

A HISTOLOGICAL STUDY OF SENSORY NERVES IN THE SPLEEN

by

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I. INTRODUCTION

Sh. ASAI, A. KUBO and many other investigators have already proved the existence of splenic sensitivity in rabbits from the physiological standpoint, while KÖLLIKER, RIEGELE, NOMURA and others have confirmed the existence of the visceral afferent nerve fibers in the spleen. But few of them described the sensory nerve endings in full details histologically. Recently, H. SETO (Tohoku-University), Ch. KIMURA and others of our clinic have found sensory nerve endings in many various visceral organs, most of which end freely and can be distinguished from the end-apparati of the autonomic nervous system. In this histological study, sensory nerve endings were sought in the spleen in human beings and dogs, and then an experimental observation was attempted for the determination of the course of these sensory nerves.

II. MATERIALS AND METHODS

The materials used in this study were the spleens of human beings and adult dogs. The author used only fresh specimens taken from the spleen which were resected operatively. After fixation for 3-4 weeks in 10% neutral solution, the specimens were frozen, sliced in thickness of 30-40 μ , fixed again in 10% neutral formol solution for more than 5 months, and then stained.

The axis-cylinder was stained with SETO's or SUZUKI's modification of BIELSCHOWSKY's silver impregnating method, and JABONERO's silver carbonate method, while the myelin sheath was stained with EHRLICH's acid hematoxyline method.

For experimental observations, adult dogs were used. Operations were carried out under general anesthesia with the injection of isomytalsodium, and thoracotomy was performed under positive pressure breathing.

The spinalnerves or the vagus were sectioned at various points in my study in order to determine the course of the afferents nerves. Operations were carried out

on the dorsal and ventral roots of the spinal cord distal to spinal ganglia, and on the vagus nerve. Secondary degeneration of the nerve fibers in the spleen was observed mainly by EHRlich's method as well as by BIELSCHOWSKY-SUZUKI's method. Degenerated nerve fibers were sought for in the peripheral layer where autonomic nerves have already changed their neurons.

According to the results of many experiments performed by investigators of our clinic, peripheral nerves demonstrate the secondary degeneration 5~6 days after the section of the roots of spinal cord. Considering these results, the spleen of dogs were extirpated 5~6 days after rhizotomy. But after vagotomy, degenerated nerve fibers were sought in the spleen which was extirpated 7~10 days after operations.

Operations were performed as follows;

- 1) Section of the dorsal roots on both sides Th. 3-Th. 4
- 2) Section of the dorsal roots on both sides Th. 5-Th. 6
- 3) Section of the dorsal roots on both sides Th. 7-Th. 13
- 4) Section of the dorsal roots on both sides Th. 13-L. 3
- 5) Section of the dorsal roots on both sides L. 2-L. 5
- 6) Section of the dorsal roots on both sides S. 1-S. 3
- 7) Section of the ventral roots on both sides Th. 10-L. 1
- 8) Section of the dorsal roots on right side Th. 10-L. 1

Next, vagotomies were performed as follows;

- 9) Bilateral vagotomy in the thorax.
- 10) Cervical vagotomy on the left side at a point distal to the ganglion nodosum.
- 11) Cervical vagotomy on the right side at a point distal to the ganglion nodosum.

III. MICROSCOPIC OBSERVATIONS OF THE SPLEEN

Many investigators have maintained, from the physiological point of view, that the sensitivity of the spleen is mediated through the sympathetic nerves, while others from the histological standpoint, have confirmed that visceral afferent nerve fibers are certainly contained in these nerves. But few of them described the peripheral figures of the sensory nerve in the spleen of human beings and dogs.

They described that the myelinated nerve fibers which enter the splenic parenchym from the hilum lose most of the myelinsheath. According to the results of my experiments of human beings and dogs, myelinated nerve fibers are observed abundantly in the hilum and a few of them, together with the non-myelinated nerve bundles accompanied by the blood vessels, enter the splenic parenchym and are traced to the splenic pulp and to the branches of the blood vessels.

Observing the silver stained preparations, axis-cylinders show a similar distribution as myelinated nerve fibers. Axis-cylinders enter the splenic parenchym from the hilum and spread over the peripheral layer. In all portions the autonomic nerve fibers can be observed. They are fine and never terminate in free endings, but their neurofibrils form a fine network (Figs. 1 and 2).

Besides the autonomic nerve fibers mentioned above, thick nerve fibers are found in the spleen. These thick nerve fibers show characteristic varicosities and are easily distinguished from the fine networks of the autonomic nerve fibers. They run accompanied with the autonomic nerve fibers or separately from them and show a wavy appearance in the peripheral layer. A few of them run in the splenic pulp and seem to terminate freely along the wall of the smallest branches of splenic artery (Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12).

