Ultrastructural Studies of the Gray Platelet Syndrome

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The gray platelet syndrome (GPS) is a rare inherited disorder in which peripheral blood platelets are relatively large, vacuolated, and almost devoid of cytoplasmic granulation. In the present study we have evaluated the ultrastructure and cytochemistry of platelets from 2 patients with the GPS to determine precisely which organelles are missing from their cells. The findings indicate that gray platelets contain normal numbers of mitochondria, dense bodies, peroxisomes, and lysosomes but specifically lack α-granules. Preliminary studies of megakaryocytes from 1 of the 2 patients suggest that the defect in granule formation may lie at the level of the Golgi zone. (Am J Pathol 95:445–462, 1979)

IN 1971 Raccuglia [1] described an unusual qualitative platelet disorder which he termed the "gray platelet syndrome" (GPS). The male patient was originally referred because of thrombocytopenia which was corrected by steroid therapy and splenectomy. Examination of his platelets revealed a high percentage of relatively large cells which were nearly devoid of granules, resulting in a peculiar gray color on Wright-stained blood smears. Biochemical studies revealed decreases in the levels of platelet ATP and phosphatides and an absence of phosphatidyl serine. Relatives of the child were unaffected and, subsequently, no additional cases of this syndrome have been reported.

A great deal has been learned about the nature of platelet organelles since the GPS was described. Investigations in several laboratories have shown that, in addition to mitochondria, normal platelets contain a small number of dense bodies which store serotonin, calcium, and the nonmetabolic pool of adenine nucleotides,[2-8] a few peroxisomes which contain catalase,[9] and a large number of organelles which have been called α-granules.[10] However, the α-granule population is not uniform and appears to consist of at least two types of organelles. One variety contains hydrolases such as acid phosphatase and aryl sulfatase and is, therefore, a lysosome.[11,12] The other organelle is believed to be a storage site for platelet factor 4, fibrinogen, and possibly other constituents important to

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platelet physiology.[13] It is possible that platelets contain yet other varieties of storage organelles, but the technical problems involved in isolating specific populations have made it extremely difficult to solve this problem.[14,15]

The GPS, therefore, provides a unique experiment of nature in which to identify whether platelets contain specialized subfractions among the populations of cytoplasmic organelles. We have studied the ultrastructure, cytochemistry, biochemistry, and physiology of platelets from the original patient with GPS on several occasions and have completed similar studies on a second, unrelated child with this disorder. Results of these studies demonstrate that gray platelets are deficient in a single population of granules. The present report will describe the ultrastructure and cytochemistry of gray platelets from the 2 patients.

Materials and Methods

Two patients were available for the present study. The history, clinical findings, and laboratory results obtained from the male patient were presented by Raccuglia in his original report.[1] The patient is now 22 years of age and in excellent health. He has led a normal life and has participated in contact sports. One episode of large hematoma formation secondary to a football injury might have been related to defective platelet function, but in all other respects his bleeding history has been negative. The second patient is a 3.5-year-old girl with Goldenhar's syndrome [16] (oculoauriculovertebral dysplasia) referred because of a recurrent pettechial rash and unusual platelets on Wright-stained blood smear. Her parents and siblings are unaffected; examination of 2 other patients with Goldenhar's syndrome did not reveal abnormal platelets. The platelet counts of both patients ranged between 100,000 and 150,000 during the course of the investigation.

The procedures used to obtain blood from normal donors and patients; mix the samples immediately with 3.8% trisodium citrate or citrate-citric acid, pH 6.5, in a ratio of 9 parts blood to 1 part anticoagulant; separate citrate platelet-rich plasma (C-PRP); and prepare the cells for study in the electron microscope have been described in several recent publications.[17-20] Most samples were fixed initially in an equal volume of 0.1% glutaraldehyde in White's saline, pH 7.3, for 15 minutes at 37 C or 23 C, sedimented to pellets, combined with 3% glutaraldehyde in the same buffer for 60 minutes at 4 C, washed in buffer, and subsequently exposed to 1% osmic acid in buffered veronal acetate. The usual osmic acid step for paired samples from both patients was replaced by addition of 1% osmic acid in distilled water with 1.5% potassium ferricyanide to enhance membrane contrast.[21]

Cytochemical studies, including the reactions for peroxidase,[20] catalase,[9] acid phosphatase, and aryl-sulfatase,[12] and a variety of special procedures, including exposure to the electron-dense stain, lanthanum nitrate,[22] the uranaffin reaction,[23] and freeze-fracture analysis,[24] were carried out on samples of platelets from the 2 patients. Specific details of each technique have been described in previous reports from this laboratory or the other publications indicated from the recent literature.[9,12,20,22-24] After completion of cytochemical and fixation procedures, all samples were dehydrated in an ascending series of alcohols and embedded in Epon 812. Thin sections were usually stained with uranyl acetate and lead citrate, but sections from samples incubated for specific enzymes or exposed to electron-dense particles were examined in the Phillips 301 electron microscope without enhancement of contrast by surface stains. The male subject has been studied on

seven occasions over the past 8 years; the female patient has been studied five times in the past 2 years.

Results

Ultrastructure

The morphology of gray platelets viewed in thin sections of plastic-embedded samples from the 2 patients differed strikingly from similar samples prepared from normal donors or patients with other inherited platelet disorders (Figure 1). At low magnification, gray platelets were highly variable in size and form. The smaller cells were relatively discoid; the larger cells appeared more spherical and irregular. Even at low magnification, the marked decrease in cytoplasmic granulation and increase in the number and size of vacuoles could be recognized. The vacuolated appearance of many of the peripheral blood platelets was nearly identical to the morphology of large areas of cytoplasm in bone marrow megakaryocytes from one of the patients (Figure 2). Other areas of the cytoplasm in megakaryocytes were virtually devoid of vacuoles or organelles; this appearance was also reflected in the morphology of platelets in the peripheral blood (Figures 3 through 6). Many platelets in thin section appeared to be formless sheets of cytoplasm with no discrete internal structures. Others contained mitochondria, dense bodies, and a few elements of the dense tubular system, originating from rough endoplasmic reticulum of the megakaryocyte, but no granules. Occasional platelets contained rough endoplasmic reticulum or unusual structural elements which have not been observed previously in human platelets (Figures 7 and 8).

The vacuolization of gray platelets was striking (Figures 9 through 14). In some platelets only a few vacuoles could be identified; in others the cytoplasm was virtually replaced by empty sacs. Many of the vacuoles appeared to be dilated channels of the surface-connected open canalicular system; others may represent granule membranes whose contents have been lost or never received. In a few platelets the vacuoles appeared to enclose debris or recognizable cytoplasmic structures and may, therefore, be autophagic vacuoles (Figure 12).

