COMPARATIVE STUDY ON THE MORPHOLOGICAL STRUCTURE OF PERIPHERAL NERVE FIBERS, WITH USE OF PHASE CONTRAST MICROSCOPE AND WITH SILVER IMPREGNATION METHOD

by

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INTRODUCTION

Since a new method of staining for the nerve tissue, in which the nerve fiber was bound first with silver salt and the latter was further reduced to the metalic silver in position, was established by BIELSCHOWSKY, STÖHR and SCHULTZE, the morphological studies in this field developed very rapidly. Also with the ceaseless technical improvement thereafter, the silver method became a really indispensable tool in the morphological studies of the nervous tissue.

Still, however, many investigators have been forcussing their efforts in getting the more remarkable contrast, i. e., the more different argentophility between the nerve fibers and the surrounding tissue, in their modified method.

Thus even with the most carefully prepared specimens of silver impregnation, it is often very difficult or impossible to reach the unequivocal conclusion on the finest structures of the nerve fiber or on the mode of its termination. It is sometimes dfficult even to distinguish the nerve fibers from the surrounding structures which possess the affinity to silver.

Also, it is always open to question to what extent the real structure in the body can be preserved through the process of this staining technique. All these problems are to be inquired carefully.

I have been studying on the morphology of epidermis layer of the skin for many years. Nerve fibers in the epidermis is widely considered to be very hard to detect. It is rather strange that only a very few neuronal structures can actually be found in the skin, in spite of the fact that it has various complicated sensory functions as a sense organ.

I began to think that these nerve fibers in the skin might be very vulnerable to the post-mortem changes and loose their actual structures very quickly when the skin was taken out from the body. Thus, in the present study, I tried to

catch the changes of nerve fibers somewhat dynamically with the use of phase contrast microscope.

A) Dynamic changes of nerve fibers enclosed in various kinds of medium

Materal and Method

Frogs were used in this study. As a premedication 0.5mg of Wintermin (Chlorpromazine) was injected, and frog was fixed in the prone position. Through the incision of the skin at the dorsal side of the thigh, sciatic nerve was very carefully dissected from the surrounding tissue. The nerve was sectioned as distally as possible. Holding this cut end the sciatic nerve trunk of approximately 2.0cm was pulled out of wound, then the proximal part was also cut. A piece of nerve thus obtained was immediately put on an objective glass and enclosed with several drops of physiological saline solution, auto-serum, alcohol, 10% neutral formalin solution and 1% osmic acid solution respectively. Materal was manually dissected with thin needle in each medium and examined chronologically with phase contrast microscope. (D. M., B. M., & N. D. L. phase plates were used). Whole these procedures were carried out within 2–3 minutes.

PHASE CONTRAST MICROSCOPICAL FINDINGS

1) Materials prepared with physiological saline solution

In the materials prepared with physiological saline solution, considerable changes in nerve fibers, such as indentation or segmentation of myelin sheath, already began to appear within the period of preparation. 10 minutes later, these changes became more significant. After 20 minutes, spherical swelling or abnormal folding, and further after 30 minutes, a defnite fragmentation of myelin sheath became to be observable. Up to this stage, axis cylinder still kept its normal appearance fairly well, and was seen as the homogeneous or shiny colloidal mass. No neural subfibril or a structure of such sort could be noticed in this stage. As the time elapsed, however, structure in axis cylinder became less homogeneous and revealed the phase differences. Further fine granular, fibrous or latice-like structures began to appear in axis cylinder. Among the features of segmentation, symmetrical indentation of the myelin sheath was most commonly observed in the earlier stage. In the later stage, however, such indentation became more marked and deeper.

At the torn sites of nerve fibers (by the mechanical facter during the manipulation), so-called myelin figure or escape of axoplasm from the ruptured hole was observed. Figs. (1—9).

2) Materials enclosed with serum

In the materials enclosed with auto-serum, the similar regressive changes of the sciatic nerve as seen in the material prepared with saline solution, were also noticed. The rate of progress of these changes, however, was much slower in the former than in the latter. This was true, either in fibers whose normal

structure was considerably preserved or in fibers at the mechanically torn sites. Thus, even after 3 hours, changes in the nerve fibers enclosed in auto-serum were fairly slight (Figs. 10—16).

3) Materials enclosed with alcohol

In the specimens prepared with alcohol. changes in myelin sheath were relatively slight, but the axis cylinder revealed the considerable regressive changes. The myelin sheath showed the features of indentation or segmentation only in a slight degree, or in parts even no such changes was observable and a sharp-cut linear outline of myelin sheath was preserved. Also, the myelin figure or the escape of axoplasm at the torn site was very scarcely found.

Axis-cylinder, on the other hand, underwent the more marked changes, i. e. as a whole it became unhomogeneous or granulated, and even a fibrous appearance of axis-cylinder began to appear (Figs. 17—21).

4) Materials enclosed with formalin solution

The sciatic nerve enclosed with 10% neutral formalin solution, underwent very marked regressive changes either in axis cylinder or in myelin sheath. Axis cylinder showed a rosary-like appearance, fragmentation of flying-swallow shape and a coarse granular or fibrous appearance of axoplasm. Also in myelin sheath various changes, such as constriction, twisted protrusion or swelling, were seen. Very obvious myelin figure or leakage of axoplasm at the mechanically torn place was noticed (Figs. 22—30).

5) Materials enclosed with osmic acid

In the specimens prepared with 1% osmic acid buffer solution (Palade's solution) the fewest changes were observed in nerve tissue, compared to those prepared with above-mentioned enclosing materials.

After 30 minutes, outline of myelin sheath was still linear and uninterrupted. Axis cylinder remained homogeneous and there were found only slight changes at the mechanically torn end. However, with the lapse of time, even in the preparation with osmic acid some changes in the nerve tissue were inevitable. Thus, when the material was fixed with osmic acid as long as 3 hours, fairly marked changes could be seen both in myelin sheath and in axis cylinder (Figs. 31—34).

6) Biomicroscopy of nerve tissue

An anesthetized frog was placed on the microscope-stage, and its mesenterium was pulled out to strech on the objective glass. Microscopic structures of the peripheral nerve fibers running through the mesenterium were examined. Changes occured in the nerve fibers were nearly equal to those seen in the materials enclosed with various medium.

Particularly at the peripheral portion, a feature of fragmentation in nerve fibers were obviously seen. Club-shape or globular fragments thus developed gradually reduced their size and finally disappeared.

For the next step, at the thigh of a frog only the distal end of the sciatic nerve was sectioned, and the nerve was placed on the objective glass leaving

the continuity of nerve towards the proximal portion.

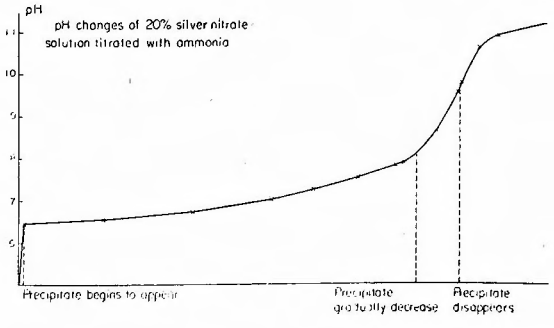
In this case, changes in the nerve tissue were even less severe, except at the cut end of the nerve. Outline of myelin sheath was entirely linear and neither indentation nor swelling could be found. Also in axis cylinder, regressive changes were rather negligible and numerous fibers with homogeneous axoplasm were running paralleled or in somewhat interlacing manner. It was very regrettable that these unmanipulated materiials were not suitable for the micrsocopic photographs due to the poor phase contrast (Figs. 35 & 36).

B) Histological pictures of nerve fibers stained by silver impregnation method

As the materials for this study, normal human skin at the sole of foot, skin with a circular tattoo around the wrist joint (in which pain sensation was preserved and tactile sensation was lost), skin at the sole of foot in a patient of leprosy in which all sensations were lost, skin at the site of amputation neurinoma and also nerve fibers of the animals, were used. These materials were stained by silver impregnation according to the BIELSCHOWSKY's method, SUZUKI's method and also my own method.

Fixation :

In the silver impregnation method, 10% neutral formalin solution are most widely used for the fixation of the materials. pH of the commercially available formalin solution is between 3. 5—4. 5. When it is strictly corrected with precipitated calcium carbonate or other chemicals between the pH range of 7. 2—7. 4 and stored in a sample bottle, there occurs a very slight change in pH even in the several months duration. (It shows a slight tendency to change toward acid-side). If, however, a piece of specimen is put in the formalin solution thus prepared, pH of the solution decreases very rapidly. Already on the next day the solution is found acid and this tendency becomes even more marked as time goes on. It is considered that any chemical treatment of the amphoteric substances, such as tissues or cells of the body, should be performed in a medium in which pH value is corrected as closely as possible to that of the body fluid. Especially, if we need to use a solution with a very unstable and changeale pH as formalin, its pH value has to be determined and corrected each time with glass eletrode. Also, it is very desirable to put a small piece of



tissue into the plenty amount of fixative solution and shake it enough to promote the penetration of solution into the specimen.

Preparation of Ammoniated silver :

When 20% silver nitrate solution is titrated with ammonia, pH of the solution changes in a fashion illustrated in graph.

