The Fine Anatomy of the Optic Nerve of Anurans—An Electron Microscope Study*

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PLATES 35 TO 42

ABSTRACT

In the optic nerve of Anurans numerous myelinated and unmyelinated axons appear under the electron microscope as compact bundles that are closely bounded by one or several glial cells. In these bundles the unmyelinated fibers (0.15 to 0.6 μ in diameter) are many times more numerous than the myelinated fibers, and are separated from each other, from the bounding glial cells, or from adjacent myelin sheaths, by an extracellular gap that is 90 to 250 A wide. This intercellular space is continuous with the extracellular space in the periphery of the nerve through the numerous mesaxons and cell boundaries which reach the surface. Numerous desmosomes reinforce the attachments of adjacent glial membranes.

The myelinated axons do not follow any preferential course and, like the unmyelinated ones, have a sinuous path, continuously shifting their relative position and passing from one bundle to another. At the nodes of Ranvier they behave entirely like unmyelinated axons in their relations to the surrounding cells. At the internodes they lie between the unmyelinated axons without showing an obvious myelogenic connection with the surrounding glial cells. In the absence of connective tissue separating individual myelinated fibers and with each glial cell simultaneously related to many axons, this myelogenic connection is highly distorted by other passing fibers and is very difficult to demonstrate. However, the mode of ending of the myelin layers at the nodes of Ranvier and the spiral disposition of the myelin layers indicate that myelination of these fibers occurs by a process similar to that of peripheral nerves.

There are no incisures of Schmidt-Lantermann in the optic myelinated fibers.

INTRODUCTION

The optic nerve of Anurans offers several advantages for an electron microscope study of central tracts. Notably, it has no nerve cell bodies and/or dendrites—a condition that facilitates the recognition of axons and glial cells that are fre-

quently difficult to identify in electron micrographs of other parts of the central nervous system (CNS). This fact, together with the average parallel arrangement of the axons, simplifies the cellular relations and facilitates the unraveling of the fine anatomy of the nerve.

Three basic anatomical questions can be studied with advantage in this nerve. These refer to: (*a*) the myelination of central medullated axons; (*b*) the organization of central nodes of Ranvier; and (*c*) the relations of unmyelinated axons to each other, to glial cells, and to myelinated fibers. The first of these questions is of particular interest because it has been contended (Luse, 1956; De Robertis *et al.*, 1958) that myelination in the CNS occurs by cellular mechanisms entirely different from those found in peripheral nerves (Geren, 1954; Robertson, 1955). The last two questions, in addi-

tion to their particular anatomical interest, are of special significance in the study of impulse conduction and in the question of independence of impulse transmission in central tracts (Howland *et al.*, 1955).

Also, the optic nerve of Anurans has an additional interest because of its notable regenerative capacity (Sperry, 1951). This property makes it a valuable tool in the experimental study of the anatomy of the CNS, given an adequate knowledge of the structure of the nerve and of its fiber population.

In a previous communication (Maturana, 1959) I reported that the number of axons in the optic nerve of Anurans had been underestimated by a factor of 30, and that this was due mainly to the peculiar anatomy of the nerve. In this paper I shall describe in detail this fine anatomy as observed under the electron microscope. I shall especially consider the organization of nodes of Ranvier and the disposition of the unmyelinated axons, and shall present evidence indicating that myelination occurs in the CNS by a process similar to that of peripheral nerves. I shall show that the differences are mainly the result of the absence of connective tissue and the absence of exclusive relationships between the glial cells and the myelinated and unmyelinated axons.

Materials and Methods

The optic nerves of various adult Anurans (*Hyla cinerea, Rana pipiens, Bufo americanus,* and *Bufo terrestris*) and of tadpoles of *Rana catesbeiana* were fixed in 1 per cent aqueous solution of OsO_4 immersed in an ice and water mixture. The fixative was buffered at pH 7.35 with veronal-acetate buffer (Michaelis, 1931), modified to make the final solution istonic to 0.75 per cent NaCl. The tissues were kept in the fixative at 0°C. for 4 hours and then washed in distilled water, dehydrated, and embedded in methacrylate. Thin sections were examined with an RCA, EMU-2D electron microscope. Conventional paraffin sections were also prepared after fixation of the nerve in a 10 per cent formol saline solution. These sections were stained by Weigert's and Holmes's methods.

OBSERVATIONS

The following description of the anatomy of the optic nerve applies to all of the Anurans that were studied. There are minor differences of detail in the various species, but these will not be considered in the present account.

A. General Remarks; Light Microscopy:

In the Anurans studied the optic nerve constitutes a single cylindrical tract about 450 μ in diameter, not grossly subdivided into bundles (Fig. 1). Myelinated and unmyelinated axons are, on the whole, uniformly distributed across the nerve, and there are no major variations in density of fiber population from one point to another. At the surface the glial cells are in direct contact with the pia mater, and usually separate it from the underlying axons. The blood supply to the nerve is provided by a loose net-work of capillaries that ramify within the pial sheath and, for the most part, remain superficial. The few capillaries that penetrate the nerve do so unaccompanied by a pial sheath.

The medullated axons (0.7 to 5 μ in diameter, but mostly below 1.5 μ) have a grossly parallel course. In detail, however, they follow a sinuous path, shifting their relative position along the nerve. As a result of this winding course each myelinated axon makes frequent contacts with other medullated fibers. At the regions of contact, which may be several microns long, the myelin sheaths of the adjacent fibers are firmly attached to each other (Figs. 4, 6) and when submitted to stress they can be deformed or torn instead of separated. No glial cytoplasm is present between the fibers at these regions, and the touching myelin sheaths appear as if they were continuous from one fiber to the other. Differences in intensity of coloration after myelin stain, however, frequently permit one to recognize the line of confluence of the two sheaths. Several myelinated axons may be simultaneously in contact (Fig. 4).

As is the case in central myelinated fibers in general, these axons do not have neurilemma, and no individual relationship can be observed between a fiber and a glial cell when examined under the light microscope.

The density of the population of myelinated fibers is relatively low, and between them there is abundant space that is entirely occupied by glial cells and unmyelinated axons. In fact, medullated axons occupy less than 20 per cent of the cross-section area of the nerve.

Nodes of Ranvier in the optic nerve (and in the central nervous system of Anurans in general) are several times longer than peripheral nodes, measuring 3 to 7 or more microns in length. Because of the thinness of the myelin sheaths (mostly between 0.15 and 0.25 μ, measured in electron micrographs) these nodes lack the usual appearance of a marked constriction (Fig. 21). They occur at intervals of 60 to 150 μ, and any internodal length within this range seems possible for any adult optic fiber. No incisures of Schmidt-Lantermann have been observed.

The unmyelinated fibers cannot be properly studied with the light microscope. They can be stained with a variety of methods (such as the vital stain with methylene blue or several silver stains), but because of their thinness and special arrangement in compact bundles (see Section B) they can be individually resolved only if a small proportion of them is stained, and only if these stand isolated amid unstained ones. Light microscopy does not allow for the resolution of the interfiber relations of the unmyelinated axons or of the relations between these and the surrounding glial cells, but with such stains it can be detected that these axons also follow a sinuous path, constantly changing their position along the nerve.

Only one type of glial cell has been observed.

B. Electron Microscopy:

The electron microscope reveals the optic nerve as a compact epithelial structure in which there are no large intercellular spaces and all cellular membranes appear in direct contact with each other, separated only by an extracellular gap of about 90 to 250 A wide. The width of this extracellular gap varies continuously within these limits in each preparation—a fact that strongly suggests that the amount of extracellular space may depend on the physiological state of the various cells that delimit it.

Unmyelinated Axons.—The unmyelinated fibers appear in transverse sections as closed circular or sinuous profiles of 0.15 to 0.6 μ in diameter, but mostly between 0.2 and 0.3 μ (Figs. 6, 7, 9). They form large irregular bundles of many closely packed axons surrounded by glial cell expansions and myelinated fibers. In these bundles the membranes of the unmyelinated axons appear separated from each other by a variable gap of extracellular space of 90 to 250 A wide. A similar gap of extracellular space separates them from the outer myelin layer of adjacent myelinated axons and from the surrounding glial membranes (Figs 6 to 8). This intercellular space is continuous with the extracellular space at the periphery of the nerve through the mesaxons formed by the glial cells (Figs. 6, 19; Text-fig. 1). It seems that several bundles of unmyelinated axons are enclosed by a single glial cell, and that often several of these groups are enclosed within the same mesaxon (Fig. 6 and Text-fig. 1).

As a result of their thinness, their sinuous path, their frequent shifting of relative position, and their passing from one bundle to another, the unmyelinated axons can be studied in longitudinal
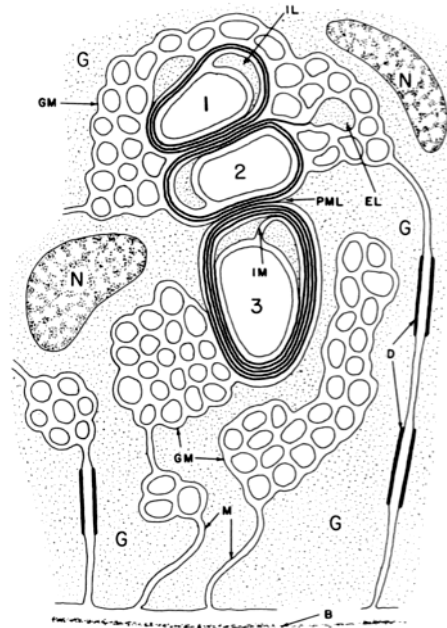
TEXT-FIG. 1. Reconstruction of the relationships between axons and glial cells in a transverse section of the optic nerve of Anurans. Thin lines represent single glial or axonal membranes; thick lines represent myelin layers that result from the fusion of two glial membranes. Axons *1* and *2* are depicted entirely in the intercellular space, surrounded by unmyelinated axons and glial cells. The line of cleavage between them passes through an interlayer space continuous with the extracellular space between the surrounding unmyelinated axons (see Fig. 8). Between axons *2* and *3* a partial myelin layer has been drawn (arrow *PML*). This condition seems to occur rather frequently, but it is difficult to demonstrate. Axon *3* was drawn as if it had an external mesaxon, but the two glial membranes that should form it are shown separated by two groups of unmyelinated fibers; again, a condition difficult to demonstrate but one that appears to occur frequently as a factor of distortion of the relations between myelin sheath and myelogenic glial cell. Care was taken to show that the distance between myelin layers is smaller than that between single membranes. In axon *3* the first myelin layer is shown split and forming a large cytoplasmic island, a condition that contributes to the persistence of the internal mesaxon. The rest of the reconstruction is self-explanatory. *IL:* internal loop; *EL:* external loop; *PML:* partial myelin layer; *IM:* internal mesaxon; *M:* mesaxon; *G:* glial cytoplasm; *GM:* glial membrane; *D:* desmosomes; *N:* glial nucleus; *B:* basement membrane.

