Journal of Neurocytology **12**, 767–784 (1983)

Abnormal cell relationships in Jimpy mice: electron microscopic and immunocytochemical findings

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Received 4 January 1983; revised 25 April 1983; accepted 4 May 1983

Summary

The mutant mouse strain Jimpy is characterized by a deficiency of myelin formation throughout the C.N.S. The cause of this hypomyelination is unknown. Based on previous reports, astrocytes, axons and oligodendrocytes are all altered, but no single cell type can be unequivocally defined as the primary target. Jimpy and age-matched normal mice were investigated using thin sectioning, freeze-fracturing and immunocytochemistry. We examined optic nerves and cervical spinal cords of Jimpy to determine which cells were morphologically altered during the period which precedes the onset of myelination and which cellular alterations persisted during myelinogenesis.

Abnormalities of astrocytes and axons were frequently observed in Jimpy not only during myelination but also in early postnatal development before mature oligodendrocytes were present. The early astrocytic changes included hyperplasia and alterations of both cytoplasm and plasma membrane. An unusually complex network of astrocytic processes divided the axons into very small groups. During myelination, astrocytic processes were found insinuated between the axons and myelin sheath and/or within the myelin lamellae. Immunocytochemical investigations also revealed a complex network of glial fibrillary acidic protein-positive processes in contact with the majority of the axons. At stages prior to myelination axonal alterations were detected. Most of the axons were not in close contact with one another and individual axons had an undulating and irregular course. In areas where axon separation by astrocytic processes occurred, axonal diameters were more variable than the homogeneously sized axons of the normal mice. Our immunocytochemical results at stages during myelination showed not only many myelin basic protein-positive processes around axons in Jimpy but also clearly immunostained myelin sheaths. This indicates that the myelinating glia present not only produce myelin basic protein but can also incorporate it into the myelin spiral. The presented results suggest that the mouse mutant Jimpy

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0300-4864/83 $03.00 + .12

could be a model for disturbed cell interactions in the C.N.S. Therefore, the hypomyelination may not be attributed to a defect of a single cell but rather to a deficiency in both macroglial types and, perhaps, the axon as well.

Introduction

In the C.N.S., neurites, astrocytes and oligodendrocytes are thought to interact before and during myelin formation (Varon & Somjen, 1979; Mugnaini, 1982). Mouse mutants have been useful models for studies of these interactions (Baumann, 1980). The house mouse Jimpy is a sex linked, recessive, lethal mutation (Phillips, 1954). This strain is characterized by a deficiency of myelin formation throughout the C.N.S., whereas the P.N.S. appears to be normally myelinated (Sidman *et al.*, 1964). The complexity of the hypomyelination in Jimpy is illustrated by the descriptions of abnormalities associated with axons (Hirano *et al.*, 1969; Robain & Mandel, 1974; Webster *et al.*, 1976), oligodendrocytes (Privat *et al.*, 1972; Meier *et al.*, 1974b; Webster & Sternberger, 1980; Skoff, 1982) and astrocytes (Skoff, 1976; Omlin & Bischoff, 1980).

This involvement of several cells, with different functions and structures, makes it difficult to identify which cell, or failure of what function, reflects the primary defect in Jimpy mice. A nonaccomplishment in normal cellular interactions possibly underlies the myelin deficiency of this mutant. Therefore, we investigated which cell or cells are morphologically altered during the period that precedes onset of myelination and which alterations persist during myelinogenesis.

Abnormal astrocyte–axon formations were present early in development. They persisted during the period of myelin formation and affected the axon–oligodendroglial interactions that are thought to be required for normal myelination. A preliminary report of these findings has been published (Omlin & Webster, 1982).

Materials and methods

Jimpy mice (C 57 BL/6J-W A-J) were obtained from Jackson Laboratories (Bar Harbor, USA). Affected mice were males (+jp/y) and were offspring of carrier females (Ta+/+jp) and Tabby males (Ta+/y). Tabby syndrome carriers had two supraorbital vibrissae by which Jimpy males can be distinguished from non-Jimpy mice (Sidman *et al.*, 1964). Due to crossing over (7–14 units) between the Tabby and Jimpy loci (Wolf & Holden, 1969), the determination of a Jimpy male at an early stage can only be made with great certainty by examining the presence or absence of myelin in the spinal cord. Optic nerves of 4, 6 and 21 day old animals, 5–7 mice for each age, were studied as examples of a late myelinating C.N.S. area. For both freeze-fracturing and thin sectioning, an optic nerve segment was cut close to the chiasm and 1–1.5 mm distal to it. The cervical spinal cord represents an early myelinating region and was investigated at 4 and 15 days after birth. Age matched, normal males of a wild mouse strain (C 57 BL/6J) served as controls.

Freeze-fracturing

After decapitation, optic nerves of 4 and 21 day old Jimpy and normal animals were exposed quickly and fixed *in situ*, for 3–4 min at 36° C. The fixation solution contained 0.5%

paraformaldehyde, 0.5% glutaraldehyde, Eagle's minimum essential medium (EMEM) and 0.1 M phosphate buffer (pH 7.4) (Omlin *et al.*, 1980). A series of glycerol concentrations (15, 20 and 25% in EMEM; 7 min each, 36° C) was then instilled into the base of the cranium. The optic nerves were dissected and then frozen on small gold discs by quenching in liquid nitrogen cooled Freon 22. The freeze-fracturing was performed in a Balzers BA360 M apparatus. The stage temperature was maintained at −110° C in a vacuum of about 6×10^{-7} torr. The temperature of the knife during fracturing and etching was −180° C. The fractured specimen was etched for 2–3 min and then replicated with platinum–carbon and carbon. Membrane faces in our replicas are named according to the terminology of Branton *et al.* (1975).

Thin sectioning and estimation of axon diameters

Jimpy and normal control mice were fixed by transcardiac perfusion at 6 and 15 days after birth. The fixative contained 3.0% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6, 36° C). Postfixation of optic nerves and cervical spinal cord was carried out in 2% osmium tetroxide (4° C, 2–4 h) followed by dehydration in ethanol and embedding in Epon or Araldite. Semithin as well as thin sections were cut with glass knives on an LKB IV ultramicrotome. The thin sections were mounted on Formvar-coated slot grids and examined with a Philipps 201 or 400 electron microscope.

From optic nerves of one 6 day old Jimpy and one age matched normal mouse, 210 axons were measured for the mutant and 210 for the control. The method of measuring each axon consisted of calculating the mean of two diameters taken perpendicular to each other (Arees, 1978). Seventy axons from the Jimpy mouse were measured from the negative (× 21 500 primary magnification) of Fig. 1 and 70 axons from the control were measured from the negative (× 21 500 primary magnification) of Fig. 4. Measurements of the remaining 140 axons of each animal were made from six other negatives, which were scattered over the whole cross-section of the nerve segment. This small number of measured axons represents an estimation and is thought to show a trend of axonal growth in relation to both astrocytic–axon and axon–axon contacts.

