THE ROLE OF SCHWANN CELLS IN THE FORMATION OF "ONION BULBS" FOUND IN CHRONIC NEUROPATHIES*, **, †

HENRY DEF. WEBSTER, M.D.,††
J. MICHAEL SCHRÖDER, M.D.,***
ARTHUR K. ASBURY, M.D.,
AND
RAYMOND D. ADAMS, M.D.
(*Boston, Mass.*)

Peripheral nerves can react to disease in only a limited number of ways. The two best known patterns of cellular response occur in both acute and chronic lesions and are called segmental demyelination and Wallerian degeneration. In segmental demyelination, the axons remain normal. Scattered internodes of myelin degenerate, and mild Schwann cell proliferation is apparent along the denuded axons before remyelination occurs. In Wallerian degeneration, destruction of the axon is accompanied by breakdown of all of its myelin segments. The Schwann cells proliferate in long columns (bands of Büngner), guide the regenerating axon distally, and then remyelinate it. If regeneration fails after severe axon and myelin destruction, nerve atrophy and fiber loss are apparent. Connective tissue lies between the few, widely separated fibers that remain. Usually, in both segmental demyelination and Wallerian degeneration, the longitudinal orientation of Schwann cells is well maintained during destruction and repair.

A third pattern of reaction is seen in transversely sectioned nerves from patients with a variety of chronic neuropathies which contain "onion bulbs" (in remainder of report to read: onion bulb). This term is used to describe a collection of overlapping cell processes and connective tissue arranged concentrically around one or several myelinated fibers. In longitudinal sections, this arrangement is not circular but forms a large tube around the fiber or fibers making up the central core. These formations, although nonspecific, are most numerous in the enlarged nerves of patients with the syndrome, hypertrophic interstitial radiculoneuropathy (2, 12). Whether the interdigitating processes forming the onion bulbs belong to Schwann cells, fibroblasts, or both, has been debated since they were first described (8, 4, 5, 12, 2). Also, the role of each of these cells in collagen synthesis remains poorly

* From the Experimental Neuropathology Laboratories, Massachusetts General Hospital, and the Department of Neurology, Harvard Medical School, Boston, Mass.

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†† Present Address: Bascom Palmer Eye Institute, University of Miami School of Medicine, 1638 N.W. 10th Ave., Miami, Florida

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understood. Since some structures in nerves from patients with neurofibromatosis may resemble onion bulbs (4), it is not surprising that there are many different concepts concerning the mechanism of their formation and their relation to the accompanying nerve fiber loss, to the presence or absence of hypertrophy, and to the clinical features of illnesses in which they occur (2, 3, 5, 1, 11, 7, 13).

This report presents histological, phase, and electron microscopic observations of onion bulbs in 5 nerve biopsies from 4 patients with chronic neuropathy. The main goals of this study were to identify electron microscopically the cells in onion bulbs, to record the accompanying alterations in myelinated and unmyelinated axons, and to correlate them with measured changes in the nerve's size and cellular constituents. Suitable control material and another biopsy, which contained no onion bulbs, were studied and included for comparison.

MATERIAL AND METHODS

Table 1 includes each patient's main clinical features which are described below. The clinical and histological findings in Cases 1 and 4 have been reported elsewhere in greater detail (1).

Case 1. History: M. H., a fifty-year old housewife, first had pain, paresthesias and weakness in both hands 4 years prior to her initial evaluation at the age of 37 years. Previously there had been 3 episodes of left lateral rectus palsy; one was marked by weakness of a hand and foot. Family history was negative. On examination, nerves in the patient's neck, anterior portion of her thorax, upper extremities, and the upper and anterior aspects of her thighs were enlarged and tender. She had mild weakness, minimal atrophy and diminished sensation in her hands; the tendon reflexes were diminished or absent. Spinal fluid protein was 204 mg. per cent. Motor nerve conduction velocities were reduced to 15 to 26 meters/sec. A brief course of intravenous ACTH was followed by improvement for a few months. A year later, she was given prednisone for more severe and widespread symptoms of neuropathy. Since then, nerve tenderness and enlargement have persisted. Minimal clinical progression has occurred while on 7 to 15 mgm. of prednisone *per* day. In 1954 and 1955, biopsies of her right sixth intercostal and right greater occipital nerves

TABLE 1

Case no.	Age	Sex	Clinical diagnosis	Duration years	Distribution
1	50	F	Multifocal hypertrophic neuropathy*	17–26	Asymmetrical, neck, shoulder-girdle, arms, now legs.
2	62	F	Chronic progressive neuropathy ? Familial	20	Symmetrical, Legs, arms.
3	9	F	Progressive system degeneration	8	Symmetrical, distal portions of limbs and cortico-spinal tracts.
4	33	F	Multifocal hypertrophic neuropathy*	9	Asymmetrical, neck, shoulders, arms.
5	65	M	Carcinomatous sensory neuropathy	1	Symmetrical, legs, arms.

* See Reference 1.

showed numerous onion bulbs, some perineurial thickening, and scattered lymphocytes and eosinophiles.

Case 2. History: M. W., aged 62 years, developed post partum panhypopituitarism at the age of 27 years. Thirteen years later, atrophy of her hands was noted. Paresthesias and weakness of her hands appeared, and 5 years later, she had transient difficulty in gait during an Addisonian crisis. At the age of 54 years, progressively increasing weakness of her feet developed and was later associated with a slapping, ataxic gait. Except for a brother with hammer toes and a milder but similar gait disorder, there was no family history of neurological disease. On examination, her median, ulnar, radial and peroneal nerves were found to be enlarged, spongy and non-tender. She had distal weakness, atrophy, and sensory loss in her upper and lower extremities. Gait was severely ataxic, and tremor of her outstretched hands was present. Her tendon reflexes were reduced or absent. Measurements of her motor nerve conduction velocities were unsuccessful.

Case 3. History: S. M., aged 9 years, was the third of 5 otherwise normal siblings. Maternal toxemia complicated the final week of pregnancy and labor was prolonged, but without noticeable consequences. Walking was delayed until 18 months; the child was clumsy and fell frequently. At the age of 3½ years, she was given orthopedic shoes to correct ankle inversion and cavus deformities which have slowly worsened. Dexterity, coordination, and intellectual ability have been below normal for her age; she repeated the second grade. A paternal aunt's gait was described as clumsy since childhood and a maternal aunt, at the age of 40 gave birth to a mongoloid child. On examination, the patient was found to have marked, bilateral cavus deformities. A slapping gait was associated with a severe foot drop, bilaterally. Foot eversion and dorsiflexion were very weak, and she had moderate weakness of her hands. Rapid alternating movements were performed slowly, without tremor. Sensory testing showed distal reduction in two point discrimination and vibration sensation. She had extensor plantar responses and absent tendon reflexes. Cerebrospinal fluid was normal. Motor nerve conduction velocities were reduced to 16 to 25 meters/sec., where testable.

Case 4. History: M. B., aged 33 years, developed weakness and paresthesias in her left hand at the age of 22 years which progressed for several years and then improved slightly. Three years later, biopsy of a left brachial plexus mass revealed an enlarged nerve with numerous onion bulbs and perivascular lymphocytic infiltration. Corticosteroid therapy was begun. There was no family history of neurological disease. On examination at the age of 26 years, distal weakness and sensory loss were found in both upper extremities. Tendon reflexes were diminished or absent. Cerebrospinal fluid was normal. Motor nerve conduction velocities were either normal or slightly reduced. A biopsied right anterior cervical nerve was enlarged and contained numerous onion bulbs as well as collections of perivascular lymphocytes.

