The Mikulicz Cell in Rhinoscleroma

Light, Fluorescent and Electron Microscopic Studies

Ernesto O. Hoffmann, MD, Leland D. Loose, PhD and
James C. Harkin, MD

The stages in the development of the Mikulicz cell in human rhinoscleroma were studied in biopsy specimens obtained from 10 patients using light, immunofluorescent and electron microscopy. The Mikulicz cell was identified morphologically as a macrophage, not a plasma cell. Acutely inflamed areas of rhinoscleroma presented abundant bacteria with a slime layer. The microorganism was infrequent and the mucopolysaccharide was scanty in rhinoscleromal tissue, where plasma cells predominated, and in cicatricial fibrous tissue. In the granulomatous stage of rhinoscleroma, the mucopolysaccharide was found within the Mikulicz cells. The vacuoles observed in the Mikulicz cells were considered to be phagosomes containing, principally, bacterial mucopolysaccharide and few bacteria and, to a lesser extent, swollen mitochondria. It was concluded that the slime layer of *Klebsiella rhinoscleromatis* plays an important role in the pathogenesis of the disease. It is postulated that this material is a nondigestible mucopolysaccharide that resides in the phagosomes of macrophages, increases the osmotic pressure and forms multiple hydropic vacuoles that rupture not only the phagosomes but also the cells, resulting in the liberation of the mucopolysaccharide. This would initiate a cycle that would prolong the disease in the absence of the bacteria (Am J Pathol 73:47–58, 1973).

RHINOSCLEROMA is a progressive, granulomatous infectious disease, usually limited to the upper respiratory tract and produced by *Klebsiella rhinoscleromatis* (*Klebsiellae* tribe III).[1,2] Sporadic cases and endemic foci of the disease have been found in 68 countries.[3,4] Clinically, the disease has been described in progressive stages; however, overlapping of the stages occurs frequently in the individual patient.[3,5–7] The *first* stage is the rhinitic (cataharral, mucopurulent) or atrophic stage, where the histopathology is nonspecific. Usually there are abundant polymorphonuclear leukocytes and cellular debris; a meticulous examination may also reveal some Mikulicz cells and plasma cells. The *second* stage is the infiltrative (nodular, granulo-

From the Department of Pathology, Louisiana State University Medical School, the Departments of Physiology and Pathology, Tulane University School of Medicine and Laboratory Service, Veterans Administration Hospital, New Orleans, La.

Supported in part by Grant AI 10050 from the National Institutes of Health awarded to the International Center for Medical Research and Training, Tulane University, New Orleans La and Universidad del Valle, Cali, Colombia.

Accepted for publication Apr 12, 1973.

Address reprint requests to Dr. Ernesto O. Hoffmann, Department of Pathology, Louisiana State University Medical Center, 1542 Tulane Ave, New Orleans, LA 70112.

matous) or florid stage, in which Mikulicz cells are abundant and a characteristic infiltrate of plasma cells with prominent Russell bodies (Cornil or Mott cells), lymphocytes and foci of polymorphonuclear leukocytes also appears. Granulation tissue rich in blood vessels is also present in this stage. The *third* stage is the cicatricial, fibrous or scarring stage, in which connective tissue is prominent, while Mikulicz cells and plasma cells are rare.

Much of the enigma concerning the relative importance of the bacteria and the tissue response in the development of this chronic disease concerns the Mikulicz cell. This cell has been described as a large, foamy, mononuclear cell containing numerous gram-negative bacilli [3,8,9] and is considered to be characteristic of the disease. The pathogenesis of the Mikulicz cells remain uncertain.[9,10] Some investigators have suggested that this cell arises from plasma cells,[11] while others have suggested that this cell is a macrophage.[9,12]

The present morphologic study of proven cases of human rhinoscleroma, using light, electron and immunofluorescent microscopic technics, concerns the pathogenesis of this peculiar cell, the Mikulicz cell.

Materials and Methods

Nasal biopsies were obtained from 10 patients with rhinoscleroma in different stages of evolution, diagnosed by histologic and bacteriologic criteria. The tissue was processed for light, fluorescent and electron microscopic studies. The patients were from the clinic and hospital of the Universidad del Valle in Cali, Colombia, an area of endemic rhinoscleroma.

Light Microscopy

Specimens were fixed in 10% buffered formalin, embedded in paraffin; 5-μ thick sections were stained with hematoxylin and eosin, periodic acid–Schiff (PAS) and Warthin and Starry stain for bacteria. Sections 1 μ in thickness were embedded in Maraglas (see electron microscopy below) and stained with Paragon Multiple Stain (Paragon, C. and C. Co., Inc., New York) for examination by light microscopy.

Fluorescent Microscopy

The procedures have been reported in previous publications [13,14] and are described here briefly. Antibodies against *K rhinoscleromatis* were prepared in rabbits by using the whole microorganism mixed with complete Freund's adjuvant. Globulins were separated from the serum with half-saturated ammonium sulfate and labeled with fluorescein isothiocyanate. The conjugates were absorbed in rat liver powder, and pure cultures of strains of *K pneumoniae* were grown. Five-micron sections of biopsy tissues were "stained" by direct fluorescent antibody technic. A Zeiss fluorescent microscope was used and photographs were taken on Ektachrome film (Eastman Kodak Co, Rochester, NY).

