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A Light and Electron Microscopic Study of Peripheral Nerves in an Armadillo with Disseminated Leprosy¹

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Recently, Kirchheimer and Storrs^(8,9) and Kirchheimer *et al*⁽¹⁰⁾ have shown that some experimentally infected armadillos (*Dasypus novemcinctus* Linn.) are highly susceptible to *Mycobacterium leprae* and develop the disseminated form of the infection. The close resemblance to human lepromatous leprosy has been well established in their reports.

The present communication describes the light and electron microscopic findings in the peripheral nerves of an experimentally infected armadillo with lepromatous leprosy. It is concluded that neural involvement in the armadillo bears a close resemblance to human leprous neuritis of the lepromatous type, thus strengthening the case for developing the nine-banded armadillo into a model for the study of the human disease.

MATERIALS AND METHODS

Armadillo number 34-C, a mature⁴ female, was inoculated 16 March 1972 in the skin of the lower abdomen with 2.0×10^8 viable leprosy bacilli (mouse foot pad multiplication) obtained from a leproma of a leprous nine-banded armadillo. These bacteria had been identified as *M. leprae* by their failure to grow on mycobacterial culture media, their pattern of multiplication in mouse foot pads and by positive dopa-oxidase reaction^(6,13).

The animal was kept under the usual conditions employed at Carville at an ambient temperature of 24°C and was daily fed a diet consisting of one raw egg, Purina cat chow soaked in water, ground meat, bone meal, raw liver, a cupful of mud, and water *ad libitum*.

On 13 June 1972, a walnut-sized subcutaneous swelling which gradually had developed on the left thigh was biopsied with a 6 mm skin punch. Histologically and bacteriologically this was shown to be a leproma⁽⁷⁾.

Approximately 190 days after infecting this armadillo, an upper respiratory tract disorder became apparent. At that time numerous acid-fast bacilli were present in smears made from its voluminous nasal discharge. The animal was euthanized 196 days after infection and autopsied at once.

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⁴Captured in wild, exact age unknown.

TABLE I. Numbers of *M. leprae* per gm of tissue.

Tissue	Number of <i>M. leprae</i>
Skin at site of inoculation	$3.6 \pm 0.7 \times 10^8$ (15) ^a
Skin (uninoculated)	$9.5 \pm 0.1 \times 10^7$ (16)
Lymph node (inguinal)	$1.9 \pm 0.3 \times 10^{10}$ (18)
Leproma (left thigh)	$1.5 \pm 2 \times 10^{10}$ (21)
Liver	$1.4 \pm 1 \times 10^9$ (21)
Spleen	$3.5 \pm 0.3 \times 10^8$ (12)

^aNumbers in parentheses are percentage of solidly staining rods (Morphologic Index).

Peripheral nerve specimens were obtained at autopsy of the armadillo from two levels of the ulnar nerve, the median nerve at the forepaw, three levels of the peroneal nerve, and two levels of the posterior tibial nerve, and were fixed in 3% glutaraldehyde in 0.05M phosphate buffer at pH 7.4 for two hours. The nerve specimens were subsequently post-fixed in 2% osmium tetroxide in 0.1M phosphate buffer, dehydrated, and embedded in epoxy plastic blocks. Thick sections of three microns were cut using a glass knife, and stained with Paragon⁵ stain

⁵Paragon multiplex stain for frozen sections. Paragon C. & C. Co., Inc., 190 Willow Avenue, Bronx, New York 10454.

for viewing with light microscopy. Ultrathin sections of 600 Å to 700 Å were cut with a diamond knife, stained with 10% uranyl acetate in methanol for four minutes and 0.2% lead citrate solution for one minute. Finally, the stained grids were viewed with a Phillips 300 electron microscope.

RESULTS

General pathology. There were no grossly visible lesions on the body or in the internal organs (aside from the walnut-sized leproma on the left thigh). The histopathologic findings were very similar to those described by Kirchheimer *et al* (10) and must have resulted from a vascular seeding. There was