Ammoniated silver solution used in the silver method is that in which the precipitate disappeared following the titration with ammonia. However, pH values of these solutions vary condiderably. We can roughly tell the degree of pH in a certain solution by looking the color tone of turbidity of the solution right before the last drop of ammonia is added and by which all precipitates disappear. If the color of a solution is light yellow immediately before the disappearance of precipitation, pH in the ammoniated silver solution at time when the precipitates completely disappear by adding a last drop of ammonia, may be high. If the solution is brown in color, on the other hand, its pH after the disappearance of precipitates must be comparatively low. This is the most important points in the course of silver impregnation, because the higher the pH value of solution is, the stronger reduciability it demonstrates. This, in turn, may give a serious influence upon the tissue which has already been infiltrated with silver salt. So-called "tricks" or a key in making a good slide of silver impregnation, may probably lie in the preparation of proper ammoniated silver solution for each particular purpose. Generally speaking, in the silver impregnation of a tissue, it is advisable to use a solution with the low pH value for the strong reducing agent, and a solution with the high pH value for the weak reducing agent. In actual processes, however, it is often very difficult and complicated to decide the most proper condition of each time because so many factors, such as kind of tissues to be stained or temperature and duration of the reduction, may take part in the process. Therefore, it may a sound policy to make the various conditions, such as pH value, duration and temperature of the precedure, constant as much as possible, following the principle of developing in photograph.

FINDINGS

1) As shown in photographs, numerous nerve fibers, originating from the subcutaneous nerve bundles and via the MARTINEZ-PÉREZ's nerve plexus in the corium, extended to the MEISSNER's corpuscles, corpuscles in the corium, hair follicles, sweat glands, sebacious glands, PACCINIAN corpuscles around the large blood vessels. In the epidermis layer of stratum MALPIGHII, even fewer number of fibers comming up to the granular layer can be seen. These findings had long been recognized by many other investigators. If, however, the fresh materials was fixed with quick-freezing method and examined phase contrast microscopically, many fibers extending to the granular layer could easily be observed. Also, if the materials were fixed with osmic acid, even in the slides prepared by the silver impregnation method, many nerve fibers running in

stratum germinativum could be found. These fibers were distinguishable from the meniscus of bassl layer, and their courses or mode of ramification were entirely unrelated to the cells in the epidermis (Figs. 37—47) (Figs. 71—81).

2) In the area of a circular tattoo, the pain sensation was preserved and the tactile sensation was lost. In the slides prepared by the silver method, features of degeneration of devastation of the corium and subcutaneous layer were seen around the points of pigment deposition. In these areas some degenerative fibers innervating the adjacent hair follicles, were also noticed. Even in the same slide, nerve fibers running remote the points of tattoo, remained relatively intact. In the affected area, nerve fibers with large caliber underwent the changes such as swelling or fragmentation, and the finer nerve fibers lost their continuity and only a linear arrangement of the interrupted granules noticed (Figs. 48—51).

3) In the skin specimens taken from the patient of leprosy, in which the all sensation had been completely lost, the degenerative changes of the tissue was so severe that the nerve fiber could never be identified. Marked infiltration of the round cells were also noticed. Only in the corium, cells which were star shape and bore some resembrance to the nerve cell, with their processes were found. These cells were 2–3 times greater than the pickle cells in size. In the specimen of skin taken from a place wherein the leprous erythema occasionally developed later, marked degenerative changes were found in the subcutaneous nerve bundles (Figs. 52—57).

4) In a patient who underwent the amputation at the upper arm and an auto-skin transplantation according to the REVERDIN's method on the wound surface, an amputation neurinoma developed postoperatively. Neurionma was excized with overlying skin. In the skin specimen thus obtained, a vigorous regeneration of the nerve fibers was noticed (Figs. 58—61).

5) Even in the normal skin material prepared by the silver method, some degenerative features of the nerve fibers such as vacuole formation, fragmentation not due to the mechanical factors, or rosary-like appearance were often observed. Also with the use of P. C. M., destruction in myelin sheath, escape of axoplasm or globular change of the axis cylinder were clearly found (Figs. 62—70).

DISCUSSION

The fact that the skin is generally regarded as a sense organ, indicates an intimate relation between the skin and the nervous system. Recently, in fact, it has become clear that a series of cell elements in the epidermis and the corium is originated and differentiated from the neural crest. The skin not only protect the outer surface of the body mechanically, but through its various functions such as body temperature regulation, metabolism, sweating, secretion and sensory perception, it plays an important role in the physiological operations of the body mechanisms.

Further, it is worthwhile to note these functions are all carried out in con-

nection with those of the nervous system no matter whether the consiousness participates in it or not.

On the morphology of the nerve elements in the skin, many excellent studies have been made.

Still, however, it may be not too much to say that the definite correlation between each of these nerve elements and the certain physiological function of the skin is entirely left unknown. It has been believed that, with the silver method, it is quite easy to examine the nerve element of the skin up to the layer of corium, but becomes very difficult to follow them beyond the basal layer into the epidermis. Considering the sensory sensitivity at the Frey's test with hair, it is supposed that a considerable number of nerve fibers should exist also in the outer layer of the skin, but actually they are only very scarcely observable in the epidermis. Here, I felt that the reinquiry in the methodology was necessary, and followed the dynamic changes of the nerve fibers by the chemical substances applied and the lapse of time, with the use of P. C. M.. Also these findings were always compared with these by the silver impregnation method. As a result of this study it become clear that the sciatic nerve of the frog could be preserved in a condition most similar to that in the body in a medium of 1% osmic acid buffer solution. Still, however, it was unevitable that the material underwent some changes following the manipulation or with the lapse of time. In the nerve specimen freed from the body, these changes started immediately after the nerve sectioning and rapidly progressed to the considerable degrees within a few minutes. In the specimen in which only the distal end was sectioned, on the other hand, structures of the nerve in the proximal portion remained fairly intact. When the specimen of this portion was examined by P. C. M., the nerve fiber was seen as a linear simple structure. There were found neither swelling nor indentation in myelin sheath. Axis cylinder was found homogeneous or shiny in appearance, and there was found no structure like the neurofibril. It was also confirmed that various structures which had been described by many investigators, such as constriction of RANVIER, SCHMIDT-LANTERMANN's creft, neurokeratin nets or GOLGI's funnel, began to appear the lapse of time after the specimens were taken out from the body. Structures of perineurim, SCHWANN's cell or lymph space could not be examined clearly. Myelin sheath underwent the graduall regressive changes and features of swelling, abnormal indentation, or fragmentation were found. Also at the torn site, well-known myelin-figure or escape of axoplasm could be seen.

It is natural to consider that since the pH value of physiological saline solution is around 5.7, specimen enclosed in this solution may undergo more rapid regressive changes compared to that enclosed with auto-serum. Thus, for the long period observations of nerve fibers, it is better to use auto-serum and in unavoidal case to use the TYRODE's solution. In choosing a enclosing material for the observation of living material or dynamic changes of a tisse, more emphasis should be put in its tissue preserving nature than in the refractive index.

Then based on the results of above-mentioned experiments, frozen sections of a normal skin specimen, taken from the sole of foot, were made and examined by P. C. M..

In this way, I could clearly observe many nerve fibers extending up to the granular layer. These fibers arose from the subcutaneous nerve bundles and penetrated through the basal layer into the epidermis. In stratum MALPIGHIi, they extended through the intercellular space ramifying freely on the way, but they never showed the direct connection with the cell elements. Thus, they seemed quite different from other nerve fibers of the skin which terminated in a certain cell such as MEISSNER's corpuscle or MERKEL's tactile meniscus.

Concerning the mode and details of the termination, however, it is probably better expect in the findings of electron-microscopical studies on this project.

At the next step, similarly skin material was fixed in 1% osmic acid buffer solution, then it was stained by silver method lightly. In the slides thus prepared, there were found numerous long argentophile nerve fibers running in stratum MALPIGHIi, and these had not previously been observed. They extended beyond the basal layer up to the outer layer of stratum MALPIGHIi, but were entirely different in appearance from the processes of the stellar cell. On a function of these nerve fibers, it can be assumed that these probably perticipate in the tactile and pain sensation, considering their location and results in clinical sensory examination.

It is generally believed from the histological or clinical studies that in the skin area covered with hair, the latter may probably play an important part in the tactile and pain perception. And even in the hairless skin area, considerable number of nerve fibers, for the tactile and pain sensation should exist, considering the existence of many other encapsulated corpuscles in the deeper layer.

To a question whether the nerve fiber of same form and shape may conduct only one kind of sensation, I have no clear-cut answer at the present step.

Even in normal skin specimens, some regressive changes of nerve fibers, such as fragmentation, vacuole formation, rosary-like appearance, can very often be observed. These changes were similarly noticed in the slides examined with P. C. M.. Obvious myelin figure, escape of axoplasm and granular change of axis cylinder were also observable. Thus, in examining a certain pathological materials, we can not be too much careful to differentiate the changes due to the actual pathological processes of the tissue from those due to the artifact during the preparation of the specimen. In this sense, we could assume that the degenerative changes seen in the skin area adjacent to tattoo, had something to do with the sensory disturbances, by comparing these to the results of clinical sensory examination and through a careful histological examination of the surrounding tissue. As to the degenerative changes in leprosy, however, definite conclusion could not be drawn whether these originated from the actual pathological process or artifacts during the preparation, since the round cell infiltration in the surrounding area rather scanty. Also, as to the genesis of starshape cells

observed in the skin area with loss of sensation, no definite comment can be made. There are many discussions about the difference between LANGERHANS's cell and pigment cells of the skin and also about the relationship between these and a series of cell elements of the skin which originated from the neural crest and belongs to the nerve system, but they all are not really settled yet. However, it is hard to believe that in a skin area in which the subcutaneous and corium nerve fibers completely disappeared and all sensations are completely lost, still normal nerve fibers remain intact only in the epidermis layer. If we assume that cytoplasmic processes stained by silver in the epidermis layer in a patient of leprosy is a neural origin, the cell itself cannot be other than the nerve cell. But the existence of a neuron in the epidermis layer of the skin rather difficult to think of. Thus, this large cell in the epidermis is not a nerve cell but probably a kind of chromatophore cell. It has been also advocated that the pigment cell may have something to do with the nerve tissue and with development of the hair root.

Regeneration of nerve fibers at the amputation neurinoma, was really vigorous. Tremendous number of regenerated nerve fibers extended in the narrow area of the skin. Patients very often complain about the local pain arround the amputation neurinoma, and these can enough be explained from above mentioned morphological findings.

