Ultrastructural study of the neuroglial and macrophagic reaction in Wallerian degeneration of the adult rat optic nerve

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Summary. The Wallerian degeneration of the optic nerve of adult rat has been studied after destroying the retina. Animals were sacrificed between 4 days and 1 year after the lesion. Different cell types of the optic nerve have been studied ultrastructurally. Our results demonstrate the existence of a population of macrophages, probably of microglial origin, responsible for scavenging degenerated myelin. Astrocytes suffer a process of proliferation and hypertrophy, and are massively stuffed by gliofilaments, leading to a glial scar. These cells apparently do not participate in phagocytic phenomena, while some cytoplasmic inclusions (e.g. lipid droplets) suggest some implication in the local metabolization of some tissue degradation products. Oligodendrocytes do not undergo ultrastructural changes, showing a rather quiescent appearance.

Key words: Optic nerve, Wallerian degeneration, Macrophages-microglia, Astrocytes, Oligodendrocytes

Introduction

Either the experimental sectioning of the intraorbital segment of the optic nerve or the mechanical destruction of the retinal ganglion cells can easily be used as a model for producing a Wallerian degeneration of the Central Nervous System (CNS). Thus, we can observe subsequent cell reactions under a lesional model devoid of vascular damage and thereby avoiding or at least minimizing the potential affluence of blood macrophages.

The Wallerian degeneration of the optic nerve has been studied in adult animals concerning the sequence of the myelin breakdown (Lassman et al., 1978), the nature and ultrastructure of the macrophages (Ling, 1978; Liu

and Shen, 1985; Stoll et al., 1989; Ludwin, 1990a), or the general alterations undertaken by both nerve fibres and neuroglial cells (Vaughn and Pease, 1970; Vaughn and Skoff, 1972; Cook and Wisniewski, 1973, 1987; Skoff, 1975; Cook, 1978; Wender et al., 1981; Shen and Liu, 1984; Ludwin, 1990b).

Since the first investigations on the injured optic nerve, controversy arose about the nature of the scavenger cells of myelin debris. Microglial cells, blood-borne macrophages, less often astrocytes (Vaughn and Pease, 1970; Vaughn and Skoff, 1972; Skoff, 1975; Fulcrand and Privat, 1977; Lassman et al., 1978; Wender et al., 1980; Shen and Liu, 1984), and even oligodendrocytes (Cook and Wisniewski, 1973, 1987; Cook, 1978; Lassman et al., 1978) have been highlighted as responsible for this process.

Our present paper aims to study ultrastructurally the reaction of neuroglial cells and macrophages within the injured optic nerve. The process has been studied chronologically with emphasis on a close description of long term changes.

Materials and methods

Forty adult albino Wistar rats, three to four months old were used for this study. Under ether anesthesia, right eye phachectomy was performed after opening the cornea. The anterior retinal surface at the back of the eye within the globe, was gently rubbed with a cotton swabp inserted through the corneal opening. Repeated movements were performed to assure a complete destruction of the retinal surface thus avoiding enucleation. With this method, the optic nerve was free of excessive stretching and hemorrhages. Finally, a palpebral cerclage was performed for a perfect closure of the orbit cavity.

Animals were sacrificed with the following schedule after the operation: 4, 10, 15, 30 and 45 days and 2, 3, 5, 8 and 12 months (four animals per phase). Tissue perfusion was made through the ascending aorta with 250 cc

of either 3% glutaraldehyde or 2% glutaraldehyde-2% paraformaldehyde. After perfusion, animals were stored refrigerated for 2-3 hours. Both optic nerves with the chiasma were extracted in block after carefully removing the cranial vault and separating the brain, and immersed in the same fixative. About 1 mm of the distal segments of both optic nerves were scised and rejected. Right and left optic nerves were separated with their corresponding half chiasma. Tissue samples were washed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide and embedded in Vestopal. Ultrathin transversal sections were obtained from the most distal part of the optic nerve, using an LKB ultramicrotome. They were stained with uranyl acetate and lead citrate and examined in a Philips EM-201 electron microscope.

Results

Four days after the lesion, only minor modifications of the nerve fibres could be appreciated. Some fibres showed dilatation and/or densification of the axoplasm. The microtubular skeleton was often blurred, yet myelin remained well preserved. In general, cell changes were barely evident. A few macrophage-like cells showing small dense bodies and occasional lipid droplets were seen. These cells showed ultrastructural similarities to microglial cells e.g. chromatin-dense nuclei, and long, isolated and scarce cisternae of rough endoplasmic reticulum (RER). Astrocytes showed their usual ultrastructure with a clear hyaloplasm hidden by the characteristic gliofilament bundles. Both control and injured optic nerves occasionally showed dense bodies in astrocytes. Mitotic astrocytes only appeared in the injured nerves. Oligodendrocytes were ultrastructurally normal with a well developed Golgi apparatus, abundant polyribosomes and typical packed short RER cisternae.