Membranous structures other than vacuoles often dominated the cytoplasm of gray platelets (Figures 15 through 20). They included elements of the dense tubular system, the surface-connected open canalicular system, and the membrane complexes formed from the two channel systems. In some platelets the open canalicular system predominated (Figures 15 and 16); in others the dense tubular system virtually replaced the cytoplasm (Figures 17 and 18). Unusual linear arrays of dense tubular system

membranes were seen on occasion in gray platelets (Figures 19 and 20). Similar organizations of dense tubular system membranes have not been observed previously in human platelets. The excess of dense tubular system membranes in gray platelets may have led to the development of areas of apparent cytoplasmic sequestration or autophagic vacuole formation (Figures 21 and 22).

Freeze-Fracture

Freeze-fracture studies confirmed the massive content of membranous structures in gray platelets as indicated by thin section material (Figures 23 through 26). It was hoped that comparison of replicas of gray platelets with normal platelets might reveal differences in the size and number of intercalated particles on internal membranes, providing a way to distinguish granule membranes from other surfaces. However, no such differences could be defined in the present study.

Electron-Dense Tracers

Lanthanum nitrate was added to samples of gray platelets during fixation to determine if all cytoplasmic vacuoles in the cells were connected by channels of the open canalicular system to the cell surface. The exterior coat of gray platelets and channels of the open canalicular system were covered by a layer of lanthanum particles, and interior surfaces of many vacuoles were also coated by electron-dense tracer (Figure 27). Membranes of some vacuoles in gray platelets, however, were not stained by lanthanum.

Cytochemistry

Enzyme localization and other specialized studies were carried out to identify the structures in the cytoplasm of gray platelets. As indicated above, there are only a few granules in gray platelets. Most of these organelles appeared to be lysosomes, since either acid phosphatase (Figure 28) or aryl sulfatase (Figure 29) could be localized in them. The organelles reacting positively for either enzyme may be from the same population of granules, since the incubations for each reaction had to be carried out on separate platelet samples. The modification of the peroxidase reaction specific for platelet catalase introduced by Breton-Gorius [9] demonstrated the presence of small numbers of peroxisomes in gray platelets (Figure 30). Thus, two types of organelles, in addition to mitochondria and dense bodies, can be accounted for in gray platelets: lysosomes and peroxisomes.

The uranaffin reaction was recently introduced by Richards and Da

Prada.[23] Incubation of glutaraldehyde-fixed platelets in a solution containing uranyl acetate resulted in selective deposition of metal on the inner half of membranes enclosing dense bodies. In our hands, this procedure also tains the inner half of the membranes of a small number of normal platelet granules.[25] The uranaffin reaction was positive in gray platelets from both patients. The inner membranes of vacuoles containing dense bodies were stained by uranium deposits, as were a few of the small complement of granules in the cells (Figure 31).

The peroxidase reaction on gray platelets revealed the presence of reaction product in the dense tubular system and its elements, forming complexes with channels of the open canalicular system (Figure 32). No reaction product was evident in the large or small vacuoles or other organelles of gray platelets.

Discussion

The gray platelet syndrome (GPS) is an exceptionally rare inherited disorder of blood patelets.[1] Only one other described patient appears to have a similar condition but is also storage-pool–deficient and, therefore, must lack dense bodies as well as α-granules in her platelets.[25] Since the only reported case and the second child with GPS described briefly in this study have had few problems with clinical bleeding, the disorder might be considered a curiosity not worth the effort of intensive study. However, as has been the case in many areas of human physiology, most of our understanding of normal mechanisms has resulted from thorough evaluation of pathologic defects. Our appreciation of blood coagulation and the role of platelets in hemostasis, for example, has stemmed mainly from work on patients with varieties of hemophilia or prolonged bleeding due to platelet dysfunction. Thus, the GPS, although not of great interest as a serious disease of platelets, may be of significant importance in our attempts to improve knowledge of normal platelets. Based on this premise, we have carried out an extensive investigation of the physiology, biochemistry, and morphology of gray platelets from the initial case described by Raccuglia [1] and a second patient recognized by our laboratory.

An important part of this effort has been directed toward the definition of precisely what organelle or group of organelles are deficient in gray platelets. Electron microscopic studies of gray platelets were carried out in the original study, and Raccuglia recognized that the bizarre cells contained normal numbers of mitochondria and glycogen stores, many vacuoles, and an absence or paucity of granules.[1] However, it was not generally appreciated at that time that platelets contain several types of

organelles which were referred to collectively as granules or α-granules.[10] Studies in recent years have shown that serotonin and the nonmetabolic pool of adenine nucleotides are stored in a specialized organelle referred to as "the dense body" which may be derived from one type of α-granule.[7,26] Hydrolytic enzymes such as acid phosphatase, n-acetyl glucosaminidase, and aryl sulfatase are confined within a small population of α-granules now called "lysosomes."[11,12,27] Catalase has been localized to a population of small α-granules called "peroxisomes."[9] Other stored products, including β-thromboglobulin,[28,29] platelet factor 4,[30] fibrinogen,[13] thrombin-sensitive protein,[31] and the mitogenic factor which stimulates proliferation of smooth muscle cells,[32,33] are believed to reside in a separate group of α-granules or in several subpopulations. In view of the complexity of the organelles known to reside in the cytoplasm of normal platelets, the GPS cannot be adequately characterized by its definition as a defect of α-granules.[1]

The present investigation has evaluated by electron microscopy and cytochemistry the organelles and other structures in the cytoplasm of gray platelets from 2 patients with the disorder. As reported by Raccuglia, the mitochondria of gray platelets are normal. Because of the striking decrease in granules, the number of mitochrondria in some cells may appear excessive. However, the increase is more apparent than real, since mitochondria are often mistaken for granules in the crowded cytoplasm of normal platelets.[34]

The dense bodies of gray platelets are also normal in number and distribution in the cells. Exposure of glutaraldehyde-fixed gray platelets to uranyl acetate according to the procedure introduced by Richards and Da Prada [23] resulted in the deposit of uranium on the inner half of dense body membranes, just as described by the authors, and on the inner leaflet of a few granule membranes, as we have observed previously.[25] Thus, the mechanism for forming dense bodies, the vacuoles in which they develop, and the small population of granules from which they may originate are present in gray platelets.

Incubation of gray platelets in the medium described by Breton-Gorius [9] for the demonstration of catalase resulted in the deposit of reaction product in a few small granules. The population of peroxisomes in gray platelets appears comparable to that of normal cells. Two cytochemical techniques specific for hydrolytic enzymes revealed the presence of acid phosphatase and aryl sulfatase in granules of gray platelets. The methods are not quantitative but provide qualitative evidence that most, if not all, of the small complement of α-granules in gray platelets are lysosomes.

The cytochemical and electron microscopic studies indicate that gray platelets contain normal numbers of mitochondria, dense bodies, peroxisomes, and lysosomes. By elimination, the only morphologically distinguishable organelle in which they could be deficient is the granule storing β-thromboglobulin,[28] fibrinogen,[13] thrombin-sensitive protein,[30] platelet factor 4,[29] and mitogenic factor.[31] Therefore, it seems reasonable to suggest that these substances and others which may be missing from gray platelets are stored in a single type of storage organelle which is not formed in the megakaryocyte precursor of this cell.

