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Fine Structure of Endocrine Hindgut Cells of a Lepidopteran, *Ostrinia nubilalis* (Hübn.)*

NANCY J. ALEXANDER and WOLF H. FAHRENBACH
Oregon Regional Primate Research Center, Beaverton, Oregon (USA)

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Summary. Cells of the hindgut of the European corn borer, *Ostrinia nubilalis*, have two functions, namely, ion transport and secretion of a hormone called proctodone. In this species, proctodone is an essential requisite to the prepupal molt in conjunction with brain hormone synthesis. Apical infoldings and their associated mitochondria form mitochondrial pumps for ion transport from the gut lumen to the hemocoel. The endocrine function of the cells is evidenced by rhythmical formation and discharge of inclusion bodies every 8 hours. These measure from 800 Å to 3 μ in diameter and vary in composition from bodies with whorled, myeloid content to inclusions with densely granular matrix. During the discharge phase, granular material appears in the basal infoldings and accumulates in large quantities underneath the basal lamina and in the hemocoelic clefts adjacent to the active cells.

Introduction

The cells of the hindgut of the European corn borer *Ostrinia nubilalis* secrete a hormone called proctodone (BECK and ALEXANDER, 1964a). The periodic release of proctodone enables the brain of a mature larva to secrete the brain hormone that initiates the pupal molt. Thus, if a mature larva is subjected to a posterior ligation, metamorphosis will be halted because the brain has not yet been exposed to proctodone. But an injection of an aqueous supernatant of hindgut homogenate ahead of the ligature will allow the pupal molt to proceed (BECK and ALEXANDER, 1964b). Similarly, if brains that had been previously exposed to proctodone are implanted into the anterior part of posteriorly ligated larvae, pupation will then be possible. Cells of the hindgut epithelium, when active, contain a secretory material which exhibits a pale green autofluorescence (BECK *et al.*, 1965b). This secretory material appears at the termination of larval feeding and is regularly released every 8 hours until just before pupation. The phase of the secretory cycle is set by the onset of darkness (BECK *et al.*, 1965a). A recent paper by HASSEMER and BECK (1968) describes cyclical changes in a variety of histochemical characteristics in the cells of the hindgut.

The morphology of the *Ostrinia* hindgut, especially of the proctodeal region called the anterior intestine, is in keeping with its primary functions of transport of ingesta and resorption of ions and water. The walls of this straight, unspecialized tube are longitudinally infolded from the pyloric valve to the rectum into six ridges, which consist of the intima and the epithelial cells. The ampullae of the Malpighian tubules enter the hindgut by way of one of the internal ridges. The

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surrounding muscle layers are scanty. Tracheoles are found scattered under the epithelial cells and occasionally in the folds. During the early larval stages, the epithelial cells are cuboidal with an apical striated border, typical of absorptive epithelia. In mature larvae the striated border becomes less prominent and the nuclei enlarge. This paper considers the ultrastructure of these hindgut cells during their phase of prepupal endocrine activity.

Materials and Methods

Larval corn borers were obtained from the U.S.D.A. Corn Borer Investigations Center, Ankeny, Iowa, and maintained on an artificial diet until the fifth larval instar. The larvae were kept under conditions of 12 hours light alternating with 12 hours darkness. This regimen serves to entrain the intrinsic 8-hour secretory rhythm, thereby providing an invariable coincidence of release of secretion with the onset of darkness. The hindguts of larvae, which had completed feeding preparatory to the pupal molt, were removed at various stages of the light cycle and fixed for 2 hours in a mixture containing 0.75% glutaraldehyde, 5% formalin, 3.5% sucrose and 0.05% calcium chloride in s-collidine buffer at pH 7.4. The tissue was washed in buffer, postfixed with 1% osmium tetroxide, and embedded in Araldite. Sections were stained with uranyl acetate or potassium permanganate, and lead citrate.

