Pathobiologic Significance of the Subcellular Organelles of Lepra Cells[1, 2]

Tomas I. Aquino and Olaf K. Skinsnes[3]

The polar types of leprosy, tuberculoid and lepromatous, are characterized by two different and well-defined forms of cellular response ([25]). Tuberculoid lesions consist of circumscribed granulomas made up of epithelioid cells which contain few leprosy bacilli. Lepromatous lesions, in contrast, are composed of diffuse aggregates of foamy macrophages (Virchow cells) laden with large numbers of bacteria and lipid debris. Epithelioid and Virchow cells constitute the two extremes in the cytologic spectrum of leprosy. Electron microscopy has emphasized the basic morphologic differences between the two cell types. The cytoplasm of epithelioid cells is occupied by abundant mitochondria and a prominent ergastoplasmic reticulum ([27]). There are no cytoplasmic components in epithelioid cells that differ significantly from the types of organelles found in ordinary macrophages. Virchow cells, on the other hand, are packed with leprosy bacilli which are frequently fragmented and seem to be in a state of degeneration. The foamy appearance of these cells has been shown by electron microscopy to be due to the presence of large clear cytoplasmic vacuoles which have been referred to as "leprosy inclusions" ([5]). The electron microscope has also revealed in lepromatous cells, an additional type of dense cytoplasmic organelle given the descriptive name of "opaque droplets' ([35]). These opaque droplets sometimes have a vesiculated or foamy appearance and are then called "foamy structures" ([8]). The opaque and foamy organelles are often found in intimate contact with phagocytized leprosy bacilli. No clear line of demarcation has been drawn between opaque droplets, foamy structures, and leprosy inclusions and these organelles have been said to evolve into one another ([13]).

The unique morphology of the cytoplasmic components of Virchow cells has attracted considerable attention and has been subject to different interpretations ([5]). The question seems to have been partly resolved by Brieger ([5, 6]) who proposed that the dense and vesiculated organelles, and probably the leprosy inclusion itself, are lysosomes.

Lysosomes have been defined as subcellular components which contain a high concentration of hydrolytic enzymes and play an important role in phagocytosis and cellular digestion ([9, 10, 11, 13, 16]). Acid phosphatase holds a special position among these enzymes because of its use in the cytochemical identification of lysosomes ([29]).

Brieger's ([6]) interpretation of the organelles of lepra cells was based on the demonstration of acid phosphatase in Virchow cells. However, his study was conducted at the light microscopic level and did not determine the exact localization of the enzyme in opaque droplets, foamy structures and leprosy inclusions which are only visualized by electron micrographs. Indirect support for the presence of lysosomes in lepra cells came subsequently from the demonstration of acid phosphatase activity in a type of dense peribacillary body observed in electron micrographs of murine leprosy lesions ([1]). The significance of the latter finding stems from the biologic similarities between human and murine leprosy and from the

1 Received for publication 5 May 1969.

2 From the Department of Pathology, University of Chicago, Chicago, Ill. Supported by grants No. 5T1-GM93 and No. AI-03627, National Institutes of Health, Bethesda, Maryland 20014.

3 T. I. Aquino, M.D., Ph.D., Department of Pathology, Woman's Medical College, 3300 Henry Ave., Philadelphia, Pa. 19129; O. K. Skinsnes, M.D., Ph.D., Department of Pathology, University of Hawaii School of Medicine, Leahi Hospital, 3675 Kilauea Ave., Honolulu, Hawaii 96816.

marked morphologic resemblance between human and murine leprosy and from the marked morphologic resemblance between the peribacillary bodies of murine leprosy and the opaque droplets of Virchow cells. Finally, a recent study of Imaeda ([24]) has shown the presence of acid phosphatase in the opaque droplets of Virchow cells.

Despite the above studies, the distribution of acid phosphatase in foamy structures and leprosy inclusions has not yet been determined and the interrelation between these organelles and opaque droplets remains to be elucidated. The investigation reported here is motivated by the apparent need for further definition of the unique ultrastructural components of lepra cells. By cytochemical demonstration of acid phosphatase in electron microscopic preparations, the present study shows the relation of lysosomal enzymes to the various subcellular components of Virchow cells, and makes possible an interpretation of the role of opaque droplets, foamy structures and leprosy inclusions in the digestion of leprosy bacilli by macrophages.

MATERIALS AND METHODS

The study consisted of the electron microscopic examination of skin biopsies from a group of lepromatous patients. A modification of the cytochemical method of Novikoff and Essner ([31]) was used to demonstrate acid phosphatase in electron microscopic preparations. Morphologic evaluation was accomplished by light microscopic examination of paraffin embedded tissue sections stained with hematoxylin and eosin and by electron microscopic examinations of epoxy embedded thin sections stained with uranyl acetate and lead citrate.

Source of material. Punch biopsies from active skin lesions were obtained from seven lepromatous patients at the University

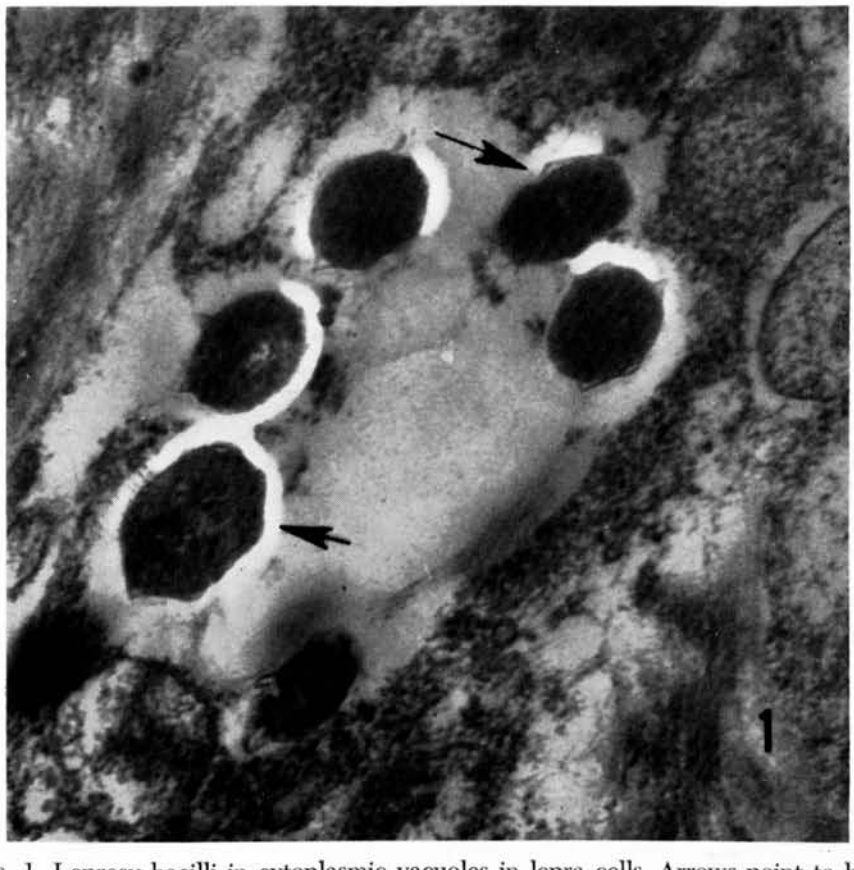

FIG. 1. Leprosy bacilli in cytoplasmic vacuoles in lepra cells. Arrows point to bacilli. Magnification: X 57,500 Ar. (aralite embedded tissues)

