FURTHER OBSERVATIONS ON THE STRUCTURE OF MYELIN SHEATHS IN THE CENTRAL NERVOUS SYSTEM

A. PETERS, Ph.D.

From the Department of Anatomy, University of Edinburgh, Scotland

ABSTRACT

Direct evidence has been presented to confirm the existence of a spiral in the myelin sheaths of the central nervous system. An account of some of the variations in structure of central myelin sheaths has been given and it has been shown that the radial component of myelin sheaths has the form of a series of rod-like thickenings of the intraperiod line. These thickenings extend along the intraperiod line in a direction parallel to the length of the axon. The relative position of the internal mesaxon and external tongue of cytoplasm has been determined in a number of transverse sections of sheaths from the optic nerves of adult mice, adult rats, and young rats. In about 75 per cent of the mature sheaths examined, these two structures were found within the same quadrant of the sheath, so that the cytoplasm of the external tongue process tends to lie directly outside that associated with the internal mesaxon. The frequency with which the internal mesaxon and external tongue lie within the same quadrant of the sheath increases both with the age of the animal and with the number of lamellae present within a sheath. The possible significance of these findings is discussed.

In 1957, Fernández-Morán and Finean (6) suggested that the myelin sheaths of the central nervous system have a spiral structure, similar to that proposed by Geren (7) and demonstrated by Robertson (21) for the myelin sheaths of peripheral nerves. A spiral structure for central sheaths was also proposed by Maturana (12) and Peters (15), who studied the optic nerves of anurans. They based their conclusions upon counts of lamellae in different regions of individual sheaths and upon the disposition of the membranes leaving and entering the sheath. Peters and Maturana showed that the essential difference between peripheral and central myelin sheaths lies in the amount of cytoplasm on the outside of the sheath. In peripheral nerves, each myelin sheath is completely surrounded by an outer layer of Schwann cell cytoplasm, but in the central nervous system this cytoplasm is confined to a small tongue process (15) or outer loop (12).

Metuzals (13, 14) was also led to the conclusion that the lamellae of central myelin sheaths form a spiral. He studied the sheaths in the diencephalon of the frog, and pointed out that the existence of a spiral structure provides an adequate explanation for the appearances obtained in sections of the nodes present in the central nervous system.

Other evidence in favour of a spiral structure in central myelin was obtained from a study of myelinogenesis in the optic nerves of rats and toads (16, 18). It was found that, in the early phases of myelin formation, an axon became enclosed by a glial cell process in such a way that the lips of the enveloping glial cell process come together to form a mesaxon. Such a mesaxon appeared to elongate in a spiral manner around

the enclosed axon, and loosely spiralled mesaxons of a few turns were demonstrated. As more turns were added, the cytoplasm became lost from between them, so that in mature sheaths cytoplasm was generally present only on the inside and outside of a sheath. Similar conclusions about the formation of central myelin were also reached by Bunge, Bunge, and Ris (1), who studied the remyelination taking place after the formation of a lesion in the spinal cords of cats.

On the basis of the similarity with peripheral

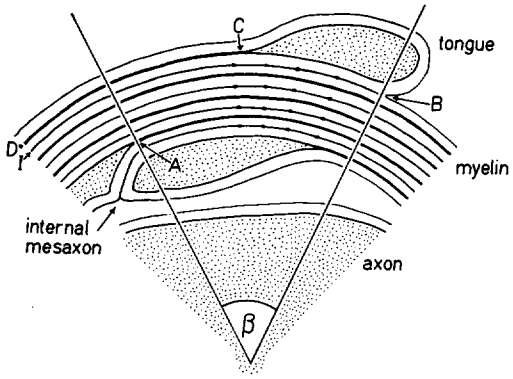


FIGURE 1 Diagram of part of a central myelin sheath to show the angle, β, subtended at the centre of the sheath, or axon, between the fixed points, *A* and *B*, related to the internal mesaxon and outer tongue of cytoplasm, respectively. The extent of the tongue process was measured between points *B* and *C*.
The cytoplasm is stippled. *D*, major dense line, and *I*, intraperiod line of the sheath.

myelin, Peters (16) and Bunge, Bunge, and Ris (1) postulated that central myelin is formed in internodal lengths. It was proposed that the body of a myelin-forming glial cell is situated some distance away from the site of myelin formation, but connected to the external loop, or tongue, of cytoplasm of the sheath, by a process. Such connections were later demonstrated by Bunge, Bunge, and Pappas (2).

This concept of the existence of a spiral wrapping is contrary to two other hypotheses. Thus, Luse (10, 11), on the basis of a study of myelin sheaths in the spinal cord, medulla oblongata, and cerebral cortex of rats and mice, denied the existence of a spiral structure by indicating that the myelin may be thicker on one surface of an axon than on another. According to Luse, central sheaths have a multiple origin, being derived from the overlapping processes of many glial cells. In the later stages of myelinogenesis, these flattened processes are considered to fuse together. On the basis of Luse's theory there is no single mesaxon, as would be expected from the spiral-wrapping concept, "but rather multiple points at which glial membranes are continuous with the lamellae of the myelinated sheath" (10).

De Robertis, Gerschenfeld, and Wald (4, 5) also deny the existence of a spiral structure. From a study of myelination in the brain and spinal cord of kittens and rats, they concluded that myelin is formed in the central nervous system from membranous material laid down within the cytoplasm of oligodendrocytes. This material, which they described as having the form of vesicles and tubules, is considered to fuse together and become deposited around the axon. Contrary to the view put forward by Luse (10), these workers found a few membranes that could be described as mesaxons, but they considered their incidence to be infrequent.

From the above account, it is apparent that it should be possible to decide whether the spiral-wrapping concept is tenable by the direct demonstration of the presence or absence of a continuous spiral structure in the mature myelin sheaths of the central nervous system. This appears to be the only means of deciding the point, since the present indirect evidence based upon the appearance of mature sheaths, as well as the evidence from studies of myelin formation, is open to dispute (see 25). The structure of central myelin sheaths has, therefore, been re-examined with the aim of settling this question of the existence of a spiral. At the same time, some of the structural variations that occur in central myelin sheaths have been considered, along with new observations on the radial component present in these sheaths (17).

MATERIALS AND METHODS

Preparation of Tissue

The optic nerves of 7-, 14-, and 20-day postnatal rats and of adult rats, mice, and toads (*Xenopus leavis*) were used in this study. The optic nerves were removed and fixed for 1 to 2 hours, at 4°C, in the potassium dichromate–osmium tetroxide mixture of Dalton (3), after which they were washed in 10 per cent alcohol and dehydrated in ethanol. The optic nerves were embedded in Araldite (8), and transverse sections cut on a Servall, Porter-Blum microtome. The sections were mounted on grids and stained with either potassium permanganate

