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Electron Microscopic Studies on the Larval Hemocytes of *Drosophila melanogaster*

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초파리 유충의 혈구에 대한 전자현미경적 연구

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요 약

초파리(*Drosophila melanogaster*)의 마지막 유충에서 혈액을 채취하여 미세구조를 관찰하였다.

Prohemocyte는 가장 미분화된 상태였고, Plasmatocyte는 보다 분화된 상태이며 어느 정도 풍부한 소기관을 가졌다. Granular 세포는 세포질내에 여러 형태의 과립을 가지는 것이 특징이고 특히 lysosome이 발달하였다. Crystal 세포내에는 커다란 crystal이 수개씩 존재하며 free ribosome이 충만되어 있다. Oenocytoid에는 과립상은 거의 없으며 mitochondria와 Golgi complex가 발달하였다.

INTRODUCTION

Light microscopic studies on insect hemocytes have been carried out in numerous species, and five to six types of hemocytes have been identified, *e.g.*, prohemocyte, plasmatocyte, granular cell, spherular cell, oenocytoid and adipohemocyte (Jones, 1962; Wigglesworth, 1965). Ultrastructural observations of insect hemocytes have also been done by Grimstone *et al.* (1967), Whitten (1969), Stang-Voss (1970), Akai (1971), Devauchelle (1971), Hagopian (1971), Akai and Sato (1973) and Ratcliffe and Price (1974).

In *Drosophila williston*, Shatoury (1955) reported that no blood cells were observed in larval stages, but only three types of cells, *i.e.*, hexagon, spheroid, platelet, were released from the lymph glands. Rizki (1953, 1957), however, has grouped the hemocytes into six types, such as, plasmatocyte, podocyte, spheroidocyte, oenocytoid, nematocyte and crystalloid cell in *D. williston* and *D.*

melanogaster.

In the present paper, the general ultrastructures of each type of hemocytes are described in the last stage larvae of *D. melanogaster*.

MATERIALS AND METHODS

Wild types of *Drosophila melanogaster* were reared on corn-medium at 25~27°C. The last instar larvae were collected in a petri dish and washed thoroughly with 6% NaCl solution to avoid contamination. Each larva was incised with a pincette and the hemolymph was collected in a centrifuge tube and fixed in 4% buffered glutaraldehyde in cold bath for one hour. After fixation the cells were centrifuged at 1,500 rpm for about 10 minutes and then the pellets were washed in several changes of phosphate buffered saline (PBS) of pH 7.4. The pellets were post-fixed with 1% OsO_4 in PBS for one hour. These pellets were washed again in two changes of PBS, dehydrated in graded concentrations of acetone and finally embedded in Epon 812.

Sections were cut with glass knives and were double-stained in saturated uranyl acetate in 30% ethanol for five minutes and in lead citrate for two minutes. The ultra thin sections were examined with Hitachi HS-7S electron microscope.

OBSERVATIONS

Each cell type was identified according to its intracellular organelles and inclusions with their shape and size. Five types of hemocytes were found in the last instar larvae, although transitional types between cells of the same type were observed.

Prohemocytes are the least common among the cell types, comprising less than 5% of total cell numbers. Prohemocytes are the smallest (4~5 μ in diameter), round or oval form and their nucleo-cytoplasmic ratio is high. They are characterized by a low concentration of intracellular organelles such as granular and agranular endoplasmic reticula, Golgi complexes, mitochondria and vacuoles. Inclusions and pseudopod-like projections were not found, while free ribosomes were relatively abundant (Fig.1). Some cells of this type were undergoing mitosis and resulting cells were found to be attached each other.

Two kinds of plasmatocytes were noticed; spindle-shaped and ovoid cells with numerous cellular projections. They are larger (6~12 μ) than prohemocytes in size and their nucleo-cytoplasmic ratio is low. The cytoplasmic organelles such as granular endoplasmic reticula, Golgi complexes and mitochondria are more conspicuous than those of prohemocytes. They also contain vacuoles and lysosomes but no any intracellular granules. Some of the plasmatocytes, believed to be one of the oldest forms, contain abundant vacuoles and myelinated lysoso-

mes. However, it was somewhat difficult to distinguish the cell type from the earlist forms of granular cells (Figs. 2, 3).