Gradual reduction and omission of corticosteroids a few months later was followed by a recurrence of neuropathy which improved after a brief course of prednisone. Numerous mild focal attacks have occurred since; these usually subsided spontaneously or after a short course of prednisone. Nerve tenderness has persisted.

Case 5. History: R. D., aged 65 years, developed rapidly progressive numbness and ataxia of feet and hands with some weakness during the 6 months prior to evaluation. There was no family history of neurological disease. On examination, a left supraclavicular lymph node was found to be enlarged and firm; a biopsy revealed undifferentiated lung carcinoma. The patient had severe ataxia and sensory loss in all extremities, with less severe atrophy and weakness. Tendon reflexes were absent. The nerves were not enlarged or tender. Measurements of motor nerve conduction velocities were unsuccessful.

Preparation of Biopsies: Anatomic variations in the origin, branching, and course of the sural nerve were studied at the post mortem examinations of 6 adults. Lack of branching and relatively constant size were found at the level that was proximal to the ankle by a distance equal to one third of the fibular length. Selection of this biopsy site for each patient helped to minimize anatomic variations and also made results from sural nerves in patients of different ages and heights more comparable.

Approximately 3 centimeters of sural nerve at this level were removed, stretched to resting length on a white card, and divided into 3 portions. Each end was fixed in formalin or osmium tetroxide, and embedded in paraffin for histological study. The central portions from Cases 2, 3, and 5, as well as a similar length of a left cervical cutaneous nerve from Case 1, were immersed for 1 hour in 3.64 per cent glutaraldehyde (Union Carbide, biological grade) containing .05M Sorenson's phosphate buffer at pH 7.6. Fixation was continued for 3 to 4 hours in 2 per cent osmium tetroxide containing .1M Sorenson's phosphate buffer at pH 7.6 (This osmium tetroxide solution was the only fixative used for the sural nerves from Cases 1 and 4). After ethanol dehydration, the nerves were cut into blocks suitable for cross and longitudinal sectioning before they were embedded in epon. Sural nerves, which served as controls, were removed at the post mortem examination of 9 patients; they did not have clinical or pathological evidence of diabetes, malnutrition, hepatic, renal or neurological disease. The endoneurial areas were measured in all 9 cases and portions of 2 were embedded in epon according to the above procedure and sectioned for phase microscopic study and counting.

Counting Methods: In the transverse, osmium fixed, paraffin sections of each sural nerve, the fascicles were counted. The endoneurial area was measured by cutting out high magnification, camera lucida tracings of each nerve's fascicles and comparing their total weight to that of a standard area. Neither these sections, nor others, stained with hematoxylin and eosin or phosphotungstic acid hematoxylin, permitted adequate differentiation, for counting purposes, between the nuclei of Schwann cells and fibroblasts. Identification of each nuclear type was possible in phase photomicrographs of transverse, two micron, epon sections. Nuclei and myelinated fibers were counted in 5 representative areas, totalling .1mm², and using the total endoneurial area, an estimate of the total for each nerve was calculated. Onion bulb estimates were calculated in similar fashion from counts in the same photomicrographs.

RESULTS

Histology and Phase Microscopy: Table 2 presents the endoneurial areas from the transversely sectioned sural nerves; also included are the myelinated fiber, onion bulb, and nuclear totals which were calculated from counts in epon sections since they could not be obtained with sufficient accuracy from cross sections of the entire nerve embedded in paraffin. Promi-

TABLE 2*

	Number of fascicles	Total endoneurial area sq. mm.	Myelinated fibers		Nuclei		Onion bulbs
			Total	Large (10–15u)	Schwann	Fibroblast	
Control							
A	13	1.0	6100	2400	1400	110	—
B	11	1.2	9600	3100	2000	30	—
Case							
1	9	2.9	2000	500	8300	870	3900
2	11	1.3	4500	260	2700	90	1000
3	11	1.1	5300	—	3800	150	700
4	12	1.0	7200	1300	3500	180	50
5	14	.8	140	10	2100	60	—

* Endoneurial cross sectional areas and counts of myelinated fibers, onion bulbs, and nuclei in sural nerves from five patients with chronic neuropathy. The control nerves were obtained at autopsies of two neurologically normal patients.

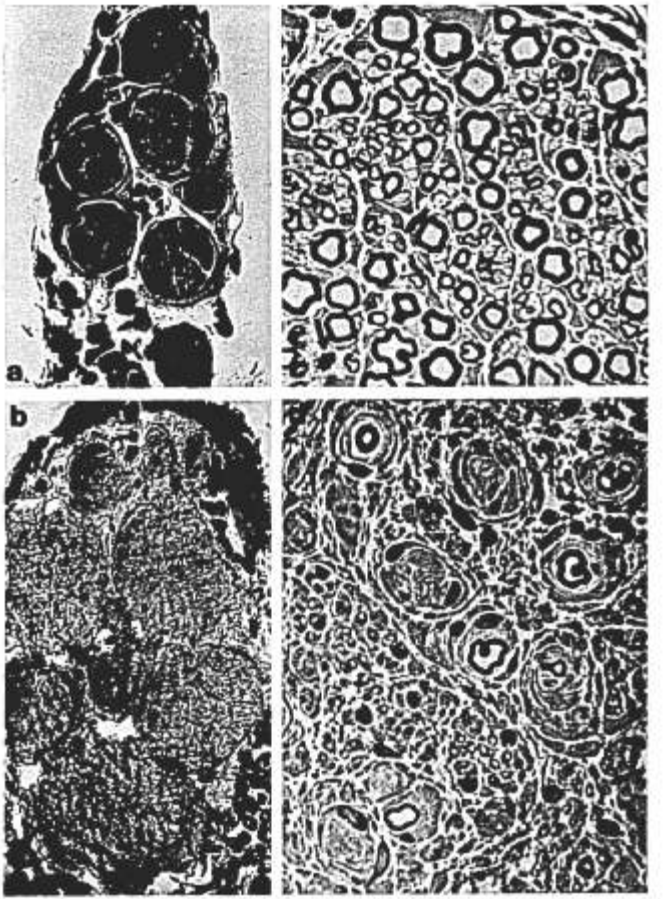
nent nerve enlargement was only present in Case 1. Onion bulbs, as well as nuclei of Schwann cells and fibroblasts were most numerous in this nerve, which also showed a severe loss of myelinated fibers (fig. 1). Fewer onion bulbs, better preservation of myelinated fibers, and less Schwann cell and fibroblastic proliferation were apparent in the nerves of normal size from Cases 2 to 4. In contrast, the slightly atrophic, sural nerve from Case 5 (which was included because it had no onion bulbs) contained normal numbers of Schwann cell and fibroblastic nuclei although the myelinated fiber loss was extremely severe.

The histological features of the onion bulbs were similar to those reported by others and have been reviewed by Wolf *et al* (17), Krücke (12), and Austin (2).

In our paraffin sections, the identification of Schwann cells and fibroblasts was difficult and frequently impossible. Most areas between onion bulbs, nerve fibers, or their remnants were occupied by collagen. No regions containing metachromatic or PAS positive material were identified. Stains for amyloid were negative. The vessels appeared normal without perivascular infiltrates. Perineurial thickening was prominent in Case 1; measurements in the remainder fell within the control range.