Electron Microscopy

Fragments of tissue 1 to 2 mm in diameter were fixed for 2 hours at 4 C in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.20) containing one drop of of 1.0% $CaCl_2$/10 ml of fixative. Tissues were washed overnight in the same cold buffer with 5% sucrose added, postfixed in 1% osmium tetroxide in phosphate buffer for 1 hour and then dehydrated in a graded series of cold ethanol, followed by propylene oxide and embedded in Maraglas. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead nitrate and examined in a Phillips EM 300 or an RCA EMU-4 electron microscope.

Results

All specimens examined contained Mikulicz cells described below. In the following sections the morphology of rhinoscleroma is limited to these cells without repeating established observations.[3,9,10,12]

Light Microscopy

The Mikulicz cell was identified as a large (10 to 100 μ) mononuclear cell with an irregular outline. The cell was sometimes fragmented and difficult to delineate from other cells. With hematoxylin and eosin staining, the cytoplasm in the well-developed large Mikulicz cell was vacuolated and frequently contained a single, irregular vesicle with granular and fibrilar debris. Coalition of the vacuolated cells was frequent, with formation of multicellular microcysts. The smaller, less altered Mikulicz cells, usually localized around blood vessels, contained few vacuoles, had a granular cytoplasm and an oval bean-shaped nucleus, sometimes with an identation. With PAS staining, slightly positive material could be seen within the majority of the vesicles and was concentrated in the periphery of these structures. With Warthin and Starry staining, bacteria could be demonstrated in some of the vacuoles and microcysts. The bacteria were more abundant in areas of acute inflammation. In thick sections of Maraglas-embedded tissues, multiple vesicles could be seen in the cytoplasm of Mikulicz cells with well-preserved intervesicular bridges containing granules. Some nuclei were stellate and some were pyknotic; in some cells nuclei were absent. In the granulomatous stage of rhinoscleroma, Mikulicz cells were abundant but less numerous in places of heavy plasma cell infiltration and vice versa. In the cicatricial areas, occasional Mikulicz cells were present in the middle of bundles of collagen fibers. In each case, especially where large or multiple biopsies had been taken, it was possible to recognize areas peculiar to each of the so-called stages of the disease.

Fluorescent Microscopy

Granular fluorescent material of nonuniform size was demonstrated in Mikulicz cells, multicellular microcysts and interstitial tissues. This fluorescent material was condensed at the periphery of the vesicles and was present in the majority of these vacuoles. Bacteria were demonstrated in a limited number of the vacuoles of the Mikulicz cell and microcysts. In the areas of acute inflammatory infiltration the fluorescent material was more abundant and the bacteria were more numerous; both fluorescent material and bacteria were located in the interstitial tissues and within the cells. In the granulomatous areas, the fluorescent material was limited to the vacuoles of the Mikulicz cells and to microcysts, very little appeared in the interstitial tissues. The fluorescent material appeared to be less abundant in the granulomatous areas with heavy plasma cell infiltrates. In the fibrous areas, occasional Mikulicz cells contained a few fluorescent granules and very rarely bacteria.

Electron Microscopy

The cell boundaries of the Mikulicz cell were usually difficult to see, and many of the cells appeared fragmented. The organelles in the cytoplasm were located in the intervesicular bridges and presented a variety of alterations according to the extent of the vacuolization. Mitochondria were usually oval or round with marked vacuolization and loss of cristae. The dense bodies of these organelles were sometimes very prominent. The smooth endoplasmic reticulum was not prominent, especially in the well-developed Mikulicz cell. The Golgi apparatus and primary lysosomes were seldom visualized. Rough endoplasmic reticulum and isolated ribosomes were sometimes found in the intervacuolar bridges. Smooth membrane vesicles of different sizes were numerous; in the majority of cases, they were interpreted as phagosomes containing a fine granular and fibrilar material similar to the slime layer of *K rhinoscleromatis,* which was also present at times within these vesicles. The ultrastructure of the microorganism [12,15] is illustrated in Figure 5. The limiting membrane of the phagosomes was frequently ruptured and fragmented, resulting in the close contact of the bacteria and their slime layer with the cytoplasm. Some phagosomes contained myelin figures and the cellular debris of other cells which consisted of Russell bodies, fragments of polymorphonuclear leukocytes and nuclear debris. In some other cases, the smaller vesicles were considered to be swollen and vacuolated mitochondria. Fusion of vesicles was frequent, forming larger vacuoles and

occasional multicellular cysts. The nuclei of the Mikulicz cells were round, oval, or stellate and pyknotic. In the small less altered cells, the chromatin was light and uniformly distributed, with some condensation to the nuclear membrane. Nucleoli were seldom observed. In the large well-developed Mikulicz cell, the nucleus was frequently absent. At times, plasma cells contained cytoplasmic vacuoles that appeared to be dilated rough endoplasmic reticulum. The abundant presence of rough endoplasmic reticulum, the Russell bodies and the peculiar distribution of the nuclear chromatin material were used to differentiate these cells from the Mikulicz cells. Bacteria were never observed in the intact plasma cell. Fragments of plasma cells, debris of other cells and bacteria or slime layer material were seen sometimes loose in the interstitial tissues.

