REDUNDANT MYELIN SHEATHS AND OTHER ULTRASTRUCTURAL FEATURES OF THE TOAD CEREBELLUM

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ABSTRACT

Some of the myelin sheaths in the cerebellum of normal adult toads exhibit extensive evaginations of their full thickness. These redundant flaps of myelin are collapsed; i.e., they contain no axon and have no lumen. They extend away from the parent axonal myelin sheaths and tend to enfold other myelinated fibers or granule cell perikarya, producing bizarre configurations of myelin and what appear to be partially or completely myelinated cell bodies. In some instances, only the redundant flap of myelin appears in the plane of section, and its attachment to an axonal myelin sheath in another plane is only inferred. Single lamellae of myelin also tend to invest cerebellar granule cells and other processes, and these too appear to fold on themselves producing two- or four-layered segments. It is suggested that there are two phases of myelinogenesis: an initial "wrapping" phase, followed by a prolonged second phase during which internodes of myelin increase in both length and girth by a process other than wrapping, and that the occurrence of redundant myelin sheaths may reflect overgrowth of myelin during the second phase. Observations on the general organization of the toad cerebellum and on the ultrastructural cytology of its layers are also presented.

INTRODUCTION

Studies of myelinogenesis in the peripheral nervous system have provided convincing evidence that compact myelin consists of the plasma membranes of Schwann cells wrapped around axons in the form of a tight spiral (11, 12, 35). The spiral genesis of the myelin sheath accounts in a simple manner not only for its periodic structure (8), but also for the structure of Schmidt-Lantermann clefts (36) and for the structure of terminal and paranodal regions of the sheath (6, 37). In general, each internodal segment is myelinated by a single Schwann cell. However, in acoustic ganglia some of the perikaryal myelin sheaths are derived from more than one Schwann cell and are more complex than a simple spiral (39). Studies of developing and remyelinating central nervous tissue indicate that, in comparable fashion, segments of myelin in the central nervous system are derived from single (4, 5, 31, 47) or multiple (23, 44) glial cell processes prolonged around axons.

The wrapping hypothesis does not, however, account for myelin irregularities of the type observed by Webster and Spiro (50) in the sciatic nerves of guinea pigs. There the paranodal portions of myelin sheaths exhibit prominent inpouchings and outpouchings which do not conform to the shape of the enclosed axon. Such redundancies occur in approximately 75% of the largest myelin sheaths. The present paper reports irregularities of this same type in the *central* nervous

system of an amphibian, where they are sometimes remarkably extensive and where, because of the architecture of central nervous tissue as compared with that of peripheral nervous tissue, they produce very complex patterns. These configurations can be explained by the relatively simple mechanism of "secondary envelopment" as will be described below.[1]

The observations reported here and those of Webster and Spiro do not controvert the hypothesis that myelin is derived from processes of supporting cells wrapped around axons, but they indicate that this is only one part of the process and that additional mechanisms also operate to bring about the final configuration of myelin in the adult animal. Moreover, in the light of current information on myelin ultrastructure, several earlier studies of the elongation of myelin sheaths during axonal growth (2, 18, 20, 46, 48) also suggest that the process of myelinogenesis entails more than the wrapping mechanism.

MATERIALS AND METHODS

Adult toads (*Bufo marinus*) weighing approximately 200 g were either anesthetized with 5 ml of 10% ethyl carbamate intraperitoneally or subcutaneously, or pithed, depending on which portion of the nervous system was wanted for study. The heart was exposed and the animal perfused through the aorta with approximately 5 to 10 ml of saline (see below) followed by approximately 50 ml of fixative. The procedure is in principle the same as that published by Palay et al. (28); however, it was found that in these animals a number of steps, including tracheotomy with artificial respiration, heparinization, and sodium nitrite instillation, could be omitted without apparent detriment. Presumably, in cold blooded animals the requirements for satisfactory fixation of nervous tissue are less rigorous than in mammals.

The saline was used at room temperature and the fixative was either chilled or used at room temperature. The perfusion apparatus consisted of a 50-ml syringe attached either to a glass cannula by rubber tubing or to a polyethelene tube with a flanged end. In the former case the saline occupied the tubing and the fixative the syringe; in the latter, the saline was introduced by a separate syringe through a three-way stopcock.

The saline is a balanced salt solution whose pH, osmolarity, and ionic balance approximately match that of the animal's blood. It was assumed that such a solution would be less apt to introduce artifactual changes in the tissue prior to its fixation than would an isotonic sodium chloride solution.

The composition of the saline is:

NaCl	5.10	g/L	
$NaHCO_3$	1.65	g/L	
KCl	0.30	g/L	
$NaH_2PO_4 \cdot H_2O$	0.13	g/L	
$CaCl_2$	0.18	g/L	
$MgCl_2 \cdot 6H_2O$	0.16	g/L	
Glucose	1.00	g/L	(optional)

In order to prevent precipitation of calcium and magnesium salts during preparation of the solution, it may be necessary to reduce the pH of the solution by exhaling through it or by bubbling CO_2 through it. The saline also tends to lose CO_2 and become alkaline on standing. It is therefore necessary to check the pH just before each use and to bring the pH of the amount used down to 7.3–7.5 in the same manner.[2]

The fixative consists of 1% osmium tetroxide in sodium acetate–sodium Veronal buffer (0.028 M for each salt, reference 26) adjusted to pH 7.3–7.5 with HCl, and with the following salts added:

NaCl	2.90	g/L
KCl	0.23	g/L
$NaH_2PO_4 \cdot H_2O$	0.13	g/L
$CaCl_2$	0.18	g/L
$MgCl_2 \cdot 6H_2O$	0.16	g/L

Approximately an hour or an hour and a half after the perfusion was begun, the fixed brain was dissected out and sliced transversely behind the olfactory lobes, at the anterior and posterior margins of the optic lobes, behind the cerebellum, and at the approximate junction between the medulla and spinal cord. The cerebral hemispheres were divided midsagittally. The slices were then rinsed in isotonic saline, dehydrated in a graded series of methanol solutions, and embedded flat in Epon or Araldite according to the recommendations of Luft (22). In one instance the brain was embedded whole and then cut into transverse slices with a fine jeweler's saw.

After polymerization of the plastic, the brain slices were trimmed and mounted on plastic plugs. The larger slices were divided midsagittally before mounting. It was then possible to cut whole (or half) brain transverse sections, at 1 to 2 μ, by using glass knives with a Porter-Blum microtome, to stain them with

[1] A preliminary report of the data reported in this paper was presented at the annual meeting of the American Association of Anatomists, April, 1965 (43).

[2] These considerations apply also to the preparation and use of the counterpart mammalian balanced salt solution, the composition of which is included in the paper on perfusion fixation by Palay et al. (28).

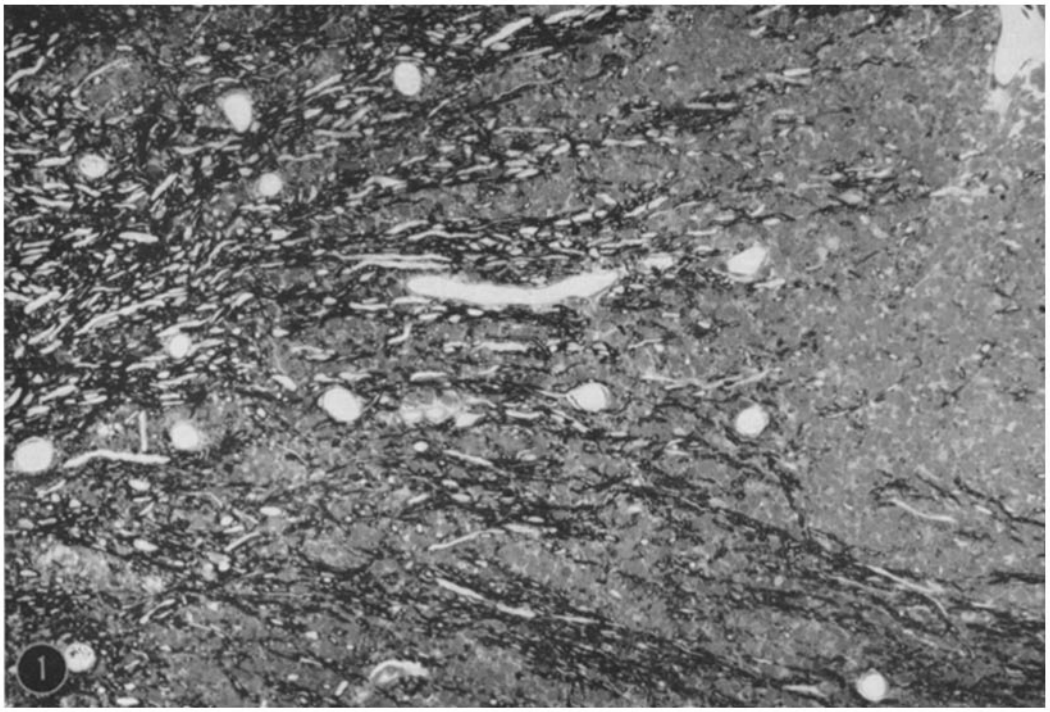


FIGURE 1 Photomicrograph of toad cerebellum showing myelinated fiber bundles at the left extending into the granule cell layer at the right. The myriad granule cell nuclei are homogeneous in size and appearance. Large blank spaces are capillaries. × 200.

toluidine blue according to the method of Richardson et al. (34), and finally to trim the block for thin sectioning of the area of interest. By using this method, it is possible to be sure of the plane of the thin sections and to identify the structures for thin sectioning with accuracy. Furthermore, the whole (or half) brain 1 to 2 μ sections are themselves very useful preparations for study by light microscopy (Fig. 1).

Thin sections were stained with lead (21) or uranyl (49) salts and examined with an RCA EMU 3E microscope.

OBSERVATIONS

GENERAL APPEARANCE OF THE PERFUSED TOAD BRAIN: Toad central nervous tissue corresponds to that of mammals in most respects (28). Cellular processes are separated from one another by relatively narrow extracellular spaces which are free of formed elements. The width of the space is variable. Although usually 100 to 200 A, (Fig. 3), the space is widened about tenfold around nodes of Ranvier (Fig. 15; cf. reference 25) and axon terminals (Fig. 4; cf. reference 38), and is obliterated at "tight junctions" (Figs. 4 and 6; cf. references 1, 7). Synapses, myelin sheaths, and the usual intracellular constituents are easily recognizable. The only apparent difference from the central nervous tissue of mammals is that glial cells are sparse, in accordance with Friede's observation (10) that the neuron-to-glia ratio is low in amphibians; in addition, there are substantial numbers of glycogen particles throughout the toad central nervous system: in axon terminals (Figs. 6, 11 and 15), dendrites (Fig. 6), nerve cell bodies (Fig. 10), and glial cells (not illustrated). The occurrence of glycogen in spinal ganglion cells and satellite cells of this animal was reported previously (41, 42).