sections in short segments only (Figs. 16, 17, 20). In these the unmyelinated axons exhibit, in addition to the characteristics already described, a cytoplasm that is rich in neurofilaments. As judged
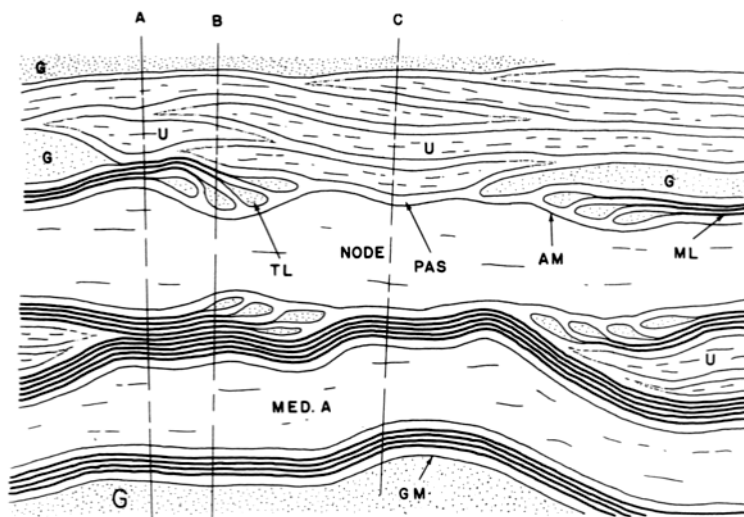
TEXT-FIG. 2. Reconstruction of a longitudinal section of a node of Ranvier to show its relations with the mye-linated and unmyelinated adjacent axons. Note that the circumnodal space is continuous with the periaxonal space under the myelin and with the intercellular space between the surrounding fibers. At the nodes the myelinated axons behave like the unmyelinated ones in their relations with the surrounding cells. *U:* unmyelinated fiber; *Med. A:* medullated axon; *PAS:* periaxonal extracellular space; *AM:* axolemma; *GM:* glial membrane; *ML:* myelin layer; *TL:* terminal loop; *G:* glial cytoplasm.

by their usually greater contrast, the unmyeli-nated axons appear to have a greater concentration of neurofilaments than the myelinated ones. In very thin sections these filaments appear to be about 70 A in diameter and of indefinite length. In longitudinal sections it can also be seen that axo-lemmas of myelinated and unmyelinated fibers, as well as glial membranes, frequently break to form small vesicles. The significance of this observation is unknown, and it is uncertain whether the vesicles originate in a normal process of vesiculation or are the result of an artefact of fixation.

Myelin Sheath.—In the medullated axons the myelin sheath is only a few myelin layers thick, rarely less than five or six in the internode. These myelin layers form a compact myelin system with a period of about 80 to 110 A and an interlayer distance of 40 to 60 A (Figs. 6 to 9, and 15). No intraperiod line has been observed. Similar to those in peripheral nerves (Geren, 1954; Robertson, 1955, 1959; Luxoro, 1956, 1958), these myelin layers result from the fusion of two membranes; in this case, from two glial membranes, that fuse when facing each other by their cytoplasmic sur-faces. This is made obvious at those points where the myelin layers split and form islands or terminal loops that contain glial cytoplasm, as occurs at the most internal and external myelin layers of the sheath and at the nodes where the myelin ends

(Figs. 7, 9, 10, 15, 18, and Text-figs. 1 to 3). Fre-quently, outside the compact myelin and occasion-ally in the myelin sheath itself, two glial mem-branes may approach each other by their cytoplasmic surfaces to distances of about 30 A without fusing (Figs. 2, 11, 12). This occurs often at the most internal myelin layer immediately around the axon and when the external myelin layer is separated from the rest of the sheath (Figs. 2, 12, 15).

Single glial or axon membranes may approach each other by their extracellular surface to about 90 to 100 A, but not closer; they have never been observed to fuse in these circumstances. Nor do single membranes and myelin layers approach each other more closely than about 90 A, a condition in clear contrast with the interlayer distance of about 40 A, which is found in the compact myelin. This can be seen at the surface of the myelin sheath, where its most superficial layer is adjacent to the single membranes of adjacent glial cells or unmye-linated axons (Figs. 2, 6, 7, 20). The distance that separates the axon membrane of the medullated fiber from the compact myelin, when no glial cyto-plasm is left between them, is also about 100 A.

These differences in the closeness between single and double membranes have also been observed in peripheral nerves (Robertson, 1958).
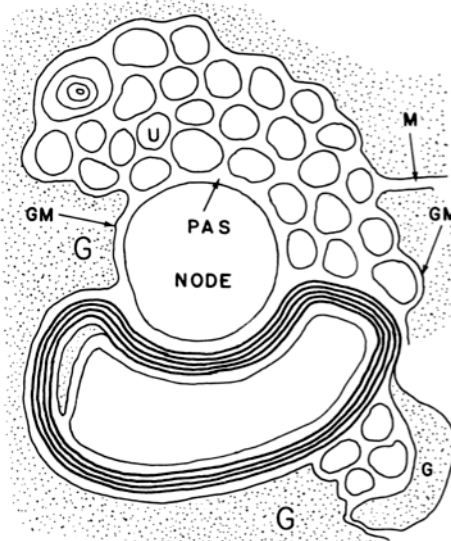
Myelinated Axons.—In transverse sections, mye-
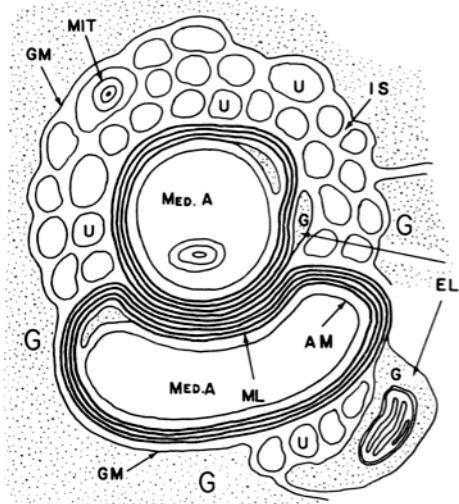
TEXT FIG. 3 *A*
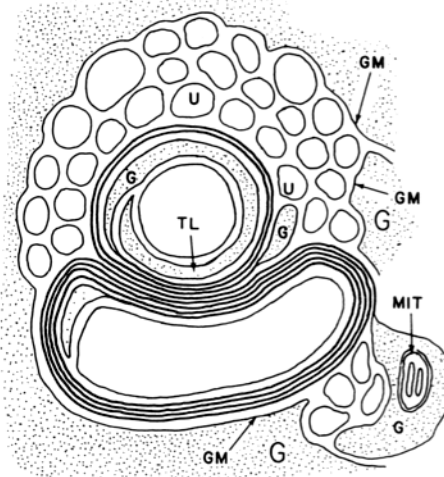
TEXT FIG. 3 *B*

TEXT-FIG. 3. Reconstructions of the fiber relations in transverse sections at the planes *A*, *B*, and *C* in Text-fig. 2; Fig. 3 *A*, a section at the level of the internode in a region of apposition of two myelinated axons; Fig. 3 *B*, a section passing at the level of a single complete turn of the terminal cytoplasmic spiral of the myelin layers; Fig. 3 *C* represents a section at the level of the node and shows the axon surrounded at that level by both other axons and glial cells. Lettering as in Text-Fig. 2. *MIT:* mitochondria; *M:* mesaxon; *IS:* intercellular space; *EL:* external loop.

TEXT FIG. 3 *C*

linated fibers may be surrounded by glial cells or unmyelinated axons or other medullated fibers (Figs. 6, 7). The extracellular gap that separates single membranes from the outer layer of the compact myelin is continuous with the intercellular space between the other cellular elements. Most medullated axons appear entirely between unmyelinated axons and glial cells without showing any myelogenic connection with the latter (Figs. 7, 9, 15). In these myelinated fibers it can be shown that the outer layer of the compact myelin spiral ends by forming a lateral loop that contains glial cyto-

plasm. This loop may lie in any position around the myelin or among the surrounding unmyelinated axons (Figs. 9, 11, 12, 15). Unfortunately, these loops, which may be extremely small, are difficult to preserve and often appear only as a small mass of cytoplasm attached to the surface of the compact myelin.

Frequently, the most internal myelin layer also forms a loop that lies between the other myelin layers and the axon membrane (Figs. 9, 12, 15). The two loops, internal and external, always point in opposite directions, as expected from the two

ends of a spiral. This condition will be discussed later in connection with the process of myelination.

A transverse section at the level of the contact between two adjacent medullated axons shows that at the region of maximal contact the two myelin sheaths form a single unit of compact myelin (Figs. 6, 8). No alteration in the spacing of the myelin layers can be observed at the line of confluence of the two sheaths. The myelin layers, however, do not mix or interchange at this point. On the contrary, in most cases, the line of cleavage between the two myelin systems passes through a clear space between two myelin layers. This space becomes the cleavage space and is continuous with the intercellular space between other neighboring structures (Figs. 8, 15, and Text-figs. 1, 3 A). This indicates that the two myelin sheaths have approached each other by the extracellular surface and that the intercellular distance between them has been reduced to the usual distance between myelin layers in the compact myelin.

It seems possible that in other cases the line of cleavage between the two myelin sheaths passes at the level of a myelin layer as suggested in Text-fig. 1, PML. This could result from squeezing off the cytoplasm in a flattened sheet of glial cell between two myelin sheaths until its two membranes fuse to form a partial myelin layer. Such fusion could occur in the structure between axons A_0 and A_1 in Fig. 11.

The loose myelin spiral of axon A_0 in Figs. 11 and 12 shows that myelin layers can exist outside the compact myelin, although in a less compact manner. Fig. 2 possibly corresponds to a longitudinal section of such a case.

Nodes of Ranvier.—Under the electron microscope, nodes of Ranvier in the optic nerve show the same basic features that have been described for peripheral nodes (Luxoro, 1956, 1958; Uzman-Geren and Nogueira-Graf, 1957; Robertson, 1957), but at the same time they show notable differences in their relations with adjacent cells, mainly as a result of the special epithelial organization of the optic nerve.

