorled bodies, chromatin in

Send offprint requests to: Dr. G.M. Chippendale, Department of Entomology, 1-87 Agriculture Building, University of Missouri, Columbia, MO 65211, U.S.A.

* Supported in part by grant no. PCM 74-18155 A01 from the National Science Foundation. Contribution from the Missouri Agricultural Experiment Station as journal series no. 8234. We thank Ms. L. Yin for her skillful assistance, and Dr. M.F. Brown of the College of Agriculture Electron Microscope Facility for his advice and the use of equipment.
** Present address: Department of Entomology, University of Massachusetts, Amherst, MA 01002, U.S.A.

0302-766X/79/0197/0453/$01.80

large chunks attached to the nuclear envelope; *non-diapause:* similar to late diapause except for less extensive smooth endoplasmic reticulum, more abundant mitochondria, fewer intercellular deposits. Although these observations suggest that the smooth endoplasmic reticulum, and possibly mitochondria, and Golgi bodies are involved in juvenile hormone production, specific sites of synthesis or storage of the hormone were not revealed.

Key words: Larval diapause – Active and inactive corpora allata – Southwestern corn borer.

The ultrastructure of the corpora allata (CA) which secrete juvenile hormone (JH) continues to receive considerable attention (Scharrer, 1971; Guelin and Darjo, 1974; Deleurance and Charpin, 1975, 1978; Elliot, 1976). Such studies provide the most relevant information when carried out in relation to the secretory activity of the CA. This activity has now been clarified for diapausing and non-diapausing larvae of the southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera, Pyralidae), used in the present study. Earlier work based on allatectomy, CA implants, and JH titer measurements showed that last instar, pre-, early, and mid-diapausing larvae retain active CA, whereas late diapausing and last instar non-diapausing larvae have inactive CA (Bergot et al., 1976; Yin and Chippendale, 1976, 1979). The CA of these various groups of larvae were now examined, thereby providing the first comprehensive account of their ultrastructural changes during larval diapause.

Materials and Methods

Experimental Larvae

Non-diapausing, pre-diapausing, and diapausing larvae of *D. grandiosella* were reared on an agar-based diet (Chippendale, 1975). Non-diapausing larvae were obtained from cultures reared at 30° C 16L : 8D, whereas 23° C 12L : 12D produced diapausing larvae. Non-diapausing larvae completed their feeding around 16 days and pupated 2 to 3 days later, whereas pre-diapausing larvae completed their feeding around 35 days, and entered diapause. At the onset of diapause, around 40 days, each larva molts from a spotted to an immaculate morph which lacks cuticular pigments. Beginning at 45 days diapausing larvae were either retained at 23° C or transferred to 30° C 12L : 12D to accelerate the completion of diapause (Chippendale and Reddy, 1973).

Microscopic Techniques

Paraffin sections (4–10 μm) of larval heads were stained with hematoxylin and Alcian blue (Steedman, 1950). Alternatively, 1 μm sections of CA, embedded in epon (Luft, 1961), were stained with a 0.1% aqueous toluidine blue solution, and mounted in oil.

Larval CA were quickly fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for electron microscopy. They were post-fixed after 4 h at 4° C in 1% buffered osmium tetroxide for an additional 3 to 4 h at 4° C. Epon sections (0.06 to 0.09 μm) were stained with 2% alcoholic uranyl acetate for 25 min and alkaline lead citrate for 5 min at room temperature. Electron micrographs were taken with a RCA EMU-3G instrument operating at 100 kV. Sections of at least 5 glands were examined from larvae of each stage. No sex-related differences were observed.

