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Identification of mitotic cells in the central nervous system by electron microscopy of re-embedded semithin sections

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INTRODUCTION

Most differentiated glial cells can be identified in semithin, resin-embedded, sections stained with toluidine blue using the criteria established by Griffin, Illis & Mitchell (1972) and Ling, Paterson, Privat, Mori & Leblond (1973). Morphological criteria for identifying immature glia in semithin sections have also been described (Sturrock, 1976). It is not yet clear whether the criteria established for identification of inter-phase glial cells can be applied to mitotic glial cells. Mitotic cells are present in the central nervous system at all ages (Sturrock, 1979; 1982*a*; 1983*a*; Korr, 1980, 1982) but only in very small numbers, even during the phase of rapid increase in glial number which occurs immediately before and during the early stage of myelination (Roback & Scherer, 1935; Schonbach, Hu & Friede, 1968; Matthews & Duncan, 1971; Sturrock, 1979). It is therefore very difficult to find mitotic glial cells in the small areas of tissue which it is practical to examine electron microscopically. It is, however, now possible to examine relatively large semithin sections which have been cut with Ralph knives and if a sufficient number of sections are examined mitotic cells can always be found.

In semithin sections of developing spinal cord of mouse (Sturrock & McRae, 1980) and rabbit (Sturrock, 1982*b*) and corpus callosum of mouse (Sturrock, 1983*b*) four types of mitotic cell have been identified. By far the most common type of mitotic cell has a cytoplasmic density similar to the surrounding neuropil and the cytoplasm usually contains moderately darkly staining narrow threads tentatively identified as mitochondria. Shortly after the onset of myelination mitotic cells with processes in contact with myelin sheaths are present in small numbers. The cytoplasmic density of these cells varies from moderately light to dark but is almost always denser than the surrounding neuropil (Sturrock & McRae, 1980; Sturrock, 1983*b*). These cells have been identified as oligodendrocytes and at least one example has been observed electron microscopically (Sturrock, 1981). A definite connection between the processes of these cells and myelin sheaths can only be unequivocally demonstrated electron microscopically. The third type of mitotic cells has a dark cytoplasm and might be either dark oligodendrocytes with no processes visible in the plane of the section, or dark glioblasts similar to those shown in mitosis in the early postnatal mouse corpus callosum (Sturrock, 1976). The fourth type of mitotic cell, which has a very pale cytoplasm similar to that of differentiated astrocytes, is present from the onset of myelination and can still be found in old age. These cells are probably mature astrocytes.

It would be useful if the morphological features of mitotic cells observed in semi-

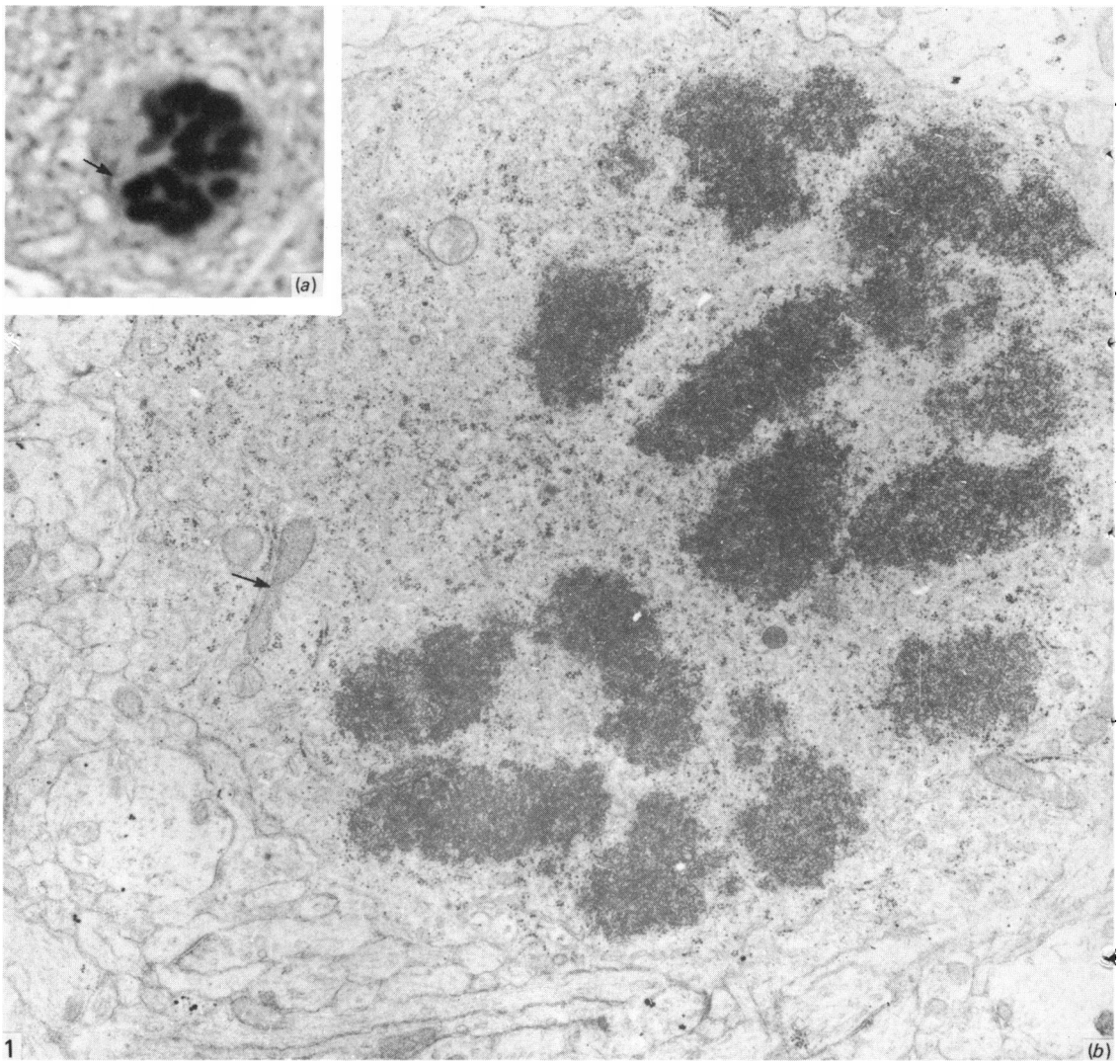


Fig. 1 (*a–b*). (*a*) A semithin section of 5 days old mouse spinal cord stained with toluidine blue. This mitotic cell has a cytoplasmic staining density similar to that of the surrounding neuropil of the dorsal grey matter. The moderately darkly stained thread-like structure (arrow) can be seen in the electron micrograph (Fig. 1 *b*) to be a mitochondrion. (*b*) Electron micrograph of an ultrathin section of the re-embedded semithin section. The cytoplasm contains a few mitochondria, some short strands of granular endoplasmic reticulum and numerous free ribosomes. (*a*) ×2400; (*b*) ×18900.

thin sections could be correlated with their ultrastructure. The most obvious feature is cytoplasmic density: it is not certain that this remains constant throughout mitosis, although cells with different cytoplasmic densities have been found in similar phases of mitosis. There is also the question of whether or not the cell processes in contact with myelin sheaths in semithin sections are involved in myelination. If it were possible to compare the same mitotic cell in a semithin section by light microscopy and in an ultrathin section by electron microscopy many of the identification problems could be solved. The alternate semithin–ultrathin section technique

