THE FORMATION AND STRUCTURE OF MYELIN SHEATHS IN THE CENTRAL NERVOUS SYSTEM

A. PETERS, Ph.D.

From the Department of Anatomy, University of Edinburgh, Scotland

ABSTRACT

The development and structure of myelin sheaths have been studied in the optic nerves of rats and of *Xenopus laevis* tadpoles. Both potassium permanganate- and osmium-fixed material was examined with the electron microscope. In the first stage of myelinogenesis the nerve fibre is surrounded by a cell process which envelops it and forms a mesaxon. The mesaxon then elongates into a loose spiral from which the cytoplasm is later excluded, so that compact myelin is formed. This process is similar to myelinogenesis in the peripheral nervous system, although in central fibres the cytoplasm on the outside of the myelin is confined in a tongue-like process to a fraction of the circumference, leaving the remainder of the sheath uncovered, so that contacts are possible between adjacent myelin sheaths. The structure of nodes in the central nervous system has been described and it is suggested that the oligodendrocytes may be the myelin-forming cells.

INTRODUCTION

Studies with polarised light (1), x-ray diffraction (2, 3), and electron microscopy (3, 4–6) have shown that, in both the central and peripheral nervous system, myelin is made up of regular concentric lamellae arranged around the enclosed axon. Geren (7) first suggested that in the peripheral nervous system these lamellae are arranged in a spiral, formed from many turns of the Schwann cell membrane, and this concept has been confirmed and extended by Robertson (6, 8, 33). Since it is relevant to the following account, it will be advantageous to consider briefly how peripheral myelin is formed. Initially, a Schwann cell surrounds a length of nerve fibre and encloses it. When the axon is completely enclosed the outer surfaces of the membrane bounding the enveloping lips come into apposition, so that a double membrane, termed the mesaxon (9), is formed. The mesaxon then elongates and forms a loose spiral around the enclosed axon. At this stage, Schwann cell cytoplasm is present between each of the turns of the mesaxon, but as the mesaxon elongates further the cytoplasm is eliminated so that the cytoplasmic surfaces of the membranes of adjacent turns come into contact, and a thick or major dense line (6, 33) is formed by this apposition. Compact myelin results; and the major dense line, which separates the lamellae, alternates with the intraperiod line which is formed within the mesaxon where the external surfaces of the membrane come together. This arrangement is shown diagrammatically in Fig. 1 *A*. Schwann cell cytoplasm persists on both the inside (Fig. 1 *A*, C_1) and the outside (Fig. 1 *A*, C_0) of the myelin lamellae and it is in the outer cytoplasm that the nucleus of the Schwann cell is found. The ends of the spiral persist as the inner (Fig. 1 *A*, M_1) and outer (Fig. 1 *A*, M_0) mesaxons.

In their electron microscope studies on osmium-fixed material, Luse (10) using young rats and mice and De Robertis, Gerschenfeld, and Wald (11) using young cats and rats, suggest that cen-

Received for publication, February 6, 1960.

tral myelin is formed in a different manner to peripheral myelin. Luse (10) concludes that within a length of central myelin the lamellae are not produced by the elongation of a single membrane as within an internode in the peripheral nervous system, but by the plication of the membranes of several glial cells, and, in particular, those of the oligodendrocytes. Her evidence is inconclusive and contrary to that of De Robertis, Gerschenfeld, and Wald (11), who, although they consider that the oligodendrocytes form the myelin, put forward the hypothesis that the lamellae arise within the cytoplasm of these cells, which have a well developed endoplasmic reticulum. These theories imply the absence of both a spiral and an internal mesaxon, although De Robertis, Gerschenfeld, and Wald (11) find that internal mesaxons are occasionally present.

In an earlier electron microscope study based on potassium permanganate–fixed optic nerves and spinal cords from tadpoles of the toad *Xenopus laevis* Daudin, the present author (12) presented evidence against the above theories. In these tadpoles the myelin lamellae are arranged in a spiral and internal mesaxons are always present. Further, no discontinuities in the myelin lamellae have been observed, though these would be expected, at least during the formation of the lamellae, if the mechanisms suggested by either Luse (10) or De Robertis, Gershenfeld, and Wald (11) are correct.

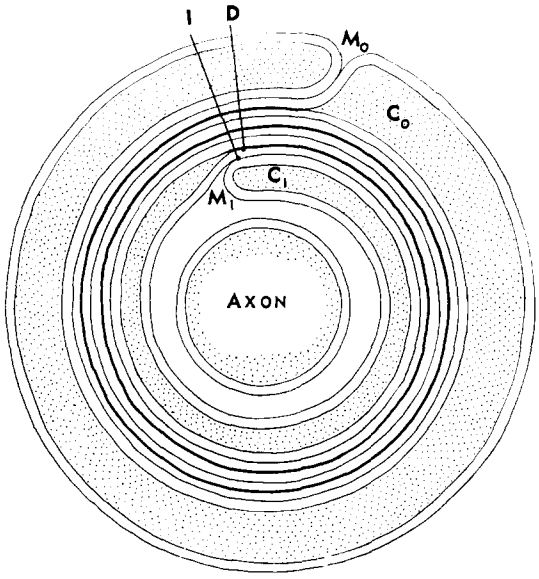


FIGURE 1*A*

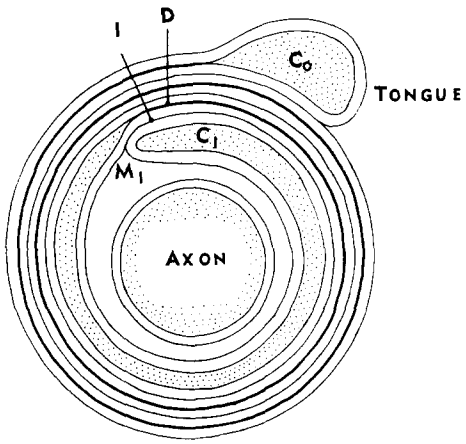


FIGURE 1*B*

Diagrams to show the structure of peripheral (Fig. 1 *A*) and central (Fig. 1 *B*) myelin sheaths. The cytoplasm of the axon, the Schwann cell (Fig. 1 *A*) and the myelin-forming glial cell (Fig. 1 *B*) is stippled. In both, cytoplasm (C_1) lies internal to the first turn of the spiral which begins at the internal mesaxon (M_1). The intraperiod line (*I*) arises within the internal mesaxon, which is formed by apposition between the external surfaces of the myelin-forming cell membrane. The major dense line results (*D*) from contact between the cytoplasmic surfaces of the same membrane. Both lines continue in a spiral and alternate throughout the thickness of the myelin sheath. In peripheral nerves (Fig. 1 *A*) a continuous layer of cytoplasm (C_0) surrounds the myelin spiral, which terminates at the external mesaxon. In the central nervous system. (Fig 1 *B*) the outer cytoplasm is confined to a tongue so that the major dense line is the outermost line of the sheath, except in the region covered by the tongue.

These figures are reprinted from the following article: Peters A., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 121.

The observed structure of the myelin sheaths in the central nervous system of *Xenopus* tadpoles is interpreted diagrammatically in Fig. 1 *B*, and the basic difference from peripheral myelin (Fig. 1 *A*) lies in the amount of cytoplasm present on the outside of the sheath. In central myelin, the outer cytoplasm (Fig. 1 *B*, C_0) is confined to a tongue, which varies in size, so that in a very few cases it may be as extensive as that of peripheral nerves, when an external mesaxon (Fig. 1 *A*, M_0) is present. However, the tongue is usually small and because cytoplasm is absent over most of the outer surface of the myelin, adjacent sheaths come into contact. Such contacts have not been recorded in the peripheral nervous system where the outer layer of cytoplasm always separates the sheaths.

In the present account these observations have been extended to the optic nerves of young rats and a study of the development of the central myelin sheath has been made in both rats and *Xenopus* tadpoles.

MATERIALS AND METHODS

The material used was the optic nerves of 18 day postnatal rats and of tadpoles of the toad *Xenopus laevis* Daudin. The tadpoles were between stages 50 and 57 of Nieukoop and Faber (13). Small pieces of tissue were fixed at 0°C. in either (*a*) the potassium dichromate–osmium tetroxide mixture of Dalton (14) for 1 hour, or (*b*) the veronal acetate–osmium tetroxide mixture of Palade (15) for 1 hour, or (*c*) 1 per cent potassium permanaganate (16) in 0.9 per cent saline for 2 hours. After fixation, pieces of tissue were washed in 10 per cent alcohol and dehydrated. The osmium-fixed material was then embedded in an 8:1 butyl-methyl methacrylate mixture and the potassium permanganate material in araldite. Sections were cut with glass knives on a Porter-Blum Servall microtome, then mounted on carbon films and examined in a Metropolitan-Vickers EM 6, electron microscope.

