Ultrastructure of the Radial Components of the Mouse Optic Nerve and its Changes during Wallerian Degeneration

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Summary. Radial components of the optic nerve of the mouse were studied by using thin sections and freeze-fracture replicas. The investigations were performed on normal optic nerves, as well as on those undergoing Wallerian degeneration following eyeball enucleation. Normal radial components in thin sections were observed as a series of light lines composed of small electron-lucent dots situated in the interperiod lines across the myelin sheath. They are frequently found in those parts of the myelin sheath lying near the outer and inner processes of the oligodendroglia. Radial components in freeze-fracture replica were observed as a parallel array of many ridges composed of a row of particles.

The particles of radial components located in the deeper part of the myelin sheath lose their linear arrangement and fall into disorder in a relatively early post-operative period. The parallel array of rows of particles located closely beneath the outer processes of the oligodendroglia remained intact for a long period, even in a markedly distorted myelin sheath.

The present observations suggest that the radial components are resistant against the disintegration of the myelin lamellae during Wallerian degeneration.

Earlier ultrastructural studies of the central myelin sheath revealed the existence of a radial component in materials fixed with osmium and then stained with potassium permanganate (HONJIN, 1959; PETERS, 1961, 1964, 1968; HONJIN et al., 1963; HONJIN and CHANGUS, 1964). Subsequently, a concept of myelinic tight junction was proposed for the rows of particles located in the freeze-fractured myelin lamellae in the central myelin sheath (DERMIETZEL, 1974; REALE et al., 1975) and the peripheral myelin sheath (INOKUCHI and HIGASHI, 1980; SHINOWARA et al., 1980). According to their location in the myelin sheath, the myelinic tight junctions were classified into two kinds, marginal and interlamellar tight junctions. The former is located along the outer, inner or lateral margins of a myelin segment (MUGNAINI and SCHNAPP, 1974), and the latter in the middle part of the myelin sheath (DERMIETZEL, 1974; REALE et al., 1975).

The so-called tight junction found in the epithelial tissue is generally considered to be an impermeable barrier (STAEHELIN, 1974) and a site of cell to cell attachment (WADE and KARNOVSKY, 1974). However, the functional significance of the radial component or myelinic tight junction is still a matter of discussion, though it is considered to be a permeability barrier and to have a role in auto-immune phenomena (MUGNAINI and SCHNAPP, 1974; TETZLAFF, 1978). The purpose of the present study is to clarify the
fine structure of the radial component in the optic nerve of the mouse and its ultrastructural changes during Wallerian degeneration, by using thin sections and freeze-fracture replicas.

MATERIALS AND METHODS

The optic nerves of adult mice, pure strain KH-1 (*Mus wagneri* var. *albula*) were used. Besides observations on normal optic nerves, observations were made on the optic nerves of mice in which the eyeball was cut from the orbit with scissors. The injured animals were allowed to survive for differing lengths of time, ranging 2–60 days.

For the light and electron microscopy of sections, the optic nerves were excised and fixed with a mixture (a modification of Karnovsky, 1971) composed of 4% osmium tetroxide 1 ml, 25% glutaraldehyde 0.1 ml, sucrose 0.4 g, 0.2 M phosphate buffer 5 ml and 3% potassium ferrocyanide 2 ml for 90 min at 4°C. The materials were then dehydrated in ethanol, passed through propylene oxide, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate, and examined in an HU-12 type electron microscope. Sections about 1 μm thick were stained with toluidine blue and examined by light microscopy.

For electron microscopy of freeze-fracture replication, the optic nerves were rapidly frozen on a copper block cooled with liquid helium or liquid nitrogen by an RF-20 or RF-2 type freezing apparatus. The frozen materials were fractured at −110°C by an FD-2A type freeze specimen processing device, and then immediately shadowed by evaporation of platinum and carbon. The materials beneath the replica was digested with Kao bleach. The replicas were washed in distilled water, mounted on a copper grid, and examined in an H-500 electron microscope.

RESULTS

1. General appearance of the radial component in normal optic nerves

In thin sections fixed with the mixture of potassium ferrocyanide, glutaraldehyde and osmium tetroxide, the radial components are usually observed as a series of light lines composed of small electron-lucent dots situated in the interperiod lines of the myelin lamellae (Fig. 1). The electron-lucent dots stand in rows arranged radially, either partially or totally running across the thickness of the myelin sheath to form light lines. They are frequently found in those parts of the myelin sheath which are lying near the outer and inner processes of the oligodendroglia. In addition, the radial components are also found in that part where the myelin sheath comes into contact with another myelin sheath belonging to adjacent nerve fibers. In its distribution pattern, the radial components of the mouse optic nerve usually belong to the so-called diffuse type, as classified by Honjin and Changus (1964). The individual light lines of the radial components lie scattered at approximate intervals of 400 Å. These lines show a width of about 80 to 100 Å.