CEREBELLAR ORGANIZATION AND ULTRASTRUCTURE: Although not foliated, the cerebellum here, as in mammals (9, 14, 27), displays typical stratification into:

1. A molecular layer (Fig. 3) in which fascicles of tiny granule cell axons travel among stout Purkinje cell dendrites. The latter are distinguished by their large concentration of

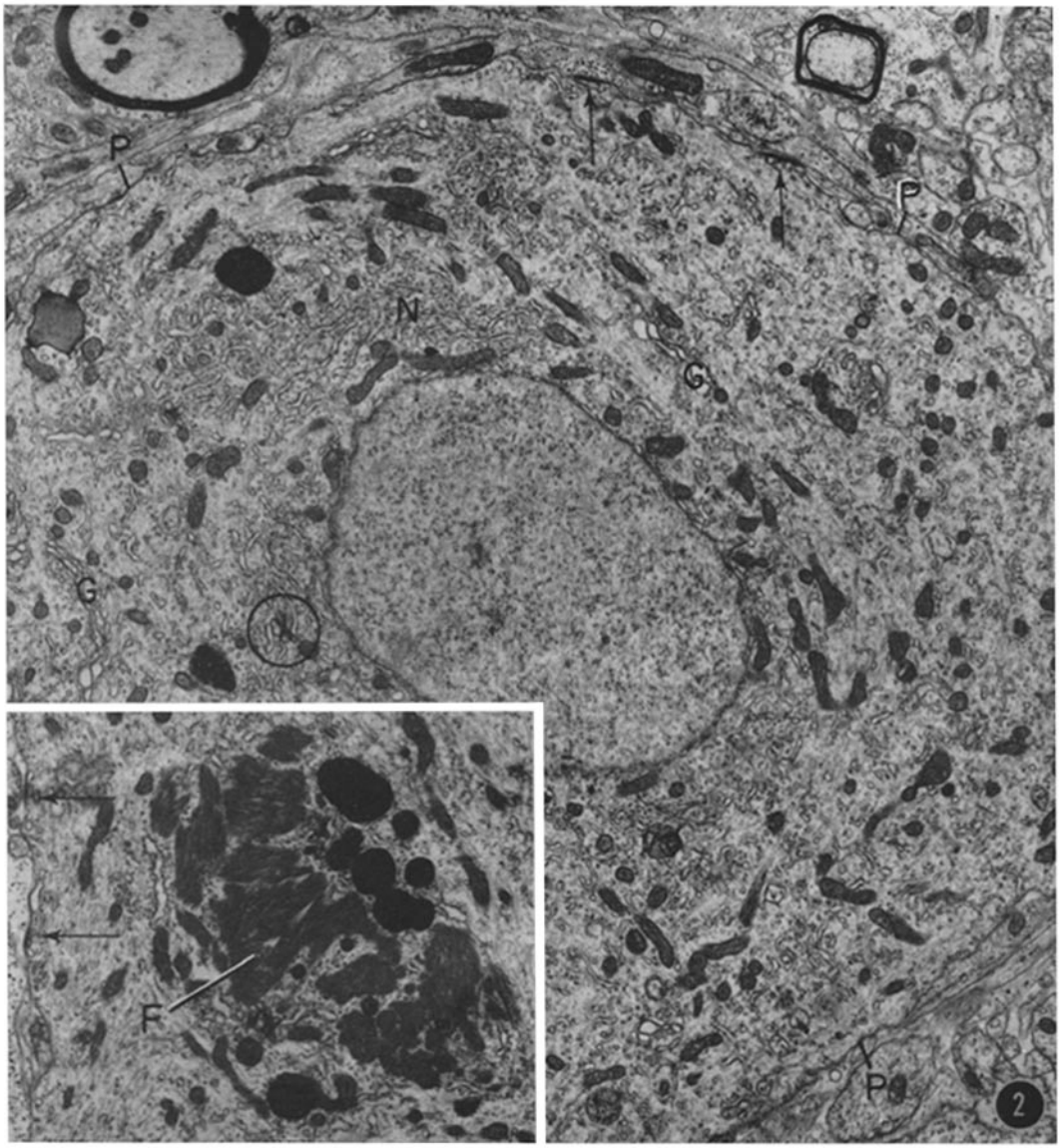


FIGURE 2 Purkinje cell. The nucleus is surrouned by a thick band of cytoplasm which contains large amounts of granular endoplasmic reticulum, or Nissl substance (*N*), Golgi apparatus (*G*), and mitochondria. The "empty" cytoplasmic areas contain innumerable microtubules. Arrows indicate subsurface cisterns; circle shows flattened membranous sacs deep in the cytoplasm. *P*, neuronal plasma membrane. Inset shows the base of a Purkinje cell dendrite. The plasma membrane is at the left. In addition to microtubules, the cytoplasm here contains bundles of tightly packed filaments (*F*). Fig. 2, × 8600; inset, × 8200.

microtubules and by the presence of "spines" upon which granule cell axons terminate.

2. A layer of Purkinje cell bodies (Fig. 2) which also contain an abundance of microtubules and, in addition, prominent Nissl bodies and Golgi network (29). There are as well, flattened, agranular membrane complexes resembling "spine apparatus" (13),

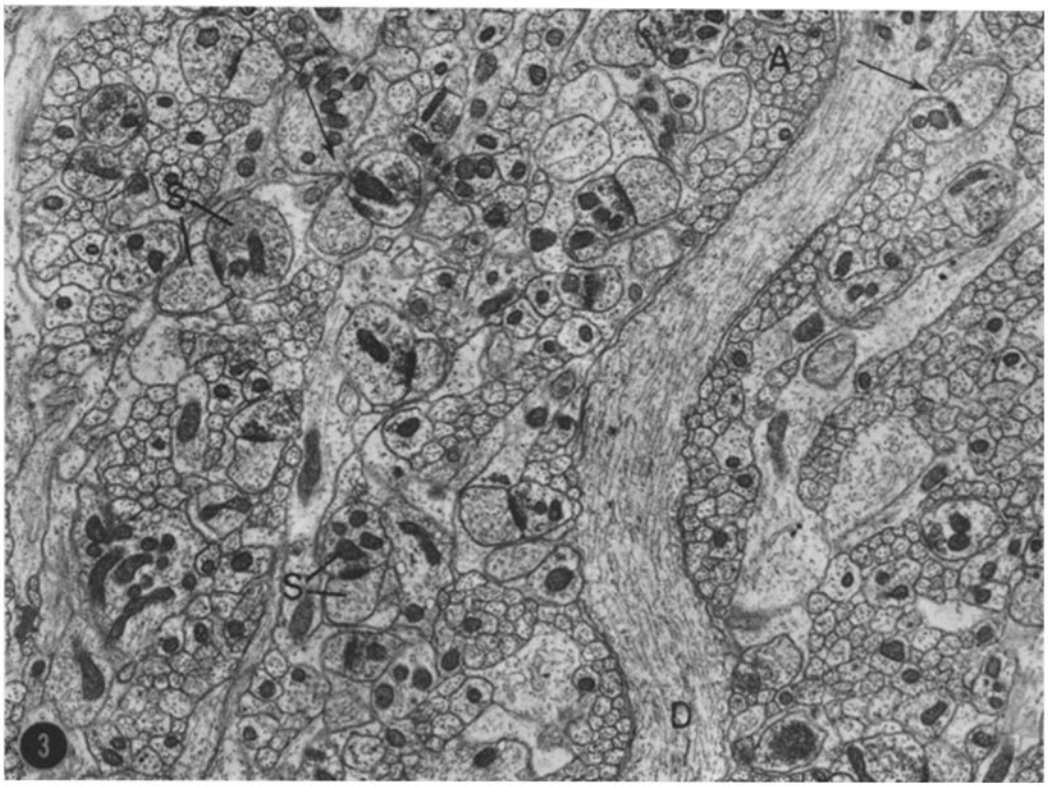


FIGURE 3 Molecular layer. A Purkinje cell dendrite (*D*) filled with microtubules lies in the plane of the section. Fascicles of tiny granule cell axons (*A*) are cut transversely. Several synapses are visible (*S*). Arrows show the origin of "spines" from Purkinje cell dendrites. × 8600.

numerous subsurface cisterns (16, 40), dense inclusions resembling lysosomes and lipofuscin, and tightly packed bundles of filaments (Fig. 2, inset).

3. A broad granule cell layer (Fig. 1) superimposed on the white matter of the cerebellum. The granule cell (Figs. 10 to 16) is characterized by a nucleus $\sim 5\ \mu$ in diameter, which, except for the presence of the nucleolus (Figs. 15 and 16), is relatively homogeneous in appearance. The thin rim of perinuclear cytoplasm contains glycogen particles and scattered rosettes of ribosomes, some of which are membrane-bound. There is, however, little organized ergastoplasm in these cells, in contrast to the much larger Purkinje cell bodies. Granule cell cytoplasm also contains inclusions which are not surrounded by membranes and which are composed of filamentous and granular material resembling that which comprises the nucleoplasm (Figs. 10 and 13). This kind of inclusion was noted previously in acoustic ganglion cells of the rat (39). Golgi apparatus, subsurface cisterns (Fig. 15), lysosomes, neurofilaments, and microtubules also occur in these cells but are not nearly so conspicuous as in Purkinje cells.

The several layers of the cerebellum are uniformly fixed by the method used.

