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The Relation of Nerve Fibers to *Mycobacterium leprae*

I. The Phagocytic Activity of Cells in Damaged Nerve[1]

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The peripheral neuritis of leprosy seems to be most intense at sites where nerves run a superficial course and are subject to repeated minor trauma. Sites such as the ulnar nerve just above the elbow and the lateral popliteal nerve at the neck of the fibula are commonly affected. In these situations the nerves may become grossly hypertrophic as a result of the uptake of the leprosy bacillus associated with a cellular reaction in and around the nerve. This is paralleled by the intensity of the Mitsuda reaction, which is a reflection of tissue sensitivity to the presence of the bacillus. The predilection of this organism for areas of minor trauma has made us examine the changes that occur in a nerve when it is slightly damaged.

The present paper is concerned with the changes that occur in segments of the median popliteal nerve of the mouse 1 cm. proximal to the site of injection of 0.002 ml. of polystyrene consisting of a suspension of spherical particles 1,380Å in diameter in normal saline. The only trauma to the nerve was that associated with exposure of the nerve and the injection of this material.

MATERIALS AND METHODS

In order to establish the pathway taken by material injected into a nerve, 0.002 ml. of a 0.5 per cent aqueous solution of Evans blue was injected into the median popliteal nerve in the lower third of the thigh of 15 anesthetized mice. The syringe was directed with the needle pointed proximally. It was found that the dye traveled in a proximal direction and reached the spinal nerve roots four hours later. After determination of the pathway followed by the injected material in a second series of 15 mice, 0.002 ml. of a solution of polystyrene (particle diameter 1,380Å) suspended in normal saline was injected into the median popliteal nerve of anesthetized mice. The extent of the incision and handling of the nerve were kept to a minimum. Wounds were closed under sterile precautions. Forty-eight hours later the power of the muscles in the leg was tested and segments of the nerve 1 cm. proximal to the site of injection were fixed in 10 per cent formol-saline for light microscopy, or fixed in 2.5 per cent glutaraldehyde, postfixed in 1 per cent osmium tetroxide, and processed for electron microscopy with the use of araldite as an embedding medium. Sections that appeared silver-gray by reflected light were obtained by use of an L.K.B. microtome and stained in uranyl acetate and then with lead citrate by Reynolds' method ([5]). An Elmiskop I electron microscope was used to examine the sections.

Because of the small number of cells in the endoneurium of this nerve ([3]) extensive searches were necessary in order to find and identify cells and polystyrene particles.

RESULTS

The changes that occurred in nerves 48 hours after the injection of polystyrene were examined under three headings. The first related to alterations in function of the muscles supplied by the nerve. The second related to the alteration in structure of the nerve. The third related to the fate of the polystyrene particles.

Functional changes. The power of the muscles supplied by the median popliteal

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nerve was assessed by comparing the movements of normal animals, or animals denervated by cutting the sciatic nerve, and of those 48 hours after injection. In the case of denervated mice there was restriction of movement of the affected leg. The performance of mice in which the median popliteal nerve had been injected compared favorably with that of normal mice. It was concluded that there was little impairment of nervous function following the injection of polystyrene.

Structural changes. All nerves showed typical degenerative changes similar to those described for Wallerian degeneration at 48 hours ([2, 8]). Nuclear counts, obtained by use of the technic of Abercrombie and Johnson ([1]), revealed no significant difference in the number of nuclei compared to the number in normal nerves.

With the electron microscope it was seen that the Schwann cells associated with myelinated fibers had increased in volume. Figure 1 is a transverse section showing part of the cytoplasm of a Schwann cell (S) associated with a myelinated nerve fiber. The cytoplasm contains bodies filled with dense particulate material as at (D inset). Distended sacs of a smooth-surfaced endoplasmic reticulum (ER) and part of a microtubule are seen at (M). The cell margin shows invaginations of the plasmalemma to form pinocytotic vesicles (see inset P). A well defined basement membrane is seen at (B). Material of similar appearance (Y) often occurred in the endoneurial space and was continuous with the basement membrane as at (X). The axoplasm (A) was separated from the first layers of the myelin by a clear space. A reentrant mesaxon is seen at (R).

It was difficult to detect any change in volume of the cytoplasm of the Schwann cells associated with nonmyelinated fibers.

