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Structure and Function of Prothoracic Glands and Oenocytes in Embryos and Last Larval Instars of *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera)*

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Summary. 1. Active prothoracic glands and oenocytes of last larval stage are both characteristized by well-developed smooth and rough endoplasmic reticulum (ER). Prothoracic glands also show plasma membrane infoldings, but not oenocytes which contain a large number of pleomorphic vesicles.

2. The fine structure of embryonic oenocytes corresponds after blastokinesis with that of active larval and adult cells. Thus, an activity in the late embryo can be assumed. Embryonic prothoracic glands reveal no signs of activity: smooth and rough ER are absent. The subcellular structure resembles that of organ anlagen, i.e. not yet fully differentiated tissue. Hormone synthesis is not likely.

3. Ecdysone titer was determined throughout embryonic development and in mature adults. Although prothoracic glands break down during adult ecdysis, imagines contain in the *Calliphora*-bioassay active factors: females 0.9 CU/g and males 0.5 CU/g. As sites of synthesis the oenocytes are suggested.

4. A relatively high ecdysone titer of 7 CU/g is measured in newly deposited eggs. The hormone is presumably of maternal origin. Subsequent to blastokinesis the hormone content increases dramatically up to about 180 CU/g, apparently due to endocrine function of the embryo. Oenocytes are proposed as the source of ecdysone during late embryonic development.

5. The function of ecdysone during early and advanced embryogenesis, especially in view of "embryonic molts", is discussed.

Key words: Ecdysial glands – Ecdysone – Embryos, larvae – *Oncopeltus fasciatus* (Insecta, Heteroptera) – Ultrastructure.

Introduction

Few specific data exist concerning the occurrence of hormones in insect eggs and their possible role in embryogenesis and "embryonic molts". Previous studies on

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this matter have produced conflicting results (for summarized discussion, see Doane, 1973, and Dorn, 1976). There are indications that newly deposited eggs contain ecdysone of maternal origin (Boohar and Bucklin, 1963; Ohnishi et al., 1971; Kaplanis et al., 1973; Novák and Zambre, 1974; Mizuno and Ohnishi, 1975). However, it is also probable that the embryo itself can synthesize molting hormones (Kaplanis et al., 1975). The goal of the present study was to determine the ecdysone titer of eggs during development, and to ascertain the location of its synthesis. Therefore, parallel to the titer determination, electron microscopic observations of the embryonic prothoracic glands (PTGs) were undertaken. Since it has been shown that oenocytes (OENs) also produce a molting hormone (Romer et al., 1974) and are probably the only source in the egg laying female, they have been included in the investigations. Possible correlations between hormone level, function of ecdysial glands (PTGs and OENs) and developmental processes in the egg of *Oncopeltus* will be pointed out.

Since ecdysone is not histologically demonstrable, it is impossible to judge from electron micrographs if PTGs or OENs are synthesizing hormones. To interpret the findings on the embryo, where it is uncertain whether hormone synthesis exists, it was necessary to compare glands which are already known to be productive. Feir and Winkler (1969) reported titer measurements of the last larval stage of *Oncopeltus fasciatus*, where a constant level of 1.7 CU/g ecdysone was found from day one to day six. The present studies on the fine structure revealed during the middle third of the last larval stage a rather constant appearance of PTGs and OENs with respect to development of the various cell organelles. The following description of the active glands refers to this developmental stage. Imagines were also included in the case of OENs.

Material and Methods

Eggs of *Oncopeltus* were collected 12 or 14 h after deposition and exposed to a temperature of 30° C. Under these conditions embryogenesis lasts 123 ± 3 h. Eggs of different developmental stages were either fixed for ultrastructural studies or preserved in methanol (60%) at −20° C for titer determination.

For electron microscopic investigations the embryo was dissected from the chorion, cut in two halves (between thorax and abdomen) and immersed in fixative. Fixation consisted of 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, with addition of 0.2 M sucrose, followed by 1% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.4. Only Fig. 8b is derived from a specimen fixed solely in osmium tetroxide. After dehydration the specimens were embedded in araldite. Most sections were double-stained in uranyl acetate and lead citrate.

Molting hormones were extracted either according to the method of Karlson and Shaaya (1964) or as follows. After triple extraction with methanol, supernatants were collected, evaporated to a few ml and extracted four times with water saturated butanol. The collected butanolic phases were evaporated until dry. The residue was diluted in small amounts of 100% ethanol, and applied to a 0.5 mm thin layer plate of silica gel 60 F 254 (Merck, Darmstadt), which was developed with dichlormethane-methanol (80:20), α-ecdysone (Fluka, Neu-Ulm) and β-ecdysone (Rohto Pharmaceuticals, Osaka) served as control substances. The hormone-containing bands were scraped off and the hormone eluted from silica gel. Extracts, received from both methods, were subjected to the *Calliphora*-test. A plotting was made in accordance with Adelung and Karlson (1969).

The degree of polyploidy was determined on 10 µm thick sections of embryos, whole mount preparations of PTGs and smears of OENs containing fat bodies. The specimens were fixed in 10% neutral formol, hydrolized for 10 minutes in 1 N HCl, at 60° C, and subjected to Feulgen's nuclear reaction. Single nuclei were measured with the cytophotometer MPE Leitz, Wetzlar (whole-nucleus-

method). Measuring diaphragms, together with lenses of differing magnification, were adjusted to the size of the nucleus being tested. Diploid nuclei of ganglion cells and haploid spermatids served as standards.

Results

1. Cytophysiological Studies on the Development of PTGs and OENs

a) Embryonic Growth. PTGs and the first OENs differentiate from the ectoderm at approximately the same time, shortly before blastokinesis.

After blastokinesis the PTGs occupy their final position, between labium and salivary glands, and are attached by a ligament to the trachea. The single cell layered, band-shaped gland shows two rows of alternately arranged nuclei. Mitotic activity soon ceases, but the volume of nuclei and cytoplasm increases considerably. Nevertheless, all nuclei remain diploid until hatching (Fig. 1).

The OENs differentiate successively until hatching and immediately become polyploid (Fig. 1). They migrate into the fat body where they are solitary or in small groups and become relatively voluminous. After polyploidization the OENs no longer divide.

b) Postembryonic Growth. Tissue growth is considerably different in PTGs and OENs. PTGs have the same number of nuclei toward the end of the last larval stage as in the older embryo, but a pronounced enlargement of the gland occurs, which is due to cell enlargement and polyploidization (Figs. 1, 2). On the day before final ecdysis signs of degeneration are clearly visible, such as described for other species (Wigglesworth, 1955; Scharrer, 1966; Osinchak, 1966). Most nuclei of glands from freshly hatched adults are pyknotic and fuse with others. The cytoplasm becomes thin and thread-like. The areas near labium and salivary gland remain intact for the longest period. Two days later the glands disappear completely. Whereas the cells of the PTGs can obviously recover after a larval activity cycle, this apparently is not the case for the OENs. Therefore, OENs must continuously be produced from the epidermis during larval and adult stages. Figs. 1 and 2 show further that neither nuclear volume nor DNA content increase during postembryonic development. Therefore, considering the growth of the animal, the necessity of a permanent differentiation of new OENs arises.

2. Fine Structure of PTGs

a) PTGs of the Last Larval Stage. A basement membrane of 1000–2000 Å thickness loosely covers the whole gland. Cell surfaces show deep invaginations, which are not lined by the basement membrane (Figs. 3a, 4a). Outermost tips of neighboring infoldings often form desmosome-like structures (Fig. 4b). Sites of developing coated vesicles can be observed (Fig. 4b). Lamellated bodies found extracellularly in invaginations may represent extruded remnants of autophagic vacuoles.