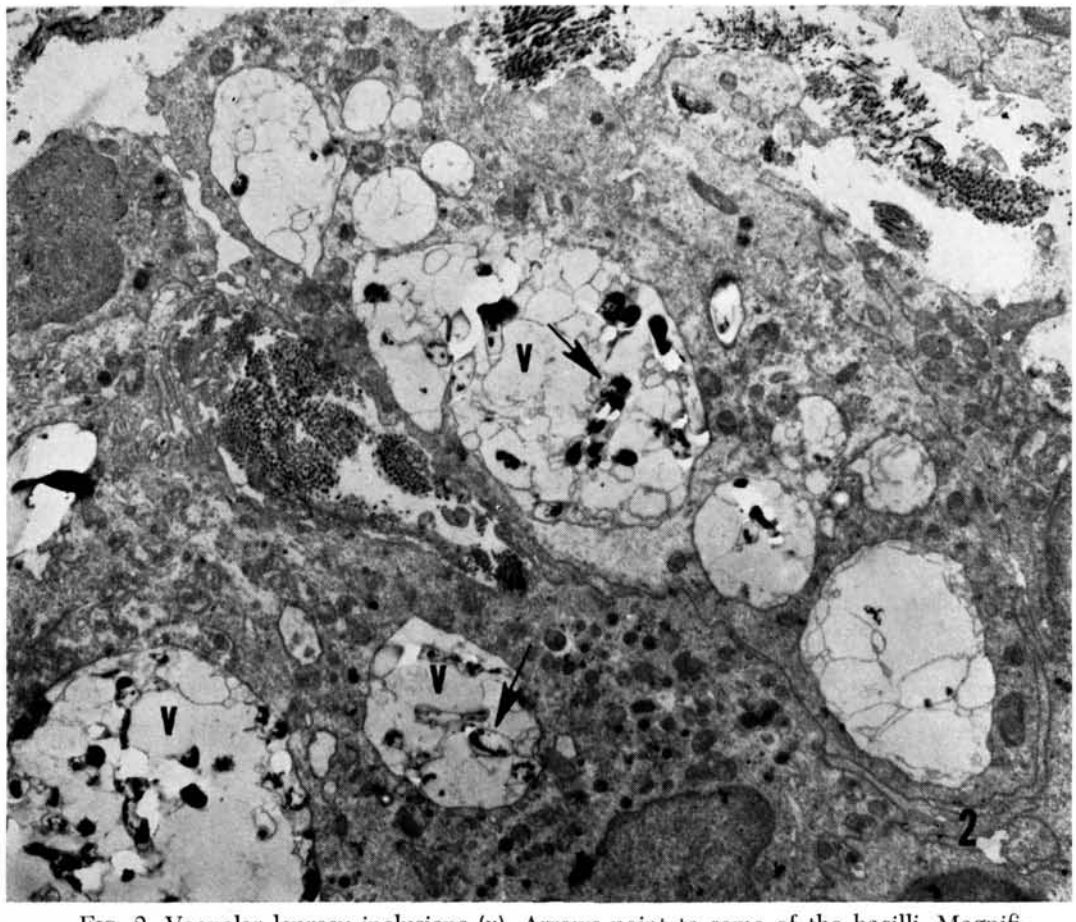

FIG. 2. Vacuolar leprosy inclusions (v). Arrows point to some of the bacilli. Magnification: X 7,500 Ar.

of Chicago and at Hong Kong. The patients were classified as lepromatous on the basis of the appearance of their skin lesions, a negative lepromin reaction, and the histologic characteristics of the skin biopsies. All patients had advanced clinical disease and had been treated with various courses of sulfone medication. The biopsies were cut into two pieces of about equal size. One of the pieces was fixed in 10 per cent buffered formalin for histologic studies. In preparation for electron microscopy, the epidermis was separated from the other piece and several small fragments of dermal lesions (1 to 2 mm. in largest dimension) were immediately fixed in OsO_4. The remaining portion of dermis was fixed in formol calcium for histochemistry.

Histology. Formalin-fixed tissues were embedded in paraffin, cut and stained with hematoxylin and eosin and acid-fast stains. These served to confirm the pathologic diagnosis, to evaluate the extent of the lesions and the presence of Virchow cells, as well as to determine the number and condition of the bacilli.

Histochemistry ([31]). Portions of lepromas were fixed overnight in cold formol calcium containing 5 per cent sucrose. On the following day, the tissues were cut at 50 microns sections in a freezing microtome. These were then incubated for one hour in acid phosphatase medium containing sodium betaglycerophosphate (Eastman) as substrate. The sections were then washed in acetate buffer and water and subsequently fixed in OsO_4.

Electron microscopy. Portions, about 1 cu.mm. in volume, of fresh tissue material, in addition to the acid phosphatase incubated preparations, were fixed for one to four hours in 1.33 per cent OsO_4 in collidine

buffer ([4]). After dehydration through increasing alcohol concentrations, the tissues were embedded in Araldite (Durucupan: Fluka) and in Epon (Shell). Sections were cut at 400 to 700 Å in an MT-2 Porter-Bloom ultramicrotome. Diamond knives were used for thin sectioning. Saturated uranyl acetate in 50 per cent ethanol and lead citrate ([33]) were used for staining. Acid phosphatase preparations were examined unstained. The sections were examined in an RCA EMU 3D and in a Siemens Elmiskop 1A electron microscope.

RESULTS

All biopsies showed extensive infiltration of the dermis by macrophages containing large numbers of bacilli. In the electron microscope, these bacilli were grouped inside phagocytic vacuoles (Fig. 1). Single bacilli were only rarely found. The bacilli seemed to be in various stages of degeneration, and many of them showed partial loss of their cytoplasmic contents. A moderate amount of uniform clear substance was present around the bacilli. This clear substance was arranged concentrically, like a halo, around individual bacillary bodies (Fig. 1) and resembled the "electron transparent substance" described by Nishiura ([27]).

In addition to well-defined groups of bacilli, the macrophages contained a number of large, round, membrane-bound cytoplasmic vacuoles which measured up to several microns in diameter (Fig. 2). The vacuoles were occupied by a lace-like network of fine membranous trabeculae and contained scattered leprosy bacilli. Irregular small osmiophilic granules and fibrils were also present in some portions of the vacuoles. These vacuolar structures were identical to the leprosy inclusions described by other authors ([5]).

