Electron Microscopic Study of Demyelination in an Experimentally Induced Lesion in Adult Cat Spinal Cord*

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ABSTRACT

Plaques of subpial demyelination were induced in adult cat spinal cords by repeated withdrawal and reinjection of cerebrospinal fluid. Peripheral cord was fixed by replacing cerebrospinal fluid available at cisternal puncture with 3 per cent buffered OsO₄. Following extirpation, surface tissue was further fixed in 2 per cent buffered OsO₄, dehydrated in ethanol, and embedded in araldite.

Normal subpial cord consists mainly of myelinated axons and two types of macroglia, fibrous astrocytes and oligodendrocytes. Twenty-nine hours after lesion induction most myelin sheaths are deteriorating and typical macroglia are no longer visible. Phagocytosis of myelin debris has begun. In 3-day lesions, axons are intact and their mitochondria and neurofibrils appear normal despite continued myelin breakdown. All axons are completely demyelinated by 6 days. They lack investments only briefly, however, for at 10 and 14 days, macroglial processes appear and embrace them. These macroglia do not resemble either one of the normally occurring glia; their dense cytoplasm contains fibrils in addition to the usual organelles.

It is proposed that these macroglia, which later accomplish remyelination, are the hypertrophic or swollen astrocytes of classical neuropathology. The suggestion that these astrocytes possess the potential to remyelinate axons in addition to their known ability to form cicatrix raises the possibility of pharmacological control of their expression.

INTRODUCTION

Histological studies have established that a neurological lesion may occur in spinal cord and medulla of adult cats subjected to repeated withdrawal and reinjection of cerebrospinal fluid (1). The neurological syndrome induced by such cerebrospinal fluid exchange, the topographic extent of the lesion, and the time course of the histopathology may be found in previous publications (1–3). In these studies it was shown that the repeated cisternal withdrawal and reinjection of cerebrospinal fluid may produce a neurological deficiency expressed in varying degrees of posterior extremity paralysis. Neuropathological studies demonstrated demyelination in a shallow band of white matter in the periphery of the upper spinal cord and lower medulla. In animals sacrificed during later stages there was evidence of regenerative phenomena in these areas, and these regenerative changes were attended by an improvement in the neurological condition of the animal.

It was clear from light microscopy that myelinated axons appeared in areas of white matter which in earlier stages had been completely demyelinated. It was not clear, however, whether these myelinated axons were fibers which had retained their continuity for a time free of myelin sheaths and had been subsequently remyelinated, or...
whether they represented a regrowth of anatomically interrupted axons. Absence of ascending or descending degeneration from the area of the lesion suggested that the axons remained intact. When it became apparent that the lesion was, indeed, a demyelination without axon interruption, the study assumed added significance in relation to the pressing problem of understanding the naturally occurring demyelinating conditions.

In certain human demyelinating diseases, notably multiple sclerosis, it has been generally agreed that some myelin sheaths are selectively destroyed leaving many axons traversing the characteristic plaque free of enveloping myelin (4). No experimental correlate of this phenomenon in central nervous system tissues has been established and, therefore, it has not been systematically studied. The kindred question of whether the myelin sheath can regenerate provided the axon remains viable has been raised (4–6). Lichtenstein (5) has stated that he does not believe such sheath regeneration can occur. Again, the experimental model for answering this question did not exist. For these reasons, an electron microscopic study of this lesion seemed advisable despite the fact that the mechanism of damage by cerebrospinal fluid exchange is unknown.

This paper, then, presents an electron microscopic study of changes occurring during an in vivo process of demyelination in the white matter of adult cat spinal cord. The process described is a true demyelination; i.e., the majority of axons remain intact during all phases of the lesion. The axon is first demyelinated, exists for a time free of ensheathing myelin, and is subsequently remyelinated by glial cells. The present description is concerned with the remyelinating phase of the lesion up to the point at which remyelination begins. The ultrastructural aspects of cellular mechanisms of remyelination will be presented in a subsequent paper (7). A third study, utilizing autoradiographic methods to follow incorporation of nucleic acid and protein precursors into cellular constituents, will be concerned with the metabolic changes in the glial cells during demyelination and remyelination (8).

Materials and Methods

Cats of either sex, weighing 2.0 to 3.3 kg, and therefore considered to have reached adulthood, were selected for this study.

Production of Lesions:

Lesions were produced by repeated withdrawal and reinjection of cerebrospinal fluid (c.s.f.). The method described previously (1) was modified to yield a more standardized procedure and a higher percentage of histologically useful lesions (43 per cent). Following intraperitoneal injection of sodium pentobarbital (42 mg per kg body weight), the animal was placed in a chamber and its head positioned in a holder. After sterile cisternal puncture, the needle was connected to a reservoir syringe outside the chamber with polyethylene tubing. The chamber was closed. By pumping air into the chamber and adjusting outflow, pressure was raised to +15 mm Hg within the chamber. This maneuver caused about 3 ml c.s.f. to flow slowly into the reservoir syringe. After 5 minutes of withdrawal, the chamber pressure was lowered to -20 to -25 mm Hg by pumping air out of the chamber and adjusting inflow. This reversal of pressure caused return of c.s.f. to the cisterna magna. Reinjection was stopped when completed or at the end of 5 minutes. This cycle was repeated 9 more times.

