Microsphere Embolization of Nerve Capillaries and Fiber Degeneration

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Polystyrene microspheres, the size chosen to plug capillaries and precapillaries, were injected into the arterial supply of rat sciatic nerves. They produced widespread segmental occlusion of capillaries in lower limb nerves. The clinical and pathologic effect was dose-related. One million microspheres produced selective capillary occlusion but no nerve fiber degeneration; approximately 6 million microspheres also produced selective capillary occlusion and associated foot and leg weakness, sensory loss, and fiber degeneration, beginning in a central core of the distal sciatic nerve; 30 million microspheres caused both capillary and arterial occlusion and a greater neuropathologic deficit. From these observations it is inferred that 1) occlusion of isolated precapillaries and capillaries does not produce ischemic fiber degeneration; 2) occlusion of many microvessels results in central fascicular fiber degeneration, indicating that these cores are watershed regions of poor perfusion; and 3) stereotyped pathologic alterations of nerve fibers and Schwann cells are related to dose, anatomic site, and time elapsed since injection. (Am J Pathol 1984, 115:275–287)

IN VARIOUS peripheral nerve disorders fiber degeneration has been attributed, often without sufficient reason, to ischemia associated with vessel pathology. In early medical reports, neuritic pain, distal leg and foot muscle weakness, and sensory loss was attributed to lower limb arterial occlusion and nerve damage.[1–6] Few physicians now accept the existence of an ischemic neuritis from peripheral vascular disease (PVD), but rejection of the entity may have been premature, because convincing evidence linking distal nerve fiber degeneration and severe and extensive lower limb PVD has been reported.[7,8] In addition, ischemia is reported to play a role in nerve degeneration in necrotizing angiopathy,[9,10] various types of diabetic neuropathy,[11,12] compression neuropathy,[13] and amyloidosis[14] and in various other neuropathies. In our view, however, the evidence is convincing only for vasculitis and, possibly, oculomotor diabetic neuropathy.[15,16]

In necrotizing angiopathy, as may occur in polyarteritis nodosa, Wegener's granulomatosis, rheumatoid arthritis, and Churg-Strauss syndrome, the association of a multiple mononeuropathy, widespread epineurial arteriolar occlusion, and patchy fiber degeneration suggests that the fiber degeneration is due to an inflammatory arteriolar occlusion.[9,17–19] This association has been evaluated further in a three-dimensional pathologic study of sections from serially taken blocks along the length of ulnar, median, sciatic, tibial, and peroneal nerves from a patient with polyarteritis nodosa who had multiple mononeuropathy and multifocal regions of fiber degeneration.[20] Whereas segmental arteriolar occlusion was widespread and even included proximal segments of limb nerves, the onset (from proximal to distal) of fiber degeneration began near mid thigh and mid-upper arm levels in central fascicular cores of widely distributed fascicles. These observations, confirmed in later studies of two additional cases[21] provided evidence supporting the following ideas: 1) Epineurial arterioles are not end arteries, and occlusion of individual arterioles does not cause a nerve infarct. 2) The multifocal central fascicular degeneration of fibers is characteristic of ischemic damage. 3) This degeneration begins at levels approximately equidistant between the points of entry of major nutrient arteries to nerve, suggesting that the cores are watershed regions of poorest perfusion. 4) The circulation of the nerve must be considerably compromised before damage begins.

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Early experimental studies, in which blood vessels to nerve were ligated for the purpose of producing fiber degeneration, gave conflicting results.[22,23] Mobilization of nerves, and thus interference with their blood supply, was found not to cause damage, presumably because of well-developed anastomatic arteries within nerve.[24] Korthals and Wisniewski[25] were the first to demonstrate a reliable model of ischemic nerve damage. When the abdominal aorta and femoral artery in a cat were ligated, unequivocal evidence of partial damage in distal sciatic nerve and of panfascicular necrosis in tibial and peroneal nerves developed. These severe neuropathologic changes could be produced without accompanying foot or leg gangrene. In a second paper, Korthals et al[26] emphasized the finding of organelle accumulation in distal sciatic nerve and lower portions of tibial and peroneal nerves and the special vulnerability of large fascicles and central fascicular regions. They attributed the organelle accumulation to a possible interruption of fast axonal transport.

Hess and co-workers[27] produced mild ischemic nerve fiber damage and severe muscle degeneration by ligating the common and internal iliac or internal and external iliac arteries of the rabbit. These studies provided unequivocal evidence that paranodal segmental demyelination and remyelination, in addition to axonal degeneration, were encountered in these lesions.

Parry and Brown[28,29] combined intraarterial injection of arachidonic acid and large vessel ligation (of the femoral artery) to produce platelet aggregation and vessel occlusion. Selective occlusion of capillaries and precapillaries and central fascicular fiber degeneration of the proximal portion of the posterior tibial nerve were produced.

In this study, we have injected polystyrene microspheres, the size chosen to plug capillaries and precapillaries (hereafter often referred to simply as capillaries) into the arterial supply of rat sciatic nerves 1) to test whether selective occlusion of capillaries could be induced; 2) to ascertain whether ischemic nerve degeneration would ensue; 3) to evaluate the three-dimensional distribution of capillary occlusion and its anatomic relationship to fiber damage; and 4) to assess pathologic abnormalities which developed as a result of this procedure. These studies will be compared to the effect of large artery ligation at a later date.

Materials and Methods

Male Sprague–Dawley rats, weighing 250–300 g, were anesthestized with sodium pentobarbital. The right common iliac artery and the bifurcation of external and internal iliac arteries (nomenclature of arteries and nerves of rats according to E. C. Greene, Anatomy of the Rat, Transactions of the American Philosophical Society, Philadelphia, 1935) were surgically exposed. Polystyrene microspheres (15 ± 0.8 μ OD, 3M, St. Paul, MN) were injected into the arteries of supply to lower limb nerves as described below. Rats with anomalous arteries were not included.

Distribution of Radiolabeled Microspheres in Nerve

To assess where microspheres lodged along the course of the sciatic nerve and its branches from injection of different arteries, and whether they remained there, different arteries of supply were injected with radiolabeled microspheres (51 Cr, 15 ± 0.8 μ OD, 3M, St. Paul, Minn). The external iliac, internal iliac, or superior gluteal arteries were tested. Consecutive 5-mm lengths of the sciatic nerve and its branches, from proximal to distal ends, were cut, desheathed, and the radioactivity and dry weight of the endoneurial portion were determined. This procedure was repeated in different animals at various time intervals after injection.

Preparation of Microsphere Suspension and Injection Approach

For histologic studies, nonradiolabeled microspheres were added to an isotonic saline solution containing 0.05% Tween 80 to provide a suspension of 100 mg/ml. Immediately prior to injection, the suspension was shaken in a vortex mixer and placed in a sonicator bath. Using a dissecting microscope, a PE-10 tubing (Clay Adams, Parsippany, NJ) was cannulated into the external iliac, internal iliac, and superior gluteal arteries for injection of microspheres after temporary ligation of proximal common iliac and superior vesical arteries. The dose most frequently employed (5.6 million) was injected in doses of 2.6, 2.1, and 0.9 million microspheres into the external iliac, internal iliac, and superior gluteal arteries, respectively. After the injection the arterial puncture site was sutured with 9-0 silk, and the ligation was then released. In a small number of rats microspheres were injected into the abdominal aorta with temporary occlusion of the left common iliac artery.