The course of these fibers are similar to those of the myelinated nerve fibers. (Fig. 13). Therefore, it can be deduced that these thick nerve fibers are identical with the sensory nerves described by H. Sero.

In the specimens taken from a patient suffering from BANTI's disease, a considerable number of myelinated nerve fibers in the hilum of the spleen are sometimes broken or show ampule-shaped swellings in places (Figs 14 and 15).

Nerve cell could not be observed in the hilum and parenchym of the spleen of human beings and dogs.

IV. DEGENERATION EXPERIMENT OF THE SENSORY NERVES IN THE SPLEEN

Considering the results of various experiments performed by many investigators from the anatomical or physiological standpoint, it can be assumed that the afferent nerves of the spleen must be derived from any of the thoracolumbar, vagal and sacral nerves.

Therefore, operations were performed as follows, in adult dogs.

Laminectomy was performed under general anesthesia with sodium isomylal. The spinal canal was opened, and the dorsal and ventral roots were separated carefully from each other and only the ventral or the dorsal roots were cut at a point distal to their ganglia on both sides or on one side. The spleen were resected 5~6 days after rhizotomy. Vagus nerves were cut on one side in the neck distal to the ganglion nodosum or on both sides in the thorax under positive pressure breathing. Specimens were taken out more than 6~10 days after vagotomy, and stained with EHRLICH's hematoxyline method. After experimental posterior rhizotomy myelinated nerve fibers in the spleen were broken at places giving a beadlike appearance, or stained unhomogenously, and sometimes degeneration granules were observed in them. All the degenerated nerves, demonstrated in this study, were easily distinguishable from normal myelinated nerve fibers.

- 1) Section of the dorsal roots on both sides Th. 3-Th. 4

No degenerated nerve fibers are found in the spleen.

- 2) Section of the dorsal roots on both sides Th. 5-Th. 6

A few degenerated myelinated nerve fibers are found in nerve bundles around the blood vessels in the spleen (Figs 16, 17 and 18).

- 3) Section of the dorsal roots on both sides (Th. 7-Th. 13).

In all cases, many degenerated nerve fibers are found in nerve bundles in the splenic hilum and around the blood vessels in the splenic parenchym. The myelin

sheaths of these degenerated nerves are stained unequally, broken in places and look like granules (Figs. 19, 20, 21, 22, 23 and 24).

4) Section of the dorsal roots on both sides Th. 12-L. 3

There are a few degenerated myelinated nerve fibers (Fig. 25 and 26).

5) Section of the dorsal roots on both sides L. 2-L. 5

6) Section of the dorsal roots on both sides S. 1-S. 3

In all cases, no degenerated nerve fibers are discovered in any portion of the spleen.

7) Section of the ventral roots on both sides Th. 10-L. 1

No degenerated nerve fibers are found in the spleen.

8) Right Vagotomy (in the neck distal to the ganglion nodosum)

9) Left vagotomy (in the neck distal to the ganglion nodosum)

10) Bilateral vagotomy (in the thorax)

No degenerated nerve fibers are observed in each case.

V. DISCUSSION

It is clinically accepted that splenalgia arises frequently as a symptom of the diseases of the spleen. Many investigators have reported histological and physiological findings which suggest the existence of sensitivity in the spleen. But from the anatomical point of view, the sensory nerve endings in the spleen has not yet clearly proved.

According to LANGLEY, SHEEHAN, BOEKE, STOEHR Jr., JAEONERO, SETO and others the autonomic nerve fibers usually form a fine network in the periphery without terminating freely and never degenerate, even if the preganglionic nerve fibers of them are sectioned.

The visceral afferent nerve fibers pass through the sympathetic trunk with cell-stations in the dorsal root ganglia and reach the effector organ without changing their neurons on the way. Therefore, when the spinal nerves are cut at a point distal to the dorsal root ganglia, these visceral afferent nerves should be degenerated throughout their course. They are free-ending thick nerves, and their peripheral structures are morphologically similar to those of the somatic sensory nerves. They are distinguishable from the autonomic nerves according to SETO.

In agreement with these opinions, many other investigators of our laboratory have described many studies in regard to the sensory nerves in various visceral organs.

The author also sought for the sensory nerves in the spleen of human beings and dogs.

In various portions of the spleen, the peripheral structure of the autonomic nerves are observed. As STOEHR Jr., JAEONERO and others have described, the autonomic nerve fibers usually form a fine network in the periphery of the spleen.