Granular cells are slightly larger (8~14μ) than plasmatocytes and their shapes are round, oval or amoeboic (Figs. 4, 5, 6). In all cases they have many pseudopod-like projections. Granular cells are rich in cytoplasmic organelles such as Golgi complexes, granular endoplasmic reticula, mitochondria, lysosomes, and are characterized by intracellular granules and inclusions. And various granules were noticed in this type of cells (Figs. 4—17). All of these cells contain large granules (to 1.5 μ in diameter) whose electron opacities are low but not enclosed by a membrane (Figs. 4, 12). Some of the granular cells possess relatively small granules (to 0.7μ in diameter) whose electron densities are very high (Fig. 5). And in high magnification, each granule is composed of several unit lamella (about 50 *nm*) which are concentrically arranged. They are not enclosed by a membrane (Figs. 5, 10).

Some large granules (to 2 μ in diameter), whose electron opacities are simillar to those of the granules mentioned above, were found in few cells. Simillar unit lamella are also noticed at the periphery of the granules, but each granule is enclosed by a thin membrane (Figs. 16, 17).

Other large granules different from those described above were also found in few granular cells (Fig. 13). Another large granules which are concentrically structured exist in some granular cells (Fig. 7).

Some cells contain small granules which are 0.1 μ in diameter at the largest. Each granule is enclosed by a membrane and its electron opacity is relatively high (Fig. 4, arrows). In addition to the granules mentioned above, granular cells contain multivesicular bodies (Fig. 4), myelinated lysosomes (Figs. 8, 9, 14), masses of ribosome-like particles (Fig. 11) and secretory vesicles (Fig. 15).

Crystal cells are relatively large (8~15 μ) and are round or oval. They are characterized by crystals (1~5 μ in length, 0.3~3 μ in diameter) in their cytoplasm (Fig. 18). In high magnification the crystals are composed of fine granules (about 5 *nm*) arranged regularly (Fig. 19). They have relatively small nuclei and free ribosomes which are heavily packed, while other cytoplasmic organelles are not usually developed. No pseudopod-like processes were observed in crystal cells (Fig. 18).

Oenocytoids are the largest (10~20 μ) of all cell types and are oval shapes with no pseudopod-like projections. In this cell type, mitochondria, granular endoplasmic reticula, free ribosomes and Golgi complexes are fully developed but granules and vesicles were not found, while occasionally several small electron dense particles (0.2 μ) were detected (Fig. 20). In some cases, two Golgi complexes pair in a set (Fig. 21).

DISCUSSION

Rizki (1953, 1957) observed the hemocytes in *D. williston* and *D. melanogaster* and classified them into six groups; plasmatocyte, podocyte, spheroidocyte, nematocyte, crystalloid cell, oenocytoid. Shatoury (1955) mentioned that blood cells did not exist in larva stages in *D. melanogaster*. Whitten (1964) grouped the hemocytes in *D. melanogaster* into five type; plasmatocyte, crystal cell, podocyte, lamellocyte and spindle-shaped hemocyte. All these results were based on light microscopic observations. But descriptions based on electron microscopic level, at least in *D. melanogaster*, are not available.

Akai and Sato (1973) studied the hemocytes in *Bombyx mori*, and Ratcliffe and Price (1974) in Dictyoptera. In both cases the observations were made light and electron microscopically, and the hemocytes were grouped into five types, such as prohemocyte, plasmatocyte, granular cell, spherular cell and oenocytoid. In the present work, all types were identified apart from the spherular cells. Crystal cells exist in *D. melanogaster* instead of the spherular cells.

In prohemocytes, because of their undifferentiated structures, any indications of activities of phagocytosis and capsule formation were not revealed. However, plasmatocytes contain relatively plenty of cytoplasmic organelles and have many pseudopod-like projections. In *Bombyx mori*, the plasmatocytes formed a capsule arround a nylon thread inserted into hemocoel (Akai and Price, 1973). In *D. melanogaster*, from their shape and existance of lysosomes, it is assumed that this cell type takes part in phagocytosis and capsule formation.