Another facet of this study has been the attempt to determine if the large number of vacuoles in gray megakaryocytes and platelets were meant as storage sites for missing products but never received them or lost the substances in transit. The fact that small vacuoles similar in size to granules are present in large numbers in the megakaryocyte suggests that they were destined to receive some product(s). Evaluation of gray platelets with an electron-dense stain revealed that a number of vacuoles in the peripheral blood cells are closed and do not communicate with the surface-connected open canalicular system. Although it is possible that some vacuoles may have developed by pinching off from channels of the OCS, it is just as likely that they developed as putative granules in the megakaryocyte. The vacuoles did not develop from channels of the dense tubular system, because every element of the DTS stains for peroxidase while the vacuoles never do.[20]

On the basis of these studies, it is possible to speculate that the abnormality in formation of α-granules in gray platelets is not due to a defect in the ability of the parent megakaryocyte to generate membrane to enclose them. The defect is unlikely to be related to membranes of the endoplasmic reticulum. Several different proteins are deficient in gray platelets, but only one type of organelle is missing from megakaryocytes as well as platelets. Since both cell types have abundant endoplasmic reticulum, the defect appears to lie somewhere in the mechanism involved in transfer of proteins from endoplasmic reticulum through the Golgi apparatus of the megakaryocyte to the membranes destined to become granules. The number of Golgi zones appears to be reduced in megakaryocytes from 1 patient with GPS, but more material will be required from the 2 patients before this possible mechanism for defective granule formation can be established.

The present investigation has employed a range of ultrastructural and cytochemical techniques to define the nature of the organelle missing from platelets of 2 patients with the gray platelet syndrome. The results indicated that all organelles except the α-granules storing fibrinogen,

platelet factor 4, mitogenic factor, and thrombin-sensitive protein are present in normal numbers in gray platelets. It is speculated that the defect may lie at the level of the Golgi apparatus in the parent cell. Biochemical investigations confirming the observations made in this study will form the basis of a separate report. Investigations into the defect in granule formation in megakaryocytes from the 2 patients with GPS are in progress.

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[*Illustrations follow*]

Legends for Figures

Figure 1—Representative low-magnification electron photomicrograph of a thin section of platelets from 1 of the 2 patients with the gray platelet syndrome. Although many platelets appear relatively normal in size, others are distinctly enlarged (*arrows*), approaching the diameter of the lymphocyte (*L*) in the same section. Few organelles are evident in the cytoplasm of the platelets. Vacuoles are the dominant structures in most cells. Fixed with glutaraldehyde and osmium. (×4800)

Figure 2—Typical area of cytoplasm in a megakaryocyte from the bone marrow of the patient whose platelets are shown in Figure 1. The cytoplasm is virtually replaced by small vacuoles. A few dense bodies (*DB*) and putative granules (*G*) are apparent, but the area is otherwise devoid of the granulation characteristic of megakaryocytes at this stage of development. Golgi zones are not evident in this section and are uncommon in GPS megakaryocytes compared with normal cells. Fixed with glutaraldehyde, paraformaldehyde, and osmium. (×42,500)

Figure 3—Gray platelets. Two of the cells in this example are nearly devoid of formed organelles but contain masses of membrane (*arrows*) resembling elements of the dense tubular system. Fixed with glutaraldehyde and osmium. (×14,000)

Figure 4—Gray platelet. This platelet is discoid, and microtubules (*T*) of the circumferential band are evident at the two poles. A mitochondrion (*M*) and a few dense bodies (*DB*) are the only formed organelles apparent in the cytoplasm. Fixed with glutaraldehyde and osmium. (×22,000)

Figures 5 and 6—Gray platelets. Both cells in the two examples are devoid of formed organelles in the cytoplasm. Elements of the dense tubular system (*DTS*) are prominent in the cell platelet of Figure 5. Two areas of the cytoplasm in the platelet of Figure 6 are filled with membranous channels which are coated by particles identical to ribosomes; therefore, the channels represent residual elements of rough endoplasmic reticulum (*ER*). Fixed with glutaraldehyde, osmium, and ferricyanide. (**Figure 5**, ×13,000; **Figure 6**, ×15,400)

Figures 7 and 8—Unusual structures of gray platelets. **Figure 7**—The cytoplasm of the cell contains a bundle of parallel filaments (*1*) and a mass of dense material (*2*) resembling nuclear debris. **Inset**—An area of rough endoplasmic reticulum (*arrow*) in the cytoplasm from another platelet. **Figure 8**—Cell contains clumps of loosely associated dense material (*arrow*) arranged in a roughly circular configuration. Structures such as these have not been identified previously in normal platelets. Fixed with glutaraldehyde and osmium. (**Figure 7**, ×24,900; **Inset**, ×52,000; **Figure 8**, ×13,600)

Figure 9—Gray platelet. The cell contains a few dense bodies (*DB*) but no granules. The rest of the subcellular organelles in the cytoplasm are mitochondria (*M*). Fixed with glutaraldehyde and osmium. (×17,000)

Figure 10—Gray platelet. This cell contains a few organelles but is filled mainly with elements of the open canalicular system (*OCS*) and dense tubular system (*DTS*). The relationship of elements of the OCS and DTS in membrane complexes is evident just below the dilated channel of the OCS. The other large vacuole (*V*) may also be a component of the OCS. Fixed with glutaraldehyde, osmium, and ferricyanide. (×11,300)

Figure 11—Gray platelet. In this platelet the association of the OCS and DTS forms a large membrane complex (*MC*). The rest of the cytoplasm is filled with vacuoles of various sizes. Fixed with glutaraldehyde, osmium, and ferricyanide. (×8850)

Figure 12—Gray platelet. Numerous vacuoles fill the cytoplasm of this platelet. some of them (*V*) contain cellular debris and may represent autophagic vacuoles. Fixed with glutaraldehyde, osmium, and ferricyanide. (×12,600)

Figures 13 and 14—Gray platelets. The cytoplasm of the cells in these two examples is virtually replaced by vacuoles of various sizes. A mitochondrion (*M*) is the only organelle evident in the two cells. Fixed with glutaraldehyde and osmium. (**Figure 13**, ×12,600; **Figure 14**, ×16,400)

Figure 15—Gray platelet. The cell contains a few granules (*G*), numerous vacuoles, and a large membrane complex (*MC*) formed by elements of the OCS and DTS. Fixed with glutaraldehyde, osmium, and ferricyanide. (×16,500)

Figure 16—Gray platelets. Mitochondria and a few granular elements are present in the large cell. Most of the cytoplasm is filled with clear canalicular elements resembling channels of the OCS. Fixed with glutaraldehyde, osmium, and ferricyanide. (×11,200)

Figures 17 and 18—Gray platelets. The cytoplasm of one of the platelets in Figure 17 contains a large mass of channel elements resembling the DTS. The cell in Figure 18 is completely filled with channels of the DTS. Fixed with glutaraldehyde, osmium, and ferricyanide. (×13,000)