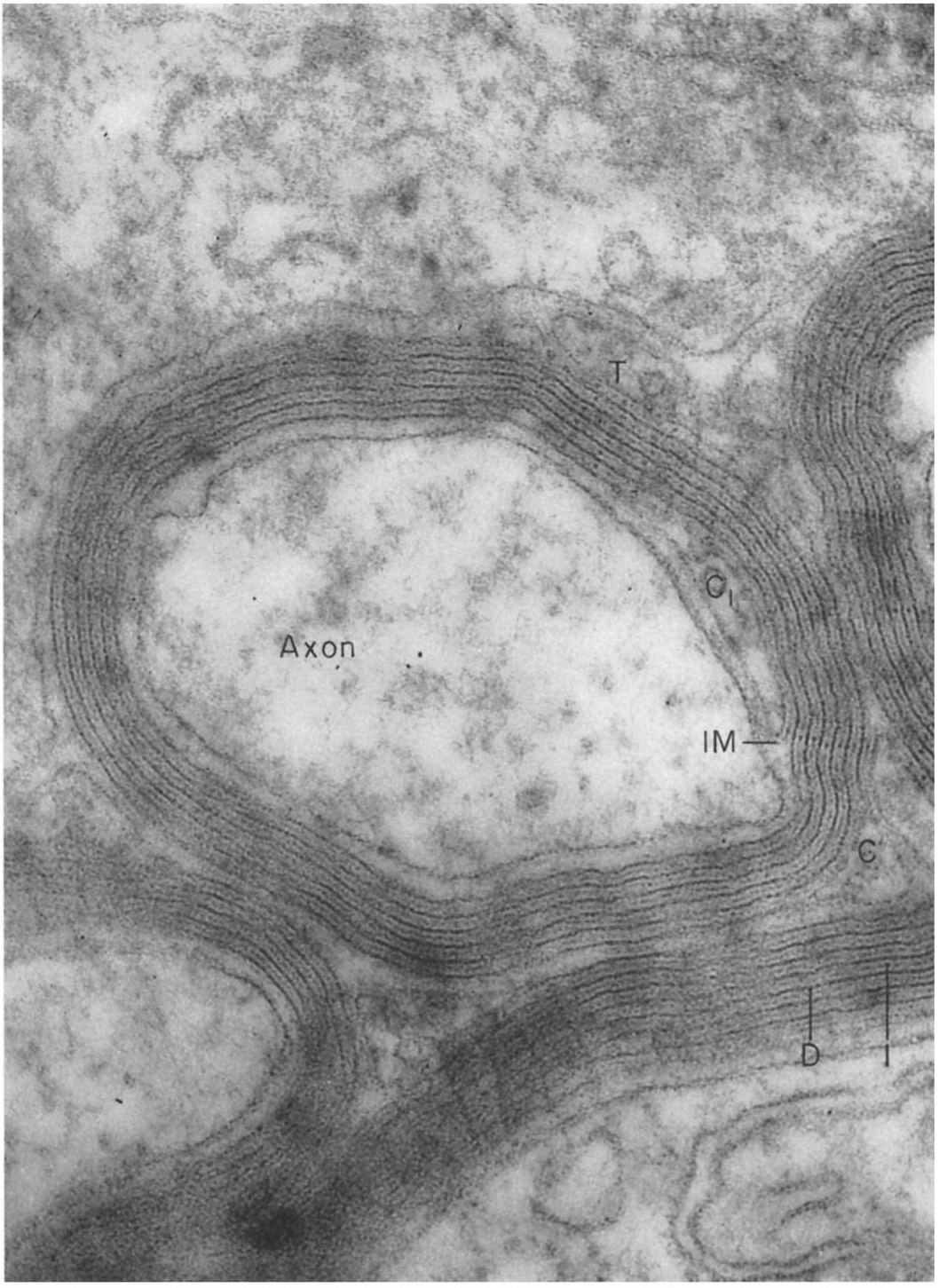


FIGURE 2 Transverse section of a myelin sheath from the optic nerve of an adult mouse. The spiral of the lamellae starts on the inside of the sheath at the internal mesaxon (*IM*), where cytoplasm (C_1) is present, and continues in an anti-clockwise direction to terminate on the outside of the sheath at the tongue of cytoplasm (*T*). The outer turn of the sheath also encloses cytoplasm at *C*. Within a sheath, the major dense line, *D*, alternates with the intraperiod line, *I*. Section stained with potassium permanganate. × 200,000.

(9), lead acetate (27), or uranyl acetate (28) before examination in a Metropolitan Vickers, EM 6.

Examination of Myelin Sheaths

The relative positions of the internal mesaxons and external tongue processes of a number of myelin sheaths were determined from photographs of transverse sections of the optic nerves. The site at which the internal mesaxon meets the inside of the myelin sheath (*A* in Fig. 1) and the site at which the cell membrane bounding the tongue of cytoplasm turns off the outside of the sheath (*B* in Fig. 1), at the outer end of the intraperiod line (*I*, Fig. 1), were taken as fixed points. The angle (β, Fig. 1) that these fixed points subtend at the centre of the axon, or the geometrical centre of the sheath, was then measured (Fig. 1). When a sheath was almost circular in cross-section, the angle was measured with a protractor, but when the cross-section of a sheath was less regular, a map measurer was used. In the latter case, the distance between the fixed points was determined as a fraction of the total circumference of the sheath, and this fraction then converted to an angle on the assumption that the total circumference of the sheath was equivalent to 360°. In all cases, the fixed point related to the internal mesaxon was taken to be 0° and the angle between this point and the fixed point related to the external tongue of cytoplasm was measured in the direction of the spiral followed by the lamellae, in other words, in the direction towards which the free end of the tongue process points (Fig. 1). In no case were measurements taken at the nodal region of a sheath, where the lamellae begin to decrease by turning off the sheath.

Although the fixed points from which the measurements were taken are arbitrary, it will be appreciated that they are equivalent to the two ends of the spiral giving rise to the compact lamellae. The results, therefore, give an indication of the relative positions of the ends of the spiral and, indirectly, the amount of overlap between the cytoplasm associated with the internal mesaxon and the cytoplasm of the external tongue process. Thus, a value of 0° implies that the inner and outer ends of the spiral of the lamellae are exactly opposite each other and on the same radius, while a value of 50° for the angle β, as in Fig. 1, implies that there is some overlap between these structures.

The extent of each outer tongue of cytoplasm was also determined. This was measured in the manner given above. As before, one of the fixed points was taken to be the site at which the cell membrane bounding the tongue process turns off the outside of the sheath (*B* in Fig. 1), and the other fixed point was the site at which the membrane bounding the tongue process becomes apposed to itself (*C* in Fig. 1) to form the major dense line of the sheath (*D*, Fig. 1).

The other factor determined for each sheath was the number of complete lamellae present.