SUMMARY

1) Using P. C. M., dynamic changes of the nerve fibers enclosed with various mediums were examined in the sciatic nerve of frog.

2) With 1% osmic acid buffer solution, the nerve fibers could be preserved in a condition most similar to that in the body fluid.

3) In the epidermis layer of the normal skin specimen fixed by 1% osmic acid buffer solution and stained by silver method, many nerve fibers extending up to the outer layer of stratum MALPIGHII, were noticed. This finding corresponded to that in the fresh frozen sections examined by P. C. M..

4) Through the comparative studies of nerve fibers using either silver method and P. C. M., it becomes clear that in the slides prepared by the silver method, it is often very difficult to differentiate the degenerative changes due to the actual pathological process from those due to the artifact or time factor during the preparation of the specimen. Therefore, these findings should carefully be interpreted in the reference to the results of sensory examination and to the morphological findings in the surrounding tissues.

5) Star-shape cell in the epidermis found in the skin of leprosy, was probably not a nerve cell, but seemed to be a chromatophore cell.

6) As to the technical problems of the silver method, some considerations were made from the view point of pH of the solution.

7) In this study, only the changes in nerve fibers were discussed. As to the mode and details of terminatin of the nerve fibers, we better wait until the

study with electron microscope may give some answer in near future.

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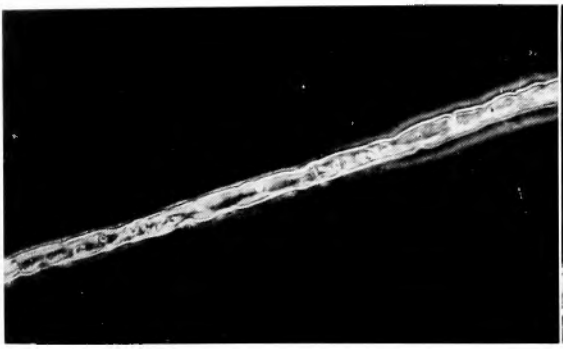

Fig. 1 Frog's sciatic nerve enclosed with physiolgical saline solution. 10 minutes after the nerve section slight changes already observable. 40×7

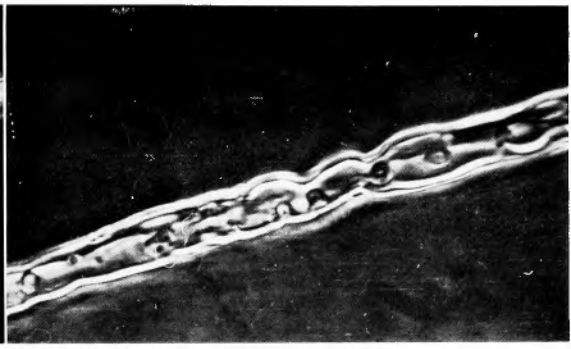

Fig. 2 After 20 minutes. Note irregular undulation of fiber and spherical recess of myelin sheath. 90×7

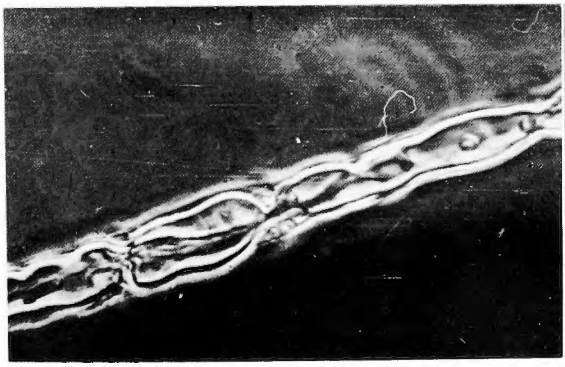

Fig. 3 After 35 minutes. Note feature of segmentation and Schwann's cell.

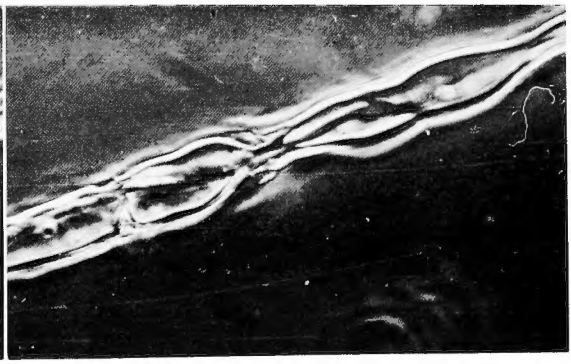

Fig. 4 After 1 hour.

Fig. 5 After 80 minutes.

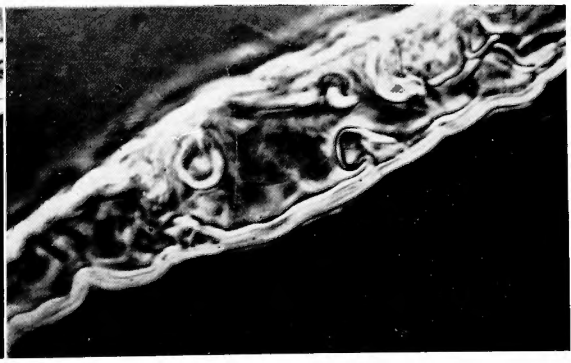

Fig. 6 After 2 and half hours. Note spherical protrusion of myelin sheath towards axoplasm.

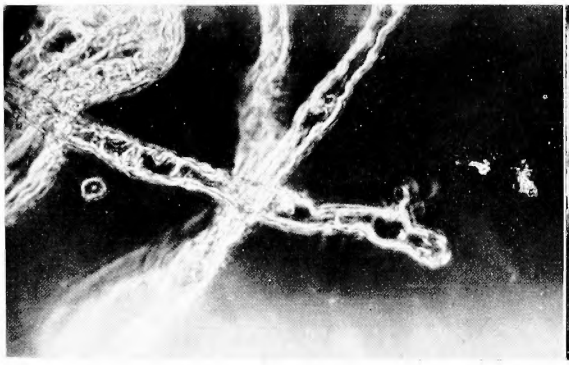

Fig. 7 At the cut end of fiber. 15 minutes after the nerve section.

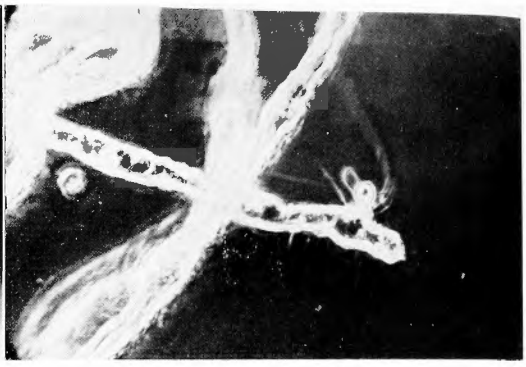

Fig. 8 30 minutes after the section.

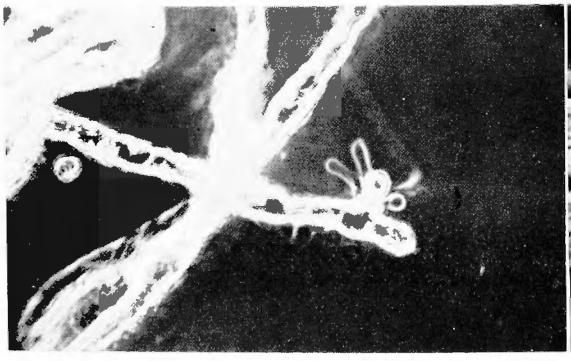

Fig. 9 60 minutes after the section. Note myelin figure and escape of axoplasm.

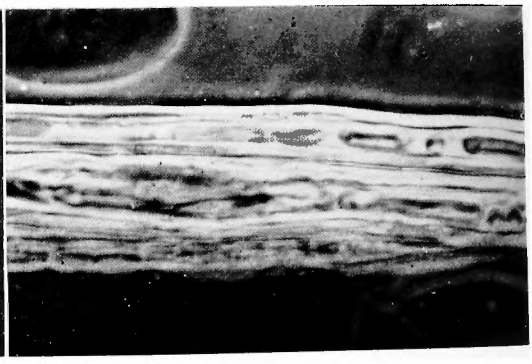

Fig. 10 Changes in chronological sequence of Frog's sciatic nerve enclosed with auto-serum. After 10 minutes.

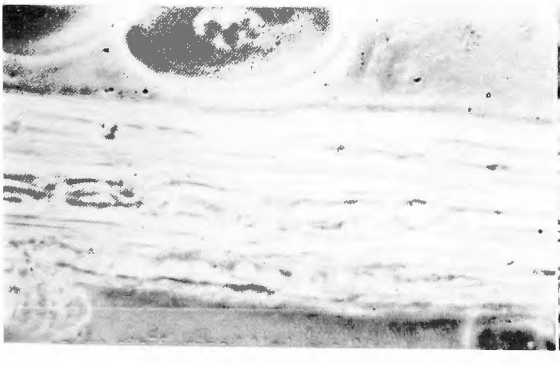

Fig. 11 After 30 minutes.

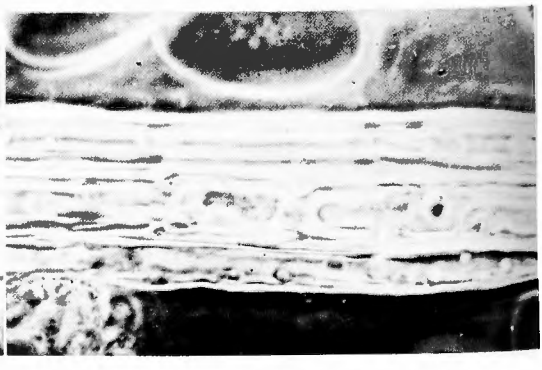

Fig. 12 After 60 minutes.