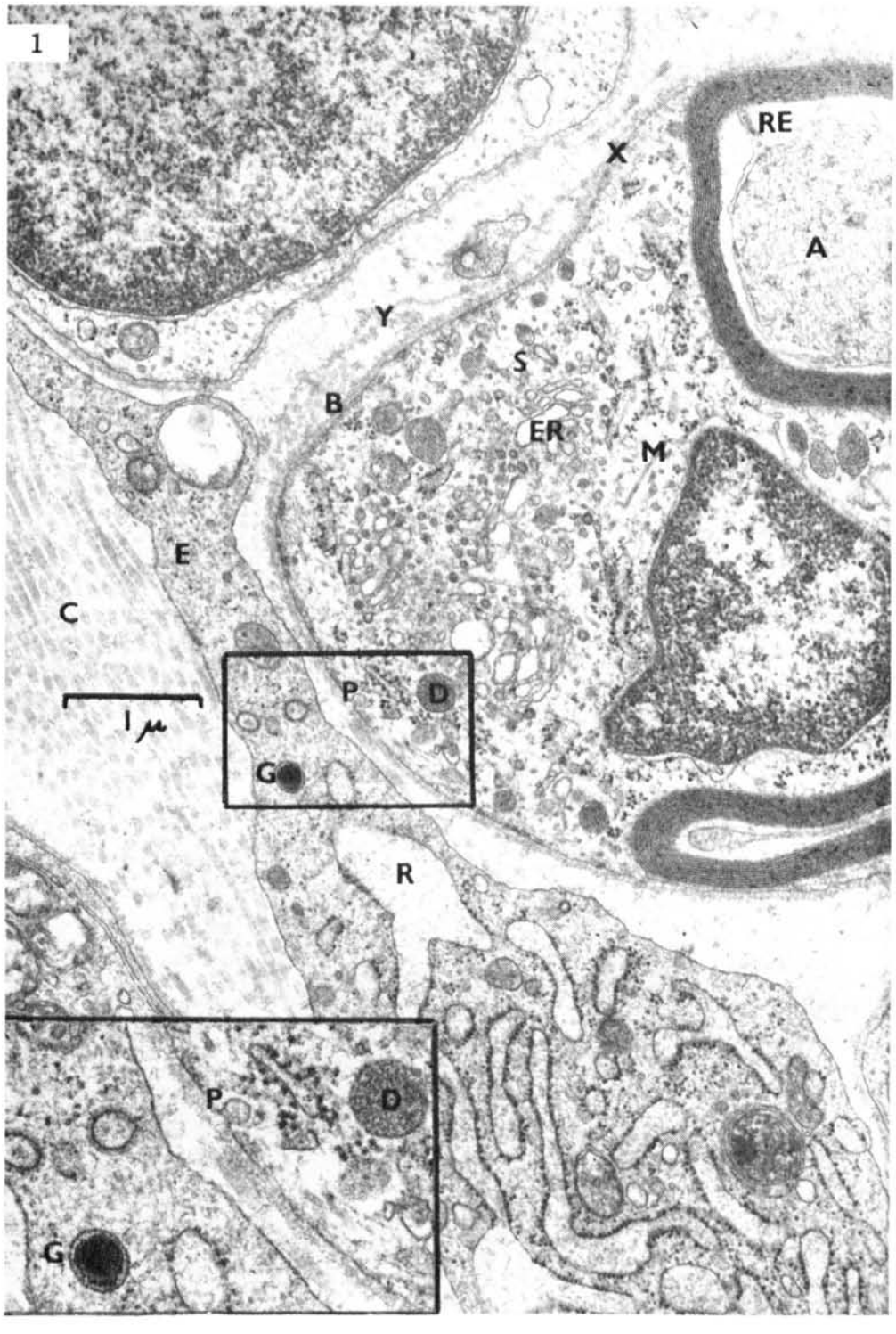
Polystyrene particles. The polystyrene particles were seen as spherical or elliptic structures with an electron-dense edge. When elliptic the long axes lay parallel with one another, as in Figure 3, and at right angles to the direction of sectioning. It was concluded that elliptic shapes were due to the compression of spherical particles during sectioning.