Longitudinal sections at the nodes show that the ending layers of the compact myelin split to form terminal loops that contain cytoplasm. The myelin layer closest to the axon is the first to come to an end, forming its loop farthest from the unmyelinated portion of the node. The more superficial layers are longer and end, one after the other, close to the latter region, forming a series of loops in contiguity with the axon membrane (Figs. 10, 16,

17, and Text-fig. 2). Occasionally, the more superficial myelin layers are shorter, and their terminal loops are formed on the underlying myelin and not on the axon (Figs. 10, 17). When glial cytoplasm is present around the myelin in the internode, it forms an open loop which may extend to cover the nodal axon membrane (Text-fig. 2). The membranes of the terminal loops do not fuse with the axon membrane, but leave a clear periaxonal space, 90 to 250 A wide, communicating with both the extracellular space around the node and the periaxonal extracellular space under the myelin of the internode.

The unmyelinated portion at the node may measure 3 to 7 or more microns in length. The axon here may or may not be covered by glial cells. In some cases the glial cells that surround consecutive internodes may touch each other over the node; in other cases a nearby glial cell may attach itself to the node or, more frequently, most of the node is covered by passing unmyelinated and myelinated fibers (Figs. 16, 17). Also, nodes of neighboring axons may be in direct contact with each other. Whatever the case, the circumnodal space between the axon membrane and adjacent structures is rarely more than 250 A wide, and it appears comparable to the periaxonal space around the unmyelinated axons.

The relations between the nodes and the surrounding structures are also obvious in transverse sections, especially if examined at various points at the transition from the nodes to the ending myelin. At the node the axon appears as a large unmyelinated fiber which may be surrounded by other medullated fibers, non-medullated axons, or glial cells (Fig. 13 and Text-fig. 3 C). Thus, at this level the myelinated fibers behave in their relations to the surrounding structures like any unmyelinated axon. Transverse section through the prenodal region at the ending myelin may show the axon surrounded by a ring of glial cytoplasm, which, in turn, is surrounded by a few myelin layers. In these cases, one oblique myelin lamella unites the not-yet-ended myelin layers with the glial membrane that surrounds the axon (Fig. 3 and Text-fig. 3 A). This myelin lamella is usually very difficult to preserve. If the section passes obliquely through several turns of the ending spiral, several internal mesaxons can be seen (Fig. 14).

Glial Cells.—Glial cells behave similarly in any part of the optic nerve and form a uniform population of satellite cells that fill all space between groups of fibers. They do not appear to hold ex-

clusive satellite relations with individual myeli-
nated axons or bundles of unmyelinated ones; on
the contrary, each glial cell appears to be involved
with many of them simultaneously. At the surface
of the nerve the glial membrane is accompanied by
a basement membrane 200 to 300 A thick, which
follows the irregularities of the glial membrane at a
distance of about 400 to 500 A. Numerous mesax-
ons and lines of confluence of adjacent glial cells
reach the surface of the nerve, but the basement
membrane does not penetrate them and remains
strictly superficial (Fig. 19). Beyond the basement
membrane one finds the collagen of the pia mater
forming a continuous sheath around the nerve.

Pairs of attachment plates or desmosomes occur
frequently in adjacent membranes of contiguous
strands of glial cytoplasm. These structures are
similar to the attachment plates observed in other
tissues (Fawcett and Selby, 1958; Odland, 1958).
Each plate consists of an oval or circular thicken-
ing of the glial membrane (to about 150 A) with a
diameter of 0.4 to 0.7 μ. The plates are separated
from each other by a very regular intercellular
space that is about 180 A wide and is continuous
with the intercellular space that separates the un-
modified membranes beyond them (Fig. 5 and
Text-fig. 1). The fibrillar components of the glial
cytoplasm adopt a layered disposition in front of
the plates, which may extend a few hundred A
beyond them in a less orderly fashion. In the skin,
fibrils like these were thought by Odland (1958) to
be the same as the tonofibrils of light microscopy.

Attachment plates seem to occur regularly between
neighboring glial cells, but they also seem to occur
between two arms of the same glial cell. The latter
possibility is suggested by the presence of desmo-
somes in pairs of membranes which appear as mes-
axons because they enclose bundles of axons (Fig.
6). However, myelinated and unmyelinated axons
are also retained between neighboring glial cells,
as is shown in Text-fig. 1.

Text-figs. 1 to 4 are reconstructions of the fiber
relations and general organization of the optic
nerve of Anurans; they do not correspond to the
drawing of any particular section. They were
drawn after study of many sections and are offered
as a summary of the anatomy of the nerve.

DISCUSSION AND CONCLUSIONS

Myelination.—From the preceding observations
it is obvious that in the myelinated axons of the
optic nerve the arrangement of the myelin layers
at the level of the nodes of Ranvier (as observed in
longitudinal sections) is identical with the arrange-
ment of such structures described by Luxoro (1956,
1958), Uzman-Geren and Nogueira-Graf (1957),
and Robertson (1957) for peripheral nerves. Since
this arrangement of the ending myelin is accounted
for by the process of myelination found in periph-
eral myelinated fibers by Geren (1954) (namely,
the spiral wrapping of the satellite cells or their
expansions around the axons, and the expulsion of
cytoplasm between the several turns of the spiral
to allow membrane fusion and the formation of
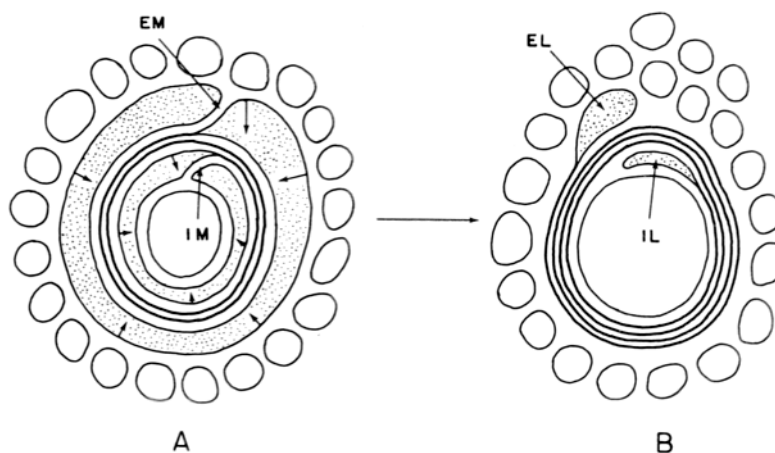
Text-Fig. 4. The purpose of this figure is to show how the external (*EL*) and internal (*IL*) loops result from
a myelinated axon in which both internal and external mesaxons are present. The transformation is from case *A*
to case *B*, by means of membrane fusion in the zones indicated by the unmarked arrows. *EM:* external mesaxon;
IM: internal mesaxon.

compact myelin), its presence in the optic nerve strongly indicates that myelination of central fibers occurs in a manner similar to that in peripheral axons. This mode of myelination is also indicated by the disposition of the myelin layers in the inter-node, with the loops of the internal and external myelin layers pointing in opposite directions, as expected of the two ends of a spiral (Figs. 9, 12, 15; Text-figs. 1, 4). Counts of myelin layers also show that their number at various points around the axon is as expected of such disposition. Also in agreement with this, we find that a cut through the prenodal region shows the periaxonal cytoplasm crossed either by the expected internal mesaxon (if the section passes through a single turn of the ending spiral loop (Fig. 3 and Text-fig. 3 C)) or by a few myelin lamellae (if the cut passes obliquely through several turns of the ending spiral loop (Fig. 14)).

The fact that typical mesaxons are extremely rare does not contradict the previous statements. Text-fig. 4 shows that the mesaxons can be partly incorporated in the compact myelin and partly in the terminal loops by an almost complete elimina-tion of the glial cytoplasm around the myelin and around the axon in the internode. When this oc-curs, one membrane of the external mesaxon fuses with the single membrane that initially surrounds the compact myelin and contributes to form a new, more superficial, myelin layer, while the other membrane remains as part of the external loop in which the new myelin layer ends. This also occurs more or less completely in peripheral nerves, as can be seen in Fig. 1 of Robertson (1955) and Fig. 8 of Robertson (1959). This loop is occasionally sep-arated from the underlying myelin by passing unmyelinated axons (Fig. 15) or glial cells (Figs. 11, 12), but more often it is not, and because of its small size it frequently appears as a poorly pre-served cytoplasmic bag on the surface of the myelin (Fig. 7). Occasionally, the most superficial myelin layer may contain an island of cytoplasm, which should not be confused with the external loop. In the internal mesaxons one of the membranes is in-corporated in the first myelin layer when it passes over the internal loop to become the second mye-lin layer (Fig. 9 and Text-fig. 4).

In transverse sections these medullated axons with an external loop appear entirely in the inter-cellular space, among other axons, glial cells, or both (Fig. 7). (A peripheral medullated fiber in similar circumstances appears surrounded by col-lagen and is thus separated from the other fibers.)

No obvious myelogenic relation could be detected between these axons and the surrounding glia. This is only because the connection between the myelin and the body of the myelogenic cell lies at some distance along the fiber beyond the section. One can easily imagine, and visualize by means of a reconstruction, how the superficial and internal loops of the myelin observed in trnasverse sections extend along the sheath as cytoplasmic cords. In other words, the myelin forms a sort of blanket with thickened edges wrapped around the nerve. The lateral edges form the terminal spiral loops that unite the internal thickening (internal cyto-plasmic cord) with the external thickening (ex-ternal cytoplasmic cord). The internal cytoplasmic cord runs uninterruptedly along the surface of the axon and unites both terminal spirals, while the external cytoplasmic cord is connected to the body (or to an expansion) of the myelogenic cell.

This arrangement can result from longitudinal growth of myelin at the internode, spreading along the axon inside the bundle. In the optic nerve mye-lination begins after most of the axons have reached their end-organs and are already arranged in bundles, but before the nerve attains its complete length (Maturana, unpublished observations). The total elongation of the myelin at the internodes during growth depends, then, upon: (a) how long the nerve becomes after the onset of myelination; (b) the length of the original internodes; and (c) the distance between internodes when they are formed initially. Before myelination starts, axons to be myelinated cannot be distinguished from those that will remain unmyelinated and, like these, they form part of the bundles without following a preferential course. Because of the sinuous path followed by the axons and because of their great number in each bundle, a particular fiber to be myelinated can make contact with the glial cells only for short lengths at a time (a few microns). If myelination begins at these regions, then, in all probability the internodes are initially very short, and must elon-gate considerably to attain their final length. During this elongation the compact myelin has to follow the axons into the bundles, in favorable con-ditions for the squeezing of the glial cytoplasm around it and the formation of the longitudinal cytoplasmic cords. In such a case, a section of an axon beyond the connection of the external cyto-plasmic cord with the myelogenic cell should show no relation between the myelin of this axon and the nearby glial cells, and, indeed, this has been ob-served. It is only if the section passes through the

region of myelogenic connection between the sheath and the glial cell that one can expect to find a typical external mesaxon.

