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AN ELECTRON MICROSCOPIC STUDY OF THE CHLOROMA CELLS (PROMYELOCYTIC LEUKEMIA CELLS)

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Chloroma, a malignant green tumor associated with leukemia, was first described in 1823. Since then numerous cases have been reported in the literature and for many years chloroma was considered to be associated with leukemia of various types (granulocytic, lymphatic and monocytic leukemia), however, it is generally accepted today that chloroma is related only to myelogenous leukemia (1). Further, the intimate relationship between chloroma and promyelocytic leukemia, which is a specific form of myelogenous leukemia, has recently been emphasized. However, the precise nature of the green color remains unknown. Judging from the fact that in some cases the buffy coat is greenish as well as that seen in visceral infiltration (2), the green pigment of chloroma may be contained in the chloroma cell itself.

The purpose of this report is to describe the ultrastructural features of the chloroleukemia cell (promyelocytic leukemia cell) and the specific changes of azurophil granules and nuclear sessile nodules found in this case.

Report of the case

The patient was a 28 year old housewife. She complained of hemotaxis and gingival bleeding in March 1967. Based on findings of anemia (RBC 200 x 10^4) and leucocytosis (27,000), a diagnosis of acute myelogenous leukemia was made and she was hospitalized at the Department of Internal Medicine, Sapporo Medical College on May 9th, 1967. After admission, leukemic cells were found at a rate of approximately 60 to 70% in the peripheral blood. Due to the cytological characteristics of leukemic cells and the high percentage (80%) of the leukemic cells which were positive to peroxidase reaction, a diagnosis of promyelocytic leukemia was made. She died of severe hemorrhagic diathesis on 15, July 1967. As a result of autopsy it was found that chloroma was present. The autopsy findings were reported elsewhere (3).

Materials and methods

On June 13th, 1967 blood samples for electron microscopy were obtained from the peripheral blood as indicated in the hemogram in Table 1.

After ten ml of the patients venous blood was centrifuged at 1500 rpm for 10 minutes at 4° C, the buffy coat was pipetted into cold 2% tetroxide osmium fixative solution buffered with veronal acetate (pH 7.6). After fixation for 2 hours at 4° C, a pellet was obtained by centrifugation at 3000 rpm for 15 minutes. The pellet was gently cut into approximately 1 mm cubes which were dehydrated through graded alcohols and embedded in epon. Sections were cut with a Porter-Blum MT-1 microtome, using

Table 1 Hemogram

RBC	242×10^4
Hb	45%
CI	0.93
Thrombo.	3×10^4
Reticulo.	6%
WBC	27,100
Leukemia cell	65%
Myel.	1%
Meta.	3%
St.	0%
Seg.	9%
Lymph.	22%
Mono.	0%
Peroxid. reaction	(+)77%

glass knives. The sections were stained with uranyl acetate and lead. The stained sections were examined with a Hitachi 11-A electron microscope.

Results

Light microscopy

The majority of the leukemic cells showed an increase of nucleo-cytoplasmic ratio and revealed round or oval nuclei with hypertrophic nucleoli. The nuclei was generally located at a slightly eccentric position in the cells, revealing nuclear indentation and irregular outlines. In the cytoplasm numerous azurophil granules were seen which showed positive to peroxidase reaction. Auer bodies were not observed in this case.

Electron microscopy

Nucleus: The outlines of nuclei were irregular and were accompanied by varying sized nuclear indentations. Large nuclear indentations were found generally facing the side of the cytoplasm which contained Golgi zones. Round hypertrophic nucleoli showing distinct dissociation of granular components and fibrilar components, were generally observed near the nuclear envelopes (Figs. 1 and 3).

