# The Chediak-Higashi Syndrome

## *Fine Structure of Giant Inclusions in Freeze-Fractured Neutrophils*

James G. White, MD

**The technic of freeze-fracture and etching has been used in the present study to examine the fine structure of giant inclusions in circulating leukocytes from a patient with the Chediak-Higashi syndrome (CHS). The surface granularity of the membranes enclosing the giant inclusions differed slightly from that of normal sized organelles. Two types of giant granules were distinguished in replicas of freeze-fractured CHS neutrophils. The difference in fine structure suggests that one variety is a massively enlarged, but essentially unaltered primary lysosome, while the other develops as a result of continued fusion of small organelles with huge inclusions throughout the stages of polymorphonuclear leukocyte maturation (Am J Pathol 72:503–520, 1973).**

THE MASSIVE INCLUSIONS present in polymorphonuclear leukocytes (PMN) and other white blood cells from patients with the Chediak-Higashi syndrome (CHS) are the characteristic cytologic features of this disease.[1–4] Many previous investigations have attempted to link giant leukocyte bodies to the unusual susceptibility of patients with the CHS to recurrent bacterial infections, lymphorectiular malignancy and early death.[5–22] Recently a defect in the bactericidal activity of CHS neutrophils has been demonstrated [21–24] which may be related to the failure of the giant organelles to fuse with and degranulate into phagocytic vacuoles containing ingested bacteria.[25] The results of these studies have stimulated renewed interest in the fundamental defect responsible for the evolution of the giant organelles and their failure to function normally in host defense.[21–27] Efforts to define the specific abnormality have been frustrating and generally unsuccessful. The present study was undertaken in order to determine if the method of freeze-fracture might reveal defects in membranes of giant CHS inclusions which had eluded other approaches.

From the Department of Pediatrics, University of Minnesota Medical Center, Minneapolis, Minn.

Supported by Grants HE-11880, AM-15010 and CA-08832 from the US Public Health Service and the Cardiovascular Clinical Research Program Project.

Accepted for publication May 11, 1973.

Address reprint requests to Dr. James G. White, Department of Pediatrics, University of Minnesota Medical Center, Box 284 Mayo Memorial Bldg, Minneapolis, MN 55455.

## Materials and Methods

The procedures used to obtain blood by venipuncture; mix the samples immediately with heparin, heparin-dextran or 3.8% trisodium citrate; separate leukocyte-rich plasma by spontaneous sedimentation or differential centrifugation; wash and resuspend the cells in Hank's buffered salt solution; and prepare the cells by fixation in glutaraldehyde and osmic acid for study in the electron microscope were described in detail in recent publications.[7,8,28,29] Only 1 patient with the CHS was available for the present investigation. The child is a 6-year-old male in good health who has not developed evidence of the accelerated phase of the disorder. His past history, clinical findings and special laboratory studies were discussed previously.[29] The technic used to prepare cells for freeze-fracture and etching was described in an earlier report concerned with the morphology of freeze-cleaved platelets.[30] Leukocyte-rich samples from the patient and several normal donors were processed through the two steps of glutaraldehyde fixation usually employed to preserve samples for study in the transmission electron microscope. After fixation for at least 2 hours, the fixative was discarded, and the pellets were resuspended in buffered White's saline containing 20% glycerol. After 10 minutes exposure to the buffered glycerol solution, the pellets were cut into small pieces and transferred to special holders. The holders with the leukocyte samples were dropped immediately into receptacles containing liquid freon suspended in liquid nitrogen. After the initial step of freezing the samples were placed in liquid nitrogen until the time of fracturing. At that time, holders with the frozen normal or CHS leukocyte samples were transfered quickly to the stage of a Balzer's freeze-fracture unit cooled with liquid nitrogen and immediately exposed to high vacuum. The frozen leukocyte preparations were fractured at a −120 C, allowed to etch at that temperature for 30 seconds and coated with successive layers of platinum and carbon. The replicas were freed from tissue by soaking the samples in 30% chlorine bleach. After complete removal of organic substance the replicas were washed several times in distilled water, mounted on uncoated 200 or 300 mesh grids and examined in a Philips 200 electron microscope.

## Results

Replicas of freeze-cleaved leukocytes presented a confusing appearance when first examined in the electron microscope, but with brief experience most of the structures evident in thin sections could be identified.[7,8,29] The fracture plane followed an extremely variable course, passing through, over, or scooping out the contents of leukocytes. It is now generally accepted that fractures through frozen cells do not expose the outside or inside surface of membranes, but instead split the lipid bilayer, revealing the inside aspect of the outer half or the outside of the inner lamellae.[31–33] True outside and cytoplasmic faces of the cell wall are revealed by deep etching.[34–36] The same type of split occurs in intracellular membranes, revealing either the inner side of the outer leaflet or the outer surface of the inside layer covering granules, mitochondria, nuclei and other structures. Once this unusual effect of the freeze-fracture process is appreciated, it is possible

to determine which surface is exposed and the relationships of closely associated membranes in the replicas.

The fine structure of replicas obtained from CHS leukocytes did not differ significantly from those obtained from samples of normal cells except for the presence of the giant organelles. At low magnification it was difficult to distinguish the massive granules in neutrophils from the several lobes of the nucleus usually present in the cytoplasm (Figure 1). Closer inspection revealed distinct differences. The nucleus was enveloped by a double membrane punctuated by characteristic pores. Usually a sufficient area of the nuclear surface was exposed in replicas to permit identification of these features. When sufficient nuclear surface membrane detail was not revealed by the fracture, the absence of internal membranes and smooth granularity of the matrix suggested, but did not prove, that the massive body was a portion of the nucleus.

The cytoplasm of freeze-cleaved CHS neutrophils contained a large number of oval or round bodies corresponding to the normal size granules observed in thin sections (Figures 1, 2A and 2B). Exposed surfaces of the organelles were coated with randomly dispersed 50 to 60 Å particles. Similar particles were evident on the split layers of the cell wall. The fracture plane seldom passed through the internal substance of the small granules, tending rather to pass over or scoop out their internal contents.

Some of the giant CHS organelles were difficult to distinguish from the normal size granules because only a portion was exposed above the level of ice in the fracture plane. However, many huge granules were easily identified by their size alone. Particles coating the massive organelles were slightly larger and more numerous than those covering normal size organelles (Figures 2A, 2B, 3 and 4). The differences, however, were not uniform and probably not significant.

The variability in giant organelles previously noted in thin sections of CHS neutrophils was also apparent in replicas of freeze-cleaved cells. Some of the massive granules were relatively circular in form with regular surface contours (Figures 1 and 3). Fractures passing through this type usually revealed a finely granular matrix (Figures 1, 2A and 3). Other huge bodies were irregular, and they extended arms or knobs into the adjacent cytoplasm among the normal size organelles (Figures 1, 2B, 3, 4A, 4B, 13, 14A and 14B). Fractures passing into irregular giant organelles revealed extensive arrays of internal membranes (Figure 4A and 4B). The membranes were often twisted into irregular masses or wrapped in concentric layers resembling myelin

configurations (Figure 5A and 5B). Occasionally the massive irregular bodies contained membranous elements indistinguishable from granules in the surrounding cytoplasm (Figures 4B, 9B, and 12). Some membranes inside the huge organelles lacked the surface particles evident on fracture faces of the cell wall, small and large granules, mitochondria and nuclei (Figure 9B). The cleavage planes through giant irregular organelles revealed large areas of the split lipid bilayer enclosing the huge structure. Careful study of the exposed surfaces indicated that irregular organelles spread out among the smaller granules in the cytoplasm and were often joined to other massive bodies (Figures 3, 6, 11 and 12). Some of the massive organelles in freeze-fractured CHS neutrophils were characterized by irregular surfaces which faded into the surrounding cytoplasm (Figures 4B, 7, 9A and 11). Groups of membranes resembling twisted ropes were evident in the matrix of irregular inclusions (Figures 6, 7, 8, 9A, 9B and 10). Structures similar to normal size granules were also observed within the surrounding membrane of the massive structures (Figures 9A, 9B, 11, 12, 13, 14A and 14B).