Immunostaining

The procedure which we used was the one described by Trapp *et al.* (1981). Semithin (1 μm) and thin (80–30 nm) sections were cut alternately and assembled. The 1 μm sections were put on glass slides and the thin sections on single slot Formvar-coated copper grids. The plasic of the 1 μm thin sections was removed from the tissue with a solution of sodium ethoxide/absolute ethanol (Baskin *et al.*, 1979) followed by 0.2% hydrogen peroxide treatment. The sections were immuno-stained either for myelin basic protein (MBP, 1:500) or for glial fibrillary acidic protein (GFAP, 1:500) by the peroxidase–antiperoxidase (PAP) technique (Sternberger *et al.*, 1970). The MBP is a marker for myelin and myelinating oligodendrocyte cytoplasm (Sternberger *et al.*, 1978) whereas the GFAP is present in the astrocytes (Eng *et al.*, 1971, 1980; Bignami *et al.*, 1972). Production and specificity of the goat antiserum to rabbit MBP has been described previously (Itoyama *et al.*, 1980; Omlin *et al.*, 1982). Antirabbit GFAP was obtained from Dakopatts (Westbury, NY, USA). Immuno-stained areas were light microscopically photographed and these images transferred onto micrographs of the same region taken in the EM. This procedure allows the characterization of fine structure of both the immuno-stained cells with their processes and the myelin sheath (Trapp *et al.*, 1981).

Results

Fine structure before myelination

Optic nerves dissected out from seven Jimpy and five normal mice were compared during early postnatal development (4–6 days after birth) by use of serially cut thin sections and freeze-fracture replicas. Astrocytic processes in Jimpy mice had an abnormal and extensive pattern of branching which penetrated the axon bundles and surrounded small groups of axons or single axons (Fig. 1). These astrocytic processes contained numerous glial filaments, glycogen granules and lamellar inclusion bodies (Fig. 2). In contrast, astrocytic processes in normal mice divided the axon into large bundles consisting of many axons and the astrocytic cytoplasm had fewer clusters of glial filaments and glycogen (Fig. 4). Gap junctions between the astrocytic processes were more prevalent in Jimpy mice and often joined processes which completely encircled a single axon. In the Jimpy optic nerves, axons were more variable in diameter and circumference and contained more lucent vacuoles than those in the control mice (Figs. 1 and 4, respectively).

In Jimpy optic nerves, at this early stage 6 days after birth, none of the cells present had features typical of a differentiated oligodendrocyte and no myelin formation was observed. In the normal mice, a few oligodendroglia were present and some axons were myelinated (Fig. 3).

In replicas of frozen and longitudinally fractured Jimpy optic nerves, most of the axons were not in close contact with each other. Furthermore, individual axons had an undulating and irregular course. In addition, most of these naked axons were separated by clearly identifiable astrocytic processes of variable size. These observations indicate that in Jimpy there is a disorganized arrangement of the axons. Some of the axons could be followed over a length of up to 40 μm. Several of these single axons had a highly variable diameter along their course. Vacuoles within the axoplasm could be observed, too, according to the findings in thin sections. The number and distribution of individual axolemmal intramembranous particles were similar in the control and Jimpy mice. In contrast, longitudinal fractures of control optic nerves revealed areas with much higher density of axons. These homogeneously sized (diameter) axons had a well organized and regular, parallel course, and no vacuoles were present within the axoplasm.

Figs. 1 & 2. Electron micrographs of the optic nerve from a 6 day old Jimpy mouse.

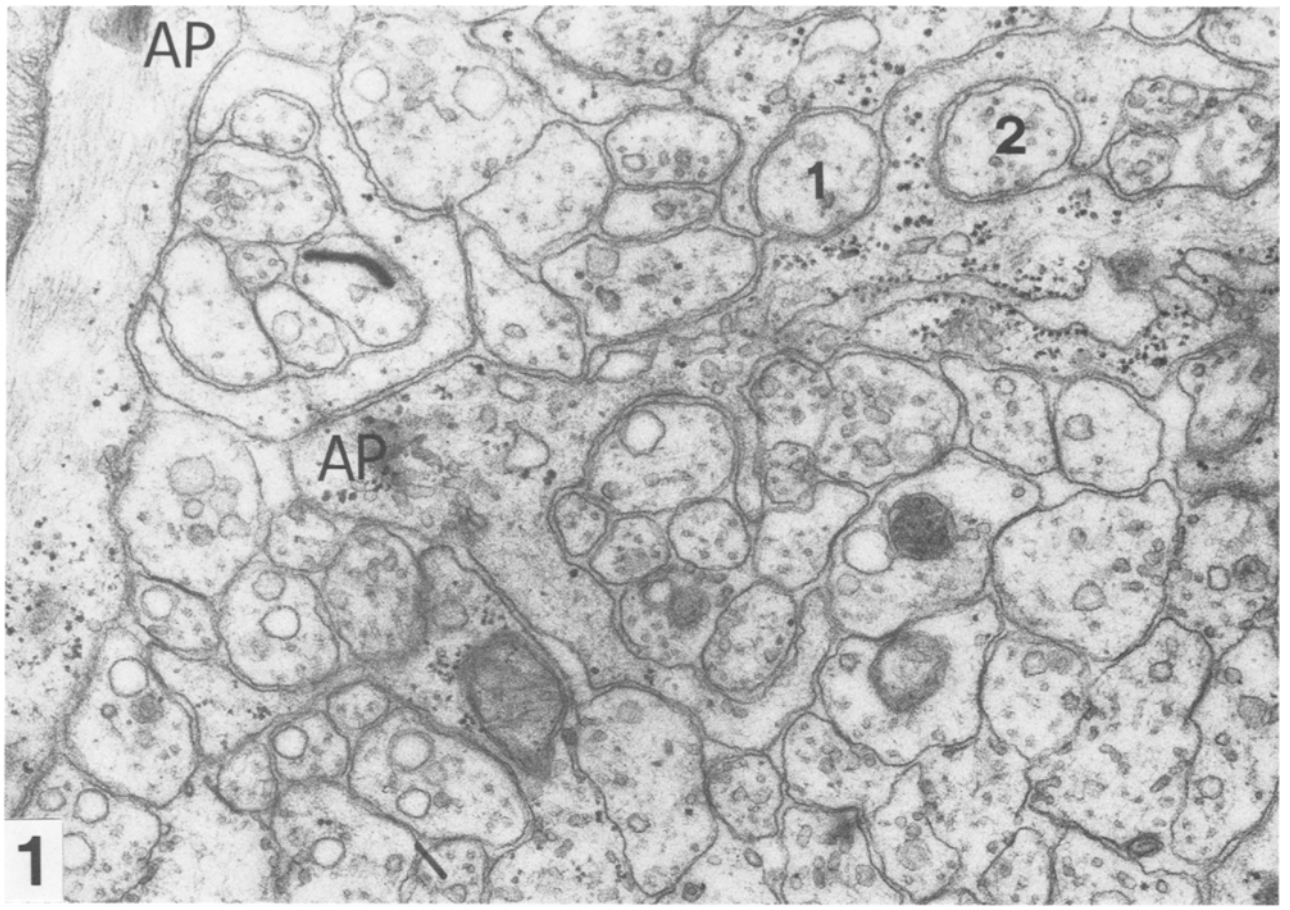
Fig. 1. Astrocytic processes (AP) surround small groups or single axons (1 and 2). Note the variability of axonal diameter. × 45 000.