Electron Microscopy:

Fixation.—Animals were sacrificed at 29 hours, 3, 6, 10, and 14 days postoperative (d.p.o.). (Radioactive tracers had been injected intrathecally into the 1, 3 and 14 d.p.o. animals a few hours prior to sacrifice for autoradiographic study (8).) Spinal cord from normal animals was also fixed. After considerable experimentation, the most satisfactory method of fixing the peripheral spinal cord was found to be the following. A needle was inserted into the cisterna magna and connected to a syringe with polyethylene tubing. Using gentle aspiration, all readily available c.s.f. was withdrawn. A similar or slightly larger volume of veronal acetate buffer (pH 7.5) containing 3 per cent osmium tetroxide (OsO4) was then injected into the subarachnoid space. This increased concentration of OsO4 was arbitrarily chosen to compensate for dilution of the fixative by c.s.f. Respiratory movements of the animal stopped immediately. A cycle of withdrawing and reinjecting fixative was accomplished 3 times to facilitate its circulation within the intrathecal space. Within 30 to 45 minutes, a portion of cervical spinal cord (cervical roots 2 to 8) was extirpated and its surrounding dura mater removed. At this time, the outer surface of the cord is black as is a shallow area just beneath it all around the cord. This length of cord was further fixed in buffered 2 per cent OsO4 for 30 minutes at 4°C. Better fixation of myelin sheaths in normal cord was obtained in later experiments with Dalton's fixative (9) which contains K2Cr2O7 in addition to OsO4. (Two, then 1 per cent OsO4 was used.) Only a very shallow area of tissue around the periphery of the spinal cord is fixed but it is within this area that the lesion is located.

After rinsing in 70 per cent ethanol, the cord was
divided into 3 segments and a portion of each segment examined by frozen section to locate the lesion. The remainder of each segment was divided into transverse slices designated alternately for electron microscopy, light microscopy, and, in some cases, autoradiography. The tissue for electron microscopic study was dehydrated in graded concentrations of ethanol as usual. During dehydration in 80 per cent ethanol, very small wedges of blackened tissue were cut from desired areas and representatives of anterior, lateral, or posterior columns kept separate.

Embedding and Sectioning.—A comparison of methacrylate and araldite embedding of this tissue showed that araldite gave superior results and so it was employed exclusively. Advantages of araldite are less shrinkage, more uniform polymerization, and, after polymerization, stability in the electron beam (10, 11). At the time this project was started, components of the mixture listed by Glauert et al. (10) were not marketed in this country; therefore, a mixture suggested by the Ciba Company was substituted. The technique (12) was modified by us to improve penetration of the monomer and polymerization within the tissue. This method is outlined in Table I. White to light gold sections, cut on a Servall Porter-Blum microtome, were placed on grids previously coated with formvar or carbon films, or carbon films with large holes. In thicker sections, there was adequate contrast for useful light microscopic study.

**TABLE I**

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Staining.—Mounted thin sections were stained with aqueous uranyl acetate (13) or lead hydroxide (14) or a combined mixture of phosphotungstic acid and uranyl acetate in 70 per cent ethanol (15). Consistently best results were obtained by immersion of the grid into freshly filtered aqueous 7.5 per cent uranyl acetate for 2 hours at room temperature. Especially good contrast was obtained when stained sections were observed over a hole in the carbon film (Figs. 1 to 5, 12, 13, 16). A good quality section was stable enough in the electron beam for successful photography even if supported only at the 4 corners of a grid opening. This is in agreement with one of the advantages of araldite noted above.

Microscopy.—Sections were examined in an RCA EMU-2 (Fig. 9), a Siemens Elmiskop I (Figs. 6, 7, 11), or Elmiskop IIb (Figs. 1 to 5, 8, 10, 12, 13, 15, 16). Ilford N40 or Gevaert Scientia 19 D 50 plates were used, with development in D 19. Initial magnifications of micrographs presented here range from X 1400 to 7200.

Light Microscopy:

Transverse slices of cord were fixed in either formalin-Tyrode's or formal-calcium (16) solution following the hour-long OsO4 fixation mentioned above. After dehydration and embedding, sections were permanently mounted as no further staining was necessary. Additional 1, 3, and 14 d.p.o animals, given radioactive materials, were sacrificed by whole body perfusion with formalin-Tyrode's solution. Hematoxylin and eosin, Weil's, and thionine-stained sections from these animals were also examined.

**OBSERVATIONS**

Normal (4 Cats):

The normal tissue was from areas comparable to those chosen for investigation of the lesion. The following description, then, refers only to subpial white matter of spinal cord.

A general view of normal subpial white matter appears in Fig. 1. Axon diameters vary, the degree of variation depending upon the location within the spinal cord. This variation is less pronounced in the pictured posterior columns than in lateral and anterior columns. The neurofibrils and mitochondria are seen in cross-section for they lie parallel to the longitudinal axis of the axon. The arrangement of the cristae mitochondriales can be seen in Fig. 4. The finding that these membranes are often parallel to the long axis of the mitochondrion rather than perpendicular to it concurs with previous descriptions of these organelles in neurons (17, among others). Small vesicles, probably representing endoplasmic reticulum, are sparsely scattered throughout the axon. Only a few small

1 The authors wish to thank Dr. Paul Kaesberg, Department of Biochemistry, University of Wisconsin, for the privilege of using this microscope and for technical advice.
The myelin sheaths vary in contour and thickness, the thicker sheaths surrounding the larger axons. Characteristic lamellae of the myelin sheath are visible in these preparations at higher magnifications. The axolemma follows closely the inner aspect of its surrounding sheath except in the area of the inner mesaxon. Internal mesaxons and external loops or tongues as described in amphibian central nervous system by Maturana (18) and Peters (19) are consistently seen. Illustration and discussion of these structures will appear in the description of remyelination. A few myelin sheaths contain cleft-like disruptions in the regular lamellar arrangement and, within such areas, irregular particles of varying size and density are characteristically present (Fig. 3). Similar disrupted areas containing these characteristic particles are visible when the sheath tapers to expose the axon in an occasional node-like profile. These cleft- or node-like interruptions seen in longitudinal section may account for the occasional sheath discontinuities noticeable in cross-section (Fig. 5). These disrupted areas are not due to poor fixation; when a myelin sheath is poorly preserved, only intermittent splitting is visible (arrows, Fig. 1).