Giant CHS organelles were also noted in replicas of freeze-fractured eosinophils, monocytes and lymphocytes. Topographic features of the membranes enclosing the massive granules resembled those surrounding small organelles in cells of the same type from healthy donors.

## Discussion

The present investigation has employed the technic of freeze-fracture and etching to evaluate the fine structure of the giant organelles present in the cytoplasm of leukocytes from a patient with CHS. Polymorphonuclear (PMN) leukocytes from CHS patients were of special interest. Recent studies have suggested that these cells have a defective bactericidal capacity and fail to migrate normally *in vivo* and *in vitro* in response to inflammatory stimuli.[21–27] The giant granules have been implicated in the deficient bactericidal activity and poor chemotactic response of the PMNs which may be directly related to the marked susceptibility of CHS patients to recurrent infections.

Previous investigations have shown that the huge granules in CHS neutrophils are abnormal lysosomes.[7,8] They originate from the azurophilic primary granules in the promyelocyte by enlarging beyond normal size, fusing together to form large masses, or both. The nature of development has suggested that giant CHS lysosomes may have abnormal membranes, and their affinity for vital dyes and substrates and failure to fuse with phagocytic vacuoles support this possi-

bility.[7,8,21,23,25,27] Attempts to characterize the biochemical or structural defect in membranes of massive organelles in CHS leukocytes, however, have not been successful.[9]

The method of freeze-fracture and etching introduced in 1957 [37] has provided a new approach for evaluating cell ultrastructure.[38,39] It has been of particular value for studying the morphology of membranes inside cells and intercellular junctions.[34–36] The nature of the fracture into frozen cells permits an unusual view of membrane topography. Instead of exposing the exterior or cytoplasmic side of the cell wall, it splits the lipid bilayer revealing the inner surface of the outside layer or outer aspect of the inside leaflet. The tendency of the fracture to bisect unit membranes appears to be a general feature of the freeze-cleaving process, and includes the membranes covering the nucleus and intracellular organelles. As a result the particles apparent on the surfaces of cell membranes in replicas of freeze-fractured cells are intercalated between the inner and outer leaflets of the lipid bilayer.

It was hoped that freeze-fracture of giant organelles in neutrophils and other leukocytes from CHS patients might reveal abnormalities which had not been noted previously. Particular attention was given to the intercalated particles which cover the membranes enclosing huge granules. The results of the investigation revealed a slight increase in the size and number of granules on exposed surfaces of giant CHS organelles. However, the surface granularity of massive inclusions was not uniformly different from small granules and may have been due in part to a shadowing artifact.

The morphology of massive granules in replicas of freeze-fractured CHS neutrophils did provide a new perspective of these unusual cytoplasmic inclusions. Previous studies have pointed out the pleomorphism of the massive bodies,[6–14] and a similar variability in fine structure was apparent in the replicas. Although the huge granules all originate from azurophilic granules, at least two distinct types were evident.[40] The first variety was a huge cytoplasmic organelle with an oval or rounded form and an uncomplicated internal structure. Except for its size, this type resembles normal size azurophilic granules in control PMNs. The finely granular matrix and absence of internal membranes suggest that it arose by simple enlargement or unrestricted growth of a primary azurophilic lysosome in early promyelocytes.

A second form of giant granule in CHS neutrophils was characterized by extremely irregular surface contours. The knobs protruding from the surface of the massive body extended into the surrounding cyto-

plasm among normal size organelles. Replicas in which the fracture broke through the irregular bodies revealed internal membranes in various arrangements. The surface membrane enclosing the giant organelles was particularly interesting because it could be followed for a considerable distance. Careful examination revealed that massive organelles appearing as separate bodies in the cytoplasm of CHS neutrophils were frequently connected to each other. Some of the irregular giant bodies in freeze-cleaved CHS neutrophils contained an unusual array of membrane resembling twisted rope in their matrix. The surface membrane of this type of inclusion could usually be identified as a fine line or exposed leaflet enclosing most of the organelle substance, but was often lost to view in the adjacent cytoplasm along one side. Small organelles filling the cytoplasm were frequently attached to the surface of the giant granules and occasionally appeared to be incorporated within the membrane enclosing the huge structure.

Since all of the giant organelles in CHS neutrophils originate from azurophilic primary lysosomes, the designation of two distinct morphologic types may seem arbitrary. The various kinds of irregular inclusions may represent different degrees of distortion of the first type and fall within the range of pleomophism for a single huge organelle, as suggested in previous reports on thin sections of CHS leukocytes.[10,11,19,20] Yet, structural differences evident in the organelles found in freeze-fractured cells and in thin sections suggest that all varieties are not identical.[7,8,29]

Examination of bone marrow and peripheral blood from several CHS patients has suggested a basis for the differences in fine structure observed in the giant neutrophil inclusions.[7–9,29,41] The first variety resembles a single massively enlarged azurophilic lysosome. It is present in the neutrophil promyelocyte, but the massive irregular inclusions are seldom observed in young precursor cells. PMN leukocytes from peripheral blood contain occasional examples of the first variety, but giant irregular bodies are far more common. In the past this observation suggested that the giant granules formed in promyelocytes underwent various degrees of internal degeneration during maturation,[7,8] resulting in transformation to irregular inclusions. A more likely explanation, based on recent investigations employing improved methods of fixation and cytochemistry,[41] is that the first type of giant organelle remains relatively unaltered after development in the promyelocyte stage. Irregular inclusions evolve during the late promyelocyte and early myelocyte stages by a continued fusion of azurophilic granules

into massive bodies. This process is not completed even in mature cells.

Freeze-fracture has been particularly helpful in demonstrating the extent of interaction between massive irregular organelles and the surrounding cytoplasm. The complex membrane structures in giant irregular granules most likely result from the process of fusion with small cytoplasmic organelles. Exposure of large areas of surfaces enclosing huge irregular inclusions revealed extensive ramifications between the CHS granules and other small and large cytoplasmic organelles. Although the technic failed to show characteristic differences between CHS inclusions and normal granules, it has been helpful in further clarifying the basis for the pleomorphism of giant granules in mature CHS neutrophlis.