Results and Discussion

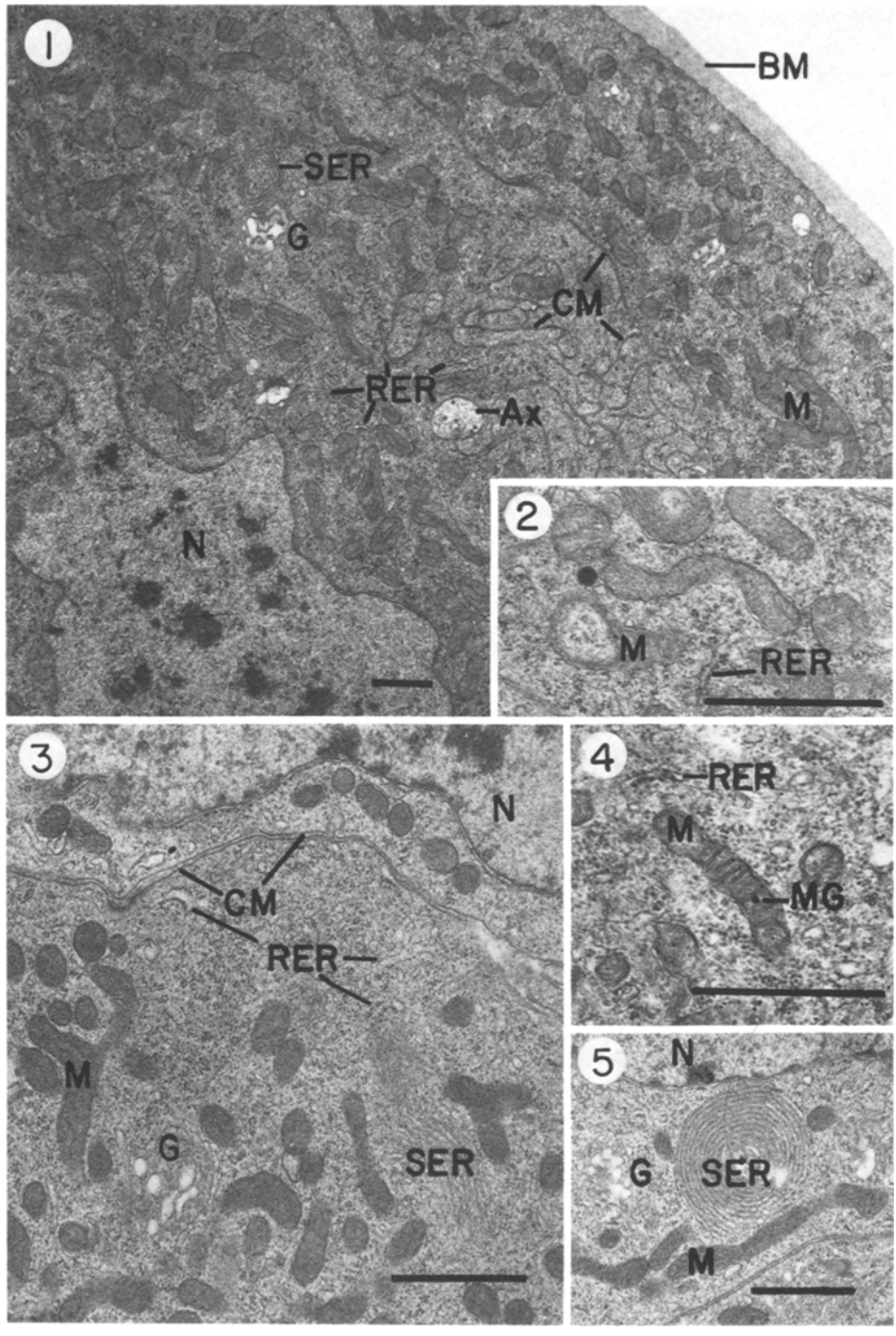
The simple lateralized CA of *D. grandiosella* are separated from the corpora cardiaca by long nervi corporis allati. Each corpus allatum is spindle-shaped, 150 to 200 μm long and 30 to 48 μm in dia. at its center, and is loosely attached to the duct of the mandibular gland. Serial sections showed that each gland contains 13 to 18 closely packed cells within a connective tissue sheath. This sheath stains green with the Alcian blue technique and, therefore, contains glycosaminoglycans. The nucleus of the cells contains 1 to 3 nucleoli, and the cytoplasm contains microtubules which presumably provide structural support. Each corpus allatum is supplied by regular and neurosecretory axons. In addition to their apparent neurosecretory control over the CA cells, neurosecretory granules were observed within the acellular stroma, suggesting that the CA may function as a neurohemal organ.