Besides them, there are also thick nerve fibers, which terminate freely in the periphery and can be easily differentiated from the autonomic nerves by their thickness. And they are considered myelinated until near the ending. Their course are

identical with those of the myelinated nerve fibers, which show clear degenerations when they are sectioned at the posterior roots. Judging from these facts, these thick nerves must be sensory in nature.

RIEGELE, KÖLLIKER, EDGEWORTH and others described such nerves like them in the splenic pulp of the different animals, but none in the splenic pulp of human beings and dogs.

These myelinated sensory nerve fibers enter the splenic cords from the hilum accompanied with the non-myelinated nerve bundles together with the blood vessels, and a few of them are found in the splenic pulp, and seem to terminate freely to the smallest blood vessels. They show the characteristic varicosities and are easily distinguished from the autonomic nerve fibers. (Fig. 27 and 28).

Most of these myelinated sensory nerves lose the myelin sheath near the endings. A few of them are large-sized nerves in the splenic pulp and the course of them are identical with those of the sensory nerve fibers. RIEGELE, NOMURA, SAKURAI and others described myelinated nerve fibers in the spleen, but none in the splenic pulp.

In the cortex few myelinated fibers and few thick nerve fibers were found.

In the spleen of human beings and dogs, these sensory nerve endings terminate freely or distribute to the smallest vessels. The author could not find a complicated structure or specific end-apparati, such as the VATER-PACINIAN corpuscle described by KUNTZ. These sensory nerve endings show simple-shaped or arborized terminations.

In the spleen of BANTI's disease, myelinated nerve fibers in the hilum are more abundant than in the normal spleen, and some of them are broken or swollen in places.

No ganglion was found in any part of the spleen of human beings and dogs.

KUNTZ, ISHIKAWA, ASAI and others have proved, from the physiological standpoint, that visceral afferent nerve fibers in the spleen are mainly derived from the dorsal roots of the spinal segments between Th. 9-Th. 12. According to the histological experiments by SAKURAI, SHISHIDO and others, the afferent nerve fibers in the spleen arise from the dorsal roots of the spinal cord between Th. 7-L. 2.

The results of histological experiments performed by the author as follows:

Sensory nerves of the spleen are derived from the dorsal roots of the spinal segments between Th. 5-L.1; most of them from Th. 7-Th. 10.

In the cases of section of the dorsal roots on both sides from Th. 7-Th. 10, degenerated nerve fibers are observed abundantly. In the case of section of the dorsal roots on the right side degenerated nerve fibers are not observable.

No degenerated nerve fibers are observed in all cases of vagotomies. Vagal afferent nerves must be few or do not exist in spleen. According to von EULER the spleen is innervated only by the adrenergic nerves and none of the cholinergic ones. The author's finding on the afferent innervation of the spleen proves that they run together with the adrenergic nerves and cause sympathetic reflex or visceral pain.

VI. SUMMARY AND CONCLUSION

Using BIELSCHOWSKY-SETO's and BIELSCHOWSKY-SUZUKI's silver methods, JABONERO's

silver carbonate method and EHRlich's acid hematoxylin method, the author studied the afferent nerves and their endings in the spleen of human beings and dogs. Examining the secondary degeneration of nerves in the spleen, the course of the afferent innervation of the spleen was determined.

Summarizing the results of the experiment, the following conclusion are obtained.

- 1) Myelinated sensory nerve fibers and their endings are observed in the spleen of human beings and dogs.
- 2) Sensory nerve endings are simple tapering of bifurcated terminations.
- 3) Some sensory nerves have myelin sheaths even near the termination.
- 4) No sensory nerve endings with a complicated structure or specific end-apparati are observed in the spleen.
- 5) Most of the sensory nerves of the spleen are derived from the dorsal roots between Th. 5-L. 1 spinal segment.
- 6) No degenerated nerve fibers are observed in any vagotomy case.
- 7) No nerve celles are found in the spleen.

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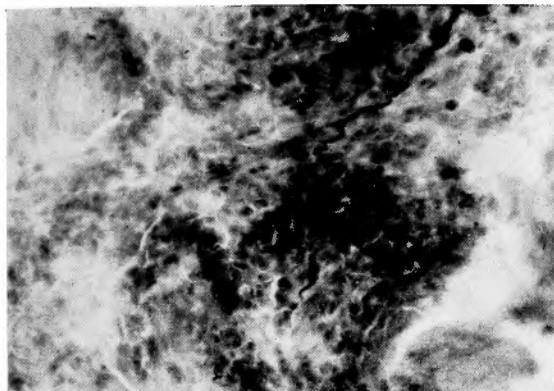


Fig. 1 A myelinated nerve fiber entering the splenic pulp from the cord (H) $\times 400$ E

