NEUROPATHOLOGICAL STUDY ON THE EXPERIMENTALLY PRODUCED DEGENERATION OF THE NERVE FIBER WITH USE OF PHASE CONTRAST MICROSCOPE

by

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I. INTRODUCTION

The experimental study on the nerve degeneration was used to be observed on the specimens which were fixed in formol solution and impregnated with silver. The results gotten with this method could not exclude the artifact accompanied by post-mortal changes of the nerve elements and others produced by the formol fixation and the complicated procedures of impregnating process. Thus some finding was maintained by an author, which was neglected by another as an artifact or failure in the impregnating technic. The author described in previous report the degeneration phenomena which occurred in the myenteric plexus of dogs after local application of alcohol and other drugs. However, most of the degeneration process gave common figures in all cases except a few specific changes after the application of these drugs.

The author then, tried to get the delicate and specific degeneration change occurring after the section of nerve fibers or the application of the Quinapon or croton oil, under the phase contrast microscope.

II. MATERIALS AND METHODS

The bullfrog (Rana esculenta) was used as experimental animal.

0.5 mg of chlorpromazine was given into the subcutaneous tissue of the frog and the sciatic nerve was exposed on one side. The nerve was cut and the section of the peripheral fragment was burried into the muscle of the leg. About 1 cm of the peripheral fragment of the sciatic nerve was cautiously removed 4, 7 and 14 days after the nerve section and several drops of 1% osmic acid solution were poured immediately on the piece of the nerve and nerve fibers were manually dissected with a thin needle each other, enclosed, and the change of the nerve fibers was examined with phase contrast microscope following the passage of time. As the control, sciatic nerve on the other side was observed after the same procedure.

Next, a piece of the normal sciatic nerve of the bullfrog was taken out and the several drops of the croton oil or Quinapon were poured on the piece of the nerve, enclosed, and the change of the nerve was observed. These procedures were operated as quickly and cautiously as possible to protect from artifact.

Ⅲ. PHASE CONTRAST MICROSCOPIC FINDINGS

1. The peripheral fragment of the sciatic nerve 4 days after the nerve section showed clear change comparing with the control. 10 minutes after enclosing with the osmic acid, a neurofibril like structure began to appear in the axoplasm. 20 minutes after, the myelinsheath showed segmentation and then fragmentation, and the axoplasm a granular degeneration. These changes correspond with those of controls shown 18 hours after enclosing (Figs. 1~7).

The change of the osmic acid affinity was observed even 7 days after enclosing, i. e. the nerve fibers which had already been in Wallerian degeneration before the removal of the sciatic nerve fragment poorly combined with osmic acid (Figs. 8 and 9).

A spinous process, which seemed to grow out of the myelinsheath, i. e. the gliocyte, appeared in the nerve on the sectioned side, but it looked indistinct and somewhat poor comparing with the control: in the control the process appeared with linear and clear cut figure 3 days after enclosing, became abundant in 2 weeks and then gradually fell into fragments and were destroyed (Figs. 10 and 11).

2. The peripheral fragment of the sciatic nerve 7 days after the section

The sectioned nerve fibers fell in marked changes even in the slightest degree of them, compared to the control, i. e. granular change of axoplasm with wrinkled and segmented myelinsheath. 1 hour after enclosing, these changes of nerve fibers became extensive and remarkable, and some of which were destroyed, while in the control the changes were far slighter (Figs. 12~21).

The gliocytes still kept the ability to bud the process. The affinity to osmic acid were rather stronger in the specimens 7 days after the section than those of 4 days in spite of increased deformation of the nerve fiber (Figs. 22~24).

3. The peripheral fragment of the sciatic nerve 14 days after the section

The degenerative change of the peripheral nerve fragment had already been so severe that one scarcely found a shadow of original figure. The nerve fibers in the bundle could only be traced by the globular or club shaped fragments of myelinsheath scattered here and there, because the rest had been destroyed into small granules. Besides the change of the myelinsheath, a marked leakage of the axoplasm were observed. Osmic acid did not combine with such nerve elements and the regeneration of the myelinsheath seemed to be almost hopeless (Figs. 25~34). The destroyance were far more intense than the control and equivalent to the figures found in the control 3 weeks after enclosing. In this stadium, the process which had been grown out of the gliocyte began to degenerate and the nerve fibers suggested that not only they had lost their function but their lives had passed away.

4. The change of nerve fibers by the application of croton oil

A piece of the sciatic nerve removed from the normal bullfrog was enclosed with croton oil and was observed under phase contrast microscope. 5 minutes after enclosing, wavy wrinkles appeared on the myelinsheath, which developed in 15 minutes and projected toward inside. The number of the projections increased with the lapse of time and became enlarged. In some parts the axis cylinder seemed to

be closed by the projections and looked like beads as a whole, but the change of the axoplasm itself was not observed.

2 days after enclosing, these morphological changes seemed to become fixed.

The morphological results suggested that croton oil did not cause the degeneration of the axoplasm by its chemical stimulus, but the mechanical pressure by the projected myelinsheath toward inside seemed to have some influence on the nerve fiber (Figs. 35～45).

5. The change of nerve fibers by the application of Quinapon

A piece of the sciatic nerve of the bullfrog was enclosed with Quinapon. 5 minutes after enclosing, a slight degree of granular change began to appear in the axoplasm, which increased the number and became more marked in 15 minutes.

At the same time, there appeared the projections of the myelinsheath toward inside, but in this case, without showing any tendency of wavy wrinkle building in the myelinsheath. The projected myelinsheath toward inside resembled LANTERMANN's cleft or RANVIER's nods. These figures reminded the author the specimen enclosed with formol solution. The projections of the myelinsheath changed into globular swellings, increased in number and became larger in 40 minutes.

These phenomena once resembled the change of the nerve fibers enclosed with croton oil, but both the myelinsheath and the axon fell into severe damage in 20 hours (Figs. 46～53).

Ⅳ. DISCUSSION

As described in the previous report the author observed degenerative changes of nerve elements in AUERBACH'S plexus after the local application of chemical agents.

However, he could not accept each figure of nerve change in the specimen without hesitation as a specific reaction of these chemical stimuli, because the complicated procedures for silver impregnation of specimens in addition to the fixation of them in the formol solution gave indefinite morphological features including artifacts.

Furthermore, it was very difficult to get ever changing degenerative process with so trouble-some method.

Then the author wished to find other method to cover the shortage of silver impregnation and made a plan with the use of phase contrast microscope.

Recently H. OKAWA published a comparative study on the morphological structures of peripheral nerve fibers with use of phase contrast microscope and with silver impregnation method. He described a critical opinion on the artifacts produced by fixation of specimen in formol solution. He detected LANTERMANN's cleft and neurofibrils in the axoplasm was nothing but artifacts and even RANVIER's nod must be reexamined on its real existence. The present study supports his opinion.