Figures 19 and 20—Gray platelets. In the two examples, channels of the DTS are organized in linear arrays (*arrows*). The arrangement of DTS channels in this manner has not been observed previously in human platelets. Mitochondria (*M*) are the most frequent organelles observed in these platelets. Fixed with glutaraldehyde, osmium, and ferricyanide. (**Figure 19**, ×11,000; **Figure 20**, ×16,500)

Figures 21 and 22—Gray platelets. Membranes communicating directly with the DTS have formed a circular array (*arrow*) in the cytoplasm of the cell in Figure 21. Its central zone may represent an area of cytoplasmic sequestration. An area of cytoplasmic sequestration is evident in the platelet of Figure 22. The enclosing membrane surrounds cytoplasmic organelles and may be an autophagic vacuole (*AV*). Fixed with glutaraldehyde, osmium, and ferricyanide. (**Figure 21**, ×30,000; **Figure 22**, ×16,500)

Figures 23 through 26—Freeze-fractured gray platelets. Replicas of the fractured cytoplasmic surfaces reveal the immense wealth of membrane material in the abnormal cells. The cell in Figure 23 resembles the platelets in Figures 10 through 14; the fractured surface in Figure 24 is similar to cells in Figures 17 and 20. The replica in Figure 25 demonstrates the enormously complex interaction of vacuoles, vesicles, OCS, DTS, and membrane complexes in the gray platelet. The replica in Figure 26 contains membrane-enclosed area strikingly similar to the autophagic vacuole (*AV*) in Figure 22. Communication of a channel of OCS with the cell surface is apparent in this example. Fixed with glutaraldehyde. (**Figures 23 and 24**, ×16,500; **Figures 25 and 26**, ×22,000)

Figure 27—Cytochemistry of gray platelets. The cells in this illustration are from a sample of gray platelets exposed to the electron-dense tracer lanthanum nitrate during fixation. Lanthanum has deposited on the cell surface and lines channels of the *OCS*. Some vacuoles (*V*) inside the cells have not been coated by the tracer and, therefore, do not appear to communicate with the cell surface. Fixed with glutaraldehyde and osmium. (×9700)

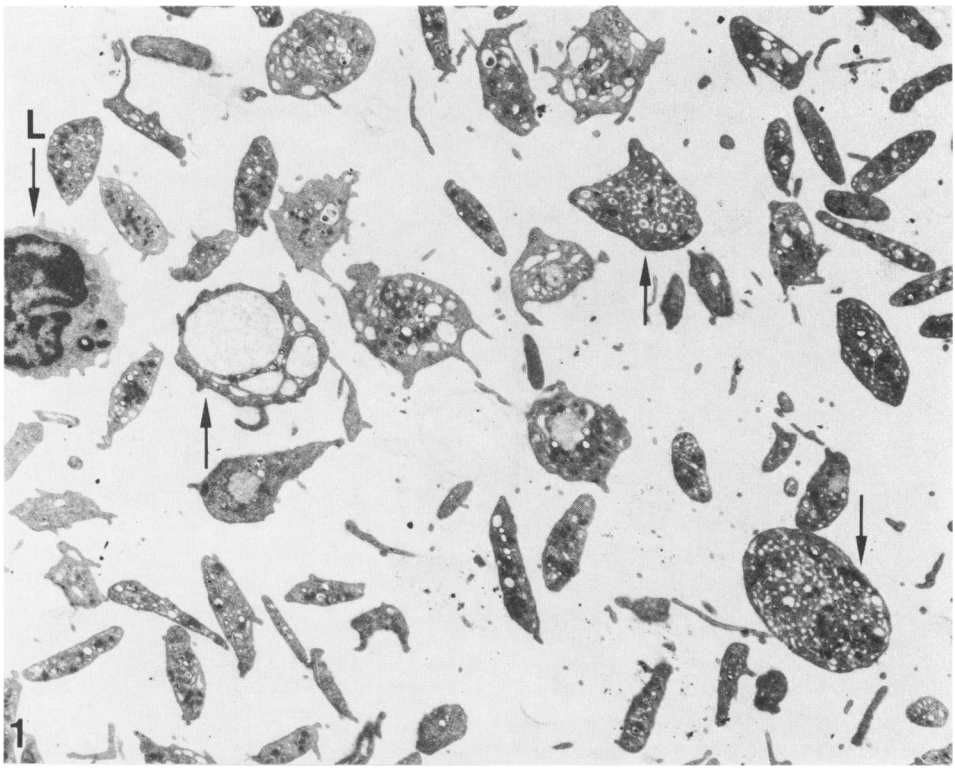
Figure 28—Cytochemistry of gray platelets. Platelet from a sample incubated for acid phosphatase activity. Enzyme reaction product, lead phosphate, is deposited inside two granules (*G*) and at the edge of a third. A dense body (*DB*) is also apparent in the cell. Fixed with glutaraldehyde, paraformaldehyde, and osmium. (×33,000)

Figure 29—Cytochemistry of gray platelets. Platelets from a sample incubated for aryl sulfatase activity. Granules are stained by the reaction product, while mitochondria (*M*) are not. Fixed with glutaraldehyde, paraformaldehyde, and osmium. (×16,500)

Figure 30—Cytochemistry of gray platelets. The cell is from a platelet sample incubated for the demonstration of catalase. Reaction product is selectively deposited in small organelles (*arrows*), which are called "peroxisomes." Fixed with glutaraldehyde and osmium. (×18,000)

Figure 31—Cytochemistry of gray platelets. Uranaffin reaction. The cell is from a sample fixed in glutaraldehyde and incubated with uranyl acetate. Uranium has deposited selectively on the inner surface of the lipid bilayer enclosing dense bodies (*arrows*). **Inset**—A few granules in gray platelets are stained in an identical manner. (**Figure 31**, ×21,500; **Inset**, ×91,000)

