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Ultrastructural Changes of the Oenocytes of *Gryllus bimaculatus* DEG (Saltatoria, Insecta) during the Moulting Cycle*

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Summary. 1. The oenocytes of *Gryllus bimaculatus* are characterized by an abundant smooth-surfaced *ER* (*ATER*). In spite of the great cell size the plasma membrane never shows extensive infoldings during the moulting cycle. In addition to mitochondria there are very large numbers of microbodies containing peroxidase but apparently not uricase. Within the second part of the instar the microbodies lie along the clefts which run through the whole cell.

2. The following changes are observed in the course of a moulting cycle: Immediately after hatching the *ATER* is scarcely developed, some liposomes are located within areas of *ATER* disappearing some hours later. 20 hours after emergence glycogen deposits appear in two forms: large deposits reaching some μm in diameter, and in distinct rosettes dispersed between the tubules of the *ATER*. 30 hours post-moult profiles of *RER* appear, which disappear together with the large glycogen deposits one day later. At the same time *ATER* is increased and the clefts develop within areas of elongate granules smaller than ribosomes. The number of such clefts subsequently becomes reduced probably as a result of confluence. Towards the end of the moulting cycle numerous autophagosomes appear. These digest parts of the agranular reticulum, many microbodies, and to a lesser extent, mitochondria. Residual bodies are extruded during moult whereas the clefts remain.

3. The ultrastructural features parallel those of steroid-producing cells in vertebrates. Besides this it is possible that oenocytes also engage in detoxication processes as shown for vertebrate liver.

Key words: Oenocytes, *Gryllus bimaculatus* — Moulting-cycle — Ultrastructure and Moulting-hormones.

Introduction

The oenocytes of insects have recently become of interest to endocrinologists because of the parallels between their ultrastructure and that of steroid-hormone-producing cells of vertebrates (Delachambre, 1966; Locke, 1969; Cassier et Fain-Maurel, 1969, 1973; Rinterknecht *et al.*, 1969; Gnatzy, 1970; Romer, 1972, 1973). In addition, physiological investigations in butterflies have shown a possible interaction between prothoracic glands and oenocytes in the synthesis of the moulting hormone ecdysone (Weir, 1970). Extraction procedures have also been used to show the presence of greater quantities of moulting hormones in oenocytes and prothoracic glands than in other tissues (Romer, 1971 b). Recently the synthesis of ecdysone by isolated oenocytes of *Tenebrio* has also been demonstrated (Romer, Emmerich, Nowock in press). Such an experiment is only possible in holometa-

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bolic insects whose oenocytes can be isolated. Therefore in hemimetabolic insects a corresponding function of oenocytes can only be assumed on the basis of similarities in ultrastructure. The present investigations will consider the ultrastructure and changes in the ultrastructure of oenocytes during a moulting cycle of *Gryllus bimaculatus* of the orthoptera.

Material and Methods

First instar larvae of *Gryllus bimaculatus* were fixed at intervals of 10 respectively 5 hours with 2% OsO_4 dissolved in Michaelis buffer (pH 7.2) for 2 hours at 4°C. The first instar takes approximately 72 hours (for breeding conditions see Romer, 1972). Alternatively a fixation with 5% glutaraldehyde dissolved in Sorensen buffer (pH 7.2) was used. Finally the specimens were dehydrated in increasing concentrations of ethanol, transferred to propylene oxide and embedded in Araldite. Silver to gold sections were counterstained with lead citrate (Reynolds, 1963) and investigated with the Siemens Elmiskop Ia.

Observations

a) The Plasma Membrane

Differentiated oenocytes of *Gryllus* are polyploid cells of polygonal shape. In contrast to the oenocytes of *Calpodes* (Locke, 1969) or *Culex* (Gnatzy, 1970) they have straight cell boundaries with few infoldings. Only in 54 hours specimens were infoldings, reaching almost to the nucleus found. These animals also showed few pinocytotic vesicles.

The basement membrane is very thin, and is compressed to blocks between adjacent cells (Fig. 5a). It is not possible to decide whether these condensations are fixation artefacts or whether they are of physiological significance for facilitating the exchange of metabolites. At open cell boundaries, in the epidermis for example, the basal lamina never shows a similar pattern.

b) The Endoplasmic Reticulum

In oenocytes of *Gryllus* the endoplasmic reticulum is mainly of the agranular type (*ATER*) that is occasionally dilated to vesicles. Rough surfaced *ER* (*RER*) is only developed at well defined stages. In both types of *ER* the following changes are to be observed during a moulting cycle.

The *ATER* is not distributed at random but occurs in groups lying mainly in the periphery of the cell (Fig. 1a, 3a). It consists of a net of ramified tubules. The diameter of the tubules is about 200 Å. In places slight dilatations are visible (Fig. 1a, 5c). The position of the clefts (structures observed by light microscopy) determines the distribution of the *ATER* separating areas of *ATER* that are of distinct compactness (Fig. 3a, 8a). There is little *ATER* close to the nucleus

Fig. 1. a Detail of an oenocyte 12 hours after hatching. The tubules of the *ATER* lie close to numerous lipid droplets (*Li*). The reticulum is smooth-surfaced, partly tubular and partly vesicular (*AER*). *N* nucleus, *M* mitochondrion, *Mb* microbody, *Cyl* cytolysosomes, *G* Golgi area. Magnification 12000. b Survey of an oenocyte showing indications of autolysis. *N* Nucleus (sectioned at the periphery), *Chr* chromatin mass, *M* mitochondrion, *Pm* plasma membrane, the arrows indicate vacuoles showing various degrees of degradation. Magnification 7500

