OBSERVATIONS ON A TRANSIENT PHASE OF FOCAL SWELLING IN DEGENERATING UNMYELINATED NERVE FIBERS

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ABSTRACT

This study describes the nature and time-course of a swelling phase during the degeneration of unmyelinated nerve fibers, as observed in highly organized cultures of rodent sensory ganglia. Observations were made on nerve fascicles after they were cut and during nutritional deprivation. About 12 hr after nerve transection, large, clear vacuoles appear throughout fascicles distal to the cut. These vacuoles are most numerous at 24 hr and then gradually subside; after 48 hr, only small granules mark the severed fascicles. Electron microscopy shows that the vacuoles are, in fact, massive focal dilations of unmyelinated axons. Similar focal dilations in unmyelinated axons are observed if cultures are not refed for 5–7 days; under these conditions glucose concentrations fall below 20 mg/100 ml and degenerative changes begin to appear in neuronal somas. If the gas-tight assembly is opened and the culture refed, there is rapid disappearance of axonal dilations (usually within 1 hr) and recovery of many of the damaged neurons. Cooling (4°C) prevents this reversal, suggesting that an active process is involved. It is postulated that the swellings result from the failure of active axolemmal ion-pumping mechanisms prior to loss of selective permeability in the axon membrane. The reasons for the focal nature of the swellings is unknown. A literature review indicates that a phase of focal swelling has frequently been observed during the degeneration of unmyelinated nerve fibers in vivo.

INTRODUCTION

In 1895, in a detailed description of the structure of normal and degenerating unmyelinated nerve fibers, Tuckett (1) observed that “through diffusive changes taking place in the dying tissue, the fibers of Remak swell up in places so as to become varicose; or, rather, that part which . . . is the core of the fibers becomes varicose, while the outer part of the fibers mechanically follows the excursions of this inner part.” Tuckett thus noted, as had others before him, that unmyelinated fibers may swell dramatically under certain adverse conditions; he emphasized that this swelling occurred if fixation was not initiated immediately after death. This early report, and others which followed, have raised many questions regarding the mechanisms of degeneration in unmyelinated nerve fibers. How regularly does a swelling phase occur during the degeneration of these fibers, and what basic axonal properties might this alteration reflect? Is it a stage in the fragmentation of the degenerating fibers? How long does the amputated axon survive in various types of nervous tissue?
In general, it appears that workers who have employed methylene blue staining have emphasized the rapidity of the loss of damaged unmyelinated nerve fibers (within 2 days) and have depicted the degenerating fiber with prominent intermittent varicosities (e.g., Tuckett (1) studying unmyelinated fibers of the rabbit autonomic system, and Weddell and Glees (2) studying cutaneous nerves in rabbit ear). Ramon y Cajal (3), on the other hand, relying on silver techniques, reported that unmyelinated peripheral nerve fibers appear essentially normal 48–56 hr after nerve transection, with substantial changes occurring only after 4–7 days.

Recent electron microscopic (EM) studies have similarly reported a wide variety of responses in the degeneration of unmyelinated nerve fibers. Roth and Richardson (4), studying fiber degeneration in the iris, and Nathaniel and Pease (5), studying peripheral nerve, have reported almost complete disappearance of unmyelinated nerve fibers in distal segments within 48 hr after nerve transection. In another case, unmyelinated nerve fibers were reported to be present for as long as 21 days after nerve sectioning (6). Other authors have reported changes consisting of occasional swellings of unmyelinated fibers distal to nerve section (7), or the transient focal accumulation of axonal mitochondria in both myelinated and unmyelinated fibers after sciatic nerve transection (8).

Kapeller and Mayor (9) have presented electron micrographs depicting axons with alternating swollen and constricted regions distal to a constriction (by ligature) of sympathetic nerve fibers. These swellings were particularly prominent 24 hr after application of the ligature and contained few axonal organelles. In contrast, focal swellings near the site of ligature were abnormally rich in organelles. It should also be mentioned that stains selective for degenerating fibers in the central nervous system, such as the Nauta stain, can often be applied weeks or even months after fiber transection.