The most prominent of the macroglial processes generally contain little more than dense bodies, bundles of fibrils, and granules (Fig. 5) and so are considered to be processes of fibrous astrocytes. These are the processes which fill most of the area not taken up by myelinated axons and cell bodies. These fibrous processes, which can sometimes be followed for a considerable distance, group axons into fascicles. Every visible bundle of fibrils is enclosed within a cell process. Fibril-containing processes lie, in varying numbers, between the pial membrane and the subjacent myelin sheaths forming an astrocytic sheath around the outermost aspect of the cord, perhaps analogous to a sheath of contiguous astrocytic processes in subpial rat cortex (20, 21). Fibrous astrocyte cell bodies, from which processes are seen to emerge and run interiorly, are also found at the cord surface. Astrocytic nuclei appear elongated and irregular in outline (Fig. 1). A nucleolus is occasionally visible. The cytoplasm of the cell body contains a small amount of endoplasmic reticulum, the membranes of which are studded with granules, clusters of granules presumed to be ribonucleoprotein particles, regions of small agranular vesicles considered to be Golgi areas, and, as in the processes of this cell type, dense bodies and bundles of fibrils (Figs. 2, 3). Many fibrils lie near the nucleus and some even indent it. The fibrils, visible in unstained as well as stained sections, average about 100 A in diameter. The dense bodies are generally round or oval in shape in transverse or longitudinal section but occasionally exhibit an elongated and irregular outline. They vary greatly in size and density (Fig. 3). It is difficult to decide which of these inclusions are mitochondria for the identifying internal membranes so clearly seen within neighboring axonal mitochondria are not apparent. Identifiable mitochondria within processes of cortical fibrous astrocytes have been demonstrated (22).

One other type of macroglia, the oligodendrocyte, is present (Fig. 1). Some characteristics observed in electron micrographs correspond to those known from light microscopic work; i.e., they are seen to lie adjacent to each other in longitudinal section and, in contrast to the fibrous astrocyte, the nucleus is round to oval rather than generally irregular in shape, cell size is smaller, and fewer processes emerge from the cell body. In cross-sections of many oligodendroglia, only a moderate amount of cytoplasm surrounds the nucleus. A nucleolus is seen occasionally. Mitochondria, Golgi complex, grouped particles presumed to be ribonucleoprotein granules, and granule-covered membranes, comprising cisternae of endoplasmic reticulum, are contained within the cytoplasm. Many of these cisternae, of roughly similar length, are oriented parallel to one another such that 2 to as many as 15 of them may be adjacent. Also present are peculiar spindle-shaped or ovoid bodies containing a central area of low density (Fig. 4). The few stout processes of oligodendroglia can be readily distinguished from the large number of slender fibrous astrocyte processes by the lack of fibrils, the contained packets of cisternae, spindle-shaped bodies, and a more dense cytoplasm generally. Additional consideration of identification of these macroglia is included in the discussion.

It appears that microglia are generally absent from this area of the spinal cord. At any rate, neither cells like those considered to be microglia by Luse (23), Farquhar and Hartmann (20), or Schultz et al. (24), nor non-macroglial cells containing phagocytosed inclusions were observed.
That fewer cells of this type reside in white matter than in gray matter is known (25).

Very few cells are seen in the pia mater in contrast to the pia which overlies lesion tissue. In agreement with Watson's report (13), electron density and banding of collagen were greatly intensified by staining.

The Lesion:

The number of cats from which satisfactory preparations for ultrastructural study were obtained is indicated for each time interval. The appearance of the lesion in different animals sacrificed at the same time interval showed little variation.

Lesion, 29 Hours Postoperative (2 Cats).—A few axons are completely demyelinated 29 hours after completion of c.s.f. exchange (Fig. 6). That they were previously myelinated is certain for their size is larger than those which normally lack myelin sheaths, they are not located immediately beneath the pial membrane, and tell-tale, thread-like remnants of former sheaths often surround them (Fig. 6). The circumferential outlines of some of these axons have become irregular and the axons appear to be more dense than their myelinated companions suggesting that these axons have undergone some dehydration. Neither neurofibrils nor mitochondria manifest noticeable morphological change, however, suggesting continued viability despite demyelination and shrinkage.

While the majority of axons are still ensheathed, their myelin investments are deteriorating (Figs. 6, 7). That sheath breakdown starts within such a short time was observed in the preceding light microscopic study (1). In some of these axons, there is an increase in the number of vesicles seen in cross-section and vacuolated formations appear in the axolemmal region. The demyelinating axons appear normal in size and shape, or irregular in outline and slightly reduced in size, or markedly shrunken. In some instances, an area of low density appears between the axolemma and the deteriorating sheath. A possible explanation for this observation is that during myelin breakdown there is an increase in osmotic pressure in this area due to formation of a larger number of smaller molecules and so fluid is drawn from the axon into this area. Many times an axon is found within a "sheath" of much greater diameter, a few "lamellae" stretching between them. Following electron microscopic examination of many different pieces of tissue, two axons in which mitochondria had greatly increased in number were seen. One axon had been completely demyelinated while the other one was undergoing demyelination.