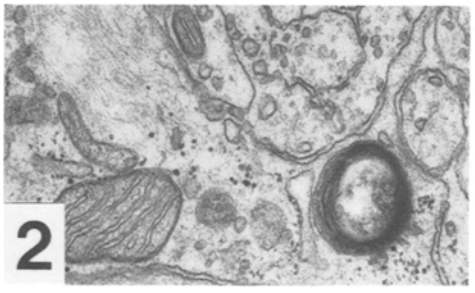
Fig. 2. Multi-lamellar inclusion body within an astrocytic process. × 34 000.

Figs. 3 & 4. Electron micrographs of the optic nerve from a 6 day old normal mouse.

Fig. 3. Oligodendrocytic process (OL) surrounds an axon. At this stage such processes are absent from the optic nerve of Jimpy animals. × 70 000.

Fig. 4. Astrocytic processes are not forming a complex network of branches as in the mouse mutant. The axonal profiles are relatively uniform in diameter. × 45 000.


AP
1
2
AP
1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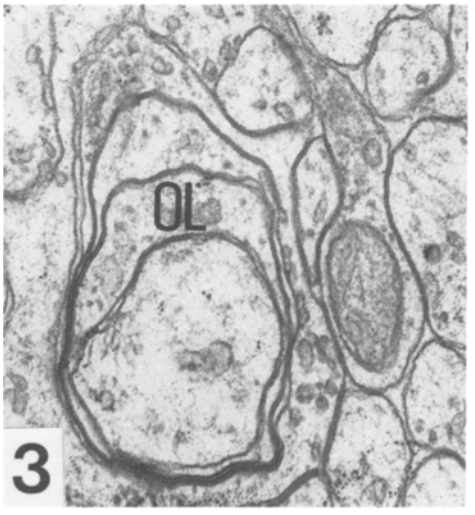
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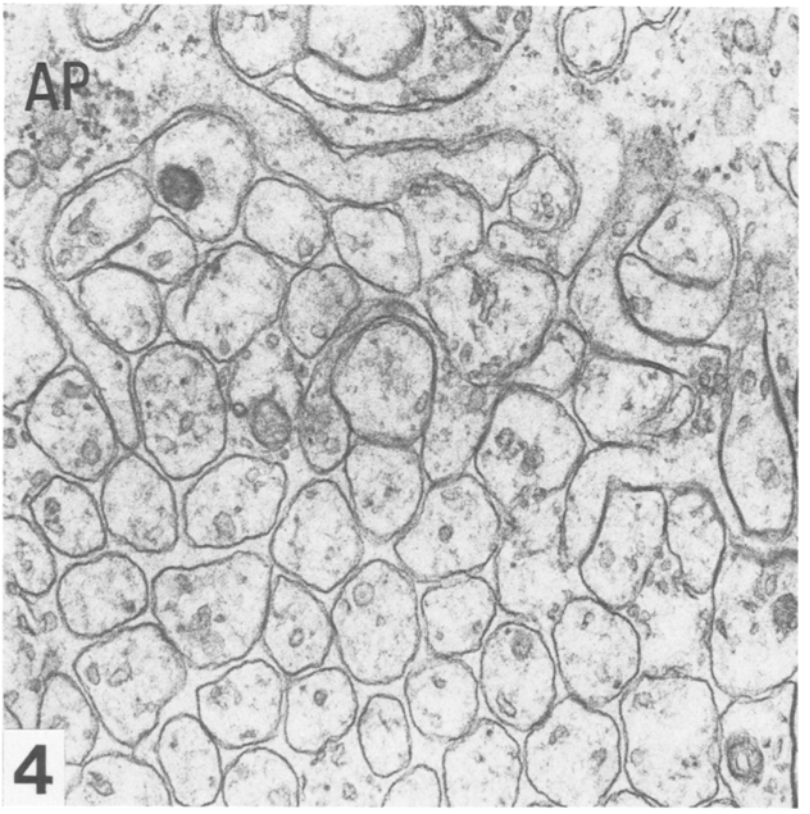
AP
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Astrocytic plasma membranes of Jimpy C.N.S. were different from those of the controls. In the Jimpy tissue, orthogonally arranged groups of 5–7 nm particles, or assemblies, characteristic of astrocytic plasma membranes, were present in the processes and cell bodies (Fig. 5). They were absent at this stage of development in the controls (Fig. 6). Gap junctions were present in astrocytic plasma membranes from both control and Jimpy optic nerves (Figs. 5, 6), but were more frequent and larger in Jimpy mice.

Quantification of axon diameters resulted in a normal, unimodal distribution curve for the control animal and a non-normal curve for the Jimpy mouse (Fig. 7). The mean diameter of the 210 control axons (0.27 μm ± 0.05 S.D.) was statistically not significantly different from that of the 210 Jimpy axons (0.32 μm ± 0.12 S.D.). But the variance of the diameters as a mean of variability of the Jimpy axons was more than four times greater than that of the controls. Another interesting trend was revealed in the Jimpy mice when axons, which were in contact only with each other (19 out of 210), were considered and measured separately from axons which were in contact with astrocytic processes (191 out of 210). The mean diameter of these 19 axons (0.28 μm ± 0.06 S.D.) corresponded to that of the 210 axons from the control mice (0.27 μm ± 0.05 S.D.). The authors are aware of the limitation of their data about alteration of axonal diameter, although the trend, which shows that changed axonal fasciculation might have a negative influence on the normal axonal growth, is important enough to be mentioned.

