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An Ultrastructural Study on Larval Hemocytes of *Orthoptera*

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메뚜기류幼虫의 血球에 대한 微細構造

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적 요

방아깨비, 사마귀, 섬서구메뚜기의 종령유충을 재료로 하여 전자현미경적 관찰을 하였다.

Prohemocyte는 세포중 가장 작고 구성비도 낮으며 그 구조상 다른 혈구의 기원이 되는것 같다.

Plasmatocyte는 나비류에서와는 달리 그 형태가 대부분 卵型이고 허족과 소기관을 가장 많이 가진다.

Granular Cell은 종에 따라 그 형태가 뚜렷이 구분되며 어느 경우나 구성비는 높았다. 같은 세포의 과립이라 할지라도 그 미세구조가 몇 가지로 구분되고 이것은 아마도 축적단계에서 오는 결과인듯 하다.

Adipohemocyte는 방아깨비에서만 나타났고 구성비는 대단히 낮았다.

INTRODUCTION

Numerous structural studies on insect hemocytes based on light microscopy have been made in various insects (Jones, 1962; Wigglesworth, 1965; Gupta et al., 1967), and some 6 types of hemocytes were noticed. Some observations on insect hemocytes based on electron microscopy have also been made (Hoffmann. 1966; Akai, 1969, 1971; Akai and Sato, 1973; Raina, 1976; Yu et al., 1976, 1977a, b) and 7 types of hemocytes were described; prohemocytes, granular cells (or granular hemocytes), spherule cells, oenocytoids and adipohemocytes. However, ultrastructural studies concerning the hemocytes of Orthoptera, except for rare cases by Hoffman (1968) and Yu et al. (1977), are not available. And the present study was undertaken to observe ultrastructures of hemocytes of mature last-instar larvae of *Acrida cinerea* Thunberg, *Paratenodera sinensis* and *Atractomorpha lata*, and to examine if any functional interpretations could be derived from their fine structural organizations.

MATERIALS AND METHODS

The larvae of three species of Orthoptera *Acrida cinerea* Thunberg, *Paratenodera sinensis* De Sauassure and *Atractomorpha lata* Hotschulsky) were reared at 25°C and mature last-instar larvae were used as materials. Each hemolymph obtained by cutting hind legs was collected directly in each centrifuge tube containing cold 4% glutaraldehyde in phosphate buffer (pH 7.4). After 1 hr. the hemolymphfixative mixture was centrifuged at 1,500 rpm. for 10 min. and then the pellets were washed in several changes of phosphate buffer (pH 7.4).

These pellets were post-fixed with 1% OsO_4 in phosphate buffer (pH 7.4) for 2 hr. and washed again in three changes of phosphate buffer. Then the pellets were dehydrated in graded concentrations of acetone and finally embedded in Epon 812 mixture. Thin sections were double-stained with uranyl acetate saturated in 70% ethanol for 20 min. and with Reynolds lead citrate for 10 min. Observat-

ions were made with Hitachi HS-7S electron microscope.

OBSERVATIONS

Three common types of hemocytes, namely prohemocytes, plasmatocytes and granular cells, were observed in all species used, and one additional type, i. e., adipohemocytes, was confirmed only in *Acrida cinerea* Thunberg. Spherule cells and oenocytoids reported mainly in Lepidoptera and crystal cells reported only in *Drosophila* were not found in the present study.

Prohemocytes were least common of all cell types accounting for less than 1% of all cell numbers. They were oval or round with a centrally placed nucleus. They were about 5×7μ in dia. and had not any cytoplasmic projections. Cytoplasmic organelles, such as endoplasmic reticulum (ER), Golgi complexes, mitochondria and vacuoles were less developed, while free ribosomes were relatively abundant (Yu et al., 1977a). Any considerable differences were not present between three species.

Plasmatocytes were more or less spherical and were 7~12μ in dia. with a centrally located nucleus and with different composition ratios (5% in *A. sinensis*, 20% in *P. sinensis*, 9% in *A. lata*). Almost of the plasmatocytes had numerous cytoplasmic projections. And the cytoplasmic organelles, such as ER, Golgi complexes, vacuoles, lysosomes were relatively well developed.

The granular cells were the most pleomorphous of all cell types and the composition ratios were very high in all cases, accounting for more than 80%.

In *Acrida cinera* Thunberg, three kinds of granular cells were noticed; oval, filiform and spindle-shaped, the last being the commonest. Spindle-shaped cells measured about 5×20μ. while the filiform cells being about 5×30 or even 3×50μ (Fig. 1). In both cases, the cells had elongated nucleus and few cytoplasmic projections, but cytoplasmic organelles were relatively well developed.

The oval cells were about 12μ in dia. with several cytoplasmic projections. The granules of the granular cells of *A. cinera* were round or oval and sometimes they were seen as crystals.