In contrast to the illustrations of Herman (1967), the ER in the active gland is very prominent, this being true for smooth ER, which occupies the largest part

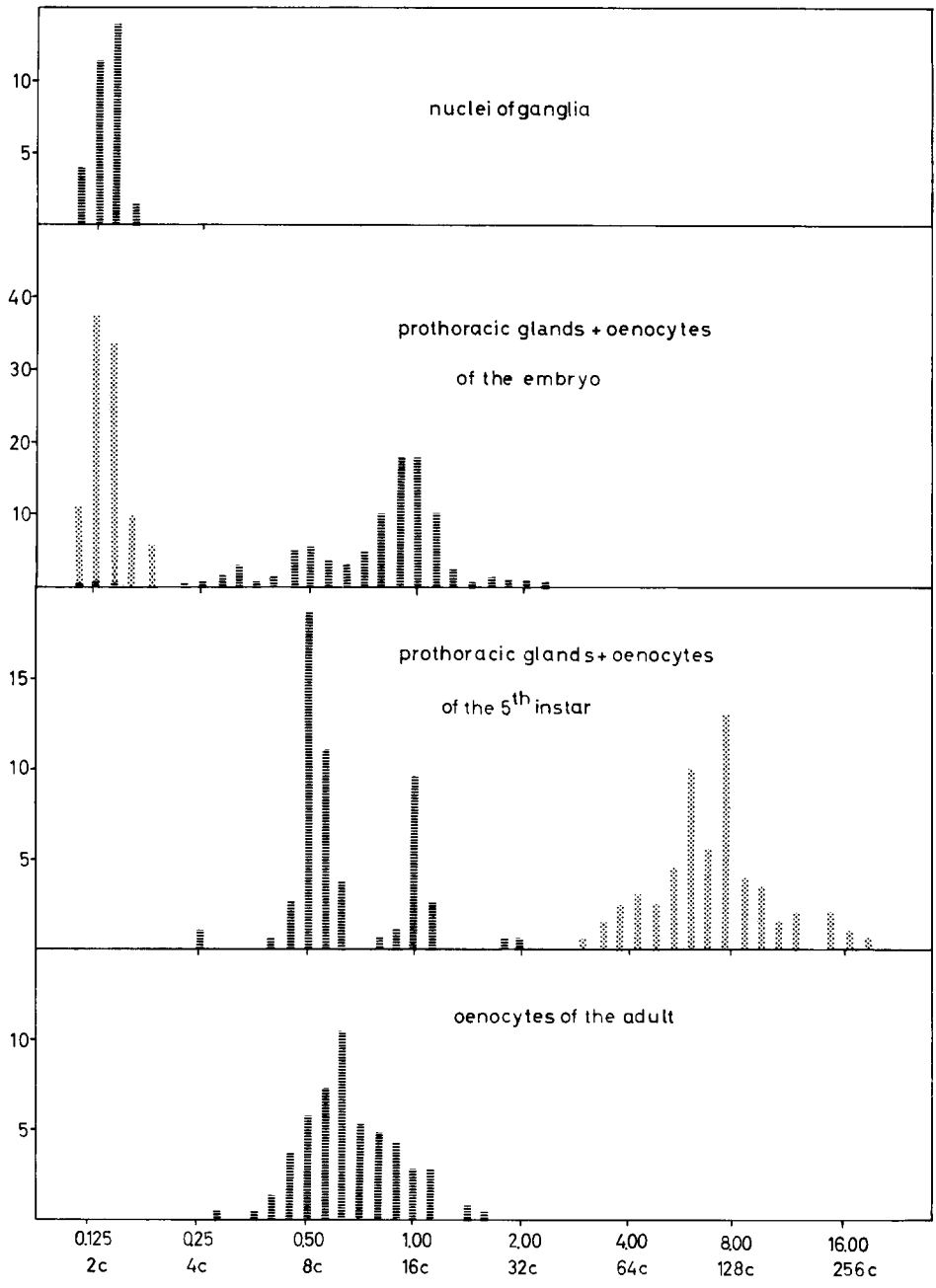


Fig. 1. DNA content of nuclei from ganglia, prothoracic glands (PTGs), and oenocytes (OENs) during embryogenesis, 5th larval stage and adult life. Ordinate: number of measured nuclei (during embryognesis expressed in per cent). Abscissa: extinction in working units (based on nuclei of ganglia) and estimated degrees of polyploidy. PTGs dotted; OENs striped

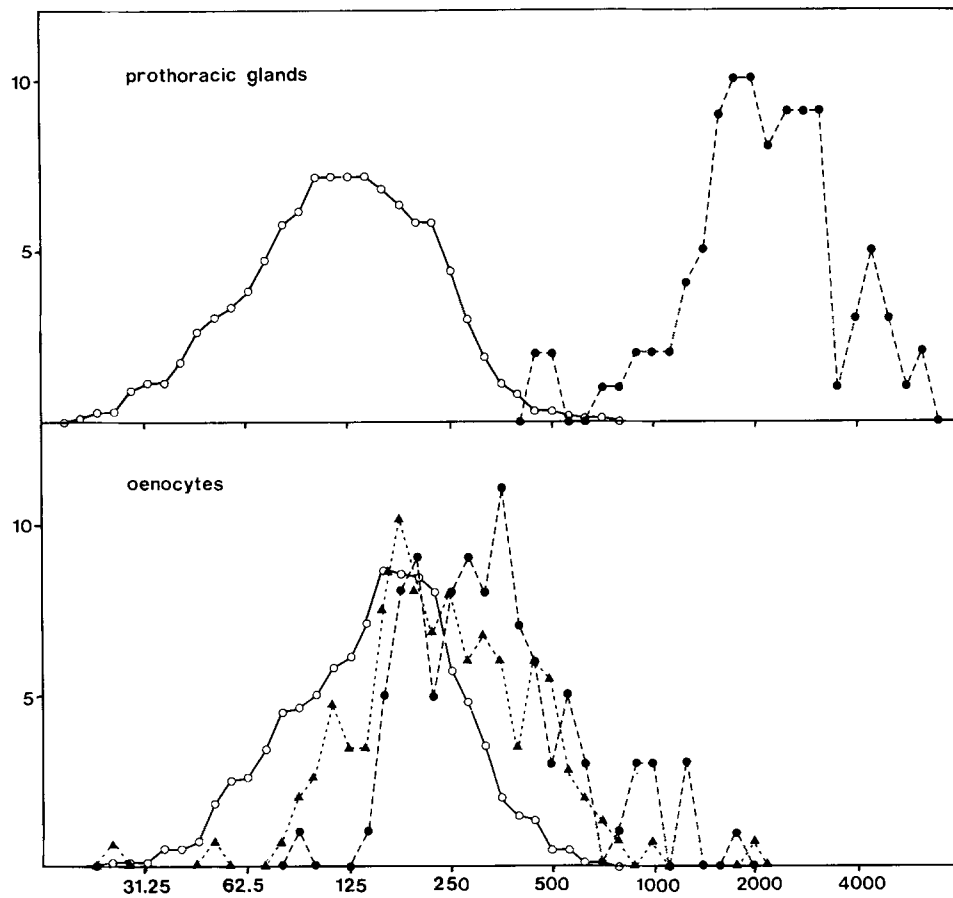


Fig. 2. Volume of nuclei from PTGs and OENs during embryogenesis (○ ——— ○), 5th larval stage (● – – – ●), and adult life (▲------▲). Ordinate: measured nuclei in per cent; abscissa: volume in μm^3

of the cytoplasm, as well as rough ER, which is oriented parallel to the nucleus (Fig. 3b).

Golgi complexes are well-developed (Fig. 3b). Their cisternae contain an electron dense substance especially in newly molted larvae. Lysosomal bodies are present but not numerous (Fig. 3b). Mitochondria are abundant (Figs. 3b, 4a). Elongated profiles show longitudinally running cristae (Fig. 4c). Microbodies are primarily found between plasma membrane infoldings (Fig. 4b) and are associated with large membrane bounded electron dense granules. Microtubules are rare and are mostly found near the surface of the gland.

Single rosettes and small clusters of glycogen are always present. Romer (1971a) pointed out the close relationship between glycogen and lipids in the PTGs of *Tenebrio*. In *Oncopeltus* no lipids were found intracellularly. However, similar to *Tenebrio*, lipid droplets are observed attached to the basement membrane. Whether or not they are absorbed by the gland cells remains unclear. The nuclei have