Substantial differences were observed in the frequency and organization of several organelles of the CA cells in pre-diapausing, diapausing, and non-diapausing larvae. Especially notable were differences in smooth endoplasmic reticulum, mitochondria, Golgi bodies and lysosomes suggesting that these organelles are related to the activity level of the CA.

A cell of a highly active CA from a 33-day-old pre-diapausing larva has a smoothly contoured nucleus, abundant irregularly-shaped mitochondria (Fig. 2), Golgi bodies, and smooth endoplasmic reticulum of both the sac and whorled type (Figs. 1–3). Short pieces of rough endoplasmic reticulum and free ribosomes are also present, and axons, some carrying neurosecretory granules, may extend into the cell (Fig. 1). A CA cell from a 45-day-old early diapausing larva is similar (Figs. 4–5). Some of the mitochondria contain electron-dense granules (Fig. 4) of the kind apparently binding bivalent cations, usually Ca^{++} and Mg^{++}, which are necessary cofactors for several enzymes (Novikoff and Holtzman, 1970). Whorled smooth endoplasmic reticulum is typical (Fig. 5).

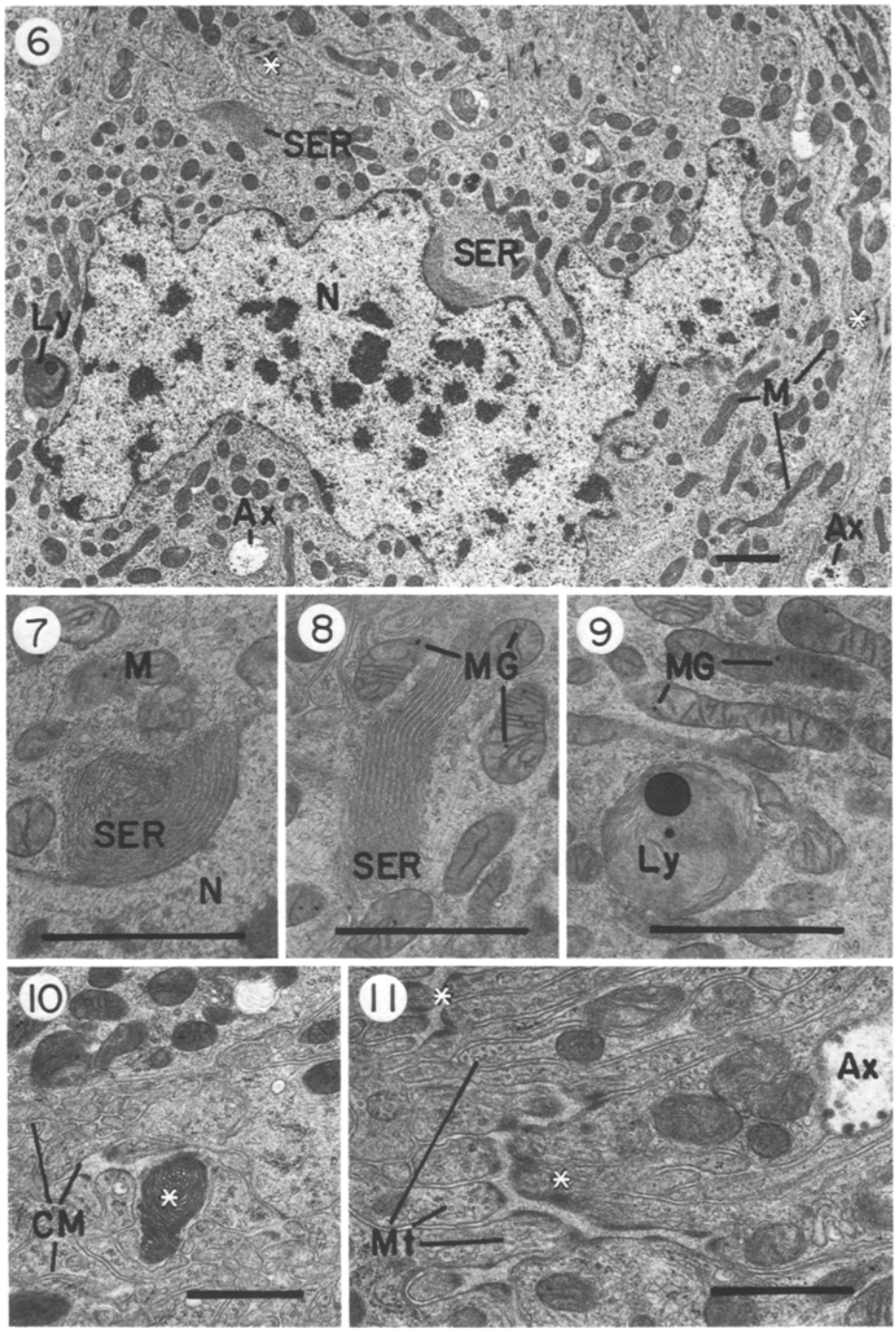
The cell of a moderately active CA from a 65-day-old mid-diapausing larva shows the following important features: a rather irregularly contoured nucleus; abundant smooth endoplasmic reticulum, whorled-type near the nuclear envelope and stacked-type near the plasma membrane; moderately dense mitochondria containing distinct cristae and dark granules, and only a few Golgi bodies and lysosomes (Figs. 6–11). Stacked smooth endoplasmic reticulum typically is close to the plasma membrane and may be formed by a fusion of the short tube reticulum (Fig. 8). Membranous lysosomes, mitochondria containing electron-dense granules, and membranous electron-dense material or bodies (see also, Scharrer, 1971) located within intercellular spaces are also characteristic of the CA cells of mid-diapausing larvae (Figs. 9, 10). Electron-dense material may accumulate at the tips of extended cell processes (Fig. 11).