OKAWA recommends 1% osmic acid solution as the best buffer solution to keep nerve fibers in a condition just as they are in the body fluid. Following his opinion, the nerve fibers operated for the observation of Wallerian degeneration and the

control specimens were always enclosed with 1% osmic acid solution.

In the present experiments, as most of enclosed specimens are still alive, degenerative change progress every moment under cover in contact with osmic acid or others. Therefore the author observed not only the fresh specimen immediately after enclosing but he followed the change of figures with the lapse of time. Thus the degree of degeneration can be expressed in following ways,

1) The morphological changes in fresh specimen of operated nerve immediately after enclosing are observed and compared with the control in the same condition.

2) The figures of the nerve specimens on the operated side are compared with the control at the same passage of time after enclosing.

3) One measures the period of time which is necessary for the control to give the same figure as the operated nerve specimen.

4) When the degenerative change progress no more under cover, the nerve specimen is regarded to be dead.

A. WALLERIAN DEGENERATION

Under the phase contrast microscope, fresh and non-operated nerve specimens do not reveal the figure of neurofibrils in the axoplasm, LANTERMANN'S cleft and even the sign of RANVIER'S nod. In the axoplasm of the peripheral nerve fragment 4 days after the nerve section, neurofibrils and granules begin to appear within 20 minutes after enclosing, which correspond to the figures in the control 18 hours after enclosing.

Thus the degree of Wallerian degeneration can be expressed with the change in the control in contact with osmic acid solution for 18 hours under cover.

The operated specimens 7 days after the nerve section reveal more marked changes than those 4 days after the nerve section not only in the state immediately after enclosing but in the rapid progress of degenerative change under the action of osmic acid. This suggests that the peripheral fragment of sciatic nerve decreases resistance against acid with the progress of Wallerian degeneration.

As for the affinity to osmic acid the same tendency is observable. Nerve fibers in Wallerian degeneration are gradually deprived of osmic affinity.

The spinous process which grows cut of the myelinsheath appears in the control 3 days after the enclosure. They grow in a straight line and increase the number in 2 weeks and then are destroyed gradually. In the nerve, in which Wallerian degeneration comes to an end, they grow no more.

The spinous process was considered first as an artifact by the author, however, there is the fact that the time of their growth and destruction is coincidental with the beginning and the end of myelinsheath degeneration, which suggests him they belong to SCHWANN'S cell element.

At any rate, when the Wallerian degeneration comes to an end, any further change occurs in the enclosed specimen. Observing the control, this final stage is reached 3 weeks after enclosing.

B. APPLICATION OF CROTON OIL AND QUINAPON

The principal object of the experiment here is in the discovery of specific degeneration process in the action of these two chemical agents. The croton oil, when applied on the nerve fiber, causes destruction of the myelinsheath, but not of axiscylinder. The change of axon is mainly due to the projected myelinsheath toward inside. The axonal canal becomes obstructed by the swellings of myelinsheath and then the change of axoplasm begins to occur. Therefore, the invasion of croton oil on the axoplasm must be regarded to be indirect.

The invasion of Quinapon to the nerve fibers is in striking contrast with the mechanism of croton oil. The nerve fibers enclosed with Quinapon produces granules in the axoplasm in 5 minutes and the swellings of the myelinsheath toward inside in 15 minutes. Quinapon acts, therefore, first on the axoplasm and then on the myelinsheath.

When the myelinsheath degeneration proceeds so far as to make beads with globular swellings, the specific figures of degenerative change by these chemical substances become somewhat indistinct, but they still be recognized even 20 hours after enclosure.

V. SUMMARY

Using the phase contrast microscope, the degenerative change of the sciatic nerve of the bullfrog was observed and came to following conclusion.

1) In the fresh nerve specimen observed immediately after enclosing, the neurofibrils in axoplasm, LANTERMANN's cleft and even RANVIER's nod are not recognized.

2) The progress of Wallerian degeneration accelerates the occurrence of neurofibrils and granules in the axoplasm in addition to the segmentation and fragmentation of the myelinsheath, while it inhibits the affinity of nerve fibers to osmic acid under cover.

3) Wallerian degeneration comes to an end 2 weeks after the nerve section.

4) On the course of Wallerian degeneration the spinous process grows out of the myelinsheath, which is regarded to belong to SCHWANN's cell element.

The spinous process grows in the control specimen, 3 days after enclosing with osmic acid solution, with distinct and linear form. They proliferate in 2 weeks and then are gradually destructed. They are somowhat indistinct and look weaker on the specimen in which Wallerian degeneration has been progressing.

5) The croton oil causes the primary destruction of the myelinsheath and then it invades the axoplasm with the swellings of the myelinsheath.

6) The Quinapon act primarily on the axon and then on the myelinsheath.

I should like to express my heartfelt gratitudes to assist. Prof. Dr. Ch. KIMURA and to Dr. H. OKAWA for their kind guidance and valuable advices throughout this study.

REFERENCES

1) Borowski, M. L.: Nervenregeneration und Troghik. V. G. Fischer, Tena, 1956.
2) Kiga, K.: Application of phase contrast microscope. Abstr. of Gynecology, No. 2, Igaku

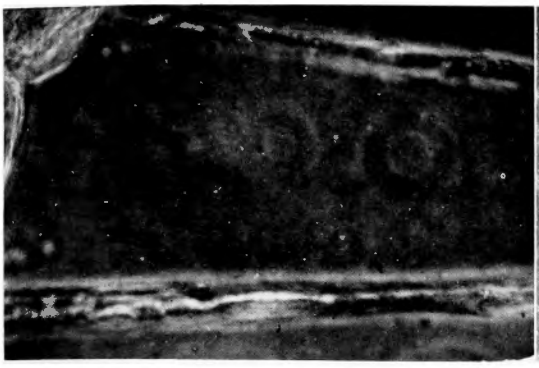

Fig. 1. Wallerian degeneration on 4th day (10 minutes after enclosing)

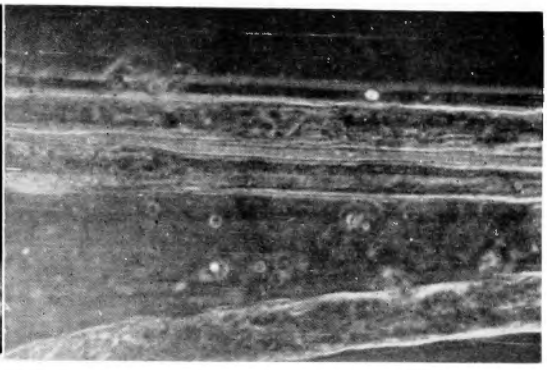

Fig. 2. Control specimen (10 minutes after enclosing)