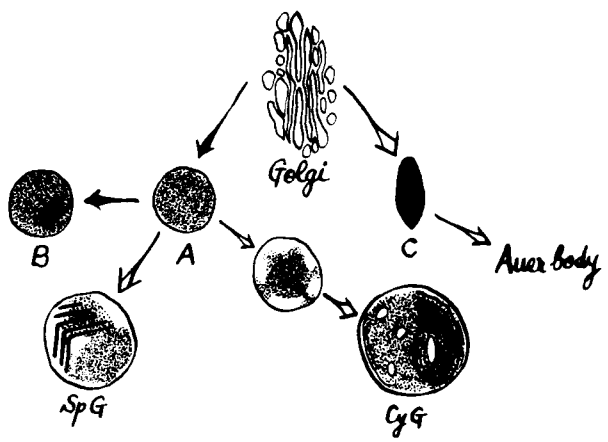
The most remarkable finding of the nuclei were the presence of nuclear sessile nodules (Figs. 1, 4, 5 and 6) and nuclear pockets (Fig. 7). In these sections, about one third of the nuclei contained sessile nodules or nuclear pockets. The frequency of sessile nodules were approximately four times that of nuclear pockets. The contents of sessile nodules were nucleoplasm, but the contents of nuclear pockets were cytoplasmic material. The nucleoplasm contained in the sessile nodules showed a general apperance of degradation accompanied by a reduction of chromatin granules (Figs. 4 and 5). The cytoplasm within the nuclear pocket usually exhibits cytoplasmic organellae such as mitochondria, and azurophil granules (Fig. 7). Both sessile nodules and nuclear pockets were surrounded by a double membrane. The double membranes of the sessile nodule consisted of an infolding nuclear inner membrane in which the nuclear outer membrane was not involved. In the case of nuclear pockets, the double membranes themselves were inversed nuclear envelopes.

An increase of nucleo-cytoplasmic ratio was usually observed. Some of the leukemic cells have only thin cytoplasms while some have large cytoplasms revealing the Golgi zone (Fig. 11). The variability of nucleo-

cytoplasmic ratio occured due to the omnipresence of the nuclei and due to the cutting direction of the cell.

Cytoplasmic organellae: Rough endoplasmic reticulum were seen with a vacuole like appearance filled with fine granular contents of moderate electron density scattered over the cytoplasm with the exception the Golgi zone (Figs. 1, 2 and 4). The Golgi apparatus were located to the side of the large cytoplasm and large nuclear indentations. Centrioles were frequently observed in the center of the Golgi zone arranged in a circle (Fig. 11). The majority of smooth endoplasmic reticulum mainly found in the vicinity of Golgi zones were seen scattered in vesicular forms in the cytoplasm. Such rudimentary development of SER was in striking contrast to the excellent development of RER. Numerous round or rod shaped mitochondria were seen scattered in the cytoplasm. Free ribosomes were abundantly seen scattered in the cytoplasmic matrix, but no glycogen particles were observed.

Schema 1



Azurophil granules: Azurophil granules were seen scattered in the cytoplasm. From the ultrastructural features, these granules were divided into three basic types. Spherical dense granules of 0.2 to 0.3μ in diameter were surrounded by a single membrane and their contents were homogenously dense without any inner structure. Some of these showed a clear zone between the limiting membrane and dense contents (A type). The granules were similar to those of A type, but they contained laminated needle like crystal structures (B type, Figs. 3 and 5). The third type of granules were not spherical, but rod shaped and usually smaller and less dense than those of A and B granules. Their contents revealed an inner structure of parallelly arrayed fibrilar arrangement along the long axis (C type, Fig. 3). The granules of A type were most numerous and the number of C granules was approximately one fourth of the A granules. B granules were rarely found in the cell. Some granules of A type showed varying features, increasing in size and decreasing in contents. Occasionally, cytolysosomal granules of various size in which laminated or vesicular membranous components were included were observed (Fig. 10). The fine structural transition between A type granules and these cytolysosomal granules were detected.

In addition to the granules above described, large granules of 0.4 to 0.7μ in diameter were observed. They were surrounded by a single membrane and showed relatively wide clear zones between the limiting membrane and their contents. The contents consisted of amorphous spherical bodies of low electron density and electron dense laminated crystaloid material (Figs. 2, 8 and 9). These dense laminated

crystaloids showed a varying appearance of linear, bended linear or arc shape. The distance between the center of the dark line and that of adjacent dark line was constantly about 200 A. The width of the dark line was 120 A and the distances of light zones among dark lines were 80 A.

Discussion

Sessile nodule and nuclear pockets

In the thin sections, one third of the nuclei of leukemic cells showed sessile nodules or nuclear pockets. This fact may suggest that the majority of nuclei in this case showed sessile nodules or nuclear pockets taking into consideration the three dimentional shape of the nuclei. In this investigation, a nucleus with both sessile nodules and nuclear pockets or a nucleus associated with two or more sessile nodules or nuclear pockets was not observed. However, considering the high frequency of sessile nodule formation in nuclei, it may be possible that a nucleus has two or more sessile nodules. The nuclear pockets were found in leucocytes of normal individuals and it was assumed that the nuclear pockets were not special diagnostic structures (4). On the other hand, sessile nodules were surmised to be a mainfestation of chromosomal abnormalities. Heuhus et al (5) reported that sessile nodules were found in neutrophils of the D_1 (13 - 15) -trisomy syndrome and that sessile nodules corresponded to the anomalous nuclear projections seen under light microscopy. In this case, sessile nodules were observed to be an abnormal separation of some of the nucleoplasm from the main part of the

nucleus disorganizing the separated nucleoplasm. The precise significance of sessile nodules in this case remains to be resolved, because no investigation on karyogram was performed.

