ULTRASTRUCTURAL STUDY OF THE REGRESSING PROTHORACIC GLANDS OF BLATTARIAN INSECTS*

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With 14 Figures in the Text

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Summary. The prothoracic glands, source of the molting hormone ecdysone, regress within a few days after the final molt, a process which was analyzed with electron microscopic methods in the cockroaches Leucophaea and Blaberus. This strictly timed event is accompanied by drastic alterations in cellular fine structure. Early signs of breakdown appear in groups of nuclei whose substance becomes segregated into patches of contrasting electron density characteristic of pyknosis.

The most conspicuous change in the cytoplasm of parenchymal cells concerns the appearance of large, heterogeneous inclusion bodies in which various cellular elements become segregated. These compartments seem to represent autophagic vacuoles within which the gradual degradation of much of their contents takes place, presumably under the influence of lysosomal enzymes. Undigested swirls of membranous character may remain sequestered within these packets for some time.

At advanced stages of cellular atrophy, plasma membranes and nuclear envelopes have gradually disappeared, and masses of protoplasm undergoing autolysis become invaded by a greater number of hemocytes than are present in nymphal glands. These phagocytic elements appear to engulf debris of parenchymal cells as well as some degenerating connective tissue elements. After the completion of the regressive process, the axial band of musculature characteristic of the nymphal gland persists on its own. Whether or not some parenchymal cells (or possibly their precursors) capable of reactivation persist in the proximity of this muscle is unknown.

The resorption of the prothoracic gland in the newly emerged insect is the result of physiological autolysis and seems to be aided by the activity of phagocytic hemocytes.

The molting hormone, ecdysone, is all important for the growing and developing insect but is no longer needed after the terminal molt which results in the adult stage. Therefore, the prothoracic gland, which in immature forms furnishes this endocrine substance, becomes inactive and regresses after the completion of metamorphosis. In the cockroach Leucophaea maderae the prothoracic gland disappears within a few days after emergence of the adult. In short, this endocrine organ has its own predictably limited life span. The process of its physiological regression was analyzed with methods of light microscopy at an earlier date (Scharrer 1948). A re-examination of the phenomenon in this and a related species, with more adequate, primarily ultrastructural, techniques yielded new insights that are the subject of the present report (see also Scharrer 1965a).

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Material and Methods

Twenty one male and female adult specimens of the blattarian *Leucophaea maderae* and four of the related *Blaberus craniifer* were fixed at intervals ranging from one hour to five days after the emergence of the adult. For the sake of simplicity, these glands will be referred to as 2, 3, etc. days “old” in this paper. The region of the prothoracic glands was exposed under a dissecting microscope and a drop of chilled fixing fluid was placed on it and allowed to act for several minutes. The glands were then dissected free, divided into pieces of suitable size, and placed into the fluid which was either a 1 or 2% solution of osmium tetroxide buffered at pH 7.4 and 7.8 respectively, or 5% glutaraldehyde (see OsiNchAk 1964) followed by osmium tetroxide, or 1% sodium permanganate buffered at pH 7.4 with veronal acetate. The Epon-embedded specimens were sectioned on an LKB ultratome. The “staining” procedure included uranyl acetate in 50% ethanol, and lead citrate (REYNolDS 1963), the latter being omitted after permanganate fixation. The sections were then studied in an RCA EMU 3 E or 3 G at 100 KV.

One-micron thick Epon-embedded sections were stained with toluidine blue or PAS for comparison with the electron micrographs. For cytochemical reactions, whole mounts of unfixed prothoracic glands were frozen immediately after their removal from the animal and kept in cold storage until the time of enzyme incubation. Among a number of tests carried out with this material only those for acid phosphatase (Gomori method) and thiamine pyrophosphatase activity (Novikoff-GoldfischEr method) (see Novikoff 1963; Novikoff and GoldfischEr 1961) will be mentioned in the context of the present paper.

The observations made in specimens in various stages of regression were compared with those in active prothoracic glands taken from a variety of nymphal stages at graded intervals throughout the intermolt period (SCHARRER 1964b). The results in the two species studied were remarkably similar. Unless otherwise stated, the description which follows will apply equally to both of them.

Observations

The prothoracic glands of newly emerged adults of *Leucophaea* and *Blaberus* largely resemble those of large nymphs soon after molting (SCHARRER 1964b, 1965c). Whole mounts (Fig. 1 a) and sections examined under the light microscope show as yet no reduction in the width of the organ. Electron micrographs (Fig. 2) reveal that the cellular architecture is still largely intact. Ovoid nuclei are closely spaced, and slender cytoplasmic processes extend toward the surface of the organ. Golgi elements, mitochondria, and endoplasmic reticulum show about the same distribution as in comparable nymphal glands (SCHARRER 1964b). Centrioles may be found in undisrupted cells of 1- or 2-day-old specimens. Dense bodies which, because of their morphological (SCHARRER 1964b) and cytochemical characteristics (OsiNchAK 1965), can be identified as lysosomes are present in prothoracic glands of nymphs as well as young adults. One may assume that these characteristic inclusion bodies seen in the electron microscope are the same as the granules showing activity with the Gomori method for acid phosphatase (Fig. 1) and the Novikoff-Goldfischer method for thiamine pyrophosphatase, as observed with the light microscope.

Shallow pits in the plasmalemma, caveolae, and micropinoeytotic vesicles comparable to those in nymphal parenchymal cells (see Figs. 8 and 9 in SCHARRER 1964b) are present in young adult specimens of both species. In *Blaberus* the cells are rich in accumulations of glycogen in 1-day-old adults (Fig. 6), as they are in the nymph.

Changes in the parenchyma

Early conspicuous signs of change in glands of specimens after emergence occur in the nuclei. A small number of cells may show them within the first day after the