In the epon sections which were studied by phase microscopy, it was possible to differentiate the Schwann cell and fibroblastic nuclei, define the arrangement of cell processes in onion bulbs, identify lesions in myelinated fibers, and also evaluate the unmyelinated axons and their Schwann cells (figs. 1c, d, 2, 3).

In the onion bulbs, many of the myelinated fibers forming the cores appeared normal although the myelin sheath occasionally was thin relative to the axon diameter (fig. 2a). Alterations in these core fibers included those of Wallerian degeneration, namely, granular densities and vacuoles in axons surrounded by myelin ovoids or adjacent to myelin discontinuities. Myelin breakdown was not observed around intact axons (segmental demyelination). Schwann cells containing unmyelinated axons were centrally located in many onion bulbs (figs. 1d, 2a, b). The size of these cells, the contour of their processes, and the number of axons indenting their surface varied a good deal. The peripheral zone of onion bulbs consisted of overlapping Schwann cell processes which were arranged circumferentially in transverse sections. These processes often contained a few unmyelinated axons. The size of each onion bulb, in a single cross section, depended on the number and radial width of these processes as well as the amount of interspersed collagen. Serial cross or longitudinal (fig. 3) sections demonstrated the variable orientation and arrangement of these Schwann cell processes along the longitudinal axis of the nerve. Fibroblasts, identified by their nuclear shape and extremely thin, tortuous processes, were not incorporated in onion bulbs but were found either between them or at their outer margins. The number and size of onion bulbs in different fascicles of the same nerve varied. They were not related to the fascicle diameter or perineurial thickness.



KEY TO FIGURES. Figure 1a and 1b are photomicrographs of paraffin embedded cross sections of osmium fixed sural nerves from a control and Case 1. Figures 1c, 1d, and 2 are phase photomicrographs of epon embedded, sural nerve cross sections, and serial longitudinal sections from Case 1 are shown in Fig. 3. Figures 4–21 are electron micrographs of transverse sections. The anterior cervical nerve from Case 1 is illustrated in Figures 4, 5, and 17; the remainder are of the sural nerve from Case 3.

FIG. 1. In comparison with the control shown in (a), the sural nerve from Case 1 shown in (b) is severely enlarged and contains very few large myelinated fibers (× 430). The normal endoneurial constituents are illustrated in (c). The myelinated axons are numerous and vary in diameter; many of the Schwann cell nuclei (arrows) are surrounded by smaller, unmyelinated axons. There is a fibroblast in the upper right corner. In (d), from Case 1, there are 9 large onion bulbs, many Schwann cell nuclei, and very few myelinated or unmyelinated axons. In the collagen between onion bulbs, there are occasional fibroblasts (arrow) (× 800).

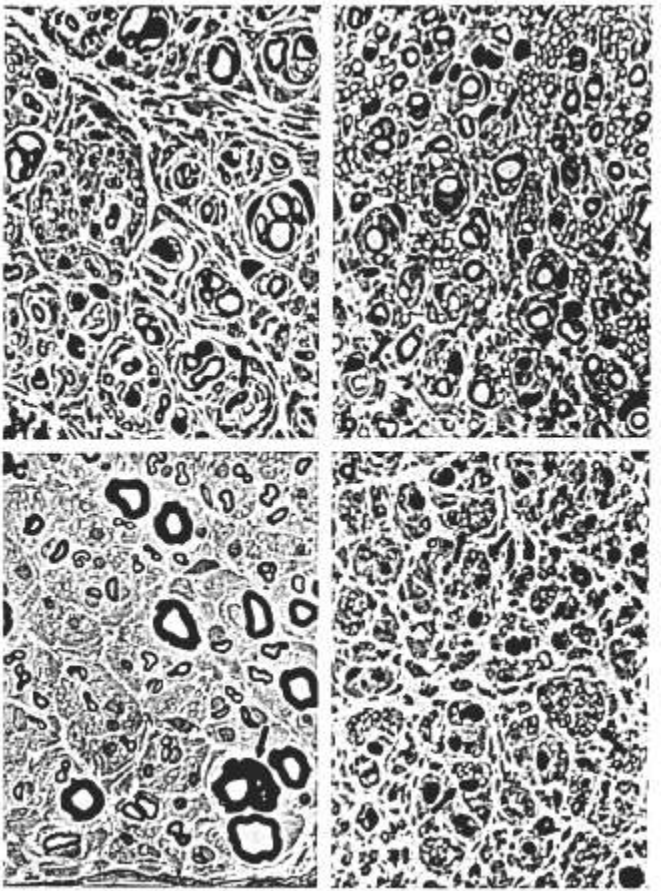


FIG. 2. In (a) from Case 2, there are degenerating (arrow) as well as multiple normal myelinated axons in the onion bulb cores. Schwann cell nuclei and processes form the peripheral zones. Both myelinated and unmyelinated axons are reduced in number. In (b) from Case 3, Schwann cells with unmyelinated axons are centrally located in two onion bulbs (arrows). Fiber loss is less severe; Schwann cell proliferation is apparent. In the upper portion of (c) from Case 4, Schwann cells and their axons appear normal. Below, there is a degenerating myelinated fiber (arrow); Schwann cell nuclei are numerous and a few processes surround several small myelinated axons. (d) from Case 5 contains only one myelinated axon but many Schwann cells and their unmyelinated axons appear normal (arrows); (× 800).

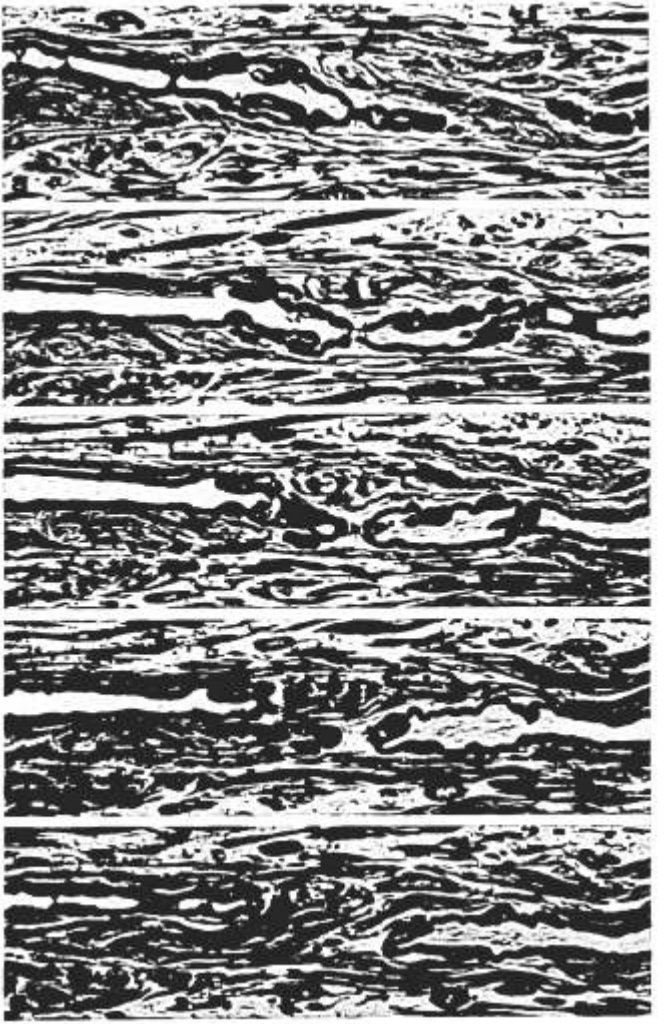


FIG. 3. In (a), above the left paranodal myelin, the Schwann cell processes are parallel to the fiber axis; some form a whorl (arrow) above the tangentially sectioned node; to the right, there is a diagonal band of processes separating the ovoids that represent surface sections of the right paranodal myelin. In (b) and (c), a wider band of Schwann cell processes, differing in orientation, lies below the left paranodal myelin and is seen above the node as a larger whorl. In (d) and (e) this band of processes continues diagonally to join the whorl above the node, an appearance that is consistent with a spiral arrangement of some of these Schwann cell processes around the myelinated fiber in the onion bulb core (× 760).