Experiments and Histologic Processing

The number of animals used, the dose of microspheres injected, the vessels injected, and the time between injection and removal of tissue are summarized

Table 1—Experimental Protocol for Microsphere Embolization

Dose of microspheres* injected	Duration of experiment	Number of rats
5.6 × 10^6†	6 hours	3
	12 hours	3
	18 hours	3
	24 hours	5
	48 hours	3
	7 days	8
	6 weeks	6
1 × 10^6†	7 days	2
3 × 10^6†	7 days	2
12 × 10^6†	7 days	2
30 × 10^6†	7 days	2
30 × 10^6‡	7 days	3
50 × 10^6‡	—§	2

* Polystyrene microspheres (15 μ) were obtained from 3M, St. Paul, Minnesota, and suspended as described in text.

† Injected into the external iliac, internal iliac, and superior gluteal arteries.

‡ Injected into the abdominal aorta after temporary occlusion of the contralateral common iliac artery.

§ The rats died within several days.

in Table 1. Rats were clinically observed daily during the first week and twice weekly thereafter. Notes were kept on abnormalities of gait, weakness of thigh adduction in walking, foot drop, toe spreading and decreased grasp. Surgical forceps were used to elicit a nociceptive response. After depilation, the hind limbs were observed for color, coldness, and trophic changes.

Nerves were fixed in situ for 60 minutes with 4% glutaraldehyde in 0.025 M cacodylate buffer at pH 7.40. The entire length of the lumbosacral plexus and the sciatic nerve and its branches were taken in continuity. The sural and tibial nerves were removed to the ankle level and the peroneal nerve to its point of bifurcation. The nerves were immersed overnight in 2.5% glutaraldehyde in the same buffer. Consecutive 2-mm lengths of nerve were cut, postfixed in 1% osmium tetroxide for 2½ hours, dehydrated in ethanol, immersed in propylene oxide, and embedded in epoxy. The periods of fixation for teased fiber studies were 12 minutes *in situ* and 2½ hours in osmium tetroxide. The left (non treated side) sciatic nerve was removed as a control. In a series of control experiments, an equal volume of saline solution (containing 0.05% Tween 80) was injected under identical conditions. Nerves were taken at 7 days and processed in a similar manner.

In 6 animals, consecutive segments of the regional arteries, including the common iliac, external iliac, internal iliac, superior gluteal, internal pudendal, inferior gluteal, femoral, and saphenous arteries were also excised for morphologic evaluation.

Transverse semithin sections (0.75 μ in thickness) were cut from the consecutive blocks along the length of the excised sciatic nerve and stained with 1% phenylenediamine or 1% methylene blue. Areas where ischemic nerve damage was becoming apparent were additionally investigated by use of serial semithin sections of the appropriate blocks of tissue.

To ascertain the distribution of microsphere embolization and vascular densities, groups of 3 rats from control and 7 days after injection were examined. Serial transverse semithin sections were taken for a distance of 50 μ from three different levels for each of the following: 1) lumbosacral trunk, 2) gluteal sciatic nerve (sciatic nerve in the pelvis minor), 3) upper sciatic nerve, 4) lower sciatic nerve, and 5) upper tibial and peroneal nerves. The number of capillaries, including occluded ones, arterioles, and venules in the endoneurium, perineurium, and epineurium were counted and related to area. The transverse area of the fascicle was divided into the subperineurial region (periphery) and central fascicular region. The area of each region was equal to one-half the total fascicular area, and the number of vessels was counted separately in each region. The endoneurial vascular densities have been reported in detail elsewhere.[30]

Results

Distribution of Radiolabeled Microspheres Within Nerves

At different time intervals (5 minutes to 7 days) after intraarterial injection of the radiolabeled micro-

Table 2—Radioactivity (cpm/mg Dry Fascicular Nerve Weight) at Different Levels of Lumbar and Sacral Plexus and Sciatic and Tibial Nerves From Injection of 51 Cr Microspheres Into Different Arteries

	Vessels		
Level of nerves	Superior gluteal artery* (n = 2)	Internal iliac artery† (n = 2)	External iliac artery‡ (n = 2)
Lymbosacral plexus	420 ± 448	10 ± 1	13 ± 3
Pelvic sciatic	2237 ± 464	664 ± 34	820 ± 243
Upper sciatic	2153 ± 424	2053 ± 326	2562 ± 394
Lower sciatic	1587 ± 153	2536 ± 1461	4165 ± 843
Proximal tibial	110 ± 58	1292 ± 29	2026 ± 231
Distal tibial	17 ± 4	469 ± 512	1022 ± 87

* When the superior gluteal artery is given radioactive polystrene microspheres, most of the radioactivity is in the pelvic sciatic and upper sciatic nerve.

† When the internal iliac artery is given an injection, most of the radioactivity is in the upper and lower sciatic nerve.

‡ When the external iliac artery is given an injection, radioactivity is greater in the sciatic nerve but extends further distally.

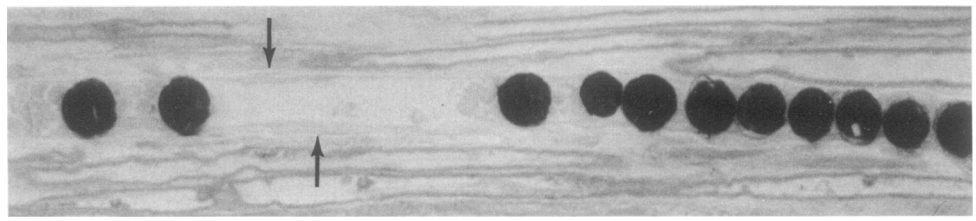


Figure 1—A longitudinal section of an endoneurial capillary showing a train of microspheres entrapped in the lumen. Notice the single layer of endothelial cells characteristic of capillaries (*arrows*). (Semithin section of glutaraldehyde- and osmium-tetroxide-fixed nerve, unstained, ×450)

spheres, segments of desheathed sciatic nerve were shown to contain a high level of radioactivity, which had not decreased by 7 days (as judged from an evaluation of 6 levels of nerve from animals at 5 minutes, 6 hours, 24 hours, and 7 days). The distribution of radioactivity along the course of the nerve was different, but overlapping, depending on which artery was given the injection (Table 2).