MYELINATED FIBERS: The myelin sheaths of the toad brain, like those in the central nervous system of other animals, consist of multilayers of compact membranous lamellae. The innermost layer immediately adjacent to the axon is usually a "loose" layer, i.e. one that contains a small residue of glial cell cytoplasm (Figs. 5 *a* and 7), while the outermost layer is compact except for a small loop of cytoplasm at its terminal edge (24, 30). The latter characteristic distinguishes myelinated fibers of the central nervous system from those of

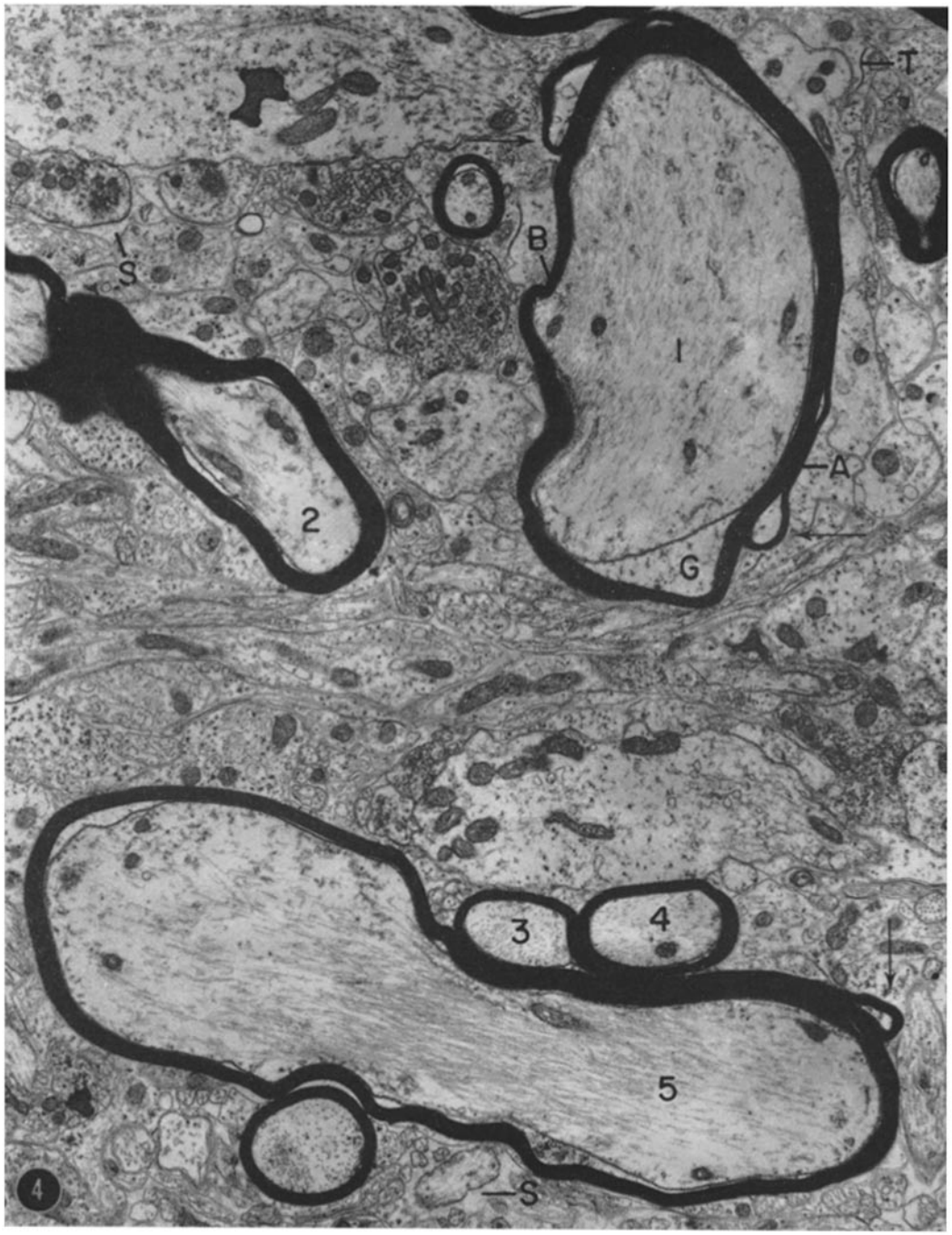


FIGURE 4 Myelinated axons. Axon *1* is about half-enveloped by a redundant myelin sheath whose bulbous ends are indicated by horizontal arrows. At *A* roughly twice as many myelin lamellae invest this axon as at *B*. The sheath around axon *2* also appears thickened in one region, but this is due to oblique sectioning rather than secondary envelopment. The sheath of axon *3* has a redundant portion which extends between the sheaths of axons *4* and *5* and terminates at the vertical arrow. *G*, glial cell cytoplasm; *T*, tight junction; *S*, widened extracellular space around axons. × 9400.

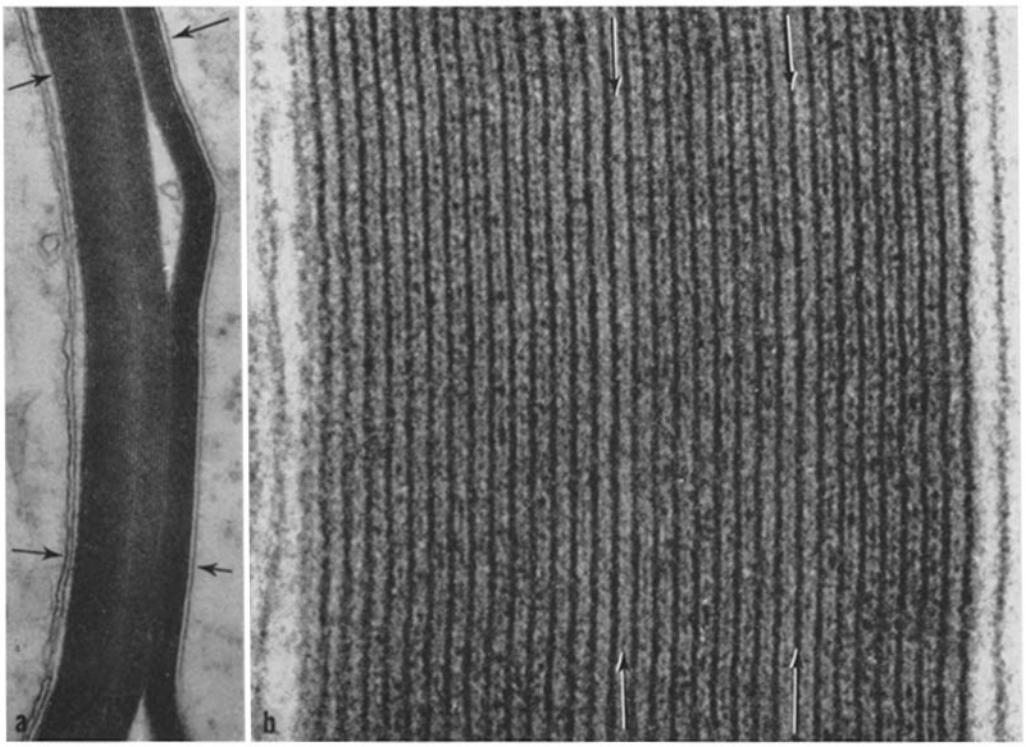


FIGURE 5 *a* Enlargement of the region indicated by *A* in Fig. 4 showing two "light seams" in the complex sheath (between the lower arrows). Here, the redundant sheath is fused onto the sheath of axon *1*, and the loose lamella in the middle of the redundant flap has become compact. Between the upper arrows, the loose lamella persists as such. × 45,500.

FIGURE 5 *b* Further enlargement of the region between the lower arrows in Fig. 5 *a* showing the structural basis for the "light seams." The vertical arrows at the right indicate the major dense line derived from the loose lamella in the middle of the redundant sheath. The intermediate lines on either side of this major dense line are delicate and contain no heavy deposits of osmiophilic material. The vertical arrows at the center of the figure indicate the intermediate line along which the redundant sheath and the sheath of axon *1* have fused. This intermediate line, the one to its right, and the three to its left are also virtually devoid of heavy osmiophilic deposits. The same observation also applies to the intermediate line at the extreme right of the figure and to the three at the extreme left. All of these regions represent myelin lamellae which are, or were at one time, near either the inner or outer surface of a myelin sheath. × 282,000.

the peripheral nervous system where the outermost layer of a myelin sheath always contains cytoplasm over the whole surface of the internode; the nucleus and major cytoplasmic organelles of the Schwann cell are located in this layer. Peripheral myelinated fibers are separated from their neighbors not only by this cytoplasmic layer, but also by the basement membrane which covers the outer surface of the Schwann cell and by a variable amount of connective tissue. Thus the compact portions of peripheral myelin sheaths never come into direct contact with one another, whereas in the central nervous system, because of the absence of the connective tissue space, the basement membrane, and cytoplasm in virtually all of the outermost layer of the sheath, compact myelin sheaths often contact one another directly. As Peters (30) and Maturana (24) have pointed out, apposed myelin sheaths in the CNS typically fuse together in such a way as to produce a continuous thickness of myelin with no intervening space or break in periodicity. Hence it is sometimes difficult to

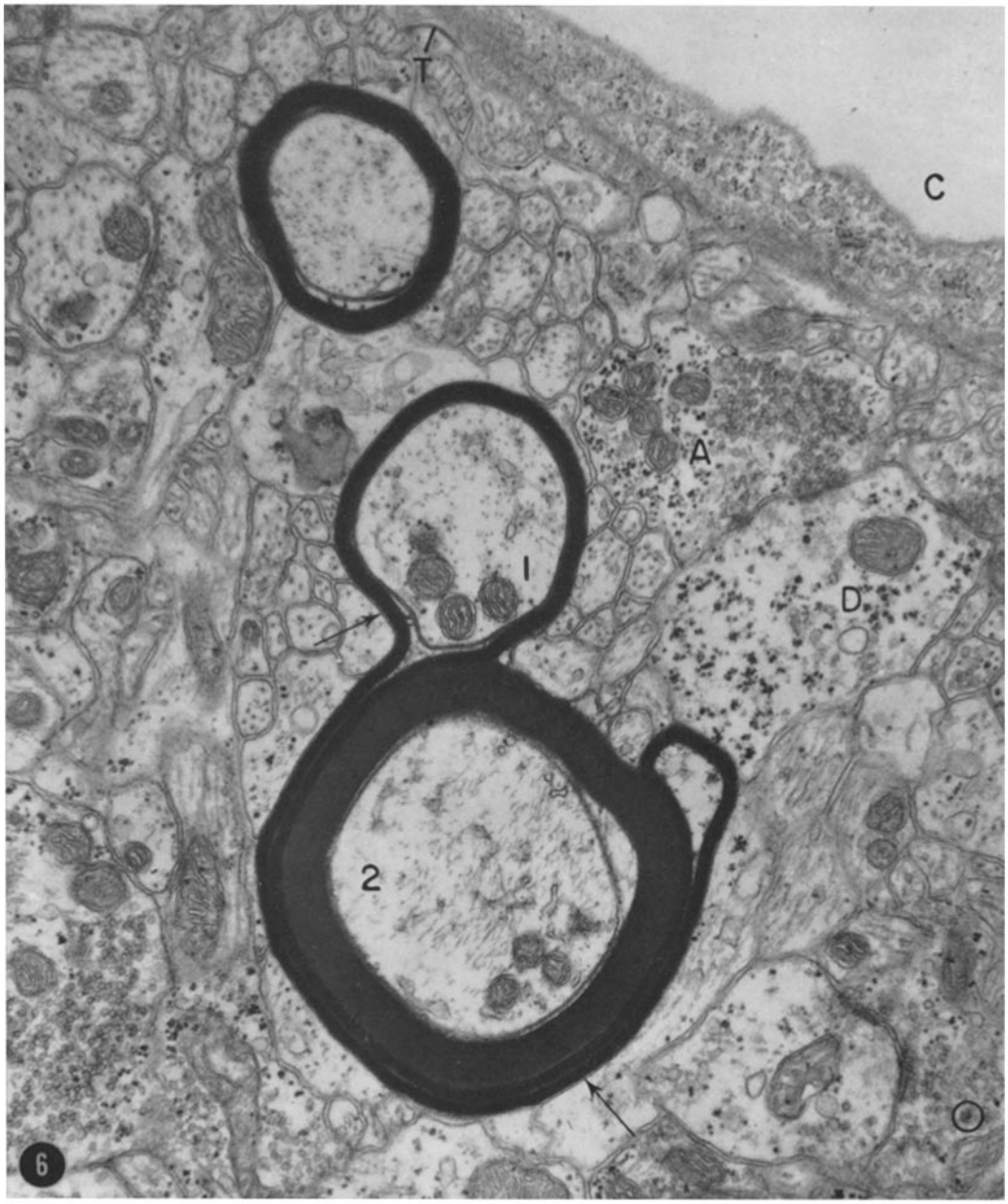


FIGURE 6 Secondary envelopment of a small myelinated axon. The redundant portion of the sheath of axon *1* extends about three-fourths of the way around axon *2*. Axon *1* looks entirely normal. Upper arrow indicates region of internal mesaxon. Region of lower arrow is enlarged in Fig. 7 *b*. Note glycogen particles in both dendritic (*D*) and axonal (*A*) processes. These particles should not be confused with dense-cored or granular vesicles (circle) that occur in axonal terminals. *C*, capillary lumen; *T*, pericapillary "tight junction"; *A*, axon terminal; *D*, dendrite. × 21,900.

know where one sheath ends and the next begins unless the complete circumference of a myelin sheath can be followed.