Discussion

Previous investigators [9,12] have considered the Mikulicz cell as a macrophage, a concept that is confirmed by our findings. The foamy mononuclear cell in rhinoscleroma has all the basic morphologic structures characteristic of macrophages.[16] Although plasma cells may at times appear vacuolated, the distinct ultrastructural features of these cells—*ie*, abundant rough endoplasmic reticulum, distribution of the nuclear chromatin and presence of Russell bodies—allowed them to be distinguished from macrophages. Bacteria were not seen in plasma cells. The presence of bacteria and mucopolysaccharides in the vacuoles of the Mikulicz cell has been previously reported.[3,8–10,12,15,17] As demonstrated in the present study using electron microscopy and fluorescent antibody technics, the cytoplasmic vacuoles of the Mikulicz cell contained few bacteria but abundant material that was structurally similar to the slime layer of *K rhinoscleromatis*. This material reacted specifically with fluorescent antibodies directed against the microorganism and was visualized as granules, which probably represent the vacuoles of the Mikulicz cell. Bacteria were more abundant in the microcysts and the interstitium of acutely inflammed areas.

An interesting finding, previously mentioned by other investigators in rhinoscleroma,[12] is the frequent fragmentation of the limiting membrane of the phagosomes. Similar disruption of membranes has been observed in leprosy [18] and was interpreted as mechanical rupture resulting from the large numbers of bacteria and their metabolic products present within the phagosomes. In listeriosis,[19] it has been postulated that this microorganism releases hemolysins containing lecithinase or phospholipase, which are responsible for the lysis of the phagosomal

membranes. In the case of other intracellular parasites, the disruption of these membranes has also been demonstrated, but the cause of this rupture is not clearly understood.[20–22] It is suggested here that in rhinoscleroma the disruption of phagosomal membranes may be mechanical, a result of increased osmotic pressure within the vacuole caused by the mucopolysaccharide of the bacillus. It has been demonstrated in experimental animals and clinical material that macrophages and other cells may become vacuolated and foamy by exposure to nondigestible polysaccharides.[23] Monosaccharides with a molecular weight of up to 200,000 apparently do not produce vacuolization. The slime layer of *K rhinoscleromatis* is a polysaccharide with an estimated molecular weight of more than 300,000.[24,25] It is suggested that the bacilli and/or the slime layer penetrate the macrophages by endocytosis and reside in phagosomes that have a membrane which is probably impermeable to these mucopolysaccharides but permeable to water.

Water may be drawn into the phagosome, resulting in increased intravacuolar pressure, sometimes to the point of rupture and fragmentation of the limiting membrane; if extreme, the cell itself could rupture. A number of vacuoles in the Mikulicz cell appeared to be swollen and vacuolated mitochondria; the cause of this is unknown.

Our osmotic theory would explain certain puzzling aspects of this peculiar disease. It would explain the difficulty encountered in attempting to produce experimental lesions with cultured microorganisms, since it has been demonstrated that bacilli from artificial culture media have small amounts of the slime layer [12,26] compared with bacilli obtained from human lesions. On the other hand, it is easy to produce antibodies with these cultured bacteria [13,14] and to produce experimental lesions, if adjuvants are added to the inoculum.[27] It would also explain the characteristic prolonged evolution of the disease and the fact that sometimes, even though the histology of the lesions is characteristic, bacteria cannot be grown from them in artificial media. In addition, it would explain the slow regression of the lesions after antibiotic treatment. At present, it is obvious that we cannot omit the possibility of lysosomal enzyme inhibition or deficiency in these macrophages. The role of plasma cells and specific circulating antibodies in the pathogenesis of rhinoscleroma and the peculiar tendency to produce abundant collagen remain to be ascertained.