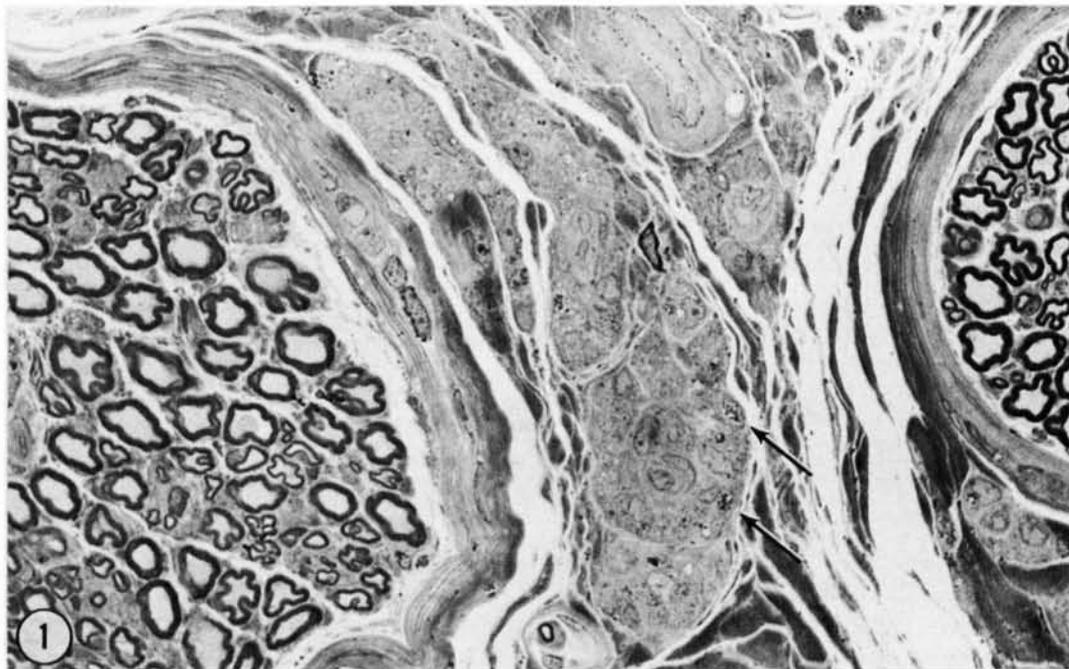


FIG. 1. Light microscopic view of median nerve from the forepaw of the armadillo showing epineurial blood vessels between two nerve fascicles. There are clusters of macrophages containing darkly staining bacilli (arrows) in a perivenular distribution. Paragon stain (600X).⁶

⁶Magnifications of figures are given as of the original.

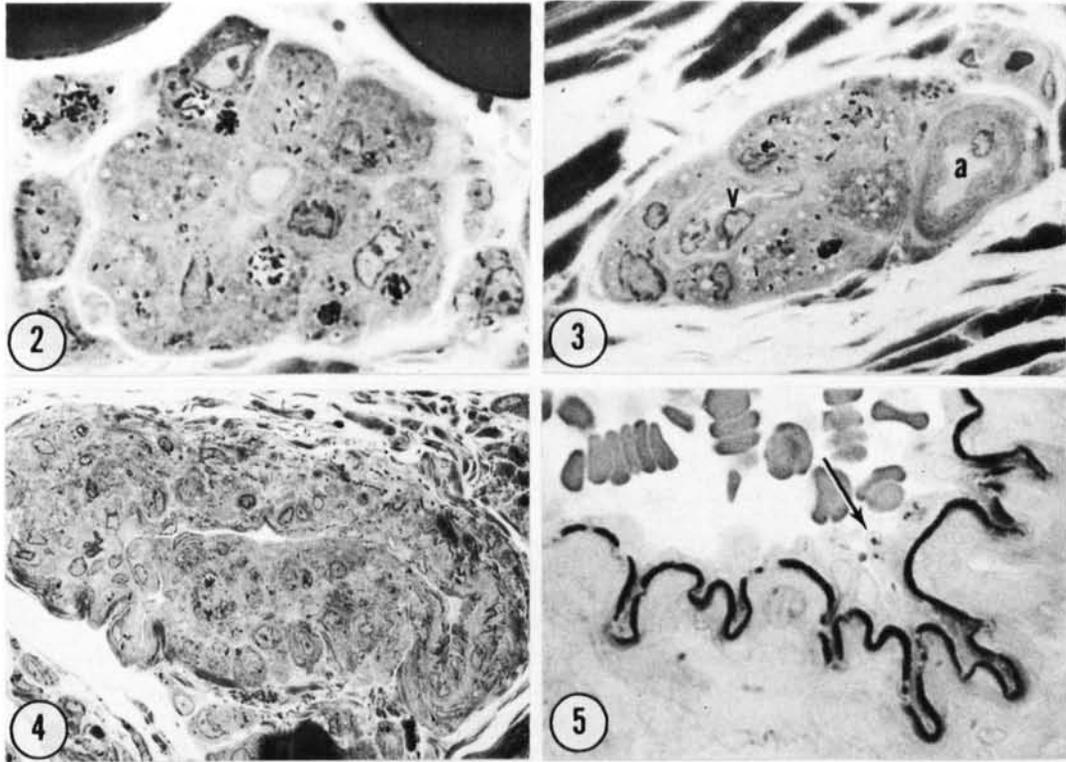


FIG. 2. High power view of an epineurial capillary in median nerve surrounded by macrophages filled with darkly staining bacilli in globi. Paragon stain (900X).