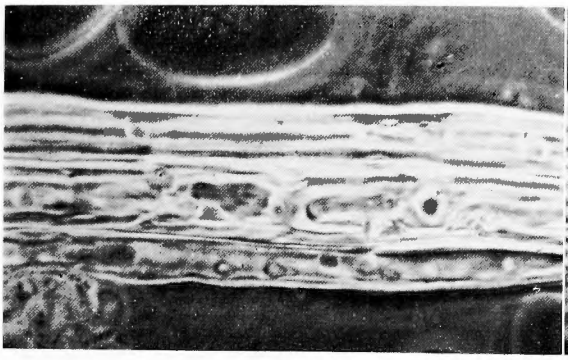

Fig. 13 After 90 minutes.

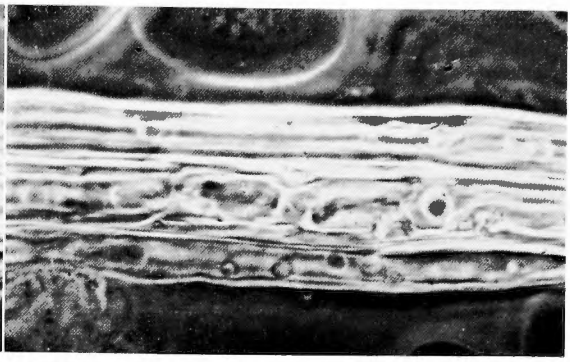

Fig. 14 After 120 minutes.

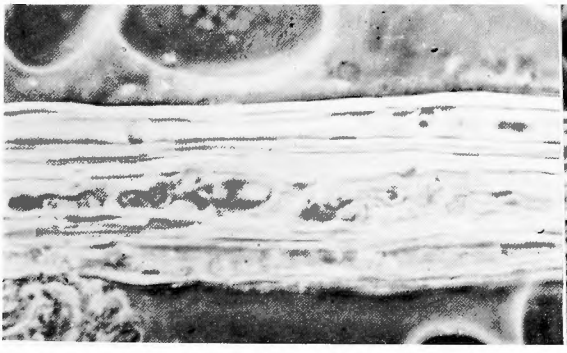

Fig. 15 After 150 minutes.

Fig. 16 After 180 minutes. Changes are still slight.

Fig. 17 Frog's sciatic nerve enclosed with alcohol. At the torn end of fiber. 20×7

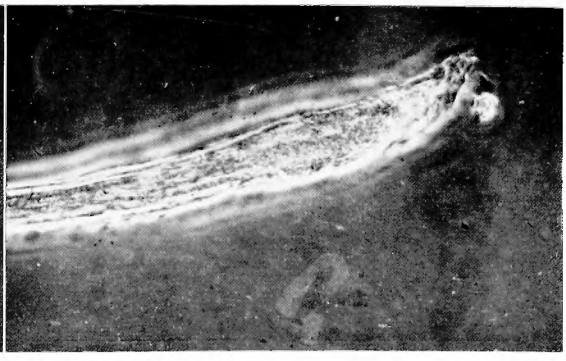

Fig. 18 Myelinfigure is less obvious. 40×7

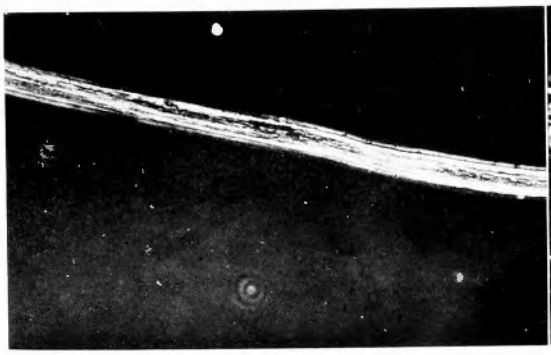

Fig. 19 Nerve fiber. 20×7

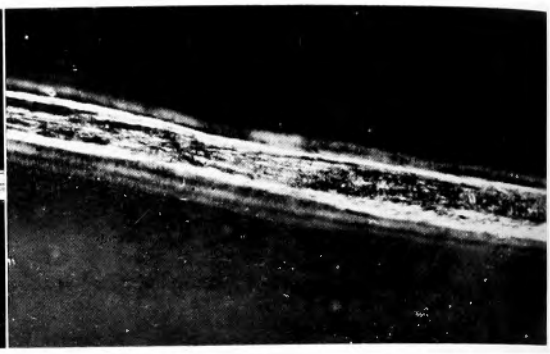

Fig. 20 40×7 Granular appearance and fibrous arrangement of axis cylinder.

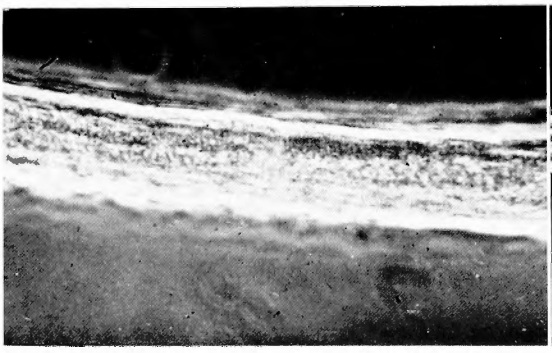

Fig. 21 90×7

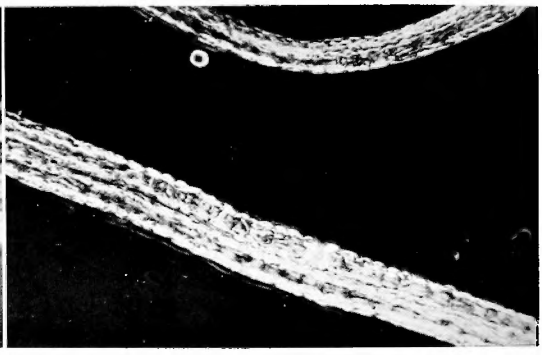

Fig. 22 Formalin fixed sciatic nerve of frog. Severe changes are observable. 20×7