While not contributing directly to the problem of the total survival time of amputated unmyelinated axons, previous studies of living nerve do suggest that these fibers undergo a swelling phase during their degeneration. Speidel (10) studied living nerves in the frog tadpole tail and reported an alveolar or vacuolar stage in the degeneration of a nerve (composed of several unmyelinated axons) occurring about 6 hr after nerve transection. Rhines (11) damaged neurites in cultures of Amblystoma spinal ganglia and noted irregular enlargements developing distal to the site of damage.

In the present paper we report observations on the time-course and patterns of degeneration of unmyelinated axons in highly organized cultures of rat sensory ganglia. Nerve injury in these cultures, either by transection or by nutritional deprivation, results in a transient phase of marked, focal swelling in unmyelinated axons. The resupplying of nutrients can rapidly reverse the swelling reaction brought about by nutritional deprivation. This latter observation suggests that the swelling results from the failure of an active transport system; several possible mechanisms which could explain this sequence of changes are considered. We have found that, for the system studied, this transient focal swelling is a useful marker of axonal degeneration and that both its time-course and pattern are regularly reproducible.

**MATERIALS AND METHODS**

Dorsal root ganglia were removed from 18-21-day-old rat fetuses and explanted onto collagen-coated glass (No. 1) or plastic (5 mil Aclar 33C) carrying coverslips (diameter 22 mm) in standard Maximow double-coverslip assemblies (12, 13). They were washed and refed twice weekly with one drop of medium containing 50% Eagle's Minimum Essential Medium, 25% human placental serum, and 25% 9 day chick embryo extract. Glucose was added to this mixture to give a final concentration of either 600 or 1200 mg/100 ml.

In experiments involving treatment of cultures in the cold (4°C), the culture in its Maximow chamber was first cooled to 4°C before it was opened for microsurgery and/or refeeding with cold (4°C) medium. We have observed that cultures may be kept at 4°C for several hours without visible effect on their long-term survival.

**Cutting of Nerve Fascicle**

Individual nerve fascicles were sectioned after the carrying coverslip was placed over a black background under a dissecting microscope. The previously selected fascicle was severed with hand-held No. 11 or No. 22 scalpel blades and the culture was re-fed and reincubated. By this method it was possible to section selected fascicles near the neuronal area (Fig. 1). A $\frac{1}{2}$–1 cm length of fascicle was then available distal to the cut for direct microscopic observations.
FIGURE 1 Explant region of a living culture illustrating the result of fascicle transection. Two major fascicles ($f_1$ and $f_2$) were originally attached to the neuronal mass ($n$). The borders of the living neuronal somata are discernible throughout the central explant region. The fascicle $f_2$ has been completely severed by the cut, and the tissue and collagen have retracted, leaving an open area ($c$). $\times 45$.

**Nutritional Deprivation**

Nutritional deprivation was accomplished by not replenishing the medium. Cultures receiving 600 mg glucose/100 ml of medium were starved until fascicle swellings appeared, usually a period of 6 days. At the peak of this reaction cultures were opened and refed either with fresh medium or by the addition of 1.5 $\lambda$ of a 20% glucose solution to the spent medium. The latter procedure raises the glucose concentration to normal levels without otherwise altering the chemical composition of the medium. Cultures receiving 1200 mg glucose/100 ml required up to 9 days to develop the same fascicle changes. At the time of refeeding, the nutritive drop being replaced was collected. The glucose concentration in the fresh medium and in the collected drops was measured by the glucose oxidase peroxidase reaction with a Zeiss PQuII spectrophotometer (14, 15).

**Microscopic Examinations**

Cultures were observed and photographed in the living state with a Bausch & Lomb 40X fluorite oil objective with a 1 mm working distance. Whole mounts of the cultures were prepared by fixing and staining the culture still attached to its carrying coverslip by either (a) fixation in 2% OsO$_4$ buffered with veronal acetate followed by staining in Sudan Black B or (b) fixation in 10% formaldehyde in basic Earle's salt solution with subsequent silver staining according to Holmes (16). The OsO$_4$ fixation, Epon embedding, and staining for light and electron microscopic examination followed procedures previously described (12). Most of this material was prepared by sectioning in a plane parallel to the plane of the coverslip, i.e., longitudinally through the nerve fascicles.