Figure 32—Cytochemistry of gray platelets. The cells are from a sample incubated for peroxidase activity. Enzyme reaction product is localized specifically to channels of the DTS and delineates its association with the OCS in membrane complexes (*MC*). Reaction product does not occur in vacuoles. Fixed with glutaraldehyde, paraformaldehyde, and osmium. (×11,300)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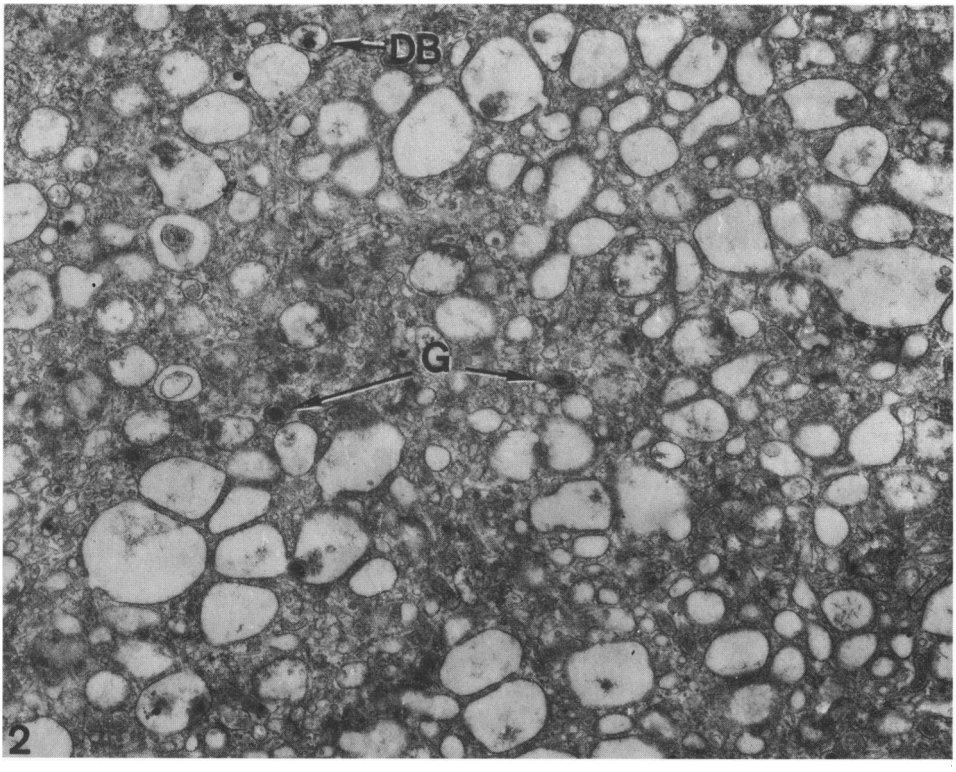
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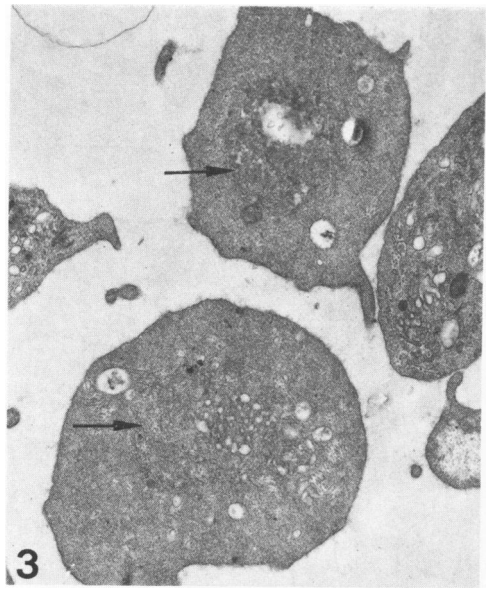

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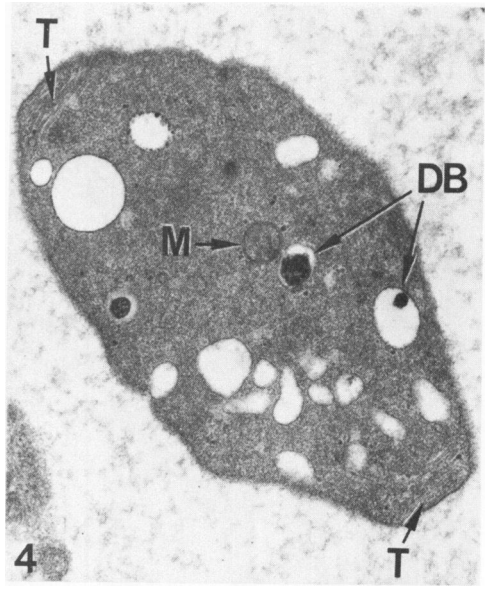

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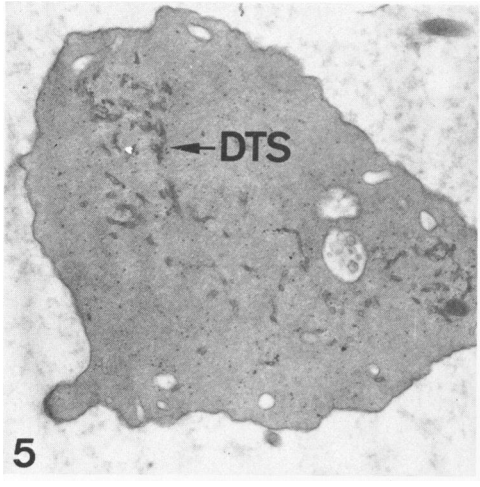

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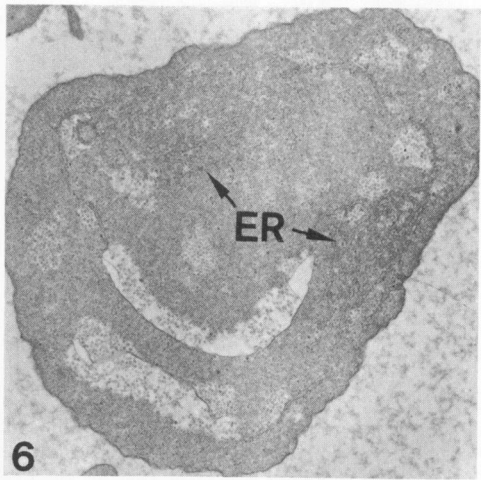

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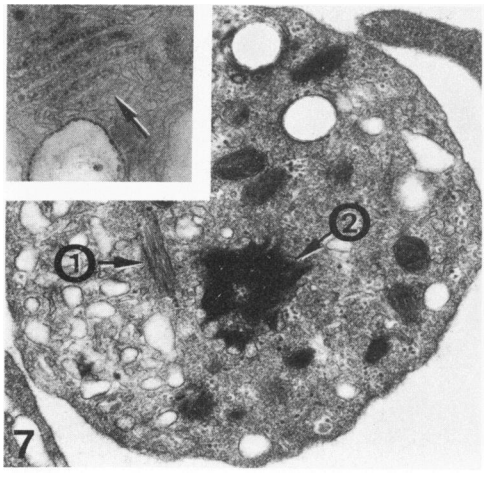

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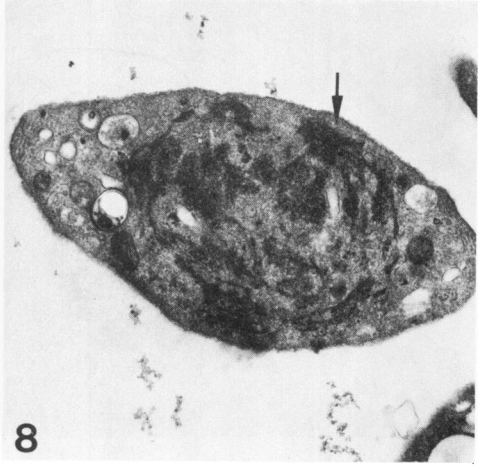