Azurophil granules

In human normal promyelocytes azurophil granules of three types are observed. However, compared with those of normal cells (6), a marked increase of C type granules and various ultrastructural changes of A type granules were noted in leukemia cells. Since azurophil granules are a certain type of lysosome (7) and promyelocytic leukemia cells cease further maturation and break at this immature stage, it seems reasonable that various features of A granules are seen shifting to cytolysosomal granules in leukemic cells.

The most characteristic finding in this case is the occurence of the specific granule (SpG) consisting of the amorphous low electron density spherical bodies and laminated crystal materials. Crystal materials in SpG are completely different from that of B granules. Therefore, it may be assumed that SpG is produced by an alteration of A granules rather than B granules. The nature of the green pigment in chloroma is still obscure. Judging from the fact that the buffy coat in this case was greenish, the green pigment may be included in the leukemic cells. Concerning this point, Schulz et al (8) reported a close relationship between the green color of chloroma and porphyrin and peroxidase. Thus, it is possible that the green color may be du to a certain change of azurophil granules which are the only cytoplasmic organelles contained peroxidase enzyme (7).

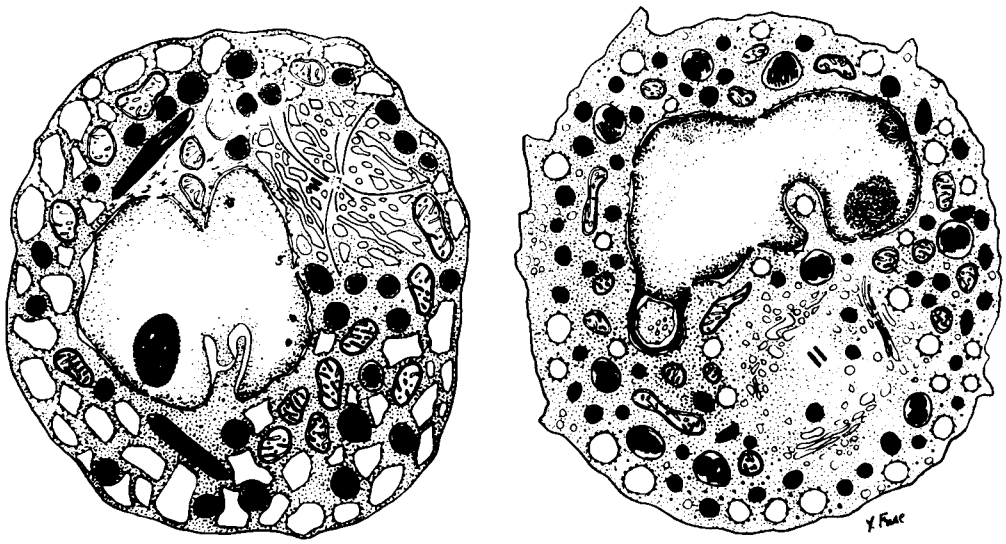
But the relationship between the specific granules (SpG) in the schematic diagram (1) observed in this case, and the green pigment of chloroma must be studied further.

The shape and size of C granules are quite different from those of A and B granules. Therefore, a transition between C granules and A or B granules are not considered. The number of C granules increased extremely in these leukemia cells as compared with that of normal promyelocytes. All C granules showed a fibrilar inner structure. It is well known that the presence of a purified single protein, as a rule, shows a crystalloid pattern as its ultrastructural feature. It is apparent that normal azurophil granules contain several lysosomal enzymes (7). Agglutination or fusion of C granules develops into Auer bodies (9). From these facts, C granules are considered to contain only one or two lysosomal enzymes which develop along their long axis and it also may be considered that C granules are imcomplete azurophil granules. A similar condition may be found in pericanalicular rod shaped bodies in rat hepatocytes (10). It is also reazonable to assume that promyelocytic leukemia cells have a large number of incomplete granules which reflect the incomplete function as a tumor cell deviated from the corresponding normal cell.