Dose and Clinical and Histologic Effect

To assess the effect of dose, initially small groups of rats were given injections of 1, 3, 5.6, 12, and 30 million microspheres into the three arteries of supply to sciatic nerve (Table 1). The neurologic deficit, density of occluded capillaries, and distribution and extent of pathologic nerve damage were related to the dose. Injection of 1 million microspheres resulted in widespread capillary occulusion but no fiber degeneration. A transitory and variable degree of muscle weakness was observed, possibly due to muscle involvement, which is known to precede nerve damage.[31] More capillaries appeared to be occluded following the injection of 5.6 million microspheres. Unilateral leg weakness and sensory loss without distal limb gangrene and central fascicular fiber degeneration were reproducibly observed. Injection of 30 million microspheres resulted in distal limb gangrene and panfascicular degeneration in the distal sciatic nerve in 3/3 animals, and the superior gluteal artery was occluded in 1/3 rats. A dose of 5.6 million microspheres was chosen for most studies because only capillaries were occluded, and it reproducibly resulted in leg weakness, sensory loss, and central fascicular ischemic nerve fiber damage.

Injection of 30 million microspheres into abdominal aorta, with temporary occlusion of the contralateral common iliac artery (Table 1), produced a quite variable clinical and morphologic deficit. Rats receiving 50 million microspheres into the abdominal aorta

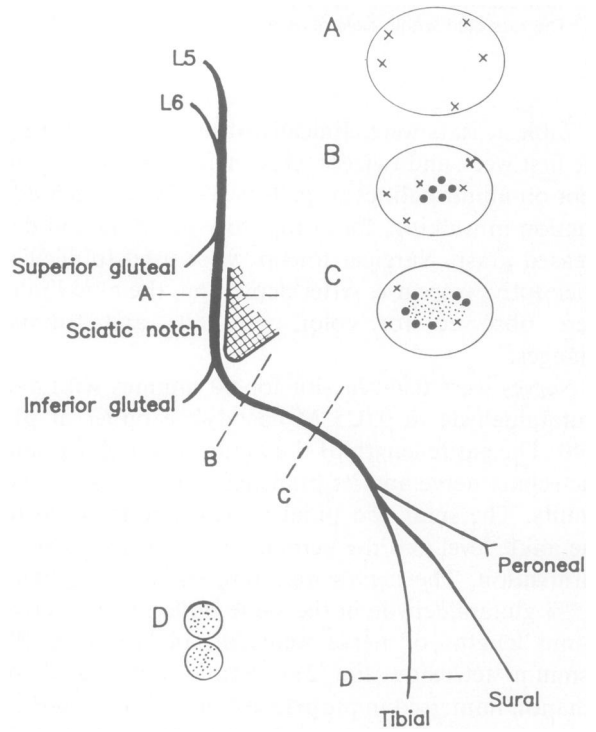
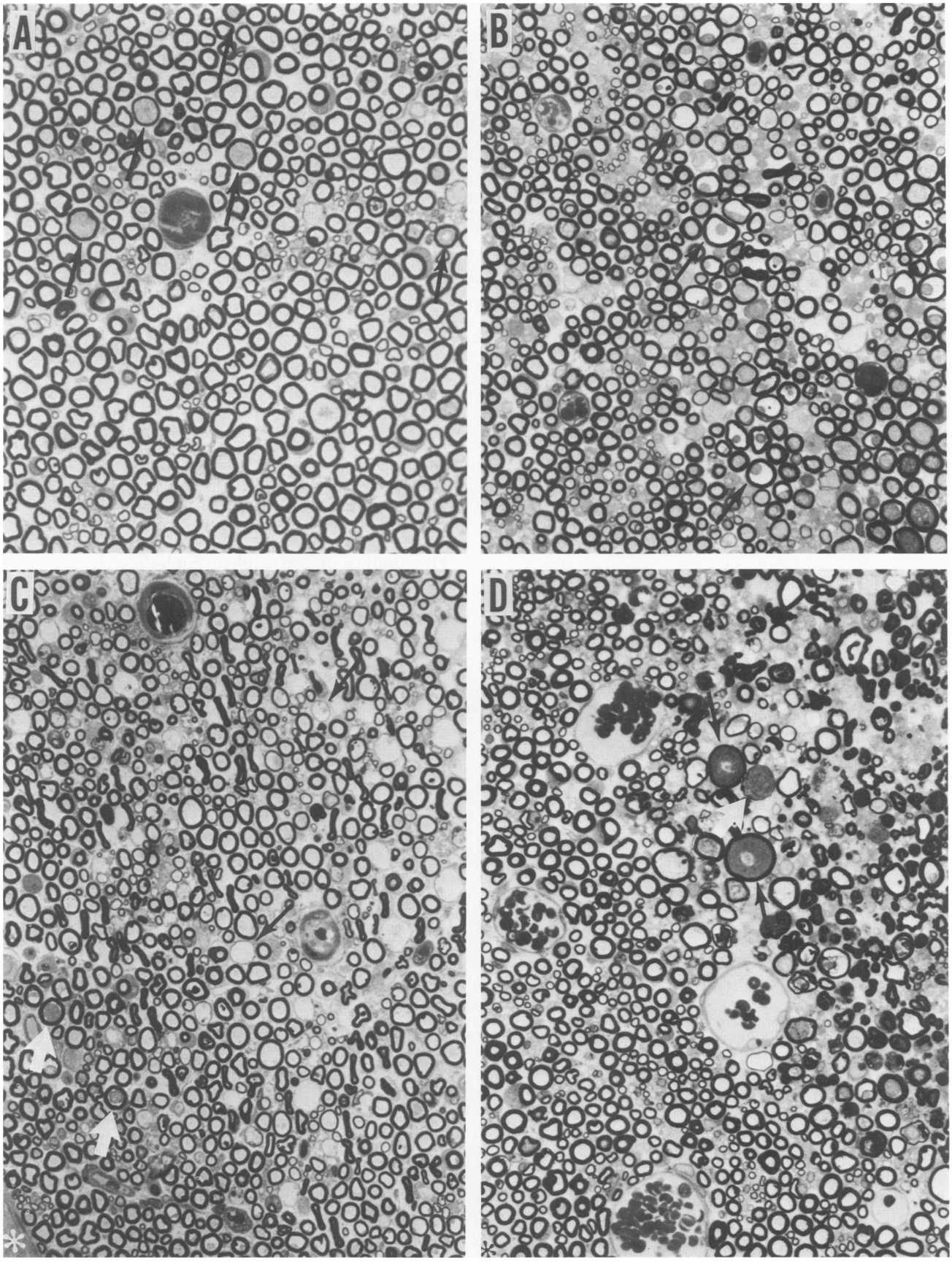


Figure 2—Distribution of the ischemic lesion in rat sciatic nerve following capillary microembolization. The distribution of abnormalities is shown on transverse sections of sciatic nerve (**A**, **B**, **C**) and tibial nerve at the ankle level (**D**). The capillaries occluded by microspheres are indicated by *crosses*, the "dark axons" by *closed circles*, and the "ischemic cores" by *dots*. See the text for explanation of pathologic changes.

