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An Ultrastrnctural Study on Larval Hemocytes of *Acrida cinerea* Thunberg

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방아깨비 종령유충의 혈구에 대한 전자현미경적 연구

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摘 要

방아깨비의 종령유충을 재료로 하여 혈구의 미세구조를 관찰한 바 4가지의 혈구 형태를 구분해 냈다. Prohemocyte는 크기가 가장 작으며 미분화된 소기관을 가졌고, Plasmatocyte는 그 수가 비교적 많고 발달된 소기관을 가졌으며, Granular cell은 방추형 또는 난형으로 세포질내에 몇가지 과립을 가진 것이 특징이다. Adipohemocyte는 많은 지방과립을 함유하고 microtubule 특징적으로 나타났다.

INTRODUCTION

The structures and functions of insect hemocytes based on light microscopic studies have been described and the hemocytes have been classified into 5 or 6 groups(Jones, 1962; Wigglesworth, 1965). Electron microscopy has greatly faciliated the study of insect hemocyte structure (Hoffmann et al., 1968; Harpez et al., 1969; Scharrer, 1972; Akai and Sato, 1973; Lai-Fook, 1973; Neuwirth, 1973; Ratcliffe and Price, 1974; Raina, 1976; Akai and Sato, 1971) and at least 4 types of hemocytes have been confirmed. However, ultrastructural studies concerning the hemocytes of Orthoptera, except for one by Hoffmann(1968), are not available. And the present study was undertaken to observe ultrastructure of hemocytes of mature last-instar larvae of *Acrida cinerea* Thunberg and to examine if any functional interpretations could be derived from their fine structural organization.

MATERIALS AND METHODS

The larvae of *Acrida cinerea* Thunberg were fed *ad libitum* at 25°C and mature last-instar larvae were used as materials. Hemolymph obtained by cutting hind legs was collected directly in a centrifuge tube and fixed in cold, buffered 4% glutaraldehyde(pH 7.4) for 1 hr. After fixation the hemolymph-fixative mixtre was centrifuged at 1,500 rpm. for 10 min. and then the pellets were washed in several changes of phosphate beffer(pH 7.4). These pellets were post-fixed with 1% OsO_4(pH 7.4) in phosphate buffer for 2 hr. and were washed again in two 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 Rey nolds lead citrate(Reynolds, 1963) for 10 min. Observations were made with Hitachi HS-7S.

OBSERVATIONS

Four types of hemocytes were observed in last-instar larvae of *Acrida cinerea* Thunberg, and the hemocytes observed were prohemocytes, plasmatocytes, granular cells, and adipohemocytes. Prohemcytes and adipohemocytes were least common, while granular hemocytes were most

abundant. Spehrule cells and oenocytoids reported mainly in lepidoptera were not found in these larvae.

Prohemocytes were ovoid or amoebic cells with a large centrally placed nucleus. They were smallest($5\times7\mu$) of all cell types and had not any pseudopodium-like projections. Cytoplasmic organelles, such as endoplasmic reticulum, Golgi complexes, mitochondria and vacuoles were less developed, while free ribosomes were relatively abundant(Fig. 1).

Plasmatocytes were more or less spherical and were $7\sim12\mu$ in diameter with a centrally located nucleus. All most of the plasmatocytes had numerous pseudopodium-like processes and myelinated bodies. Cytoplasmic organelles, such as Golgi complexes, enoplasmic reticulum, vacuoles and free ribosomes were well developed(Figs. 2, 3)

Two kinds of granular cells were noticed; spindle-shaped and oval. Spindle-shaped cells measured about 5×20 or even $3\times40\mu$ with an elongated nucleus centrally located(Figs. 6, 7). This cell type had several cytoplasmic processes and cytoplasmic organelles were relatively well developed. The second type is oval or round with few pseudopodium-like projections(Figs. 4, 5, 8, 9, 10).