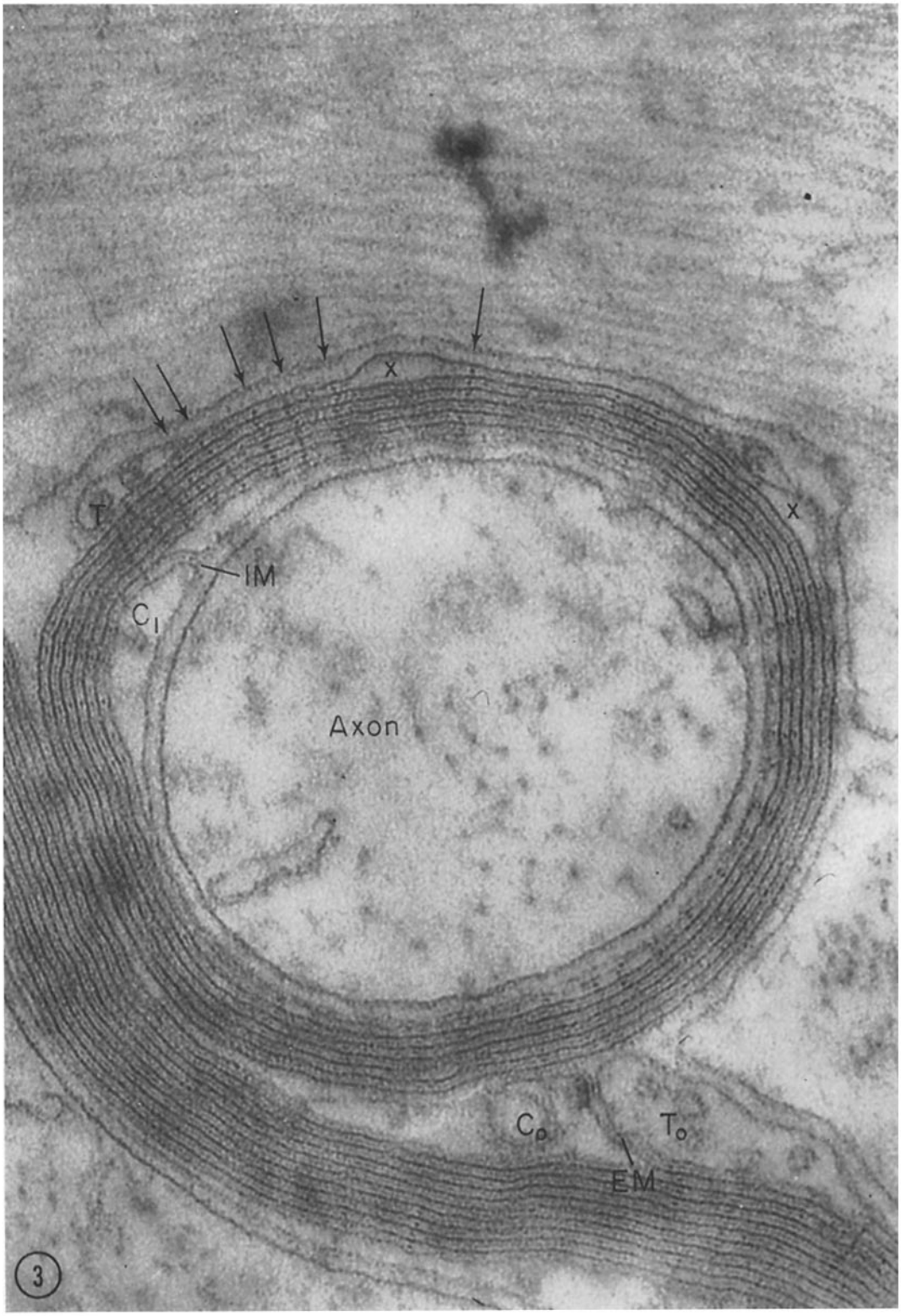
OBSERVATIONS

Disposition of Myelin Lamellae

In a few cases, it has been possible to obtain transverse sections from the optic nerves of adult mice in which the spiral arrangement of the lamellae is apparent. Two examples are shown in Figs. 2 and 3, where the spiral can be readily traced by following the major dense line of the sheath. The major dense line (*D*, Fig. 2) is more prominent than the intraperiod line (*I*, Fig. 2), with which it alternates, and is formed by apposition of the cytoplasmic surfaces of the cell membrane forming the sheath (see 19). The major dense line begins on the inside of the sheath where the membrane bounding the inner process of cytoplasm (C_1 in Figs. 2 and 3) comes together to form the lamellae and terminates on the outside of the sheath where the membranes separate to enclose the tongue of cytoplasm (*T*, Figs. 2, 3, and 6).

In Fig. 2, the spiral of the lamellae is in an anticlockwise direction and the major dense line completes 6 turns, so that the sheath is composed of

FIGURE 3 Transverse section of a myelin sheath from the optic nerve of an adult mouse. The spiral of the lamellae is anti-clockwise. It commences on the inside of the sheath at the internal mesaxon (*IM*), which has cytoplasm on both sides (C_1), and terminates on the outside of the sheath at the tongue process (*T*). In some parts of the sheath there are radial thickenings of the intraperiod line (arrows), and in two regions (*X*) the surfaces of the membranes forming the intraperiod line have separated from each other. In the lower half of the figure, part of a second myelin sheath is visible. On the outside of this sheath a definite external mesaxon (*EM*) is formed between the membranes bounding the tongue process (T_0) and the cytoplasm (C_0) within the underlying turn of the sheath. Section stained with potassium permanganate. × 180,000.


x
T
x
IM
C_I
Axon
C_o
T_o
EM
3

6 complete lamellae. In Fig. 3, the spiral is also anti-clockwise, and the major dense line completes almost 7 turns. Thus, 7 lamellae are visible within the sheath, although the outer lamella is not complete, because the site at which the membrane separates to enclose the outer tongue of cytoplasm is not exactly opposite the beginning of the major dense line on the inside of the sheath.

In these two examples there is no doubt of the spiral disposition of the myelin lamellae, and complete spirals have also been observed in a number of other sheaths. However, for technical reasons it is not usually possible to demonstrate the existence of a spiral directly. For example, if a section through a sheath is not absolutely transverse, overlapping of lamellae occurs at various sites, with the result that in these regions the major dense and intraperiod lines cannot be followed. In other cases, sectioning results in either a compression or a smearing of the lamellae. Such smearing or compression of a section is found particularly on the sides of the sheaths where the lamellae have been oriented parallel to the edge of the knife during sectioning, in other words, on the sides of the sheaths that lie at right angles to the direction of cutting. This effect is apparent in Fig. 6, in which the direction of cutting is indicated, although it will be observed that not all of the lamellae oriented at right angles to the direction of cutting are affected.

In the many hundreds of sections of well preserved myelin sheaths that have been examined from the optic nerves and brains of rats, mice, and toads, no appearances have been observed that are inconsistent with the existence of a spiral structure. Thus, it has been found that:

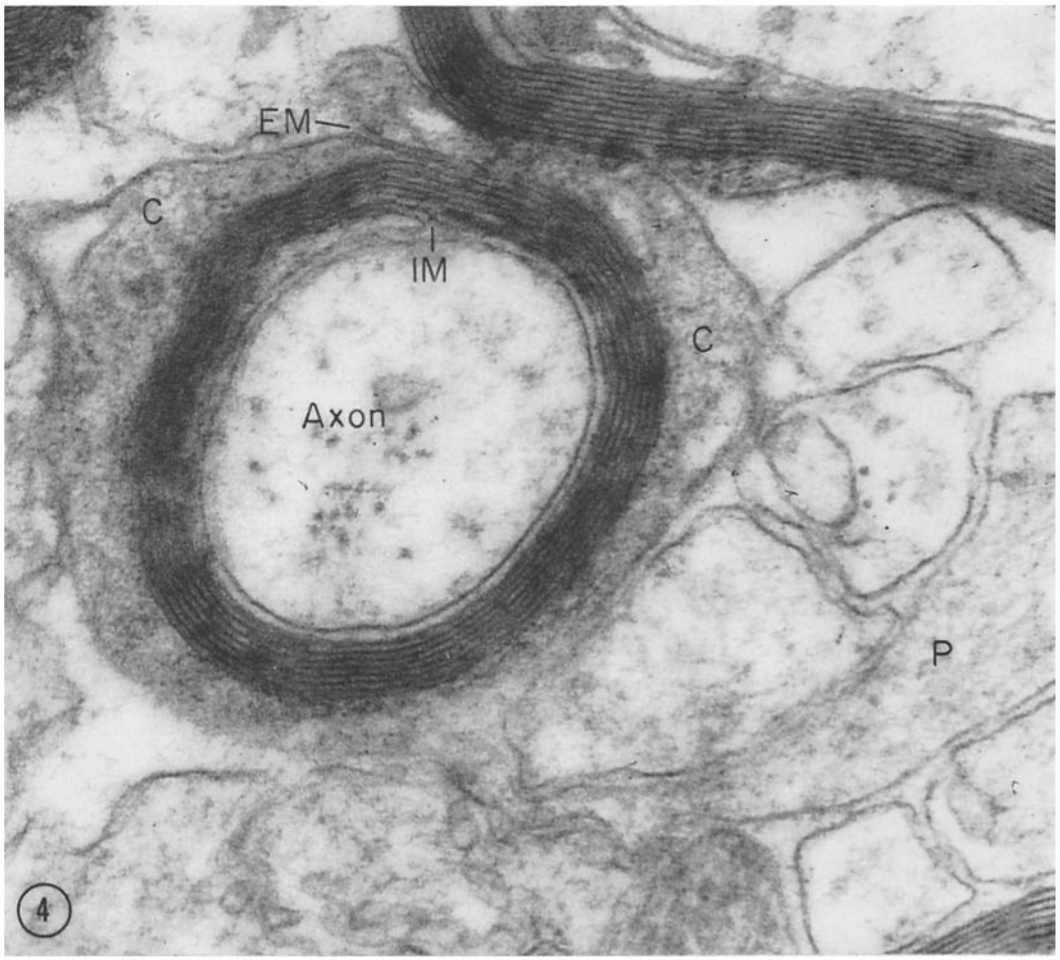
(*a*) An internal mesaxon (*IM*, Figs. 2, 3, 6, and 9) is always present. The extent of the internal mesaxon varies with the amount of cytoplasm on the inside of the sheath. Sometimes, as in Fig. 3, the internal mesaxon (*IM*) is a definite structure, but, in other cases, where the cytoplasm on the inside of the sheath is confined to a loop as in Fig. 2, the internal mesaxon is very short. Such internal loops have been described by Maturana (12) in the optic nerves of some Anurans, but Bunge, Bunge, and Ris (1) find that they are rarely seen in re-myelinating nerves from the spinal cords of cats. In the optic nerves of mice, internal loops of cytoplasm are quite common, as can be seen in Fig. 6, although they are rarely present in the optic nerves of rats.