Two types of endoneurial cells were seen: those that did contain polystyrene and those that did not. Neither cell was associated with a basement membrane. Examples of the first type of cell, which contained polystyrene particles enclosed within vesicles (V, Figs. 2 and 3) bounded by a unit membrane, are seen in Figures 2 and 3. The cell, part of which is seen in Figure 1, lies in the endoneurial space adjacent to Schwann cells enclosing myelinated and nonmyelinated nerve fibers and polystyrene particles. Figure 2 shows another example at a higher magnification. Vesicles containing these particles often contain dense material, as at (V). The second type of cell may be seen in Figure 1 (E). It possesses long processes and contains a well defined endoplasmic reticulum with distended cisternae. The cytoplasm contains vesicles filled with amorphous material (Fig. 1, inset). There is no basement membrane. In no case was polystyrene enclosed in Schwann cells or nerve fibers.

DISCUSSION

Little change in the behavior of the muscles supplied by the nerve injected with polystyrene was detected, and using the light microscope we were unable to differentiate injected nerves from normal nerves. These findings parallel those of Abercrombie and Johnson ([1]), who found little alteration in the numbers of nuclei of a nerve 48 hours after neurectomy. These authors used the distal stump while we used part of the nerve 1 cm. proximal to the site of the injection, where changes might be expected to be even less, for changes proximal to the site of nerve injury are minimal ([9]).

With the electron microscope, however, it was seen that there were degenerative changes in the axoplasm of the myelinated nerve fibers. Such changes are similar to those seen in the distal stump of a nerve in Wallerian degeneration after 48 hours ([2, 8]). This was a surprising finding, for examination by light microscopy revealed little indication of any damage to the nerve. It would be of interest to see if similar degenerative changes occur in the fine structure of nerves subject to other forms of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minor trauma and most particularly nerves such as the great auricular nerve in man, which are known sites for leprosy infection.

Polystyrene was phagocytosed by endoneurial cells and was readily distinguished from vesicles in the cell cytoplasm, which contained other kinds of particulate matter. The fact that polystyrene particles are phagocytosed by endoneurial cells and not by Schwann cells is at variance with the results obtained by Palmer *et al.* (4). Their results, however, were obtained by use of colloidal carbon and crushed or cut nerves and, while the morphologic features of Schwann cells in normal nerve are well known (6), the identification of these cells in inflammatory masses is difficult. Thomas (7) has investigated these problems recently and produced evidence suggesting that macrophages, and not Schwann cells, are responsible for phagocytosis in damaged nerve. Our own results are seen to be more closely aligned to those of Thomas (7) than those of Palmer *et al.* (4). An explanation of this difference may lie in the size of the particle, for, although the surface area of the polystyrene particles (.06 square μ) was considerably less than that of *M. leprae* (1.5 square μ), it was greater than that of the colloidal carbon (0.001 square μ) used by Palmer *et al.* (4). It is also possible that different cellular mechanisms are responsible for the disposal of materials as different as carbon, polystyrene, degenerating nerves, and *M. leprae*. Furthermore, the nature of the stimulus to the nerve may be important. Most previous investigations were based on the cellular response to injury after complete transection of the nerve. We have attempted to minimize damage in order to maintain the functional integrity of the nerve and to simulate the changes that occur as it travels subcutaneously in a vulnerable site.

In our experiments the number of particles injected into the nerve was presumably well within the phagocytic capacity of the cells already present in the nerve; in fact many of the endoneurial cells did not contain polystyrene. If the inoculum were greater perhaps more cells would be involved by migrations from the lumen of blood vessels and epineurial tissues, or by division by cells already present in the nerve. Finally, an overwhelmingly large inoculum might result in the failure of the normal mechanisms responsible for removing particulate matter from the nerve. This would parallel the findings in lepromatous leprosy, where numerous bacilli are present in the endoneurial space and cellular infiltration is small.