In both cases, the granular cells are characterized by numerous granules embedded in cytoplasm. The granules are round or oval and sometimes they were seen as crystals. Granular cells are very pleomorphic in having granules. Earlier forms have few granules, and under the light microscope they might be seen as plasmatocytes (Figs. 7, 8, 9), while in late forms they contain many granules(Figs. 6, 10). In certain cells the granules themselves show a polymorphism. The commonest granules are made up of dense material and peripheral less dense material(Fig. 11-a). The second types are composed of inner fine granular material and peripheral less dense layer (Fig. 11-b, c). And the third types are made up of inner flocculent material and outer less dense material(Fig. 11-d), while fourth types are composed of microtubules arranged regularly(Fig. 12-e). In addition to the granules described above, certain cells contain lipid droplets(Fig. 11-arrow), myelinated bodies(Figs. 13, 14), vacuoles(Figs. 5, 14) and masses of flocculent material(Fig. 13).

One of the infrequent cell types is adipohemocyte with rich lipid droplets in its cytoplasm(Figs. 15, 16, 17). Adipohemocytes are oval and are about 12μ in dia. Owing to the accumulation of lipid droplets, the nucleus was pushed aside(Figs. 15, 16). 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 endoplasmic reticulum(Fig. 16-arrows) and microtubules located in peripheral cytoplasm (Figs. 16, 17).

DISCUSSION

Crossley(1964) suggested that classification based solely on morphology could be misleading. However, some recent studies favor the concept of ultrastructural integrities of various hemocyte types(Akai and Sato, 1973; Lai-Fook, 1973; Neuwirth, 1973; Raina, 1976).

The prohemocytes, because of their poor cytoplasmic organelles, were considered as immature cells(Wigglesworth, 1965) or as stem cells (Lai-Fook, 1973). And Gupta and Sutherland(1966) considered prohemocytes as young plasmatocytes. In *Acrida cinerea* Thunberg the prohemocytes had not any lysosomes or pinocytotic vacuoles. So the absence of such cytoplasmic organelles may indicate that these cells are not involved in phagocytosis.

Two kinds of plasmatocytes have been noticed in various insects(Akai and Sato, 1973; Raina, 1976; Ratcliffe and Price, 1974). However, in the present observations the spindle-shaped forms were not identified. Nittono(1960) 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 forms. Gupta and Sutherland(1966) suggested that plasmatocytes are the basic type from which other types are deriv-

ed by secondary transformations. Clark and Harvey(1965) reported that plasmatocytes are involved in the formation of cellular membranes during wound healing and Wigglesworth(1973) indicated that basement membrane in *Rhodnius* was formed by plasmatocytes. The plasmatocytes in *A. cinerea* have many pseudopodium-like processes, vacuoles and several lysosomes, and it may be possible that the plasmatocytes are involved in some activities, such as formation of basement membranes, healing of wound tissues or even phagocytosis.

Granular hemocytes have often been called coagulocytes, amoebocytes, phagocytes, adipohemocytes, or hyaline cells(cf Lai-Fook, 1973). The usual forms of granular cells are round or oval with external cytoplasmic processes(Akai and Sato, 1973; Gupta and Sutherland, 1966; Raina, 1976). However, in the present work two kinds of granular cells were noticed; spindle-shaped and oval. Spindle-shaped types are about 3μ wide and 30μ long or even to 40μ in length with few cytoplasmic processes and they might be considered as plasmatocytes at the light microscopic level. While oval-shaped types are about 7 X 10μ with no cytoplasmic processes. In both cases the characteristic granules are contained. One of the undisputed functions of the granular hemocytes is phagocytosis(Raina, 1976). In *A. cinerea* the vacuoles, Golgi complexes and lysosomes are relatively well developed, and because of such organelles, it is supposed that the granular cells in this insect are also involved in phagocytosis.

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 hemocytes into cells that are often called adipohemocytes. And in *A. cinerea* the adipohemocytes were noticed, though no progressive accumulation of lipid droplets was found. Few lipid droplets were contained in granular cells, while all of the adipohemocytes had very large quantity of lipid droplets. And it might be impossible, at least in *A. cinerea*, that adipohemocytes are transformed from the granular cells by progressive accumulation of lipid droplets.

SUMMARY

The ultrastructures of hemocytes of *Acrida cinerea* Thunberg were studied and 4 types of hemocytes were noticed; prohemocytes, plasmatocytes, granular cells and adipohemocytes.

