THE FINE STRUCTURE OF CEREBRAL FLUID ACCUMULATION

IX. Edema Following Silver Nitrate Implantation

Asao Hirano, M.D.*; H. M. Zimmerman, M.D., and Seymour Levine, M.D.

From the Henry and Lucy Moses Research Laboratories of the Laboratory Division, Montefiore Hospital, New York, and the New York Medical College Center for Chronic Disease, New York, N.Y.

Many forms of intracerebral fluid accumulation have in common a predilection for white matter. Nevertheless, electron microscopic studies have revealed ultrastructural differences in the appearance and distribution of the different types of edema fluid. The present communication concerns electron microscopic observations on edematous white matter following the intracerebral implantation of a necrotizing agent, silver nitrate.

Material and Methods

Pellets composed of a mixture of equal parts by weight of silver nitrate and graphite were implanted in the anterior end of the hemispheric white matter (callosal radiation) in 10 adult female rats. Two rats were sacrificed 1 and 2 days after implantation. Brains were fixed by immersion in 5 per cent glutaraldehyde, buffered with phosphate at pH 7.4. Paraffin embedded sections were stained with hematoxylin and eosin and the periodic acid-Schiff (PAS) luxol fast blue stain. The brains of 2 rats, also killed 1 and 2 days after implantation, were fixed by immersion in glutaraldehyde, followed by immersion in Dalton's fixative, dehydration, and embedding in Epon. The remaining rats were sacrificed in pairs, 1, 2 and 3 days after implantation. These brains were fixed by perfusion with 5 per cent glutaraldehyde in phosphate buffer pH 7.4, followed by immersion in Dalton's solution, dehydration and embedding in Epon. Thick sections from all the Epon embedded material, both immersion fixed and perfusion fixed, were stained with toluidine blue, cresyl violet or silver for light microscopy. Thin sections were cut with a Porter-Blum ultramicrotome, stained with lead hydroxide or uranyl acetate and examined with an RCA EMU-3F electron microscope. Further technical details have been presented before.[1,2]

Observations

Light Microscopy

The site of implantation was marked by a well localized black mass of graphite which served as a chemically inert excipient. It was surrounded

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* Visiting Scientist, Epidemiology Branch, National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service.

by a well demarcated zone of silver infiltrated brain tissue in which almost all vessels, regardless of size, were heavily impregnated with silver.[3] Adjacent brain tissue exhibited perifocal suppuration, severe necrosis, hemorrhage and edema. Pools of eosinophilic and PAS-positive amorphous edema fluid resembling plasma surrounded vessels and extended among the white matter bundles of the homolateral callosal radiation and corpus callosum.[1] Perfusion fixed, Epon embedded specimens revealed distended tissue spaces filled with fluid in the distant white matter. The edema fluid was diffusely and lightly stained with cresyl violet, toluidine blue or silver in contradistinction to the empty and widened vascular lumens (Figs. 1 and 2). The fluid appeared to accumulate around the vessels and extend into the parenchyma, especially into the white matter. The fluid exhibited no layering. There were only a few neutrophils and phagocytes, usually close to the implant.

Electron Microscopy

The outstanding feature of the distant white matter was the presence of abundant fluid. The fluid had a fine, granular texture of moderate electron density, without reticulation or discrete morphologic units (Fig. 3). The texture and electron density varied from area to area, but there was a considerable resemblance to blood plasma. Direct comparison to plasma was impossible in specimens from perfused animals because the vessel lumens though widened were empty. In the vessels of immersion fixed tissues, the plasma varied somewhat in texture and density from one area to another, probably due to different degrees of concentration and preservation. The intravascular plasma appeared to be morphologically identical with the pools of edema fluid (Figs. 4 and 5). In addition, both the plasma and the edema fluid increased in density with either lead or uranyl acetate staining, as did the general background of cerebral tissue. There was no differential staining, with reversal of relative intensities, as has been observed in fluid accumulation rich in polysaccharides.[4]