Some investigators (11, 12, 13) consider that chloroma cells belong to the monocytic leukemia cell series. Tanaka et al (11) reported that the lack of RER and the existence of ϕ granules (Tanaka) together with the presence of phagosomes in chloroma cells were considered to be the electron microscopic specificity of the monocytic leukemia cell series. However, the differences between SER and ERE, between azurophil

granules and Φ granules were not apparent in his figures from the specimen embedded in methacrylate. Further, the phagosomes in Tanaka's figure are identical to the cytolysosomal granules (Fig. 10), which are derived from the A granules. Therefore the phagosomes in Tanaka's figure may not be due to phagocytosis.

Schema 2 Schematic representation of promyelocytic leukemia cell (left) and chloro-leukemia cell (right).



It is known that azurophil granules are formed in the stage of promyelocytes and that promyelocytes have well-developed RER, motochondria and a large Golgi zone. On the other hand, myeloblasts lack granules and have only rudimentary RER, motochondria and Golgi apparatus. These ultrastructural characteristics between myeloblasts and promyelocytes are found in myelobastic leukemia and promyelocytic leukemia.

Hence, leukemic cells revealing a certain number of azurophil granules and which have ultrastructurally well-developed RER, mitochondria and Golgi apparatus, should be classified as promyelocytic leukemia, regardless of the light microscopic cytology of the lekuemic cells.

Summary

Electron microscopic features of chloroma cells (promyelocytic leukemia cells) were presented. Nuclear sessile nodules and various features of azurophil granules found in this case were described in detail and the relationship between promyelocytic leukemia and chloroma was discussed.

(Recieved May 20, 1969)

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Explanations of figures

Fig. 1 Golgi zone (G) is located in the center of the cytoplasm. The eccentrically located nucleus shows a sessile nodule formation (arrow) and a nucleolus consisting of dense fibrilar strands and diffuse granular areas. A moderate number of azurophil granules varying in size and density are mainly observed around the Golgi zone. Mitochondria, rounded rough ER and free ribosomes are seen scattered in the cytoplasm. x 14,000

Fig. 2 Azurophil granules are seen scattered numerously in the cytoplasm. Most of them are round, but some are rod shaped. Several large granules which are specific in this case, are indicated by arrows. x 14,000

Fig. 3 An large number of azurophil granules are observed are the Golgi zone. Rod shaped azurophil granules of C type (arrows), and round azurophil granules of B type (double-headed arrows) are seen. x 21,000

Fig. 4 Chromatin granules inside the sessile nodules tend to be disorganized, and vacuole like structures appear. x 28,000

Figs. 5 and 6

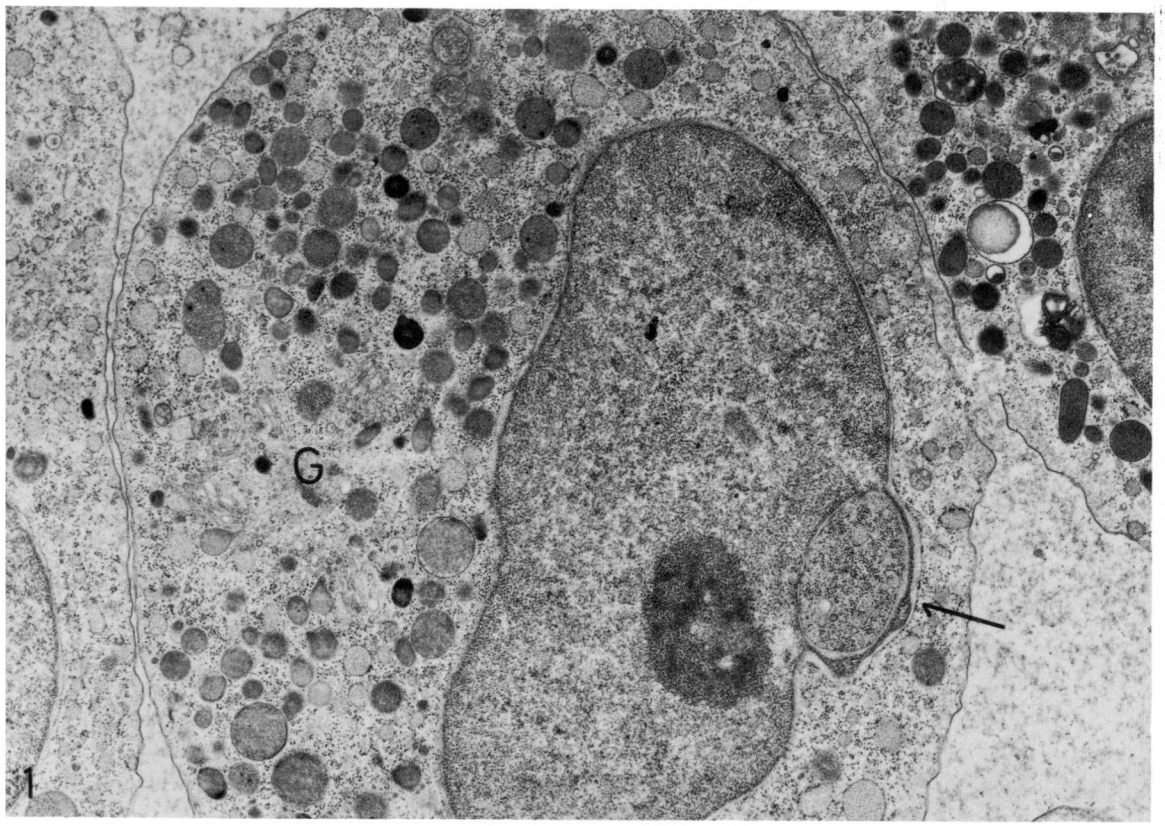
The nucleoplasm in sessile nodules show a marked reduction of chromatin granules. Arrow indicates an azurophil granule of B type. Nuclear pores are clearly observed when cut tangentially in Fig. 6. x 28,000