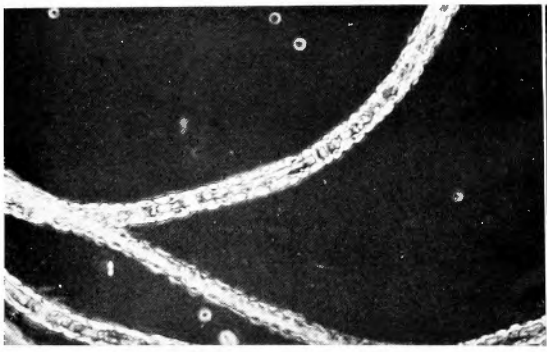

Fig. 23 Fragmentation of flying swallow shape. 20×7

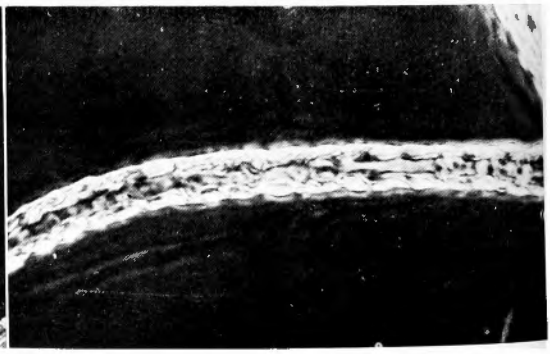

Fig. 24 Lantermann's creft. 40×7

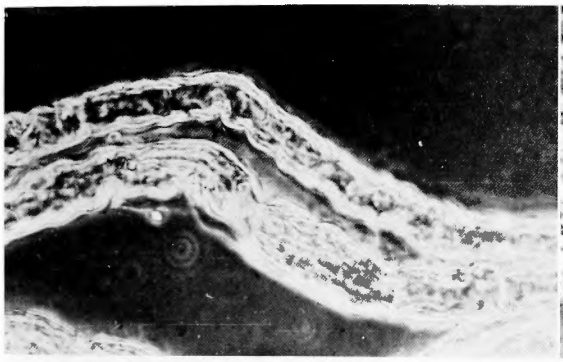

Fig. 25 40×7

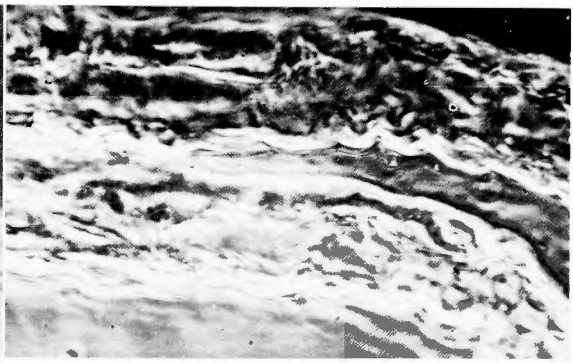

Fig. 26 90×7

Fig. 27 Note myelinfigure at the torn end and escape of axoplasm. 20×7

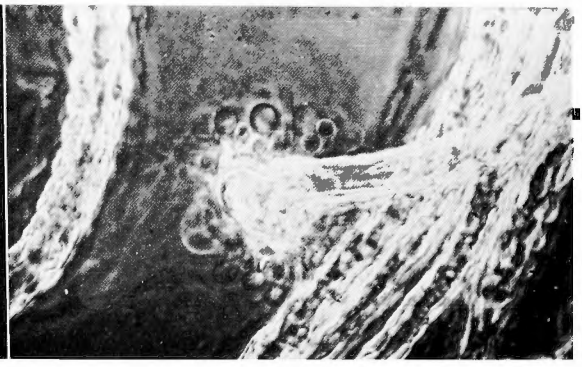

Fig. 28 Note very marked myelinfigure and escape of axoplasm. 40×7

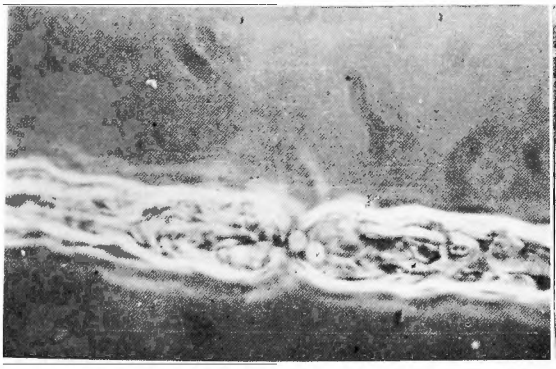

Fig. 29 90×7

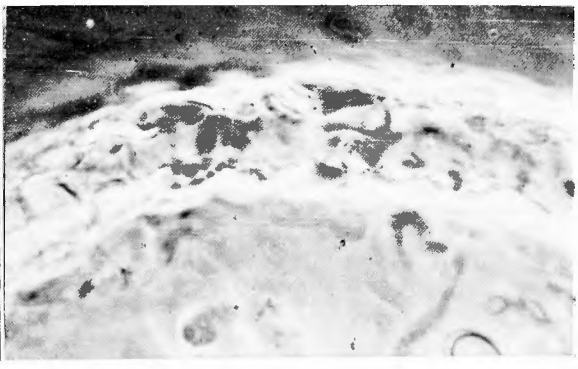

Fig. 30 90×7

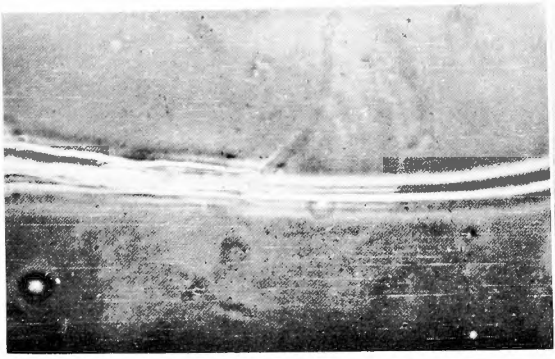

Fig. 31 Frog's sciatic nerve enclosed with osmic acid. After 30 minutes. 40×7

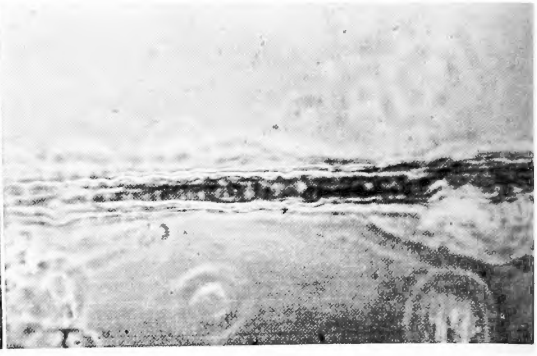

Fig. 32 After 30 minutes. Changes are very sight. 90×7

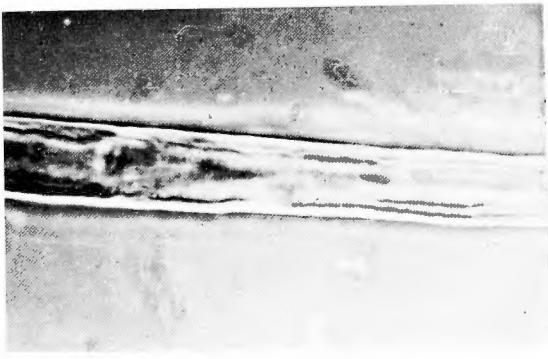

Fig. 33 After 3 hours. 40×7

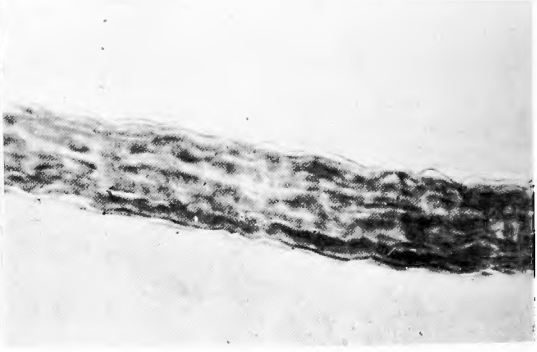

Fig. 34 After 3 hours. Changes progressed more marked. 90×7

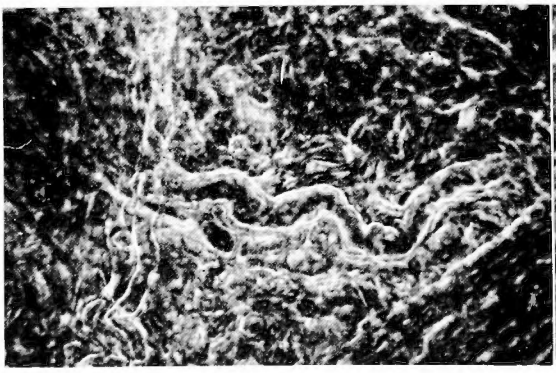

Fig. 35 Nerve fiber in the mesenterium.

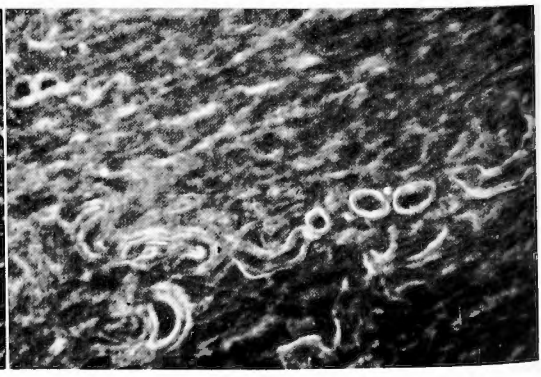

Fig. 36 Club-shape and globular fragments.

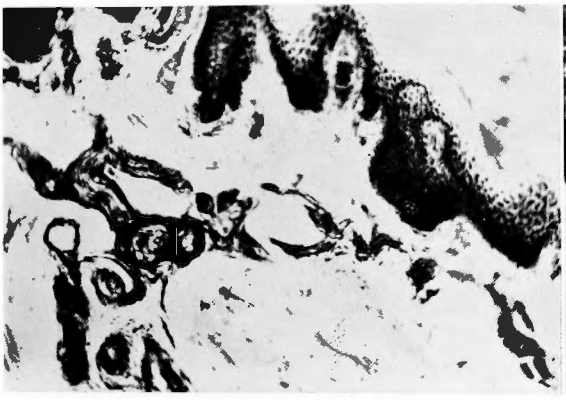

Fig. 37 Martinez-Pérez's nerve plexus.

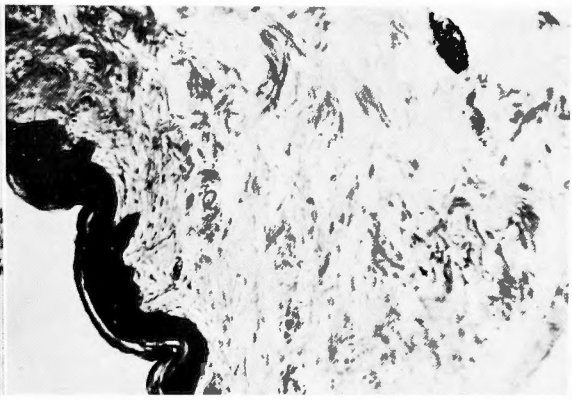

Fig. 38 Encapsulated corpuscle in the cutis.

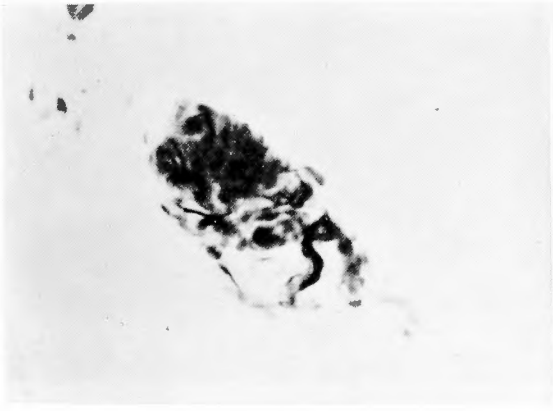

Fig. 39 enlarged figure of encapsulated corpuscle in cutis.

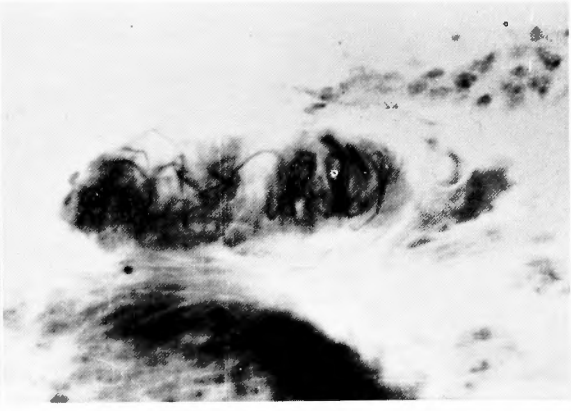

Fig. 40 Meissner's corpuscle.

Fig. 41 Paccinian corpuscle.

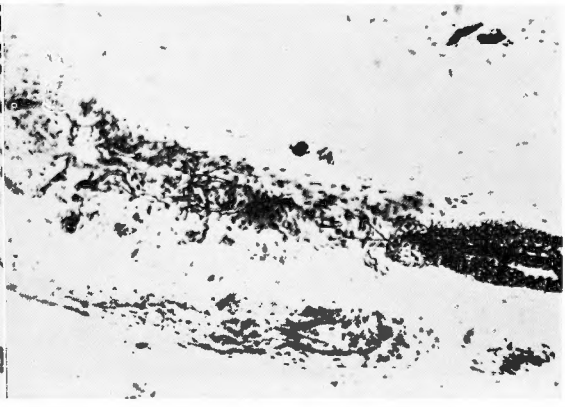

Fig. 42 Nerve fibers of hair root.