On close examination, the membranous trabeculae of leprosy inclusions were found

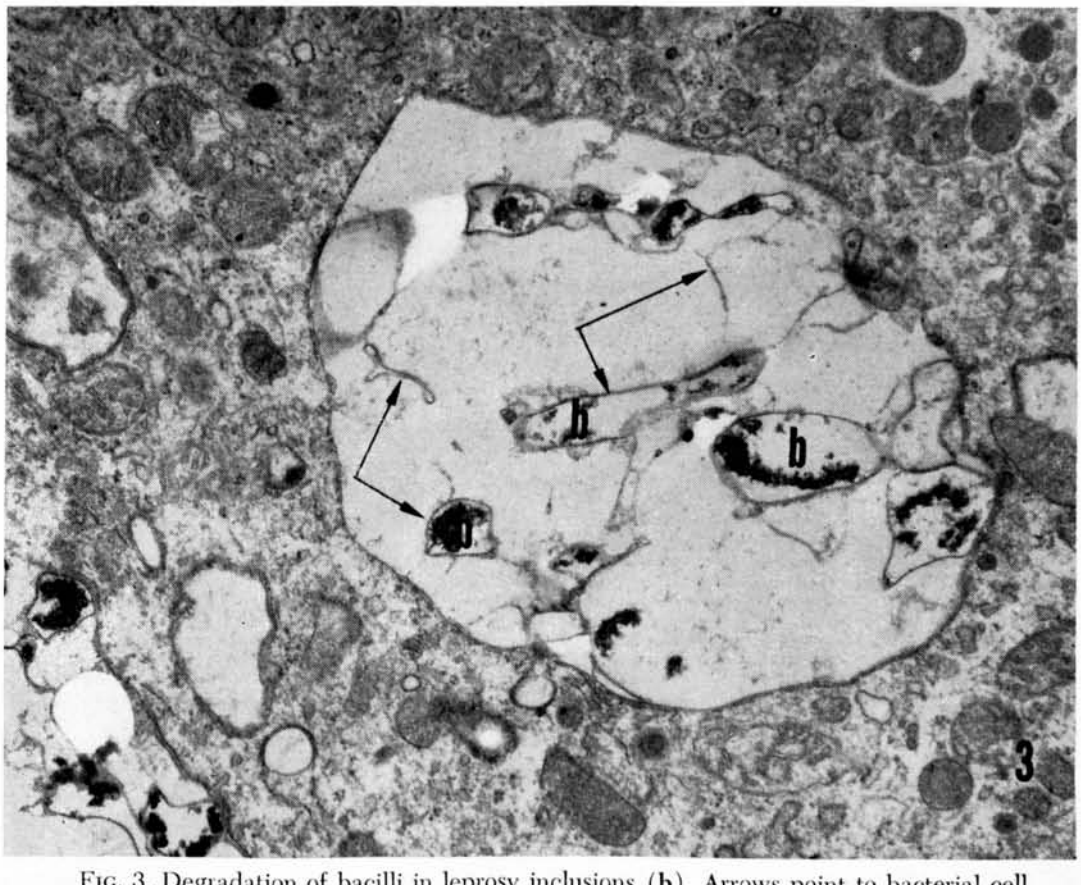

FIG. 3. Degradation of bacilli in leprosy inclusions (**b**). Arrows point to bacterial cell walls. Magnification: X 32,000 Ar.

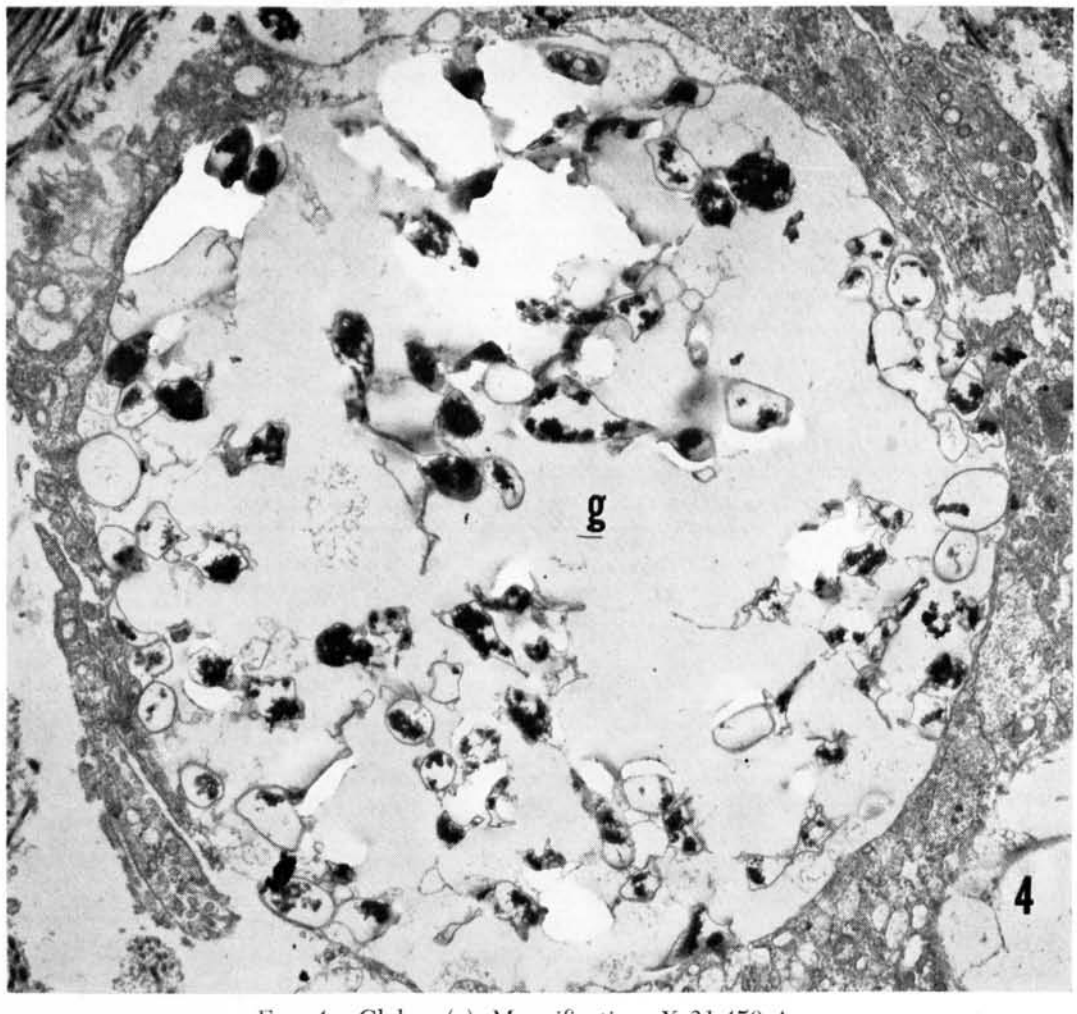

FIG. 4. Globus (g). Magnification: X 21,450 Ar.

to have the same morphologic appearance as the cell walls from adjacent bacilli (Fig. 3). Moreover, transitional stages could be found between fairly well preserved bacilli and groups of membranous trabeculae. Thus, it appears that the so-called inclusions constituted an altered form of phagocytic vacuoles in which the bacilli were in an advanced state of degeneration and appeared extremely distorted. These bacilli had lost all of their cytoplasmic contents and appeared as empty bacterial cell walls which were irregularly disrupted and distended. The space inside the vacuoles was occupied by a homogeneous substance of low electron density. This substance was present around the preserved bacilli and both inside and around the frame of disrupted bacterial cell walls. This substance was identical to the peribacillary electron transparent substance discussed above.

Occasional groups of bacilli attained considerable size and were no longer enclosed within a single macrophage (Fig. 4). Instead, they were surrounded by several adjacent macrophages. These extracellular groups were the equivalent of the "globi" of light microscopic preparations ([12, 25]).

Mitochondria, endoplasmic reticulum cysternae, ribosomes, and Golgi elements were present, in discrete numbers, in the cytoplasm of Virchow cells. Two additional types of cytoplasmic organelles were also observed. One was a round, membrane-bound, dense cytoplasmic body of uniform granular appearance (Figs. 5, 6, 7). The