FIG. 3. High power view of an arteriole (a) adjacent to a venule (v). There is marked clustering of bacilli-filled macrophages around the venule and the relative absence of clustering around the arteriole. Paragon stain (900X).

FIG. 4. Epineurial artery in median nerve. Macrophages filled with bacilli have infiltrated the adventitia as well as the media of the artery. There is preservation of some remnants of elastica within the right side of the vessel wall. Paragon stain (750X).

FIG. 5. High power view of a portion of a medium sized artery with red blood cells in the lumen. The endothelial cells of the vessels internal to the thick elastic membrane harbor aggregates of bacilli (arrow). Paragon stain (1,200X).

massive invasion of lymph nodes, spleen, liver and skin remote from the site of inoculation with *M. leprae*. In peripheral nerves one could see, with the light microscope, macrophages stuffed with acid-fast bacteria mainly in the connective tissue between the nerve fascicles. Acid-fast bacilli were also present in peripheral blood macrophages. There were accumulations of macrophages containing enormous numbers of acid-fast rods in the nasal mucosa. There also were accumulations of macrophages with acid-fast bacteria in the mucosa of the larynx. Although there were no grossly recognizable lung lesions, microscopically the animal had a widespread macrophagic pneumonitis with numerous acid-fast bacilli in these macro-

phages. The bacteria recovered from the organs were dopa-oxidase positive, which identifies them as *M. leprae* (6,13).

As previously noted (8,9,10), the number of leprosy bacilli in the various tissues was much greater than that usually found in human lepromatous leprosy. Data concerning bacterial number per gram of the respective tissues removed at autopsy are shown in Table 1. The viability of these bacteria is now being assessed by their ability to multiply in mouse foot pads.

Light microscopy of nerve. Only the median nerve as it entered the forepaw was found to be involved. The nerve trunk is superficially situated in this location. The most obvious feature was the presence of thick

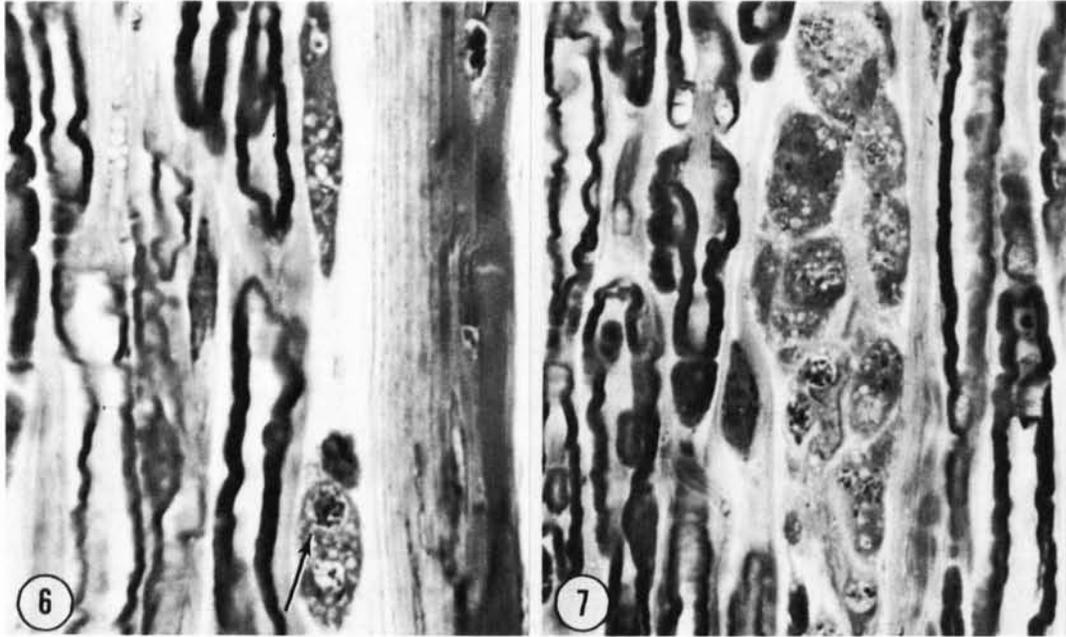


FIG. 6. Longitudinal section of a fascicle from median nerve. Bacilli (arrows) are present within vacuoles in perineurial cells, and within subperineurial, spindle-shaped macrophages or fibroblasts. Paragon stain (1,000X).