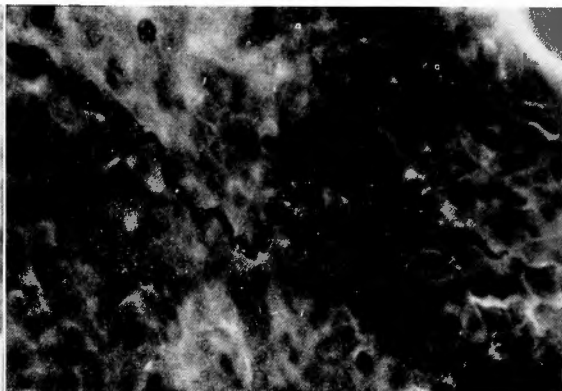


Fig. 2 A myelinated nerve in the splenic pulp (H) $\times 600$ E



Fig. 3 Autonomic nerves in the splenic cord (D) $\times 600$ B

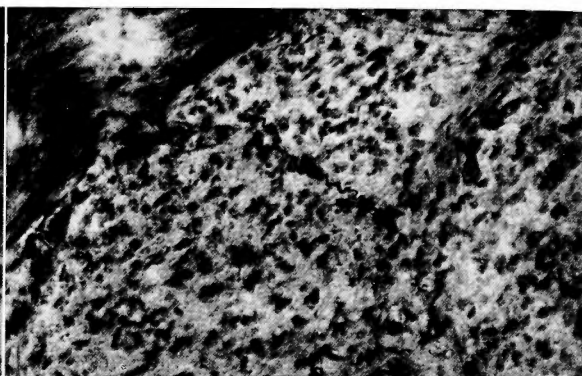


Fig. 4 Sensory nerve fibers entering the pulp from the cortex (D) $\times 400$ B

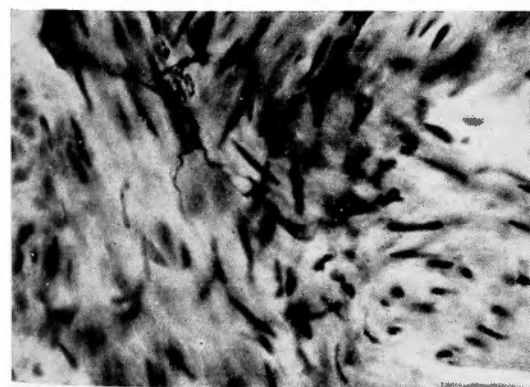


Fig. 5 Sensory nerve fibers near the blood vessels (D) $\times 400$ B

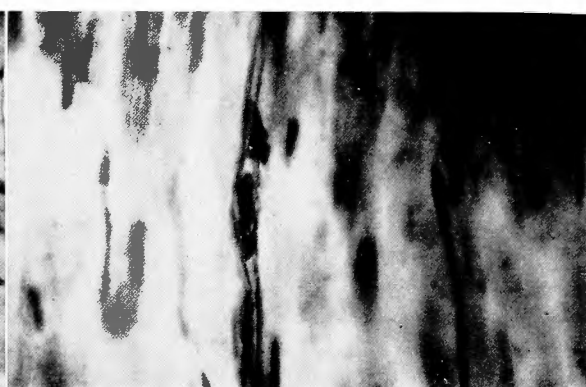


Fig. 6 A large myelinated nerve fiber in the splenic cord and autonomic nerves (D) $\times 600$ B