The process discussed above requires the elongation of the compact myelin of the optic axon. The growth in length of the compact myelin has been shown by several authors (Hess and Young, 1952; Hiscoe, 1947; Thomas and Young, 1949; Thomas, 1955), who found that the internodal length in peripheral nerves increases with the size of the animal, while the number of internodes remains constant. Although very little is known about this growth, it seems reasonable that active cytoplasm is necessary to supply the material for the formation of new myelin. This cytoplasm is present only at two places in the compact myelin sheaths: at the terminal spiral end formed by the ending myelin in the prenode, and at the incisures of Schmidt-Lantermann (Luxoro, 1956, 1958; Robertson, 1958). In these circumstances, one could expect the elongation of the sheath to occur by growth of the compact myelin at the prenode and the incisures, rather than by elongation of the compact myelin itself. If this supposition is correct, then, in the optic nerve, in the absence of incisures, such elongation should occur only by growth at the terminal cytoplasmic spiral cord.[1]

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[1] Robertson (1958) has suggested that the incisures of Schmidt-Lantermann represent shearing defects of the compact myelin, produced by stresses and strains on the nerve fibers. This strikes me as highly unlikely because: (a) Incisures are also abundant in the dorsal and ventral roots of spinal nerves and in the white substance of the spinal cord, notwithstanding their great protection from mechanical stresses and strains. (b) Incisures are abundant in the peripheral nerves of *Amphibia*, e.g. oculomotor nerves, but they are not present in the optic nerve which is exposed to similar mechanical stresses and strains. (c) In general, incisures seem to be abundant in nerves and tracts that are submitted to a considerable elongation after the onset of myelination. It seems to me that it is more likely that incisures should represent centers of longitudinal growth of the myelin layers, and that they should be specially useful in rapidly elongating nerves as in the limbs. In slowly elongating bundles, or in fibers which myelinate after most of the elongation has been produced, growth at the level of the terminal spiral loops could account for the elongation of the compact myelin. I think that this is the case in the optic nerve of Anurans and in other parts of their CNS where incisures are absent. This hypothesis could be tested experimentally by an adequate study of the incorporation of radioactive tracers in the cytoplasm of the incisures, in the terminal loops, and in the elongating compact myelin itself.

Since there is only one type of glial cell in the optic nerve of Anurans, the problem of identifying the myelogenic cell does not appear. Nor is it possible to differentiate myelogenic from non-myelogenic glial cells if the latter ever occur. To the extent that the boundaries of individual glial cells are extremely difficult to identify completely, and to the extent that these cells have numerous expansions in all directions, it has been impossible to estimate how many myelinated and unmyelinated axons are simultaneously related to any single glial cell. I feel, although I have no direct evidence, that these relations are multiple and that each glial cell is myelogenically related to several myelinated axons simultaneously. That it can be related to several hundreds of unmyelinated ones is apparent. In this respect the glial cells behave very differently from the Schwann cells in peripheral nerves that separate individual axons. This special behavior of the glial cells and the absence of connective tissue from the optic nerve are probably the most important factors in determining the anatomy of the optic nerve. It may be that the exclusion of connective tissue from the nerve is also a result of the behavior of the glial cells.

Sheath Contacts.—The apposition of myelin sheaths in the CNS appears to be a very common phenomenon and can be easily observed with light microscopy, in both the white and the gray substances, after myelin stain. It has also been observed by electron microscopists (Fernández-Morán, 1955; Luse, 1956; Schultz et al., 1957; Wyckoff and Young, 1956), but its existence has thus far appeared baffling and has created some difficulty in understanding the mechanism of myelination of central fibers. This is, perhaps, due to the absence of an alteration in the myelin period in the region of contact.

As mentioned above, in the optic nerve this apposition of myelin sheaths occurs without interchange of myelin layers between the sheaths in contact. This is shown by counts of the myelin layers of the touching sheaths, and by directly observing that the line of cleavage between them passes through an interlayer space that is continuous with the extracellular space between the surrounding structures (Fig. 8). The absence of the interchange of myelin layers is the general rule in the optic nerve and in other parts of the CNS of Anurans.

Bearing in mind the absence of connective tissue, the sinuous path of the axons, and the absence of exclusive relationship between the axons and the

glial cell, one can easily imagine how these contacts between myelinated fibers come about as they continuously shift their relative position along the bundles (axons A_0 and A_1 in Figs. 11 and 12). It is also obvious that in order for these contacts to occur the surrounding cytoplasm of the myelogenic cell has to be squeezed along the sheath to form the most superficial myelin layer. These contacts have been represented in the reconstructions shown in Text-figs. 1 and 2. Text-figs. 3 A to C show this in several sections along a bundle. The touching myelin sheaths approach each other as the myelin layers do and attain some of the stable characteristics of the compact myelin, like closeness and adhesiveness. The fact that it appears easier to tear the myelin sheath than to separate two touching fibers speaks in favor of the strength of these attachments and suggests that they may have an indirect supporting role. Whatever the factors are that determine the distance between myelin layers and their adhesiveness, these factors do not appear to depend upon the spiral myelin formation but upon structural changes of the membranes when they fuse to form the myelin layers.

I have not observed an obvious intraperiod line in the myelin of the optic nerve (or in other parts of the central nervous system of Anurans), nor have I observed its enhancement in particular regions of the sheath as has been observed by Fernández-Morán (1955). This line is usually not very clear after fixation with solutions of osmium tetroxide and is fully apparent only after fixation with permanganate. Its absence in these observations is possibly due to the fixation used. Its absence, even at the place where two myelin sheaths touch, also indicates that at these places the outer myelin layers of the sheaths behave as any other pair of layers of the compact myelin.

Since the optic nerve is a central tract, it can be expected that its pattern of anatomical organization represents the group to a large degree. If this is so, it can be expected that the process of myelination in other parts of the CNS may be the same as it is in the optic nerve. Also, it can be expected that distortions similar to those found here disturb and obscure the typical cellular relations. For example, the difficulties encountered by Schultz et al. (1957) in finding the myelogenic connections between myelinated axons and glial cells in the corpus callosum, may possibly have been due to a situation similar to that discussed above for the optic nerve. This situation can be expected to be especially striking in heavily myelinated bundles bceause of the great abundance of myelinated axons in direct contact with each other. In these cases it can be expected that most, or all, of the medullated axons of any particular section will show no myelogenic relations with the glial cell present in the section. A sufficiently careful study should demonstrate that in all of these fibers the myelin sheath shows an external loop more or less modified by separation from the compact myelin. This I have observed in the heavily myelinated optic nerve of *Anolis carolinensis*. In peripheral nerves the situation is simplified by the connective tissue that separates the neighboring fibers.

In the gray matter, the situation is complicated by the numerous dendrites, unmyelinated axons, terminals, and expansions of various types of glial cells that pass in every direction. This often results in a loss of clarity in the picture due to the frequent oblique sectioning of the membranes, a condition that complicates the unraveling of the cellular relations. In my opinion it is this kind of difficulty which led both Luse (1956) and De Robertis et al. (1958) to suggest for the CNS a process of myelination different from that found in peripheral nerves. It seems to me that their pictures can best be understood in terms of the discussion presented above. In the tectum of Anurans, at least, I have observed typical transverse sections of internodes and prenodal regions, as well as longitudinal sections of the ending myelin at the nodes. I should recall here that in crustaceans that have fully myelinated axons the compact myelin and nodes of Ranvier are similar to those found in vertebrates (McAlear, 1958). All of this speaks in favor of the unity of myelogenic processes.

An additional difficulty found by other authors has been the presence of what appeared as partial myelin layers (Luse, 1956). I think that, in most cases, this appearance may be conveyed by the confluence of myelin sheaths discussed above. It may also be due to partial separation of the most superficial myelin layer by other passing structures, as often occurs in the optic nerve (Figs. 11, 15). It is also possible that partial myelin layers are formed by local internal fusion of the membranes of the surrounding glia. This is suggested in Text-fig. 1, arrow *PML*, and is shown in Fig. 2.

Intercellular Spaces.—Electron microscopy has revealed the optic nerve as a compact epithelium in which there are no large intercellular spaces, all adjacent membranes being close to each other and

separated only by a narrow extracellular gap 100 to 250 A wide. At the nodes the myelinated axons behave as the unmyelinated fibers in their relations to the surrounding elements. The most notable aspect of this behavior is the close contact of the nodal membrane with any of the possible surrounding cells: unmyelinated or myelinated axons, glial cells, and even other nodes of Ranvier. In this behavior, nodes in the optic nerve (and for that matter, central nodes in general) differ radically from peripheral nodes. In these, the Schwann cells of consecutive internodes extend over the nodal region, directly covering the unmyelinated portion of the axon (Uzman-Geren et al., 1957; Luxoro, 1956, 1958; Robertson, 1957). They contact each other over the node, and completely separate the axon membrane from the surrounding sheath of Henle. Thus, by means of Schwann cells and connective tissue, peripheral nodes are entirely separated from other neighboring myelinated and unmyelinated axons. This arrangement seems to minimize the possibility of interaction between functional nerve membranes. Contrariwise, in the optic nerve the anatomical organization secures the closest contacts between them and creates what appears, morphologically, as an optimal situation for ionic and electrical interaction during impulse conduction.

In the close epithelial structure of the nerve, the narrow intercellular spaces seem to offer a great resistance to rapid ionic movements between the active nerve membranes and the periphery of the nerve and blood vessels. In addition, glial cells and nerve fibers can be expected to constitute collectively a diffusional barrier having the properties of at least a plasma membrane isolating nerve fibers from the circulation with respect to the ionic interchange. In these circumstances it can be expected that at least for short time-course fluctuations, the ionic environment in the narrow intercellular spaces will be mostly or entirely independent of the circulation and completely controlled by the active ion-transport (Hodgkin and Keynes, 1955) of nerve and glial membranes. To the extent that all nerve fibers have similar properties and dependence on the surrounding ions for impulse conduction, one cannot but realize that in the bundles the activity of any axon will modify the ionic environment of the adjacent ones, and thus may interfere with their activity. However, for two particular fibers, which because of their sinuous course remain in contact only for short lengths (from a

fraction of a micron to a few microns), the magnitude of this interaction will be minimal. Furthermore, it has been shown (Lettvin and Maturana, unpublished observations, 1959) that adjacent axons in the nerve may come from widely separated retinal areas—a condition that reduces the chance of simultaneous activity of many axons in each bundle and minimizes the possibility of interaction. The possibilities of ephaptic interaction also seem minimal at the level of these short contacts because of the electrical properties of the nerve membranes (Horstmann and Meves, 1959).