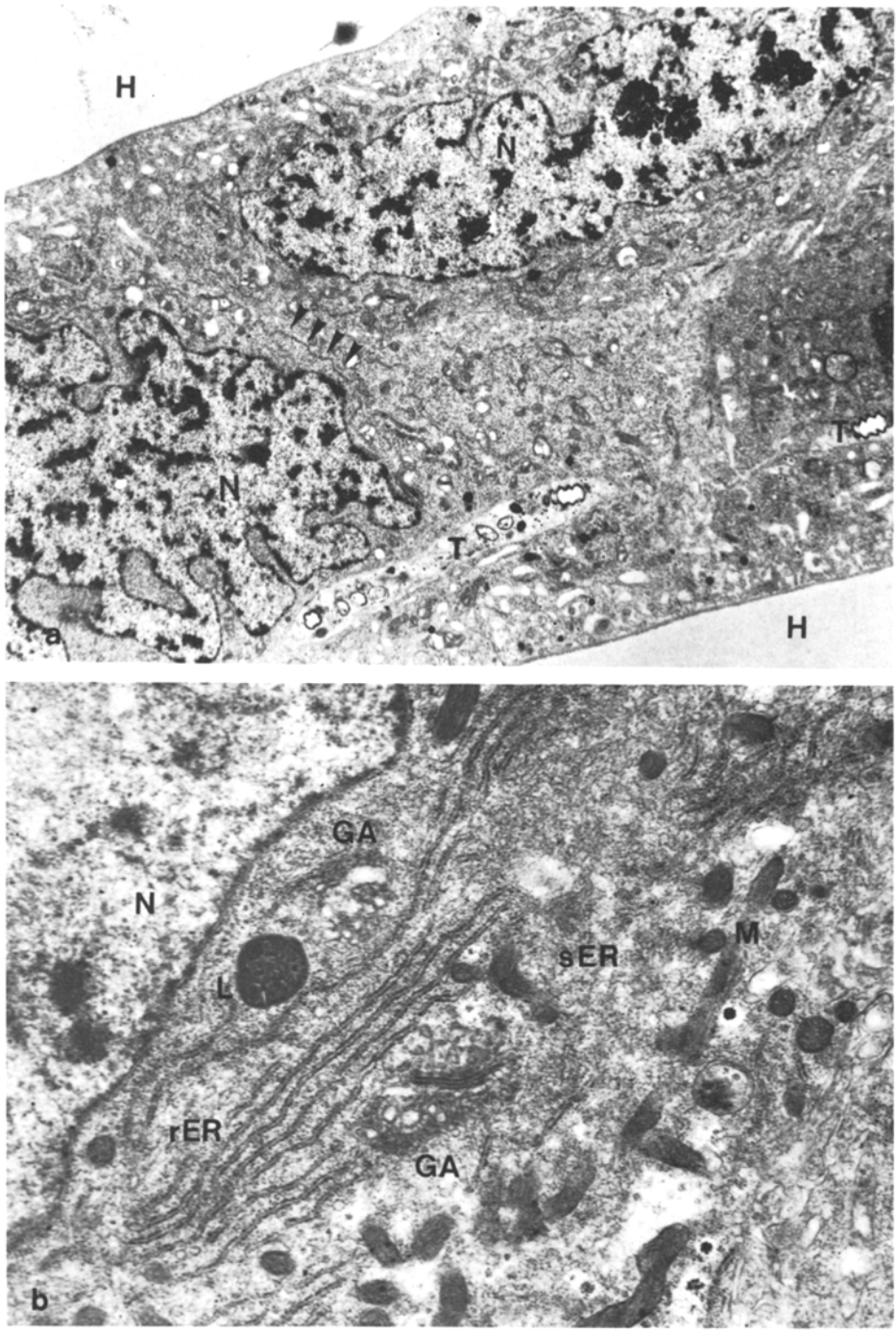


Fig. 3a. Part of PTG from young 5th larval instar; *H* hemocoel; *N* nucleus; *T* tracheole; *arrows* point to desmosome. ×4000. **b** Active cell from PTG; smooth and rough ER (*sER*, *rER*) well-developed; *GA* Golgi apparatus; *M* mitochondrion; *N* nucleus. ×20,000

irregular outlines with prominent protrusions (Fig. 3a). They possess several large nucleoli and round electron dense granules.

Toward the end of larval life the PTG cells change markedly. The plasma membrane infoldings disappear and extensive, irregular, membrane bounded vacuoles take their place (Fig. 4d). Rough ER disintegrates into single strands, and smooth ER is only present in isolated vesicles (Fig. 4d). Isolation bodies appear and are transformed into autophagic vacuoles.

The innervation of PTGs does not seem to be uniform in insects (Romer, 1971a). In some species nerves extend into the gland. In *Oncopeltus* neither "conventional" nor neurosecretory axons are observed. Tracheoles are noted frequently.

b) PTGs of the Embryo. Gland cells of an embryo close to hatching are illustrated in Fig. 5a. They contact each other loosely by means of cell projections. The complicated cell periphery (Fig. 5b) resembles the cell membrane infoldings of the active gland to some extent. However, both do not seem to express a similar functional state, since the labyrinth of the embryonic gland cells is much more irregular and lined by the basement membrane. The intercellular system often presents membranous material. Electron lucent vacuoles in the peripheral cytoplasm (Fig.5b) are characteristic of cells of organ anlagen generally.

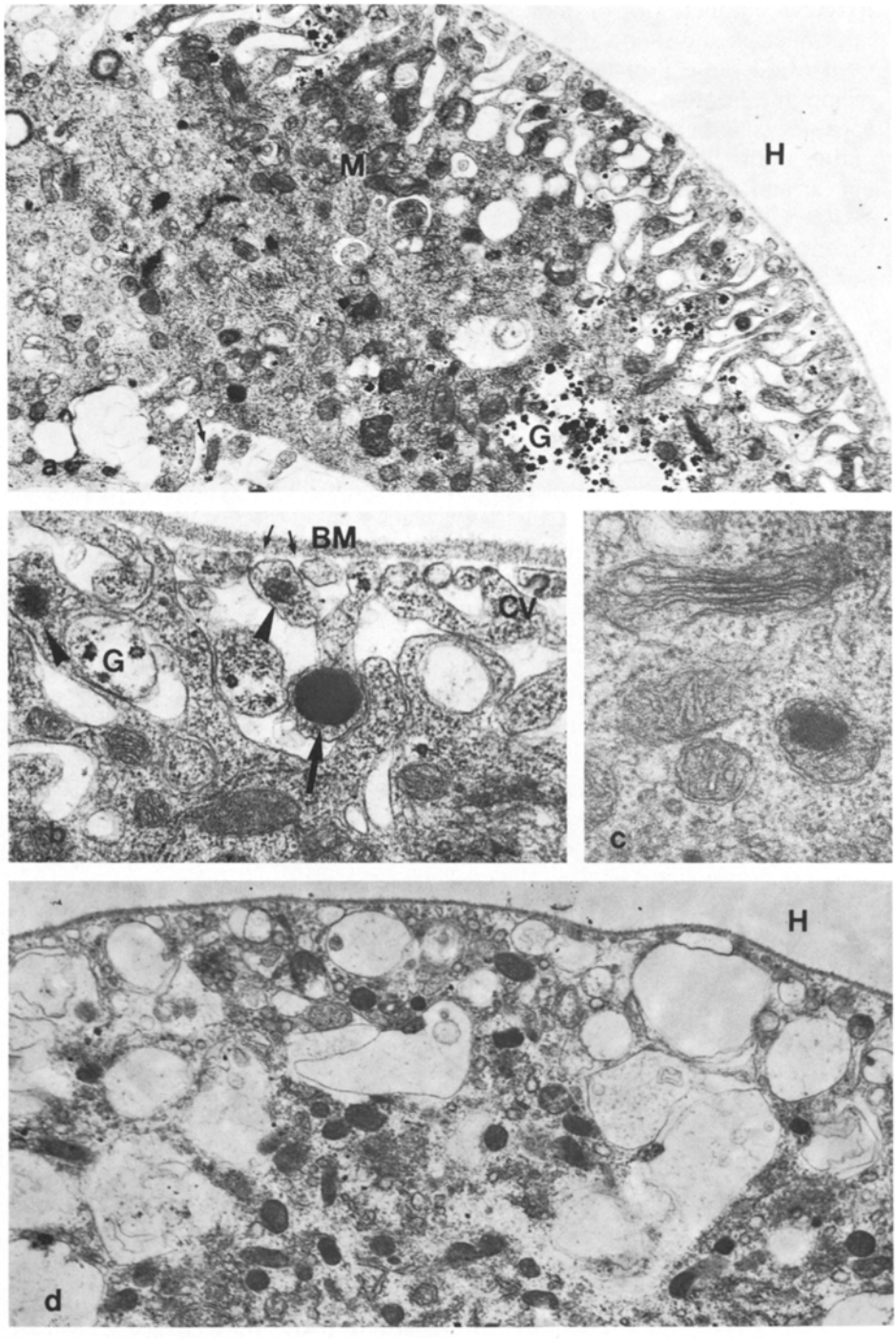
The cytoplasm is relatively scant and densely populated with free ribosomes (Fig. 6). Only rough ER is present in single strands. Golgi complexes are inconspicuous; lysosomes are rare. Mitochondria of the crista type have predominantly round profiles. Microbodies are not found. Rosettes of glycogen are seen in rare cases, lipids never. The abundance of pigment granules, which is somewhat surprising, indicates the ectodermal origin of the PTG. It should be mentioned that the corpus allatum cells also possess these pigment granules during this stage of development, although juvenile hormone (JH) is already synthesized (Dorn, 1975a). OENs, however, also derivates of the ectoderm, never form pigment granules. The nuclei are round or oval, and their envelopes show many indentations (Fig. 5a). Nucleoli are single in the nucleus.

On the whole, the PTGs offer no signs of a specific activity (i.e. production of ecdysone) before hatching in comparison with glands of the fifth larval stage.

3. Fine Structure of OENs

a) OENs of the Fifth Larval Instar. The outline of OENs is determined by the surrounding fat body, the lipid droplets of which often strongly dent the plasma membrane. A basement membrane is only then present, when the cell comes in contact with the body cavity (Fig. 9a). Plasma membrane infoldings are never developed.