DESCRIPTION

In the optic nerves examined, myelin sheaths were still being formed, so that many early stages of myelinogenesis were present. As seen in the thin sections, the external surfaces of sheaths are frequently in contact with those of adjacent nerve fibres and at the same time they come into close proximity to the surrounding non-myelinated nerve fibres and the glial cells (Figs. 2 and 3). There are no nerve cells in the optic nerves and the majority of glial cells are oligodendrocytes. In the rat optic nerve there are large numbers of these cells arranged in columns which run the length of the nerve, but in the tadpole, the oligodendrocytes are relatively fewer and appear to be distributed in a more random manner. Astrocytes are less numerous than oligodendrocytes and no microglia have been recognised in the tissue which has been examined.

Identification of the different varieties of glial cells has been based on the work of Farquhar and Hartmann (17) and Schultz, Maynard, and Pease (18), the difference between these cells being relatively well defined in osmium-fixed material (Fig. 3). The oligodendrocyte nucleus has clumped chromatin (Fig. 3, *ONu*) and the cytoplasm, in which the endoplasmic reticulum is well developed (Fig. 3, *OC*), stains darker than that of either the astrocytes (Fig. 3, *AC*, AC_1) or the surrounding nerve fibres (Fig. 3, *N* and *NN*). The darker staining of the cytoplasm is more marked after treatment with Dalton's fixative (Fig. 14) than after Palade's fixative (Fig. 3). In direct comparison to the oligodendrocytes, the astrocytes have a darker nucleus in which the distribution of the chromatin is more homogeneous (Fig. 3, *ANu*). Their cytoplasm is lightly stained (Fig. 3, *AC* and AC_1) rather like that of the nerve fibres, and has a very poorly developed endoplasmic reticulum. Both types of cell send out processes between the surrounding nerve fibres, but while those of the oligodendrocytes can be traced for only short distances, the astrocyte processes are long (Fig. 2, 3 and 15, *AC* and AC_1) and frequently come close to the processes of other astrocytes (Fig. 3, *AC* and AC_1). In material fixed in potassium permanganate, these differences between the cells are less marked, since only the membranes are stained appreciably. However, the oligodendrocytes can again be identified by the well developed endoplasmic reticulum (Fig. 2, *OC*), the astrocytes having a cytoplasm which is relatively devoid of inclusions other than mitochondria.

The process of myelin formation in the tadpole and the rat is so similar that the following description applies equally well to both animals.

The first stage in myelination is one in which a nerve fibre is enclosed by a process of the myelin-forming cell, and an early phase in this enclosure is shown in Fig. 4. Here a nerve fibre (*N*) is partially surrounded by the cell process (*P*), the cytoplasm of which contains more vesicles and is stained rather darker than that of the enclosed

(N) and adjacent nerve fibres (N_1, N_2, N_3). A slightly later stage in which a nerve fibre has been further enclosed is seen in Fig. 5. This is from a permanganate-fixed block of tissue and shows the nerve fibre (N) almost completely engulfed by the process (P), although its lips (arrows) have not yet come together. When the lips of the process meet they become apposed (Fig. 6) and form a mesaxon (M). This mesaxon then elongates in a spiral manner around the enclosed nerve fibre. Two stages in the elongation are shown in Figs. 7 and 8. In Fig. 7 the mesaxon (M) describes half a turn around the nerve fibre (N) and in this osmium-fixed section it will again be observed that the cytoplasm of the process (P) is distinct from that of the enclosed axon (N) and surrounding non-myelinated nerve fibres (N_1, N_2, and N_3) in that it contains many more vesicles. In Fig. 8 the mesaxon (M) has completed two-thirds of a turn around the nerve fibre (N).

During these preliminary phases, the external surfaces of the cell membrane come into contact within the mesaxon (Fig. 8) to form the intraperiod line, so that no appreciable gaps exist between the membranes (Fig. 7). Since cytoplasm is present between the turns, the spiral is loose, but usually by the time 3 or 4 turns have been produced, most of the cytoplasm has been excluded. This results in apposition of cytoplasmic surfaces of the spiralled membrane, so that compact myelin is formed. Apposition of the cytoplasmic surface leads to the formation of the major dense line, which in compact myelin alternates with the intraperiod line arising within the mesaxon (6, 12)

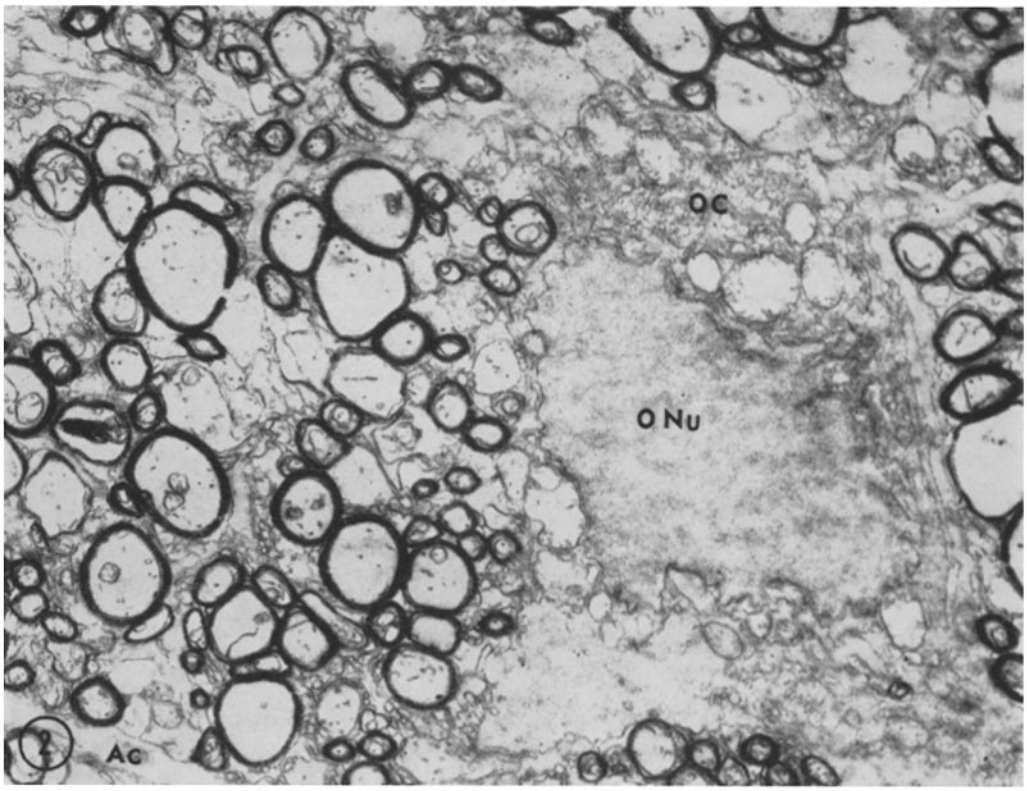
Three early stages with compact myelin are shown in Figs. 5, 6, and 9. The nerve fibre in Fig. 9 is from a tadpole optic nerve (fixed in veronal-acetate OsO_4) and here, most of the cytoplasm between the turns has disappeared so that compact myelin is present throughout the sheath in which only 2 or 3 lamellae exist. In such osmium-fixed material only the major dense line is consistently visible; the intraperiod line may show faintly, but usually it is not visible. Both lines are more completely visible after potassium permanganate fixation, and two fibres fixed by this means are shown in Figs. 5 and 6. In Fig. 6 cytoplasm is still present between the turns at x_1 and x_2, and as expected, it is the major dense line that is interrupted by this cytoplasm. However, in Fig. 5 the gaps z_1 and z_2 are produced by separation of the external surfaces of the membrane, so that the breaks have been produced in the intraperiod line. When the sheath is thicker, such gaps are usually absent (Figs. 14 and 15), but when cytoplasm intervenes it is found most frequently between the outermost lamellae. As described previously (12), the intraperiod and major dense lines alternate within the sheath, and while the major dense line terminates (Fig. 5, D) as the surfaces of the membranes separate to enclose the cytoplasm of the tongue (T) the intraperiod line (Fig. 5, I) ends where the membrane of the tongue diverges from the surface of the sheath (Fig. 5, S).