Accordingly, electrical recordings from single myelinated and unmyelinated fibers in the optic nerve of the intact frog (Lettvin and Maturana, 1959), using physiological stimulation, show no obvious interaction between neighboring fibers. If most axons are somehow simultaneously stimulated repetitively, it can be expected that they will modify the ionic environment enough (e.g., by external accumulation of potassium ions) to interfere with each other. That this may be the case is shown by the reversible block of the unmyelinated fibers (conducting at less than 0.3 meters per second) when the whole nerve is stimulated at frequencies of 30 to 40 impulses per second for several seconds (Lettvin and Maturana, unpublished observations) although a single fiber, firing alone at such a frequency under physiological stimulation, remains unblocked. In these circumstances, the sinuous path of the axons and the fact that adjacency in the nerve does not mean adjacency in the retina appear as very significant anatomical features maintaining the independence of function in the closely packed axons.

Number of Fibers.—The question of the number of fibers in the optic nerve of Anurans has already been presented in another communication (Maturana, 1959) and will not be further discussed here. It was mentioned there that the arrangement of the unmyelinated axons in compact bundles precluded the counting of all unmyelinated axons by means of light microscopy. Since the failure of light microscopy in revealing the total number of unmyelinated axons present in the optic nerve of Anurans is due to its peculiar anatomy, a similar situation is likely to occur in the optic nerve of other vertebrates. This may be especially true in poorly myelinated nerves, where light microscopy reveals an abundant matrix between the medullated axons. The optic nerve of man may be a case of this kind (Chacko, 1948).

Desmosomes.—The presence of desmosomes in the optic nerve of Anurans seems related to the moving of the nerve by the action of the muscle retractor bulbi and its obvious exposure to mechanical disturbances through the roof of the mouth. The presence of desmosomes in mesaxons suggests that these occur as frequently between two separated arms of a glial cell as between neighboring ones. If this is so it would appear that the factors that govern their formation depend more upon the mechanical and geometrical conditions of the system than upon cytoplasmic discontinuity between the cellular expansions that form them. Whether or not desmosomes are of general occurrence in the CNS is not known, but I have not found them in the tectum of Anurans, which is little exposed to mechanical deformation (Maturana, 1958). It is possible, however, that they may be present in larger vertebrates in parts of the CNS more exposed to movement or to gravitational deformation.

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EXPLANATION OF PLATES

PLATE 35

(All figures are electron micrographs, except Figs. 1, 4, and 21, which are light photographs.)

FIG. 1. Transverse section of the optic nerve, *Bufo bufo*. Holmes' silver method. Both myelinated and unmyelinated axons are stained. Notice that the nerve constitutes a single unit, not grossly subdivided into bundles. Magnification, 100.

FIG. 2. Longitudinal section at the surface of a myelinated axon with some of the surrounding structures, *Bufo americanus*. The axoplasma is at the right side of the myelin sheath. *Arrow A* points to the first myelin layer in which the forming glial membranes are only partly fused. Notice that the distance that separates these partly fused membranes is smaller than the distance between single membranes approaching by their external surfaces. *Arrow C* also shows an incomplete internal fusion of two glial membranes, outside of the myelin sheath. *Arrow B* shows a complete internal fusion forming a partial compact myelin layer. This figure possibly corresponds to the longitudinal section of a partly unwound myelin sheath (see Fig. 11). Magnification, 68,000.

FIG. 3. Transverse section of a prenodal region at the level of a single turn of the terminal spiral of an optic fiber (*Rana catesbeiana*). The internal mesaxon (*IM*) and the external loop (*EL*) are visible. *U:* unmyelinated axons; *G:* glial cytoplasm. Magnification, 29,000.

FIG. 4. Transverse section of the optic nerve, toad *Bufo americanus*. Myelin stain. The myelinated axons form groups in which the myelin sheaths are in direct contact with each other and no glial cytoplasm intervenes between them. Unmyelinated axons are not visible and, at most, they faintly increase the contrast of the background around the myelinated axons. *N:* glial nucleus. Magnification, 1000.

FIG. 5. Section of an attachment plate or desmosome of the optic nerve, *Rana catesbeiana*. Magnification, 57,000.
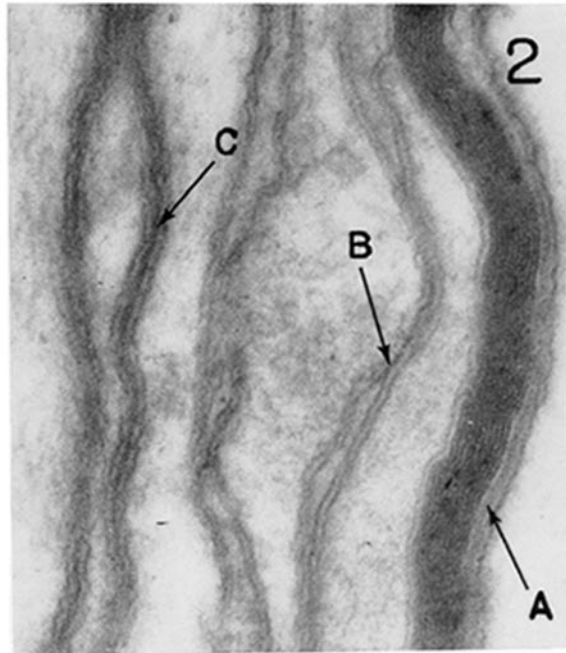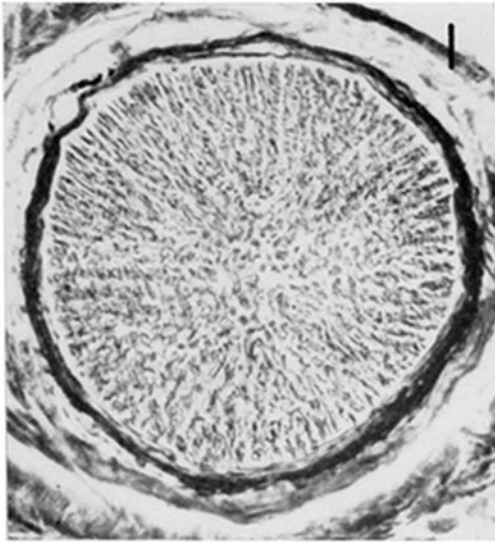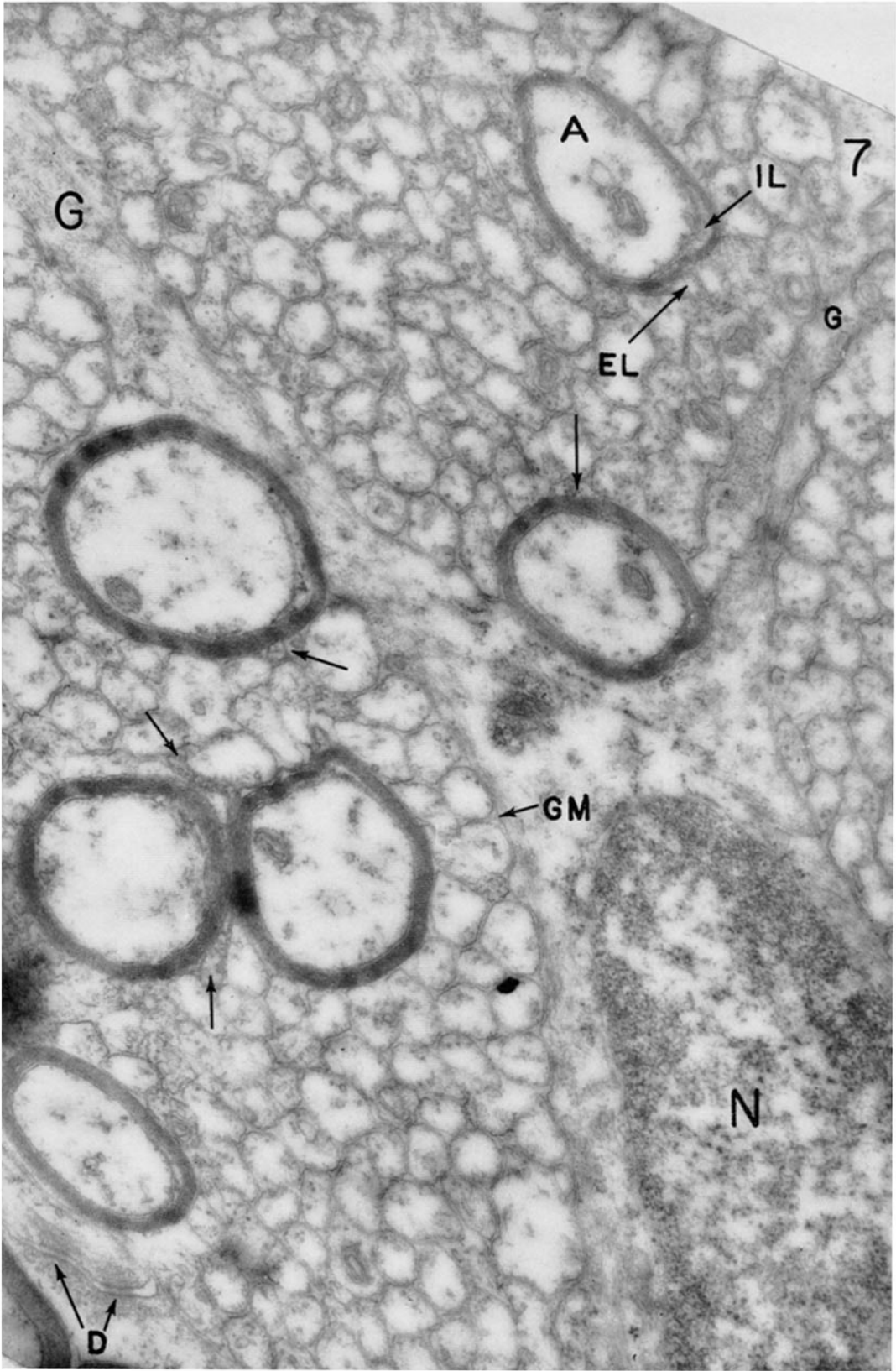
(Maturana: Anatomy of optic nerve)
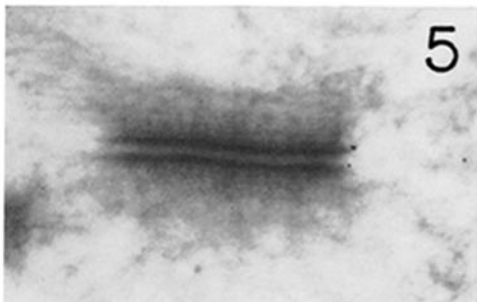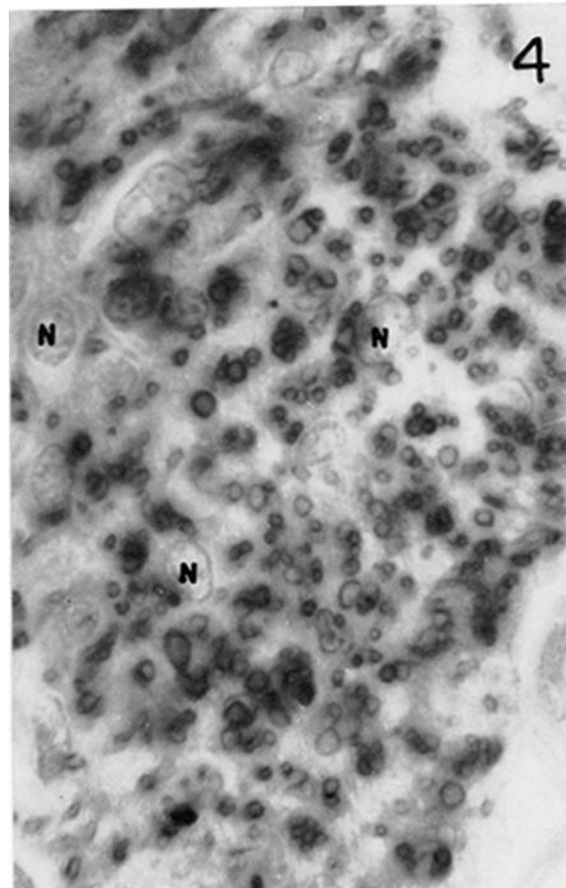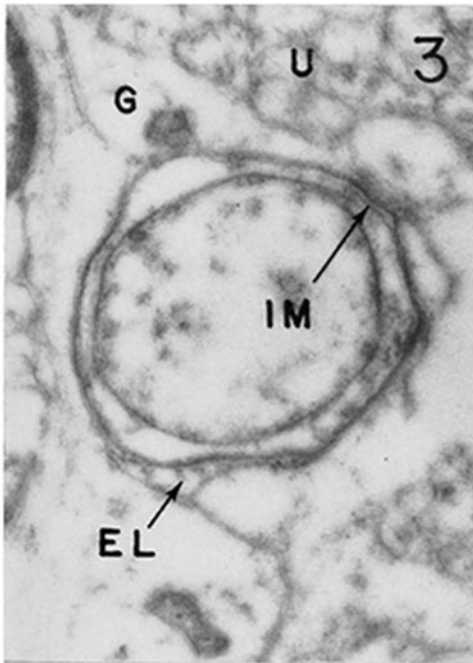
PLATE 36