FIG. 7. Longitudinal section of a fascicle in median nerve. There are a large number of bacilli-filled macrophages, clustered between myelinated axons. The macrophages are probably surrounded by an endoneurial blood vessel which is not seen in this plane of section. Paragon stain (900X).

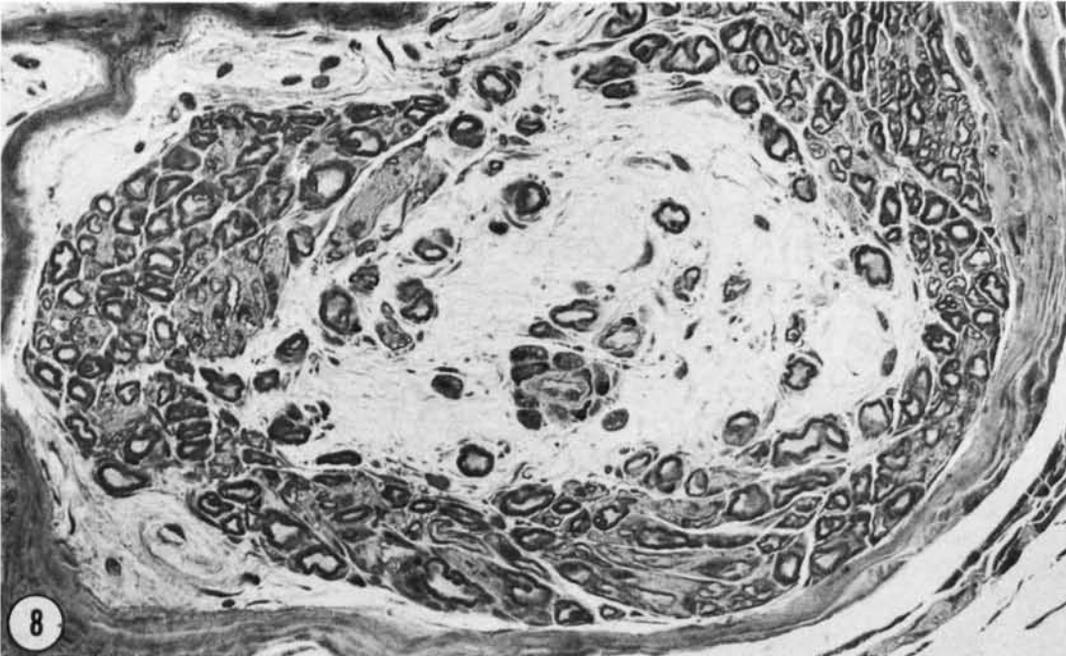


FIG. 8. Transverse section of a whole fascicle of median nerve. A blood vessel surrounded by a few macrophages occupies the center of the fascicle. There is a concentric zone of nerve destruction, fibrosis, with a few scattered axons, around the blood vessel. The peripherally situated axons near the perineurium appear intact. Paragon stain (350X).

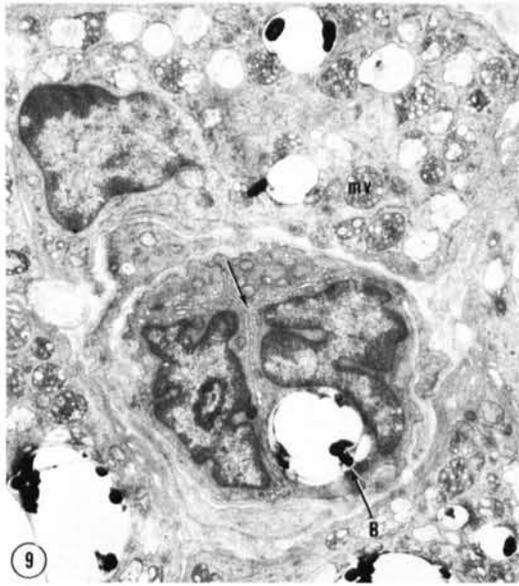


FIG. 9. Electron micrograph of a capillary in the epineurial tissue of the median nerve. The lumen is collapsed (arrow). Bacilli (B) are seen in a vacuole in an endothelial cell, and in the vacuoles within the macrophages surrounding the capillary. The surrounding macrophages lack a basement membrane, but possess numerous filopodia. Macrophage cytoplasm contains mitochondria, darkly staining multivesicular bodies (mv) and lucent secondary lysosomal bodies with and without bacilli. Note: the large transparent holes surrounding bacilli are artifactual. Epon does not appear to "adhere" to the bacilli and forms tears around the bacilli during sectioning as well as during viewing with the electron microscope (6,500X).