With respect to the previous phase, few ultrastructural changes were detected on the 10th day after lesion. The degeneration of the optic nerve fibres continued progressively.

On the 15th day after lesion, large fragments of degenerated myelin could be identified among groups of thin astrocyte cell processes. Macrophages were laden with a variable number of lipid droplets and presented long, isolated cisternae of RER (Fig. 1) and sometimes unequivocally englobed segments of degenerating nerve fibres (Fig. 2). Astrocytes presented an increased cytoplasmic volume with abundant gliofilaments, some small dense bodies scattered throughout the somatic cytoplasm and some lipid droplets of notable size (Fig. 3). Oligodendrocytes showed no ultrastructural changes compatible with phagocytic activity.

Thirty days after the lesion, macrophages showed some residual bodies of complex laminar structure (described below). An intense astrocyte reaction was evidenced by thick astrocyte cell processes stuffed with glial filaments. These cell processes were seen running parallel or transversal to the major axis of the optic nerve, often grouped in bundles delimiting areas where degenerating fibres were placed (Fig. 4). In addition to normal-appearing dense bodies, some elongated and membrane-bound dense bodies with a very regular laminated content could be seen within the astroglial soma (Figs. 5, 6). The astrocytic glia limitans lining the meningeal space appeared thickened and with numerous superficial irregularities seldom seen in the glia limitans of control non-injured nerves (Fig. 7). Except for the appearance of some nuclear indentations, no changes were recorded in oligodendrocytes.

Abundant debris of degenerated myelin still remained in the optic nerve 45 days after the lesion. Axonal remnants were undetectable. Occasionally, cell processes seemed to enter degenerating nerve fibres, dilating and deforming the myelin ring. In most fields, the cross sectional area was occupied by cell processes and somata outweighing those filled with degenerating myelin. Myelin was localized exclusively in extracellular spaces or within macrophage-like cells. Macrophages in this stage showed characteristic lipid droplets and residual dense bodies of crystaloid laminated content and angulous contour (Fig. 8). Astrocytes presented large somata, cytologically similar to those described above, including the presence of lipid droplets. The glia limitans appeared widened, with complex interdigitations among the astrocyte cell processes. The superficial irregularities sometimes appeared as closely packed astrocyte tongue-like projections, leaving between them narrow clefts occupied by a basal lamina (Fig. 9). Oligodendrocytes remained unchanged (Fig. 10).

At the second month after lesion, astrocyte gliofilaments increased considerably in number (Fig. 11). Lipid droplets were still present in these cells, and dense bodies containing a crystaloid material more electrondense than the matrix were sometimes seen. Astrocyte processes showed some microtubules and were frequently joined by gap-like cell junctions (Fig. 12). In some instances we saw aberrant myelinizations, i.e. cells with a soma probably oligodendroglial (Fig. 13), surrounded by a thin, though well defined, myelin layer.

Three months after the lesion, there were no significant changes with respect to the previous phase. Myelin debris still remained throughout the tissue section.