FIG. 6. Transverse section of the optic nerve, *Rana pipiens*. The unmyelinated axons form large groups in direct contact with each other and with myelinated fibers. Glial cytoplasm (*G*) separates the bundles. At the lower right several mesaxons are visible; they end at the surface of the nerve just outside of the picture. Notice that the mesaxon that exhibits a desmosome (*D*) is enclosing a small group of three unmyelinated axons outside the main bundle. At the lower left there are two myelinated axons in direct contact, with no alteration of the myelin period at the apposition of the two myelin sheaths (see Fig. 8). *M:* mesaxons. Magnification, 22,000.

(Maturana: Anatomy of optic nerve)

PLATE 37

FIG. 7. Optic nerve, transverse section, *Rana catesbeiana*. The unmyelinated axons are clearly visible; they form large bundles separated by strands of glial cytoplasm (*G*). Notice that the distance between single membranes and myelin layers is greater than that between the myelin layers themselves, but a little smaller than that between single membranes. Small circular profiles (probably related to the endoplasmic reticulum of the axoplasm) and membranous bodies (possibly mitochondria) fill the axoplasm of both myelinated and unmyelinated fibers. In axon *A* it is possible to see the external (*EL*) and the internal (*IL*) loops of the myelin spiral pointing in opposite directions. This axon is entirely in the extracellular space, surrounded by unmyelinated fibers. The other myelinated axons are also in the extracellular space, but two of them are partly surrounded by the neighboring glial cell without being myelogenically related to it. Their external loops are marked with arrows. *D:* desmosomes; *GM:* glial membrane; *N:* glial nucleus. Magnification, 39,000.

PLATE 37
VOL. 7

(Maturana: Anatomy of optic nerve)

PLATE 38

FIG. 8. Detail of Fig. 6 to show the region of confluence of two myelin sheaths. Notice that the distance between myelin layers is about 50 A, while the distance between pairs of single membranes or between these and the myelin layers is about 90 to 150 A. There is no interchange of myelin layers, and the line of cleavage between the two sheaths passes at the level of an interlayer space which is continuous with the intercellular space between the surrounding unmyelinated axons (U). There is no alteration in the myelin period when passing from one fiber to the other, and the two of them constitute a single myelin unit at this level. Magnification, 114,000.

FIG. 9. Detail of Fig. 7 showing the spiral disposition of the myelin system of axon A. Notice that the myelin layers adjacent to the external and the internal loops are split and form small islands of cytoplasm (unmarked arrows). G: glial cytoplasm. Magnification, 52,000.

FIG. 10. Optic nerve, longitudinal section, *Bufo americanus*. Ending myelin at a node of Ranvier. Notice that the myelin layers end in loops which contain cytoplasm. Although many membranes have been cut obliquely and there is poor resolution in many places, it can be seen that the myelin layers split to form the terminal loops and that these do not fuse with the underlying axolemma. Notice the neurofilaments in the axoplasm A. D: desmosome. Magnification, 46,000.
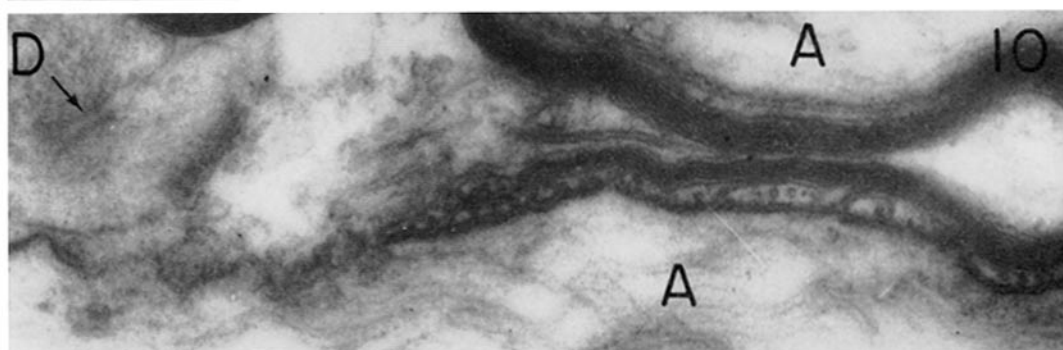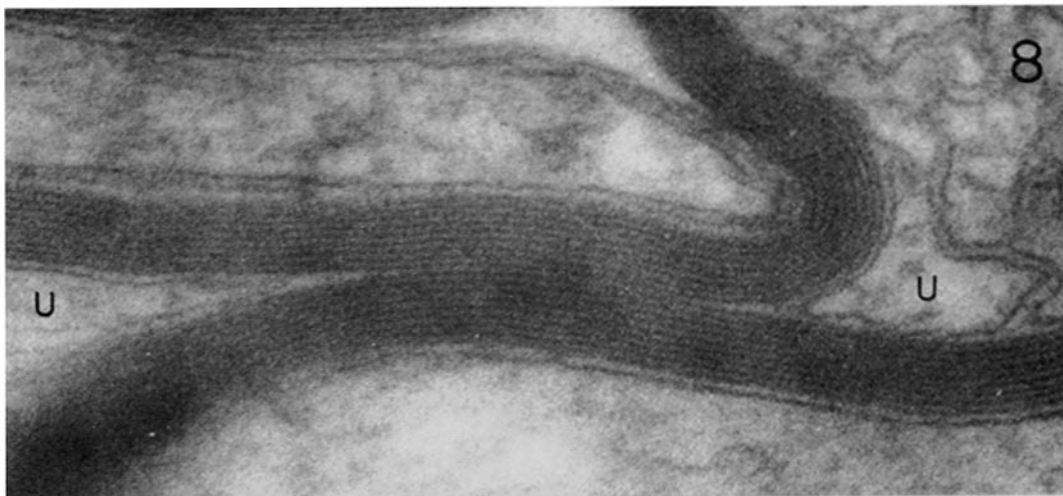
PLATE 39