In granular cells, there are many characteristic lysosomes and pseudopod-like projections, and they may take part in phagocytosis. Since this type of cells has secretory vesicles containing secretory granules, it is suggested that it may have a secretory function. The most characteristic granules which exist in all granular cells are thought to be lipid and are sometimes released into the hemolymph.

In crystal cells the cytoplasmic organelles are the least developed of all cell types, and it presumed that these cells may be responsible mainly for storage or transportation of blood protein.

According to Nittono (1960) oenocytoids contain protein deposits visible at the light microscope. Akai and Price (1973) observed that these deposits were consisted of fibres with several subfibrils of 5 *nm* in diameter. However, in oenocytoids of *D. melanogater* other deposits were not found, although cytoplasmic organelles, such as granular endoplasmic reticula, Golgi complexes, mitochondria were highly developed.

SUMMARY

The hemocytes of *Drosophila melanogaster* were observed with electron microscope, and five types of the cells were identified; prohemocyte, plasmatocyte, granular cell, crystal cell and oenocytoid, accounting for about 5%, 35%, 45%, 10%, 5% respectively of total cell numbers.

Prohemocytes are characterized by a low concentration of intracellular organelles. Plasmatocytes are spindle or oval in shape and have relatively plenty of organelles and lysosomes. Granullar cells are the most polymorphic. They have numerous pseudopod-like projections and contain various granules and inclusions. In this cell type, intracellular organelles are fully developed. Crystal cells are characterized by numerous crystals composed of fine granules arranged regularly. Oenocytoids are the largest one among all cell types and contain relatively developed organelles.

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EXPLANATION OF FIGURES

Abbreviations

Cr: crystal G: Golgi complex Gr: granule L: lysosome M: mitochondrion
N: nucleus Nu: nucleolus Ps: pseudopod V: vacuole

Fig. 1. Prohemocyte.

Figs. 2, 3. Plasmatocytes: They have pseudopod-like projections (Ps) and myelinated lysosomes (arrows).

Fig. 4—17. Granular cells:

4. A characteristic granule (Gr), many small dense granules (arrows) and multivesicular bodies (V) are seen.

5. A granular cell containing concentrically structured granules (arrows), lysosomes (L) and a pseudopod (Ps).

6. A granular cell showing fully developed vacuoles(V).

7. A large secretory granule (arrow) are embedded in cytoplasm.

8—9. Myelinated lysosomes (arrow) are found in most of the granular cells.

10. In high magnification small dense granules (as Fig.5) are composed of several lamella sets.

11. Ribosome-like particles are packed in a special region.

12. Characteristic granules found in all granular cells.

13. A large dense granule enclosed by limiting membrane.

14. Myelinated lysosomes are composed of several lamella sets.

15. Secretory particles are surrounded by double membrane.

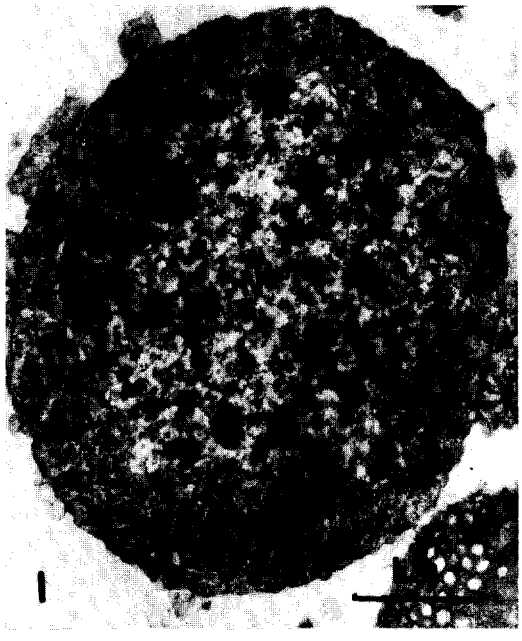
16, 17. A large dense granules have several lamella sets at the periphery.

Fig. 18. Generalized crystal cell: Crystals are cross sectioned.

Fig. 19. High magnification of a crystal.

Fig. 20. Oenocytoids: Cytoplasmic organelles are fully developed.

