Giant Organelles Containing Tubules in Chediak-Higashi Lymphocytes

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A giant inclusion filled with structures resembling microtubules was observed in the cytoplasm of a small percentage of circulating lymphocytes from 4 children with the Chediak-Higashi syndrome (CHS). The organelles were similar in many respects to tubule-containing inclusions described previously in normal and abnormal lymphocytes. Tubule-filled organelles appear to develop by cytoplasmic sequestration, and their massive size in CHS cells may result from fusion of small organelles to form a single giant inclusion. Acid phosphatase was found only in giant tubular inclusions which had fused with lysosomal granules. The basis for the existence of these organelles in normal and abnormal lymphocytes remains obscure (Am J Pathol 69:225–238, 1972).

AN UNUSUAL CYTOPLASMIC INCLUSION filled with tubular elements in 8% of the circulating lymphocytes from an elderly female with chronic rheumatoid arthritis and leukopenia was observed by Hovig *et al*[1] in 1968. The inclusions were not found in lymphocytes from members of her family and had not been described in previous reports on the ultrastructure of normal and abnormal blood cells. Hovig and his coworkers suggested that the new lymphocyte organelle was probably related to the patient's disease or treatment. Subsequently, however, Huhn[2] noted strikingly similar organelles composed of tubular elements in 1 to 5% of peripheral blood lymphocytes from 12 healthy individuals.

The fine structure of the giant inclusions found in the cytoplasm of blood leukocytes from patients with the Chediak-Higashi syndrome (CHS) has been discussed in several reports,[3–13] and recent investigations have detailed the ultrastructural features of massive granules in white blood cells from animal models of the human disease.[14–17] Huge granules with a complex internal structure were frequently observed in the cytoplasm of CHS lymphocytes, but tubule-filled organelles

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Supported by Grants HE-11880, AI-05153 and CA-08832 from the US Public Health Service and the Cardiovascular Clinical Research Program Project.

Accepted for publication May 25, 1972.

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of the kind described by Hovig *et al*[1] and by Huhn[2] were not discussed, although they were probably identified.[5,8] Evaluation of a new patient with CHS and reexamination of leukocyte samples from earlier investigations indicate that massive organelles filled with tubules are often present in CHS lymphocytes and occur infrequently in other leukocytes. The unusual form of giant CHS organelle will be described in this report.

Materials and Methods

Four patients with the characteristic abnormalities of the Chediak-Higashi syndrome provided blood samples for the present investigation; three were evaluated in previous reports.[5,6,7] The fourth child, a new patient, is a 6-year-old male who has been followed for 3 years. He was diagnosed shortly after birth, after the development of a parotid abscess. Upper respiratory infections were frequent during his first year of life, and vericella, contracted at 2½ years of age, was considered unusually severe. He often had fevers of undetermined origin, but the frequency and severity of bacterial infection in the past 2 years have not been unusual. His physical findings, except for a slight dilution of skin pigmentation, have consistently been within normal limits; his intelligence is normal for his age. He has not developed organ enlargement or other clinical evidence of the accelerated phase of the disease. Abnormal inclusions characteristic of the CHS have been observed in leukocytes from his bone marrow and peripheral blood and in the cells of other tissues. The parents are unrelated, and an older sister has no signs of CHS.

The methods used for separating leukocytes from samples of peripheral blood, fixing the cells for study in the electron microscope, and carrying out the cytochemical procedure for the demonstration of acid phosphatase activity were described in earlier reports.[5,6] Some of the leukocyte samples for the present study were fixed in unbuffered 4% osmic acid in distilled water, pH 6.8, for 2 hours before dehydration and embedding. All observations were carried out in a Philips 200 electron microscope on thin plastic sections stained with uranyl acetate and lead citrate to enhance contrast.

Results

General

Organelles containing masses of tubular elements were identified in circulating lymphocytes from each of 4 children with Chediak-Higashi syndrome. Assessment of the frequency of inclusions was difficult because the blood samples were obtained primarily for studies on granulocytes. However, lymphocytes were frequent contaminants of the leukocyte preparations due to the relative granulocytopenia of CHS patients. Determination of the frequency of lymphocytes containing tubule-filled organelles in thin sections was based on evaluation of fourteen leukocyte preparations obtained at different times over a span of 3 years from the new patient. Although the number varied in each sample, lymphocytes containing inclusions filled with tubules were present in every specimen and appeared to comprise from 1 to 4% of

the total population of lymphocytes. Since the plane of thin section may pass through lymphocytes without intersecting the organelles, the frequency may be substantially higher. However, because of the small number of lymphocytes encountered in single thin sections, it was not possible to apply confidence limits to the values obtained. The frequency of lymphocytes containing tubule-filled organelles in thin sections of leukocyte preparations from the three other patients appeared similar to that observed in samples from the new patient in whom the occurrence rate was evaluated.