FIGS. 11 and 12. Two transverse sections of the same group of axons at two levels along the nerve. Fig. 12 is closer to the node. The myelin spiral of axon A_0 has been unwound by other structures; in this case by what appears to be glial cytoplasm. The spiral myelin layer appears double most of the time, as if the extreme fusion observed in the compact myelin were possible only for short lengths outside such a system (see Fig. 2). The two ends of the spiral are indicated by EL and IL arrows. Notice also that in Fig. 11 axons A_0 and A_1 have their myelin systems practically touching each other (there is an axon or a glial cell process compressed between), while in Fig. 12 they are separated by several passing unmyelinated fibers. Axons A_2 are the same in both figures. EL: external loop. IL: internal loop; G: glial cytoplasm. Magnification: Fig. 11, 50,000; Fig. 12, 45,000.
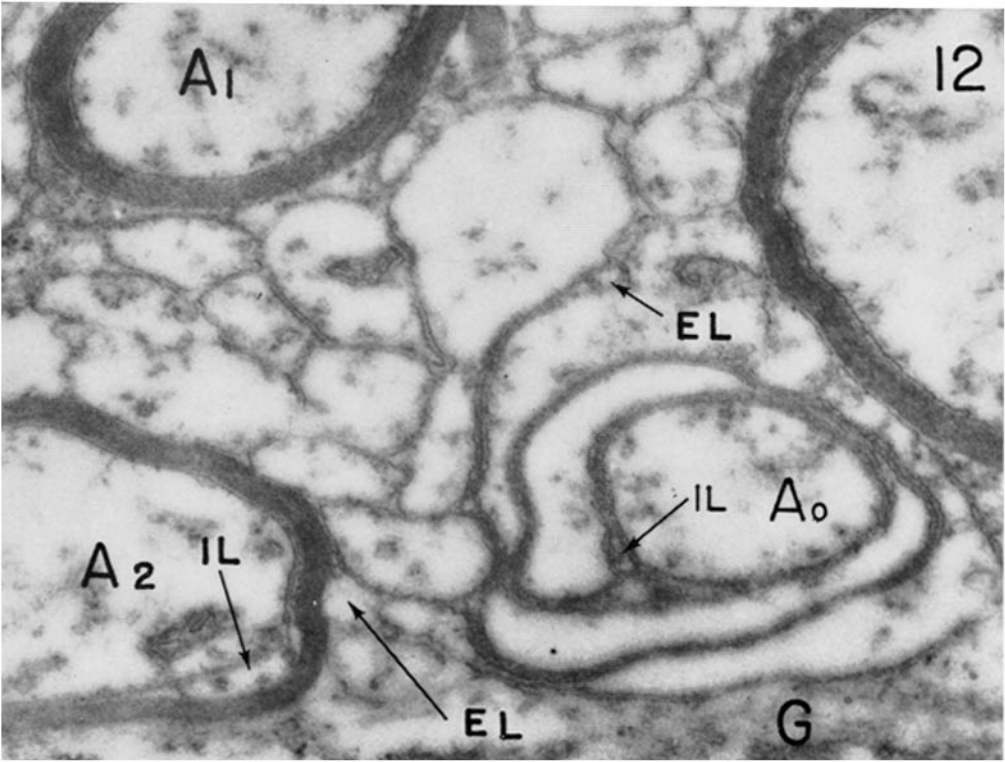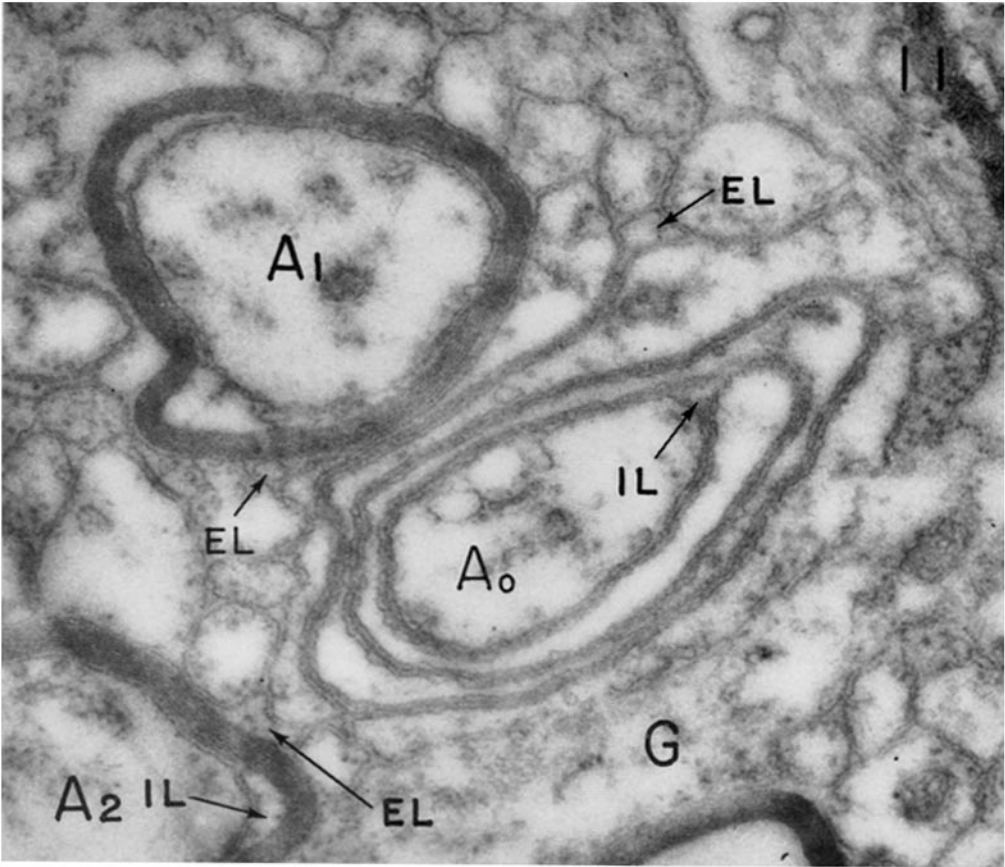
FIG. 13. Transverse section at the level of a node, optic fiber, *Rana catesbeiana*. The node is directly surrounded by unmyelinated axons and a myelinated fiber. The perinodal extracellular space is continuous with the intercellular space between the surrounding unmyelinated axons. Since the medullated fibers do not follow a special course, at the nodes they behave entirely like unmyelinated axons in their relations with the surrounding cells. *EL:* external loop. Magnification, 39,000.

FIG. 14. Transverse section at the prenodal region, optic fiber, *Rana catesbeiana*. This section has passed obliquely at the prenodal region, cutting through two turns of the terminal spiral loop of the myelin. In accordance with this, two internal mesaxons *(IM)* can be seen. *G:* glial cytoplasm. Magnification. 29,000.

FIG. 15. Optic nerve, transverse section, *Rana catesbeiana*. The external loop *(EL)* of myelinated axon *A* has been separated from the compact myelin by a passing unmyelinated axon. Counts of the myelin layers at various points around the axon show that there is no interchange of myelin layers with the adjacent myelinated fibers. These counts are also in agreement with the spiral disposition of the myelin layers. *IL:* internal loop. Magnification, 63,000.

FIG. 16. Node of Ranvier, longitudinal section, *Bufo terrestris*. The node appears surrounded by unmyelinated axons *(U)*. At the lower end the ending myelin has been cut tangentially and it is not possible to see the individual layers ending in the terminal loops. At the upper end the fiber has been cut near the axis and the myelin layers appear forming terminal loops. Both appearances are typical. When the myelin layers split to form the terminal loops the contrast of the membranes is markedly reduced and they are difficult to see, but a careful study of the picture will show that the splitting of the myelin layers occurs and that the loops do not fuse with the surrounding membranes. *U:* unmyelinated axons; *AM:* axon membrane; *TL:* terminal loops. Magnification 31,000.
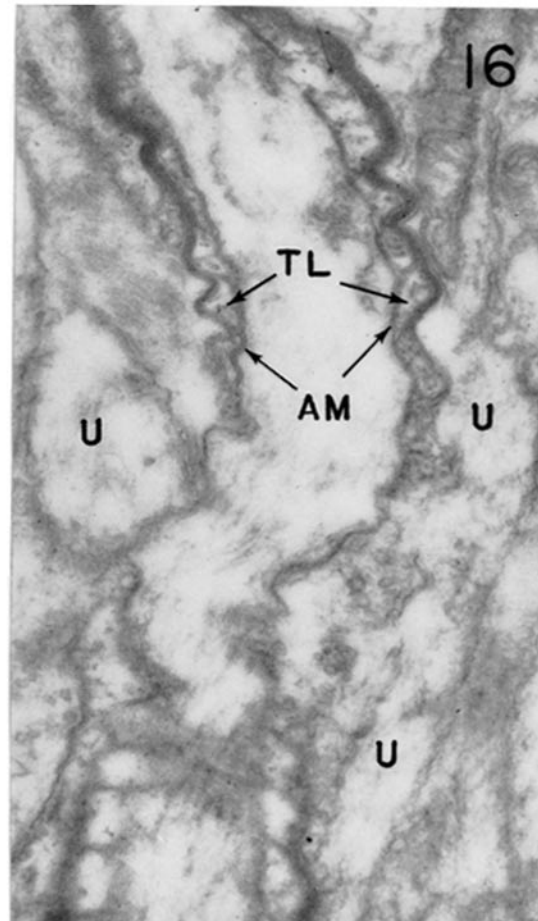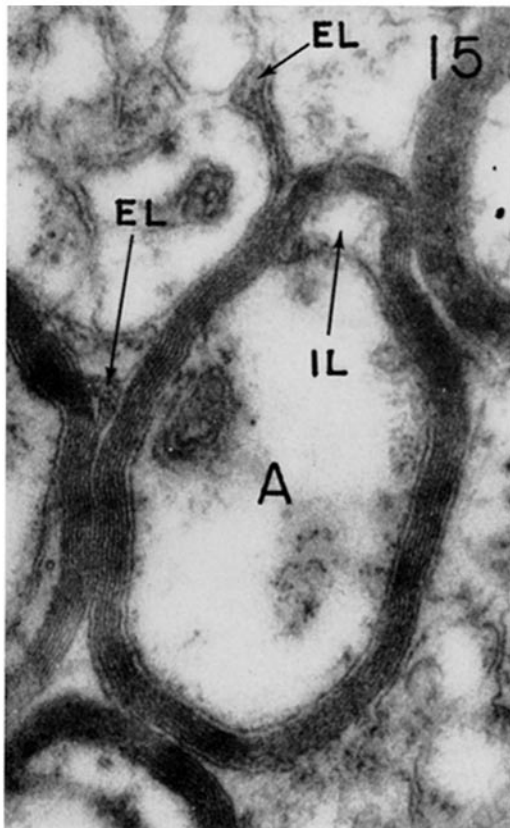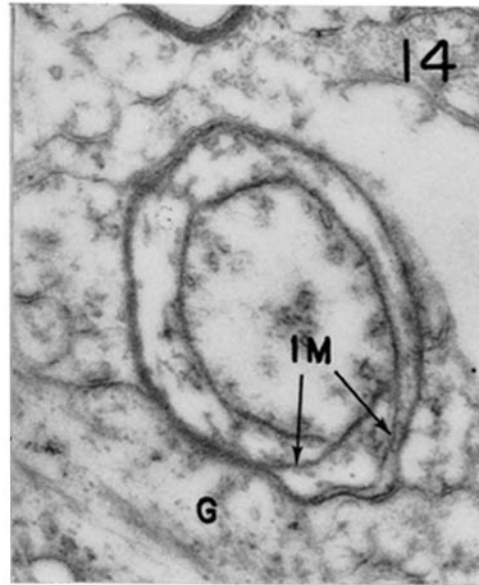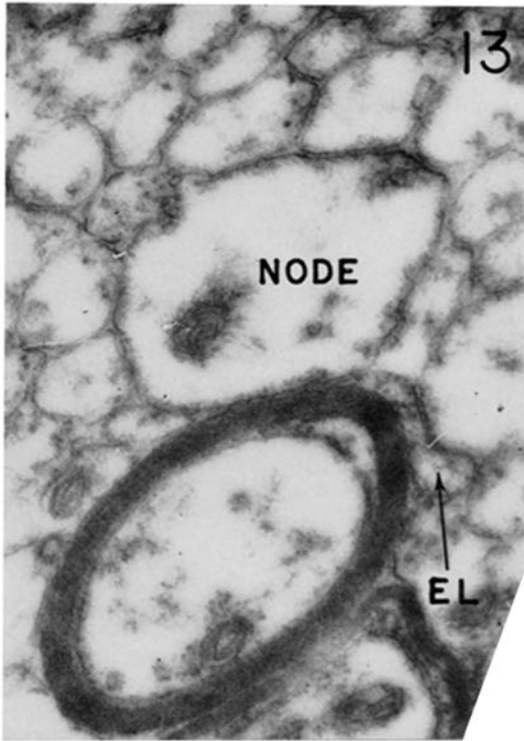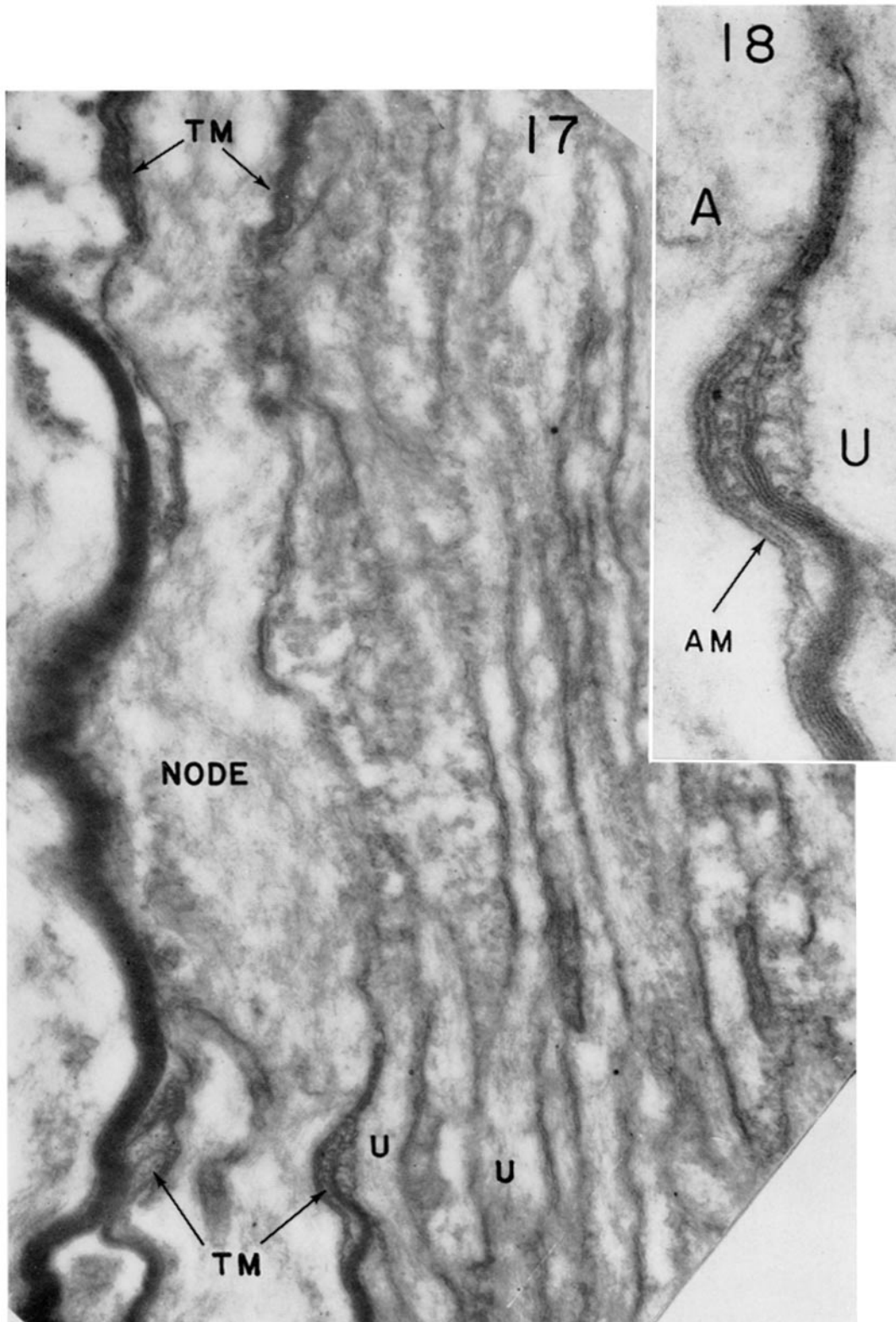
(Maturana: Anatomy of optic nerve)