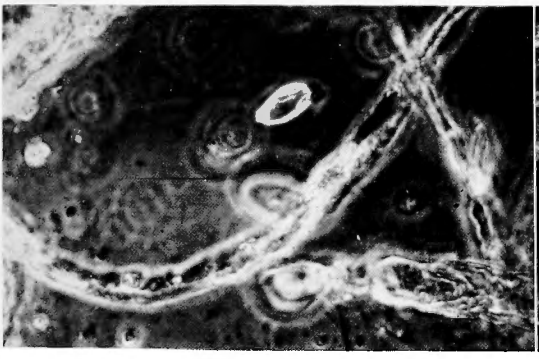

Fig. 3. Wallerian degeneration on 4th day (20 minutes after enclosing)

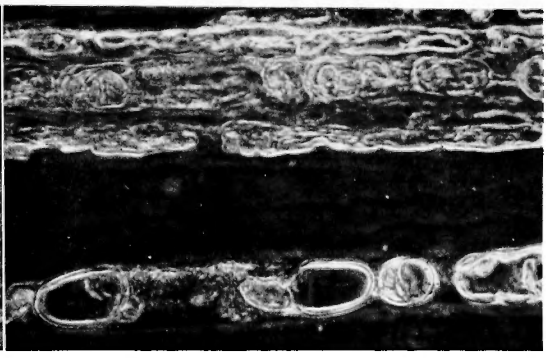

Fig. 4. idem

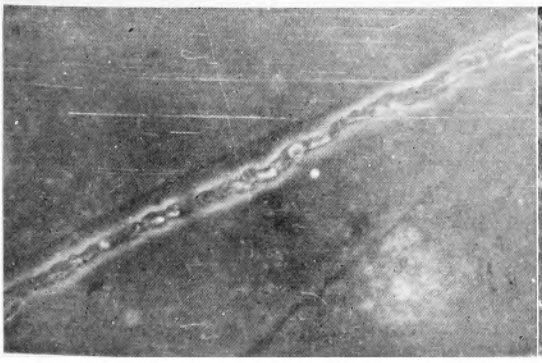

Fig. 4′. idem. (30 minutes after enclosing)

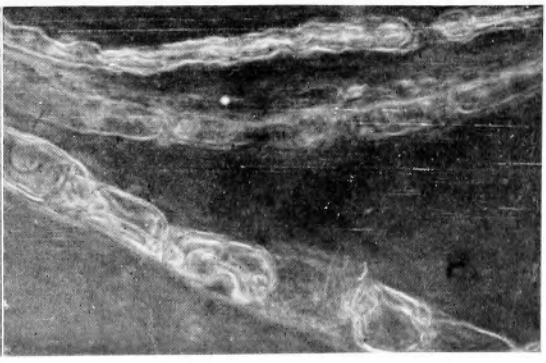

Fig. 5. Control specimen (18 hours after enclosing)

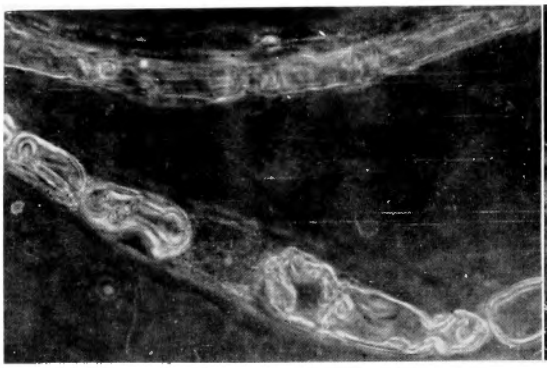

Fig. 6. Control specimen
(3 days after enclosing)

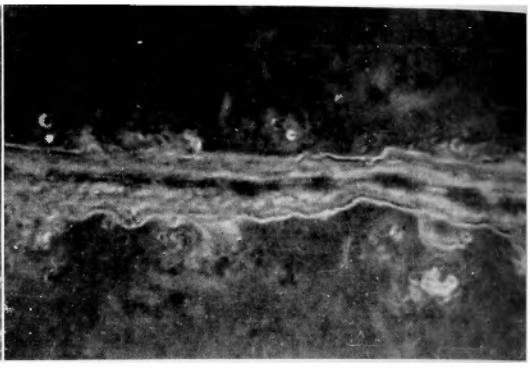

Fig. 7. Walleriandegeneration on 4th day
(3 days after enclosing)

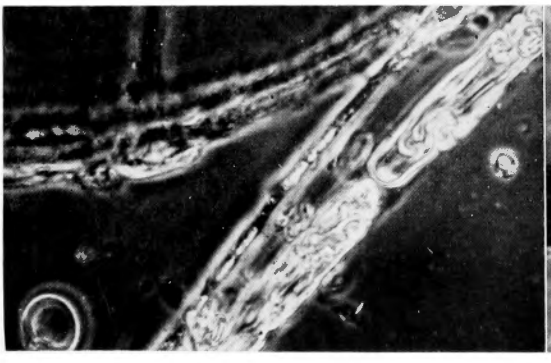

Fig. 8. W. d. on 4th day
〔7 days after enclosing (Lowered affinity to osmic acid)〕

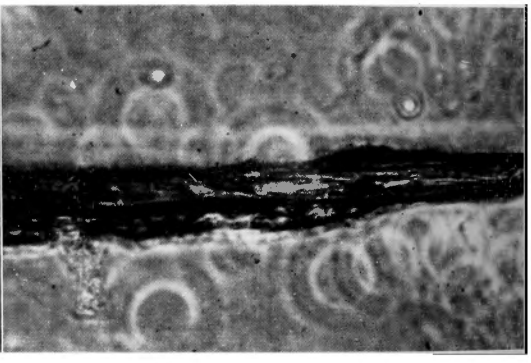

Fig. 9. Control specimen
〔7 days after enclosing (Normal affinity to osmic acid)〕

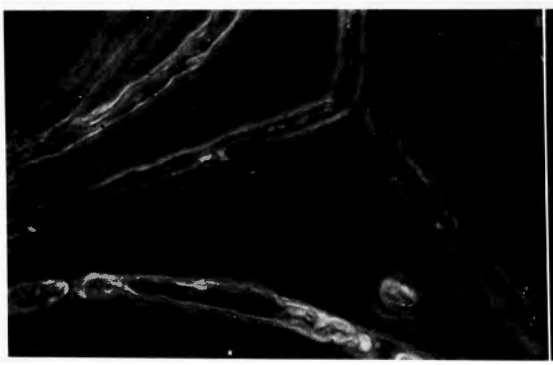

Fig. 10. W. d. on 4th day
〔3 days after enclosing (Process out of myelinsheath looks indistinct and poor.)〕

