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# Annulate Lamellae in Prothoracic Gland Cells of Brainless Pupae of the Swallowtail, *Papilio xuthus* (Lepidoptera)

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**ABSTRACT**—In 2 individuals out of 33 brain extirpated diapause pupae of the swallowtail, *Papilio xuthus,* the prothoracic gland cell (PGC) was particularly characterized by abundant annulate lamellae, of which some showed an unusual open flower arrangement. In addition, the cytoplasm contained numerous agranular membranes, which seemed to originate from the annulate lamellae and which were in close contact with dense masses that appeared to be liberated from large denser cytoplasmic spherules encapsulated by a nest of mitochondria. The PGC was also characterized by a multilobulated nucleus and remarkable perichromatin granules surrounded by massive condensed heterochromatin. These cytological features may account for the peculiar fact that some brainless pupae occasionally break their dormancy during a long storage period.

## INTRODUCTION

Ultrastructural studies on insect prothoracic glands have been carried out on Lepidoptera [1–14], Diptera [15, 16] and Coleoptera [17, 18] as examples of holometabolous insects, and on Orthoptera [19], Dictyoptera [20, 21] and Hemiptera [see 22], as examples of hemimetabolous insects. In prothoracic gland cells (PGC's) of two species of silkworm, *Antheraea pernyi* and *Bombyx mori,* Beaulaton [6] observed annulate lamellae and their disorganization in the later half of the 4th larval period. Scharrer [20] reported a cytomembrane system like annulate lamellae having a close relation to microtubules in cockroach PGC's.

In the previous paper [23], we reported a peculiar fact that 30% of artificial diapause pupae induced by a combined short day and brainless treatments could not maintain dormancy during a storage period as long as 8 months and occasionally differentiated into adults. Examining PGC's of such brainless pupae under the light microscope, we found that 10% of the individuals showed cytological features which supported the conclusion that the dormant PGC's of brainless pupae had recovered their secretory activity. The recovery in diapause pupae of *Hyalophora cecropia* has been reported by McDaniel *et al.* [12], but fine structural detail in the spontaneous recovery of dormant PGC was unknown. The present study deals with electron microscope observations of the PGC's of brainless pupae of the swallowtail and reports that the annulate lamellae may be cytologically related to the secretory activity of the gland cell.

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## MATERIALS AND METHODS

Brainless pupae were prepared from the spring generation by rearing them in a dark room with only 7 hr of light every day throughout their whole embryonic and larval period. The brain together with the suboesophageal ganglion was extirpated within 24 hr after pupation to ensure diapause. The wound was sealed immediately with melted paraffin as already reported [23]. A hundred

brainless pupae were prepared early in June, then stored in natural light at room temperature as long as 5 months until October. Starting from the 20th day after pupation, several samples were randomly selected every 20 days during the storage, and small pieces of the prothoracic segment containing one of the pair of prothoracic glands were fixed in 1/3 strength Karnovsky's aldehyde mixture diluted with 0.1 M phosphate buffer, pH 7.4. At the time of fixation, the pupal fat body was examined under the dissecting microscope to confirm that it was still in pupal form and had not initiated imaginal differentiation. The prothoracic glands were dissected out, immersed in fresh fixative for an hour, and then postfixed in 1% aqueous solution of osmium tetroxide for another hour. Thin plastic sections were cut with a Sorval MT-1 microtome and examined under Hitachi HU-11E electron microscope with acceleration voltage of 75 kv. Sections were stained with 2.5% aqueous uranyl acetate followed by 2.5% lead citrate.