Giant Tubule-Filled Organelles in CHS Lymphocytes

At low magnification, the giant tubule-filled inclusions in CHS lymphocytes were relatively homogenous and difficult to distinguish from other forms of giant inclusions that occur in these cells (Figure 1A–D). Higher magnification revealed the characteristic tubular substructure of the organelle matrix (Figures 1E, 2B). Completely formed inclusions were enclosed by typical unit membranes and packed tightly with tubular elements sectioned in various planes (Figures 1C–E, 2B). Cross-sections of the tubules revealed circular profiles surrounding hollow cores which often contained central filaments (Figures 1E, 2B, 4A). The diameter of the tubules varied between 150 to 300 Å, with a wall thickness of approximately 65 Å. The size and morphology of the tubular elements filling the giant granules in CHS lymphocytes were similar to microtubules found in nearly all cells,[18] but some differences were apparent. Microtubules are rarely found in wall to wall contact, and, due to their inherent rigidity, are straight or slightly curved over long distances. Tubular elements in the CHS organelles were packed tightly together and followed irregular courses, producing a vermiform appearance in the compact matrix.

Membrane-bound inclusions containing tubular elements in normal and CHS lymphocytes may have originated directly from cytoplasmic constituents (Figure 2A, C–E). Loosely associated groups of tubular elements were observed which lacked a unit membrane or were only partially enclosed (Figure 2C, D). The tubular complexes without membranes may represent a precursor stage in the formation of the giant organelles. Some of the tubule-filled organelles contained structures which usually exist independently in the cell (Figures 2E, 3A–C). Their presence may have resulted from sequestration with tubular elements when the involved area of cytoplasm was enveloped by a membrane.

Although most of the fully developed organelles appeared to consist entirely of tubular elements, some examples in which homogenous substance, separate from the tubules, occupied a portion of the matrix were observed (Figures 3A–C, 4A, B). The homogenous substance resembled the material in giant lysosomal granules identified previously in CHS lymphocytes,[5–8] suggesting the possibility that fusion of two types of organelles had produced a complex inclusion.

Examination of lymphocytes from samples incubated for acid phosphatase activity supported this possible association. The giant organelles which consisted exclusively of tubular components did not reveal acid phosphatase activity. In some lymphocytes, however, lead phosphate, indicative of acid phosphatase activity, was deposited in giant organelles which contained tubules in areas of matrix separate from the circular hollow profiles (Figure 4B).

Tubular Elements in Giant Granules of Other CHS Leukocytes

Massive organelles consisting entirely of tubules, similar to those found in lymphocytes, were not evident in other blood cells from the CHS patients. Occasionally, however, a few tubules resembling those in lymphocytes were apparent in the giant organelles of monocytes and polymorphonuclear leukocytes (Figure 4C–F). The microtubules present in the neutrophil inclusions occurred only in giant organelles manifesting internal disorganization (Figure 4C, D). In monocytes, tubular elements were found in the matrix of giant organelles and as loosely associated bundles, without a membrane, in the cytoplasm (Figure 4E, F).

Discussion

Huge membrane-bound masses of tubular elements were observed, in the present investigation, in a small percentage of circulating lymphocytes from 4 children with the Chediak-Higashi syndrome. Similar inclusions were probably identified in previous investigations of the CHS,[5,8] but the presence of hollow-cored circular profiles resembling microtubules in giant lymphocyte organelles was not discussed. A lattice of parallel lines separated by intervals of 150 Å was noted by Douglas *et al* in the matrix of some massive lysosome-like organelles in CHS lymphocytes;[8] the structures may correspond to the tubule-containing bodies described in this report. However, the organized matrix substance was defined as crystalloid material by the authors, and a similar periodic organization has been observed in giant CHS

leukocyte granules by others.[7] Thus, the massive tubule-filled organelles in CHS lymphocytes appear to have escaped notice in previous studies, and their relationship to giant inclusions which contain crystalloid material is uncertain.

The membrane-enclosed masses of tubules in CHS lymphocytes resemble the tubule-containing organelles found in the cytoplasm of normal and abnormal lymphoid cells in earlier investigations.[1,2] Tubular elements observed by Hovig *et al*,[1] in lymphocytes from a patient with leukopenia and rheumatoid disease, were approximately 420 Å in diameter, nearly twice the size of tubules in CHS lymphocyte organelles, and occurred as isolated bundles in swollen vacuoles rather than as compact masses filling their enclosing membranes. However, the tubule-containing vacuoles observed by Hovig *et al* appeared to develop by sequestration of loosely associated masses of tubules within membranes; this process often resulted in the incorporation of organelles and other cytoplasmic components, in addition to the hollow polymers. Giant tubule-filled inclusions in CHS lymphocytes appear to evolve in a similar manner from the cytoplasm, and frequently contain cytoplasmic material as well as tubules. It is probable that the two phenomena, the one observed by Hovig and his coworkers and the other identified in our study of CHS lymphocytes, are the same. Differences in the disease states of the patients studied and methods used to prepare lymphocytes for examination in the electron microscope may have affected the size of the tubules and appearance of the organelles in the separate investigations.