SECONDARY ENVELOPMENT OF AXONS BY REDUNDANT MYELIN SHEATHS: In the toad brain the usual configuration of myelinated fibers is complicated in certain cases by sheaths which appear to be a great deal larger than the axons they enclose and which, as a result, have extensive slack or redundant portions.

Typically, such an axon contains the normal complement of filaments and mitochondria and occasional microtubules (Fig. 6). The investing myelin sheath hugs the axon and conforms exactly to its contour except at one point along its circumference where an extension of the sheath buckles outwards away from the axon. The protruding flap consists of two thicknesses of the myelin sheath separated from each other by a thin layer of glial cell cytoplasm, a continuation of the innermost loose lamella of the sheath (Figs. 6 and 7). The axon itself does not extend into the flap and there is no lumen in the redundant part of the sheath. Frequently the loose lamella in the middle of the flap becomes compact except at the bulbous end of the flap (Figs. 4, 5 *a*, and 8), and in these instances, the flap consists of a solid wall of compact myelin twice as thick (less one lamella) as the sheath from which it is derived.

Generally these redundant flaps do not curl on themselves forming isolated knots of myelin, but rather follow and surround other myelin sheaths, fusing with them and thereby causing other axons to appear more heavily myelinated along some parts of their circumference than along others (Figs. 4, 6, and 8). Envelopment in this manner by redundant flaps of myelin will be referred to henceforth as "secondary envelopment" or "secondary myelination." The length of a redundant flap of myelin may be very great and the degree of secondary myelination very extensive (Fig. 8). Indeed, in one instance, a flap was so long that it extended a full 360° around another myelinated nerve fiber and then continued back on itself and partially enveloped the axonal sheath from which it was itself derived. In some sections it is possible to see the continuity between a redundant sheath and the axonal sheath from which it originates; i.e., the nonredundant part of the sheath and the enclosed axon are present in the same section as the redundant flap (Figs. 6 and 8). However, often only the redundancy itself is visible, and at either end it contains a loop of glial cell cytoplasm but no trace of either a lumen or an enclosed axon (Fig. 4).

The fusion of a redundant flap to another myelin sheath may be complete, with the extracellular space between them totally obliterated and with formation of an intermediate line (Fig. 5), or a thin residue of the extracellular space may persist (Fig. 7 *b*). In the latter case there is no difficulty in distinguishing the respective components, except at low magnification. Even when the fusion is complete, however, there usually remains a light "seam" along the line of fusion; and similarly, when the loose lamella in the middle

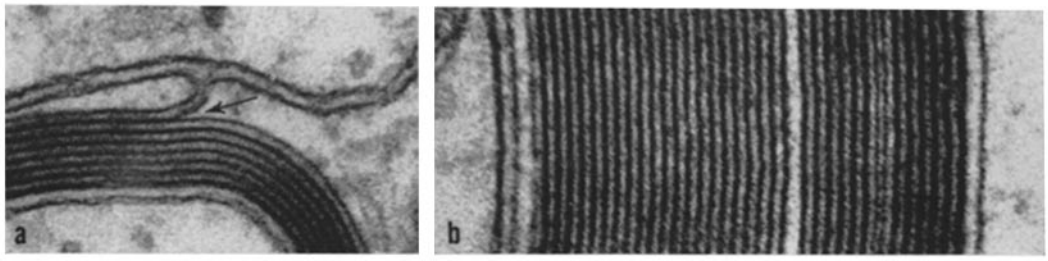


FIGURE 7 *a* Enlargement of the region indicated by the upper arrow in Fig. 6. Six compact lamellae extend downwards into the redundant part of the sheath. The loose lamella which extends into the middle of the redundant flap also forms a compact lamella (arrow) adjacent to the internal mesaxon. × 120,000.

FIGURE 7 *b* Enlargement of the region indicated by the lower arrow in Fig. 6. Unlike the complex sheath shown in Fig. 5 *b*, this one shows a clear separation between the redundant sheath and the sheath of the axon being secondarily enveloped. In addition, the loose lamella in the middle of the redundant sheath persists as such. The redundant sheath consists of six compact lamellae on either side of this loose lamella whose origin is shown in Fig. 7 *a*. × 150,000.

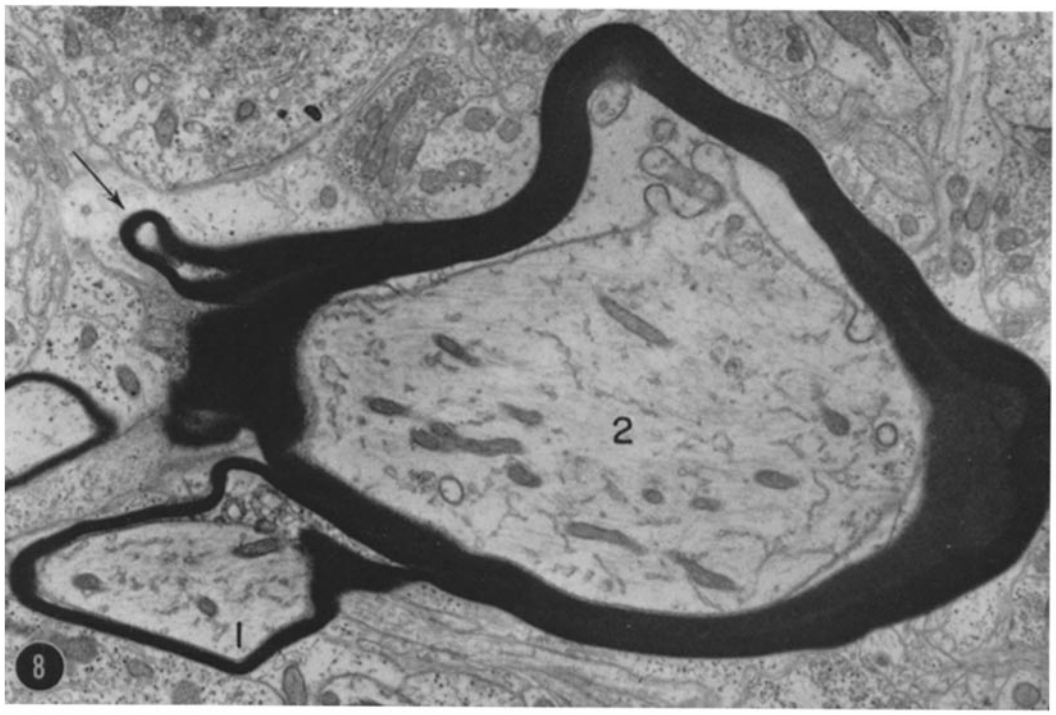


FIGURE 8 Almost complete secondary envelopment of axon *2* by a redundant flap originating from the sheath of axon *1*. The bulbous ending of the flap is indicated by the arrow. Light seams are visible along most of the length of the complex sheath. × 10,100.

of a redundant flap becomes compact, a comparable "seam" occurs here (Fig. 5). These vestiges of separation within such complex sheaths can be used to establish their origin from several components, even when the individual components cannot be traced to their sources in a given section.

The structural basis for the light "seams" appears to be a relative paucity of dense osmiophilic material along the intermediate lines of the lamellae in the region where fusion has occurred (Fig. 5 *b*). In normal sheaths as well, there is a gradient in the concentration of osmiophilic material along intermediate lines, which increases from either the innermost or outermost surface of the sheath to its middle. This fact is not obvious, however, except when two sheaths become fused together.

Presumably bizarre patterns, such as those in Fig. 9, in which one myelin sheath has an apparently concentric defect and the other has a thick dense band in it extending most of the way around the sheath, arise by secondary envelopment, but the section does not happen to pass through a portion of the sheath that indicates the basis for its configuration. One might guess that process *1* in Fig. 9, judging by its content, is not an axon but rather a glial cell process and that the innermost part of its sheath is really the telescoped outermost part of a myelin sheath belonging to an axon in another plane.

SECONDARY MYELINATION OF NERVE CELL BODIES: The same process may also involve nerve cell bodies, in particular, cerebellar granule cells. Myelinated axons which abut directly against the bodies of granule cells, as in Fig. 10, may have slack portions of their sheaths, which follow the granule cell plasma membrane and are separated from it by a gap of ~ 150 A. The degree to which the granule cell is thus secondarily enveloped may be minimal as in Fig. 10. However, it is not difficult to find examples of granule cells half-enveloped by redundant myelin sheaths (Figs. 11 and 12), and on occasion (Fig. 13) the envelopment is almost complete, save for a "pore" through which a process emerges from the neuron.

