# The Structure of Myelin Sheaths in the Central Nervous System of *Xenopus laevis* (Daudin)

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PLATES 43 TO 47

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ABSTRACT

The structure of myelinated nerve fibres has been studied in the spinal cord and optic nerve of the tadpoles of *Xenopus laevis*. Potassium permanganate-fixed material was examined with the electron microscope. The myelin sheath itself is made up of spirally arranged lamellae in which the intraperiod and dense lines alternate. Inside the myelin sheath an inner cytoplasmic process surrounds the axon and where the external surfaces of its bounding membrane come together an internal mesaxon is formed. The intraperiod line begins within the mesaxon and the dense line usually begins in the same region by apposition of the cytoplasmic surfaces of the membrane. The width of each lamella is 140 A. The outer line in the sheath is the dense line and this terminates in a tongue where the cytoplasmic surfaces of the myelin-forming glial cell separate. Thus, central myelin in *Xenopus* tadpoles is arranged in the same way as peripheral myelin, the only difference being that in central fibres, cytoplasm on the outside of the sheath is confined to that present in the tongue. For this reason adjacent central sheaths come into apposition without any intervening material being present. When this occurs an intraperiod line is formed between them.

Studies on the structure of peripheral and central myelin using x-ray diffraction (1, 2), polarised light (3), and electron microscopy (2, 4–6) show that in both cases the myelin has the same basic structure; it is made up of regular concentric lamellae. In the peripheral nervous system Geren (7) showed that these lamellae, formed by the Schwann cells, are disposed in a spiral around the axon and this helical structure is now generally accepted (4, 6). Much less information is available about the formation and arrangement of the lamellae of central myelin and the two electron microscope studies which have been carried out by Luse (8) on young rats and mice and by De Robertis, Gerschenfeld, and Wald (9) on young cats and rats appear to be inconclusive and have produced conflicting results. Luse (8) suggests that in the central nervous system a single cell does not form the lamellae, as does a Schwann cell within an internode in the peripheral system, but rather that the lamellae are formed by a plication of the membranes of many glial processes, in particular, those of the oligodendrocytes. The theory of De Robertis, Gerschenfeld, and Wald (9) is somewhat contrary to that of Luse, for while they also state that the myelin is formed by the oligodendrocytes, they consider that it arises within the cytoplasm of these cells, where large numbers of membranes and vesicles are present, membranes being laid down in concentric layers around the axon.

The results of these two electron microscope studies, which are based on osmium-fixed material, are so much at variance that it was decided to reinvestigate the problem using potassium permanganate as the fixative (10). This latter fixative produces much better staining of membranes than osmium and has been used to great advantage in the study of peripheral myelin (6, 11). Although potassium permanganate stains membranes effectively, it is a poor fixative for other cellular components, with the result that it is difficult to differentiate between axonal and glial processes. The results of the present investigation are therefore confined to a consideration of the arrangement of the lamellae within the myelin sheaths of the central nervous system.

### *Materials and Methods*

The material used was the optic nerve and spinal cord of tadpoles of the toad *Xenopus laevis* (Daudin). The tadpoles examined were at stages 55 to 57 of Nieukoop and Faber (12). Small pieces of tissue were removed and fixed at 0°C. in 1 per cent potassium permanganate in 0.9 per cent saline for 2 hours. They were then washed in 10 per cent alcohol, dehydrated, and embedded in araldite (13). Sections were cut with glass knives on a Porter-Blum "Servall" microtome and examined, without the use of a supporting film, in a Metropolitan Vickers, EM 6, electron microscope.

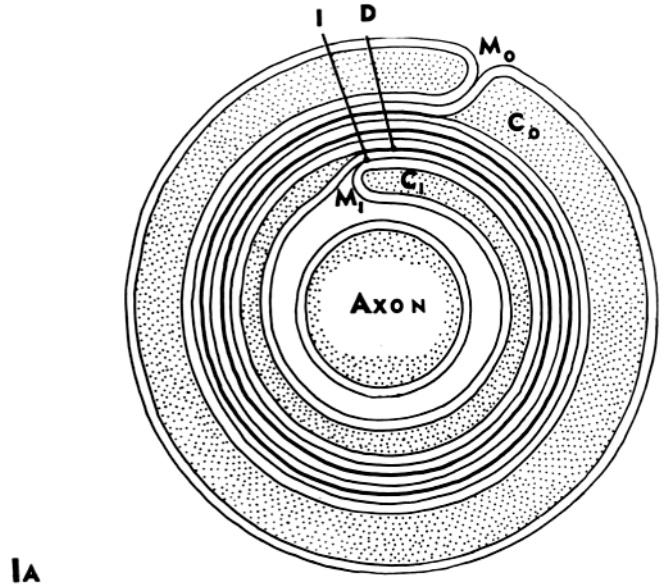
### DESCRIPTION

In the stages of *Xenopus* tadpoles examined, both the peripheral and central myelin are at an early phase of development. This can be seen in Fig. 1, which shows the typical arrangement of myelinated fibres in part of the spinal cord and peripheral nerve root in a stage 56 tadpole. The most striking differences between the two types of tissue are the close proximity of adjacent central fibres in comparison with the well separated peripheral fibres, and the absence between the central fibres of nuclei corresponding to those of Schwann cells in the peripheral nerve (Fig. 1 *N*). Processes of cells are present between the central fibres, but as previously pointed out, since potassium permanganate is a poor fixative for cellular components other than membranes, it is difficult to determine whether these processes belong to either glial cells or neurons. Some of the processes do, however, contain vesicles (Fig. 1 *V*) which are characteristic of adult neuronal cytoplasm in the region of a synapse (14), but in the present state of our knowledge it would be inappropriate to use these vesicles as a means of certain identification at this early stage of development. The above remarks on the structure of the spinal cord apply equally well to the optic nerve, although no vesicle-containing processes have been seen within this nerve.