The characteristic appearance of myelin breakdown in this lesion is shown in Fig. 6. Layers of varying thickness split apart more or less regularly, the edges of these layers yielding subsequently to a honeycomb- or alveolar-like pattern of dispersion. This pattern is distinctly different from that which follows inadequate fixation or characterizes occasional sheath discontinuities in comparable normal areas. Breakdown may start within or at the inner or outer surface of the sheath. Additional investigation is needed to determine whether myelin breakdown results from splitting of the major dense or intraperiod lines or by some other process. Serial sections reveal that local enlargements shift gradually about the circumference of the deteriorating sheath. Irregularly shaped, highly dense particles, ranging from about 50 to 200 A in size, appear at a certain stage in breakdown of myelin in unstained as well as stained preparations. These may be liberated lipid droplets.

The lesion is markedly edematous regardless of how much demyelination has occurred. Products of myelin degeneration fill much of this extracellular space. Although extracellular space has not been observed in any of the ultrastructural studies of normal central nervous tissue, the work of Schultz and Pease (26) as well as the present observations indicate that extracellular space may develop rapidly in pathological conditions. It is likely that this space results from degeneration of glial processes as Schultz and Pease also suggest.

Some of the products of myelin breakdown have already been phagocytosed by cells. Typical of phagocytes, these cells display long, sheet-like processes. Such processes may embrace the entire axon and its ensheathing myelin which is removed from the axon after its fragmentation (Fig. 7). Or, they may spiral around one object as many as 3 times, and as many as 6 different processes may converge on the same object. Or, they may invade the degenerating sheath itself. Degenerating cells, no longer identifiable, appear within the phagocytes, too. Not as many nuclei of phagocytosing cells contain nucleoli as in later stages. The cell bodies from which the long, sheet-like processes extend seem to be of at least two types or stages—some cells, a few of which contain phagocytosed
material, are similar to the many macrophages observed in the pia mater at this stage; other cells resemble lymphocytes (Fig. 7). Cells containing only a small amount of phagocytosed substance do differ in appearance. Nevertheless, all cells containing ingested material within their cytoplasm will henceforth be labeled “gitter” cells, the name for phagocytes of the central nervous system.

Whether or not gitter cells derive from microglia cannot be stated at this time. Whereas no microglia fitting previous description (23, 20, 24) exist in the lesion at this stage, these cells are known to undergo morphological change with phagocytic activity (25), thus complicating identification. It seems likely that at least some of the gitter cells originate from macrophages which have migrated from the pia mater into the adjacent lesion area. Many cells appear to be lined up just below the pial membrane in the cord. Many neutrophils, identifiable by characteristic polymorphous nuclei and specific granules, are present in the lesion as well as in the pia and whereas, in a few cases, they assume intimate contact with fragmenting myelin, no debris was ever seen within them. Schultz and Pease (26) suggest that in a puncture wound in rat cortex, phagocytes arise from both microglia and hematogenous macrophages but, as in this investigation, found that determination of the origin of any one gitter cell does not seem to be possible by ultrastructural study alone. Adams (27) has called attention to the similar dilemma facing the light microscopist.

Completely lacking in this array of cellular processes are the extensive macroglial processes, notably those of fibrous astrocytes, so characteristic of normal spinal cord. One manifestation of their absence may be the difficulty with which the lesion is sometimes preserved intact during preparation for light microscopy. Some of the thread-like debris may represent degenerating astroglial fibrils. A few macroglial cell bodies may have survived as rounded and modified forms but the loss of the distinguishing inclusions precludes identification. It is also difficult to say with certainty whether some of the disintegrating cells present at this time were formerly glial cells or some glial cells have turned to phagocytosis. Whether degenerating macroglial cells are phagocytic has long been debated (28). Study of shorter time intervals is needed to learn the fate of the macroglia.

Lesion, 3 Days Postoperative (2 Cats).—Although more axons have been completely demyelinated, disintegrating myelin still invests many axons at 3 d.p.o. (Figs. 8 to 10). As in the earlier stage, the diameters of some axons are much smaller than the “sheaths” encircling them (Fig. 9). Longitudinal sections reveal varying degrees of irregularity in the axonal outline. Adjacent vacuoles within the degenerating myelin can be seen to indent the axon (Fig. 10)—this is, then, one factor contributing to the typical irregular profiles seen in cross-section. Though many axons are tortuous, they are thought to remain continuous because their contained mitochondria and neurofibrils appear normal (Fig. 10) although more concentrated than in axons of normal tissue. That every axon is maintained is questionable; those few which appear much more dense, very small in diameter, and stellate-shaped in cross-section may be degenerating. Comparing the number of axons within identically sized areas in normal and lesion tissue is inadvisable because the lesion is edematous, axons are smaller and closer together following demyelination, and enlarging gitter cells further alter the normal relationships. A few elongated processes containing a remarkably increased number of mitochondria traverse the lesion as noted earlier. That these axons seem likely because, in longitudinal section, their dimensions are similar to those of adjacent still myelinated axons, they do not emerge from a cell body, and their cytoplasm contains fibrils. Many of the mitochondria do not lie parallel to the long axis of such an axon. In one of these processes, vesicles and clustered granules were abundant.

Myelin breakdown continues in the manner described for the earlier stage. Sheaths in different stages of disintegration are still visible (Figs. 8, 9). Observation of longitudinal sections reveals that the deterioration of the sheath seen locally in a transverse section does extend for some distance along the length of an axon. Freed myelin appears as large, partly collapsed but, otherwise, sheath-like structures, irregular whorls, or less oriented dense clumps, and these formations occur free in tissue spaces or within gitter cells.

The way in which long, thin processes of gitter cells have penetrated disintegrating sheaths is striking (Fig. 8). In the gitter cell are granule-studded membranes of endoplasmic reticulum, Golgi complex, and mitochondria. A few gitter cells appear to be entering the pia. A few neutrophils are present. Many cells, presumably macro-
phages from overlying pia, are lined up immediately beneath the pial membrane. Nucleoli are present within their nuclei and phagocytic activity has been initiated by some of them.