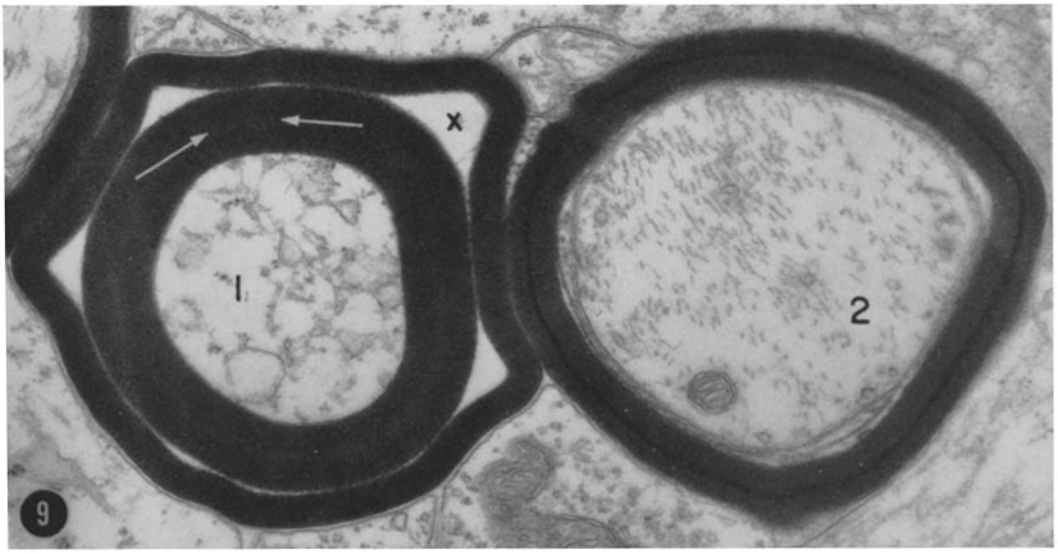


FIGURE 9 Unusual myelin configurations. Although process *1* looks at first like a myelinated axon, it does not have the cytological characteristics of axoplasm. It contains no neurofilaments and few microtubules. The innermost layer of the enveloping sheath is compact all the way around and no mesaxon is visible. The sheath has a prominent concentric defect (*x*) and, in addition, a concentric light seam is visible (arrows). This process may be either a terminal loop of glial cell cytoplasm, like those indicated by arrows in Fig. 4, whose sheath has telescoped on itself, or a dendrite which has been secondarily enveloped by an axonal myelin sheath. Process *2* has the usual characteristics of an axon. Its sheath is distinctive in that it has a dark seam separating the inner 14 lamellae from the outer 9. The seam does not extend quite all the way around the axon. This sheath is reminiscent of that surrounding a cerebellar granule cell in Fig. 13 with the "terminal loops" fused. × 27,600.

The redundant sheath may be manifestly derived from an axonal sheath (Figs. 10, 11, and 15) or its source may not be clear (Figs. 12 and 13). In the latter case, both ends of the redundant sheath are bulbous and contain what is presumably glial cell cytoplasm. Such sheaths, like those in Figs. 4 and 9, are probably connected to axonal sheaths in other planes of section, although the possibility remains that they are isolated entities which nowhere enclose an axon. Although the granule cells are closely packed together, a redundant sheath typically follows the contour of a *single* granule cell; it does not meander at random among granule cells and relate to several of them along the way.

In addition to partially ensheathed nerve cell bodies, it is also possible to find examples of granule cells which appear to be completely surrounded by myelin, with no interruption of any kind in the sheath. Sometimes, as in Fig. 14 *a*, such a sheath offers little hint as to its origin except, as in this case, for the barest suggestion of a light seam in the middle of the sheath. In other cases, however, there is an obvious seam in the middle of the sheath as well as frank separation of the sheath into two components at the points where it is angulated (Fig. 14 *b*). Thus, even when a nerve cell body appears to be completely ensheathed by myelin, it is still possible to find evidence for secondary envelopment by myelin which is derived from an axonal sheath.

The three-dimensional structure of such a myelin sheath around a nerve cell body can only be surmised from an individual section. Serial thin sections through a structure as large as a granule cell would be virtually impossible. However, such sheaths have been followed through serial 1-μ sections, and it is clear that in some instances, at least, the granule cell is not surrounded by a narrow equatorial band of myelin but is almost completely covered by myelin. In such a series it is also usually possible to identify the axonal myelin sheath from which the redundant segment arises (Fig. 14 *c*).

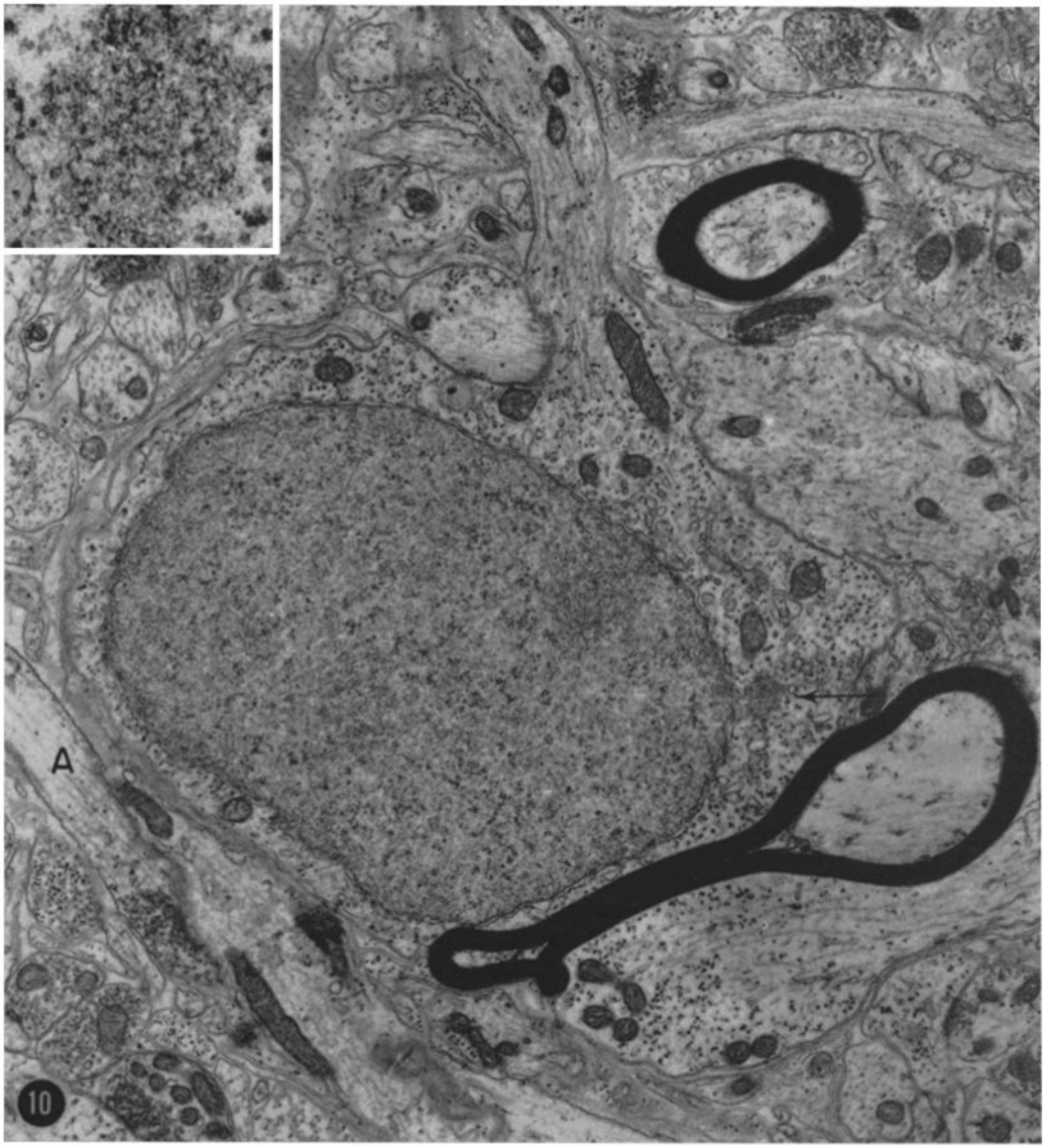


FIGURE 10 Cerebellar granule cell. At the lower right a collapsed segment of an axonal myelin sheath is closely applied to the plasma membrane of the cell, separated from it by about 150 A. A process containing many microtubules extends from the granule cell body toward the top of the figure. Arrow shows a cytoplasmic inclusion resembling nucleoplasm. *A*, preterminal region of axon. Inset: detail showing the cytoplasmic inclusion indicated by the arrow. The inclusion is composed of finely granular and thready material and is not surrounded by a membrane. Fig. 10, × 12,500; inset, × 89,700.

ISOLATED MYELIN LAMELLAE: Cerebellar granule cells may be partially enwrapped not only by thick folds of axonal myelin, as described above, but also by individual lamellae of myelin. An example is illustrated in Fig. 15 where it can be seen that the upper granule cell is separated from the cellular process to its right only by a cleft of ~ 150 A bounded on either side by the respective plasma membranes of the cell and the process. On the left side, however, the junction between this

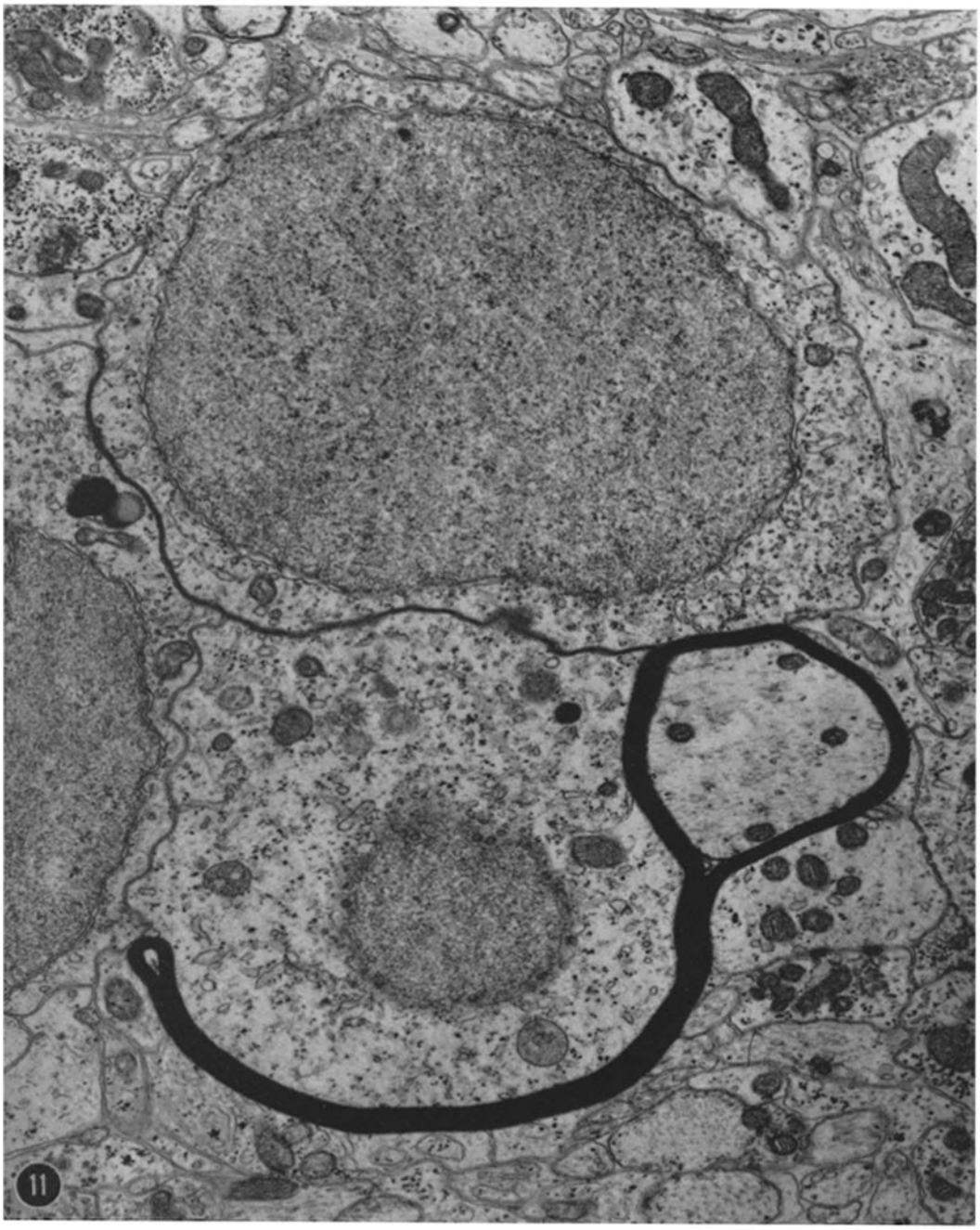


FIGURE 11 Parts of three contiguous cerebellar granule cells. The lowermost cell is about half-enveloped by a redundant axonal myelin sheath which appears completely compact. The uppermost neuron is separated from the other two by a single lamella of myelin (cf. Figs. 15 and 16). × 14,900.