The best way to understand the structure of central myelin is to compare it with that of the peripheral nervous system which has been studied extensively. It will therefore be advantageous to consider briefly how peripheral myelin is formed. At an early stage, the Schwann cell forms a spiral around the axon and by obliteration of the cytoplasm between the turns, the membranes of the Schwann cell come together to form compact myelin. The inner and outer ends of the spiral persist as the inner and outer mesaxons (Text-fig. 1 A, $M_1$ and $M_o$). Schwann cell cytoplasm remains in the first turn of the spiral between the compact myelin and the axon, the inner cytoplasmic process (Text-fig. 1 A, $C_1$), and also in the last turn of the spiral, the outer cytoplasmic process (Text-fig. 1 A, $C_o$). It is in this latter that the nucleus of the Schwann cell is found (Fig. 1, *N*). As the membranes of the Schwann cell come together to form compact myelin, it has been shown by high resolution electron microscopy (2, 6, 11) that alternating thick and thin lines are formed. These will be referred to as dense lines and intraperiod lines respectively. The dense lines arise as the cytoplasmic surfaces of the Schwann cell membrane become apposed and the intraperiod lines as the external surfaces of the membranes come into contact (Text-fig. 1 A).

Throughout this description the term "mesaxon" is retained to describe the double membrane formed by the apposition of the external surfaces of the myelin-forming cell where it is not producing compact myelin. Although this term is not necessarily correct, it is now generally accepted even though Robertson (6) has used the terms "surface-connecting membrane" and "meso" to describe this structure, defining "meso" as the double membrane leading from some included structure to the outside. These latter terms, though more appropriate, have not been widely employed by other workers.

Inside the compact myelin, central fibres show similar structures to those present in peripheral fibres. The axon is bounded by an internal cytoplasmic process (Figs. 2 to 5, and 8, *C*), which surrounds the axon between it and the internal lamellae of compact myelin. As in peripheral nerves, the process forms the first turn of a spiral and at the point where the turn is completed an internal mesaxon arises by the apposition of the external surfaces of the membrane bounding the process (Figs. 2 to 5, and 8, *M*). Within the internal mesaxon, the thin, intraperiod line arises (Figs. 3 and 4, *I*). It is usual for the dense line (Figs. 3 and 4, *D*) to be formed as the mesaxon approaches the innermost layer of compact myelin, for at this point the cytoplasmic surfaces of the membrane bounding the internal cytoplasmic process come together. Although an internal mesaxon is always present, it is not so readily visible when the cross-sectional area of the axon approaches that of the innermost lamella of compact myelin, for then the limiting membranes of the

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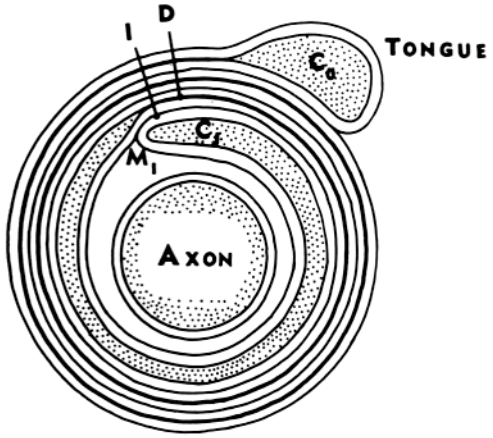


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TEXT-FIG. 1. Diagrams to show the structure of peripheral myelin, Text-fig. 1 A, and central myelin, Text-fig. 1 B. Cytoplasm of the axon, Schwann cell (Text-fig. 1 A, $C_1$ and $C_0$) and glial cell (Text-fig. 1 B, $C_1$ and $C_0$) is stippled. In both nerves the inner cytoplasmic process, $C_1$, surrounds the axon and its membrane comes together to form the inner mesaxon, $M_1$, in which the first intraperiod line, $I$, arises. The first dense line, $D$, is formed by the apposition of the cytoplasmic surfaces of the same membrane. These lines continue in a spiral and terminate at the outside of the sheath. In the peripheral nerve the intraperiod line terminates in the outer mesaxon, $M_0$, while the dense line terminates by a separation of the cytoplasmic surfaces to enclose the cytoplasm of the outer process, $C_0$, of the Schwann cell. In the central sheath, the dense line terminates as the membranes separate to enclose the cytoplasm, $C_0$, of the tongue, and the intraperiod line ends as the tongue membrane turns away from the outside of the sheath.

inner cytoplasmic process approach each other, sometimes with the result that the dense line begins before the intraperiod line is formed.

Although the myelin sheaths of peripheral and central fibres have the same internal structure, very obvious differences occur at the outsides of the sheaths. Thus, in the central nervous system processes of adjacent cells may be as close as 100 to 150 A to the external surface of the myelin sheath (Figs. 1, 3, 5, and 8). Such a situation never occurs in peripheral nerves, where the myelin sheaths are well separated by the presence of the outer cytoplasmic process of the Schwann cell, external to the myelin lamellae (Fig. 1 and Text-fig. 1 A, $C_o$). Examination of the outer region of central myelin shows that the external, or last, line in the sheath is a dense line (Figs. 2, 3, 5, and 8) which is formed by the apposition of the cytoplasmic surfaces of the cell membrane. That such is the case, is seen in Figs. 5 to 8, where the outer dense line ends in a tongue applied to the surface of the myelin sheath. In Fig. 5, such a tongue is seen in association with each of the three myelin sheaths and the structure of the tongue on the surface of the lower sheath is shown in Figs. 6 and 7. No breaks in the myelin lamellae have been observed in the present study and the outer dense line always terminates in the same manner, only one tongue being present on the external surface of a myelin sheath (see also Figs. 8 and 10). In this material, the distance from centre to centre of two adjacent dense lines, *i.e.* the width of each lamella, is $\sim$140 A.