References

1. Godoy GA: Etiologia y fisiopatologia del escleroma. Arch Col Med Salvador 19:158–163, 1966

2. Hoffmann E: The etiology of rhinoscleroma. Int Pathol 8:74–77, 1967
3. Kerdel-Vegas F, Convit J, Gordon B, Goihman M: Rhinoscleroma. Springfield, Ill, Charles C. Thomas, Publishers, 1963
4. Muzyca MM, Gubina KM: Problems of the epidemiology of scleroma. J Hyg Epidemiol Microbiol Immunol 16:8–20, 1972
5. Barten JJC: Scleroma respiratorium in Indonesia. Doc Med Geograph Trop 8:101–116, 1956
6. Furnas DW: Recognition of scleroma (rhinoscleroma). Laryngoscope 78:1948–1952, 1968
7. Shaw HJ, Martin H: Rhinoscleroma: a clinical perspective. J Laryngol Otol 75:1011–1039, 1961
8. Smith DT, Conant NF: Zinsser Microbiology. New York, Appleton Century Crofts, Inc, 1968, pp 632–633
9. Woyke S, Domagala W, Olszewski W: Electron microscopic studies of scleroma granulomatous tissue. Acta Med Pol 10:231–242, 1969
10. Shokeir AA, Osman M: Rhinoscleroma: an electronmicroscopic study. J Hyg Epidemiol Microbiol Immunol 16:1–7, 1972
11. Fisher EE, Dimling C: Rhinoscleroma: light and electron microscopic studies. Arch Pathol 78:501–512, 1964
12. Welsh RA, Correa P, Herran R: Light and electron microscopic observations of scleroma. Exp Mol Pathol 2:93–101, 1963
13. Godoy GA: Identification de *Klebsiella rhinoscleromatis* en frotis de cultivos puros y tejidos de raton utilizando tecnicas de anticuerpos fluorescentes. Rev Lat Am Microbiol 8:119–127, 1966
14. Hoffmann E, Correa P, Lujan R: Immunofluorescencia en el Rhinoscleroma, Rev Lat Am Patol 7:67–76, 1963
15. Gonzales-Angulo A, Marques-Monter H, Greenberg DS, Cerbon J: Ultrastructure of nasal scleroma: emphasizing the fine structure of *Klebsiella rhinoscleromatis* within the lesion. Ann Otol Rhinol Laryngol 74: 1022–1033, 1964
16. Pearsall NN, Weiser RS: The Macrophage. Philadelphia, Lea and Febiger, 1970, pp 5–16
17. Larralde C, Marquez-Monter H: Polisacaridos en el escleroma nasal: estudio histoquimico. Rev Lat Am Patol 9:205–211, 1970
18. Imaeda T: Electron microscopic approach to leprosy research. Int J Lepr 33:669–688, 1965
19. Armstrong VA, Sword CP: Electron microscopy of *Listeria monocytogenis* infected mouse spleen. J Bacteriol 91:1346–1355, 1966
20. Edwards GA, Edwards MR, Hausen EL: Electron microscopic studies of *Histoplasma* in mouse spleen. J Bacteriol 77:429–438, 1959
21. Karlsbad G, Kessel R, de Petri S, Monaco L: Electron microscopic observations of *Brucella abortus* grown within monocytes *in vivo*. J Gen Microbiol 35:383–390, 1966
22. Miller MG, Wilson RB: *In vivo* and *in vitro* observations of *Leptospira pomona* by electron microscopy. J Bacteriol 84:569–576, 1962
23. Cohn ZA, Ehrenreich BA: The uptake, storage and intracellular hydrolysis of carbohydrates by macrophages. J Exp Med 129:201–226, 1969
24. Rojas-Espinosa O, Estrada-Parra S: Immunochemistry of the capsular

polysaccharide of *Klebsiella rhinoscleromatis*. Rev Lat Am Microbiol 10: 7–10, 1968

25. Wheat RW, Dorsh C, Godoy G: Occurrence of pyruvic acid in the capsular polysaccharide of *Klebsiella rhinoscleromatis*, J Bacteriol 89:539, 1965
26. Gonzales-Angulo A, Marquex-Monter H, Greenberg SD, Cerbon J: Observaciones sobre la ultraestructura de *Klebsiella rhinoscleromatis* en cultivo. Rev Lat Am Microbiol 6:15–26, 1963
27. Hoffmann E, Duque E: Rhinoscleroma experimental. Rev Lat Am Patol 10.57–70, 1971

Legends for Figures

Fig 1—Section from formalin-fixed rhinoscleromal tissue has a predominance of Mikulicz cells with vacuolated cytoplasm and intravacuolar granular debris. The nuclei are pyknotic and in many cells are absent. Formation of microcysts by coalescence of vacuolated cells is also observed (H & E, × 800).

Fig 2—Maraglas-embedded tissue stained with Paragon multiple stain. Notice the delicate foamy appearance of the cytoplasm in the Mikulicz cells with granules in the intervacuolar bridges. A pyknotic and stellate nucleus is characteristic of the well-developed Mikulicz cell. Less altered macrophages and few plasma cells are observed around small blood vessels. Two large Russell bodies are also prominent in this photomicrograph (Paragon, × 1000).

Fig 3—Electron microphotograph of an early stage of vacuolization of a Mikulicz cell (*M1*) presenting the characteristics of a macrophage. This cell is surrounded by other intensively vacuolated cells (*M2*). Notice the absence of dense bodies (primary lysosomes) and the presence of a finely granulofibrillar material within the vacuoles of these cells. This material appears ultrastructurally similar to the slime layer around the bacteria, present in the interstitial space (*B*) (Uranyl acetate and lead citrate, × 10,000).

