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# ELECTRON MICROSCOPIC STUDY ON WALLERIAN DEGENERATION OF THE SPINAL CORD AFTER SECTION

by

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## INTRODUCTION

It is a well known fact that when a neuron is divided the nucleus-containing central segment does not die, but the axon and myelin sheath of the peripheral segment developes disintegration. This kind of degeneration of nerve, known as Wallerian degeneration or the secondary degeneretion, has mainly been studied on the peripheral nerves.[1, 2, 3, 7, 22, 30, 31] Wallerian degeneration has been widely applied for the determination of nerve pathways in the central nervous system. Although the process and mechanism of the degeneration has been extensively studied, there is no concord of opinions as to details because of disadvantages in light microscopy as follows.

On light micoscopic studies of the nerve tissue done so far, specific structural componenent could be observed on the slide specially stained; therefore, an entire view must have been reorganized by means of collected slides obtained individually. It must be permitted that during the slide processing some morphologic damage can not be avoided.

Recently, an electron microscope has been applied to the studies of the central nerve tissue, and new findings have been obtained which are somewhat different from those of light microscopic studies. The greatest advantages of electron microscopic study are that by its high resolving power each structural element can be observed simultaneoualy under relatively unchanged condition similar to vital state, and so the fine changes unable to be observed with previous methods are also demonstrated.

This article deals with the secondery degeneration of the rabbit spinal cord occuring after transverse section studied elsctron microscopically.

## MATERIALS AND METHODS

Thirty rabbits, weighing 2.0 to 2.5 kg, were used. The spinal cord was exposed under laminectomy at the first lumbar vertebra and severed tranversely with a sharp knife. The specimens were obtained in 12 hours, 20 hours, 24 hours, 44 hours, 4 days, 5 days, 7 days, 10 days, 21 days, 30 days, 61 days, and 166 days postoperatively in the following method.

The spinal canal was opened extending above and below the site where the spinal

cord had been severed. The tissue was taken from the dorsal funiculus 2 to 3 cm cranially from the site of section, then put into the fixative and immediately divided into the small pieces of approximately 1.0 × 1.0 × 0.5 mm in the fixative solution. Specimens for light microscopic study were also obtained in several cases.

### Processing of Specimens for Electron Microscopic Studies

White matter of the mammalian central nerve is one of the most difficult tissues for successful processing for electronmicroscopic study; that is, mylin sheath in the central nerve is easily destroyed during specimen processing.[8, 28] Various ways of fixation and embedding have been, therefore, recently devised.[5, 8, 28] The author also investigated the several methods of the specimen processing and finally came to a conclusion that the tissue is well preserved with the following procedure.[42, 47]

1) Fix in a mixture of chrome-osmium solution (2.5 % $OsO_4$ in DALTON's buffer[10] of pH 7.4) 2 ml and 1 drop of 1 % aqueous solution of saponin. At 3°C for 1 hour.
2) Dehydrate with each 10 minutes in graded series of ethanol (50 %, 75 % and 95 %).
3) Two changes of absolute ethanol 15 minutes each.
4) Two changes of propylene oxide 10 minutes each.
5) Immerse in the mixture of propylene oxide and embedding material (1 : 2) 3 hours.
6) Polymerize in oven, at 37°C for first 20 hours and 60°C for following 10 hours. Embedding material was a mixture of the followings.

| | |
|---|---|
| Epon 812 | 5 ml. |
| Epon 815 | 5 ml. |
| D. D. S. A. | 16 ml. |
| D.M.P.-30 | 0.45 ml. |

Ultrathin sectioning was made with glass knives on an J. U. M. -5 ultramicrotome. For electronmicroscopy, Hitachi HS-6 microscope was chiefly used and HU-11 was occasionally used. Micrographs were obtained at original magnification of 2,000 to 20,000 and thereafter enlarged photographically.

### Phase Contrast Microscopic Studies of Electron Microscopic Specimens.

One of the disadvantages of electron microscopy is an extremely limited visual field; therefore, the same specimen was sectioned into 0.5 × 1 mm in size and studied under a phase contrast microscope.

### Specimens for Light Microscopic Studies

Some of the rabbit spinal cord specimens were used for light microscopic study, being fixed with formalin and stained with Hematoxylin-Eosin, Sudan III and Marchi's methods.

## OBSERVATIONS

Electron microscopic appearance of the normal dorsal funiculus of spinal cord was a dence structure composed of myelinated fibers of various diameter and a complicated meshwark of the glial processes filling remaining space (Fig. 1).

In myelinated fibers of the central nervous system, being essentially same as peripheral nerve,[13, 19, 20] the axon was wrapped by layered myelin sheath (Fig. 2).

Myelin sheath consists of alternating dense line and light layer, and in the center of the latter an intermediate line was visible (Fig. 3, Text-Fig. 1).

Inside the axon numerous axonfilaments of approximately 100 Å in diameter scattered throughout homogeneous matrix of low electron density and scanty mitochondria and endoplasmic reticulum could be seen.

### Change in the Axon

In the specimen 12 to 24 hours after spinal cord section, some fibers showed vacuolization or granular change of endoplasmic reticulum and mitochandria (Fig. 4).

Axonfilaments also underwent changes simultaneoualy or somewhat later. Their arrangement first became irregular (Fig. 4) and then they were shortly cut, demonstrating interwined or coalescended appearance (Fig. 7).

The early change of axoplasm as mentioned above, were later followed by formation of a gap between the axon and myelin sheath in 20 hours after section (Fig. 5). Such a change of the axon was initially conspicuous in the vicinity of node (Fig. 6). The axon did not show a simple constriction, but partly beame very different in size if seen in longitudinal section. Between the axon and myelin sheath, there were different kinds of area; for example, one was with matrix of very low density and another was apparently devoid of matrix. All of these structures were clearly limited by the membranous structures (Fig. 5, Fig. 6, Fig. 7) which partly originated from the myelin lamella separated and projected inward.

The axoplasm began to show marked change in 44 hours after spinal cord section in the small fibers (Fig. 7) and within 10 days in the large fibers. The axon-oligocytic membrane, so-called A. O. membrane, analogous to the axon-schwann membrane of myelinated fiber in the peripheral nerve (Text-Fig. 1), disappeared and organellae such as mitochondria were no longer able to be observed. There observed were what changed from the axonfilament through chopping up and conglomerating, and what looked like indistinct granules (Fig. 10). These degenerating products of the axon diminished as morphologic changes of the myelin sheath progressed in nearly all of degenerating fibers approximately in 10 days postoperatively. They finally disappeared showing a picture as if compressed under the deformed myelin sheath (Fig. 15). In some of the extra-large fibers, however, change in the myelin sheath progressed slowly and degenerating products remained for a considerably long period (Fig. 21).

### Change in the Myelin Sheath

Observation in the longitudinal section first demonstrated changes in diameter of the myelin sheath at some portion (Fig. 6).

And then, myelin lamellae irregularly dissociated and projected inward forming the separating wall at the narrowing portions of the sheath where the nerve fiber finally come to pieces called ovoid[3, 24, 31, 33, 34, 49] or ellipsoid[2, 44] in which debris of the degenerated axon was contained (Fig. 10). In cross section of the early specimen, small fibers demonstrated flattening or protrusion of the myelin sheath (Fig. 5, Fig. 7), whereas in large fibers those changes took place rather late presenting some peculiar changes in the myelin sheath such as formation of double or triple layers, flattening and infolding (Fig. 15), or assembly of more than two of them (Fig. 16).

In extra-large fibers, protrusion or invagination of the myelin sheath was locally observed (Fig. 20). Some of the myelin sheathes included structures of other myelin sheathes inside (Fig. 20, Fig. 21).

As mentioned above, in the degenerating process the myelin sheathes showed various pictures according to sites of the ultrathin-section. The lamellar structure, however, was still maintained until this time of process (Fig. 17). The myelin sheathes then lost their lamellar constitutions becoming electron dence conglomerates (Fig. 12, Fig. 18, Fig. 22, Fig. 23). This process corresponds to Marchi stadium[44] in light microscopic finding. (Fig. 19). The dense conglomerates collapsed, sooner or later, into the dense granules (Fig. 24).

### Interstitial Changes

Approximately at 20 hours after spinal cord section, interspaces of the glial processes partly began to collect fluid (Fig. 5) and the fluid accumulation reached its maximum in 4 to 7 days, associated with formation of vacuoles and mitochondrial destruction in the glial processes (Fig. 9, Fig. 13). On the other hand, proliferation of macroglia was observad on the 5 th postoperative day (Fig. 11).

In relatively early period; that is, 44 hours postoperatively, the cells began to appear in which dense granules were phagocytosed (Fig. 8).

## DISCUSSION

### Running Direction of the Nerve Fibers

Running direction of the nerve fibers should be considered when degeneration of the nerve tissue is discussed. Light microscopic study already disclosed that majority of the posterior funiculus is made of the tract originating cells of which being in the spinal ganglia and terminating in the dorsal fascicular nuclei of the medulla oblongata.[46, 53] Descending fibers, however, are also included. It is impossible to discriminate the descending fibers only from their diameter. In this experimental study, fibers which degenerated on account of spinal cord section were investigated.

### Traumatic Degeneration

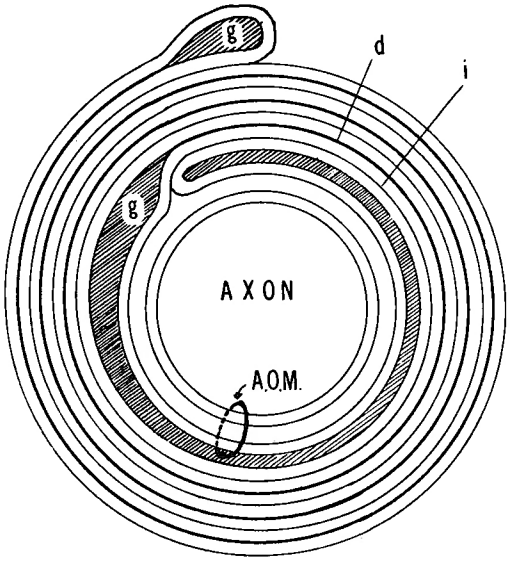
Besides the Wallerian degeneration, traumatic degeneration also occurs following spinal cord section and is known to be limited within a few milimeters from the site of section[7]. The probable area of traumatic degeneration was of course excluded from the

specimen for study.