Fig. 21. Two Golgi complexes pair in a set in oenocytoids.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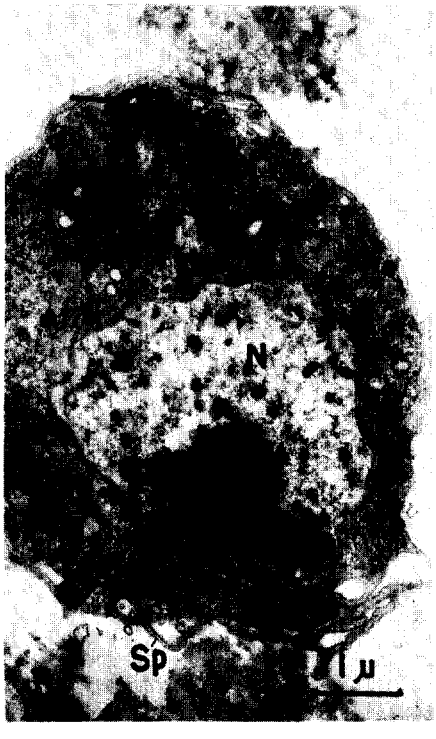
Sp
2
1μ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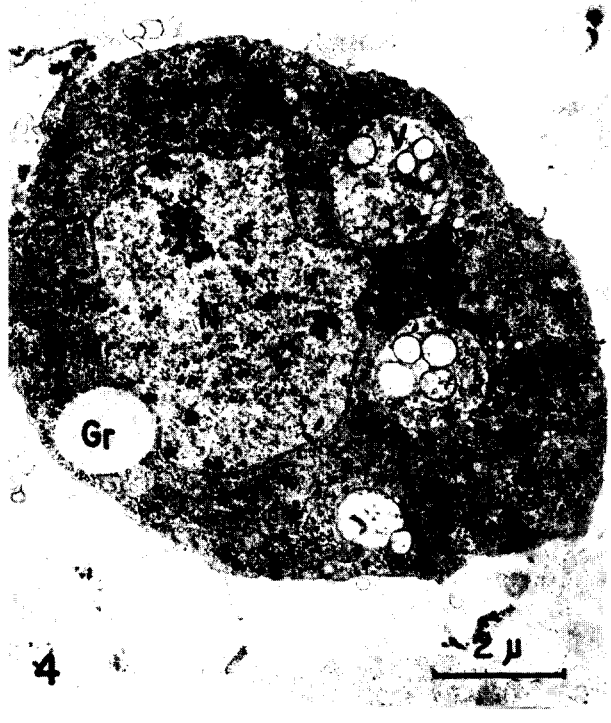
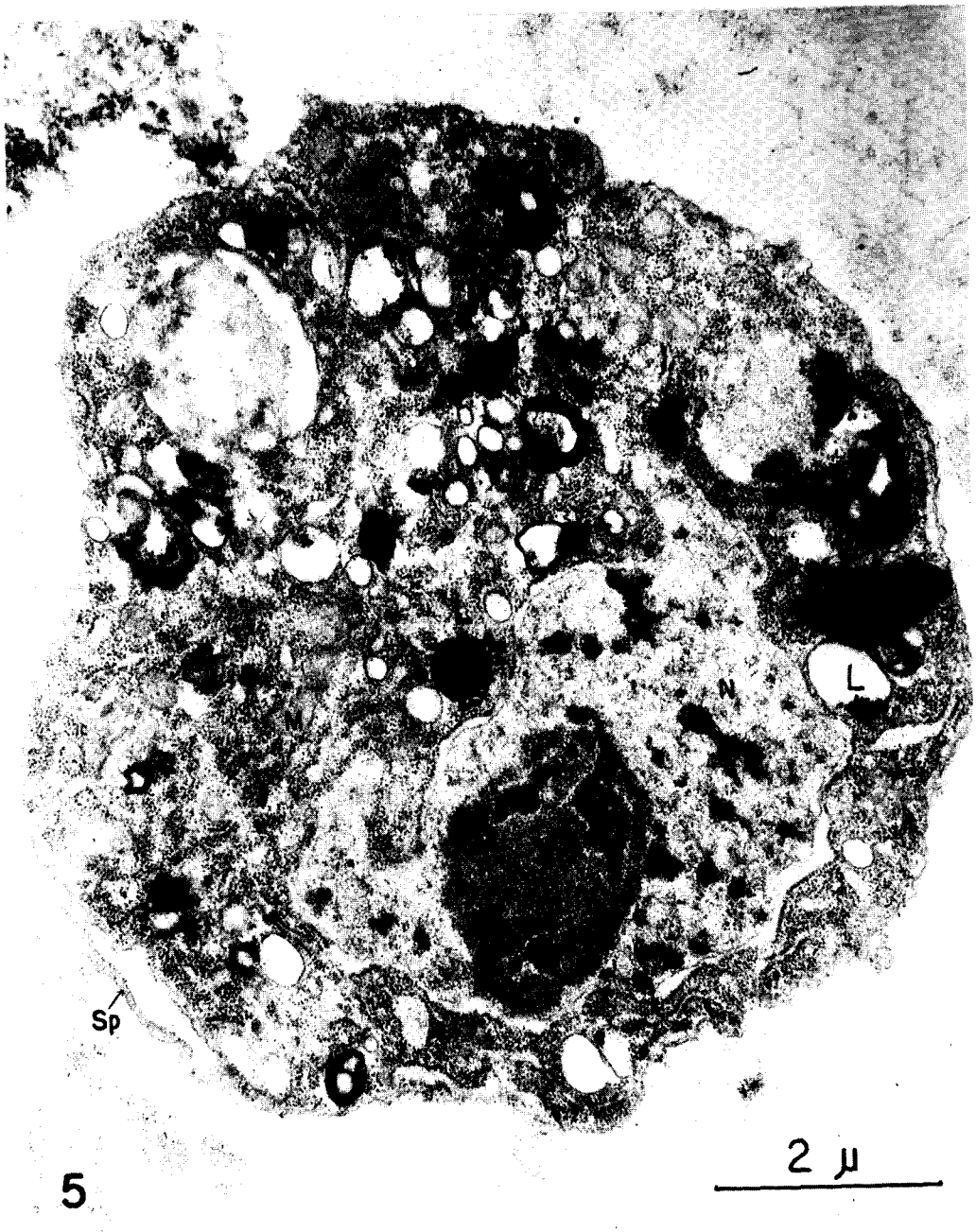