Figure 3—Transverse sections of rat sciatic nerve after capillary occlusion. **A**—Most proximal section of the ischemic lesion twelve hours after embolization showing "dark axons" (*arrows*). Notice a homogeneous mass of red cells in the capillary. (Methylene blue, ×560). **B**—Proximal end of an "ischemic core" 12 hours after embolization, showing severe "attenuated axons" (*arrows*) and "dark axons." Flattened myelin profiles and empty axons are less prominent. (Phenylenediamine, ×440) **C**—Midthigh level of the "ischemic core" 18 hours after embolization, showing flattened myelinated fibers, "tubular" profiles (*black arrows*), and empty axons. Notice the "dark axons" at the border zone (*white arrows*) and the entrapped microsphere in the capillary. *, perineurium. (Phenylenediamine, ×440) **D**—Prominent "dark axons" and "dark axons with light cores" (*black arrows*) at the border zone of an "ischemic core" 48 hours after embolization. Notice that one "dark axon" is devoid of myelin (*white arrow*). *, perineurium. (Methylene blue, ×440)


A
B
C
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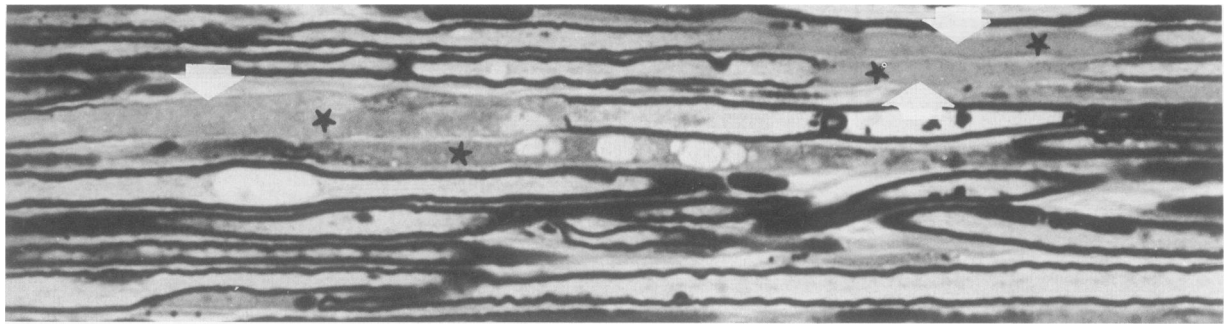

Figure 4—Longitudinal section of rat sciatic nerve 24 hours after capillary embolization, showing paranodal demyelination (*white arrows*), "dark axons" (*stars*), vacuoles, and myelin irregularity. Notice that demyelinated segments are "dark axons." (Phenylenediamine, ×760)

usually died within several days. In these rats, microspheres were found in both sciatic nerves. One animal developed ischemic gangrene of the ipsilateral foot and leg. Arterioles were occluded predominantly at bifurcation points. These studies were not pursued because the clinical deficit was variable, bilateral lower limb effects occurred, and more than capillaries were occluded.

Clinical Observations

Immediately after injection of 5.6 million microspheres, the foot and leg of the injected side was noticeably cooler and bluer. After recovery from anesthesia a strong pinch of the toes did not result in withdrawal of other pain response. The toes were closely apposed, hung limply downward, could not be spread, and extension and flexion of the toes and feet was not possible. Although weakness of toe spreading, extension, and flexion was still present, some improvement had occurred within a few days. By 6 weeks, only a slight degree of foot and leg muscle weakness remained. No clinical deficit was observed on the contralateral side or in rats given the diluent.

Distribution of Microsphere Emboli

After injection of the 5.6 million dose, microspheres, which were easily recognizable by their brownish black color and spherical shape, were ob-

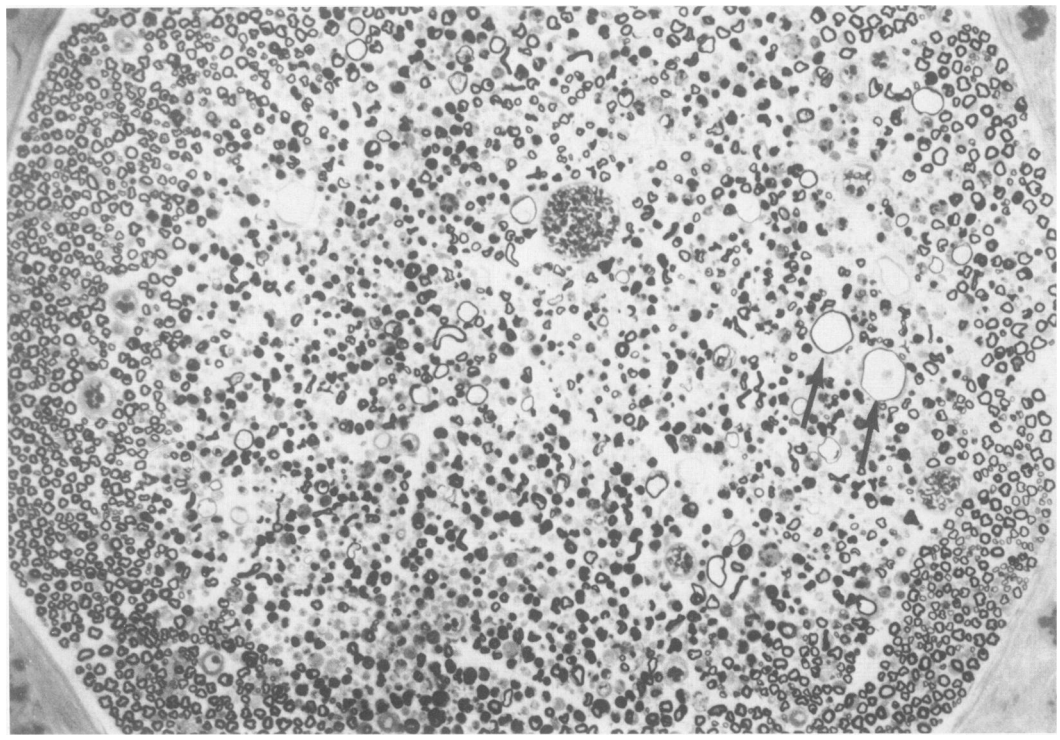

Figure 5—Transverse section taken at the midthigh level of rat sciatic nerve 7 days after microsphere embolization, showing central fascicular fiber degeneration ("ischemic core"). Notice the swollen axon with watery axoplasm (*arrow*). (Phenylenediamine, ×180)