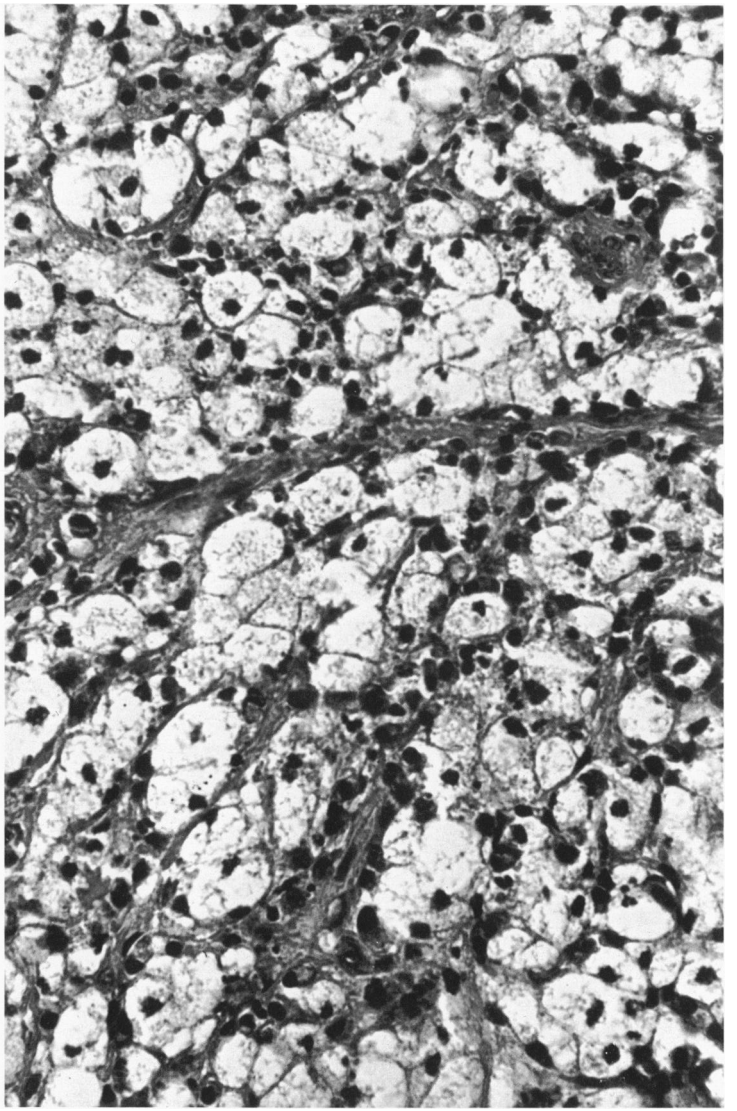
Fig 4—Coalescence of cytoplasmic vacuoles probably due to rupture of the phagosomal membrane is observed in two Mikulicz cells (*M*). The vesicles present a finely granulofibrillar material similar to the capsular mucopolysaccharide of the bacteria. Few mitochondria are seen but apparently no dense bodies (primary lysosomes). Rough endoplasmic reticulum of surrounding plasma cell (*P*) is dilated and contains small Russell bodies (Uranyl acetate and lead citrate, × 17,000).

Fig 5—A large vacuole in a Mikulicz cell contains two bacteria (*B*), one of them apparently dividing. The thick slime layer of the microorganisms is granulofibrillar and fills the vesicle. Few remnants of the phagosomal membrane are left (*arrow*). Coalescence with a smaller vacuole (*V*) is observed at the lower right hand corner (Uranyl acetate and lead citrate, × 50,000).

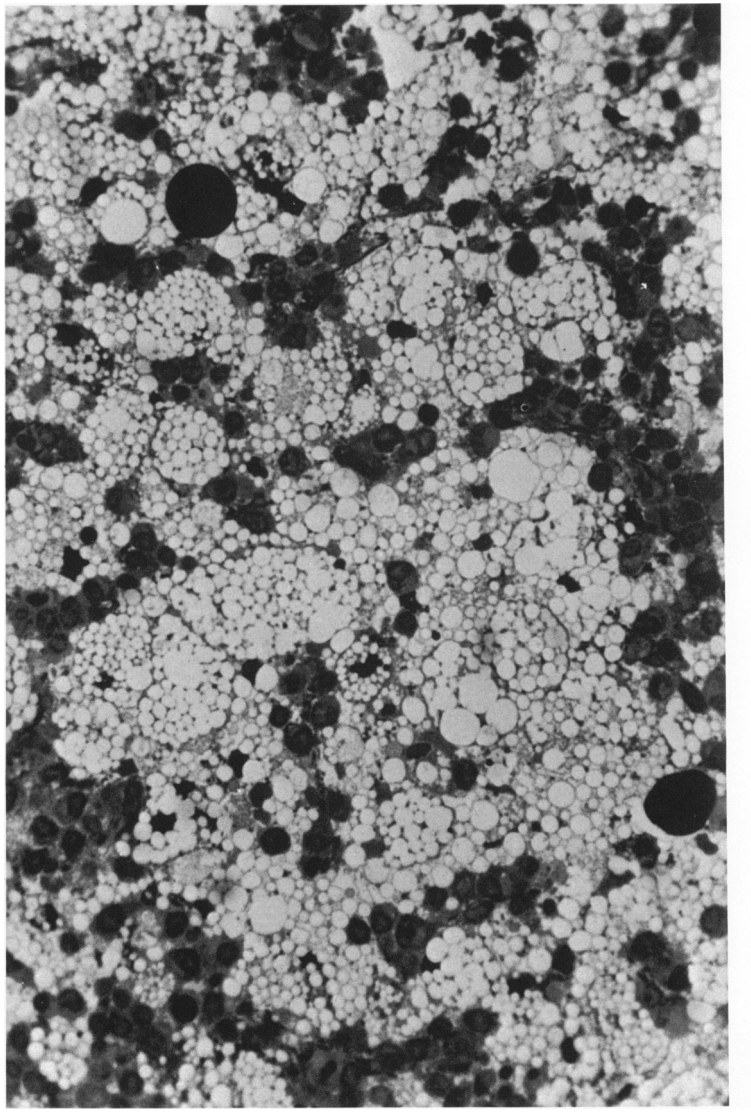
Fig 6—Electron micrograph of the cytoplasm of a Mikulicz cell presents several small vacuoles, many of them with recognizable mitochondrial structures (*m*). Some of the large vacuoles (*v*) show rupture of their limiting membrane and coalescence with other vesicles (*arrows*). Dilated rough endoplasmic reticulum (*ER*) is also observed (Uranyl acetate and lead citrate, × 50,000).