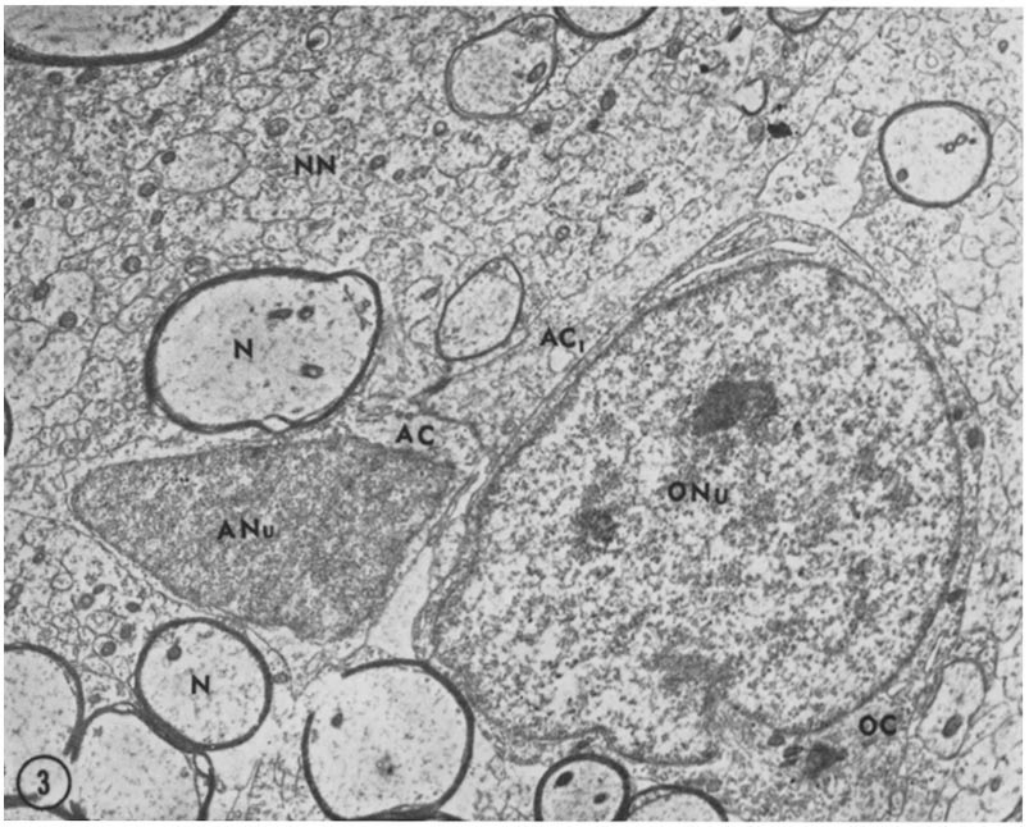
FIGURE 2

Micrograph of a transverse section through a permanganate-fixed optic nerve from an 18-day postnatal rat. The nerve is made up of both non-myelinated and myelinated nerve fibres and frequent contacts occur between adjacent myelin sheaths. An oligodendrocyte is present within the field and its nucleus (ON_u) is surrounded by a cytoplasm (OC) in which the endoplasmic reticulum is well developed. At the bottom of the figure is an astrocyte process (AC). × 4,800.

FIGURE 3

Transverse section of a Palade-fixed optic nerve from a stage 51 tadpole. The large oligodendrocyte nucleus (ON_u) has clumped chromatin and is surrounded by a darkly stained cytoplasm (OC) in which the endoplasmic reticulum is well developed. In contrast, the chromatin of the astrocyte nucleus (AN_u) is more homogeneous and the nucleus is stained darker. The cytoplasm (AC) of the astrocyte contains very few inclusions and comes into close proximity with a process (AC_1) from another astrocyte. The cytoplasm of this process, which extends to the top of the micrograph, contains rather more inclusions, but is easily distinguished from the cytoplasm of the oligodendrocyte (OC). Surrounding the glial cells are both myelinated (N) and non-myelinated (NN) nerve fibres. × 6,800.


OC
ONu
Ac


NN
N
AC
AC1
ONu
ANu
N
OC
3

Infrequently, cytoplasm is retained after many turns of the spiral have been completed and such a loose spiral is shown in Fig. 10. In this spiral, which has 6 turns, cytoplasm is present between all turns except in one small region (*X*) where the membranes have come together to form compact myelin. However, this may be a section through a node, for here cytoplasm would also be present between the turns.

Although cytoplasm is excluded from between the turns of the spiral during the formation of compact myelin, it is retained to varying degrees on both the inside and the outside of the sheath. Usually cytoplasm is present between the first turn of the mesaxon and the membrane ensheathing the axon, but occasionally it may either extend to part of the first turn of the spiral (Fig. 5), or be eliminated in places between the axon and the inside of the sheath (Fig. 6, *Y*). The amount of cytoplasm on the outside of the sheath is very variable, so that the size of the tongue differs from sheath to sheath (Figs. 5, 6, 14, and 15), but usually the tongue is quite small, particularly in the more mature sheaths. However, in rare instances cytoplasm persists around the entire sheath, when an external mesaxon, such as occurs in peripheral fibres, is present. In this latter situation, which seems to occur invariably in the peripheral nervous system, the outer cytoplasm (Fig. 1 *A*, C_0) maintains a separation of adjacent sheaths.

The general lack of cytoplasm on the external surfaces of the sheaths in the central nervous system allows adjacent sheaths to come into contact (Figs. 2, 5, and 15). Similar contacts may also occur between sheaths and tongues (12), as well as sheaths and the external surfaces of cell processes surrounding axons which have not yet myelinated (Figs. 5 and 6, *C*). In all these situations, where the external surfaces of membranes of myelin-forming cells come together, an intraperiod line is produced.

Both this and the previous study (12) have shown the similarity in the structure of peripheral and central sheaths, the essential difference being the amount of cytoplasm on the outside of the sheath. The similarity is further emphasised by the structure of the nodes in the central nervous system (Figs. 11 to 13). The nodes shown here are from the optic nerve of an 18-day postnatal rat, so that they are at a relatively early stage of development. At a node, two segments of myelin terminate, leaving a bare length of nerve fibre which comes into close proximity to the surrounding non-myelinated nerve fibres and glial cell processes (Figs. 11 and 12). The nerve fibres show no special features in the nodal region, although mitochondria (Figs. 11 to 13, *M*) are often present in the axoplasm near the terminal part of the sheath.

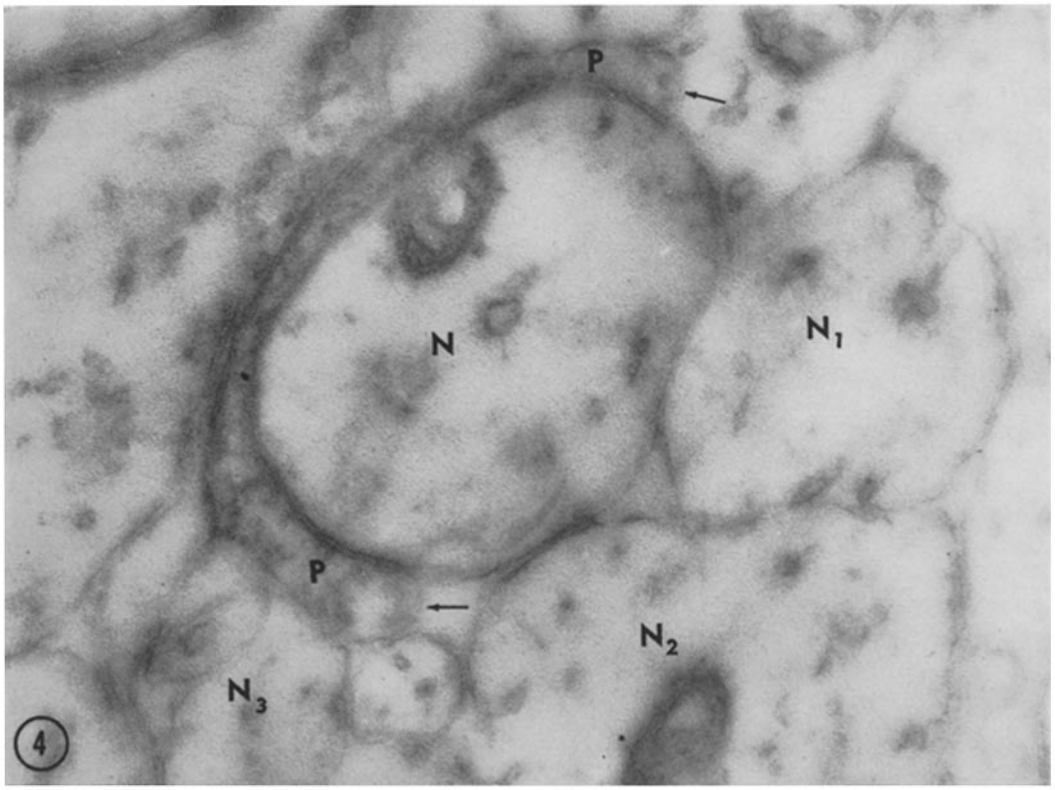
The termination of a length of myelin is accomplished by the lamellae of the sheath ending in a helix, so that the sheath gradually becomes thinner (Figs. 11 to 13). In this terminal helix, the lamellae separate along the major dense lines (Fig. 13, arrows) and the double membranes con-