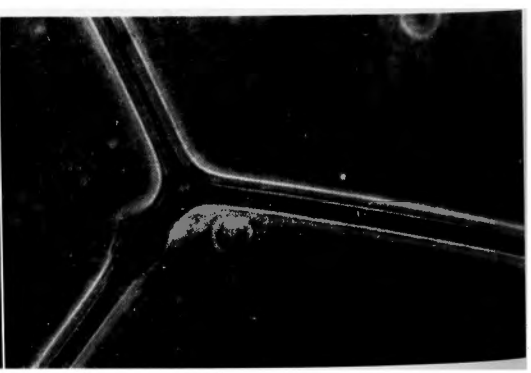

Fig. 11. Control specimen
〔3 days after enclosing (Process of gliocyte grows straint with a clear cut figure.)〕

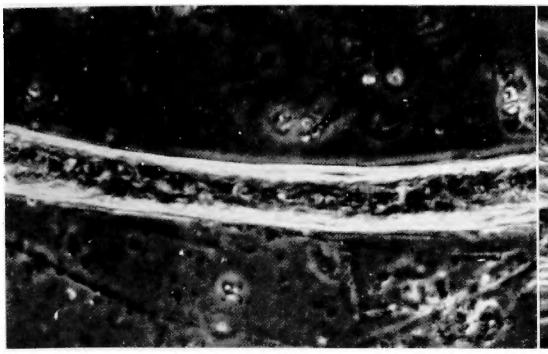

Fig. 12. Wallerian degeneration on 7th day (10 minutes after enclosing (Unhomogeneous axoplasm is shown.))

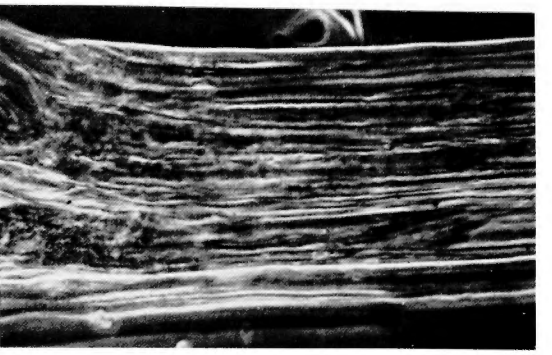

Fig. 13. Control specimen (10 minutes after enclosing)

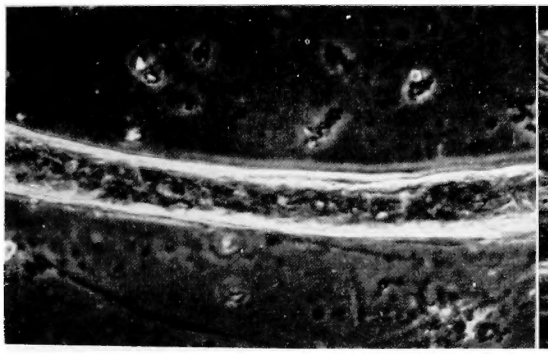

Fig. 14. W. d. on 7th day (15 minutes after enclosing)

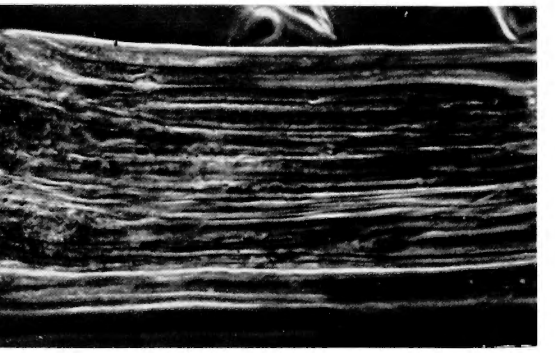

Fig. 15. Control specimen (15 minutes after enclosing)

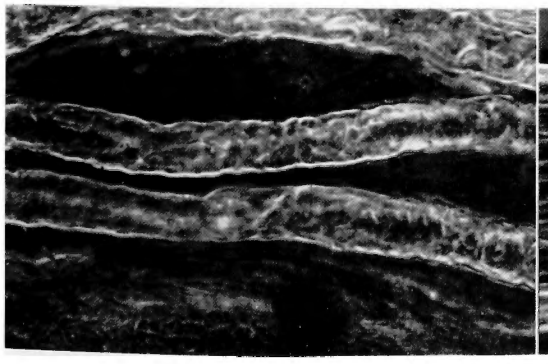

Fig. 16. W. d. on 7th day (30 minutes after enclosing (Wrinkled myelinsheath are shown.))

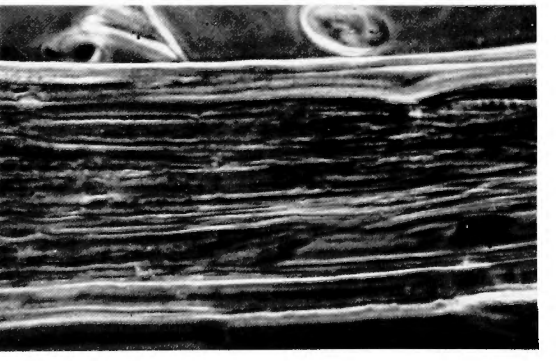

Fig. 17. Control specimen (30 minutes after enclosing)

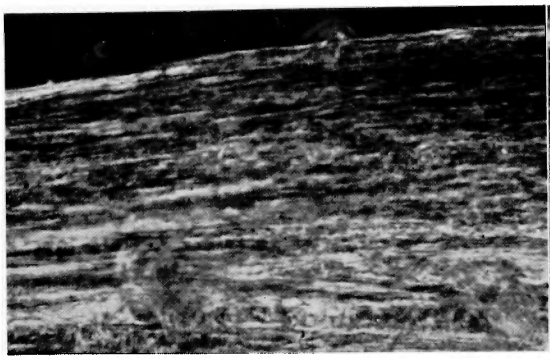

Fig. 18. W. d. on 7th day
〔1 hour after enclosing (Globules in axoplasm with wrinkled myelinsheath are shown.)〕

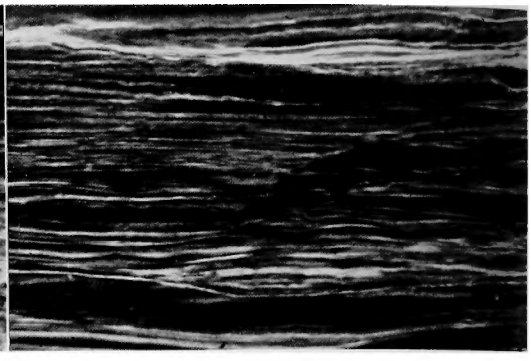

Fig. 19. Control specimen
(1 hour after enclosing)

