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Structural Correlates of Hormone Production by the Corpora Allata in the Pine Moth, *Dendrolimus spectablis* Butler, during Larval-Pupal-Adult Transformations

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松蟲變態에 따른 알라타體의 호르몬生産과 그 構造的變化의 相關

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摘　　要

昆蟲의 알라타體의 호르몬生産과 그構造的變化를 밝히기 위하여 松蟲을 對象으로 變態 各時期의 알라타腺細胞의 微細構造의 變化를 電子顯微鏡으로 追究했다.

미토콘드리아는 越冬에서 最終齡幼蟲까지와 蛹化直後에서 20日蛹까지는 活動相을 보이는 反面 營繭期부터 蛹化直前의 前蛹까지는 不活性相을 보인다.

알라타體의 변두리細胞는 幼蟲期 특히 越冬幼蟲에서는 滑性小胞體의 液胞속에 電子密度가 높은 顆粒을 가졌고 管狀粗性小胞體의 一端이 膨張하여 큶은 滑性小胞體의 液胞로 되며 그中 얼마는 纖維性蛋白質을 含有한다. 한편 두細胞사이의 空腔에도 液胞가 나타나는데 그들은 서로 融合하여 커지지만 이들兩者는 蛹化直前에 없어진다. 그러나 蛹化後 細胞質內液胞는 다시 나타나므로 分泌活動은 再開된다고 생각된다.

알라타體에 와있는 軸索속에 神經分泌顆粒이 羽化直前인 20日蛹부터 5日되는 成蟲까지에서만 나타난다는 事實은 알라타體의 分泌가 이時期에는 腦의 支配下에 있음을 알려주고 幼蟲期부터 早期蛹까지는 그런顆粒이 나타나지 않으므로 그分泌가 腦와는 아무런 關係가 없음을 意味한다.

3日되는 成蟲에서 電子密度가 높은 顆粒이 細胞質속 核부근의 큰液胞속에 나타나고 그液胞는 數와 크기가 增加하므로 核은 5日成蟲에서 畏縮한다. 이것은 아마 腺細胞의 機能退化相일 것으로 생각된다.

따라서 알라타體는 호르몬生産과 그分泌機構에서 볼때 年齡에따라 적어도 두가지 호르몬 即最終齡幼蟲까지 腦의 直接的刺戟없이 幼若호르몬을, 그다음 늦은 蛹期부터 成蟲까지 腦의 刺戟을 받아서 生殖腺刺戟호르몬을 分泌한다고 보며 老熟幼蟲에서 前蛹까지에 觀察되는 分泌相은 아마 ecdysone에 의한 蛋白質合成과 관계가 있을 것이고 또 腦의 支配下에 있지 않는 早期蛹에서의 分泌相은 前胸腺刺戟活動과 관계있을 것으로 생각된다.

INTRODUCTION

It is generally accepted that the juvenile hormone, the secretion of the corpora allata, possesses three kinds of activities; morphogenetic (Wigglesworth, 1954), prothoracotropic (Wigglesworth, 1955; Gilbert, 1962) and gonadotropic (Wigglesworth, 1964; Krishnakumaran and Schneiderman, 1965), but the structural correlates of hormone production by the gland of insects are not still well understood, particularly in the different view of the endopterygous and the ectopterygous insects. Preliminary observation using electron microsope was reported by Schultz (1960) in the hawk moth, *Celerio lineata,* and the more detailed studies on the ultrastructural changes in the allatum cells of the wild silk moth, *Hyalophora cecropia,* during the pupal-adult transformation by Waku and Gilbert (1964). They reported that not only the smooth-surfaced but the rough-surfaced endoplasmic reticula were concerned with the hormone secretion at the active gland of pupa in these endopterygous insects. On the other hand, ultrastructural properties in relation to the secretory product, particularly of adult, were studied in the ectopterygous insects, i.e., in a cockroach, *Leucophaea maderae,* by Scharrer (1961, 1964, 1971) and in a locust, *Schistocerca paranensis,* by Odhiambo (1966a, 1966b). The latter noted that the active allatum cells in *Schistocerca* were dominated by existence of the smooth-surfaced endoplasmic reticulum, while the rough surfaced one took place only in limitted distribution. So he concluded that the smooth-surfaced endoplasmic reticulum was intimately associated with the biosynthesis of the allatum hormone and its extrusion into the haemocoel.

All of these observations were carried out on pupae and/or adults. And hitherto pupae and adults were also used as the sources of all extracts for isolation and identification of the juvenile hormone.