At the 5th month after lesion, myelin debris was less frequently observed, though easily detected in the extracellular space. Macrophages were less evident and less numerous. The number of intrastrocytic lipid droplets decreased though some of large size could be occasionally detected. Glial filaments occupied most of the volume of the astrocyte cell processes and large areas within the soma. Complex interdigitations among astrocyte cell processes or even among somata and processes were frequent (Fig. 14).
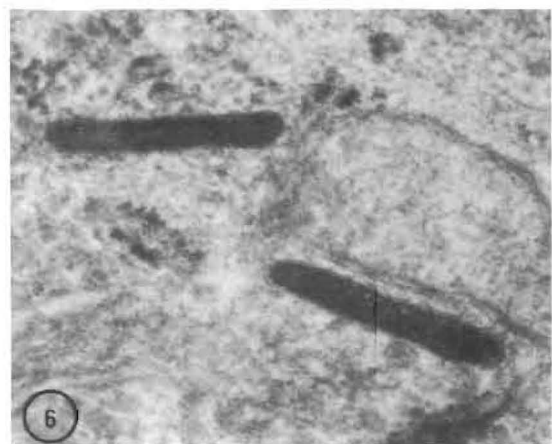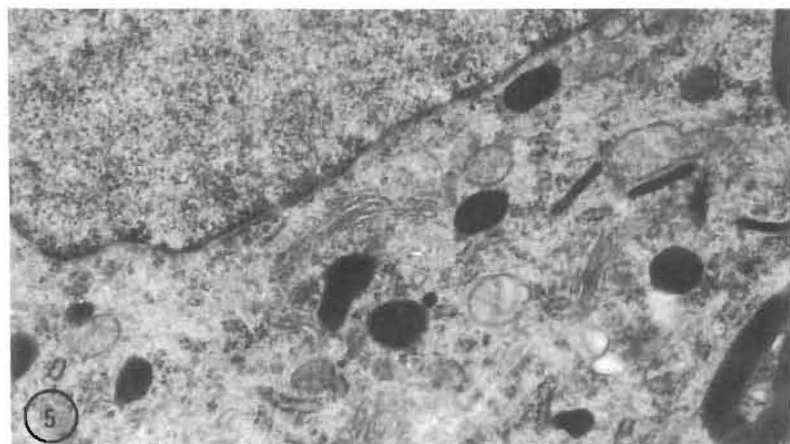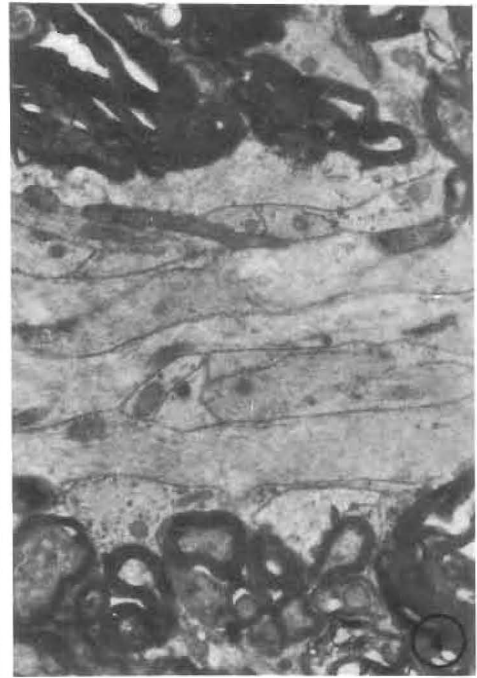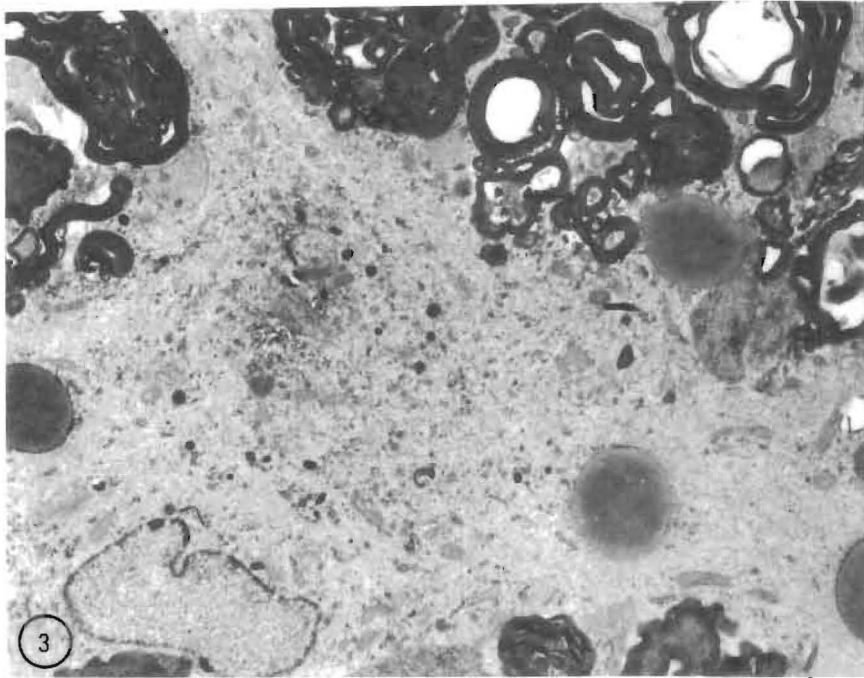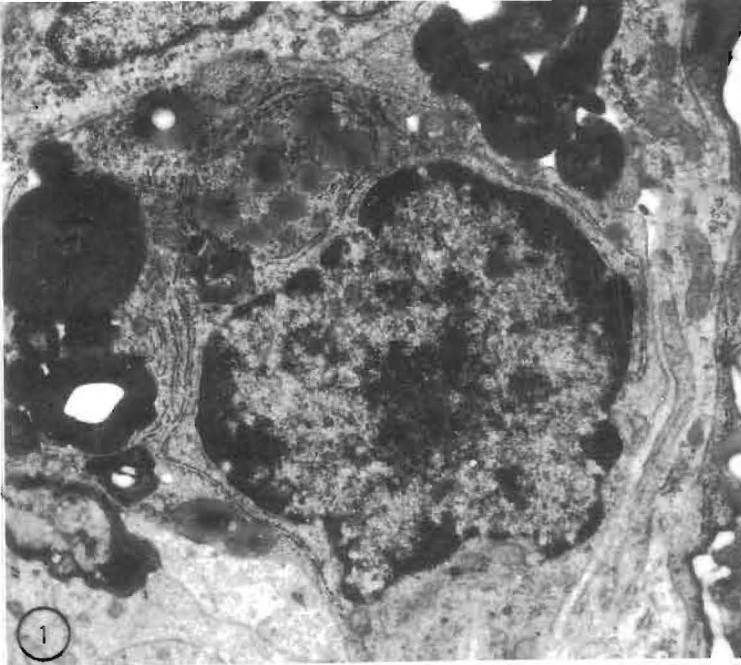
Fig. 1. Postlesional day 15. Macrophage cell with cytoplasmic lipid droplets and long, narrow RER cisterns. × 14,080