OENs in the process of differentiation are characterized by a poorly developed ER and a high concentration of free ribosomes. In active OENs free ribosomes are restricted to certain areas, whereas smooth ER occupies the main part of the cytoplasm (Fig. 7b). Rough ER forms characteristic short stacks, which are irregularly scattered. Rough ER is not as well-developed in OENs as in the PTGs, whereas the content of smooth ER is similar in both.


M
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Mitochondria are numerous, relatively large and often round or elongated and irregular in profile. In general the matrix is rather electron dense (Fig. 7a). Mitochondria are normally of the crista type, although the tubulus type was also found (Fig. 8b). Since the latter was very rare a correlation between mitochondrial structure and functional state was not possible. Golgi complexes could not be clearly identified. The appearance of the active gland is characterized by populations of pleomorphic vesicles (Fig. 7a). Some of them may contain readily soluble lipids (Dorn, 1972), others may represent lysosomes and autophagic vacuoles. The OENs contain more glycogen than the PTG cells (Fig. 7a, b). Microbodies are rather frequent (Fig. 7b) and have round to elongated profiles. Large, round, electron dense granules are present (Fig. 7b). The nuclei are round and never irregular (Fig. 8a). Several large nucleoli are the rule; accessory nucleoli occur.

b) OENs of the Embryo. An OEN which has just differentiated and still lies in the hemocoel is presented in Fig. 8a. A thin (100 Å) basement membrane is already formed. Free ribosomes are scattered throughout the cytoplasm. Rough and smooth ER are poorly developed. Golgi complexes are extremely rare. The mitochondria are large in comparison with those of the active gland cell, but not as numerous. It should be pointed out that centrioles were observed in embryonic OENs (Fig. 8a).

After migration of the OENs into the fat body, pronounced changes in cellular structure occur. The smooth ER occupies almost the whole cytoplasm (Fig. 8b). It consists mainly of vesicles, which often show dilatations of variable extent. Vacuoles, electron lucent or with a flocculent content, in the cell cortex are probably derived from pinched off vesicles (Fig. 9a) and released into the hemocoel. Omega-shaped indentations of the plasma membrane were observed. It can not be ruled out that the vacuoles indicate pinocytotic activity. They can lie singularly or in a row with open connections to each other like a string of pearls. They only occur in embryos and adults, not in larvae. Microbodies are numerous, more so than in the fifth larval stage. Their profiles are round, dumbbell or horseshoe-shaped, sometimes even branched (Fig. 8b). Due to their shape and other structural aspects, a derivation from smooth ER can be considered. Pleomorphic vesicles, as described for the cells during the last larval stage (Fig. 7a), are also present in the embryonic OENs and apparently undergo similar processes. Glycogen is rare. Anchorage of OENs to fat body cells by desmosomes is occasionally seen in the embryo (Fig. 9b), but not in larvae or imagines.

Fig. 4a. Apical zone of an active PTG cell with numerous plasma membrane infoldings and mitochondria (*M*); *G* depots of glycogen; *H* hemocoel. × 12,000. **b** PTG cell; plasma membrane infoldings form short desmosome-like structures (*small arrows*) close to basement membrane (*BM*); profiles of coated vesicles (*CV*), glycogen (*G*), microbodies (*arrow heads*) and larger electron dense bodies (*large arrow*) in cytoplasm between infoldings. × 20,000. **c** Mitochondria of PTG cells; one with electron dense inclusion, another with longitudinally running cristae. × 40,000. **d** Apical zone of a PTG cell from an old 5th larval instar; plasma membrane infoldings are no longer present; irregular electron lucent vacuoles are numerous; mitochondria are frequent; short singular profiles of rough ER and vesicles of smooth ER can be recognized; *H* hemocoel. × 12,000

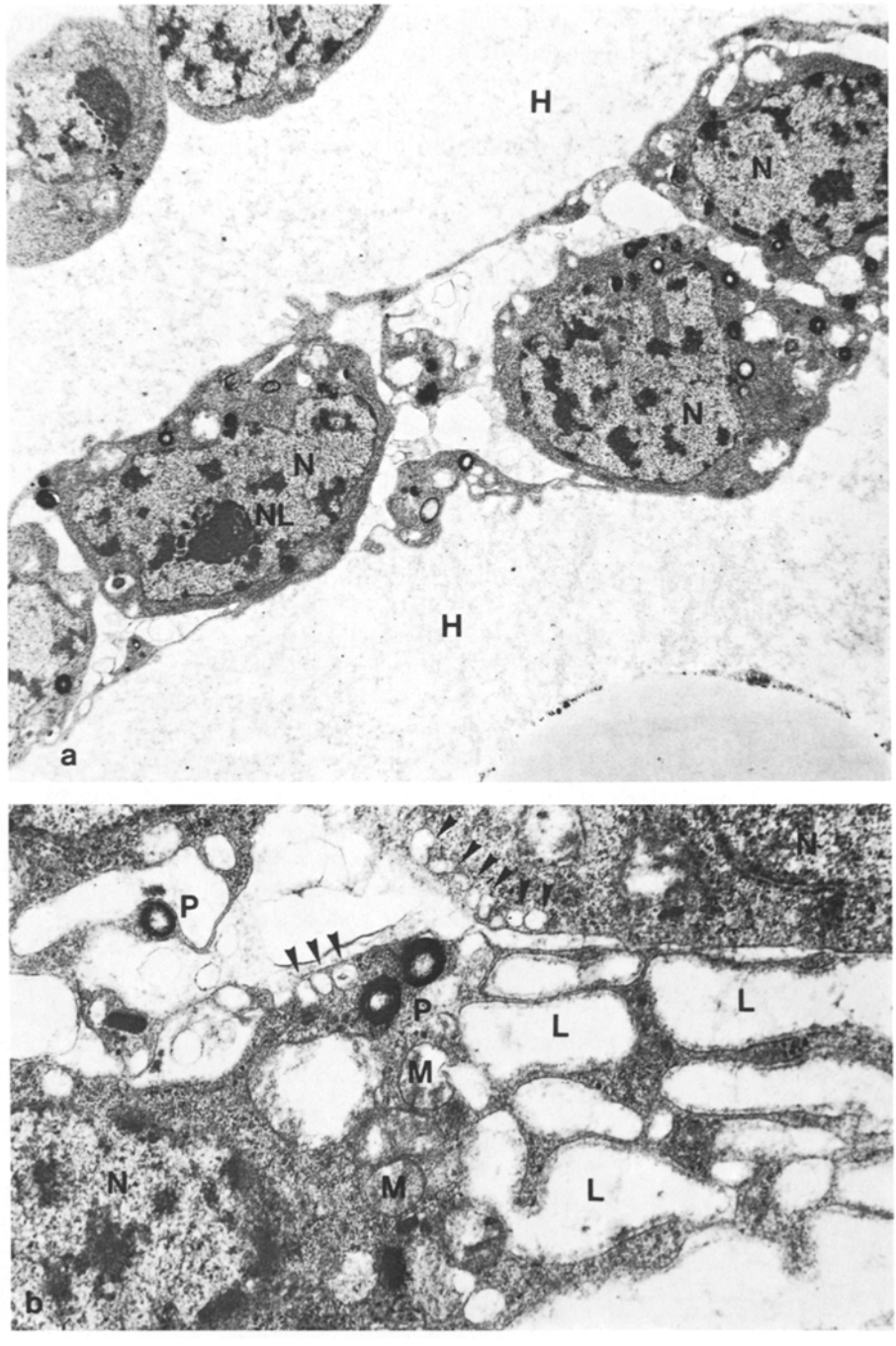

Fig. 5a. Part of PTG from an embryo 110 h old; cells are in loose contact, mostly by cell protrusions; *N* nucleus of PTG cells; *NL* nucleolus. × 6000. **b** PTG cells from 110 h old embryo showing labyrinth-like formation of cell periphery; invaginations (*L*) lined by basement membrane; *arrows* point to electron lucent vesicles near plasma membrane; *M* mitochondrion; *N* nucleus; *P* pigment granule. × 20,000