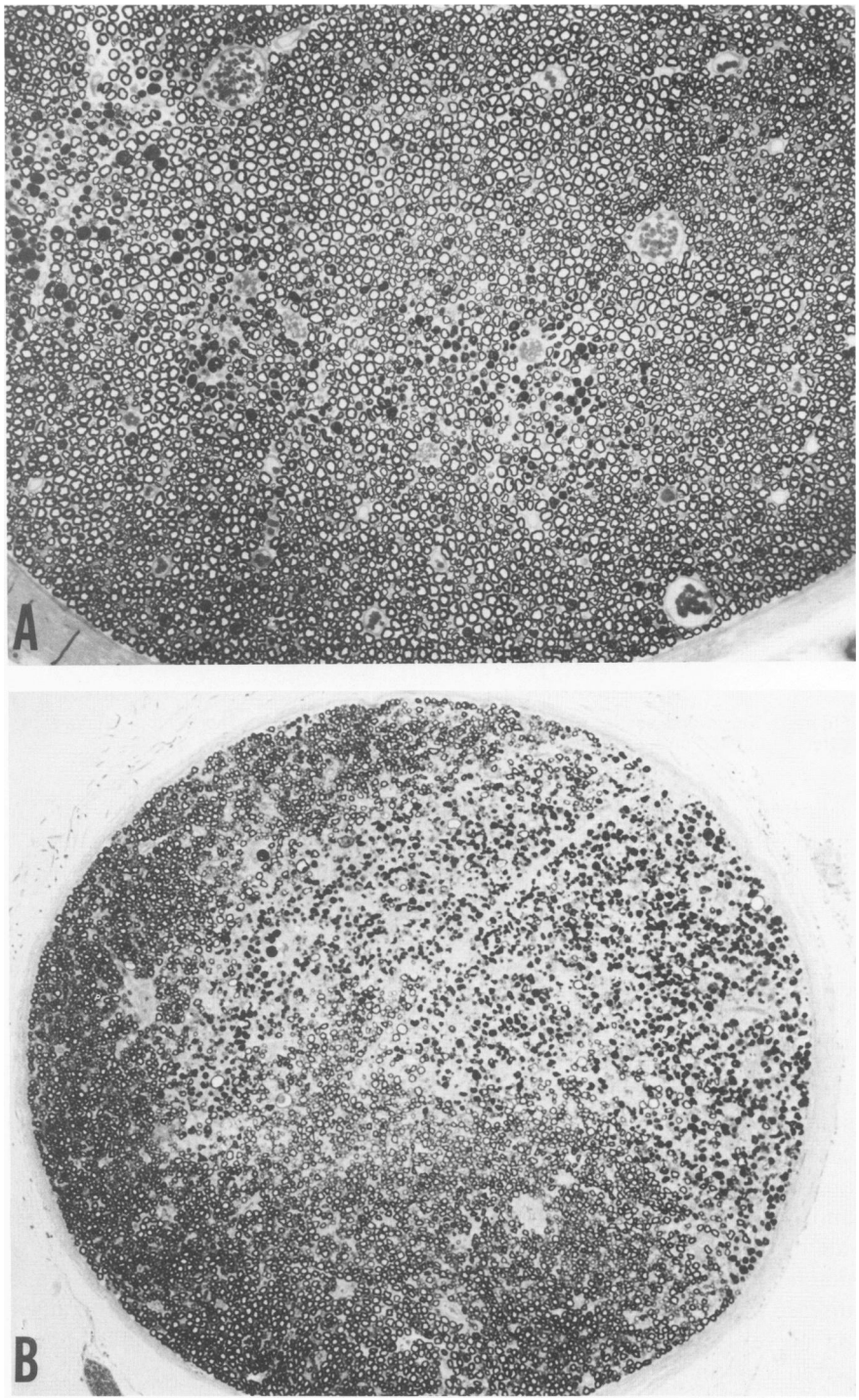

Figure 6—Transverse section of rat sciatic nerve 7 days after microsphere embolization, showing various patterns observed at the proximal region of the "ischemic core." **A**—Multiple small clusters of degenerating fibers. (Phenylenediamine, ×160) **B**—A "piece-of-pie" shaped lesion. (Phenylenediamine, ×100)

served in capillaries but not in arteries or arterioles. The capillaries were occluded by single or sometimes by multiple (clumped) microspheres (Figure 1).

Approximately 15% of sampled capillaries contained microspheres. A significant difference in the density of occluded capillaries along the course of the nerve was not demonstrated ($p > 0.05$ between the levels evaluated). Congested capillaries (packed with blood cells) were more frequent in ischemic than in comparable levels of control nerves (mean values 30.4% and 6.0%, respectively, $p < 0.001$). More microspheres (mean, 76.3%) were found in the subperineurial than in the central (mean, 23.7%) region of fascicles. This ratio is, however, approximately that of the diverse capillary densities encountered between outer and inner shells of fascicles (data that will be

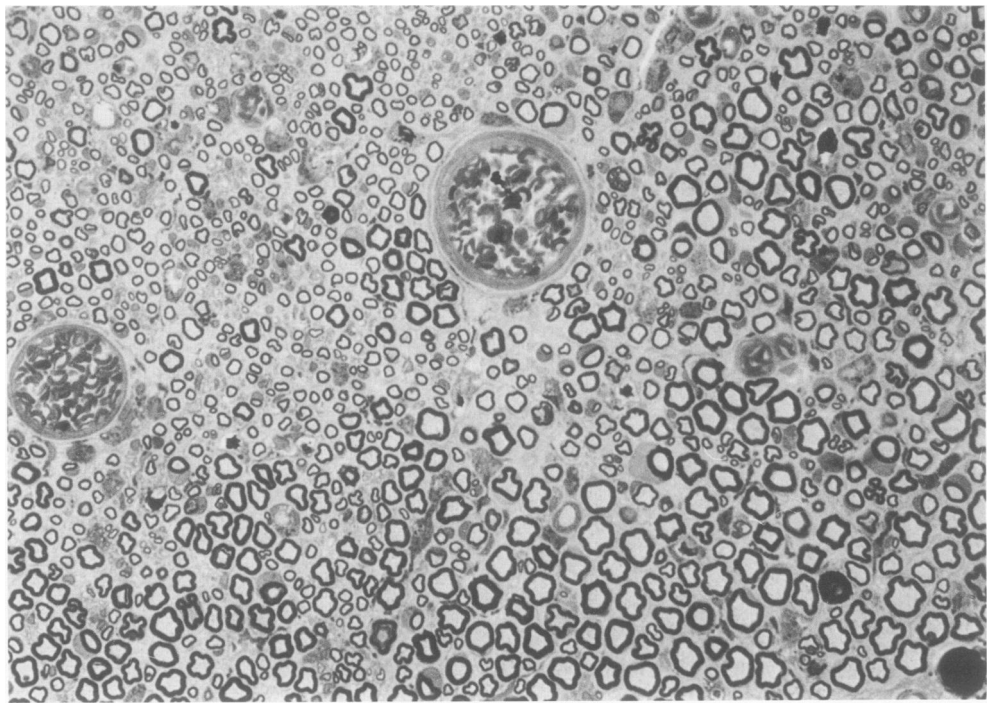

Figure 7—Transverse section of rat sciatic nerve 6 weeks after microsphere embolization, showing small regenerating fibers in the "ischemic core." Notice the myelin debris and the microsphere. (Phenylenediamine, ×430)

reported in another communication).[30] Stagnated microspheres were also seen in 1.2% of the postcapillary venules. However, these vessels did not appear to be occluded.