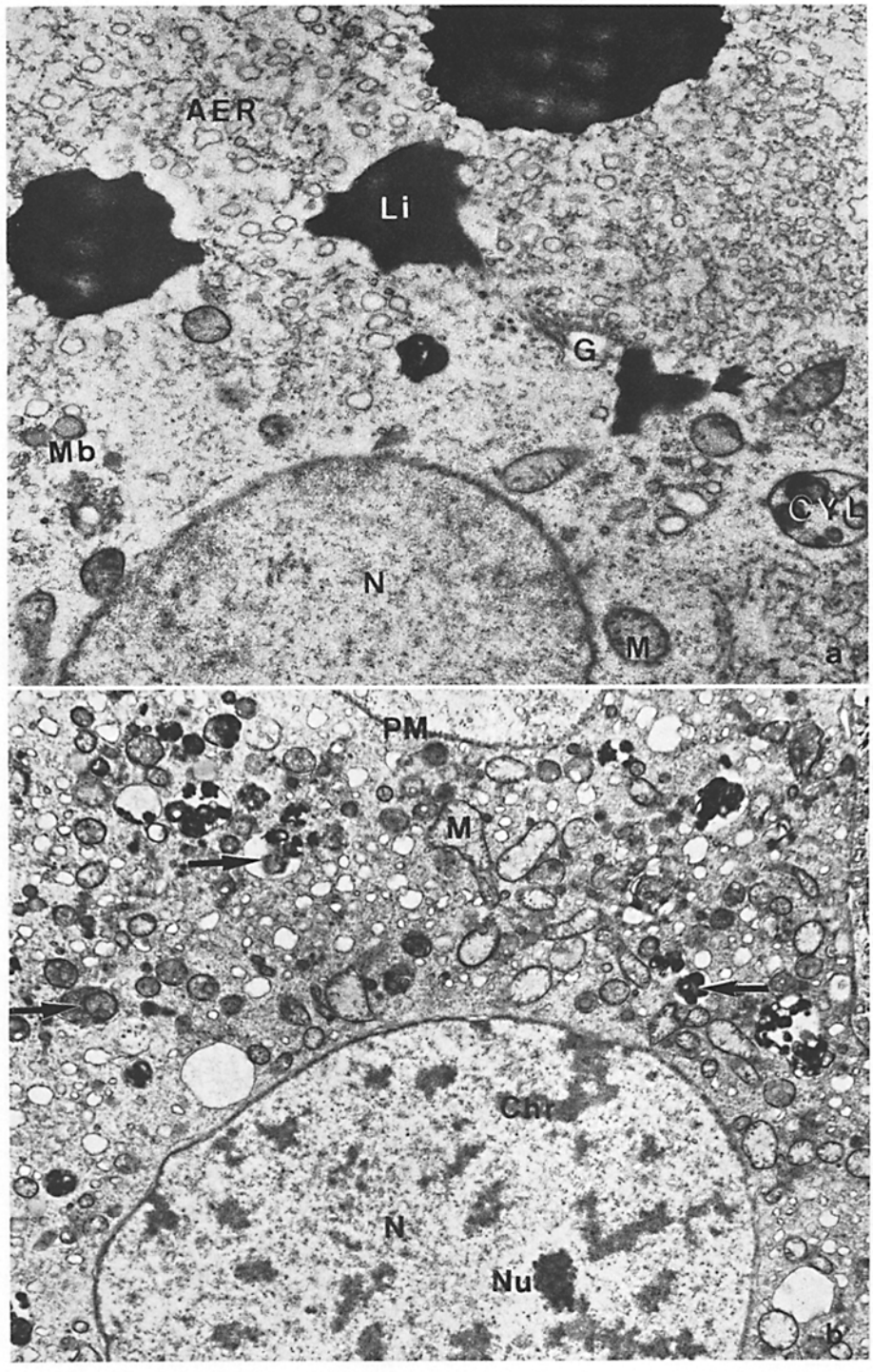

Fig. 1

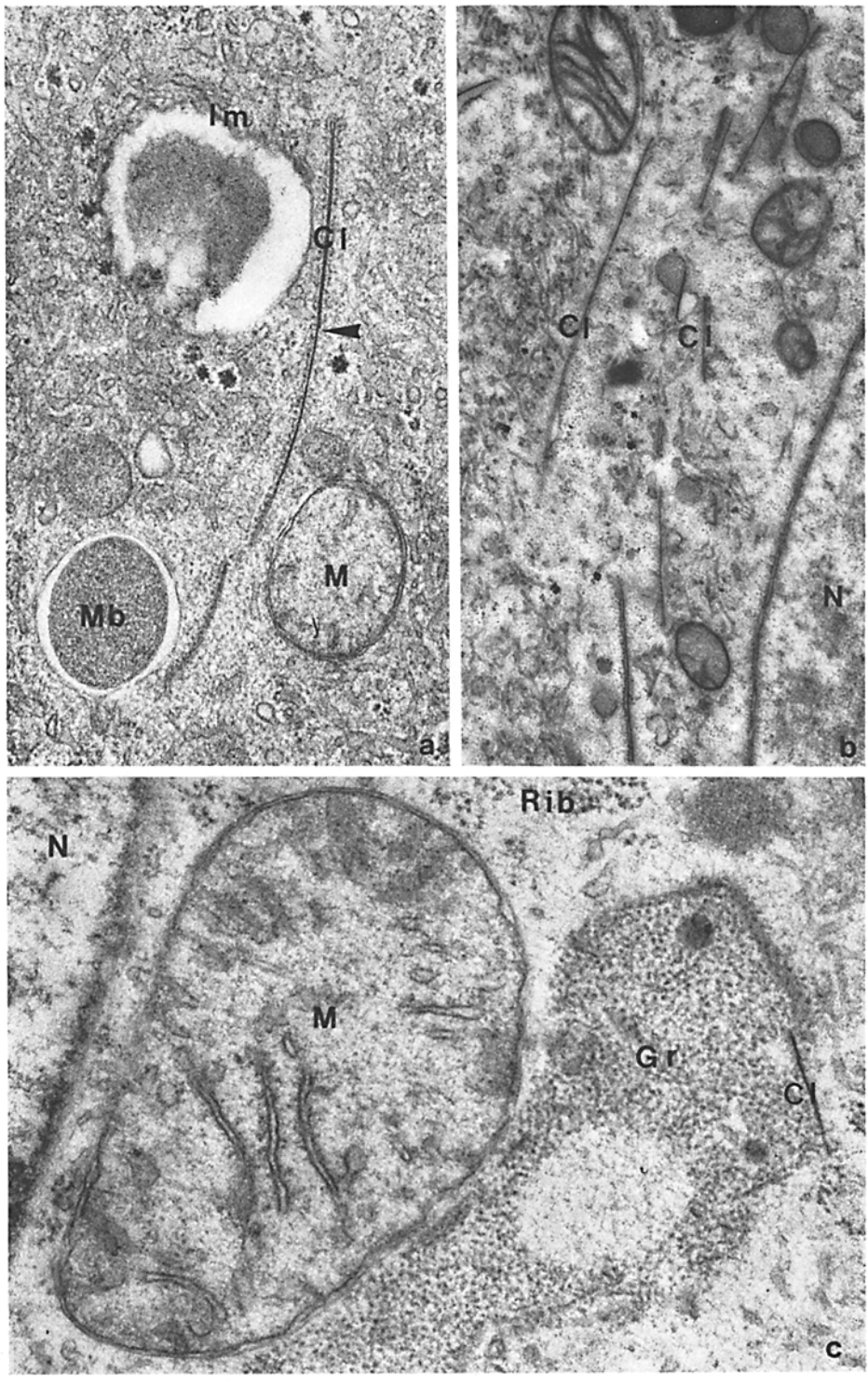

Fig. 2

(Fig. 1a, 3a, 4) or only a few elements. During further development the islet-like distribution of *ATER* is obscured by enlargement of the distance between tubules. 37 hours post moult parallel, ribosome-rich cisternae appear close to the nucleus. Subsequently the *RER* increases further. Parallel cisternae bend inward and give rise to concentrically arrayed *ER*-lamellae. The lumen of the cisternae appears to be empty or to be filled with a homogeneous, slightly electron dense substance. Connections with Golgi areas could not be observed. In some cells the rough surfaced *ER* increases to an extent that the oenocytes could only be identified by virtue of their large size or by the presence of strands of *ATER*.

At this time the first clefts occur (Fig. 2a, b). These are not in connection with the *RER*. They occur within areas of *ATER* but do not show clear continuity with the tubules of the latter. 60 hours post moult the *RER* has disappeared, and the *ATER* is strongly dispersed (Fig. 3b, 5a). At this stage the diameter of the tubules is between 170 and 340 Å.

Direct continuity between *ATER* and plasma membrane does not occur. However tubules show a close relationship with the cell membrane along considerable distances (Fig. 5a). They are also in close relation to mitochondria (Fig. 6a) and microbodies (Fig. 2a, 3b).

During moult the tendency for vesicles to be formed in some cells is considerable particularly in those having many lysosomes (Figs. 5c, 7a). The lumen of the vesicles shows no electron dense content, and reaches a diameter of up to 0.5 μm. All transitions from tubules to vesicles were found.

c) Golgi Areas

A close correlation is to be observed between the moulting cycle and the differentiation of Golgi areas. Immediately after hatching several Golgi areas are found in each section of a cell. They consist of circular cisternae surrounded by numerous vesicles with electron dense content (Figs. 1a, 4). Certain Golgi areas show coated vesicles which appear to originate in this region. Usually such vesicles are thought to be associated with the resorption and transport of proteins at cell membranes (Roth and Porter, 1964; Locke, 1969). As rough-surfaced *ER* increases the number of Golgi zones decreases, but increases again close to the end of the moulting cycle.