Fig. 2. Postlesional day 15. Macrophage cell with a phagosome containing myelin debris. Lipid droplets similar to those of Fig. 1 can be observed. × 11,200

Fig. 3. Postlesional day 15. Hypertrophic astrocyte with cytoplasmic homogeneous dense bodies and lipid droplets. Phagosomes are absent. × 7,450

Fig. 4. Postlesional day 30. Gliofilament-rich bundle of astroglial processes placed transversally to the nerve fibres. × 11,520

Figs. 5 and 6. Postlesional day 30. Juxtanuclear region of the cytoplasm of an astrocyte. Fig. 5: Golgi's dictyosomes and lysosome-like dense bodies. × 21,024. Fig. 6: Higher magnification of Fig. 5. Membrane bound bodies of lamellar content. × 73,670
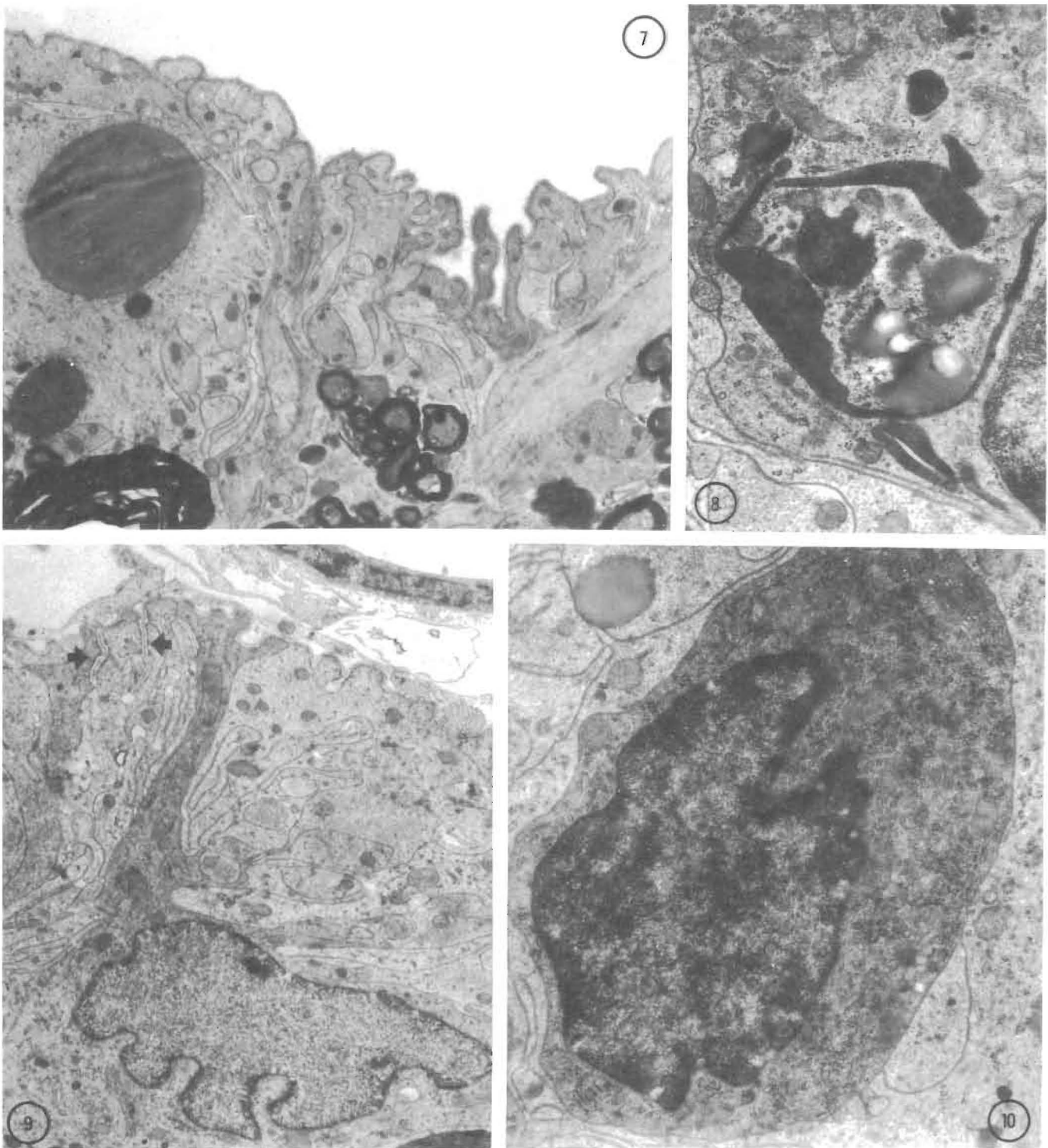
Fig. 7. Postlesional day 30. Irregular glia limitans on the optic nerve surface. × 10,530