Prohemocytes were the smallest of all cell types with poor cytoplasmic organelles, such as endoplasmic reticulum, Golgi complexes, vacuoles and lysosomes. Plasmatocytes were round or oval with many cytoplasmic processes, vacuoles, endoplasmic reticulum and even myelinated bodies. Granular cells were spindle-shaped or oval. In both cases, they are characterized by various granules. Adipohemocytes were very rich in lipid droplets and microtublles.

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EXPLANATION OF FIGURES

(Abbreviation)

Cy : cytoplasm
L : lipid droplet
M : mitochondria
My : myelinated body
Ps : cytoplasmic process
Gr : granule
Ly : lysosome
Mt : microtubule
N : nucleus
V : vacuole

Fig. 1. Prohemocyte with a large nucleus.

Figs. 2, 3. Plasmatocytes with many cytoplasmic processes. They contain lysosomes (Ly) and myelinated bodies (My).

Figs. 4-14. Granular cells.

4-7. Granular cells showing pleomorphism.

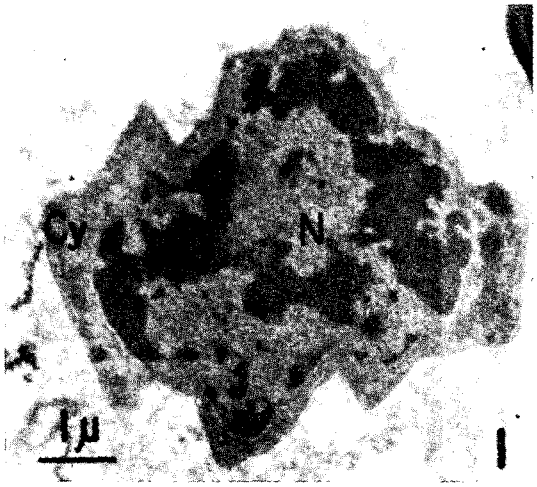
8-10. Progressive accumulation of characteristic granules is noticed.

11, 12. At least 4 types(a, b, c, d, e, f)of granules are seen.

13, 14. Certain granular cells contain myelinated bodies and flocculent material (arrows).

Figs. 15-17. Adipohemocytes.

The nucleus was pushed aside by the heavy accumulation of lipid droplets. And numerous microtubules(Mt) are seen in the cytoplasm.



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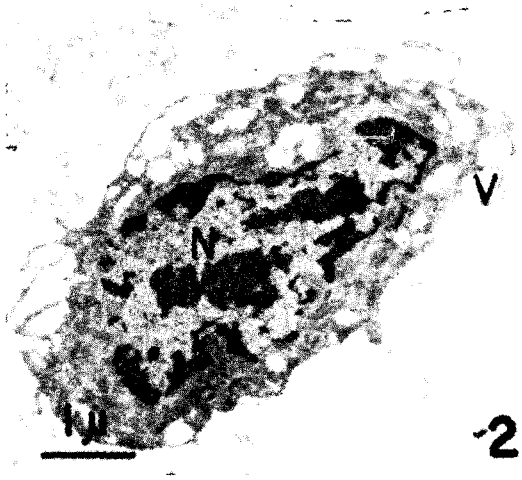

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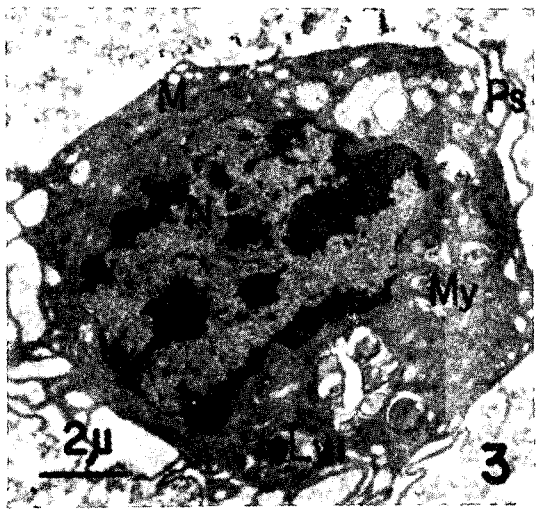