Fig 7—Granules that react positively with fluorescent anti-*K rhinoscleromatis* antibodies are present in many Mikulicz cells and the interstitial spaces. Few bacteria are also seen (*arrows*) (FA, × 800).

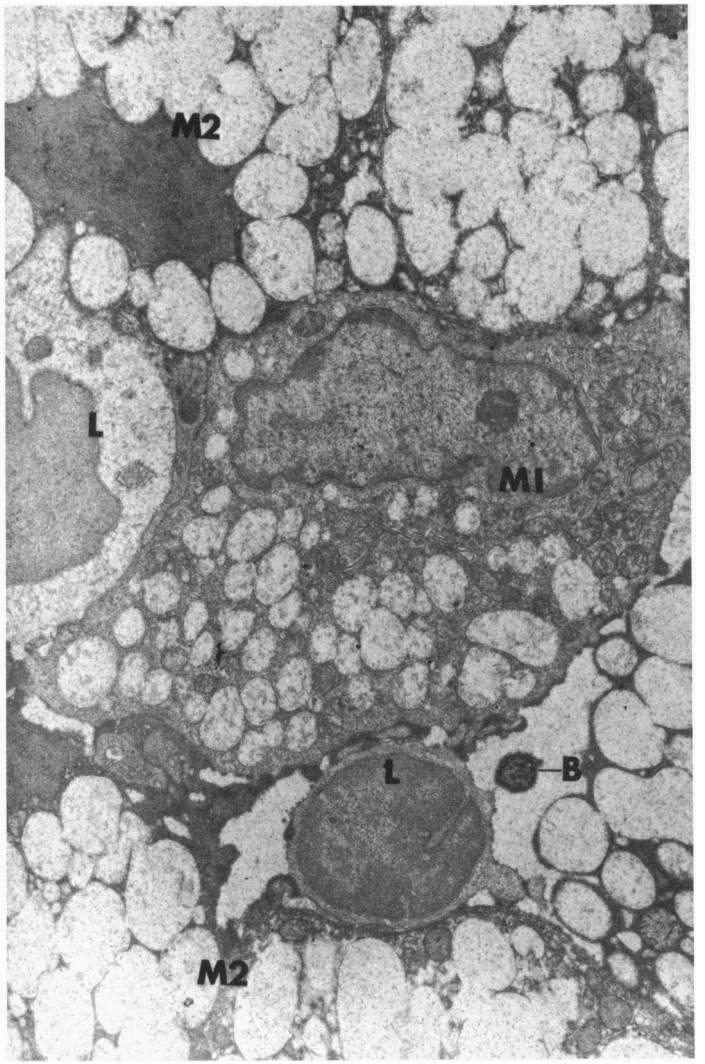
Fig 8—Three Mikulicz cells contain granules that reacted positively to anti-*K rhinoscleromatis* fluorescent antibodies. Notice the negative reaction around these cells where an intense infiltrate of plasma cells was found (FA, × 1000).