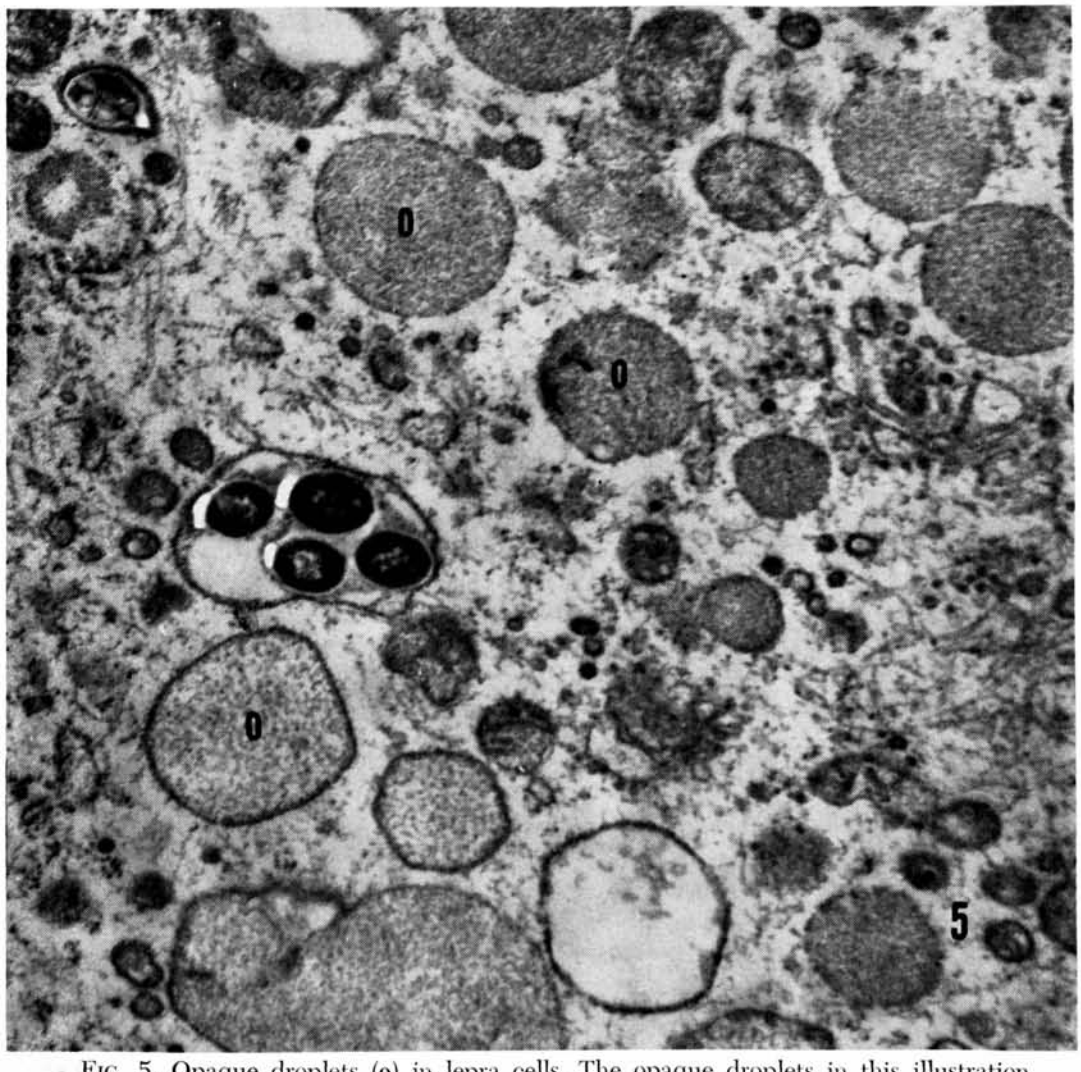

FIG. 5. Opaque droplets (o) in lepra cells. The opaque droplets in this illustration are free in the cell cytoplasm. Magnification: X 31,700 Ar.

other was similarly dense; but instead of being homogeneously granular, it was speckled by groups of small vesicles which measured from 500 to 2,000 Å in diameter (Figs. 6, 7). These organelles corresponded to the previously described opaque droplets and foamy structures and were, in the majority of cases, intimately associated with bacilli. Transitional forms could be found between leprosy inclusions and foamy structures and between foamy structures and opaque droplets. Apart from the transitional forms, occasional opaque droplets were found to be attached to phagocytic vacuoles that contained well preserved leprosy bacilli (Fig. 8). Such opaque droplets seemed to be emptying themselves into the phagocytic vacuoles.

A mixture of opaque droplets, foamy structures, and leprosy inclusions was present in the macrophages of every biopsy; however, each individual biopsy showed its own characteristic blend of these cytoplasmic components. In some cases, the majority of phagocytic cells were filled with clear vacuoles; and the opaque and foamy organelles were only moderately prominent. In other cases, the opaque and foamy bodies predominated, while the clear vacuoles were smaller, less numerous, and globi were lacking. It seemed as if conditions, including the age of the lesion,

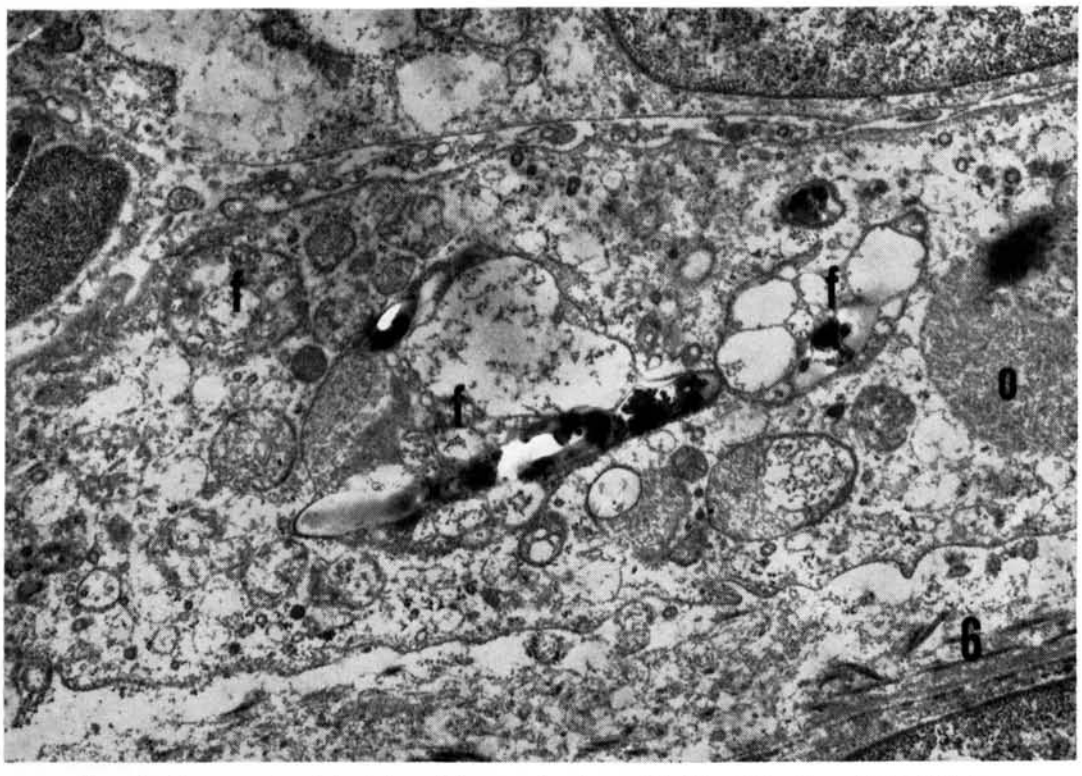

FIG. 6. Opaque droplets (o) and foamy structures (f). Magnification: X 16,800 Ar.

peculiar to each individual case determined the morphologic make-up of the lesions down to the ultrastructural level of the macrophages.

In the acid phosphatase preparations, the enzymatic sites appeared as lead phosphate deposits which were easily distinguished in the electron microscope due to their density (Fig. 9). These deposits were found to be selectively present inside opaque droplets and foamy structures. An additional site of enzyme localization was the vacuolar leprosy inclusions. The pattern of deposition in these vacuoles was especially interesting, since the enzyme was concentrically related to the periphery of the vacuole. In some places where the enzyme appeared to penetrate deeper into the vacuole, evolution into a foamy structure seemed to be taking place. One characteristic of acid phosphatase distribution in foamy structures was that the enzyme was abundantly present in the dense part of the structures but was absent from the clear vesicular spaces within the structures. The foamy pattern was, thus, enhanced by the lead phosphate deposits which stopped abruptly outside the clear vesicular spaces. Control preparations incubated without the substrate showed no deposition of lead phosphate.

DISCUSSION

The development of cytochemical technics to demonstrate acid phosphatase sites in electron microscopic preparation has made it possible to define the lysosomal nature of a variety of subcellular organelles. Dense bodies, multivesicular bodies, phagocytic and pinocytotic vacuoles, structures derived from focal cytoplasmic degredation, and certain pigment deposits have been found to contain acid phosphatase and are included within the general group of lysosomes ([15, 17, 21, 22, 28, 30]). In the present study, the same technics have enabled us to identify three morphologic types of lysosomes in Virchow cells. These are the organelles previously referred to as opaque droplets, foamy structures, and leprosy inclusions ([5, 8, 35]).