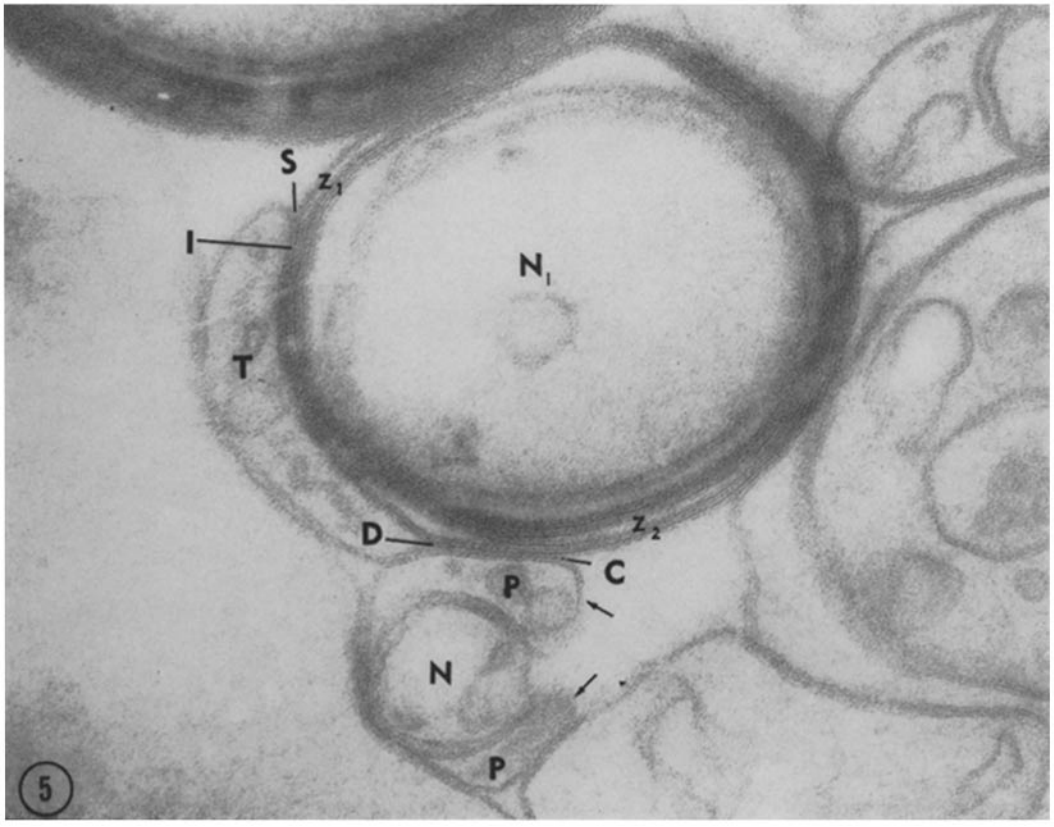
FIGURE 4

An early stage in myelination taken from a Palade-fixed optic nerve of a stage 51 tadpole. The nerve fibre (*N*) is partially surrounded by a cell process (*P*) the cytoplasm of which contains more vesicles and is more darkly stained than that of the enclosed axon (*N*) and surrounding non-myelinated nerve fibres (N_1, N_2, and N_3). The process extends as far as the arrows. × 74,000.

FIGURE 5

Transverse section of part of an optic nerve of an 18-day postnatal rat fixed in potassium permanganate. The nerve fibre (N_1) has a myelin sheath of 4 lamellae in which there are gaps (z_1 and z_2) where the intraperiod line is interrupted. The major dense line of the sheath terminates (*D*) where the membranes of the spiral separate to enclosed the cytoplasm of the tongue (*T*). The intraperiod line (I) ends where the inner membrane of the tongue turns away (*S*) from the outside of the sheath. At the bottom of the micrograph is a smaller nerve fibre (*N*) which is surrounded by a cell process (P) whose lips (arrows) have not met to form a mesaxon. This cell process (*P*) is in contact with the sheath of the upper fibre and an intraperiod line (*C*) is present at the point of contact. × 120,000.


P
N
N1
P
N2
N3
4


S
z1
I
N1
T
D
z2
C
P
N
P
5

taining the intraperiod lines (mesaxons, Figs. 12 and 13, *Z*) turn inwards towards the axolemma. When they reach the axolemma the individual membranes of the pairs separate, each to turn on itself and enclose a part of the cytoplasmic helix. Thus, the membranes form a tunnel which is filled with cytoplasm containing numerous round or oval profiles. Longitudinal sections through the helix of the cytoplasm give the appearance of a series of pockets of cytoplasm (Figs. 11 to 13, *C*) lying between the myelin sheath and the nerve fibre. This expanded end of the spiraled sheath is wrapped around the nerve fibre in a tight coil, approaching the axolemma on its internal surface, in which each turn is sandwiched between the previous and succeeding turns. Sometimes the membranes bounding the cytoplasmic helix on its inside appear to come into contact with the axolemma (Fig. 13, *A1*), but in other cases (Fig. 12) a well defined gap is present. Intimate contact between the axolemma and the Schwann cell membrane has been described at peripheral nodes by Robertson (27).

As the double membranes pass towards the axon, the angle that they make with the axolemma varies from node to node, but, in general, the innermost membranes come off at an oblique angle so that in longitudinal sections the pockets of cytoplasm are flattened between the axon and the sheath (Figs. 11 and 12). However, as the helix progresses towards the node, the diameter of the sheath increases while that of the nerve fibre decreases, so that the spiral tunnel widens. Thus, the middle pairs of membranes come off the sheath almost at right angles, but they begin to tilt again in the juxtaterminal region as the myelin sheath constricts and the nerve fibre bulges out into the nodal region, where the sheath is absent.

The type of glial cell that forms the myelin sheath in the central nervous system has not been determined. The choice appears to lie between the oligodendrocytes and the astrocytes, since in these young optic nerves no microglial cells have been identified. As pointed out previously (12), nuclei have not been found in the tongues on the outside of the sheath, so that it is unlikely that the nucleus of the myelin-forming cell is situated in the outer cytoplasm (Fig. 1 *A*, C_0), immediately adjacent to the sheath as it is in peripheral nerves. It seems most probable that a myelin sheath in the central nervous system is formed by cell processes at some distance from the cell body, although no such connections between cell bodies and any point of the sheaths have been found. Identification of the