cuffs of heavily bacillated macrophages surrounding blood vessels (Figs. 1-8), particularly veins and venules in the epineurium (Figs. 1-3), and endoneurium (Figs. 7, 8). Macrophages were present not only in the perivascular spaces and adventitia but also insinuated themselves within the media as well (Fig. 4). In some small arteries and arterioles, little perivascular macrophage infiltration was evident, but clusters of bacilli were often found in adventitial and endothelial cells (Fig. 5). Capillaries were often heavily cuffed by macrophages containing large clusters of organisms (Figs. 2, 7).

Sharp contrast in the degree of macrophage cuffing between veins and arteries was frequently observed (Fig. 3). A ring of bacilli-laden macrophages was seen surrounding the venule with virtually no macrophage cuffing of the adjacent arteriole. Clusters of bacilli were present within some perineurial cells and subperineurial cells, the latter identified as macrophages or fibroblasts (Fig. 6). Aside from the bacillation of some perineurial cells, the perineurium maintained its integrity (Figs. 1, 6).

Foamy cells were scattered within endoneurial spaces between myelinated nerve fibers. Myelinated fibers appeared to be present in normal numbers in most fascicles (Figs. 1, 7), but in one fascicle a broad zone of central clearing with apparent loss of most nerve fibers was observed (Fig. 8). An endoneurial vessel with a cuff of macro-

phages was centrally placed in this loosely-textured cleared zone, suggesting that nerve fiber destruction had occurred in an ever-widening spread from the vessel. It was not possible to evaluate the state of the unmyelinated nerve fibers by light microscopy.

Electron microscopy of nerve. Examination of the perivascular cuffs of macrophages seen by light microscopy confirmed the presence of large cytoplasmic membrane-bound vacuoles containing bacilli (Figs. 9-12). The cells containing the bacilli-laden vacuoles were easily identifiable as macrophages by their finger-like filopodia, absence of basement membrane, and numerous darkly and lightly staining vacuolated bodies in the cytoplasm, presumably representing lysosomal bodies (Fig. 9). The presence of endothelial cell bacillation seen by light microscopy was confirmed by electron microscopy (Fig. 9).

Schwann cell infestation was frequently encountered in a widespread distribution in many fascicles, but was almost exclusively confined to Schwann cells associated with unmyelinated nerve fibers. Bacilli in varying stages of degeneration were contained along with amorphous debris in vacuoles which at times almost completely replaced the entire Schwann cell cytoplasm (Figs. 11, 12). These vacuoles appeared to displace the unmyelinated axons to the periphery of the Schwann cell. A thin rim of Schwann cell cytoplasm separated the contents of the vacuoles from the axons (Figs. 11, 12). Most unmyelinated axons appeared to be intact.

Only rarely were bacilli found within the Schwann cells of myelinated nerves. Myelinated axons appeared to be intact in the