Inactive CA cells from late diapausing larvae show the following important features (Figs. 12–14): smoothly contoured nucleus; lipid droplets; many lysosomes; intercellular spaces frequently containing dark deposits or bodies. Although these extracellular deposits appear to be more common in the CA of mid- and late diapausing larvae than in those of other stages, their function remains unknown. As found in the CA of mid-diapausing larvae electron-dense material may accumulate at the tips of extended cell processes (Fig. 12). Notable differences were observed in

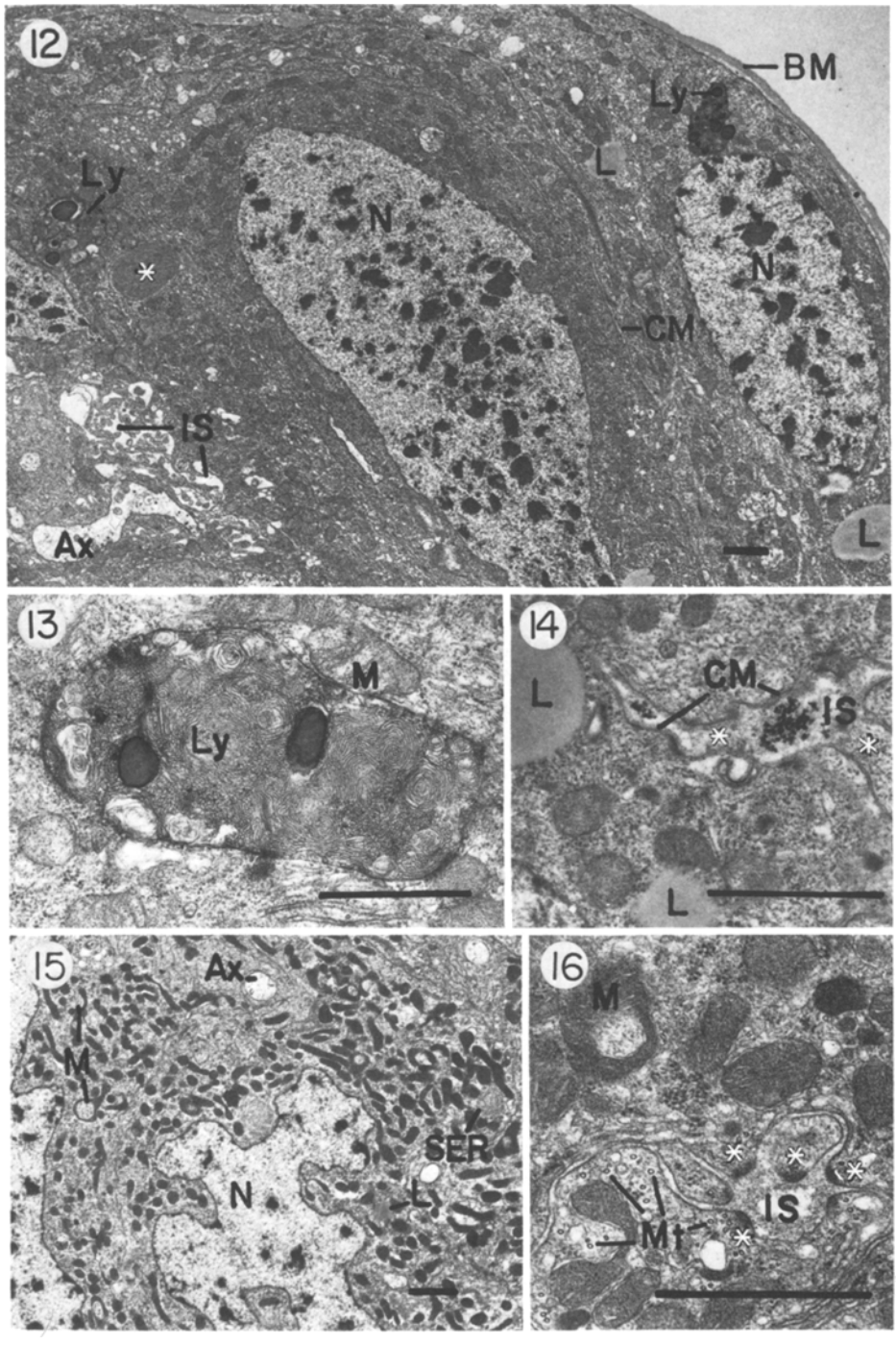


Figs. 1–3. Highly active corpora allata from 33-day pre-diapausing larvae of *D. grandiosella* showing sac-type and whorled smooth endoplasmic reticulum (*SER*), numerous mitochondria (*M*), Golgi bodies (*G*), smoothly contoured nucleus (*N*), rough endoplasmic reticulum (*RER*), plasma membrane (*CM*), axon (*Ax*), and acellular stroma (*BM*)

Figs. 4 and 5. Active corpora allata from 45-day early diapausing larvae of *D. grandiosella* showing mitochondrial granule (*MG*), and whorled SER. Scale bars equal 1 μm