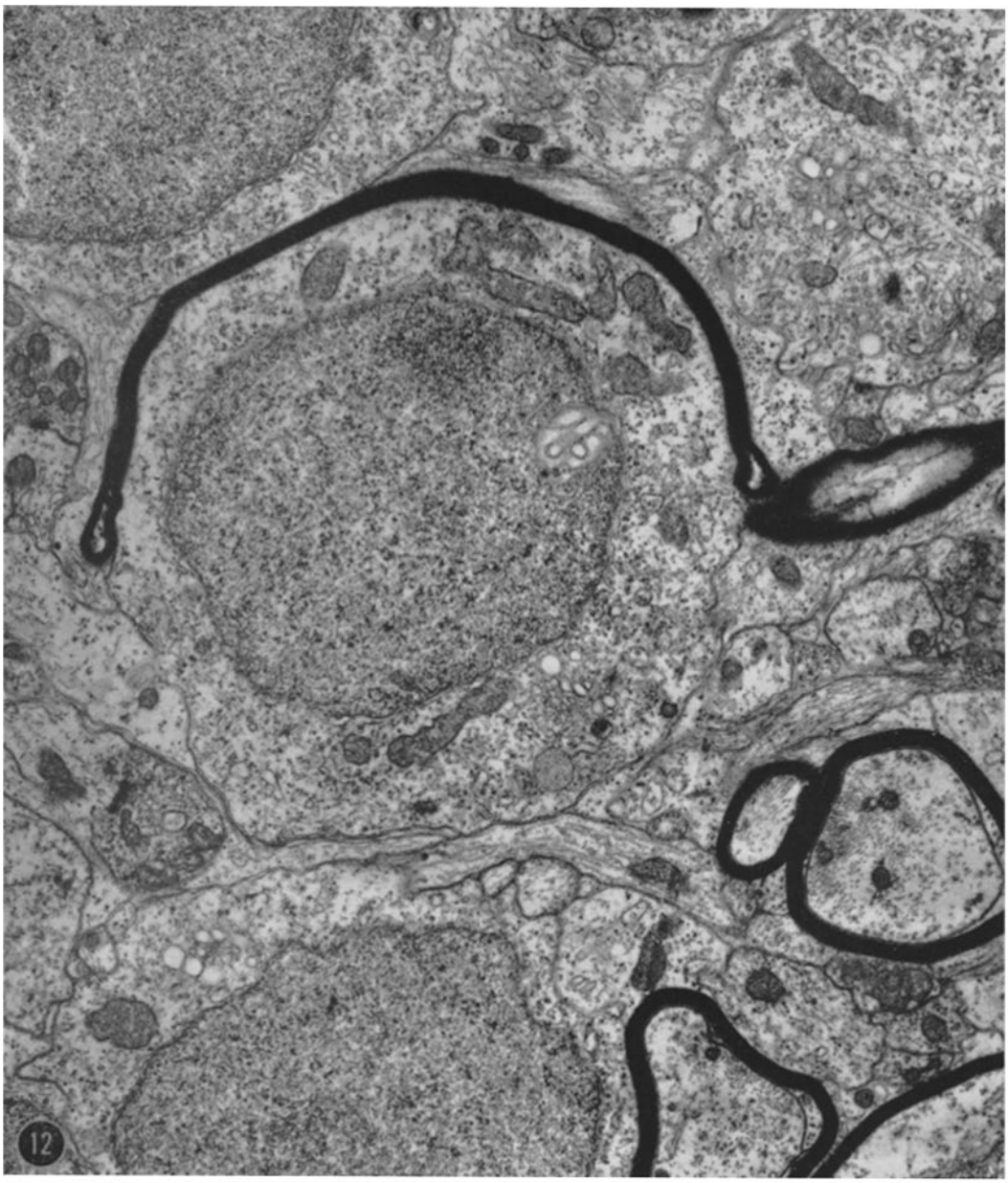


FIGURE 12 Cerebellar granule cell about half-enveloped by myelin. In this instance the collapsed myelin sheath is not attached to an axonal myelin sheath in the plane of the section. The lower right corner of this field overlaps that shown in Fig. 13. × 11,500.

cell and the neighboring process appears unusually dense and distinct owing to the presence of an extra membrane in the extracellular space between them. This membrane amounts, in effect, to a single layer of myelin which extends about a third of the way around the cell. At higher magnification (Fig. 16), such a single lamella interposed between two cell membranes can be seen to split to enclose a thin layer of cytoplasm both at its end and along its length. This observation establishes the origin of the single lamella from two plasma membranes fused along their inner surfaces quite like those comprising multilaminate myelin sheaths. Indeed, in some in-

stances such a single lamella appears to be nothing but an extension of the outermost layer of a thicker myelin sheath (Fig. 11).

Occasionally, single lamellae of myelin surrounding adjacent granule cells come together to form two myelin lamellae separating the two cells. The mode of their apposition is quite like that which occurs between thicker myelin sheaths in the central nervous system.

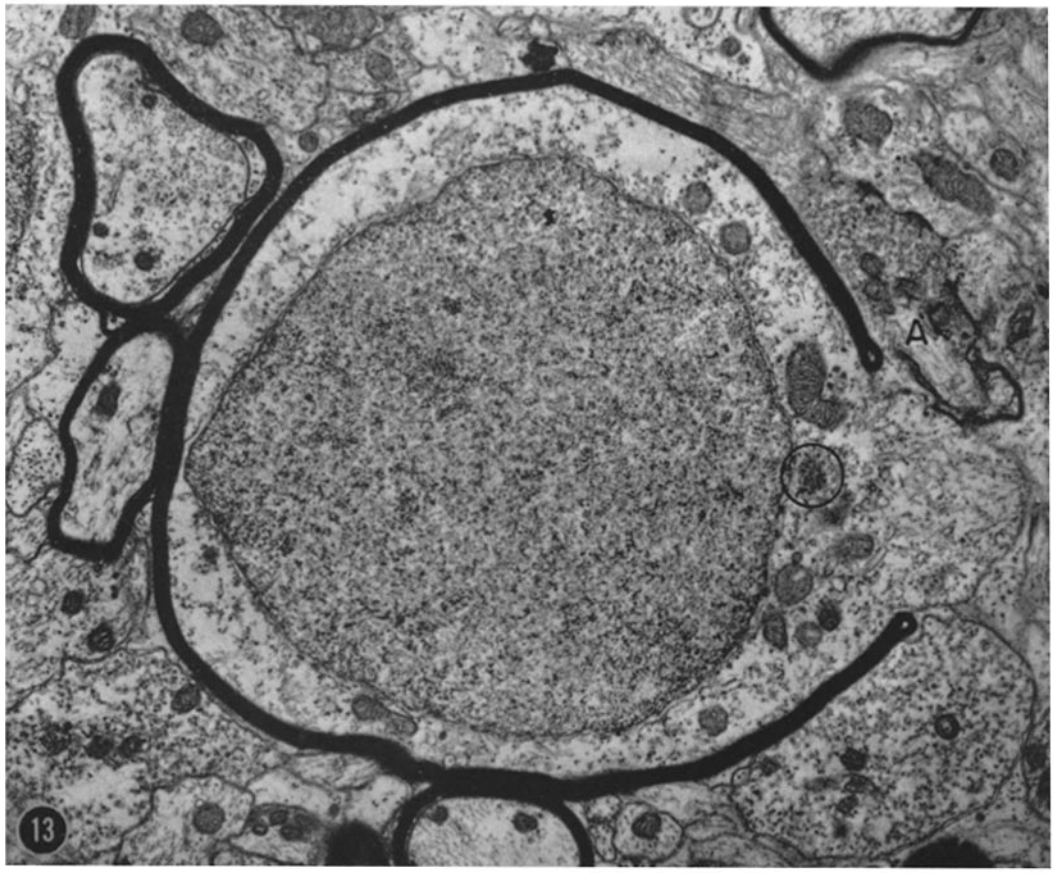
Apparently, such single and double lamellae of myelin are also capable of the same kind of buckling and folding as occurs in the thicker myelin sheaths. Fig. 18 shows a neuronal process, a short segment of which is ensheathed by a collar of myelin four lamellae thick. From the open loop formation at the ends of the sheath, it is apparent that this is really a two-layered sheath which has doubled on itself in just the same manner as illustrated in Fig. 12 for a thicker sheath.

Isolated myelin lamellae may enwrap granule cells almost completely. They have also been found around Purkinje cell dendrites and have been seen as well in the forebrain, not only around cell bodies but also around processes of small caliber (Fig. 17).

DISCUSSION

In summary, the observations show that some of the myelin sheaths in the central nervous system of normal adult toads have slack or redundant portions quite like those described by Webster and Spiro in the peripheral nerves of normal adult guinea pigs. In the toad brain, the slack portions of the sheaths tend to enwrap other already myelinated axons, small nerve cell bodies, especially cerebellar granule cells, and probably also segments of dendrites and glial cell processes. Isolated lamellae of myelin also occur and appear to undergo the same kind of folding. In addition, it is shown that lamellae of myelin are not identical throughout the thickness of a sheath. Accumulations of osmiophilic material along intermediate

FIGURE 13 Granule cell almost completely enveloped by myelin. Terminal loops of glial cell cytoplasm occur at both ends of the sheath. No separation or light seam is visible within the sheath. From the unensheathed portion of the granule cell, a process emerges. Immediately adjacent to the nucleus there is another example (circle) of a nonmembrane-bounded cytoplasmic inclusion which resembles nucleoplasm. *A*, axon terminal. × 11,500.