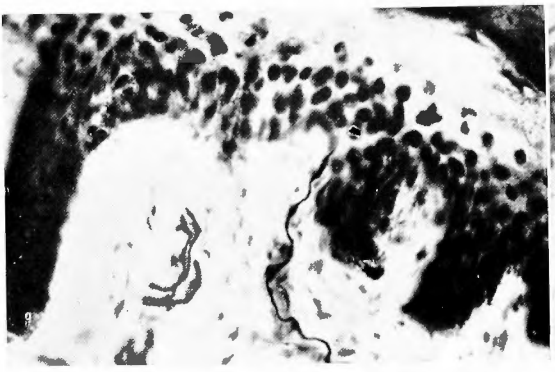

Fig. 43 Nerve fibers in the basal layer of epidermis.

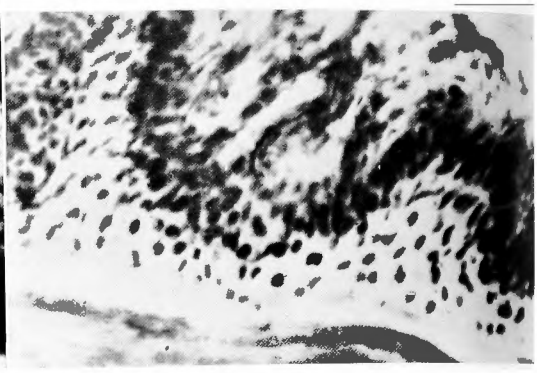

Fig. 44 Nerve fibers just beneath the basal layer of epidermis.

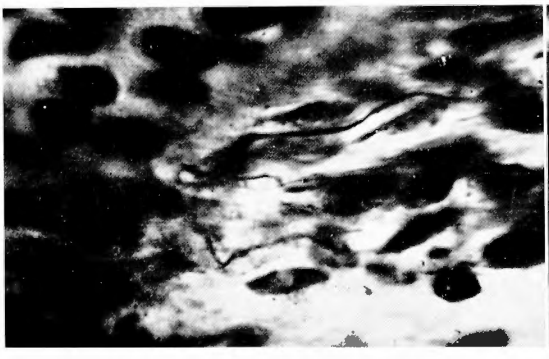

Fig. 45 idem.

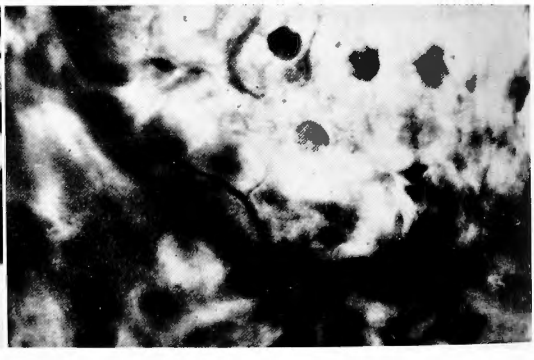

Fig. 46 idem.

Fig. 47 idem.

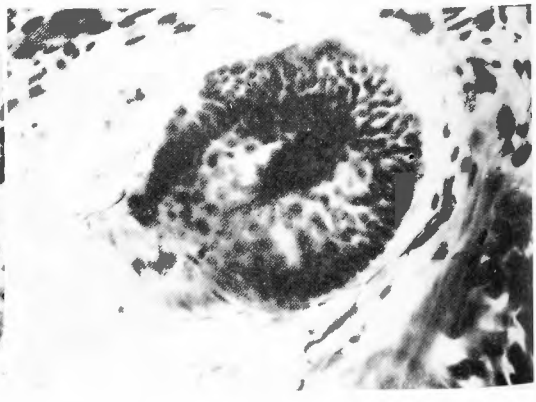

Fig. 48 Fragmentation of nerve fibers surrounding the hair follicle.

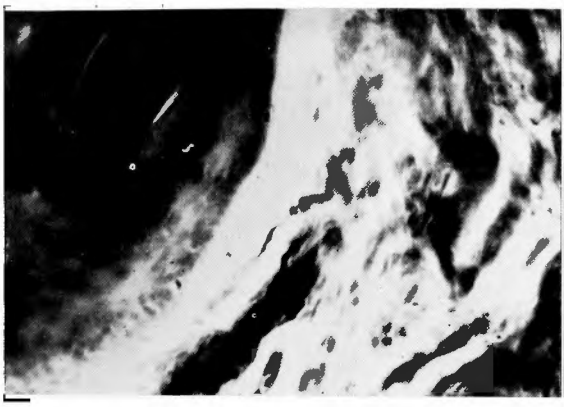

Fig. 49 Hair follicle in the tattooed area. Note fragmentation and spherical swelling of nerve fiber.

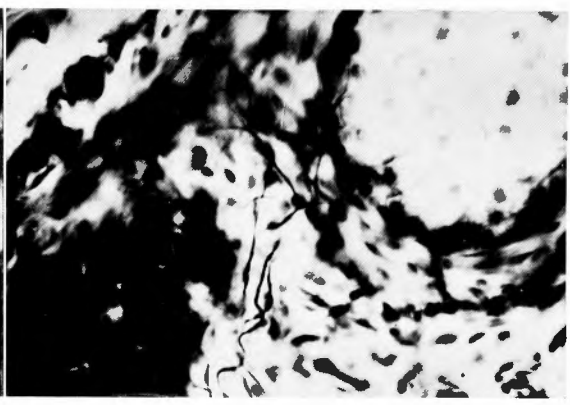

Fig. 50 Nerve fibers surrounding sebaceous gland.

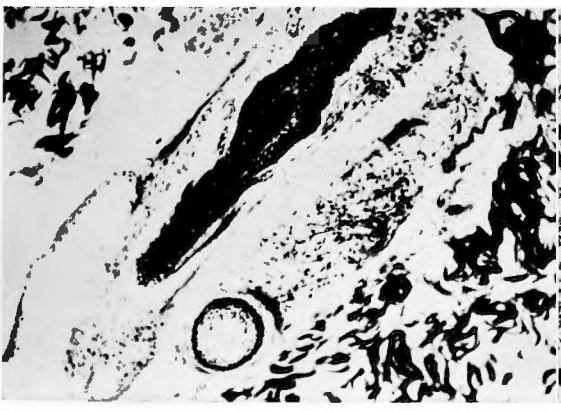

Fig. 51 Nerve fibers surrounding hair follicle.

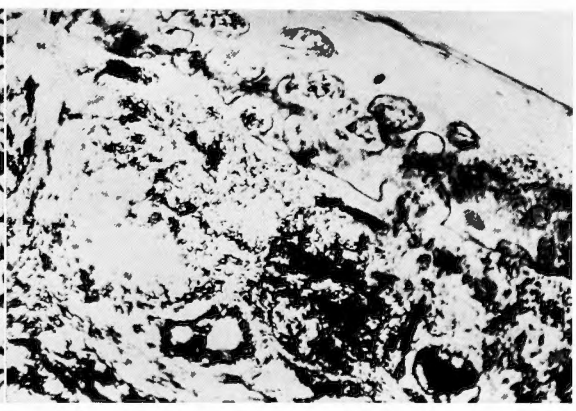

Fig. 52 Destructive changes in the skin of leprosy.

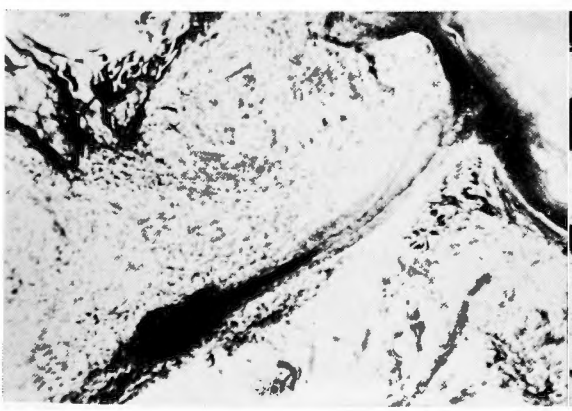

Fig. 53 idem.

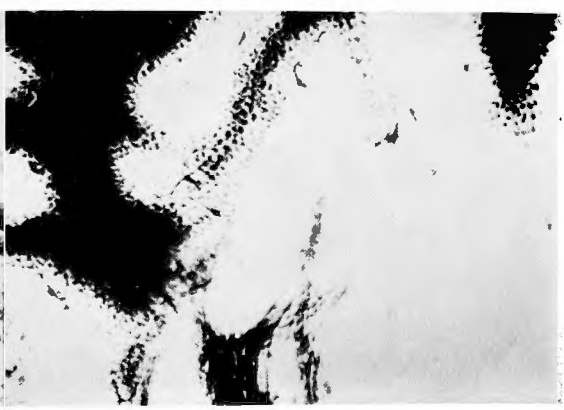

Fig. 54 Star-like cells in the stratum Malpighii of leprosy.

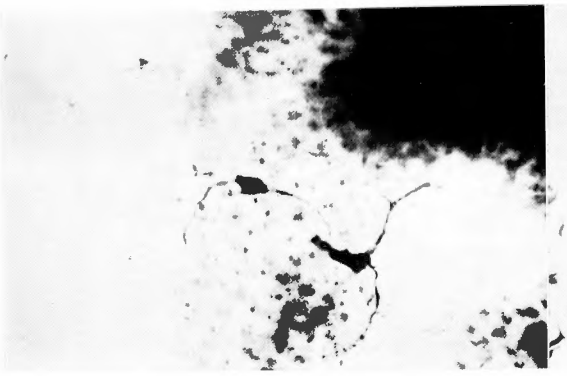

Fig. 55 Enlarged starshape cells.