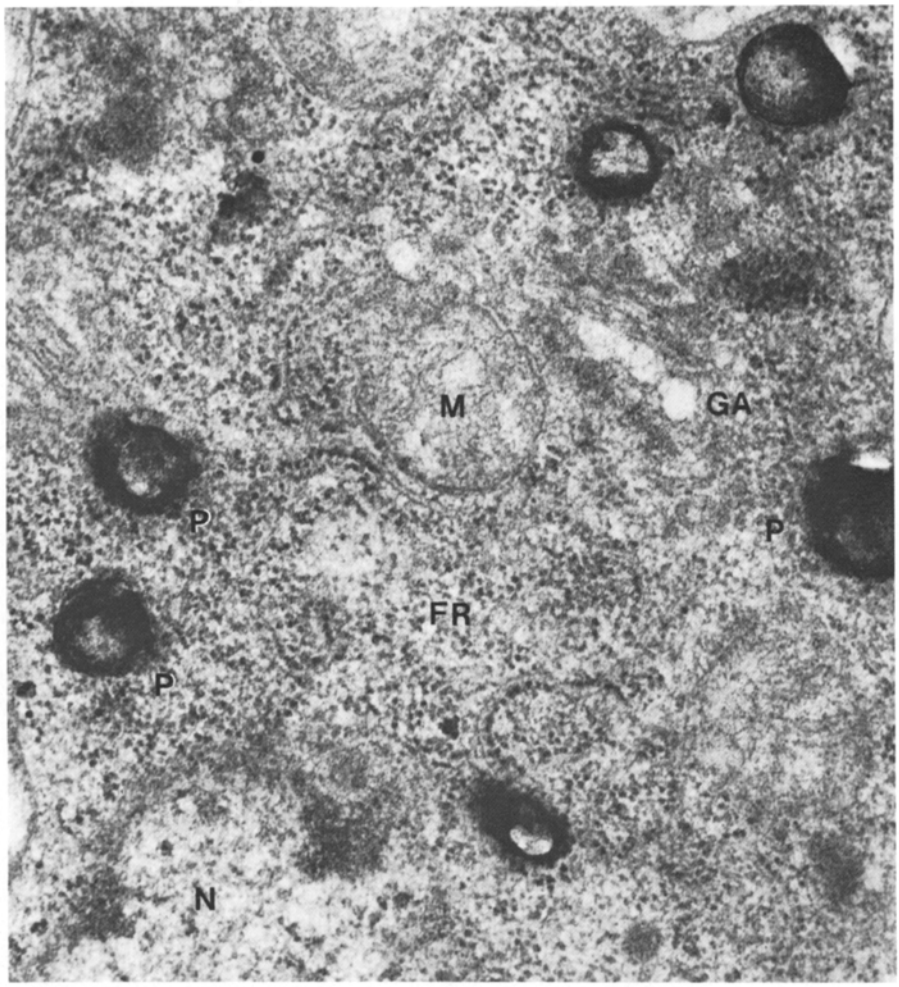

Fig. 6. PTG cell from an 110 h old embryo; free ribosomes (*FR*) are abundant; *GA* Golgi apparatus; *M* mitochondrion; *N* nucleus; *P* pigment granule. × 60,000

4. Determination of Ecdysone Titer

As already shown, the PTGs break down during adult molt and already one day later they are no longer detectable. The males and females used for determination of hormone titer were between 20 and 40 days old. Nevertheless, an active factor(s) could be demonstrated in the *Calliphora*-bioassay (Table 1). Furthermore, females show almost twice as much CU per gram body weight as males. The values from our investigation lie somewhat higher than those given by Feir and Winkler (1969) for young adults of *Oncopeltus*, but they are still quite comparable. According to the above-mentioned authors no ecdysone is traceable in newly hatched imagines, while a titer from 1.7 CU/g remains constant from the first to the 14th day of

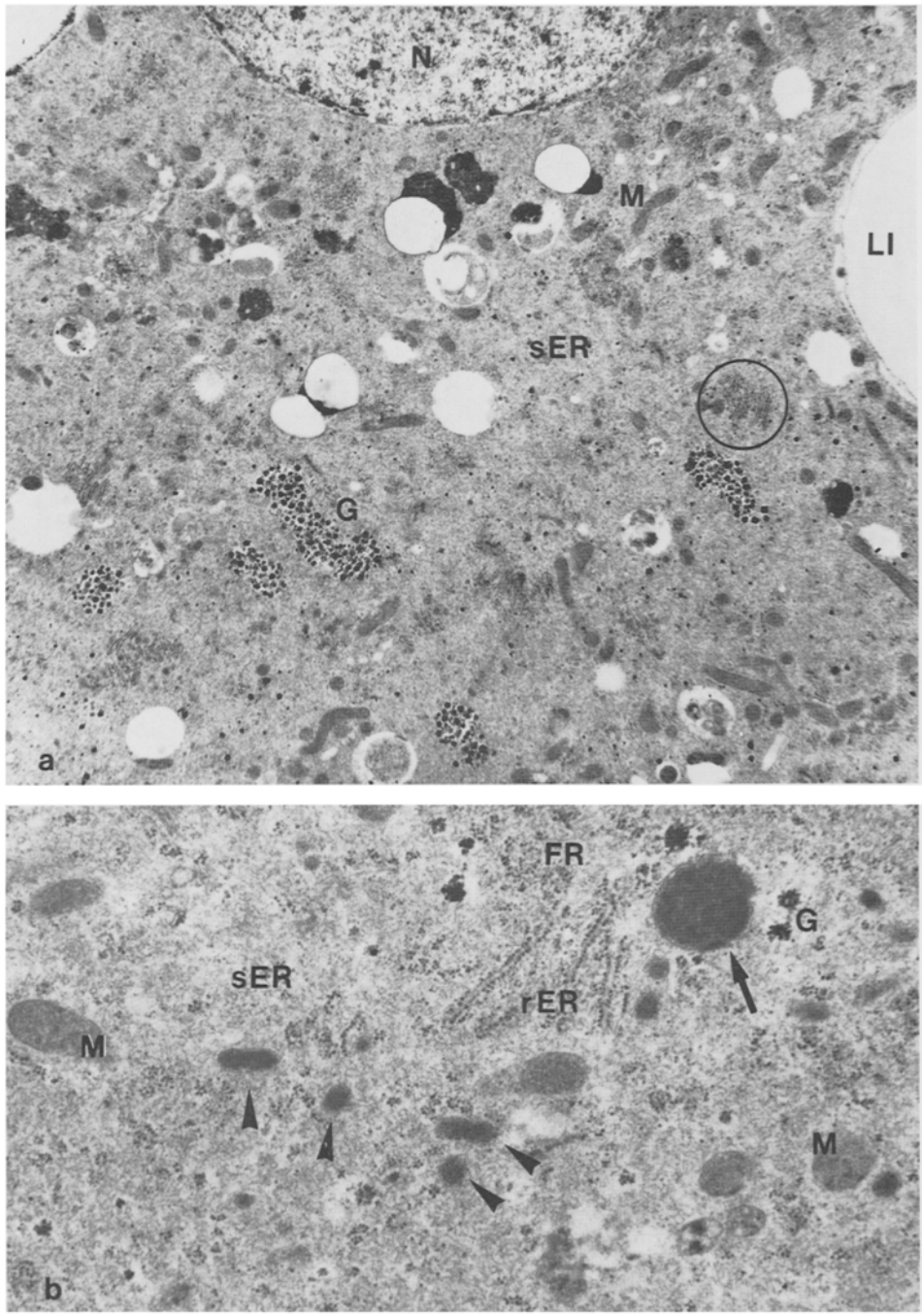

Fig. 7a and b. Active OENs from 5th larval instar. **a** Cell showing abundance of pleomorphic vesicles; rough ER (*encircled*) is arranged in short stacks; *sER* smooth ER; *G* glycogen; *M* mitochondrion; *N* nucleus; *LI* lipid droplet from fat body cell. ×6000. **b** *Arrow heads* point to profiles of several microbodies; *arrow* points to a large electron dense body; *sER* and *rER* smooth and rough ER; *G* rosette of glycogen; *M* mitochondrion. ×20,000