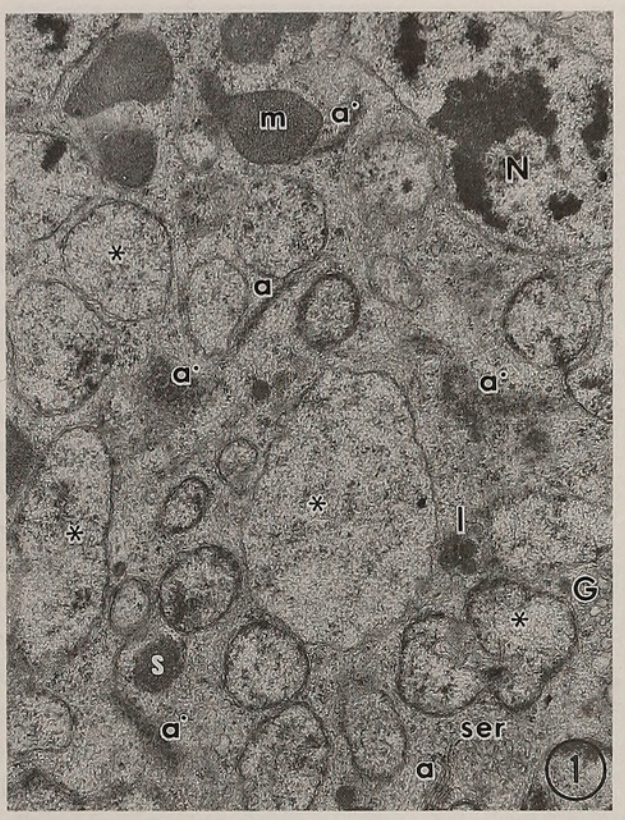
## RESULTS

In the prothoracic glands of 2 individuals fixed on the 60th day and the 80th day after pupation, processes of the multilobulated nuclei were observed all over the cell, and annulate lamellae were conspicuous in almost all regions of the cytoplasm, especially around the nucleus (Fig. 1). They were arrayed in clusters of several or ten at most, having a length of more than 15 $\mu$m. Except for a feature which will be mentioned below, the annulate lamellae in PGC's are essentially similar to those reported in oocytes and other tissues of many vertebrates and invertebrates [see 24]. The individual lamella consists of a pair of relatively parallel membranes, separated from each other by about 30–40 nm, and interrupted by pores about 60 nm in diameter (Figs. 2 and 3). The lamella lies in close contact with the outer nuclear membrane, from which it appears to originate (arrows in Fig. 2). Perichromatin granules surrounded by prominent halos were observed within dense heterochromatin masses (Fig. 3). A noticeable feature in the annulate lamellae, of the PGC's, is an appearance of an open flower with a central granular structure at the end of the stacks (Fig. 2). Numerous vesicular, agranular membranes are supplied from the open flower arrangement (af in Fig. 4) and have contact with masses of dense substance (s in Fig. 4) liberated from a denser granulofilamentous cytoplasmic spherule encapsulated by a nest of mitochondria (S in Fig. 4). PGC's from the remaining individuals, on the other hand, showed rather quiescent looking (Fig. 5). The cytoplasm is essentially characterized by abundant ribosomes, and by sparse granular (RER) as well as agranular endoplasmic reticulum (SER). No annulate lamella nor agranular membrane system was observed. Nuclei are not much lobulated, and contain less heterochromatin with no perichromatin granules, in contrast with the cases of the other 2 pupae. Cytoplasmic spherules are tightly encapsulated by mitochondria, and do not show any morphological change to liberate dense masses into cytoplasm (Fig. 5).

FIG. 1. A prothoracic gland cell (PGC) in a pupa on the 60th day of diapause. Numerous profiles of cut processes (*) of the multilobulated nucleus (N) are visible. A few annulate lamellae cut normally (a) or tangentially (a˙) are observed. ser: smooth endoplasmic reticulum, G: Golgi complex, l: lysosomes, m: mitochondria, s: fragment of cytoplasmic spherule (×12,000).

FIG. 2. A PGC with conspicuous annulate lamellae (a) found in a limited pupa fixed on the 60th day of diapause. The plane of section cuts the annulate lamellae parallel to the axis of the stack. Perinuclear stacks appear to be continuous with the outer nuclear membrane (arrows). Another feature of annulate lamellae, of an appearance of open flower (af) with central dots, can be seen. Sections of nuclear processes with nucleolus (n), and those containing dense heterochromatin (h) can be seen. G: Golgi complex. (×20,000).