The report by Huhn,[2] in which he identified tubule-filled organelles in 1 to 5% of normal circulating lymphocytes from twelve healthy persons, has not been followed by supporting publications from his or other laboratories. However, routine evaluation of blood samples by electron microscopy in this laboratory over the past 10 years supports the observation made by Huhn. Lymphocytes containing one or several tubule-filled organelles were found in small numbers in every leukocyte sample when an effort was made to search for them. The frequency of normal lymphoid cells with tubular inclusions was not determined in our material, but the rate suggested by Huhn [2] must be reasonably accurate since they could be found without difficulty in random sections of leukocyte preparations. In contrast to the single giant organelles in CHS lymphocytes, normal cells usually contained several of the tubule-filled structures which were less than 0.7 μ in diameter, while huge CHS inclusions sometimes exceeded 3 μ. However, tubular elements in organelles of normal and CHS lymphocytes

were similar in their dimensions, and the process of organelle formation in both cell types appeared to be the same (Figure 2A). As a result, the giant tubule-filled organelles in CHS lymphocytes resemble closely the small inclusions of tubular elements in normal lymphoid cells and most likely represent an aberration of normal organelle formation rather than a unique phenomenon.

The origin, nature and function of tubule-filled organelles in normal and CHS lymphocytes are obscure. The cells which contain them may represent a special class of lymphocytes, or the structures may be present in all normal lymphoid cells at some specific stage in their life cycle, and disappear with increasing maturity. The process of granulopoiesis in lymphocytes has not been studied as thoroughly as it has in other cell types,[19] and it is possible that lymphoid cells develop more than one, and possibly several, kinds of storage granules. Tubule-containing inclusions may represent a specialized type of cytoplasmic structure or a particular step in the evolution of one form of lymphocyte organelle. Further experiments will be required to evaluate these possibilities.

The inclusions filled with tubules appear to form in the cytoplasm, rather than through the sequential steps involved in the packaging of most secretory and storage organelles. Small and large groups of loosely associated tubules were found free in lymphocyte cytoplasm, and stages in formation of sequestering membranes were identified. In normal lymphocytes the process involved only tubular elements, but cytoplasmic debris and organelles were often isolated with tubules inside sequestration membranes in abnormal cells. The several small organelles in normal cells remained discrete, but Hovig noted a tendency for tubule-containing inclusions to fuse in abnormal lymphocytes.[1] Interaction and fusion of small organelles could provide a mechanism for the origin of the single, massive, tubule-filled organelles in CHS lymphoid cells.

The process of cytoplasmic sequestration, isolation of organelles, and fusion of granules are ordinarily associated with autophagy,[6] but development of tubule containing organelles in normal and CHS lymphocytes did not resemble a destructive process. In addition, acid phosphatase, a marker enzyme for lysosomes and autophagic vacuoles, was not found in tubule-containing organelles in normal cells or in the giant inclusions that were filled with tubules in CHS lymphocytes.[2,5–7] Acid phosphatase activity was present in giant lysosomes of CHS lymphoid cells,[5–7] and in the amorphous matrix of tubule-containing organelles which had fused with lymphocyte lysosomes. Fusion

of organelles is not unusual in CHS cells and is considered the basic mechanism involved in formation of giant azurophilic inclusions in CHS granulocytes.[3-7,16,17] The observation in CHS lymphocytes indicates that giant lysosomes can fuse with nonlysosomal organelles to produce compound inclusions, a finding which supports previous studies on the phenomenon of cytoplasmic sequestration in CHS cells.[6]

Endoplasmic reticulum may be the source of the tubular elements ultimately sequentered in the giant tubule-filled organelles in CHS lymphocytes. Groups of loosely associated or branched tubules similar to the tubular elements in giant CHS lymphoid organelles have been found in a variety of cells and tissues obtained from patients with malignant and nonmalignant diseases.[20-24] Similar aggregates of tubules have been identified in cultured lymphoid cells from patients with cancer,[23,25] systemic lupus erythematosus,[26] idiopathic thrombocytopenic purpura,[27] infectious mononucleosis,[28] Chediak-Higashi syndrome [29] and persons without evidence of disease.[25] The clumps of cytoplasmic tubules have also been observed in circulating lymphocytes from individuals with systemic lupus erythematosus [26] and a patient with chronic lymphocytosis.[30] Several workers have considered the tubular aggregates to be viral in origin and related to the etiology of the diseases in which they occur.[21,24,26-28] Others have provided evidence suggesting that tubules originate from endoplasmic reticulum of pathologic and normal cells. Though they may represent an aberrent response of the cells to infection or malignant transformation, they are not viruses.[22,25] Since tubule-containing organelles are regularly found in a small percentage of normal circulating lymphocytes, it is doubtful that the massive tubule-filled inclusions in CHS lymphocytes are related to the action of viral agents. It is more likely that the tubular elements in CHS lymphocytes originate from endoplasmic reticulum, aggregate in the cytoplasm and then become sequestered into giant organelles. The proposed sequence must remain speculative, however, for no direct evidence for the origin of tubular elements from endoplasmic reticulum in CHS lymphocytes was obtained in the present investigation.