Outside the cord, in the frankly thickened pia mater, there are many small gitter cells, fibroblasts, macrophages, lymphocytes, and neutrophils. The inner surfaces of endothelial cells, comprising pial blood vessels, are nearly completely lined with white blood cells. Many of these cells had been caught in the act of escaping from blood vessels at the time of fixation. In two cases, a neutrophil was clearly within an endothelial cell, possibly leaving the vessel by this route rather than migrating between such cells.

**Lesion, 6 Days Postoperative (1 Cat).**—All axons are completely demyelinated by 6 d.p.o. They appear in groups, possibly because the continually enlarging gitter cells have pushed them into such a configuration, and are flattened slightly but, otherwise, appear normal (Fig. 11). Many axons have lost the markedly indented profiles noted earlier but will assume a typical ruffled contour as macroglial processes embrace them. Like the ensheathed axons in normal cord, there is greater variation in the size of demyelinated axons in lateral and anterior columns than in posterior columns. At this stage, too, are noted a few axons, varying in size, containing a large number of mitochondrial processes.

**Macroglia** have entered the lesion by this stage. Thus, the lesion exists for only a few days without macroglial cells. Processes from these cells start to invest some of the axons. In one longitudinal section, a particularly large gial cell accompanied an axon for some distance, its very elongated nucleus oriented parallel to the axon axis. Additional description of these macroglia appears below.

As a result of continued phagocytic activity, nearly all the myelin debris is within gitter cells which have, accordingly, become further enlarged (Fig. 11). The cytoplasm is so engorged with vacuoles that many gitter cell nuclei are pushed aside and indented by adjacent phagocytosed inclusions and are, therefore, very irregular in shape. Neutrophils have disappeared. Fibroblasts are present only rarely. Of equally rare occurrence in the lesion are cells which resemble the lymphocytes and macrophages present in the pia at this time. Many gitter cells are also present in the pia mater and they are larger in size than those observed here at 3 d.p.o.

**Lesion, 10 and 14 Days Postoperative (3 Cats).**—Axons, despite demyelination, lack investments only briefly for, as noted above, macroglial cell processes start to envelop them by 6 d.p.o. This process continues so that by 14 d.p.o. nearly all groups of axons have been invaded by glial cell processes varying in thickness. Some axons lie within the perinuclear cytoplasm.

Many macroglia are present (Figs. 13, 14). They are equipped with a large, oval, and sometimes irregularly shaped nucleus, in which a nucleolus is almost always visible, and a copious amount of very dense cytoplasm containing endoplasmic reticulum, free and clustered granules, Golgi complex, mitochondria, and dense inclusions (Figs. 13, 16). Endoplasmic reticulum is represented by nearly always unoriented cisternae, the membranes of which are covered with granules. Many of these cisternae contain moderately dense material and sometimes appear engorged. Dense rod-shaped structures are presumed to be mitochondria even though cristae mitochondriales are not always visible. Added to this array are fibrils like those contained within fibrous astrocytes in comparable normal tissue but different in that they do not lie in the large and very compact bundles seen normally (Figs. 15, 16). These fibrils are arranged in smaller bundles or are more dispersed such that they are sometimes difficult to resolve. Fibrils occur near nuclei, throughout the cytoplasm, and within some of the many processes seen to emerge from these cells. Since this cell type is clearly different from both macroglial types in normal adult cord, it will be called tentatively "reactive macroglia." One cell differed from its neighbors in that only a little cytoplasm surrounded a large, irregularly shaped nucleus which contained a greatly enlarged nucleolus (~3 μ).

That gitter cells are still very numerous can be seen in Fig. 12. Despite the engorged state of these cells as a whole, areas containing the usual cytoplasmic organelles are still visible occasionally. A striking feature of gitter cells in this lesion is the heterogeneous appearance of the contained products of demyelination. These are found in small or large vacuoles, the contents of which range from homogeneous substance of low density to variously contorted sheath fragments differing greatly in size (Fig. 12). Lamellae are still visible in a few of the fragments. Though a few large distorted whorls of myelin remain outside gitter cells, most extracellular debris is gone. Increased extra-
Etiology of Lesion:

Recent publications indicate that glial cells: (a) form myelin (29, 30), (b) exhibit substantial levels of metabolic activity in adult tissue after myelination has apparently ceased (31–34), and (c) may be concerned in water-ion balance (35). These observations suggest an intimate myelin-glial relationship. The process of demyelination may be initiated by a disturbance of this relationship. But until additional data are available in this underexplored area, any discussion of the causes of demyelination remains only speculative.

The etiology of demyelination in cat spinal cord has not been systematically studied. The following speculations are based largely on theoretical mechanisms proposed by Lumsden in a recent review of experimental anoxic and toxic demyelinating processes (36). It may be postulated that the combination of deep anesthesia, negative chamber pressure during the injection phase of c.s.f. exchange, and relatively increased pressure on the cord surface causes a transitory anoxia either by decreased oxygen saturation or by blood vessel collapse. Oxygen-dependent elements in this region, the glial cells, then degenerate leaving the more resistant axons intact. Either concurrently or subsequently the myelin sheaths degenerate.

An alternate theory is that venous collapse on the cord surface leads to an increased pressure in the venous end of subpial capillaries. Thus, with capillary hydrostatic pressure equal or greater than oncotic pressure, the normal flux of fluid into the capillary would not take place and excess fluid would accumulate in the tissue either in or between cells. By this or other methods of edema formation, the normal glial-myelin sheath relationships would be disrupted, or the myelin sheath itself damaged, and myelin breakdown would result.