FIG. 3. A mass of annulate lamellae cut normally (a), tangentially (a′) and obliquely (a″). Perichromatin granules (PCG) with remarkable halos are observed within dense heterochromatin. A PGC of an individual fixed on the 80th day of diapause. s: fragments of cytoplasmic spherule with lower density, h: mass of heterochromatin, p: nuclear pores cut to reveal the en face view (×33,000).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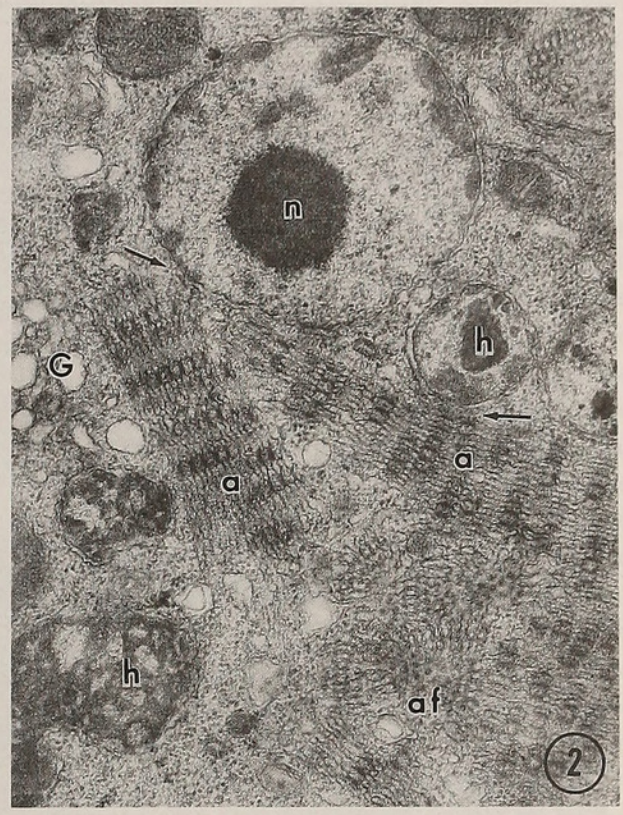
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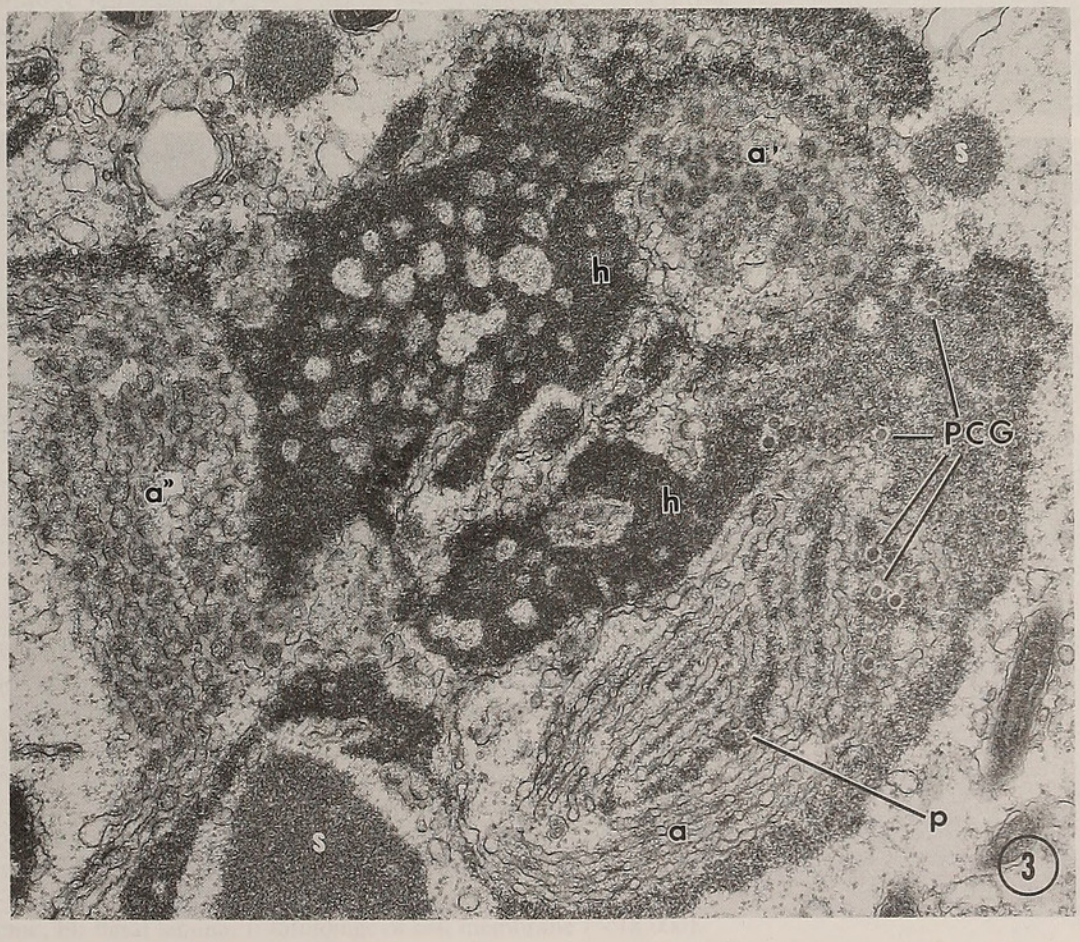
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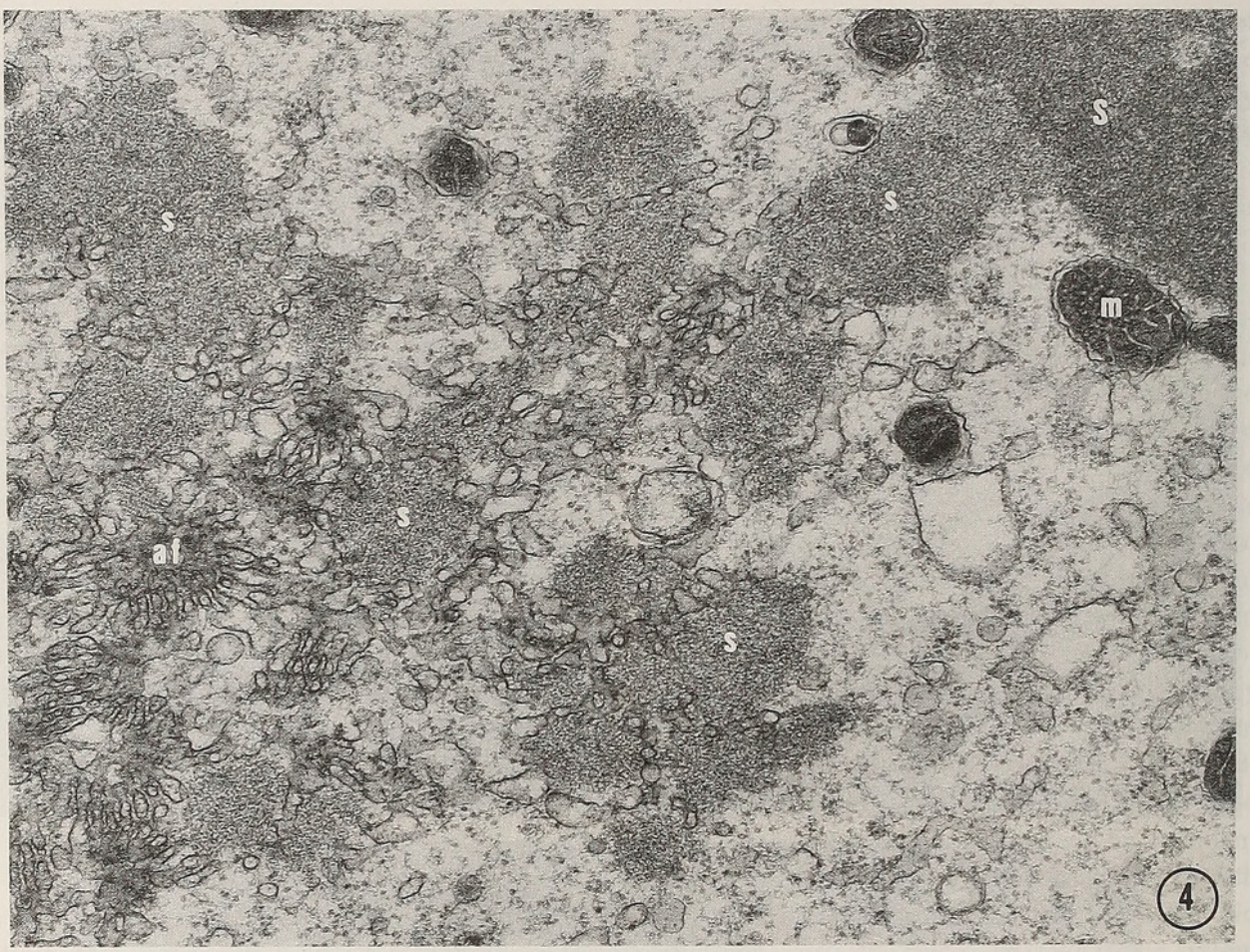
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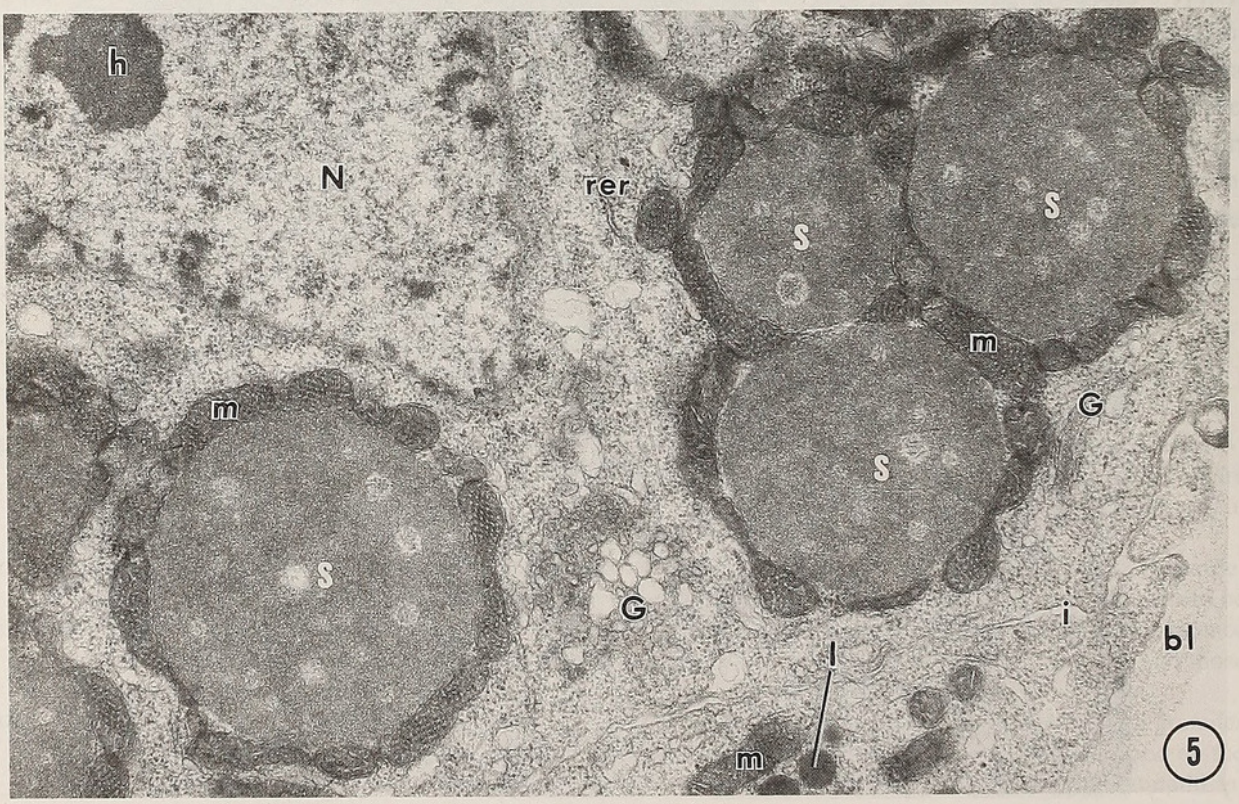
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## DISCUSSION