Gr
4
2μ



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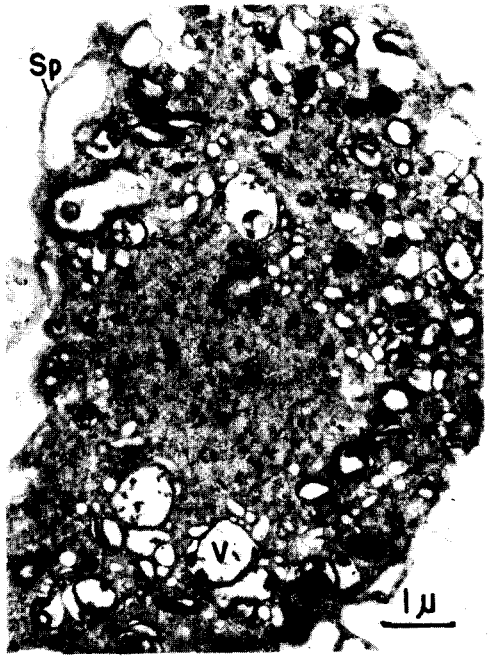
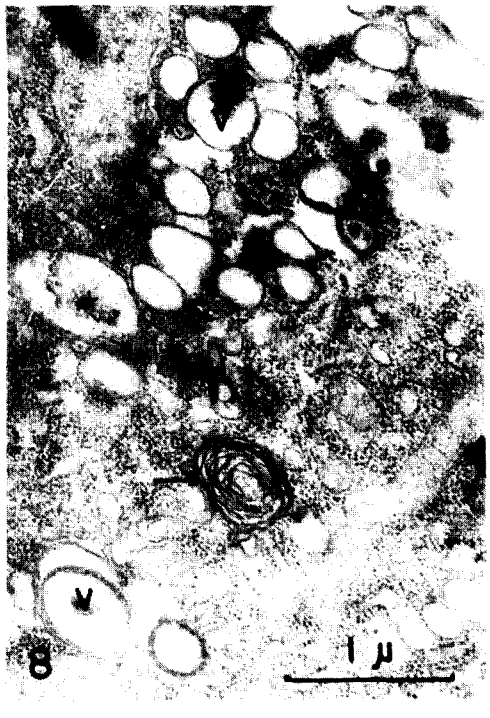