## References

1. Béquez-César A: Neutropenia chronica maligna familiar congranulaciones atipicas de los leukocitos. Bol Soc Cuba Pediat 15:900–922, 1943
2. Chediak M: Nouvelle anomalie leucocytoaire de charactere constitutionnel et familial. Rev Hematol 7:362–367, 1952
3. Higashi 0: Congenital giagantism of peroxidase granules. Tohoku J Exp Med 59:315–332, 1954
4. Steinbrink W: Uber eine neue Granulation-Anomolie der Leukocyten. Dtsch Arch Klin Med 193:577–581, 1948
5. Bernard J, Bessis M, Seligmann M, Chasigneux J, Chome J: Un cas de maladie de Chediak-Steinbrink-Higashi. Presse Med 68:563–574, 1960
6. Bessis M, Bernard K and Seligmann M: Etude cytologique d'un cas de maladie de Chediak. Nouv Rev Fr Hematol 1:422–425, 1961
7. White JG: The Chediak-Higashi syndrome: a possible lysosomal disease. Blood 28:143–156, 1966
8. White JG: The Chediak-Higashi syndrome: cytoplasmic sequestration in circulating leukocytes. Blood 29:435–451, 1967
9. Windhorst DB, White JG, Zelickson AS, Clawson CC, Dent PB, Pollara B, Good RA: The Chediak-Higashi anomaly and the Aleutian trait in mink: homologous defects of lysosomal structure. Ann NY Acad Sci 155:818–846, 1968
10. Douglas SD, Blume RS, Wolff SM: Fine structural studies of leukocytes from patients and heterozygotes with Chediak-Higashi syndrome. Blood 33: 527–540, 1969
11. Douglas SD, Blume RS, Glade PR, Chessin LN, Wolff SM: Fine structure of continuous long term lymphoid cell cultures from a Chediak-Higashi patient and heterozygote. Lab Invest 21:225–229, 1969
12. Blume RS, Glade PR, Gralnick HR, Chessin LN, Wolff SM: The Chediak-Higashi syndrome: continuous suspension culture of peripheral blood leukocytes. Blood 33:821–832, 1969
13. Efrati P, Danon D: Electron microscopical study of bone marrow cells in a case of Chediak-Higashi-Steinbrink syndrome. Br J Haematol 15:173–184, 1968

14. Fortunato RA: Estudio electromicroscopico de la anomalia de Chediak-Higashi-Steinbrink. Acta Cient Venez 18:73–78, 1967
15. Moran TJ, Estevez JM: Chediak-Higashi disease: morphologic studies of a patient and her family. Arch Pathol 88:329–339, 1969
16. Bedaya V, Grimley PM, Duque O: Chediak-Higashi syndrome. Arch Pathol 88:340–349, 1969
17. Padgett GA: The Chediak-Higashi syndrome. Adv Vet Sci 12:239–284, 1968
18. Lutzner MA, Tierney JH, Benditt EP: Giant granules and widespread cytoplasmic inclusions in a genetic syndrome of Aleutian mink. Lab Invest 14:2063–2079, 1966
19. Davis WC, Spicer SS, Green WB, Padgett GA: Ultrastructure of bone marrow granulocytes in normal mink and mink with the homolog of the Chediak-Higashi trait in humans. I. Origin of the abnormal granules present in the neutrophils of mink with CHS trait. Lab Invest 24:303–317, 1971
20. Davis WC, Spicer SS, Green WB, Padgett GA: Ultrastructure of cells in bone marrow and peripheral blood of normal mink and mink with the homologue of the Chediak-Higashi trait of humans. II. Cytoplasmic granules in eosinophils, basophils, mononuclear cells and platelets. Am J Pathol 63: 411–432, 1971
21. Wolff SM, Dale DC, Clark RA, Root RK, Kimball HR: The Chediak-Higashi syndrome: study of host defenses. Ann Int Med 76:293–306, 1972
22. Blume RS, Wolff SM: The Chediak-Higashi syndrome: studies in four patients and a review of the literature. Medicine 51:247–280, 1972
23. Root RK, Rosenthal AS, Balestra SJ: Abnormal bactericidal, metabolic and lysosomal functions of Chediak-Higashi syndrome leukocytes. J Clin Invest 51:649–665, 1972
24. Clawson CC, Repine JE, White JG: Chediak-Higashi syndrome: quantitative defect in bactericidal capacity. Blood 38:814, 1971
25. Padgett GA: Neutrophilic function in animals with the Chediak-Higashi syndrome. Blood 29:906–915, 1967
26. Clark RA, Kimball HR: Defective garnulocyte chemotaxis in the Chediak-Higashi syndrome. J Clin Invest 50:2645–2652, 1971
27. Stossel TP, Root RK, Vaughan M: Phagocytosis in chronic granulomatous disease and the Chediak-Higashi syndrome. N Engl J Med 286:120–123, 1972
28. Clawson CC, White JG: Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. Am J Pathol 65:367–380, 1971
29. White JG: Giant organelles containing tubules in Chediak-Higashi lymphocytes. Am J Pathol 69:225–238, 1972
30. White JG, Conard WJ: The fine structure of freeze-fractured blood platelets. Am J Pathol 70:45–56, 1973
31. Deamer DW, Branton D: Fracture planes in an ice-bilayer model membrane system. Science 158:655–657, 1967
32. Branton D: Membrane structure. Annu Rev Plant Physiol 20:209–238, 1969
33. Tillack TW, Marchesi VT: Demonstration of the outer surface of freeze-etched red blood cell membranes. J Cell Biol 45:649–653, 1970
34. Chalcroft JP, Bullivant S: An interpretation of liver cell membrane and junction structure based on observation of freeze-fracture replicas of both sides of the fracture. J Cell Biol 47:49–60, 1970

35. Friend DS, Gilula NB: Variations in tight and gap junctions in mammalian tissues. J Cell Biol 53:758–776, 1972
36. Staehelin LA, Chlapowski FJ, Bonneville MA: Lumenal plasma membrane of the urinary bladder. I. Three-dimensional reconstruction from freeze-etch images. J Cell Biol 53:73–91, 1972
37. Steere RL: Electron microscopy of structural detail in frozen biological specimens. J Biophys Biochem Cytol 3:45–49, 1957
38. Moor H, Muehlethaler K: Fine structure in frozen etched yeast cells. J Cell Biol 17:609–627, 1963
39. Branton D: Fracture faces of frozen membranes. Proc Natl Acad Sci USA 55:1048–1056, 1966
40. Bainton D,F Farquhar MG: Origin of granules in polymorphonuclear leukocytes: two types derived from opposite faces of the Golgi complex in developing granulocytes. J Cell Biol 28:277–286, 1966
41. White JG: The Chediak-Higashi syndrome: interaction of giant organelles with small granules in polymorphonuclear leukocytes. (In press)

## Legends for Figures

**Fig 1**—Replica of a freeze-fractured polymorphonuclear (PMN) leukocyte from the peripheral blood of a patient with the Chediak-Higashi syndrome (CHS). The fracture has broken through the cell wall *(CW)* into the cytoplasm, revealing lobes of the nucleus *(N)* and a variety of randomly dispersed organelles. A double envelope of membrane and nuclear pores are evident on the lobe of nucleus on the left side of the cell, and the interior matrix has been exposed in the nuclear lobe on the right. Several sizes and types of granules are apparent. The *first arrow (1)* indicates a group of small organelles similar to those observed in normal PMN leukocytes. Granules appear convex or concave, depending on whether the fracture has passed over the organelle or scooped out its contents. Some of the small granules are round, while others are oval, rod-shaped or resemble dumbbells. The *second arrow (2)* points out a granule in the shape of a dumbbell which is more than twice the size of the organelles surrounding it. Massive inclusions typical of those found in neutrophils of patients with CHS are indicated at *3*, *4* and *6*. The huge body at *3* might be mistaken for a lobe of the nucleus, but it lacks a double membrane. It has relatively regular surface contours, while *3* and *6* are irregular in form. The organelle indicated by *arrow 5* is of intermediate size. It is difficult to determine whether it is a massive CHS inclusion or a large azurophilic primary lysosome (× 12,300).

**Fig 2A and B**—Replicas of freeze-cleaved CHS neutrophils. The fracture plane has split open two huge inclusions *(1* and *2)* in the cytoplasm of the cell in **A.** A few small vesicular elements are present in *1*, while the second organelle *(2)* has a smooth granular matrix *(M)*. The fracture has split the lipid bilayer of the membrane enclosing the giant organelle, revealing the outer surface of the inner leaflet commonly referred to as the A face. Particles 50 to 70 Å in size are diffusely spread over the exposed membrane surface. The size and number of the particles on the surface of the massive organelle appear to exceed those covering the small granules in the surrounding cytoplasm. A similar frequency and size of particles are evident on the exposed surface of the giant irregular granule in **B.** However, the difference in surface granularity of giant CHS inclusions and normal size organelles is not uniform, and is of doubtful significance (**A,** × 24,200; **B,** × 18,100).