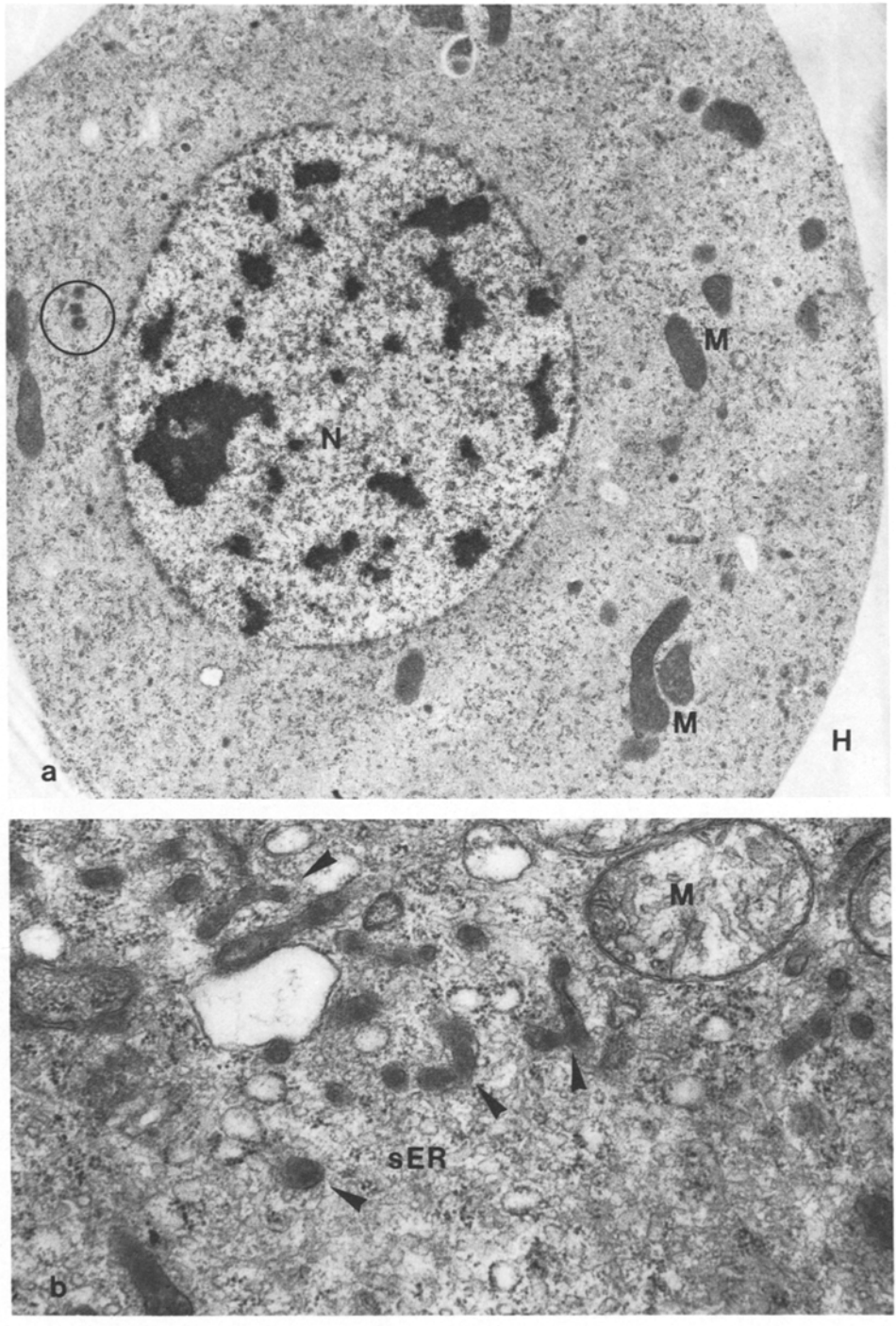

Fig. 8a. Newly differentiated OEN from an embryo; *N* nucleus; *M* mitochondrion; centrioles *encircled*; *H* hemocoel. ×7000. **b** Presumably active OEN from an embryo several hours before hatching; smooth ER (*sER*); microbodies (*arrow heads*) (connection to sER?); *FR* free ribosomes; *M* mitochondrion of the tubulus type. ×30,000

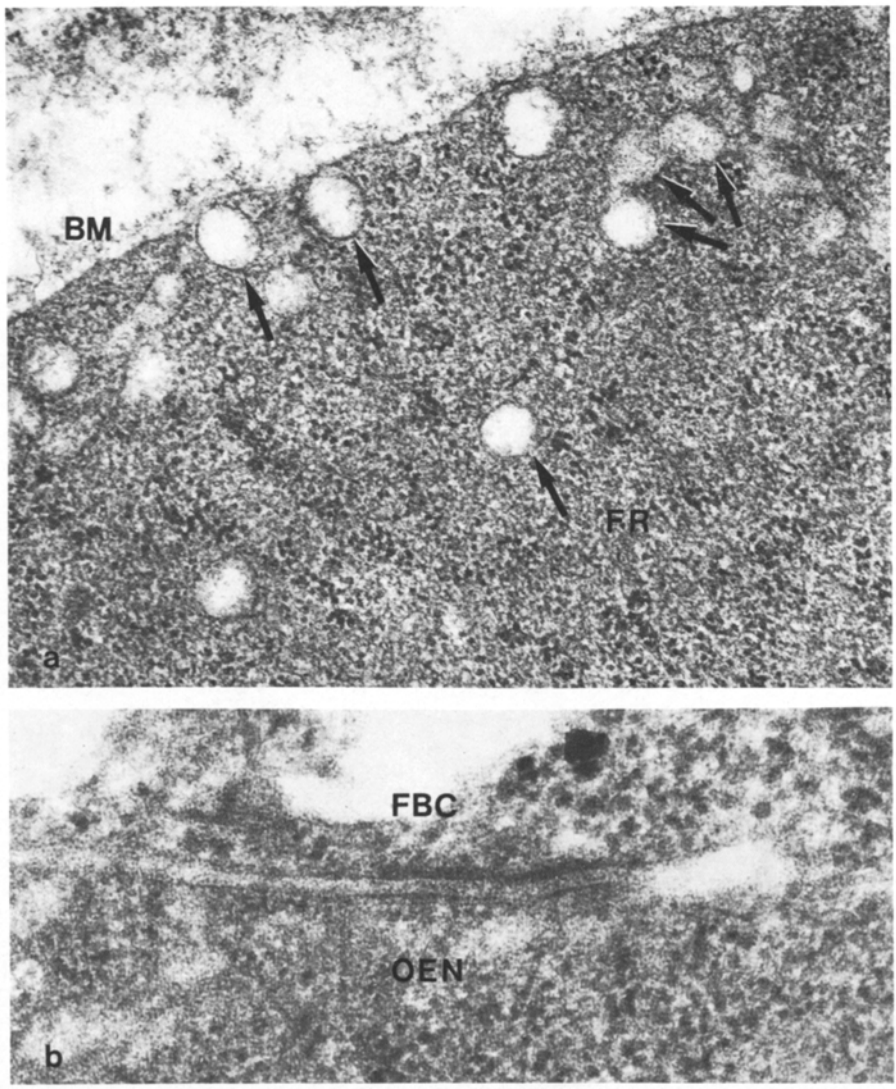

Fig. 9a. Part of an OEN from the embryo (110 h old); *arrows* point to electron lucent vacuoles (some with flocculent content); *FR* free ribosomes; *BM* basement membrane. × 60,000. **b** Desmosome between OEN and fat body cell (*FBC*). × 88,000

adult life. This makes it probable that an approximately constant molting hormone titer is maintained throughout the entire reproductive span of life in females and males.

Table 1 shows that a relatively high molting hormone titer is already present in freshly deposited eggs and amounts to a multiple of that in mature females. The values remain about the same until day four of embryonic development, when they then rise dramatically and reach a maximum at day five. The highest value

Table 1. *Calliphora*-bioassay of ecdysone extracts of embryos and adults of *Oncopeltus*

Hours after egg deposition	eggs/extract (g)	Mean weight of one egg (mg)	CU/egg	CU/g	Mean CU/g
0– 24	11.0	0.2681	0.0016	5.8	7.0
	3.0		0.0022	8.3	
24– 48	5.5	0.2574	0.0008	2.9	2.9
48– 72	3.5	0.2438	0.0026	10.8	12.9
	3.0		0.0037	15.0	
72– 96	3.0	0.2275	0.0017	7.5	7.5
90–102	12.0	0.2173	0.0098	45.0	45.0
96–120	2.0	0.2074	0.0770	371.0	178.1
	3.0		0.0280	135.0	
	2.5		0.0059	28.3	
120–144*	4.0	0.1751	0.0035	19.7	19.7
Days after adult moult	adults/ extract (g)	Mean weight of one adult (mg)	CU/adult	CU/g	Mean CU/g
♀♀ 30–40	15.0	78.1	0.070	0.9	0.9
♂♂ 30–40	13.0	46.0	0.023	0.5	0.5

* Most specimens already hatched

measured shows for one egg the same amount of active substance (0.07 CU) as in a whole female. During hatching the hormone titer declines almost to the starting quantity. The peak of the curve coincides with the apolysis of the second embryonic cuticle and the deposition of the third cuticle, which represents the definitive first larval cuticle.

Discussion

1. Development and Growth of Prothoracic Glands and Oenocytes

Due to their structure it was taken into consideration that the OENs, simulateneously or even prior to the PTGs, synthesize molting hormone (Delachambre, 1966; Locke, 1969; Romer, 1972, 1974, 1975). In the meantime in vitro studies have proven the production of α-ecdysone in PTGs (Romer et al., 1974 and others). α-ecdysone is believed to function as a "prohormone" (Moriyama et al., 1970), which is transformed in other tissues into the active compound β-ecdysone (Marks, 1973; Andries and Mounze, 1975). The OENs were found to be such a tissue (Romer et al., 1974; Romer, 1973). Agui (1974) demonstrated a "joint action of prothoracic

glands and oenocytes on cultivated wing discs". We hoped to learn more about this functional cooperation by a comparative study of both tissues.