FIGURE 6

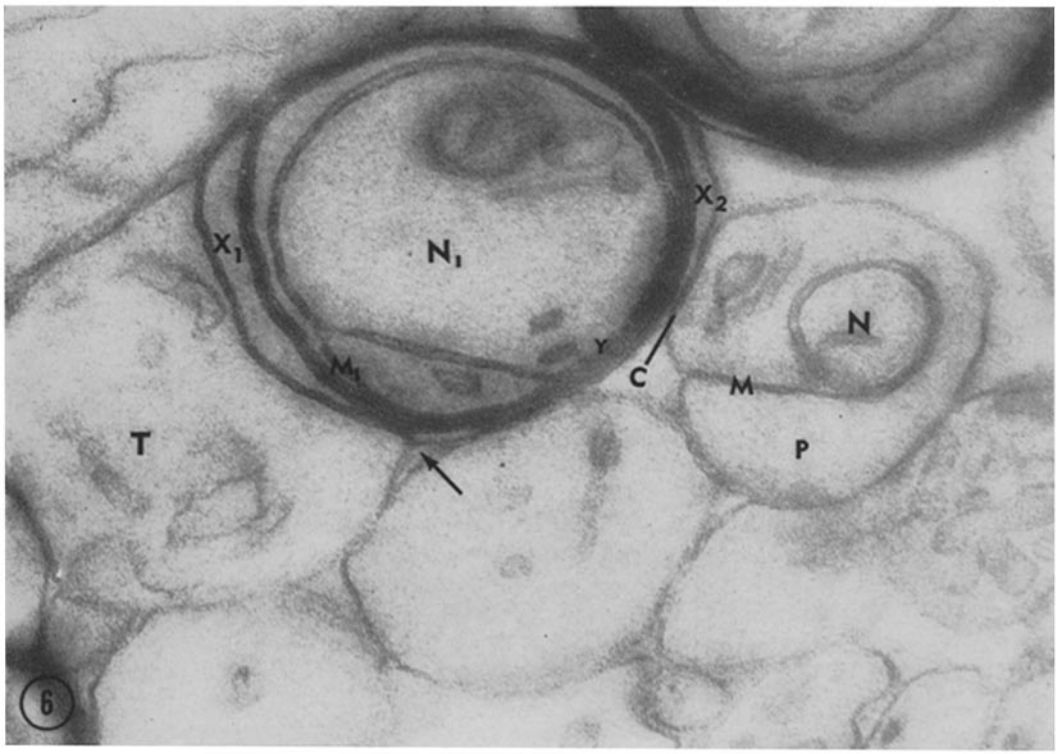
Transverse section of part of a permanganate-fixed optic nerve from an 18-day postnatal rat. The nerve fibre (*N*) is surrounded by a cell process (*P*) which has formed a mesaxon (*M*). The process (*P*) is in contact (*C*) with the outside of the sheath of an adjacent nerve fibre (N_1). This sheath is thin and in two places (X_1 and X_2) the lamellae are separated by cytoplasm, so that the major dense line is interrupted. The intraperiod line, which begins in the internal mesaxon (M_1), terminates where the membrane surrounding the tongue (*T*) turns away from the outside of the sheath (arrow). At *Y* the cytoplasm has been eliminated between the nerve fibre and the inside of the myelin sheath. × 120,000.

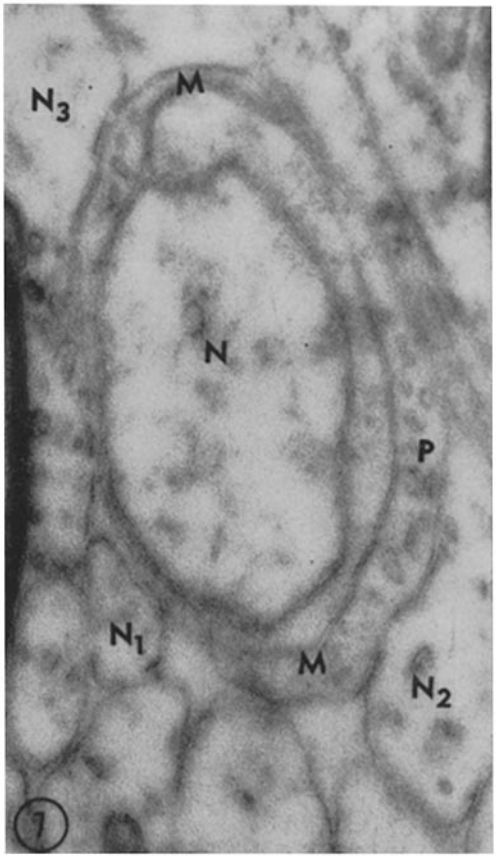
FIGURE 7

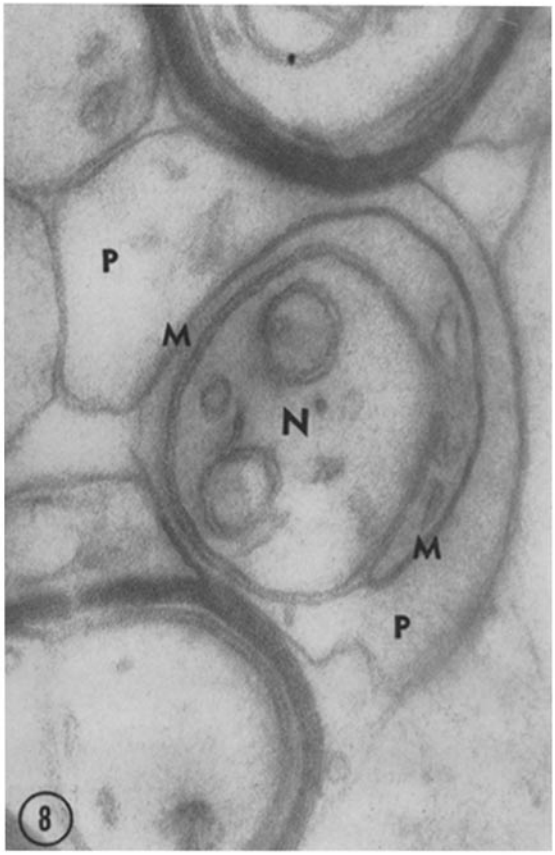
Micrograph of a stage in myelinogenesis in which the mesaxon (*M*) produced by the cell process (*P*) has completed half a turn around the nerve fibre (*N*). The cytoplasm of the cell process (*P*) contains more vesicles and is stained somewhat darker than the axoplasm of surrounding non-myelinated nerve fibres (N_1, N_2, and N_3). This is part of a transverse section of a Palade-fixed optic nerve from a stage 51 tadpole. × 64,000.

FIGURE 8

A slightly later stage in myelinogenesis than Fig. 6. This nerve fibre is from the potassium permanganate–fixed optic nerve of an 18-day postnatal rat. The mesaxon (*M*) produced by the apposition of the external surfaces of the membrane of the cell process (*P*) has completed two-thirds of a turn around the enclosed nerve fibre (*N*). × 100,000.


X1
N1
X2
N
Y
C
M1
M
T
P
6


N3
M
N
P
N1
M
N2
7


P
M
N
M
P
8

myelin-forming glial cells must depend therefore on indirect evidence.

Examination of material fixed in potassium permanganate throws little light on this problem, since only membranes are well displayed. However, a survey of a large number of sheaths and of processes at various stages of myelin formation, indicates that, on the whole, the cytoplasm of the myelin-forming cell has a better developed endoplasmic reticulum than that of the axons (see Figs. 5, 6, and 10). More information is available from the osmium-fixed material, for here cytoplasmic inclusions as well as membranes are stained. From this material it is evident that at all stages of myelin formation (Figs. 4, 7, 9, 14, and 15) the cytoplasm of the myelin-forming cell usually stains darker and contains more vesicles than that of the nerve fibres or astrocytes. The difference between the tongue and astrocyte cytoplasm is clear in Fig. 15 where the astrocyte process (*AC*) is stained lighter and contains fewer inclusions than that of the tongue (*T*) on the outside of the sheath surrounding the nerve fibre (*N*). The astrocyte cytoplasm is very similar to that of the surrounding nerve fibres. If the cytoplasm of a tongue is compared with that of an oligodendrocyte there is more similarity. This is best seen in Fig. 14, where the cytoplasm in the tongue (*T*) of the nerve fibre (*N*) can be compared with that of the oligodendrocyte (*Ol*) at the bottom of the photograph. On the basis of this indirect evidence it appears likely that the formation of myelin can be attributed to the oligodendrocytes rather than to the astrocytes.

DISCUSSION

The process by which myelin is formed in the central nervous system, as shown here in the optic nerve, is similar to that described in the peripheral nervous system (6, 7, 33), although minor differences occur. In both cases, the first step in myelinogenesis is one in which the nerve fibre is surrounded by the myelin-forming cell. In the peripheral nervous system (19, 20) each Schwann cell may surround a number of nerve fibres initially, so that in a section of a developing nerve, a Schwann cell may be seen at the centre of a group of nerve fibres, each of which is embedded in its cytoplasm. However, before a mesaxon is formed the number of Schwann cells increases, so that each contains only one myelinating nerve fibre. In the central nervous system the arrangement is somewhat different, for at any stage of myelinogenesis never more than one nerve fibre has been found embedded in a myelin-forming cell process. At present, the significance of this is not clear, because the myelin-forming cells have not been identified, although indirect evidence suggests that they may be the oligodendrocytes. However,

FIGURE 9

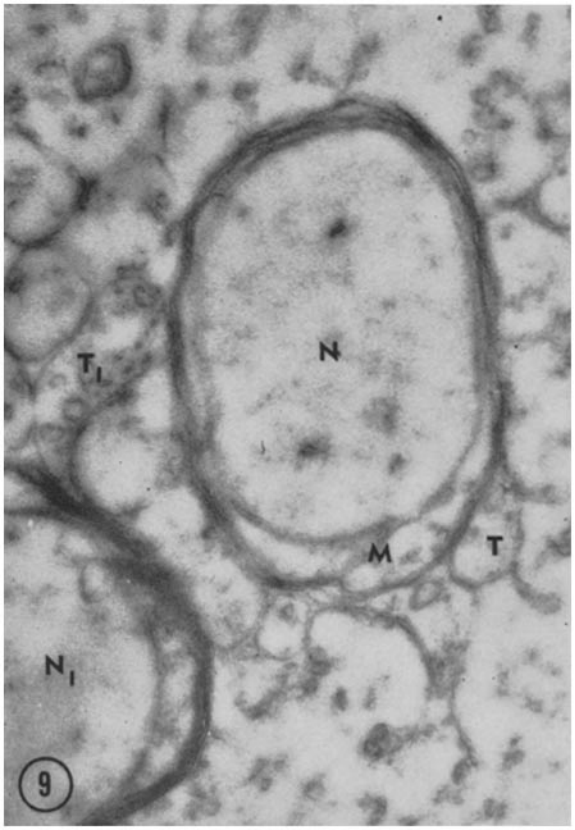
An early myelinated nerve fibre from a Palade-fixed stage 51, tadpole optic nerve. The nerve fibre (*N*) has a thin myelin sheath composed of three or four lamellae. The internal mesaxon (*M*) and the tongue (*T*) are indicated. The tongue (T_1) from the sheath of an adjacent nerve fibre (N_1) is also shown. × 43,000.