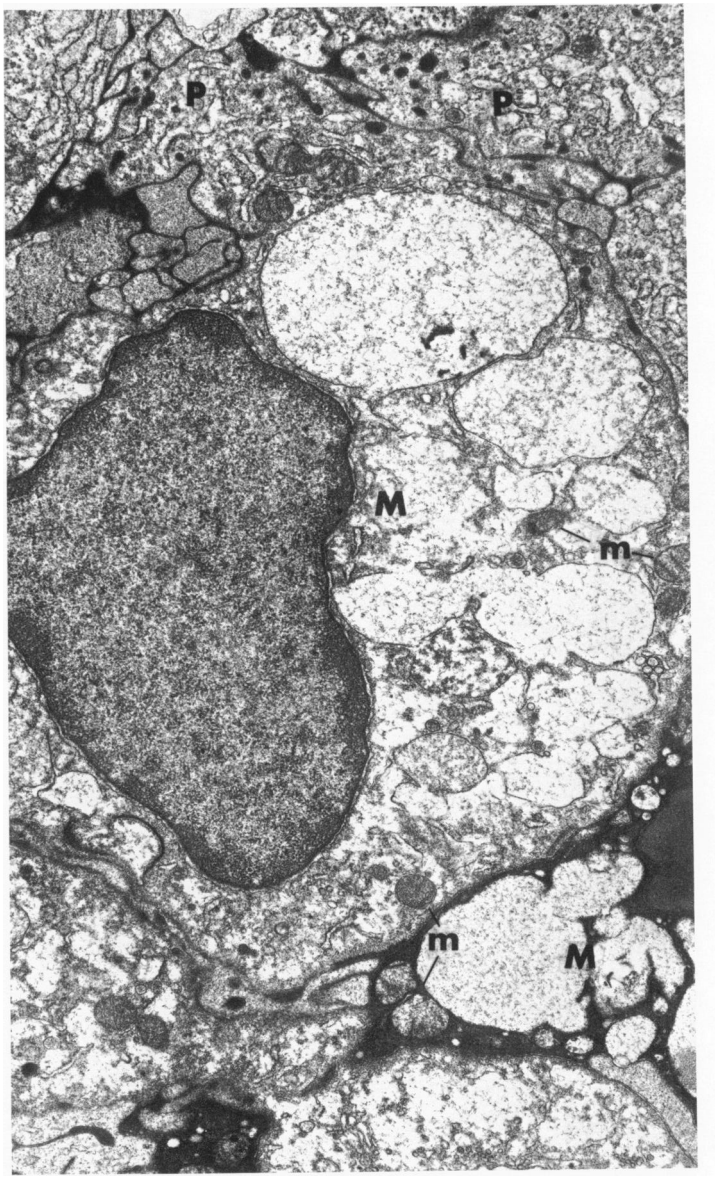
1



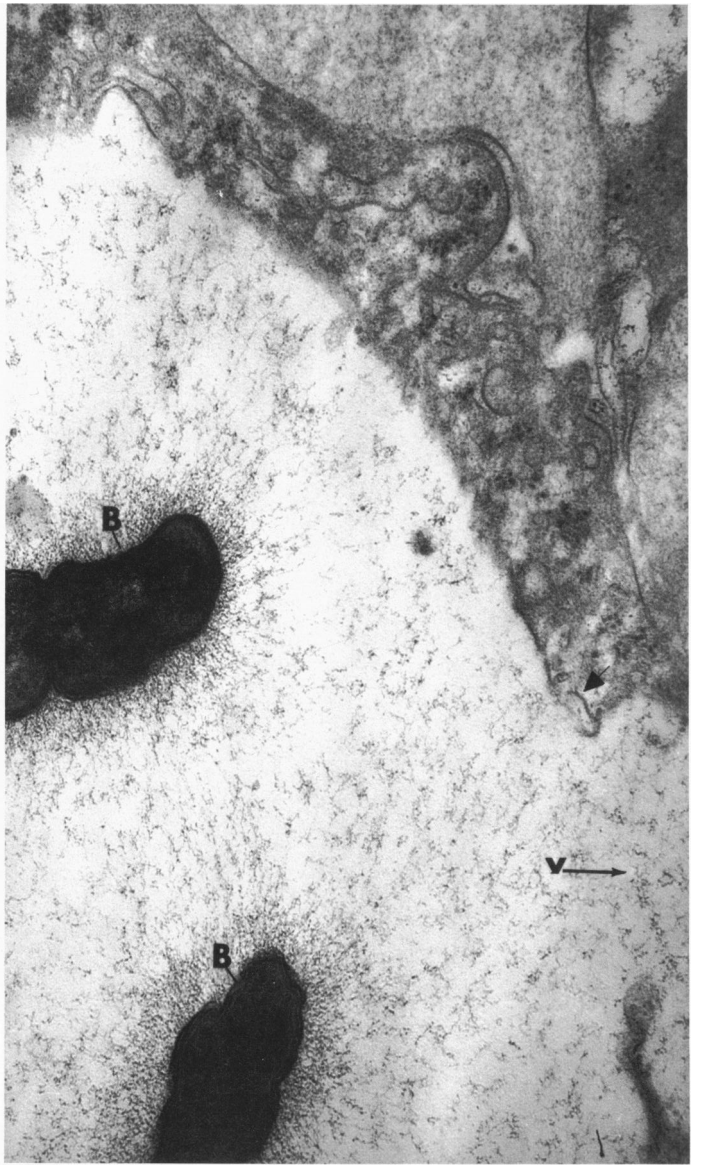
2



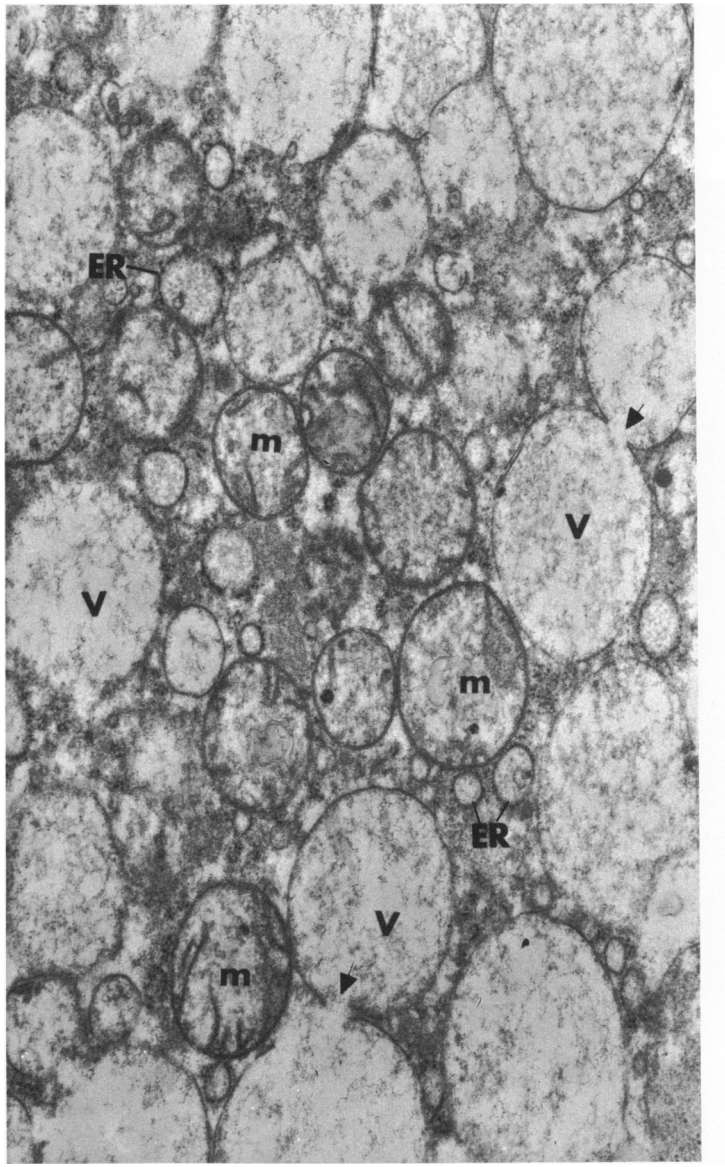