The present report, therefore, examines the ultrastructural changes in the allatum cells, particularly in emphasizing the larval-pupal-adult transformations of an endopterygous insect, the pine moth, *Dendrolimus spectabilis* Butler.

MATERIALS AND METHODS

The overwintered (6th instar) caterpillars of the pine moth, which were collected from their shelters arround the pine trees in the University campus, were reared in the laboratory at 25±1°C, each ten of which were after then used as materials in various stages of overwintered and the last mature larvae, making cocoon, prepupa, pupa just after pupation, 20-day old pupa just two days before emergence of adult and 3-and 5-day old adults.

Glutaraldehyde (Fisher Biological Grade 25% solution) at 4°C was injected into the animals's head and the corpora allata were removed from the head under stereomicroscope. The corpora allata were fixed for two hours in the glutaraldehyde at 4°C and then for an hour in the 1% osmium tetroxide.

For electron microsope the fixed materials were dehydrated in alcohol or acetone and then in propylene oxide and embedded in araldite (Epon 812, Luft, 1961). Silver sections were cut with a glass knife using the Sorvall Proter Blum MT-2 ultramicrotome. 2% uranyl acetate for ten minutes and then lead citrate for five minutes were adopted for staining. Observations were made with the Hitachi HS-7S microscope operated at 50kV.

For light microscope the materials embedded in araldite were semisectioned, following stained with Heidenhain iron haematoxylin, PAS-reagent, haematoxylin-eosin and phloxin.

RESULTS

Light microscopy

The allatum-cadiacum complex of the pine caterpillars consists of tiny ovoid corpus allatum, the covered portion of which becomes shorten as the gland grows in maturing larvae, so that the rest of the gland becomes largely bathed by haemolymph. The corpus allatum of the last mature larvae is the largest, about 72-3μ $\times$ 91-4μ in size and consists of 27 to 32 cells on the median longitudinal plane of the gland but it becomes atrophic and loses the ovoid form in the pupa of increasing age. The histological boundary between the two glands is very distinct during the larval life but becomes obscure in the late pupa.

The nuclear chromatin masses of the corpus allatum cells are homogeneously dispersed almost through the larva to pupa in the pine moth. The nucleus has an eosinophilic nucleolus and eosinophilic substances are observed in the large vacuoles in the peripheral cytoplasm. The cytoplasms of the cells show negative PAS reaction but the fibrous connective tissue layers enveloping the gland positive. The voluminous cytoplasms of the corpus cardiacum cells and the neurosecretory granules in the nerve fibres show positive reactions to the phloxin, while the contents of the allatum cells negative. In the 20-day old pupa, however, the neurosecretory granules within the axons in the corpus allatum show positive to the phloxin as well as these in the corpus cardiacum.

Electron microscopy

1) The hibernated larvae

In the hibernated larvae the inner membrane (140-150Å wide) of the nuclear envelop which is deeply infolding into the cytoplasm is thicker than the outer one (47-52Å wide) and the spherical or elongate mitochondria are numerous with well developed cristae packed in homogeneous electron dense soluble substances (Fig. 1 and 2). Poorly developed Golgi complex occurs occationally in the cytoplasm and it consists of Golgi cisternae which are relatively swollen (100-1000 Å), Golgi vacuoles and Golgi vesicles which contain high electron dense particles (about 90-110Å in size) (Fig. 2). The endoplasmic reticulum also develops inconspicuously but most of them are tubular (110-170 Å wide), while few are vesicular (270-420Å wide). These are the smooth-surfaced endoplasmic reticula but the tubular rough-surfaced ones are often observed, too. Ribosomes and polyribosomes occur free in the cytoplasm and only a few ribosmes are attached to the endoplasmic reticula. Lysosomes (0.5-20μ in size) covered by a single membrane (about 50Å wide) take place in the peripheral cytoplasm, which contain spherical or rod-shaped granules in size from 50×50mμ to 50×250mμ and small spherical bodies in size from a major diameter of 80 to 750mμ which have high electron donse periphery and homogeneous electron opaque contents. The peripheral cytoplams of the peripheral cells of the gland have large vacuoles that are extensive vesicular endoplasmic reticulum, in which electron dense particles (about 140mμ in size) are scattered (Fig. 4). The particles are eosinophilic.

The plasmalemmas between two adjacent cells are interdigited and distinct desmosomes are observed on them particularly of the peripheral cells of the gland and the membrane stacks on the basement membrane.