## Classification of Fibers According to Their Diameter

In many studies on Wallerian degeneration of peripheral and central nervous system, nerve fibers have been classified according to differance of diameter which is said to be related with the behavior of degeneration.[1, 7, 21, 33, 34, 50, 56]

In this article, nerve fibers were accordingly divided into four groups: (1) small fibers (smaller than 1 $\mu$ in diameter), (2) medium (1-3 $\mu$), (3) large (3-5 $\mu$) and (4) extra-large fibers (larger than 5 $\mu$). It must be permitted, however, that this classification is not a definite one, because fibers partly change their shape and size as degeneration advances. It is also said that fibers with different function degenerate in different rapidity.[30, 50] This seems to be especially true in the spinal cord as it contains various kinds of fiber; therefore, in this experiment, a classfication according to diameter is only for convenience.




**Text-Figure 1.**

The myelin sheath of the central nerve is formed of the oligocytic processes (g) surrounding the axon in spiral and lamellar fashion. The dense line (d) is a result of close contact between the cytoplasmic surfaces of the glial cell membrane. The intermediate line (i) is a result of apposition between the external surfaces of the same mbrane. Axon-oligocytic membrane (A. O. M.) is defined, by DE ROBERTIS, to imply the axolemma, oligocytic surface and space between them.

(The author referred to the illustration shown by PETERS, A. (1960))

## Machanism of the Myelinogenesis

According to LUSE, numerous glial processes flatten, fuse together and then form the myelin sheath[26], and this view is shared by ROSS and others.[40] DE ROBERTIS and others, on the other hand, are of an opinion that cytoplasmic membranous structures in the glial cell around the axon gather, fuse, flatten and finally form the myelin sheath.[11]

PETERS[36, 37] and MATURANA[29] emphasized also the role of glial processes, and suggested that the myelin sheath of the central nerve might be formed by spiral wrapping of glial processes (Text-Fig. 1). Their opinion was supported by BUNGE and others who demonstrated a stereographic scheme[6] which is generally accepted today.

## Change in the Axon

*Early changes in the axoplasm.*

In 12 to 24 hours postoperatively, granule or vacuole formation of endoplasmic reticulum and mitochondria began (Fig. 4). Simultaneoualy or a little later, change in the axonfilaments occured (Fig. 4, Fig. 7).

Early changes in organellae have been studied by OHMI,[33, 34] HONJIN and others[21], and VIAL[49] in the peripheral nerves, YURI and others[55] in the optic nerve; and the onset of changes differs respectively. Author's study on the spinal cord also revealed a fact that the several specimens of the same postoperative period did not necessarily exhibit the same early changes in organellae. The following explanation may be reasonable for it, i. e., the organellae are extremely sensitive to the physico-chemical conditions of the fixative[32] and are easily affected by the electron bombardment.

*Change in the shape of the axon.*

Following the change in the organellae, 20 hours postoperatively, partial constriction of the axon began (Fig. 5), resulting in the rosary appearance in longitudinal section (Fig. 6). This change in the axon is initially seen in the proximity of the node quite remarkably suggesting that the change was related with segmentation of the axon in the light microscopic level because of similarity of onest and localization.

In the axon of a peripheral nerve, morphologic change due to nerve section is said to be promoted by surface tension[43, 54] over the axolemma caused by stasis of axoplasm deriving from the originating cell[51].

In the spinal cord, however, in addition to the similar change as seen in a peripheral nerve, swelling and expansion of vacuoles due to degenerating products in the axon, and detachment and intrusion of the innermost myelin lamella were observed (Fig. 5, Fig. 7, Fig. 13).

In this study axonsegment enveloped in A. O. membrane could not be found (Fig. 10, Fig. 13). These facts indicate that segmentation of the axon of the central nerve does not depend on only the surface tension of the axon.

*Segmentation of the axon.*

The axon of rosary apearance developed progressive change thereafter. A. O. membrane disappeared in 44 hours in small fiber, whereas in 10 days in large fiber postoperatively (Fig. 10).

The fiber contained small pieces of axonfilament interwined each other and denser corpuscles with obscure contour scattered in groups over the inner space of the myelin sheath (Fig. 10). The axon lost its continuity at this stadium, as called segmentation in electron microscopic level. Degenerating products of the axon gradually disappeared from the field following segmentation.

## Change in the Meylin Sheath

As previously mentioned, myelination and cellular structures in the central nerve have several different aspects from those in the peripheral nerve,[16, 45] and so, it is supposed that the degenerating process of the myelin sheath may be different between both.

*Early Changes in the myelin sheath.*

No structural change occured in the myelin sheath until when change of shape began in the axon in the early period (Fig. 4).

Asscoisted with constriction of the axon in the early postoperative period, small and medium fibers with thin myelin sheath developed redundancy of its circumference resulting in protrusion and flattening of that part of the sheath (Fig. 5, Fig. 7, Fig. 9). The large fibers with thick myelin sheath showed a similar change much later.

Above difference in behavior between thick and thin myelin sheathes is probably due to differrent rigidity of the myelin sheathes. This fact also suggests that a promoter of morphologic change in the early stage of degeneration, particularly until the time of the axon segmentation, consists in the axon rather than in the myelin sheath. It is strongly suggested here that morphologic changes of the myelin sheath are quite passive judging from the finding in which the myelin sheath is retracted toword the A. O. membrane in association with constriction of the axon. By the way, the axon of this stage still possessed the A. O. membrane as a rule.

*Segmentation of the myelin sheath.*

When segmentation of the myelin sheath occured, that of the axon was seen precedingly (Fig. 10). It was essentially similar to what observed by OHMI in the peripheral nerve.[33, 34]

In the spinal cord, however, there was no Schwann cell and the A. O. membrane had disappeared in this stadium of degeneration. How can we explain mechanism of segmentation? Here assumed is that segmentation might be caused by intrusion of the myelin lamellae. Namely, in fibers the axon of which had already segmented, myelin sheath projected inward here and there forming the separating wall which finally resulted in small corpuscles (ovoid or ellipsoid) retaining degenerating substanec of the axon inside (Fig. 10, Fig. 13, Fig. 21).

This change began in 2 to 10 days postoperatively.

It is reasonable, therefore, to think that the promoter of the structural change is no longer the axon but the myelin sheath if after segmentation of the axon has completed.

*Formation of the myelin sheath conglomerates.*

Behavior of the myelin sheath showed following segmentation strikingly differed from that observed in a peripheral nerve; [33, 34] myelin sheath of the latter is decomposed under Schwann cell activity into large myelin globules, then further to small globules.

In the spinal cord, segmented myelin sheath, focally enclosing degenerating products of the axon, became flattened or doubled up on itself with partial projection outward or inward. Finally, it disintegrated into dense masses lacking of cavity (Fig. 12, Fig. 18, Fig. 22, Fig. 23).

YURI and others observed in the optic nerve that medium fiber is surrounded by two or three flattened large fibers the ends of which soon come together forming a concentric myelin sheath rings that decompose later and fuse together into a dense mass.[55] Similar finding was only occasionally observed in the spinal cord (Fig. 16).

A dense mass progressively disintegrated into finer and dense granules (Fig. 24), and they were finally eliminated from the tissue by means of phagocytosis.

## Change in the Stroma

Interstitial edema appeared 20 hours after spinal cord section (Fig. 5). and became striking in 4 to 7 days followed by gradual regression (Fig. 13). Simultaneously with presence of edema, glial processes focally showed degeneration.

Biochemical study already disclosed a fact that water content of the distal portion of a severed peripheral nerve increase 4 to 32 days after nerve section;[23] and this is explained as a result of increased interstitial edema.[23, 39]

In the experimental studies on peripheral nerve section genesis of water retention is attributed to vasomotoric disorders[25].

In the spinal cord, vascular damage at the time of section and increased permeability of the capillaries are also considered to act as edema-producing factors.

In experimental demyelination macroglia. is said to appear in 6 days,[5] whereas present study also noted proliferation of macroglia approximately 5 days postoperatively (Fig. 11).

## Clearing Process of the Degenerating Products

Light microscopic studies have brought an opinion that disposal of debris in the central nervous system is mainly performed by microglia having histiocytic activity.[17, 18]

It is postulated, however, that cells of macroglial origen also possess phagocytic ability.[9, 14] In this study, peculiar cell enclosing phagocytosed granules was observed at earliest 44 hours postoperatively (Fig. 8, Fig. 14). The cell had a round to oval dense nucleus of fairly smooth outline with abundant chromatin. Cytoplasm was dense, abundant, rich of organellae and of smooth outline. The cell obviously differed from macroglia but was not necessarily consistent with microglia.[12, 27, 41, 48] It may be probably so-called "gitter cell"[5, 14, 17, 52] which has arised from microglia. Morphological change of microglia leading to gitter cell is thought due to its hyperactivity.[17]

## Regeneration of the Nerve

During the entire period of this experimental study, regenerating fiber could not be observed, but, in the terminal period of degeneration, glial processes proliferated irregularly in the space from where the nerve fiber had disappeared (Fig. 25).

In degeneration of a peripheral nerve after section,[33, 34, 35] on the other hand, active proliferation of Schwann cell occurs following disappearance of nerve fiber debris, resulting in formation of "BÜNGNERsches Band"[4, 38] in which Schwann cells are arranged longitudinally in the site where nerve fibers are going to regenerate.