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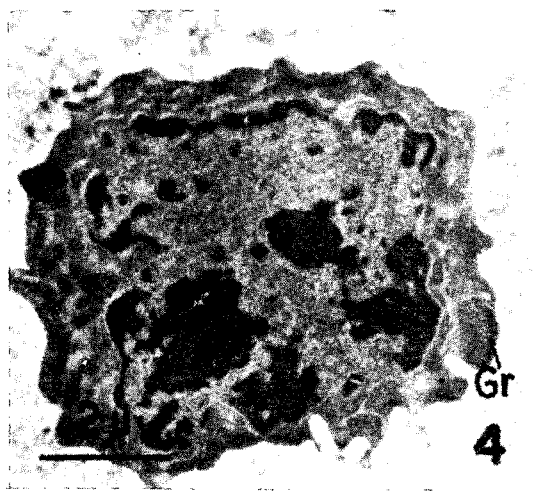

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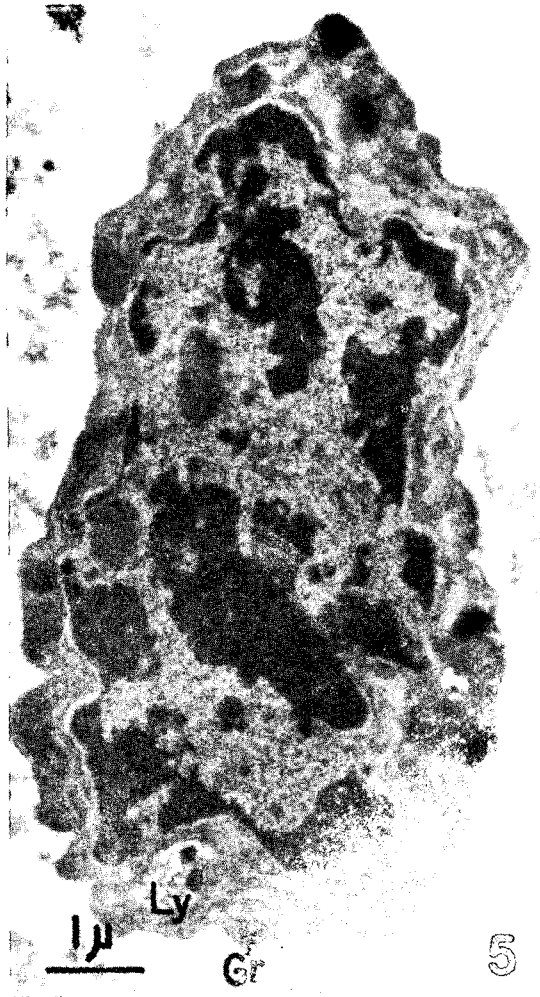