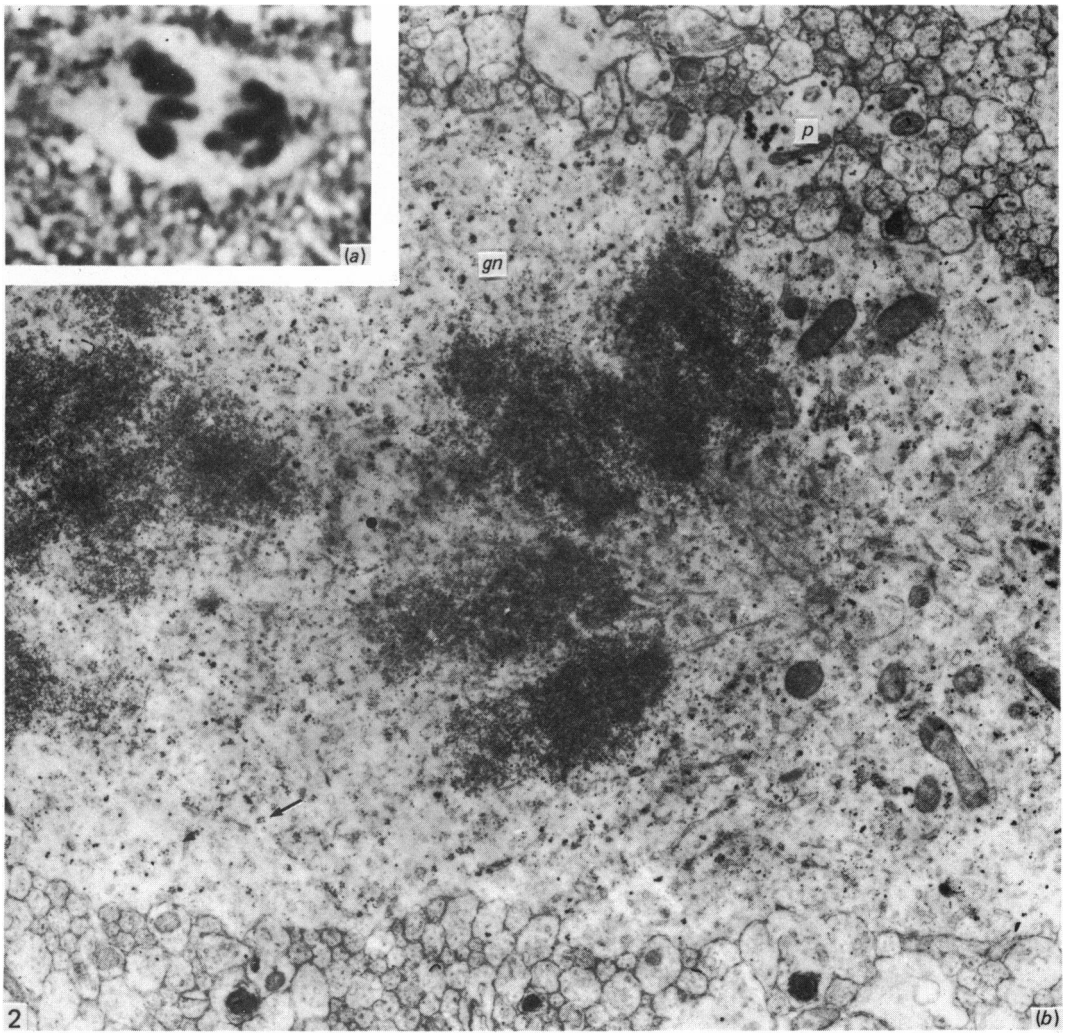
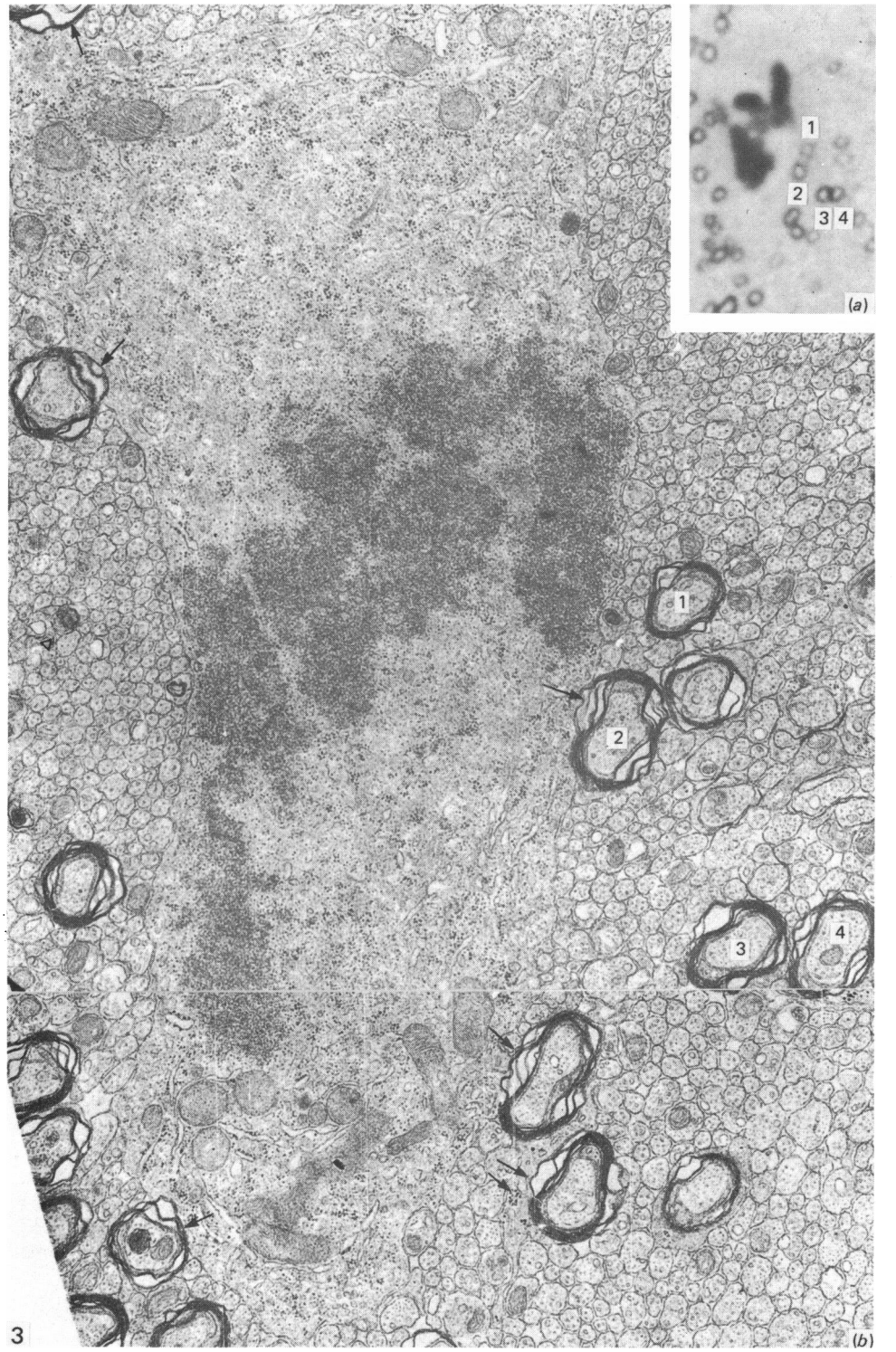


Fig. 2(*a–b*). (*a*) A section stained with toluidine blue of the corticospinal tract in the spinal cord of a 5 days old mouse. This mitotic cell has a very pale cytoplasm. (*b*) An electron micrograph of a re-embedded section of the same cell. The cytoplasm contains a number of mitochondria and short strands of both granular and smooth endoplasmic reticulum. Small glycogen granules (*gn*) are scattered throughout the cytoplasm. These are smaller than the glycogen granules in an adjacent astrocytic process (*p*) but can be differentiated from free ribosomes by their size (they are slightly larger) and by the fact that the latter are usually arranged in rosettes (arrow). (*a*) ×2400; (*b*) ×18900.

used by Griffin *et al.* (1972), Ling *et al.* (1973) and the author (Sturrock, 1976) is too time consuming to use in this instance, since mitotic cells are rarely found, even during the most rapid phase of glial acquisition (Sturrock, 1979). King, Kammlade & Murphy (1982) have recently described a technique which enables resin-embedded semithin sections to be re-embedded and sectioned for electron microscopy. The technique is simple and does not require the use of chemicals such as hydrofluoric acid (Kaplan & Hinds, 1977).