2) The mature larvae

The nucleus is expanded. The mitochondria have various shapes and ring-shaped one occurs frequently. Their tubular cristae are more well developed comparing with those in the hibernated larvae but the number of the mitochondria as well as the electron densities of the matrices within them are decreasing. And the tubular endoplasmic reticula are observed in a few numbers, while the vacuolar or vesicular endoplasmic reticula increase in the peripheral cytoplasm, within which are observed fibrous proteins or lamellated structures (Fig. 5 and 6). The most of endoplasmic reticula at this stage are smooth-surfaced, while rough-surfaced ones are very few and polyribosomes are plentiful.

A part of the boundary between two adjacent cells is swollen to make the intercellular spaces where the aggregated vacuoles in diameter from 200 to 1000Å occur (Fig.5). The pinosomal vesicles ranged from 750 to 1000 Å in diameter and associated with the pinocytosis are observed in the cytoplasms of these areas.

3) The making cocoon stage

There are no changes in the nucleus comparing with those of the other stages apart from the ribosomes attached to it's outer membrane surface. The mitochondria are mainly ovoid and larger than those of the last mature larvae but the spherical ones are very few. The cristae are vacuolated in the parts and the electron densities of their matrices are light.

The rough-surfaced endoplasmic reticula increase to form cisternae partially, while smooth-surfaced ones are very scarce and the tubular rough endoplasmic reticula are apt to expand in part to become smooth endoplasmic reticular vesicles about 2500mμ in diameter in not only the periplasms but the endoplasms and fibrous proteins and often lamellated structures appear in the lumen of such vesicles (Fig. 6). The ribosomes which are free or attached on the rough-surfaced endoplasmic reticula and polyribosomes are increasing in numbers. The lysosomes also take place in frequence and homogeneous spherical bodies surrounded by a simple membrane and ranged from 340 to 500mμ in size are seen which are presumably the microbodies. The intercellular spaces grow with vacuoles which also increase in number and fused each other to enlarge with the ages to the pupation. (Fig. 5).

4) The prepupal stage

In the prepupae, most of the mitochondria are spherical (ranging from 0.5 to 20μ in diameter) or elongated, their cristae becoming vacuolated and their matrices having high electron dense granules (5000–700Å in diameter) (Fig. 7). The membranous systems in the cytoplasms are inconspicuous. As histolysis takes place in the stage just before pupation, the large smooth-surfaced vacuoles and small vesicular rough-surfaced endoplasmic reticula are not observed, but polyribosomes increase in number.

5) The pupal stage just after pupation

The allatum cells of the pupa just after pupation have the compact cytoplasms compared with that of the prepupae. The mitochondria show various shapes, i.e., spherical (in size of 0.1μ in diameter), rod-shaped (in size of more than 2μ in length) and sometimes horse-shoe shaped, their cristae being well developed and their matrices showing relatively light electron densities. There are few tubular and vacuolar endoplasmic reticula but many vesicular ones appear place by place in various sizes, within which fibrous proteins are contained in part (Fig. 8). The Golgi complex, ribosomes, polyribosomes, microbodies, lysosomes and lamellated structure occur in this stage, too. The plasmalemmas are less interdigitated compared with those of the overwintered larvae.

6) The 20-day old pupae

The allatum cells of the 20-day old pupae about 2-days before the emergence have compact nuclei and cytoplasms. The nuclei are extensive and the chromatins are scattered homogeneously. The mitochondria are large and also various in shape, spherical (in size of 0.4μ in diameter), rod-shaped (more than 2.0μ in length) and ring-shaped but are quite different in form from that of the other stages. Their cristae are tubular and complex and their matrices electron light. The endoplasmic reticula are inconspicuous but the smooth-surfaced vacuoles grow further. There are many free ribosomes, polyribosomes and tracheoles distributed widely even into the center of the gland.

The neurosecretory granules, which are electron dense and range from 540 to 1150Å in diameter, are observed accompanied with mitochondria within the axons terminated in the corpus allatum at this stage as well as those in the corpus cardiacum (Fig. 9). These granules are of two kinds, electron dense and less dense, the former being more numerous than the latter.

7) The 3-and 5-day old adults

The electron microscopic scene of the allatum cells is quite similar with the overwintered larvae. The cytoplasms are compacted and mitochondria are more simple in shape. The swollen smooth-surfaced vesicles near the nucleus contain secretory electron less dense granules than that in the overwintered larvae (Fig. 10), enlarging in contact with the nucleus, so that the nucleus is curved in the 5-day old adult (Fig. 12). This seems to be of degenerating phases of the allatum cells.

The neurosecretory granules surrounded with simple membrane within the axons in the corpus allatum occur as same as in the late pupae but the axons appear in the endoplasm more near the nucleus than that of the late pupa (Fig. 11). The corpus cardiacum becomes atrophic in size and the neursecretory granules within the cardiacum cells also become reduced in number.