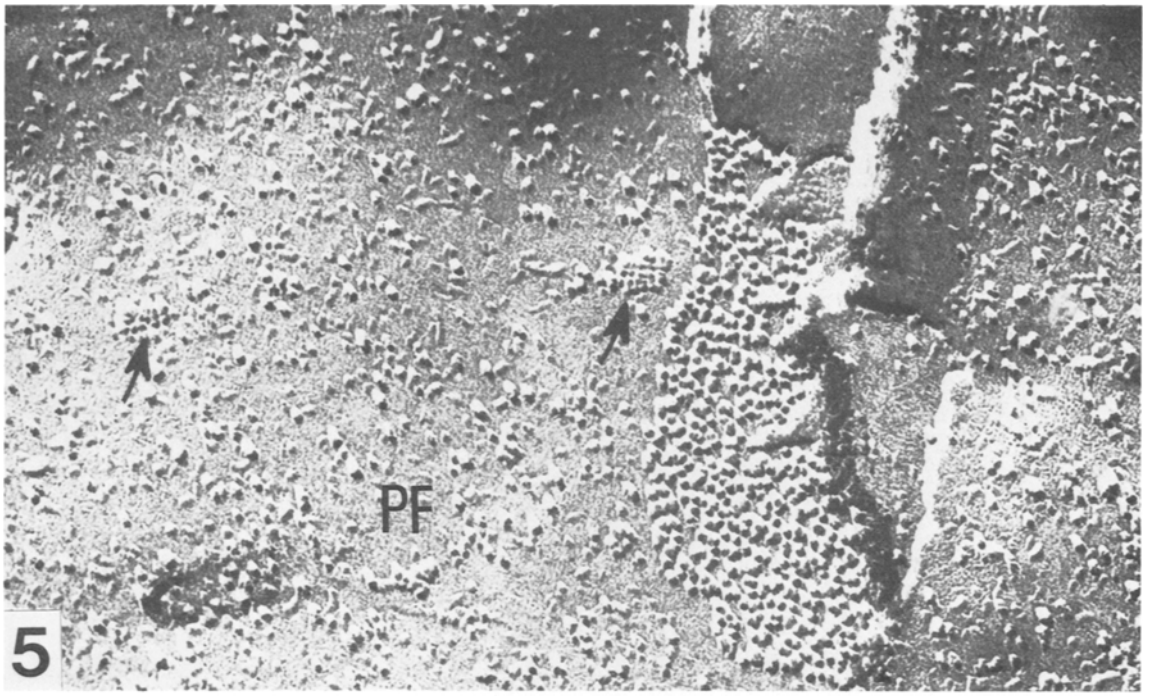
Fine structure during myelination

In the cervical spinal cord at 15 days after birth, few compact myelin sheaths were present in Jimpy mice. The increase in the number of astrocytic processes which occurred during early postnatal development of the Jimpy optic nerves continued during myelinogenesis. One or more astrocytic processes were insinuated between the axon and inner surface of the myelin sheath of 20% of these myelinated fibres. The number of interposed astrocytic branches and the degree to which they extended along the axon and wrapped around the circumference of the axon was variable (Figs. 8–10, 12). These processes, when present, covered 25–100% of the axon surface in transverse sections and extended longitudinally up to 10–20 μm. Within their cytoplasm were found mitochondria, smooth endoplasmic reticulum, free ribosomes, glial filaments, glycogen and microtubules (Figs. 8–12). Direct contact by apposition, and spacing, suggesting complexes were present between the axolemma and adjacent, interposed astrocytic plasma membranes (Fig. 11). Since no tilting (goniometer stage technique) has

Figs. 5 & 6. Freeze-fractured astrocytic plasma membrane from the optic nerves of 4 day old mice showing the protoplasmic fracture face (PF).

Fig. 5. Jimpy astrocyte, showing characteristic particle assemblies (arrows) and a high density of individual particles. × 110 000.

Fig. 6. In the normal mouse, astrocytic assemblies are very rare and few background particles, arranged in rosettes, are present. × 110 000.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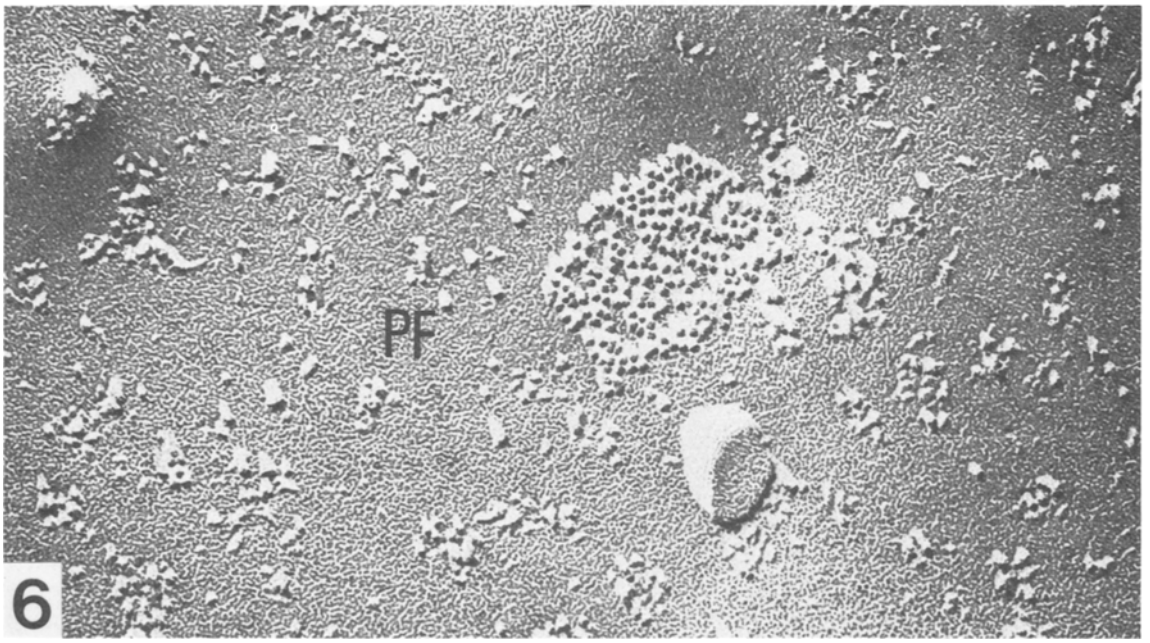
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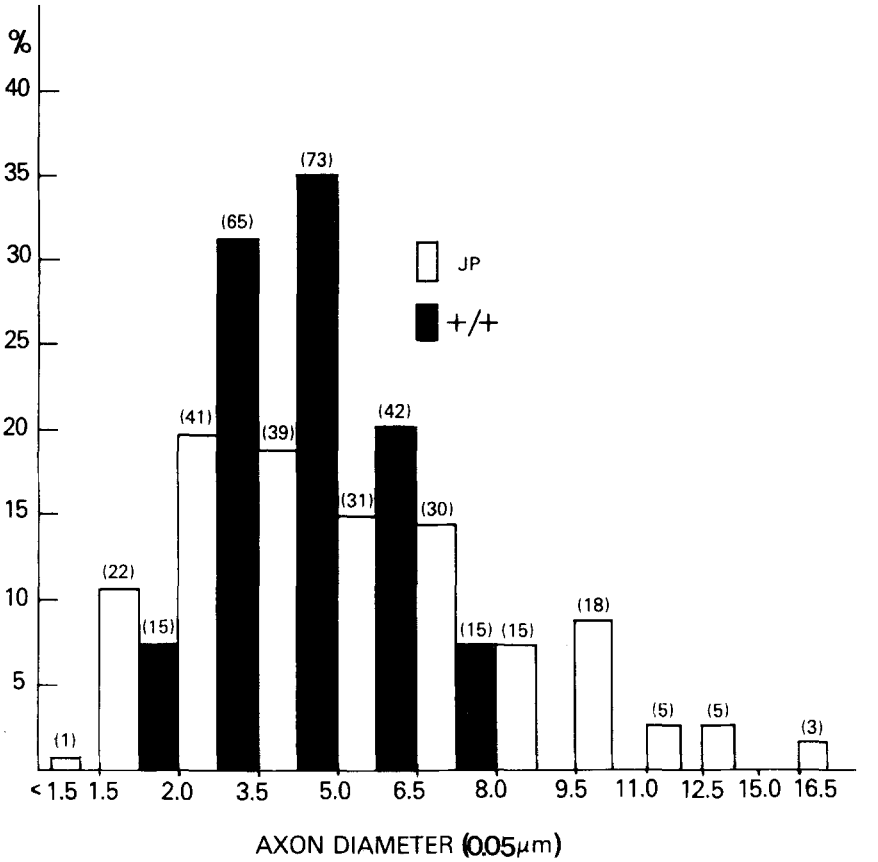