The relationship of the tubule-filled organelles in normal and CHS lymphocytes to aggregates of tubules in circulating lymphoid cells from patients with systemic lupus erythematosus [26] and chronic lymphocytosis,[30] and in cultured lymphocytes from heterozygotes and patients with CHS [29] is not clear. Although the tubules have similar dimensions (150 to 310 Å), the cytoplasmic aggregates, in cultured CHS cells and circulating lymphocytes from patients with other dis-

eases, differ morphologically from groups of tubules gathered in the cytoplasm and sequestered in the giant organelles in lymphocytes from peripheral blood of children with CHS. The differences are relatively minor, and may be induced by a particular disease, the conditions employed in cell culture or the technics used to fix and dehydrate cells for electron microscopy. On the other hand, the differences in the morphology of cytoplasmic tubular aggregates in normal and abnormal lymphocytes may reflect a pathologic alteration in the cytoplasm. Further investigations of tubular aggregates and tubule-filled organelles in lymphocytes may reveal their function in the cell and role in disease states involving lymphoid cells and tissues.

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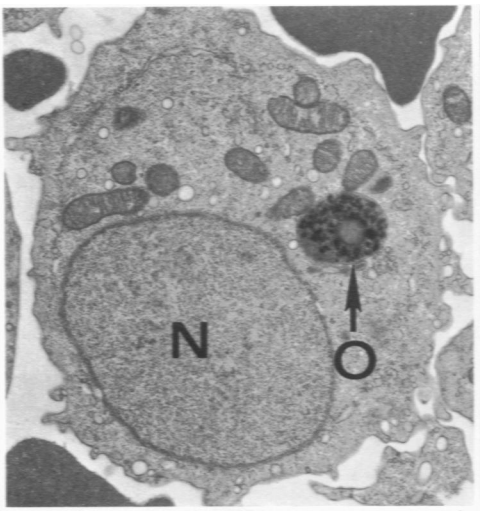
Legends for Figures

Fig 1—Lymphocytes from patients with the Chediak-Higashi syndrome. The cell in **A** was fixed in 4% osmic acid without buffer and the others in the usual manner with glutaraldehyde and osmic acid. The organelles (*O*) in the cells shown in **A** and **B** reveal the pleomorphic substructure characteristic of giant CHS inclusions. Giant inclusions (*TO*) of the lymphocytes in **C** and **D** are filled with tubules, but at this magnification they are difficult to distinguish from the homogeneous granule in **B.** In **E** a portion of lymphocyte cytoplasm viewed at higher magnification reveals the substructure of a giant tubule-filled organelle (*TO*), and the relationship of the inclusion to other cell structures. The massive organelle may indent the nucleus (*N*), but is always separated from it. A centriolar complex (*C*), Golgi saccules (*GA*), mitochondria (*M*) and microtubules (*MT*) are also distinctly separate structures (**A**, × 9,600; **B**, × 13,900; **C**, × 13,900; **D**, × 12,400; **E**, × 41,500).

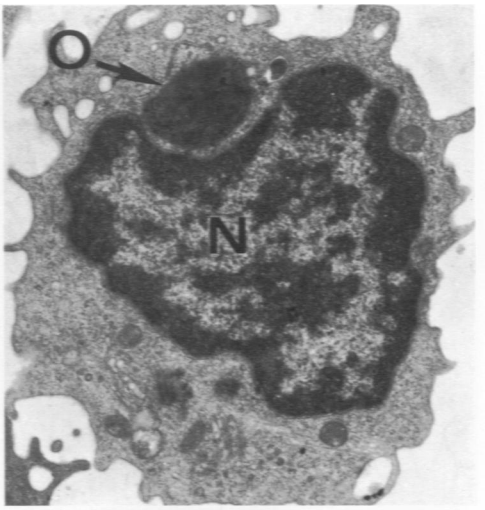
Fig 2—The cell in **A** is a normal lymphocyte with several tubule containing organelles (*arrows*) in various stages of formation. Tubules within the small organelles of the normal lymphocyte are similar in size and appearance to the tubular elements filling the giant inclusion (*TO*) of the CHS lymphocyte in **B**. The tubules which fill the massive inclusion in **B** are packed together in wall to wall contact. They are curved rather than straight, and, as a result, produce a vermiform appearance in the matrix of the giant organelle. The diameter of the tubules in cross section varies between 150 to 300 Å. The two examples in **C** and **D** reveal the cytoplasmic development of the tubular complexes. In each a portion of the enclosing membrane (*arrows*) can be delineated, but the interface on most surfaces is continuous with the surrounding cytoplasm. The example in **E** reveals sequestration of two membrane-bound granules (*G*) within the partially developed membrane (*arrows*) enclosing a complex of tubular elements. The variable diameters of tubular elements is also evident (**A**, × 32,000; **B**, × 36,300; **C**, × 41,500; **D**, × 38,200; **E**, × 74,500).

Fig 3—The area of cytoplasm from a CHS lymphocyte in **A** reveals several kinds of cytoplasmic structures. A giant organelle (*TO*) contains tubular elements (*T*) on one side of its matrix and amorphous debris (*D*) on the other. It is encircled by two multivesicular bodies (*MVB*) an area containing small granules which resembles an autophagic vacuole (*AV*), a smaller organelle (*G*) containing some tubular components and several mitochondria (*M*). The massive inclusions seen in **B** contains compressed tubular elements (*T*) surrounded by amorphous materials (*D*) of various densities and a myelin figure (*MF*). In **C**, two tubule-containing organelles are closely associated. The larger of the two (*TO*) contains some amorphous material (*D*) as well as tubular elements. The smaller, developing inclusion appears closely related to channels of endoplasmic reticulum (*ER*) (**A**, × 58,100; **B**, × 38,000; **C**, × 36,200).