Results

The empty lumen of the hindgut is surrounded by a cuticular intima composed of 2 distinct layers. The outer layer, which is very dense and about 0.15 μ thick, probably represents the epi- and exocuticle; the less dense region, 4.5—7 μ thick, is the endocuticle. The underlying cells measure up to 20 μ in maximal extent (Fig. 1). The apical plasma membrane adheres closely to the cuticle and has hemidesmosomal attachments to it. Deeply infolded leaflets of the surface are intimately associated with elongated mitochondria (Fig. 2). The lateral boundaries of the cells, joined only for a few micra, form desmosomes in the apical portion. The basal surface of the epithelial cells is infolded into a complicated network of anastomosing clefts and canaliculi similar to those of mosquito larvae (COPELAND, 1964). At frequent intervals hemidesmosomes face the adjacent thick basal lamina. This acellular sheath has a width of 0.3 μ. The hindgut epithelium appears to lack innervation; peripheral axonal bundles innervate the muscularis.

The cytoplasm of the epithelial cells abounds with endoplasmic reticulum and free ribosomes. Golgi cisternae are inconspicuous and scarce. Mitochondria are principally associated with the apical infoldings. Numerous microtubules in the cytoplasm insert on hemidesmosomal plaques. The large nucleus is located basally and exhibits the coarsely clumped chromatin structure commonly found in insect nuclei. Some nuclei contain clusters of randomly oriented, short micotubules.

Two hours before the onset of darkness, the cells of the hindgut exhibit autofluorescence in the fresh state. Their cytoplasm, particularly the region basal to the nucleus, is filled with an abundance of membrane bounded bodies (Fig. 2). These inclusions have a diameter of 800 Å to 3.0 μ and commonly consist of concentric, whorled membranes (Fig. 3). Others have a densely granular matrix, usually with embedded membranous elements (Fig. 4). The inclusions retain their appearance after osmium fixation, indicating that glutaraldehyde