Figs. 6–11. Moderately active corpora allata from 65-day mid-diapausing larvae of *D. grandiosella* showing whorled and stacked smooth endoplasmic reticulum (*SER*), frequent mitochondria (*M*) some containing granules (*MG*), irregularly contoured nucleus (*N*), plasma membrane (*CM*), membranous lysosomes (*Ly*), axons (*Ax*), and microtubules (*Mt*). *Asterisk* shows extracellular deposit. Scale bars equal 1 μm



Figs. 12–14. Inactive corpora allata from 202-day late diapausing larvae of *D. grandiosella* showing mainly rod-shaped mitochondria (*M*), smoothly contoured nucleus (*N*), plasma membrane (*CM*), axon (*Ax*), membranous lysosomes (*Ly*), lipid droplets (*L*), *IS* intercellular space, and acellular stroma (*BM*)

Figs. 15 and 16. Inactive corpora allata from last instar non-diapausing larvae of *D. grandiosella* showing similar features to those of late diapausing larvae except for more abundant mitochondria (*M*), and irregularly contoured nucleus (*N*). Microtubules (*Mt*) clearly visible. *Asterisks* show extracellular deposits. Scale bars equal 1 μm

Table 1. Ultrastructural differences in the corpora allata of pre-diapausing, diapausing, and non-diapausing larvae of *D. grandiosella**