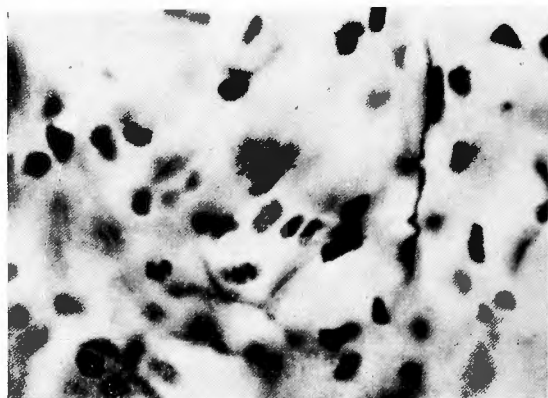


Fig. 7 A sensory nerve fiber terminating in the pulp (D) $\times 600$ B

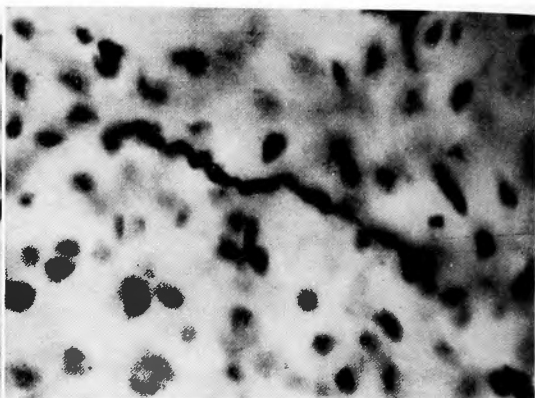


Fig. 8 A thick sensory nerve fiber in the pulp (D) $\times 600$ B

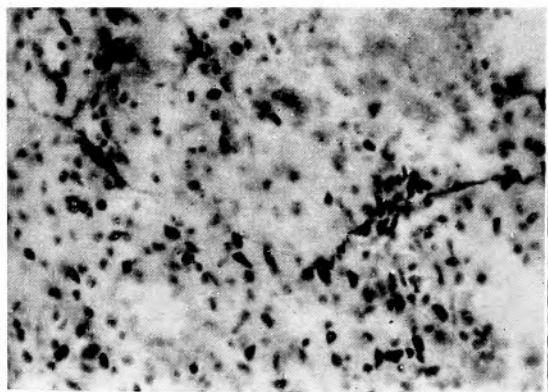


Fig. 9 A sensory nerve fiber running in the pulp from the cord to the another cord (D) $\times 400$ B

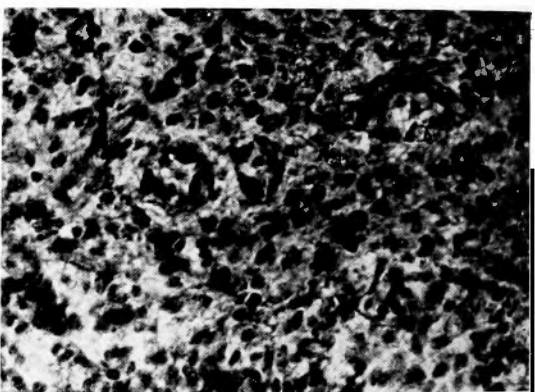


Fig. 10 A sensory nerve fiber running in the splenic pulp (H) $\times 400$ B

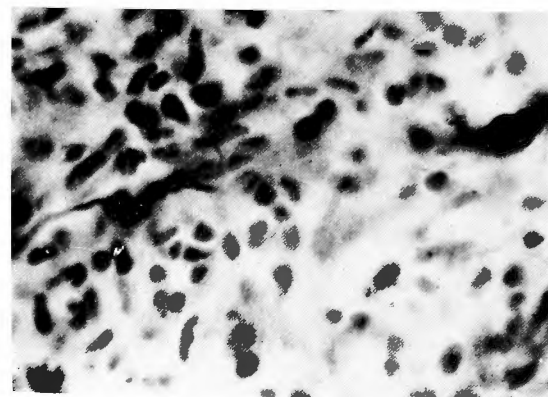


Fig. 11 Sensory nerve fibers arborized from the nerve bundle in the splenic pulp (D) $\times 400$ B

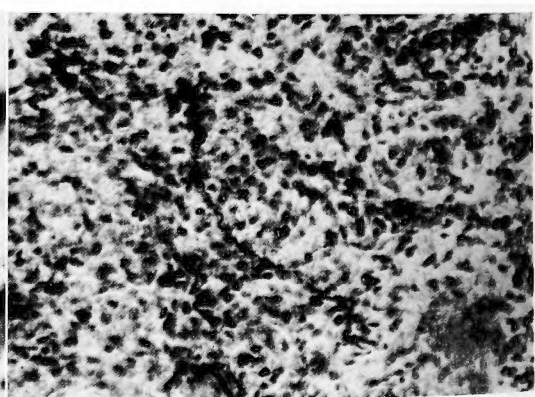


Fig. 12 Sensory nerve fibers in the pulp (H) $\times 400$ B

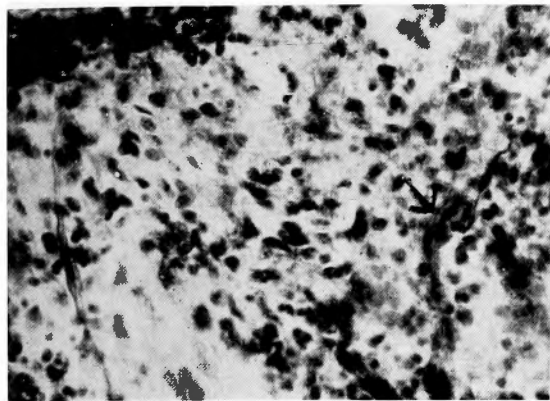


Fig. 13 A myelinated nerve fiber terminating to the wall of artery of the pulp (D) $\times 400$ B