Fig 4—The organelle in **A** is from a CHS lymphocyte fixed in 4% osmic acid in distilled water. Central filaments are evident in several of the hollow circular profiles (*T*). A portion of the organelle matrix consists of amorphous substance (*D*). The CHS lymphocyte in **B** was incubated for acid phosphotase which is indicated by specific deposits of lead phosphate. Enzyme reaction product is localized within the membrane of the giant organelle (*T*), but is almost completely excluded from the zone containing tubular elements. A neutrophil in **C** contains a giant azurophilic granule (*A*) and three massive zones formed by disintegration of the large primary granules and their interaction with cytoplasmic components (*1*, *2*, *3*). Tubular elements (*T*) are evident in the matrix of two of the giant organelles. A similar area containing tubular elements (*T*) is shown at higher magnification in **D**. A large azurophilic (*A*) nearby reveals a periodic substructure. This cell was fixed in 4% osmic acid alone, as was the monocyte shown in **E** and **F**. The monocyte has two areas (*1*, *2*) in which tubular elements are localized. Higher magnification reveals a membrane which encloses one of the tubule-containing organelles (*2*), while the other group appears to lie free in the cell cytoplasm (*1*) (**A**, × 41,500; **B**, × 39,300; **C**, × 8300; **D**, × 33,200; **E**, × 10,300; **F**, × 36,300).



1A



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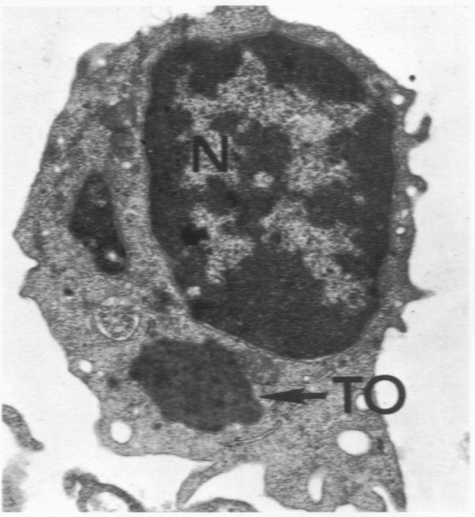

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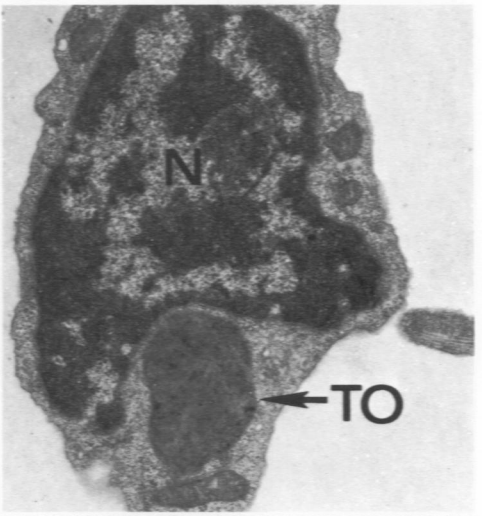

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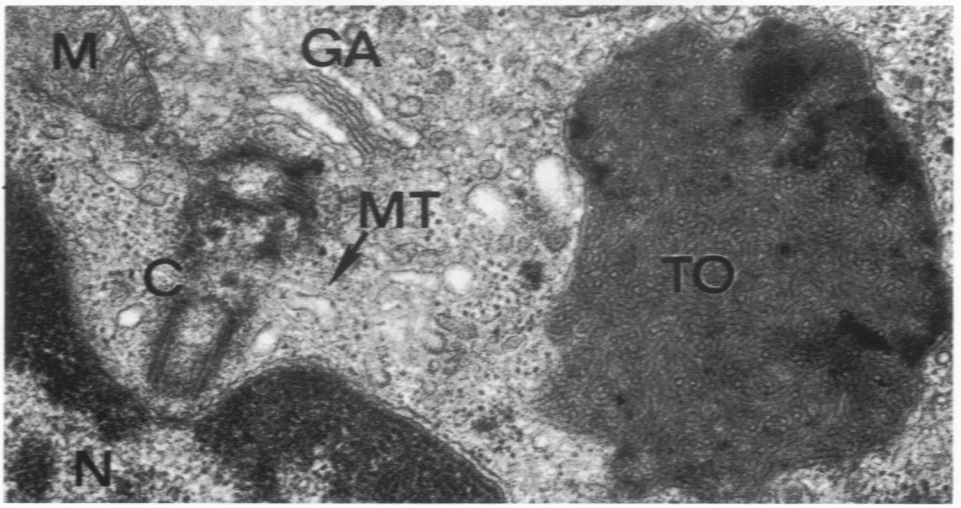