Sp
V
1μ


0.5μ


V
V
8
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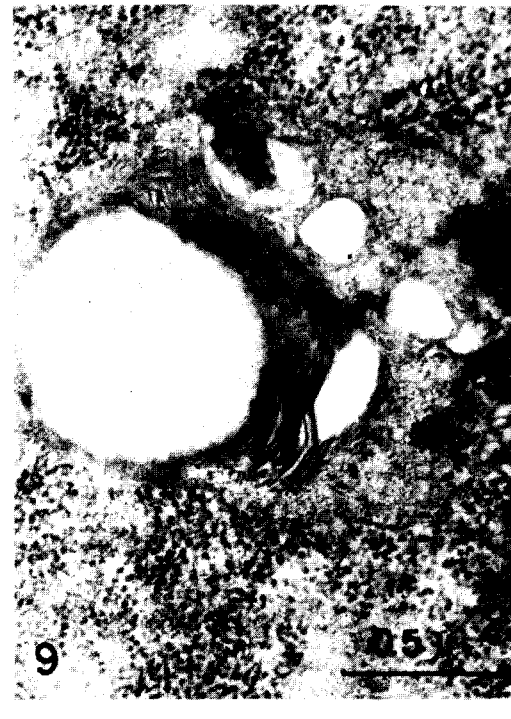
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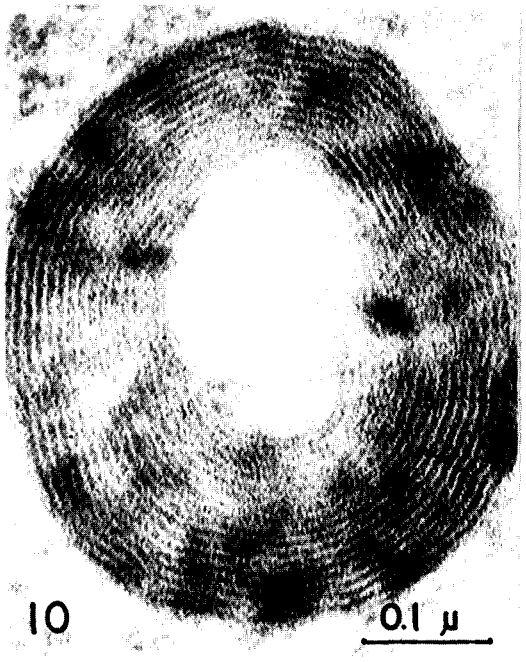
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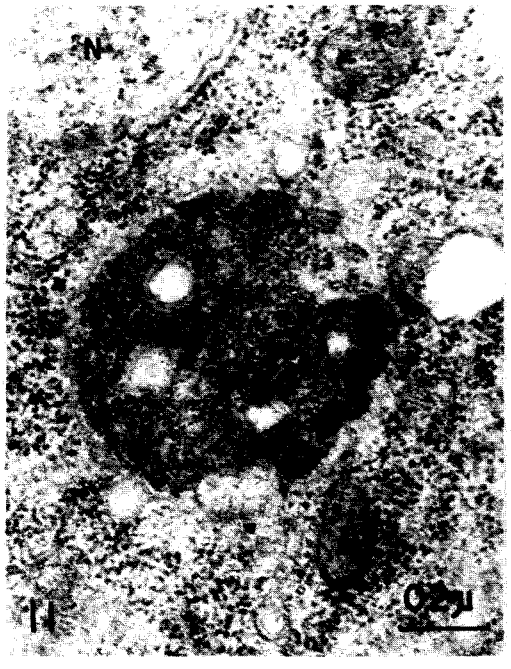
N
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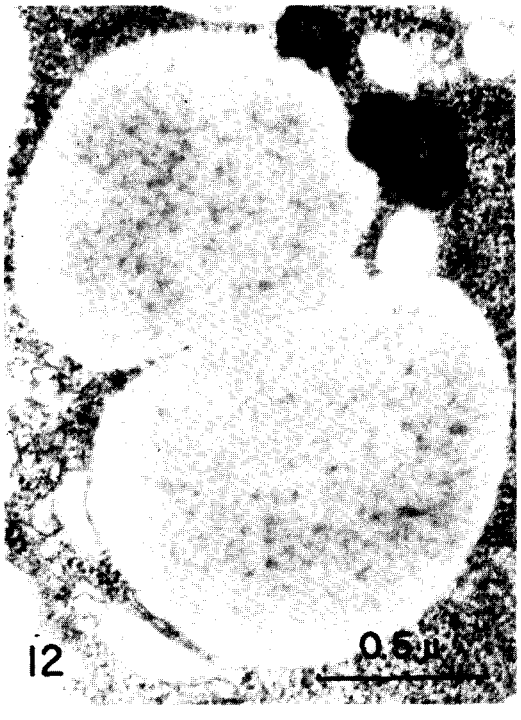