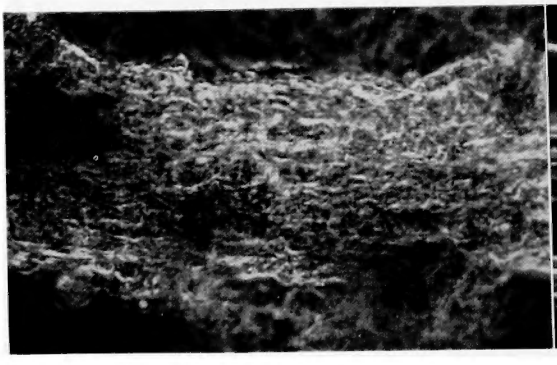

Fig. 20. W. d. on 7th day
〔1½ hour after enclosing (Myelinsheaths fall into destroyance.)〕

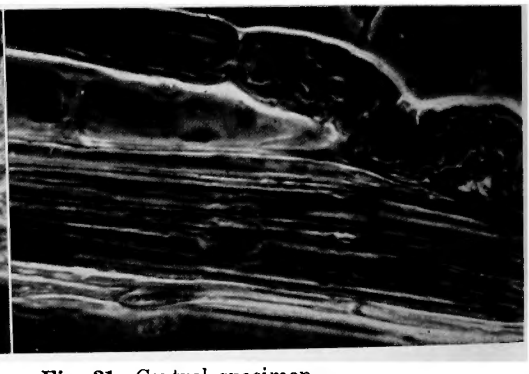

Fig. 21. Control specimen
(1½ hour after enclosing)

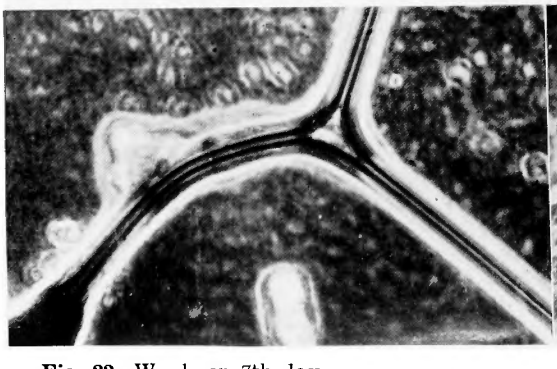

Fig. 22. W. d. on 7th day
〔4 days after enclosing (A process still buds out of the myelinsheath.)〕

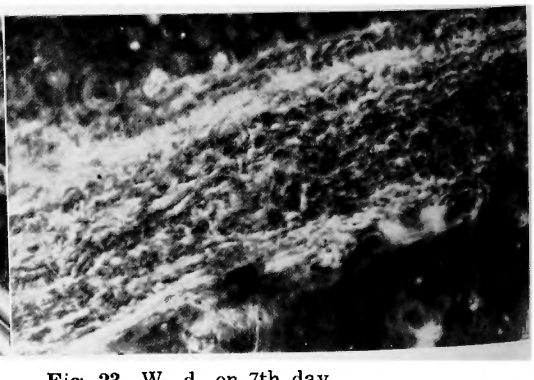

Fig. 23. W. d. on 7th day
〔4 hours after enclosing (Segmentation and fragmentation of the myelinsheath.)〕

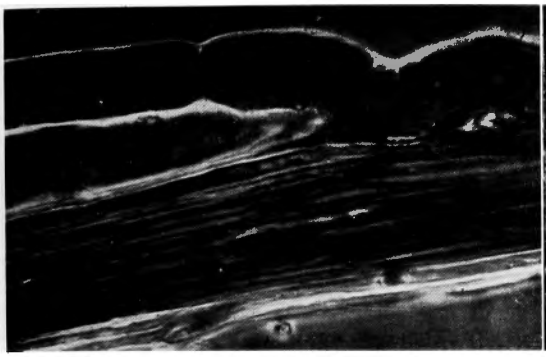

Fig. 24. Control specimen
(4 hours after enclosing)

Fig. 25. Wallerian degeneration on 14th day
〔5 minutes after enclosing (Most of nerve fibers are already destroyed.)〕

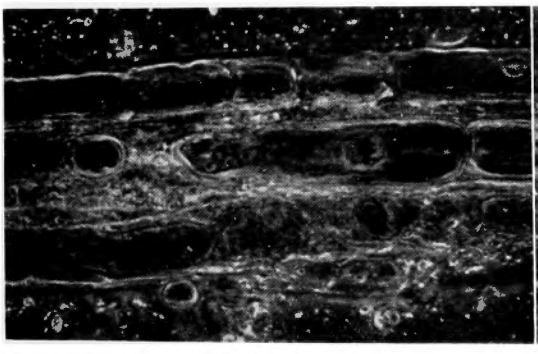

Fig. 26. Control specimen
(5 minutes after enclosing)

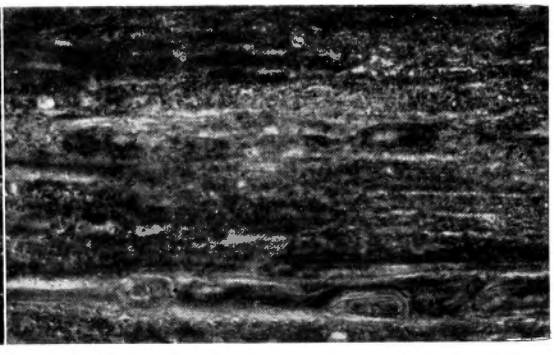

Fig. 27. W. d. on 14th day
〔15 minutes after enclosing (Almost the same figures as shown in Fig. 25)〕

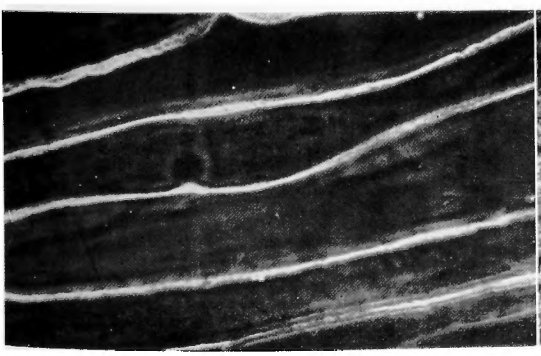

Fig. 28. Control specimen
(15 minutes after enclosing)