Fig. 8. Postlesional day 45. Detail of a macrophage cytoplasm. Dense bodies of laminated content next to lipid droplets. × 20,150

Fig. 9. Postlesional day 45. Basal lamina filling clefts (arrows) between thin tongue-like projections of the glia limitans in the surface of the optic nerve. × 11,200

Fig. 10. Postlesional day 45. Normal-appearing oligodendrocytes surrounded by abundant gliofilament-rich astrocyte cell processes. × 17,960
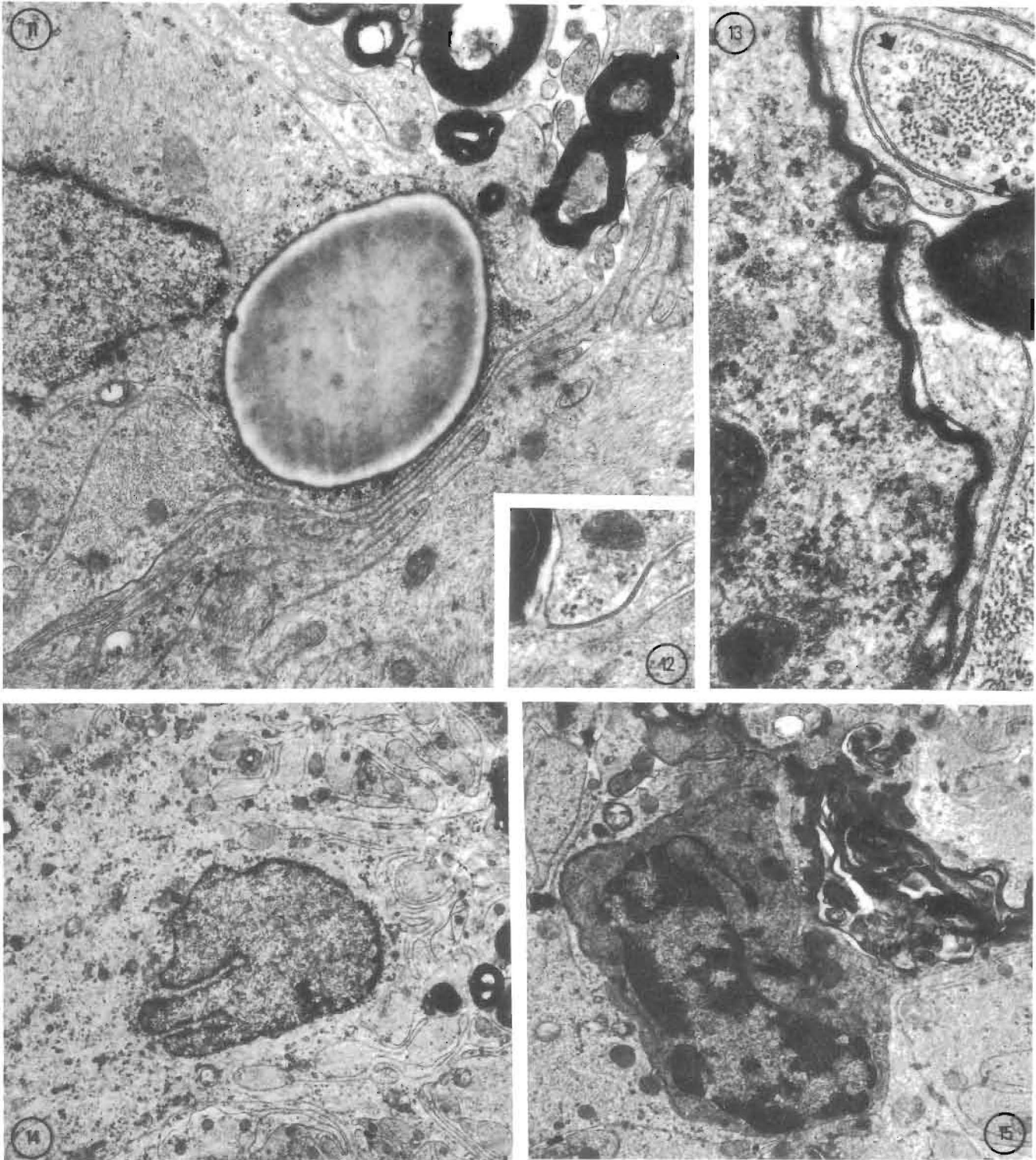
Fig. 11. Postlesional month 2. Abundance of gliofilaments in the soma and cell processes of astrocytes. Note the persistence of lipid droplets. × 21,460