**Fig 3**—A lobe of nucleus *(N)* in this replica of a CHS neutrophil is distinguished by its double surface membrane. Four huge inclusions are evident in the cytoplasm. The first *(1)* has relatively regular surface contours and an uncomplicated internal matrix. A second massive body *(2)* has a completely different structure. The surface is extremely irregular and has been outlined in order to facilitate identification of its borders. Several small organelles appear to be imbedded or incorporated into the A face on the left side of the giant irregular mass. The interior of the large organelle is filled with membranes fractured in various planes. A third organelle *(3)* has a smooth surface and is probably similar to *(1)*. Concave membranes of two small organelles appear fused to the lower right surface of *3*. A fourth massive inclusion *(4)* containing numerous smaller bodies also enclosed by membranes is apparent at the lower margin of the illustration (× 14,350).

**Fig 4A and B**—Giant inclusions in replicas of two CHS neutrophils shown in **A** and **B** contain sheets of membrane fractured in various planes. Irregular surface contours are outlined. The left border of the giant body in **B** is indistinct and merges into adjacent cytoplasmic organelles. One granule *(1)* just outside the border of the huge inclusion is essentially identical in appearance to an organelle *(2)* located within the membrane of the massive body (**A,** × 14,600; **B,** × 16,100).

**Fig 5A and B**—Giant inclusions in the replicas of CHS neutrophils in **A** and **B** contain sheets of membrane in their matrix *(M)* arranged in concentric layers resembling myelin configurations. The fracture has exposed the inner side of the outer half of the lipid bilayer, often referred to as the B face, in **A**. It is difficult to define a clear separation of this membrane from the cytoplasmic organelles along the lower side of the giant inclusion (**A,** × 45,600; **B,** × 40,000).

**Fig 6**—A replica of a freeze-fractured CHS neutrophil containing several different types of giant inclusions. Organelles *2, 6* and *7* have rounded contours and probably represent enlarged azurophilic primary granules. The first giant inclusion *(1)* contains masses of membrane resembling twisted rope. Three *(3)* may be a lobe of the nucleus *(N)*, but lacks the two layers of membrane; it is probably a massive CHS organelle. Number *4* and *5* are giant irregular inclusions which may be joined to each other (× 10,250).

**Figs 7 and 8**—Twisted masses of membrane form complexes *(C)* filling the matrix of the giant granule in **7** and a portion of the huge organelle in **8**. The B face or inner surface of the outer layer of unit membrane is exposed in both examples. It is difficult to follow the membrane isolating the huge inclusion in **7,** particularly along the left side (**7,** × 41,500; **8,** × 33,200).

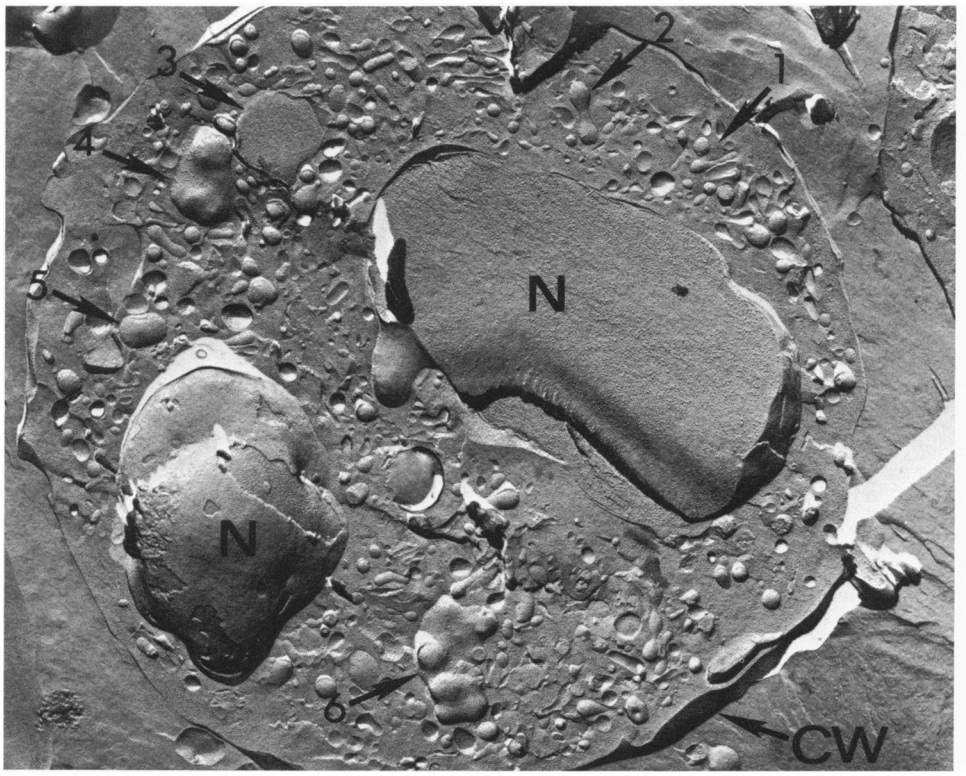
**Fig 9A and B**—Indefinite borders of two massive inclusions are outlined in the replicas of CHS neutrophils in **A** and **B**. The left border of the irregular giant body in **A** merges directly into the membranes of small cytoplasmic organelles *(arrow)*. Both left and right borders of the inclusion of 9B merge with the cytoplasm and small granules. Some of the membranous organelles *(arrow)* in the matrix of the giant inclusion in **B** resemble granules in the adjacent cytoplasm, and may contribute to the membrane complexes *(C)* evident in this type of huge CHS inclusion (**A,** × 16,400; **B,** × 13,000).

**Fig 10**—The complexity resulting from the presence of several giant inclusions in the cytoplasm is evident in this replica of a CHS neutrophil. The first inclusion *(1)* has a smooth granular matrix, but the right border of the enclosing membrane is indefinite. Two *(2)* is an irregular giant inclusion, and three *(3)* is similar to it. The fracture has exposed a complex *(C)* of membranes in *3* which appears closely associated with the enclosing membrane of the huge body along the upper surface. The three inclusions occupy a large area of the cytoplasm without appearing to crowd other structures in the cell (× 17,000).