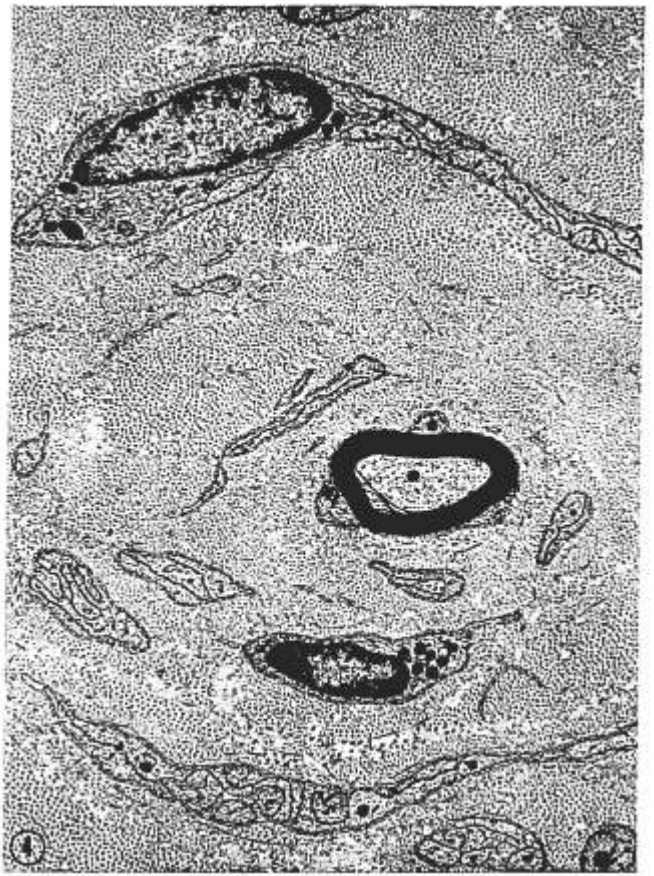


FIG. 4. In this onion bulb, a normal myelinated axon is surrounded by collagen and circumferentially arranged, attenuated processes of Schwann cells. In the processes, surface membranes surround cytoplasmic branches some of which resemble unmyelinated axons (× 10,000).

Although occasional collections were observed, the distribution of onion bulbs appeared to be random rather than subperineurial, central, or perivascular.

In regions of the nerves not occupied by onion bulbs, the loss of large myelinated fibers was moderate to severe. In those that remained, the characteristic changes of Wallerian degeneration were frequently encountered. Many of the smaller myelinated axons appeared normal. Degeneration, when present, involved both the axon and myelin sheath. There were very few macrophages.

All of the nerves containing onion bulbs showed a moderate to severe loss of unmyelinated axons (figs. 1d, 2a, b, c). Many, small, isolated Schwann cell processes containing only one or two axons were separated by large zones of collagen. Single, unmyelinated axons were also frequently encountered in the Schwann cell processes within onion bulbs. On the other hand, in spite of the severe loss of myelinated fibers in Case 5, there were few, if any changes in the unmyelinated axons and their Schwann cells; no onion bulbs were observed (fig. 2d).

Electron Microscopy: Schwann cells and their processes, identified by the presence of a basal lamina (basement membrane) at their surfaces were the only cellular constituents of virtually all of the onion bulbs observed in the five biopsies from Cases 1 to 4 (figs. 4, 7, 10). When they were located in the core, they contained myelinated or unmyelinated axons. In cross sections of the peripheral zone, the number, contour, and radial thickness of the circumferentially arranged Schwann cell processes varied. In general, they were more numerous, thinner, and were separated by more collagen in the larger onion bulbs found most often in Case 1. Closely apposed surface membranes without an intervening basal lamina bounded many overlapping processes which were often difficult to distinguish from the few unmyelinated axons that they surrounded (figs. 4, 5, 7–14, 18, 19). Other cytoplasmic branches projected from the surface as folds or large crescents (figs. 8, 9, 10, 11) and smaller, irregular tongues (figs. 4, 11, 12, 15–18). Occasionally, relatively isolated Schwann cell processes formed a complex network (fig. 5). The perinuclear cytoplasm contained granular endoplasmic reticulum, Golgi membranes, many mitochondria, and occasional lysosomes. Elsewhere, there were profiles of agranular endoplasmic reticulum, tubules, filaments, glycogen granules and fewer mitochondria. Collagen fibrils surrounded by a single membrane were not present in Schwann cells; they were observed occasionally in the processes of fibroblasts. In Schwann cells, the presence of overlapping processes or surface projections was not limited to those in either the core or peripheral zone of onion bulbs. Elsewhere, they were frequently encountered in the scattered, small processes containing unmyelinated axons (fig. 13); also in those with myelinated axons, small zones of cytoplasm or projecting tongues were observed occasionally adjacent to the origin of the external mesaxon (figs. 14–17). These alterations in the contour of Schwann cells and their processes were not present in Case 5.

In Cases 1 to 4, the reduction in the number of unmyelinated axons was proportional, in general terms, to the loss of myelinated fibers, the degree of

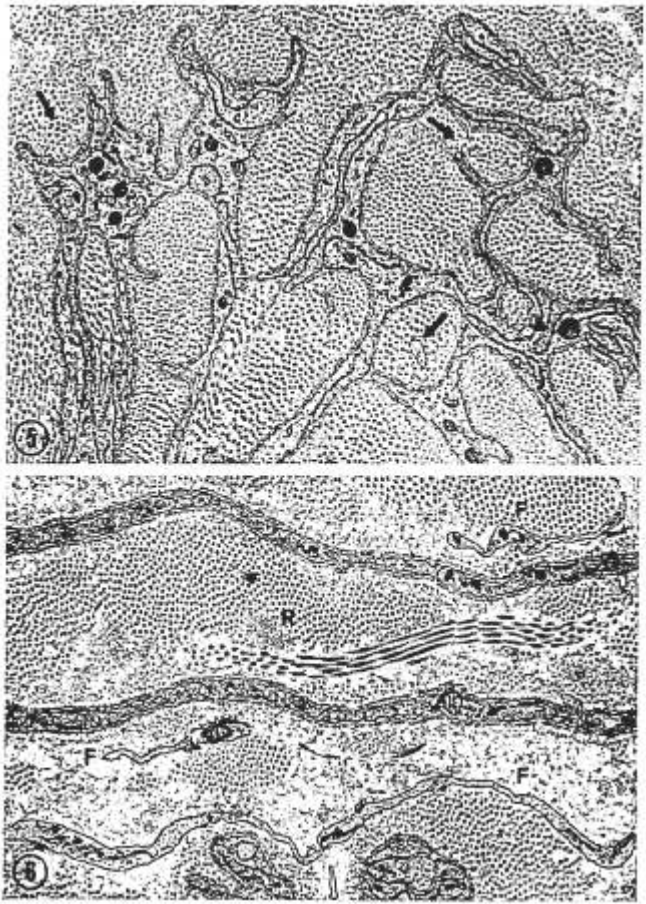


FIG. 5. Collagen fills oval indentations (arrows) of Schwann cell surfaces, one of which contains a membrane limited profile that may represent an axonal remnant (lower arrow). The flattened, overlapping processes contain occasional mitochondria and cisternae of granular endoplasmic reticulum (× 13,000).