Morphologic Changes

Light-Microscopic Features

In methylene blue and phenylenediamine-stained sections obtained from consecutive blocks, occluded capillaries were found throughout the sciatic nerve and its branches. A careful search of both transverse and longitudinal sections, however, showed that ischemic nerve damage usually began below the sciatic notch and extended downward (Figure 2). No fiber disease was seen in the lumbosacral trunk of the sciatic nerve. Central fascicular fiber degeneration, referred to as an "ischemic core," was clearly seen at the midthigh level of the sciatic nerve. The "ischemic core" tended to become more diffuse distally and extended to the ankle level of the tibial nerve.

The ischemic core of the distal sciatic nerve, 12 to 48 hours after injection, typically showed "dark axons," "dark axons with light cores," "attenuated axons," "demyelinated axons," and "vacuolated axons" in the border zone between normal fibers and the "ischemic core" and mild separation of fibers, flattened and boomerang-shaped myelin profiles, "tubular" and "honeycomb" structures, and axonal cytolysis in the "ischemic core" (Figure 3). "Dark axons," with or without light cores, often had a thinner than normal myelin sheath or no myelin ("demyelinated axons"), and these were sometimes enlarged or attenuated ("attenuated axons"). The "bare axons" were larger than unmyelinated fibers and did not appear in clusters. Myelinated profiles were often flattened, without apparent axonal content; but sometimes they contained fibrillar or vacuolar debris. The "tubular" or "honeycomb" profiles had clear centers and a thin wall which could not be resolved with the light microscope.

All of the abnormalities observed in transverse sections were also seen in longitudinal sections (Figure 4). The "dark axons" and the "dark axons with light cores" generally involved axons several hundred microns in length, and they were often situated adjacent to or through a node of Ranvier or a demyelinated segment. Occasionally, there was a large, clear, elliptical vacuole in a "dark axon." Not infrequently, a region of absence of myelin considerably longer than that observed in the normal node of Ranvier could be demonstrated. The myelin of preserved internodes sometimes exhibited excessive irregularity. The myelin adjacent to such an elongated node might be attenuated and of unequal thickness.

After becoming familiar with the well-developed pathologic changes, it was possible to recognize lesser

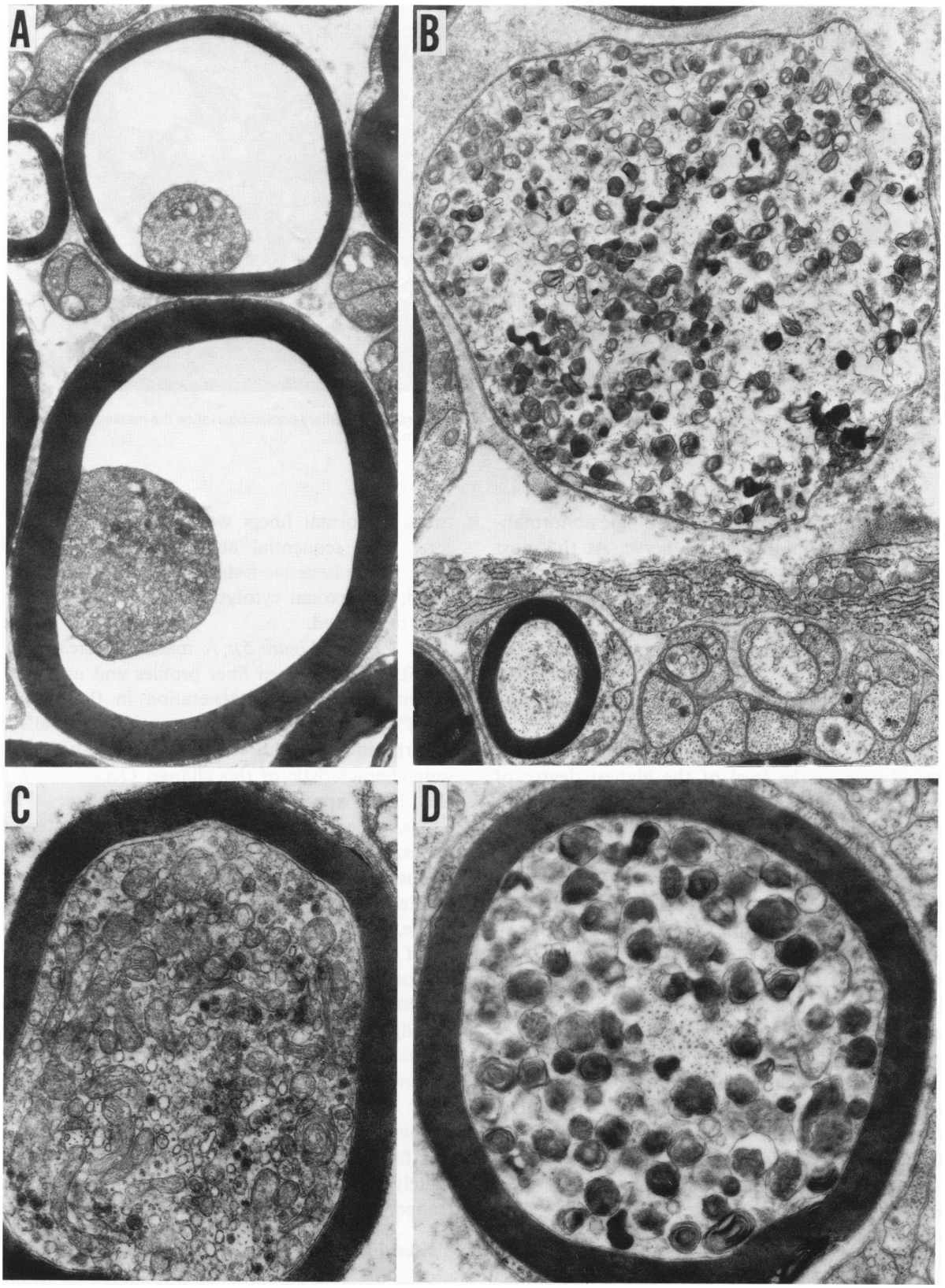

Figure 8—Transverse electronmicrographs of "dark axons" from rat sciatic nerve after microsphere embolization. **A**—Enlarged MF with distension of myelin sheath and shrunken eccentric axon 18 hours after injury. (×7500) **B**—Accumulation of organelles in demyelinated fiber 18 hours after embolization. (×7700) **C**—Accumulated mitochondria and vesicles 18 hours after microsphere injection. (×19,400) **D**—Accumulation of dense and membranous bodies in axoplasm 48 hours after embolization. Notice the normal neurofilaments and microtubules at the center of the axoplasm. (×16,400)