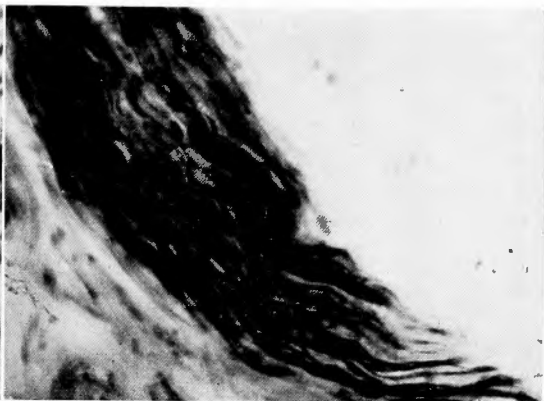


Fig. 14 Degenerated nerve fibers in the hilum of the spleen suffering from BANTT'S disease (H) $\times 400$ E

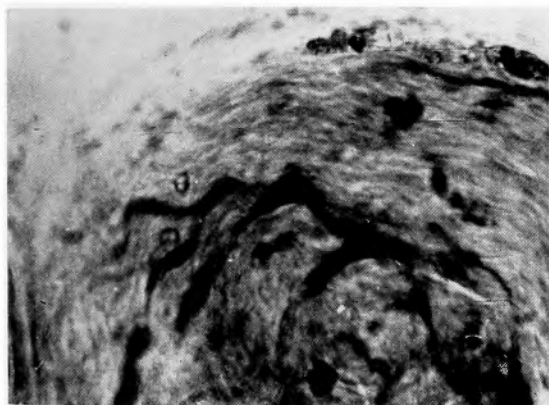


Fig. 15 Degenerated nerve fibers in the bundle of the splenic cord suffering from BANTT'S disease (H) $\times 400$ E

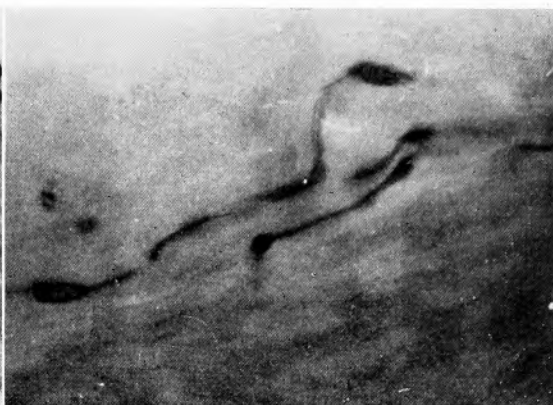


Fig. 16 Many degenerated fibers in a nerve bundle along the blood vessel with post. rhizotomy on both sides (Th₅-Th₆) (D) $\times 400$ E

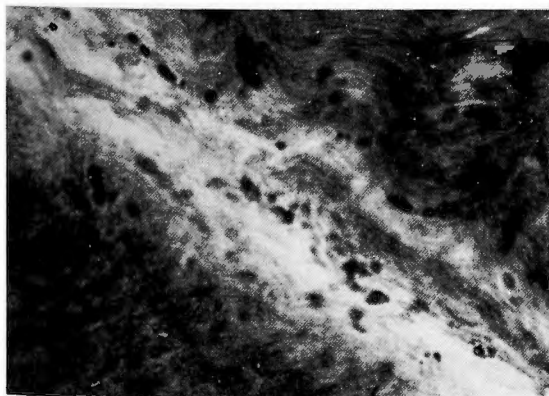


Fig. 17 Degenerated fibers in the splenic cord with post. rhizotomy on both sides (Th₅-Th₆) (D) /400 E



Fig. 18 A degenerated nerve fiber in the cortex of the spleen with post. rhizotomy on both sides (Th₅-Th₆) (D) $\times 400$ E

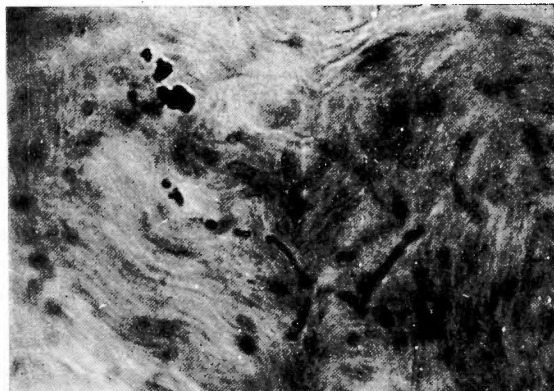


Fig. 19 A degenerated nerve fiber in the hilum of the spleen with post. rhizotomy on both sides (Th₇-Th₁₃) (D) ×400 E