Connections between Golgi zones and the *ATER* are characteristic of *Gryllus* oenocytes (Fig. 4). A substance synthesized in the agranular reticulum must be extruded by the Golgi zones. Acid phosphatases (DeDuve and Wattiaux, 1966) and esterases (Miller and Palade, 1963) are transported by Golgi vesicles, and a transport of lytic enzymes to the autophagic vacuoles can be assumed to occur close to the end of the moulting cycle. Immediately after moulting there is no indication for a lytic activity.

Fig. 2a—c. Development of clefts. a The formation of the two walls does not occur synchronously. At the right side (arrow) it is only half-developed. Elongate granules are situated at its periphery. At the top of the figure part of the cytoplasm is surrounded by an isolation membrane (*Im*). The lower part shows an isolated microbody. Magnification 38000. b Formation of numerous small clefts. Some elongated small granules are already present. Magnification 20000. c A field of elongate granules with forming clefts (*Cl*). The ribosomes (*Rib*) are distinctly arger. A giant mitochondrion (*M*) near the border of the nucleus (*N*). Magnification 42000

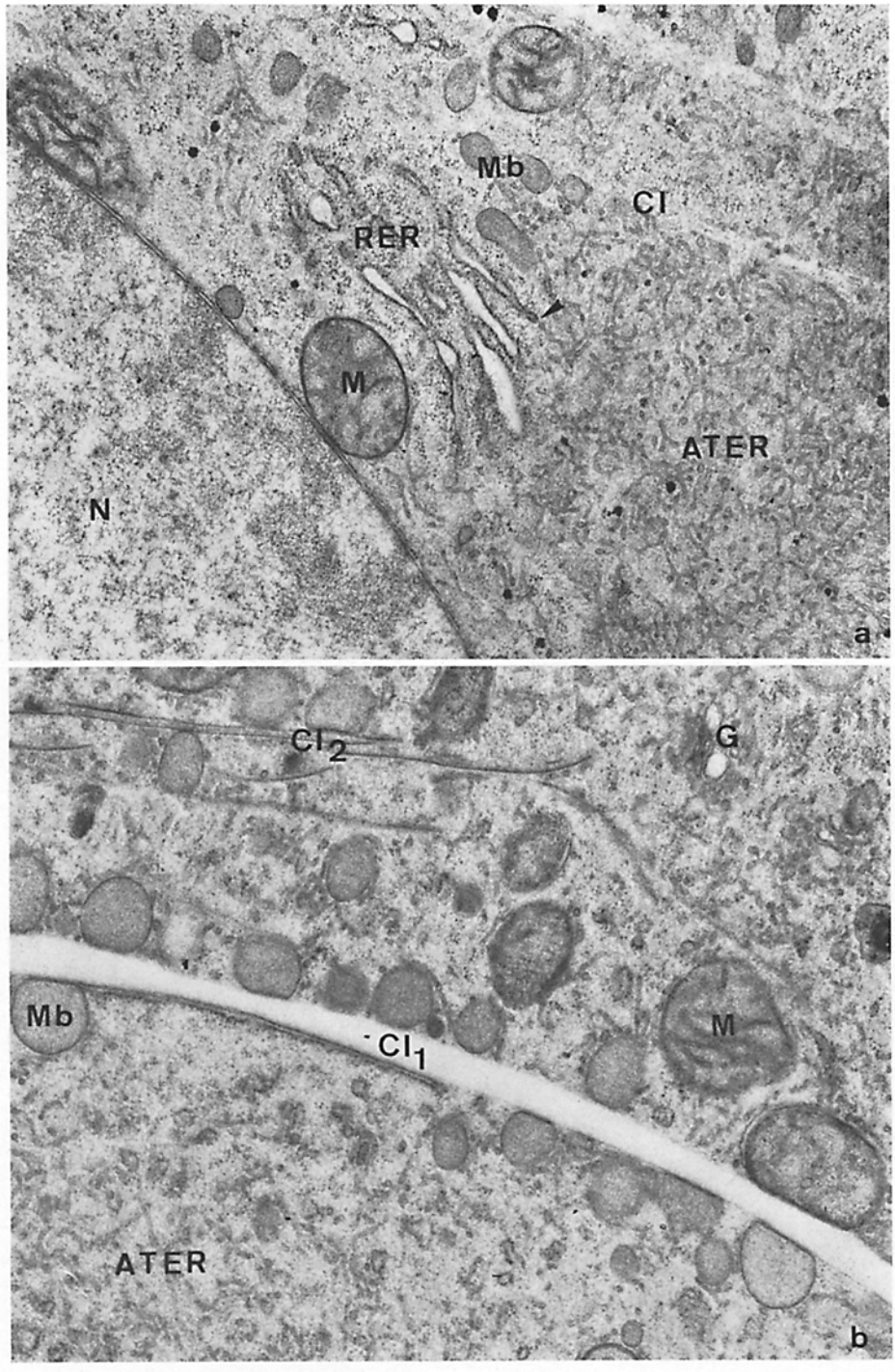

Fig. 3

Near the Golgi zones there are single microbodies. We have no evidence that they form from Golgi vesicles and the enzymes regularly found in microbodies (catalase and urate oxidase) are formed directly by the *ER* and not by Golgi zones (Hruban and Rechcigl, 1969; Locke and McMahon, 1970).

d) Ribosomes

Judging by their limited stainability with pyronine few ribosomes are to be found in oenocytes of immediately post-hatch larvae. This condition changes 12 hours post moult (Fig. 1a). Especially in the pale zone around the nucleus, there are many extended polysomes consisting of 10–15 or more ribosomes. Subsequently the regions not occupied with *ER* also accumulate ribosomes. To some extent they invade the interstices of the *ATER*. The uneven distribution explains the patchy staining found in light microscopy after pyronine staining. The concentration of ribosomes becomes maximal mainly in the regions of rough-surfaced *ER* 35 to 55 hours post moult. When the rough-surfaced *ER* is subsequently reduced, most of the ribosomes are to be found once more in the perinuclear pale zone.

Second instar oenocytes always have many ribosomes in their cytoplasm (Fig. 4). A marked decline in cytoplasmic RNA by comparison with the post-hatch stage cannot be observed.

e) Mitochondria

Mitochondria are evenly distributed in the cytoplasm except near the nuclear membrane where they are especially concentrated. Mitochondria lying close to the nuclear membrane were found in animals shortly before or after moulting. This proximity was not so marked in the intermoult. The diameter of the mitochondria varies from 1–2 μm. The profiles are round to ovoid, long, thread-like mitochondria are not numerous. The matrix is more dense than the surrounding cytoplasm. The inner membrane is folded to form irregular banded cristae. Some mitochondria show a circular arrangement of the cristae (Fig. 6b). Distinct tubular mitochondria typical of steroidhormone-producing cells, could not be found, and the number of giant mitochondria is small (Idelman, 1964; Lindner, 1964; Beaulaton, 1968). They appear in cells showing pronounced lytic activity close to moulting (Fig. 1b). Their matrix is not electron dense.

In *Culex* Gnatzy (1970) showed a close correlation between mitochondria and *ATER*. Direct connections between tubules of the *ATER* and mitochondria were never observed. However the mitochondria are surrounded by a network of *ATER* (Fig. 6a).

f) Microbodies

The microbodies are a significant component of the oenocytes of *Gryllus*. Their number depends on the moulting stage. Their diameter ranges from 0.1 to 1.0 μm. Their shape is ovoid to globular and they contain granules of variable electron density. Occasionally they have tubular inclusions.