**RESULTS**

Dorsal root ganglia develop and mature, in the tissue culture system employed, into miniaturized replicas of their in vivo counterparts (12). The nerve fibers (axons) which radiate from the central neuronal area are organized into fascicles consisting initially of numerous unmyelinated axons; some of these become myelinated as the culture matures. The nerve fascicles are often thicker near the explant (Fig. 1) and undergo a series of branchings and rebranchings as they approach the edge of the coverslip (see Fig. 1, reference 12).

**Cut Fascicle**

The sectioning of a nerve fascicle included the underlying collagen substrate which immediately retracted, leaving a gap between the severed ends of the nerve (Fig. 1). The observations reported here describe the changes in the fascicle distal to the cut. These were particularly well seen in the more peripheral branches, where the fascicles are thinner and myelinated axons are generally sparse (Fig. 2). Light microscopic observations indicated that 6–8 hr after sectioning, the smooth linear pattern of the fascicle was interrupted by the appearance of small, round, clear vacuoles intermittently spaced along its length. These vacuoles increased in size and number to reach a peak in 12–24 hr (Figs. 3, 5). Subsequently, the number and size of the vacuoles decreased until, by 48 hr, only a few remained (Fig. 4, Diagram 1). The severed fascicles could now be identified by the presence of numerous, small granules as well as degenerating myelin (Fig. 4).

Examination of this material after OsO$_4$ fixation and/or Epon embedding indicated that the vacuolar configurations were well preserved after these procedures (Figs. 5, 6 a). It was not possible, however, to ascribe these changes to a specific cellular component prior to EM examination. The
FIGURES 2-4 Living fascicles showing the sequence of events occurring when a fascicle is cut. Fig. 2 is of a normal fascicle containing three myelinated (arrows) and many unmyelinated fibers. The latter with their companion Schwann cells cause the background linear pattern within the fascicle. Fig. 3 shows a similar fascicle at the height of the vacuolar response after sectioning. The myelinated fibers are undergoing changes typical of early Wallerian degeneration. The linear pattern of the fascicle is frequently interrupted by vesicles of various sizes (arrows). Fig. 4 shows the same fascicle after spontaneous resolution of the vacuolar response. The degeneration of the myelinated fibers has progressed and the vacuoles are no longer visible. Fig. 2 X 625. Figs. 3 and 4 X 450.

FIGURE 5 A culture fixed (in buffered osmium tetroxide) at the height of the vacuolar response after nerve transection and subsequently stained with Sudan Black B. The small fascicle traversing the field is filled with small vacuoles of various sizes. The nuclei of the Schwann cells occupying the fascicle are marked by arrows. X 400.

EM observations on longitudinally sectioned fascicles demonstrated that the vacuoles were, in fact, massive swellings occurring intermittently along the length of the unmyelinated axons (Fig. 6 b). These large dilations were of low electron opacity. The axonal neurofilaments were seen either coursing linearly through the axonal enlargement or as dispersed remnants (Fig. 6 b). Mitochondria were sometimes observed within the enlargements. The Schwann cell sheath was found to be intact and complete around even the largest axonal swellings.

Concomitant with this swelling in unmyelinated fibers, the myelinated axons underwent the sequence of changes characteristic of Wallerian degeneration (Figs. 3, 4) (17). Despite the proximity of the cut to the neuronal area and the large number of axons severed, the neuronal somata rarely showed alterations visible in the light microscope.
We have observed that if a fascicle is cut in a culture that has been precooled to 4°C, refed cooled medium, and maintained in the cold, the swelling reaction does not occur, even after 30 hr (Diagram 1). Upon subsequent incubation, however, the swelling reaction appeared in the time-course expected.