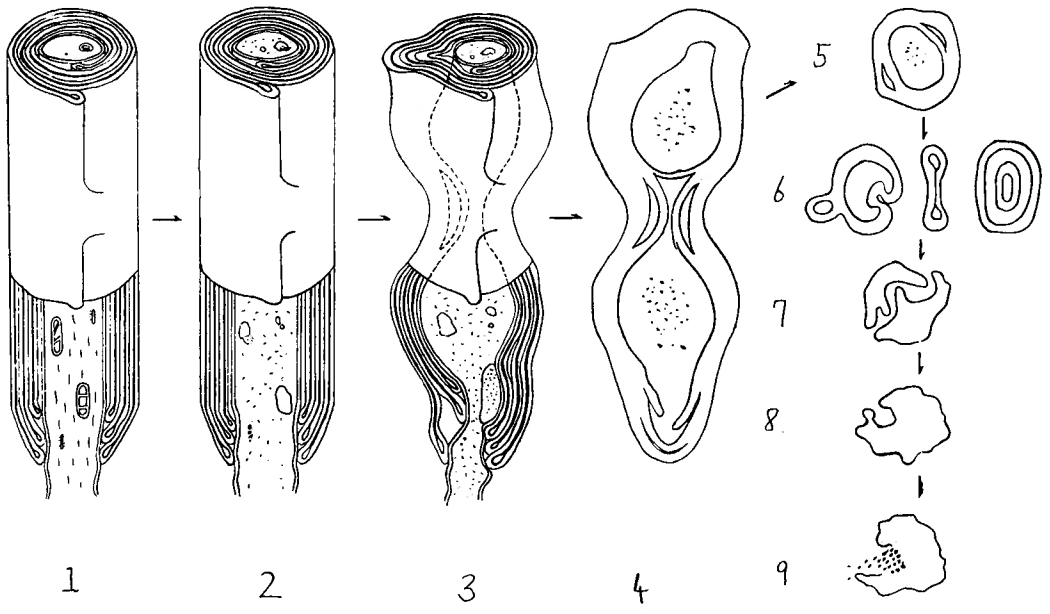
Both glia in central nerve and Schwann cell in peripheral nerve are sustentacular tissue, nevertheless, they behave differently in case of degeneration; i. e. the former inhibits, whereas the latter conducts the growth of regenerating fiber.

As pointed out by GERALD and other,[15] based on their light microscopic study, regenerating ability of nerve fiber differs according to whether it is central or peripheral.

Regeneration of the central nerve fiber might be expected if it is enabled to control, by any means, glial proliferation which occurs in degeneration.

**Text-Figure 2**

Scheme of Secondary Degeneration of Spinal Cord Fiber




1. Normal.
2. Early changes in axoplasm.
3. Constriction of the axon. Separation of myelin lamellae.
4. Advanced degeneration of the axon.
   Fiber undergoes rosary change, segmentation and ovoid formation.
5. Segmented fiber, ovoid.
6. Various structural changes in ovoid.
7. Myelin conglomerate.
8. Myelin conglomerate.
9. Disintegration of conglomerate into small dense granules.

## SUMMARY AND CONCLUSION

Wallerian degeneration of posterior funiculus fibers of rabbit spinal cord was studied mainly with electron microscope.

1. Early change in axoplasm was manifested by degeneration of endoplasmic reticulum and mitochondria seen 12 to 24 hours after section of nerve fiber, and it was followed by degeneration of axonfilament usually seen within 44 hours.

These organellae developed complete destruction at latest on the 10 th postoperative day.

2. Following degenerative change in the organellae of axon, approximately in 20 hours after nerve section, the axon became of rosary appearance due to constriction probably caused by action of surface tension over the axon.

Segmentation of the axon developed in 44 hours to 10 days associated with disappearance of axon-oligocytic membrane. Change in shape of fiber up to this stage is thought to be promoted by the axon.

3. Following segmentation of the axon, the myelin sheath began to segmentate by intrusion of myelin lamellae resulting in formation of short segments of fiber called "ovoid". After this stadium, promoter for morphological change is thought to be the myelin sheath.

4. Segments of fiber lost the lamellar structure of myelin sheath and finally became dense conglomerates. These conglomerates disintegrated to the dense granules which later disappeared due to phagocytosis by gitter cells probably derived from microglia.

5. On the 166 th day after spinal cord section, no more degenerating product was recognized, and degenerated fibers were completely replaced by glial processes.

6. Throughout the study, regenerating fiber was never observed.

7. Nerve fibers were, for convenience, classified according to their diameter; and the smaller the fiber was, the more rapid progress in degeneration was observed. This is thought due mainly to influence of surface tension over the axon and also to difference in rigidity of the myelin sheath.

8. In stroma, transient edema and proliferation of macroglia were observed.

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## REFERENCES

1) Abercrombie, M., and Johnson, M. L.: Quantitative Histology of Wallerian Degeneration. I. Nuclear Population in Rabbit Sciatic Nerve. J. Anat., **80**, 37, 1946.
2) Bethe, A.: Allgemeine Anatomie und Physiologie des Nervensystems. Georg Thieme. Leipzig. 1903.
3) Bucy, P. C.: Studies in Degeneration of Peripheral Nerves. J. Comp. Neur., **45**, 129, 1928.
4) v. Büngner, O.: Über die Degeneration und Regenerationsvorgänge am Nerven nach Verletzungen. Beitr. Path. Anat., **10** 321, 1891.
5) Bunge, R. P.; Bunge, M. B., and Ris, H.: Electron Mioroscopic Study of Demyelination in an Experimen tally Induced Lesion in Adult Cat Spinal Cord. J. Biophysic. Biochem. Cytol., **7**, 685, 1960.
6) Bunge, M. B., Bunge, R. P., and Ris, H.: Ultrastructural Study of Remyelination in an Experimental Lesion in Adult Cat Spinal Cord. J. Biophysic. Biochem. Cytol., **10**, 67, 1961.
7) Cajal, R. Y.: Degeneration and Regeneration of the Nervous System. (translated by May, R. M.) Hanfner. New York 1959.
8) Condie, R. M., Howell, A. E. Jr., and Good, R. A.: Studies on the Problem of Preservation of Myelin Sheath Ultrastructure: Evaluation of Fixation Dehydration and Embedding Techniques. J. Biophysic. Biocchem. Cytol., **9**. 429. 1961.
9) Cramer, F., and Alpers, B. J.: The Function of the Glia in Secondary Degeneration of the Spinal Cord. The Oligodendroglia as Phagocytes. Arch. Path., 13, 23, 1932.
10) Dalton, A. J.: A Chrome-osmium Fixative for Electron Microscopy. Anat. Rec. **121**, 281, 1955.
11) De Robertis, E. and Gerschenfeld, H. M.. and Wald, F.: Cellular Mechanism of Myelination in the Central Nervous System. J. Biophysic. Biochem. Cytol., **4**, 641, 1958.
12) Farquhar, M. G., and Hartmann, J. F.: Neuroglial Structure and Relationships as Revealed by Electron Microscopy. J. Neuropath. Exp. Neur., **16**, 18, 1957.
13) Fernandez-Moran, H., and Finean. J. B.: Electron Microscope and Low-Angle X-ray Diffraction Studies of the Nerve Myelin Sheath. J. Biophysic. Biochem. Cytol., **3**, 725, 1957.
14) Ferraro, A.: Acute Swelling of the Oligodendroglia and Grape-Like Areas of Disintegration. Arch. Neurol. Psychiat., **20**, 1065, 1928.
15) Gerard, R. W., and Grinker, R. R.: Regenerative Possibilities of the Central Nervous System. Arch. Neurol. Psychiat, **26**, 469, 1931.