Using a slightly modified version of this technique ultrathin sections were prepared from sections 1–3 μm thick containing various types of mitotic cell. The material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(a)
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used was either 5 days postnatal mouse spinal cord or 17 days postnatal mouse corpus callosum and hippocampal commissure. These regions and ages were selected, since previous studies (Sturrock & McRae, 1980; Sturrock, 1983*b*) have shown that all the types of mitotic cells described above are present, albeit in small numbers, and tissue blocks were already available.

MATERIALS AND METHODS

Blocks of spinal cord from 5 days postnatal mice and corpus callosum and hippocampal commissure from 17 days postnatal mice were used in this study. All material was fixed by perfusion-fixation with mixed aldehydes, post-fixed in buffered osmium tetroxide and embedded in Spurr's resin. Full details of the methods of fixation and embedding of both spinal cord (Sturrock & McRae, 1980) and corpus callosum (Sturrock, 1976) have already been published.

Semithin sections, 1–3 μm thick, were cut using a Spencer rotary microtome and Ralph knives. Each section was picked up dry using fine watchmakers' forceps and placed in a drop of distilled water on a microscope slide. There were six drops of water at approximately equal distances apart on each slide. After six sections had been placed on the slide it was placed on a hot plate at 90 °C and after the water had evaporated the slide was left for a further two to three minutes to attach the sections to the slide. The sections were then stained with 1 % toluidine blue for 25–35 seconds at 90 °C.

Sections were examined under oil immersion without a coverslip at × 640. Once a mitotic cell had been identified, it was photographed at ×400 under oil immersion and then the complete section was photographed at × 20. The micrographs were printed at × 2400 and × 120 respectively. The ×120 print was compared with the section and the position of the mitotic cell was carefully marked on the print. After this had been done the immersion oil was removed from the section by gently blotting with tissue paper. The slides containing sections selected for re-embedding were placed on a hot plate at 120 °C for two minutes. In turn, each slide was inverted and a cylindrical mould filled with freshly prepared Spurr's resin was placed beneath the selected section (or sections). The section plus mould was re-inverted and placed on a flat surface in an oven at 70 °C for 18–24 hours. The slides, complete with moulds, were removed from the oven and placed on a hot plate at 90 °C for two minutes. Each slide was removed from the hot plate in turn and put into a holder, which was a simplified version of that described by King *et al.* (1982) and the mould was snapped off the slide using pliers. The semithin section was now embedded in the surface of a cylinder of Spurr's resin. This cylinder was cured for a further 24 hours at 70 °C.

The re-embedded sections were trimmed with the aid of the low magnification

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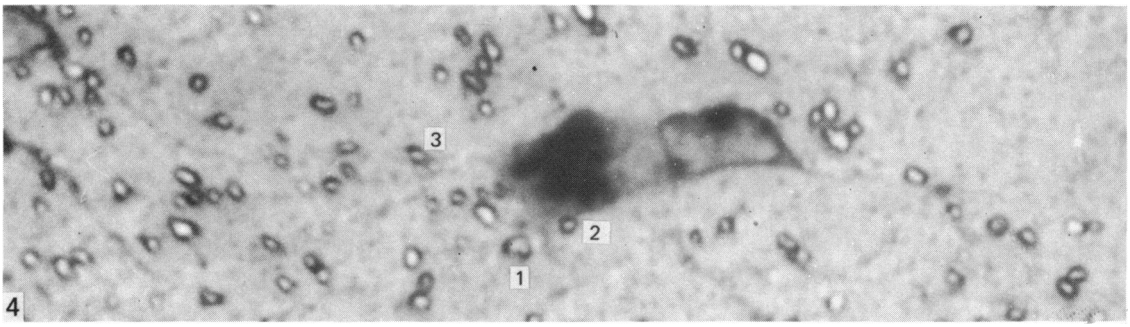
Fig. 3(*a–b*). (*a*) A section stained with toluidine blue of the hippocampal commissure of the 17 days old mouse. The cytoplasm of this mitotic cell is slightly lighter than the surrounding axons but darker than that of the astrocyte in Fig. 2(*a*). (*b*) Electron micrograph of the corresponding ultrathin section. The mitotic cell has a number of mitochondria and strands of granular endoplasmic reticulum of varying length at each pole. As well as being more numerous, the mitochondria are larger than those in Figs. 1(*b*) and 2(*b*). There are also numerous free ribosomes throughout the cytoplasm. Five myelinated axons (arrows) are in contact with the cell membrane and a process (double arrow) appears to pass from one of these to another axon. In both (*a*) and (*b*), the myelin sheaths, 1–4, are numbered to facilitate orientation of semithin and ultrathin sections. (*a*) ×2400; (*b*) ×18900.



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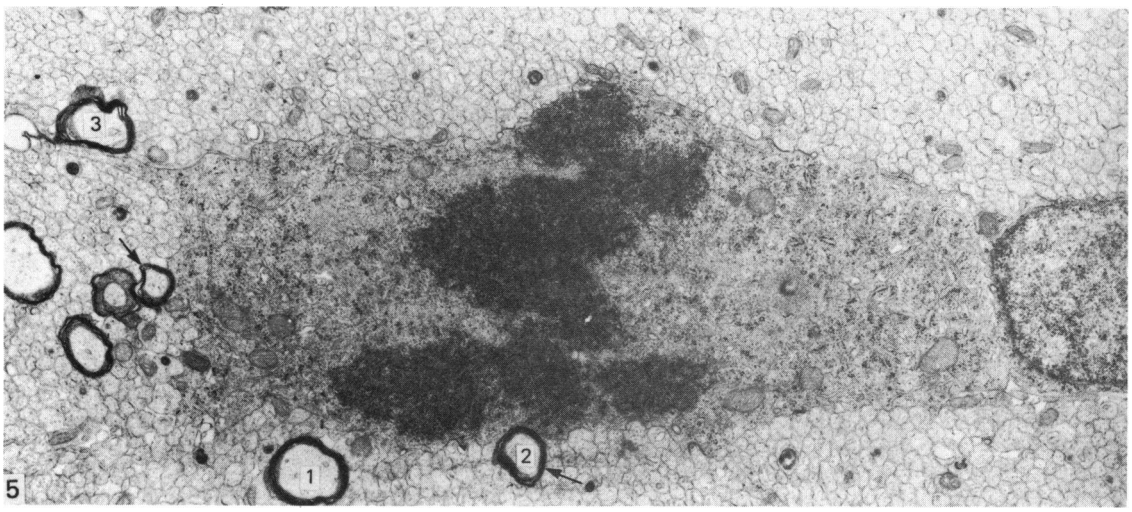