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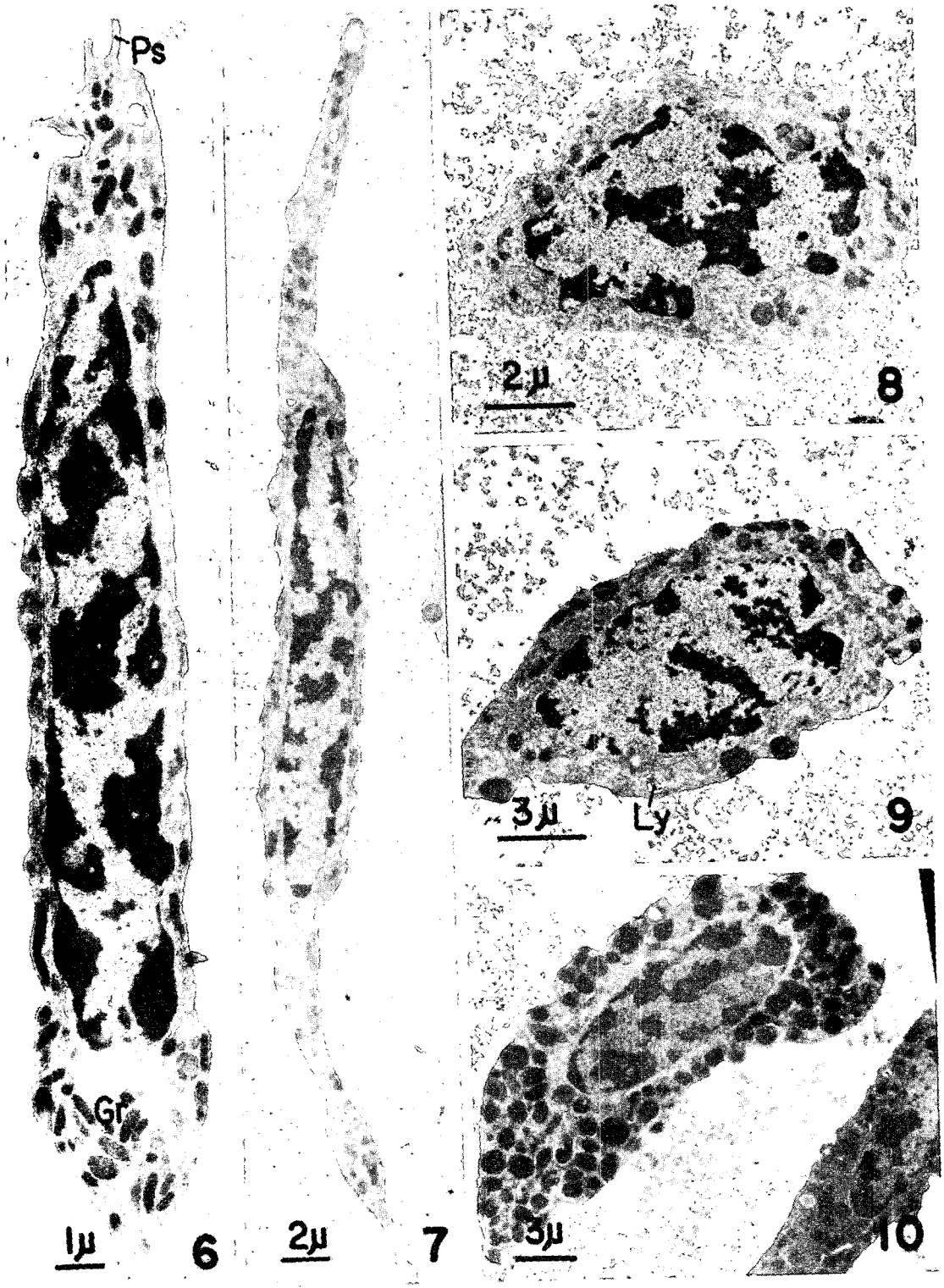
Ps
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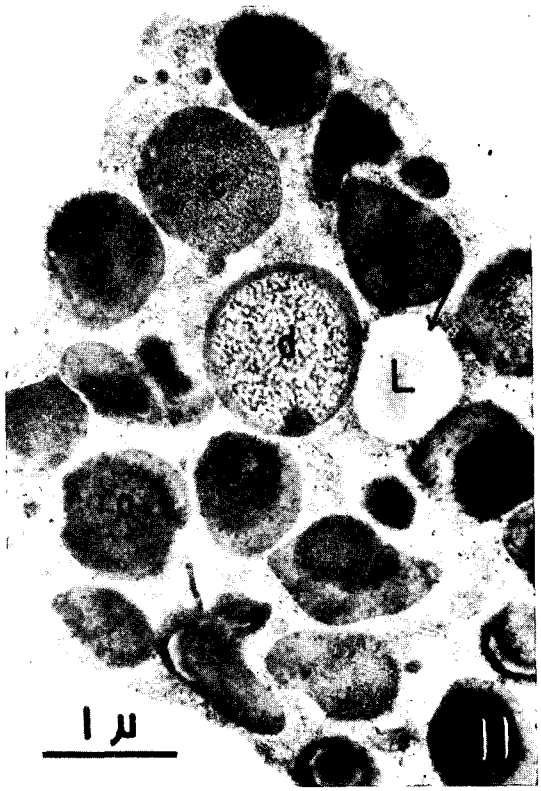
1μ
6
2μ
7
2μ
8
3μ
Ly
9
3μ
10