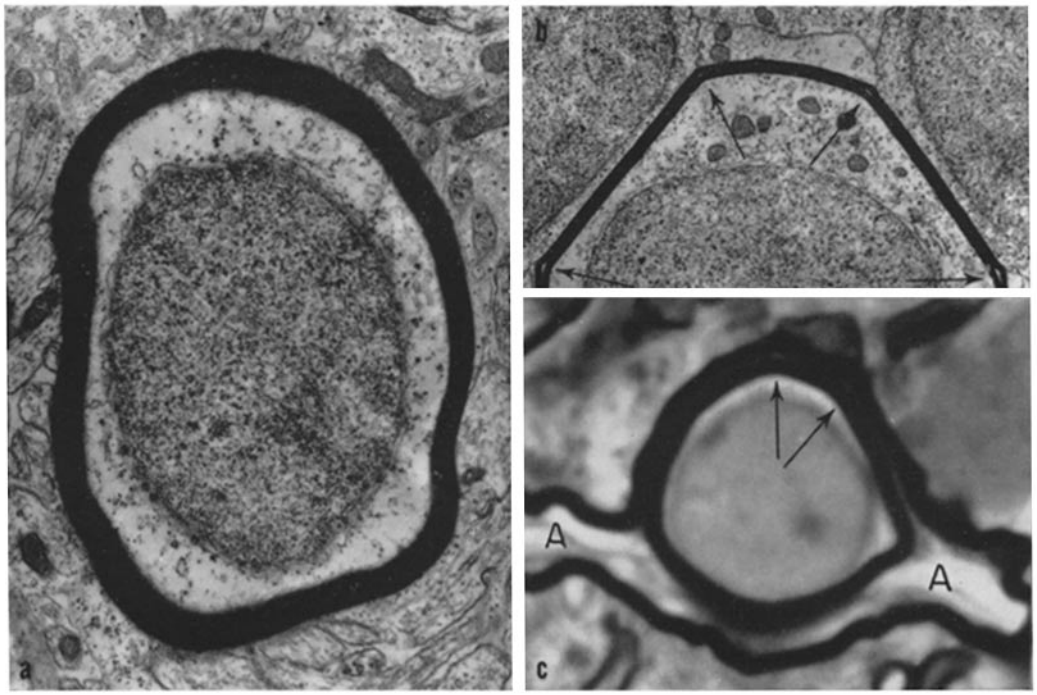


FIGURE 14 *a* Cerebellar granule cell apparently completely enveloped by myelin. There is a vague suggestion of a light seam in the middle of the sheath. × 11,600.

FIGURE 14 *b* Another such cell. Here the enveloping sheath manifestly consists of two components which separate at arrows, where the sheath is angulated. × 6700.

FIGURE 14 *c* Photomicrograph showing a cerebellar granule cell completely enveloped by a redundant flap of myelin. The flap is clearly a continuation of the myelin sheath covering an axon (*A*). Separation of the redundant sheath into its two components is clear, particularly at the points where the sheath is angulated (arrows). × 3600.

lines are more common in the deeper layers of the sheath than in those near its inner or outer surfaces.

The postulated process of secondary envelopment by flaps of redundant myelin is of importance because it accounts for the occurrence of certain bizarre patterns of myelin in the central nervous system and for the occurrence of myelin in unexpected places such as around nerve cell bodies and glial cell processes. Examples of apparently myelinated cell bodies have been seen in the brain stem of a rat (52) and in tissue cultures of rat cerebellum (19), midbrain (19), and spinal cord (3). Redundant myelin sheaths have likewise been noted in the central nervous system of adult (17, 33) and developing (23, 32) mammals and in cultured specimens (51) as well. The phenomenon of secondary myelination is probably a general one and, although not extensive enough to have much effect on conduction velocity, it may have significant theoretical implications, several of which will be considered here.

DECREASE IN AXONAL SIZE: The slack portions of axonal myelin sheaths could develop as a result of reduction in either the caliber or length of large myelinated axons, resulting in buckling and folding of their investing sheaths and in the development of redundancies. Presumably, the envelopment of nearby processes by the slack portions of the sheath would then occur in a

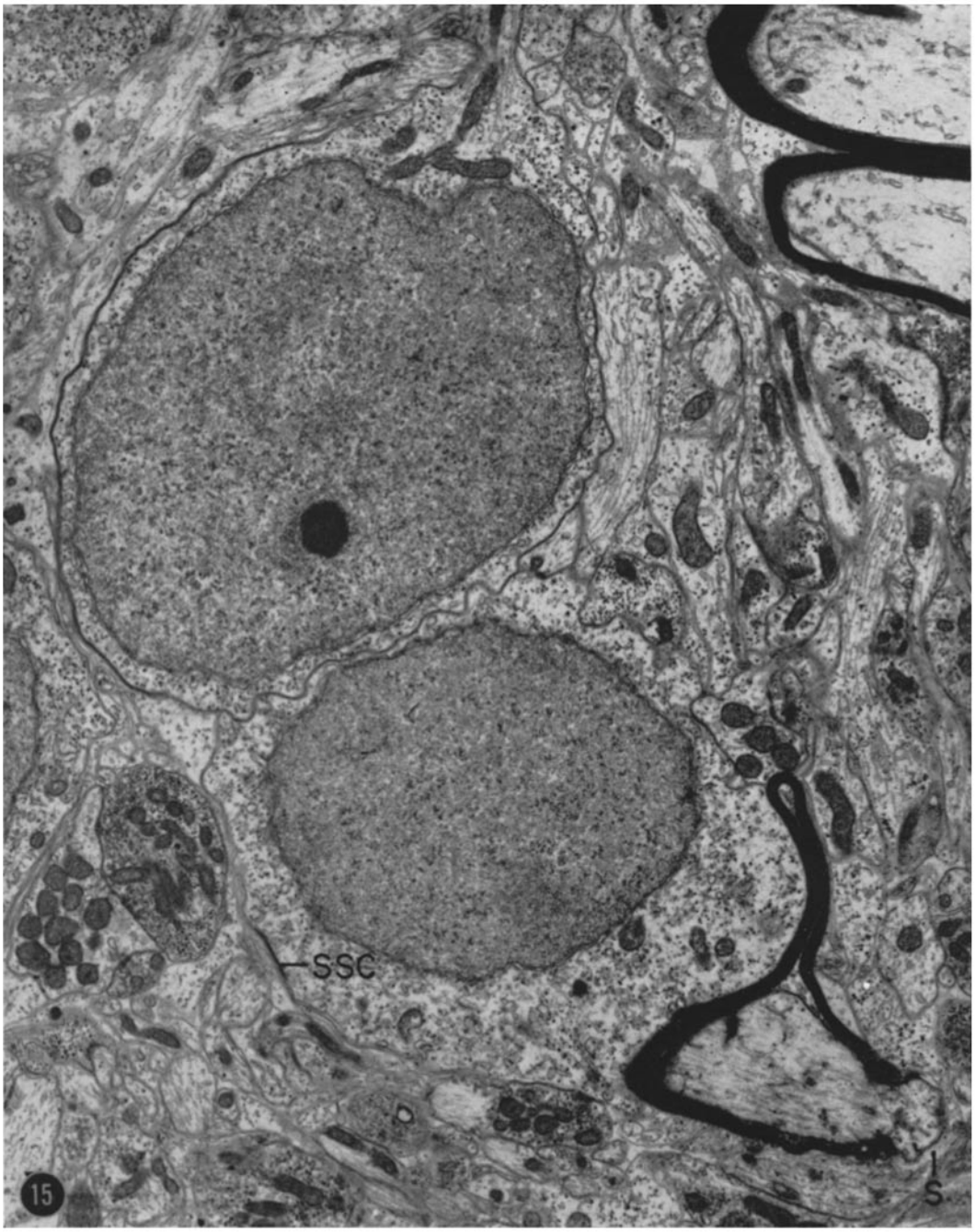


FIGURE 15 Adjacent cerebellar granule cells. The right border of the upper cell is separated from the neighboring process by the usual cleft of ~ 150 A. At the left side of the cell, the intercellular cleft contains a single lamella of myelin. The lower neuron is partially enwrapped by a flap of myelin which arises from the paranodal portion of an axonal sheath. Axodendritic synapse at the lower left shows many mitochondria in both pre- and postsynaptic processes. Note widened extracellular space (*S*) around node of Ranvier. *SSC*, subsurface cistern. × 9600.

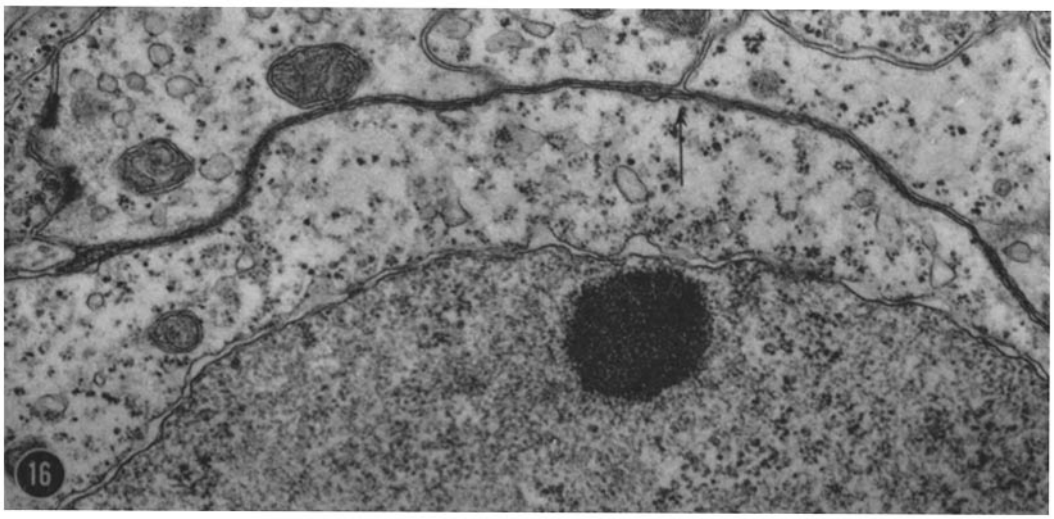
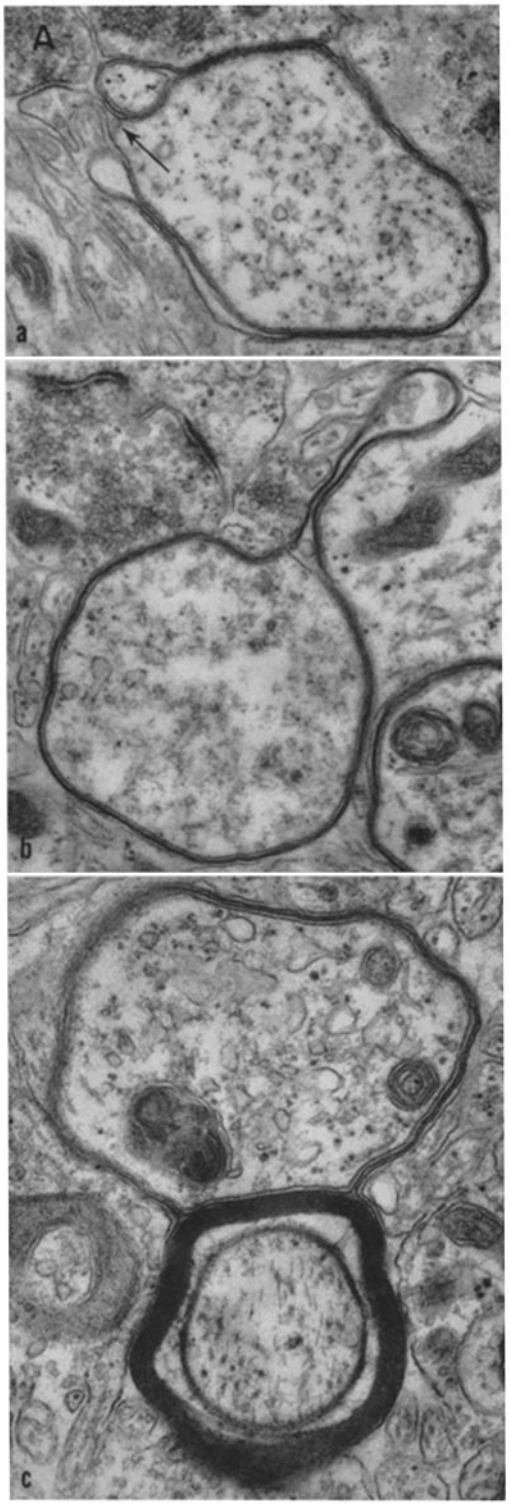