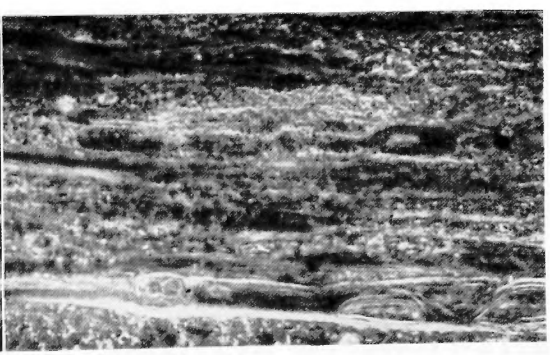

Fig. 29. W. d. on 14th day
〔30 minutes after enclosing (The change after enclosing does not proceed.)〕

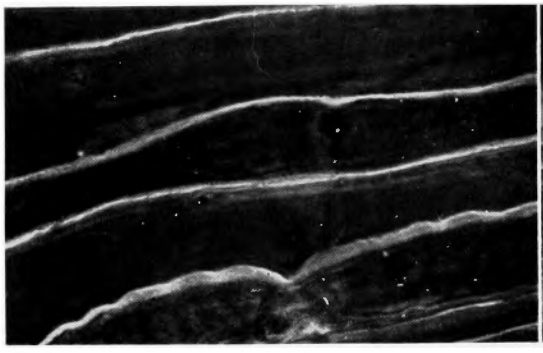

Fig. 30. Control specimen
(30 minutes after enclosing)

Fig. 31. W. d. on 14th day
〔1 hour after enclosing (Fragmented meylinsheath with globulated axoplasm in thick nervefibers)〕

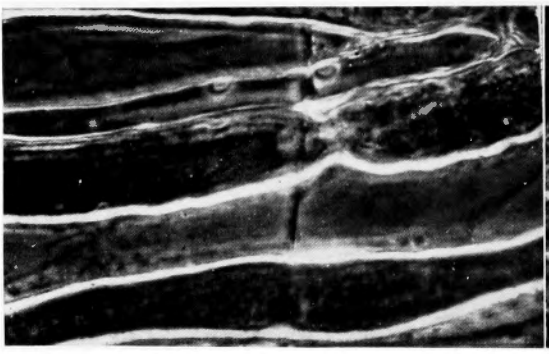

Fig. 32. Control specimen
(1 hour after enclosing)

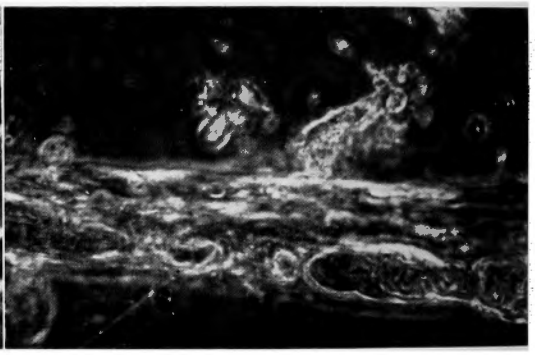

Fig. 33. W. d. on 14th day
〔30 hours after enclosing (Segmented myelinsheath and destroyed process of myelinsheath of thick nerve fibers are shown.)〕

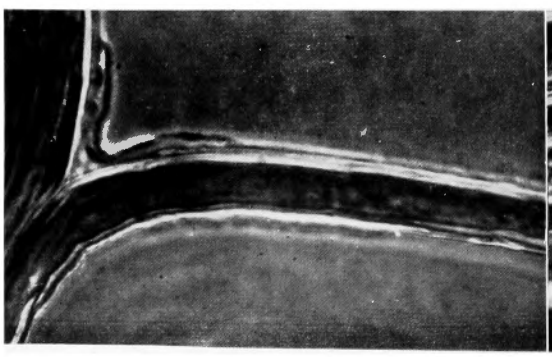

Fig. 34. Control specimen
(30 hours after enclosing)

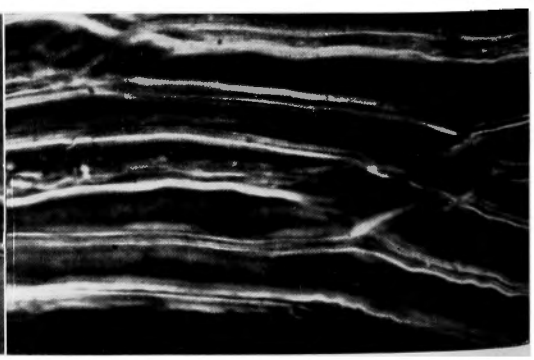

Fig. 35. Nerve fibers enclosed with croton oil
(5 minutes after enclosing)