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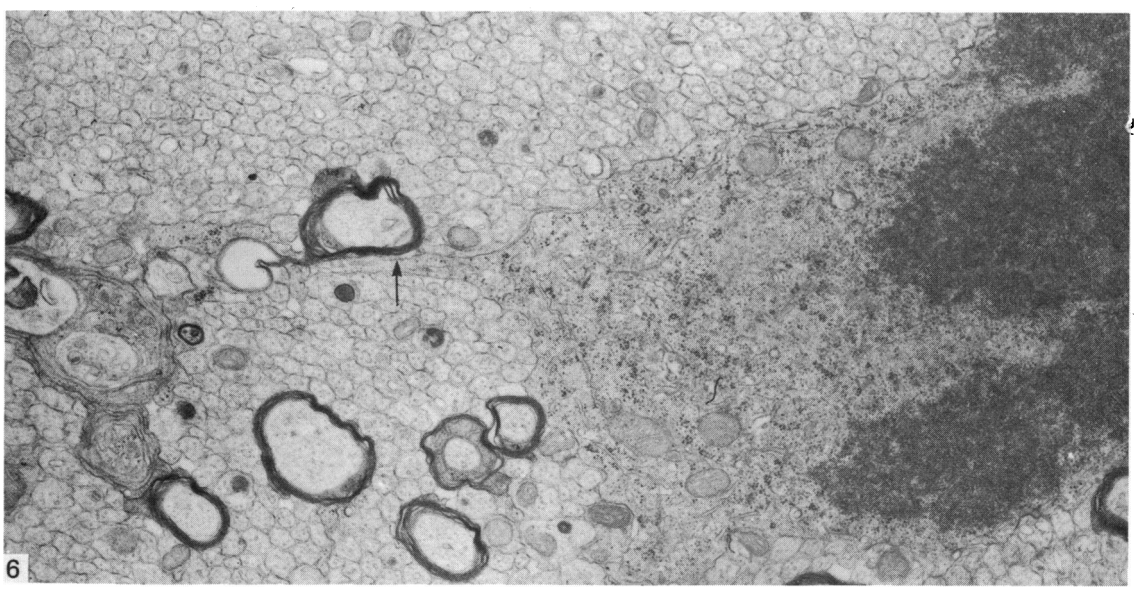
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micrographs. Ultrathin sections were cut with a Reichert ultramicrotome, mounted on copper mesh grids and stained with uranyl acetate and lead citrate and examined in an AEI 801 electron microscope.

RESULTS

Initial attempts at re-embedding semithin sections failed because the section did not remain adherent to the slide during re-embedding and was found 'floating' in the cylinder of re-embedding resin. It was only after a number of trial procedures that this problem was solved by re-heating the slide after removal of the immersion oil. Even with re-heating, there was a tendency for a slight degree of wrinkling to occur at the edge of the tissue. This wrinkling was more marked if the original section had a rim of resin around the tissue. Where the original block had been trimmed to include tissue only the wrinkling at the tissue/clear resin interface was less apparent. The extra processing, staining and heating appeared to have some effect on the material. Myelin sheaths were more disrupted than normal. Similar disruption of myelin was present in illustrations of re-embedded rat cerebral cortex (Kaplan & Hinds, 1980). Staining properties with uranyl acetate and lead citrate were affected, resulting in a reduced staining. Contrast was poorer than usual and the ultrathin sections had to be examined at 40 kV instead of the usual 60 kV. Contamination built up in the sections more rapidly than normal and all these factors produced micrographs which were less than ideal. Nevertheless the results were good enough to allow identification of mitotic figures and comparison of their structure by light and electron microscopy.

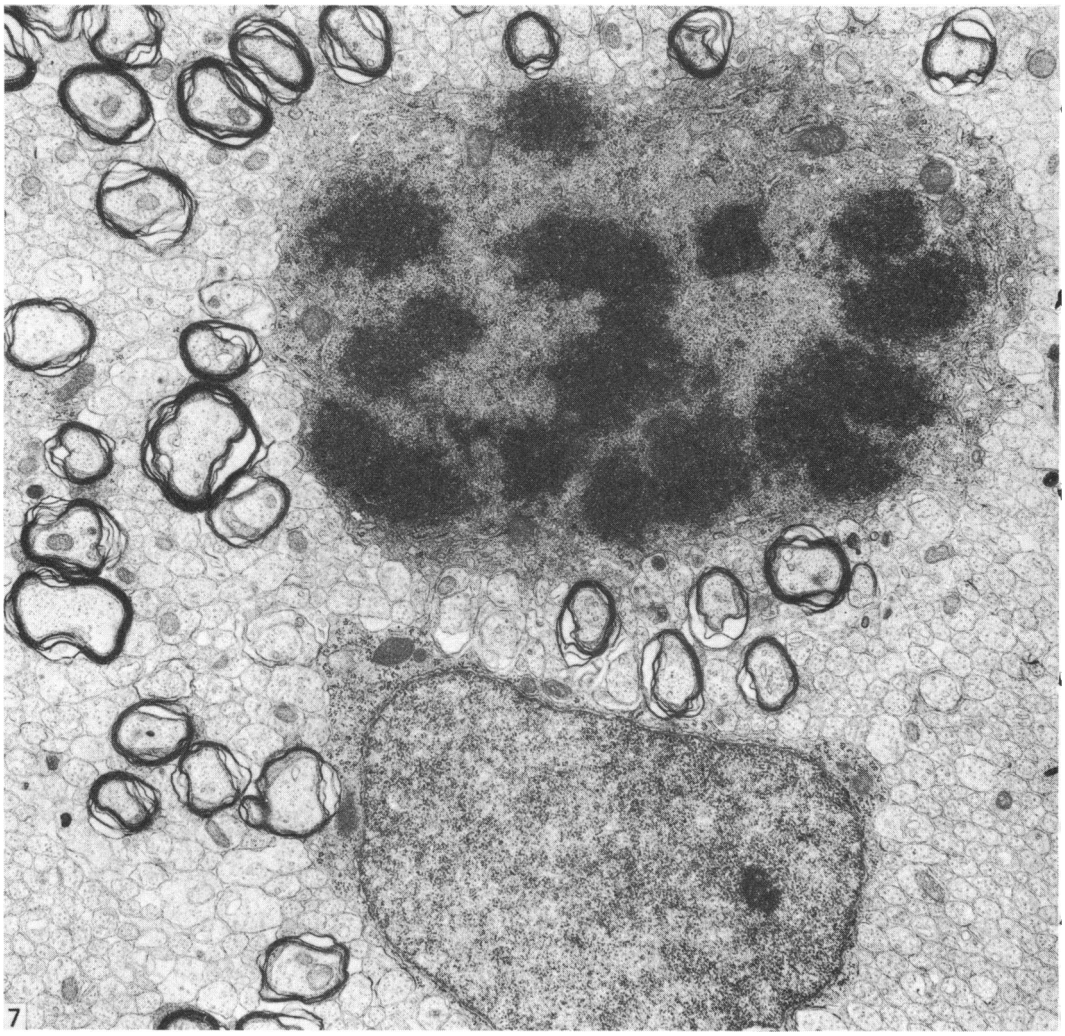
The most common type of mitotic figure found in semithin sections of the spinal cord (Fig. 1*a*) and forebrain during the early stages of myelination was found in ultrathin sections to have a cytoplasmic staining density similar to, or only slightly denser than, the surrounding neuropil (Fig. 1*b*). Organelles, apart from abundant free ribosomes, were sparse and consisted of short strands of granular endoplasmic reticulum and mitochondria. Comparison of the light and electron micrographs confirmed that the fine dark lines in the cytoplasm observed at the light microscope level were mitochondria.

Pale mitotic figures (Fig. 2*a*), which were present only after the onset of myelination appeared to be astrocytes. The cell illustrated in Figure 2*b* had a pale cytoplasm containing numerous glycogen granules. The glycogen granules in the mitotic astrocyte cytoplasm were smaller than those visible in an adjacent astrocyte process. Strands of both granular and smooth endoplasmic reticulum were present as were a number of mitochondria. Most of the organelles were concentrated at the poles of the dividing cell (Fig. 2*a*, *b*). There were numerous small rosettes of free ribosomes which were smaller and less darkly stained than glycogen granules. Microfilaments were not obvious in this cell.

Fig. 4. Semithin section of the corpus callosum of a 17 days old mouse. This shows a mitotic cell in close proximity to an interphase cell, probably an oligodendrocyte. The myelinated axons indicated (1–3) are the same as those shown in Fig. 5. Axon 1 appears to be connected to the mitotic cell by a process which is not visible in Figs. 5 or 6. × 2400.

Fig. 5. Electron micrograph of a re-embedded section shown in Fig. 4. Two myelinated axons (arrows) are in contact with the mitotic cell membrane. There are many short strands of granular endoplasmic reticulum and a few mitochondria at each pole. × 12000.