Silver-stained preparations of cultures fixed at 1, 3, and 7 days after cutting suggest that there is a gradual decrease in the number of stainable axons distal to the cut following the natural resolution of the swelling phase. At 7 days after cutting, few axons were demonstrable distal to the cut.

**Nutritional Deprivation**

If healthy cultures were not refed at their usual 3-4 day interval, a similar sequence of vacuole formation was observed (Fig. 7). Explants receiving 600 mg glucose/100 ml of nutritive medium usually began to show degenerative changes on the 5th or 6th day after the last feeding. In cultures receiving 1200 mg glucose/100 ml these changes occurred on the 9th or 10th day postfeeding (Fig. 8). In the case of nutritional deprivation, the reaction was seen in all the fascicles. Again the first changes observed in the light microscope consisted of the appearance of small, clear vacuoles which increased in size and number for 12-18 hr, until the fascicles were packed with vacuoles (Figs. 7, 8). Holmes' silver staining at the height of this reaction suggested that these vacuoles were axonal swellings (Fig. 9).

Electron microscopic examination of longitudinally sectioned fascicles confirmed that these vacuoles resulted from massive dilations of unmyelinated axons (Figs. 10, 13, 14). In some electron micrographs the axonal membrane in the region of the dilations appeared as a partially interrupted line in contrast to the adjacent membranes of other cells (Fig. 14).

Glucose measurements were made on the medium collected from the cultures at the height of this response. At the end of the usual feeding interval of 3-4 days, the glucose level was normally over 50 mg/100 ml. In starved cultures the glucose level was always found to be less than 30 mg/100 ml, and was observed on several occasions to be lower than 10-20 mg/100 ml (the lower limit of accuracy for the test employed).

At the time vacuoles began to appear in the fascicles, light microscopic examination of these deprived cultures showed that the neuronal nuclei were distended and surrounded by an abnormally prominent nuclear membrane. As the syndrome progressed, the nuclei often became eccentric and the cytoplasm became acutely granular. As these granules aggregated adjacent to the nucleus, a clear area developed near the periphery of the perikaryon (compare Figs. 11 and 12). This degree of degenerative change was not seen in all neurons.

There was considerable degeneration of the myelinated fibers which proceeded in a Wallerian pattern. Subsequent to our observation of this swelling phase during the degeneration of unmyelinated axons, we have come to recognize that the appearance of a series of vacuoles along the length of a nerve fascicle in untreated cultures accompanies the occasional neuronal loss that occurs in healthy cultures.
Reversal of the Deprivation-Induced Axonal Swelling

If the vacuolar response was allowed to progress, the cultures continued to degenerate slowly and the vacuoles gradually disappeared. About 24 hr after the peak of vacuole formation, the fascicles appeared granular and disorganized and distinct vacuoles could no longer be clearly discerned. In marked contrast, the vacuoles were observed to disappear rapidly if the deprived culture was re-supplied with normal nutrient at the height of the swelling response. Rapid disappearance of 90–100% of the vacuoles could be obtained in 30–90 min by the following manipulations: (a) opening the chamber and refeeding with normal medium (compare Figs. 15 and 16), (b) supplementing the expended drop of medium with a glucose solution calculated to bring the glucose concentration to the usual value, or (c) opening the culture to room air for 2–4 min, allowing gaseous equilibration. In addition to O₂ replenishment, opening the culture chamber results in a pH shift towards the alkaline caused by a loss of accumulated CO₂. All of these procedures were followed by incubation at 35°C. On several occasions it seemed that the third method of reversal was more temporary than the reversal induced by complete refeeding or by the addition of glucose. Small granules characterized the fascicle after reversal (Fig. 16).