FIGURE 16 Segment of a granule cell which is approximately three-fourths–surrounded by a single lamella of myelin. The lamella splits to enclose cytoplasm at the arrow and terminates in a small loop of cytoplasm at the extreme left. × 24,100.

random manner. Reduction in length or girth of an axon might result from a low-grade degenerative process, and the occurrence of myelin redundancies might then be an early sign of a pathological or aging process characterized by a waning of axonal diameter. In addition, it is possible that axonal caliber changes in response to level of physiological activity, and that animals maintained in the laboratory, protected from the vicissitudes of nature, may develop a kind of disuse atrophy of certain parts of the brain. Finally, axons may not be rigidly fixed in position, but may migrate. An axon which establishes a connection by a slightly devious route may in time shift its position and become shorter and straighter. Thus there are a number of possible reasons for reduction in either length or diameter of a myelinated axon and for the development of slack in its sheath.

Although this hypothesis is a tenable one, it does not seem likely, for the redundant flaps of myelin do not in fact enwrap other processes at random, but, particularly in the case of granule cells, seem to stick to one cell body. Furthermore, when the origin of a redundant sheath from an axonal sheath is visible, the latter can be seen to fit the enclosed axon snugly except for the one point along the circumference of the axon where the redundant portion of the sheath arises. It is not a generally loose-fitting sheath.

OVERGROWTH OF MYELIN: An alternative possibility is that redundant myelin sheaths develop as a result of active overgrowth of myelin, in response to an appropriate stimulus. During the past ten years there has been much emphasis on the derivation of myelin from wrappings of Schwann cell or glial cell processes around axons. Yet it is clear that myelin formation of another sort must continue beyond the wrapping phase in order to account for the appearance of myelin in the adult animal. Several studies (2, 18, 20, 45, 48) have shown that, as nerve fibers grow, the number of internodes per fiber remains the same, but the length and girth of each internode increases. The inference that the segment of myelin covering each internode is "stretched" (2, 20, 48) by the growing fiber is, however, untenable. Obviously, since there is no thinning of the sheaths, or reduction in the number of lamellae, there must be in fact a net increase in the quantity of myelin, and it is therefore necessary to postulate synthesis of new myelin as the axon elongates. Since all of the myelin lamellae, including the innermost one, elongate as the axon grows, this second phase of myelin "growth" cannot be accounted for merely by further wrapping of glial or Schwann cell processes. It probably entails direct enlargement of the lamellae either at the paranodal edges (24, 45) or at Schmidt-Lanter-



mann clefts (24). In both locations, cytoplasm persists. Thus, in short, there is experimental evidence to support two phases of myelinogenesis: (*a*) a primary phase during which lamellae are laid down rapidly by a wrapping process, and (*b*) a prolonged secondary phase during which lamellae increase in both length and girth to keep pace with the growth of the axon.[3]

Quantitatively, much more myelin may be formed during the second phase than during the first phase. In a sciatic nerve fiber, for example, if an internode increases fivefold in length and twofold in diameter (2) from the time it is originally myelinated until the animal is fully grown, and if the thickness of the myelin remains the same, then the quantity of myelin of this internode must increase about tenfold during this period. In other words, 90% of the myelin covering this internode is synthesized during the secondary phase of myelinogenesis, and only 10% during the primary, or wrapping, phase.

An overgrowth of myelin during the secondary phase would account for the myelin redundancies described here and for those in the peripheral nervous system described previously by Webster and Spiro. Whatever the stimulus for this kind of growth is normally, it may, under certain circumstances, lead to excessive production of myelin beyond the needs of the enclosed axon, and thus result in the formation of oversized sheaths. Or the stimulus may persist beyond the period of axonal growth, and result in continued enlargement of myelin sheaths in the fully grown animal.

The vigor with which specimens of cerebellum

[3] The two phases may not be mutually exclusive; i.e., some lamellar growth may occur during the wrapping phase, as Robertson suggests (35), and some additional wrapping may occur during the secondary growth phase, resulting in a gradual increase in sheath thickness.

FIGURE 17 *a* Dentritic process from the forebrain, almost completely surrounded by two lamellae of myelin. At the arrow a tiny process of the dendrite extends out and establishes synaptic contact with an axon terminal (*A*). × 24,800.

FIGURE 17 *b* Unknown process, probably glial, from the forebrain, completely enveloped by two lamellae of myelin. × 27,400.

FIGURE 17 *c* Another such process surrounded by two lamellae of myelin. In this instance the origin of these lamellae from the paranodal portion of a compact myelin sheath is manifest. × 38,200.

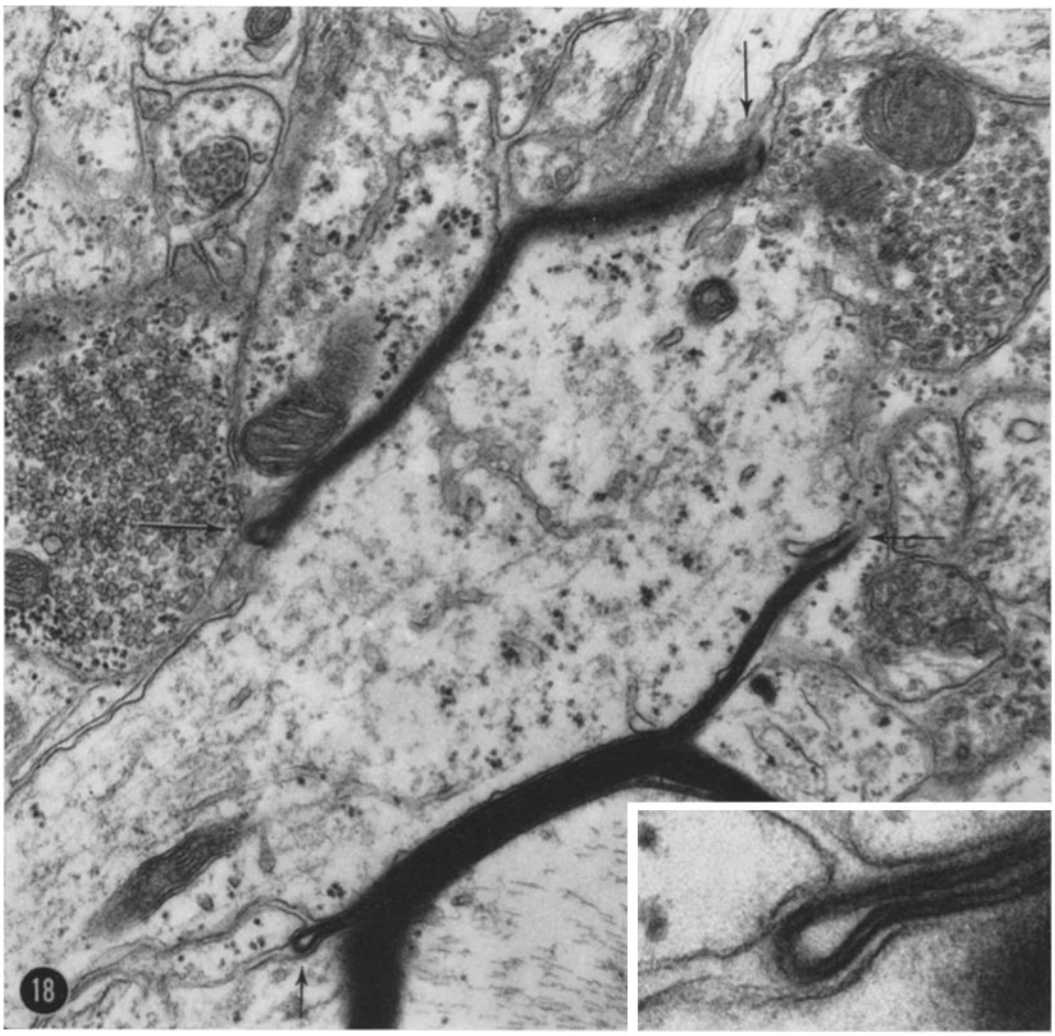


FIGURE 18 Neuronal process which bears a collar of myelin (arrows) four layers thick. The terminal loop indicated by the lower left arrow is enlarged in the inset. Inset: terminal loop of the collar showing two myelin lamellae doubling on themselves to form a four-layered sheath. Fig. 18, × 29,500; inset, × 121,000.

acquire myelin in culture as compared with other parts of the nervous system (15) correlates with the prominence of redundant myelin sheaths in the cerebellum in vivo and suggests that the cerebellum may provide a particularly favorable climate for myelinogenesis. The faithfulness with which redundant flaps of myelin follow the contour of granule cells suggests in addition that these cells might be a source of the stimulus.

The question of what the nature of the myelinogenetic stimulus might be is merely part of the more general question of what accounts for the production of myelin in some locations but not others, around axons but not dendrites (except those of sensory ganglion cells), around large axons (except their initial and terminal segments) but not small ones, around the axons of vertebrates but not those of most invertebrates, around the perikarya of acoustic ganglion cells but not around those of other peripheral ganglion cells.

From the data presented here, it is not possible to decide which, if either, of the two general mechanisms discussed above, i.e. diminution in axonal size or overgrowth of myelin, is responsible

for the occurrence of myelin redundancies. Since configurations of this kind have been seen in specimens of cultured nervous tissue, it might be feasible to resolve the question by serial observation of cultured specimens. In any case, it is clear that a mechanism other than simple glial cell wrapping is operating to produce the configurations described here.

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