FIG. 6. The fibroblastic processes (F) do not have a basal lamina and contain fewer organelles than the two Schwann cell processes between them. There are reticulin filaments (R) adjacent to the longitudinally sectioned collagen fibrils with normal periodicity (× 13,000).

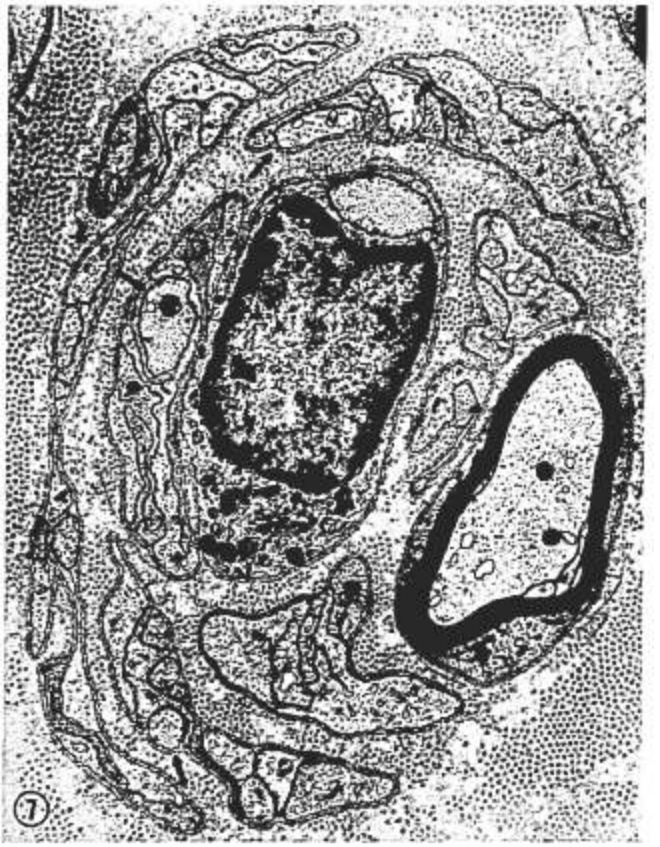


FIG. 7. A Schwann cell, with normal organelles, nucleus, and an unmyelinated axon, is surrounded by attenuated, overlapping Schwann cell processes that envelop only 3, clearly identifiable axons (arrows). The adjacent myelinated fiber appears normal (× 15,000).

Schwann cell proliferation, and the number of onion bulbs. Schwann cell surfaces contained numerous oval pockets which were filled with collagen instead of containing an unmyelinated axon. Those axons that remained were often small, contained granular material, or poorly defined tubules and fila-

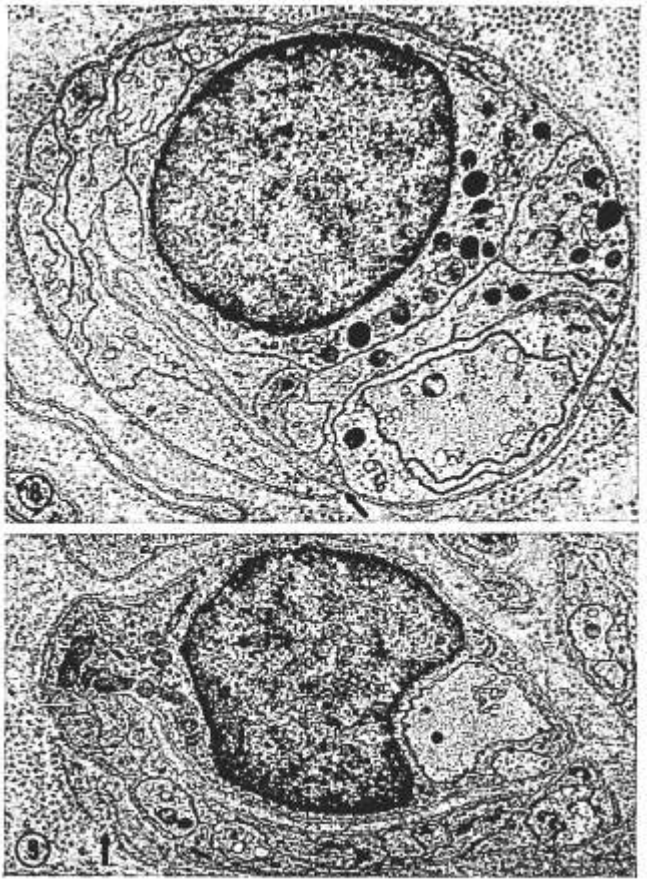


FIG. 8. The basal lamina is duplicated (right arrow) adjacent to a small indentation in the surface of a Schwann cell with many closely opposed processes. A much larger indentation (lower arrow) forms the upper margin of a series of processes which extend below the main portion of the cell (× 17,000).

FIG. 9. Irregular fragments of basement membrane are apparent (arrow) adjacent to many overlapping Schwann cell processes which form a tongue-shaped projection below that part of the cytoplasm that envelops two unmyelinated axons (× 21,500).

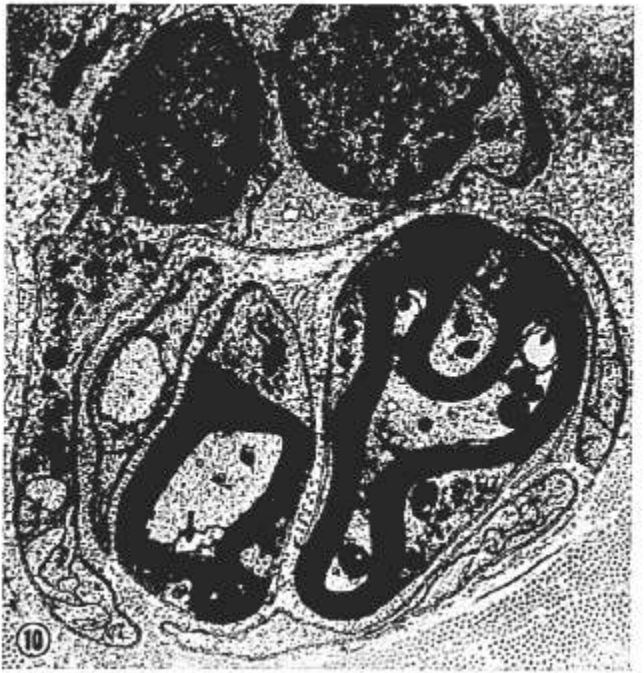


FIG. 10. Adjacent Schwann cell surface membranes are located between two nuclear profiles. To the left, a cytoplasmic process and others, below and to the right, surround 2 myelinated axons and a Schwann cell process containing an unmyelinated axon. Glycogen granules, Golgi membranes, mitochondria, lysosomes, and endoplasmic reticulum are present in Schwann cell cytoplasm. The spaces (arrows) adjacent to the axon membranes are probably related to the preparative procedure (× 12,500).

ments (figs. 7, 12, 13), and their appearance was often similar to that described by Taxi (14). In general, the unmyelinated axons that were centrally located in larger Schwann cell processes had fewer alterations than those at the cell surfaces.