Silver-stained preparations of deprived cultures fixed either 45 min after reversal by refeeding with
Figures 7-10  The vacuolar development induced by nutritional deprivation. Fig. 7 shows a living fascicle at the height of the reaction. Numerous clear dilations (arrows) fill the fascicle. × 750. Fig. 8 is a whole mount preparation fixed in OsO₄ and stained with Sudan Black B. This illustrates myelin changes as well as the vacuolar response in a branching nerve fascicle. × 500. Fig. 9 is a Holmes’ silver stain showing in one instance four dilations along a single axon (arrows). The other vacuoles sometimes appear axonal; in other cases the origin of the vacuoles is not clear. These preparations are difficult to evaluate because it is not always clear which axons are myelinated; and not all small axons stain. × 500. Fig. 10 shows two dilations developing along the same axon. A neurofilamentous aggregate traverses the dilation on the left. The myelinated fibers above do not show definitive signs of abnormality at this time. × 8,000.

normal medium or 24–36 hr after the peak of the swelling response (i.e., when vacuoles could no longer be seen) showed no discontinuity in the axons. In both cases the fascicles appeared surprisingly normal (Diagram 2).

Electron microscopic examination of the cultures fixed immediately after reversal (established by light microscopic observation) demonstrated only modest periodic swellings of unmyelinated axons (Figs. 17, 18). These areas may represent regions previously involved in the more substantial dilations present at the height of the reaction (see Figs. 7, 15). The electron micrographs showed an abnormal number of heterogeneous dense bodies predominantly within the Schwann cell cytoplasm ensheathing the unmyelinated axons (Figs. 17).
These structures could be lysosomal and could account for the granularity observed in the light microscope. Those cultures which were refed and maintained for longer periods retained a substantial population of neurons. In this situation the myelin degeneration in progress at the time of reversal continued and vacuoles did not recur.

It is important to emphasize that similar manipulations of nutrients applied to cultures in which fascicles had been cut had no effect; that is, neither the time-course nor the extent of the swelling response and its disappearance could be influenced.

If a starved culture at the peak of vacuole formation was cooled (to 4°C), then opened in the refrigerator, and/or refed cold medium, the expected rapid reversal did not occur, even after 24 hr (Diagram 2). If this same culture was then incubated at 35°C, complete reversal occurred within 2 hr (Diagram 2). Any small degree of warming during this procedure resulted in a confusing partial resolution of the vacuolar change.

**DISCUSSION**

The experiments described demonstrate a similar reaction of unmyelinated axons after nerve transection and after nutritional deprivation. In each case the unmyelinated axon undergoes a period of focal swelling during the early phases of degeneration. These massive swellings reach their maximum within about 12 hr after their first appearance and then gradually recede during the next 24 hr. The swellings are characterized by their early appearance, transient course, and focal nature.

A review of past reports of unmyelinated fiber degeneration makes it apparent that this type of reaction has frequently been depicted but variously interpreted. Speidel illustrated an apparently similar reaction occurring in living nerves of the frog tadpole about 6 hr after a fascicle containing unmyelinated axons was cut (Fig. 4, reference 10). Taxi's (7) electron micrographs of cross-sectioned autonomic fibers distal to a cut illustrate some axons that are markedly swollen. These dilated regions appear empty and may well represent the focal swellings reported in the present paper. The type of axonal swelling described by Kapeller and Mayor (9) (see Introduction) as occurring in the distal portions of axons isolated by ligature seems directly akin to that reported in the present study. On the basis of these observations and the light microscopic observations discussed earlier, it seems reasonable to conclude that a transient swelling phase may mark the early phases of unmyelinated fiber degeneration in a variety of species and regions of the nervous system. Because of the early transient nature of the response and its focal nature, it may be missed in studies that either do not include the first hours of the course of degeneration or that rely primarily on analysis of cross-sections of nerve. The dispersion of the neurofilamentous content of the axon in the swollen regions may make this phase of axonal degeneration difficult to recognize in conventional silver stains.

This type of reaction involving a clear swelling of the axon must be distinguished from other conditions that involve axonal dilation with organelle accumulation. These differ in that (a) they do not appear to be transient and (b) the dilated axon is filled with a variety of normal or abnormal axoplasmic constituents. The term “beading” has been applied in some of these cases. In living

**FIGURES 11 and 12** In normal cultures, the neuronal somata display central nuclei, with a mildly granular but generally homogeneous cytoplasm, as seen in Fig. 11. In Fig. 12 the same neurons as in Fig. 11 (n and n') are shown at the height of the vacuolar response in the fascicles. If reversed at this point some neurons will recover; others will not. × 700.