In longitudinal fracture faces of the myelin sheath, many ridges of particles are found along the axis of the fiber. They correspond to the series of light lines of the radial components observed in the thin sections. The spacing between the ridges is calculated to be about 400 Å. The ridges are frequently present just beneath the processes of the oligodendroglia. Each of the ridges appears as a row of particles measuring approximately 100 Å in diameter (Fig. 2, 3). In a transverse fracture face, a
layer-to-layer arrangement of particles, about 100 Å in diameter, are found. In a longitudinal face of the myelin sheath, many slender grooves are found along the axis of the fiber (Fig. 3, arrows). The locus of the ridge and that of the groove are in complementary sites in two different fracture faces, P- and E-faces. The particles are confined to the P-face, while the grooves, devoid of particles, correspond to the E-face.

Fig. 1. A cross section of the normal optic nerve of the mouse. The radial components (R) are seen in the myelin sheath lying near the outer process (O) and inner process (I) of the oligodendroglia. ×75,000

Fig. 2. A freeze-fracture replica of the normal optic nerve. The rows of particles of the radial component located near the outer process (O) of the oligodendroglia are seen on P-face (P). ×75,000

Fig. 3. A freeze-fracture replica of the normal optic nerve. The rows of particles in parallel array in the radial component are seen on P-face (P) and the grooves (arrows) in parallel array are seen on E-face (E). ×72,000
When the materials are frozen with liquid helium, the particles are found exclusively in the P-face, while the grooves are found in the E-face. When the materials are frozen with liquid nitrogen, several particles are found in the E-face intermingling with the grooves, and a few grooves are also observed in the P-face. The shape of the particles produced in freeze-replica shows no fundamental difference between the materials frozen with liquid helium and those with liquid nitrogen.

2. Behavior of the radial component during Wallerian degeneration after eyeball enucleation

Fine structural changes of the radial components during Wallerian degeneration were studied at a site approximately 2 to 4 mm from the severed part of the optic nerve. Thin sections and freeze-replicas were prepared by those techniques used for the normal optic nerve study. Edematous degeneration in the axons of the large myelinated fibers and a slight distortion of the myelin sheath appear within 2 days after the operation. The distortion of the myelin sheath and the hypertrophy of glial cells become more conspicuous with each day. The distortion of the myelin sheath becomes markedly apparent 14 to 21 days after the operation. At 45 to 60 days post-operative, almost all of the nerve fibers have undergone degeneration. While a few fibers retained their normal appearance at 60 days post-operative, they belonged to the efferent fibers derived from the brain.

In thin sections, even in the markedly distorted myelin sheath, the light lines of...
radial components are still observed beneath the outer process of oligodendroglia (Fig. 4). The radial components are also seen in the cortical layer of markedly degenerated myelin sheath (Fig. 5). The radial components located in the cortical layer of the myelin sheath and beneath the outer processes of the oligodendroglia remained observable even at 60 days post-operative.

In the freeze-fracture replica, the rows of particles of radial components show an

Fig. 6. A freeze-fracture replica of the degenerated myelin sheath of the optic nerve fiber in the 7th post-operative day. The rows of particles in the radial component (R) show an undulating pattern. The particles lose their regular arrangement and are dispersed throughout the myelin lamellae. ×48,000

Fig. 7. A freeze-fracture replica of the degenerated myelin sheath of the optic nerve fiber in the 14th post-operative days. The ridges of the radial component (R) located in the deeper part of the myelin sheath show a distortion of the normal parallel course of the particles. ×54,000

Fig. 8. A freeze-fracture replica of the degenerated myelin sheath of the optic nerve fiber 45 days after enucleation of the eyeball. The rows of particles and grooves of the radial components (R) are seen under the outer process (O) of the oligodendroglia remaining without significant changes. ×75,000
undulated pattern in the distorted myelin sheath of the large myelinated fiber in which the axon undergoes edematous degeneration. The particles of radial components separate from the rows and dispersed throughout the myelin lamellae (Fig. 6). The particles of radial components on the inner side of degenerated myelin sheath lose their linear arrangement and fall into disorder (Fig. 7). However, the parallel array of rows of particles in radial components located beneath the outer processes of oligodendroglia remained intact even in a markedly distorted myelin sheath 45 to 60 days post-operative (Fig. 8).