These observations on the structure of myelin sheaths of central fibres have been brought together in Text-fig. 1 B, which shows the suggested arrangement of the myelin sheath. For comparison, the generally accepted arrangement of peripheral myelin is shown in Text-fig. 1 A. The only part of this arrangement for which no evidence has been presented is that of the existence of a spiral in central myelin. Owing to compression in the direction in which the sections have been cut, it is not possible to trace the myelin lamellae throughout the entire sheath, but in some sheaths a few lamellae can be followed, and in these there is no doubt that the lamellae follow a spiral course (see Fig. 10). It is also significant that the direction in which the outer dense line terminates in the external tongue is that which would be expected on the basis of a spiral commencing at the internal mesaxon (Figs. 5 and 8). Indirect evidence for a spiral is obtained by counting the number of dense lines in different parts of the sheath. The number is constant except within the region between the beginning of the first dense line at the internal mesaxon and the termination of the last dense line in the tongue (see Text-fig. 1 B and Fig. 8). Assuming the myelin lamellae to be continuous, the only arrangement which can account for this result is a spiral. More direct evidence has been presented by Fernández-Morán and Finean (2) who, in an electron micrograph of an early myelinated fibre from the thalamus of a mouse, show the presence of a well defined spiral.

Thus, peripheral and central myelin is arranged in a similar manner around the axis cylinder, the main difference being in the termination of the spiral at the outside of the sheath. While in peripheral myelin the spiral terminates where the external mesaxon leaves the sheath (Text-fig. 1 A), in central myelin the sheath terminates by the separation of the cytoplasmic surfaces of the membrane bounding the tongue. However, the two methods of termination, though superficially different, are very similar, for it can be seen that should the external cytoplasmic process of a Schwann cell incompletely surround the myelin sheath the external mesaxon will disappear and an external tongue will result. The cytoplasmic surfaces of the outer membrane of the Schwann cell will thus become applied to the surface of the compact myelin to form an outer dense line, exactly as in central myelin. In confirmation of the essential similarity of the two arrangements is the infrequent finding of outer mesaxons in the central nervous system, where cytoplasm is still present in the outer turn of the spiral.

As pointed out previously, when two adjacent myelin sheaths come together intimate contact is made between them (Figs. 4, 5, and 8). The result is that an intraperiod line is formed at the point of contact (arrow, Fig. 8). Thus, the outer surfaces of the myelin sheaths show exactly the same property that is associated with the formation of lamellae of compact myelin. Furthermore, the external surfaces of the tongues can become apposed to the outsides of adjacent myelin sheaths. These situations all occur in Fig. 8 in which the tongue belonging to the small upper fibre is applied to the external surface of the lower fibre on the right of junction of the two sheaths. If this junction is analysed, it can be seen that the two sheaths come together at the right; 8 dense lines are present in the upper sheath and 9 in the lower. At the point

where the cytoplasmic surfaces of the tongue membrane become apposed, an extra dense line (*D*) is formed, together with the intraperiod line (arrow) produced by contact between the sheaths. On the left where the sheaths separate, the lower one still has 9 dense lines, while the upper sheath now has an extra dense line making a total of 9. Such contacts, which produce an extra intraperiod line, occur between the external surfaces of sheaths and sheaths, as well as tongues and sheaths, but have not been found to exist between these structures and any other processes.

Although the early stages in central myelinogenesis have not been studied extensively, those fibres which have been observed are similar in many respects to peripheral nerve fibres in the same stage of myelination. Two such examples are shown in Figs. 9 and 10. Fig. 9 is an early stage in which the process (*C*) of the myelin-forming cell encloses the axon (*Ax*) with the resulting formation of a short mesaxon (*M*). Fig. 10 is a later stage in which 2 or 3 myelin lamellae have been formed. The internal mesaxon (*M*) leaves the axon (*Ax*) and spirals between it and the formed myelin. An external tongue is present (*T*).

## DISCUSSION

These observations on the myelin sheaths in the spinal cord and optic nerve of *Xenopus* tadpoles suggest that both central and peripheral myelin sheaths have the same basic structure. In each an internal mesaxon is present and there is evidence, both here and in the work of Fernández-Morán and Finean (2), that the myelin lamellae are arranged in a spiral. The basic difference between the structure of the two types of sheath resides in the mode of termination of the spiral on the outside of the sheath. Since an external cytoplasmic process does not surround the myelin sheath in central fibres, no external mesaxon is present, in the sense used in peripheral myelin structure.

This interpretation is contrary to those of either Luse (8) or De Robertis, Gerschenfeld, and Wald (9). Luse does not mention the existence of internal mesaxons in the central nervous system and suggests that myelin is formed by the plication of the membranes of a number of glial cell processes on the surface of the axon, so that at any one point on the fibre "many glial cell processes and their plasma membranes may be involved rather than a single cell and its processes." Further, Luse suggests that the sheath about a central axon need not have the same number of lamellae in all regions. De Robertis, Gerschenfeld, and Wald (9) agree with Luse that the oligodendrocytes play the major part in the formation of central myelin, but put forward the hypothesis that the "process of myelination consists in the laying down, within the cytoplasm of the oligodendrocyte and around the axon, of concentric membranous myelin layers." The existence of mesaxons is not refuted by these workers for they state that although in some cases membranes which could be described as mesaxons are present, relative to their frequency of occurrence in the peripheral nervous system, they are scarce.

The above authors relied on osmium-fixed material for their evidence, and it is well known that the resulting picture of the relations of membranes is not so well defined as after fixation with potassium permanganate. From the present observations there is no doubt of the existence of internal mesaxons in the central nervous system and they have been found to occur as frequently as in the peripheral system of *Xenopus* (15). The continuity of the internal mesaxons of central fibres with the internal myelin lamellae of the sheath has also been demonstrated, a situation which would not be expected if either of the above theories applied to *Xenopus*. Furthermore, there is no evidence of discontinuity in the myelin lamellae, as would be expected from the work of Luse (8) and De Robertis, Gerschenfeld, and Wald (9). The pictures of De Robertis, Gerschenfeld, and Wald indicate that their discontinuities in the myelin lamellae on the outside of the sheath are in fact points of approximation between the sheath and surrounding cell processes and between such cell processes themselves. As to the existence of a variable number of lamellae in different regions of the sheath, this has never been found in the present study; the number in any part of a given sheath is constant, except in the region between the internal mesaxon and the external tongue, where one extra lamellae is present. It is certainly true, however, particularly in the early stages of myelin formation, that cytoplasm is often present between the lamellae in some parts of the sheath, and in osmium-fixed material, such a separation of the lamellae will give the appearance of a varying number in different regions. Separations also occur in more mature sheaths (Fig. 2), but they are less frequent than in the early stages.