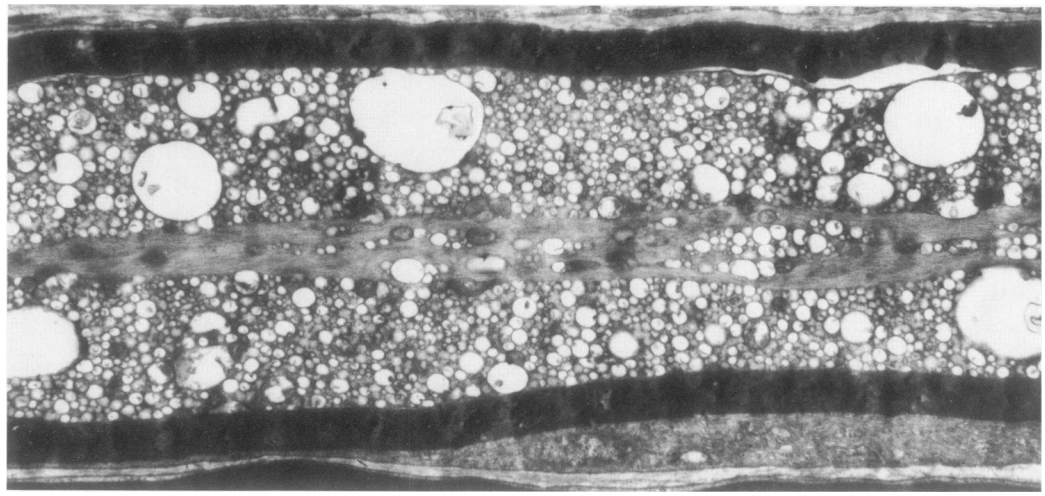

Figure 9 — Longitudinal electron micrograph of "dark axon with light cores" 18 hours after capillary occlusion. Notice the marked accumulation of vesiclelike profiles with relative sparing of the central regions of the axoplasm. (×5800)

degrees of similar, but milder, pathologic abnormalities at more proximal levels of nerve. At the most proximal level of the "ischemic core," the only pathologic abnormalities encountered were a small central fascicular grouping of fibers having "dark axons" and "dark axons" with light cores." At more distal levels of the same lesion "demyelinated axons" and "attenuated axons" predominated (Figures 2 and 3A and B).

Coagulative necrosis of all tissue elements was not observed even at the level of the highest degree of fiber destruction. Nuclear profiles and numbers seemed reasonably preserved.

Temporal Profile of Pathologic Changes

At 6 Hours: No nerve fiber abnormalities were recognized despite capillary occlusion by microspheres.

At 12 Hours (Figures 3A and B): The "ischemic core," although not clearly delineated, could be recognized by rarification of fibers and the presence of "dark axons," "dark axons with light cores," "attenuated axons," and "demyelinated axons." Occasionally, flattened myelin profiles, empty axons, and "tubular" profiles were also seen.

At 18, 24, and 48 Hours (Figures 3C and D and 4): At these times, the changes described above were especially well developed. There were many "dark axons," "dark axons with light cores," "attenuated axons," "demyelinated axons," and axons with thin myelin at the proximal and lateral border zone of the "ischemic cores." Particularly at 24 and 48 hours, flattened myelin profiles, clear axon contents, "tubular" profiles, and decreased fiber density and edema between abnormal fibers were seen in the "ischemic core." The sequential alterations of unmyelinated fibers in the ischemic lesions were not critically evaluated, but axonal cytolysis and decreased numbers were recognized.

At 7 Days (Figure 5): A marked decrease in the density of myelinated fiber profiles and unequivocal evidence of axonal degeneration in the "ischemic cores" were observed. Myelin ovoids and balls and hyperplastic Schwann cell cytoplasm within a nerve were characteristic of this change. Occasionally, myelin debris appeared to be in macrophages. "Dark axons," "dark axons with light cores," "attenuated axons," and flattened myelin profiles were much less frequent than at the earlier times studied. The proximal end of the "ischemic core" usually showed a single, small cluster of degenerating fibers at the center of the fascicle, but sometimes multiple, small clusters of degenerating fibers were seen (Figure 6A). In two rats, ischemic fascicular damage was restricted to a wedge-shaped "piece-of-pie" area in which the base of the triangle extended to an arc of the perineurium (Figure 6B).

At 6 Weeks (Figure 7): The former site of the "ischemic core" could readily be recognized because it was filled with small regenerating fibers all approximately 2–5 μ in diameter. Microspheres were still trapped in capillaries.

Teased Fibers

One hundred teased fibers were prepared from the proximal border of the "ischemic core" of the sciatic nerve of 3 rats, 24 hours after embolization. Approximately 60% of fibers showed paranodal segmental

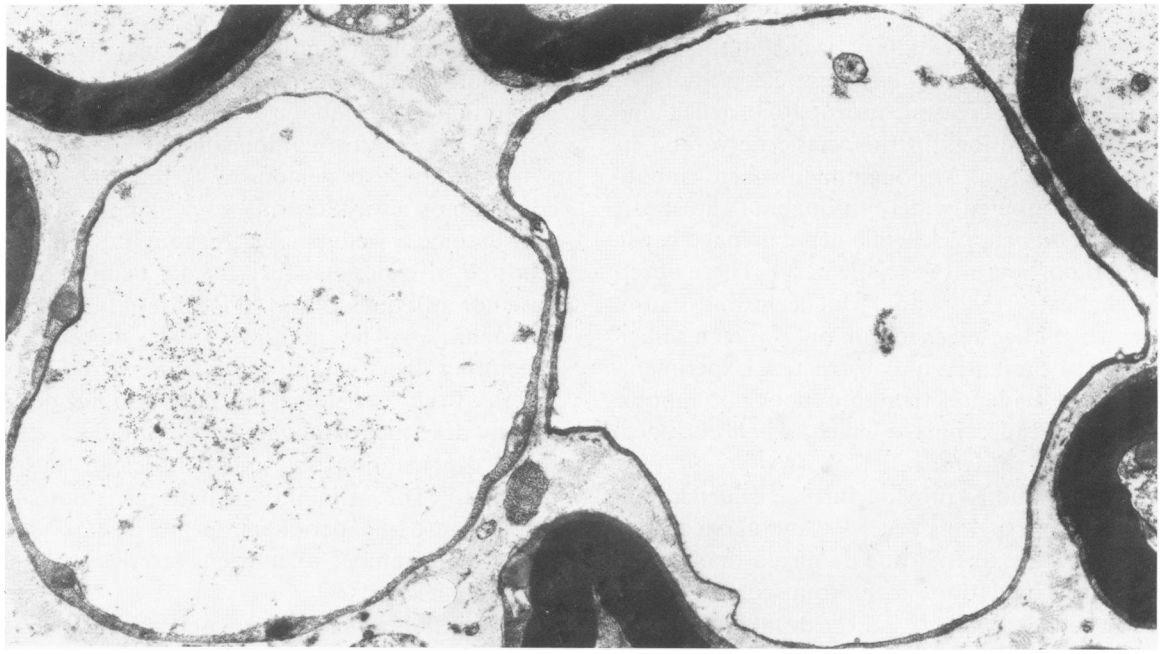

Figure 10—Electron micrograph of "tubular" or "honeycomb" structure 24 hours after microscope embolization, showing remaining rims of Schwann cell cytoplasm and basement membrane with cytolysis of axon, axolemma, and most of the myelin. (×6400)

absence of myelin for varying distances (condition C), while about 20% showed early axonal degeneration (condition E).[47]