16) Geren, B. B.: The Formation from the Schwann Cell Surface of Myelin in the Peripheral Nerve of Chick Embryo, Exp. Cell. Res., **7**, 588, 1954.
17) Glees, P.: Neuroglia Morphology and Function. Blackwell, Oxford, 1955.
18) Greenfield, J. G. Blackwood, W., McMenemey, W. H., Meyer, A., and Norman, R. H.: Neuropathology. Edward Arnold. London 1958.
19) Hess, A., and Lansing, A. I.: The Fine Structure of Peripheral Nerve Fibers. Anat. Rec., **117**, 175, 1953.
20) Honjin, R.: On the Ultrastructure of the Neurons. Symp. Soc. Cell. Chem., **5**, 109, 1957. (in Japanese)
21) Honjin, R., Nakamura, T., and Imura, M.: Electron Microscopy of Peripheral Nerve Fibers, Ⅲ, On the Axoplasmic Changes during Wallerian Degeneration. Okajimas Fol. Anat. Jap., **33**, 131, 1959.
22) Howell, W. H., and Huber, G. C.: A Physiological, Histlogical and Clinical Study of the Degeneration and Regeneration in Peripheral Nerve Fibers after Severane of Their Connection with the Nerve Center. J. Physiol., **13**. 335, 1893.
23) Johnson, A. C., McNabb, A, R,. and Rossiter, R. J.: Chemical Studies of Peripheral Nerve During Wallerian Degeneration. I. Lipid, Biochem. J., **45**, 500, 1949.
24) Johnson, A. C., McNabb, A. R., and Rossiter, R. J.: General Review. Chemistry of Wallerian Degeneration. Arch. Neur. Psych., **64**, 105, 1950.
25) Krücke, W.: Erkrankungen des Peripheren Nervensystems. Handbuch der Speziellen Pathologischen Anatomie und Histologie. (Lubarsch, O., Henke, F., and Rösse, R.) Bd. **13**, T1. 5, 1955.
26) Luse, S. A.: Formation of Myelin in the Central Nervous System of Mice and Rats, as Studied with the Electron Microscope. J. Biophysic. Biochem. Cytol., **2**, 777, 1956.
27) Luse, S. A.: Electron Microscopic Observations of the Central Nervous System. J. Biophysic. Biochem. Cytol., 2, 531, 1956.
28) Luse, S. A.: Fixation and Embedding of Mammalian Brain and Spinal Cord for Electron Microscopy. J. Ultrastr. Res., **4**, 108, 1960.
29) Maturana, H. R.: The fine Anatomy of the optic Nerve of Anurans—An Electron Microscope Study. J. Biophysic. Biochem. Cytol., **7**, 107, 1960.
30) Möckeberg, G., und Bethe, A.: Die Degeneration der markhaltigen Nervenfasern der Wirbelthiere unter hauptsächlicher Berücksichtigung des Verhaltens der Primitivfibrillen. Arch. f. Mikr. Anat., **54**, 135, 1899.
31) Noback, C. R., and Montagna, W.: Histochemical Studies of the Myelin Sheath and its Fragmentation Products during Wallerian (secondary) Degeneration. I. Lipids. J. Comp. Neur., **97**, 211, 1952.
32) Ogura, M. and Sato, A.: Studies on the Effect of pH of Immersion Medium on the Structure of Peripheral Nerve with an Electron Microscope. J. Electronmicroscopy, **7**, 25, 1958. (in Japanese)
33) Ohmi, S.: Electron Microscopic Study of Wallerian Degeneration of the Peripheral Nerve. Arch. Jap. Chir., **28**, 1396, 1959.
34) Ohmi, S.: Electron Microscopic Study on Wallerian Degeneration of the Peripheral Nerve. Zeitschr. f. Zellforsch. **54**, 39, 1961.
35) Ohmi, S.: In press.
36) Peters, A.: The Structure of Myelin Sheaths in the Central Nervous System of Xenopus laevis (Daudin). J. Biophysic. Biochem. Cytol., **7**, 121, 1960.
37) Peters, A.: The Formation and Structure of Myelin Sheaths in the Central Nervous System. J. Biophysic. Biochem. Cytol., **8**, 431, 1960.
38) Ranson, S. W.: Degeneration and Regeneration of Nerve Fibers. J. Comp. Neur., **22**, 487, 1912.
39) Rosenbluth, A., and Dempsy, E. W.: A Study of Wallerian Degeneration. Am. J. Phys., **128**, 19, 1939.
40) Ross, L. L., Bornstein, M., and Lehrer, G.: Electron Microscopic Observation of Myelin Sheath Formation in Tissue Cultures of Developing Rat Cerebellum. Anat. Rec., **136**, 268, 1960.
41) Schultz, R. L., Maynard, E. A., and Pease, D. C.: Electron Microscopy of Neurons and Neuroglia of Cerebral Cortex and Corpus Callosum. Am. J. Anat., **100**, 369, 1957.
42) Shinagawa, Y., and Uchida, Y.: On the Specimen Damage of Spinal Cord due to Polymerization of Embedding Media. J. Electronmicroscopy., **10**, 86, 1961.
43) Spiegel, E. A.: Die Physikalischen Veränderungen der Markscheide im Beginne der Wallerschen Degeneration. Beitr. Path. Anat., **70**. 215, 1922.
44) Spielmeyer, W.: Degeneration und Regeneration am Peripherischen Nerven. Handbuch der

Normalen und Pathologischen Physiologie. (Bethe, A., andere) Springer, Berlin. **9**, 285, 1929.
45) Terry, R. D. and Harkin, J. C.: Regenerating Peripheral Nerve Sheathes Following Wallerian Degeneration. Exp. Cell. Res., **13**, 193, 1957.
46) Tsuzaki, T.: Zikken Dobutsu Kaibogaku. Kanehara-shuppan. Tokyo. 1954. (in Japanese)
47) Uchida, Y.: in press.
48) Uozu, T.: Electron Microscopic Studies on the Ultrastructure of the Neuroglia Cells. Juzen-igakukai-zassi. **64**, 289, 1960. (in Japanese)
49) Vial, J. D.: The Early Changes in the Axoplasm during Wallerian Degeneration. J. Biophysic. Biochem. Cytol., **4**, 551, 1958.
50) Watanabe, R., Sato., T., and Ito, T.: Experimental Studies on the Myelin-Degeneration on the Peripheral Nerve Fibers. Fukushima Med. J. **6**, 131, 1956. (in Japanese)
51) Weiss, P.: Evidence of Perpetual Proximo-distal Growth of Nerve Fibers. Biol, Bull., **87**, 160, 1944.
52) Windle, W. F.: Biology of Neuroglia. Charles C. Thomas, Springfield. 1958.
53) Winkler, C., and Potter, A.: An Anatomical Guide to Experimantal Researches on the Rabbit's Brain. W. Versluys, Amsterdam, 1911.
54) Young, J. Z.: Surface Tension and the Degeneration of Nerve Fibers. Nature, **154**, 521, 1944.
55) Yuri, Y., Komai, T., and Murai, M.: Electron Microscopic Research for the Secondary Degeneration of the Optic Nerves of Mice. Acta Soc. Ophthalm. Jap., **64**, 1630, 1960. (in Japanese)

## 和文抄録

# 脊髄切断後の神経線維ウオーラー変性についての電子顕微鏡学的観察

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われわれの先に改良発表した独自の固定包埋法を応用することにより,家兎脊髄切断後,神経線維の *Waller* 変性を電顕的に容易に追及することが出来，次の結果をえた.

1) 切断後最初に起る変化は axoplasm の変化であつて，切断12時間～24時間後に endoplasmic reticulum, mitochondria の空胞化，顆粒化が始まり，同時に，或いはそれよりややおくれ，12時間～44時間で axon-filament に変化が始まる．そしてこれらの organellae は,おそいものでも脊髄切断後10日で完全に崩壊する.

2) organellae の変化の発現にややおくれて，切断20時間後から，主として表面張力の作用により軸索は念珠状となり，更にこれに axon-oligocytic membrane が消失して，小径線維では切断44時間後頃から，超大径のものでは同10日後頃までに軸索は分節を起し，その連続性を失うようになる．そしてこの段階までの線維の形態変化の promoter は軸索と考えられる.

3) 軸索の分節にひきつづいて，主として myelin lamellae の彎曲性によつて線維は短い断節（ovoid）となるが，以後の形態変化の promoter は髄鞘と考えられる.

4) 線維の断節（ovoid）は，種々の形態的変化を示しながら髄鞘の層構造を失つて，内腔のない dense な塊状のものとなる.

5) 次いでこれが dense な小破片に崩壊して，microglia に由来すると考えられる gitter cell に貪喰されて組織から消失して行く.

6) 切断166日後では，もはや変性産物は全く認められなくなり，変性線維の消失したあとはグリア細胞の突起がすき間なく埋めている.

7) 観察の全過程を通じて再生線維と思われるものを認めえなかつた．恐らく，増殖したグリア細胞が，新生線維の伸長に対してバリケードとして作用するためであろう.

8) 便宜上，神経線維をその直径によつて分類して観察すると，大体において小径のものほど変化の進行が速い傾向がある．これは主として，軸索に生ずる表面張力の影響と，髄鞘の rigidity の差によつて来るものであろう.

9) 間質に於いては，場所によつて切断20時間後から浮腫の像が認められ，特に4日～7日後に著明であつた．そして一方では，切断5日後から macroglia の増殖が認められて，以後間質はグリア細胞の突起によつて次第に埋められて行く.

gitter cell は早いものでは，切断44時間後から出現した.

## EXPLANATION OF FIGURES

(A bar indicates 1 $\mu$ unless otherwise explained.)

**Fig. 1** Electron-micrograph of the normal rabbit spinal cord (posterior funiculus). Myelinated fibers of various diameter are demonstrated, and glial processes fill remaining space. In axon, numerous axonfilaments of approximately 100 Å in diameter are seen but mitochondria and endoplasmic reticulum are few. (×45,000)

**Fig. 2** Myelin sheath of myelinated fiber of central nervous system has essentially similar structure to that of peripheral nerve; i. e., myelin lamellae encircle the axon making alternating dense line and light layer. (×150,000)

**Fig. 3** Higher magnification of the myelin lamellae. Intermediate line is recognized at the center of the light layer. (×450,000)

**Fig. 4** Cross section of nerve fiber 12 hours after section. There are beginning granular change of mitochonoria (m) and vacuolation of endoplasmic reticulum (v). No remarkable change in axonfilaments is noted except for some disarrangement of their course. No change in the myelin sheath. (×48,000)

**Fig. 5** 20 hours after spinal cord section. Gap between the axon and myelin sheath has occurred in small and medium fibers.

In small fibers, flattening of the sheath is remarkable (arrow). These were accurately observed in cross section specimen. (×15,000)

**Fig. 6** Longitudinal section of nerve fiber 24 hours after severance. Dissociation of the sheath from the axon is remarkable in proximity of node.

The axon presents rosary appearance in longitudinal section as a result of alteration in its diameter. Between the axon (A) and the sheath, there are two kinds of space; i. e., one is filled with matrix of low density (B) and another is almost devoid of matrix and carrying small vesicles (C). Each are clearly limited by membranous structure. (×12,000)

**Fig. 7** 44 hours after spinal cord section.

Morphologic changes take plase also in large fibers (F1, F2). As shown in Fig. 6, there are different kinds of matrix outside the axon. They are limited by a distinct membranous structure. Inside the deformed axon, vacuolation of mitochondria and numerous fine vesicles are recognized. Axonfilaments are shortly cut, and some small or medium fibers (F3, F4) present a finding as if they interwined or coalesced.

Myelin sheath, associated with change in the axon, shows changes such as flattening, partial protrusion or infolding (F3, F4, F5). (×14,000)

**Fig. 8** 44 hours after nerve section.

Gitter cell is noticeable in which a lot of dense granules are phagocytosed. Small fiber flattened is also recognized. (×33,000)

**Fig. 9** 4 days after nerve section.

Interstitial edema associated with changes in the glial processes is demonstrated. In medium fibers, constriction of the axon is seen as well as protrusion and/or duplicity of certain myelin sheath. (×16,000)

**Fig. 10** 5 days after section.

Remarkable change in the axon is shown. The axon-oligocytic membrane has disappeared, and the organellae such as mitochondria can not be recognized.