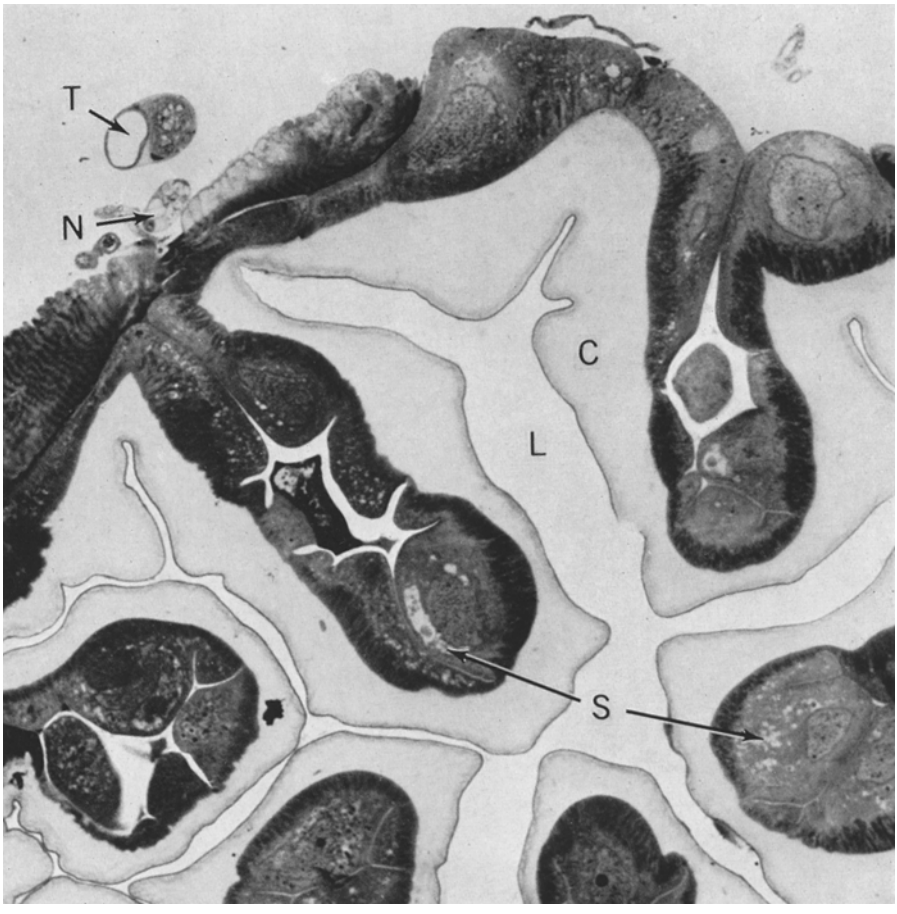

Fig. 1. Light micrograph of a 1 μ transverse section of the *Ostrinia* hindgut, fixed 2 hours before the onset of darkness. Prominent ridges of epithelial cells are covered by cuticle (*C*) and surrounded by an incomplete muscularis. The lumen of the intestine (*L*) is empty. Secretory material (*S*) is located in proximity to the nuclei. Note striated border of the epithelial cells. Nerve (*N*); tracheole (*T*). Stained with 1% toluidine blue and 1% borax. ×500

fixation or artifactual extraction does not produce these myeloid bodies, although preparative extraction undoubtedly affects their ultimate morphology.

Cells fixed at the onset of darkness are entering the discharge phase. The cytoplasm is still relatively full of inclusion bodies, but now numerous focal accumulations of a granular substance appear at all levels of the basal infoldings, often in dilatations of the canaliculi (Fig. 5). The material appears to move toward the basal surface of the cell and to be temporarily retained between the mouths of canaliculi and the adjacent basal lamina. This secretory activity is coincident with the appearance of similar opaque masses in the clefts between the hindgut cells, i.e., on the hemocoelic side of the basal lamina.

Forty-five minutes to one hour later, that is, after the start of darkness, the cells are almost empty of inclusion bodies (Fig. 6) and the lumina of the basal