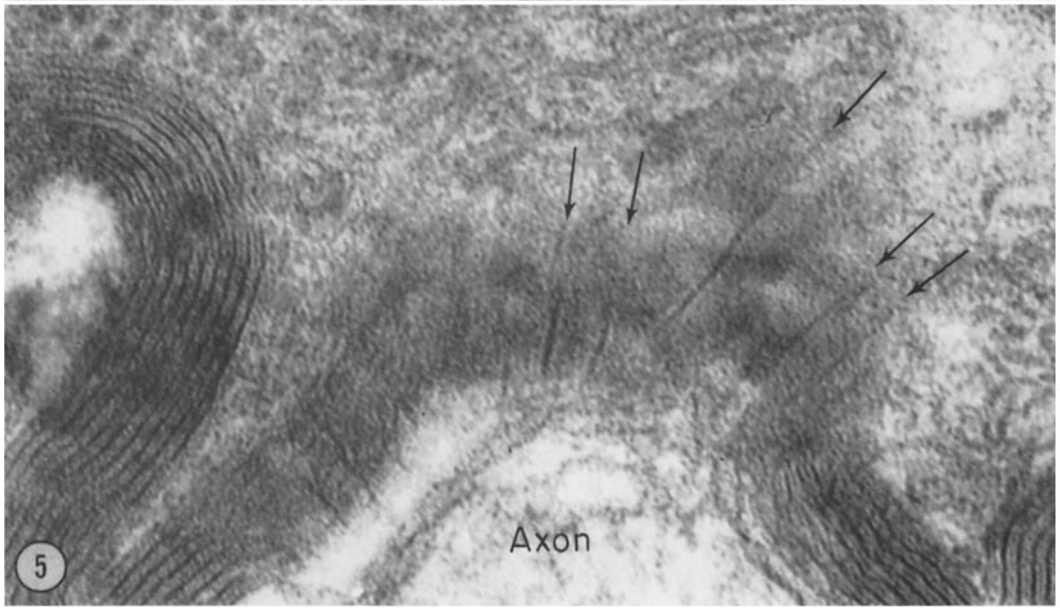
(*b*) An external tongue of cytoplasm is always present. In the majority of sheaths from the optic nerves of mature mice (Figs. 2, 3, and 6) and rats, the external tongue occupies about 5 per cent of the circumference of a sheath cut in transverse section, and rarely extends for more than 20 per cent of the circumference. The intraperiod line on the outside of the sheath terminates where the membrane bounding the tongue of cytoplasm turns off from the outside of the sheath. This point is, therefore, equivalent to the site at which the external mesaxon occurs in peripheral nerves. Indeed, true external mesaxons do sometimes occur in the central nervous system. Such an external mesaxon (*EM*) is present on the outside of the sheath that is partially visible at the lower edge of Fig. 3 and is formed between the cell membrane bounding the tongue process (T_0) and that bounding the cytoplasm (C_0) present in the underlying turn of the sheath.

In developing sheaths, the external tongue of cytoplasm is usually larger than in mature sheaths (compare Figs. 6 and 9). Thus, examples may be found, such as that illustrated in Fig. 4, where cytoplasm (*C*) completely surrounds the outside of the myelin. In such sheaths a definite external mesaxon (*EM*) is present, and the sheath has an appearance exactly similar to that normally found in peripheral nerves. However, a nucleus has never been observed in this external layer of

FIGURE 4 Section of a myelinated fibre from the optic nerve of a 14-day postnatal rat. A complete layer of cytoplasm (*C*) is present around this sheath, and at one point this extends away from the sheath as a process (*P*). Both internal (*IM*) and external (*EM*) mesaxons are present, so that the sheath has a structure similar to that of the sheath of peripheral nerves. Section stained with uranyl acetate. × 95,000.

FIGURE 5 Oblique section through part of a myelin sheath from the optic nerve of an adult mouse. In this oblique section the radial component of the sheath has the form of dark lines (arrows). Section stained with potassium permanganate. × 180,000.


EM
C
IM
C
Axon
P
4


Axon
5

cytoplasm in central sheaths, but, as in Fig. 4, a process (*P*) of cytoplasm may sometimes be seen to extend away from the sheath.

Although cytoplasm is frequently present between the turns of the spiral in developing sheaths, in mature sheaths it is rarely found except within the outer lamella (*C*, Fig. 2). No structures similar in appearance to the Schmidt-Lanterman clefts of peripheral nerves (23) have ever been observed in the central nervous system.

(*c*) At the internal mesaxon and outer tongue process, the membranes always turn onto the inside and off the outside of the sheath in a direction consistent with the existence of a spiral.

(*d*) Counts of lamellae in different parts of the same sheath are always consistent with the existence of a spiral. In developing sheaths, where cytoplasm may be present between the turns, it is less frequently possible to count the number of lamellae than in mature sheaths. Nevertheless, inner mesaxons and outer tongue processes are present and their disposition is always consistent with the existence of a spiral (see Fig. 9).