Fig. 7 Nuclear pockets are seen protruding into the cytoplasm. The nuclear pocket contains an azurophil granule. x 28,000

Figs. 8 and 9
Specific large granules with concentric laminated structures are seen. x 45,000

Fig. 10 Double headed arrows indicate swollen azurophil granules and the arrows indicate cytolysosomal granules. x 28,000

Fig. 11 A large Golgi zone and centriole are seen in the paranuclear cytoplasmic region. x 25,000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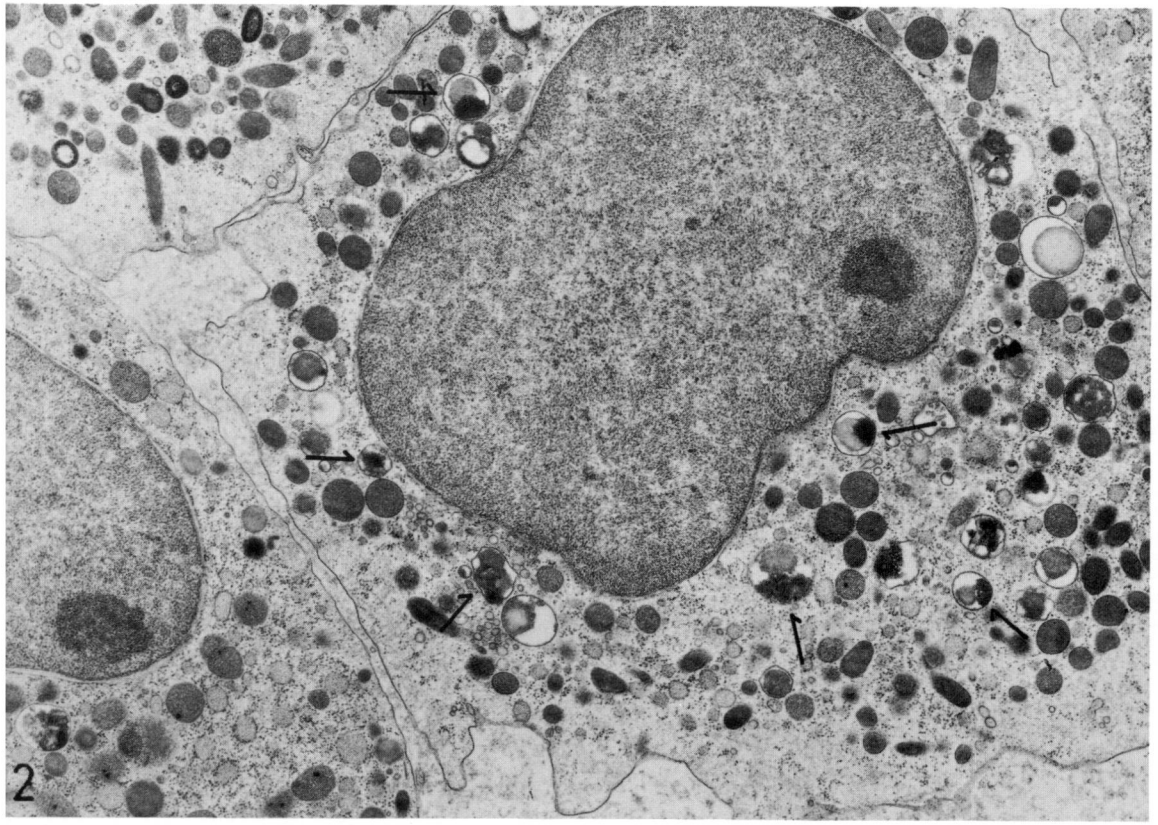
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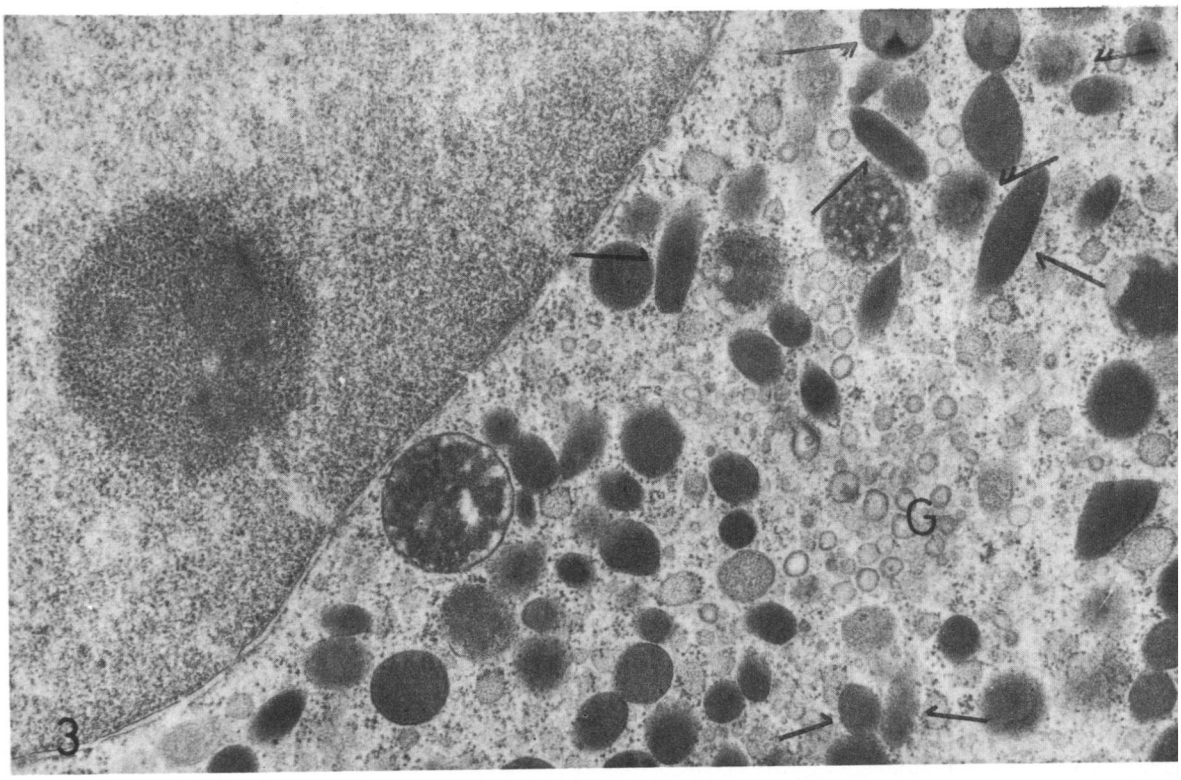
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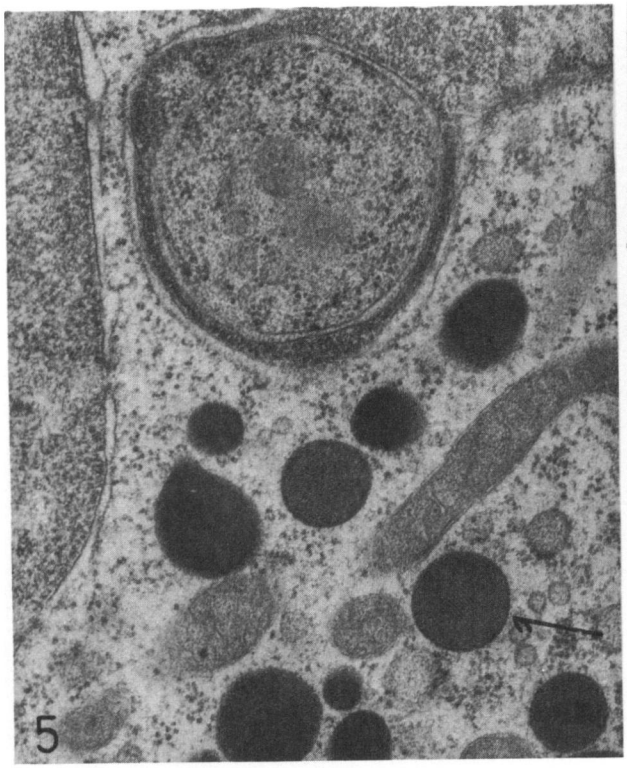