The fluid was particularly abundant in proximity to vessels in the distant white matter. Vascular basement membranes were infiltrated by the plasma-like fluid which often extended into greatly distended extracellular spaces of the white matter. Perivascular rings of astrocytic foot processes were frequently disrupted. Fluid extended from the vessels to the extracellular spaces through the gaps between adjacent astrocytic processes (Fig. 6). Separation of astrocytic vascular feet was frequently so extensive that only endothelial cells separated vascular lumens from perivascular edema fluid over considerable distances. In areas of most severe fluid accumulation, completely naked endothelial rings were ob-

served surrounded by the pools of edema fluid. Endothelial cells often became very thin and irregularly folded. Direct communication between intravascular plasma and perivascular edema fluid was, however, not observed. Vessels of this sort had indistinct basement membranes. Astrocytes in edematous areas were often watery and voluminous, and contained increased numbers of glycogen-like granules, as seen in other experiments.[5]

Edema fluid spread into extracellular spaces among the myelinated nerve fibers (Fig. 7). Despite wide separation of individual myelinated nerve fibers, the myelin sheaths and outer loops were usually well preserved. Most often, the outer loop of the myelin-forming cells retained its normally close anatomic relationship with the underlying myelin sheath (Fig. 8). In areas with severe edema, however, separation of the outer loop and of the outer myelin lamella was occasionally observed (Fig. 9). Separation occurred at the intraperiod line and was widest in the area of the outer loop; it tapered gradually until the intraperiod line was restored. The inner layers of the myelin sheath usually remained intact. Separation was occasionally not so regular, however, and alternating lamellas were affected (Figs. 9 and 10). In such cases, normally preserved intraperiod lines alternated with intraperiod separations in a regular pattern among the intact major dense lines (Fig. 10). The spaces that resulted from intraperiod separations were not optically empty, but were filled with fluid identical in appearance to the pools of extracellular fluid elsewhere, and direct communication was usually evident.

Occasionally, fibrin and erythrocytes were seen in the edema fluid, especially around vessel walls in the vicinity of the implant (Fig. 11). The silver-impregnated tissue on the margins of the implant appeared coarsely granular and failed to show the usual fine structural preservation, probably because of the coagulation necrosis as well as the partial fixation effect of the implanted silver nitrate. In addition, there were accumulations of granular silver deposits around blood vessels especially in the region of the basement membrane, as described previously.[3]

Discussion

Pools of PAS-positive edema fluid have been observed by Feigin and Popoff [6] in human cerebral edema, and by others in experimental cerebral edema caused by experimental cold injury [7-9] or subcortical implants of various necrotizing agents.[1] In all these cases, edema and increased permeability of the blood-brain barrier were more severe in white matter than in gray matter. The edema fluid surrounding vessels and extending among the myelinated fibers resembled plasma in its staining reactions and appearance. Even electron microscopy failed to reveal

morphologic differences between the edema fluid and plasma. The tendency to accumulate around blood vessels, and the occasional appearance in the edema fluid of fibrin and erythrocytes, also support the hematogenous origin of the edema fluid.

One of the noteworthy features of the present experiment was the separation of perivascular astrocytic foot processes from each other and from the vascular basement membrane. In the most severely involved areas, vessels were often completely stripped of attached glial processes and appeared to float in a pool of edema fluid. This observation can probably be related to the marked increase of permeability of the blood-brain barrier in edematous white matter following implants of silver nitrate. It is interesting to contrast these findings with those obtained after implantation of cryptococcal polysaccharide. Extracellular fluid in white matter following polysaccharide implantation differed from plasma in its reticulated appearance, in the presence of layering, and in the reversal of electron density following heavy metal staining. The astrocytic vascular foot processes were rarely disrupted, and there was relatively little alteration of blood-brain barrier permeability. The polysaccharide-rich fluid did not appear to be of hematogenous origin. Following polysaccharide implantation, only small amounts of hematogenous fluid appeared in the early stages, close to the implant, where mechanical trauma was probably responsible.[4]

In white matter at a distance from the silver nitrate implant, myelinated and non-myelinated nerve fibers were usually well preserved despite the distention of extracellular spaces by edema fluid. The occasional alterations observed in myelin sheaths are of considerable interest. These findings are quite unlike the large clefts within myelin sheaths illustrated by Aleu, Katzman and Terry in triethyltin intoxication of rabbits.[10] Our observations are more similar to those of Lampert, who found a regular widening of the space between myelin lamellas in rats with allergic encephalomyelitis.[11] Our material, however, had less regular separation as well as alternating separation, and the spaces were filled with edema fluid in continuity with the extracellular fluid; no mononuclear cells were adjacent to the altered myelin sheaths in our experiments.