Relative Positions of Internal Mesaxons and External Tongue Processes

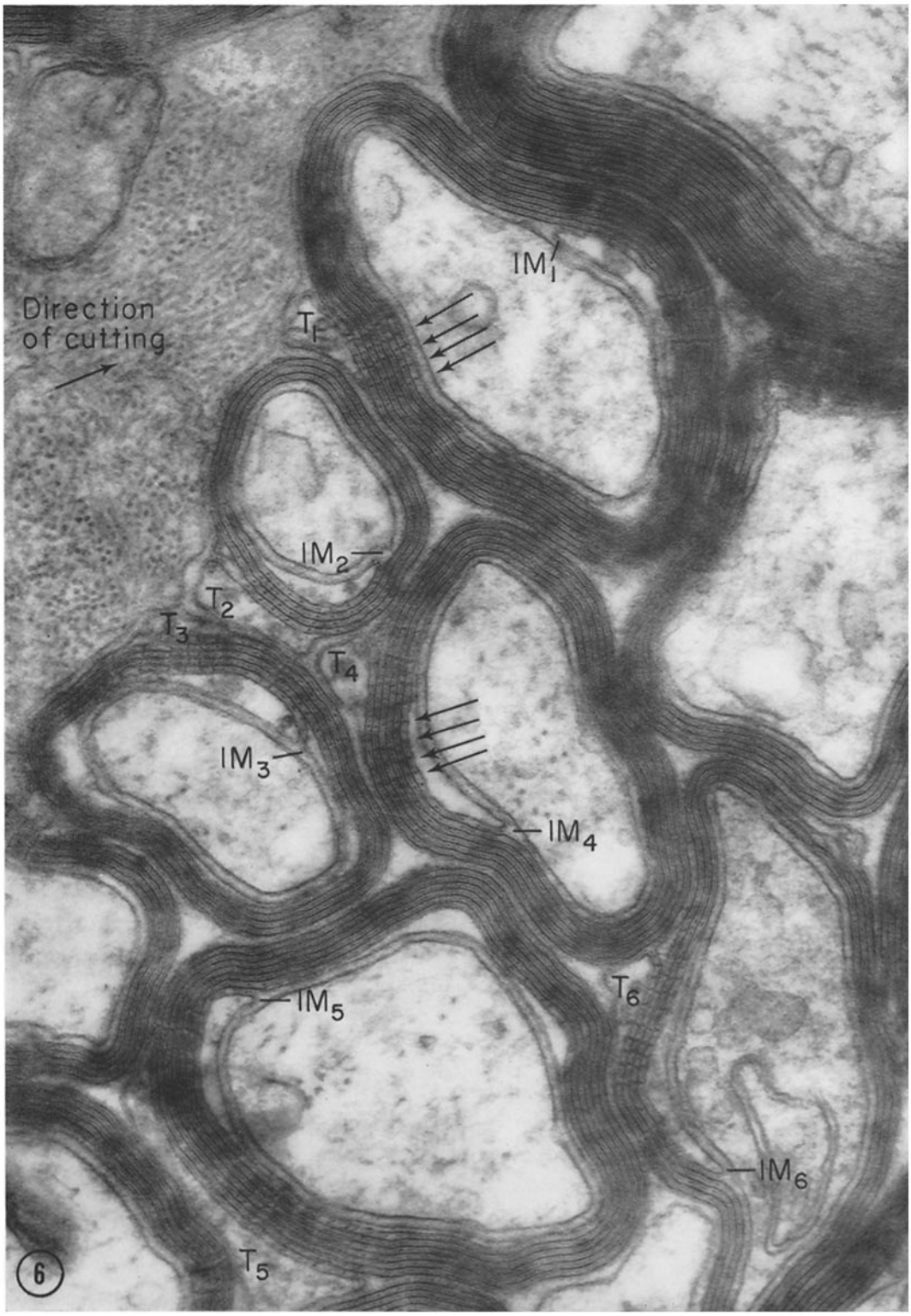
Whilst examining transverse sections of myelin sheaths from the central nervous system of adult animals, it became apparent that the internal mesaxon and external torgue of cytoplasm were frequently to be found within the same sector of a myelin sheath. Thus, if the internal mesaxon is located, it is common to find the external tongue of cytoplasm on the outside of the sheath immediately opposite to it (Figs. 2, 3, and 6). At first, it was assumed that this could be attributed to the effects frequently produced by sectioning. For example, if the section is compressed, there is the greatest chance that these two structures will be visible in any one sheath when they are located in the same region of that sheath. However, careful examination of sections in which a minimal amount of damage was apparent showed that this assumption was incorrect. Thus, in Fig. 6, although the internal mesaxons (IM_1 to IM_6) and external tongue processes (T_1 to T_6) of different sheaths are visible and randomly oriented with respect to one another, the internal mesaxons and external tongue processes of the same sheath are most frequently to be found within the same sector of that sheath.

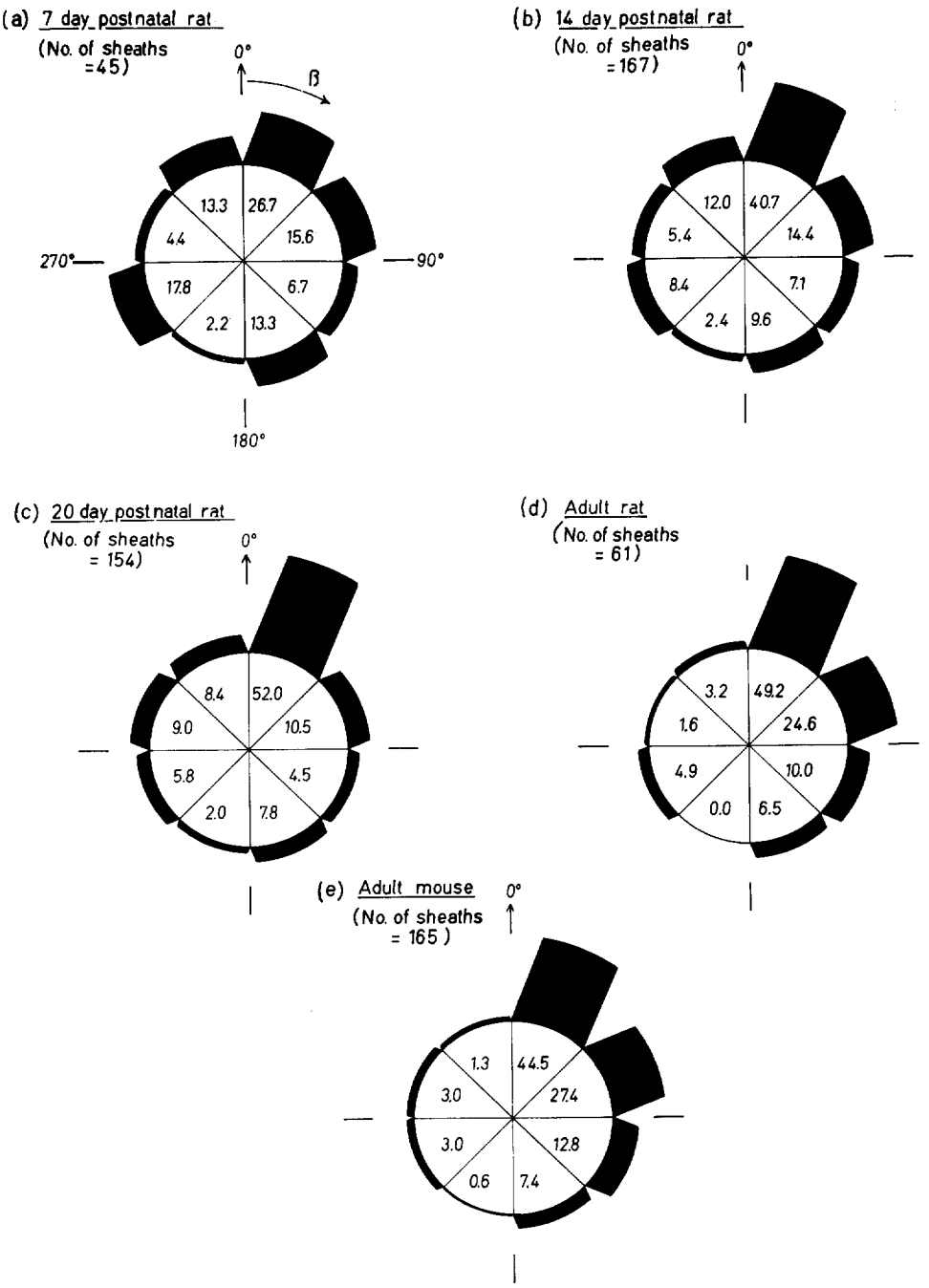
The relative positions of the internal mesaxons and external tongue processes of a number of sheaths from the optic nerves of rats and mice have, therefore, been analysed in the manner described in the previous section. The results are shown in Figs. 7 and 8. In constructing these figures, it has been assumed that each sheath is circular in cross-section. These figures give the frequency with which the angle (β in Fig. 1) subtended at the centre of the axon (or sheath) between fixed points related to the internal mesaxon (point *A* in Fig. 1) and external tongue process (point *B* in Fig. 1) falls into each octant of a circle. This frequency is given as a percentage in the octants in the middle of each figure, and, to facilitate interpretation of the figures, the frequencies are also indicated by the size of the black area at the periphery of each octant.

If the results for the optic nerves of the adult rat (Fig. 7 *d*) and adult mouse (Fig. 7 *e*) are considered first, it is apparent that, in 75 per cent of the sheaths examined, the internal mesaxon and external tongue process are situated within 90° of each other, that is, in the same quadrant of the sheath. Further, in about 90 per cent of the sheaths, these structures lie within the same hemicircle of the sheath, and it is only rarely that they are situated at diametrically opposite points on the sheath.