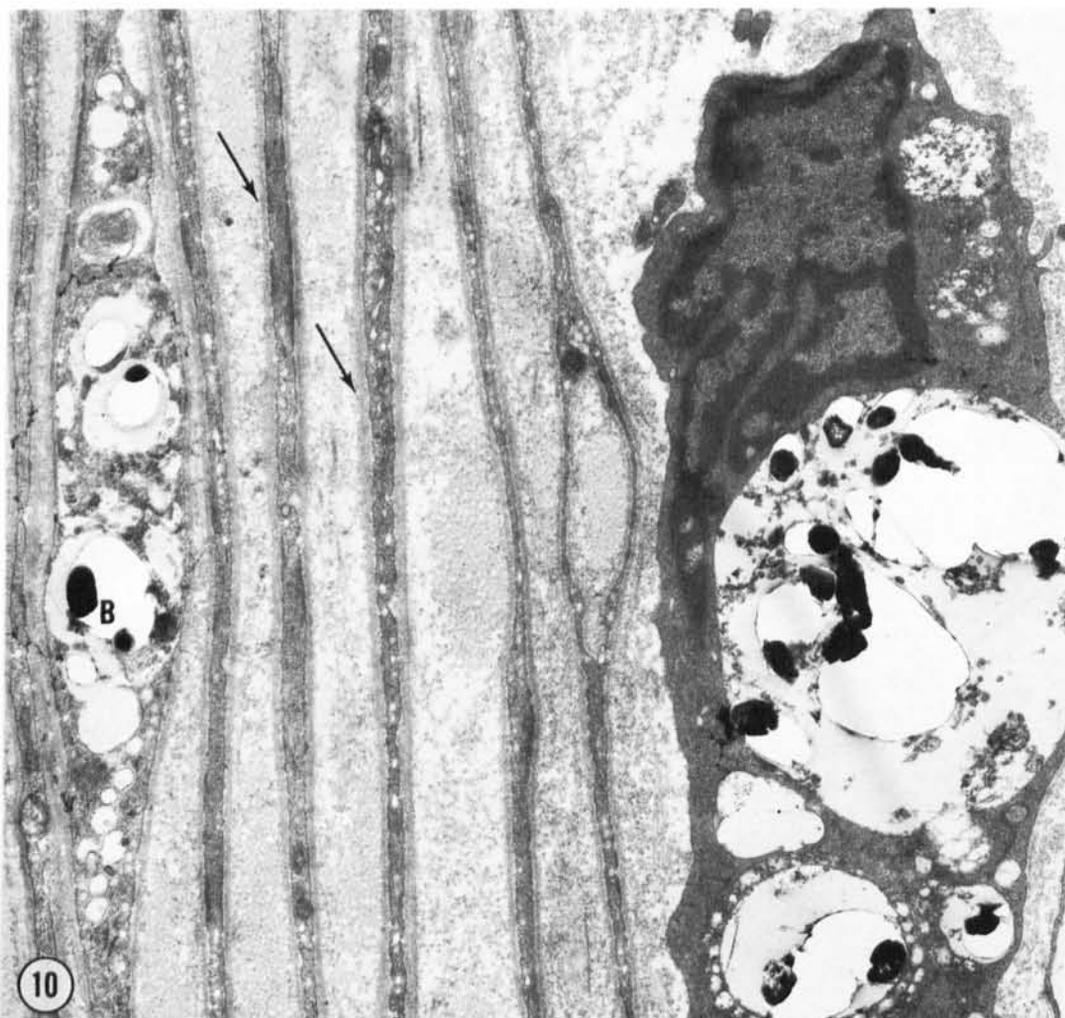


FIG. 10. Electron micrograph of perineurium in median nerve. Perineurial cells have a basement membrane (arrows). Collagen fibers lie between the perineurial cell layers. One perineurial cell is widened and contains cytoplasmic vacuoles with bacilli (B). In the right field a darkly staining macrophage is seen in the subperineurial space. The cytoplasm of the macrophage contains several vacuoles filled with electron lucent material and bacilli. The transparent holes surrounding the bacilli are artifactual (7,500X).