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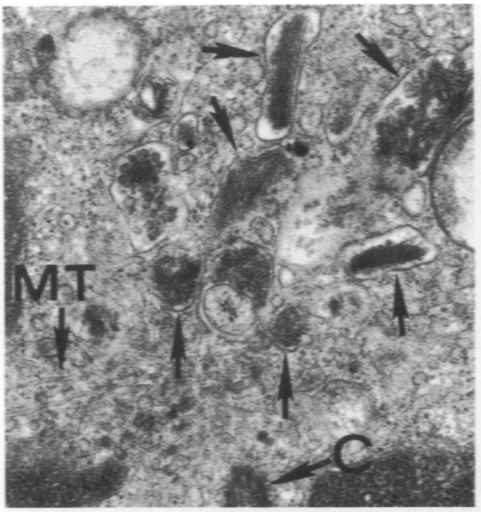
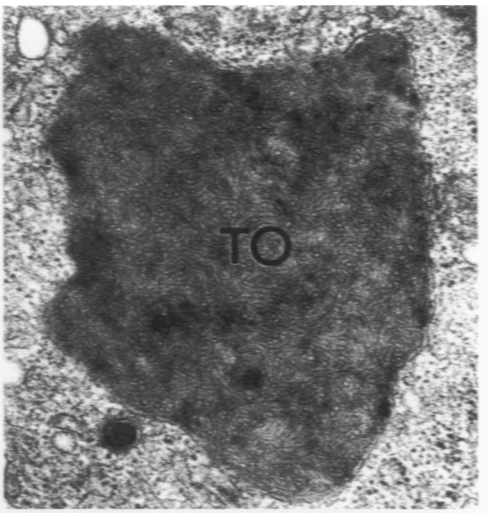


2A



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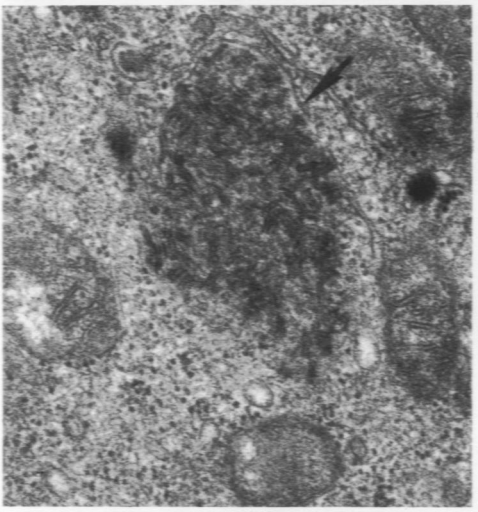

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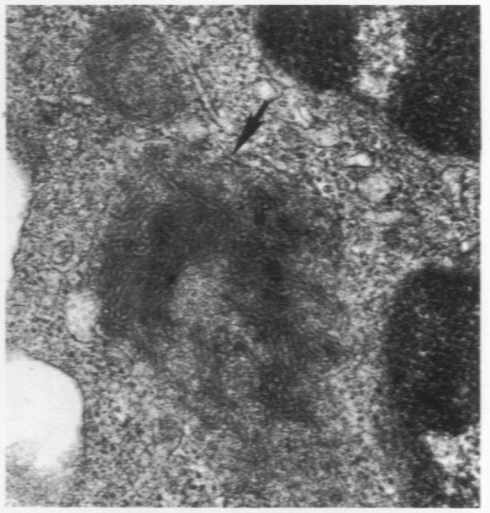

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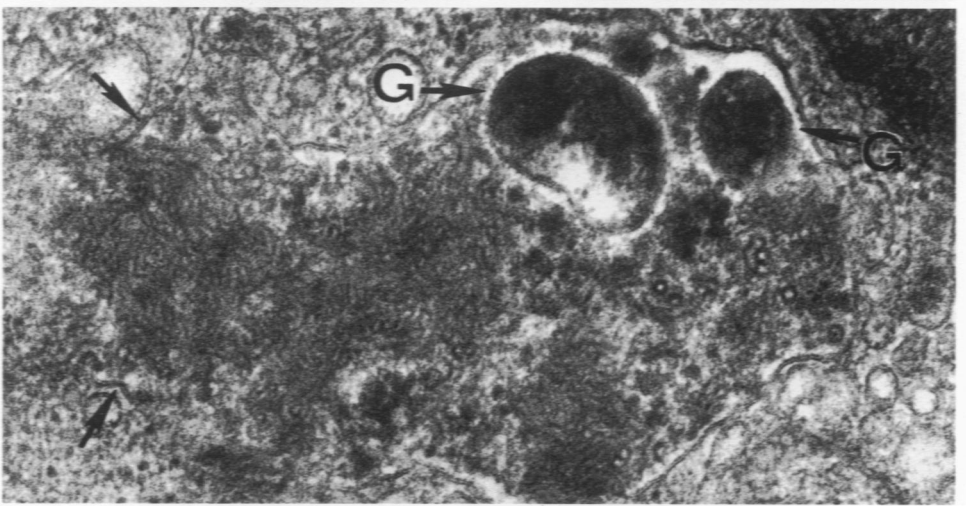