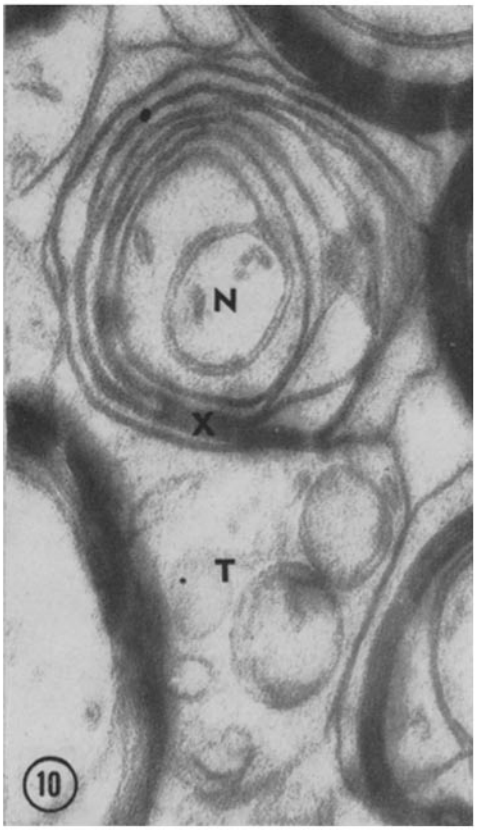
FIGURE 10

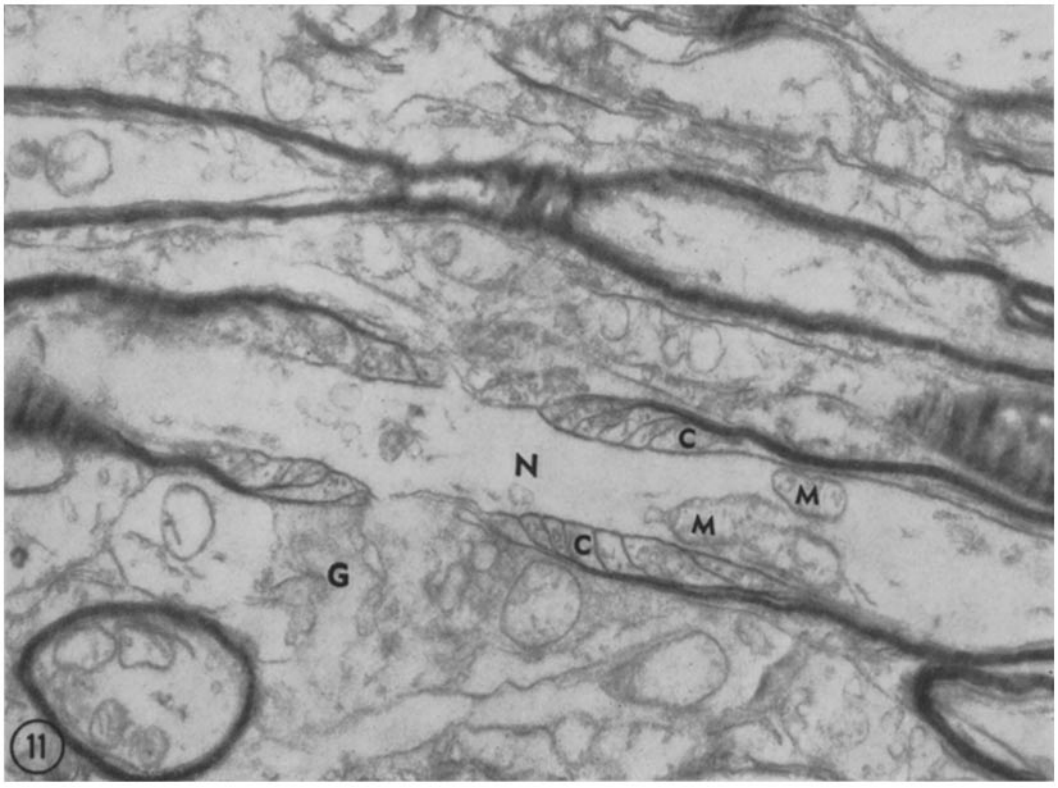
A nerve fibre (*N*) surrounded by a loosely spiralled mesaxon. Cytoplasm is present between all the turns of the spiral except in the region *x*, where a small area of compact myelin has been formed. The tongue (*T*) is quite large and its cytoplasm contains vesicles. 18-day postnatal rat optic nerve, fixed in permanganate. × 50,000.

FIGURE 11

A longitudinal section through a permanganate-fixed optic nerve from an 18-day postnatal rat. A node is shown at which two adjacent lengths of myelin terminate. At the node, the nerve fibre (*N*), which contains mitochondria (*M*), is naked and comes into close proximity to a glial cell (*G*). As the spiraled lamellae of myelin turn inwards from the inside of the sheath, the individual membranes of the lamellae separate and turn back on themselves to enclose part of the cytoplasmic helix (*C*) derived from the myelin-forming cell. × 21,000.


N
T1
M
T
N1
9


N
X
T
10


C
N
M
M
C
G
11

no nuclei have been located in the tongues within the immediate vicinity of the sheath, as they are in the peripheral nervous system, so that the disposition of the myelin-forming cells must certainly be different from that of the peripheral nervous system where the nuclei of the Schwann cells are encountered within the outer cytoplasm. In the optic nerve of the rat, for example, the glial cells are arranged in columns between which run bundles of nerve fibres completely devoid of nuclei. Therefore on structural grounds alone it is probable that the nucleus-containing body of the myelin-forming cell is some distance from the site where the myelin is formed. This arrangement could account for both the absence of nuclei within the tongues as well as the failure to find connections between tongues and glial cells, since the thin sections so essential to electron microscopy would tend to preclude the possibility of obtaining the whole length of a connection in one section.