Axonfilamants shortly cut and coalesced, and dense granular structures with indistinct outline are present. Complicated dissociation and inward projection of the myelin sheath lamellae partly lead to rosary appearance; a, b, c and d show the process the fiber is divided at the narrow neck into short ovoids (O). (×10,000)

**Fig. 11** 5 days after section.

Extensive observation with a phase contrast microscope shows proliferation of macroglial cells (g), although whether they are oligocytes or astrocytes can not be determined.

Numerous dense particles were also observed. They are probably conglomerates coming from small fiber as a result of degeneration (arrows).

**Fig. 12** 5 days after section.

Conglomerates resulting from degeneration of nerve fibers with small diameter. (×9,000)

**Fig. 13** 7 days after section.
Both F1 and F2 are the early ovoids. Their unusual figures probably depend upon the site where ultra-thin sectioning hits. (×18,000)

**Fig. 14** 7 days after section.
A gitter cell contains phagocyting granules. Arrows indicate the cytoplasmic border which is quite smooth. (×30,000)

**Fig. 15** 10 days after section.
Ovoids of large or extra-large fibers. Some of them do not have degenerating products inside. There are sheathes flattened, duplicated or triplicated. (×12,000)

**Fig. 16** 10 days after section.
Degenerating products are still present inside the sheath of the big fibers. There is an interesting finding that a double layered sheath is surrounded by the flattened and curved sheath. (×23,000)

**Fig. 17** 10 days after section.
Lamellar structure of myelin is well maintained at this time. (×68,000)

**Fig. 18** 10 days after section.
Myelin conglomerate is made of one or several aggregated ovoids in which degenerative products of the axon have disappeared. At this stage, lamellar structure of myelin is already lost. (×13,000)

**Fig. 19** Light microscopic finding of 10th day specimen (Marchi staining)
Numerous black-stained granules are present.

**Fig. 20** Ovoid of extra-large fiber on 21th postoperative day. In extra-large fibers, changes progress slowly, therefore, the space is still present in the myelin sheath which partly shows complicated projection outward or inward. Inside the space, there are other structures looking like myelin sheath. (×6,800)

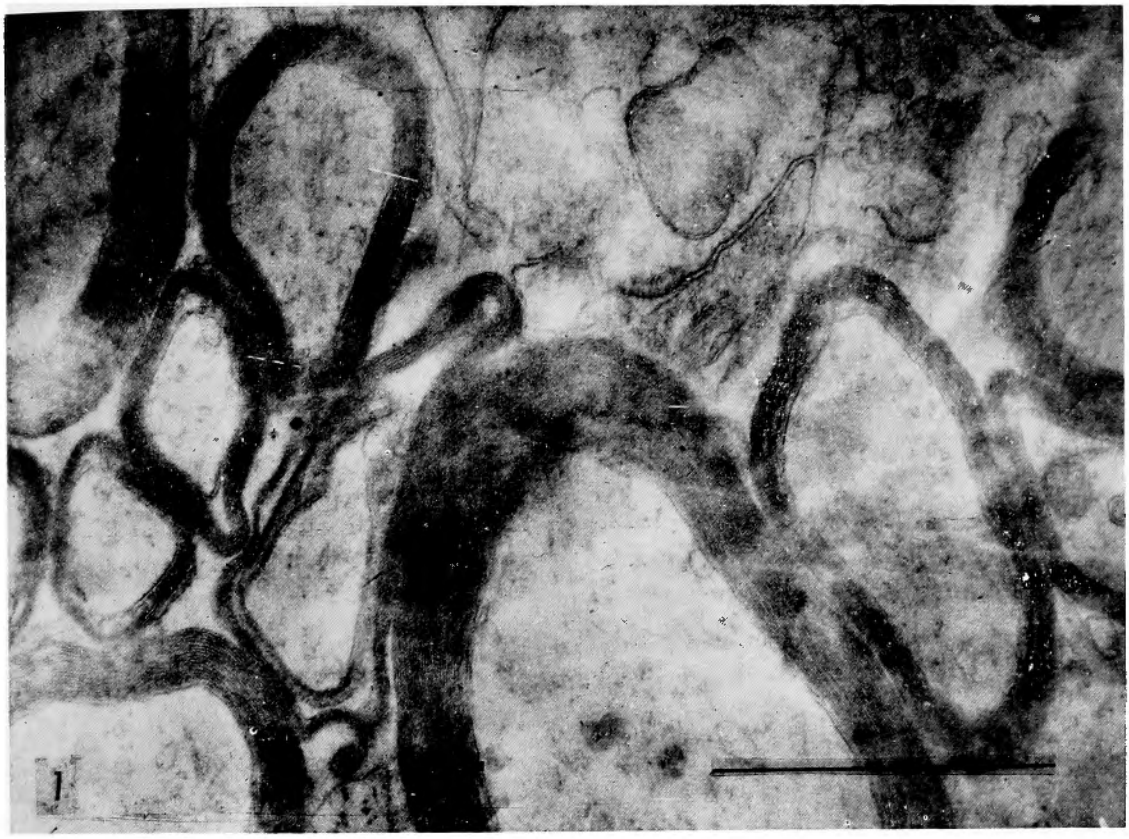
**Fig. 21** 21 days after section.
Ovoid of the extra-large fiber in which degenerative products, myelin structures and vacuoles are included. (×11,000)