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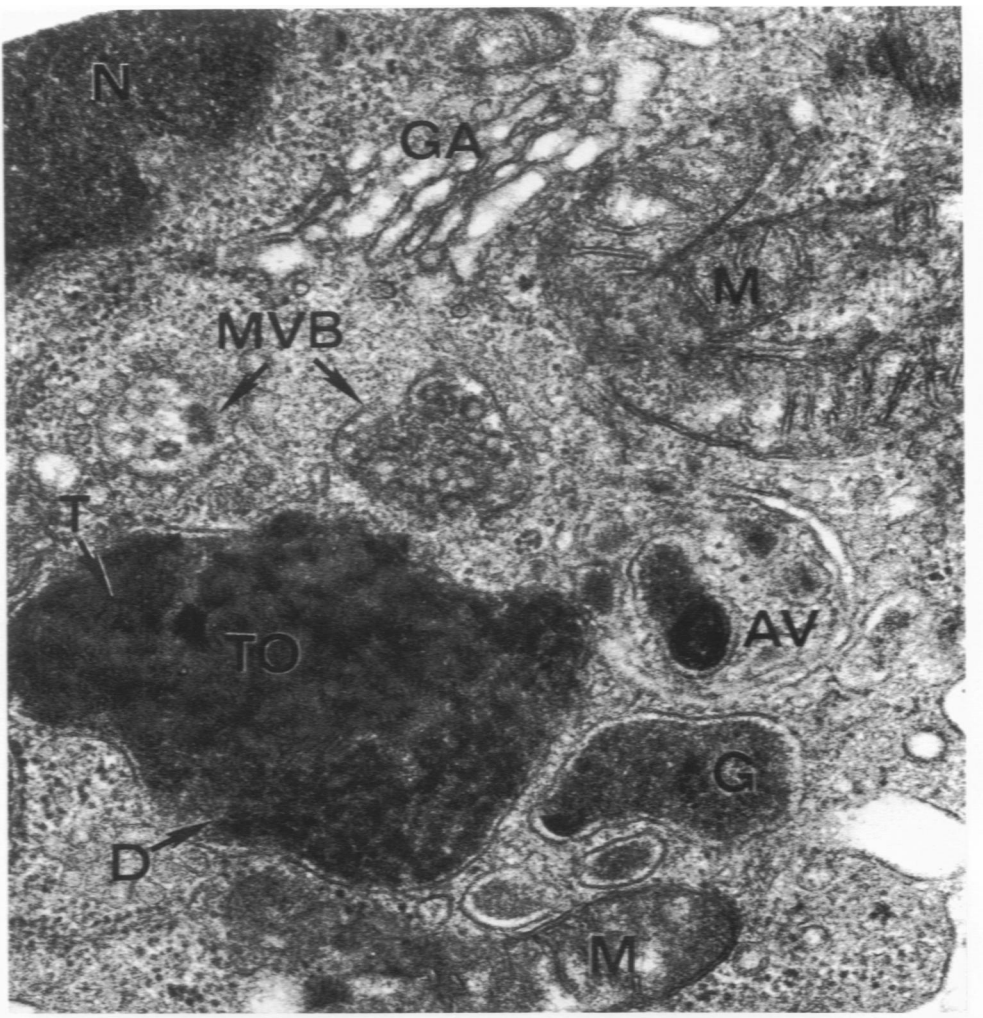
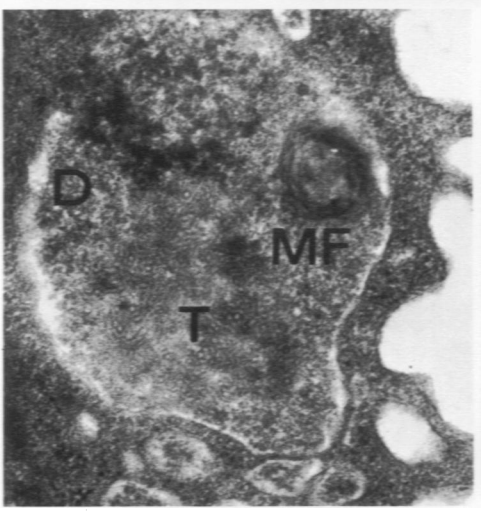
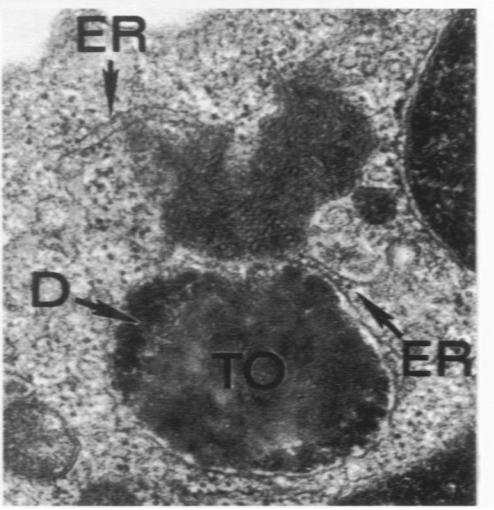