Fig. 7. Pattern of axon diameters (in 0.05 μm) and frequency (in %) of 420 axons in all, of optic nerves from 6 day old mice (JP = Jimpy, +/+ = age matched control). The absolute number of each class is indicated in parentheses at the top of the bars. The diameter classes were formed after measurements of all 420 axons.

Figs. 8–12. Electron micrographs of the cervical spinal cord from a 15 day old Jimpy mouse. All fibres illustrated show an insinuated astrocytic process (AP) between myelin sheath and axolemma.

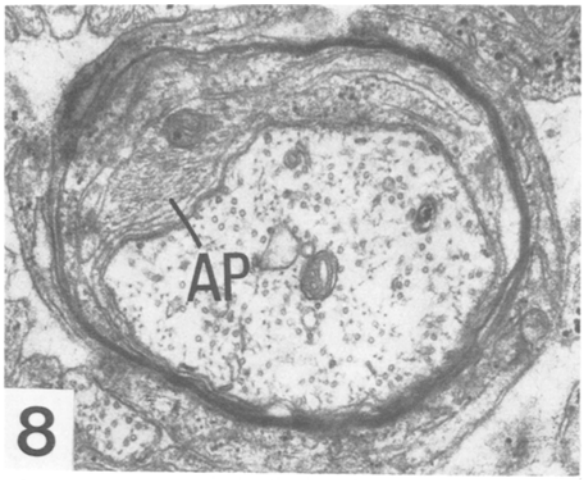
Fig. 8. Myelinated fibre with three compact lamellae. AP contains many glio-filaments. × 36 000.

Fig. 9. Myelinated fibre with eight compact lamellae. Some sign of myelin-breakdown is indicated (arrow). AP contains both glio-filaments and glycogen. × 20 000.

Fig. 10. An astrocytic process (AP) containing microtubules and glio-filaments surrounds almost the whole circumference of the axon. Arrows point at signs of myelin breakdown. × 42 000.

Fig. 11. Higher magnification micrograph of part of Fig. 10, showing junction-like specializations (arrows) between the axon and the astrocytic process, which contains glio-filaments (F) and glycogen (G). × 98 000.

Fig. 12. Eighteen myelin layers surround this axon. The insinuated astrocytic process (AP) between myelin and axolemma shows a bundle of glio-filaments, many microtubules in cross section and granules of glycogen. The inner tongue process of the oligodendrocyte is indicated by an O. × 50 000.



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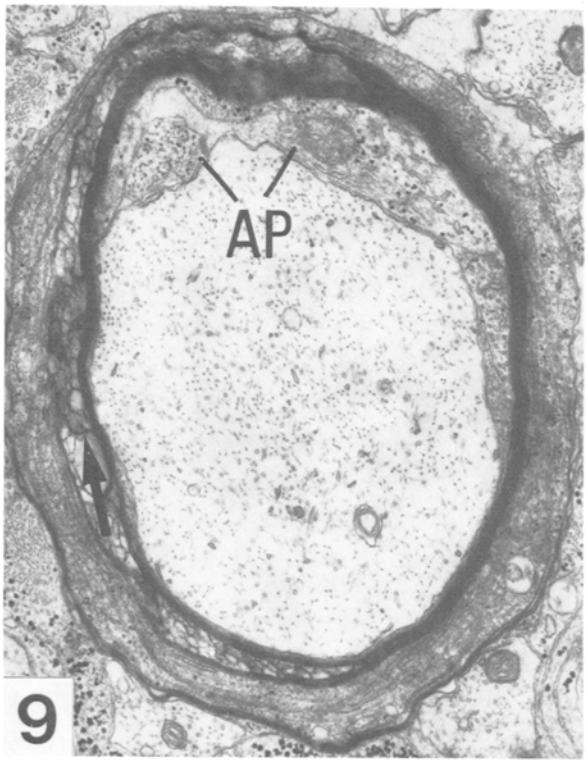

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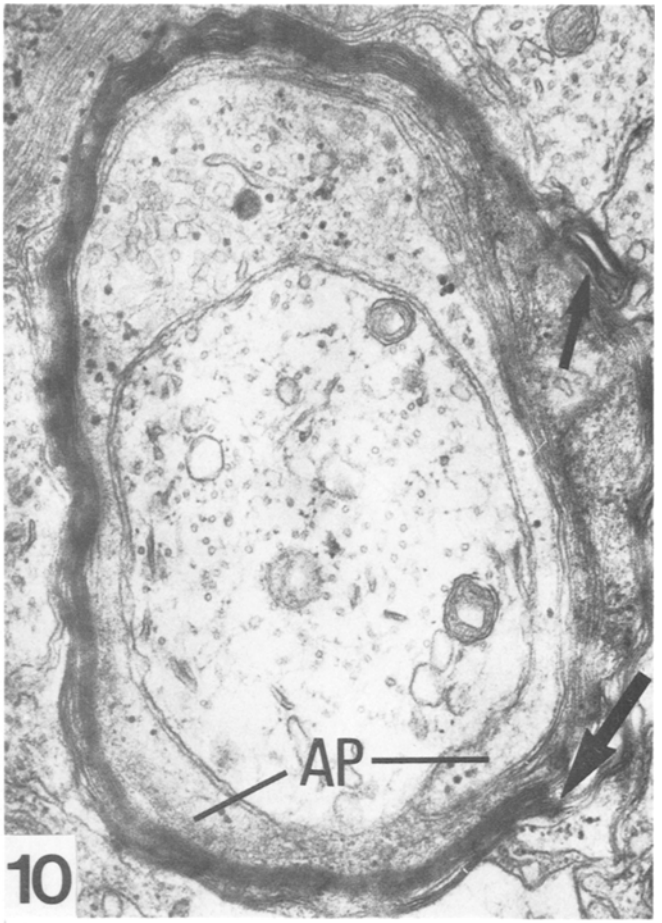