Granular cells were very pleomorphic in having granules. Younger forms had few granules, and under the light microscope they might be seen as plasmatocytes (Fig. 7), while in older forms they contained many granules (Fig. 8). In certain cells the granules themselves showed a polymorphism. The commonest granules are made up of denser materials and peripheral less dense materials (Fig. 9-a). The second type is composed of inner fine granular materials and peripheral less dense layer (Fig. 9-b). And the third types are made up inner flocculent materials and outer less dense material (Fig. 9-c), while fourth types are composed of tiny microtubules arranged regularly (Fig. 10). In addition to the granules described above, certain cells contain several lipid droplets(Fig.. 9).

In *Paratenodera sinensis* De Saussure, the granular cells could be subdivided into two forms; spindle-shaped and oval. The majority of the granular cells is spindle-shaped with elongated nucleus, small number of granules, several lipid droplets and relatively well developed cytoplasmic organells (Fig. 2). The oval ganular cells have lobulated nucleus large number of granules, many lipid droplets and poorly developed cytoplasmic organelles (Fig. 3). Sometimes the granular cells contain may microtubules believed to be cytoskeleton (Fig. 11). Three kinds of granules were noticed in this species; dense granules composed of homogenous materials, granules composed of microtubules and granules made up of two materials of different opacity. The first resemble the usual granules occuring in many insects (Fig. 2) and the second in similar to the granules of the cells of *A. cinerea* described above (Fig. 11). The third form, as in *A. cinerea*, are composed of two kinds of materials of different opacities, inner and peripheral.

In *Atractomorpha lata* Hotschulsky, the granular cells could be subdivided into three forms; dark cells, light cells and younger cells rich in ER. The granules of these cells are smaller than those of *A. cinerea*, while their opacities are fairly similar to those of the latter (Fig. 4, 5, 6). All of the

granules of *A. lata* are composed of homogenous materials and the two material containing granules were never found.

Adipohemocytes were noticed only in *A. cinerea*. They are oval (about 12μ in dia.) and rich in lipid droplets. Owing to the heavy accumulation of lipid droplets, the nucleus was pushed aside. In certain cells small vacuoles and spherules composed of flocculent materials were noticed. And some cells have thick cytoplasmic processes compacked with concentrically arranged rough ER and have microtubules located in peripheral cytoplasm (Yu et al., 1977a).

DISCUSSION

It is well known that the common cell types occuring in all insects are prohemocytes, plasmatocytes, and granular cells. In many cases, the insects may have additional cell types, such as spherule cells, oenocytoids, adipohemocytes and crystal cells, each-species having one or more of them (Akai and Sato, 1973; Lai-Fook, 1973; Raina, 1976; Yu et al., 1976, 1977a, b).

The prohemocytes, because of their poor cytoplasmic organelles, were considered as innature cells (Wigglesworth, 1965) or as stem cells (Lai-Fook, 1973). And they were often considered as young plasmatocytes. In the present study the prohemocytes had not any lysosomes or phagosomes indicating that these cells are not involved in phagocytosis. Two kinds of plasmatocytes have been noticed in various insects (Akai and Sato, 1973; Raina, 1976). Nittono (1960), however, pointed out that the plasmatocytes are spindle-shaped for only a few minutes after withrawal and they rapidly undergo extensive transformations into spherical, oval, pear-like and amoebic froms. Gupta and Sutherland(1966) suggested that the plasmatocytes are the basic type from which other types are derived by secondary transformations. In the present observation almost of the plasmatocytes were oval or amoebic with numerous cytoplasmic projections and well developed cytoplasmic organelles, perhaps indicating phagocytotic activities. Most frequently occuring cell types in the present study were granular cells, which have often been called coagulocytes, hyaline cells (Grégoire et al., 1950), amoebocytes, adipohemocytes (Wigglesworth, 1956) and phagocyte (Ögel. 1955). The usual forms of granular cells are round or oval with external cytoplasmic processes (Gupta and Sutherland, 1966; Aaai and Sato, 1973; Raina, 1976). In the present work, however, the majority of the granular cells were spindle-shaped or filiform with few cytoplasmic projections. In *Pieris rapae*, the components of the granules have been described as polysaccharide and lipid (Kim et al., 1968). And Arnold et al. (1967) reported that the granules showed strong PAS reaction and negative reaction for acid mucopolysaccharide thus indicating neutral polysaccharide. In the present study the granules differed in morphology and opacity according to species. The granular cells have been described as the most active cell types in many insects. According to Akai et al. (1973) and Neuwirth (1974), the granular cells have phagocytic activity. Other functions, such as formation of basement membranes (Wigglesworth, 1965), controlling of prothoracic glands (Highman (1958b) and coagulation (Grégoire, 1955, 1970) were described in several insects.