These ultrastructural studies of the various types of cerebral edema have revealed differences not only in the nature and origin of the fluid, but in its effect on the myelin sheaths in areas of involvement.

Summary

Implants of silver nitrate in the rat brain produced local necrosis and widespread edema of white matter associated with increased permeability of the blood-brain barrier. Electron microscopic examination of the

edematous white matter demonstrated pools of moderately electron dense fluid which infiltrated the basement membranes, filled the perivascular spaces and extended into the distended extracellular spaces. The texture of the fluid was indistinguishable from blood plasma. The perivascular ring of astrocytic vascular feet had discontinuities, with direct communication between vascular basement membrane and extracellular fluid. In severely edematous areas, blood vessels floated in pools of edema fluid, completely stripped of attached glial processes. In the distal portion of the involved white matter, myelinated and nonmyelinated nerve fibers were usually well preserved. Occasionally, however, there was separation of outer loops and outer myelin lamellas with extension of edema fluid between the major dense lines of the myelin sheath.

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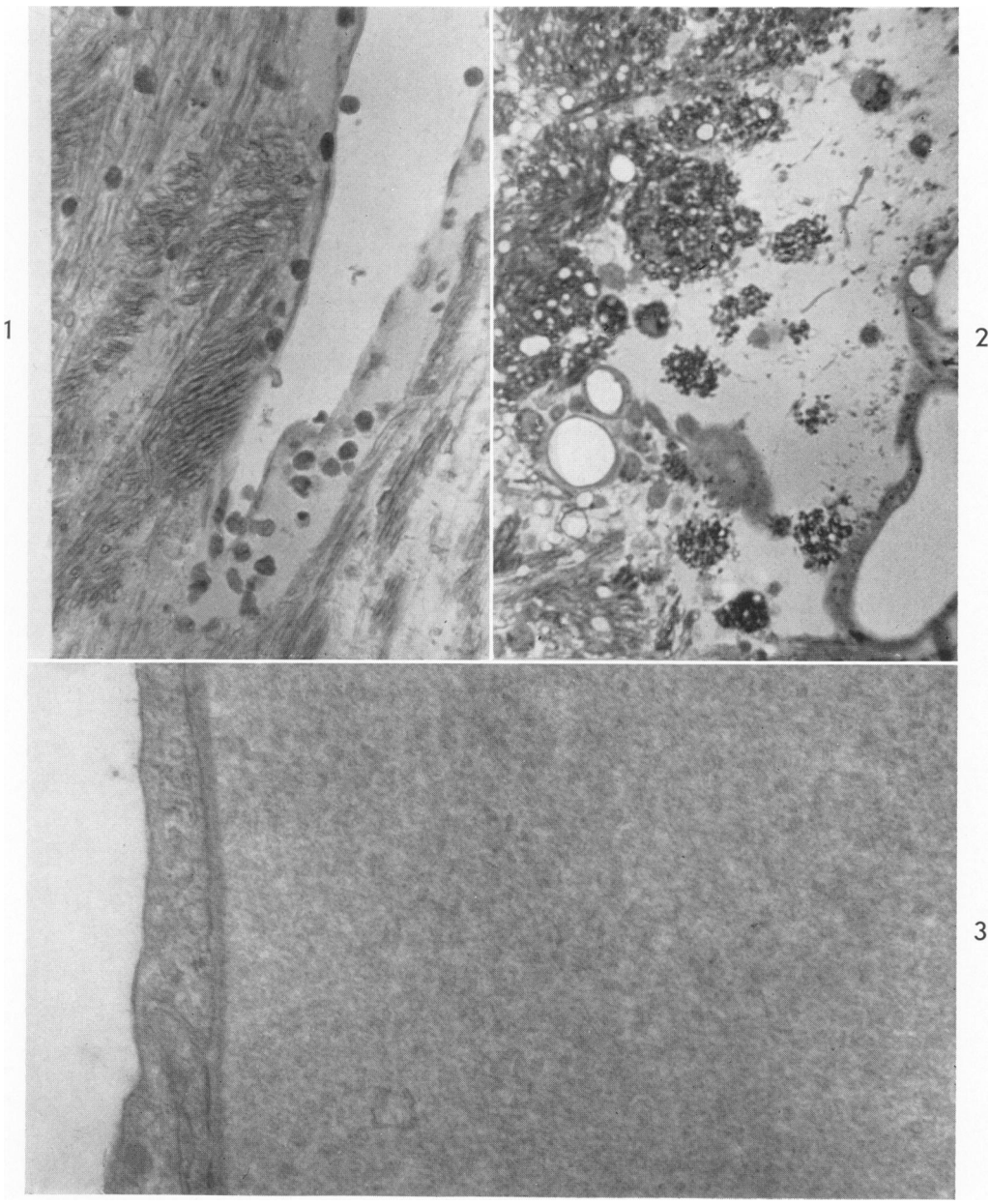
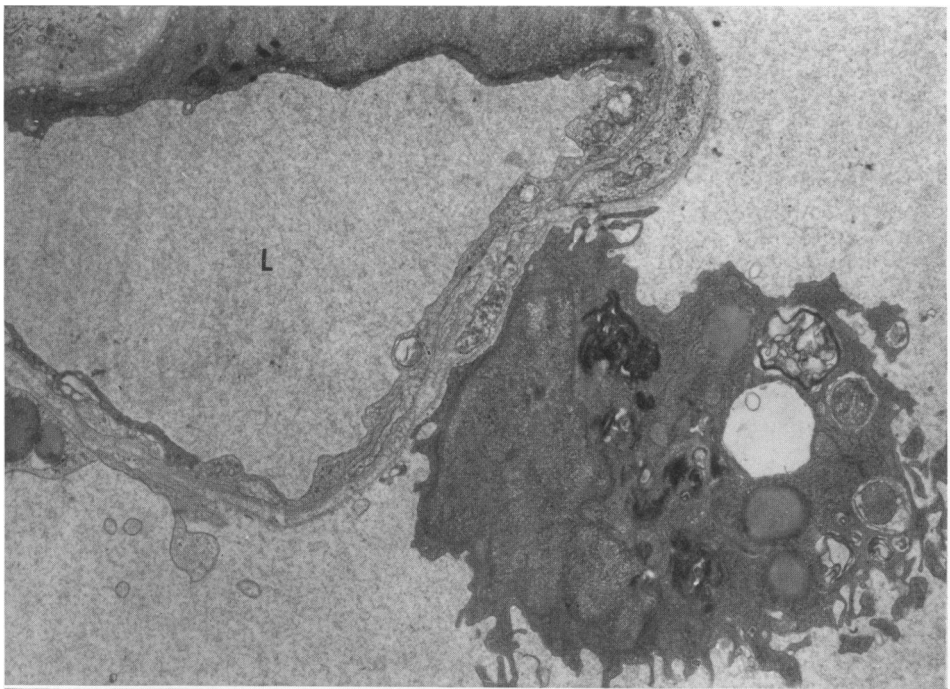
Legends for Figures

Figs. 1 and 2. Callosal radiation, 24 hours (Fig. 1) and 72 hours (Fig. 2) after implantation. Lumens of vessels are clear and empty as a result of perfusion. Perivascular regions and white matter contain accumulations of edema fluid. Epon embedding, cresyl violet stain. × 460.

Fig. 3. A portion of a vessel wall in the callosal radiation, 72 hours after implantation. On the left is the lumen, empty because of perfusion. On the right is a pool of edema fluid. × 33,500.



FIGS. 4 and 5. Blood vessels floating in edema fluid in white matter, 48 hours after implantation. Immersion fixation. Edema fluid appears identical to the plasma within the lumen (L) of the vessel. A phagocyte is seen in Figure 4 (lower right). Figure 4, × 9,000; Figure 5, × 29,000.