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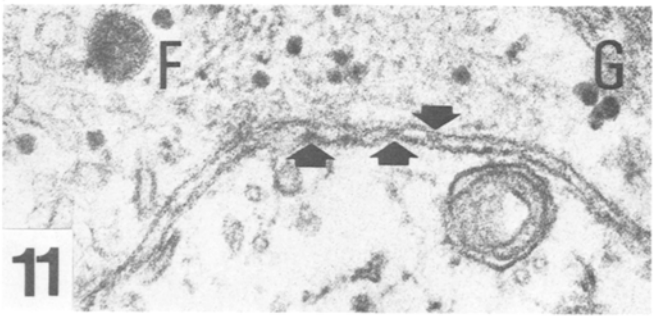

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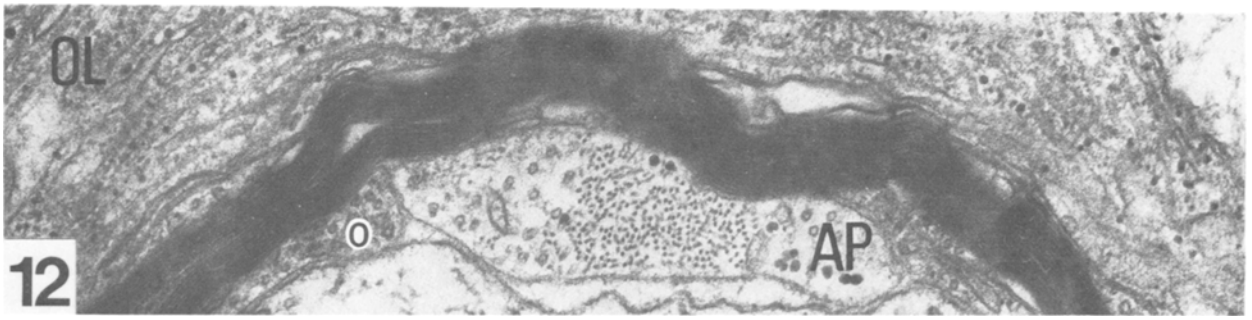

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been applied, the possibility that these structures are cutting artefacts remains open. Evident are the structural similarities with the membrane specializations between axons and astroglia in demyelinated lesions of the C.N.S. described by Raine (1978).

The myelin sheaths of axons with interposed astrocytic processes were of different thicknesses. Some consisted of only 3–4 compact lamellae (Fig. 8), some had eight compact lamellae (Figs. 9, 10); while others were thicker with 14 or more lamellae (Fig. 12). Signs of myelin breakdown occurred along these axons (Figs. 9, 10).

There were few oligodendrocytes present in Jimpy tissue and they appeared morphologically normal. Approximately 10% of all glial cells present were microglia. The characteristics of these cells included: a small nucleus with scattered chromatin, cytoplasm with variable lamellar and crystalline inclusions; and freeze-fracture replicas revealed a plasma membrane, which shows a different particle distribution compared to that of astrocytes, oligodendrocytes and myelin.

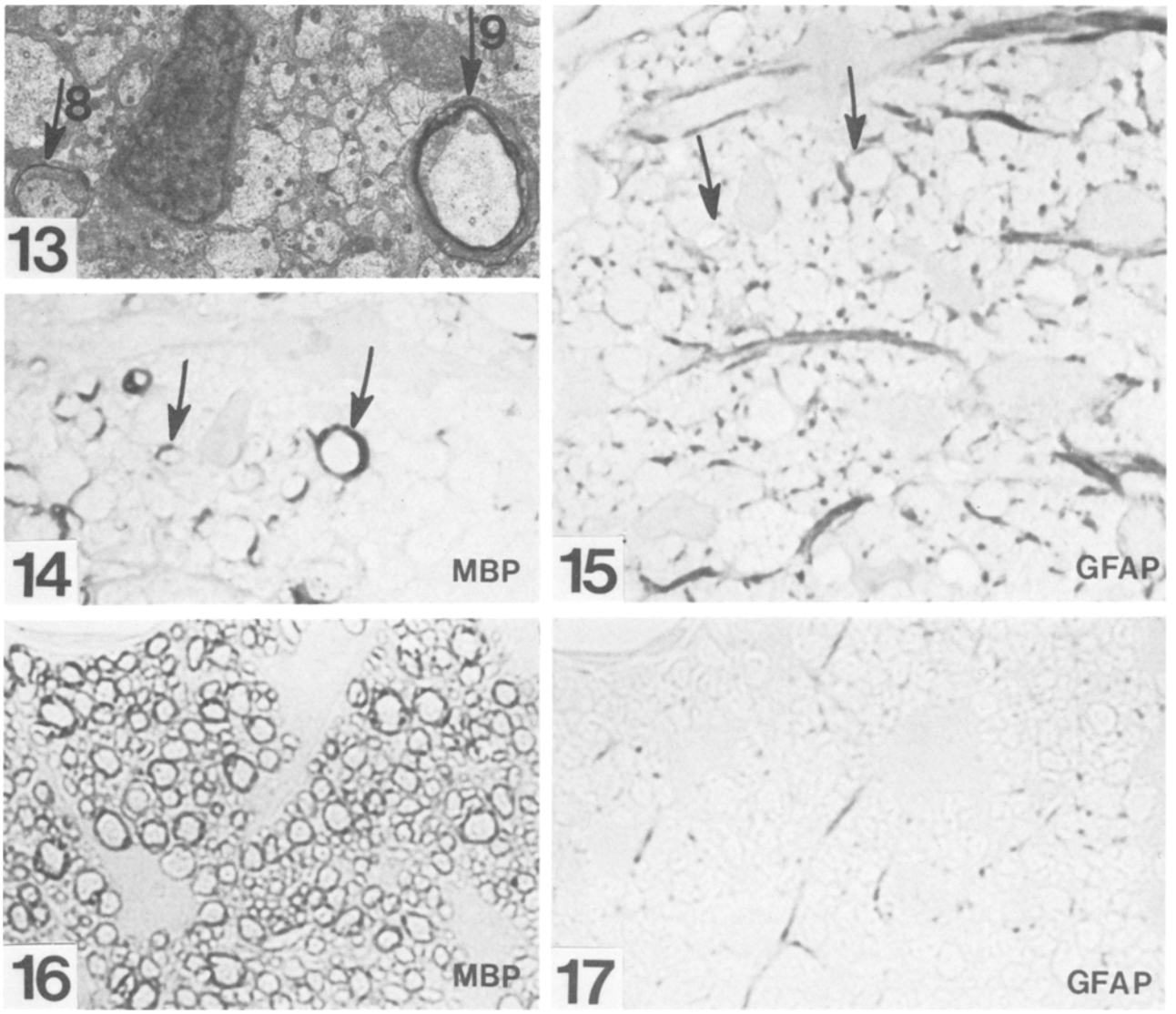
Immunocytochemistry during myelination

To examine and confirm further the abnormal relationship between the axons, astrocytes and myelin sheaths in Jimpy mice, immuno-stained 1 μm thin sections were compared with adjacent thin sections. Cervical spinal cord of 15 day old mutant and normal mice were used for this investigation.