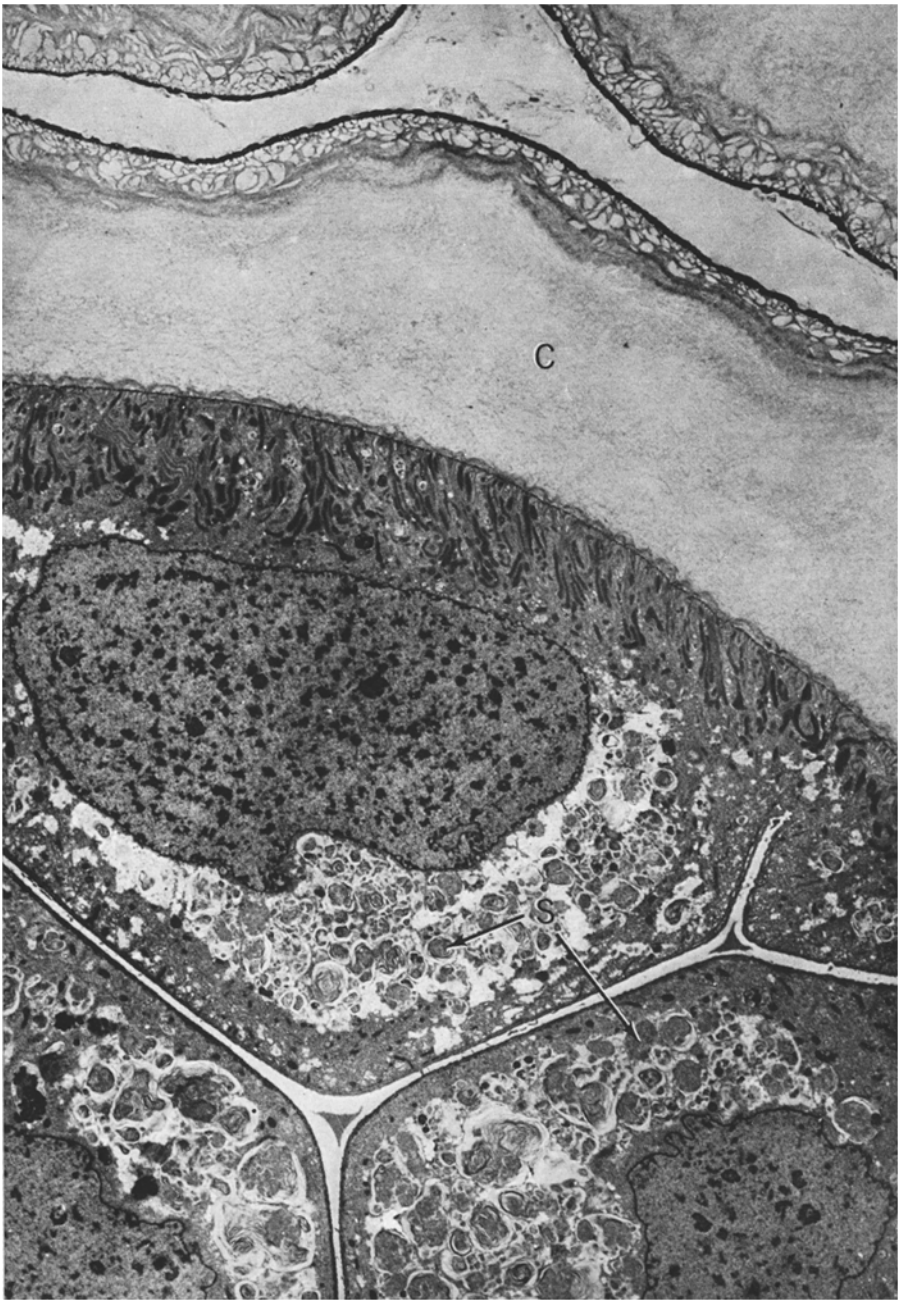

Fig. 2. Three adjacent cells fixed 2 hours prior to the onset of darkness, their cytoplasm filled with secretory inclusion bodies (*S*). The nucleus contains several nucleoli and dispersed chromatin clumps. The apical zone of the cells, underlying the cuticle (*C*), is elaborated into mitochondrial pumps. ×6,500

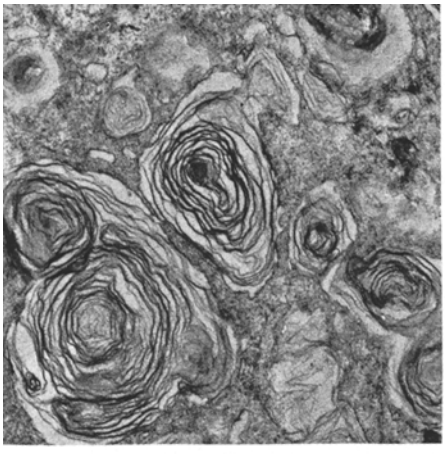

Fig. 3

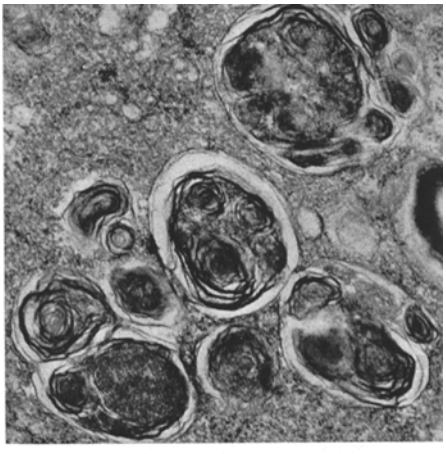

Fig. 4

Figs. 3 and 4. Examples of the polymorphic appearance of the secretory granules. Usually one type of inclusion dominates in a given cell. Fig. 3: ×20,000; Fig. 4: ×40,000