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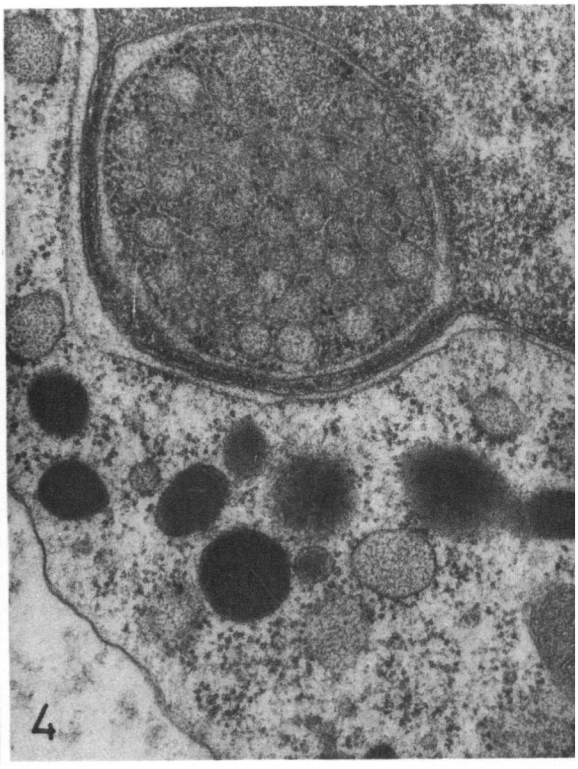
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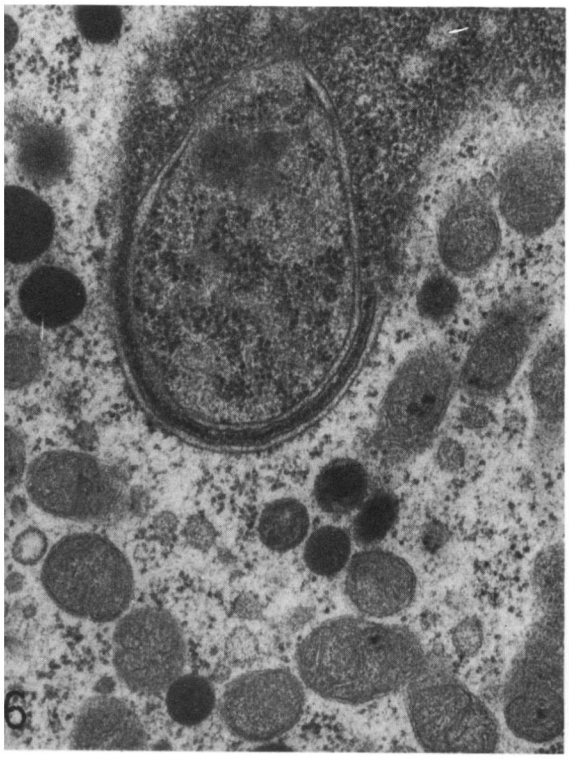
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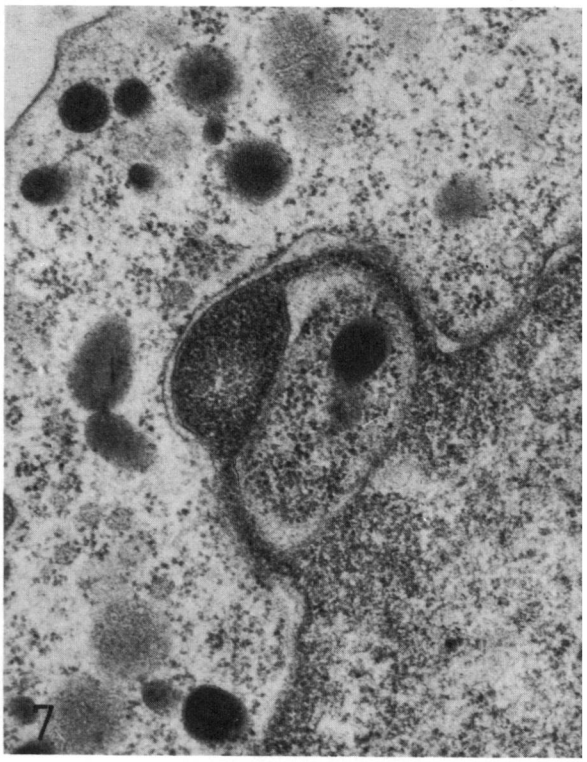
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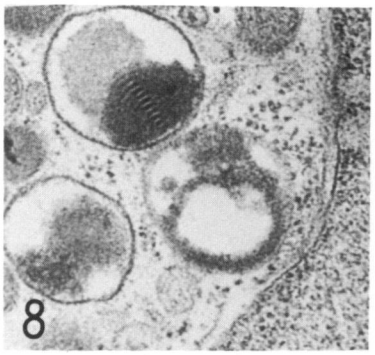