The low magnification electron micrograph (Fig. 13) of the Jimpy tissue shows parts of the area in two adjacent semithin sections (Figs. 14, 15), immuno-stained, respectively, for MBP (Fig. 14) and GFAP (Fig. 15). The two myelinated fibres (Fig. 13, arrows) had an interposed astrocytic process between the myelin sheath and the axon. This identification can be confirmed by the fine structure of these two fibres (Figs. 8, 9) as well as by the immunocytochemical findings. The myelin sheaths and the oligodendrocytic processes were immuno-stained for MBP (Fig. 14, arrows), while the astrocytic branches of the same area were labelled for GFAP (Fig. 15, arrows).

It is of interest that in overviews, MBP immuno-stained collars were visible around many axons. This light microscopic appearance did not show if the immuno-stained collars were oligodendrocytic processes or myelin; however, the adjacent thin sections revealed that most of these labelled structures were processes of oligodendrocytes that had not formed a myelin spiral; a few were compact myelin (Fig. 14, arrows). In contrast, the cervical spinal cord of normal mice contained many MBP immuno-stained myelin sheaths (Fig. 16).

The difference in GFAP immuno-stained sections between Jimpy (Fig. 15) and age matched normal animals (Fig. 17) is evident. The mutant presented a complex network of astrocytic branches, the courses of which were either parallel or perpendicular to the axons. Almost all axons of the mutant were in contact with one or more astrocytic branches. Immuno-stained astrocytic processes appeared in cross-sections as black dots of variable diameters (Fig. 15). Also, the processes were more intensely immuno-stained compared to those of the normal animals.



Figs. 13–17. Electron and light micrographs of the cervical spinal cord from 15 day old Jimpy mice (Figs. 13–15) and normal animals (Figs. 16, 17).

Fig. 13. Area of cervical spinal cord from a 15 day old Jimpy mouse, containing the two myelinated fibres, which were presented in Figs. 8 and 9. × 6000.

Figs. 14 & 15. One-micron thin Epon sections cut adjacent to the thin section of Fig. 13. The section of Fig. 14 was immunostained for MBP. Few MBP-immunostained structures are visible (arrows). The section in Fig. 15 was immunostained for GFAP. GFAP-positive structures (arrows) are insinuated between axons and myelin sheaths. Note the complex network of cross and longitudinally sectioned astrocytic processes. × 2600.

Figs. 16 & 17. One-micron thin Epon sections immunostained for MBP (Fig. 16) or GFAP (Fig. 17). In the normal animal, many MBP-positive myelin sheaths are present. GFAP positive processes show a less dense network compared to the Jimpy mutant. × 1300.

Discussion

Astrocytes in optic nerves and cervical spinal cord of Jimpy mutant mice display a number of abnormalities not only during myelination but also in early postnatal development before mature oligodendrocytes appear. The early astrocytic abnormalities include hyperplasia and alterations of the cytoplasm and plasma membranes. The astrocytes form an extensive and complex network of processes that divide the axons into very small groups. During myelination, astrocytic processes insert between the axons and myelin sheath and/or within the myelin lamellae, an observation confirmed by our immunocytochemical investigations which revealed a complex network of GFAP-positive processes in contact with the axons.

These findings, which all involve astrocytic abnormalities raise two important questions. Can the astrocytes in Jimpy mice be defined simply as 'reactive' or could the astrocytic abnormalities explain the hypomyelination?

Earlier investigations of Jimpy tissue have illustrated alterations in astrocytes, including an increase in the number of astrocytic branches (Skoff, 1976), the presence of giant astrocytes (Kraus-Ruppert *et al.*, 1973), increased numbers of glycogen granules (Privat *et al.*, 1972) and glial filaments (Skoff, 1976), as well as inclusion bodies (Meier & Bischoff, 1975). Some of these changes took place when myelination was proceeding. Therefore, they were interpreted as a secondary phenomenon and part of an extrinsic response. However, an intrinsic defect of Jimpy astrocytes is suggested by a profound functional alteration; K^+-induced stimulation of oxygen uptake by astrocytes is absent both *in vitro* (Hertz *et al.*, 1980) and *in vivo* (Keen *et al.*, 1976). In our opinion, this functional abnormality of astrocytes, together with our observations of alterations before myelination occurs, indicate that the astrocyte changes are probably not simply 'reactive' nor secondarily affected. This leads to the second question: to what degree are astrocytes responsible for the hypomyelination?

Investigations of astrocytes in Jimpy and normal mice before and during myelination (Skoff, 1976, 1981) showed a more complex network of astrocytic processes in optic nerves of two day old Jimpy animals compared to the age matched normal controls. It was suggested that these branches formed a mechanical barrier between axon and oligodendrocytic processes so that the myelin-forming process of oligodendrocyte could not reach the axon (Skoff, 1976). Although our observations were similar, semi-thin sections immunostained for MBP indicate that some of the axons in Jimpy are surrounded by oligodendrocytic processes, even though they do not form a myelin sheath. This finding can be interpreted that the astrocytic barrier suggested by Skoff (1976) is incomplete and, therefore does not prevent all oligodendrocytic processes from reaching the axolemma.

The astrocytes in Jimpy mice divide the whole axon population into many small groups of axons. This division appears to be related to both the axon diameter and the irregular course. In areas where the separation of axons is distinct, the axon calibres were much more variable than the homogeneously sized fibres of the normal animals.

This variability in axon calibre may be a reflection of an alteration in the axonal growth process due to a highly reduced axon–axon contact and due to a change in the local environment caused by the abnormal network of the astrocytic branches. There is strong evidence that normal astrocytic development is necessary for the guidance of outgrowing axons: Silver *et al.* (1982) found in normal mouse foetuses, that the first callosal axons grow along the surface of the astrocytes which form a cellular bridge or 'sling'. Furthermore they found in a mouse mutant suffering from hereditary agenesis of the corpus callosum that the astrocytic 'sling' does not form and the axons cannot cross the midline. The finding that axons in Jimpy mice have an irregular course might be attributed to a malformation of the axonal fasciculation caused by abnormal astrocytic branching.