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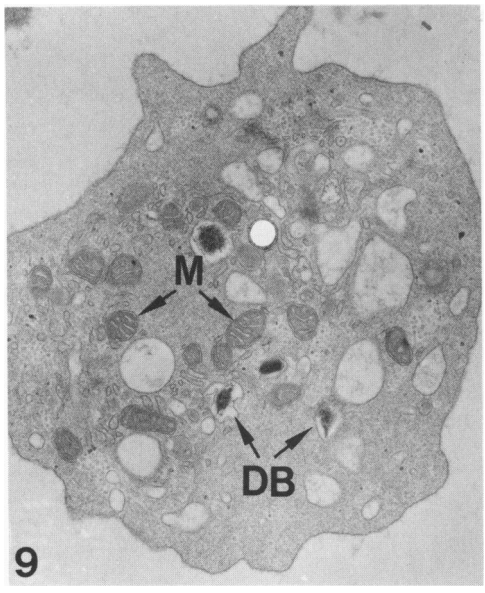
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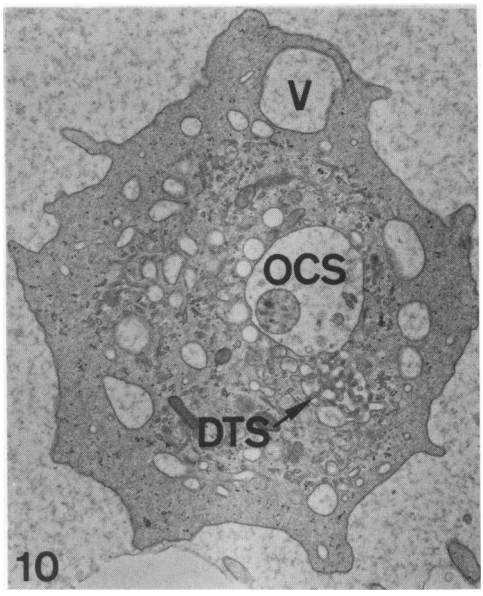
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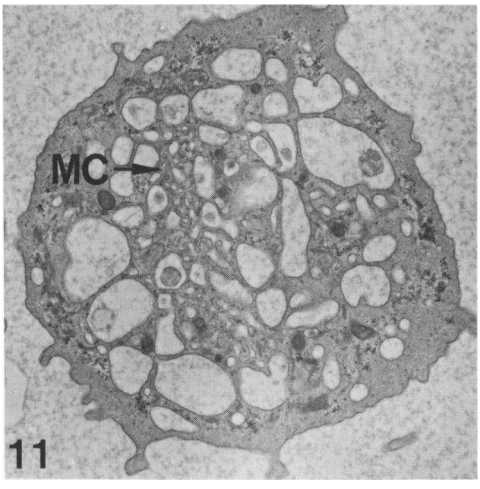
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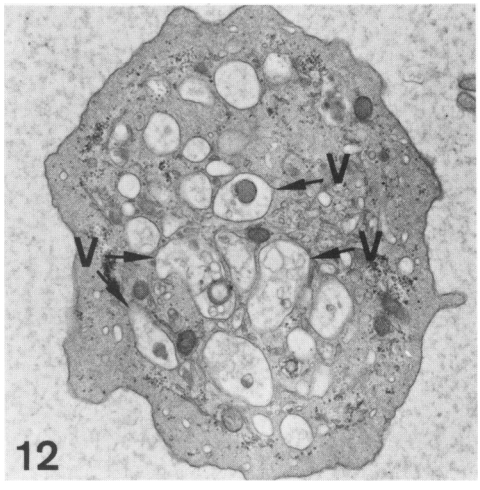
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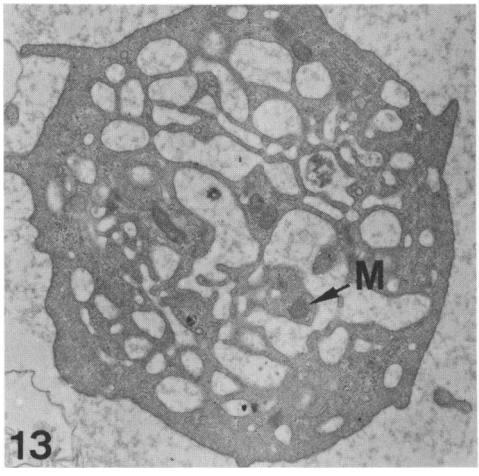
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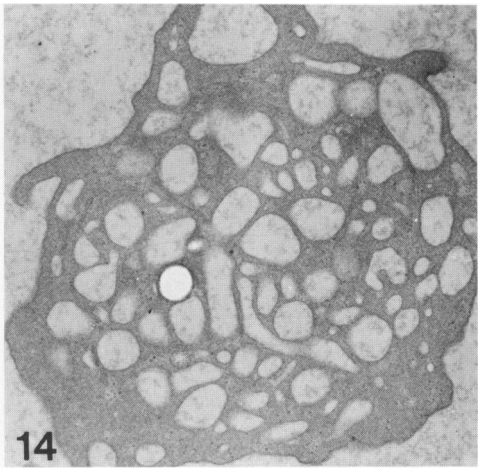
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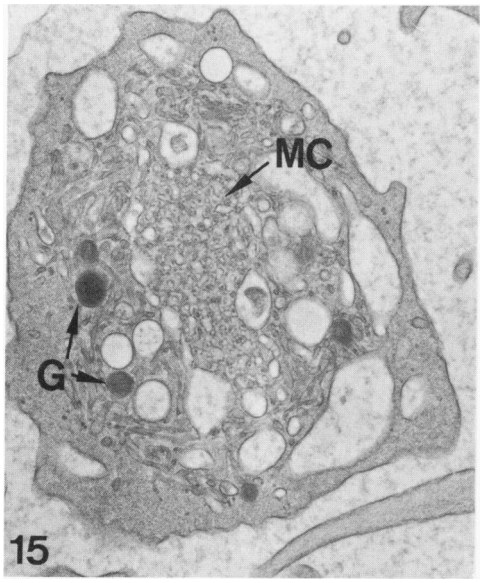
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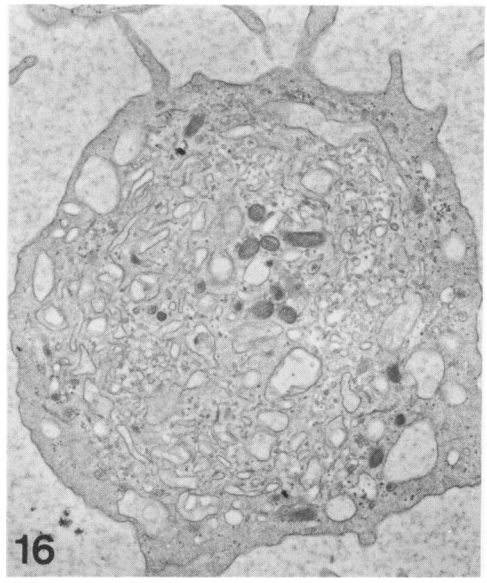