Fig. 6. Electron micrograph of part of the cell shown in Fig. 5. Note the process (arrow) extended to a myelinated axon. × 18900.



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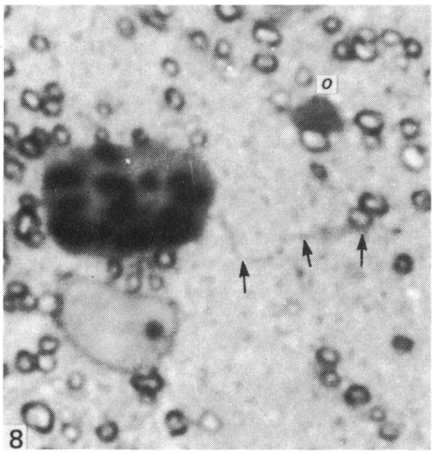

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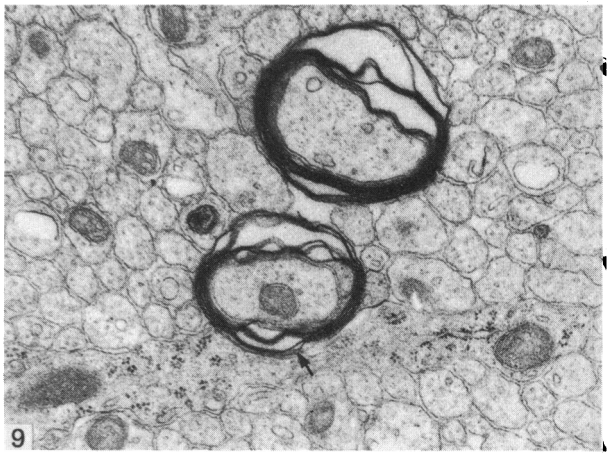

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Figure 3 shows a mitotic cell with a cytoplasmic density which appeared to be slightly less than that of the surrounding axons in semithin sections (Fig. 3*a*) and similar to the axons in electron micrographs (Fig. 3*b*). Five myelinated axons were in contact with the cell membrane (Fig. 2*b*). Free ribosomes were numerous. Short strands of granular endoplasmic reticulum and mitochondria were present in larger numbers at either pole of this cell than in either of the cells shown in Figures 1*b* and 2*b*. The relationshp of the cell membrane to myelinated axons, the low cytoplasmic density and the quantity of mitochondria and endoplasmic reticulum suggested that this cell might have been a light oligodendrocyte.

A mitotic cell with a cytoplasmic density greater than that of the surrounding axons is shown in Figures 4–6. Like the mitotic cell in Figure 3*b*, this mitotic cell had some mitochondria and a large number of short strands of granular endoplasmic reticulum at each pole, as well as free ribosomes (Fig. 5). At least two axons were in contact with the cell membrane and Figure 6 shows a process in contact with a myelinated axon. This cell process was not prominent in the semithin section (Fig. 4).

Another mitotic cell of medium density was found (Figs. 7–10) which had several myelin sheaths in contact with its cell membrane. In the semithin section (Fig. 8) a cell process was visible extending towards two myelin sheaths lying at some distance from the cell body. Electron microscopy (Fig. 9) showed that this process was in contact with at least one of the sheaths. The cell process followed a fairly tortuous course from the cell body to the myelin sheaths and, although a number of serial electron micrographs were examined, it was not possible to follow its complete course in any one micrograph (Fig. 10). The process was much less electron-dense than the mitotic cell body although this was not apparent in the semithin section (Fig. 8). The mitotic cell contained several mitochondria and short strands of granular endoplasmic reticulum, mainly around the periphery (Figs. 7, 10). The cytoplasmic density of the mitotic cell was similar to that of an adjacent cell which was also in contact with a number of myelin sheaths and also to that of a fairly characteristic segment of oligodendrocyte cytoplasm (Figs. 8, 10). This segment, as well as being in contact with myelin sheaths, contained parallel strands of granular endoplasmic reticulum.

Dark mitotic cells were rare and that illustrated in Figures 11–14 was almost certainly an oligodendrocyte. A cytoplasmic dense body seen in the semithin section (Fig. 10*a*) was found to be a myelin body by electron microscopy (Figs. 10*b*–14). Serial electron micrographs of this cell showed that some myelin sheaths remained in contact with the cell membrane in all the sections whilst others were in contact in only one or two of the micrographs. The cytoplasm contained mitochondria, strands of granular endoplasmic reticulum and numerous free ribosomes. The myelin sheath, cut longitudinally, which is visible in Fig. 11*a*, was not present in any of the electron micrographs. Measurement of this sheath in Fig. 11*a* indicated it was about 1 μm in diameter; since the semithin section was 3 μm thick, the sheath could have been absent from the majority of the ultrathin sections cut from the re-embedded section.

Fig. 7. Electron micrograph of a medium dark mitotic cell. A number of myelinated axons are in contact with the cell membrane of this cell. × 18900.

Fig. 8. Semithin section of the pair of cells shown in Figs. 7 and 10. A convoluted process (arrows) appears to be winding its way from the mitotic cell towards two myelinated axons. The dark piece of tissue (*o*) is shown to be part of an oligodendrocyte in Fig. 10. × 2400.

Fig. 9. Electron micrograph showing the continuity between the process arrowed in Fig. 8 and one of the myelin sheaths (arrow). × 30000.