**Fig. 22** 30 days after section.
Conglomerate of myelin. (×15,000)

**Fig. 23** 30 days after section.
Conglomerates. (×18,000)

**Fig. 24** 61 days after section.
Destruction of conglomerate into dense and fine granules. (×16,500)

**Fig. 25** 166 days after section.
Destructive debris of fibers are cleared away from the tissue which is replaced by glial processes. Intact fibers, probably descending, are scattered. (×22,000)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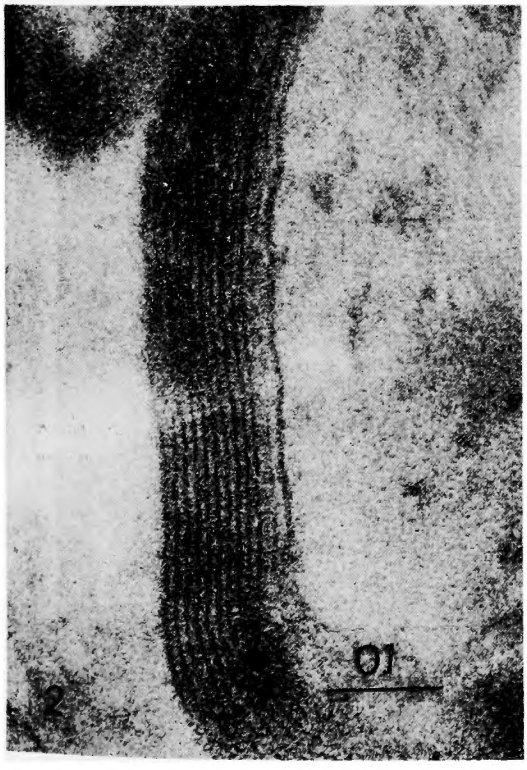
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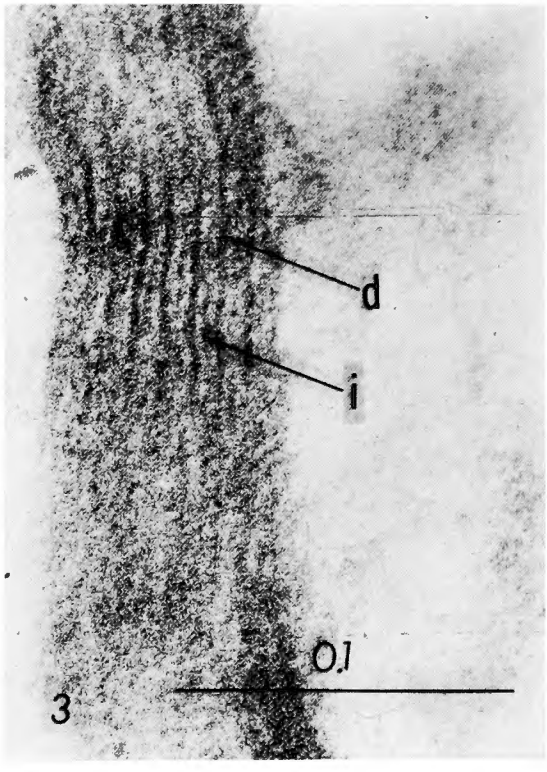
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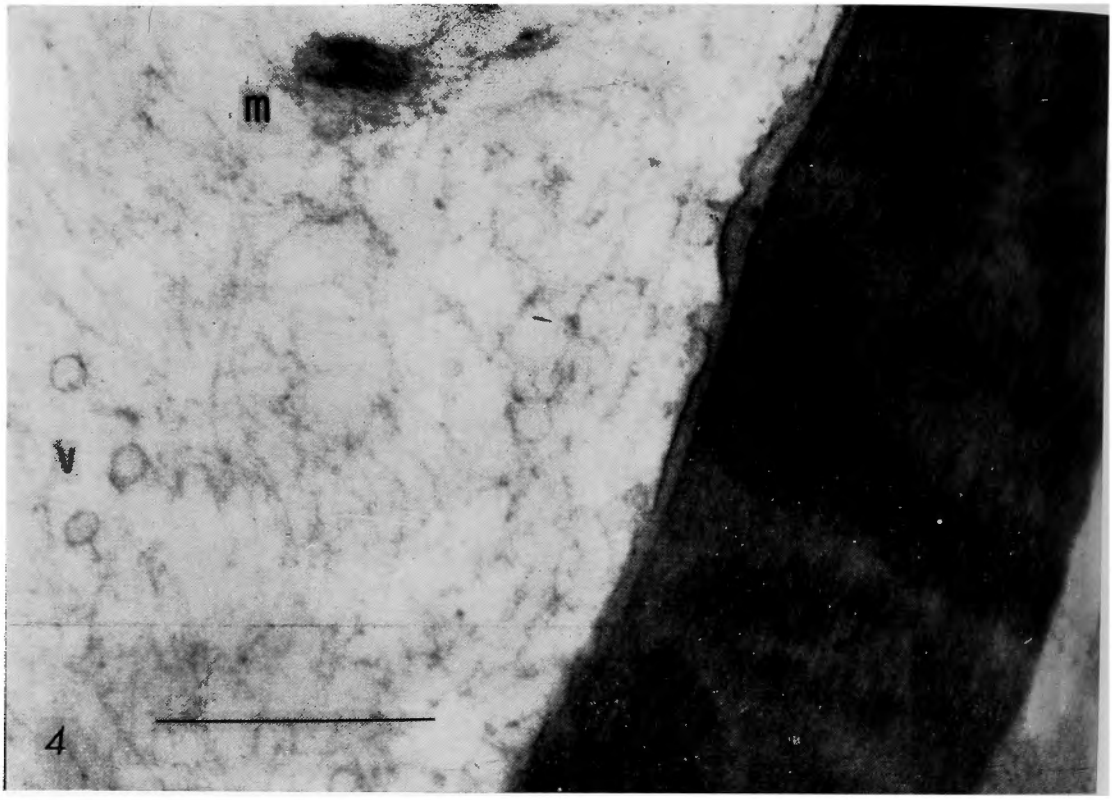
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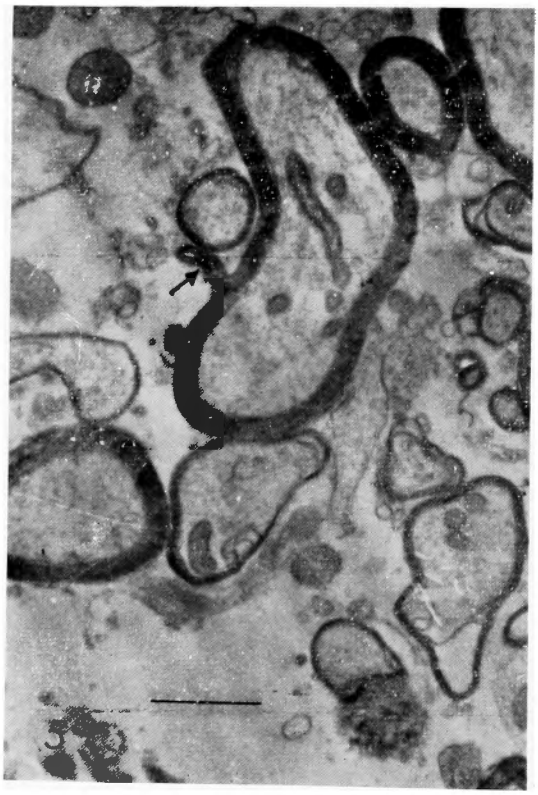