Axon Continuity versus "Fragmentation":

"Fragmentation" of axons has been described in light microscopic studies of certain demyelinating diseases (37). In the light microscopic study of the c.s.f. exchange lesion (1), apparent interruptions in Bodian-stained axons observed on the 4th to the 14th day were interpreted as axon fragmentation and, for this reason, it was assumed that axon degeneration was occurring. The present study indicates that these observations reflected only an accentuated axon tortuosity secondary to vacuolization of deteriorating myelin sheaths and gitter cell enlargement. Ultrastructural characteristics of the axons are unchanged, and this finding is consistent with the absence of secondary degeneration above or below the lesion. Ultrastructural integrity is considered to be proof of continuity. The demonstration that the stage initially interpreted as axon fragmentation is, in fact, a
state of total demyelination may be of general neuropathological interest.

**Relation to Other Demyelinating Conditions:**

To define a neurological lesion one must describe both the characteristic distribution and fundamental pathological process occurring in the lesion. The discussion of the relationship of the c.s.f. exchange lesion to other demyelinating conditions is limited by disagreement over the nature of the processes involved and the lack of other ultrastructural studies dealing specifically with demyelination. Condic and Good (38) report a limited ultrastructural study of pathological changes in spinal cords of guinea pigs with experimental allergic encephalomyelitis. Emphasized in this study is the finding that the myelin sheaths remained intact while initial changes were occurring in nerve fibers. There was first an increase, then vacuolization of axonal mitochondria. This would suggest a different process from the demyelination of structurally intact axons that we have observed in cat spinal cord. Assuming the initial change in the major demyelinating diseases to be in the myelin sheath, these studies taken together would indicate that although the distribution of lesions in experimental allergic encephalomyelitis and some demyelinating diseases is similar, this plaque of demyelination in cat spinal cord may present a more comparable initial process. Any such statement can only be speculative until methods become available for ultrastructural study of early stages in human demyelinating diseases. When such studies become possible it is hoped that the present efforts will help provide a basis for their intelligent interpretation.

**Macrogial Cell Types:**

The problem of electron microscopic identification of macrogial cells in subpial white matter of adult cat spinal cord is simplified by the presence of only two cell types, the fibrous astrocyte and the interfascicular oligodendrocyte. The most important distinguishing characteristic of the *fibrous astrocyte* is the presence of compact bundles of fibrils throughout the cell body and numerous slender processes. The processes are further characterized by a paucity of inclusions other than fibril bundles. Also helpful in distinguishing this cell type from the oligodendrocyte are larger cell size, more slender and frequent processes, elongated and irregularly shaped nuclei, and less dense cytoplasm. Furthermore, the generally equidistant spacing of fibrous astrocytes contrasts to the occurrence of the oligodendroglia in rows between axons. Luse (23, 39) was the first to demonstrate electron microscopically the presence of bundles of fibrils within astrocytes in adult rodent spinal cord. Gray (22) has observed similar fibril-containing processes in rat cortex and believes that they belong to fibrous astrocytes.

In contrast to the fibrous astrocyte, the *oligodendrocyte* displays a relatively sparse but more dense cytoplasm. While granule-studded endoplasmic reticulum is invariably present, the amount and degree of aggregation varies considerably from cell to cell. This would be consistent with Kryspin-Exner's demonstration of variation in amount of Nissl stainable substance in oligodendroglial cytoplasm (40). It is of interest that granular endoplasmic reticulum is more prominent in oligodendroglia than in fibrous astrocytes and that there is greater amino acid incorporation into the former than into the latter (32). The cell identified as oligodendrocyte corresponds in most respects to that so designated by Farquhar and Hartmann (20) and Schultz, Maynard, and Pease (24). While both light (41) and electron microscopic (20) studies have revealed transitional forms between oligodendroglia and astrocytes, we have observed these cell types to be quite distinct in subpial white matter of normal adult spinal cord.

**Macrogia in Pathological Conditions:**

The two distinct macrogial cell types are not observed when pathological conditions prevail. The macroglial cell appearing in the lesion by the 6th day cannot be identified as either oligodendrocyte or fibrous astrocyte. In the light microscope, the cell is large in size and its cytoplasm quite uniformly eosinophilic. The large, oval nucleus is of medium density and contains a prominent nucleolus. The electron microscope reveals a dense cytoplasm abounding, both in the perikaryon and distal processes, in granule-studded membranes of endoplasmic reticulum and fibrils. We have elected to call these cells *reactive macroglia*.

Schultz and Pease (26) found intermediate glial types in an ultrastructural study of cicatrix formation accompanying wound healing in rat cortex. Because glial cells displayed features of both astrocytes and oligodendroglia, they suggested that under these conditions there may possibly be reversion of macroglia to a more primitive (em-
bryonic) state. (They note that the presence of intermediate types is not surprising for all macroglia are thought to arise from the same stem cell, the spongioblast.) In the borders of these traumatic lesions they noted unusually heavy myelin formations and concluded that a strong stimulus for myelination develops. Neither the source nor the mechanism of formation of this myelin was ascertained. Our observation that demyelinated white matter is invaded by a similar type of macroglia prior to the onset of remyelination and that this cell is responsible for myelin formation would help to explain their observations.

In an ultrastructural study of reactive and neoplastic astrocytes, Luse (39) did not observe any cells identical to the reactive macroglia in demyelinated cat cord. She investigated in rabbits cerebral stab wounds 4 weeks after production and areas of secondary degeneration in optic nerve at various intervals following enucleation of the eye. In the first case, the 4th week may have been too late for finding this cell. She did observe an increase in the amount of cytoplasm and cytoplasmic inclusions in astrocytes, but this was at a time when compact fibril bundles predominated. In the secondary degeneration of optic nerve, conditions may not be ideal for the appearance of reactive macroglia.