So far neither a tongue containing a nucleus,

nor a connection between a tongue and a glial cell have been observed. Whether one glial cell is related to a length of one axon, as is the Schwann cell to an internode in the peripheral nervous system, or whether, as De Robertis, Gerschenfeld, and Wald (9) suggest, each myelin-forming glial cell forms myelin around a number of axons, is not known. However, should the latter prove to be true, so that a number of processes from each glial cell form myelin around different axons, the geometrical relations will be such that the myelin is unlikely to be formed by a spiralling of the whole process of the glial cell around the axon, though it is conceivable that the free edge of the process could do so.

I wish to thank Professor G. J. Romanes for his interest during the course of this work, Mr. G. Wilson for his skillful 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.

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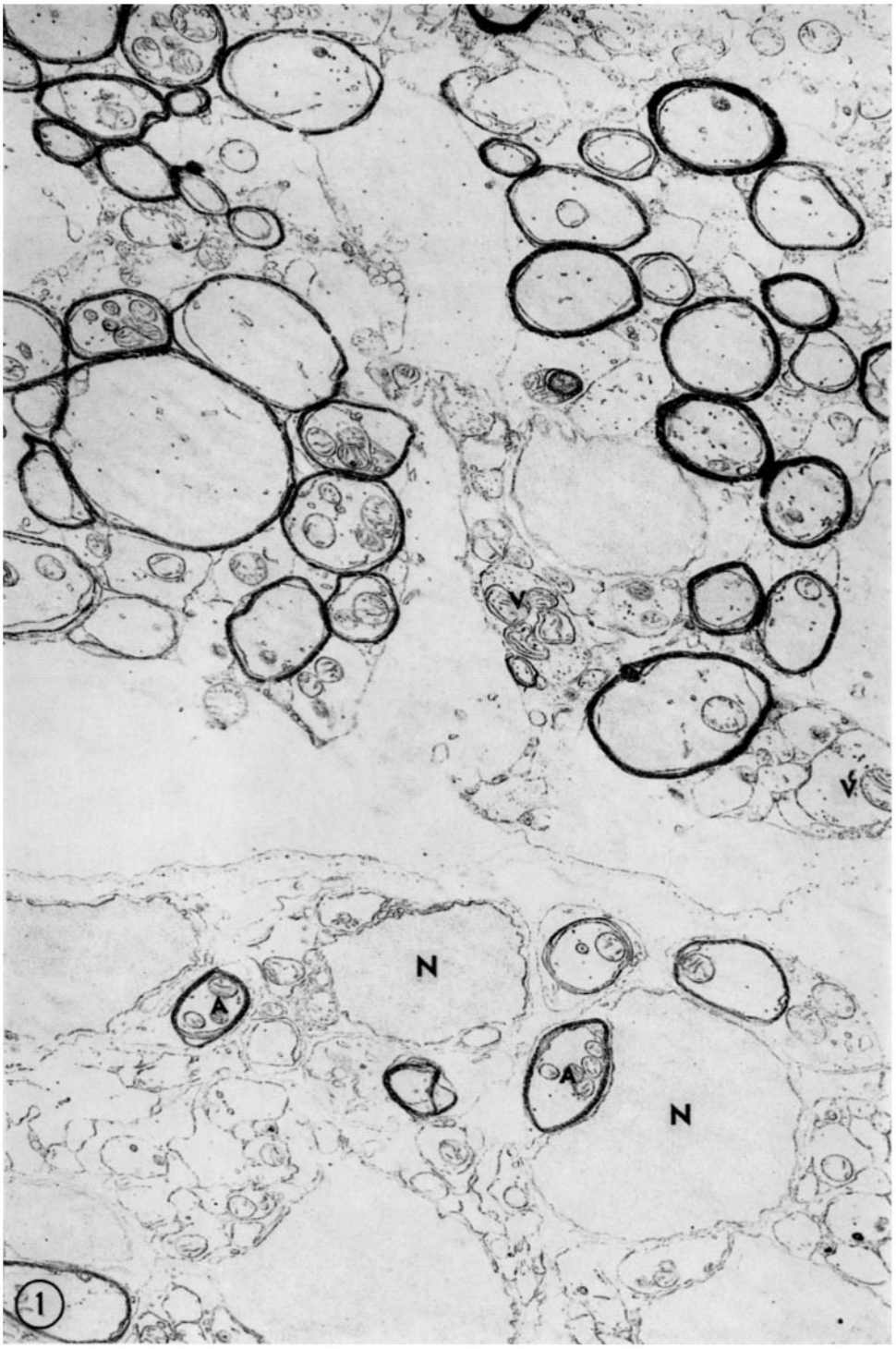
## Explanation of Plates

### Plate 43

Fig. 1. Micrograph of part of the spinal cord, top of picture, and peripheral nerve root, bottom of picture, from a stage 56 *Xenopus* tadpole. The sheaths of peripheral axons (*A*) are well separated and are related to Schwann cell nuclei (*N*). This arrangement is in contrast to that of the myelinated axons of the spinal cord, which are frequently in contact with each other and are not associated with nuclei equivalent to those of the Schwann cells. Some of the structures in the spinal cord contain vesicles (*V*). × 7,000.