DISCUSSION

In the pine moth, the mitochondria also vary in their numbers, sizes and structures with the ages. The small and simple forms of mitochondria are numerous in the cytoplasms of the hibernated caterpillars, whose cristae are relatively well developed and matrices contain electron dense soluble substances. In the last instar larvae the ring-shaped mitochondria occur frequently, while the number of the mitochondria are decreasing and electron density of the matrix becomes light. In the making cocoon stage they are mainly ovoid and larger than those of the last instar, while still decreasing in numbers. At the prepupae most of the mitochondria are large spherical and their cristae are vacuolated in parts and matrices contain electron dense granules. In the pupa just after pupation, small and large mitochondria appear in a large number, their matrices being electron dense and their cristae well developed just like those of the hibernated larvae. In the 20-day old pupae the mitochondria decrease in number but have various forms. In the corpus allatum of the pine moth, therefore, the mitochondria are in active phases from the hibernation to the last instar and from the pupae just after pupation to the 20-day old pupae, while they are in inactive phases from the making cocoon to the prepupal staeg just before pupation.

The endoplasmic reticula are in general inconspicuous and most of them are smooth-surfaced but the rough-surfaced ones occur more numerous in the making cocoon and prepupal stages than in the other stage of the pine moth. The tubular and vacuolated endoplasmic reticula are also observed in the cytoplasms of the overwintered larvae and the tubular endoplasmiic reticula seem to expand in part to become vesicular ones, within which are contained electron dense secretory granules which are identical with the eosinophilc substances seen under the light microscope. These vesicular endoplasmic reticula from the last instar to the prepupa contain not the electron dense granules such like those of the hibernated larvae but the fibrous proteins.

Scharrer (1964) and Waku and Gilbert (1964) reported that not only the smooth-surfaced endoplasmic reticula but the rough-surfaced ones are concerned with the hormone secretion at the active gland in *Leucophaea* and *Hyalophora*, respectively. And Odhiambo (1966a, 1966b) noted that in active gland, mitochondria are small, more or less spherical and simple in structure, while in inactive gland the mitochondria are numerous but only very rarely do they exhibit intra-mitochondrial granules. The results obtained in the pine moth agree with these observations in parts.

The fact that free ribosomes and polysomes appear in a large number in addition to the rough-surfaced endoplasmic reticulum containing fibrous proteins within from the making cocoon to the prepupal stages means that the biosynthesis of protein takes place in these stages, as the secretion of ecdysone increase gradually. On the other hand, in addition to increase of vacuolated or vesicular endoplasmic reticula in the cytoplasms, the aggregated vacuoles increase also in number in the intercellular spaces from the mature larva and then are fused each other to enlarge until the prepupa (Kim *et al*, 1972). In actively secreting corpora allata of the locust, Odhiambo (1966a,b) noted that progressively swollen vesicles of smooth-surfaced endoplasmic reticulum could be traced toward the basement membrane materials and appear to empty there, so that he thought the content would be juvenile hormone. The present author also explained these vacuoles might contain the product of biosynthesis in the allatum cells, having prothoracotropic, activity, at the 14 International Congress of Entomology at Canberra but after reexamination, he guess this may be of the atrophic phases of thegland cells. The properties of the products within the intra-and inter-cellular vacuoles of the pine moth, whether they are same material or not, will be examined by histochemical methods in future.

The fact that these vacuoles above mentioned disappear during the pupation and new endoplasmic reticular vesicles develop from the new pupa means that the gland begins to product the secretions which retain presumably the prothoracotropic activity, because it is well known that in Lepidoptera, at least, the juvenile hormone activates the prothoracic gland and mimics the brain hormone, and that if the secretion of the jovenile hormone ceases at the pupal stage, the prothoracic gland atrophies.

The fact that the neurosecretory granules appear only in the late pupa and early adult suggests that the secretion from the allatum cells of the pupa and adult is under the control of the brain, while the secretion of the juvenile hormone during the larval life has no relation with the brain hormone, because such granules are not observed within axons in the larval gland. Even though the same secretory granules occur in the smooth-surfaced vesicles in the allatum cells of the overwintered larvae and of the early adult, the juvenile hormone of larval life is supposed to be different from that of the adult in the aspect of production and secretion mechanisms. The gonadotropin seems to be released by getting stimuli or precursory materials from the neurosecretory granules in the axons as suggested by Wigglesworth(1954), although the juvenile hormone of the larval life does not so.