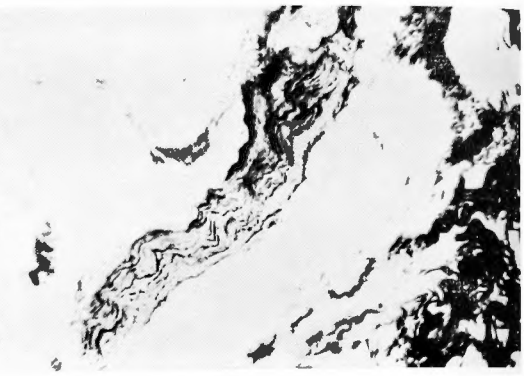

Fig. 56 Degeneration of nerve fibers in subcutaneous tissue.

Fig. 57 Enlarged figure of it.

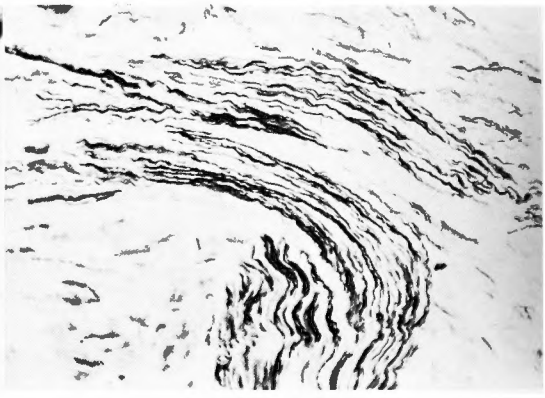

Fig. 58 Regeneration of subcutaneous nerve fibers in patient of causalgia.

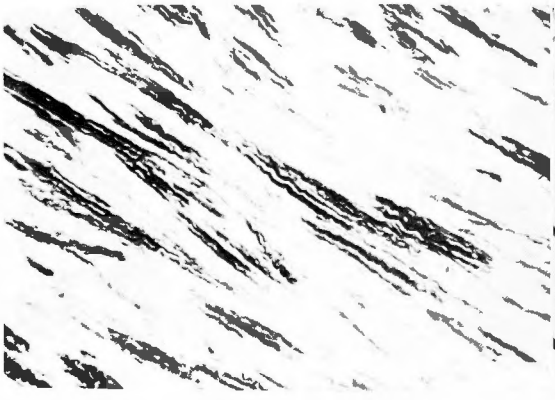

Fig. 59 idem.

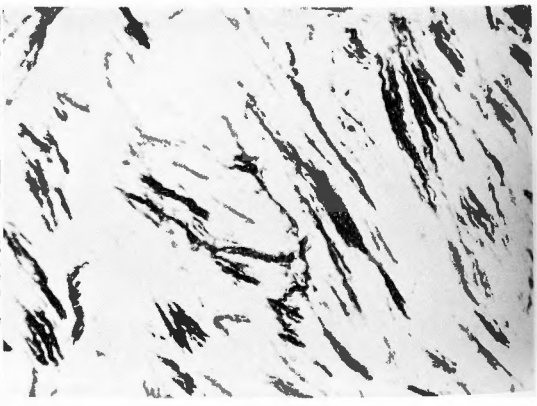

Fig. 60 Regeneration of nerve fibers in the cutis in patient of causalgia.

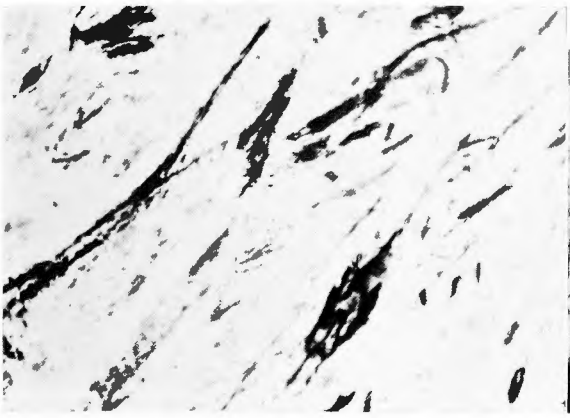

Fig. 61 idem.

Fig. 62 Fragmentation of frog's nerve fibers enclosed with physiological saline solution. After 15 minutes.

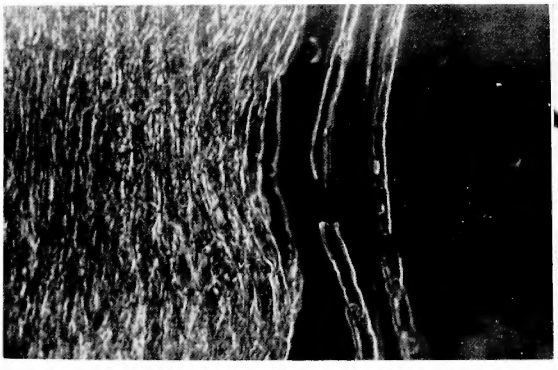

Fig. 63 30 minutes after the section.

Fig. 64 Formalin fixed nerve fiber beneath the epidermal layer of human foot sole. Segmentation seen in the preparation stained by silver method. Also vacuole formation is observable.

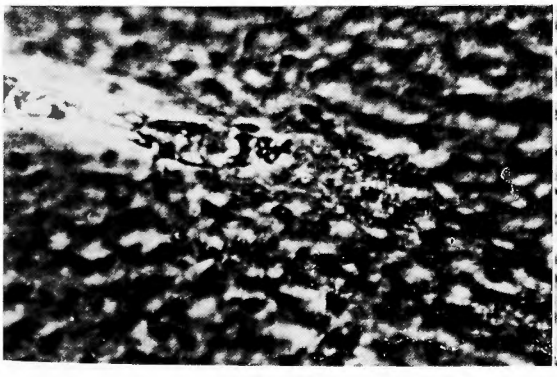

Fig. 65 P. C. M. figure of formalin fixed nerve fibers in tooth germ of calf. Note destructive changes of nerve fibers.

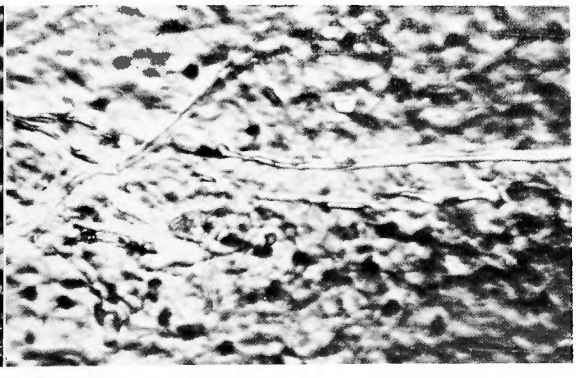

Fig. 66 idem.

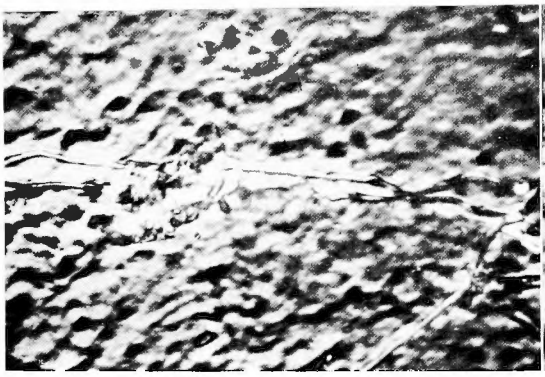

Fig. 67 idem.

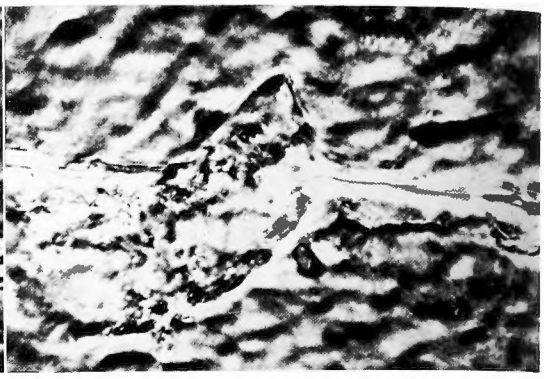

Fig. 68 90×7 Note destruction in myelin sheath, escape of axoplasm or globular changes of the axiscylinder.

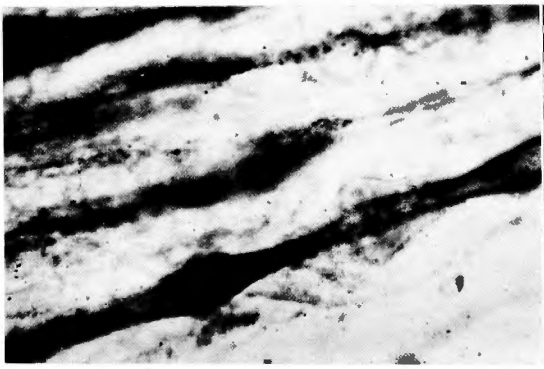

Fig. 69 Frog's sciatic nerve fibers fixed with formalin and stained by silver method. Rosary-like appearance of axis cylinder. P. G. M. Figure. 90××77

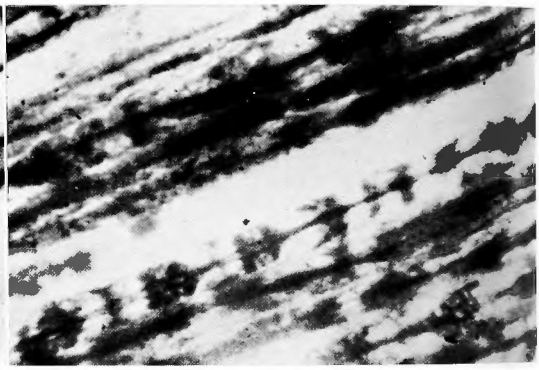

Fig. 70 idem. Typical feature of flying-shape segmentation in myelin sheath. 90×7

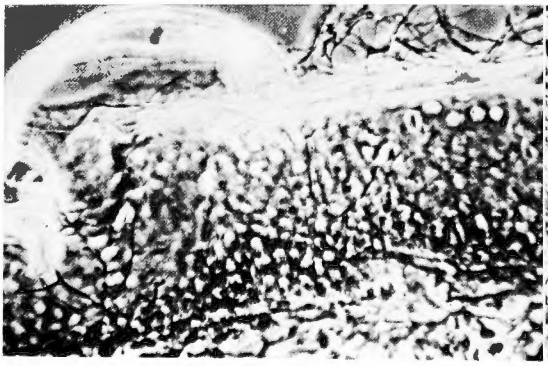

Fig. 71 P. C. M. picture of nerve fibers extend from basal layer to stratum lucidum in the epidermis without staining.