Although annulate lamellae have been described in adult somatic cells, they are more frequently observed in various rapidly growing, developing and differentiating cells. In many types of cells, annulate lamellae are formed in connection with or in close proximity to the nuclear membrane [24–29]. Another mode of formation has been described by Kessel and Beams [30] and by Halkka and Halkka [31], who noted annulate lamellae within dense masses in the oocyte cytoplasm of different species of dragonfly. Our observations, which showed annulate lamellae in close contact with the nuclear membrane, possibly support the former mode of formation. A marked concentric arrangement of annulate lamellae has been reported by Bawa in the Sertoli cells of the human testis [25], and by Harrison in the alligator and sea gull adrenal cortical cells [26]. Bawa [25] also observed another conspicuous type, the half-moon shaped paired annulate lamellae, in the Sertoli cells. Annulate lamellae in PGC's of the swallowtail showed another unusual type of arrangement, like an open flower with central dots.

Blazsek and Mala [9] observed extracellular micro vesicles at the time of cocoon formation in *Galleria mellonella.* Sedlak *et al.* [13] reported morphological changes of multivesicular sacs which occured within cell cytoplasm and within intercellular spaces at the time of major ecdysone peak in *Manduca sexta,* suggesting that these sacs were likely involved in the packaging of a glandular product exocytosed into intercellular space. Gersh *et al.* [10] reported an increase in size of mitochondria in active PGC phase in *Galleria mellonella.* The extreme irregulation of multilobulated nuclear surface [1, 8, 13, 16], which were often encountered in cases of our 2 pupae, too, may be interpreted as the induction of the transport of the nuclear material into cytoplasm. Regarding to the cytoplasmic agranular membrane system, which was also characteristic in our 2 pupae, Akai and Kiuchi [2] showed slit-like vesicles distributed in cytoplasm before the spining stage in which ecdysone titers rapidly increase in *Bombyx mori.* McDaniel *et al.* [12] reported the filled vesicles at the periphery of PGC just prior to their secretion in *Hyalophora cecropia.* Takeda [14] and King *et al.* [16] suggested that ecdysone was synthesized by SER in *Monema flavescens* and in *Drosophila melanogaster,* respectively. The SER was thought to be the relation site for the side-chain alternation and hydroxylation of the cholesterol molecules in the course of the elaboration of ecdysone [14, 17]. SER has also been proposed as an important site of steroid hormone synthesis in vertebrates [32–34]. In our 2 individuals, PGC showed numerous agranular vesicular membrane system supplied by annulate lamellae, suggesting that the gland cells were in an active phase of hormone elaboration.