Adams (27) has recently reviewed the reaction of glia to pathological processes, outlining those conditions in which astrocyte proliferation and hypertrophy are prominent. Conditions appear to be most ideal in diseases which destroy parenchyma, whether this be predominantly nerve cells or myelinated fibers, or whole tissue. The description of these cells and the time course of their formation is given thus: "... the astrocytes which proliferate are usually those that have escaped destruction in the margin of the lesion. At first, they enlarge in all dimensions and their nuclei swell, a change which may be noted within a few days of the injury. Nuclear hyperplasia... is evident by the 4th and 5th days and becomes increasingly prominent in the following weeks (astrocytosis). The dividing cells have an abundant, eosinophilic cytoplasm. These are the swollen or plump astrocytes of Nissl (27)." These cells exhibit the same time course of formation and an identical light microscopic picture as the reactive macroglia forming in demyelinating cat cord and, subsequently, remyelinating the tissue. We submit that reactive macroglia are identical to the hypertrophic or swollen astrocyte of classical neuropathology.

These hypertrophic or swollen astrocytes have long been assumed to be precursors of the fibrous astrocyte (42), their abundant and richly eosinophilic cytoplasm merely marking the initial proliferative phase of their development as the chief cells of nervous cicatrix. That these cells may function in remyelination has not been suggested. We believe, from our observations and review of the literature, the most pertinent conclusion to be drawn is this: the reactive macroglia, responsible for remyelination in demyelinated cat spinal cord, are equivalent to the hypertrophic or swollen astrocytes common to many neuropathological processes. The hypertrophic astrocytes have formerly been considered to be merely progenitors of the inevitable and obstructing fibrous astrocytes, but the possibility now emerges that these astrocytes possess the potential to remyelinate. In those neuropathological conditions in which they appear, their ability to remyelinate must be considered.

The prominence of the astrocytic reaction in demyelinating diseases, especially multiple sclerosis, has been emphasized by McAlpine et al. (4). It is conceivable that in multiple sclerosis the presence of the hypertrophic astrocyte is related to remyelination and thereby to the characteristic clinical remissions. The shadow plaque of multiple sclerosis is described as containing, in addition to the numerous hypertrophic astrocytes, groups of uniformly thin myelin sheaths (4, 43). This recalls the light microscopic picture of the partially remyelinated lesion following c.s.f. exchange (1). We suggest that this shadow plaque may not be a region of partial demyelination, as has been thought, but may represent an area of remyelination. Since we have observed demyelination to involve always the entire thickness of the sheath, we think it unlikely that the thin sheaths represent a stage in deterioration. It is more likely that the thin sheaths represent newly formed structures and the prominent hypertrophic astrocytes may be the instruments of their formation.

If these interpretations are correct and human nerve fibers can be demyelinated and remyelinated as has been observed in adult cat spinal cord, there arises the possibility that the multipotential hypertrophic astrocyte can be pharmacologically con-
trolled to foster remyelination rather than scarring. If a substance could be found to turn this cell away from fibrous astrocyte formation and toward remyelination, this drug might be of clinical usefulness in treating demyelinating conditions.

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**EXPLANATION OF PLATES**

All plates are photographs of subpial white matter of adult cat spinal cord. One picture, Fig. 14, is a photomicrograph; all the other figures are electron micrographs. Each bar indicates 1 micron.

**PLATE 340**

**Figs. 1 and 2. Normal white matter from posterior columns; cross-section.**

**Fig. 1.** In this area, the axons, containing characteristic neurofibrils and mitochondria, are surrounded by myelin sheaths of relatively uniform thickness. Coursing between the sheaths are slender processes of fibrous astrocytes. These numerous processes are clear except for compact bundles of fibrils. A fibrous astrocyte (fa) appears at the upper right. Its nucleus, which contains a nucleolus, is elongated and irregularly shaped. Rounded nuclei surrounded by dense cytoplasm from which few processes emerge typify the three oligodendroglia shown below the astrocyte. Intermittent splitting (see arrows) within the myelin is thought to result from inadequate fixation. X 4500.

**Fig. 2.** This figure contains a fibrous astrocyte from an adjacent area. More perinuclear cytoplasm is present than in the astrocyte shown in Fig. 1. Fibril bundles in the cell body are seen to continue into three of the processes. X 4500.
FIG. 3. Individual fibrils, comprising the discrete bundles seen in the two previous figures at lower magnification, are visible within the perinuclear cytoplasm of this fibrous astrocyte. Characteristic bodies varying in size, shape, and density, Golgi complex (c), and ribonucleoprotein granules, free and in association with membranes, are present. Portions of axons (a) are included in this longitudinal section. In the sheath surrounding one of them is a cleft-like structure (at arrow) briefly described in the text. × 14,500.

FIG. 4. This cross-section contains a portion of oligodendroglial (ol) cytoplasm. Contained within this cytoplasm are oriented cisternae of granular endoplasmic reticulum, free ribonucleoprotein particles, mitochondria, and spindle-shaped bodies exhibiting a central area of low density. Cristae mitochondriales can be readily resolved in one of the axonal mitochondria. A portion of the pia (p) is present. × 17,000.
(Bunge et al.; Demyelination in spinal cord lesion)
PLATE 342

FIG. 5. Normal white matter from posterior columns; cross-section. At the right is a fibrous astrocyte (fa) process, containing portions of a fibril bundle and dense body. The limiting membrane of this process can be seen at the arrow. An axon is invested by a modified sheath displaying a peculiar pattern found typically in cleft- or node-like areas. This pattern is distinctly different from that observed during demyelination (see figure directly below) or following inadequate fixation (see Fig. 1). × 20,500.