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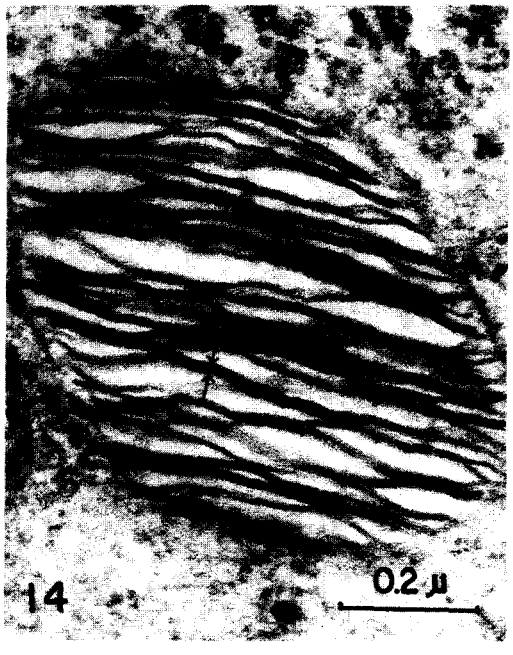
13
1 μ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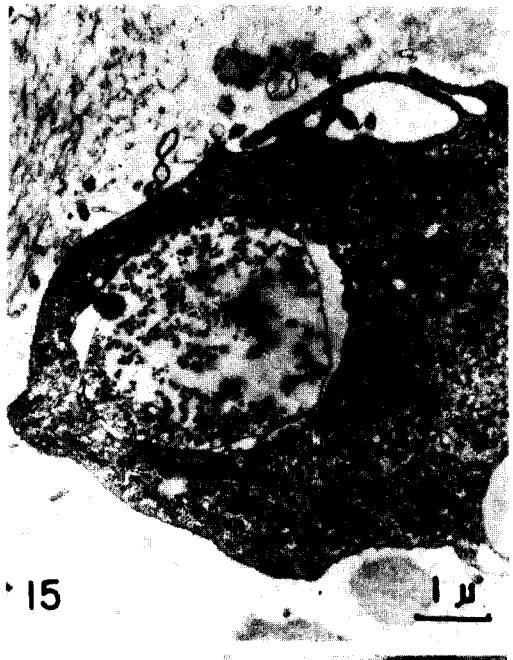
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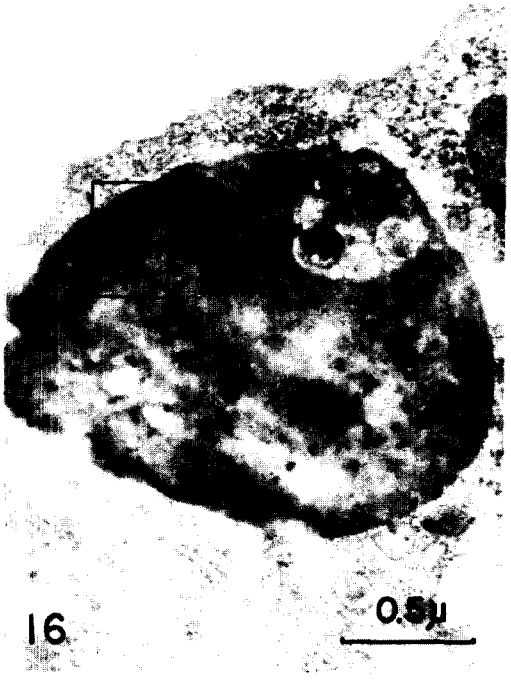
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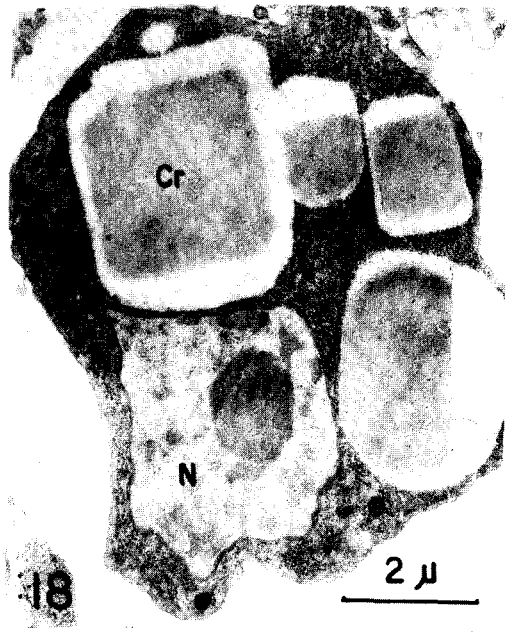
0.5μ