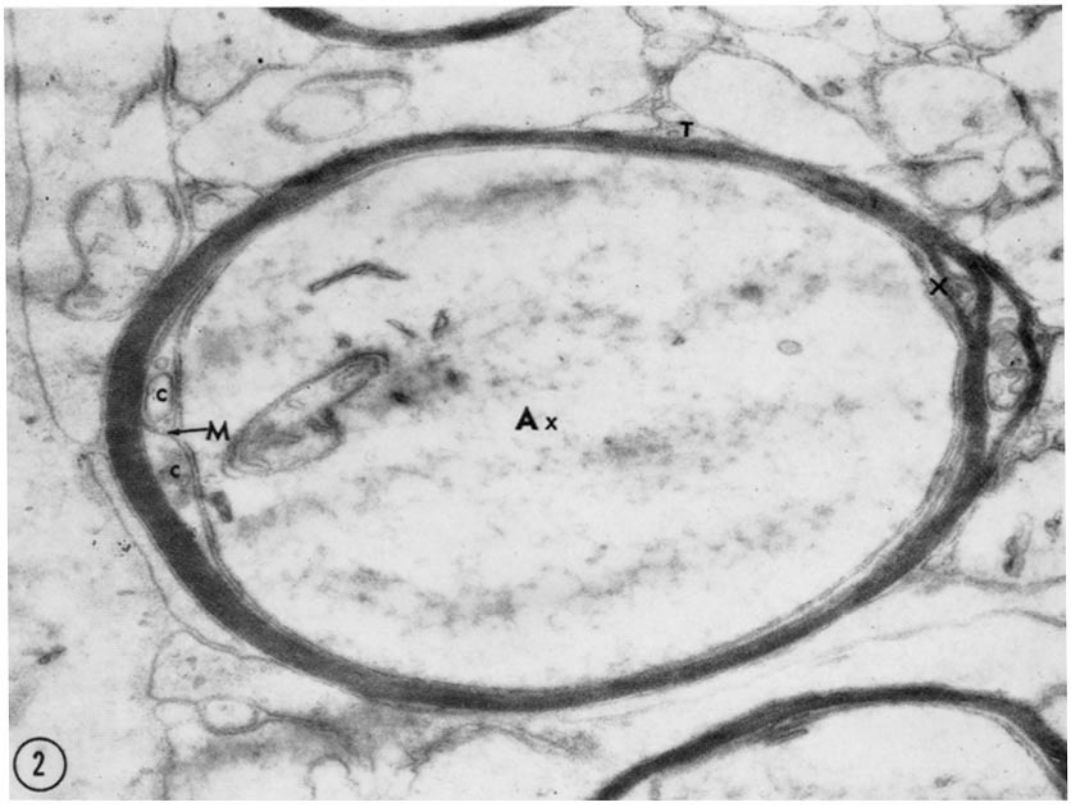


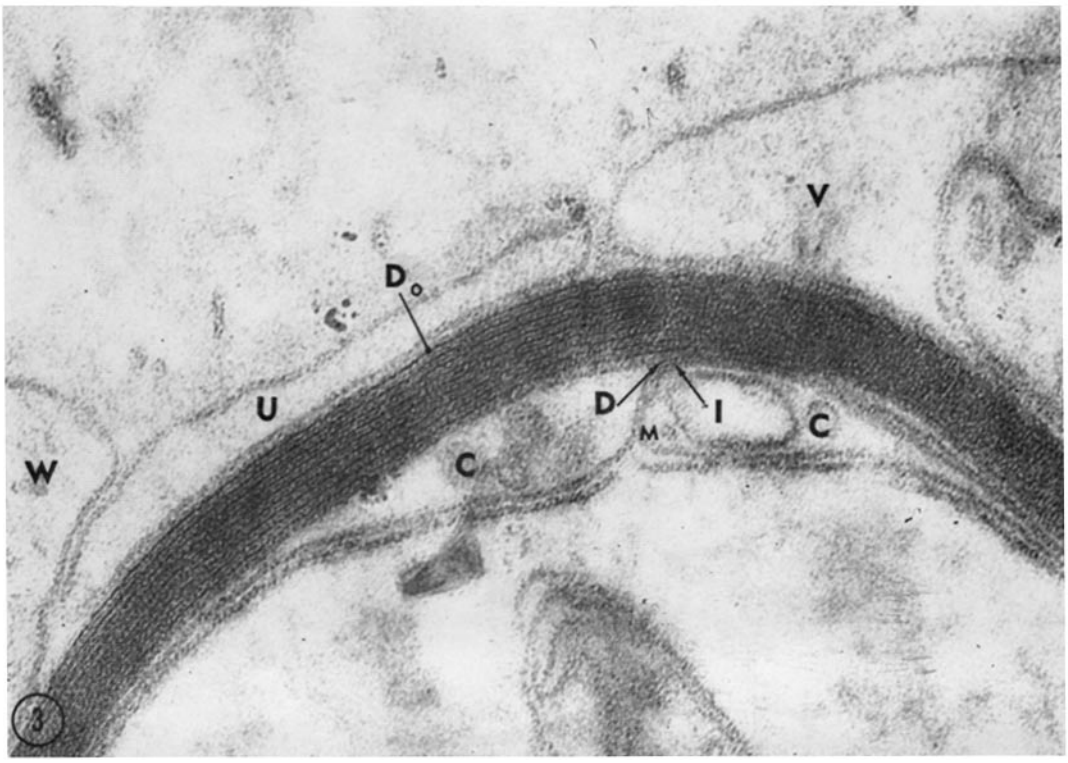

(Peters: Myelin sheaths in central nervous system)

PLATE 44

FIG. 2. Micrograph of a myelinated fibre from the optic nerve of a stage 57 tadpole. The axon (*Ax*) is surrounded by a cytoplasmic process (*C*) whose membranes come together to form the internal mesaxon (*M*). The probable position of the external tongue is indicated (*T*). Note the presence of cytoplasm between some myelin lamellae. × 38,000.

FIG. 3. Enlargement of part of Fig. 2 showing the origin of the internal mesaxon (*M*). The intraperiod line (*I*) is formed at the point where the mesaxon comes into contact with the inside of the myelin sheath. In the same region the dense line (*D*) arises by the apposition of the cytoplasmic surfaces of the membrane surrounding the inner cytoplasmic process (*C*). Three processes (*U*, *V*, *W*) approach very close to the outside of the myelin sheath, the outer line of which is a dense line, ($D_0$). × 103,000.