Fig. 12. Postlesional month 2. Gap-like cell junction between astrocyte cell processes. × 44,590

Fig. 13. Postlesional month 2. Aberrant myelinization surrounding an oligodendroglial soma. Numerous gliofilaments and some microtubuli (arrows) are seen in adjacent astrocyte cell processes. × 68,110

Fig. 14. Postlesional month 5. Numerous interdigitations and infoldings on the surface of an astroglial soma. × 10,300

Fig. 15. Postlesional month 12. Macrophage cell closely associated to interstitial free-lying myelin debris. × 14,720
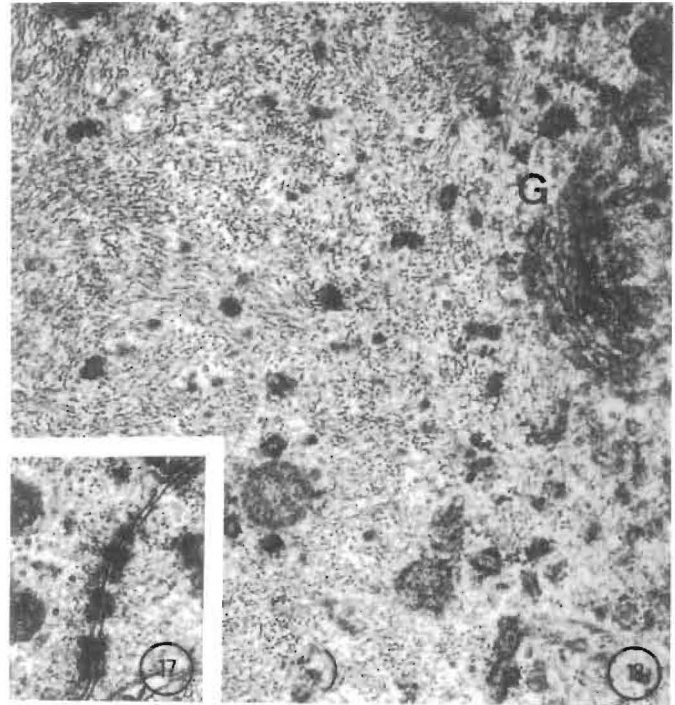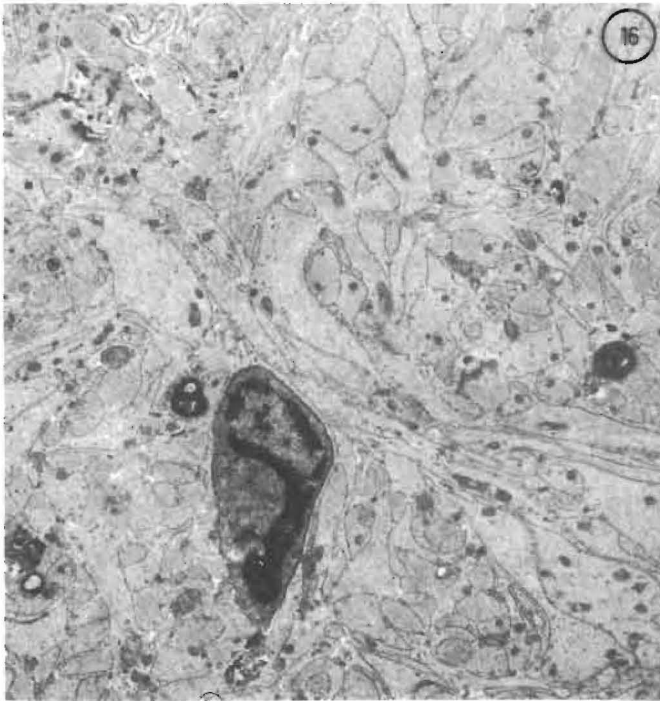
Fig. 16. Postlesional month 12. Gliotic appearance of the optic nerve parenchyma. × 4,485