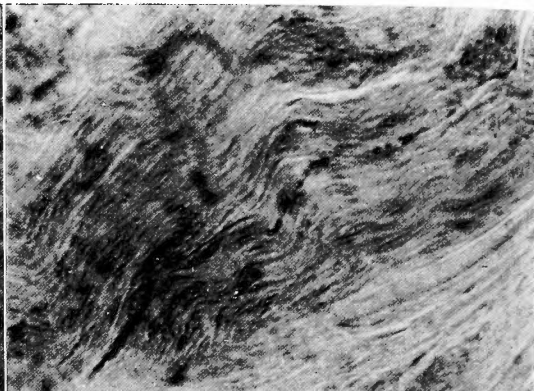


Fig. 20 A degenerated nerve fiber in a bundle of splenic hilum with post. rhizotomy on both sides (Th₇-Th₁₃) (D) ×400 E

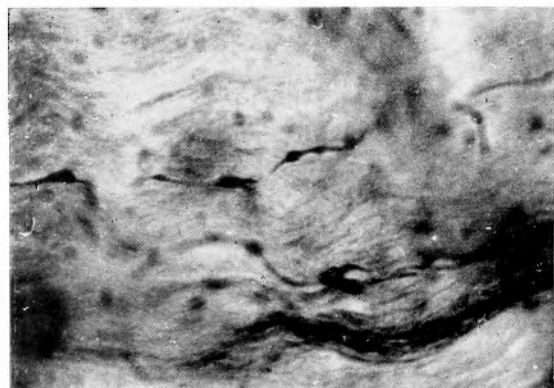


Fig. 21 Degenerated nerve fibers in a bundle along the splenic blood vessels with post. rhizotomy on both sides (Th₇-Th₁₃) (D) ×400 E

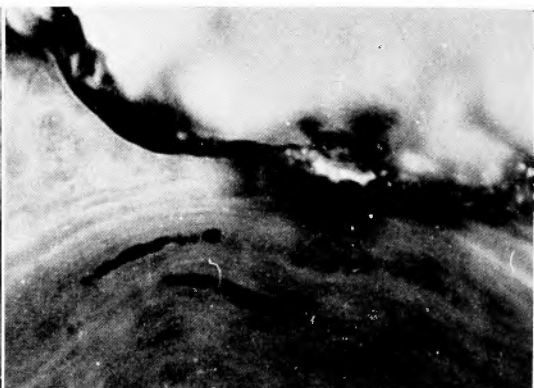


Fig. 22 A degenerated nerve fiber under the splenic cortex with post. rhizotomy on both sides (Th₇-Th₁₃) (D) ×400 E

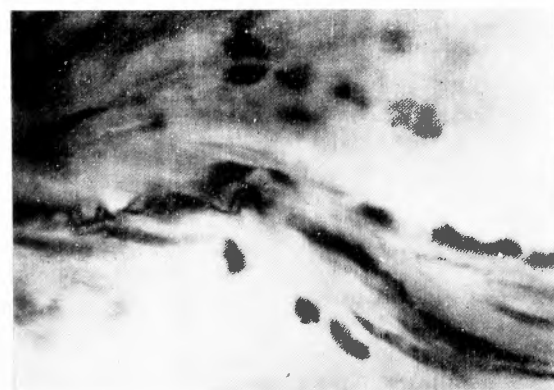


Fig. 23 Degeneration of axis-cylinder in the splenic cord along the blood vessel, accompanied by autonomic nerve, with post. rhizotomy on both sides (Th₇-Th₁₃) (D) /400 B

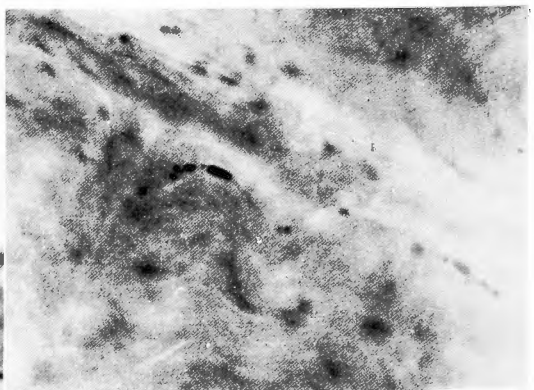


Fig. 24 A degenerated nerve fiber in a bundle of hilum with post. rhizotomy on the left side (Th₁₀-L. 1) (D) ×400 E

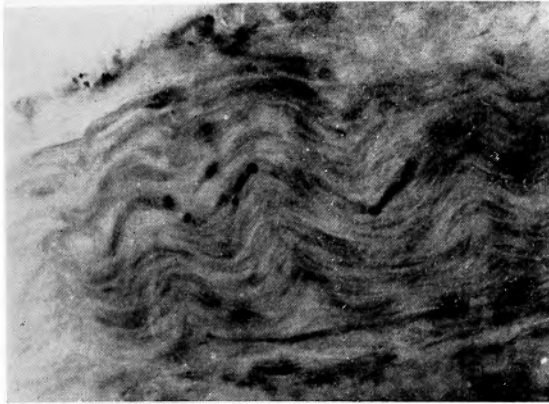


Fig. 25 Degenerated nerve fibers in a nerve bundle along the blood vessels with post. rhizotomy on both sides (Th₁₃-L. 3) (D) $\times 400$ E.