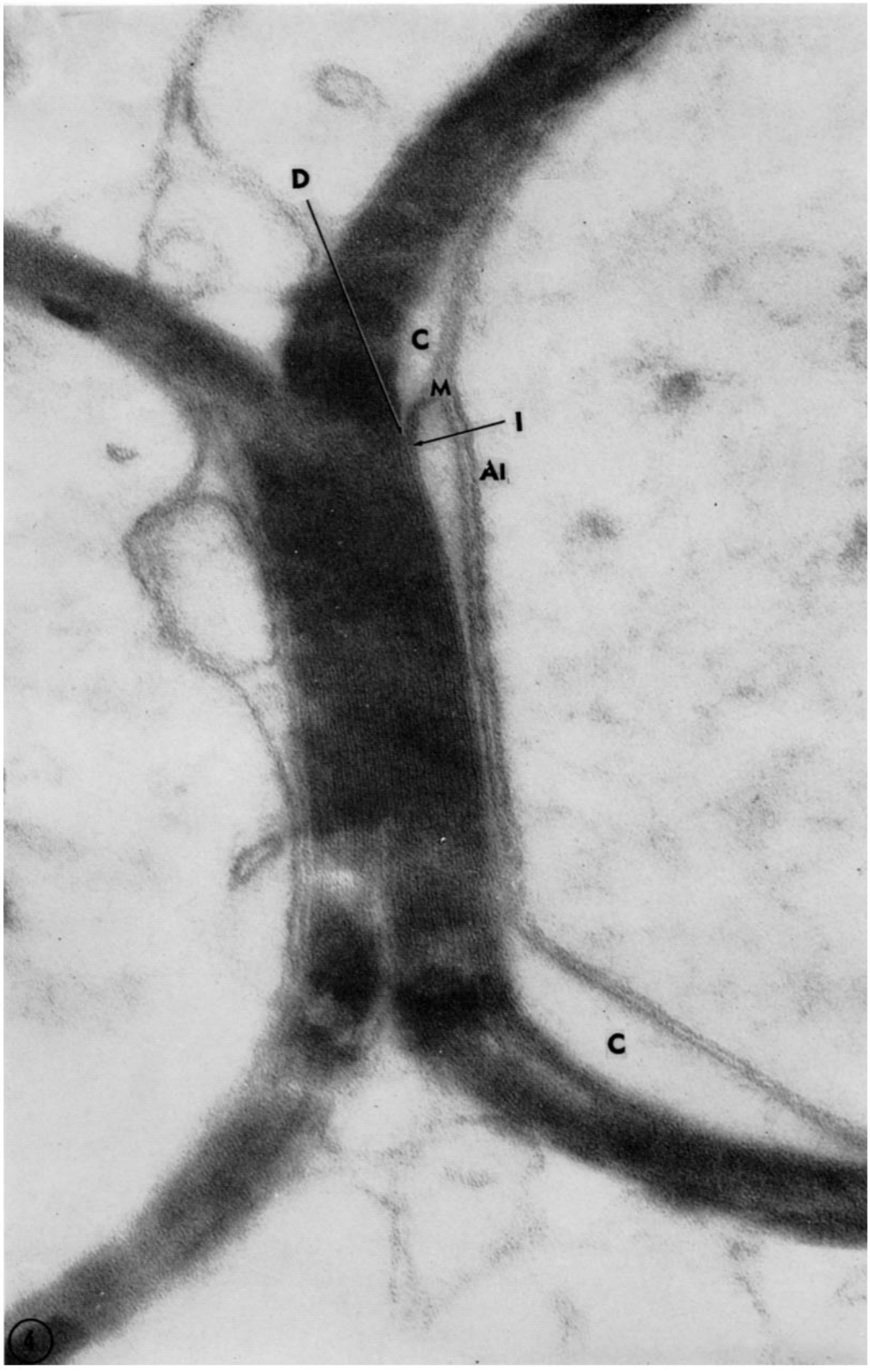



(Peters: Myelin sheaths in central nervous system)