Fig. 3. a Nuclear border of an animal 37 hours post-moult. Microbodies and mitochondria are associated with the nuclear envelope. A connection between *RER* and *ATER* is shown (arrow). Near the clefts (*Cl*) several microbodies lie in a row. Magnification 22000. b Oenocyte of a specimen shortly before moulting. In addition to dilated clefts (Cl_1) there are several collapsed ones (Cl_2) Magnification 21000

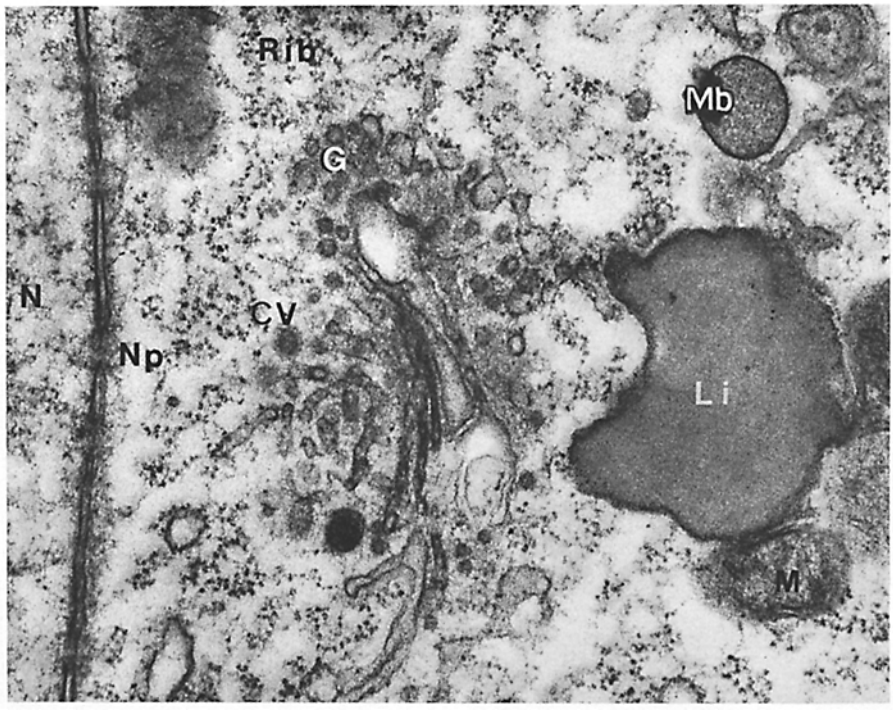

Fig. 4. Golgi zone close to the nucleus. It is in continuity with some tubules of the *ATER*. In addition to Golgi vesicles there are also some "coated vesicles" (*CV*). Lipid droplets associated with mitochondria are common shortly post-moult. *Np* nuclear pore. Magnification 41000

The following *changes* within the 1st instar are to be observed in microbodies: 30 minutes after emergence there are few along the poorly developed clefts. 12 hours post-moult their distribution is similar but their diameter at this time is about 2500 Å. From 25 hours onwards they are also found close to the nucleus. Subsequently they increase in size and number, reaching a diameter of 4000 Å. In areas of *RER* they are extremely rare, whereas they are numerous within the *ATER*. In moulting stages they reach their greatest number and size (Figs. 3b, 5, 7a, b). At this time they are present in greater quantities than mitochondria, but already show degenerative features. Their content becomes coarse grained and they develop 1 or 2 vacuoles. Isolation membranes surround microbodies to form isolation bodies in which parts of the *ATER* may also be included. Mitochondria may also be enclosed, but less frequently. The diameter of microbodies before moulting is about 5500 Å (Fig. 3b), in animals of the second instar this is reduced to about 1750 Å (Fig. 5b).

Genesis of the Microbodies. According to the investigations of Locke (1969) the microbodies of *Calpodes* oenocytes are formed from the smooth-surfaced *ER*. Gnatzy (1970) confirmed the findings of Locke in *Culex*. Some pictures of *Gryllus* suggest a connection between *ATER* and microbodies; we can thus conclude that they have the same origin as in other species. In a study in the fat body of *Calpodes*, Locke and McMahon (1970) demonstrated the formation of microbodies from the

RER. There was no indication for this in *Gryllus*. From a few hours post-moult up to the second instar small microbodies appear along the collapsed clefts indicating connections with the *ATER*. As the majority of the microbodies becomes eliminated by autophagy, it is assumed that the small ones arise at the beginning of the second instar. *Gryllus* shows the same mode of elimination that Locke and McMahon demonstrated for the 5th instar fat body of *Calpodes*. A degeneration as found in the 4th instar could not be confirmed.

According to De Duve and Baudhuin (1966), microbodies of the mammalian liver contain catalase and urate oxidase. Locke and McMahon were able to demonstrate both enzymes in the fat body of *Calpodes* by light microscopical histochemistry and catalase by electron microscopy. In the oenocytes of *Gryllus* peroxidase was demonstrated by benzidine oxidation. The result was a precipitate close to the clefts. Because microbodies are predominantly located along the clefts we interpret these precipitates as peroxidase possibly contained in the microbodies.

A number of microbodies are situated among the tubules of the *ATER*. They probably originate from *ATER*. The majority however lie close to the clefts. In *Calpodes* microbodies also have an intimate relation to the "crystal inclusions" (Locke, 1969). This favoured position could not be found in *Culex* (Gnatzy, 1970).

g) Clefts

As mentioned above, fissures form in the cytoplasm shortly before moulting. These fissures may extend through the entire cell. Similar structures were found in *Rhodnius* (Wigglesworth, 1933) and *Dacus* (Evans, 1967). In *Locusta migratoria* Rinterknecht *et al.* interpret them as rigid tubules covered like coated vesicles. They are common in the adult. Already in very young *Gryllus bimaculatus* ($^1/_2$ after hatching) structures identical with clefts are present. They extend for considerable distances within the cell, curve suddenly, and terminate in a spiral. According to the plane of the section one or two closely parallel membranes are distinguishable. In young animals there is little or no interspace between them. Usually a tightly packed *ATER* is located on one side, and hyaloplasma without organelles or containing dispersed *ATER* on the other.

The accumulation of clefts in areas with *ATER* is in agreement with the findings of Rinterknecht *et al.* (1969) and Cassier and Fain-Maurel (1973). Within the first instar the number of clefts remains approximately constant. Their number shows an increase in larvae 54 hours old (Fig. 2b). Peculiarly the newly formed clefts do not connect with the preexisting clefts but originate simultaneously in different regions of the cell. This is in agreement with the fact that in younger animals there are numerous small clefts, whereas in older specimens there are fewer, larger clefts. It is therefore reasonable to assume that the smaller clefts fuse to give rise to larger ones. Indeed the clefts arise in areas consisting mainly of *ATER*, but do not apparently form directly from the smooth-surfaced tubules. Fig. 2a demonstrates their mode of development. During development the membrane surrounding a cleft is not yet completed. Elongate granules with a diameter of up to 100 Å are located at the cytoplasmic surfaces of the membranes. The diameter of ribosomes in the same material is about 150 Å. In areas where clefts are forming, these granules are very numerous.