To determine whether this phenomenon occurs throughout development, the optic nerves of 7-day (Fig. 7 *a*), 14-day (Fig. 7 *b*), and 20-day (Fig. 7 *c*) postnatal rats have been examined. As will be seen from a comparison of the results, in the nerves of 20-day postnatal rats the disposition of

FIGURE 6 Transverse section of part of an optic nerve from an adult mouse. In six of the sheaths, the tongue of cytoplasm (T_1 to T_6) on the outside of the sheath and the internal mesaxon (IM_1 to IM_6) are indicated by corresponding numbers. The direction of cutting of the section is indicated on the left, and some of the radial components of the sheaths are shown by arrows. For further explanation, see text. Section stained with potassium permanganate. × 100,000.


Direction
of cutting
IM'1
T1
IM2
T2
T3
T4
IM3
IM4
IM5
T6
IM6
T5
6



FIGURES 7 AND 8 Diagrams to show the relative positions of internal mesaxons and external tongues of cytoplasm in transverse sections of sheaths from the optic nerves of rats and mice of different ages (Fig. 7), and in sheaths from 7-, 14-, and 20-day postnatal rats, on the basis of the number of complete lamellae within the sheath (Fig. 8).

The relative position of the external tongue process and internal mesaxon of each sheath was determined by measuring the angle β subtended at the centre of the sheath, between the fixed points A and B, respectively, in Fig. 1. In measuring the angle β, the position of the fixed point related to the

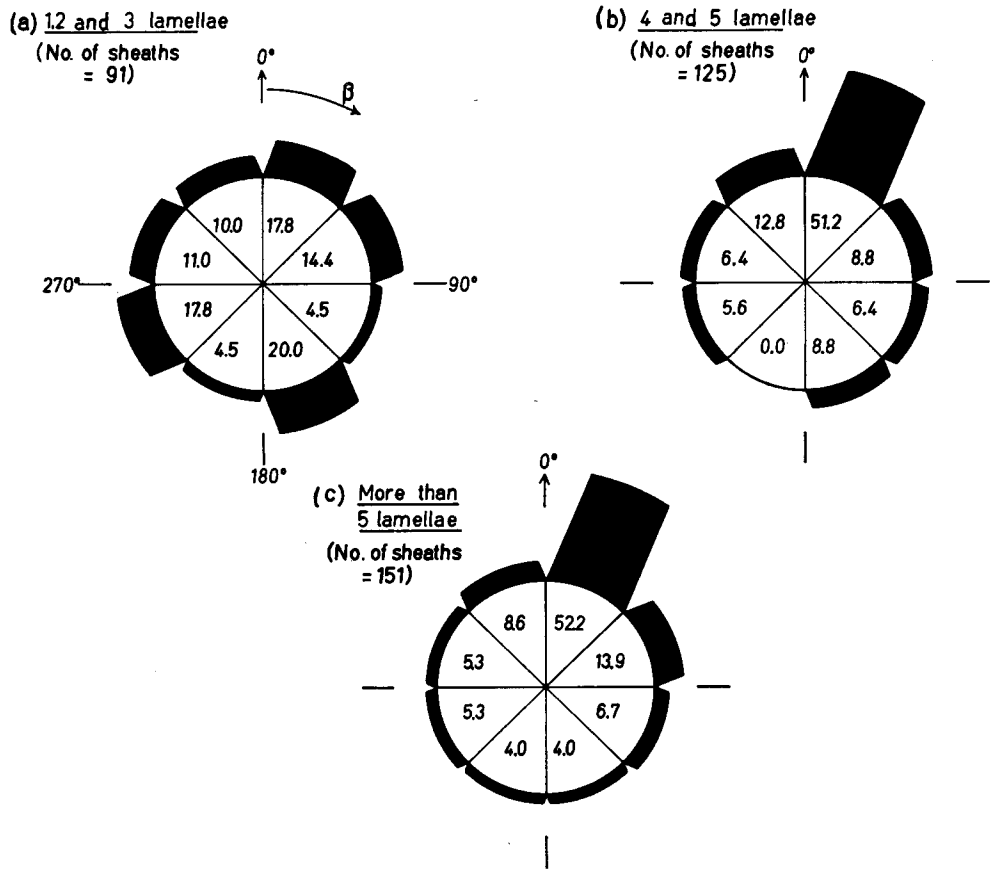


FIGURE 8

internal mesaxon was taken to be 0° and the angle between this point and that related to the external tongue process was measured in the direction taken by the spiral of the lamellae of the sheath. The frequency with which the angle β fell into each octant of a circle is given as a percentage in the middle of each diagram, and this frequency is also indicated by the size of the blackened area at the periphery of each octant. The number of sheaths measured during the construction of each diagram is given. The figures given within the sections of the sheaths are in per cent. For further details, see text.

the internal mesaxons and external tongue processes is somewhat similar to that found in adult rat sheaths. However, the tendency for these structures to lie within the same quadrant of the sheath becomes less in younger animals. Thus, while the angle (β) between the fixed points related tc the internal mesaxon and external tongue process is within 90° in 63 per cent of the sheaths examined in 20-day postnatal rats, the corresponding value for the 14-day postnatal rat is 55 per cent, and this is reduced to only 42 per cent in the 7-day postnatal rat.

These results suggested that some correlation may exist between the relative position of the internal mesaxon and external tongue process and the thickness of the sheath. To investigate this point, all the measurements obtained from the 7-, 14-, and 20-day postnatal rats were grouped together and analysed on the basis of the number of lamellae present within the sheaths. The results are shown in Fig. 8, which shows the situation in sheaths with 1, 2, and 3 lamellae (Fig. 8 *a*), in sheaths with 4 and 5 lamellae (Fig. 8 *b*), and in sheaths with more than 5 lamellae (Fig. 8 *c*). From these results, it is apparent that, in the sheaths with 4 and 5 lamellae, and in those with more than 5 lamellae, the relative positions of the internal mesaxons and external tongue processes

are similar to those in the adult rat (Fig. 7 *b*). On the other hand, in sheaths with 1 to 3 lamellae, the relative positions of these structures are more random.

From the above results, then, it is apparent that in the early stages of myelin formation the internal mesaxon and external tongue process of each sheath are randomly oriented with respect to each other, but that, as the sheaths become more mature, these structures tend to become preferentially oriented, so that they most frequently lie within 90° of each other in the direction taken by the spiral. This situation can be appreciated in Fig. 9, which is a transverse section of part of the optic nerve of a 14-day postnatal rat. Within the sheaths around nerve fibres *4* to *6*, where only two to three complete lamellae are present, the internal mesaxon (*IM*) and external tongue process (T_4 to T_6) of each sheath are randomly oriented, while in the more mature sheaths around nerve fibres *1* and *2*, as well as around nerve fibre *3*, these two structures lie within the same region of the sheath. For comparison with Fig. 9, in which the greater proportion of nerve fibres are still unmyelinated, the situation obtaining in an adult nerve is shown in Fig. 6.

Although the above results are confined to an analysis of the optic nerves of rats and mice, the same situation in respect of the relative positions of the internal mesaxons and external tongues of cytoplasm also appears to obtain both within their spinal cord and cerebral cortex, as well as within the optic nerves of *Xenopus* toads. However, the number of observations made upon these latter structures have not been sufficient for an analysis to be carried out.

Radial Component of the Sheath

In the myelin sheaths of the central nervous system a radial component is present (17, 19). In transverse sections, this component is apparent as a radial series of thickenings of the intraperiod line, and is most commonly observed in that part of the sheath underlying the tongue of cytoplasm on the outside of the sheath (arrows in Figs. 3 and 6), although it may also be found in the other regions of a sheath. This component is most prominent when sections of osmium tetroxide–fixed material are stained with potassium permanganate; it has occasionally been observed after lead acetate staining, but has not yet been seen after uranyl acetate staining.