Fig. 17. Postlesional month 12. Group of desmosome-like junctions between astrocyte cell processes. × 39,130

Fig. 18. Postlesional month 12. Portion of an astrocyte soma showing numerous gliofilaments as predominat cytoplasmic elements. G: Golgi dictyosome. × 39,130

Fig. 19. Postlesional month 12. Two oligodendrocytes of normal ultrastructure surrounded by abundant and interdigitating thin astrocyte cell processes. × 14,720

The gliosis remained while myelin debris slowly disappeared. At the 8th month after lesion, astrocytes were virtually devoid of lipid droplets. We could observe some images of aberrant myelinization surrounding oligodendrocytes, as described in previous phases.

Finally, 12 months after the lesion, small amounts of degenerating myelin, lying free in the extracellular space, were still detected. Myelin debris was seen among astrocyte cell processes and sometimes near to macrophage-like cells (Fig. 15). Macrophages were scarce and of inactive appearance, with some dense bodies, occasional residual bodies and absence of lipid droplets. Most of the cross sectional area of the optic nerve was occupied by a dense network of astroglial cell processes stuffed with gliofilaments (Fig. 16). Surface irregularities, interdigitations and frequent gap-like cell junctions among the astrocyte cell processes were common in the glia limitans. In other areas we detected interastrocytic desmosome-like junctions sometimes linearly grouped (Fig. 17). Gliofilaments were the predominant structure within the somatic cytoplasm of astrocytes; organoids were more scarce than in previous phases (Fig. 18). Microtubules and gliofilaments coexisted within the astrocyte cell processes. Oligodendrocytes were homogeneously distributed throughout the optic nerve. These cells presented an ultrastructure within normal limits (Fig. 19); their cytoplasm was scarce, their appearance quiescent and sometimes discrete irregularities were seen in the nuclear membrane.

Discussion

After the retinal destruction a progressive degeneration of the nerve fibres of the optic nerve follows. Though the axon undergoes relatively quick damage and promptly disappears, myelin remains for a prolonged time, though seriously damaged. Thus, in the latest phase studied, (1 year after lesion), unphagocyted extracellular myelin debris was still detected. Long term studies have been performed on adult rats (up to a year: Trimmer and Wunderlich, 1990; and up to 22 months: Ludwin, 1990a,b) and in other species (monkeys and cats, up to 413 days: Cook and Wisniewski, 1973). These authors coincide in signalling the slow elimination of the degenerated myelin. Cook and Wisniewski (1973) and Ludwin (1990a,b) found variable amounts of myelin debris in the latest phases, while according to Trimmer and Wunderlich (1990), most of the degenerated myelin had virtually disappeared after 150 days.

Macrophages

In our study we found macrophagic elements with a notable lipid and lysosomal content at the 15th day after lesion. After the 30th day, peaking at the 45th, residual crystaloid bodies could be seen within the cytoplasm. These cells are far fewer at the 5th month. By this time, these cells showed a more quiescent appearance. In the final stages, they were devoid of lipids and only conserved part of their former lysosomal endowment.

The presence of crystalloid lamellar bodies in the cytoplasm of macrophages in the Wallerian degeneration of the optic nerve was described by Lassman et al. (1978), Shen and Liu, (1984) and Liu and Shen (1985). Similar inclusions were described in the cytoplasm of «M»-cells (Matthews and Kruger, 1973) throughout thalamic degeneration in the rabbit brain. Nevertheless, such inclusions are not found in macrophages appearing in experimental brain stab wound lesions (unpublished observations). Thus, these bodies could be indicative not only of the different type of lesion produced, but also of the different chemical composition of lipids in the phagocytized tissue debris.