This is quite sure by the well known facts that the juvenile hormone is not concerned with the development of the ovary in the larval life but when the secretion of the juvenile hormone falls off, the differentiation in the gonads takes place and then in the adult the corpus allatum again becomes active to have a gonadotropic effect and to promote the deposition of yolk. Sägessor (1960) reported that the corpus allatum in *Leucophaea* seemed to product at least two different hormones: a juvenile hormone and a gonadotropic hormone. Krishnakumaran and Schneiderman (1965) noted that the lipid extracts of the pupa in cecropia moth also had morphogenetic prothoracotropic and gonadotropic activies and that the corpus allatum was the source of at least two of these three activities. The results observed in the pine moth are identical with those facts.

It is, therefore, suggested that at least two kinds of hormones are released with the ages as far as concerned with the production and secretion mechanisms of the allatum hormone: juvenile hormone is released until the last instar larvae without any direct stimulation of the brain and gonadotropic hormone is secreted from the late pupa to the adult by getting of the brain's stimulation and that the secretory phases observed from the mature larvae to prepupae are presumbly concerned with the biosynthesis of protein owing to the ecdysone and those from the early pupal stage under uncontrolled condition by the brain with the prothoracotropic activity.

SUMMARY

Ultrastructural changes in the cells of the corpora allata of the pine moth, *Dendrolimus spectabilis* Butler, were studied by electron microscope to know the structural correlates of hormone production by the gland during the larval-pupal-adult transformations.

Mitochondria are in active phases from the overwintered to the last instar larvae and from the pupae just after pupation to the 20-day old pupae, while they are in inactive phases from the making cocoon stage to the prepupae just before pupation.

The peripheral allatum cells have electron dense granules in the intracellular vacuoles of smooth-surfaced endoplasmic reticulum in the larval life, particularly in the overwintered larvae and in the early adults but the swollen smooth-surfaced intracytoplasmic vacuoles made by expansion of an end of the tubular rough endoplasmic reticulum, some of which contain fibrous proteins, are observed in addition to the vacuoles in the intercellular spaces in which the vacuoles grow by fusing each other from the mature larvae to the prepupae, both of them disappearing during just before pupation. After pupation the cytolasmic vacuoles develop again in the allatum cells so that they seem to begin the secretory activity.

The fact that the neurosecretory granules stored within the axons terminated in the corpus allatum are visible only from the 20-day old pupa about two days before abult emergence to the 5-day old adult means that the secretion from the allatum cells is under the control of the brain from the late pupal stage, while the secretion during from the larval to the early pupal life has no relation with the brain, because such granules are not observed within the axons.

It is, therefore, suggested that at least two kinds of hormone are released with the ages as far as concerned with the production and secretion mechanisms of the allatum hormone: juvenile hormone is released until the last instar larvae without any direct stimlation of the brain and gonadotropic hormone is secreted

from the late pupa to the adult by getting brain's stimulation and that the secretory phases observed from the mature larvae to prepupae are presumably concerned with the biosynthesis of protein owing to the ecdysone and those from the early pupal stage in uncontrolled condition of the brain with the prothoracotropic activity.

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EXPLANATION OF THE PLATES

Abbreviations on Plates

AX neurosecretory axon — ED electron-dense body
g Golgi complex — M mitochondria
N nucleus — NE nuclear envelope
ngs_1 neurosecretory granule (electron transparent)
ngs_2 neurosecretory granule (electron opaque)
DRB polyribosome — PM plasmamembrane
RB ribosomes — RER rough endoplasmic reticulum
SER smooth surface endoplasmic reticulum — SV secretory vacuole (cytoplasmic vacuole)
T tracheole — V vesicle
Bar lines of Fig. 2 and 4 indicate 0.5μ and others 1μ.

Fig. 1—3. Electron micrographs of the corpus allatum from the overwintered larva. Fig.1 shows the central cells of the corpus allatum, having infolded nuclear envelope (×14,080) and Fig. 2 Golgi complex, ribosomes, vesicles, and cisternae of the rough endoplasmic reticulum (×36,024). Fig.3 reveals the portion of a peripheral cells from the corpus allatum: Note high concentration of mitochondria and electron dense granules in intracellular vacuoles (×7,975).

Fig. 4. The allatum cell from the 8th instar larva, showing the intercellular space at the adjacent region of two gland cells, within which vacuoles take place. Note pinosomes poring at the plasmalemma (arrow). (× 26,960).