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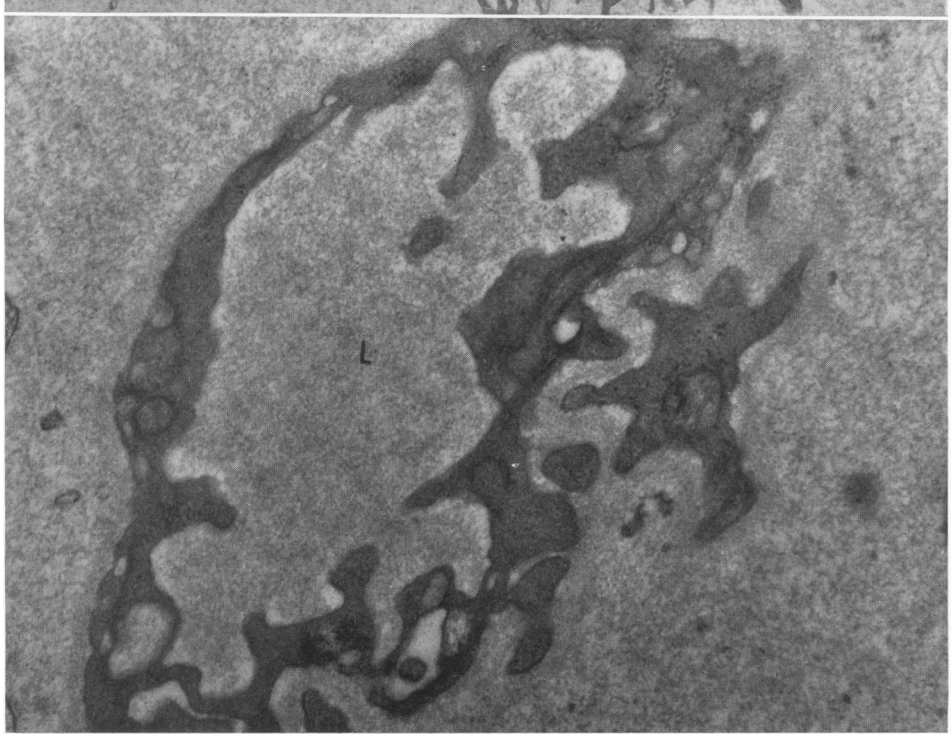

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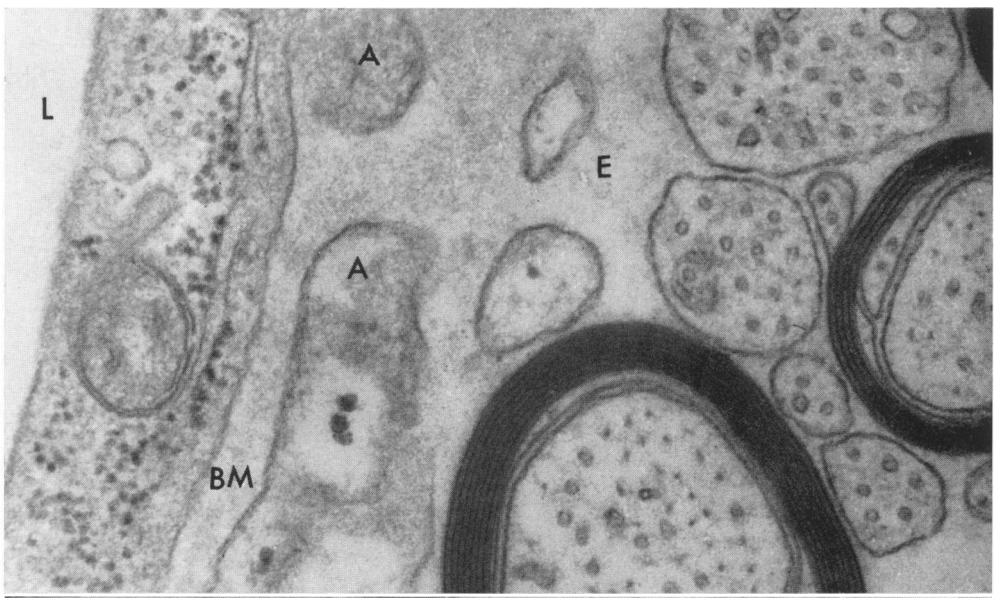