A progressive accumulation of lipid droplets was noticed in the granular cells of pink bollworm (Raina, 1976). Gupta and Sutherland (1966) and Neuwirth (1973) also noticed a similar transformation of granular cells into cells that are often called adipohemocytes. And in *A. cinerea* the adiopohemocytes were noticed, though no progressive accumulation of lipid droplets was found. And it might be impossible, at least in this species, that adipohemocytes are transformed from the granular cells by progressive accumulation of lipid droplets.

SUMMARY

An electron microscopical study on hemocytes of last instar larvae of Orthoptera (*Acrida cinerea* Thunberg, *Paratenodera sinensis* De Saussure, *Atractomorpha lata* Hotschulsk was performed to

observe cell types, structures and their origins.

Prohemocytes are the smallest round cells with large nucleus, poorly developed cytoplasmic organelles and lower composition ratio, accouting for less than 1% of all cell types. Little remarked differences were revealed among different species.

Plasmatocytes are relatively large cells with higher composition ratio, accounting for 5 to 20%. The majority of the plasmatocytes are oval forms with numerous external cytoplasmic projections., relatively well developed cytoplasmic organelles.

Granular cells are characterized by various granules in cytoplasm. Some considerable remarked differences were revealed among different species. Furthermore the granules themselves differ in fine structures. Adipohemocytes were found only in *Acrida cinerea* Thunberg.

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Explanation of Figures

Abbreviation

ER ; Endolpasmic Reticulum
L ; Lipid
M ; Mitochondria
Nm; Nuclear membrane
Gr ; Granule
Ly ; Lysosome
N ; Nucleus

Fig. 1. An elongated granular cell of *Acrida cinerea* Thunberg. ×4,000.

Fig. 2. A spindle-shaped granular cell of *Paratenodera sinensis* De Saussure including granules and lipid droplets. ×10,400.

Fig. 3. A granular cell of *Paratenodera sinensis* De Saussure, heavily packed with granules and lipid droplets. ×8,900.

Fig. 4~6. Granular cells of *Atractomorpha lata* Hotschulsky.

4. including electron dense granules. ×7,000.
5. showing well developed rough ER. ×7,500.
6. showing two types of cell, light (whole appearence) and dark (arrow). ×6,700.

Fig. 7~12. Granular cells of *Acrida cinerea* Thunberg.

7. including small numbers of granules. ×8,700.
8. heavily packed with electron dense granules, ×5,900.
9. Higher magnification, showing numerous granules of various shape. ×18,500.
10. Higher magnification, showing another granules (arrow) build up of microtubules. ×19,700.
11. Granular cell of *Paratenodera sinensis* De Saussure, including microtubules(arrow). ×3,600.
12. Granular cell of *Atractromopha lata* Hotschulsky with specialy structured ER (arrows). ×16,500.