**FIGURE 13** Ensheathed unmyelinated axon dilating dramatically as in Fig. 10. The filamentous content of the axon is dispersed but remains centrally disposed within the vacuole. × 14,000.

**FIGURE 14** This region of an axon dilation (a) induced by nutritional deprivation illustrates the continuity of the Schwann cell sheath (sc) surrounding the dilation. The axolemmal image in the region of the dilation is not clearly a continuous line (as in the Schwann cell membrane) and is marked by a subjacent flocculent material. × 21,000.

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FIGURES 15 and 16 Serial photographs of the same living fascicle illustrating the rapid reversal of the vacuolar syndrome when a nutritionally deprived cultures is refed. The branching fascicle contains a single myelinated fiber. The prominent vacuolar content seen in Fig. 15 disappeared within 45 min (Fig. 16) and the fascicles are now characterized by small granules. X 1,000.

material, dilations occur near the terminal tips of naked growth cones (18). Organelle-filled dilations have been observed in axons immediately adjacent to nerve section (19) or ligature (9), and in several pathological conditions of the peripheral nervous system (20). Webster (8) noted focal accumulation of axonal contents in myelinated fibers and also organelle-filled enlargements of unmyelinated axons after crush injury. Focal enlargements of axons in the central nervous system which involve organelle accumulation have been described in recent EM studies in various disease processes (e.g., 21) and after the administration of certain drugs (e.g., 22, 23).

It should also be noted that many unmyelinated nerve fibers of the autonomic nervous system normally have varicosities near their effector ends;

DIAGRAM 2 Course of axonal degeneration after nutritional deprivation.
Figures 17 and 18. Fascicles altered by nutritional deprivation (as in Fig. 7) are shown here after rapid reversal brought about by refeeding the culture. The regions of increased diameter are abnormal and may represent the sites of previous axonal swelling. The heterogeneous dense bodies (arrows) seen in the cytoplasm of the Schwann cell were frequently observed after reversal and may account for the granularity seen in the light microscope (see Fig. 16). Fig. 17, X 12,500. Fig. 18, X 15,000.

This is observed in both adrenergic (e.g., 24) and cholinergic (25) fibers. These dilations are occupied by groups of vesicles and sometimes mitochondria, and there are several observations which suggest that, when swelling occurs in these fibers, it tends to occur at these points (26). There is a single description indicating that unmyelinated fibers in peripheral nerve show elongated dilations normally (27); these dilations are much less marked than the abnormal dilations observed in the present study.

Mechanism of Axonal Swelling

It seems a reasonable working hypothesis that the empty appearance of the axonal swellings described above results from the intra-axonal accumulation of ions and water. The axolemma of many axons, in common with other membranes, is known to contain a mechanism for the active extrusion of Na ions. This mechanism, which involves a Na-K-activated ATPase, requires adenosine triphosphate (ATP) as its energy source (reviewed in 28). In nerve, this activity is required to provide for Na extrusion after the Na influx that accompanies the action potential as well as the influx from Na leakage. In the sensory ganglia cultures employed in the present study, it seems likely that we are dealing primarily with Na leakage into the fibers, for these cultures are not known to be spontaneously active (29). If active extrusion of Na should fail, it might be expected that Na (and secondarily water) would accumulate in the axon, thus diluting the axoplasm and causing a clear swelling.

After both nerve sectioning and nutritional deprivation (as emphasized in the present study), the supply of ATP might be expected to decrease. In nutritional deprivation the glucose levels have
been demonstrated to be severely depleted; the O₂ supply may also be deficient. It is known that the isolated rabbit retina responds rapidly (with swelling of all organelles) to a combined deprivation of both glucose and oxygen (30). Glucose deprivation alone produces few changes, whereas oxygen deprivation produces similar alterations but at a much slower rate. Recovery is possible only if the deprivation is not prolonged. It is also known that isolated sciatic nerve rapidly becomes depleted at ATP and other high energy phosphate compounds after a short period of anoxia (31, 32).