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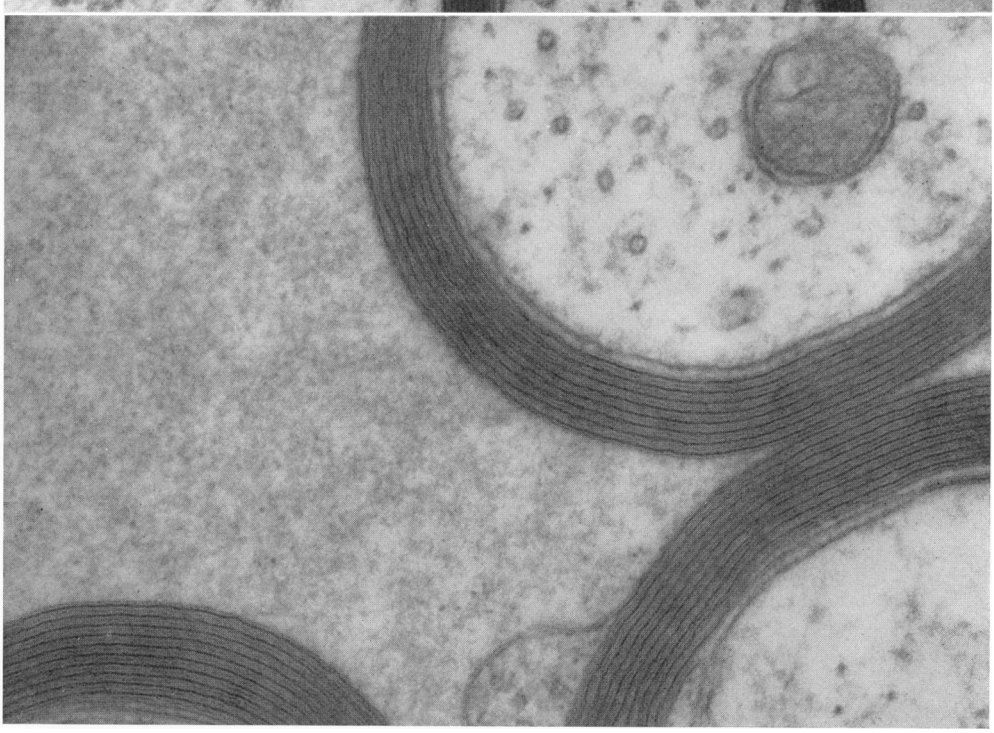

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FIG. 6. Vessel wall (left) and adjacent callosal radiation, 48 hours after implantation. Perfusion fixation. The basement membrane (BM) is indistinct as the result of infiltration with edema fluid; it communicates freely with the distended extracellular spaces (E) through the gap between adjacent astrocytic processes (A). × 73,500.

FIG. 7. Callosal radiation, 48 hours after implantation. Electron dense fluid fills distended extracellular spaces among myelinated nerve fibers. × 99,200.