L
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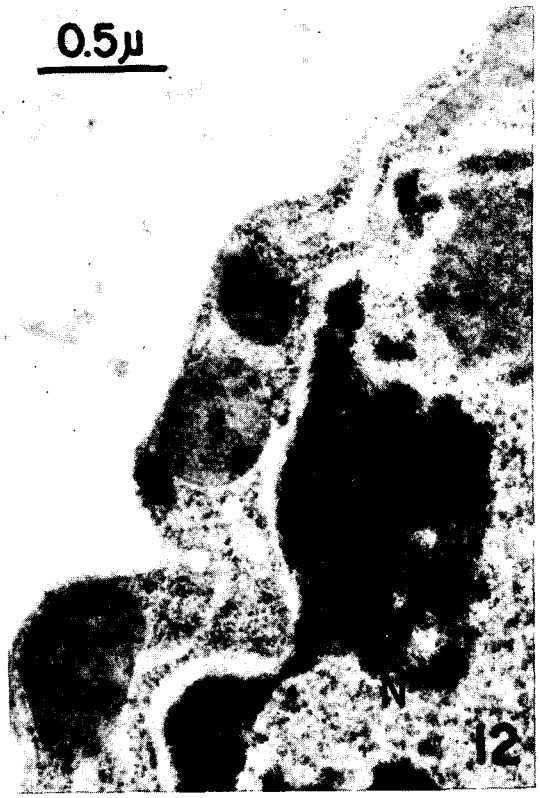
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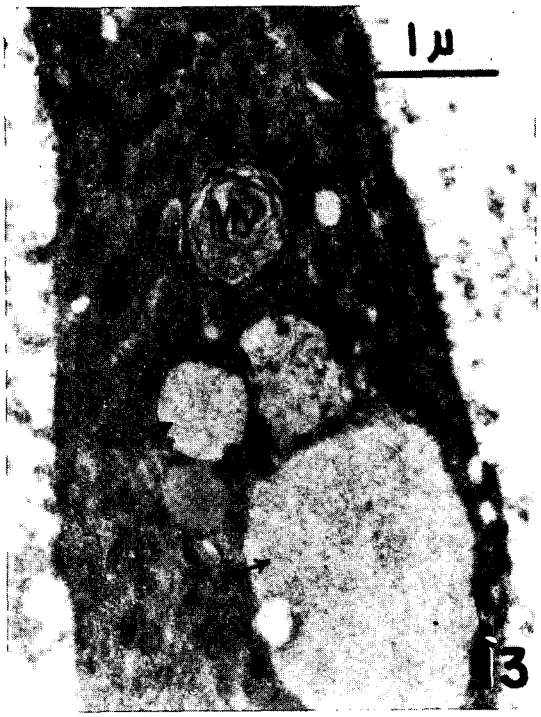
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M
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1 μ
My