Ultrastructural Features

The "dark axons" encountered under the light microscope consisted of axons filled with vesicular profiles, vacuoles, dense bodies, mitochondria, membraneous bodies, and multivesicular bodies (Figure 8). Variation in the number and type of accumulated organelles were noted among individual fibers. The "dark axons with light cores" had central (spherical) regions of more normal appearance which contained microtubules, neurofilaments, and other organelles (Figure 9). The "demyelinated axons" were often surrounded by a thin perimeter of Schwann cell cytoplasm but exhibited similar ultrastructural features to the "dark axons" described above. Myelinated fibers with "attenuated axons" showed either an enlarged periaxonal space, which sometimes contained variable electron-dense material or myelin debris, or separation of the inner mesaxon from the innermost myelin major dense line. The latter was less common. The axons were attached to one part of the inner perimeter of the myelin sheath and often showed organelle accumulation (Figure 8A). The structure of myelin appeared to be normal in those fibers with flattened, collapsed, or boomerang-shaped profiles. However, the axoplasm lost its normal appearance and was watery or floccular. The "tubular" and "honeycomb profiles had the same diameter as myelinated fibers but were found to consist of one of the following (Figure 10): 1) basement membrane without other cellular contents or 2) basement membrane with a ring of Schwann cell cytoplasm with or without a few outer layers of myelin or sometimes with myelin ovoids in the cytoplasm. The "tubular" and "honeycomb" profiles are, therefore, the basement membrane and outer Schwann cell cytoplasmic remnants of myelinated fibers from which the axon and myelin have disappeared because of ischemic damage.

Discussion

Polystyrene microspheres have been used to study the effect of vascular occlusion in heart, kidney, lung, gut, brain, retina, and muscle.[32] By choice of the appropriate size to plug capillaries and by injecting the arteries of supply to sciatic nerve, it has been possible to produce a model of selective ischemic damage of the sciatic nerve without arterial occlusion and without development of foot or leg gangrene. The favorable characteristics of this model include the following: capillaries alone are occluded; their sites can be identified; the ischemic effect varies with the dose, and reproducible focal ischemic fiber degeneration of sciatic nerve can be induced.

An important finding of our study concerns the relationship of the site of capillary occlusion to ische-

mic nerve fiber degeneration. A small dose was found to result in widespread capillary occlusion, but nerve fiber degeneration was not demonstrated. With use of higher doses, an increasing neuropathic deficit and typical ischemic lesions in the sciatic nerve and its branches were shown. Although microsphere embolization was distributed widely throughout the sciatic nerve and its branches, ischemic nerve damage began in the distal portion of the sciatic nerve. These observations indicate that capillary occlusion causes ischemic nerve fiber degeneration only when a sufficient number of capillaries are obstructed. Experimental and human evidence, therefore, does not support the idea of an end capillary which, when occluded, can cause ischemic damage.

The present studies provide further evidence that certain levels and regions of the sciatic nerve and its branches are more likely to be damaged than others when the limb circulation is compromised. In this and other models of ischemia,[25,27,28] the distal foot and leg nerves appear to sustain the greatest damage. A second area of vulnerability is the distal sciatic nerve and the proximal aspect of the tibial and peroneal nerves. This presumably is approximately midway between arteries of supply to these nerves.

The central fascicular fiber degeneration extending over a lengthy distance of sciatic nerve with relative sparing of the subperineural fibers, as seen in the present model but also reported in other models of ischemic neuropathy[25,27,28] and in necrotizing angiopathy,[20] might be due to increased vulnerability of the central fascicular fibers or decreased perfusion to this region. Fewer capillaries in these fascicular regions (reported here) may be an anatomic explanation for decreased perfusion in these areas, as has been reported, without quantitation, by Lundborg.[33] Sladky et al,[34] however, have reported no difference in blood flow between superficial and deep portions of the endoneurial fascicle in normal rat sciatic nerve except when blood flow is reduced. The discrepancy may be explained by the fact(s) that 1) the vulnerable levels of the sciatic nerve may not have been studied and/or 2) the difference in blood flow between outer and inner zones may not be revealed except when overall blood perfusion is reduced and when "reserve vessels" are functional. It has been confirmed that these "reserve vessels" can frequently be observed in the normal endoneurium and start to function immediately when the nerve is traumatized.[35] After ischemic injury, a decrease in blood flow through the subperineurial region could be less than that in the central region because of these "reserve vessels." It has been demonstrated that blood flow to the central region of rat sciatic nerve decreased more than that to surrounding regions following femoral artery ligation.[34] Using the same ligation, however, no morphologic changes were found in rat[36] or rabbit[27] sciatic nerves. Mild reduction of endoneurial blood flow may not produce morphologic changes in nerve fibers. It has been suggested that the metabolic demand of peripheral nerve is small relative to the blood flow.[37]

Our study also demonstrates that the "ischemic core" is not always central fascicular in pattern but may assume a wedgeshape. Although not previously reported in experimental ischemic neuropathies, we have not infrequently seen this pattern in angiopathic neuropathy.[20] The multiple small clusters of degenerating fibers within one fascicle at the proximal region of the "ischemic core" have also not been previously described although, in human disease, multifocal degeneration in several fascicles has been reported.[20] The variability of patterns is not surprising, because the peripheral nerve deviates in size, origin, and number of nutrient arteries and anastomatic connections.

The pathologic changes of ischemic nerve fibers at the border zone are clearly different from those in the "ischemic core." At the border zone, "dark axons," "attenuated axons," and "demyelinated axons" were the prominent features, whereas within the "ischemic core," flattened myelin profiles, "tubular" profiles, and axonal cytolysis predominated, particularly at early stages after capillary embolization. "Dark axons" at the proximal and lateral border zones were seen in normal myelinated or demyelinated fibers and normal, swollen, or attenuated axons. Such focal accumulations or organelles in axons have been described at the border zone after multiple arterial ligation.[26] It is widely accepted that these accummulations may be attributed to selective redistribution of axoplasmic organelles toward the lesion.[38-40] "Attenuated axons" may be the result of decreased neurofilament transport, as has been described following β, β'-iminodipropionitrile administration.[41] With the use of a ligature model, acute attenuated axons have been produced for distances that seem to be longer than can be explained by decreased slow axonal flow.[42] Demyelination has been demonstrated in relatively less severe ischemic models such as after multiple ligation of arteries in the rabbit[27,43] and in human sural nerves of patients with mild rheumatoid neuropathy[44,45] and PVD.[46] Segmental demyelination may be produced in mild cases or border zone regions after acute nerve ischemia.

We consider that pathologic alterations of nerve fibers and Schwann cells can be related to severity of ischemia, anatomic site, and time elapsed since the ischemic injury.

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