The main point of controversy refers to the nature of these macrophages. No ultrastructural markers are available to disclose the endogenous (microglial) or exogenous (monocytic) origin of a given activated macrophage. This led to one of the most controversial discussions in the last decades of neurobiology: the origin of macrophages crowding the lesions of the CNS. Even modern techniques such as immunohistochemistry for monocyte/macrophage markers or lectin histochemistry are unable to accurately differentiate microglial from monocytic elements (Stoll et al., 1989; Ludwin, 1990a). We agree with Ling (1981) that in acute traumatic lesions of the CNS, e.g. the experimental brain stab wound lesion

(Boya et al., 1986), both sources may participate. However, in degenerative lesions such as the retrograde neuronal degeneration or the Wallerian degeneration, microglia can be the predominant macrophagic source, with a negligible participation, if any, of blood-borne macrophages. Recently, Cook and Wisniewski (1987), described a monocytic infiltration of the distal part of the optic nerve near the section surface after ocular enucleation. For this reason, we preferred to destroy the retina rather than enucleating it, always rejecting the distal part of the lesioned nerves extracted.

Astrocytes

Astrocytes stuff the spaces left by the degeneration of the nerve fibres. Fifteen days after the lesion, we detected a clear increase in the cytoplasmic volume of the astrocytes and a larger amount of gliofilaments. These filaments consist of glial fibrillary acidic protein and vimentin (Dahl et al., 1981a,b, 1982; McLoon, 1986; Calvo et al., 1990). In our experimental model, the amount of gliofilaments increased progressively throughout the phases studied, whereby residual gliosis should be considered permanent. Different authors generally aggree on this point; however, Cook and Wisniewski (1973) detected some atrophy of the astrocyte cell processes after 200 days of evolution of the lesion.

Many authors accept phagocytic ability for astrocytes both under physiological (Ronnevi, 1978) and pathological conditions (Gonatas et al., 1963; Fernando, 1973; Lemkey-Johnston et al., 1976; Nathaniel and Nathaniel, 1981; Barret et al., 1984; Al-Ali et al., 1988). Some researchers admit phagocytic capacity for astrocytes in the degeneration of the optic nerve (Vaughn and Pease, 1970; Vaughn and Skoff, 1972; Skoff, 1975; Fulcrand and Privat, 1977; Lassman et al., 1978; Wender et al., 1980; Shen and Liu, 1984). In our study, we observed lipid droplets in the astrocytes from the 15th day onwards, disappearing after the 8th month, as well as some dense bodies of a peculiar morphology during intermediate phases. Since we never observed in astrocytes unequivocal phagocytic images of degenerated nerve fibres, we suggest that there is no evidence to assign a clear phagocytic role to these cells. In the experimental brain stab wound lesion we never observed inclusions in the astrocytes as those described herein (unpublished observations). In our opinion, the astrocytes do not play any role as phagocytes, while they can probably be involved in the metabolic management of some breakdown products of tissue degeneration.

A last observation deserves mention regarding the progressive irregularity of the surface of the degenerated optic nerve and the complexity of the interastrocytic interdigitations in the glia limitans. The glia limitans of the CNS turns irregular and complex under different situations, as has been already

described (Moore and Raine, 1986; Carbonell and Boya, 1988), in different experimental models.

Oligodendrocytes

In our study, the oligodendrocytes showed no significant ultrastructural changes. Images of aberrant myelinization described here seem to be common in this lesion type as described by other authors (Vaughn and Pease, 1970; Vaughn and Skoff, 1972; Ludwin, 1990b; Trimmer and Wunderlich, 1990).

In the lesions of the CNS, Maxwell and Kruger (1965) first described «reactive oligodendrocytes» with phagocytic capacity. Other authors also admit this ability (Colonnier, 1964; McMahan, 1967; Triarhou et al., 1985). With respect to the degenerating optic nerve, Cook (1978) and Cook and Wisniewski (1973, 1987), considered the oligodendrocyte as the single phagocytic cell scavenging all degenerated myelin. Lassman et al. (1978) and Ludwin (1990a), admit only a small contribution of oligodendrocytes to phagocytosis. We never found any of these cells with lipid droplets, lysosomes nor residual bodies and therefore we do not consider the phagocytic ability of these cells for axonal or myelin debris. Apparently, oligodendrocytes adopt a rather passive attitude throughout the degeneration, without histochemical signs of enhanced biological activity (Wender et al., 1981) nor suffering irreversible damage or alterations leading to their degeneration (Ludwin, 1990b). This quiescence could suggest that these cells simply release their processes, thus making the oligodendroglial soma independent from the nerve fibre.

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