FIG. 6. Lesion, 29 hours after c.s.f. exchange, in posterior columns; cross-section. Axons appear normal although their myelin sheaths either have disappeared (a1, a2) or are breaking down. Remnants of a former sheath are still present around axon a1 at this level and at other levels viewed in a series of sections. Another demyelinated axon, a2, lies adjacent to a cell (ph) which must be considered to be a phagocyte because myelin debris is contained within its cytoplasm at other levels. The processes (at arrows) of this cell lying close to the axon are longer and are wrapped around the axon about one and a half times in serial sections. Part of the pia (p) is included. × 11,500.
(Bunee et al.: Demelination in spinal cord lesion)
PLATE 343

Fig. 7. Lesion, 29 hours after c.s.f. exchange, in posterior columns; cross-section. As in the directly preceding figure, both demyelinated (a₁, a₂) and demyelinating axons are present. One axon, a₁, displays fragmented myelin which is surrounded by a cellular process; this is a typical picture. Another axon (a₂), completely demyelinated, lies adjacent to a phagocyte which contains myelin debris within its cytoplasm (gitter cell, g). A second cell, a lymphocyte (l), is present. × 8500.

Fig. 8. Lesion, 3 days after c.s.f. exchange, in lateral columns of cat 1; cross-section. Although the large axon exhibits an irregular profile, neurofibrils and mitochondria are morphologically unchanged. Sheath breakdown is seldom so uniform as it appears here. Long, sheet-like processes (at arrows) of phagocytes invade a deteriorating myelin sheath. × 15,000.
(Bunge et al.: Demyelination in spinal cord lesion)
**PLATE 344**

*Fig. 9. Lesion, 3 days after c.s.f. exchange, in lateral columns of cat 1; cross-section.* Some axons (a₁, a₂) lack ensheathing myelin, while others are invested with varying amounts of myelin in different stages of breakdown. The sizes of demyelinated axons vary considerably (cf. a₁ and a₂). In the lower left corner of the picture, an axon (a₃) is surrounded by a “sheath” of much larger diameter. Gitter cells (g) are present. Very rarely, intact appearing sheaths, similar to the one (i) shown in part, are seen to contain no traces of enclosed axons. Whether the axons have degenerated or the sheaths have rejoined following removal from axons is not known. The pia (p) contains a variety of cell types. × 8000.
(Bunge et al.: Demyelination in spinal cord lesion)
FIG. 10. Lesion, 3 days after c.s.f. exchange, in lateral columns of cat 2; longitudinal section. Axonal neurofibrils and mitochondria appear normal. Cristae mitochondriales run along the longitudinal axes of the two mitochondria contained within this axon. Myelin breakdown is evident. Resulting from this process are vacuolar formations which indent the axon. X 12,000.

FIG. 11. Lesion, 6 days after c.s.f. exchange, in lateral columns; longitudinal section. The four axons, a₁ to a₄, lack investing myelin. All myelin debris has been removed, too, so that some of them now lie in apposition. A portion of a gitter cell (g) separates two of the axons. X 8000.
(Bunge et al.; Demyelination in spinal cord lesion)
PLATE 346

Fig. 12. Lesion, 14 days after c.s.f. exchange, in lateral columns of cat 1; cross-section. Demyelinated axons, debris-laden gitter cells, and small portions of reactive macroglia are present. Only one gitter cell nucleus (n) appears in the area pictured. Arrows point to the outline of one gitter cell. Reactive macroglial (rm) cytoplasm is visible, and processes of this cell type are seen to course throughout the group of axons at the upper left. A very small part of the pia (p) is included. X 4500.
(Bunge et al.: Demyelination in spinal cord lesion)
Figs. 13 to 15. Lesion, 14 days after c.s.f. exchange.

Fig. 13. Anterior columns of cat 2; cross-section. Scattered among the axons are three reactive macroglia (rm). Their nuclei contain prominent nucleoli. Within the dense cytoplasm of the upper left reactive macroglial cell, an enlargement of which appears in Fig. 16, are cisternae of granular endoplasmic reticulum and small fibril bundles. Centrioles are visible in the lower left glial cell. Part of a gitter cell (g) is present. Collagen occupies an area near the middle of the figure. A portion of the pia (p) is visible. × 5500.

Fig. 14. This photomicrograph of formalin fixed lateral columns from cat 3 shows the appearance of three reactive macroglia in the light microscope following staining with hematoxylin and eosin. The cytoplasm is eosinophilic. The area is from the lesion-normal tissue border. × 1800.

Fig. 15. Anterior columns of cat 2; cross-section. This figure is an enlargement of portions of two reactive macroglial processes, delineated at the arrows. In the larger process, both longitudinally cut fibrils and cisternae of granule-studded endoplasmic reticulum are visible. The other process contains fibrils in oblique section. × 28,500.
(Bunge et al.: Demyelination in spinal cord lesion)
Fig. 16. Lesion, 14 days after c.s.f. exchange, in anterior columns of cat 2; cross-section. This is a reactive macroglial cell. Its nucleus exhibits a large nucleolus. Contained within its dense cytoplasm are granule covered cisternae of endoplasmic reticulum, free ribonucleoprotein granules, mitochondria, and small aggregations of fibrils. Most fibrils appear in cross- or oblique section. A few fibrils, at the right of the nucleus, are longitudinally oriented. The membranes of axons (a) lying adjacent to glial cytoplasm manifest a ruffled silhouette. Collagen is present at opposite corners of the figure. Portions of the pia (p) and a pial blood vessel are present. × 19,500.
(Bunge et al.: Demyelination in spinal cord lesion)