SUMMARY

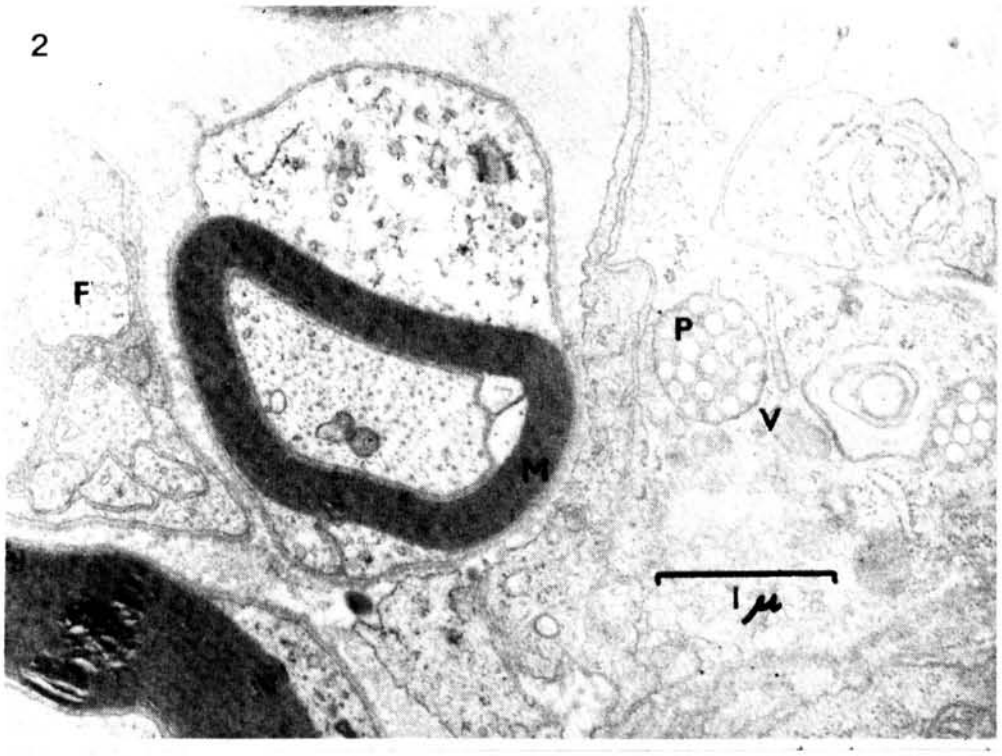
The functional changes caused by injecting polystyrene particles into a nerve are slight at 48 hours. Light microscopy revealed no alteration in the numbers of nuclei within the nerve. With the electron microscope, however, degenerative changes were seen in the myelinated nerve fibers. Particles of polystyrene were phagocytosed by endoneurial cells, but not by Schwann cells. These results seem comparable to the lesions of nerve in lepromatous leprosy, where there is little change in cell numbers, and bacilli are confined to the endoneurial space.

RESUMEN

Los cambios funcionales causados inyectando partículas de polystyreno en un nervio, son

FIG. 1. A transverse section of a nerve injected 48 hours previously with polystyrene latex. An endoneurial fibroblast with distended sacs of endoplasmic reticulum (**ER**) lies in the endoneurial space surrounded by collagen fibers (**C**). The cytoplasm contains bodies filled with particulate matter (**D**). The Schwann cell (**S**) contains part of a microtubule (**M**) and a pinocytotic vesicle (**P**). It is surrounded by a basement membrane (**B**). This membrane is continuous with material of similar appearance (**Y**) at point (**X**). The axoplasm (**A**) is separated from the myelin. A re-entrant mesaxon is seen at (**R**).