Lysosomes are abundantly distributed in

phagocytic cells and play an important role in the digestion of bacteria and other particles engulfed by these cells ([9, 16, 20]). By a combination of electron microscopic, autoradiographic, and cytochemical technics, Cohn *et al.* ([10]) traced the origin of lysosomal granules in macrophages. The enzymes seem to be synthesized by the cell's endoplasmic reticulum and reach the granules probably via the Golgi system of channels and vesicles. Nonenzymatic components of the granules derive from pinocytosis. The use of heavy metal labelling and electron microscopy by Gordon *et al.* ([16]) uncovered the sequential stages in the digestion of foreign particles by macrophages and correlated these stages with the morphology of acid phosphatase containing organelles. As shown by these investigators, the particles are initially contained inside phagocytic vacuoles or phagosomes. Hydrolytic enzymes are soon incorporated into the vacuole that, thus, becomes a phagolysosome. As particles are hydrolyzed, the vacuoles go through a series of morphologic transformations; and finally, by condensation, they become dense bodies or telolysosomes. Undigestible products may remain inside vacuoles as residual bodies. Reutilization of the enzyme reaching the stage of dense body occurs by incorporation of these dense bodies into other phagocytic vacuoles.

The three types of acid phosphatase containing organelles of Virchow cells may be interpreted as representing different stages in the interaction between leprosy bacilli and macrophages. This interpretation is based on their morphologic and histochemical similarity with the organelles described in the previous paragraph. Thus, the bacteria-laden vacuoles are phagosomes or early phagolysosomes in which the bacilli show a fair degree of preservation of their integrity. Leprosy inclusions and perhaps globi, on the other hand, represent late phagolysosomes in which the bacilli have undergone degeneration and have become surrounded by large amounts of an electron transparent substance.

The occurrence of bacterial degeneration in leprosy inclusions and in globi deserves special consideration. Large numbers of intracellular bacteria in lepromatous lesions indicate that the host is not capable of killing or checking the multiplication of leprosy bacilli. Another indication of the inefficiency of the host's antibacterial mechanism, as shown in the present study, is the inability of lysosomal enzymes to penetrate beyond the periphery of leprosy inclusions, into the electron transparent substance in which the microorganisms are

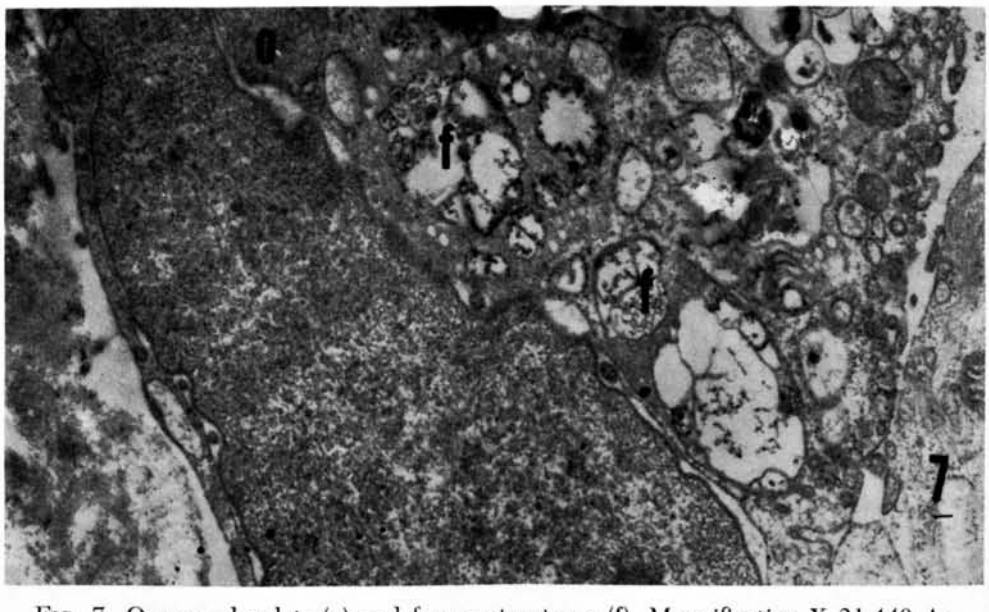

FIG. 7. Opaque droplets (o) and foamy structures (f). Magnification X 21,440 Ar.

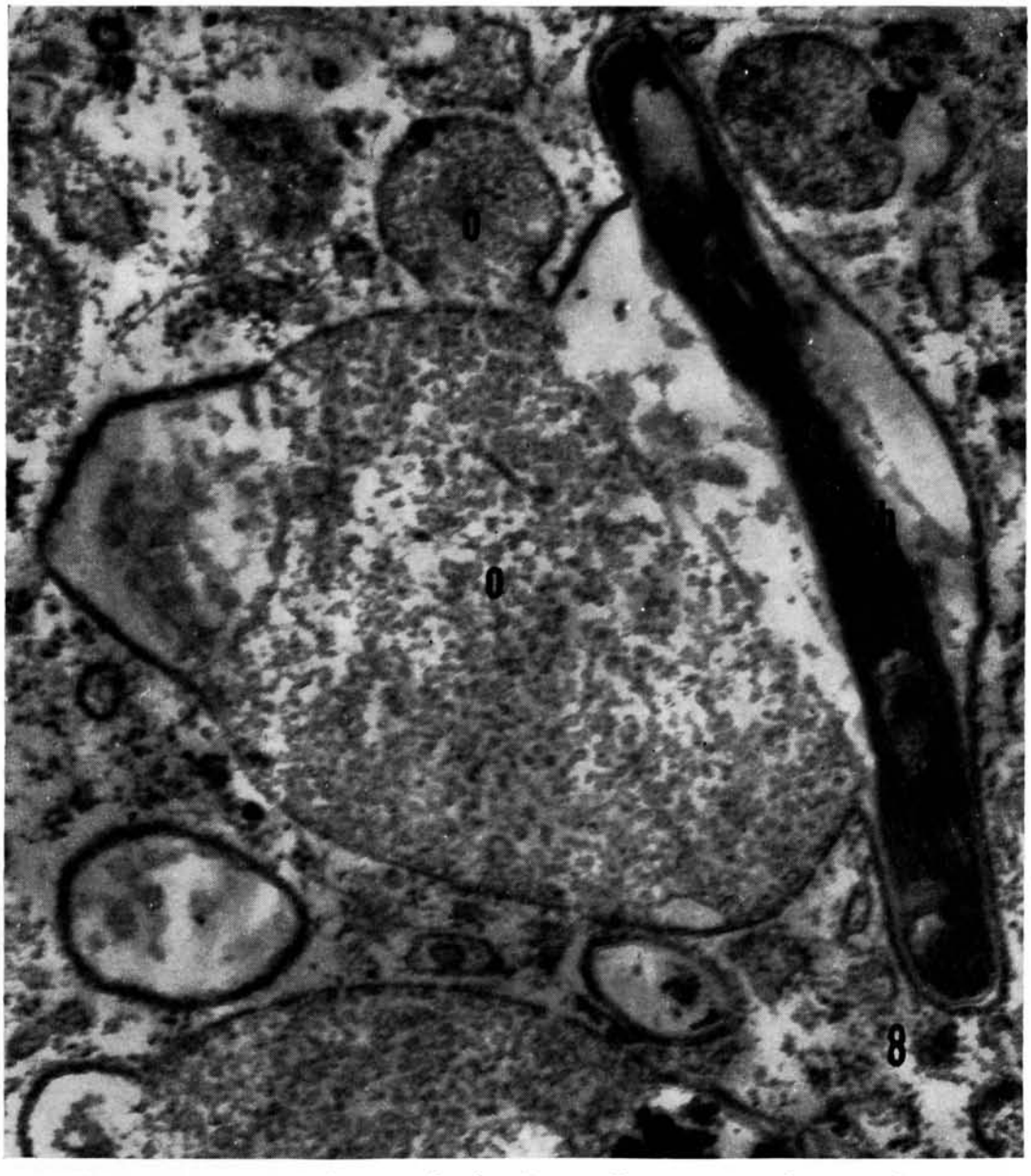

FIG. 8. Incorporation of opaque droplets (**o**) into phagocytic vacuoles. Magnification: X 80,000 Ar. (**b**) leprosy bacilli.