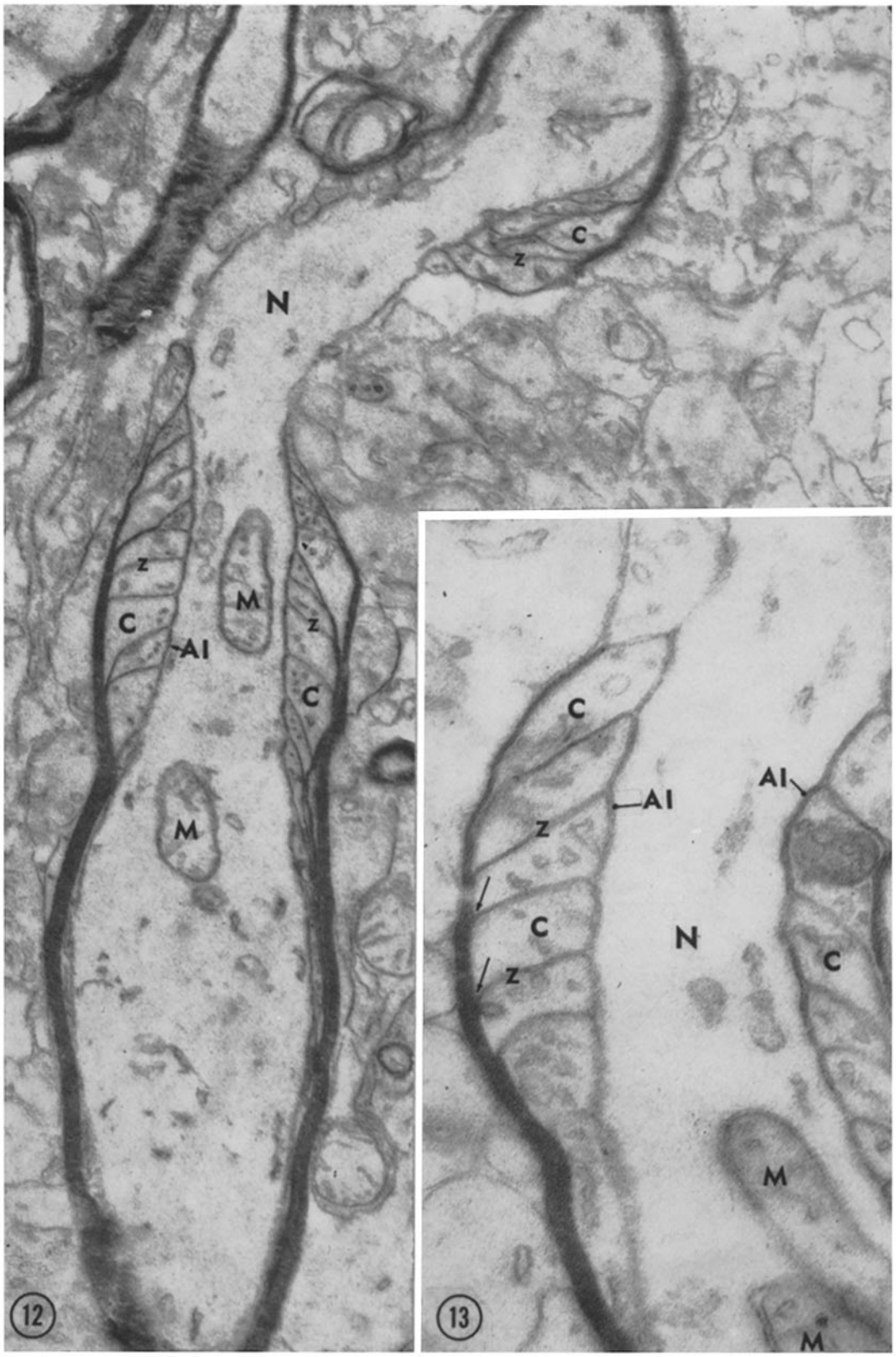
The cytoplasm of the myelin-forming processes is akin to that of the oligodendrocytes, but differs from the astrocyte cytoplasm, which suggests that the former may be responsible for the formation of myelin. Other evidence to suggest that the oligodendrocytes may give rise to the myelin is the preponderance of these cells in the optic nerve. In addition, the oligodendrocytes have a well developed endoplasmic reticulum, which is consistent with an active metabolism, such as would occur during the sythesis of cell membranes necessary for the formation of myelin. The results of other workers have not clarified this problem which has been debated unsuccessfully by neurologists and histologists for many years. Both astrocytes and oligodendrocytes (21, 22) have both been held responsible and the formation of myelin has even been attributed to the axons themselves (23).

The later stages in myelinogenesis are similar in the peripheral and central nervous system. In both, the mesaxon, formed by contact between the lips of that part of the cells which engulfs the nerve fibre, elongates in a loose spiral, in which the cytoplasmic surfaces of the membranes are held apart by the cytoplasm of the myelin-forming cell. Later, cytoplasm is eliminated from between the turns so that the cytoplasmic surfaces of the membranes come into contact to form the major dense line. In the peripheral nervous system Schwann cell cytoplasm completely covers the internal and external surfaces of the spiral myelin sheath, but in the central nervous system the cytoplasm is much less extensive, so that although it forms a complete internal layer, externally it is confined to a tongue, which covers only a fraction of the outside. This structure of central sheaths which was first described in *Xenopus* tadpoles (12), has in the present study been confirmed in the optic nerve of the rat.

The structure of the nodes of Ranvier in the peripheral nervous system has been described by Uzman and Nogueira-Graf (24), Robertson (25–27) and Engstrom and Wersall (28). However, there has been no previous information about the structure of central nodes, although on the basis of light microscopy their presence has been recorded by a number of workers (29, 30). Electron microscope photographs of nodes have been published recently by Gray (31), but unfortunately these are not clear enough for a detailed analysis of their structure to be made. The present study shows that the nodes resemble those of the peripheral nervous system, a fact which serves to re-emphasise the similarity between the sheaths. In both cases the sheath terminates as the spiralled lamellae turn off from the inside to enclose a helix of cytoplasm derived from the myelin-forming cell.

FIGURES 12 and 13

Sections of nodes from the permanganate-fixed optic nerve of an 18-day postnatal rat. At a node the spiraly arranged lamellae of myelin turn off from the inside of the sheath which becomes progressively thinner. The lamellae separate along the major dense lines (Fig. 13 arrows) and form pairs of membranes (*Z*) analogous to mesaxons. The individual membranes of each pair separate as they reach the axolemma (*Al*) and enclose the helix of cytoplasm (*C*) in which there are vesicles. When the membranes meet the axolemma, they either come into contact with it (Fig. 13) or remain separate (Fig. 12). Near the termination of the sheath, mitochondria (*M*) are usually found within the nerve fibre (*N*) which at the node itself is naked and comes into close proximity to adjacent cell processes and non-myelinated nerve fibres. Fig. 12, × 33,000. Fig. 13, × 75,000.


N
C
z
z
C
AI
M
z
C
M
12
C
AI
AI
z
C
N
z
C
M
13
M

This structure is very characteristic. It should be pointed out that the helix of cytoplasm present at the node is continuous with the cytoplasm on both the inside and the outside of the sheath. The only other place where such a continuity appears to exist is at the Schmidt-Lanterman clefts (32) in which the lamellae are separated by a shearing defect, but whether or not these clefts are present during life appears to be uncertain. In the remainder of the sheath the lamellae are in such close contact that the possibility of continuity of cytoplasm is precluded.

One feature in which these young central nodes differ from mature peripheral nodes is related to the bare part of the nerve fibre. In both cases the nodal fibre is completely free of myelin, but in mature peripheral nerves the fibre is not uncovered, for as Robertson (27) has shown, it is surrounded by a "collar of minute finger-like processes of the two Schwann cells." These processes are derived from the outer cytoplasmic process and may serve to isolate the bare nerve fibre from surrounding nerves. In the central nervous system, where the outer cytoplasm is confined to a tongue, the nodal nerve fibre from an 18-day postnatal rat optic nerve has no such covering, so that it is in close proximity to the surrounding nerve fibres and glial cells. Whether this situation persists in the more mature myelinated nerves has not yet been investigated.

I wish to thank Professor G. J. Romanes for his interest during the course of this work, Mr. G. Wilson for his skilful maintenance of the electron microscope, which is on loan from the Wellcome Trust, and Mr. H. Tully for his able technical assistance. The *Xenopus* tadpoles were kindly provided by Dr. B. Hobson.

ADDENDUM

Since the manuscript of this paper was submitted for publication, the investigation of Maturana into the structure of the optic nerves of various Anura has appeared (*J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 107). This work was published alongside that of the present author (*J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 121) in which the structure of the myelin sheaths in the optic nerve and spinal cord of tadpole of a toad, *Xenopus laevis* Daudin was considered. The interpretation of the structure of the myelin sheaths is the same in both cases. Maturana also discussed the structure of nodes in the optic nerves of anurans and the results from the present work are in complete agreement with his findings.