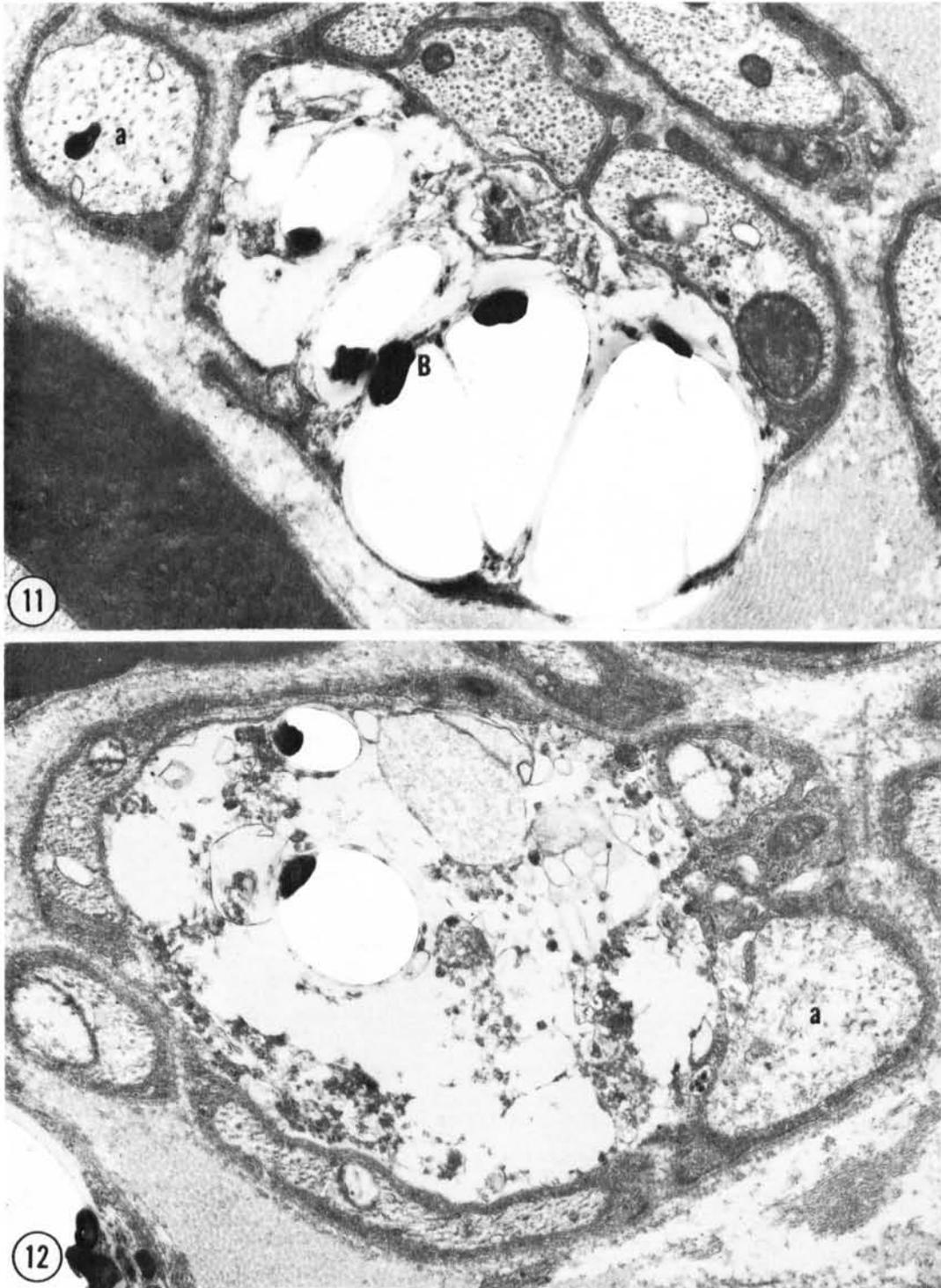
areas of unmyelinated nerve involvement as well as in other areas. Electron micrographs of the area of nerve destruction illustrated in Figure 8 revealed only rare instances of myelinated nerve involvement, and no unequivocal examples of intra-axonal bacillation were observed.

DISCUSSION

Research and experimentation in leprosy in the past has been limited because of the absence of a valid experimental model representative of the human disease. *M. leprae*

still remains to be cultured *in vitro*, and until recently has not been reported to have caused disseminated disease in unaltered experimental animals. The limitations of this type of animal experimental model for the study of human leprosy has been discussed by Kirchheimer and Storrs (8).

In 1971 and 1972, Kirchheimer and Storrs (9) reported disseminated leprosy in some experimentally infected armadillos (*Dasypus novemcinctus*, Linn.) without immunosuppression. *M. leprae* was found in clumps of macrophages in biopsies of the dermis



FIGS. 11 and 12. Electron micrographs of unmyelinated median nerve in transverse section. Schwann cell cytoplasmic processes and basement membrane surround the unmyelinated axons (a). Microtubules, microfilaments and mitochondria are present within the axoplasm. The cytoplasm of the Schwann cell contains large vacuoles with an electron lucent matrix, cellular debris and bacilli (B). A large area of Schwann cell cytoplasm is occupied by the vacuoles. Figure 11 (7,500X); Figure 12 (7,000X).

and dermal peripheral nerves both in inoculated and distant areas. Acid-fast bacilli were found in smears made from the buffy-coat of the blood. Subsequently, Kirchheimer *et al* (¹⁰), reported extensive tissue involvement in other organs and in the peripheral nerves of experimentally infected armadillos with disseminated (lepromatoid) leprosy.

In this study, the distribution of *M. leprae* in the various cellular elements of peripheral nerve from an experimentally infected armadillo closely parallels the distribution of bacilli in peripheral nerve from patients with lepromatous leprosy. In human lepromatous leprosy, the presence of *M. leprae* in endothelial cells, perineurial cells, endoneurial macrophages, Schwann cells of myelinated and unmyelinated axons, in axons, and in macrophages surrounding blood vessels have all been observed by light and electron microscopy, and extensively documented by Khanolkar (⁵), Dastur (¹), Nishiura (¹²), Imaeda (²) and Job (^{3,4}). In our study of the armadillo, *M. leprae* were found in all of the same cellular elements in the experimentally infected armadillo model. Some features seen in the armadillo such as bacillary invasion of the adventitia and media of blood vessels may also occur in human lepromatous leprosy but has not yet been reported. In addition, the nerve destructive lesion seen in Figure 8 has a perivascular topography. This impressive involvement of blood vessels in the armadillo coupled with the observed bacillemia and endothelial cell bacillation strongly suggests a hematogenous spread of the disease.