17



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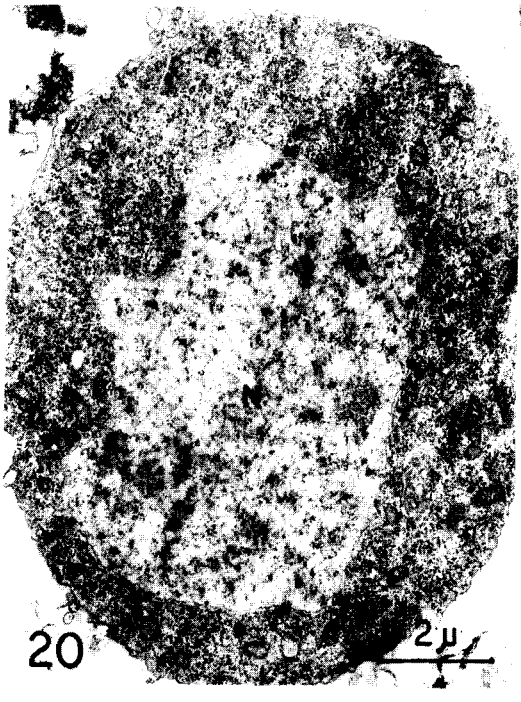

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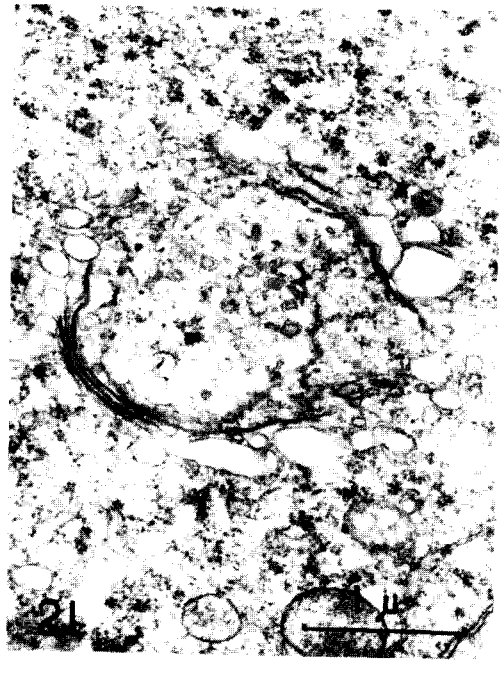

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