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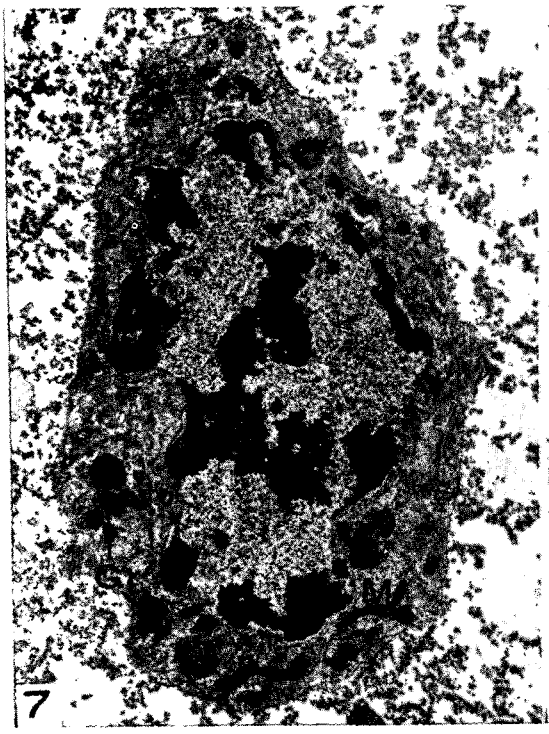
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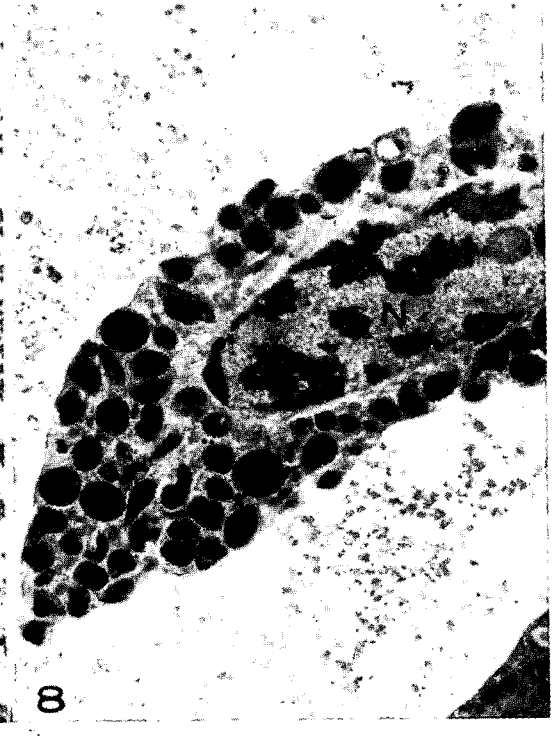
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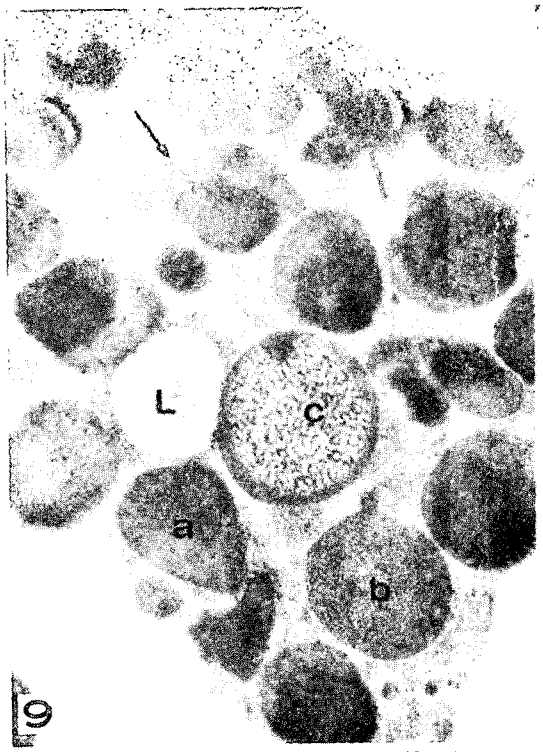

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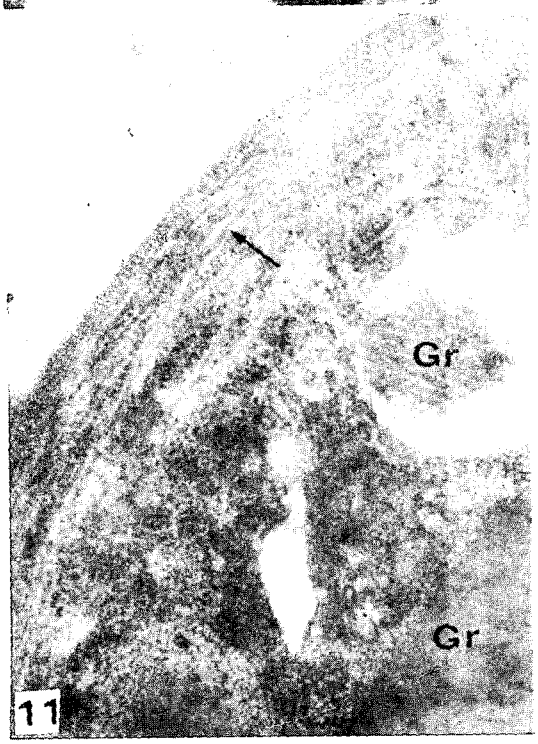

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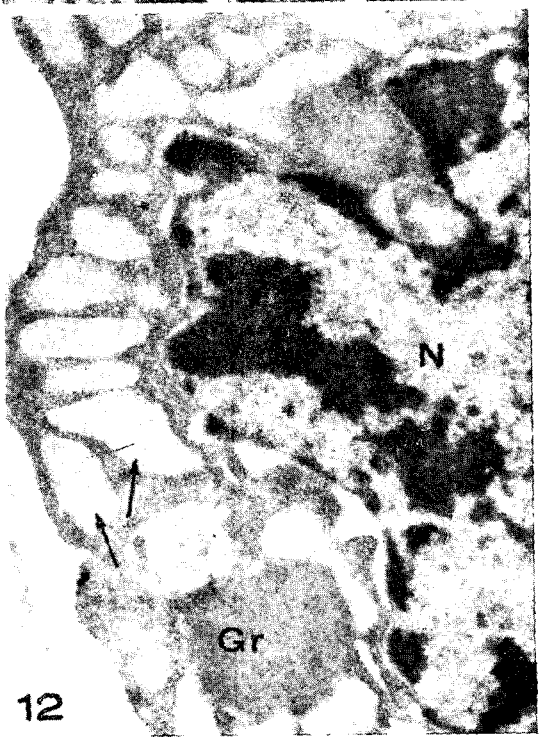

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