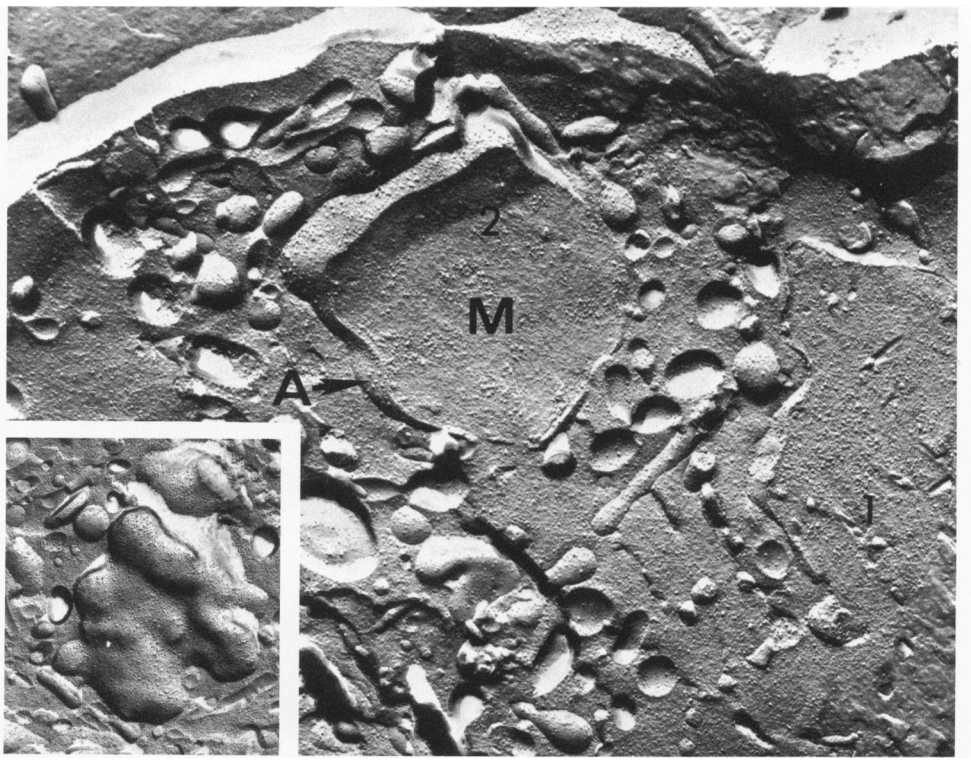
**Figs 11 and 12**—The huge inclusions outlined in the replicas of CHS neutrophils in these illustrations reveal evidence of membrane fusion. In **11** the massive body appears to consist of two separate inclusions *(1* and *2)* joined together by several layers of fused membranes *(arrow)*. The apposition *(arrow)* of two large inclusions *(1* and *2)* in **12** suggests that they are fused. A massive organelle outside the perimeter of the huge compound inclusion contains a granule in its matrix *(arrow)* resembling small cytoplasmic organelles (**11,** × 13,400; **12,** × 12,300).

**Fig 13**—Two large inclusions were fractured in this cell. One appears to have a double membrane enclosing it *(arrow)*, but is obviously a giant granule and not a lobe of the nucleus. The contents of the second giant organelle were scooped out by the fracture process. Its membrane appears to merge into cytoplasmic granules along the lower side (× 16,300).

**Fig 14A and B**—The massive inclusions shown in the two replicas of CHS neutrophils appear to be compound structures. In **A** the exposed B face of one portion of a giant organelle *(1)* appears to merge with a second large inclusion *(2)*. A cytoplasmic granule indicated by the *lower arrow* is interposed at the junction of the two lobes of the huge body. At least two and possibly three CHS organelles are outlined in **B**. It is difficult to determine whether the spindle-shaped inclusion on the left is a single body or a product of fusion by several organelles. A deeper fracture might reveal that the huge inclusion on the right is connected at some point to the larger body of granular material on the left (**A,** × 12,300; **B,** × 13,700).