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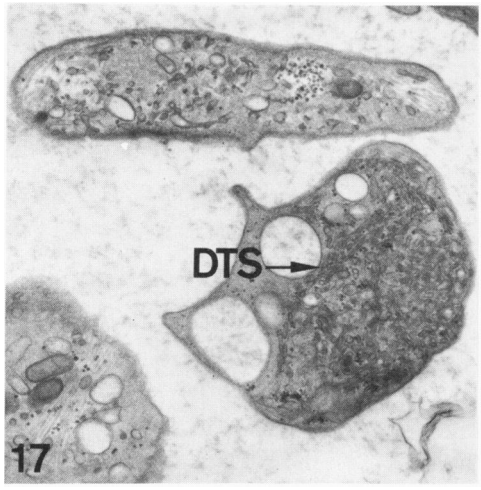
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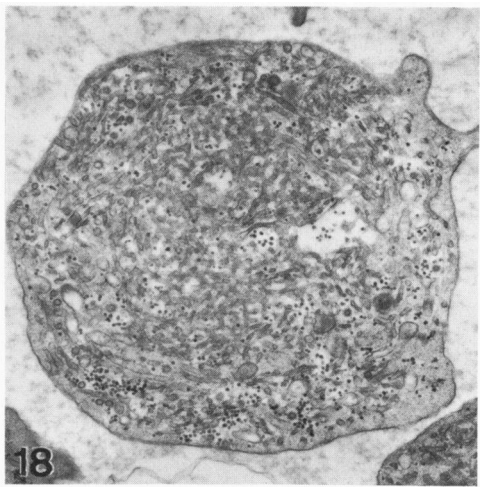
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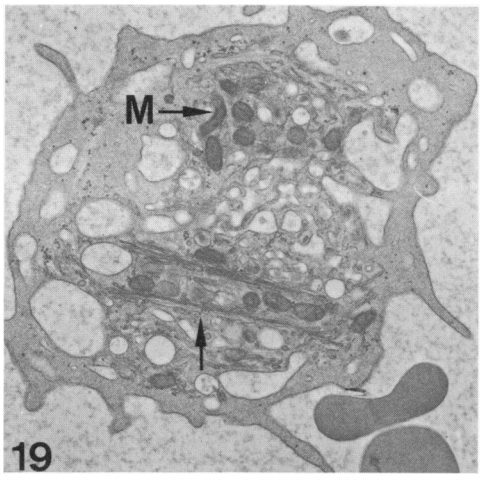
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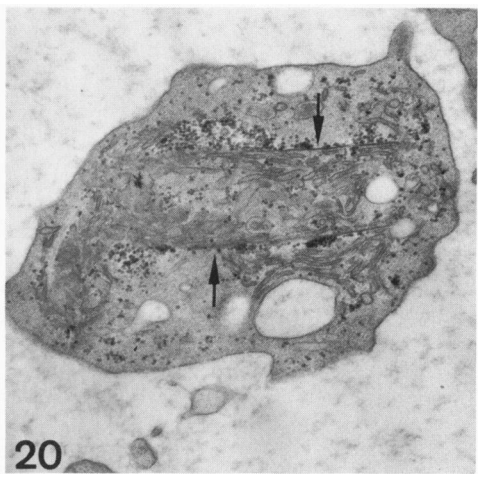
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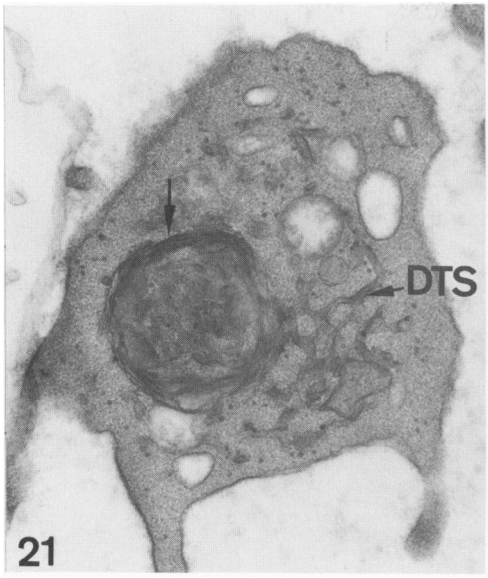
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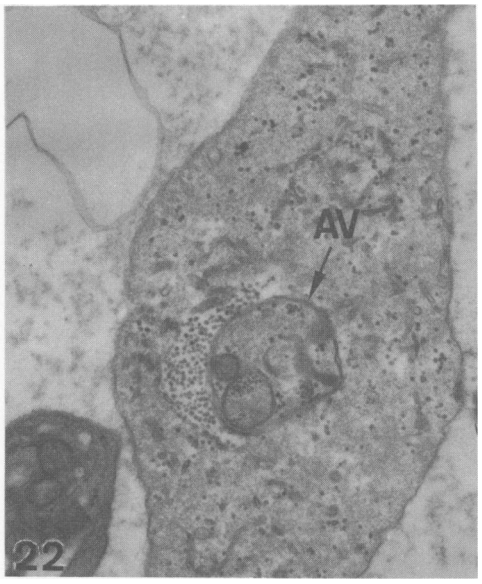
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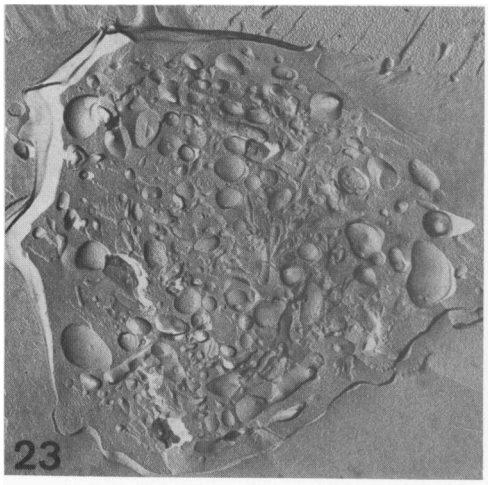
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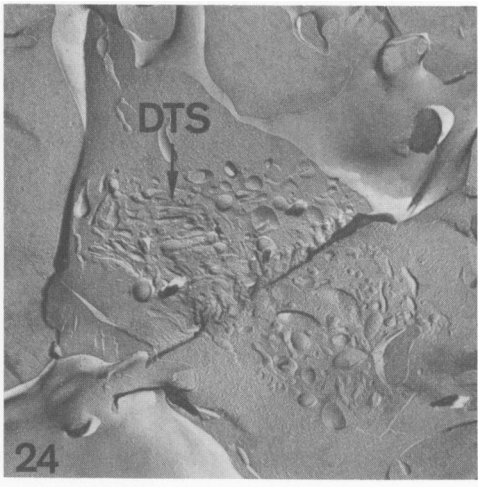