Fig. 5. The making cocoon stage, showing cytoplasmic electron dense bodies, extensive vacuolar smooth-surfaced and lamellated rough surfaced endoplasmic reticula, particularly vesicles made by expansion of an end of the rough-tubular endoplasmic reticulum (×15,360).

Fig. 6. The prepupal stage. Note numerous ribosomal aggregates including polyribosomes, rough-surfaced endoplasmic reticulum, lamellated structure and spherical

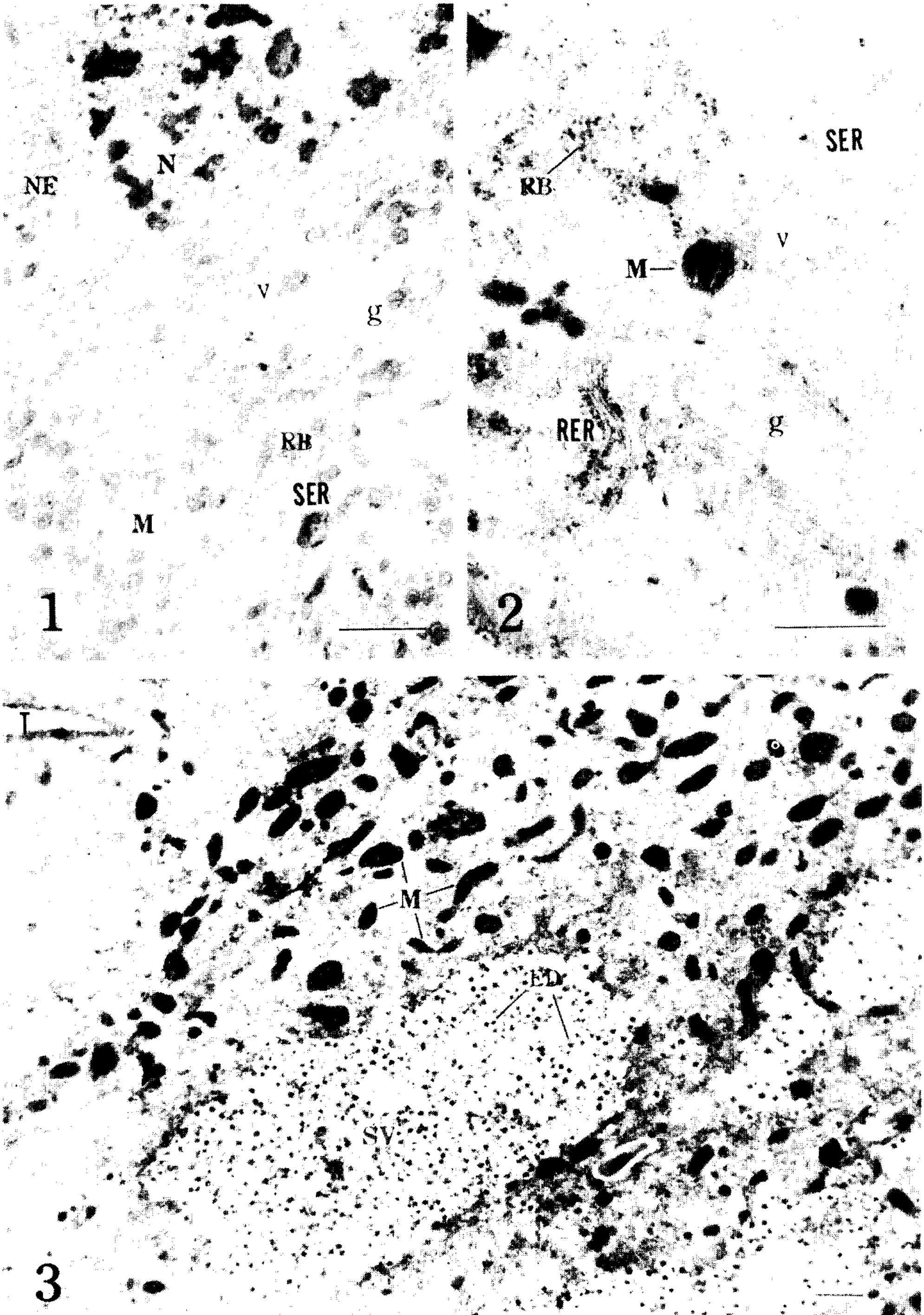
mitochondria containing partial bulbed cristae with intramitochondrial electron dense granules (×15, 600).

Fig. 7. The pupa just after pupation, revealing numerous mitochondrial and extremely extensive smooth-surfaced intracytoplasmic vacuoles in the endoplasm (×11, 235).