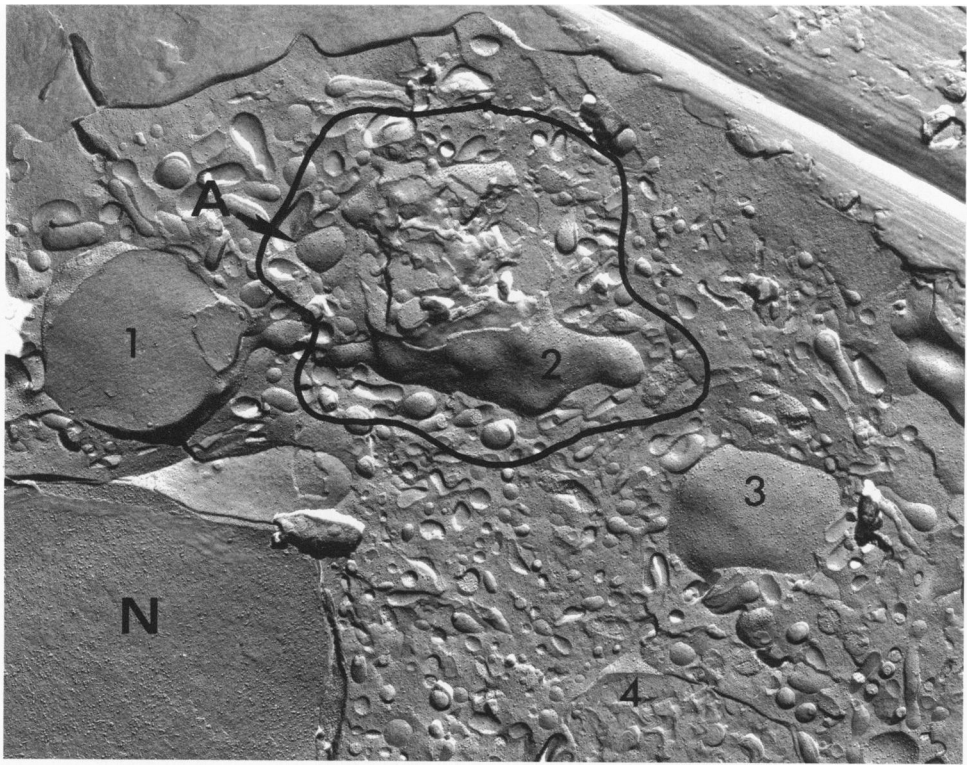

1



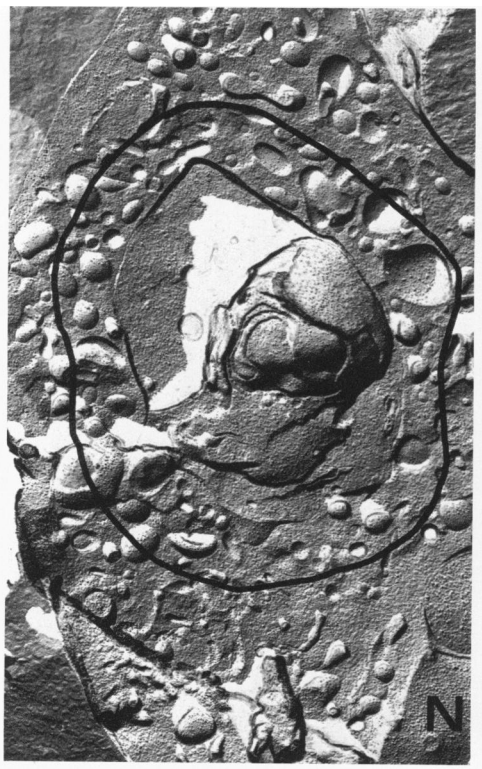

2A



2B

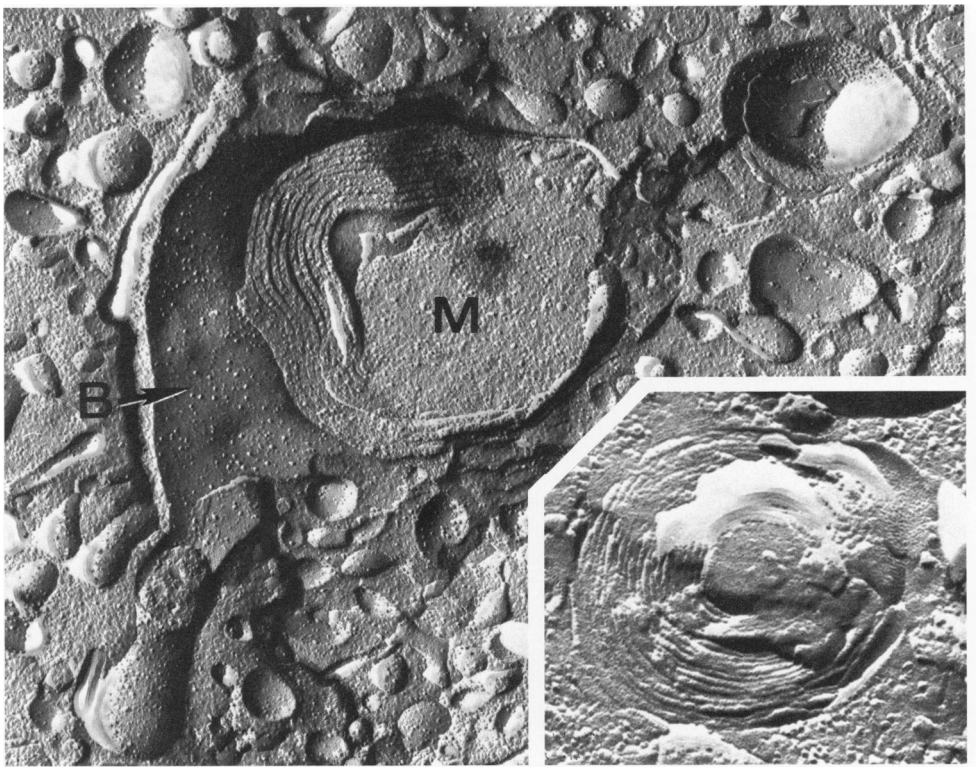



3



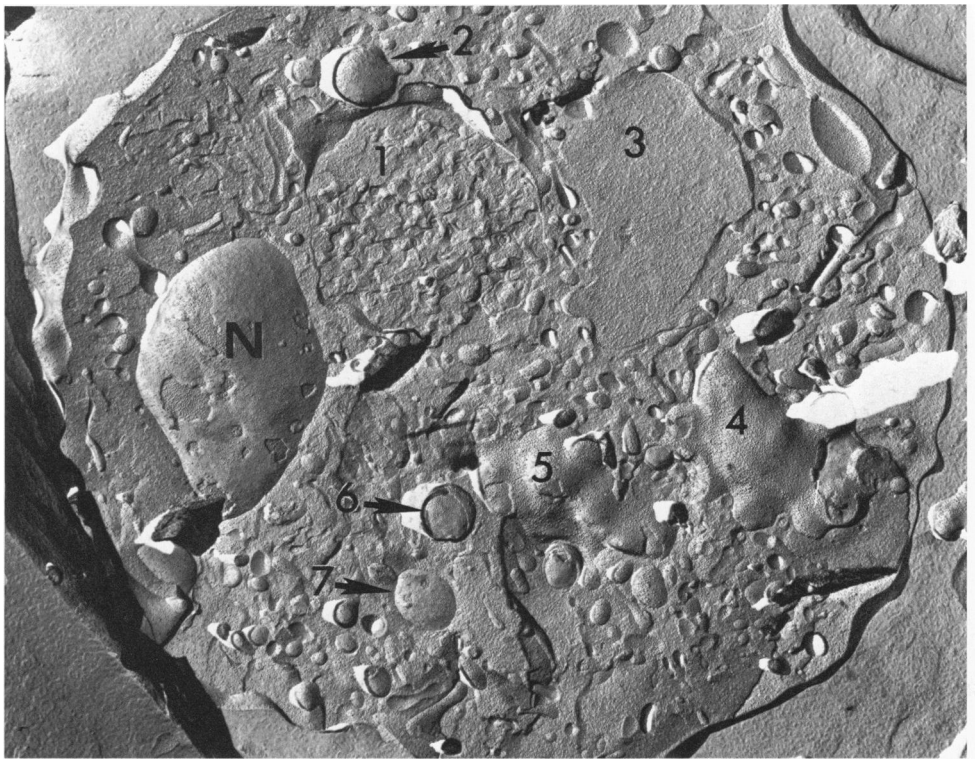

4A


4B

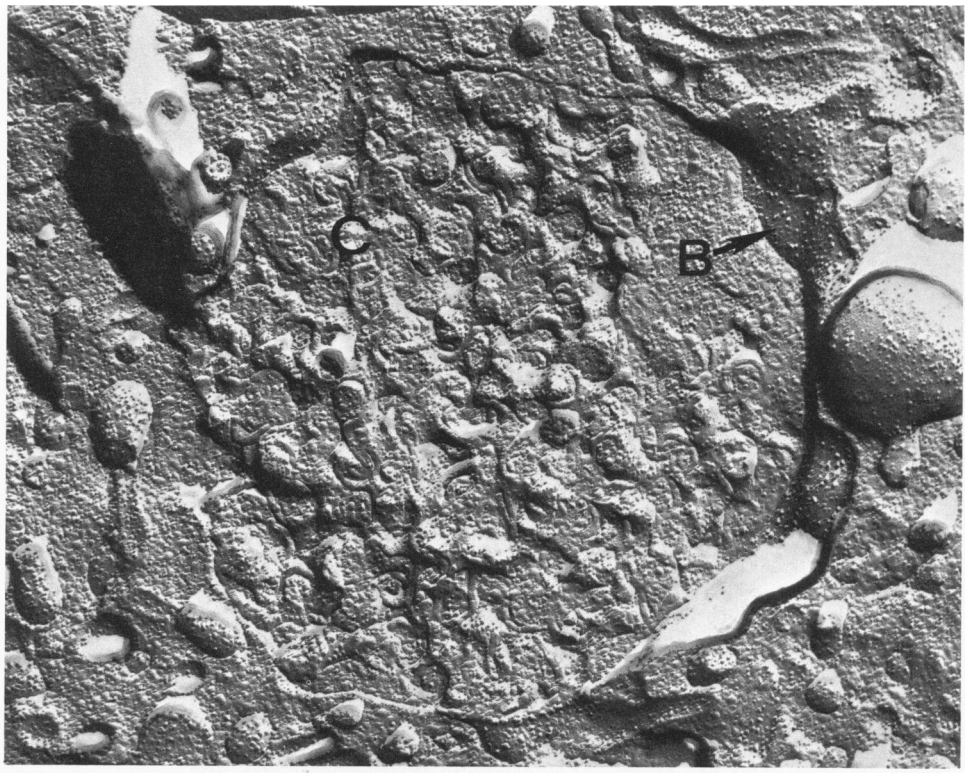