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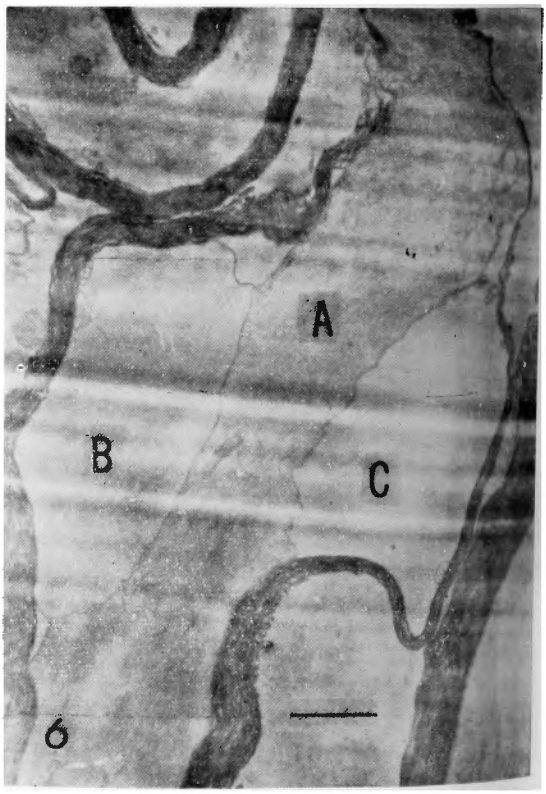
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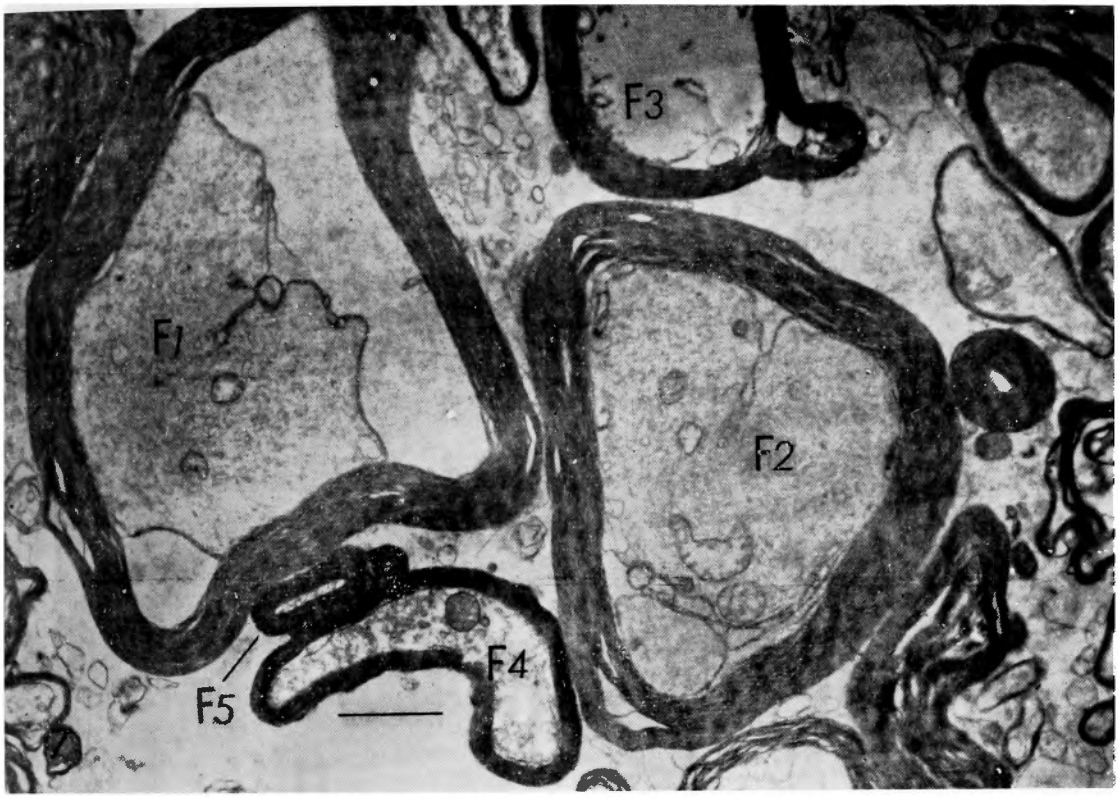
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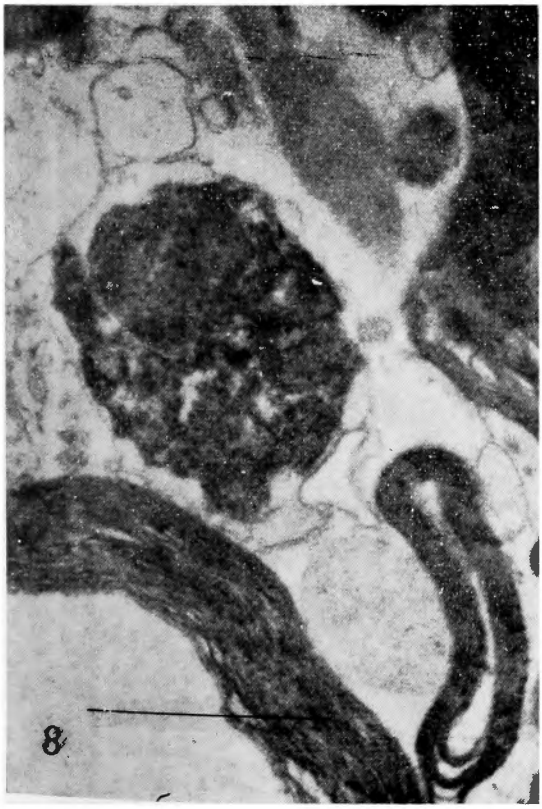
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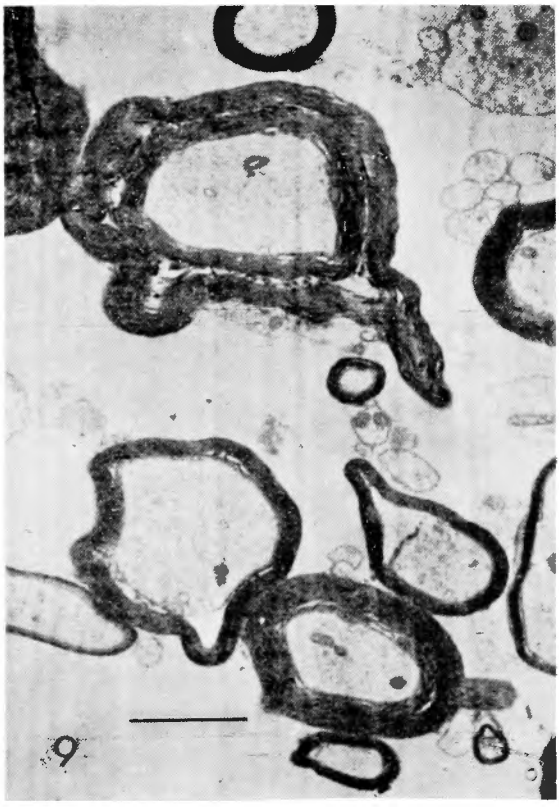
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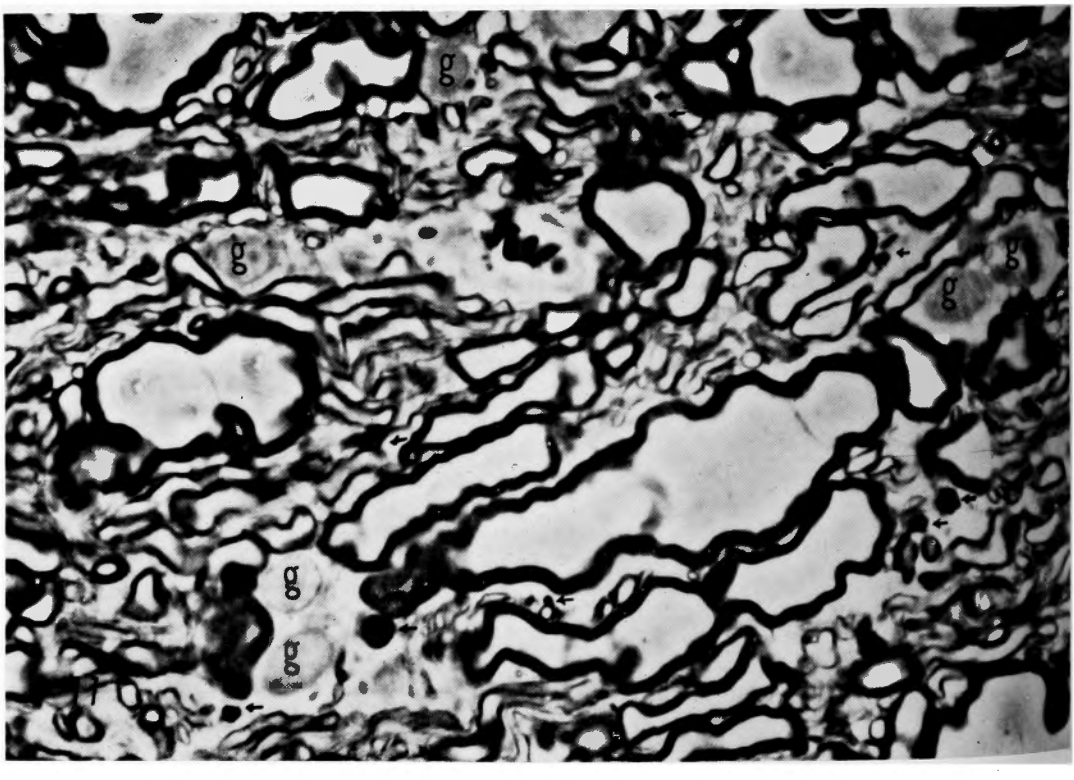
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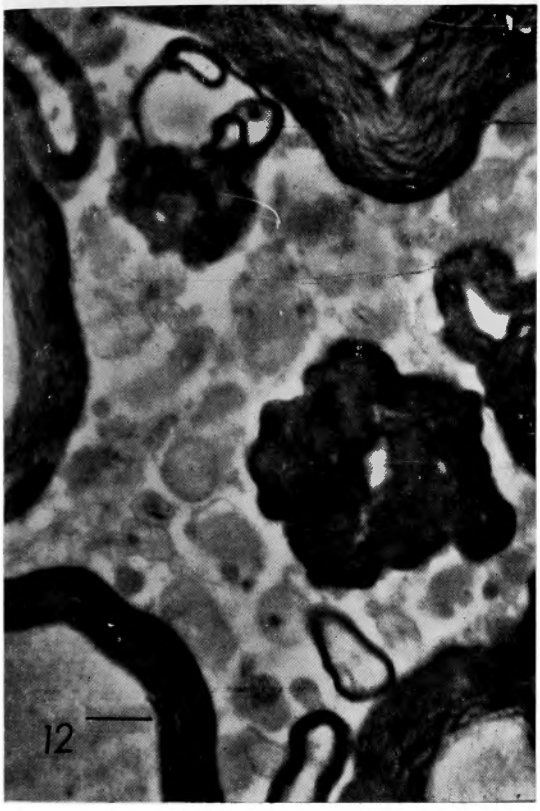
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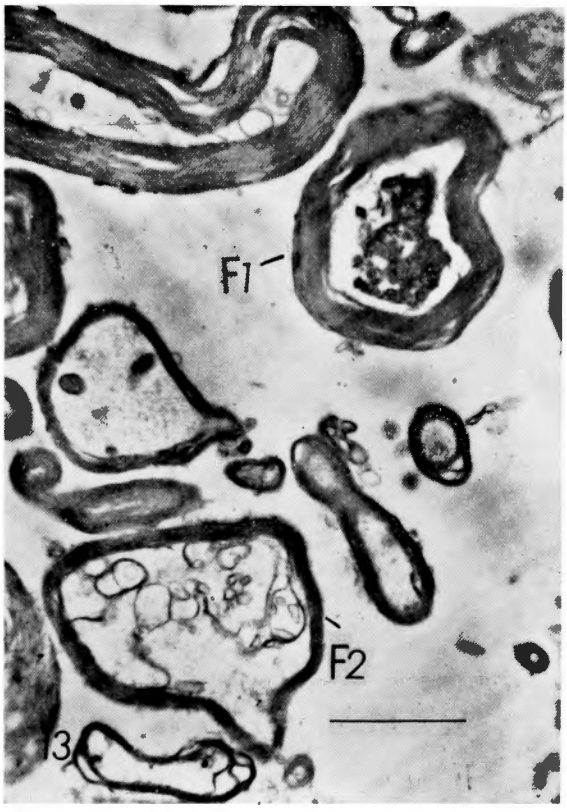
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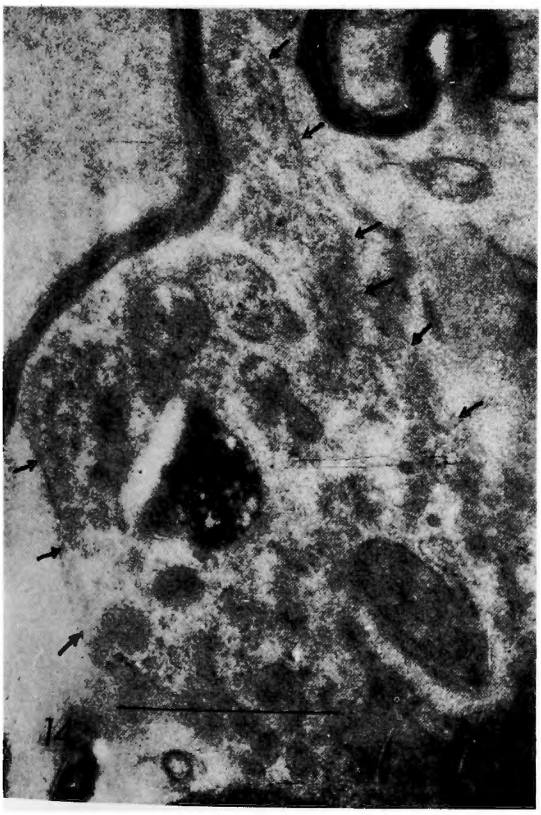