embedded. This suggests that the degeneration of bacteria in leprosy inclusions is not the result of digestion by the host's enzyme, but is due to some other mechanism. The studies of Rees [32] on *M. leprae* inoculation into the foot pads of mice give clues on an alternative mechanism. Rees showed a numerical limit to the multiplication of *M. leprae* in the foot pads. When an inoculum of less than 10^6 bacteria was used, there was progressive multiplication until the total bacterial count per pad was 10^6. This same end point was reached regardless of the size of the inoculum. On the other hand, when an inoculum of 10^6 or more bacteria was used, no multiplication took place. It seems possible that a part of the mechanism that causes degeneration of *M. leprae* in Virchow cells and which limits its multiplication in mouse foot pads is an

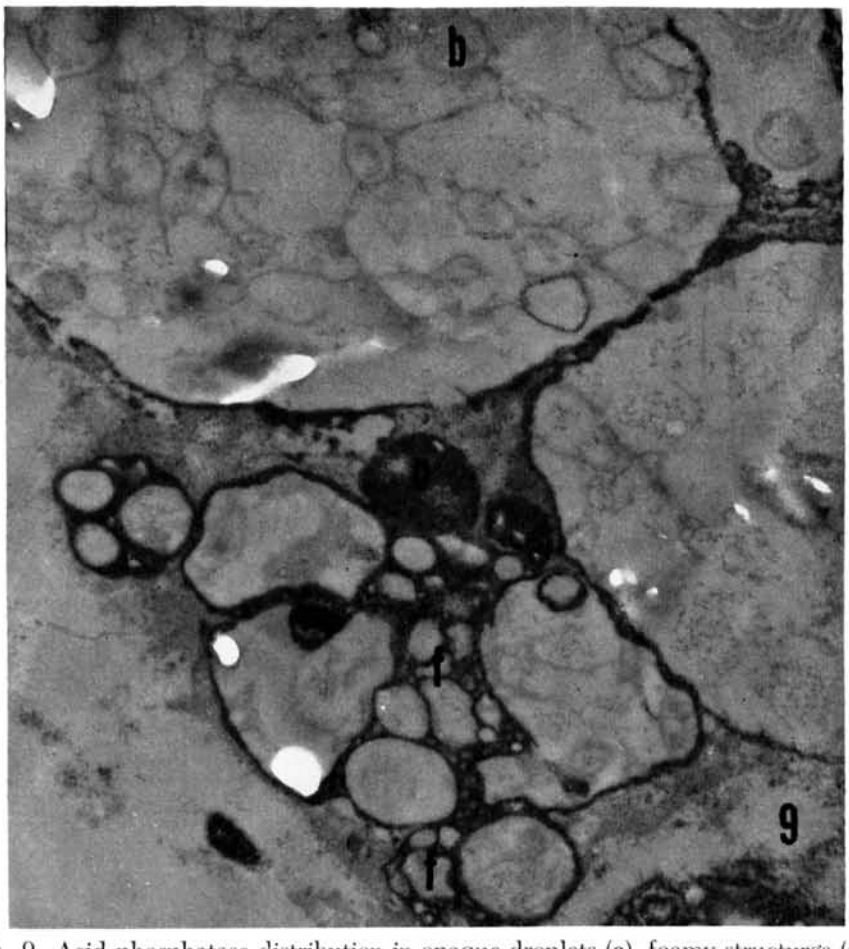

FIG. 9. Acid phosphatase distribution in opaque droplets (**o**), foamy structures (**f**) and leprosy inclusions (**b**). Magnification: X 36,450 Ar.

imbalance between the magnitude of a growing bacterial population and its limited nutritional supply. In other words, the bacilli are affected by a sort of starvation rather than by the direct antibacterial action of the host. Another possible mechanism has been proposed by Nishiura ([27]) who suggests that the peribacillary electron transparent substance interferes with the metabolic activity of the bacilli and affects their viability.

Another important question raised by electron microscopic studies of lepra cells is that of the nature of the electron transparent substance that surrounds the bacilli. Nishiura ([27]) indicated that the peribacillary electron transparent zones were "intimately related to the metabolism of the bacilli in the cytoplasm of lepra cells." Hanks ([19]) subsequently suggested that this clear zone or halo represented a bacterial capsule. Hanks ([18]) also demonstrated a clear peribacillary halo around leprosy bacilli negatively stained with nigrosin dyes.

The present investigation reveals a well defined tendency for acid phosphatase to remain at the periphery of the leprosy inclusion and a lack of penetration into the electron transparent substance or into the clear vesicular areas of foamy structures. This finding suggests that the electron transparent substance is nonaqueous matter, most likely of bacterial origin rather than of host cell origin. It would seem probable that an aqueous medium would allow the water soluble enzyme to diffuse freely and to be randomly found in all portions of the leprosy inclusion or of the foamy structure.

The abundant lipid components of mycobacterial cell walls offer a likely explanation for the nonaqueous electron transparent substance that surrounds leprosy bacilli. The lack of electron density of this material does not argue against a lipid composition since marked variation in osmiophilia and electron density occurs between different lipid compounds ([3, 23, 34]). Noteworthy is the resemblance between the clear lipid inclusions of Gaucher cells and the leprosy inclusions. In both cases, large cytoplasmic vacuoles are occupied by electron transparent substances ([14]). The types of lipids in Gaucher cells are no doubt different from those found in Virchow cells. However, they seemingly exemplify intracellular lipid deposits which are not opaque to the electron beam. The above considerations are consistent with an interpretation of the electron transparent substance of leprosy inclusions and globi as representing a bacterial capsule or as being derived from capsular material. Moreover, of all the components of lepra cells, this substance seems to be the only one that corresponds in amount and distribution to the "slimy wax-like substance" or "gloea" that has been repeatedly observed by leprosy investigators throughout the years ([12, 26]).

The two other types of acid phosphatase containing bodies in Virchow cells, namely opaque droplets and foamy structures, can also be interpreted within the framework of lysosomal physiology. Opaque droplets can be found free in the cytoplasm of lepra cells, in which case they are obviously identical to the dense bodies or telolysosomes discussed by Gordon *et al* ([16]). These organelles represent end products of phagocytosis, focal cytoplasmic degradation or pinocytosis and no doubt may also result from the digestion of some leprosy bacilli. Another type of opaque droplet is found inside the phagocytic vacuoles that contain leprosy bacilli. The distribution of acid phosphatase in these droplets coincides with that of the electron dense material. We have found evidence suggesting that the density of peribacillary opaque droplets is not only due to electron opaque enzymatic protein but is also due to the presence of electron dense partly digested substances within the phagocytic vacuoles ([2]).

Foamy structures seem to originate from the interaction between opaque droplets and the electron transparent substance that surrounds the bacilli. We cannot agree with the view of Yamamoto ([35]) and others ([7]) that foamy structures are derived from the enzymatic digestion of opaque droplets. If that were the case, acid phosphatase would be expected to fill up the clear vesicular spaces of foamy structures. The presently observed segregation of acid phosphatase outside those vesicular spaces suggests that the electron transparent substance and the opaque droplets form independently and that under some circumstances, the enzyme-rich electron opaque material permeates the electron transparent substance to cause the development of foamy structures.