5A

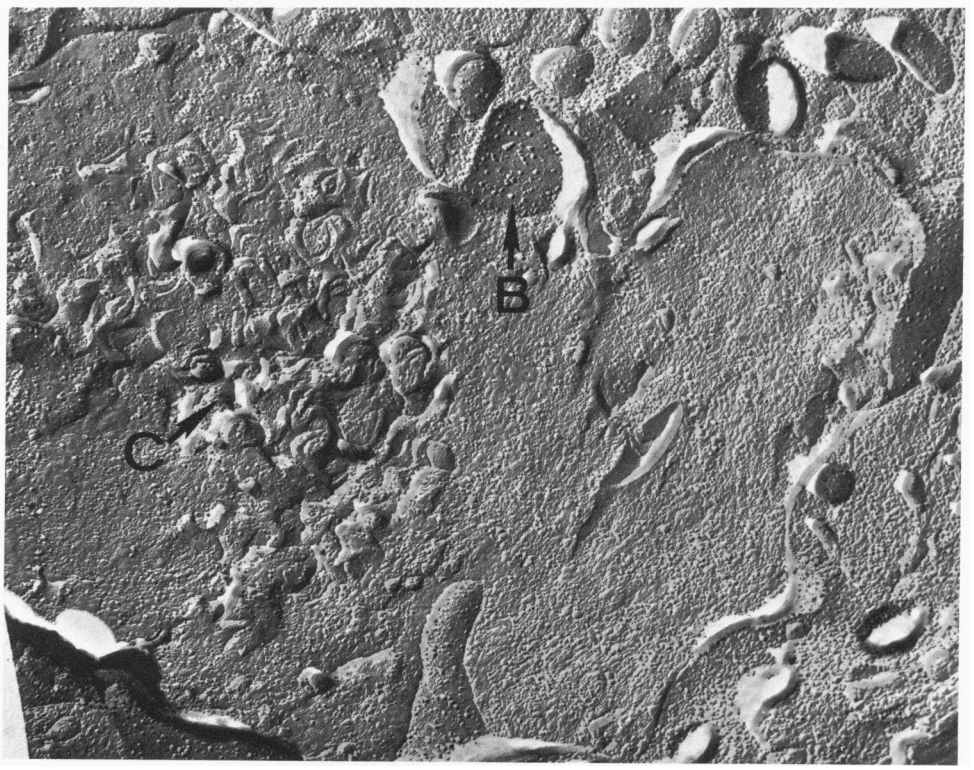
5B



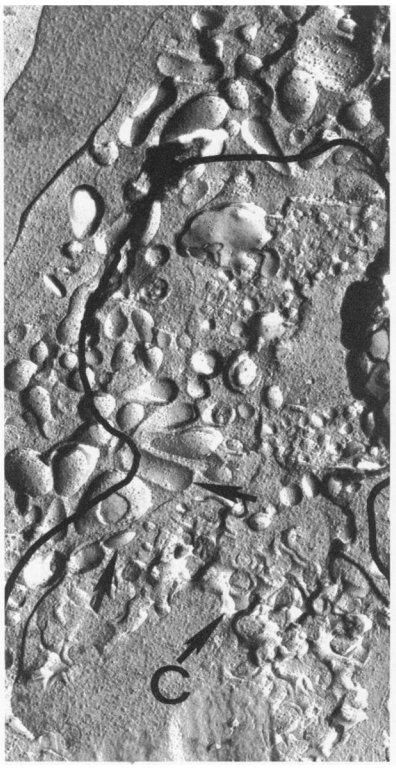

6



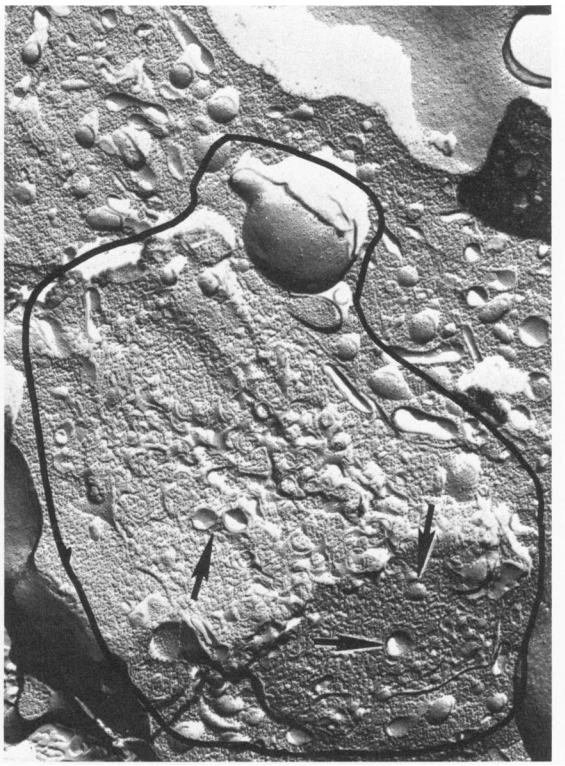

7



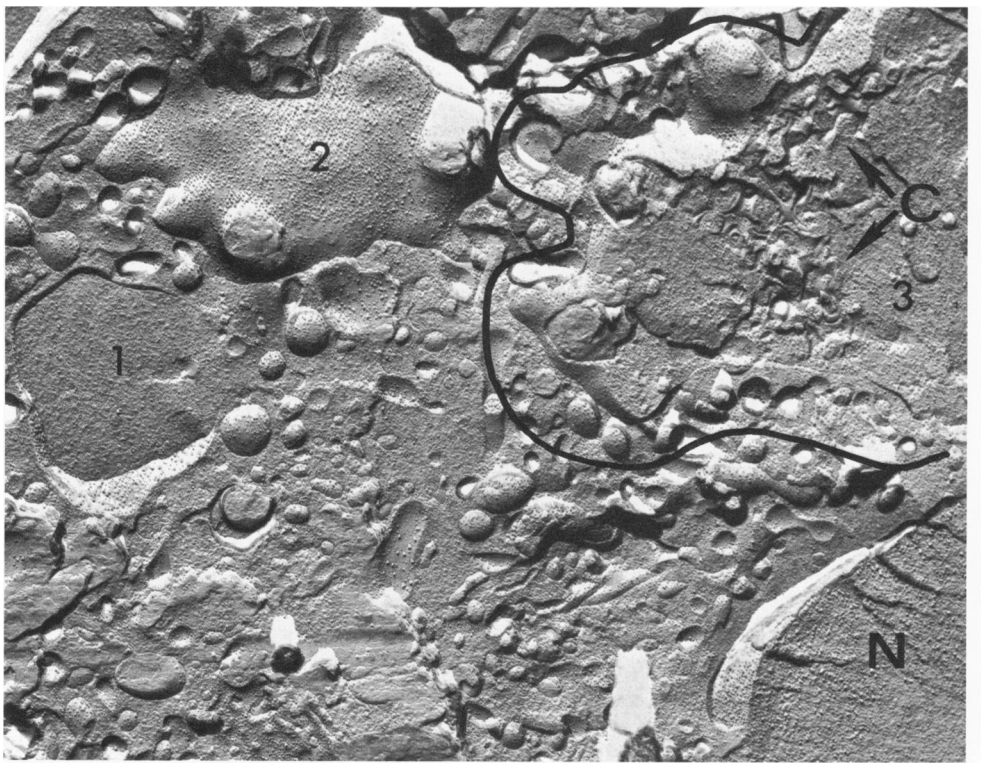

8




9A