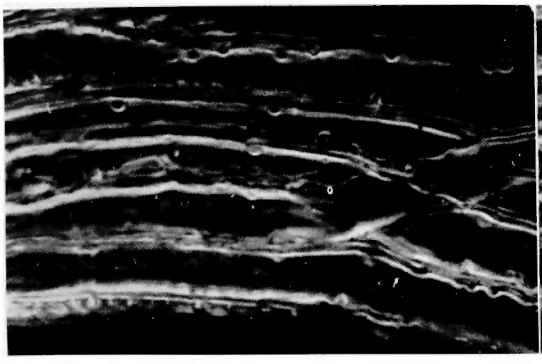

Fig. 36. After 15 minutes

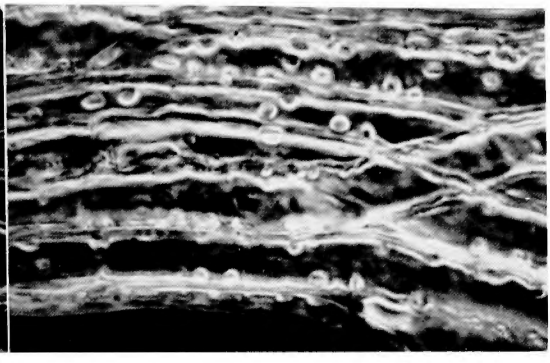

Fig. 37. After 30 minutes

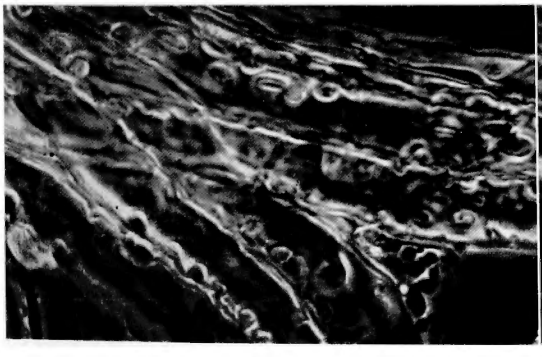

Fig. 38. After 1 hour

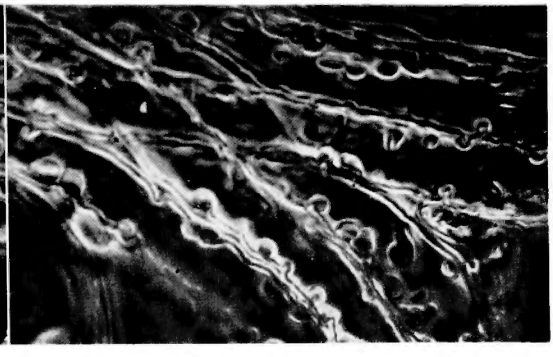

Fig. 39. After 1½ hour

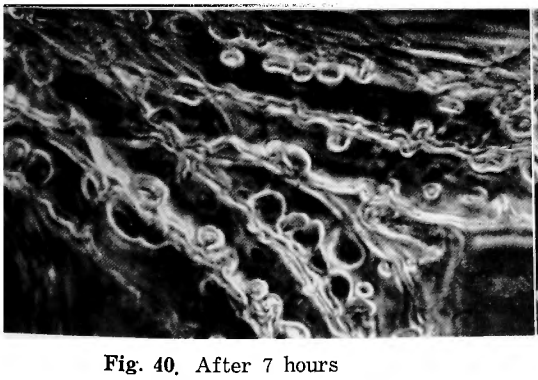

Fig. 40. After 7 hours

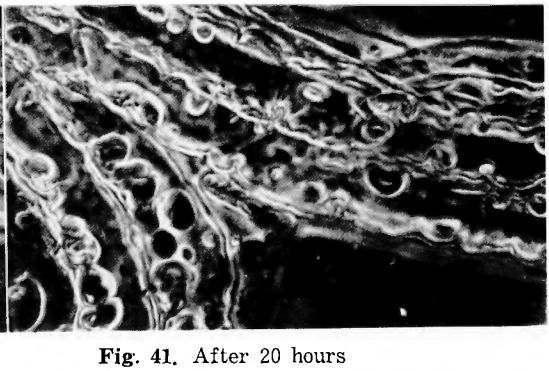

Fig. 41. After 20 hours

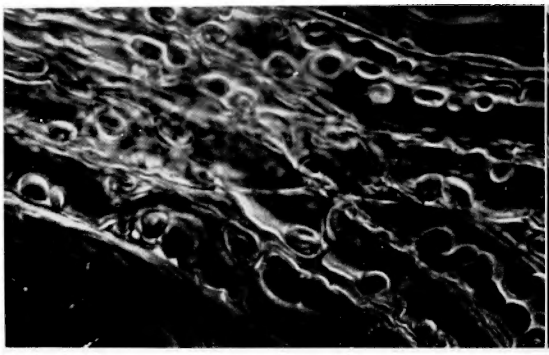

Fig. 42. After 27 hours

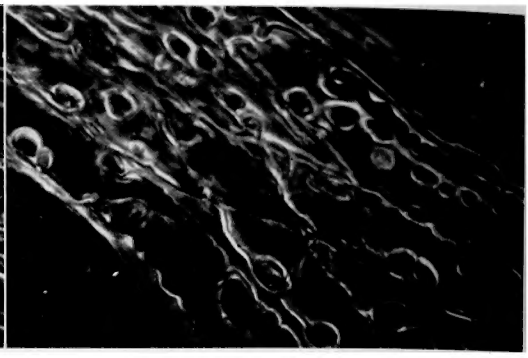

Fig. 43. After 2 days

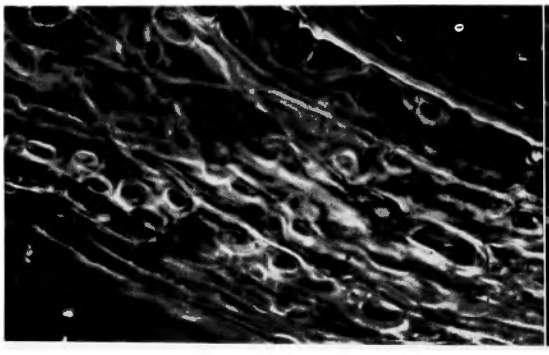

Fig. 44. After 3 days

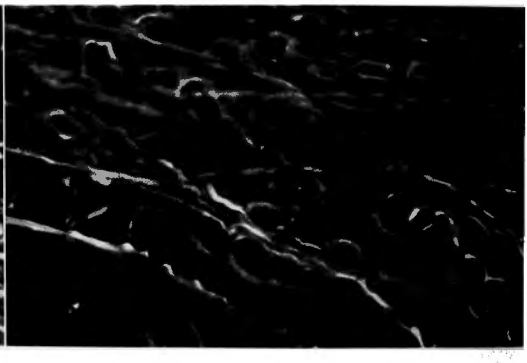

Fig. 45. After 4 days
(Spherical swellings)

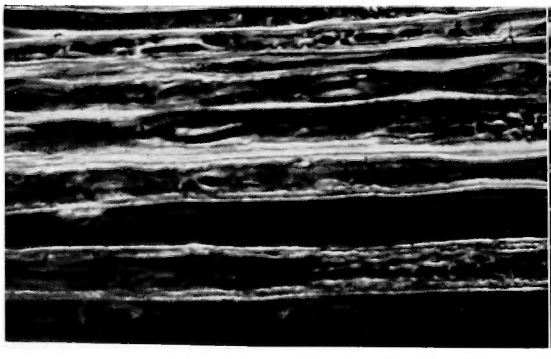

Fig. 46. Nerve fibers enclosed with Quinapon
〔5 minutes after enclosing (Change of axoplasm beginns to appear.)〕

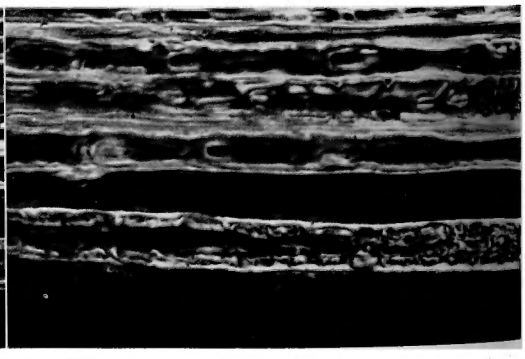

Fig. 47. After 15 minutes
(Unhomogeneous axoplasm and spinous projections of myelinsheath)