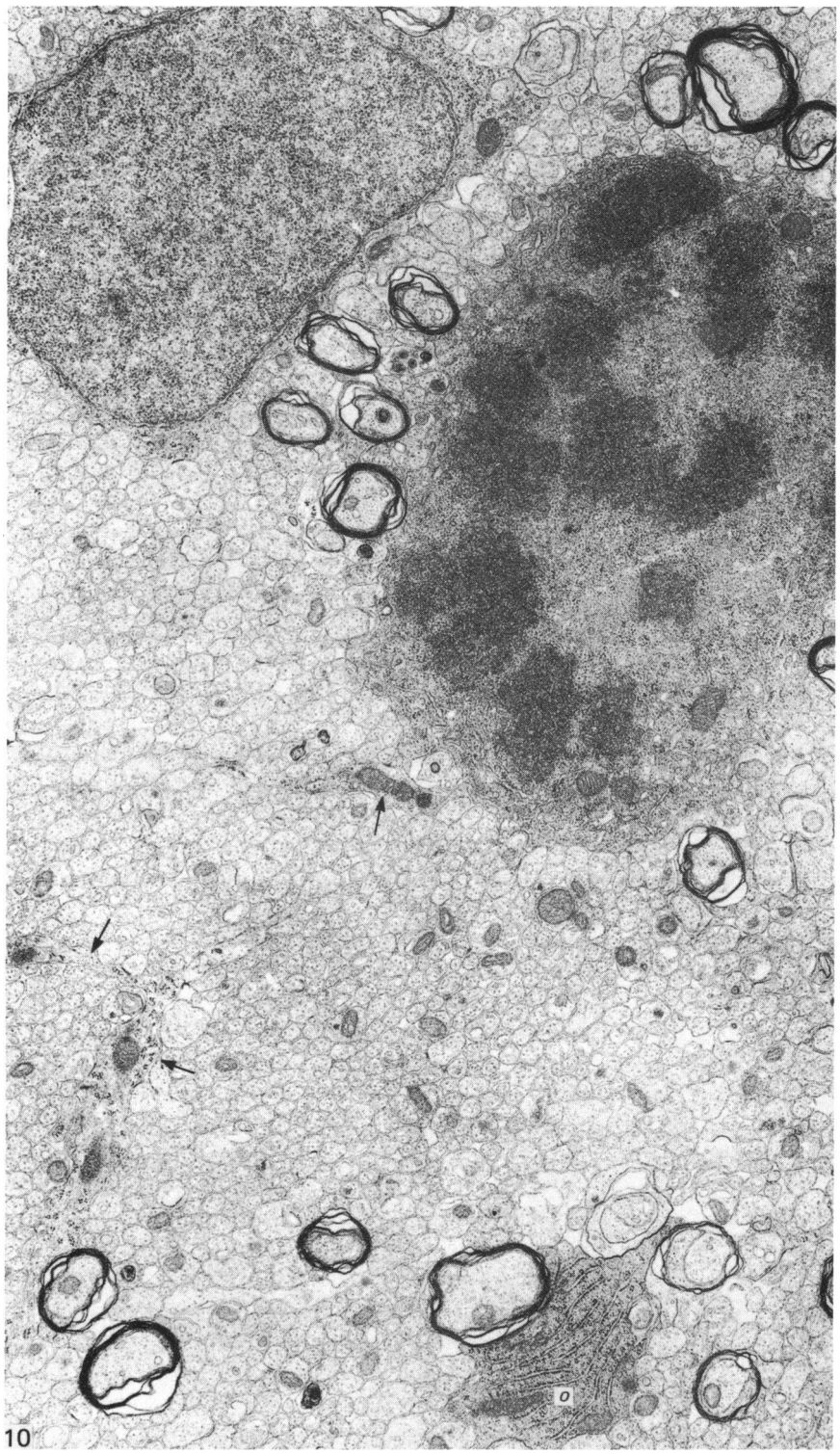


Fig. 10. Electron micrograph montage of a serial section of Fig. 7. The process (arrows) is less electron-dense than the rest of the cytoplasm and only parts of it are in the plane of section. The dark piece of cytoplasm (*o*) in contact with two myelinated and one pre-myelinated axon is identifiable as part of an oligodendrocyte by the numerous parallel sheets of granular endoplasmic reticulum. × 18900.

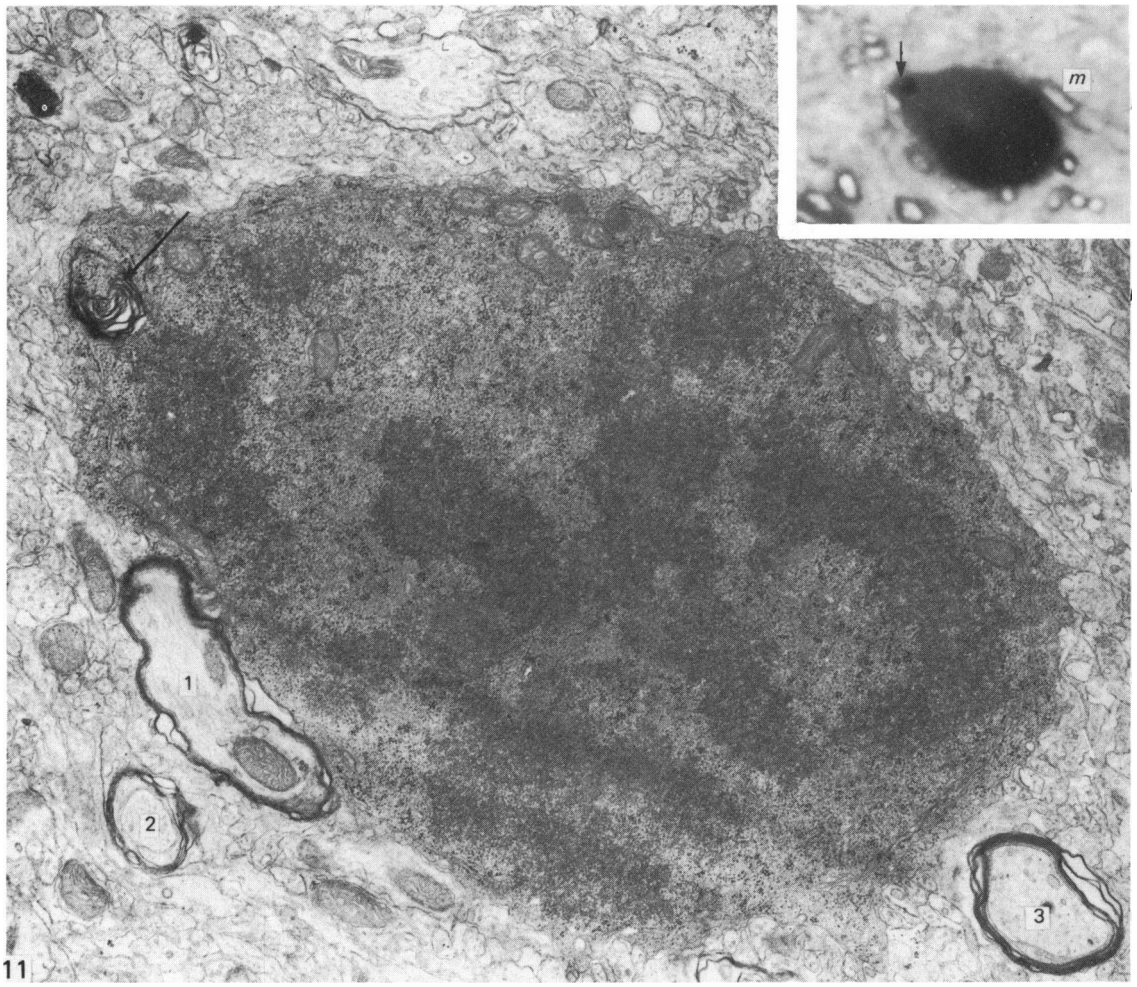
DISCUSSION

The results of the present study indicate that in most cases mitotic glial cells can be reliably identified in semithin sections. Indeed, in the case of oligodendrocytes, it is often easier to follow their processes in semithin sections than in ultrathin sections. The use of semithin sections for identification of mitoses has the advantage that if there is any doubt regarding the identity of a particular cell it can be further examined electron microscopically. The results of electron microscopy of re-embedded material are not as good as might be expected from material prepared directly for electron microscopy but are more than adequate for identification of mitotic cells.

Mitotic glial cells retain their cytoplasmic staining characteristics throughout mitosis. The most numerous type of mitotic cell is that previously described as a pale mitotic cell (Sturrock, 1983*b*), its cytoplasmic density being similar to the surrounding neuropil. In semithin sections the neuropil and tracts made up largely of unmyelinated axons do take up stain to some extent (Fig. 1*a*) and are markedly darker than astrocyte cytoplasm which does not appear to be stained (Fig. 2*a*). With the electron microscope, the pale mitotic cell appears to be identical to mitotic cells previously described in the subependymal plate of the rat (Blakemore, 1969; Privat & Leblond, 1972) and the mouse (Sturrock & Smart, 1980) and also in the neostriatum of the 10 days postnatal mouse (Sturrock, 1980) where it was identified as a glioblast. The presence of similar mitotic cells in the subependymal plate suggests that this cell is an undifferentiated glial precursor and this is supported by the relatively sparse cytoplasmic organelles. Probably this type of mitotic cell should be called a mitotic glioblast rather than a 'pale mitotic cell' to avoid possible confusion with a mitotic astrocyte or a mitotic light oligodendrocyte.

Electron microscopy confirms that the very pale mitotic cell, previously tentatively identified as an astrocyte (Sturrock & McRae, 1980), is in fact an astrocyte. This particular cell does not appear to be fully differentiated, as microfilaments could not be identified, but microfilaments are not a prominent ultrastructural feature of astrocytes in the early postnatal mouse central nervous system (Sturrock, 1974, 1976). This is in keeping with the scarcity of immunocytochemical staining of glial fibrillary acidic protein in early postnatal rodent forebrain (Bignami & Dahl, 1974). Fully differentiated astrocytes with numerous microfilaments in the cytoplasm have been observed in mitosis in the normal young adult rat (Mori & Leblond, 1969) and human embryo (Sturrock, 1975).