In the case of axons distal to a point of sectioning, failure of active Na⁺ pumping could also occur if the neuron soma is responsible for a continuing supply of components for the active Na⁺ pumping mechanism.

It is known that there is a substantial decrease in ATPase activity in peripheral nerve tissue within 2 days after nerve injury (33).

The experimental reversal of the axonal dilations can be discussed in the same context. If a deprived culture is refed after maximal axonal swelling has occurred but before the culture has deteriorated further, a rapid disappearance of the swelling occurs. Placing the cultures in the refrigerator at 4°C prevents this rapid reversal, suggesting that an active process is involved. Does this rapid reversal involve participation of the neuronal soma or does it depend entirely upon local axonal mechanism? The rapidity of the reaction (30–90 min) suggests the latter possibility. With axon transport rates of 40 mm/day (34) to 500 mm/day (35), it is conceivable that perikaryon products could reach the distal regions of the axons in the cultures within 30–60 min. The reversal which occurs within a few minutes, however, is difficult to explain on this basis. The alternative explanation is that the effect is mediated by local ATP generation within the axon. With ATP available, Na⁺ pumping is resumed and the dilations are reduced. Because it has not been possible to separate the effects of oxygen, glucose, and pH, the basis for the rapid reversal is not clear. The Na⁺ conductance has been shown to be rapidly and reversibly altered at the nodes of Ranvier by changes of pH (36). In the system employed, pH changes unavoidably occur when the cultures are handled to change nutrients.

The axonal swellings occurring after both glucose deprivation and fascicle sectioning disappear spontaneously about 24 hr after their maximum development. This appears to involve not immediate axonal fragmentation, but a return towards normal size first, with total axonal loss (after fascicle section) occurring only after several days. This slow, spontaneous loss of the swelling may occur due to a complete loss of selective permeability in the axolemma, permitting an equilibration of the axonal contents both inside and outside the axon.

The effects of temperature alterations are puzzling. It has been noted above that cooling the culture after nerve transection prevents the expected appearance of axonal swelling 12 hr later. We have also observed that cooling per se, i.e., maintenance of a normal culture at 4°C for several days, does not cause the swelling phenomenon. Perhaps lower temperatures stabilize the membrane (37), thereby decreasing the passage of all ions across the cell membrane (38). Under these conditions the ion influx may not exceed the output and thus swelling does not occur.

The stress in the above discussion is on the failure of active Na⁺ extrusion. Perhaps the input of ions becomes abnormal and overwhelms the extrusion mechanisms. This has been the general thesis of Van Harreveld (discussed in 39, 40) in studies of central nervous tissues during anoxia and during fixation. The apparent focal swelling of all processes illustrated in certain central tissues after aldehyde fixation (39) bears some resemblance to the axonal dilations observed in the present study. Van Harreveld and collaborators have postulated an abnormal accumulation of Cl⁻ and water in swollen cell processes, which occurs secondarily to an instability of the plasma membrane induced by fixatives or by anoxic conditions.

The relation between Van Harreveld's observations on central nervous tissue and the present study on the peripheral nervous system is not yet clear.

Pattern of Axonal Swelling

We do not know why the axon swells focally rather than uniformly along its length. In contrast, the large axons of lobsters and squids appear to swell and shrink uniformly with changes in the external NaCl concentration (41). Could focal swelling mean widely spaced pumping sites along the axolemma (42) or regions where Na leakage occurs most readily? Are the elastic properties of the axolemma involved? There is some evidence that the axolemma of certain very large nerve fibers possesses specialized regions, as evidenced by
local thickenings and reaction to detergents (43, see also 44). There is also some indication that certain ion channels involved in the generation of the action potential occupy only a small fraction of the axolemma surface; some calculations place them as much as 10 μ apart (37). From these considerations and the present results, it appears that the unmyelinated axon should not be considered a uniform structure along its length.

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