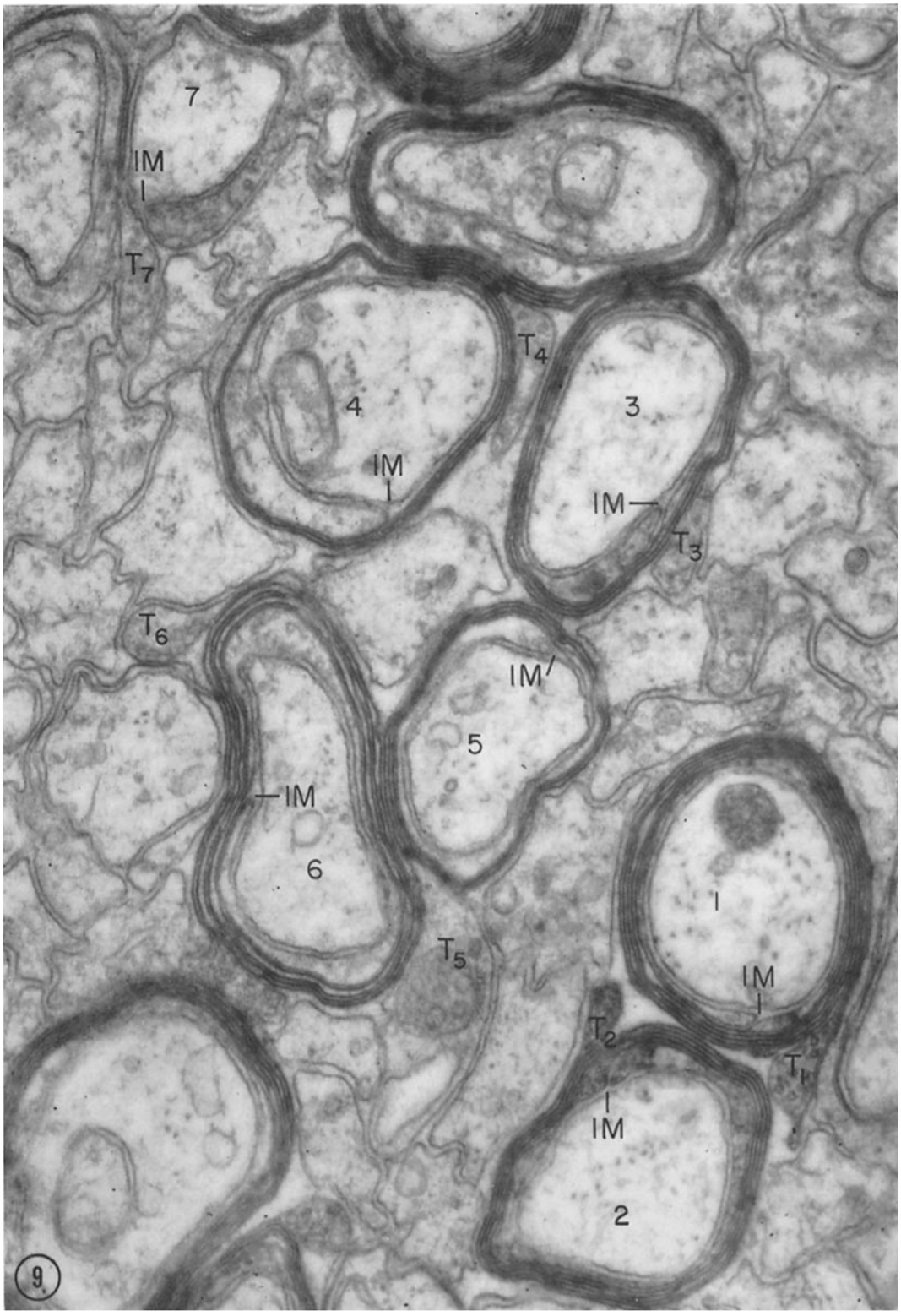
When this radial component was first observed, it was considered to be produced by a series of disc- or plaque-like thickenings of the intraperiod line, which, in transverse sections of sheaths, increase the width of the intraperiod line to 40 to 50 A and extend along it for a distance of 60 to 90 A. A disc-like structure was proposed because similar thickenings have also been observed in longitudinal sections of sheath.

After further study, it became apparent that in transverse sections this radial component appears too regularly throughout the thickness of a sheath for it to have a disc-like structure. The chances of cutting an isolated series of discs so frequently appeared to be unlikely. Transverse serial sections have, therefore, been taken through a number of sheaths, and examination shows that even in sections as much as 3,000 A apart, the same sets of radial components are apparent. Consequently, the radial thickenings of the intraperiod line observed in transverse sections appear to extend along the length of the intraperiod line in a direction parallel to the length of the nerve fibre. This conclusion is borne out by the fact that in oblique sections of sheaths, as shown in Fig. 5, a series of dark lines is sometimes observed (arrows). The width of such lines is 50 to 70 A, which is similar to the extent of the radial components along the direction of the intraperiod line in transverse sections of sheaths. In oblique sections (Fig. 5) these lines may be as long as 2,000 A, although their true extent is probably greater, and they may extend from node to node.

DISCUSSION

On the basis of the present observations on the structure of central myelin sheaths, there is little doubt that the lamellae have a spiral arrangement. Thus, as suggested previously (1, 12–16), the basic structure of central myelin sheaths is exactly

FIGURE 9 Transverse section of part of the optic nerve of a 14-day postnatal rat. In the sheaths around seven of the axons (*1* to *7*), the position of the internal mesaxon (*IM*) and external tongue of cytoplasm (T_1 – T_7) is indicated. Section stained with urany acetate. × 70,000.


7
IM
T7
T4
4
3
IM
IM
T3
T6
IM'
5
IM
6
1
T5
IM
T2
T1
IM
2
9

similar to that of peripheral sheaths. In adult animals, the amount of cytoplasm on the outside of the sheath is usually small, but, as shown, it is possible, in sections of developing optic nerves, to find sheaths where the cytoplasm on the outside forms a complete layer. The structure of such sheaths is exactly similar to that of a peripheral sheath, in which the spiral arrangement of the lamellae appears to be generally accepted (see 24). As pointed out, the theories that Luse (10, 11) and De Robertis, Gerschenfeld, and Wald (4, 5) have put forward to explain the formation of central myelin deny the existence of a spiral at all stages, and cannot account for the presence of either an internal mesaxon or the termination of the lamellae on the outside of the sheath at the external tongue of cytoplasm. These latter structures are consistently observed in sections of well preserved sheaths. Luse (10, 11) has suggested that different numbers of lamellae may be present in different parts of the same myelin sheath, and Robertson and Vogel (25) have also mentioned that this may be true of some sheaths, but in the present and earlier (15, 16, 18) studies, as well as those of Maturana (12) and Bunge, Bunge, and Ris (1), no such inconsistencies have been observed.

On the basis of their structure, it was postulated (1, 12, 16) that the myelin-forming glial cells are connected to the tongue of cytoplasm on the outside of the sheaths by a process of cytoplasm. Such connections have now been demonstrated in the spinal cords of kittens (2) and in the optic nerves of young rats (20). Both sets of results show that although the sheath and glial cell may be connected by a long process, in other cases the sheath lies immediately adjacent to the cell. Further, Bunge, Bunge, and Pappas (2) have illustrated an example in which two sheaths are connected to the same glial cell, so that it appears likely that each glial cell may be responsible for the formation of internodal lengths of myelin around a number of nerve fibres. Such relations between sheaths and glial cell are of course consistent with the existence of a spiral (see 2). Nevertheless, as pointed out by Ross, Bornstein, and Lehrer (26), the existence of a spiral does not preclude the possibility that more than one glial cell may participate in the formation of an internodal length of central myelin. They find that, in tissue cultures of rat and mouse cerebellum, the developing sheath may be composed of more than one spiralling process, these processes overlying and interdigitating with each other to form a multi-lamellated structure. Such multiple contributions to the formation of a central myelin sheath are probably abnormal, and have not been observed in the present and earlier (16, 1) studies concerned with the formation of a spiral in normal tissue. However, as Ross, Bornstein, and Lehrer (26) suggest, but do not demonstrate, if more than one glial cell contribute to the formation of a length of myelin, there must be both longitudinal and circumferential fusion of multiple processes in order to produce a final, even distribution of lamellae.