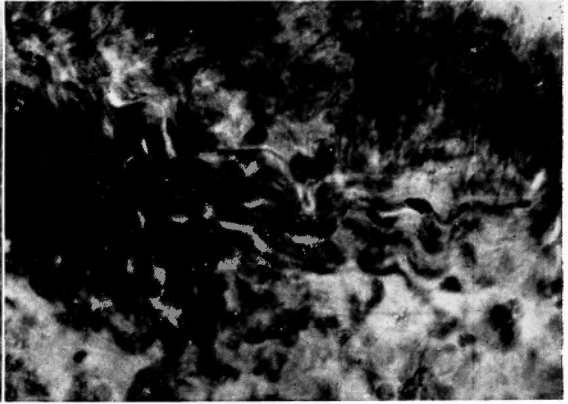


Fig. 26 Degenerated nerve fibers in a bundle along the blood vessel of the spleen with post. rhizotomy on both sides (Th₁₃-L. 3). (D) $\times 400$ E

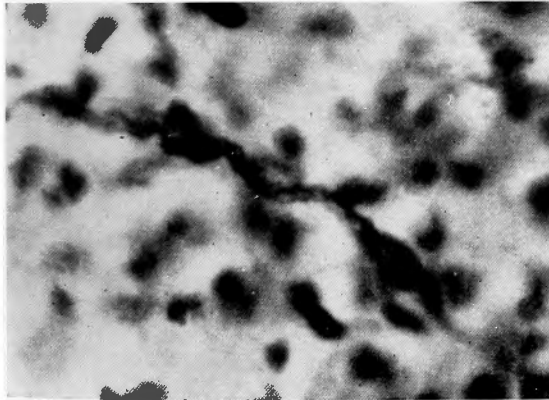


Fig. 27 A sensory nerve in the splenic pulp accompanied by autonomic nerves. (H) $\times 600$ B

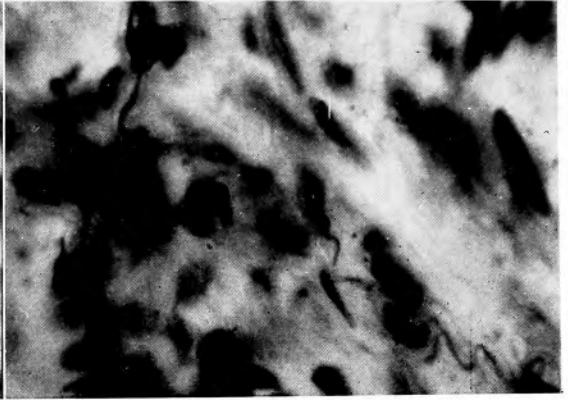


Fig. 28 A sensory nerve in the splenic cord accompanied by autonomic nerves. (D) $\times 400$ B

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Abbreviation

- B.....BIELSCHOWSKY's method.
 E.....EHRlich's method.
 H.....Human being.
 D.....Dog.

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脾臓の知覚神経に関する組織学的研究

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Bielschowsky 氏神経鍍銀法の瀬戸氏変法, 鈴木氏変法, Jabonero氏神経鍍銀法及びEhrlich氏神経髄鞘染色法を用い, 人及び犬の新鮮なる脾臓標本で知覚神経の形態及び分布を検索し, 更に迷走神経, 脊髄後根, 脊髄前根を両側或は偏側に於て実験的に切断した犬で, 脾臓に於ける末梢神経の二次的変性を追及し, 求心性神経経路を決定した。

そして実験結果から次の結論を得た。

- 1) 人及び犬の脾臓には有髓知覚神経とその終末が認められた。
- 2) 知覚神経の終末形式は, 非分岐性並びに単純性

樹枝状である。

- 3) 知覚神経は終末の近く迄髄鞘を保有している。
- 4) 複雑な構造, 特殊終末をもつた知覚終末は脾臓では観察されなかつた。
- 5) 犬の脾臓に於ける知覚神経の大部分は脊髄後根のTh.5-L1を通る。
- 6) 迷走神経を如何なる部分で切断しても変性は認められなかつた。
- 7) 人及び犬の脾臓に於いては神経細胞は認められなかつた。