In addition to the loss of myelinated axons, atrophy of many that remained was suggested by the abnormally large and numerous folds in their myelin sheaths. Frequently, the axons contained collections of granular material, glycogen, vacuoles, and mitochondria as well as a highly variable distribution of filaments, tubules, dense bodies and endoplasmic reticulum profiles

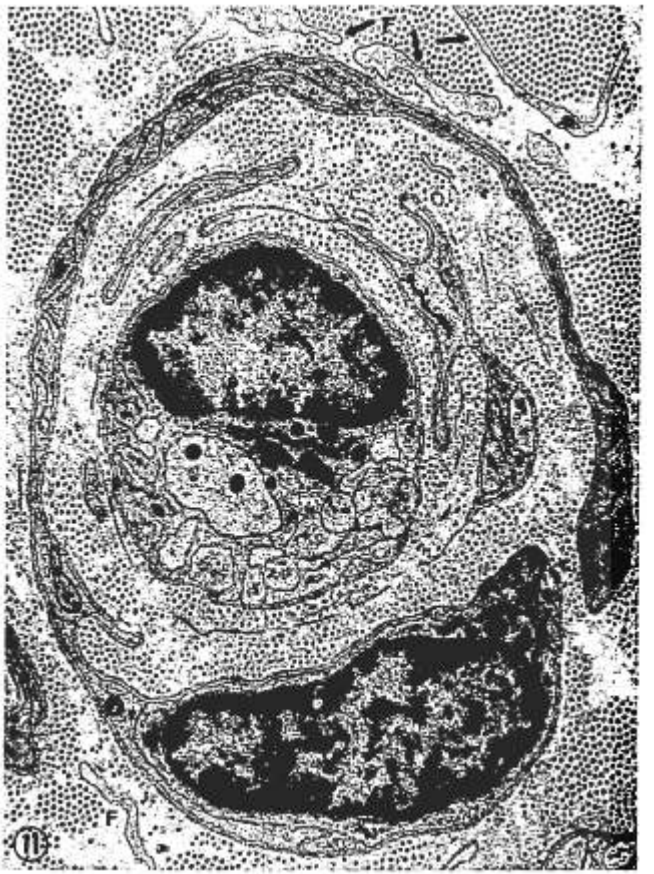


FIG. 11. The centrally located Schwann cell contains a large unmyelinated axon. Other oval profiles include cytoplasmic processes, 2 smaller unmyelinated axons, and perhaps, remnants of others. Attenuated Schwann cell processes, with pockets containing collagen, are arranged circumferentially in the peripheral zone of this onion bulb. The processes of fibroblasts (F) do not have a basal lamina (× 17,500).

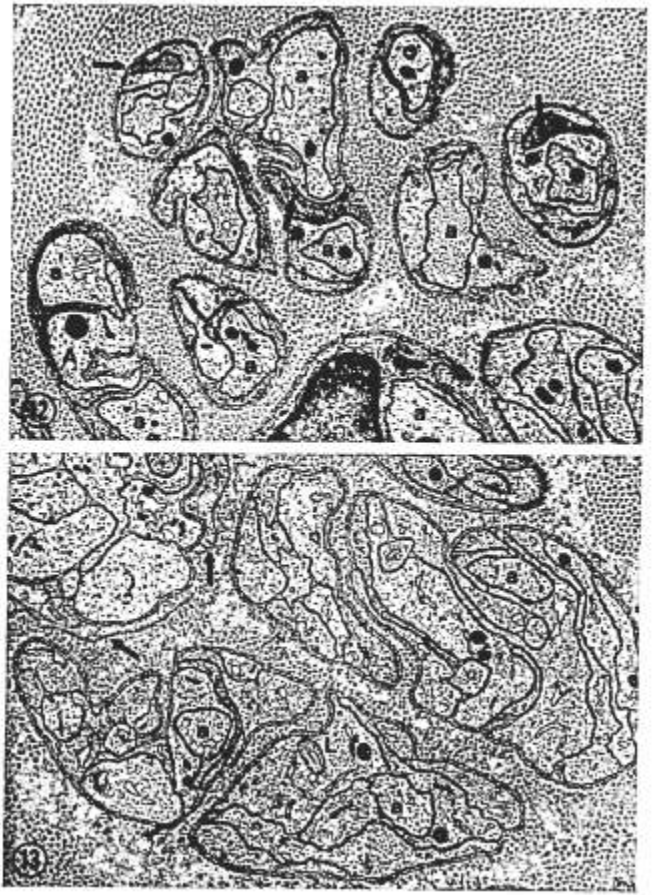


FIG. 12. Normal unmyelinated axons (a), containing filaments, tubules, and mitochondria are enveloped by Schwann cell processes. Other unmyelinated axons (arrows) are small and flattened; they contain dense collections of filaments and a few glycogen granules. The surface membrane of a profile (A) thought to be an axon is poorly defined and indented (× 13,500).

FIG. 13. Reduplication of the basal lamina (arrows) is apparent adjacent to Schwann cell processes containing glycogen. Tubules, filaments, and a lamellar body (L) are present in other Schwann cell processes which surround a few normal unmyelinated axons (a) (× 13,500).

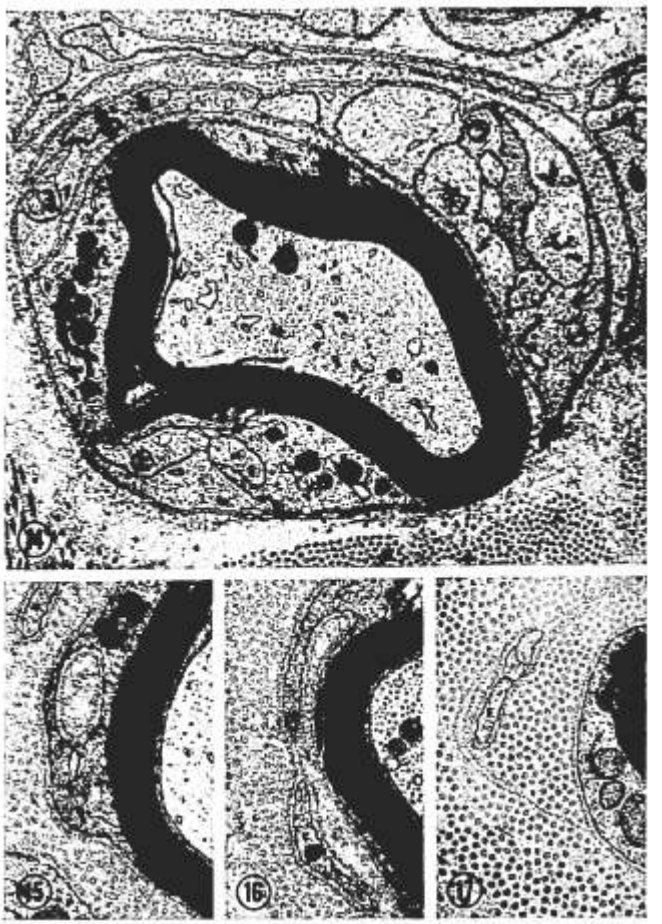


FIG. 14. Schwann cell processes partially surround a myelinated fiber. In addition to the mesaxon (arrow), other extensions of the surface membrane partition the cytoplasm in this region. Aggregates of filaments and numerous profiles of agranular endoplasmic reticulum are located in the axon (× 16,000).