Although cells described as mitotic oligodendrocytes have been identified in semithin sections by the presence of processes in contact with myelin sheaths (Silberberg, Dorfman, Latovitzki & Younkin, 1980; Sturrock & McRae, 1980; Sturrock, 1983*b*), such cells have only rarely been observed in ultrathin sections (Sturrock, 1981). The validity of the assumption that cell processes apparently in contact with myelin sheaths in semithin sections are in fact responsible for myelination can be questioned, but all such processes re-examined in ultrathin sections during the course of this study nevertheless appeared to be involved in myelination. It is much easier to find processes extending some distance from oligodencrocyte perikarya out to myelin sheaths during the early stages of myelination when relatively few axons are myelinated and oligodendrocyte processes are broader than in older animals. Some earlier studies implicating oligodendrocytes as the cells responsible for myelination relied on this feature as a criterion (Bunge & Glass, 1965; Bunge, 1968). As myelination proceeds, the processes become narrower and more convoluted, making it difficult,



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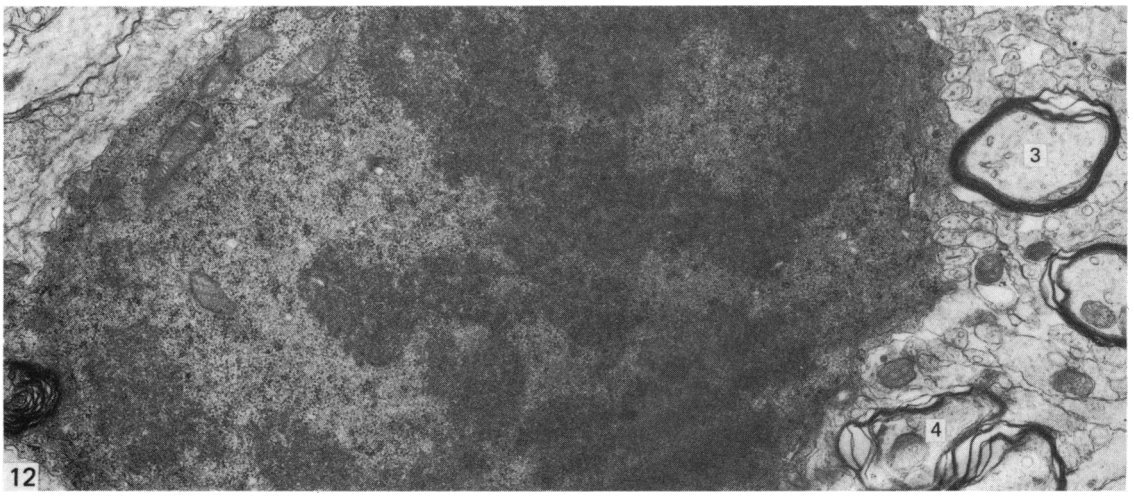

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and often impossible, to follow them in semithin sections. Even during development, oligodendrocyte processes are so narrow and convoluted that they can be followed only in semithin sections and not in ultrathin sections. This is not the case with astrocyte processes in parts of the central nervous system such as the optic nerve (Sturrock, 1975), where bundles of axons are separated by astrocyte processes which can be identified by both light and electron microscopy. These astrocyte processes probably take the form of sheets rather than cylinders.

Light, medium and dark cells attached to myelin sheaths have been identified undergoing mitosis in both semithin and ultrathin sections. It is, nevertheless, still possible to argue that these cells may not be fully differentiated oligodendrocytes. Mitotic oligodendrocytes of each cytoplasmic density appear to contain fewer organelles than interphase oligodendrocytes of the same density, except for free ribosomes which are abundant. Although free ribosomes are a characteristic feature of undifferentiated cells (Leblond, 1981) they are also a prominent feature of all mitotic cells from prophase to telophase (Erlandson & de Harven, 1971), probably being released into the cytoplasm from the nucleus when the nuclear membrane breaks down in early prophase (Mitchison, 1971). The ultrastructural features of mitotic oligodendrocytes, therefore, are not necessarily a reliable guide to the stage of differentiation.

There is still considerable disagreement as to whether differentiated oligodendrocytes proliferate. Privat, Valat & Fulcrand (1981) and Korr (1980, 1982) support the view that oligodendrocytes can divide. Kaplan & Hinds (1980) and Korr, Schilling, Schultze & Maurer, (1983) have both illustrated labelled, differentiated, oligodendrocytes, the former by electron microscopy of re-embedded semithin autoradiographs and the latter by metallic stained autoradiographs. In both cases, however, the animals had been killed some weeks after injection of radio-labelled thymidine so that the identity of the cell *at the time of division* is not proven. It is possible that the labelled cells could have differentiated between the time when they incorporated the label and when the animals were killed. In rat optic nerve, Skoff (1975) illustrated a labelled cell which appears to be a fairly well differentiated oligodendrocyte but in a study of gliogenesis Skoff, Price & Stocks (1976) concluded that oligodendroblasts divide but oligodendrocytes do not. They did believe, however, that oligodendroblasts are capable of myelinating axons. This interpretation has recently been supported by Paterson (1983) in an autoradiographic study of adult rat. Imamoto, Paterson & Leblond (1978), however, report that once cells have differentiated as far as light oligodendrocytes they lose the capacity to divide.

Perhaps the best evidence for oligodendrocyte division is that presented recently by Bologa *et al.* (1983) and Roussel *et al.* (1983). Both groups studied differentiating oligodendrocytes in tissue culture using a combination of immunocytochemistry and autoradiography. Oligodendrocytes were identified by the presence of either myelin basic protein (MBP) and galactocerebroside (Bologa *et al.* 1983) or by myelin basic

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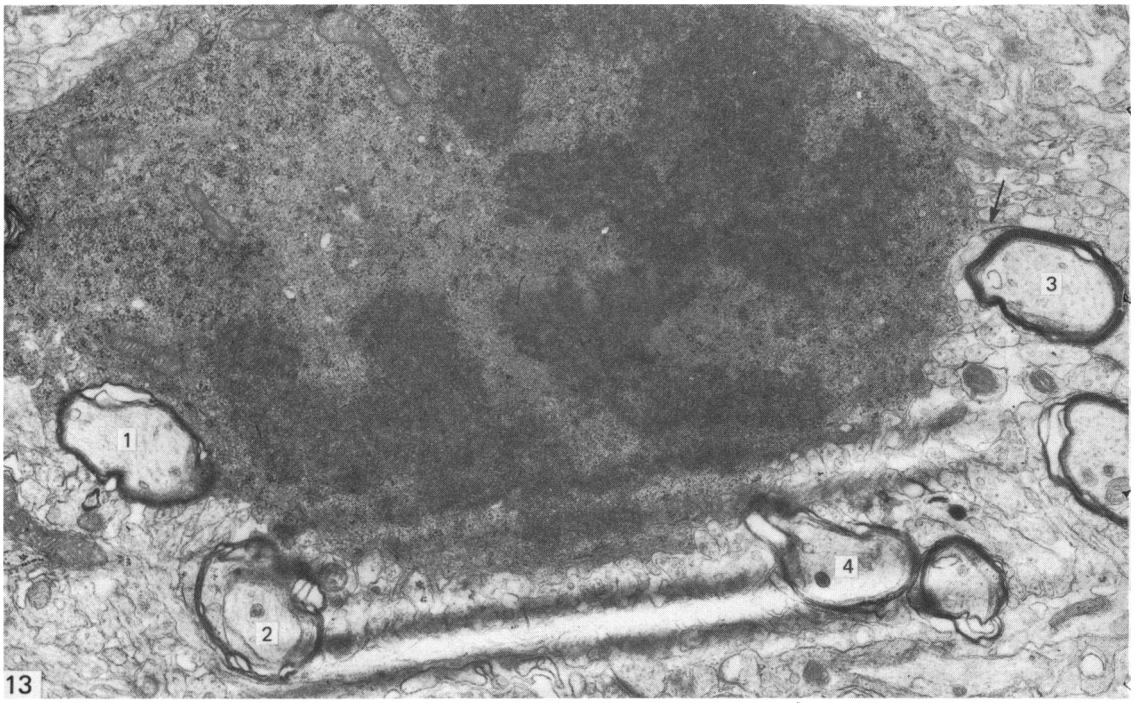
Fig. 11 (*a–b*). (*a*) Semithin section of a dark mitotic cell with a dense body (arrow). Serial ultrathin sections are illustrated in Figs. 11*b*–14. Note that the myelin sheath (*m*) does not appear in any of the ultrathin sections. (*b*) Electron micrograph of re-embedded semithin section showing three myelin sheaths (1–3). The dense body is seen to be a myelin body (arrow). At this level, myelinated axon 1 is in contact with the mitotic cell membrane. The artefactual ridges at the bottom of the micrograph can also be seen in Figs. 12–14; since they are present at all four levels, they may be due to damage sustained during re-embedding. (*a*) ×2400; (*b*) 18900.