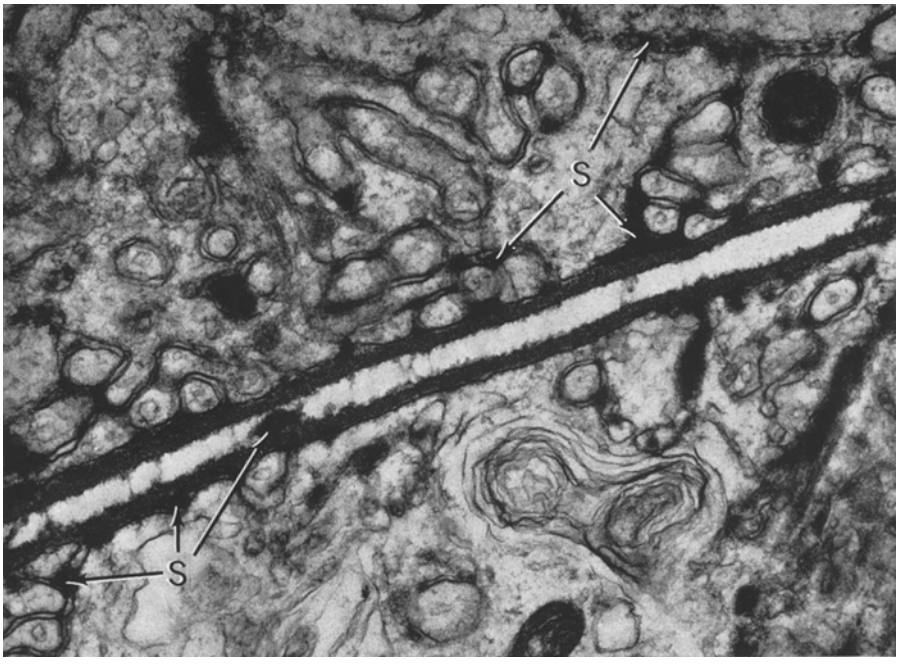

Fig. 5. Basal surfaces of two adjacent epithelial cells, fixed at the onset of darkness. Opaque secretory material (*S*) fills many of the basal infoldings in addition to lying on either side of the basal lamina. ×40,000

infoldings have cleared. The greater opacity of the cytoplasm is a function of the abundance of granular endoplasmic reticulum and free ribosomes, compacted at this stage without intercalated granules. This "empty" phase persists for only