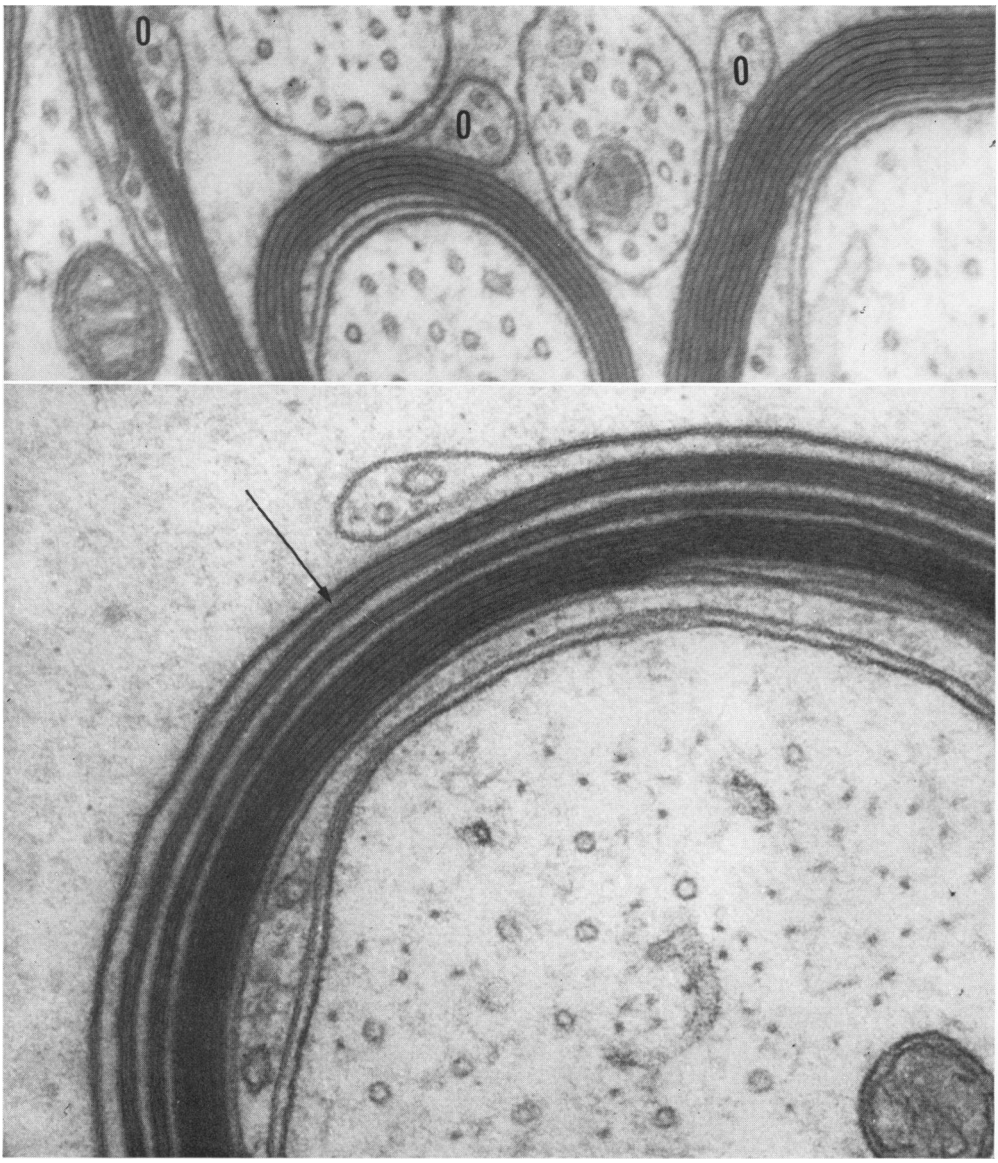

FIG. 8. Callosal radiation, 48 hours after implantation. Three outer loops (O) are evident in three myelinated nerve fibers. The outer loops of myelin-forming cells retain their normal close anatomic relation without separation from myelin lamellas. × 80,000.

FIG. 9. A portion of a myelinated nerve fiber in severely edematous white matter 48 hours after implantation. Separations of outer loop and outer myelin lamellas are seen. Widened space between major dense lines is filled by electron dense fluid. A point of fusion, with restitution of an intraperiod line is indicated by an arrow. × 105,000.