PTGs and OENs are of ectodermal origin. But already during differentiation important differences were observed: the OENs very soon become polyploid, have no pigment and already show signs of activity in the embryo; the PTGs remain diploid in the embryo, contain pigment like the epidermal cells and appear to be inactive at least until hatching. Whereas the OENs are reproduced during the course of development and their degree of polyploidization never exceeds that of the embryo, the PTGs remain cell constant and experience an increasing polyploidization. Finally the PTGs degenerate during adult molt in contrast to the OENs, which are present until death. Hence, the fate of both organs is quite different and the "joint action" can only concern a sector of their tasks, at least with respect to the OENs. Regarding glands in other insect species, however, studies have shown that their mode of growth is not generally determined. Thus, in *Tenebrio* the OENs are cell constant and grow exclusively endomitotically (Romer, 1975), while PTGs show mitotic activity and their nuclei remain relatively small (Romer, 1971 a). Thus, the conclusion can be drawn that the mode of glandular growth is independent of function, at least concerning hormone synthesis.

The presence of molting hormone long after degeneration of the PTGs, also observed by Feir and Winkler (1969), is apparently due to an activity of the OENs. It would be desirable to know more about the chemical nature of this molting hormone, and whether it is identical with that occurring in the egg. Detailed studies were only carried out on α- and β-ecdysone. However, Kaplanis et al. (1973, 1975) isolated in addition several other molting hormones in insects (among them *Oncopeltus*), but their function is still unknown.

2. Fine Structure of Prothoracic Glands and Oenocytes

The PTGs show the essential structural features of active secretion during the fifth larval stage (compare Scharrer, 1964; Romer, 1971 a; Blaszek et al., 1975; Gersch et al., 1975). Pronounced basal membrane infoldings cause a strong surface enlargement and point to an increased exchange of substances. Rough ER, smooth ER and prominent Golgi regions were also found in *Tenebrio* (Romer, 1971 a) during the most active phase of the gland which coincides with molting. The mutante *l(2) gl* of *Drosophila melanogaster*, which fails to undergo metamorphosis, develops neither rough nor smooth ER (Aggarwal and King, 1969). Although no hormone titer determinations were carried out, the behavior of the gland indicates a developmental arrest. However, a well-developed smooth ER must not be a prerequisite for the function of PTGs, as was indirectly assumed by Locke (1970) and Joly et al. (1973). In the meantime several in vitro studies have proven hormone synthesis in the PTGs. Since the latter are also engaged in the degradation of the molting hormones (Romer et al., 1974; Hoffmann and Koolman, 1974; Willig et al., 1971), the partly well-developed rough ER might be the site of formation of the decomposing enzymes.

The embryonic PTG is distinctly different from the larval one. In the former, rough and smooth ER, mitochondria with an electron dense matrix, striking

Golgi complexes, glycogen and liposomes are absent. The cytoplasm is characterized by an electron dense matrix and an abundance of ribosomes, but the organelles, which are typical for the active larval glands, are scarse. Therefore, synthesis of molting hormones does not seem likely.

In contrast to the PTGs, OENs show typical "larval" features soon after differentiation, which makes the onset of a function, already in the 74 h old embryo, quite probable. As in other species, short stacks of rough ER are signs of activity. However, the smooth ER prevails in the embryo as well as in advanced stages. Especially during larval and adult stage voluminous clusters of glycogen were seen (compare with *Gryllus*, *Kalotermes*, *Heliothrips* and *Apis;* Romer, 1973). To what extent they are subjected to metabolism remains to be elucidated. Microbodies are frequent, as in other insect species (Romer, 1973; Locke, 1969; Gnatzy, 1970), which parallels other steroid hormone-producing cells (Reddy and Svoboda, 1972). They might develop from smooth ER. Particularly the OENs in adults give indications for a strong lytic activity. But the origin of these enzymes can not be traced, since similar to *Tenebrio* (Romer, 1975), Golgi complexes were rarely found.

3. Function of Ecdysone during Embryonic Development

Titer determinations and ultrastructural studies suggest that the high ecdysone level during the second half of embryogenesis is due to the activity of embryonic glands, i.e. OENs. The hormone present during the first half of embryogenesis is most probably of maternal origin. Since mature females are devoid of PTGs, the OENs might produce the hormone, which is added to the eggs; however, according to Hagedorn et al. (1975) the ovaries must also be drawn into consideration.

The function of ecdysone during early development is obscure. The hormone could be necessary for DNA synthesis (Krishnakumaran et al., 1967) or for the regulation of cyclic nucleotide concentration (Applebaum and Gilbert, 1972), which determines the developmental path of a cell (McMahon, 1974). It could also have an influence on "control centers" from where directing factors diffuse to morphogenetic areas.

The peak of hormone level after blastokinesis coincides with apolysis of the second embryonic cuticle and deposition of the definitive first larval cuticle. Thus, the "second embryonic molt" offers the characteristics of a larval molt. The "first embryonic molt" occurs already before most endocrine glands start functioning (Dorn, 1975a, b, c). However, the structure of the first embryonic cuticle differs considerably from the second. While the latter is quite similar to a larval one, the former is similar to a membrane (Dorn, 1972, 1975a) and probably does not represent a "true" cuticle (Louvet, 1974).

The temporal distribution of ecdysone is comparable with that of other hormones in the embryo of *Oncopeltus*. Whereas the curve for JH runs about parallel with molting hormone (Dorn, 1975a), the secretion of neurohormones starts somewhat earlier (Dorn, 1975b) and might well play a dominant (controlling) role. Although it seems obvious that embryonic and larval molts are both governed by hormones in a similar manner (for references, see Dorn, 1972; Bullière, 1973), some

in vitro observations on embryonic tissue reject the necessity of ecdysone for cuticle deposition and apolysis (Schneider and Bucklin, 1958; Takami, 1963; Mueller, 1963; Mueller and Bucklin, 1965; Sbrenna Micciarelli and Sbrenna, 1972). Nevertheless, there is proof that molting hormones and JH are capable of influencing embryonic tissue growth (Kuroda, 1971; Chihara and Fristrom, 1973; Chihara et al., 1972). The precise role of hormones in embryonic development awaits further investigation.

Especially interesting are the observations of Kaplanis et al. (1973, 1975) who found relatively large amounts of new ecdysones in eggs of *Manduca* (26-hydroxyecdysone) and *Oncopeltus* (makisterone A). The authors conclude that "...different ecdysones could function at different stages of insect development...".

References

Adelung, D., Karlson, P.: Eine verbesserte, sehr empfindliche Methode zur biologischen Auswertung des Insektenhormons Ecdyson. J. Insect Physiol. **15**, 1301–1308 (1969)

Aggarwal, S.K., King, R.C.: A comparative study of the ring glands from wild type and *l(2) gl* mutant *Drosophila melanogaster*. J. Morph. **129**, 171–200 (1969)

Agui, N.: Joint action of prothoracic glands and oenocytes on the cultivated wing discs of the cabbage armyworm, *Mamestra brassicae* L. *in vitro* (Lepidoptera: Noctuidae). Appl. Ent. Zool. **9**, 256–260 (1974)

Andries, J.C., Mouze, M.: Action in vivo d'ecdysones sur la morphogénèse imaginale d'*Aeschna cyanea* (Odonata). J. Insect Physiol. **21**, 111–135 (1975)

Applebaum, S.W., Gilbert, L.I.: Stimulation of adenyl cyclase in pupal wing epidermis by β-ecdysone. Develop. Biol. **27**, 165–175 (1972)

Blaszek, I., Balázs, A., Novák, V.J.A., Malá, J.: Ultrastructural study of the prothoracic glands of *Galleria mellonella* L. in the penultimate, last larval, and pupal stages. Cell Tiss. Res. **158**, 269–280 (1975)

Boohar, R.K., Bucklin, D.H.: Possible ecdysone activity in the embryo of the grasshopper *Melanoplus differentialis*. Amer. Zool. **3**, 496 (1963)

Bullière, F.: Cycles embryonnaires et sécrétion de la cuticle chez l'embryon de blatte, *Blabera craniifer*. J. Insect Physiol. **19**, 1465–1479 (1973)

Chihara, C.J., Fristrom, J.W.: Effects and interactions of juvenile hormone and β-ecdysone on *Drosophila* imaginal discs cultured in vitro. Develop. Biol. **35**, 36–46 (1973)

Chihara, C.J., Petri, W.H., Fristrom, J.W., King, D.S.: The assay of ecdysones and juvenile hormpne on *Drosophila* imaginal disks in vitro. J. Insect Physiol. **18**, 1115–1123 (1972)

Delachambre, J.: Remarques sur l'histophysiologie des oenocytes epidermiques de la nymphe de *Tenebrio molitor*. C. R. Acad. Sci. (Paris) **263 D**, 764–767 (1966)