To our knowledge only two papers are concerned with the annulate lamella in the PGC's of insects. Beaulaton [6] reported the appearance of distinctive annulate lamellae in PGC's, which were taken from two different species of silkworm in the later half period of 4th instar larvae. Scharrer [20] observed a membrane system similar to annulate lamellae having a close relation to microtubules in the PGC's of freshly emerged cockroach. In *Xylenborus ferrugineus,* Chu *et al.* [18] reported an existence of many microtubules in PGC of pupae which contain high titers of ecdysteroid. However, whether the annulate lamellae and microtubules are in exact relation to secretory activity of the

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FIG. 4. Substances with lower density (s) is liberated from a denser granulo-filamentous cytoplasmic spherule (S), which is surrounded by mitochondria (m), into cytoplasm to come in close contact with numerous agranular membranes supplied from annulate lamellae having an open flower arrangement with central dots (af). Eightieth day of diapause (×35,000).

FIG. 5. General profile of a PGC from one of the remaining 31 pupae. Numerous free ribosomes scatter in the cytoplasm, which has many dense spherules (S) surrounded by mitochondria (m). Agranular and granular endoplasmic reticula (rer) are sparse. No annulate lamella, whichever a stack or an open flower arrangement, has been observed in any case of the 31 pupae. Golgi complex (G) can be seen in this section but not frequently in other sections. Nucleus (N) is not so much lobulated as shown in Figs. 1 and 2. Eightieth day after pupation. l: lysosome, bl: basal lamina, i: infoldings of cell membrane (×18,000).

PGC remains an open question. McDaniel *et al.* [12] reported that the PGC's were filled with free ribosomes, but little RER and no SER in "dauer pupae" by brain extirpation and in "arrested pupae" by aminophiline, of *Hyalophora cecropia*. These characteristics are similar to those of PGC's of our remaining 31 pupae.

In our previous paper [23], large intracytoplasmic spherules in the PGC's of brainless swallowtail pupae were reported to be Feulgen positive, and encapsulated by a nest of mitochondria. They dissolve when larval brains were implanted or a crude extract of brain of *Bombyx mori* was injected into the brainless pupae. Even without any treatment, occasional individuals among a large number of brainless pupae showed dissolving spherules and vacuoles in their PGC [23]. This cytological figure, under the light microscope, presumably showed that the dormant PGC's had recovered their secretory activity previous to the imaginal differentiation, and could explain why some brainless pupae differentiated into adults during storage [23].

In the present report, numerous vesicular and agranular membranes which were shown to be derived from annulate lamellae came in contact with masses of substance liberated apparently from the large spherules. Nuclei of the PGC's with annulate lamellae also showed conspicuous intranuclear perichromatin granules. Reviewing these ultrastructural features in the swallowtail PGC's, we suggest that the formation of annulate lamellae might result from the manifestation of some genetic message stored in the nuclear perichromatin granules or in cytoplasmic spherules, and be related to the initial phase of the cell differentiation prior to the secretion of ecdysone.

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