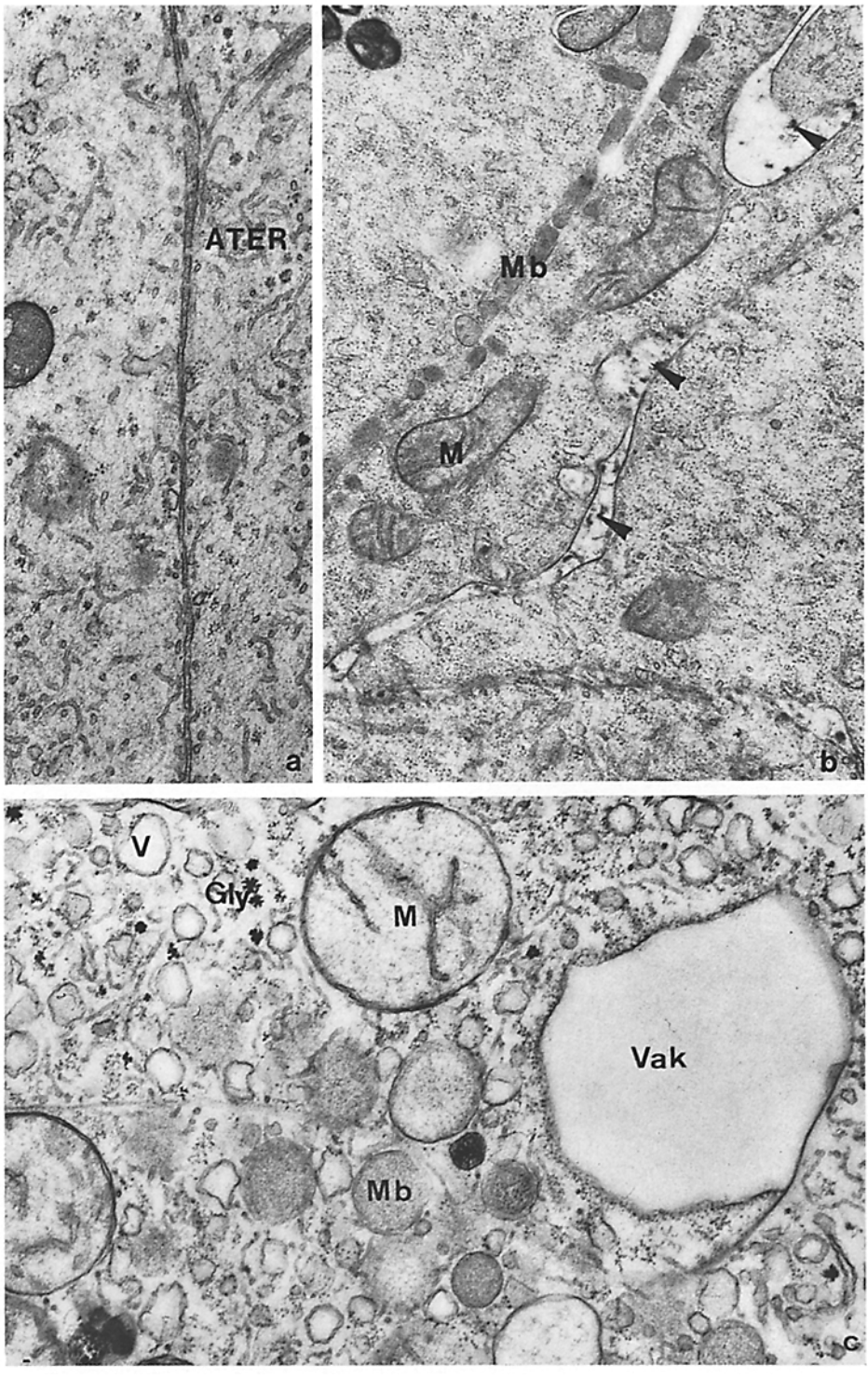

Fig. 5

In animals older than 30 hours the membranes lie further apart and the space they enclose is of lesser electron density. In moulting stages the clefts are much more distinct. In some sections they are swollen (Fig. 5c) so that the cells appear vacuolated (perhaps as a result of a low osmolarity fixative). Numerous clefts always exist in early 2nd instars, not older than 5 hours. Some of them were dilated. These findings may indicate that the clefts are structures with a long life span not eliminated during moult. Thus the clefts as such are surely not fixation artefacts, and their close relation to microbodies also argues against such an assumption. However, they apparently exist only in 50 percent of the cells investigated.

Whether the clefts reach the cell membrane could not be answered by the pictures examined in this study. Communications with the plasma membrane were never found. Because no or only few plasma membrane infoldings were seen, clefts could be interpreted as structures for enlargement of the cell surface or as structures for the elimination of degraded cell parts. A connection to the cell membrane would be necessary for this function. The few observations of lysosomes in cleft-like structures is not evidence for such a function. According to the results of the Schultz-digitonin test the clefts have a high content of cholesterol. Rinterknecht *et al.* have also favoured this opinion without having made any histochemical investigations. Locke (1969) observed these structures in *Calpodes* too. He named them "crystal inclusions". Unfortunately he did not characterize them biochemically. Gnatzy (1970) also described cleft-like structures in the 4th instar of *Culex pipiens*. Here the formation of clefts is related to the age and stainability by fat dyes. He interpreted them as lipid deposits. According to their development, the clefts of *Culex* contrast with those of *Gryllus*; whereas the former are dilatations of the *ATER*, the latter emerge as membrane bound clefts in the cytoplasm in an area of elongate granules.

h) Lysosomes

In the last instar oenocytes of *Calpodes* and *Culex*, Locke (1969) and Gnatzy (1970) described a pronounced formation of lysosomes leading to the destruction of larval cell generation. In both cases the findings relate closely to metamorphosis.

During the first instar of *Gryllus* corresponding results could not be expected because the animals are at the beginning of growth. As will be demonstrated this assumption can be verified only to a certain degree (Table 1). Up to 34 hours a marked occurrence of myelin bodies cannot be observed. They become more numerous only in cells showing a distinct *RER*. There are several fissures reminiscent of but smaller than the clefts in the cytoplasm. The closer to moulting the greater is the number of degenerating particles observed leading to myelin

Fig. 5. a Adjacent cell boundaries of two oenocytes shortly before moulting. The intercellular space is small and occupied by a discontinuous adventitia. The *ATER* contacts the plasma membrane but shows no continuity with it. Magnification 29000. b See (a) but after moulting. The intercellular space is dilated and remnants of degraded cell particles are visible (arrows). Magnification 20000. c Oenocyte of *Gryllus* during moulting. The reticulum is partly swollen (*V*). The mitochondria are enlarged as are the microbodies. The large vacuole (*Vak*) is apparently a dilated cleft. *Gly*, Glycogen. Magnification 29000