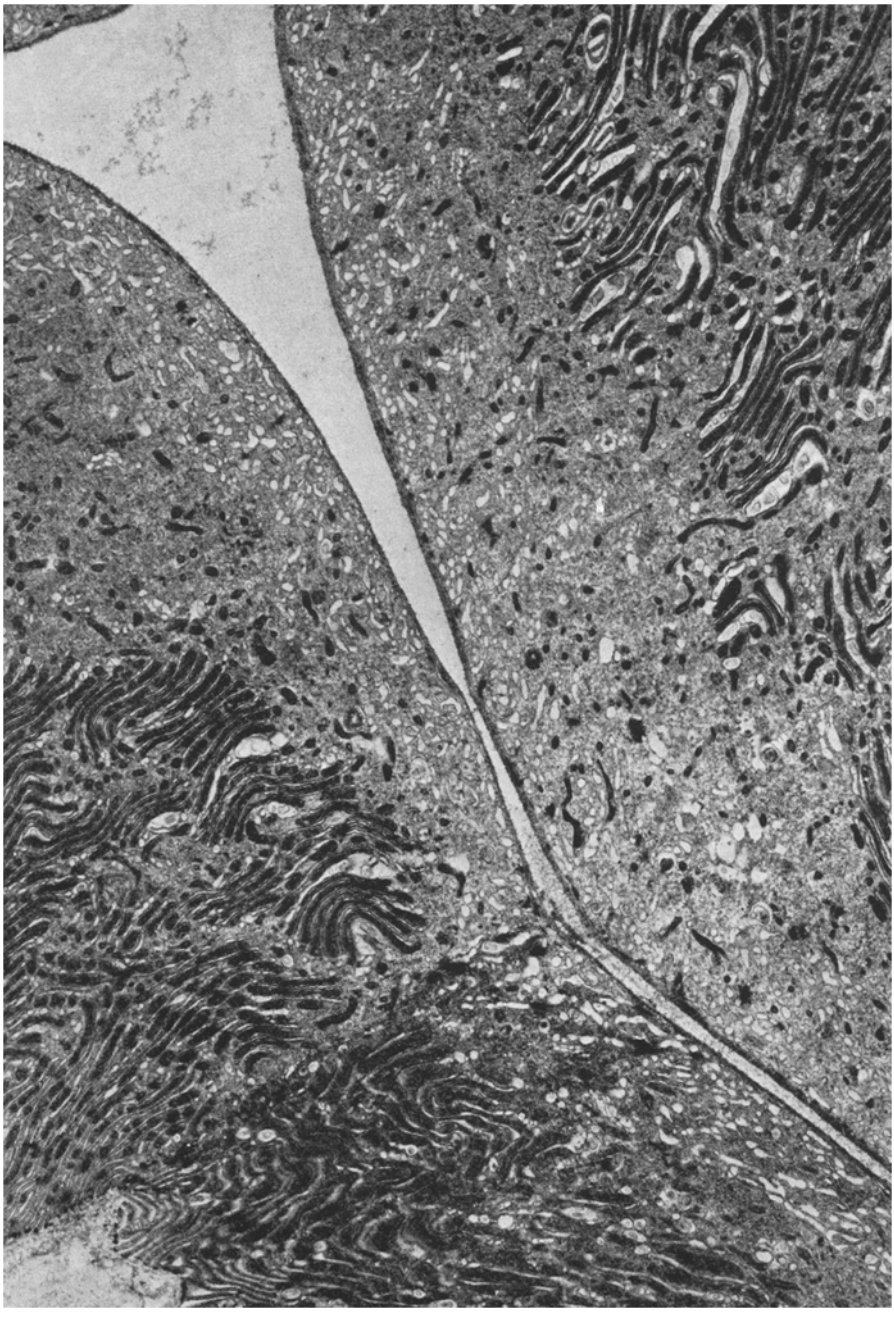

Fig. 6. Parts of two adjacent epithelial cells empty of secretory products, fixed one hour after the onset of darkness. Note empty basal infoldings. The cuticle is visible in the lower left corner. ×9,000

a brief period. About 2 hours after the onset of darkness new granules start appearing. Their formation occurs in close association with the cisternae of the endoplasmic reticulum rather than with the very rare Golgi elements. Newly forming inclusion bodies usually resemble those shown in Fig. 4, although some have a myeloid appearance (Fig. 3) from the start. The cells are at the "full" stage 4 to 5 hours after the start of granule production.

Discussion

Insects have two endocrine organs of a nonneural nature, the prothoracic gland and the corpus allatum. The parenchymal cells of the prothoracic gland, as reported for blattarian species, have sparse cytoplasmic membranes and no secretory granules (SCHARRER, 1964b, 1966). This gland secretes ecdysone, a steroid hormone of known biochemical structure (KARLSON, 1962). The cells of the corpus allatum, whose epithelioid nature is somewhat similar to that of the prothoracic gland, display remarkable changes in cytoplasmic volume in response to different states of activity and, when active, have a more conspicuous amount of granular and agranular reticulum (SCHARRER, 1964a; ODHIAMBO, 1966a, b). Corpus allatum cells secrete juvenile hormone, a methyl ester epoxy derivative of a polyunsaturated branched-chain fatty acid (RÖLLER, *et al.*, 1967). Like the prothoracic gland, the corpus allatum is devoid of any type of secretory granules. Both glands are supplied with some neurosecretory axons.