Organelle or Structure	Pre-diapausing Larvae	Early diapausing Larvae	Mid-diapausing Larvae	Late diapausing Larvae	Non-diapausing Larvae
Smooth endoplasmic reticulum					
(a) stacked	+	++	+++	++	+
(b) whorled	+	++	++	+++	+
(c) vesicular	+++	++	++	++	+
Mitochondria					
(a) cup-shaped	+++	+	NS	NS	++
(b) rod-shaped	++	+++	+++	+++	++
(c) Y-shaped	+	+	NS	NS	+
Mitochondrial granules	NS	+	+++	NS	NS
Golgi bodies	+++	++	+	++	+
Free ribosomes	++	+	+	+	+
Lysosomes	+	+	++	+++	++
Nuclear envelope					
(a) smooth	+++	++	+	+++	++
(b) convoluted	+	++	+++	+	++
Intercellular deposits					
(a) ground substance	+	+	++	+++	+
(b) granules	NS	NS	NS	++	NS
(c) bodies	NS	NS	+	+++	NS

* Pre-diapausing larvae 33 days of age (23°C); early diapausing 45 days (23°C); mid-diapausing 65 days (45 days 23°C + 20 days 30°C); late diapausing 85 days (45 days 23°C + 40 days 30°C); and non-diapausing 14 days (30°C). Observations based on at least 5 glands from each stage. Frequency of observation: + = infrequent; ++ = frequent; +++ = very frequent; NS = not seen

the inactive CA cell from last instar non-diapausing larvae (Figs. 15–16). In contrast to those of late diapausing larvae, these cells contain a convoluted nucleus, numerous mitochondria (frequently cup-shaped), and extensive interdigitations of the plasma membranes. Since cup-, rod- and Y-shaped mitochondria are present in pre-diapausing, early diapausing, and non-diapausing larvae, the presence of any one type of mitochondrial organization is not related to the secretory activity of the gland.

Since abundant stacked, whorled and vesicular smooth endoplasmic reticulum was found in the CA cells, except in the inactive cells of non-diapausing larvae, this organelle may be involved in JH synthesis (Table 1). From biochemical studies carried out on the CA of the cockroach *Blaberus giganteus,* and the tobacco hornworm *Manduca sexta,* soluble and membrane-bound cytoplasmic enzymes have been implicated in the synthesis of JH (Hammock, 1975; Reibstein et al., 1976; Lee et al., 1978). For example, the epoxidation of methyl farnesoate or farnesoic acid may occur on the smooth endoplasmic reticulum of the CA cells. Melnikova and Panov (1975) have summarized information about the relative abundance of

smooth and rough endoplasmic reticulum found in the cells of the CA of various insects. The CA cells of *D. grandiosella,* like those of the silkmoth *Hyalophora cecropia* (Waku and Gilbert, 1964), the silkworm *Bombyx mori* (Fukuda et al., 1968), and the fall webworm *Hyphantria cunea* (Melnikova and Panov, 1975), have extensively developed smooth endoplasmic reticulum.

Based on the present evidence few conclusions can be drawn about the involvement of mitochondria and Golgi bodies in JH synthesis and transport in *D. grandiosella.* Through electron microscopic autoradiography with tritiated JH as a tracer, Tobe and Saleuddin (1977) concluded that at least the last 2 stages of JH synthesis (epoxidation and carboxyl alkylation) occur in the smooth endoplasmic reticulum and possibly the mitochondria and Golgi bodies of the CA of the adult locust *Schistocerca gregaria.* If JH diffuses from the CA into the hemolymph of *D. grandiosella* as proposed for *S. gregaria,* ultrastructural studies will not reveal deposits of JH in the CA or specific subcellular adaptations necessary for its release (Tobe and Pratt, 1974). The present results suggest that JH does not accumulate in the CA cell. The hormone probably diffuses through the plasma membrane without any involvement of hemidesmosomes (Joly et al., 1968).