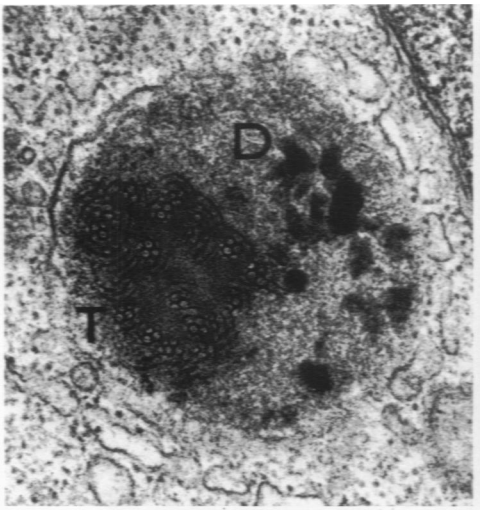
3A



3B



3C



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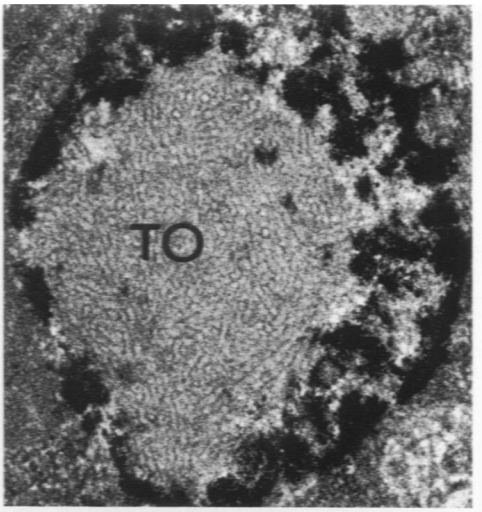

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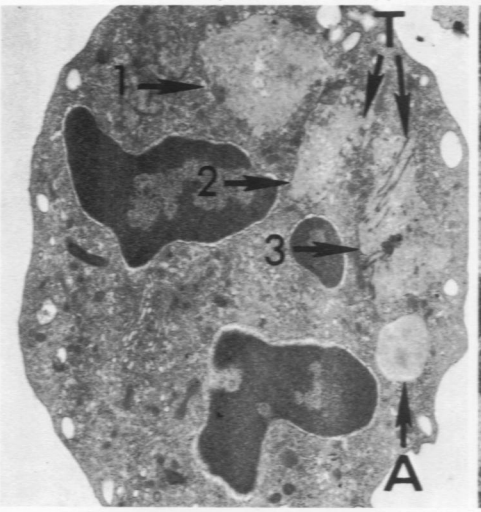

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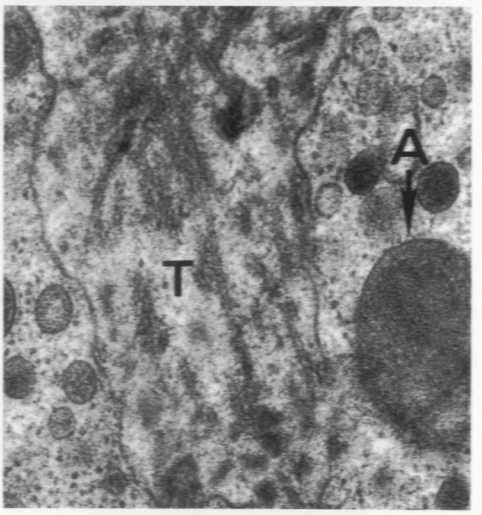

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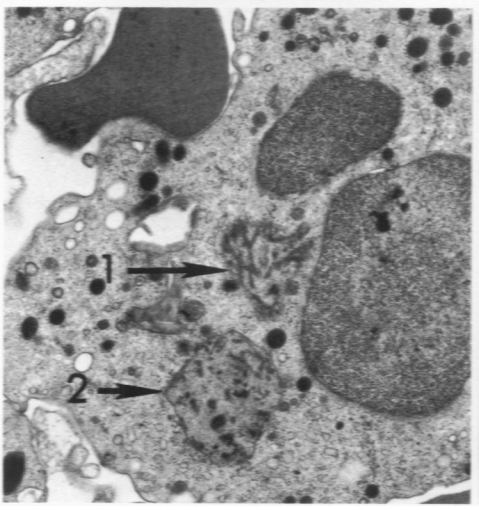

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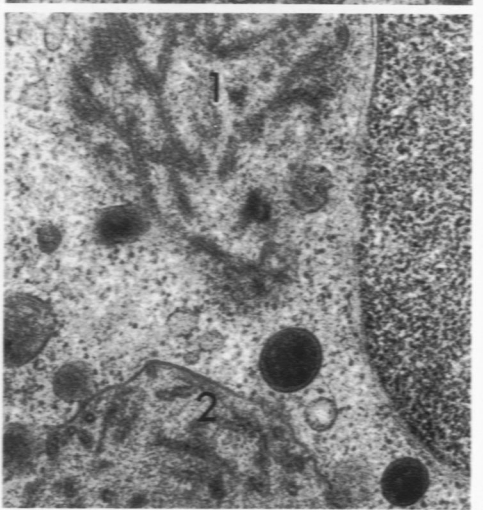

4F