It is difficult to explain the observation that Schwann cells of unmyelinated nerve are heavily bacillated while Schwann cells associated with myelinated nerve fibers are relatively free of bacilli. Nonetheless, in our experience, early involvement of unmyelinated nerve fibers and their Schwann cells with relative sparing and later involvement of myelinated nerve fibers and their Schwann cells is common to the human form of leprosy. Schwann cells were parasitized with clusters of bacilli which were in varying stages of morphologic integrity in large vacuoles within the cytoplasm. These lesions bear a close morphological resemblance to the lesions seen in the peripheral nerves of humans with lepromatous

leprosy.

Finally, no intra-axonal bacilli were observed by light or electron microscopy in the axons of the armadillo. Axonal bacillation in human lepromatous leprosy has been reported by several investigators (^{2, 11, 12}) and confirmed by our own observations in nerves from patients with lepromatous leprosy (¹⁴). Intra-axonal bacillation probably represents a late stage phenomenon of lepromatous leprosy which provides an explanation for why it was not seen in the relatively acute infection in the armadillo.

In this light and electron microscopy study there is morphologic similarity between nerve lesions in patients with lepromatous leprosy and in the armadillo which has been experimentally infected with *M. leprae*, suggesting that the armadillo is a suitable experimental model for the study of peripheral nerve lesions in lepromatous leprosy. Moreover, the armadillo has the advantage over other animal models proposed for similar purposes in that it does not require immunosuppression to facilitate the dissemination of the disease after inoculation. Further study of this model, particularly in terms of the immunological conditions requisite for dissemination of the disease, will do much to clarify the many arcane features of lepromatous leprosy.

SUMMARY

The lesion of peripheral nerve observed in an armadillo which developed lepromatoid leprosy following experimental infection with *M. leprae* was found to be similar by light and electron microscope examination to the peripheral nerve lesion of human lepromatous leprosy. Bacilli were found primarily within macrophages, endothelial cells, perineurial cells and Schwann cells of unmyelinated fibers. Destruction of nerve tissue appeared to have a perivascular distribution. The pattern of bacillation with predominant involvement of blood vessels suggests hematogenous dissemination of *M. leprae* in the armadillo. These observations taken together constitute evidence that armadillos with the disseminated form of leprosy are suitable models for the study of the neural lesions of human lepromatous leprosy.

RESUMEN

Se encontró que las lesiones de nervios peri-

féricos que presentan los armadillos que desarrollan lepra lepromatosa después de la infección experimental con *M. leprae* eran similares a las lesiones nerviosas periféricas de la lepra lepromatosa humana, al estudio tanto por el microscopio de luz como por el microscopio electrónico. Los bacilos se encontraron principalmente dentro de los macrófagos, células endoteliales, células perineurales y células de Schwann de fibras no mielinizadas. La destrucción del tejido nervioso parecía tener una distribución perivasculosa. El modelo de disposición de los bacilos, con compromiso predominante de los vasos sanguíneos sugiere diseminación hematogena del *M. leprae* en el armadillo. Estas observaciones en conjunto constituyen buena evidencia que los armadillos con forma diseminada de lepra son modelos adecuados para el estudio de las lesiones nerviosas en lepra lepromatosa humana.

RÉSUMÉ

Des études menées au microscope optique et par des méthodes de microscopie électronique ont permis de constater que la lésion des nerfs périphériques observée chez l'armadillo qui développe une lèpre lépromateuse à la suite d'une infection expérimentale par *M. leprae*, est similaire à la lésion nerveuse périphérique observée dans la lèpre lépromateuse humaine. Les bacilles sont trouvés essentiellement à l'intérieur des macrophages, des cellules endothéliales, des cellules périméurales, et des cellules de Schwann des fibres non myélinisées. La destruction du tissu nerveux semble suivre une distribution périsvasculaire. Les profils de distribution des bacilles, marqués par une atteinte prédominante des vaisseaux sanguins, suggèrent une dissémination hématogène de *M. leprae* chez l'armadillo. Ces observations, lorsqu'elles sont reliées entre elles, montrent que les armadillos atteints d'une forme disséminée de lèpre constituent des modèles appropriés pour l'étude des lésions nerveuses de la lèpre lépromateuse humaine.

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