REFERENCES

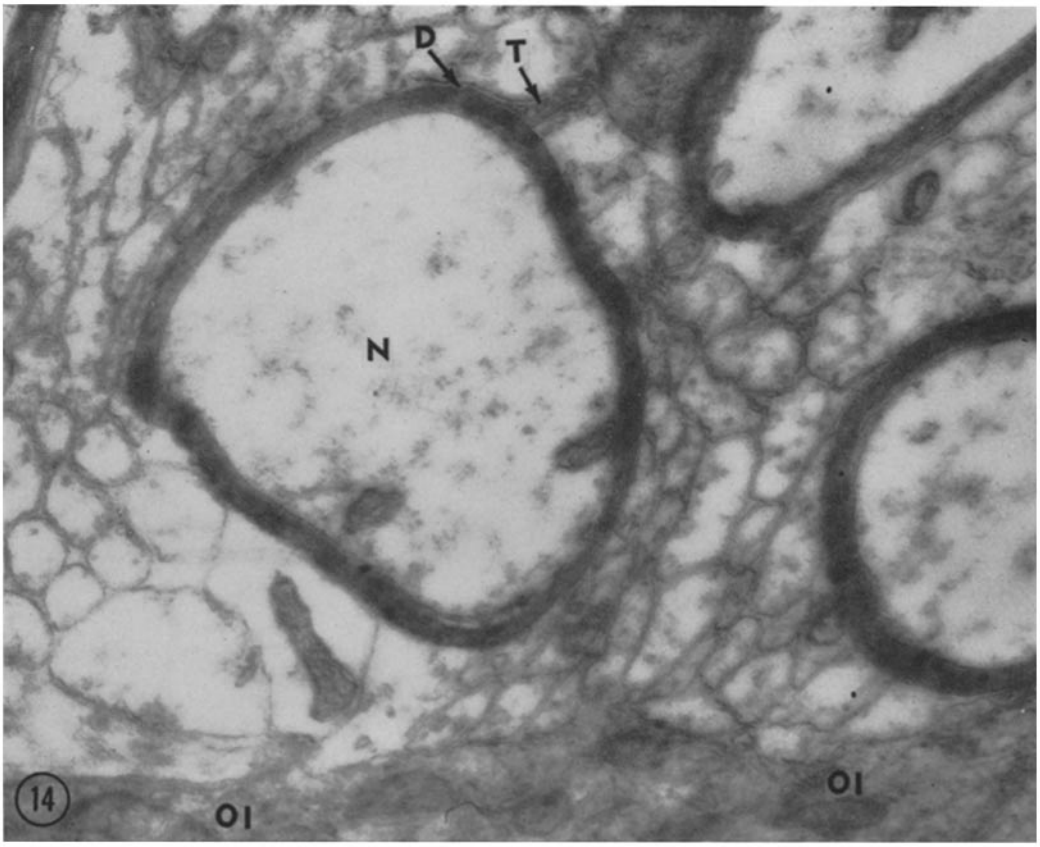
1. SCHMIDT, W. J., *Z. wissench. Mikr.*, 1937, **54**, 159.
2. SCHMITT, F. O., and BEAR, R. S., *Biol. Rev.*, 1939, **14**, 27.
3. FERNÁNDEZ-MORÁN, H., and FINEAN, J. B., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 725.
4. FERNÁNDEZ-MORÁN, H., *Exp. Cell Research*, 1950, **1**, 143.
5. FERNÁNDEZ-MORÁN, H., *Exp. Cell Research*, 1952, **3**, 283.
6. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 349.

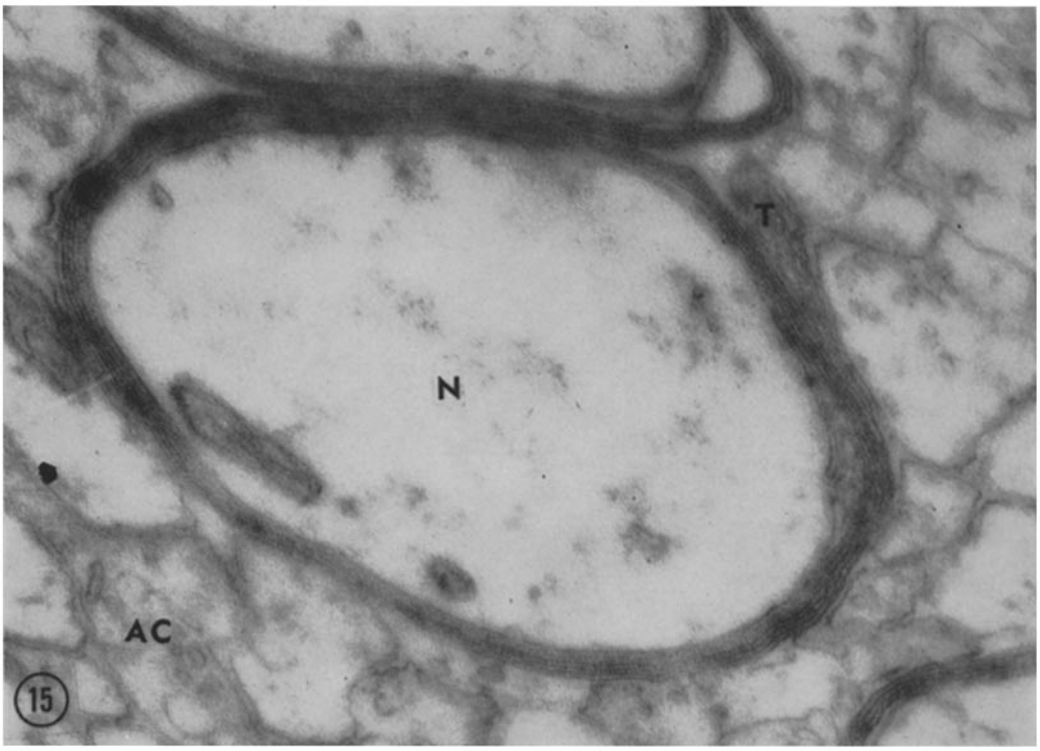
FIGURE 14

Micrograph from the Dalton-fixed optic nerve of a stage 51 tadpole. One entire sheath and parts of three others are shown. In these osmium-fixed sheaths only the major dense lines are visible and in the sheath of one fibre (*N*) the major dense line (*D*) can be seen to terminate at the tongue (*T*) on the outside of the sheath. Note the similarity between the cytoplasm of the tongue (*T*) and that of the oligodendrocyte (*Ol*) at the bottom of the photograph. × 43,000.

FIGURE 15

Micrograph of a myelinated nerve fibre from the Palade-fixed optic nerve of a stage 51 tadpole. The sheath of the nerve fibre (*N*) is in contact with that of another fibre. Only the major dense line is visible within the sheath and this terminates at the tongue (*T*). Non-myelinated nerve fibres are adjacent to the sheaths and at the bottom left hand corner of the micrograph is an astrocyte process (*AC*). Note the difference between the cytoplasm of this process and that of the tongue (*T*) the cytoplasm of which is more akin to that of the oligodendrocyte in Fig. 14. × 75,000.


D
T
N
14
OI
OI


T
N
AC
15

7. GEREN, B. B., *Exp. Cell Research*, 1954, **7**, 558.
8. ROBERTSON, J. D., Ultrastructure and cellular chemistry of neural tissue, *in* Progress in Neurobiology, (H. Waelsch, editor), New York, Hoeber-Harper, 1957, **2**, 1.
9. GASSER, H. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1952, **17**, 32.
10. LUSE, S. A., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 777.
11. DE ROBERTIS, E., GERSCHENFELD, H. M., and WALD, F. J., *Biophysic. and Biochem. Cytol.*, 1958, **4**, 651.
12. PETERS, A., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 121.
13. NIEUKOOP, P. D., and FABER, J., Normal Tables of *Xenopus laevis* (Daudin), Amsterdam, North Holland Publishing Co., 1956.
14. DALTON, A. J., *Anat. Rec.*, 1955, **121**, 281.
15. PALADE, G. E., *J. Exp. Med.*, 1952, **95**, 285.
16. LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
17. FARQUHAR, M. G., and HARTMANN, F. J., *J. Neuropath. and Exp. Neurol.*, 1956, **16**, 18.
18. SCHULTZ, R. L., MAYNARD, E. A., and PEASE, D. C., *Am. J. Anat.*, 1957, **100**, 369.
19. PETERS, A., and MUIR, A. R., *Quart. J. Exp. Physiol.*, 1959, **44**, 117.
20. PETERS, A., 1959, data to be published.
21. DEL RIO HORTEGO, P., *Arch. Histol. Norm. Path.*, 1942, **1**, 5.
22. ALPERS, B. J., and HAYMAKER, W., *Brain*, 1934, **57**, 195.
23. HILD, W., *Z. Zellforsch.*, 1957, **46**, 71.
24. UZMAN, B. G., and NOGUEIRA-GRAF, G., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 589.
25. ROBERTSON, J. D., *J. Physiol.*, 1957, **135**, 56P.
26. ROBERTSON, J. D., *J. Physiol.*, 1957, **137**, 8P.
27. ROBERTSON, J. D., *Z. Zellforsch.*, 1959, **50**, 553.
28. ENGSTROM, H., and WERSALL, J., *Exp. Cell Research*, 1958, **14**, 414.
29. ALLISON, A. C., and FEINDEL, W. H., *Nature*, 1949, **163**, 449.
30. HESS, A., and YOUNG, J. Z., *Proc. Roy. Soc. London, Series B*, 1952, **140**, 301.
31. GRAY, E. G., *J. Anat.*, 1959, **93**, 420.
32. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 39.
33. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 271.