The presence of misplaced, interposed astrocytic processes suggest to us the possibility of malfunctioning cell-to-cell interaction in the C.N.S. of Jimpy mice. A further indication for disturbed cell interactions is the described occurrence of junction-like connections between axons and astrocytes. Interposed astrocytic processes have also been reported by Griffiths *et al.* (1981) in so called shaking pups, a hypomyelinating mutant of Spaniel dogs. We suggest that these altered astrocytes have, even during myelination, an abnormal preference to contact axonal surfaces. The presence of junction-like structures between axon and astrocytic branches might be a reflection of such an abnormal affinity. On the other hand the association of the MBP-positive oligodendrocytic branches with the axons seems too weak to maintain the contact. Additionally, most of the fibres with interposed astrocytic processes show some sign of myelin disruption, which corresponds with observations of Raine (1976) in EAE and MS tissue. This could indicate that the myelin sheaths formed in Jimpy may not be maintained in a normal manner.

In a recent study, Nixon (1982) showed that axonal proteolysis increased twice as fast in retinal ganglion cells of myelin deficient mutant mice (e.g. of Jimpy) as in the controls. He suggested that this increase was due to an abnormal axon–glia interaction. The frequent occurrence of vacuoles in the axoplasm of Jimpy optic nerves could represent the morphological correlate of the increased proteolysis described by Nixon (1982). These findings also indicate that glial and axonal functions are interdependent (Foster *et al.*, 1980).

On the other hand it can also be suggested that the axons require contact with other axons to ensure simultaneous growth and synchronization of myelin formation. Furthermore, it has been reported that some of the proteins needed for stimulation of myelin production originate from the axons (Giorgi *et al.*, 1973). When most of the axons are separated from each other by astrocytic processes, as is the case for Jimpy mice, the exchange of axonal factors could be impeded. Therefore, axon-to-axon communication for stimulation of myelin production would occur between only a small percentage of the axonal population in the mouse mutant Jimpy.

It is generally accepted that the number of mature oligodendrocytes in Jimpy is decreased (Farkas-Bargeton *et al.*, 1972; Privat *et al.*, 1972; Kraus-Ruppert *et al.*, 1973;

Meier & Bischoff, 1975; Skoff, 1976, 1982). Skoff (1976) showed in an ultrastructural classification and count of glial cells, that oligodendrocytes are reduced by approximately 50%. This decrease certainly reflects the near absence of myelin in the mutant but it is not the only cause (Skoff, 1976).

Oligodendroglia produce MBP (Sternberger *et al.*, 1978), a major protein constituent of C.N.S. myelin (for review see e.g. Braun & Brostoff, 1977; Carnegie & Moore, 1980). Immunocytochemical results indicated MBP positive structures around axons in Jimpy (Sternberger *et al.*, 1979). Our findings on 1 μm thin immunostained sections compared with the adjacent thin section show that not only oligodendroglia cell processes but myelin sheaths are positive with the MBP antibody. Thus, the oligodendrocytes of Jimpy do not only produce MBP but are also able to incorporate this major myelin protein into the plasma membrane of the myelin spiral. It would appear therefore that some of the oligodendroglia cells are, in this regard, similar to normal myelinating glia.

Meier and colleagues (1974a, 1974b, 1975) identified cells which contain lipid bodies as abnormal oligodendrocytes. They interpreted these inclusions as precursors or accumulation products of myelin constituents. In conclusion they claimed that Jimpy is therefore a model for the human disease of Pelizaus-Merzbacher, which is thought to represent a condition due to a primary oligodendrocyte defect (Zeman *et al.*, 1964). However, some of the cells identified as oligodendrocytes (e.g. Fig. 2 of Meier *et al.*, 1974) have the morphological appearance of microglia or macrophages, according to our interpretation and that of Dentinger *et al.* (1982). In addition, the freeze-fractured plasma membranes of those cells with inclusion bodies clearly distinguished them from astrocytes and oligodendrocytes in Jimpy (Omlin *et al.*, 1979, 1980). These authors suggest that this type of cell is different from both the myelinating glia and astroglia (Dentinger *et al.*, 1982) and possess characteristics of a phagocytosing microglia cell. An increased number of microglia in different areas of Jimpy C.N.S. compared to the normal mouse C.N.S. has been reported (Torii *et al.*, 1971; Farkas-Bargeton *et al.*, 1972; Privat *et al.*, 1972; Kraus-Ruppert, 1973; Skoff, 1976).

Concluding remarks

The obvious characteristic of the C.N.S. of the mouse mutant Jimpy is the near absence of myelin (Sidman *et al.*, 1964). What causes this hypomyelination cannot be pinpointed until the complex intracellular interactions are better understood. But it is clear that astrocytes, axons and oligodendrocytes all show abnormalities.

Astrocytic hyperplasia in the mouse mutant Jimpy is associated with an abnormal fasciculation of axons. The direct environment of the axons may therefore be changed due to the enlarged astrocytic surface, and also due to alterations of the astrocytic plasma membrane and cytoplasm. These astrocytes have some features of reactive glial cells; however, we do not consider them as reactive since the described alterations occur before myelination starts. Recently, Noble (1982) also noted the importance of astrocytes for normal myelination. Using low density platings and cell type-specific antibodies, he

found that astrocytes supply soluble factors required by oligodendrocytes for extended survival. Furthermore, 'astrocytes can profoundly influence oligodendrocyte morphology through cell surfaces' (Noble, 1982). Astrocytes are also implicated in remyelinated axons in experimentally evoked demyelination in the C.N.S. (Blakemore, 1978, 1982). Within lysolecithin-induced lesions, remyelinated axons lie in areas containing astrocytes, while the chronically demyelinated axons occupy regions where there are but few astrocytic processes. *In vitro* findings (Noble, 1982), experimental investigations (Reir & Webster, 1974; Raine, 1976; Blakemore, 1978, 1981) and our *in vivo* results suggests that a normal functioning of astrocytes is essential for oligodendrocytic development and therefore for myelination.

Evidence from our results and from a variety of sources indicates, further, that the mouse mutant Jimpy could be a model for disturbed cell interactions in the C.N.S. The hypomyelination may not be attributed to a defect of a single cell but rather to a deficiency in both macroglial types and, perhaps, the axon as well.

Acknowledgements

FXO is grateful to Dr Henry de F. Webster for his help, discussion and support during the tenure of a Fellowship (1981 and 1982) in his laboratory. This research was supported by a Fellowship (FXO) from Janggen-Pöhn-Stiftung and Schweizerische Stiftung für med. biol. Stipendien, Switzerland. The authors thank Dr N. Herschkowitz (University of Bern, Switzerland; National Foundation Grant 3.717.30) for the Jimpy mice and Drs M. Brightman, G. Stoner and B. Trapp for their constructive criticism and improvement of this paper. Patricia Weir (NIH Bethesda) and Therese Comte (University of Lausanne) are acknowledged for the careful typing of the manuscript.

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