The above considerations suggest the formulation of the following structural pathway in the degradation of phagocytized leprosy bacilli in Virchow cells. The microorganisms are initially engulfed by lepromatous macrophages and remain unaltered inside phagocytic vacuoles where they are able to multiply. As multiplication progresses, increasing amounts of electron transparent substance are laid down around the bacilli. In the macrophages of some patients, the presence of bacilli induces only slight production of lysosomal enzymes. In other cases, however, larger amounts of enzymes are produced resulting in the development of peribacillary opaque droplets. The amount of enzyme produced is a function of the capacity of the host macrophage to respond to the intracellular presence of leprosy bacilli. Lysosomal enzymes are kept segregated to the periphery of the phagocytic vaculole by the impenetrable electron transparent substance. In time, the bacilli outgrow their nutritional supply (or are smothered by the electron transparent substance) and undergo degeneration, transforming the phagocytic vacuoles into leprosy inclusions and globi. As a result of the degenerative process, the electron transparent substance becomes slowly more and more permeable and is penetrated by the hydrolytic enzymes. As penetration progresses, the inner contents of the inclu-

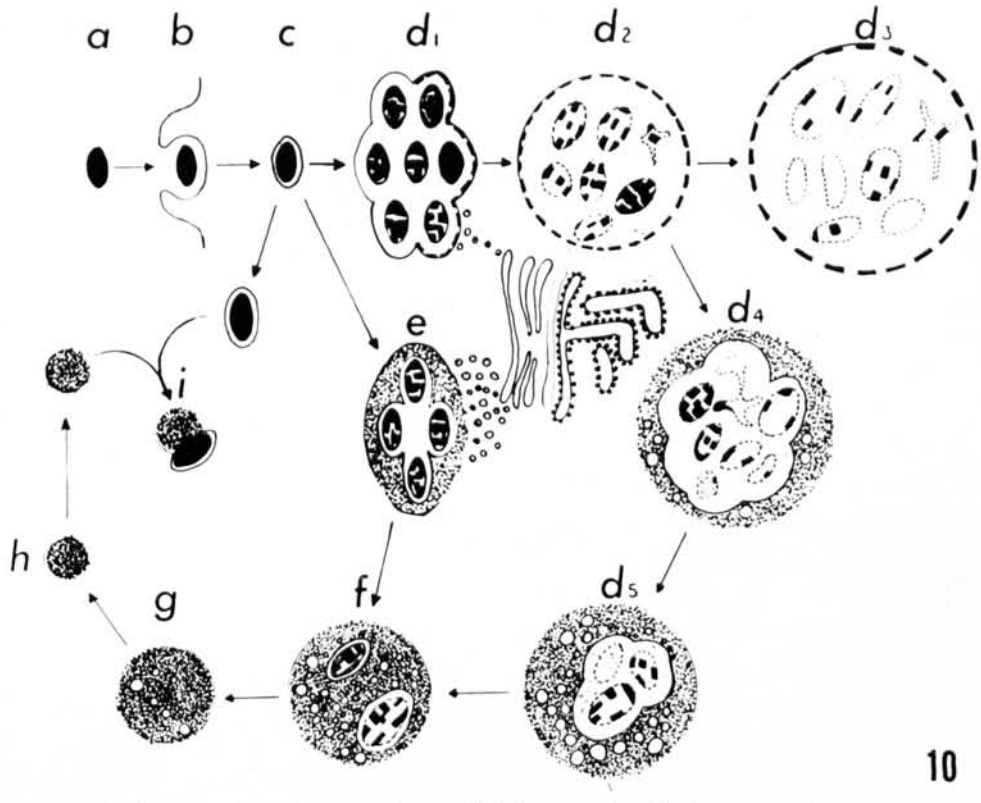

FIG. 10. Structural pathways of engulfed leprosy bacilli in macrophages. **a** to **c**—Engulfment of bacilli. d_1 to d_3—Multiplication and degeneration of engulfed bacilli to form globi. **c-e-f** and d_2-d_4-d_5-**f**—Pathways in the formation of foamy structures. **f-g-h**—Evolution of foamy structures to form free cytoplasmic opaque droplets. **i**—Incorporation of opaque droplets into phagocytic vacuoles.

sions are reached and digested. The chemical—or antigenic—composition of these contents induces the synthesis of additional lysosomal enzyme. The interaction between the partly degraded electron transparent substance and the increased amounts of electron opaque-enzyme-rich material results in the development of foamy structures. Round dense bodies appear in the cytoplasm when the digestive process is carried to completion. This proposed pathway is schematically summarized in the diagram in Figure 10.

SUMMARY

Biopsies from skin lesions of lepromatous leprosy were studied by the electron microscope. A cytochemical method was used to determine the ultrastructural localization of acid phosphatase. A structural pathway for the fate of phagocytized leprosy bacilli was formulated. The following conclusions were reached:

1. Three special morphologic types of lysosomes are present in Virchow cells. They are the organelles previously referred to as opaque droplets, foamy structures, and leprosy inclusions.

2. The degeneration of bacilli in globi and in lepromatous inclusions is not due in major part to enzymatic digestion but is primarily due to the inadequate nutrition of bacilli after they multiply in large numbers inside the phagocytic vacuoles and other possible inherent mechanisms of bacillary death.

3. Peribacillary opaque droplets are accumulations of lysosomal enzymes derived from the endoplasmic reticulum and the Golgi. Some of this material is nonenzymot-

ic and is probably derived from other sources including the digestion of some leprosy bacilli.

4. Free cytoplasmic opaque droplets are the end stage of digestion of leprosy bacilli in the macrophage. They are the equivalent of the so-called dense bodies or telolysosomes.

5. The electron transparent substance in the inclusions, globi, and foamy structures is a nonaqueous product of bacterial origin. This substance is related to the bacterial capsules. It is, most likely, of lipid nature.

RESUMEN

Se estudiaron biopsias de lesiones cutáneas de lepra lepromatosa por medio del microscopio electrónico. Se utilizó un método citoquímico para determinar la localización ultraestructural de la fosfatasa ácida. Se explicó el destino estructural de los bacilos una vez fagocitados. Se llegó a las siguientes conclusiones:

1. En las células de Virchow se encuentran tres tipos morfológicos especiales de lisosomas. Ellos son los organelos a los cuales se han referido previamente como gotas opacas, estructuras espumosas e inclusiones leprosas.

2. La degeneración de los bacilos en globis y en inclusiones lepromatosas no se deben en mayor parte a digestión enzimática, sino que se debe principalmente a la inadecuada nutrición de los bacilos después que se multiplican en grandes cantidades dentro de las vacuolas fagocíticas y otros mecanismos inherentes de muerte bacilar posibles.

3. Las gotas opacas peribacilares son acumulaciones de enzimas lisosomiales derivadas del retículo endoplásmico y el retículo de Golgi. Parte de este material es no enzimático y probablemente se derive de distintas fuentes, incluyendo la digestión de algunos de los bacilos de lepra.

4. Las gotas opacas libres que se encuentran en el citoplasma son la fase terminal de la digestión de los bacilos de lepra dentro de los macrófagos. Son los equivalentes de los llamados cuerpos densos o telolisosomas.

5. La sustancia transparente a los electrones que se encuentra en las inclusiones, globis y estructuras espumosas es un producto no-acuoso de origen bacteriano. Esta sustancia está relacionada con las cápsulas bacterianas. Es, casi seguramente, de naturaleza lipídica.

RÉSUMÉ

Des biopsies de lésions cutanées de lèpre lépromateuse ont été étudiées par la microscopie électronique. Une méthode cytochimique a été utilisée pour déterminer la localisation ultra-structurale des phosphatase acides. Le cheminement structural des bacilles de lèpre phagocytés a été tracé. On est arrivé aux conclusions suivantes:

1. Trois types morphologiques particuliers de lysosomes sont présents dans les cellules de Virchow. Ce sont: les organelles désignées antérieurement sous le terme de gouttelettes opaques, les structures spumeuses, et les inclusions lépreuses.