The inset is an enlargement of the marked area, and shows bodies containing particulate matter in the endoneurial fibroblast and Schwann cell.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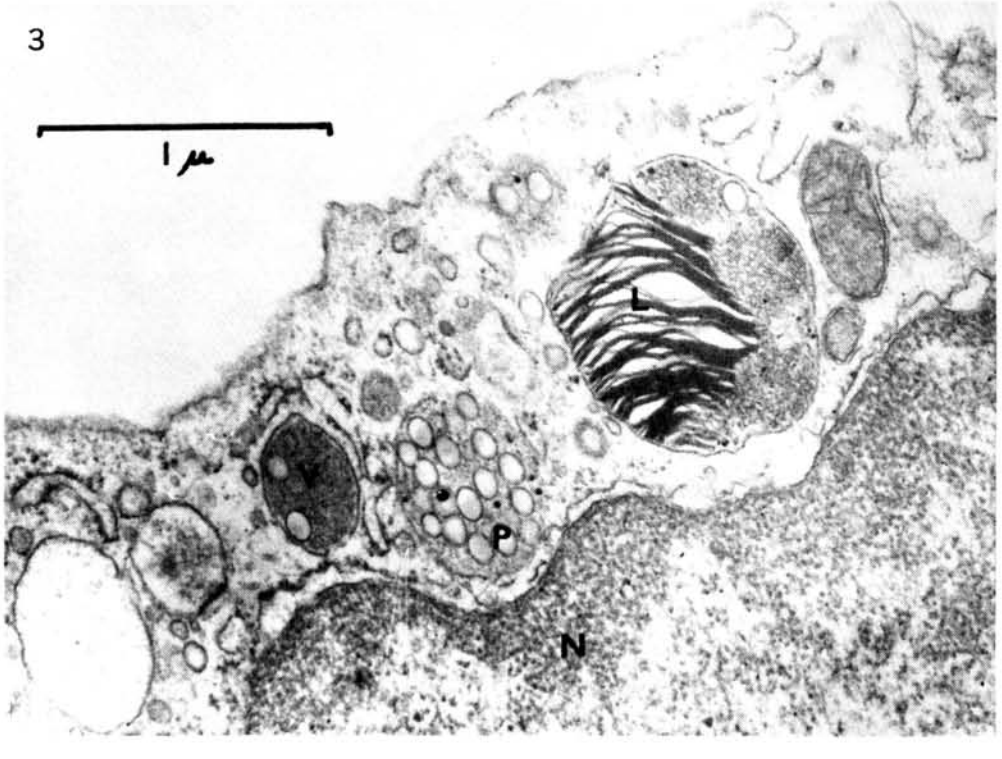
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ligeros en 48 horas. La observación al microscopio reveló no haber alteración en el número de nucleos dentro del nervio. Con el microscopio electrónico, sin embargo, cambios degenerativos se observaron en las fibras myelínicas del nervio. Las partículas de polystyreno fueron phagocytadas por las células endoneuriales, pero no por las células de Schwann. Estos resultados parecen comparables a las lesiones del nervio en lepra lepromatosa, donde hay poco cambio en el número de las células, y los bacilos están confinados en el espacio endoneurial.

RÉSUMÉ

Les changements fonctionnels que l'on peut noter après 48 heures, à la suite de l'injection dans un nerf de particules de polystyrène, sont peu notables. La microscopie optique n'a pas révélé de modifications dans le nombre des noyaux à l'intérieur du nerf. Le microscope électronique a toutefois permis de mettre en évidence des modifications dégénératives dans les fibres nerveuses myélinisées. Les particules de polystyrène étaient phagocytées par les cellules endoneurales, mais ne l'étaient pas par les cellules de Schwann. Les modifications observées paraissent similaires aux lésions nerveuses que l'on rencontre dans la lèpre lépromateuse, au cours le laquelle il se produit peu de changements dans le nombres des cellules, la présence des bacilles étant limitée aux espaces endoneuraux.

REFERENCES

1. Abercrombie, M. and Johnson, M. L. Quantitative histology of Wallerian degeneration. I. Nuclear population in rabbit sciatic nerve. J. Anat. **80** (1946) 37-50.
2. Barton, A. A. An electron microscope study of degeneration and regeneration of nerve. Brain **85** (1962) 799-808.
3. Causey, G. and Barton, A. A. The cellular content of the endoneurium of peripheral nerve. Brain **82** (1959) 594-598.
4. Palmer, E., Rees, R. J. W. and Weddell, G. Experimental studies on nerve fibers in leprosy. I. The reaction of rat Schwann cells toward carbon particles, *Mycobacterium lepraemurium* and *Mycobacterium leprae*. Internat. J. Leprosy **33** (1965) 137-159.
5. Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17** (1963) 208-212.
6. Ross, L. L. Peripheral nervous tissue. *In* Electron Microscopical Anatomy. S. M. Kurtz, Ed. New York, Academic Press, 1964.
7. Thomas, P. K. The cellular response to nerve injury. I. The cellular outgrowth from the distal stump of transected nerve. J. Anat. **100** (1966) 287-303.
8. Webster, H. de F. Transient focal accumulation of axonal mitochondria during the early stages of Wallerian degeneration. J. Cell. Biol. **12** (1962) 361-383.
9. Young, J. Z. The functional repair of nervous tissue. Physiol. Rev. **22** (1942) 318-374.

Fig. 2. Part of a nerve injected 48 hours previously with polystyrene. The endoneurial cell contains vesicles (**V**) filled with polystyrene particles (**P**). The myelinated (**M**) and nonmyelinated fibers (**F**) show degenerative changes that are transitory.

Fig. 3. Part of an endoneurial cell containing polystyrene particles (**P**), which have been compressed during sectioning to form elliptic structures. The vesicles containing polystyrene often contain dense material, as at (**V**). Another vesicle (**L**) contains dense material showing myelinic forms. Nucleus (**N**).