PLATE 45

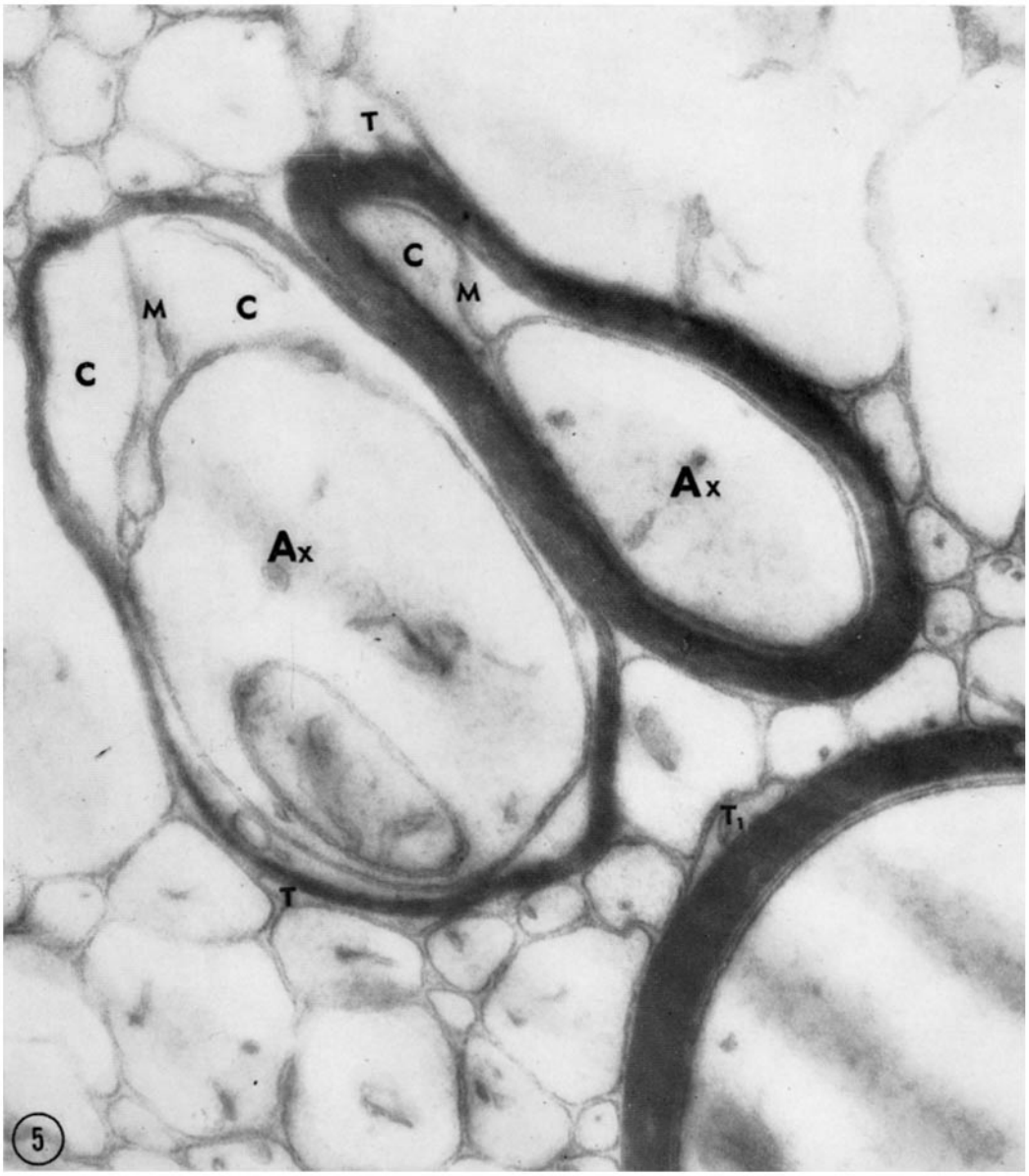
FIG. 4. Micrograph of the point of contact between two myelin sheaths from the optic nerve of a stage 57 tadpole. No space separates the sheaths, so that a regular pattern of alternating dense and intraperiod lines extends from the inner surface of one sheath to the next. An internal mesaxon (*M*) is present in the right hand fibre. The axolemma (*Al*) is separated from the membrane bounding the inner cytoplasmic process (*C*), the external surfaces of which come into contact to form the mesaxon. The intraperiod line (*I*) arises on the inner side of the sheath within the mesaxon and the dense line (*D*) is formed by contact between the cytoplasmic surfaces of the membrane bounding the inner cytoplasmic process. × 110,000.

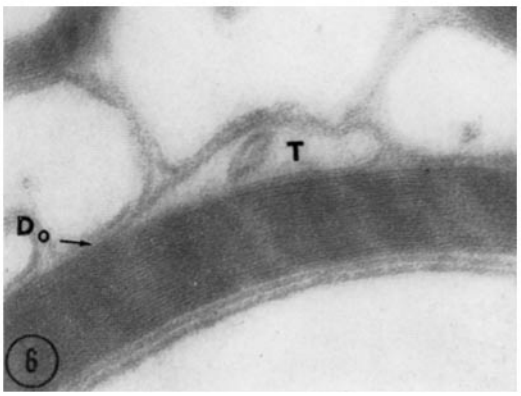


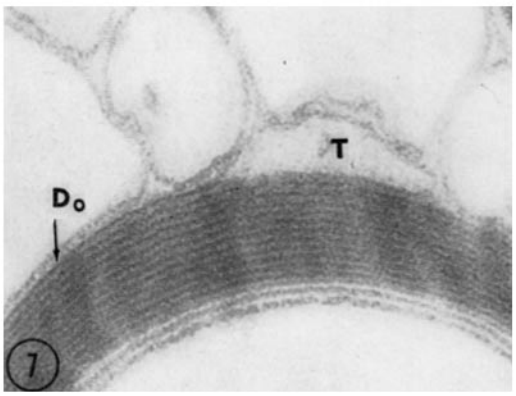

(Peters: Myelin sheaths in central nervous system)

PLATE 46

FIGS. 5 to 7. Micrograph of myelinated fibres from the optic nerve of a stage 58 *Xenopus* tadpole. Two myelinated fibres are shown completely, one of which (the right) has 10 lamellae and the other 4 lamellae. In each, the axon (*Ax*) is surrounded by a cytoplasmic process (*C*), the membranes of which come together to form the internal mesaxon (*M*). The outer surfaces of the sheaths are in contact. External tongues are present (*T*). In the lower right hand corner of the micrograph, part of a myelin sheath has an external tongue ($T_1$). The structure of this tongue is shown in the enlargement, Fig. 6. Fig. 7 is taken from an adjacent section and since it is underfocused only the dense lines are visible. The cytoplasmic surfaces of the tongue (*T*) come together to form the outer dense line ($D_0$). Fig. 5, × 50,000; Figs. 6 and 7, × 85,000.