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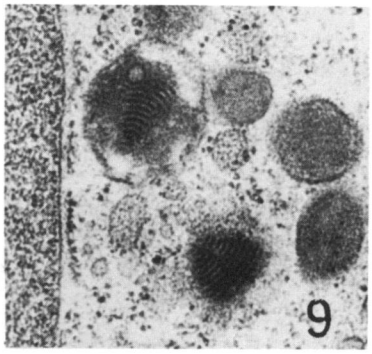
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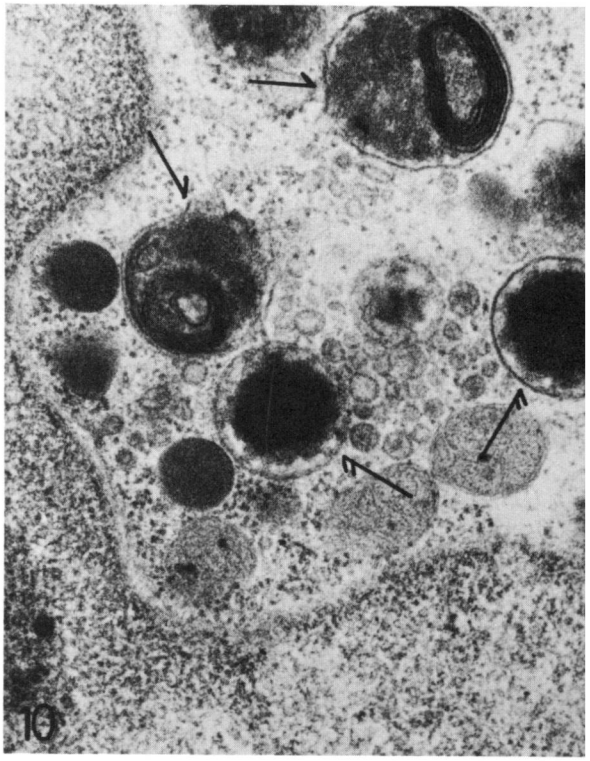
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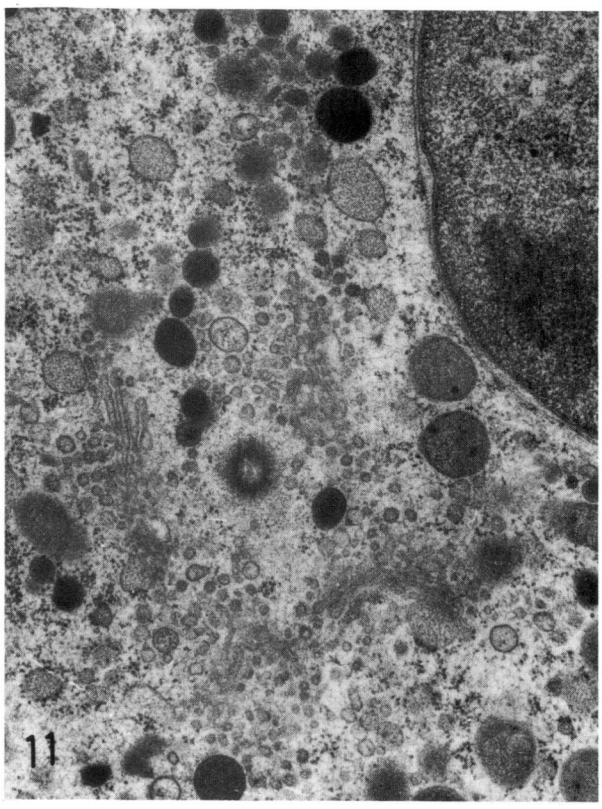
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