Fig. 8. The 20-day old pupa. Note annulated mitochondria increasing greatly in number and size and neurosecretory axon containing two types of neurosecretory granules, electron dense and less dense. (× 16, 680).

Fig. 9. The 3-day old adult, showing numerous mitochondria, enlarged smooth-surfaced intracytoplasmic vacuoles within which appear electron dense particles and neurosecretory axon(×7, 569).

Fig. 10. The 5-day old adult. Note large smooth-surfaced vesicles containing electron dense granules, in contact with the curved nucleus (×9, 459).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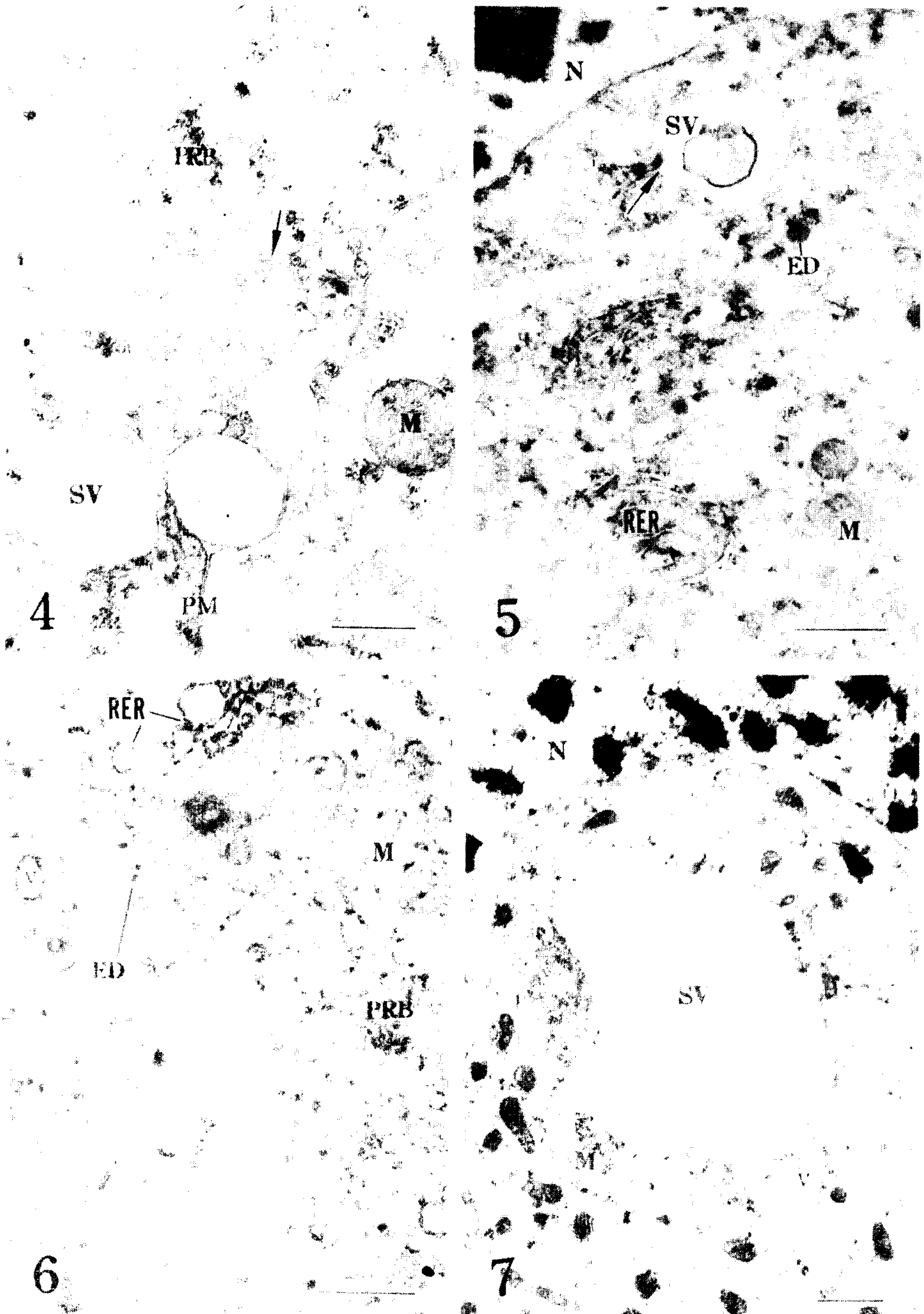
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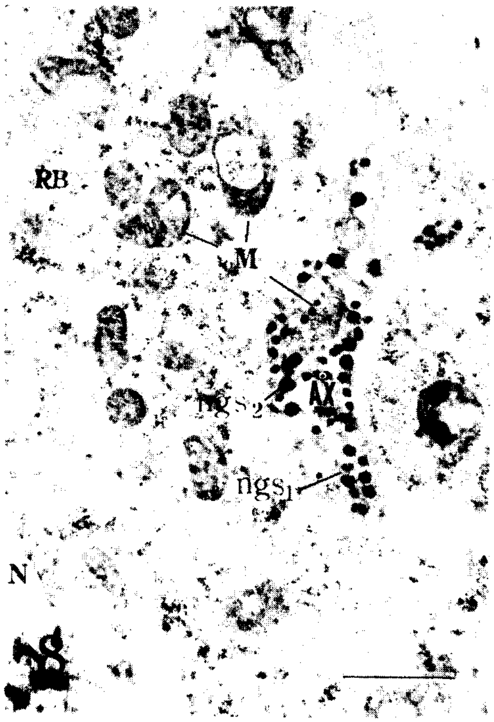
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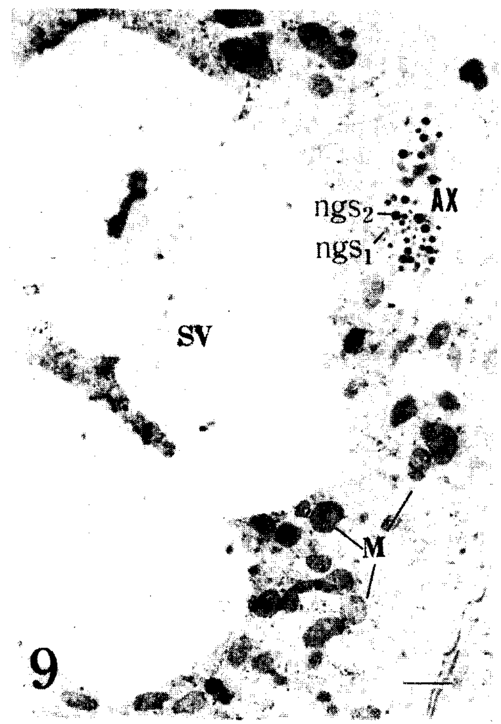
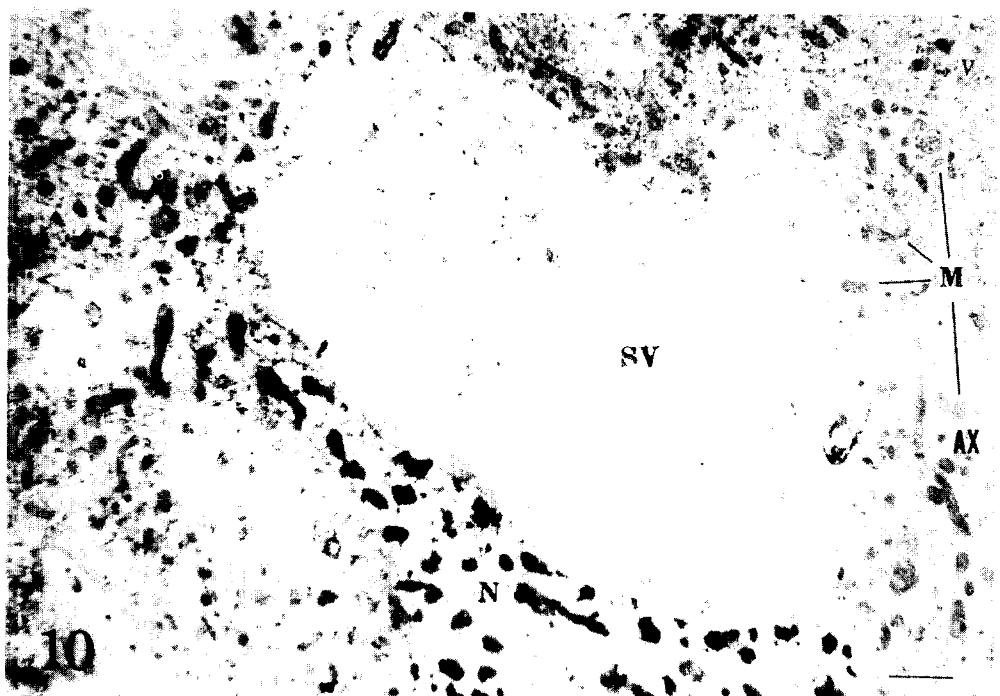
N
8


ngs2
AX
ngs1
SV
M
9


V
M
SV
AX
N
10