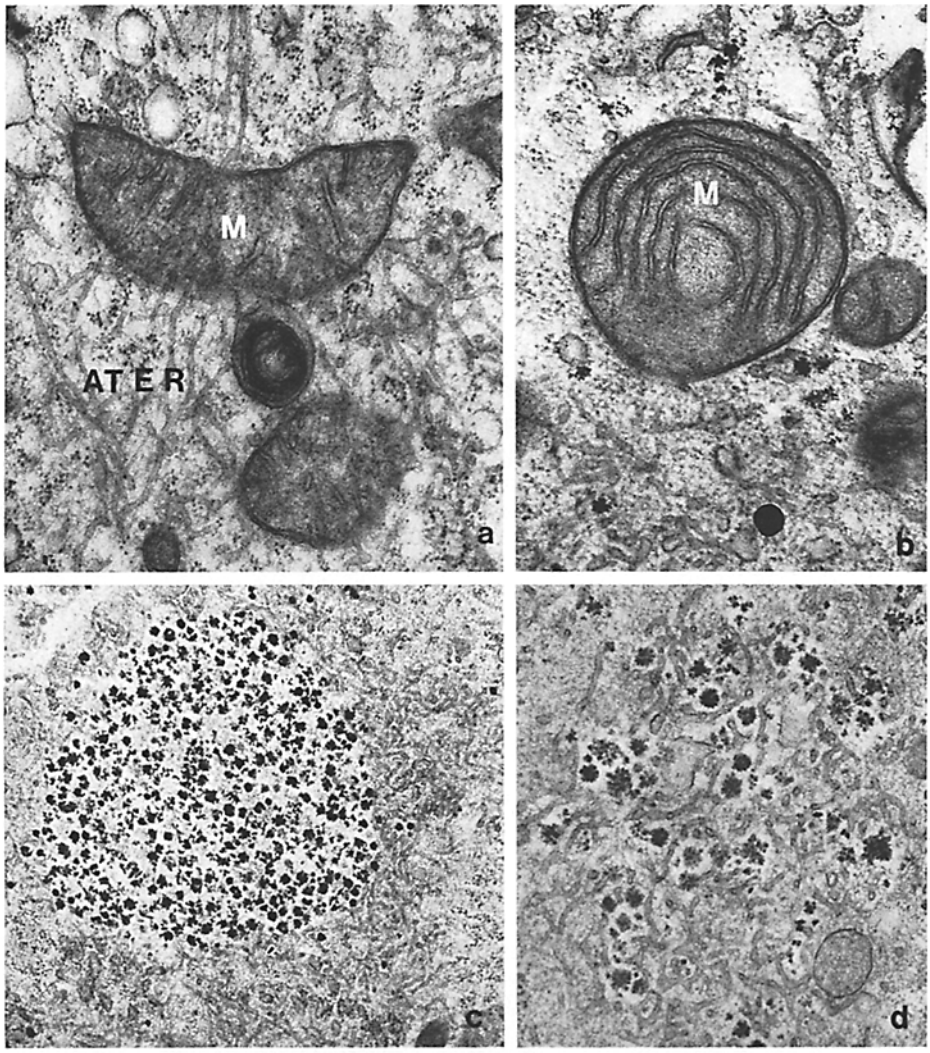

Fig. 6. a Mitochondrion in close proximity to the *ATER*. Magnification 29000. b Mitochondrion with curved cristae. Magnification 33000. c Glycogen deposit in a first instar larva of *Gryllus*. The glycogen is deposited within the *ATER* zone. Magnification 18000. d Isolated glycogen rosettes between the tubules of the agranular reticulum, 72 hours post-moult. Magnification 23000

bodies. The isolation and degeneration of parts of the cytoplasm takes place by the formation of autophagic vacuoles. First, isolation membranes appear and engulf parts of the *ATER* (Fig. 2a), microbodies, mitochondria or all three together (Fig. 7a, b), which are than digested by hydrolytic enzymes. The origin of isolation membranes remains unexplained: there are no indications that isolation membranes are formed by Golgi areas as observed in the interstitial cells of the

Table 1. Distribution of lysosomes within the first instar

Age in hours	0.5	12	25	37	54	72
Average number of lysosomes per cell-section	6.5	4.8	3.2	6.7	14.3	38.4

guinea pig by Franck and Christensen (1968). An origin of *ATER* tubules is more probable and would correlate with the findings of Locke in *Calpodes*. The autophagosomes remain the same size and do not fuse to form larger complexes.

The extent of elimination is not the same in all cells. The average number of autophagosomes per cell section is about 30, but is sometimes much greater than 50 (Fig. 1b). At these stages extensive areas of *ATER* have been isolated as well as microbodies and mitochondria. The isolated components disintegrate to small myelin figures which are partly lamellated and partly amorphous.

With the beginning of the first and second instar the number of myelin figures is negligible. Because of this observation one may assume that those residual bodies which are not absorbed, are eliminated. The possible role of the clefts in this elimination was mentioned earlier (see page 37). Only one of the hundreds of clefts observed contained degenerated cell components; an excretory function may therefore be excluded. It is more probable that disintegrated cell particles will be extruded at the cell surface into the haemolymph and will be resorbed later by phagocytes. In animals having terminated the first moult numerous small particles were found in intercellular spaces. These particles were similar to the residual bodies in the autophagic vacuoles (Fig. 5b). As adjacent cell membranes of two oenocytes do obviously not allow a rapid exchange of cell particles the transfer process is slow enough to be captured (Fig. 5b). This assumption is supported by the fact that the number of isolated particles increases at the end of the first instar, and that these particles can be observed in intercellular spaces some hours after moult.

i) Glycogen

Within the first 25 hours of the first instar no glycogen can be observed by electron microscopy. 34 hours post moult however glycogen deposits can be discovered some reaching a diameter of nearly 3 μm (Fig. 6c). They might correspond to the PAS-positive spots in the cytoplasm revealed by light microscopy. The glycogen deposits are not surrounded by membranes as in the growing glycogen vacuoles of the prothoracic glands of *Tenebrio* (Romer, 1971b). It should be pointed out that glycogen never appears in relation to *RER* but forms in areas of smooth surfaced *ER*. In addition to the large glycogen deposits, glycogen rosettes form between the dilated meshwork of the smooth reticulum (Fig. 6d). 54 hours post moult the large glycogen deposits have mostly disappeared, and have been replaced by evenly distributed glycogen rosettes. Oenocyte glycogen decreases progressively as the moult approaches. In animals shortly before moulting glycogen has completely disappeared. These observations suggest that glycogen has a function of an energy source for the increasing cell metabolism towards the end of the first instar.

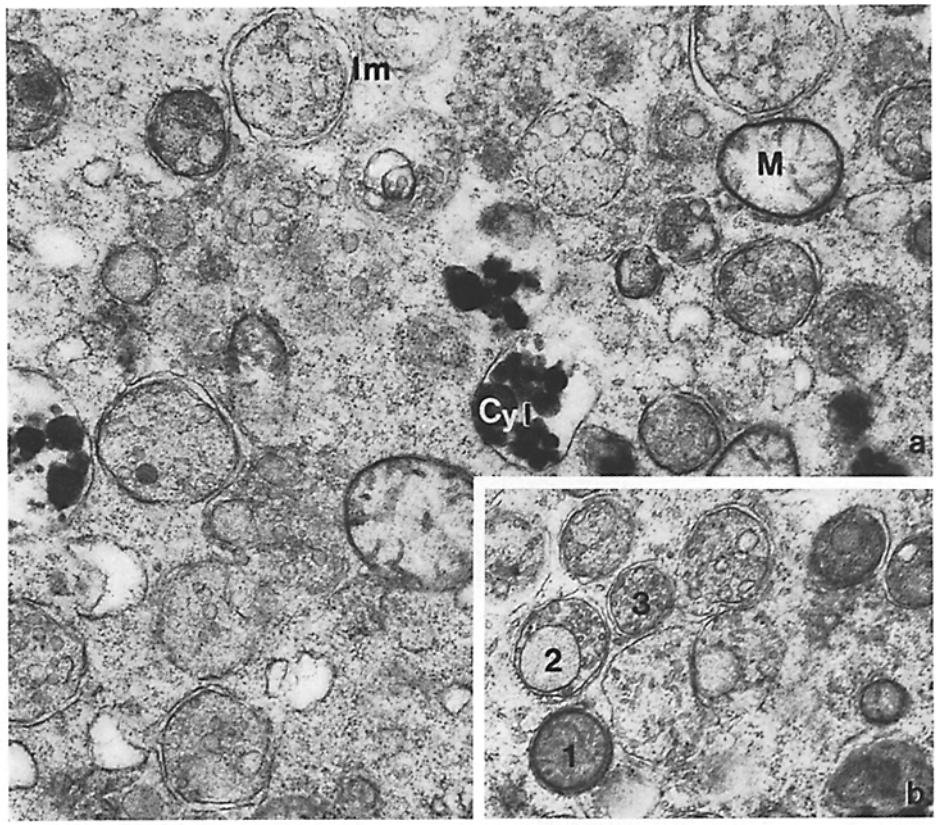

Fig. 7. a Detail of an oenocyte showing a vigorous degradation of cell organelles. In addition to autophagic vacuoles transformed into distinct residual bodies (*Cyl*) there are some areas of cytoplasm surrounded by isolation membranes. Magnification 21000. b Isolation bodies containing mitochondria (*1*), microbodies (*2*) and parts of *ATER* (*3*) lying close to one another. Magnification 23000