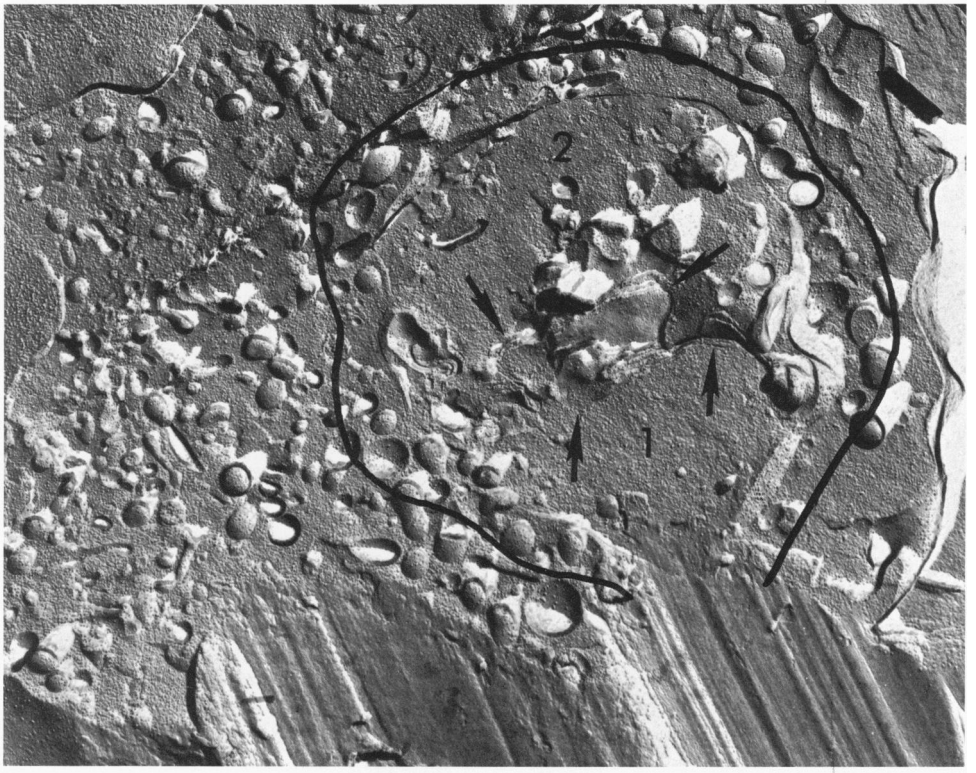


9B

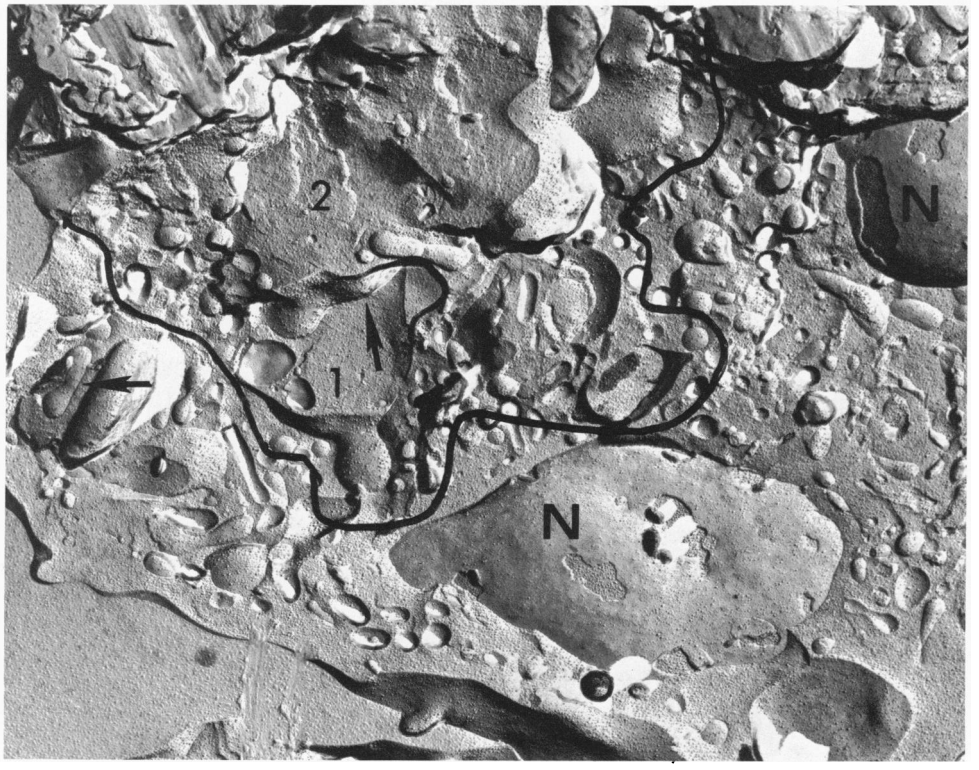


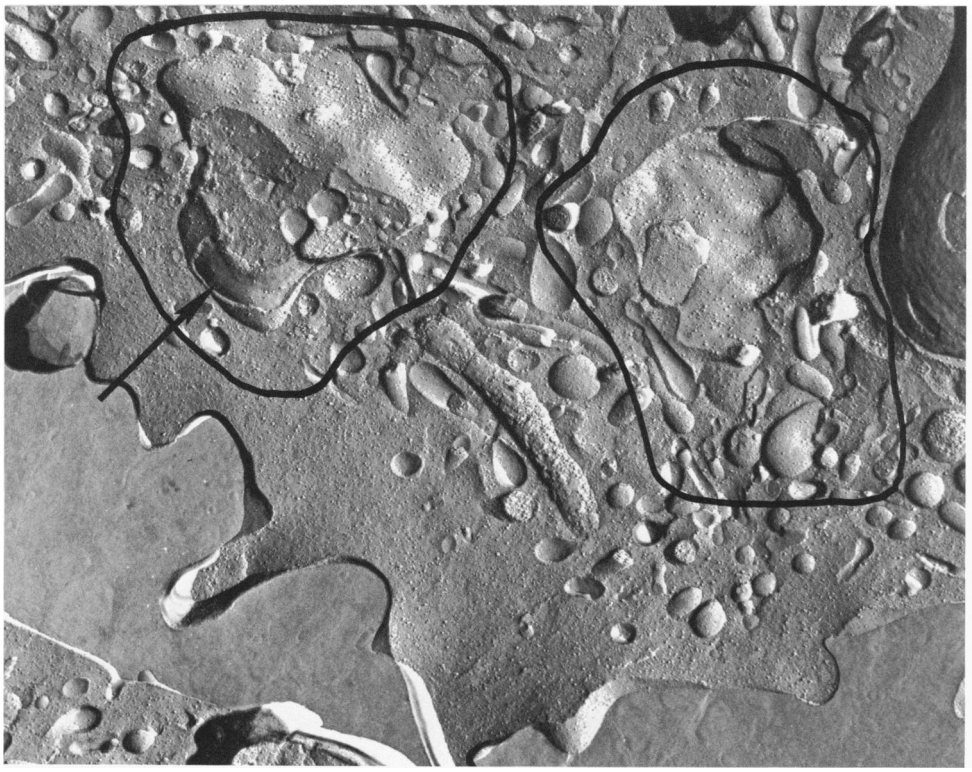

10

11



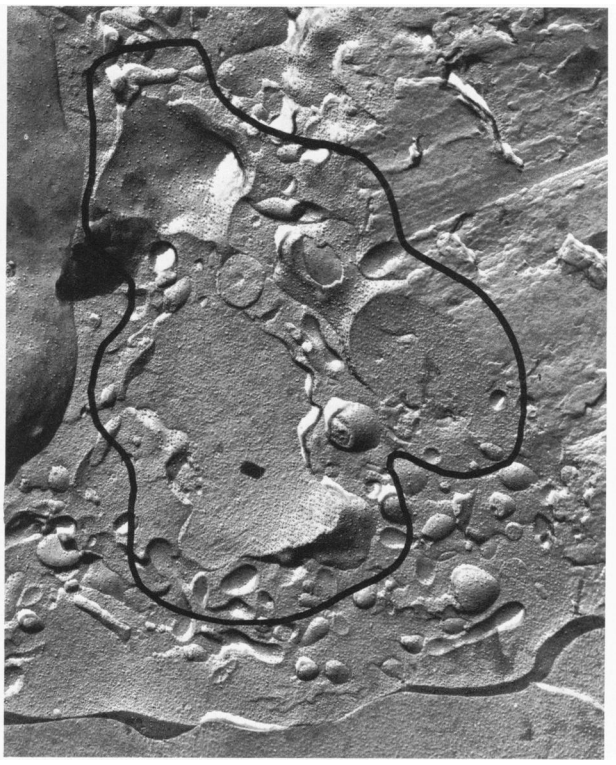

12





13


14A



14B

[*End of Article*]