References

Bergot, B.J., Schooley, D.A., Chippendale, G.M., Yin, C.-M.: Juvenile hormone titer determinations in the southwestern corn borer, *Diatraea grandiosella,* by electron capture-gas chromatography. Life Sci. **18,** 811–820 (1976)

Chippendale, G.M.: Ascorbic acid: an essential nutrient for a plantfeeding insect, *Diatraea grandiosella.* J. Nutr. **105,** 499–507 (1975)

Chippendale, G.M., Reddy, A.S.: Temperature and photoperiodic regulation of diapause of the southwestern corn borer, *Diatraea grandiosella.* J. Insect Physiol. **19,** 1397–1408 (1973)

Deleurance, S., Charpin, P.: Evolution ultrastructurale des corps allates de la larve du dernier stade de *Choleva angustata* Fab. (Catopidae). Action du jeûne, en présence ou non d'ecdystérone. C. R. Acad. Sci. (Paris) **280 D,** 2113–2116 (1975)

Deleurance, S., Charpin, P.: Ultrastructural dynamics of the corpus allatum of *Choleva angustata* Fab. (Coleoptera, Catopidae). Cell Tissue Res. **191,** 151–160 (1978)

Elliot, H.J.: Structural analysis of the corpus allatum of an aphid, *Aphis craccivora.* J. Insect Physiol. **22,** 1275–1279 (1976)

Fukuda, S., Eguchi, G., Takeuchi, S.: Histological and electron microscopical studies on sexual differences in structure of the corpora allata of the moth of the silkworm, *Bombyx mori.* Embryologia (Nagoya) **9,** 123–158 (1968)

Guelin, M., Darjo, A.: Etude ultrastructurale des corpora allata en relation avec le contrôle photopériodique de leur fonction gonadotrope chex *Locusta migratoria migratoria* L. C. R. Acad. Sci. (Paris) **278 D,** 491–494 (1974)

Hammock, B.D.: NADPH dependent epoxidation of methyl farnesoate to juvenile hormone in the cockroach, *Blaberus giganteus* L. Life Sci. **17,** 323–328 (1975)

Joly, L., Joly, P., Porte, A., Girardie, A.: Etude physiologique et ultrastructurale des corpora allata de *Locusta migratoria* L. (Orthoptère) en phase grégaire. Arch. Zool. Exp. Gén. **109,** 703–728 (1968)

Lee, E., Schooley, D.A., Hall, M.S., Judy, K.J.: Juvenile hormone biosynthesis: homomevalonate and mevalonate synthesis by insect corpus allatum enzymes. J. Chem. Soc. Chem. Comm. no. **7,** 290–292 (1978)

Luft, J.H.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9,** 409–414 (1961)

Melnikova, E.J., Panov, A.A.: Ultrastructure of the larval corpus allatum of *Hyphantrea cunea* Drury (Insecta, Lepidoptera). Cell Tissue Res. **162,** 395–410 (1975)

Novikoff, A.B., Holtzman, E.: Cells and organelles, 337 pp. New York: Holt, Rinehart, and Winston, Inc., 1970

Reibstein, D., Law, J.H., Bowlus, S.B., Katzenellenbogen, J.A.: Enzymatic synthesis of juvenile hormone in *Manduca sexta*. In: The juvenile hormones (L.I. Gilbert, ed.), p. 131–146. New York: Plenum Press (1976)

Scharrer, B.: Histophysiological studies on the corpus allatum of *Leucophaea maderae:* V. Ultrastructure of sites of origin and release of a distinctive cellular product. Z. Zellforsch. **120,** 1–16 (1971)

Steedman, H.F.: Alcian blue: a new stain for mucin. Quart. J. Micros. Sci. **91,** 477–479 (1950)

Tobe, S.S., Pratt, G.E.: Dependence of juvenile hormone release from corpus allatum on intraglandular content. Nature (Lond.) **252,** 474–476 (1974)

Tobe, S.S., Saleuddin, A.S.M.: Ultrastructural localization of juvenile hormone biosynthesis by insect corpora allata. Cell Tissue Res. **183,** 25–32 (1977)

Waku, H., Gilbert, L.I.: The corpora allata of the silkmoth, *Hyalophora cecropia:* an ultrastructural study. J. Morph. **115,** 69–96 (1964)

Yin, C.-M., Chippendale, G.M.: Hormonal control of larval diapause and metamorphosis of the southwestern corn borer, *Diatraea grandiosella.* J. Exp. Biol. **64,** 303–310 (1976)

Yin, C.-M., Chippendale, G.M.: Diapause of the southwestern corn borer, *Diatraea grandiosella:* further evidence showing juvenile hormone to be the regulator. J. Insect Physiol. **25,** in press (1979)

Accepted January 18, 1979