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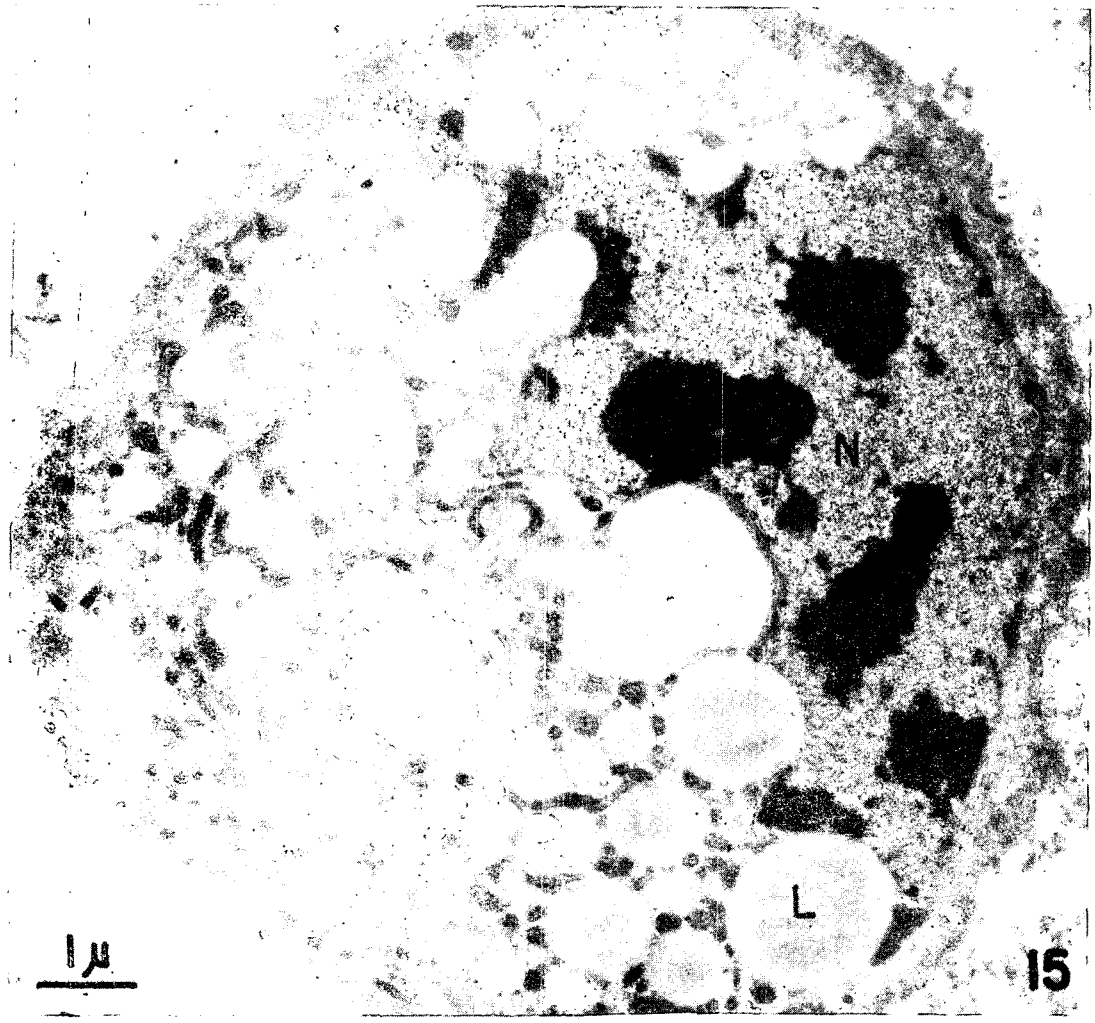
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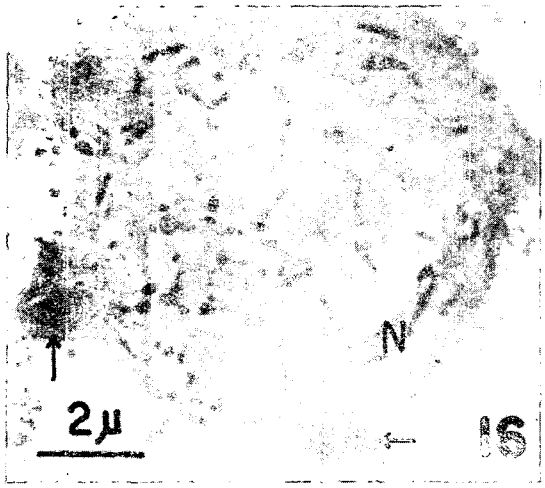
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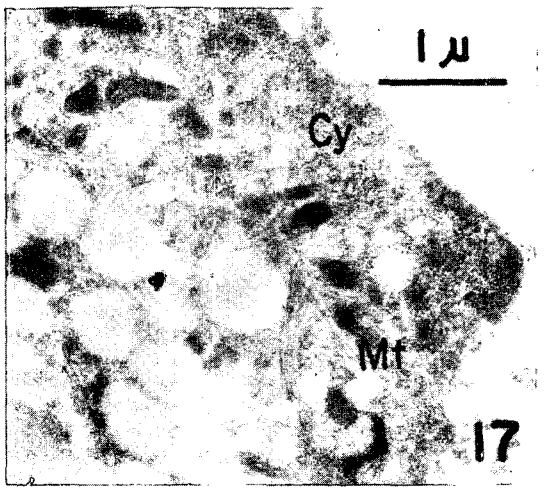
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