An interesting study is that of Robertson and Vogel (24) on the structure of the concentric laminations of glial cell processes in some oligodendrogliomas. From their excellent illustrations, there is no doubt that apart from the arrangement of the lamellae, the concentric laminations are similar in appearance to the loose myelin formed during the early stages of myelin formation in both central (15, 16, 18) and peripheral (see 22) myelin sheaths. If the cells responsible for the formation of both these concentric laminations and central myelin sheaths (see 2) are oligodendrocytes, then it is very interesting that the cells are capable, under different conditions, of forming both concentric and spiral lamellae.

In about 75 per cent of the mature myelin sheaths examined in the present study, the internal mesaxon and external tongue of cytoplasm lie within the same quadrant when sheaths are examined in transverse sections. Such a relationship is also apparent in some of the illustrations published by Maturana (12) of the optic nerves of anurans, and by Bunge, Bunge, and Ris (1) of the spinal cord of cats. No comparable study has yet been made of the peripheral nervous system, and from the illustrations that have been published of peripheral sheaths it is not possible to determine whether such a relationship also exists there.

From the analysis of myelin sheaths composed of different numbers of lamellae, it is apparent that while the internal mesaxon and external tongue process, or alternatively the inner and outer ends of the intraperiod line, are randomly oriented in sheaths with only a few lamellae, by the time that 4 or more lamellae are present there is a tendency for these structures to occur within the same quadrant of the sheath. It appears, then, that this is the most stable configuration for the sheath. This con-

figuration is also shown by the sheaths in the optic nerves of the 14-day and 20-day postnatal rat, despite the fact that, at this age, very few sheaths have anything approaching the adult complement of lamellae. The only apparent interpretation of this result is that if the number of lamellae is increased by a spiral elongation of either the internal mesaxon or external tongue of cytoplasm, then this growth must occur in phases, being interrupted by relatively long intervals of time when these two structures remain within the same region of the sheath.

This preferential configuration of the inner and outer ends of the spiralled lamellae means that, in the majority of sheaths, the cytoplasm of the tongue process on the outside of the sheath overlies the cytoplasm associated with the internal mesaxon, although these two areas of cytoplasm are separated from each other by the thickness of the myelin. Both of these areas of cytoplasm may be very small in the mature sheath. In transverse sections, it is found that the tongue process usually occupies only about 5 per cent of the circumference of the sheath, while the cytoplasm on the inside of the sheath is frequently confined to the region of the internal mesaxon (see also 2). Nevertheless, this close proximity does not appear to be of importance unless, perhaps, metabolites are transferred across the myelin lamellae in this region, from the outer to the inner cytoplasm. It is in this region of the sheath that the radial component is most commonly found. Nowhere is there continuity between the inner and outer cytoplasm of the sheath except through the terminal helix of cytoplasm at the node. No other areas of continuity, such as the Schmidt-Lanterman clefts of peripheral sheaths (23), occur within the central nervous system.

The author wishes to thank Prof. G. J. Romanes for his continued interest in this work, which was carried out on an electron microscope on loan from the Wellcome Trust.

Received for publication, May 21, 1963.

REFERENCES

1. BUNGE, M. B., BUNGE, R. P., and RIS, H., Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 67.
2. BUNGE, M. B., BUNGE, R. P., and PAPPAS, G. D., Electron microscopic demonstration of connections between glia and myelin sheaths in the developing mammalian nervous system, *J. Cell Biol.*, 1962, **12**, 313.
3. DALTON, A. J., A chrome-osmium fixative for electron microscopy, *Anat. Rec.*, 1955, **121**, 281.
4. DE ROBERTIS, E., GERSCHENFELD, H. M., and WALD, F., Cellular mechanism of myelination in the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 651.
5. DE ROBERTIS, E., GERSCHENFELD, H. M., and WALD, F., Submicroscopic morphology and function of glial cells, *in* International Review of Neurobiology, New York and London Academic Press, Inc., 1961, **3**, 1.
6. FERNÁNDEZ-MORÁN, H., and FINEAN, J. B., Electron microscope and low-angle x-ray diffraction studies of the nerve myelin sheath, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 725.
7. GEREN, B. B., The formation from the Schwann cell surface of myelin sheaths in peripheral nerves of chick embryos, *Exp. Cell Research*, 1954, **7**, 558.
8. GLAUERT, A. M., and GLAUERT, R. H., Araldite as an embedding medium for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 191.
9. LAWN, A. M., The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy-resin, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 197.
10. LUSE, S. A., Formation of myelin in the central nervous system of mice and rats, as studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 777.
11. LUSE, S. A., Ultrastructure of the brain and its relation to transport of metabolites, *in* Ultrastructure and Metabolism of the Nervous System, Association for Research in Nervous and Mental Disease, 1962, **40**, 1.
12. MATURANA, H. R., The fine anatomy of the optic nerve of Anurans—an electron microscope study, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 107.
13. METUZAIS, J., Ultrastructure of myelinated nerve fibres and nodes of Ranvier in the central nervous system of the frog, European Regional Conference on Electron Microscopy, 1960, Delft, Nederlandse Verenigig Voor Electronmicroscopie, 1961.
14. METUZALS, J., Ultrastructure of myelinated nerve fibers in the central nervous system of the frog, *J. Ultrastruct. Research*, 1963, **8**, 30.
15. PETERS, A., The structure of myelin sheaths in the central nervous system of *Xenopus laevis*

(Daudin), *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 121.

16. PETERS, A., The formation and structure of myelin sheaths in the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 431.
17. PETERS, A., A radial component of central myelin sheaths, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 733.
18. PETERS, A., Myelination in the central nervous system, *in* Proceedings of 4th International Congress of Neuropathology, 1961, Munich, (H. Jacob, editor), Stuttgart, Georg Thieme Verlag, 1962, **2**, 50.
19. PETERS, A., Plasma membrane contacts in the central nervous system, *J. Anat.*, London, 1962, **96**, 237.
20. PETERS, A., unpublished observations, 1963.
21. ROBERTSON, J. D., The ultrastructure of adult vertebrate peripheral myelinated fibers in relation to myelinogenesis, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 271.
22. ROBERTSON, J. D., Some aspects of the ultrastructure of double membranes, *in* Ultrastructure and Cellular Chemistry of Neural Tissue, (H. Waelsch, editor), New York, Hoeber-Harper, 1957, 1.
23. ROBERTSON, J. D., The ultrastructure of Schmidt-Lanterman clefts and related shearing defects of the myelin sheath, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 39.
24. ROBERTSON, J. D., The ultrastructure of cell membranes and their derivatives, *in* Biochemical Society Symposium, No. 16, The Structure and Function of Subcellular Components, Cambridge University Press, 1959.
25. ROBERTSON, D. M., and VOGEL, F. S., Concentric lamination of glial processes in oligodendrogliomas, *J. Cell Biol.*, 1962, **15**, 313.
26. ROSS, L. L., BORNSTEIN, M. B., and LEHRER, G. M., Electron microscopic observations of rat and mouse cerebellum in tissue culture, *J. Cell Biol.*, **14**, 19.
27. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
28. WOHLFARTH-BOTTERMANN, K. E., Die Kontrastierung tierischen Zellen und Gewebe in Rahmen ihrer elektronenmikroskopischen Untersuchung an Ultradunned Schnitten, *Naturwissenschaften*, 1957, **44**, 287.