DISCUSSION

By using methacrylate or araldite for embedding and potassium permanganate for staining thin sections of the central myelin sheath of the frog and mouse, Honjin (1959) and Honjin et al. (1963, 1964) demonstrated the radial component to be series of electron-dense, radially oriented, and partially thickened interperiod lines of the myelin lamellar layer. In the present study, which used a fixing mixture composed of potassium ferrocyanide, glutaraldehyde and osmium, and an epoxy resin embedding, the radial component appears as rows of electron-lucent dots standing across the thickness of the myelin sheath. Tabira et al. (1978) show the same light lines for the radial component using a fixative containing osmium ferrocyanide and Durcupan embedding. Dermietzel and Kroczeck (1980) also indicate the same electron-lucent lines using a water-miscible embedding medium (GMA). This indicates that the pattern of the radial components is modified in response to a variance in fixing, embedding and staining methods. The electron dense thickening in the radial components revealed in our previous papers is identical to the electron-lucent dots in the present study. Honjin and Changus (1964) stated that the radial components were found most commonly in that part of the myelin sheath lying under the outer processes of oligodendroglia. They also reported that the radial components appear in another part of the myelin sheath, lying near the inner process of the oligodendroglia, and coming into contact with another myelin sheath belonging to the adjacent nerve fiber. In the present study of the mouse optic nerve, the radial components are observed in the same part of the myelin sheath as in the frog central nervous system. It has been reported that radial components in lower animals, such as amphibians and fish, are more numerous than in mammals (Shinowara et al., 1980), and that the radial components are more developed in the central nervous system than in the peripheral nervous system (Shinowara et al., 1980; Tetzlaff, 1982). In a simultaneous study done by us on the mouse sciatic nerve, the radial components were very small in number as compared with those in the mouse optic nerve.

The concept of a myelinic tight junction in the central and peripheral myelin sheath was proposed by Tabira et al. (1978) and Dermietzel (1974, 1980) after using freeze-fracture replication. They suggested that the myelinic tight junctions correspond to the radial components described earlier by Honjin et al. (1959, 1963, 1964) and Peters (1961, 1964, 1968). Certainly, through freeze-fracture replication, intramembranous particles in parallel array are found, situated in the locus of the radial component in thin sections. The intramembranous particles are arranged in ridges which run radially across the myelin sheath and most prevalently beneath the outer process of the oligodendroglia. The particles appearing in replicas show a diameter of 100 Å and a spacing between the ridges of particles of about 400 Å. These numerical values in diameter and spacing correspond to those in the dots and lines of the radial
component observed in thin sections. These observations indicate that the myelinic tight junction of Tabira et al. (1978) and Dermietzel (1974, 1980) is in accord with the radial component. However, in the radial component, the arrangement of ridges of particles is linear and a lack of particles is found in the course of the ridges. These structural features of junction in the radial component are different from those of the so-called tight junction observed in the oviduct epithelial cells, which show a compactness and a continuous arrangement of ridges of intramembranous particles (Komatsu et al., 1978). It is now generally accepted that the tight junctions of the epithelial cell have a mechanical role in maintaining the structural integrity of the epithelium, and also show an impermeable barrier of small molecules and ions. The present observation indicates that the junctional structure in the radial component is a "leaky" type of tight junction as suggested by Shivers (1979).

Honjin et al. (1963) already reported that the radial components observed in thin sections remained during the deformation stage of the myelin sheath in Wallerian degeneration without marked degenerative changes after enucleation of the eyeball in the mouse optic nerve. In the present study using thin section and freeze-replica techniques, the radial component located beneath the outer processes of the oligodendroglia, which corresponds to the marginal tight junction of Mugnaini and Schnapp (1974), keeps a normal appearance for a period of up to 60 days post-operation; while the radial component located in the deeper part of the myelin sheath, which corresponds to the interlamellar tight junction of Dermietzel (1974), becomes diffused in a relatively early post-operative period. This fact strongly suggests that the radial component plays a resistant role against the disintegration of the myelin lamellae during Wallerian degeneration.

Until now, there is only one report by Tetzlaff (1982) about changes in the myelinic tight junction during Wallerian degeneration in the sciatic nerve of the chicken. He reported the loss of the parallel-running of the particles and the formation of circular or polygonal tight junctional strands. In the present study, the formation of the circular or polygonal arrangement can not be observed. Tabira et al. (1978), based on experiments of the frog optic nerve, reported a remarkable resistance of the myelinic tight junction against the vacuolating effect of hexachlorophene (HCP) intoxication. Nagra et al. (1981, 1982) described the myelinic tight junction's role as that of "stopper," preventing against the myelin splitting induced by triethyl tin (TET) in the spinal ganglia of neurological mutant mice. Recently, Mackenzie et al. (1984) reported that electron-dense tracer lanthanum nitrate passes through the marginal tight junctional system to enter the interperiod gap. These reports support our opinion that the radial component has an important role in maintaining the structure of the myelin lamellae, though it is not so solid as the epithelial tight junction.

REFERENCES


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