Fig. 17. Node of Ranvier in the optic nerve, longitudinal section, *Bufo americanus*. The node is surrounded by myelinated and unmyelinated fibers. Observe the two regions of myelin ending (*TM*) and notice that the unmyelinated portion of the node is almost 7μ long. The nodal region bears no special relation to the surrounding structures and behaves entirely as an unmyelinated axon. Notice the richness in neurofilaments of the axoplasm. *U:* unmyelinated axons. Magnification, 20,000.

Fig. 18. Detail of the ending myelin at the lower right side of the node in Fig. 17. Notice that the myelin layers split to form the terminal loops. These loops do not fuse with the nearby axolemmas. *U:* unmyelinated axon; *A:* axoplasm; *AM:* axolemma. Magnification, 71,000.
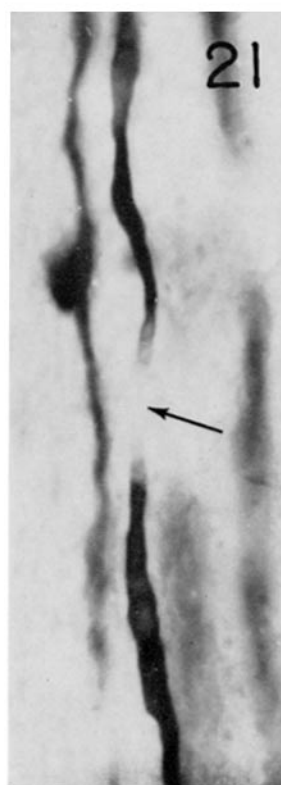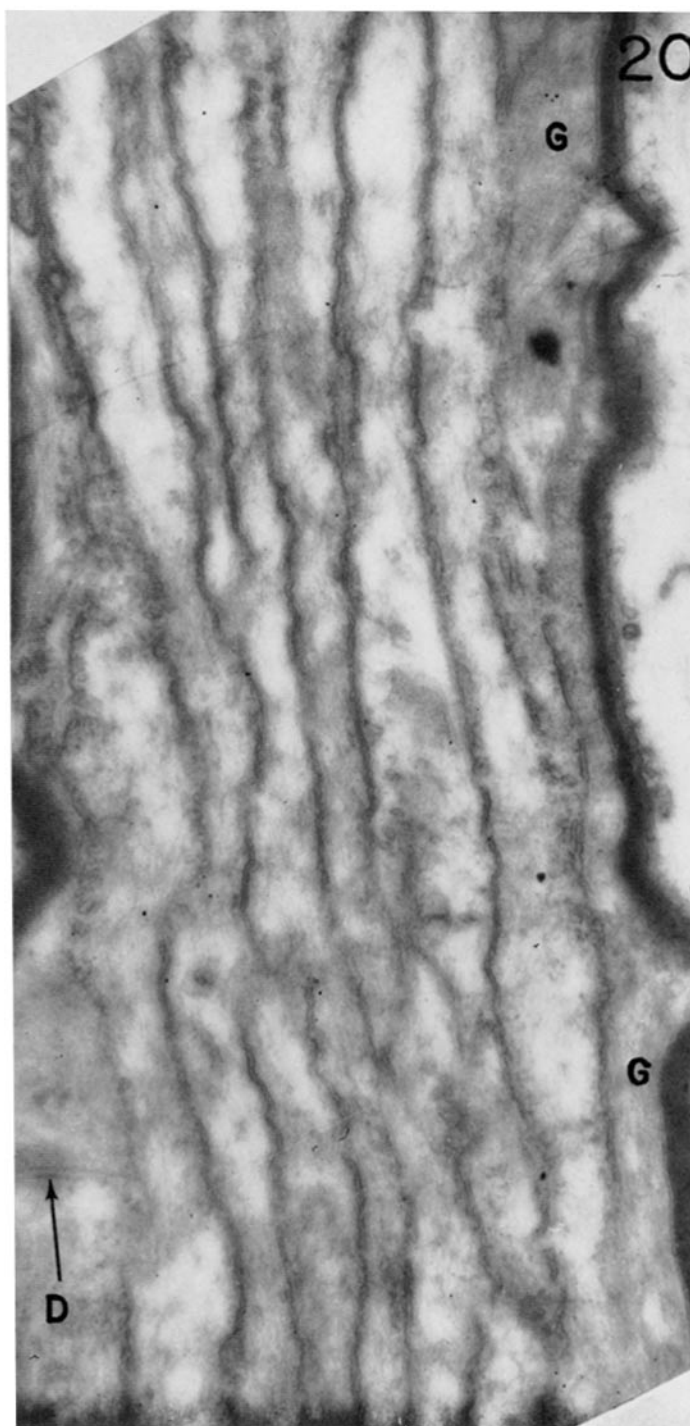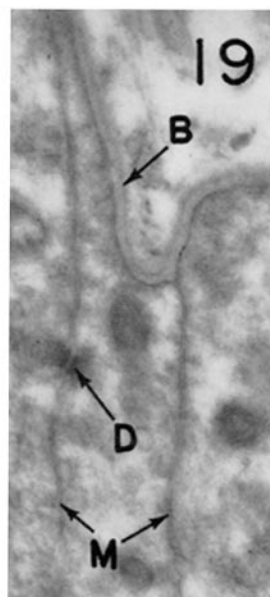
PLATE 42

FIG. 19. Transverse section near the surface of the optic nerve, *Rana pipiens*. Two mesaxons (*M*) open at the surface of the nerve. The extracellular space between the membranes which form them is continuous with that between the unmyelinated axons in the bundles. The basement membrane (*B*) follows the sinuosities of the glial membrane at the surface of the nerve, but does not penetrate into the mesaxons. A desmosome (*D*) is present in one of the mesaxons shown. A few collagen fibers of the pia mater are visible beyond the basement membrane. Magnification, 27,000.

FIG. 20. Optic nerve, longitudinal section, *Bufo americanus*. This section passed through a bundle of unmyelinated axons between two myelinated fibers. These are surrounded by glial cytoplasm (*G*). Unmyelinated axons do not branch in the optic nerve, and the appearance of confluence of some unmyelinated fibers is entirely due to the oblique sectioning of their axolemma at the point where they come out of the section. At places, the glial and axonal membranes seem to break into vesicles. *D:* desmosome; *G:* glial cytoplasm. Magnification, 31,000.

FIG. 21. Node of Ranvier, optic tract, *Bufo bufo*. Myelin stain. At the node the myelin appears less stained because of the progressive ending of the myelin layers to form the terminal cytoplasmic spiral. The axon itself is not stained. The arrow indicates the unmyelinated zone. The node is about seven microns long. Magnification, 1,300.

(Maturana: Anatomy of optic nerve)