Doane, W.W.: Role of hormones in insect development. In: Developmental systems: Insects Vol. 2, pp. 291–497 (S.J.Counce and C.H. Waddington, eds.). London and New York: Academic Press 1973

Dorn, A.: Die endokrinen Drüsen im Embryo von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). Morphogenese, Funktionsaufnahme, Beeinflussung des Gewebewachstums und Beziehungen zu den embryonalen Häutungen. Z. Morph. Tiere **71**, 52–104 (1972)

Dorn, A.: Struktur und Funktion des embryonalen Corpus allatum von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). Verh. dtsch. zool. Ges. **67**, 85–89 (1975a)

Dorn, A.: Elektronenmikroskopische Studien über das neurosekretorische System von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera) während der Embryonalentwicklung. Cytobiologie **10**, 227–234 (1975b)

Dorn, A.: Elektronenmikroskopische Studien über Differenzierung und Funktionsaufnahme der Corpora cardiaca im Embryo von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). Cytobiologie **10**, 235–248 (1975c)

Dorn, A.: Review of hormonal control of egg maturation and embryonic development in insects: In press (1976)
Feir, D., Winkler, G.: Ecdysone titres in the last larva and adult stages of the milkweed bug. J. Insect Physiol. **15**, 899–904 (1969)
Gersch, M., Birkenbeil, H., Ude, J.: Ultrastructure of the prothoracic gland cells of the last instar of *Galleria mellonella* in relation to the state of development. Cell Tiss. Res. **160**, 389–397 (1975)
Gnatzy, W.: Struktur und Entwicklung des Integuments und der Oenocyten von *Culex pipiens* L. (Dipt.). Z. Zellforsch. **110**, 401–443 (1970)
Hagedorn, H.H., O'Connor, J.D., Fuchs, M.S., Sage, B., Schlaeger, D.A., Bohm, M.K.: The ovary as a source of α-ecdysone in an adult mosquito. Proc. nat. Acad. Sci. (Wash.) **72**, 3255–3259 (1975)
Herman, W.S.: The ecdysial glands of arthropods. Int. Rev. Cytol. **22**, 269–347 (1967)
Hoffmann, J.A., Koolman, J.: Prothoracic glands in the regulation of ecdysone titres and metabolic fate of injected labelled ecdysone in *Locusta migratoria*. J. Insect Physiol. **20**, 1593–1602 (1974)
Joly, L., Weins, M.J., Hoffmann, J.A., Porte, A.: Evolution des glandes prothoraciques de larves permanentes de *Locusta migratoria* obtenues par irradiation sélective du tissue hématopoiétique. Z. Zellforsch. **137**, 387–397 (1973)
Kaplanis, J.N., Dutky, S.R., Robbins, W.E., Thompson, M.J., Lindquist, E.L., Horn, D.H.S., Galbraith, M.N.: Makisterone A: a 28-carbon hexahydroxy molting hormone from the embryo of the milkweed bug. Science **190**, 681–682 (1975)
Kaplanis, J.N., Robbins, W.E., Thompson, M.J., Dutky, S.R.: 26-hydroxyecdysone: new insect molting hormone from the egg of the tobacco hornworm. Science **180**, 307–308 (1973)
Karlson, P., Shaaya, E.: Der Ecdysontiter während der Insektenentwicklung. – I. Eine Methode zur Bestimmung des Ecdysongehalts. J. Insect Physiol. **10**, 797–804 (1964)
Krishnakumaran, A., Berry, S.J., Oberlander, H., Schneiderman, H.A.: Nucleic acid synthesis during insect development. II. Control of DNA synthesis in the *Cecropia* silkworm and other saturniids. J. Insect Physiol. **13**, 1–57 (1967)
Kuroda, Y.: Effects of substances with ecdysone and juvenile hormone activity on the growth of embryonic tissues from *D. melanogaster*. Drosophila Inf. Serv. **46**, 104 (1971)
Locke, M.: The ultrastructure of oenocytes in molt/intermolt cycle of an insect. Tissue and Cell **1**, 103–154 (1969)
Locke, M.: The control of activities in insect epidermal cells. Mem. Soc. Endocr. **18**, 285–299 (1970)
Louvet, J.-P.: Observation en microscope électronique des cuticules édifiées par l'embryon, et discussion du concept de "mue embryonnaire" dans le cas du phasme *Carausius morosus* Br. (Insecta, Phasmida). Z. Morph. Tiere **78**, 159–179 (1974)
Marks, E.P.: Deposition of insect cuticle in vitro: differential response to α- and β-ecdysone. Gen. comp. Endocr. **21**, 472–477 (1973)
McMahon, D.: Chemical messengers in development: a hypothesis. Science **185**, 1012–1021 (1974)
Mizuno, T., Ohnishi, E.: Conjugated ecdysone in the eggs of the silkworm, *Bombyx mori*. Develop. Growth and Differ. **17**, 219–226 (1975)
Moriyama, H., Nakanishi, K.H., King, D.S., Okauchi, T., Siddal, J.B., Hafferl, W.: On the origin and metabolic fate of α-ecdysone in insects. Gen. comp. Endocr. **15**, 80–87 (1970)
Mueller, N.S.: An experimental analysis of molting in embryos of *Melanoplus differentialis*. Develop. Biol. **8**, 222–240 (1963)
Mueller, N.S., Bucklin, D.H.: Development and molting of *Hyalophora cecropia* embryos in vitro. Amer. Zool. **5**, 206 (1965)
Novák, V.J.A., Zambre, S.K.: To the problem of structure and function of pleuropodia in *Schistocerca gregaria* Forskål embryos. Zool. Jb. Physiol. **78**, 344–355 (1974)
Ohnishi, E., Ohtaki, T., Fukuda, S.: Ecdysone in the eggs of *Bombyx silkworm*. Proc. Jap. Acad. **47**, 413–415 (1971)
Osinchak, J.: Ultrastructural localization of some phosphatase in the prothoracic gland of the insect *Leucophaea maderae*. Z. Zellforsch. **72**, 236–248 (1966)
Reddy, J., Svoboda, D.: Microbodies (peroxisomes) in the interstitial cells of rodent testes. Lab. Invest. **26**, 657–665 (1972)
Romer, F.: Die Prothorakaldrüsen der Larve von *Tenebrio molitor* L. (Tenebrionidae, Coleoptera) und ihre Veränderungen während eines Häutungszyklus. Z. Zellforsch. **122**, 425–455 (1971 a)
Romer, F.: Häutungshormone in den Oenocyten des Mehlkäfers. Naturwissenschaften **58**, 324–325 (1971 b)

Romer, F.: Histologie, Histochemie, Polyploidie und Feinstruktur der Oenocyten von *Gryllus bimaculatus* (Saltatoria). Cytobiologie **6**, 195–213 (1972)
Romer, F.: Moulting hormone content in prothoracic glands and oenocytes of *Tenebrio molitor*, within a moulting cycle. Experientia (Basel) **29**, 1417–1418 (1973)
Romer, F.: Ultrastructural changes of the oenocytes of *Gryllus bimaculatus* DEG (Saltatoria, Insecta) during the moulting cycle. Cell Tiss. Res. **151**, 27–46 (1974)
Romer, F.: Morphologie und Ultrastruktur der larvalen Oenocyten von *Tenebrio molitor* L. (Insecta, Coleoptera) bei Larve, Puppe und Imago. Z. Morph. Tiere **80**, 1–40 (1975)
Romer, F., Emmerich, H., Nowock, J.: Biosynthesis of ecdysones in isolated prothoracic glands and oenocytes of *Tenebrio molitor* in vitro. J. Insect Physiol. **20**, 1975–1987 (1974)
Sbrenna Micciarelli, A., Sbrenna, G.: The embryonic apolysis of *Schistocerca gregaria* (Orthoptera). J. Insect Physiol. **18**, 1027–1037 (1972)
Scharrer, B.: The fine structure of blattarian prothoracic glands. Z. Zellforsch. **64**, 301–326 (1964)
Scharrer, B.: Ultrastructural study of the regressing prothoracic glands of blattarian insects. Z. Zellforsch. **69**, 1–21 (1966)
Schneider, N.A., Bucklin, D.H.: Molting and development of cultured fragments of embryos of the grasshopper *Melanoplus differentialis*. Anat. Rec. **131**, 597 (1958)
Takami, T.: In vitro culture of embryos of the silkworm *Bombyx mori* III. Moult of brainless embryos in culture. J. exp. Biol. **40**, 735–739 (1963)
Wigglesworth, V.B.: The breakdown of the thoracic gland in the adult insect, *Rhodnius prolixus*. J. exp. Biol. **32**, 485–491 (1955)
Willig, A., Rees, H.H., Goodwin, T.W.: Biosynthesis of insect moulting hormones in isolated ring glands and whole larvae of *Calliphora*. J. Insect Physiol. **17**, 2317–2326 (1971)

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