FIGS. 15–17. Other variations in the appearance of Schwann cell surfaces at different levels are shown at the same magnification. In Fig. 15, a collagen filled, basement membrane lined, cavity is surrounded by a ribbon of cytoplasm (× 24,000). In Fig. 16, a cytoplasmic tongue and its extension are covered by a common basement membrane (× 24,000). The continuity of the isolated process and the adjacent Schwann cell in Fig. 17 is suggested by the uninterrupted basement membrane between them (× 23,200).

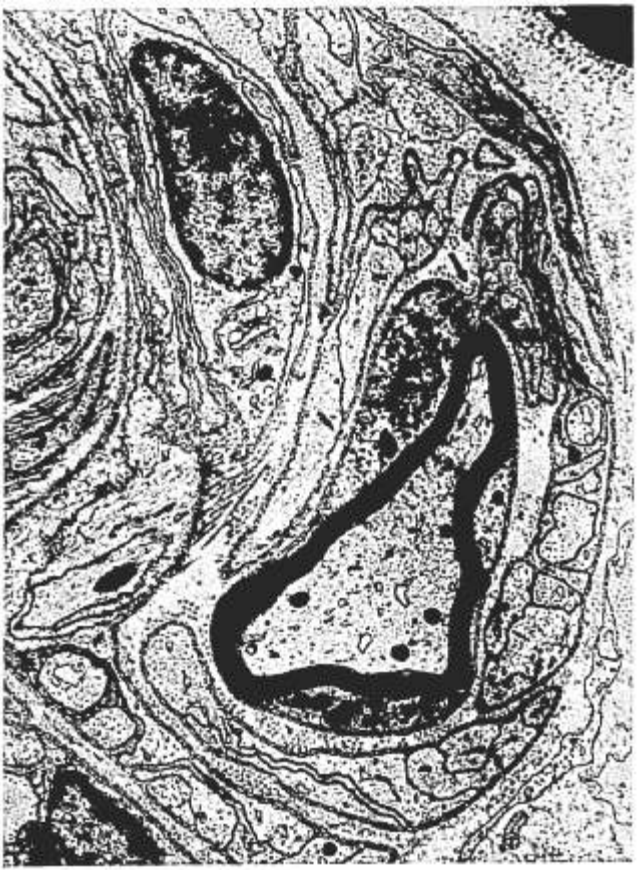


FIG. 18. A probable surface projection (arrow) of a Schwann cell containing glycogen extends along an unmyelinated axon (a). This cell's basal lamina also covers the many Schwann cell processes partially encircling it on the right. On the left, a few tangentially sectioned axons are enclosed in a complex arrangement of overlapping Schwann cell processes (× 13,000).

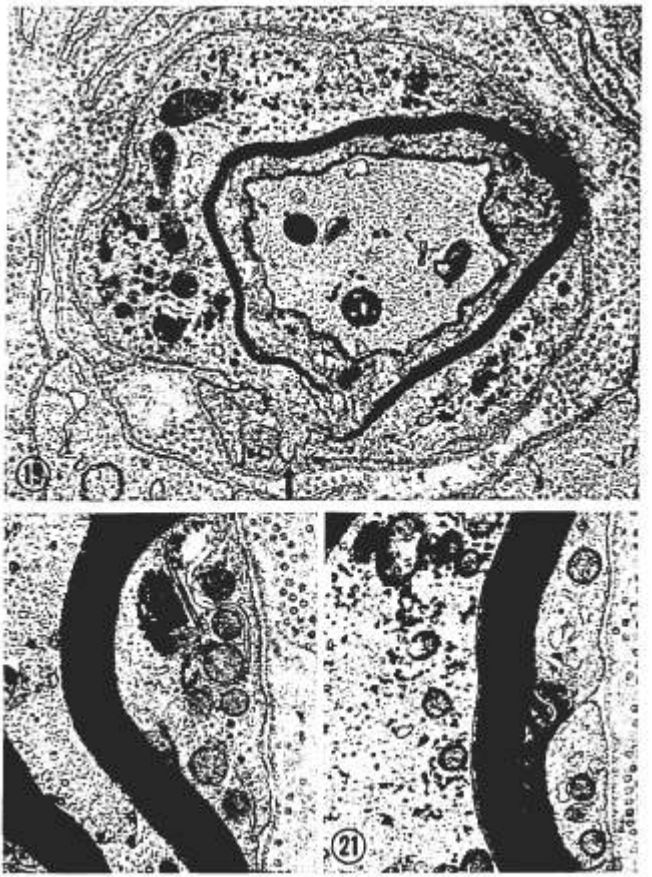


FIG. 19. The axon, limited by an irregular, poorly defined membrane, contains a dense body, 2 mitochondria, and many filaments. The inner myelin lamellae are fragmented; there are many glycogen granules and a few mitochondria in the Schwann cell cytoplasm which has a few irregular projections next to the origin of the external mesaxon (arrow) (× 22,000).

FIG. 20. The Schwann cell cytoplasm contains glycogen and mitochondria, some of which are partially surrounded by membranes. The myelin sheath and axon appear normal (× 29,000).

FIG. 21. Mitochondria and glycogen are prominent in the axon which contains few tubules. In the Schmidt-Lantermann incisure, the Schwann cell cytoplasm between the myelin lamellae contains minute dense particles which vary in size (× 29,500).

(figs. 19, 21). In zones where the axon membrane was irregular or discontinuous, adjacent myelin lamellae were fragmented (fig. 19). More advanced Wallerian degeneration was characterized by the presence of lamellar ovoids and myelin remnants around a severely altered axon. Generally, Schwann cells containing a normal axon and myelin sheath also appeared unaltered. In some, however, the cytoplasm contained collections of mitochondria and glycogen granules (figs. 14, 18, 20) or occasional lamellar bodies.

The endoneurial fibroblasts were most numerous adjacent to the perineurium and along the vessels. Their processes occasionally encircled an onion bulb but were not identified within either the central or peripheral zone. The intercellular matrix consisted almost entirely of longitudinally oriented collagen fibrils with a normal periodicity. Occasionally, tangential or transverse orientation was encountered and seemed more frequent adjacent to the occasional, redundant loops and free fragments of Schwann cell basement membrane. Fine filaments, corresponding to reticulin were also observed, but nothing was observed that might represent myxomatous material with the histological staining properties of mucopolysaccharides.