3



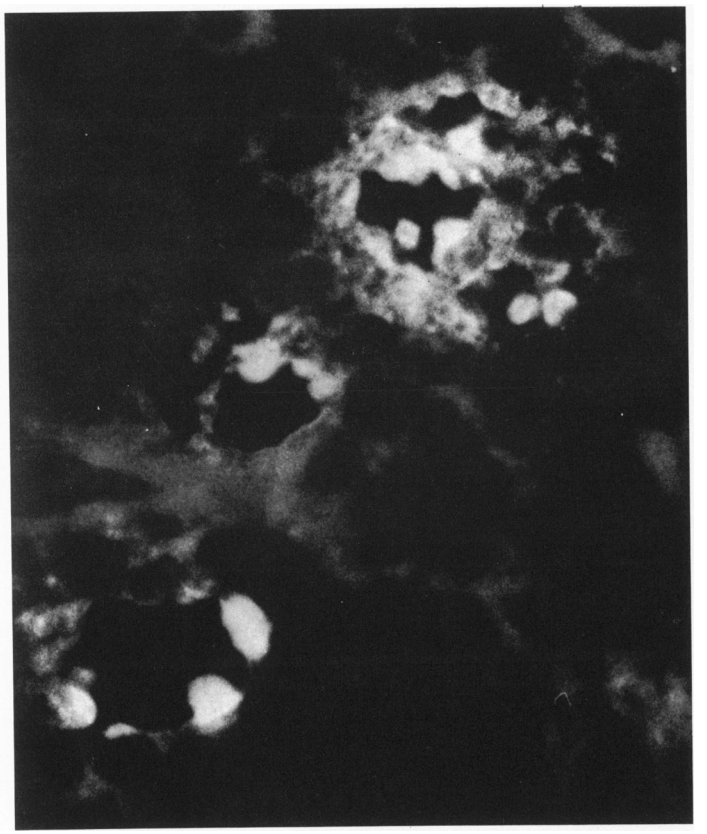
4



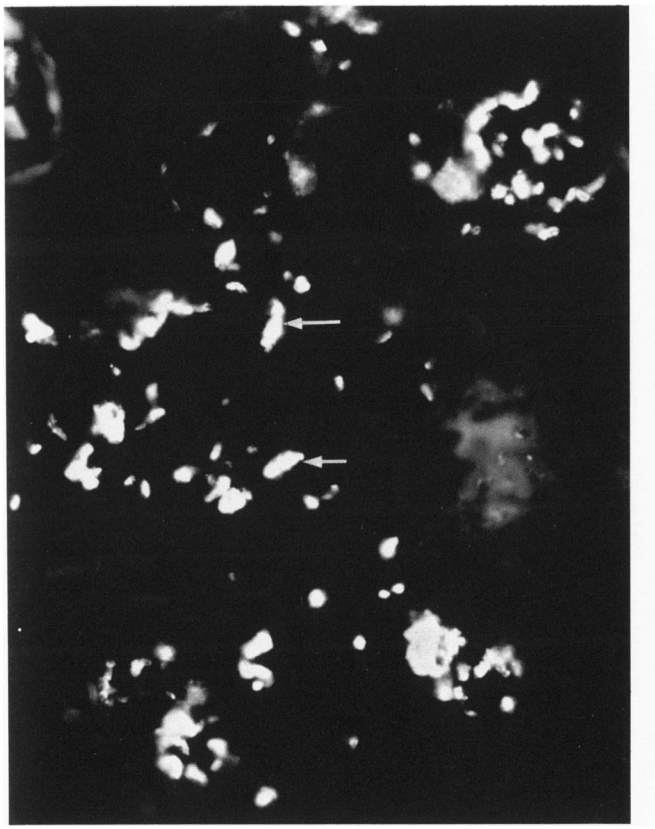
5



6



8



7