k) Lipid Inclusions

It is well known that steroidhormone-producing cells store lipids which disappear following the activation of these cells (Christensen, 1965; Berchtold, 1969; Aoki, 1970; Romer, 1971b). In the oenocytes so far investigated such lipid storage has never been observed. In the oenocytes of *Gryllus* however lipid droplets have been found within the first 12 hours of the 1st and 2nd instar. They disappeared shortly thereafter (Fig. 1a, 4). The liposomes are of varying sizes and are generally not numerous. The findings in Fig. 1a are an exception. Because of their small number the participation of liposomes in cholesterol storage for the synthesis of moulting hormones is probably not very great.

Discussion

a) Ultrastructure and Possible Sites of Hormone Synthesis

The study of the ultrastructure of oenocytes belonging to various insect species (Delachambre, 1966; Evans, 1967; Locke, 1969; Cassier and Fain-Maurel,

1969; 1973, Rinterknecht *et al.*, 1969; Gnatzy, 1970; Romer, 1972, 1973) has shown parallels to steroidhormone-producing cells of vertebrates (Christensen, 1965; Blanchette, 1966; Christensen and Fawcett, 1966; Lindner, 1966; Berchtold, 1969; Aoki, 1970 and others). Extracts of *Tenebrio* oenocytes produced positive effects in bioassay studies (Romer, 1971a). For this reason it was suggested that the extract contained a steroid hormone. Definite proof was provided by the in vitro synthesis of the hormone in isolated oenocytes of *Tenebrio*. Thus oenocytes as well as prothoracic glands are concerned in the production of the moulting hormone. The arrangement of oenocytes in *Gryllus* does not permit the easy isolation of enriched cell preparations suitable for carrying out similar bioassays. Therefore only a morphological comparison and an investigation of the ultrastructure and its changes within a moulting cycle is possible, and certainly reveals some interesting parallels.

Aoki (1970) investigated the Leydig cells of the mouse testis. In young animals, which are probably not able to synthesize greater amounts of hormones, he demonstrated numerous lipid droplets that were partly surrounded by tubules of the *ATER*. In addition considerable accumulations of glycogen granules were formed in the area of liposomes. These cells already possessed a smooth-surfaced *ATER*, but not as extensive as in the activated state. Treatment with gonadotropic hormones of 9 day-old mice not yet showing signs of gonadal activation, was followed by a marked change in the ultrastructure. Lipid droplets established close contact with mitochondria, and subsequently disappeared along with glycogen granules. The nucleoli were activated and in the cytoplasm, free ribosomes were found in abundance, while the rough-surfaced *ER*—previously hardly developed—showed a vigorous growth. Subsequently the *ATER* has increased and distinct Golgi-areas were observed.

Compared with the mouse Leydig cells there are only sporadic lipid droplets in the cytoplasm of *Gryllus* oenocytes. Some authors interpret these structures in the cytoplasm of steroidhormone-producing cells as cholesterol reservoirs (Baillie, 1964; Moses *et al.*, 1969). These reservoirs are thought to be either a precursor of the hormone or a material for the formation of the *ATER*-membranes. Other results on rats (Stein *et al.*, 1969) do not support a specific storage of cholesterol in liposomes. In the opinion of these authors cholesterol becomes incorporated in the membranes of the *ER*, in mitochondria, in liposomes, and in cytolysosomes to the same extent. If these are actual cholesterol deposits in *Gryllus* they would indicate a distribution early within the moulting cycle, reduced thereafter. This also shows parallels to Sertoli and interstitial cells, the possible sites of male sex hormones in vertebrates. Bröckelmann (1963) and Dufaure (1971) were able to demonstrate a seasonal incorporation of glycogen in Sertoli cells, reduced at the beginning of spermatogenesis.

A most obvious sign of activation, however, is the formation of a rough-surfaced *ER* and an increase in cytoplasmic ribosomes, which Leydig cells do not show to a comparable extent. Later on, both Leydig cells and oenocytes of *Gryllus* develop an extensive *ATER*. It is not clear whether *ATER* increases in amount or whether the reticulum merely becomes less compact although the marked increase in cell size (Romer, 1972) and the elimination of *ATER* at the end of the moulting cycle suggests the former. The termination of the cell cycle correlates with a