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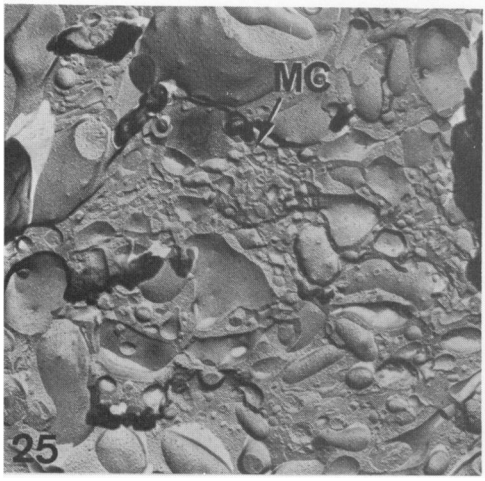
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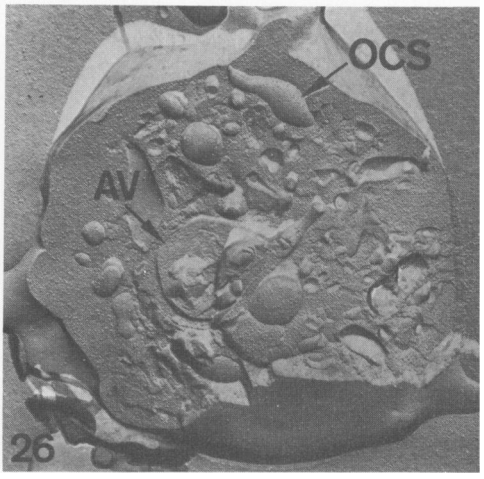
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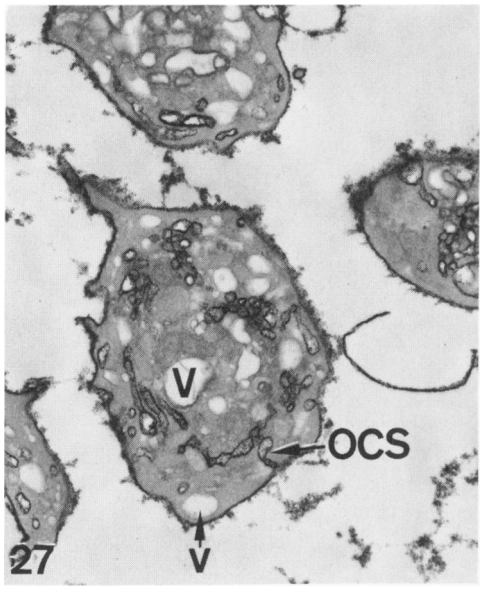
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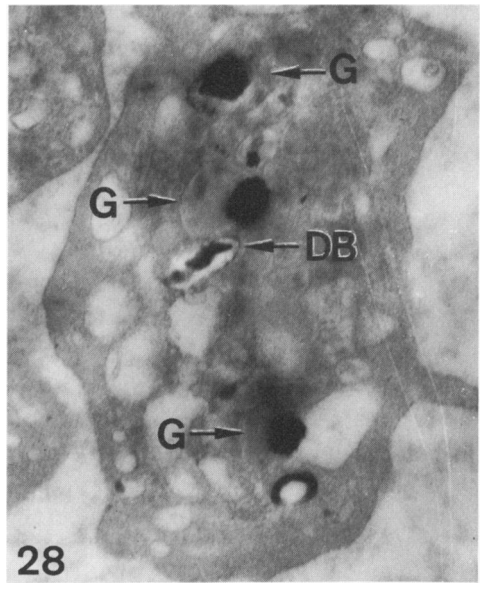
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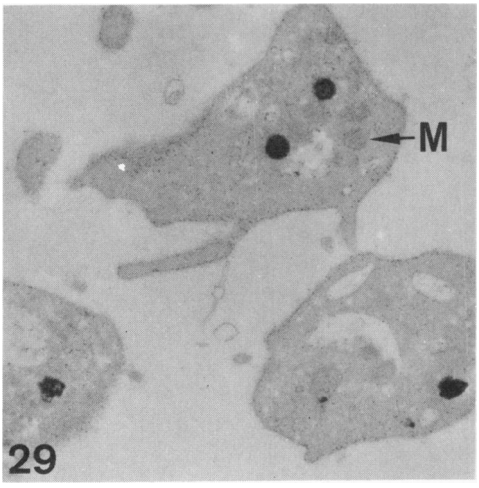
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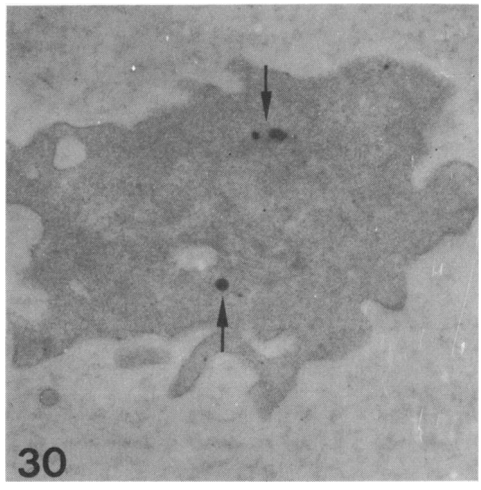
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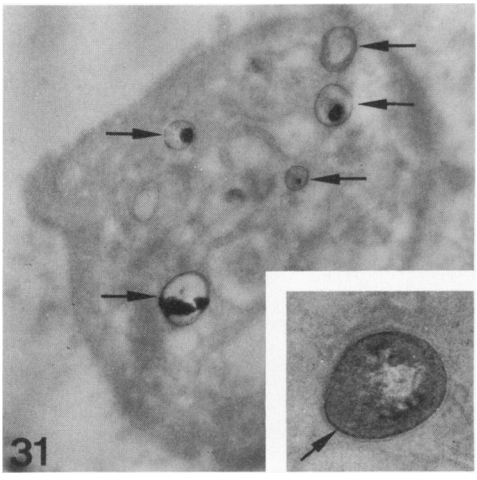
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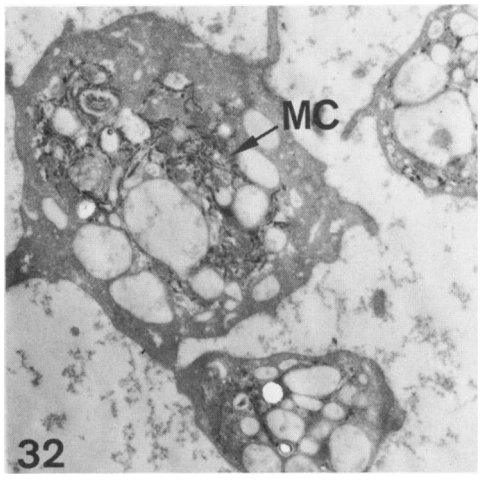
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