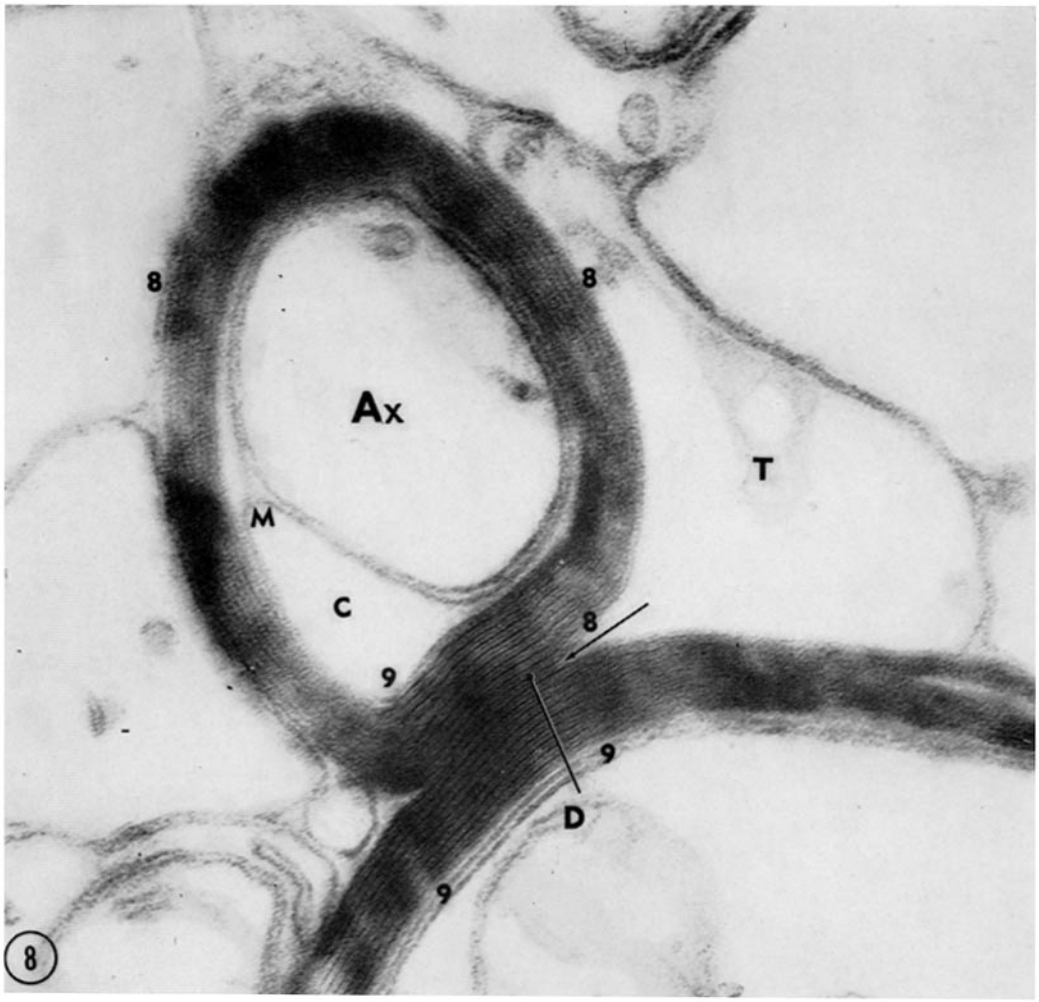

(Peters: Myelin sheaths in central nervous system)

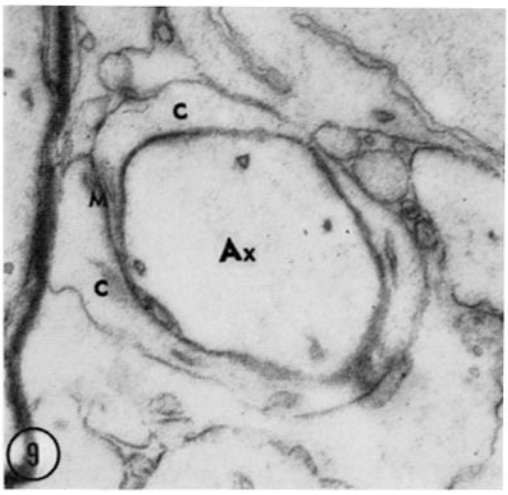
PLATE 47

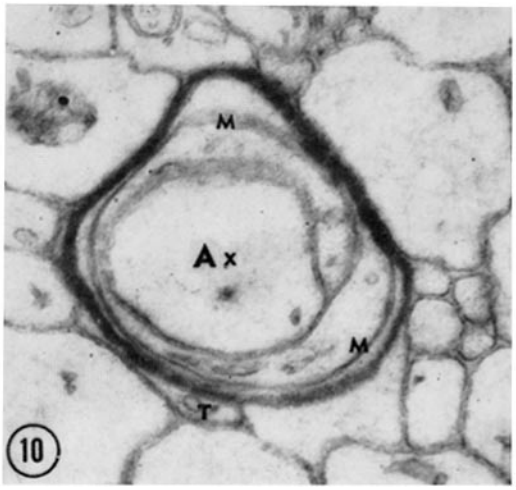
FIG. 8. Micrograph of a myelinated fibre from the optic nerve of a stage 58 *Xenopus* tadpole. The inner cytoplasmic process (*C*) circumscribes the axon (*Ax*) and forms the internal mesaxon (*M*). The external tongue (*T*) is associated with the upper fibre, but the outer surface of its bounding membrane is also applied to the myelin sheath in the lower part of the micrograph. In the region of the tongue the upper sheath has 8 dense lines and the lower one, 9. Where the sheaths meet at the right hand side, the cytoplasmic surfaces of the tongue membrane come together to form a dense line (*D*), which continues on the outside of the upper sheath so that when the sheaths separate on the left side both have 9 dense lines. An intraperiod line is formed where the two sheaths come into contact (arrow). Counts of the number of dense lines in different parts of the myelin sheath of the small fibre (see numbers) are consistent with the lamellae being arranged in a spiral. × 84,000.

FIG. 9. Micrograph of an early stage in myelinogenesis present in the spinal cord of a stage 56 tadpole. The axon (*Ax*) is embedded in a cytoplasmic process (*C*). A short mesaxon (*M*) occurs where the lips of the process (*C*) meet. × 42,000.

FIG. 10. Micrograph of an early stage in myelination from the optic nerve of a stage 58 tadpole. Two or three lamellae are present and a loosely spiralled internal mesaxon (*M*) leads from the axon (*Ax*) to the inside of the formed myelin. On the outside of the myelin is a tongue (*T*). × 35,000.




(Peters: Myelin sheaths in central nervous system)