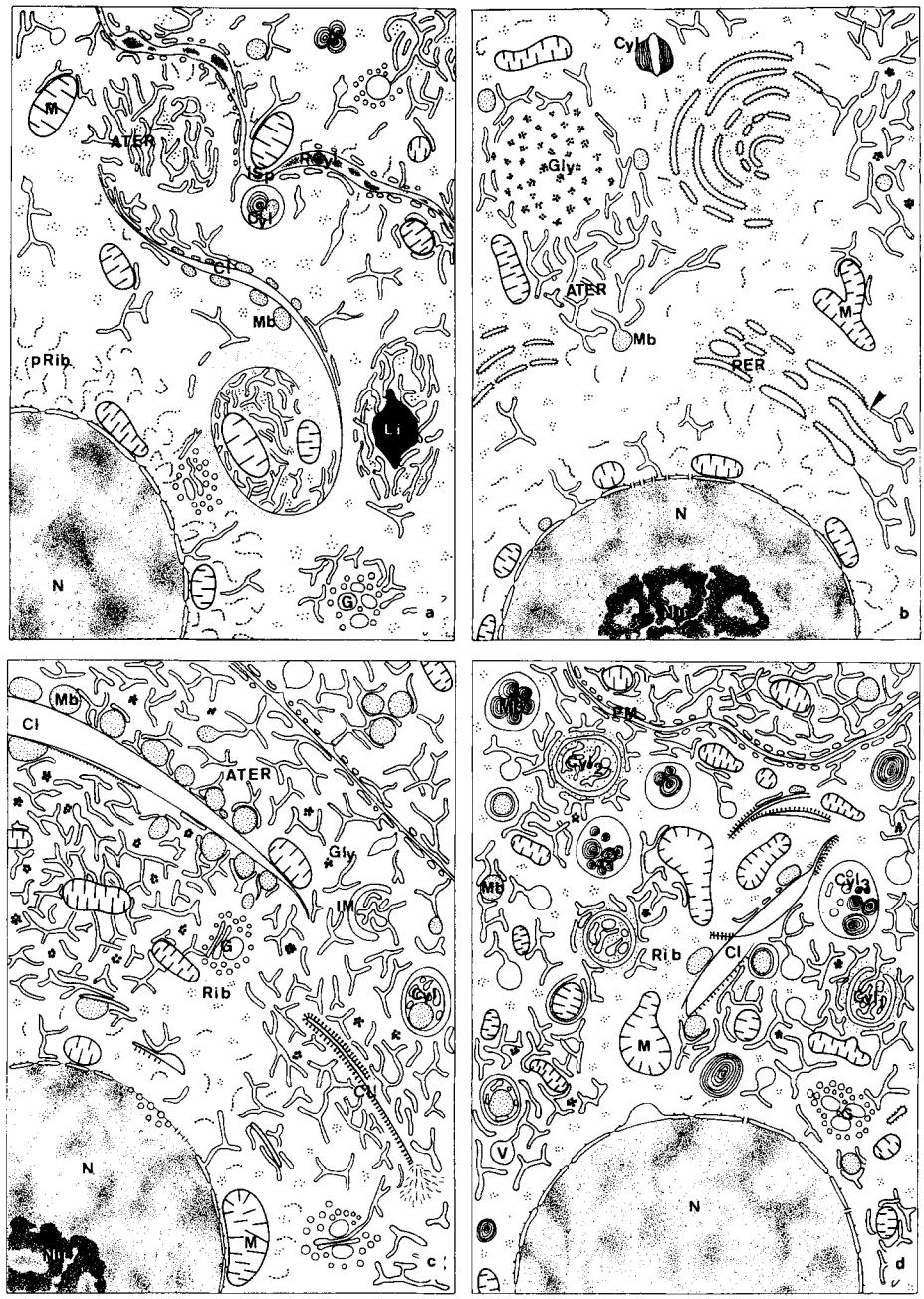

Fig. 8a—d. Cyclical changes of the ultrastructure in oenocytes of *Gryllus bimaculatus* within a moulting cycle. a View immediately after hatching. At this stage the *ER* is scarcely developed. Long fissures, the so called clefts are seen within the cytoplasm. Microbodies and agranular tubules are situated close to them. Several lipid droplets (*Li*) are located in areas of *ATER*. Numerous polyribosomes (*p Rib*) surround the nucleus. Degraded cytolysosomes (*RCyl*) are located within the intercellular space (*ISp*). b View 30 hours post-moult. This stage of develop-

decline of distinct cell components by autophagic vacuoles. In Leydig cells of the mouse a comparable mode of formation of myelin figures is observed.

In the oenocytes of *Gryllus* we have a tissue that changes periodically with the rhythm of moulting. It is also assumed that a secretion cycle also occurs within the embryonic period, as we know is the case in other insect species investigated by light microscopy (Harmsen and Beckel, 1967 in *Hyalophora*, Dorn, 1972 in *Oncopeltus*). Leydig cells in 9 day-old mice do not seem to be activated to a great extent. As we can not fix the starting point for a new secretion cycle, let us compare it with that of the prothoracic glands, the tissue thought to be engaged in the production of the moulting hormone. Prothoracic glands of *Samia cynthia* exposed to ^{3}H-uridine showed RNA-synthesis that is controlled by the brain throughout the entire pupal period (Krishnakumaran *et al.*, 1965).

The criteria of activation in vertebrate steroid cells applied to the oenocytes in *Gryllus* indicate a main activation period between 38 and 54 hours, because within this period we have the most marked elaboration of *ER*, of ribosomes, and a reduction of glycogen deposits. In comparison with the epidermis the oenocytes are activated earlier in the moulting cycle because increased *RER* is found in the latter long before it occurs in the epidermis (54 hours post moult, Romer, 1971 c). Associated with the activation of oenocytes there must be a marked increase in hormone titre. There are no results in *Gryllus* at this time, but hopefully, current investigations in our laboratory will elucidate this problem soon. Even titres of whole animals have to be interpreted with care because in addition to the activity of two hormone sources, the inactivating system (Karlson and Bode, 1969) as a third factor, will modify the titre.

The epidermal cells are known to be activated by ecdysone (Schneiderman and Gilbert, 1964). Whether this is also the case in oenocytes cannot be answered. Some authors believe the corpora allata to be responsible for activation of oenocytes (Day, 1943; Vogt, 1948; Pflugfelder, 1952; Huber, 1958; Schmidt, 1961). It may also be possible that oenocytes are activated by the prothoracic glands. The investigations of Weir (1970) with *Calpodes* are in line with this hypothesis.

b) Microbodies

According to the investigations of Hruban and Rechcigl (1969) the microbodies contain the enzymes catalase and urate oxidase. We should therefore find

ment is characterized by the accumulation of glycogen (*Gly*) in deposits reaching some micrometers in diameter, and by a well-developed rough-surfaced *ER* (*RER*) in connection with the smooth-surfaced *ER* (arrow). Numerous mitochondria border the nuclear membrane. The nucleolus comprises several units, each representing the nucleolus of a diploid cell. Cytolysosomes are only present in the form of residual bodies. c About 50 hours post-moult we find the strongest development of the *ATER*. Between the tubules there are isolated glycogen rosettes (*Gly*). At this time new clefts arise (*Cl*). *Cl* indicates the differentiation of such a structure. The membrane is only half-developed, and parallel elongate granules border it. At this time new isolation membranes (*IM*) arise enclosing distinct plasma areas and their content becomes digested (*Cyl*). d shows the culmination of the lytic activity. Great parts of the *ATER*, numerous mitochondria and microbodies become eliminated in this way. At this time the clefts are still enlarged. The stage is that of an animal shortly before moulting. *IB*, isolation body, Cyl_1–Cyl_3 diverse stages of degradation ending in myelin bodies (*MB*). The *ATER* is dilated at some points to form vesicles (*V*)

catalase in the homogeneous ground substance of the microbodies and urate oxidase in the electron dense cores. In *Gryllus* peroxidase occurrence was shown by light microscopy alone. Peroxidase has a similar mode of action as catalase. The microbodies of *Gryllus* oenocytes never show distinct cores and there is no mention of urate oxidase in the ultrastructural literature on such microbodies. Here the existence of urate oxidase is difficult to understand in relation to the physiology. The enzyme causes the conversion of uric acid to allantoic acid. Its existence in the fat body and in Malpighian tubes (Locke and McMahon, 1970) indicates that in these tissues uric acid becomes transformed to allantoic acid. Because such a function is not known for oenocytes this interpretation must be excluded.

Indications as to the function of microbodies and of catalase in vertebrates may help elucidate their function in the insect tissue studied here. In vertebrates catalase is found mainly in the liver and kidneys. An inhibition of its synthesis by allylisopropylacetamide led to an increase in the serum cholesterol level (Hruban and Rechcigl, 1969). Thus, catalase appears to be involved in the destruction of cholesterol. In presence of intact microbodies it was demonstrated that the liver was able to synthesize taurochol- and glycocholic acid. The position of microbodies near the clefts might be connected with cholesterol metabolism.

Because microbodies are also found in a great number in interstitial cells of rodent testis and because the microbodies sediment together with the microsome fraction, Reddy and Svoboda (1972) conclude that they may contain the enzymes of steroid metabolism—eg. 3-hydroxysteroid-dehydrogenase.

c) Detoxication

Another functional aspect attributed to oenocytes is detoxication. Clark and Dahm (1973) investigated the effect of phenobarbital on the oenocytes of *Musca domestica*. Application of this substance resulted in an increase of ribosomes, of smooth-surfaced *ER*, and of "scrolls". The scrolls correspond to formations found by Locke (1969) in the oenocytes of *Calpodes*. They also might correspond to the clefts of *Gryllus* or to "tubules rigides et encroûtés" in *Locusta* (Rinterknecht *et al.*, 1969). To what extent they are a consequence of phenobarbital treatment, is a question that cannot be answered here. There is no sign of the elimination of these structures during the moulting cycle.

An exact morphometric study of the membranes induced by phenobarbital in the rat liver was carried out by Bolander and Weibel (1973). Following drug treatment, the number of autophagic vacuoles, enclosing membranes but only few microbodies or mitochondria, increased rapidly. Compared with the oenocytes they must detoxicate in the course of moulting because at no other time is such an extensive indication of lytic activity observed. Compared with other tissues the number of autophagic vacuoles is nowhere as great as in oenocytes.

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