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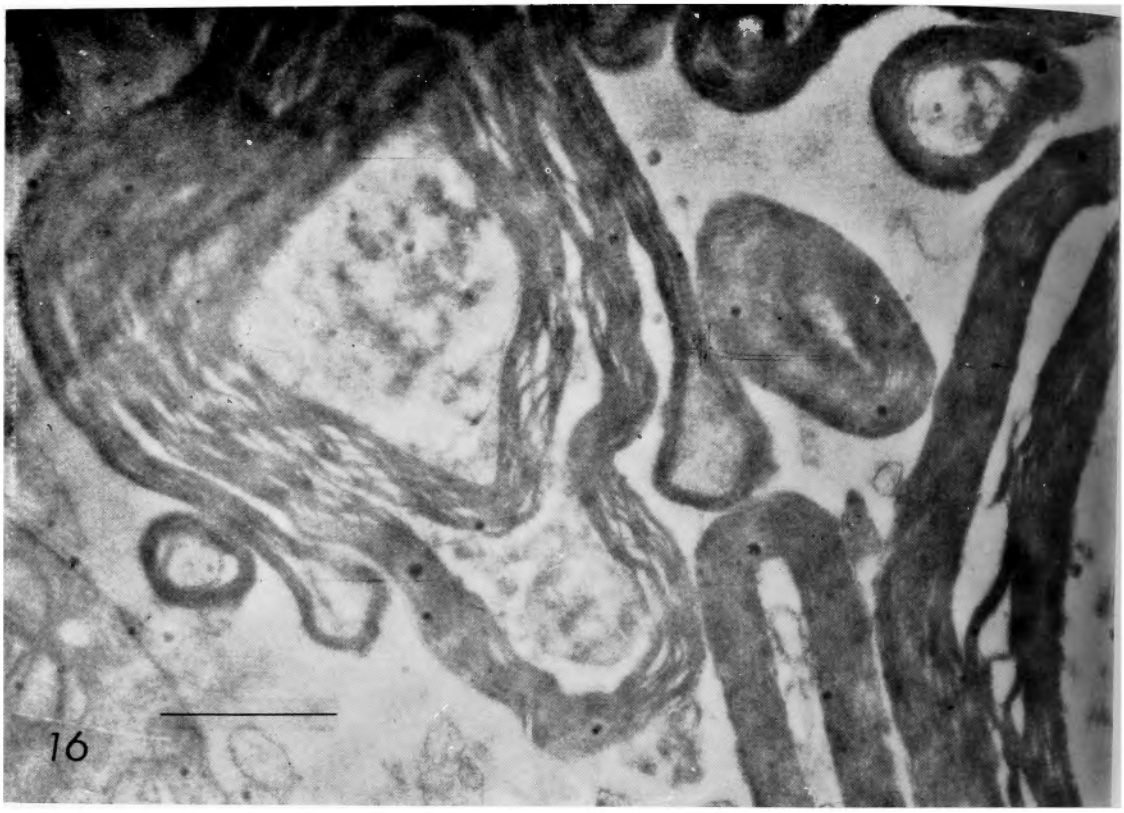
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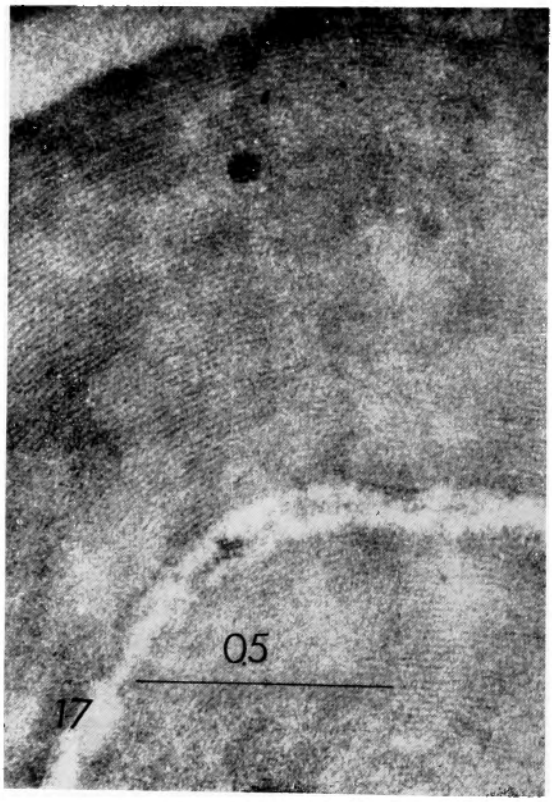
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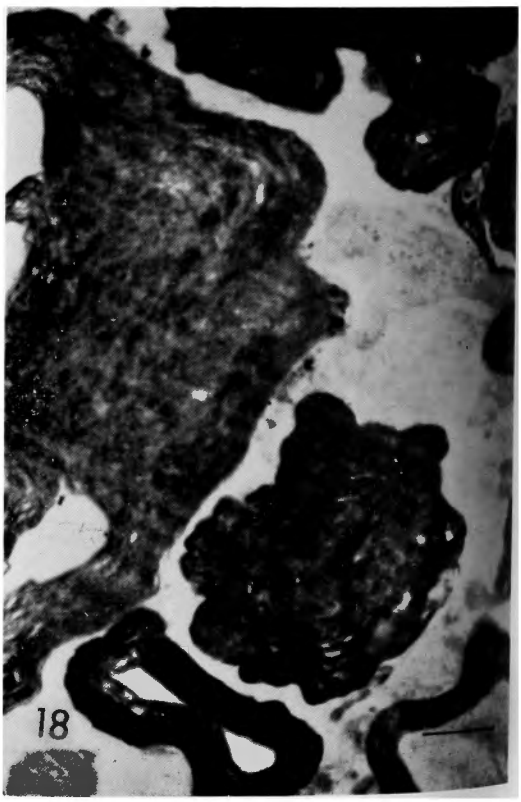
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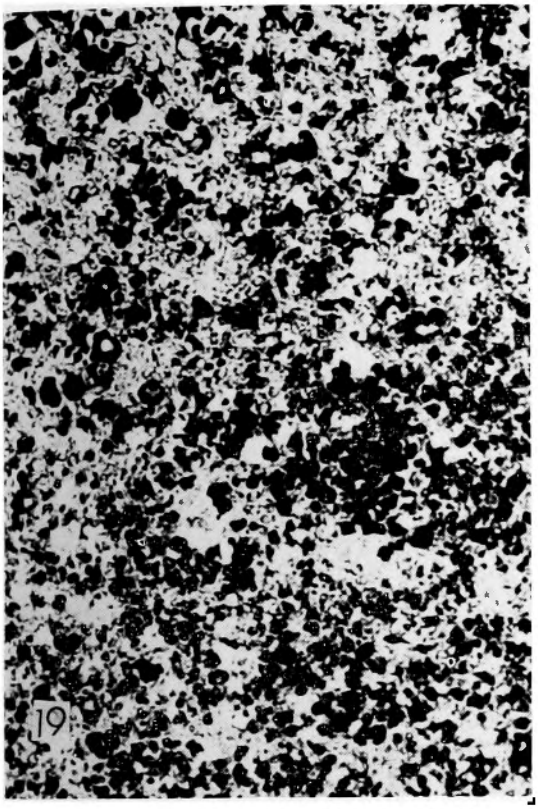
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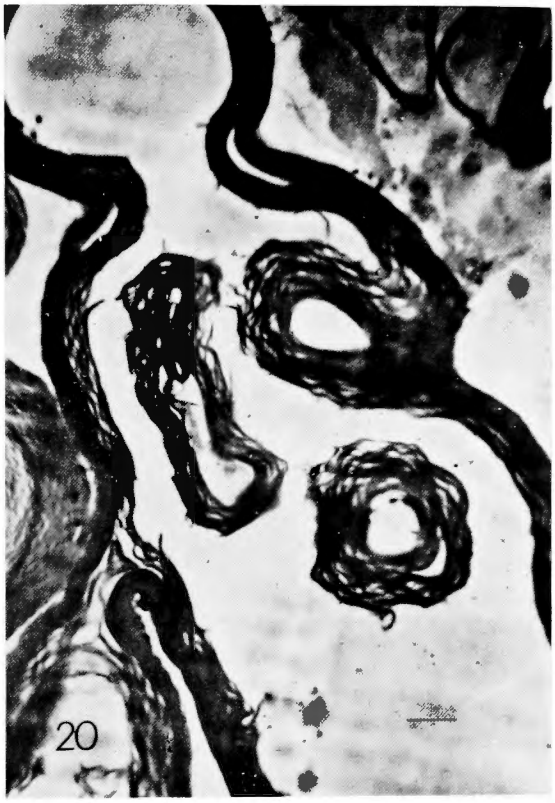
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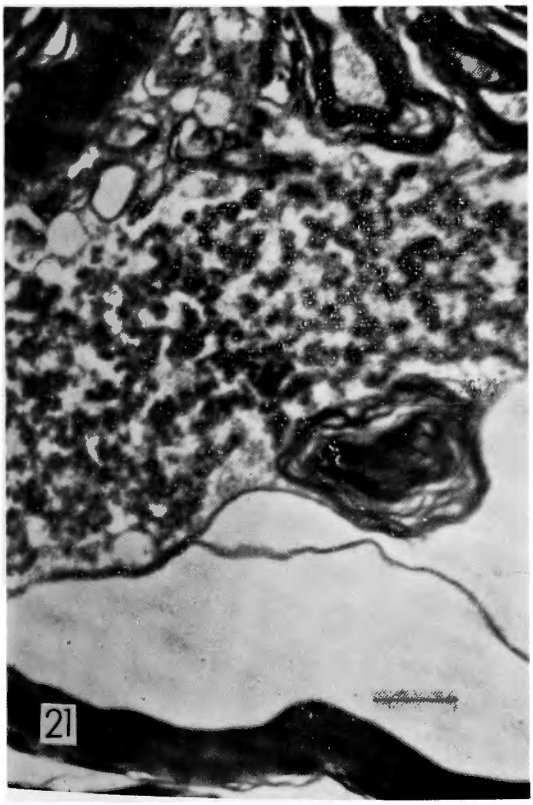
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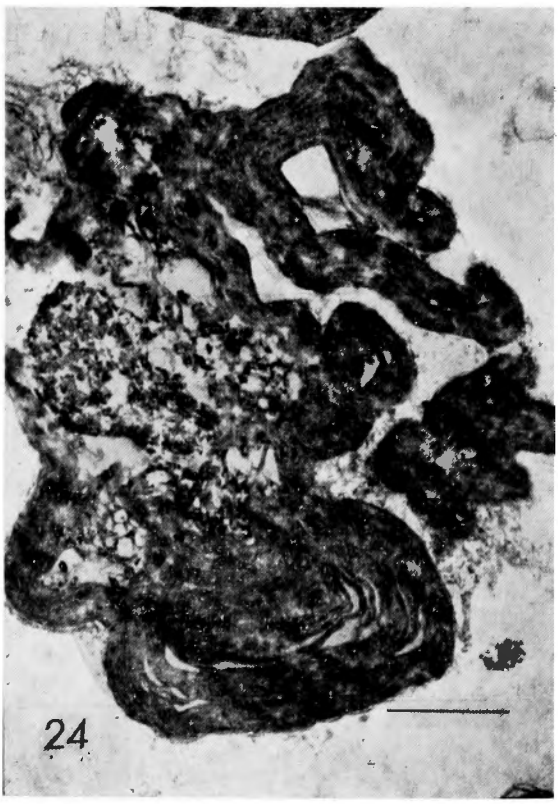
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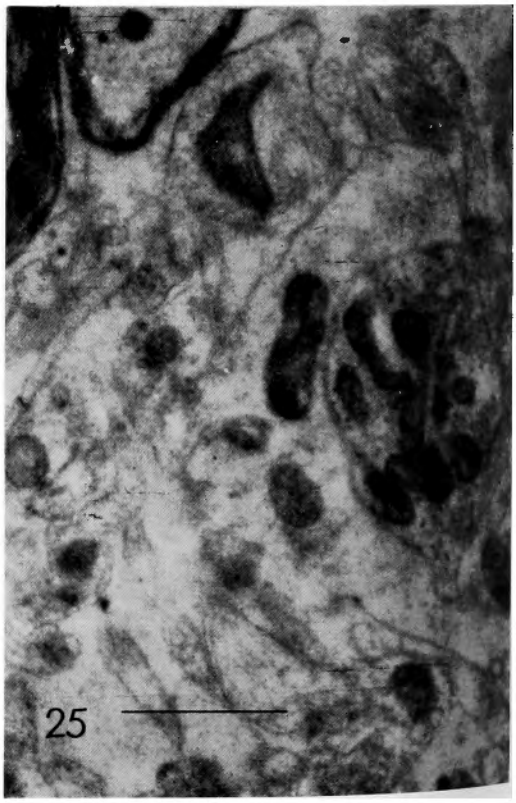
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