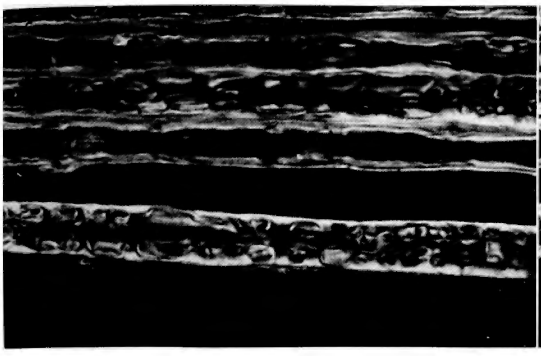

Fig. 48. After 25 minutes

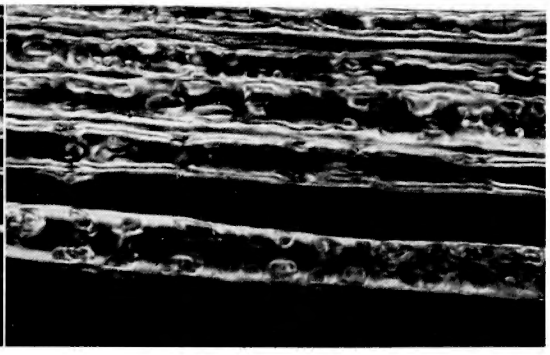

Fig. 49. After 40 minutes

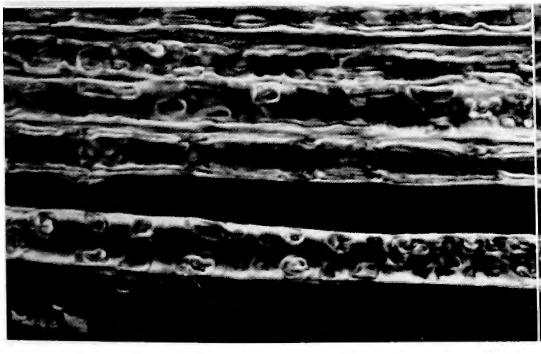

Fig. 50. After 1 hour

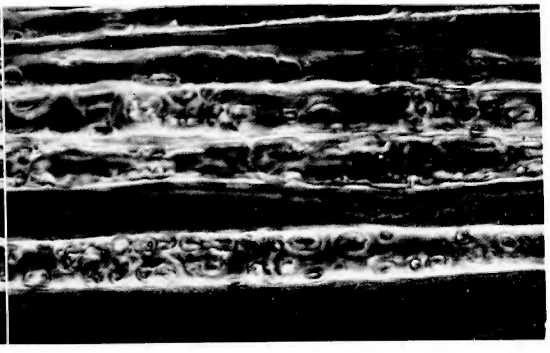

Fig. 51. After 2 hours

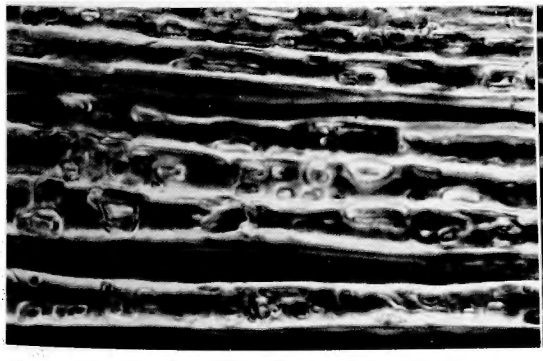

Fig. 52. After 8 hours

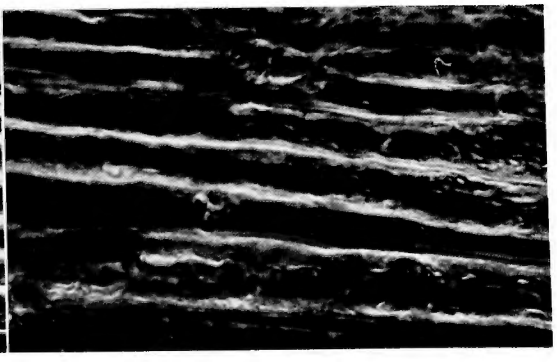

Fig. 53. After 20 hours

Shoin, 1954.
3) Mizuhira, T.: Application of phase contrast microscope. Igaku Shoin, 1952.
4) Mori, M.: Theory and techinique in histochemistry. Nanzando & Co., 1949.
5) Nash, J., Surgical physiology, C. C. Thomas, 1953.
6) Nishisaka, M. and Ito, K.: Technique in medical photograph. Shokabo, 1957.
7) Okamoto, K., Ueda, M. and Maeda, T.: Microscopic histochemistry. Igaku Shoin, 1955.
8) Okawa, H.: Comparative study on the morphological structure of peripheral nerve fibers, with use of phase controst microscope and with silver impregnation method.
9) Peterson, H.: Histologie und mikroskopische Anatomie. J. F. Bergmann, 1935.
10) Sawamura, E.: Physiological and histological studies on sensation of intestinal tract and mesenterium. Tokyo Igakukai Zasshi, 1912.
11) Stöhr, Ph.: Handbuch der mikrokopischen Anatomie.
12) Spielmeyer, W.: Histopathologie des Nervensystems. Sekundäre Degeneration.
13) Scott, D.: The response pattern of the paccinian corpuscle.
14) Takagi, B.: Clinical pathology with use of electron microscope. Rinsho Byori, No. 8, 1958.
15) Takagi, B. and Suzuki, A.: Thechnology in electron microscopy as a new method of clinical test.

和 文 抄 録

位相差顕微鏡による神経線維の変性実験に関する神経病理学的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

満 田 久 和

位相差顕微鏡を用いて食用カヘルの坐骨神経切断後に起る Waller 変性を観察し，又クロトン油並びにキナボン液を坐骨神経に作用させ，その変性像を追求した結果，次の如き所見を得た．

1) 新鮮封入坐骨神経標本には Lantermann の尖裂，軸索内神経細線維等は認められず，Ranvier の狭輪さえもみられない．

2) Waller 変性の過程に於て，初め軸索内に神経細線維，顆粒等が現われ，同時に髄鞘の分節化，断裂等が起り，又オスミウム酸に対する染色性は低下する．

3) Waller変性は2週間で終末する．

4) Waller変性の過程に於て，封入3日後Schwann細胞に属するとみられる髄鞘から棘状突起が出現し，この突起は対照標本に於ては真直ぐに直線状に伸び，その像は鮮明である．これらは2週間後迄増殖し，然る後次第に破壊される．斯かる棘状突起は Waller 変性の進展に伴い不明瞭且つ貧弱な像を与えるのみとなる．

5) クロトン油は初期にまず髄鞘に作用して，これを破壊するが軸索には作用せず，軸索の破壊は髄鞘破壊に伴う2次的変化として現われる．

6) キナボンは最初から軸索並びに髄鞘の両者に向って破壊的に作用する．