DISCUSSION

Our observations clearly demonstrated that Schwann cells and axons were the only cellular constituents of the onion bulbs observed in the nerve biopsies from 4 patients with 3 clinically and genetically different types of chronic neuropathy. The core included one or several Schwann cells; their myelinated or unmyelinated axons frequently showed degenerative changes but often were normal. In transverse sections, the peripheral zone was formed by overlapping, circumferentially arranged Schwann cell processes with a few degenerating or normal unmyelinated axons. Although the longitudinal contour and length of these processes was not defined in detail, some appeared to be spiral and together, they formed an interlacing network of processes that formed a tube around the central core. Collagen fibrils occupied the space between the Schwann processes and the onion bulbs did not contain fibroblasts.

Thus, the formation of these onion bulbs involved a change in the contour of Schwann cells. Obviously, the study of comparable biopsy material in patients at different stages during the evolution of these lesions supplemented by a similar study of a suitable experimental model would provide the best basis for defining the dynamic aspects of this change. Unfortunately, onion bulbs have not been produced experimentally, and comparable, serial biopsies from each of our patients were not available. However, our tabulation of nerve measurements and counts as well as the study of the electron microscopic alterations in Schwann cells and axons between onion bulbs added substantially to our understanding of this process even though they must be interpreted cautiously and critically.

In the normal sural nerves which were used as controls, the endoneurial

areas, myelinated fiber counts, and the relative proportions of Schwann cell and fibroblastic nuclei were similar to those reported by Dyck (10), and Causey (6). In Case 5, the changes were similar to those seen in chronic Wallerian degeneration. In Cases 1 to 4, the onion bulbs were most numerous in nerves exhibiting the greatest Schwann cell proliferation along with the smallest number of remaining myelinated and unmyelinated axons. Nerve enlargement only occurred when fibroblastic hyperplasia was pronounced. These data indicated that the formation of onion bulbs was preceded by a loss of myelinated and unmyelinated axons and a proliferation of Schwann cells in addition to a change in their usual surface contour. Unfortunately, similar counts and measurements in nerves with a variety of chronic lesions are not available in the literature for comparison. In future studies, it will be more important to test the validity of this relationship than the magnitude of each value since the number of onion bulbs is known to vary widely at different levels of the same nerve, in different nerves from the same patient, and in the many clinical variants of the neuropathies in which they are observed.

Although more prominent in onion bulbs, similar electron microscopic alterations of the Schwann cells and their axons were observed elsewhere in the nerves from Cases 1 to 4. In transverse sections, the Schwann cells surrounding unmyelinated axons did not retain their usual oval shape. Large crescentic extensions or small isolated processes contained very few axons and many of these were atrophic. Normally, the Schwann cell processes are arranged longitudinally along the unmyelinated axons which they partially or completely surround. Degeneration of many of these axons, particularly those at the surface, may have been responsible for the varied orientation of the numerous cytoplasmic branches which were seen either as surface infoldings or projections. If the length of these Schwann cells was maintained after the loss of many of their axons, the rearrangement of their processes may have been associated with an increase in the Schwann cells' surface area prior to axonal regeneration. Alternatively, a decrease in the Schwann cells' length after axonal degeneration could have changed the number and size of their transverse processes without producing an increase in their surface area. Finally, remodelling of the surface of Schwann cells, containing either myelinated or unmyelinated axons, also could have been influenced by the deposition of collagen adjacent to the basement membrane.

The lack of segmental demyelination in our material was surprising since a few attacks of neuropathy in Cases 1 and 4 were followed by rapid recovery. Also, it has been a prominent histological finding in many nerves containing onion bulbs (12). Cammermeyer described delamination of myelin sheaths, beginning with the outer layers, in nerves from patients with Refsum's syndrome (5). In a recent electron microscopic study of another case, unrolling of the myelin sheath and segmental demyelination were also reported; other findings included nerve fiber loss, Schwann cell hyperplasia and hypertrophy as well as an increase of collagen (9). These observations,

and the absence of segmental demyelination in our biopsies may reflect differences in interpretation, sampling, or disease etiology. The quantitative distribution of segmental demyelination and Wallerian degeneration along the length of a peripheral nerve with onion bulbs has not been studied in any disease. Both might occur in different regions of the same nerve or either one might predominate in each of the many diseases characterized by onion bulb formation. Perhaps, different pathogenetic mechanisms affect either Schwann cells or axons primarily and regardless of the initial target, the chronic interaction of the axons and their satellites produces onion bulbs.

Our failure to observe axonal regeneration may have been due to the current lack of reliable criteria for its electronmicroscopic identification. Since the axon diameter and number of mitochondria, endoplasmic reticulum profiles, tubules, and filaments vary widely in serial sections of normal axons and those undergoing degeneration (15) or remyelination (16), it was not possible to identify regenerating axons by a survey of these parameters. However, axonal branching and budding were not seen. Unmyelinated axons, similar in diameter to those that were myelinated were observed in Cases 1 to 4 and were examined in many sections. None of these was surrounded by a lamellar spiral, a finding which should have been encountered occasionally if the thin myelin sheaths, similar to those in Case 2, represented remyelination of axons that had regenerated.

The deposition of collagen was clearly responsible for the nerve enlargement in Case 1. The accompanying fibroblastic proliferation was also associated with an overall decrease in cell density. Both were obvious in all of the sections and were confirmed by the counts when they were expressed as the numbers of nuclei, myelinated fibers, and onion bulbs *per* square millimeter of endoneurial area and compared with similar figures for the other patients. In Case 1, these findings were even more prominent in the patient's anterior cervical nerve which had been enlarged to palpation for 13 years. Thus, the formation of collagen produced the hypertrophy of the nerves which contained many large onion bulbs and very few normal nerve fibers. The cellular origin of the collagen was not clear but the proliferation of fibroblasts during nerve enlargement suggests that they played a more important role in collagen production than the Schwann cells, which became much less numerous *per* unit area.

Our present concept of onion bulb formation is based on a study of distal sensory nerves, in which unmyelinated axons are especially numerous. Initially, unknown etiologic factors, which may be genetically determined, produce slowly progressive or episodic degeneration of the unmyelinated and myelinated axons which fail to regenerate promptly and completely. The processes of proliferating Schwann cells and those which have lost axons become arranged around a core of one or several Schwann cells that still contain axons. The surface area of Schwann cells probably enlarges as their unmyelinated axons degenerate and increasing amounts of collagen polymerize adjacent to their basement membranes. Progressive collagen deposi-

tion increases the distance between cells, may accelerate axonal degeneration, and probably hinders their regeneration; ultimately, it produces enlargement of nerves which then contain numerous onion bulbs and a few, scattered normal myelinated fibers.

SUMMARY

Onion bulbs in 5 nerves from 4 patients with chronic neuropathy were studied by light, phase, and electron microscopy. One or several Schwann cells, with myelinated or unmyelinated axons, formed the core of each onion bulb; the peripheral zone contained attenuated, overlapping, circumferentially arranged processes that also belonged to Schwann cells. Measurements of endoneurial areas as well as counts of myelinated fibers, onion bulbs and cell nuclei indicated that onion bulb formation was accompanied by degeneration of myelinated and unmyelinated axons, proliferation of Schwann cells, and rearrangement of their processes. Nerve enlargement, or interstitial hypertrophy, was produced by collagen deposition and was associated with fibroblastic proliferation.

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Addendum: Since this manuscript was submitted, additional electron microscopic observations of onion bulbs have been reported by P. J. Dyck in Mayo Clinic Proc., 41: 742, 1966.

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