2. La dégénérescence des bacilles dans les globi et dans les inclusions lépromateuses n'est pas due principalement à la digestion enzymatique, mais bien primitivement à une nutrition inadéquate des bacilles après qu'ils se soient multipliés en grand nombre à l'intérieur des vacuoles phagocytaires, ainsi qu'à d'autres mécanismes inhérents qui peuvent éventuellement intervenir dans la mort bacillaire.

3. Les gouttelettes opaques péribacillaires sont constituées par des accumulations d'enzymes des lysosomes, dérivés du reticulum endoplasmique et de l'appareil de Golgi. Une partie de ce matériel n'est pas enzymatique. étant probablement dérivé d'autres sources parmi lesquelles la digestion de quelques bacilles de la lèpre.

4. Les gouttelettes opaques cytoplasmiques libres constituent le stade terminal de digestion des bacilles de la lèpre dans le macrophage. Ils sont les équivalents de ce qu'on appelle les corps denses ou télolysosomes.

5. La substance transparente aux électrons, que l'on trouve dans les inclusions, dans les globi et dans les structures spumeuses, est un produit non-aqueux d'origine bactérienne. Cette substance est en relation avec les capsules bactériennes. Il est trés vraisemblable qu'elle est de nature lipidique.

REFERENCES

1. Allen, J. M. Brieger, E. M. and Rees, J. W. Electron microscopy of host-cell parasite relation in murine leprosy. J. Path. & Bact. **89** (1965) 301-306.
2. Aquino, T. I. Thesis for the degree of Doctor of Philosophy, Department of Pathology, University of Chicago, 1967.
3. Bahr, G. F. Continued studies about the fixation with osmium tetroxide electron stain IV. Exper. Cell Res. **9** (1955) 277-285.
4. Bennett, H. S. and Luft, J. H. S-collidine as a basis of buffering fixatives. J. Biophys. Biochem. Cytol. **6** (1959) 113-114.
5. Brieger, E. M. The life history of the

lepra cell. Transactions Leonard Wood Memorial-Johns Hopkins University Symposium on Research in Leprosy. Baltimore, Maryland; 8-10 May 1961, pp. 139-148.

6. Brieger, E. M. and Allen, J. M. Cytopathological changes in lepra cells. Exper. Cell Res. **28** (1962) 438-440.
7. Brieger, E. M. and Allen, J. M. The submicroscopical structure of *M. leprae* and the cell of Virchow (lepra cell). *In:* Leprosy in Theory and Practice. Cochrane, R. G. and Davey, T. F., Eds. Bristol, John Wright & Sons Ltd.; Baltimore, Williams & Wilkins, 2nd ed., 1964, pp 36-40.
8. Brieger, E. M. and Glauert, A. M. Electronmicroscopy of the leprosy bacillus. A study of submicroscopic structure. Tubercle **37** (1956) 195-206.
9. Cohn, Z. A. The fate of bacteria within phagocytic cells. Parts I, II and III. J. Exper. Med. **117** (1963) 27-42 and 43-53; **120** (1964) 869-883.
10. Cohn, Z. A., Fedorko, M. E. and Hirsch J. G. The *in vitro* differentiation of mononuclear phagocytes. Part V. J. Exper. Med. **123** (1966) 757-766.
11. Cohn, Z. A., Hirsch, J. G. and Fedorko, M. E. The *in vitro* differentiation of mononuclear phagocytes. Part IV. J. Exper. Med. **123** (1966) 747-756.
12. Cowdry, E. V. Cytological studies on globi in leprosy. American J. Path. **16** (1940) 103-135.
13. DeDuve, C. Pressman, B. C., Gianetto, R., Wattiaux, R. and Applemans, F. Intracellular distribution patterns of enzymes in rat liver tissue. Biochem. J. **60** (1955) 604-617.
14. DeMarsch, Q. B. and Kautz, J. The submicroscopic morphology of Gaucher cells. Blood **12** (1957) 324-335.
15. Essner, E. An electron microscopic study of erythrophagocytosis. J. Biophys. Biochem. Cytol. **7** (1960) 329-334.
16. Gordon, G. B. Miller, L. R. and Bensch, K. G. Studies on the intracellular digestive process in mammalian tissue culture cells. J. Cell. Biol. **25** (1965) 41-45.
17. Gordon, G. B., Miller, L. R. and Bensch, K. G. Fixation of tissue culture cells for ultrastructural cytochemistry. Exper. Cell Res. **31** (1963) 440-443.
18. Hanks, J. H. Significance of capsular components of *Mycobacterium leprae* and other mycobacteria. Internat. J. Leprosy **29** (1961) 74-83.
19. Hanks, J. H. Capsules in electron micrographs of *Mycobacterium leprae*. Internat. J. Leprosy **29** (1961) 84-87.
20. Hirsch, J. G. Cinemicrophotographic observations on granule lysis in polymorphonuclear leukocytes during phagocytosis. J. Exper. Med. **116** (1962) 827-834.
21. Holt, S. J. Factors governing the validity of staining methods for enzymes and their bearing upon the Gomori acid phosphatase technique. Exper. Cell Res. **7** (1959) 1-27. (Suppl.)
22. Hruban, Z., Spargo, B., Swift, H., Wissler, R. W. and Kleinfeld, R. G. Focal cytoplasmic degradation. American J. Path. **42** (1963) 657-683.
23. Imaeda, T. Electron microscopic analysis of the components of lepra cells. Internat. J. Leprosy **28** (1960) 22-28.
24. Imaeda, T. Electron microscopy approach to leprosy research. Internat. J. Leprosy **33** (1965) 669-683.
25. Khanolkar, V. R. Pathology of leprosy. *In:* Leprosy in Theory and Practice. Cochrane, R. G. and Davey, T. F., Eds. Bristol, John Wright & Sons Ltd.; Baltimore, Williams & Wilkins, 2nd ed., 1964, pp. 125-151.
26. Neisser, A. Ueber die Struktur de Le ra- und Tuberkelbacillen mit spezie ler Berucksichtigung der Rosanilin-und Pa rosanilinfarbstoffe und über Leprazelle Verb. d.d. Derm. Gesellschaft. **1** (1889 29-56.
27. Nishiura, M. The electron microscopic basis of the pathology of leprosy. Internat. J. Leprosy **28** (1960) 357-400.
28. Novikoff, A. B. Lysosomes and related particles. *In:* The Cell. Brachet, J. and Mirsky, A. E., Eds. New York, Academic Press Inc., Vol. II, 1961, pp. 423-488.
29. Novikoff, A. B. Lysosomes in the physiology and pathology of cells. Contribution of staining methods. Ciba Foundation Symposium on Lysosomes. Boston, Little, Brown & Co., 1963, pp. 36-73.
30. Novikoff, A. B. and Essner, E. The liver cell. American J. Med. **29** (1960) 102-131.
31. Novikoff, A. B. and Essner, E. Cytolysomes and mitochondrial degeneration J. Cell Biol. **15** (1962) 140-146.
32. Rees, R. J. W. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse foot pads. Internat. J. Leprosy **33** (1965) 646-655. Part 2
33. Reynolds, E. S. The use of lead citrate

at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17** (1963) 204-212.

34. Stoeckenius, W. and Mahr, S. C. Studies on the reaction of osmium tetroxide with lipids and related compounds. Lab. Invest. **14** (1965) 1196-1207.
35. Yamamoto, T., Nishimura, M., Harada, N. and Imaeda, T. Electron microscopy of ultrathin sections of lepra cells and *Mycobacterium leprae*. Internat. J. Leprosy **26** (1958) 1-18.