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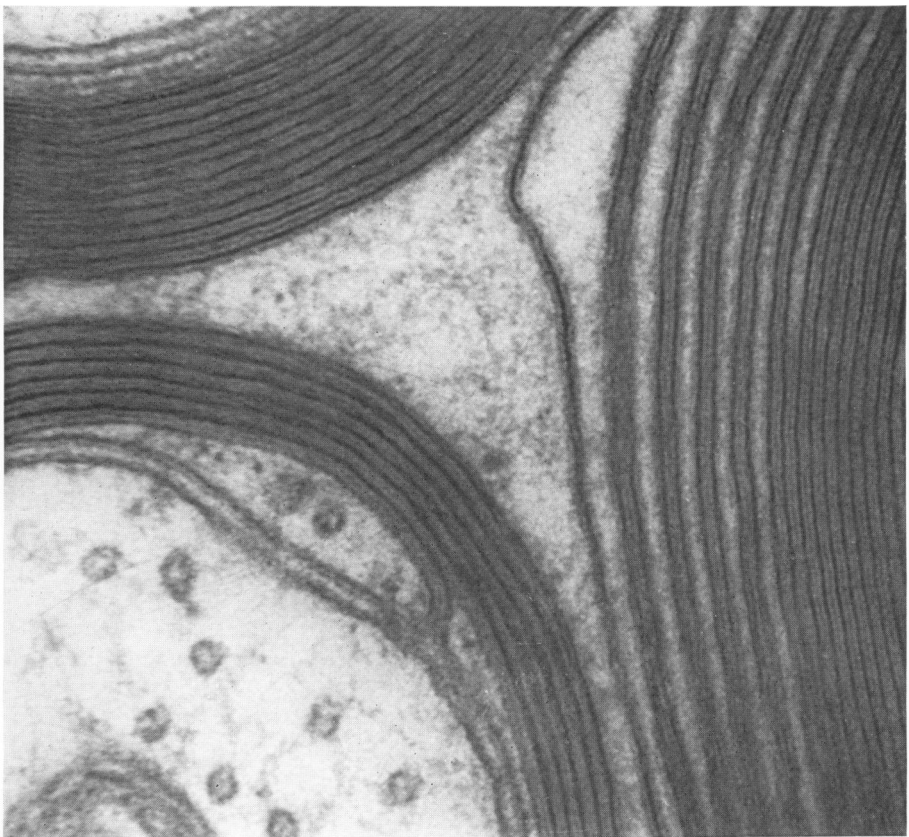

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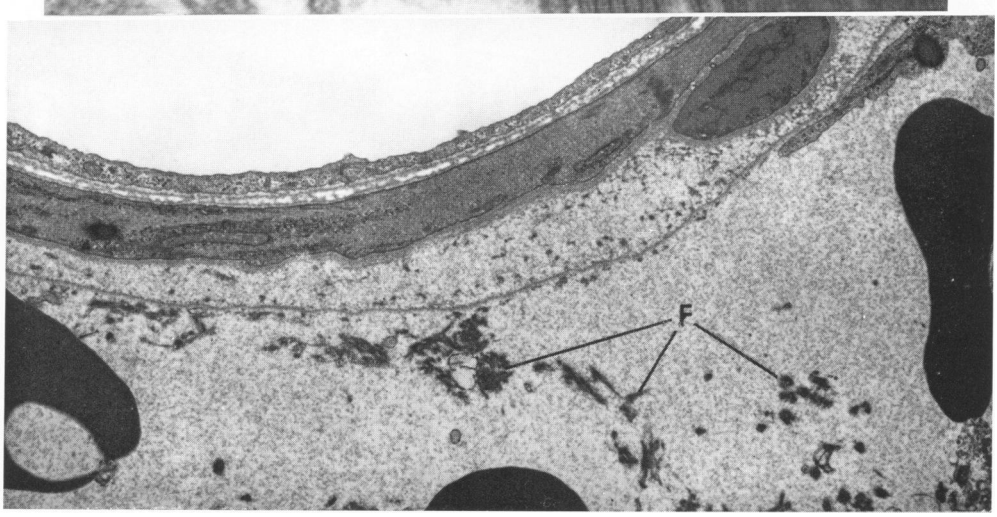

FIG. 10. Callosal radiation, 48 hours after implantation. A triangular space formed by three adjacent myelin sheaths is distended and filled with fluid. An alternating separation of outer myelin lamellas is seen. × 174,000.

FIG. 11. A vessel near the implant, 48 hours after implantation. The vessel wall is surrounded by plasma-like fluid in which scattered fibrin aggregates (F) and parts of three red cells are seen. × 9,000.