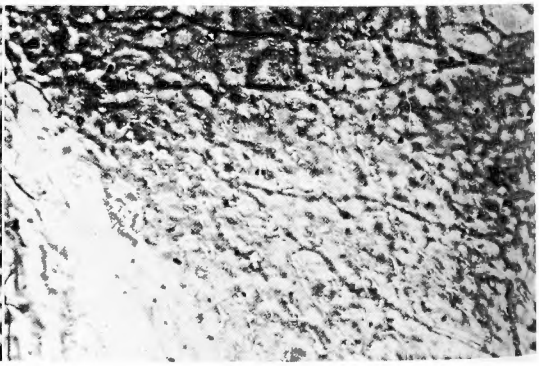

Fig. 72 Note nerve fibers comming from basal layer into stratum Malpighii.

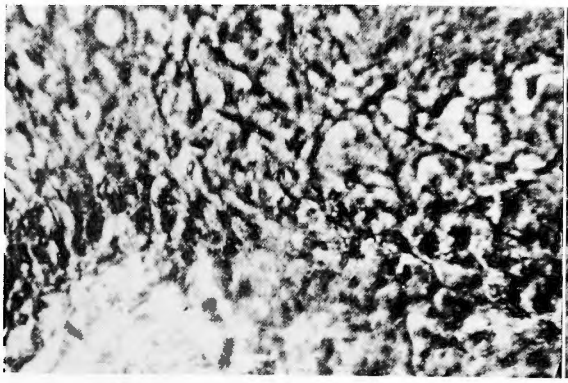

Fig. 73 idem.

Fig. 74 Nerve fibers in deep layer of stratum Malpighii.

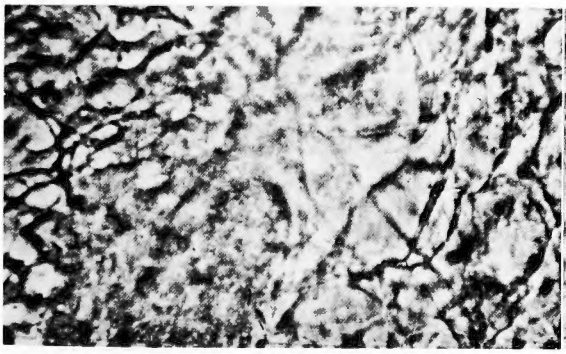

Fig. 75 idem.

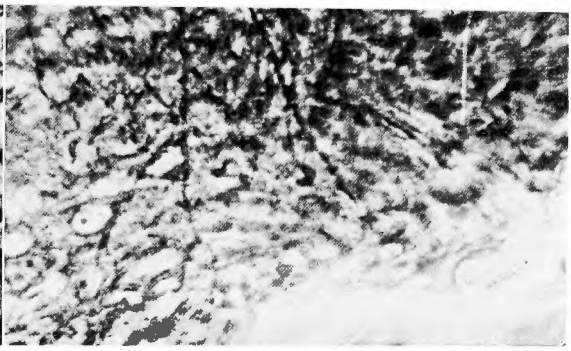

Fig. 76 idem.

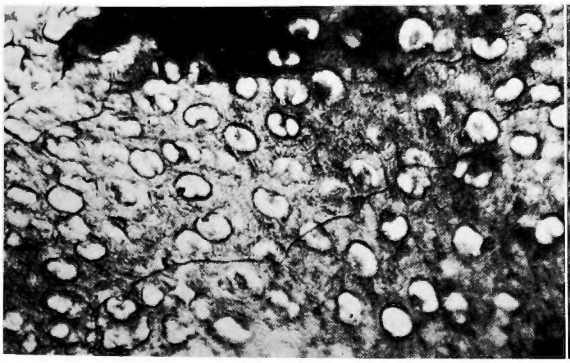

Fig. 77 Nerve fibers running through stratum Malpighii, stained by silver method.

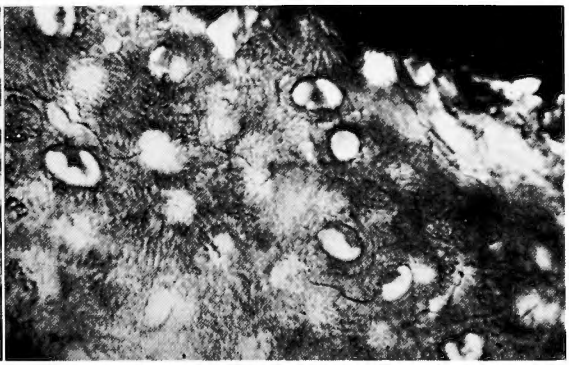

Fig. 78 idem.

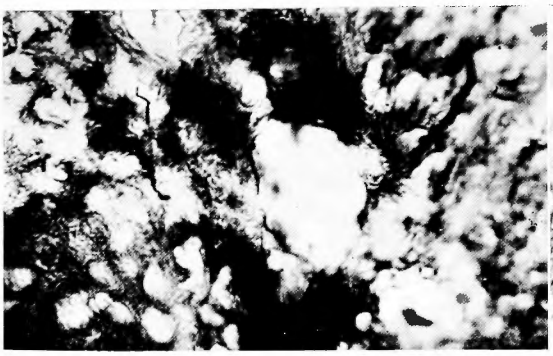

Fig. 79 idem.

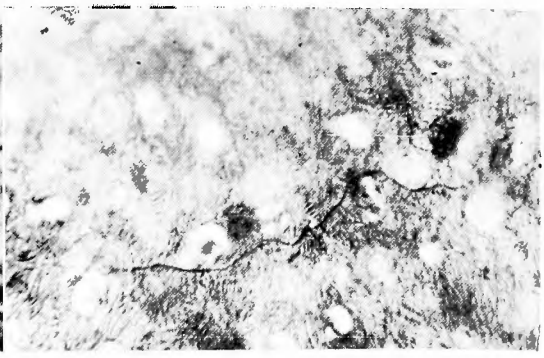

Fig. 80 idem.

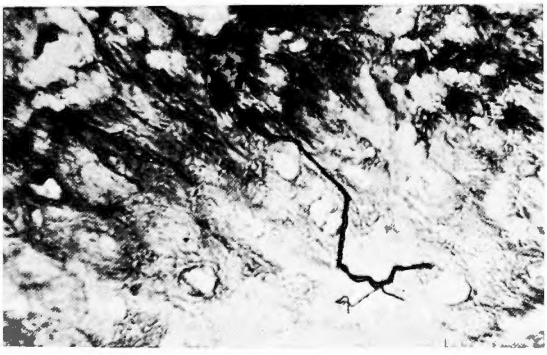

Fig. 81 idem.

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和 文 抄 録

位相差顕微鏡及び鍍銀法による神経線維の比較研究

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多年人体皮膚特にその上皮内神経を組織学的に立証すべく努力を重ねてきたが，知覚検査から推量する程数多くの神経線維を証明することは出来なかつた．即ち従来多くの人々も Frey の毛髪検査に際して有毛部に於ても無毛部においても触痛覚が高度に立証されるのにも拘らず，想像されるようには上皮内に豊富な神経支配が存在せず，極めて少ないか寧ろ上皮層内には神経線維は存在しないとの報告である．

茲に於て著者は標本採取時の化学的，機械的侵襲或は標本採取時の時間的推移に因る変化の為に，神経線維が形態学的に捉え難くなるのではないかと考え，神経線維そのものの動態を蛙の坐骨神経を用いて位相差顕微鏡により観察したところ，種々封入剤による変化及びその時間的経過を知ることができた．その結果神経線維は生体内に於ては直線状の髄鞘内に均質無構造の甚だ簡素な形態をとるものであるが，ひとたび生体外に取り出すと，切離直後乃至は操作中からすでに変化が始まり，数分で高度の変性像を呈することが判明した．即ち，従来知られていた Ranvierscher Schurring, Schmidt-Lantermannsche Einkelbung, Neurokeratingerüste あるいは Golgischer Trichter と称せられるものは悉く生体外に取り出された後に現われて来る変化像として認めることが出来た．又腸間膜中の末梢神経線維では之等の変化は更に進んで分節化，断裂を来し遂には球形，棍棒状のものとなり最後には消失するに到ることが認められた．

使用した封入剤の中では1%オスミウム酸緩衝液が最も生体内の構造に近く神経線維を保存することが出来たが，一般に神経鍍銀法の固定に好んで用いられるフオルマリンに対しては甚だしい破壊像を示すものである．

次に鍍銀法に於て認められた空泡形成，断裂像を同じく位相差法により検討したところ，組織中に髄鞘の破壊，軸索内容の洩出を認めることが出来て，之等の変化は正常組織にも認められるものであるから，病態組織の神経線維について，変性を云々する際には，臨床検査による知覚麻痺と病変部周辺の組織像とを比較し，充分に検討する必要があるものである．

発芽層下部に多く認められる Langerhans 氏細胞の本態に関しては，之が神経系細胞に属するものか或は色素細胞に含められるべきものかについて長く議論されているところであるが，既に神経線維の消失している癩の知覚脱失部の皮膚にも認められた之等の細胞群は寧ろ色素細胞に属すべきものと認めたい．

以上の様な各吟味のもとに皮膚上皮層内の神経線維についてその動態の観察を行つた結果，位相差法によつても，鍍銀法によつても上皮内には多数の神経線維が存在することを立証することができた．併し之等の終末形式に関しては今後の電子顕微鏡的検索結果に期待したい．