Fig. 12. Electron micrograph. At this level, myelin sheath 3 is in contact with the cell membrane and no outer tongue of cytoplasm is visible. Myelin sheath 4 is not in contact. ×18900.



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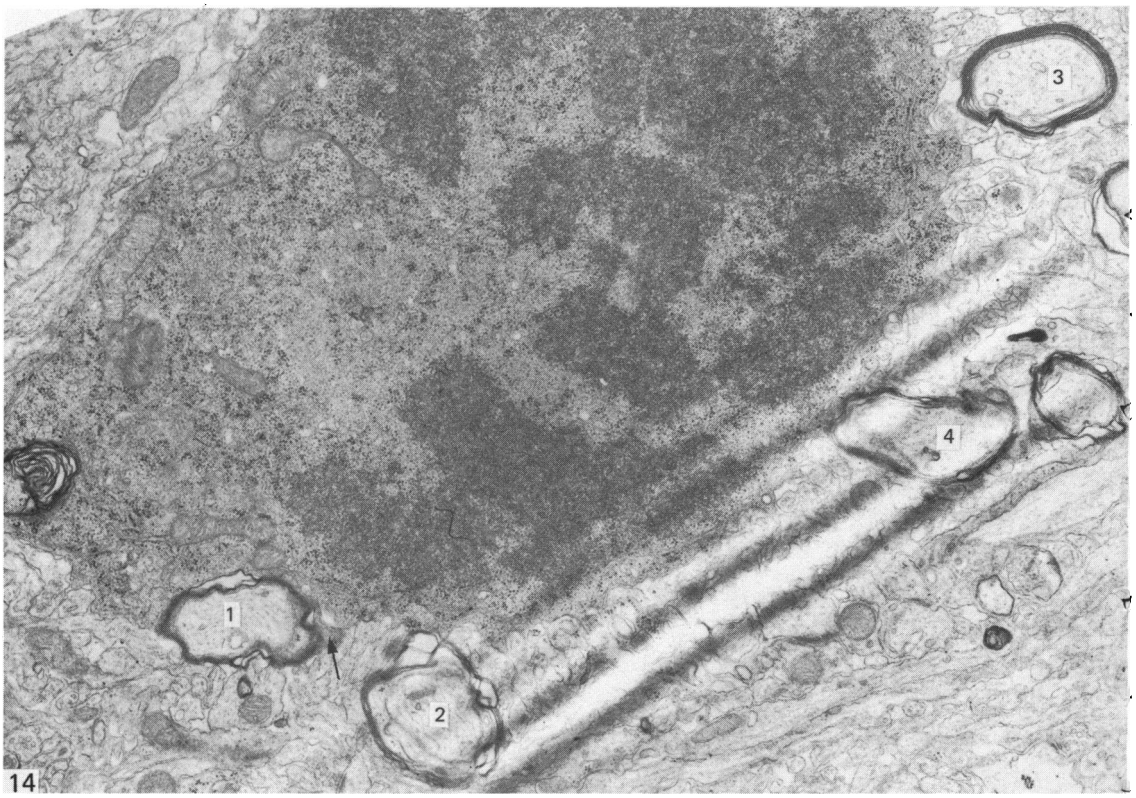

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protein and Wolfgram (W1) protein (Roussel *et al.* 1983). Both groups conclude that immunocytochemically identifiable oligodendrocytes are capable of proliferation but that as differentiation proceeds the number of proliferating cells declines. In particular, few cells which expressed myelin basic protein showed incorporation of [^{3}H]thymidine. Some caution must be observed in extrapolating results of tissue culture studies to living animals since the extracellular environment is different; it would be interesting to see these experiments repeated *in vivo*.

The current evidence indicates that myelin producing cells can divide. In semithin sections studied by light microscopy and in ultrathin sections studied in the electron microscope these dividing cells show a spectrum of cytoplasmic density similar to that of differentiated oligodendrocytes. Immunocytochemical evidence shows that proliferating cells express a number of protein markers associated only with oligodendrocytes. Whether these dividing cells are referred to as oligodendroblasts or oligodendrocytes appears to be a matter of semantics.

One interesting feature revealed by the present study is that mitotic oligodendrocytes and astrocytes are at least partially differentiated. Studies of the ultrastructure of mammalian cells during mitosis have, not unnaturally, concentrated on either cells such as HeLa cells grown in tissue culture (Robbins & Gonatas, 1964; Erlander & de Harven, 1971) or undifferentiated cells such as fetal neural or kidney epithelial cells (Jokelainen, 1967), since in both cases mitotic cells are numerous and relatively easy to find in different stages of mitosis. Mitotic oligodendrocytes and astrocytes show certain features not usually found in mitotic cells in tissue culture. For example they do not retract their processes during mitosis as do cells such as fibroblasts and amoeba (Mezia, 1961; Mitchison, 1971). The retraction of cell processes is considered to be one mechanism for adjusting the area of the cell membrane during mitosis. Experiments on single mitotic cells have shown that respiration is lowered and that there is a reduction or cessation of most normal cellular physiological processes during mitosis (Mezia, 1961; Mitchison, 1971). It is therefore surprising that myelin-producing oligodendrocytes undergo division, particularly when one considers the area of cell membrane involved. This suggests that myelin, once formed, requires relatively little maintenance. This is supported by the reduction in cytoplasmic volume and organelles observed in dark oligodendrocytes (Mori & Leblond, 1970), which form the major part of the oligodendrocyte population after myelination is completed. It is also possible that, during mitosis, differentiated or partially differentiated cells may maintain their physiological processes at a higher level than undifferentiated cells.

SUMMARY

Semithin (1–3 μm) sections of 5 days postnatal mouse spinal cord and 17 days postnatal mouse corpus callosum and hippocampal commissure were examined to find examples of mitotic glial cells. The sections were re-embedded for electron microscopy and the structure of the mitotic cells by light and electron microscopy

Fig. 13. Electron micrograph. Myelin sheaths 1, 2 and 4 are in contact with the cell membrane. Sheath 3 is in contact with process (arrow) but an outer tongue of myelin is also present. × 18900.

Fig. 14. Electron micrograph. Myelin sheaths 1, 2 and 4 remain in contact. The arrow indicates a portion of cytoplasm which may be the beginning of an outer tongue of cytoplasm around sheath 1. Sheath 3 is now completely separate and the process is no longer present. × 18900.

was compared. The most commonly found type of mitotic cell had a cytoplasmic staining density similar to, or slightly darker than, the surrounding neuropil. This was seen electron microscopically to be an undifferentiated cell, similar to mitotic cells described by other authors in the subependymal layer. This cell was classified as a glioblast. Mitotic cells with cytoplasm which appeared unstained in semithin sections were identified as partially, or fully, differentiated astrocytes. Mitotic cells with processes in contact with myelin sheaths had a wide variety of cytoplasmic staining densities in semithin sections. Electron microscopy confirmed that these cells were involved in myelination and it is possible that the mitotic cells compared to the light, medium and dark varieties of oligodendrocytes.

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