The secretory cells of the *Ostrinia* hindgut differ considerably from these two endocrine organs. The cells have two functions, namely ion transport and endocrine secretion in the prepupal phase. The morphology of the quiescent hindgut cells best demonstrates their duplicate functions. The apical infoldings and their associated mitochondria constitute mitochondrial pumps, which have been observed in various insect tissues engaged in ion transport (COPELAND, 1964; GUPTA and BERRIDGE, 1966). Basal infoldings are frequently found in the same cells (COPELAND, 1964) and in *Ostrinia* appear to subserve the endocrine secretory process as well. The discharge of material by way of basal infoldings and through the basal lamina is unusual, though not entirely unknown. A partly analogous form of endocrine discharge has been described in the corpus cardiacum of several insects (SCHARRER, 1963, 1968), in which release of neurosecretory substances into the hemocoel occurs through a thick stromal layer comparable to a basal lamina. Microtubules attached to apical and basal hemidesmosomes are commonly found in insect epithelia exposed to mechanical deformation (BASSOT and MARTOJA, 1965; NOIROT-TIMOTHÉE and NOIROT, 1966), especially in those cells underlying a cuticle.

The hindgut of *Ostrinia* also differs markedly from the above mentioned glands in that its secretory product is visible as inclusion bodies and that it cycles with a remarkably short, 8-hour period. The chemical composition of proctodone is unknown. A lipid component is suggested by the myeloid nature of the secretory granules as well as by their positive reaction to various stains for lipids (HASSEMER and BECK, 1968). The cyclical appearance and disappearance of these secretory granules argues against the possibility of the hormone being a

steroid, since steroid secretion in both vertebrates and invertebrates occurs without the intervention of secretory vesicles and is usually associated with conspicuous quantities of agranular reticulum (CHRISTENSEN and FAWCETT, 1961; CHRISTENSEN, 1965; SCHARRER, 1965). The granules are also PAS-positive and diastase resistant (HASSEMER and BECK, 1968).

The similarity of the inclusions to residual bodies as well as the positive reaction for acid phosphatase (HASSEMER and BECK, 1968) found in the cells of the hindgut, though not in register with the inclusion bodies, require comment. An appropriate comparative situation is provided by the cytoplasmic events in the degenerating prothoracic gland of cockroaches (SCHARRER, 1966). After the final molt of these insects, nuclei of the parenchymal cells of the prothoracic gland become pycnotic and, within one day, cytoplasmic contents are sequestered within large cytolysomes. Continuous degradation of their contents leads to the eventual appearance of myeloid residual bodies on the third day. During a comparable interval, the hindgut cells of *Ostrinia* cycle through the filled and depleted stage about 8 times without giving evidence of any degradative changes. Cytoplasmic components have never been observed within the inclusion bodies. Nuclei of the epithelial cells retain a morphology that is usually considered in keeping with a cell of high metabolic or synthetic activity. Even when cytolysomes subserve a metabolic storage function, their formation proceeds by gradual steps starting with cytoplasmic isolation bodies, as has been shown in the fat body of the larvae of *Calpodes ethlius* (LOCKE and COLLINS, 1965). Thus, an interpretation of the inclusions in *Ostrinia* hindgut cells as residual bodies is neither congruent with the time course encountered in the formation of similarly appearing residual bodies, i.e., structures representative of the terminal degradative step of the lysosomal sequence, nor in keeping with the fact that the larvae are apparently capable of persistent cycling up to 2.5 months under appropriate light conditions.

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Dr. N. J. ALEXANDER
Department of Electron Microscopy, Oregon Regional Primate Research Center
505 N.W. 185th Avenue
Beaverton, Oregon 97006/USA